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By Mallory Lynn Myers

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Developmental differences in hypothermic and behavioral responses to ethanol treatment in Alcohol Preferring and Non-Preferring Rats

For the degree of Master of Science

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DEVELOPMENTAL DIFFERENCES IN HYPOTHERMIC AND BEHAVIORAL RESPONSES TO ETHANOL TREATMENT IN ALCOHOL PREFERRING AND NON-PREFERRING RATS

A Thesis

Submitted to the Faculty

of

Purdue University

by

Mallory Lynn Myers

In Partial Fulfillment of the

Requirements for the Degree

of

Master of Science

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Indianapolis, Indiana

I would like to dedicate this to my parents who were always there,

in particular my mother who in all her infinite wisdom is always able to turn

“enough” into “funny” when I need her to.

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ABSTRACT

Myers, Mallory Lynn, M.S. Purdue University, December 2011. Developmental differences in hypothermic and behavioral responses to ethanol treatment in Alcohol Preferring and Non-Preferring Rats. Major Professor: Charles Goodlett.

Differences in voluntary consumption of ethanol have been negatively correlated with differences in initial sensitivity and tolerance to ethanol's pharmacological effects. From this perspective, both adolescent and adult alcohol-nonpreferring (NP) rats would be expected to be initially more sensitive to the sedative and hypothermic effects of ethanol and fail to acquire tolerance to those effects than preferring (P) rats. The first objective of this experiment was to assess alcohol-induced hypothermia and locomotor sedation in adolescent and adult P and NP rats over five consecutive daily administrations (saline, 1.5 g/kg, or 3.0 g/kg ethanol 17%v/v), testing the hypothesis that the P rats would acquire tolerance to the hypothermic response whereas the NP rats would not show changes across days. In addition, it was hypothesized that there would be age-related differences in initial sensitivity to ethanol, evident by adolescent rats displaying less ethanol-induced hypothermia and locomotor sedation than adult rats on Day 1. The second objective was to determine if conditioning was occurring between the administration environment and the hypothermic response and locomotor sedation elicited by ethanol exposure, via a sixth injection of saline. Female rats were surgically

implanted with intraperitoneal Mini Mitter telemetry probes on postnatal day 25 or 85 and experimental manipulations began five days later. Data were collected every minute; temperature data were then converted to change from baseline scores and locomotor data were totaled for each session. On Day 1, maximum temperature reduction elicited by the 3.0 g/kg dose was greater in the NP rats than the P rats, regardless of age. That dose also produced greater levels of locomotor sedation in the adult rats compared to the adolescent rats, regardless of line. The 1.5 g/kg dose of ethanol produced a greater hypothermic response in adult rats compared to adolescent rats, locomotor activity was reduced equally across the groups. With repeated administrations, NP adult rats displayed sensitization to the hypothermic response elicited from the 3.0 g/kg dose; in contrast, tolerance to the hypothermic response was found within the 1.5 g/kg dose for the adolescent P, adult P, and the adult NP rats. Repeated saline administrations also resulted in tolerance to the hypothermic response associated with administration in the adult NP and adolescent P rats. On the Day 6 saline administrations, adult rats which had previously been exposed to the 3.0 g/kg dose, maintained their baseline body temperatures better than both of the other exposure groups. Adolescent rats failed to show any signs of conditioning when administered saline on Day 6. Contrary to prediction the P rats failed to acquire tolerance to the 3.0 g/kg dose for either measure; and the line difference in ethanol-induce hypothermia was due to sensitization of the hypothermic response in adult NP rats. These results also provide further support that adolescent rats are less sensitive to the initial aversive effects of ethanol at the 1.5 g/kg dose for ethanol-induced hypothermia and the 3.0 g/kg dose for locomotor activity. The current experiment provides evidence that initial sensitivity as well as the acquisition of tolerance

to ethanol-induced hypothermia may be behavioral phenotypes correlated with selection for high and low alcohol drinking preference.

CHAPTER 1 INTRODUCTION

1.1. Objectives

Alcohol consumption is extremely common in America with over half of the population having consumed alcohol in the last year (Health 2007). While this mostly includes casual drinkers, some will develop a dependence on the drug. The number of individuals who abuse alcohol have been increasing, with approximately 10 million people meeting the DSM-IV criteria for alcohol abuse in 2002 (Grant et al 2004), costing federal, state and local governments nearly $468 billion in treatment-associated costs for 2005 (CASA 2009). It would be of great benefit to society to reduce the prevalence of alcohol use disorders. To do so, it is critical first to identify possible predispositions associated with alcohol abuse and dependence. While the mechanisms of addiction are unclear, it has been shown that alcohol abuse is influenced by genetics (Edenberg 2007, Enoch 2006) and age (Brown & Tapert 2004, Spear & Varlinskaya 2005). While these variables have been examined in isolation via human and animal experiments, a more systematic approach is needed to address how their combined impact alters alcohol use and abuse.

1.1.1. Genetic Predisposition

The strong genetic predisposition to alcohol abuse in humans has been observed. There is a by a four-fold increase in likelihood of abuse when there is a direct relative diagnosed with the disease (Goodwin et al 1974). Further evidence for a genetic link to alcoholism has come from the use of twin studies, in which genetically identical twins have a higher concordance rate for alcoholism compared to that of fraternal twins and other siblings (Grant et al 2006). The high concordance rate between identical twins is not substantially decreased when the siblings are separated soon after birth and raised in different environments (Grant et al 2006). When the general heritability of alcoholism is calculated, it is found to be between 40-60% (Schuckit 2001). Due to this strong genetic link displayed in humans, there have been attempts to selectively breed animals based upon free-choice alcohol drinking to establish an animal model of alcoholism within specific genetic groups.

Several selected lines of animals have been created based upon their willingness or unwillingness to consume alcohol voluntarily (Murphy et al 2002). Alcohol-preferring (P) and alcohol-nonpreferring (NP) rats are one set of divergent lines that were selectively bred from a closed colony of Wistar stock based upon their voluntary consumption of 10% alcohol when given a 2-bottle choice between ethanol and water. The P rat has been shown to be the most robust animal model for alcoholism that currently exists (Bell et al 2006a, Murphy et al 2002). This is due to the P rat meeting all of the criteria put forth for an animal model of alcoholism (Lester & Freed 1973, McBride & Li 1998). The P rat demonstrates a predisposition to voluntarily consume quantities of ethanol (>5.0 g/kg/day) large enough to result in a pharmacological impact

with blood alcohol concentrations (BACs) reaching 200 mg% (Lumeng et al 1982, Murphy et al 2002). NP rats consume relatively little alcohol (≤1.0 g/kg/day) under free-choice conditions. P rats will also work in an operant setting for access to alcohol as a reinforcer (Murphy et al 1989), will develop tolerance to the sedative-hypnotic effects of ethanol, as measured by performance in shock-motivated jumping task (Gatto et al 1987, Waller et al 1983) and will display signs of alcohol dependence (Waller et al 1983).

The selective breeding based upon a higher preference for alcohol (10% v/v) over water presumably resulted in line differences between P and NP rats in the pharmacological effects of the drug. However, this preference could also result from spurious variables unrelated to the oral self-administration of alcohol for its reinforcing properties, such as the need for higher caloric intake or a possible taste component. To test for alcohol preference without the confound of the oral sensory components, both P and NP rats were given the opportunity to operantly self-administer alcohol via intracranial [into the ventral tegmental area (VTA)] (Gatto et al 1994) or intragastric means (Waller et al 1984). The naïve NP rat, which metabolizes alcohol at a similar rate to the naïve P rat (Lumeng et al 1982), was markedly less likely to self-administer alcohol in either of these situations, thus demonstrating that the P rat will administer alcohol even when the oral component is bypassed (Gatto et al 1994). With initial metabolism similar between the lines, differences in the amount of alcohol consumed could be due to differences in their sensitivity to alcohol's effect.

Due to the fact that the P and NP lines were established based upon bidirectional selection for alcohol self-administration preferences, essentially two genetically similar animals with differing responses to alcohol were created. However, it is not always clear

exactly how these rats will respond to alcohol administration when assessed on measures not part of the selection criteria. The initial sensitivity of P and NP rats to alcohol has been shown to differ when rats from the two lines are administered the same dose of alcohol. For instance, following a 3.0 g/kg dose of alcohol, P rats recover from the loss of the righting reflex faster and at a higher BAC level than NP rats (Kurtz et al 1996). The NP rat has also been shown to develop a stronger conditioned taste aversion (CTA) to alcohol when compared to the P rat (Froehlich et al 1988).

While both the P and NP rat lines have a similar ethanol metabolism rate when alcohol naïve, P rats have shown the ability to acquire metabolic tolerance, as evidenced by changes in the elimination rate following chronic free-choice alcohol drinking (Lumeng & Li 1986). This difference in the acquisition of tolerance could explain why, when given free access to alcohol, P rats display drinking bouts that are prolonged and of greater frequency than those of NP rats (Files et al 1998). Differences also exist between the lines when concurrent access to multiple concentrations of alcohol is intermittently separated by periods of ethanol deprivation; in such instances, only in the P rats preference is shifted towards higher concentrations of alcohol (Rodd-Henricks et al 2000). Disparity is also seen between the P and NP lines in behavioral responding to repeated alcohol exposure, with P rats recovering from loss of the righting reflex faster following a second alcohol exposure when compared to the initial experience (Kurtz et al 1996). In contrast, NP rats sensitize to the sedative effects of alcohol between two exposures (Kurtz et al 1996). This suggests that there may be an innate difference between these lines not only in initial sensitivity to alcohol, but also in their experience following repeated administrations of the drug.

1.1.2. Age related susceptibility

In addition to genetic factors predisposing an individual to alcoholism, there are important developmental influences, with younger people being at higher risk to experiment with alcohol and potentially develop dependence than their adult counterparts (Monti et al 2005). A survey from the National Institutes of Health reported that over 30% of high school seniors had engaged in a binge alcohol experience (consuming five or more alcoholic drinks in one sitting) within the two weeks prior to the questionnaire (Johnson 2003). In another survey conducted in 2005, adolescent males (ages 15-17) self reported having consumed 36.7 +/- 2.4 alcoholic drinks in the previous 30 days (Newes-Adeyi et al 2007). In both studies the subjects were considered to be in the phase of development referred to as adolescence, which occurs from age 13 and continues into the second decade of life (Spear 2000). Although there is some debate as to the beginning and end of the adolescent period, it is typically defined by maturation of the reproductive system (Spear 2000). Adolescence also includes associated behavioral changes such as increases in risk taking and exploration (Spear 2000). Other biological changes occur, that accompany a surge in hormones, including neural plasticity, that seem to make individuals this age more accepting of drugs of abuse compared to their adult counterparts (Monti et al 2005). While adolescents are more susceptible to engaging in drug use, their experimentation has also been shown to be a good predictor of drug abuse in adulthood (Grant et al 2006). This is also true in animal models, in which adolescent animals have displayed preferential acceptance patterns compared to both adults and younger animals (Spear 2000). Adolescence in rats is considered to begin at postnatal day (PND) 28 and end on PND 42 for female rats and male rats. Similar to humans, it is

consistent with sexual maturation and also associated with profound behavioral and biochemical differences from adulthood (Spear 2000).

Experiments conducted using adolescent heterogeneous randomly-bred rats having no apparent genetic predisposition to alcohol abuse will consume quantities of alcohol (30% v/v) sufficient to yield pharmacologically relevant BACs (110 mg%) whereas their adult counterparts do not (Truxell et al 2007). Animal studies have also shown differences in the pattern of alcohol self-administration between adolescent and adult rats in both unselected rats (Vetter et al 2007) and lines selectively bred for alcohol preference (Bell et al 2006b, Bell et al 2011). Why the adolescent consumes more alcohol than the adult is not clear. One theory (Little et al 1996) postulates that the pharmacological effects of alcohol might change throughout ontogeny, resulting in adolescent animals having a differential experience during intoxication compared to adult animals (Little et al 1996). As a result, there has been an increase in experiments designed to identify differences between adolescent and adult animals that might explain this increase in alcohol self-administration during adolescence (Barron et al 2005, Spear 2004). Two different approaches have been taken in the literature, with one examining alcohol's lower aversive qualities and the other examining alcohol's greater hedonic qualities.

One aversive consequence of alcohol exposure is loss of basic body control, quantified by loss of the righting reflex as well as its return, following high dose administrations (4.0 g/kg). Little (1996) found that adolescent rats are less sensitive to the sedative effects of alcohol, evident by adolescent rats regaining their righting reflex in significantly less time than adult rats, following administration of the same dose of

alcohol. Not only did adolescents regain their righting reflex faster than adults, they were able to do so with more alcohol present in their system as evidenced by higher BACs at time of recovery. There was also an age-related change in pharmacokinetics with adult animals taking a longer time to reach peak serum concentrations (Little et al 1996). Other aversive properties of intoxication exist, such as the anxiety associated with withdrawal from a high dose of alcohol (4.0 g/kg). Anxiety is often measured using an elevated plus maze, where a higher ratio of time spent in the security of a closed arm versus time spent in open arms is an indication of increased anxiety. The same apparatus can be used to assess anxiety associated with withdrawal (Doremus et al 2003). In one such study of withdrawal-related anxiety, adult rats displayed more anxiety-specific behaviors in the elevated plus maze compared to adolescent rats, but only when differences in clearance rates were accounted for in the time course of the experiment (Doremus-Fitzwater & Spear 2007). This further strengthens the hypothesis that the reason adolescents willingly self-administer alcohol to a greater extent than adults is due to decreased initial aversion.

However, the literature also indicates that the hedonic experience of intoxication is increased for adolescents when compared to adults. Research examining heart rate increases (tachycardia) following drinking has found such physiological responses to alcohol intoxication to be appetitive, associated with increase in self administration, and to occur during the ascending limb of the BAC curve in humans (Brunelle et al 2007) and in rats (Ristuccia & Spear 2008). When given free access to alcohol, adolescent animals will self-administer large enough quantities to cause alcohol-induced tachycardia, while adult rats will not (Ristuccia & Spear 2008).

Regardless of the mechanism leading to the increased self-administration of alcohol seen in adolescents, other differences exist, such as the pattern of consumption (García-Burgos et al 2009). Previous studies in adults have shown a positive correlation between the magnitude of alcohol self-administration and the acquisition of tolerance for a 3.5 g/kg dose of alcohol (Millard 1983). This suggests that the increased consumption of alcohol by adolescent rats could be due to underlying differences in their ability to acquire tolerance.

Age-related differences in alcohol tolerance have previously been found, with quicker acquisition of tolerance to alcohol's aversive effects (loss of the righting reflex, impairments in learning ability, and ethanol-induced hypothermia) seen in adolescent rats compared to adult rats (Rajendran & Spear 2004, Silveri & Spear 2001, Swartzwelder et al 1998). However, when tolerance for the appetitive effects of alcohol are examined, adolescent rats show slower tolerance acquisition than adult rats (Silveri & Spear 1999, Varlinskaya & Spear 2004). This divergence in tolerance formation adds to the previous theory that the dissimilarity in alcohol consumption seen between these age groups is not only related to differences in the intoxication experience, but also in the acquisition of tolerance that follows.

The aforementioned experiments utilized rats from stocks that do not normally consume large quantities of alcohol in adulthood; therefore differences between adolescent and adult rats could be attributed to decreased consumption of alcohol and the subsequent associated behaviors in adults. By utilizing a selected line to model the genetic predisposition seen in human alcoholics such as the previously discussed P rat, it would be possible to create a model of alcoholism that addresses differences in initial

sensitivity and acquisition of tolerance to alcohol based upon age in those genetically predisposed to drink.

1.1.3. Genetic and Age Interactions

Relatively little work has been done up to this point using the selected rat lines to systematically analyze differences in alcohol responding at various developmental stages. Rodd-Henricks et al (2002a, 2002b) found that when adolescent and adult P rats were given similar lengths of prolonged exposure to alcohol, adolescent rats showed adaptive neurobehavioral changes compared to alcohol naïve adolescent rats; similar changes were not present in the adults. This series of experiments also showed behavioral alterations in peri-adolescent P rats following periods of alcohol pre-exposure. These alterations included quicker acquisition of operant alcohol self-administration, more difficulty extinguishing this behavior, and higher levels of responding following extinction and a period of home-cage rest compared to naïve counterparts when tested as adult rats (Rodd-Henricks et al 2002a). When the same alcohol pre-exposure procedure was conducted with adult P rats there were no differences in acquisition of alcohol self-administration, extinction, relapse, or alcohol-seeking behavior (Rodd-Henricks et al 2002a, Rodd-Henricks et al 2002b). The differences that occur in the adolescent but not the adult rat both biologically and behaviorally suggest that differences in tolerance acquisition exist between age groups in these selected lines of rats. While adolescent rats appears to differ from adult rats in selected lines as well as non-selected stocks, the mechanism underlying

these differences is still unclear, although it is possibly due to disparity in the initial quality of the experience and/or the resulting acquisition of tolerance/sensitization.

1.2. Core Body Temperature Change

Maintenance of body temperature, otherwise known as thermoregulation, is of critical importance for all living organisms considering protein integrity and enzyme activity function properly within a finite temperature range (Argyropoulos & Harper 2002). Given its importance, for endothermic creatures that must expend energy to actively modulate body temperature, research has been done to understand the neuronal mechanisms underlying thermoregulation. As early as the 1960s, the main areas responsible for thermoregulation had been identified within the central nervous system (CNS). Researchers pinpointed that the spinal cord, lower brain stem, hypothalamus, and septal regions were all involved in maintaining body temperature (Hammel & Pierce 1968). In particular, special attention has been given to the preoptic nucleus/anterior hypothalamic area (PO/AH), which displayed thermosensitivity (Satinoff 1978). Neurons in the PO/AH are designated as either warm receptive (10%), cold receptive (30%) or temperature insensitive (60%) (Boulant 1998).

When the PO/AH region is artificially heated, rats will produce corrective responses which lower body temperature. These behaviors were not elicited when this brain region was electrically stimulated, thereby demonstrating that the thermoregulatory behavioral responses elicited were specific to the application of heat to the PO/AH (Satinoff 1978). Lesions to the PO/AH region have produced rats that show deficits in

thermoregulation when put into a challenging environment (Srividya et al 2006). More recent studies have also supported PO/AH involvement, but not sole control, over alcohol-induced hypothermia as indicated by increased activation in the PO/AH region after intraperitoneal (IP) administration of alcohol, and no alterations in the hypothermic response following PO/AH lesion (Westerman et al 2010).

While the neuronal mechanism underlying hypothermia is not completely understood, the behavioral response has been extensively studied due to its dangerous and often fatal consequences. For instance, a sustained 1.5^{o}C change in body temperature is sufficient to produce brain damage (Gordon 1990). Since fluctuations from homeostasis can result in catastrophic consequences, organisms try to minimize thermal change so it is important that the method of measurement for body temperature be precise enough to detect even relatively small alterations. The surgical implantation of the Mini Mitter transponder allows for readings with accuracy of +/- 0.1^{o}C (Respironics Mini-Mitter: Bend, OR, USA). Along with advantages in the precision of data collection, the Mini Mitter also ensures a more accurate temperature reading by not increasing the stress level of the rat in comparison to the insertion of a rectal thermometer (Peris & Cunningham 1986). Increases in stress have been shown to lead to an increased rate of tolerance acquisition (Maier & Pohorecky 1985). More directly, the insertion of a rectal thermometer has been demonstrated to alter alcohol-induced hypothermia (Peris & Cunningham 1987). The repeated handlings of the animal may also alter other behaviors of interest such as locomotion and restraint stress (Trudeau et al 1990), as well as social defeat (Keeney et al 2001). When unaltered by stress through the use of a surgically implanted transponder, alcohol-induced hypothermia can serve as an accurate

measurement of the individual rat's physiological response to alcohol, which may be related to their intoxication experience (Peris & Cunningham 1987).

In addition to the stress associated with data collection through rectal probing, other experimental factors can modify the intoxication experience. For example, the dose of alcohol administered will increase the hypothermic response in a linear dose-dependent manner in the naïve rat (Lomax et al 1980), with larger decreases in body temperature being associated with greater conditioned taste aversion (Cunningham et al 1988). The ambient room temperature can also alter the intoxication experience (Finn et al 1989, Le et al 1986). Even when these variables are controlled, there is still variability in the hypothermic experience that can be attributed to factors such as genetics, age, and conditioning.

1.2.1. Genetic Predisposition

Body temperature decreases caused by alcohol administration appear to have a strong genetic component, evidenced by the ability to create selected lines of mice based upon differential hypothermic outcomes following 3.0 g/kg dose of ethanol (1.8^{o}C decrease in core body temperature in HOT mice and 3.6^{o}C in COLD mice) (Crabbe 1994). There is further support for a strong genetic versus a weak environmental component to alcohol-induced hypothermia according to studies conducted using cross-fostered animals. Rats having a genetic propensity to avoid the consumption of alcohol showed stronger hypothermic responses to alcohol administration, which remained unaltered regardless of their maternal environment. However, this was not the case for

other measures of aversion such as CTA, which was attenuated in Lewis rats cross-fostered to Fisher dams (Roma et al 2008). The measurement of alcohol-induced hypothermia also seems to be a steady predictor of future alcohol self-administration, regardless of the ambient environment's thermal impact on body temperature regulation (Cunningham et al 1992).

Alcohol-induced hypothermia is also a very sensitive measure of biological tolerance, due to the ability to detect changes within a relatively short time frame compared to other forms of biological tolerance. This is demonstrated by a reduction in the decrease in core body temperature following alcohol administration even while BACs remain unchanged (Ritzmann & Tabakoff 1976b). The degree of hypothermia is correlated with behavioral withdrawal symptoms, and can be used as a quantitative measure of the severity and time course of the withdrawal syndrome (Ritzmann & Tabakoff 1976a). Reports of a relationship between the physiological experience during intoxication and measurable hypothermic outcomes add support for our examining tolerance via core body temperature measurement.

1.2.2. Age-related susceptibility

Utilization of the alcohol-induced hypothermia measure in rats has enabled researchers to distinguish between age groups in initial hypothermic response as well as the acquisition of tolerance to ethanol-induced hypothermia. When three groups of rats ranging in age from 4 to 25 months were compared, the oldest rats displayed less of a hypothermic response to alcohol administration than the other two age groups. The oldest

rats also failed to develop tolerance to the hypothermic effects by the fifteenth administration (York & Chan 1994). The development of tolerance only by rats having a significant initial hypothermic response would support the idea that tolerance occurs in direct correlation with the initial level of disturbance to homeostasis (Kalant et al 1971).

While York and Chan (1994) used rats of various adult ages in their experiment, younger rats also experience alcohol-induced hypothermia. The research on hypothermia in very young rats is limited based upon rats being born poikilothermic, as seen with reptiles, and having complete reliance upon the mother's care to maintain homeostasis. However, adolescent rats [starting at PND 31] are able to maintain their core body temperature and are capable of being studied, even though maximum levels of resiliency are not achieved until PND 60 (Gordon 1990). A study of differences in the alcohol-induced hypothermia displayed by juvenile (PND 16), adolescent (PND 28), and adult (PND 56) rats receiving repeated administrations of alcohol was conducted by Silveri and Spear (2001). Following the initial administration of alcohol, juvenile and adult rats showed a greater decrease in core body temperature when compared to saline controls and adolescent rats exposed to alcohol. When tolerance was assessed, adult rats showed no change from their initial hypothermic response. Adult rats also had significantly higher scores than the other two age groups when body temperature was divided by BAC (determined 15, 60, or 105 minutes after injection), which was done to account for differences in ethanol metabolism. Thus, adolescent and juvenile rats displayed a decrease in this metabolism adjusted score when assessed for tolerance (Silveri & Spear 2001). Adolescent animals were not only significantly different from the other two age groups in initial responding but also in the acquisition of tolerance, supporting the

hypothesis that there are alterations occurring at this age that lead to a markedly different alcohol experience when compared to rats at other age points.

1.2.3. Genetic and Age Interactions

Experiments examining alcohol-induced hypothermia have also been conducted in lines selectively bred for alcohol preference or avoidance. These animals have a clear predisposition to either prefer or not prefer the intoxication experience, and have been tested for initial hypothermic response and the acquisition of tolerance to this effect (Stewart et al., 1992). Due to the design of the experiment, direct comparisons between P and NP rats were not conducted. However, body temperature was reported and when compared following the first administration of alcohol, the P rat had a greater hypothermic response, decreasing approximately two degrees while the NP rat's body temperature fell only one and a half degrees. Rats were then administered a second alcohol dose either 24 or 72 hours following the first administration. When the second administration occurred 24 hours later both lines of rats displayed tolerance, with their body temperatures dropping by about half a degree less. However, when the second administration was separated from the first by 72 hours, neither line showed tolerance and the NP rats actually developed sensitization, indicated by lager decreases in body temperature relative to the first alcohol dose (Stewart et al 1992). Evaluating differences in the pattern of ethanol-induced hypothermia responses in these two rat lines is valuable in determining what factors underlie their differences in alcohol consumption. While previous experiments have examined alcohol-induced hypothermia across ages, studies

addressing how the hypothermic response to alcohol intoxication of adolescent P and NP rats would differ from their adult counterparts have yet to be conducted.

1.2.4. Conditioning

Not only do alterations in the hypothermic response occur due to the acquisition of tolerance by biological adaptation, but repeated administration of alcohol can also cause alterations in body temperature as a result of a learned response. Multiple alcohol administrations in a specific context can lead to a pairing of the environmental cues and a decrease in body temperature, so that the procedure itself can produce an expectancy (resulting from associative learning) that can elicit a compensatory response even without the presence of the psychoactive drug (Le et al 1979). To examine this phenomenon, Crowell (1981) gave rats exposure to both alcohol and saline in alternate contexts for 20 experiences per condition, such that all rats experienced chronic alcohol treatment. Rats were then subjected to one of the following four conditions: alcohol in the alcohol context, saline in the alcohol context, saline in the context previously paired with saline administration, or alcohol in the saline context. The results demonstrated no change from baseline for both the saline in the saline context and the alcohol in the alcohol context groups, demonstrating the acquisition of tolerance to this effect. The rats that received alcohol in the initial saline context showed a large decrease in body temperature equivalent to that of their naïve experience. The group that received the saline injection in the alcohol context displayed a classically conditioned compensatory response, indicated by a significant increase in body temperature (Crowell 1981). This learned hyperthermic

response was in the opposite direction of the alcohol elicited hypothermic response, which allowed, at least in part, the rat to maintain baseline body temperature following alcohol administration. Measuring the amount of hyperthermia in a context without alcohol present allows for the possible determination of conditioned physiological changes following alcohol exposure, which appear to influence the expression of tolerance to alcohol's effects.

1.3. Locomotion

Behavioral activation measured by locomotor activity associated with drug administration has been suggested to be an indicator of the reward and abuse potential for drugs of abuse (Wise & Bozarth 1987). This holds true for alcohol intoxication, where the degree of locomotor stimulation observed following low-dose alcohol exposure has been shown to predict levels of future self-administration (Boerngen-Lacerda & Souza-Formigoni 2000, Chappell & Weiner 2008). The opposite is also true; the locomotor sedation resulting from the administration of a larger dose of alcohol has an aversive quality (Pohorecky 1977) and is likely associated with a decrease in operant self-administration of the drug (Worsham et al 1977). The development and expression of locomotor activity following repeated ethanol administrations also contains a learned aspect (Larson & Siegel 1998, White et al 2002a). Based upon these studies the measurement of locomotor activity, either increasing or decreasing, provides predictive information about future alcohol acceptance.

1.3.1. Genetic Predisposition

Similar to changes in core body temperature, locomotor activation resulting from alcohol administration has a genetic component, evidenced by the ability to create selected lines displaying divergent levels of this phenotype [FAST-SLOW mice (Crabbe et al 1987) and most affected (MA) or least affected (LA) rats (Riley et al 1976)].

There have also been changes in locomotor response displayed in rats bred for their ethanol consumption preferences (Waller et al 1986). A dose-response analysis was conducted for male adult P and NP rats using doses of alcohol ranging from 0.12 to 1.5 g/kg, results showed noticeably different locomotor activity patterns occurring between the lines. While the lowest doses (0.12 g/kg and 0.25 g/kg) seemed to produce some activation in both lines, it was only significant in the P rats. When NP rats were administered the 0.5 g/kg dose of alcohol or higher, they displayed a decrease in locomotor activity compared to their saline controls. The P rats never showed a significant decrease in motor activity, but also failed to show further locomotor activation, following administration of the 0.5 g/kg and higher dose (Waller et al 1986).

1.3.2. Age-related susceptibility

There are difficulties when trying to use locomotor stimulation or sedation as a measure in juvenile rats due to their limited ability to walk. Once rats become mobile, locomotor ability is relatively linear, as increases in gait and stride width occur linearly as a function of age (Parker & Clarke 1990). Use of the Mini Mitter transponder to record locomotor activity reduces gait confounds since its mechanism for measurement of

locomotion is by the transponder gaining and losing a radio frequency connection while the rat moves across a radio antenna grid (Respironics Mini-Mitter 2009), and not distance traveled per se. One disadvantage of the Mini Mitter transponder measure of locomotion is that it may not be sensitive enough to dissociate low-dose stimulatory effects. However, locomotor activity data gathered from this apparatus should be sufficient to detect motor impairment resulting from large doses of ethanol.

Research on rats of outbred origin indicate that motor impairments induced at lower doses of alcohol are greater in adults than in adolescents either measured with a tilting plane (White et al 2002b) or with activity counts (Little et al 1996). The outcome is consistent with a correlation with alcohol self-administration observed in heterogeneous/outbred rats, in that adult rats drink less than younger rats (García-Burgos et al 2009). While the adult P rat, will consume pharmacologically relevant amounts of alcohol (Bell et al 2006b) the adolescent P rat will consume even greater quantities when given access to alcohol through the drinking-in-the-dark-multiple-scheduled-access procedure (adolescent 3.4 g/kg and adult 1.6 g/kg) or continuous access (Bell et al 2011).

1.3.3. Genetic and Age Interactions

A study by Rodd and colleagues (2004) focused on the locomotor activity of adolescent P and NP rats following alcohol administration. In a paradigm similar to that used by Waller and colleagues (1986) to examine adult P and NP locomotion, Rodd and colleagues administered doses of alcohol ranging from 0.25 to 1.5 g/kg to create a dose-response curve for each line. The curve generated by the male adolescent P rats had

initial locomotor activation for the doses of alcohol ranging from 0.25 to 0.75 g/kg, as well as a significant reduction in locomotor activity with the 1.5 g/kg dose compared to saline controls. When the male adolescent NP rats were administered alcohol, no significant increase in locomotor activity occurred for any dose, and there was a significant decrease in locomotor activity at and above the 0.75 g/kg dose compared saline (Rodd et al 2004).

Based on these two different dose-response studies, several general conclusions can be made. Direct comparisons between the age groups are complicated by differences in the baseline rate of locomotion, with adolescent rats being more active both prior to and following saline administration compared to their adults. However, when the adolescent and adult NP rat are compared based upon the pattern of their dose response, it appears that the adolescent rats require a higher dose of alcohol than the adult rats to experience sedative effects, with the 0.5 g/kg dose resulting in sedation in adult but not adolescent rats.

Thus, while experiments have been conducted using age groups of rats from selected lines that best model human alcoholism, these studies were conducted at different times, which does not allow for direct comparison between the effects seen. To accurately assess if there are differences in initial locomotor sedation following alcohol administration, the current study was conducted to utilize both age groups of rats concurrently.

1.4. Purpose

Consumption of ethanol varies greatly based upon many factors. To better understand alcohol abuse and dependence, it is important to examine associated behavioral and physiological phenotypes in a controlled manner. While the adolescent P rat will self-administer ethanol in quantities producing blood ethanol levels that are pharmacologically relevant and that also exceed the intake of adult P rats, the differential mechanism of reward compared to the adult P rat remains unclear (Bell et al 2006b, Bell et al 2011). Furthermore, it has yet to be fully ascertained if the genetic selection for ethanol consumption of the P rat has altered responsiveness to other ethanol-related behaviors at different developmental stages in comparison to the non-preferring selected line of NP rats.

The current set of experiments were designed to address the role of genetics (through the comparison of P and NP selected lines) and development (by comparing adolescent rats to young adult rats) for differences in initial responding and in the acquisition and expression of tolerance to the physiological (hypothermic) and behavioral (locomotor) changes associated with ethanol administration. These experiments used Mini Mitter transponders to minimize measurement stress to the rats, thereby collecting data representative of alterations due mainly to ethanol administration, rather than stress associated with rectal probes. Lastly, these studies also examined possible differences in contextual learning of the selected lines, which may be attributed to the ethanol associated environmental context by a final administration of a vehicle injection in the environment in which repeated ethanol administrations were given.

1.5. Hypotheses

Based on studies described in this introduction, repeated administrations of 3.0 g/kg ethanol in P rats is expected to produce tolerance to ethanol-induced hypothermia, whereas the NP rats are not expected to displaying altered hypothermic response from the initial response. Tolerance to both the hypothermic response and locomotor sedation are predicted to be evident sooner in the adolescent rats compared to adult rats. Adolescent rats are also expected to show a less severe initial ethanol-induced hypothermia or sedation than adults. Lastly, repeated administrations of ethanol are predicted to result in a condition compensatory response when the animals are administered saline on the sixth day.

CHAPTER 2 METHODS

2.1. Experimental Design

This experiment utilized a between-subjects design (see Table 1). Female rats underwent experimentation as either adults (PND 90-95) or adolescents (PND 30-35; the youngest age possible due to minimum body cavity dimensions needed for surgical implantation of the transponder). Ethanol doses of 1.5 g/kg and 3.0 g/kg were used based upon previous research suggesting that these doses elicit measureable alterations in body temperature and locomotor activity. The effects of ethanol can greatly differ based upon the dose given, having an acute biphasic effect depending on when measurements are recorded (before or after the peak in the BAC curve). Low doses of ethanol can produce a stimulatory effect and correlates with greater self-administration, whereas a larger dose yields sedation and aversion (Lewis & June 1990). To examine aversion associated with ethanol, a dose of 1.5 g/kg or greater should be employed since it will result in both a decrease in body temperature (Lomax et al 1980) and locomotor sedation (Frye & Breese 1981) when administered to rats.

The concentration of ethanol remained constant for all doses at 17% (v/v), with the volume being adjusted for each dose, to limit effects of differences in concentration (Linakis & Cunningham 1979). For comparison purposes a saline control group was run with an injection volume equivalent to that of the largest dose of ethanol. Rats were pseudo-randomly assigned to one of three doses (saline, 1.5 g/kg ethanol, and 3.0 g/kg

ethanol) so that all doses were evenly represented within a cohort. Rats underwent a single injection of the designated solution per day for five consecutive days, which has been shown to be sufficient for the acquisition of tolerance to ethanol-associated effects (Bell et al 2001). On the sixth (and final) day, rats were treated as in the previous days except that all rats were given a saline injection; for a detailed experimental timeline see Figure 1. For the ethanol groups, the administration of saline on the sixth testing day in the context previously paired with ethanol allows for the assessment of possible contextual conditioning of compensatory responses that counter the expected effects of ethanol. Measurement of all physiological and behavioral data occurred through the Mini Mitter transponder and was recorded by the associated computer software package (Vital View Version 4.1: Mini Mitter: Bend, OR, USA).

2.2. Experimental Procedure

2.2.1. Subjects

The experiment used 137 female rats (33 adolescent P, 37 adult P, 34 adolescent NP, and 33 adult NP) obtained from the breeding colonies maintained at the Indiana Alcohol Research Center (School of Medicine, Indiana University, Indianapolis, IN). Rats were housed in an AAALAC accredited facility at PND 21or PND 60 and maintained in clear polycarbonate cages on ventilated racks (Lab Products Inc, Seaford, DE, USA) with food and water available ad lib. Those rats arriving at PND 21 for experimentation during adolescence were immediately single housed in preparation for

surgery, whereas the adults were double housed from arrival up until PND 80 when they were single housed prior to surgery. All manipulations were conducted during the light phase of the light/dark cycle (12 hour cycle, lights on at 7am) and in accordance with Indiana University School of Medicine IACUC approved protocols.

2.2.2. Surgical implantation

Animals underwent surgical implantation of a Mini Mitter thermal telemetry transmitter (E-Mitter: PDT-4000: Mini Mitter: Bend, OR, USA) in the peritoneal cavity. To alleviate possible discomfort associated with surgical procedures, carprofen (5.0 mg/kg) was injected subcutaneously (SC) using a 23 gauge needle two hours prior to surgery. Surgeries were conducted using sterile technique under isoflurane anesthesia with a flow rate between 18.75-27.0 cc/hr. The abdomen was shaved and rats were secured to the sterile field by tape so that they were positioned on their back. The shaved area was then cleaned with iodine (10% topical solution) and alcohol prior to the first incision which was through the skin only and approximately 30 cm in length. The skin around the incision was then stretched away from the muscle wall to create both a sufficient area for the second incision and enough flexibility in the skin for future closure. The incision in the muscle wall was a midline cut approximately 15 cm in length. A Mini Mitter telemetry probe sterilized in Cidex Plus (3.4% alkaline glutaraldehyde, Advanced Sterilization Products, Irvine, CA, USA) and stored in 70% ethanol (v/v) was coated in iodine and then inserted into the abdominal cavity. The muscle wall was sutured closed with a combination of surgeon's knots and running stitches, followed by the application

of lidocaine (0.1%) to the incision area. The skin was then closed with surgical staples and superglue. The dose of isoflurane gas was tapered off to a level less than 10 cc/hr prior to revival while the surgical tools were prepped for the next surgery. Surgeries typically took between four and eight minutes to complete and all rats were mobile within 10 minutes of the initial incision.

Following implantation of the Mini-Mitter transponder, animals were given a recovery period of five days during which they were monitored for pain (writhing, loss of weight/poor dietary intake, piloerection, etc). The day after surgery, all rats were administered a second carprofen (SC, 5.0 mg/kg) injection to relieve any possible surgical related pain. Once experimentation began animals were monitored for illness and/or infection based upon behavioral posturing, baseline body temperature and other observational means such as food and water intake by both animal care staff and the researcher.

On the last day of recovery animals were brought into the testing room and placed into an experimental chamber (44.45 X 25.4 X 38.1 cm) with opaque sides and pine shaving bedding to assess the connection between the probe, the receiver unit (E-Mitter: E-4000: Mini Mitter) and the computer software system. The Mini Mitter system works by producing an electrical loop (via radio frequency energy) between the probe and the position of the receiving area, creating several zones (three lateral planes, five longitudinal planes, and four vertical planes). These zones allow for probes to transmit locomotor information as the computer records when a signal is lost by one plane and picked up by another. However, since the Mini Mitter system does not record which

specific plane is crossed, it can only be utilized for reporting gross activity data and not specific types of movement.

To ensure these connections were functional, an animal was placed into the experimental chamber for three data cycles (temperature and locomotion recorded) before being returned to its home cage. The assessment lasted approximately three minutes. Once an animal was returned to its home cage, the next animal was placed into its specified experimental chamber for probe assessment. This process continued until the functionality of all the implanted probes had been verified.

2.2.3. Ethanol Exposure Paradigm

Following surgery animals were pseudo-randomly assigned to a solution group (saline, 1.5 g/kg ethanol, or 3.0 g/kg ethanol) so that each solution was equally represented within each cohort. Whenever possible test cohorts consisted of 12 animals comprised of four animals from each solution group. On test days, animals (PND 30-34 and PND 90-94) were weighed (scale: Sartorious GW3202, AG, Germany) in the vivarium before being transferred to the testing room (~21^{o}C) where they were immediately placed into their specified experimental chambers. The lights were turned off and animals were given 90 minutes to habituate to the chambers before receiving an intraperitoneal (IP) injection of their assigned solution. All solutions were mixed fresh daily and were warmed on a heating pad (50W 120VAC) to reach body temperature (~38^{o}C) prior to administration.

IP administration occurred between 1:00-3:00pm and took approximately 30 seconds per adolescent and one minute per adult, using a 25 gauge needle and a 23 gauge needle, respectively. Following IP administration, animals were left undisturbed for an additional 150 minutes. Throughout the 240 minutes that the animals were in the experimental chambers, locomotor activity and body temperature data were recorded in one minute increments from the telemetry probe. Upon conclusion of the test session, animals were removed from the experimental chambers and returned to their home cages, which were then transported back to the vivarium and placed in the ventilated rack. The pine shaving bedding used to line the floor of the experimental chambers was changed daily and the walls were wiped clean with soapy water. This process continued for four additional days, yielding a total of five consecutive IP injections of the assigned solution. Care was taken to alternate which side of the body cavity received the injection across days. On the sixth experimental day, animals (PND 35 and PND 95) underwent the same habituation and test procedure previously described, with the exception that all animals received an IP injection of saline equivolume to that used for the 3.0 g/kg dose.

2.3. Data Screening and Statistical Analysis

Surgery was completed on 150 rats, all of which produced reliable signal transduction when assessed prior to experimentation. The first cohort of rats (3 P and 4 NP adolescents) was removed due to incorrect carpofen dosing and six adult NP rats were removed due to health issues during the experiment. Only rats that underwent all five consecutive administrations were included in the screening process. Data screening was

done on core body temperature measurements with removal criteria established as having more than two consecutive 15 minute bins greater than two standard deviations above or below the mean on any given day. There were 11 outliers removed based upon performance during the tolerance assessment portion of the experiment (1 NP adolescent, 3 P adult, and 7 NP adults), leaving data from a total of 137 rats (33 P Adolescent, 34 NP Adolescent, 37 P Adult, and 33 NP Adult) for statistical analysis. An additional 7 outliers (1P Adolescent, 4 NP Adolescent, 2 NP Adult) were removed based upon the same criteria (thermal data) prior to analysis of data from the contextual conditioning day.

All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS: An IBM Company, 17th edition, Chicago, Illinois, USA). Thermal data were collected in one minute increments and averaged into 15 minute bins for analysis. Further data manipulation was done to assess the ethanol-induced hypothermic response, with the data being averaged across the habituation period (90 minutes) to create baseline values from which all of the post injection bins were subtracted. Maximal change from baseline scores were also created by accepting the most negative score (greatest hypothermic response) as the sole measurement for a given day. The baseline values were also compared on the first testing day (no prior ethanol experience) to ascertain if there were any initial differences in body temperature between the age groups and lines, evaluated via an ANOVA with between-subjects variables of age, line, and dose. A similar analysis was conducted as a mixed model ANOVA for Day (1-5) to compare baseline data alterations in temperature prior to injection across days.

To determine if differences in initial sensitivity to the hypothermic response existed following an acute administration of ethanol, change score data for Day 1 was

analyzed by a repeated measure ANOVA (bin 1-10) with between-subject factors of age, line and dose. Tolerance was assessed with the addition of Day (1-5) to the within-subject factors of the repeated measure mixed ANOVA. Differences in the time course of tolerance acquisition was determined for each dose and group by separate repeated measure ANOVAs where the data for each test day was compared back to that of the initial test day. Assessment of contextual conditioning on the sixth test day was determined by a repeated measure ANOVA for bin (1-10) with between-subject factors of age, line and dose.

Locomotor activity data were collected in the same fashion as thermal data (every minute), but the data were summed for the pre-injection and post-injection periods so as to create a measure depicting the total number of movements occurring prior to or following the IP administration of ethanol. It was appropriate to create these total movement scores for baseline (90 minutes) and the post injection period (150 minutes) due to the sporadic, burst-like nature of the rodent's movement. Initial differences in baseline locomotion were determined by an ANOVA for total locomotor activity prior to the IP administration on Day 1, with between factors of age, line and dose. Changes in baseline locomotion following subsequent administrations of saline or ethanol were detected through a repeated measure ANOVA for Day (1-5) with the same factors as above.

To determine if a single IP administration could elicit alterations in locomotor activity an ANOVA for the Day 1 post injection counts was analyzed. Tolerance (or sensitization) acquisition was assessed with a repeated measures ANOVA (Day 1-5) on the post injection locomotor totals. Differences in the time course of tolerance acquisition

was determined for each dose and group by paired t-tests where each day was compared back to the initial day; an adjustment to the alpha level was made based upon the number of comparisons made ($p=0.0125$). Assessment of contextual conditioning on the sixth test day was determined by examining the post injection total locomotor activity counts via a MANOVA utilizing the between-subject factors of age, line and dose.

CHAPTER 3 RESULTS

3. 1. Body Temperature

3.1.1. Day 1 baseline and ethanol induced hypothermia

On the first experimental day, significant differences in baseline core body temperature existed between the adolescents and adults ($F(1,137)=37.076$, $p<.001$) as well as between the P and NP lines ($F(1,137)=11.756$, $p=.001$). There were no other significant main or interactive effects. As shown for the pre-injection data in the upper panel of Figure 2, P rats had a higher average core body temperature compared to NP rats during the 90min baseline period. In addition, the adolescent rats had lower average baseline body temperatures compared to their adult counterparts. The injections on Day 1 resulted in a decrease in core body temperature at all three doses with the hypothermic response being dose dependent ($F(2,137)=56.880$, $p<.001$), such that the high dose of ethanol (3.0 g/kg) elicited the greatest loss in body temperature. Dose also interacted with line ($F(2,137)=6.889$, $p=.001$) and age ($F(2,137)=3.097$, $p=.049$), reflecting the stronger hypothermic response of the NP rats relative to P rats and of the adolescent rats relative to the adult rats. To facilitate the line and age comparisons (given the baseline body temperature differences), the raw temperature data were transformed into change (from baseline) scores for each of the ten 15-min post-injection intervals and the maximal

reduction in body temperature was then determined. The lower panel of Figure 2 depicts the average maximal (negative) change score, indicative of the maximal hypothermic response for each group on Day 1. For the 3 g/kg dose, the NP rats showed a significantly greater hypothermic response than the P rats, regardless of age [NP: -2.87+/-.14; P: -2.04+/-.14; $F(1,43)=11.450$, $p=.002$)]. For the 1.5 g/kg dose, there was a significant effect of age, with the adult rats showing an attenuated response relative to their adolescent counterparts ($F(1,49)=7.291$, $p=.010$), an effect more evident in the NP line. There were no line or age differences in the reduction of body temperature induced by the saline injection, which was matched to the volume of the 3.0 g/kg dose.

3.1.2. Changes across 5 days of injections

Figure 3 shows body temperature change scores (from average baseline body temperature) for the ten post-injection bins for all five days. Change scores were used because baseline differences in core body temperature were evident between the groups across the five days of testing ($F(4,500)=3.982$, $p=.003$). There were significant main effects of dose ($F(2,121)=164.616$, $p<.001$), line ($F(1,121)=13.887$, $p<.001$), age ($F(1,121)=17.818$, $p<.001$) and interactive effects of line x dose ($F(2,121)=77.240$, $p<.001$), as well as complex interactions with the day and bin repeated factors [Bin x Line x Age x Dose ($F(18,1089)=4.912$, $p<.001$), Day x Bin x Line x Dose ($F(72,4356)=1.449$, $p=.008$) and Day x Bin x Age x Dose ($F(72,4356)=1.480$, $p=.006$)]. Consequently, the body temperature effects were analyzed separately for each dose.

3.1.3. Emergence of line differences in sensitization with the 3.0 g/kg dose

For the 3 g/kg ethanol dose (Figure 3, bottom panel), across the five days of treatment the NP rats consistently showed greater hypothermic effects than the P rats ($F(1,38)=20.139$, $p<.001$). Across days, there was an unexpected yet pronounced increase in the hypothermic effects of the 3.0 g/kg ethanol injection evident only in the NP adults [Day x Bin ($F(36,1368)=2.309$, $p<.001$), Day x Bin x Age ($F(36,1368)=1.500$, $p=.030$) and Bin x Line x Age ($F(9,342)=7.354$, $p<.001$)]. As seen in Figure 3, the NP adult rats showed larger reductions in body temperature by the end of the treatment period compared to the first day. In contrast, the P adult rats did not show systematic changes over days in the profile of post-injection hypothermia following the 3 g/kg dose. These increasing body temperature reductions in adult NP rats are consistent with sensitization to ethanol's hypothermic effects. This was confirmed by follow-up paired t-tests comparing Day 1 and Day 5 change scores within each line/age combination. There was a significant main effect of Day ($F(1,90)=9.716$, $p=.011$) as well as a Day x Bin interaction ($F(9,90)=4.879$, $p<.001$) for the NP adults; the other three groups showed no significant effects of Day. Additional follow-up comparisons for the NP adults were conducted in which body temperature change scores for Days 2, 3, and 4 were compared with Day 1 scores. Significant effects were found for the Day x Bin interaction beginning with the second administration day [Day 2 ($F(9,90)=2.361$, $p=.019$), Day 3 ($F(9,90)=2.433$, $p=.016$), Day 4 ($F(9,90)=6.991$, $p<.001$].

3.1.4. Acquisition of tolerance to repeated 1.5 g/kg dose of ethanol

For the 1.5 g/kg dose (Figure 2, middle panel), adult rats showed consistently greater body temperature reductions compared to adolescent rats ($F(1,43)=22.978$, $p<.001$). However, across days for the 1.5 g/kg dose there were decreases in the hypothermic response (tolerance) displayed by all groups except for the adolescent NPs (Day x Bin x Line x Age ($F(36,1548)=2.061$, $p<.001$). When compared to their Day 1 responses, the adult and adolescent P rats and the adult NP rats all showed smaller reductions in body temperature on the 5th treatment day. To establish the first day on which tolerance was evident, body temperature change scores for each day were compared to the Day 1 body temperature change scores for each line/age combination. When compared to the first administration the NP adolescent was the only group not to show a change in hypothermic response [adolescent P rats ($F(1,9)=5.116$, $p=.047$), adult P rats ($F(1,12)=8.266$, $p=.014$) and adult NP rats ($F(1,12)=10.575$, $p=.007$)]. When follow up analyses were conducted to determine when differences from Day 1 began only the adolescent P rats showed a significant difference before Day 5 with the adjusted alpha level [Day 3 ($F(1,10)=9.371$, $p=.012$) and Day 4 ($F(1,9)=16.676$, $p<.001$)].

3.1.5. Changes over days in response to saline administration

Repeated administrations of saline also showed a change across day resulting in a significant attenuation of body temperature reduction from Day 1 (Day $F(4,160)=5.265$, $p=.001$). There were also significant interactions with age (Day x Bin x Age $F(38,1440)=1.498$, $p=.030$) and line (Bin x Line $F(9,360)=6.032$, $p<.001$). When the groups were analyzed separately, the saline injection elicited a hypothermic response on

the fifth day when compared to the response on the first day, adolescent P ($F(1,10)=10.074$, $p=.010$) and adult NP rats ($F(1,8)=11.336$, $p=.010$) showed less of a decrease in temperature on the fifth day.

3.2. Locomotion

3.2.1. Baseline (pre-injection) and post-injection locomotor activity on Day 1

Differences in baseline locomotor activity scores, shown in Figure 4, were evident prior to the first injection with the P rats having more locomotor activity counts (3364+/-94) than the NP rats (2442+/-99). Overall activity decreased following IP administration, and as expected rats given ethanol showed greater reduction in locomotion scores than saline controls ($F(2,121)=114.879$, $p<.001$). The significant interaction of Dose x Line ($F(2,121)=4.509$, $p=.013$) is likely due to differences based upon line remaining in saline exposed rats, similar to that observed in baseline, whereas the high dose of ethanol produced similar levels of sedation in the P (393+/-70) and NP rats (366+/-70). In the rats administered 3.0 g/kg ethanol, there was an age effect in which the adult rats showed less activity compared to adolescent rats [adolescent rats (2080 +/-110.) and adult rats (1628 +/-110)].

3.2.2. Acquisition of tolerance to repeated 3.0 g/kg doses of ethanol

To compare across the five treatment days, locomotor activity counts were totaled for the entire 150 minute experimental procedure, as shown in Figure 5. The dose response of ethanol-induced reduction in locomotor activity remained throughout the

experiment, with the 3.0 g/kg eliciting the most locomotor sedation ($F(8,484)=2.490$, $p=.012$). A main effect of age was also present in which adolescent rats showed more activity than their adult counterparts ($F(4,484)=2.602$, $p=.035$). There was also a significant interaction between Line x Dose ($F(2,121)=14.239$, $p<.001$). When tolerance is defined as an increase in locomotor activity from that observed on Day 1, only the NP adults that received 3.0 g/kg ethanol displayed tolerance beginning with the second day of administration and continuing on experiment Day 2 ($t(10)=-4.835$, $p=.001$), Day 3 ($t(10)=-3.127$, $p=.001$), and Day 5 ($t(10)=-5.437$, $p<.001$). The tolerance to the locomotor sedative effects of ethanol in the adult NP rats stands in striking contrast to their development of sensitization to the hypothermic effects of ethanol.

3.3. Contextual Conditioning

After receiving five daily IP injections of their assigned solution, on the sixth day all treatment groups were administered an injection of saline and then monitored for core body temperature (Figure 6) to assess expectancy effects (conditioned compensatory responses). Adult rats of both lines previously given the 3.0 g/kg ethanol dose showed a strikingly attenuated change in body temperature after the saline injection compared to the adult group previously given saline [P rats ($p=.003$), NP rats ($p=.011$)] or 1.5 g/kg ethanol [P rats ($p=.006$); and NP rats ($p=.049$)]. In fact, for the adult P and NP groups previously given 3.0 g/kg ethanol, the Day 6 saline treatment did not produce a significant change (decline or increase) in body temperature, whereas all adult groups previously given saline or 1.5 g/kg show the typical modest hypothermia in response to injection of saline on Day 6. In contrast, the adolescent groups showed no differential

effects of prior treatment, and all three prior treatment groups generated modest hypothermic responses to saline on Day 6. These differences yielded main effects of prior treatment ($F(2,120)=5.853$, $p=.004$), age ($F(2,120)=6.402$, $p=.002$) and an Age x prior treatment interaction ($F(18,1080)=2.824$, $p<.001$).

Total locomotor activity counts occurring after the Day 6 saline administration (Figure 7) show a main effect for age ($F(1,128)=11.145$, $p=.001$) and line ($F(1,128)=20.275$, $p<.001$). Previous administrations of saline, 1.5 g/kg, or 3.0 g/kg did not produce a difference in total locomotor counts when rats were administered a saline injection on Day 6. There continued to be no significant effect of solution when examined within groups.

CHAPTER 4 DISCUSSION

4.1. General Findings

The first hypothesis that line differences in ethanol-induced hypothermia following repeated administration of the 3.0 g/kg dose of ethanol would be expressed as greater acquisition of tolerance in the P rats was not confirmed. The P rats showed no significant change in hypothermia over days. However, a line difference in ethanol-induced hypothermia was present due to the striking acquisition of sensitization to the hypothermic response in the NP rats. Notably, the NP rats had greater hypothermic responses to the 3.0 g/kg dose than the P rats, consistent with greater initial sensitivity to this effect of ethanol. The second hypothesis that adolescent rats would be less sensitive than adults to the hypothermic and sedative effects of ethanol was confirmed for the 1.5 g/kg dose for hypothermia and for the 3.0 g/kg dose for locomotor activity. Finally, repeated administration of 3.0 g/kg to the adult rats of both ages was sufficient to produce a classically conditioned compensatory response in body temperature; locomotor activity on the saline test day did not show and differential effects of the prior treatments.

4.2. Ethanol-Induced Hypothermia

4.2.1. Sensitization to the 3.0 g/kg dose of ethanol

Because baseline differences in temperature among the groups complicate the interpretation of the changes following drug administration, post-injection scores were evaluated as change from baseline scores to facilitate group comparisons of the effects of the injections relative to individual baseline temperatures. The 3.0 g/kg (17%v/v) ethanol dose produced a hypothermic response differed in magnitude across the lines, with the NP rats showing a more severe hypothermia than the P rats. This line difference in initial response to ethanol is consistent with reports of other aversive effects of ethanol being more pronounced in animals that do not willingly self administer ethanol (Little et al 1996). These data further strengthen the theory one correlate of the differences in the P and NP rats voluntary consumption of ethanol is that the aversive effects of ethanol appear to be less severe in the alcohol-preferring animals compared to the non-preferring animals. The current results suggest that the converse is also valid, i.e., that the NP rats experience increased aversion with repeated ethanol exposure which may also contribute to the less ethanol consumption. The theory also postulates that P rats would acquire tolerance to the aversion of the intoxication experience and NP rats, which fail to consume large doses of ethanol, would not show tolerance after repeated exposures and could even sensitize to the aversive effects.

In further support of this perspective, the five repeated administrations of the 3.0 g/kg dose resulted in greater decreases in body temperature for the adult NP rats compared to the first hypothermic response. Evidence of sensitization to ethanol-induced

aversive effects in NP rats has been shown previously. In a study examining the loss of the righting reflex, a measure of ethanol intoxication typically considered aversive, the NP rats showed greater initial sensitivity than the P rats and were the only group to acquire sensitization. Kurtz and colleagues (1996) gave P and NP rats a 3.0 g/kg dose of ethanol and measured their time to lose the righting reflex and the duration of impairment. On the initial exposure, NP rats showed impairment earlier, took significantly longer to regain the reflex, and had a lower BAC upon recovery compared to the P rats. Following a second administration the NP rats showed sensitization in the righting reflex, displaying greater latency to regain the righting reflexes.

Ethanol-induced hypothermia has also been examined in the P and NP lines for change between two ethanol administrations. In a study by Stewart and colleagues (1992), NP rats displayed sensitization to the hypothermic effects of ethanol when 48 or 72 hours separated the first and second injection with the hypothermic response becoming more prominent with the longer time between administrations. However this same study reported tolerance in the NP rat when only 24 hours separated the injections. This reported tolerance, also observed in the P rats, might be habituation to the stress associated with rectal probing or other portions of the procedure which may produce acute adaptations, which may not persist beyond 24 hours. The stress of collecting temperature measurements by rectal probing can also lead to an enhanced hypothermic response (Peris & Cunningham 1987); this stress is eliminated in the current experiment with the implantation of Mini Mitter telemetry probes. The substantially reduced experimental stress in the current experiment may account for the ability to observe

sensitization in the NP rats even after 24 hours, rather than the tolerance seen in the Stewert et al (1992) paper.

4.2.2. Absence of tolerance to the 3.0 g/kg dose of ethanol

While the current study clearly demonstrates a robust difference in the hypothermic response between the lines based upon sensitization within NP rats, the results failed to support the predicted acquisition of tolerance to the 3.0 g/kg dose in the P rats. Experiments that previously showed tolerance to ethanol-induced hypothermia in rats, either used larger doses of ethanol (Silveri & Spear 2000, Stewart et al 1992, Swartzwelder et al 1998, York & Chan 1994) or higher concentrations (Crowell 1981), along with the more stressful rectal probe measurement (discussed above). The linear dose response curve associated with ethanol induced hypothermia, in conjunction with the theory that tolerance is influenced based upon the amount of the initial detriment (Kalant et al 1971, San-Marina 1989), suggests that tolerance acquisition may require a higher dose than was used in this experiment. Differences in concentration can also change the experience of ethanol intoxication (Linakis & Cunningham 1979) as well as higher concentration may result in a more stressful experiences due to irritation at the injection site.

Another key methodological issue of past experiments is the failure to report the temperature of the solutions prior to injection or that of the testing room. The current study sought to minimize environmental effects by heating solutions to body temperature (approximately 38^{o}C) before IP administrations occurred in a room temperature environment of (21+/-1^{o}C). It has not been determined how much of an effect the

injection of a cold solution has on the ethanol-induced hypothermic response however ethanol-induced hypothermia has been shown to be greater when administered in a cold environment (Cunningham et al 1992). Due to the failure of previous studies to report these temperatures, it is possible that the tolerance observed in the previous studies is in part due to confounding variables and the current study is a more accurate depiction of the pharmacological effects of ethanol and not those associated with the injection environment.

4.2.3. Acquisition of tolerance to the 1.5 g/kg dose and Saline

Even though the conditions for tolerance acquisition were not sufficient within the 3.0 g/kg dose, the paradigm of repeated administrations was able to show a decrease in the hypothermic response within the saline and 1.5 g/kg dose. Repeated administrations of saline resulted in changes from Day 1 administration consistent with habituation to the procedure. With the assumption that habituation to the procedure should be the same across groups then any change in the hypothermic response prior to Day 5 can be attributed to the drug's pharmacological effects.

Within the 1.5 g/kg dose, three groups showed a decrease in ethanol-induced hypothermic effect across days: the adolescent P rats, the adult P rats and the adult NP rats. The adult NP rat showed tolerance following the fifth IP administrations of the 1.5 g/kg dose of ethanol. However since the adult P and NP rats given saline also showed diminished hypothermia on that day, one cannot rule out that the tolerance acquisition to the 1.5 g/kg dose may be due to procedural habituation. Contrastingly, adolescent P rats were able to display tolerance to the hypothermic response earlier than would be

expected due solely to habituation to the administration procedure shown by a decreased hypothermic response from Day 1 following three ethanol administrations. This difference in acquisition is consistent with other reports that adolescent rats are quicker to develop tolerance to the aversive effects of ethanol compared to adults (Doremus et al 2005).

4.2.4. Conditioning effects in adults

The tolerance and sensitization observed to the hypothermic effect of ethanol could be attributed to biological changes in response to ethanol administration and/or to conditioning of the daily effects to the exposure context. Repeated administrations of ethanol can result in learning of the contingency between the experimental environment and the pharmacological effects of drug exposure. A classically conditioned compensatory response may be elicited from exposure to the environment alone and in the opposite direction of the drug manipulation (Bueno & Fachini 2007). When saline was administered to all rats on Day 6 of the experiment, possible effect of learning was evident within the adult rats of both lines given repeated exposures to 3.0 g/kg ethanol, in that both the P and NP adult rats previously given the 3.0 g/kg dose of ethanol showed significantly less hypothermia than the groups previously given saline or 1.5 g/kg ethanol.

This learning effect cannot completely be classified as a classically conditioned compensatory response, since in this case the response was to maintain their baseline temperature, not a hyperthermic effect. However, the classically conditioned compensatory response can still be shown as an increase in temperature of the 3.0 g/kg

treated rats relative to rats that previously received repeated saline or 1.5 g/kg ethanol administrations, which displayed a considerable drop from baseline temperatures on Day 6. The lack of change from baseline following saline administration is particularly interesting in the adult NP rats which showed sensitization to the 3.0 g/kg dose. This implies that the hypothermic response on Day 5 is due to a biological sensitization to the pharmacological effects of ethanol and occurs even in the presence of a possible conditioned compensatory response.

4.3. Baseline Differences

Data collected on the first day of the experiment revealed differences in baseline between the P and NP lines for both core bodytemperature and locomotor activity counts, with the P rats having higher locomotor activity compared to NP rats. Similar differences have previously been reported using a photo beam array (Rodd et al 2004). Replication of this effect with the less precise measurement of gross locomotor activity counts (combined across longitudinal, latitudinal and vertical planes) demonstrates the robustness of the line difference in locomotion. There was no difference based upon age for locomotor activity baselines, unlike previous findings (Parker & Clarke 1990). However, it is important to note that the size of the antenna array was not adjusted for body size, so in order for the adolescent rats to register a locomotor count they would need to cover a greater distance or height in relation to their body size, than the adult rats.

This is the first report of higher baseline temperatures in the P rat compared to the NP rat. Previous studies (Stewart et al 1992) examining ethanol-induced hypothermia within the P and NP lines suggested baseline differences existed, but due to limitations in

the experimental design no statistical analysis were conducted between the lines. The current study is also the first experiment to detect age-specific differences in body temperature within the lines, such that adolescent rats of both lines had lower temperatures compared to their adult counterparts. By further classification of differences between these lines a more complete understanding of behavioral phenotypes associated with their ethanol consumption preferences is possible.

4.4. Locomotor Sedation

In contrast to the multitude of changes seen with the ethanol-induced hypothermia measure, locomotor activity showed few significant results. Besides the baseline differences between the lines discussed earlier there was a decrease in activity during baseline consistent with habituation to an environment such that more activity was measured at the beginning of baseline compared to later time bins. Repeated exposures to the testing chambers showed habituation across days evident by decreases in baseline locomotor activity counts over the course of the six day experiment. It is also critical to mention that the baseline differences were not accounted for in the post-injection data due to the inconsistent burst like pattern of locomotor activity.

At first administration, both doses of ethanol produced levels of sedation equal across the lines. The lack of line differences cannot be attributed to the injection paradigm since the saline administration continued to show greater activity in the P rats. The levels of sedation produced by the 3.0 g/kg dose of ethanol in conjunction with the less precise measurements of locomotor activity could have resulted in a floor effect obscuring the ability to detect the line difference. However; since the lower 1.5 g/kg dose

was also unable to detect a line difference while producing less motor impairment it is reasonable to conclude that the mechanism is pharmacological with ethanol administration producing sedation similarly between P and NP rats.

At the 3.0 g/kg dose, when first administered, adult rats of both lines had a greater level of locomotor sedation than their adolescent counterparts. This is consistent with the theory that adolescents consume more ethanol than adults due to a decrease in initial aversive quality of the intoxication experience (Little et al 1996). The theory was not further supported by the repeated administrations data since the only group to show tolerance between Day 1 and Day 5 to the 3.0 g/kg dose was the adult NP rat.

4.5. Experimental Limitations

4.5.1. Stress Effects

While the earlier discussion highlighted that experimental stress was reduced with implantation of the Mini Mitter probes, the stress of the experimental procedure was not completely eliminated. The current procedure, while an improvement over previous studies still had stress effects due to injection and its associated restraint; the effects of this stress has been shown to differ based upon age (Ristuccia et al 2007). In a study, it was shown that familiarizing adult rats with the injection paradigm until there is a reduced corticosterone response, results in a decrease in ethanol-induced hypothermia similar to that of an injection of saline; whereas the same familiarization paradigm showed no alteration in the ethanol-induced hypothermic response seen with adolescent rats. While the Ristuccia and colleagues (2007) experiment was conducted in non-

selected rats it is possible that age-associated differences in stress are still present in the current study.

4.5.2. Sex differences in Hypothermia and Locomotion

Differences in outcomes for both locomotion and body temperature from previous studies could be due to effects of sex, given that female rats were used in the current study compared to males in previous studies (Ristuccia & Spear 2004, Stewart et al 1992). Female rats have previously been shown to differ from male rats in consumption [in comparison to body weight, g/kg (Lancaster et al 1996, Piano et al 2005)], in baseline temperatures (Webb et al 2002), and in susceptibility to ethanol induced hypothermia (Hirvonen & Huttunen 1995, Taylor et al 2009, Webb et al 2002).

Rodd and colleagues (2004) created dose-response curves for locomotor activity both female and male adolescent P and NP rats. While the male and female P rats did not differ, the female adolescent NP rats showed a decrease in locomotor activity following administration of a lower dose of ethanol than did the male adolescent NP rats (Rodd et al 2004). When compared across studies, the female adolescent NP rat, which differed from the male, showed a more similar response to the adult NP rats of the Waller study (1986). This suggests that the lack of an age effect within the 1.5 g/kg dose for locomotor sedation could be based upon the use of female rats in the current experiment.

This sex differences could also be associated with the neuronal changes which occur at different time points, with females maturing faster than males (Devaud et al 1999). Adolescence is defined by sexual maturation caused by increases in hormonal activity, and differences in hormone type and level could explain why female adolescent

rats are more similar to their adult counterpart than to their age matched opposite sex control.

While the adolescent rats should not have begun the estrous cycle, the current stage of the estrous cycle is a concern for the adult females since hormone levels in adult female rats can alter the hypothermic response (Silva 2006). Typically the different stages of the estrous cycle are randomized within group in free-cycling female rats, however it is impossible to know if the rats in this study were free-cycling or if their estrous cycles had become synchronized. It's noteworthy that the present study tested 12 cohorts, which should have minimized the effect of estrous cycle across cohorts.

4.5.3. Mechanisms controlling hypothermia

While certain brain regions and peripheral locations have been implicated in thermoregulation and possible disturbances associated with ethanol exposure; it is still unclear what physiological mechanisms induces ethanol-associated hypothermia. It has been suggested that ethanol induced hypothermia works by changing the neuronal set point (Ritzmann & Tabakoff 1976a) but it is not clear if there are also changes in peripheral thermal regulation.

A limitation of this study, and all previous studies, is that the relationship between core and brain temperatures is not completely understood and little work has been done to compare if decrease in the core temperature have similar implications on brain temperature across ontogeny. While the stabilization of core body temperature occurs during adolescence (Kalant & Lê 1983) it is still possible that brain temperature shows greater fluctuations in adolescents than adults, considering the extreme neuronal

restructuring also occurring at that age (Monti et al 2005, Spear 2004) and the possible role of differential maturing neurotransmitter systems on thermoregulation (Ferguson et al 1985). Since adolescence is characterized by changing biological systems, both in the periphery and neuronal, they may be experiencing thermal alterations more frequently than adults and therefore it is possible that the hypothermia produced by ethanol administration would not be as aversive in this respect.

4.6. Conclusions

By systematically examining physiological and behavioral differences in the P and NP lines of rats at either adolescence or adulthood on initial sensitivity to ethanol-induced hypothermia and locomotor activity as well as across repeated administrations, the current experiment was able to show behavioral phenotypes that may correlate with ethanol self administration. Initial sensitivity to the 3.0 g/kg dose of ethanol showed NP rats had a more severe ethanol-induced hypothermia compared to the P rats. In addition repeated ethanol administrations yielded sensitization in the adult NP. Although the 3.0 g/kg dose failed to produce the expected tolerance in the P rat, repeated administrations of the 1.5 g/kg dose did produce tolerance in the P rat at both age points. Repeated administrations of the 3.0 g/kg dose resulted in NP adult rats acquiring sensitization to the hypothermic response; this dose also produced a classically conditioned compensatory response in adult rats of both lines. By using the Mini Mitter and a heated injection solution the results obtained in the current experiment are more likely attributable to the pharmacological effects of ethanol than that of stress associated with the test procedures.

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TABLES

Table 1. Diagram of experimental assignment.

A between subject design was utilized to test the effects of age and line on locomotor activity and body temperature when exposed to saline, 1.5 and 3.0 g/kg ethanol. Either adolescent or adults underwent the experimental procedure at a given time and within a cohort of rats all solutions were equally represented.

		AGE					
		Adolescent Postnatal Day 30			**Adult** Postnatal Day 90		
Selected Line	P	0	1.5	3.0	0	1.5	3.0
	NP	0	1.5	3.0	0	1.5	3.0

FIGURES

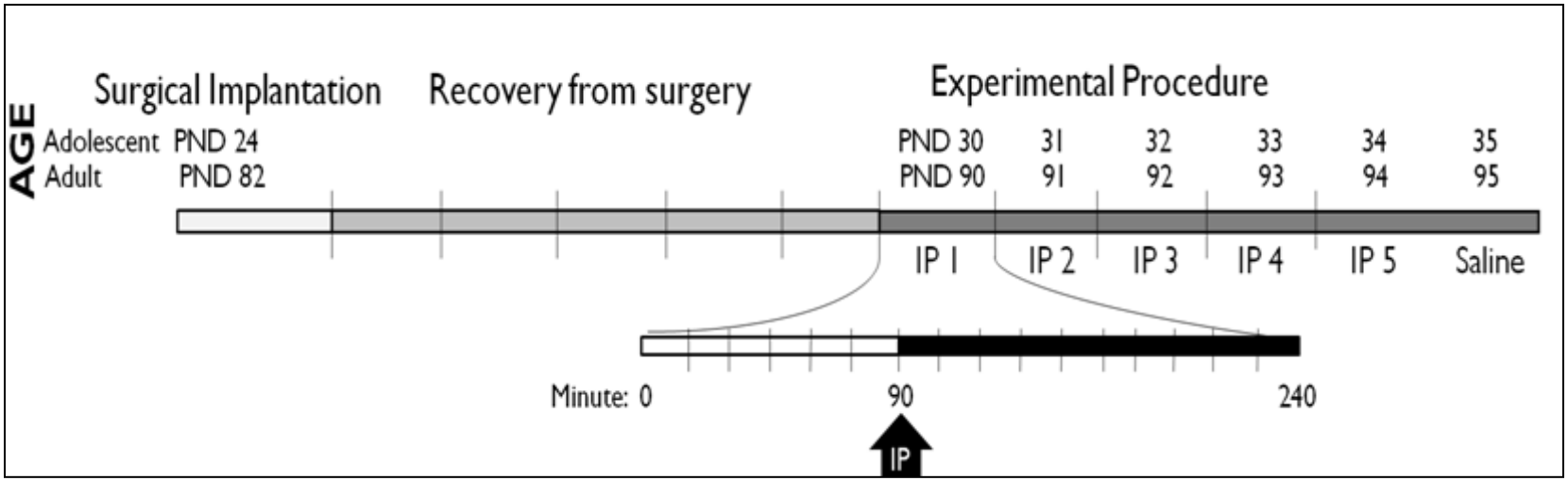

Figure 1. Summary of Experimental Procedure.

Age points for the adolescent and adult rats are displayed for each step of the procedure. After undergoing surgery (PD 24 or 82) rats are allowed 5 days to recover before beginning the 5 day repeated IP administration paradigm culminating in a saline injection on the sixth day (PD 35 or 95). Within the IP administration days the 240 minutes of data collection can be divided into a 90 minute baseline prior to the injection of a heated (~38^{o}C) solution and the 150 minutes post-injection period. During that time the Mini Mitter transponder probes recorded thermal and locomotor data in one minute increments.

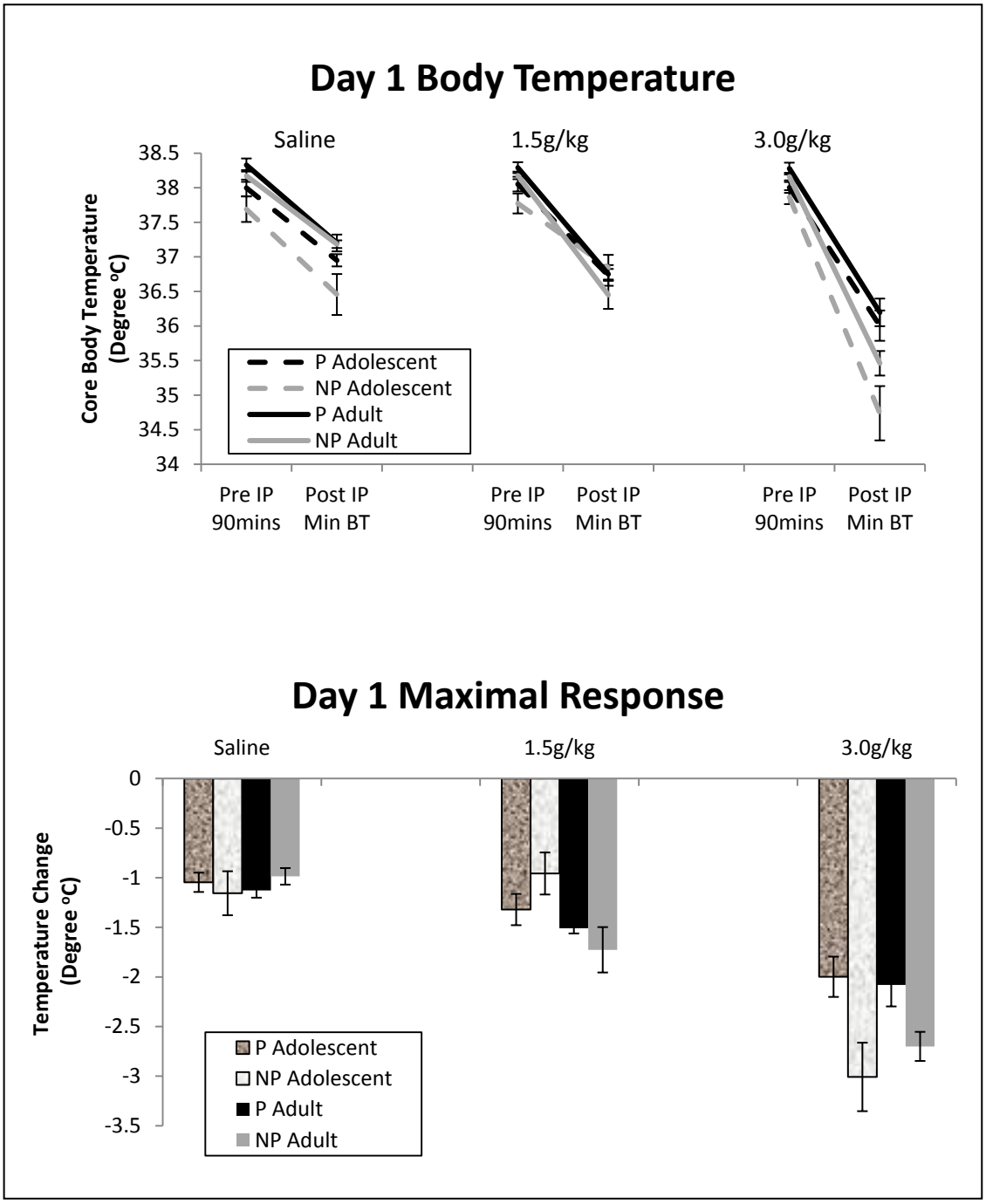

Figure 2. Day 1 Body Temperature Measures Pre- and Post-injection.

Upper Panel: IP administration resulted in a decrease in body temperature regardless of dose. However, the 3.0 g/kg dose of ethanol produced the largest hypothermic response. The NP rats showed a larger hypothermic response following IP administration when compared to P rats.

Lower Panel: Significant difference in body temperature existed at baseline with the NP rats having lower core temperatures than P rats; therefore data were converted to a maximal change from baseline score. Differences existed due to dose so each treatment group was analyzed separately. Within the 3.0 g/kg dose, an effect of line becomes evident with the NP rats having a greater hypothermic response than the P rats at both ages. This analysis for the 1.5 g/kg dose displays differences in response exist between the ages, with the adolescent rats maintaining more of their baseline body temperature compared to the adult rats.

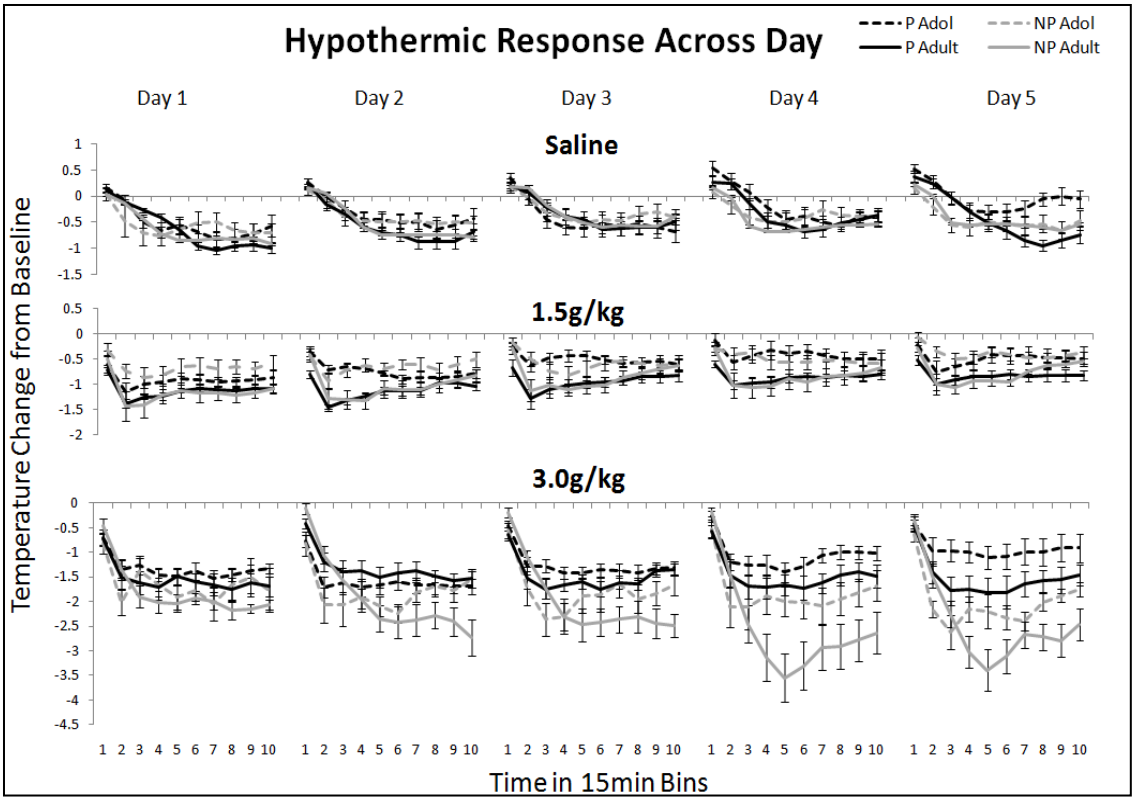

Figure 3. Hypothermic response across Day.

Changes in body temperature (from pre-injection baseline) over 150min post-injection period over five consecutive days by treatment (dosing groups= saline, 1.5 g/kg or 3.0 g/kg ethanol); one bin= 15mins. Note the striking emergence of sensitivity in the 3.0 g/kg dose to hypothermia effects in the NP adult. In contrast, groups injected with either saline or 1.5 g/kg ethanol show less of a decrease in body temperature across day.

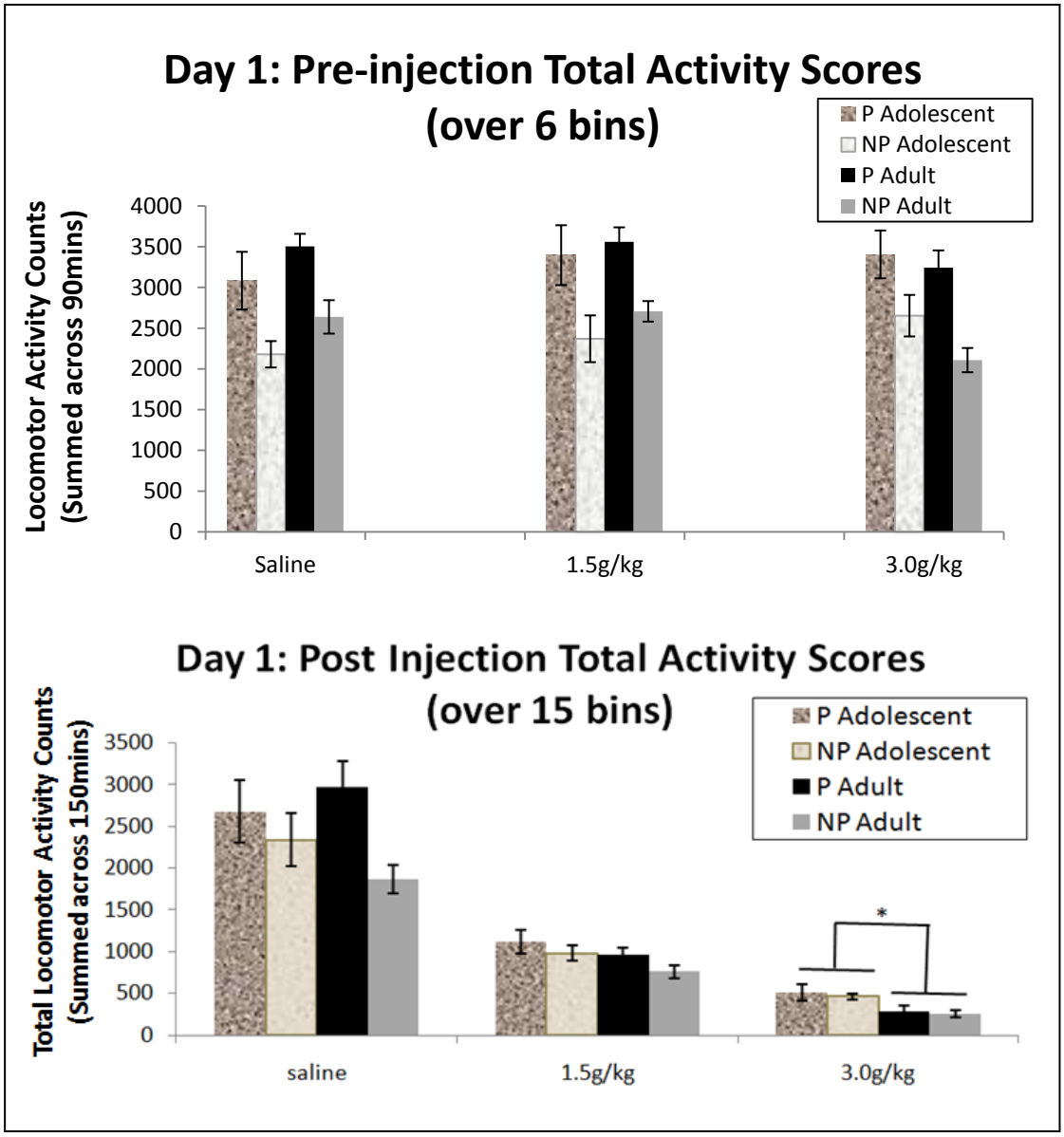

Figure 4. Average locomotor activity within a 15min bin Post- Injection on Day 1.

Upper panel: Baseline Activity and Locomotor Sedation on Day 1. Baseline locomotor activity counts showed a line difference prior to manipulation, with the P rats being more active than NP rats.

Lower panel: Total locomotion following first IP exposure. When the activity counts from the entire 150 minute experimental phase are summed there is a dose response

relationship with dose producing different levels of sedation. The line difference seen at baseline is still present in the groups administered saline but is no longer observable following ethanol exposure. While the line effect fades with the higher dose of ethanol, 3.0 g/kg ,there is emergence of an age, with the adult rats showing more sedation than the adolescent rats. * represents $p<.01$ between age effect.

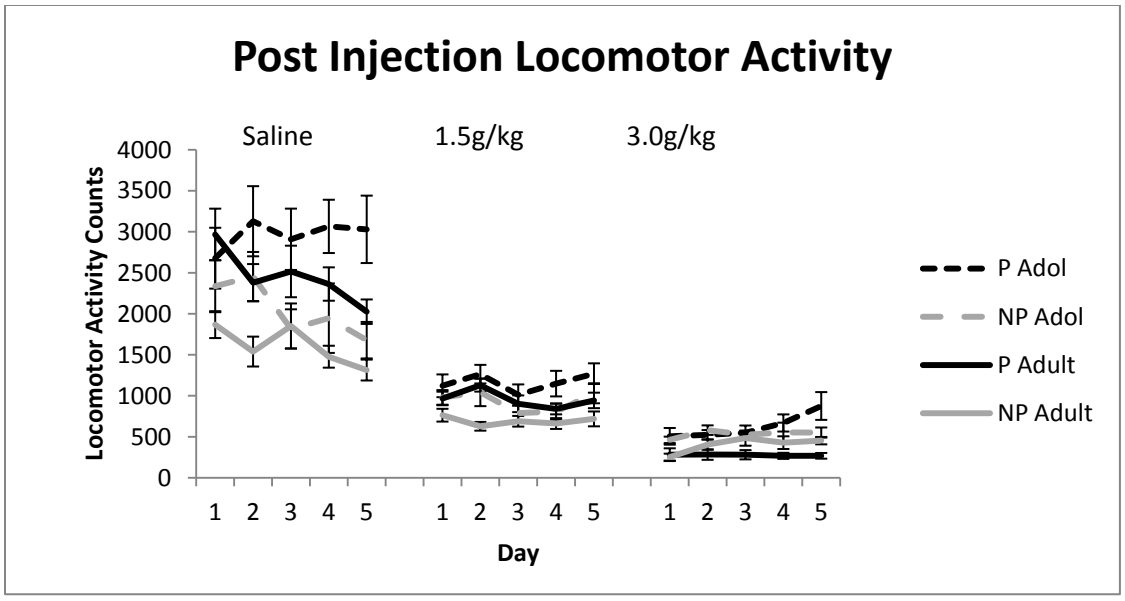

Figure 5. Locomotor sedation following repeated injections.

When total locomotor activity scores following each IP administration are compared across the five day procedure there is a significant effect of dose with ethanol groups consistently showing greater levels of sedation than the saline controls. Age had an effect on the level of sedation following injection with the adolescent rats displaying less sedation. Over the repeated exposures there was differential responding over time based upon dose, with the adult NP rats exposed to 3.0 g/kg showing increase in locomotor activity scores compared to those on Day 1.

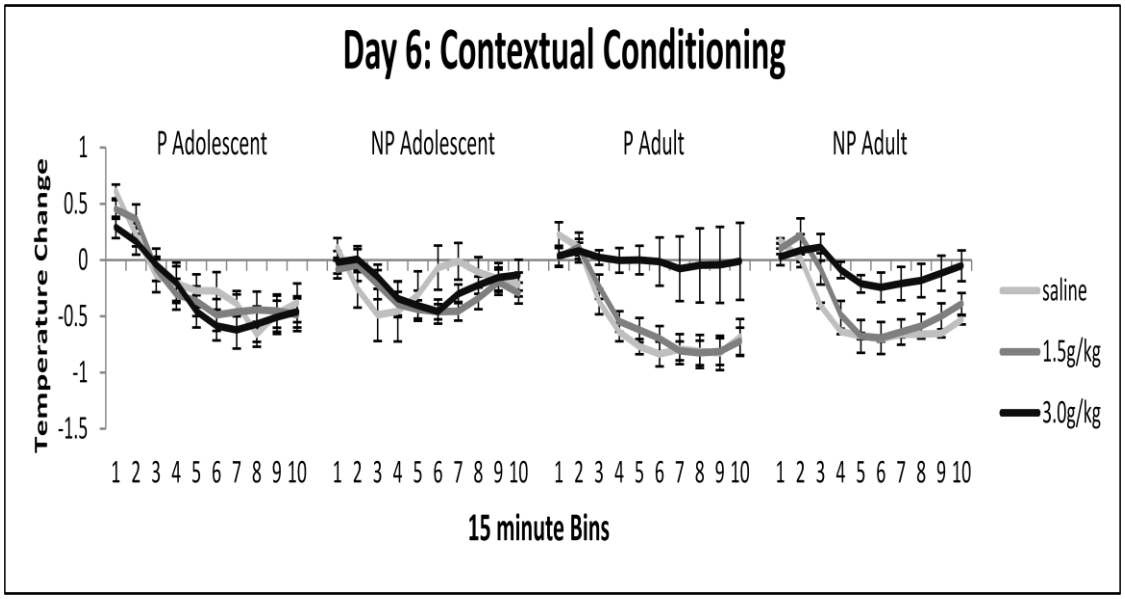

Figure 6. Core body temperature change following saline administration on Day 6. When saline was administered to adult rats which had previously received 3.0 g/kg ethanol, there was a less severe hypothermic response compared to that elicited from previous exposures to 1.5 g/kg or saline. The saline administration on Day 6 produced similar hypothermic responses in all adolescent rats regardless of previously administered solutions.

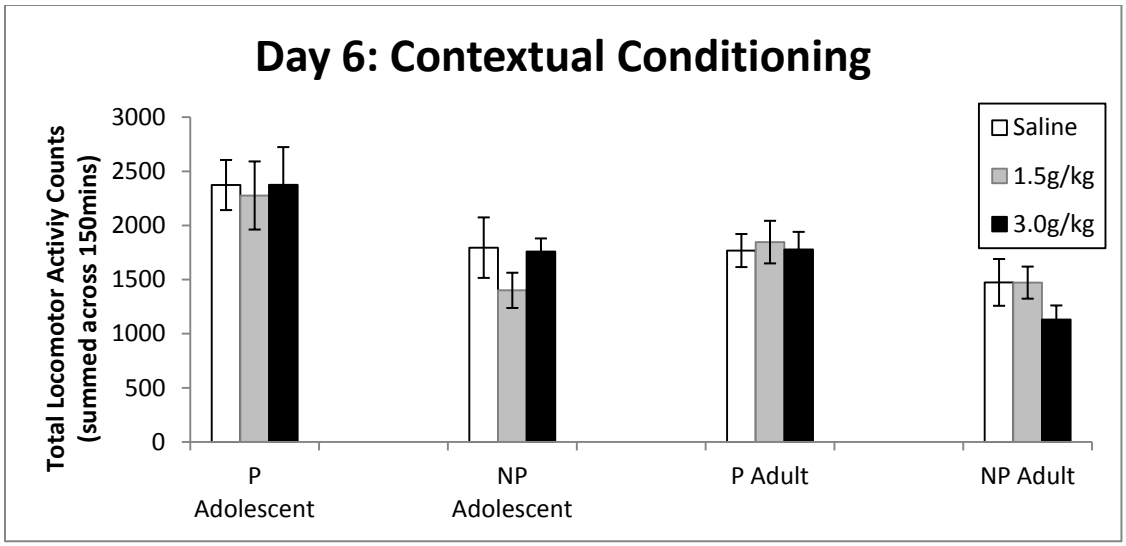

Figure 7. Locomotor activity following saline administration.

Adolescent NP rats which had previously been exposed to a 1.5 g/kg ethanol dose showed less locomotor activity compared to the saline and 3.0 g/kg groups. The adult NP rats who had received 3.0 g/kg displayed decreased activity with the saline injection versus the other two treatment groups. For P rats, locomotor activity on Day 6 was not significantly changed based upon the prior ethanol treatment dose.