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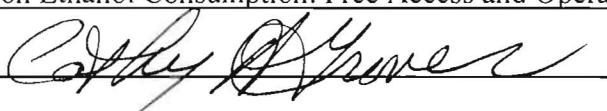
Abstract approved: 

I examined the effects of housing conditions (Isolated, Social) on ethanol consumption when the animals have the choice to work for ethanol or water in an operant situation as well as in a free access condition. Subjects were 30 male Long Evans Blue Spruce rats randomly assigned to 1 of 2 groups: isolated or socialized housing. Experimentation required a total of 8 phases. Phases 1 and 8 consisted of two-bottle (water vs. 5% ethanol) tests on the home cage. Phases 2 – 5 involved training rats to press a lever for ethanol reinforcement using a sucrose-fading procedure. Phases 6 (1 hr, 2 days) and 7 (24-hr) consisted of choice operant lever pressing for water vs. ethanol. My most important finding was that housing conditions affected ethanol consumption differently. Isolated rats consumed more ethanol in a free-access situation than socialized rats both at initial exposure and after repeated exposure. Further, in an operant situation, where the animal must press a lever for ethanol, isolated and socialized rats did not differ in ethanol reinforcement.

HOUSING EFFECTS ON ETHANOL CONSUMPTION: FREE ACCESS AND OPERANT SITUATIONS

A Thesis

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CHAPTER I

INTRODUCTION

According to the 10th Special Report to the US Congress on Alcohol and Health (2000), alcohol related problems in the U.S. cause 100,000 deaths and cost society $184.6 billion a year. Alcohol abuse is responsible for the hospitalization of 20 to 40% of patients in some urban areas, is associated with diseases of the liver and heart, and may also cause cancer (Tenth Special Report to Congress). Alcohol abuse is a serious problem that requires further investigation.

According to Higley, Hasert, Suomi and Linnoila (1991), alcohol abuse has a complex etiology that includes environmental elements such as stress. Stress increases ethanol consumption in a variety of conditions. For example, stress induced by shock, housing conditions, and dominance tend to increase ethanol consumption in laboratory rats. Research shows that not all types of stress produce increases in ethanol consumption. For example, Roske, Baeger, Frenzel, and Oehme (1994) indicate that chronic intermittent immobilization does not produce an increase in ethanol consumption, whereas social isolation significantly increases ethanol (10%) consumption in the rat model. Animals, specifically rats, have been widely used as models of environmental stress and ethanol consumption (e.g., Blanchard, Hori, Tom & Blanchard, 1987; Ellison, 1981; Parker & Radow, 1974; Schenk, Gorman & Zalman, 1990).

Of interest in recent years is the role of isolation and social housing on ethanol consumption. This research has yielded interesting but at times contradictory results. For example, numerous studies have noted that isolation increases ethanol consumption in the rat (Buckalew, 1979; Deatherage, 1972; Juarez & Vazquez-Cortez, 2003; Lodge & Lawrence,

2003), whereas other studies report no difference (e.g., Schenk et al., 1990); that socialization increases ethanol consumption, especially among subordinate rats (e.g., Blanchard et al., 1987; Rockman, Borowski & Glavin, 1986), or that socialized rats are more excessive consumers of ethanol than isolated rats (Ellison, 1981). Researchers usually attribute inconsistencies in the literature to different concentrations of ethanol (e.g., Deatherage), the age of isolation onset (e.g., Fahlke, Hard, & Erikson, 1997), gender (e.g., Brown & Grunberg, 1995), crowding in social cages (e.g., Nagaraja & Jeganathan, 2003), and environmental enrichment (e.g., Adams & Oldham, 1996; Ellison, 1981). Housing conditions impact ethanol consumption.

Although much research has been devoted to isolated and socially housed rats in a free choice condition, minimal to no research attention relates to housing effects on preference where animals work for their choice of alcohol or water. The purpose of this study is to investigate the effect of isolated vs. group housing on ethanol consumption when the animals have the choice to work for ethanol or water in an operant situation as well as in a free access condition. For the purposes of this study, I defined my socialized housing as a housing arrangement of 3 rats per cage in cages that are 24 x 17 x 20 cm. I defined isolated housing as a housing arrangement of 1 rat per cage in cages that are 24 x 17 x 43 cm. Isolated rats had no physical contact with other rats.

A great deal of prior research is inconsistent about the influence of housing conditions on ethanol consumption. Carefully reviewing the procedural differences among these studies clarifies the conflicting results. In addition, examining studies of ethanol consumption in operant situations allows for a better working model of oral ethanol consumption. This understanding can better isolate factors that significantly contribute to

ethanol consumption and support future research to develop new models of alcohol consumption that will help solve alcohol related problems.

Review of the Literature

Ethanol Consumption

Deatherage (1972) investigated the effects of housing on voluntary ethanol consumption in the rat. He randomly assigned 48 male Long-Evans hooded rats at the age of 42 days to either a socialized housing condition (6 rats per 66 x 26.7 x 18.4 cm cage) or in an individually housed condition in the same sized cage. Deatherage established 6 research groups, including 2 social ethanol groups, 2 isolated ethanol groups, a social water control group, and an isolated water control group. Rats living in the social housing condition had 3 water bottles per cage, and 1 group experienced a 10% ethanol concentration while the other group experienced a 20% ethanol concentration. Rats living in the isolated housing condition had 1 water bottle per cage and experienced the same ethanol or water presentation as the social group. Following a 30-day trial, the isolated rats consumed 20% more ethanol at the 20% concentration than their socially housed counterparts. However, Deatherage did not observe differences between these groups at the 10% ethanol concentration. This study suggests that isolated rats may consume more ethanol than socially housed rats at higher ethanol concentrations.

Under isolated or social housing conditions, the concentration strength of a liquid may play an important role in consumption. Hall, Huang et al. (1998) investigated the consumption of ethanol, sucrose, and saccharin at different concentrations by randomly assigning 22 Fawn Hooded male rats and 25 Wistar male rats, all at the age of 21 days, to either isolated living in a 20 x 20 x 20 cm cage or socialized housing conditions with 2 rats

per 45 x 20 x 20 cm cage. In this study, socially isolated rats could see, hear, and smell each other but could not physically touch other rats. Experimentation began after about 56 days in the experimental housing conditions. Researchers conducted 3 sets of two-bottle preference tests, including ethanol vs. water, sucrose vs. water, and saccharin vs. water. During the first experiment, the ethanol concentration began at 2% and increased to 4%, 8%, and lastly 16% when the consumption of each concentration stabilized. During the second experiment, the concentration of sucrose increased weekly from 0.7%, to 2.1%, followed by 7.0%, then to 21.0% and finally to 34%. The last stage consisted of an increase in saccharin concentration every four days from 0.01%, to 0.04%, followed by 0.16%, and then to 0.64%. Hall et al. (1998) concluded that, regardless of strain, isolated rats consumed significantly more sucrose, saccharine, and ethanol in higher concentrations than socially reared rats, but isolated rats did not consume significantly more ethanol at lower concentrations. This indicates that isolated rats may have more of a preference for higher concentrations than socialized rats.

Many recent studies indicate that the age of isolation may also greatly influence ethanol consumption (e.g., Buckalew, 1979; Fahlke et al., 1997; Juarez & Vázquez-Cortés, 2003; Schenk et al., 1990). Schenk et al. suggest that the age of isolation may greatly influence the self-administration of ethanol in an isolated or group housed condition. They assigned 32 male Long-Evans rats at the age of 21 days and 19 male Long-Evans rats at the age of 65 days to either a socialized housing condition (4 rats per 41 x 25 x 18 cm cage) or in an individually housed condition (20 x 25 x 18 cm cage). For 17 days the rats experienced an acquisition phase, where every other day rats had free access to water on the home cage. On the alternate days all rats could drink both ethanol and water. The concentration of ethanol

began at 2% and increased by 1% during every ethanol presentation until the researchers attained 10%. For the next 20 days, the rats experienced a two-bottle preference test between 10% ethanol and water ad libitum (ad lib). The rats that were isolated at weaning (21 days of age) consumed significantly greater amounts of ethanol at higher (10 %) concentrations. Schenk et al. did not find significant differences with rats isolated at 65 days of age. Additionally, they did not find significance between isolated and socialized rats at lower ethanol concentrations. Schenk et al. concluded that rats isolated at weaning are more likely to consume ethanol at higher concentrations than rats isolated later in life.

Rockman, Hall, Markert, and Glavin (1988) furthered this understanding by examining the effects of early weaning on adult ethanol consumption. They weaned 18 Wistar rat pups at 16 or 21 days of age and exposed them to an isolated or social housing condition. The assessment of ethanol consumption consisted of sequentially increasing ethanol concentrations from 3-9%. Rats weaned at 16 days consumed significantly more at the higher concentrations of ethanol than rats weaned at 21 days. These results seem to support the findings previously suggested by Schenk et al. (1990). However, Fahlke et al. (1996) pointed out that exposing a rat to social isolation too soon after birth may confound the influence of social isolation on ethanol consumption as in the Rockman et al. study.

Fahlke et al. (1996) bred Wistar rats and exposed the pups at birth to 1 of 3 rearing conditions: Condition A consisted of 26 pups that experienced early weaning (16 days old) and social isolation, Condition B consisted of 24 pups that experienced early weaning and were socially housed with 2 littermates, and Condition C consisted of 26 pups that experienced regular weaning (26 days old) and were housed with 2 littermates. When a litter contained 9 males, the researchers randomly assigned 3 rats to each condition. If a litter

contained 8 males and a female, researchers placed 2 males in condition A, 2 males and a female in condition B, and the remaining 3 males in condition C. Rats in all 3 conditions experienced the same type of cage (45 x 30 x 16 cm). Researchers assessed ethanol consumption beginning on day 25 using a two-bottle preference test for water and ethanol. The ethanol concentration began at 2% and increased by 2% each week until it reached 10%. Their results indicated that early weaning decreased ethanol consumption at lower ethanol concentrations (2-6%) as compared to normally weaned rats.

Buckalew (1979) reported that maternally induced ethanol exposure may lead to an offspring preference in rats. Buckalew mated 3 adult female alcohol-naïve hooded rats with a single male. Six days before mating, all females experienced a 5% ethanol and water solution. Following the initial ethanol exposure, the mothers had access to the male for 5 days. During gestation and lactation, the mothers only drank an ethanol solution. Buckalew weaned 19 pups from all 3 mothers at 28 days of age. At weaning, approximately half of the pups experienced isolation and the other half experienced social housing (2 rats per cage). Buckalew did not state the specific cage sizes for either housing condition. He then exposed the pups to a two-bottle preference test with water and ethanol. Every 3 days for a total of 30 days Buckalew measured liquid intake and switched bottle positions to prevent a possible bottle position effect. According to this study, when researchers expose prenatal rats to ethanol, isolated offspring consumed significantly more ethanol than their socially housed counterparts.

Housing Variations

Researchers have considered several variations of isolation and social housing in ethanol consumption models (e.g., Juarez & Vázquez-Cortés, 2003; Wolffgramm, 1995). For

example, a recent study explored ethanol consumption among older rats that experienced continuous or periodic exposure to a social or isolated environment from an early age (Juarez & Vázquez-Cortés). In this study, 32 males from 5 different Wistar litters were placed at weaning (22 days old) in 1 of 4 different groups. All groups had at least 1-2 rats from each litter. All rats initially experienced social housing with 8 rats in a 49 x 37 x 19 am cage. Following 10-20 days, depending on the experimental group, all rats had exposure to particular durations of isolated housing (28 x 19 x 15 cm). The isolation group experienced permanent isolation. The socialized group experienced permanent social housing. A third group was the Isolated/Socialized group that lived in isolation but had exposure to social housing for 12 hr every other day. The last group was the Socialized/Isolated group that lived socially but had exposure to isolation in the same manner as the third group. The animals experienced an 8% ethanol concentration as the only available liquid on their home cage every other day. The researchers were unable to devise a method to determine ethanol consumption per individual rat among socially housed animals; therefore, individual consumptions were only possible in the isolated condition. The ethanol consumption for each socially housed animal was determined by dividing a group's total consumption by the number of rats in that group. They concluded that ethanol consumption was significantly higher among permanently isolated rats than among partially isolated rats or than socially housed rats. Therefore, social isolation may result in higher ethanol consumption.

Stress

Recently many studies have suggested that stress caused by social isolation may be responsible for increased ethanol consumption among isolated animals (e.g., Ellison, 1981; Parker & Radlon, 1974; Rockman et al., 1988). For example, Parker and Radlon examined

rats housed socially and in isolation. They found that susceptibility to consuming ethanol may be the result of stress induced by housing conditions. Specifically, isolated rats showed higher incidence of enlarged adrenal glands and increased cortisone levels (cortisone increases the ability of a rat to metabolize ethanol). Isolated rats may show increased ethanol consumption and tolerance because they have higher cortisone levels due to the experience of isolation.

Further research indicates that not all types of stress produce increased ethanol consumption. For example, chronic intermittent immobilization did not produce increases in ethanol consumption, whereas social isolation led to a significant increase in alcohol (10%) consumption in rat models (Roske et al., 1994). Roske et al. placed an unknown number of male Wistar rats in social isolation at 8 weeks of age, or intermediate immobilization at 12 weeks of age. The rats placed in isolation were not able to see or touch any other rats for a total of 20 weeks. The rats placed in intermediate immobilization experienced alternating phases of immobilization and free mobility for 7 weeks. During the last 3 weeks of each condition, the rats experienced a two-bottle preference test for ethanol (10%) and water. In addition, all rats received the gut dependence test, a measure of endogenous opioid dependence, administered after 17 weeks of isolation or 4 weeks of intermediate immobilization. Roske et al. concluded that the stress produced by intermediate immobilization caused endogenous opioid dependence in rats whereas stress produced by social isolation did not induce this dependency. This difference may explain why isolated rats consumed more ethanol than social rats. Patterson-Buckendahl et al. (2004) measured adrenal gene expression for enzymes of the catecholamine synthetic pathway in rats after 4-7

weeks of ethanol consumption. Their results suggest ethanol may enhance the ability to respond to acute or chronic stress.

Another interesting point is that gender and crowding may affect ethanol consumption among socially housed rats (e.g., Russell & Stern, 1973). Brown and Grunberg (1995) assigned 114 male and female Wistar rats to 1 of 2 experimental conditions. In Experiment 1, researchers examined the effect of crowding and isolated housing on corticosterone levels. They assigned 7 males and 7 females to gender specific housing conditions at the age of 154 days. Four male rats (32 x 20 x 18 cm cage) and 4 female rats (27 x 15 x 13 cm cage) experienced a social housing condition. Researchers controlled for the difference in male and female rat body sizes by altering cage sizes. The remaining 3 male and 3 female rats experienced social isolation (44 x 23 x 20 cm cages). Brown et al. exposed all rats to the experimental conditions for 18 hr. After this exposure, researchers measured levels of corticosterone, a stress-related hormone. Crowded males showed increased levels of corticosterone in comparison to their isolated counterparts. In contrast, crowded females did not exhibit a stress response as noted by lower levels of corticosterone. The results indicated that male rats experience stress more when crowded and female rats experience more stress when isolated.

In Experiment 2, Brown and Grunberg (1995) investigated the difference between population density and spatial density among male and female rats. Fifty male and 50 female rats at the age of 105 days experienced 1 of 5 same sex housing conditions, including isolated housing in a 44 x 23 x 20 cm cage, 5-grouped housing with males in a 47 x 37 x 19 cm cage and females in a 35 x 30 x 15 cm cage, 10-grouped housing with males in a 77 x 37 x 19 cm cage and females in a 64 x 32 x 18 cm cage, 5-crowded housing with males in a 40 x 22 x 18

cm cage and females in a 27 x 19 x 18 cm cage, or lastly 10-crowded housing with males in a 47 x 37 x 19 cm cage and females in a 35 x 30 x 15 cm cage. They exposed the rats to experimentation 18 hr/day for 14 days. After this exposure, researchers obtained corticosterone levels as previously noted. The results indicated that females experienced population density and spatial density equally; however, males experienced more stress by spatial density than by population density. This study may explain why research findings in this area are inconsistent. Another study that investigated the extent of social crowdedness on ethanol consumption indicated that stress caused by brief social crowding (6 hr/day) did not increase ethanol consumption, whereas when the rats were stressed by social crowding for longer periods of time (24 hr/day) they tended to consume more 2% (wt/vol) ethanol than rats that were not crowded (Nagaraja & Jeganathan, 2003).

Consistent with prior findings that isolation may influence the effect of ethanol, Jones, Connell, and Erwin (1990b) indicated that social isolation in mice may decrease the brain's sensitivity to ethanol. In this study, they randomly assigned 18 male long-sleep mice and 20 male short-sleep mice at the age of 45 days to an isolated (24.5 x 12.5 x 10 cm cage) or social housing condition with 5 mice per 29 x 18 x 12.5 cm cage. After about 22 days all mice experienced an injection of ethanol (24% wt/vol in isotonic saline). Jones et al. measured sleep time to determine ethanol sensitivity in the mice and concluded that social isolation may cause a decrease in the anesthetic effects of ethanol due to shorter ethanol-induced sleep times in mice. Isolated rats generally consume more ethanol than socially housed rats because socially isolated rats may be less sensitive to the effects of ethanol than socialized rats.

Ellison (1981) supported previous suggestions that isolated rats consume more ethanol than socially housed rats. Additionally, Ellison noted that the variability in alcohol preference is more unpredictable in socially housed rats than in isolated rats. Ellison separated 66 male Long-Evans rats at an unknown age into two groups. The first group contained 30 rats reared together for 130 days in an enriched arena (6 x 4 m). The remaining 36 animals experienced social isolation in an impoverished 24 x 28 cm cage. Both groups experienced a choice of water or ethanol ad lib on their home cage in addition to a 1 hr feeding session each day during the middle of their dark cycle. The ethanol concentration began at 1% and increased gradually over 30 days to 10%. Also, the positions of the ethanol and water bottles altered every 5 days to prevent a position effect on consumption. After 6 months in this condition, researchers placed socially housed rats in individual housing in order to access individual ethanol consumption. After 7 days of habituation in the new cages, all rats experienced a two-bottle preference for ethanol (10%) and water for 14 days. This study replicated previously mentioned accounts that isolation induces more ethanol consumption than social housing. In addition, most ethanol drinking variability appeared in the socially housed rats. These results suggest that rats reared in an enriched social environment may experience more variability in ethanol consumption than rats reared in impoverished isolation, but that isolated rats still consumed more ethanol.

In contrast to Ellison (1981), Adams and Oldham (1996) found that rats living in semi-natural housing conditions (enriched) drank 2 to 3 times more ethanol than rats living in isolation. Adams and Oldham randomly assigned 31 male Maudsley Reactive rats at the age of 42 days to 1 of 3 experimental conditions, including 11 rats to isolation in 18 x 24 x 18 cm cages, 12 rats to "typical" group housing in 30 x 60 x 30 cages, and 8 rats to seminatural

housing in 10 x 20 x 10 cm in 1 of 2 groups. Two weeks after moving into the experimental housing conditions, all rats experienced a two-bottle preference test between water and ethanol (10%) for approximately 8 weeks. Researchers rinsed, refilled, and switched the bottle positions every other day. It is important to note that Adams and Oldham created a ratio of ethanol consumption by dividing the liquid consumption weight by the rat's body weight. In this manner the researchers controlled for different sized rats. Rats housed in semi-natural conditions consumed more ethanol than those housed in isolation; however, rats housed in typical social housing did not consume more ethanol than the isolated rats. These results suggest that environmental enrichment may impact ethanol consumption in rats.

Social isolation in rats may impair the development of schedule-induced polydipsia, the excessive drinking of water by food deprived animals (Jones, Robbins, & Marsden, 1989). Frustrating conditions, such as isolation rearing or shock, may also impair the acquisition of polydipsia. Therefore, isolated rats may not consume as much liquid as socially housed rats. This finding implies that stressful conditions, such as social isolation or crowding may reduce the consumption of ethanol aside from the reinforcing properties of ethanol.

Behavioral Responses

Alterations in behavioral responses of rats raised in isolation have been widely reported (e.g., Frisone, Frye, & Zimmerberg, 2002; Van den Berg, et al. 1999). For example, rats reared in isolation are more excitable, exhibit enhanced exploratory behaviors, are hyperactive in novel environments, and have increased weight gain compared to socially reared rats (Jones et al., 1989). In addition, social isolation in juvenile rats may reduce the motivation for adult social behavior (Van den Berg et al., 1999). Van den Berg et al. assigned

20 male Wistar rats to either a social housing condition (5 rats per cage) or an isolated condition for 13 days. All rats experienced the same type of cage (40 x 26 x 20 cm). A social interaction test consisted of placing an isolated and social rat together in a test cage (70 x 70 x 50 cm). Body weight was controlled so that no 2 rats differed by more than 20 g. Researchers recorded the test interactions and later each rat was analyzed for frequency, duration, and latency to social contact. Isolation before puberty may decrease the motivation for adult social behavior, but, once contact is established, normal behavior responses occur.

Frisone et al. (2002) found that social isolation-induced stress may significantly affect spatial learning when it occurs early in life. Researchers noted significant impairment of juvenile isolated rats' spatial learning ability using the Morris Water Maze. Interestingly, the same rats tested as adults displayed enhancement spatial learning.

Isolation may cause alterations in memory (e.g., Matthews & Simson, 1998), sleep patterns (e.g., Farnell & Ing, 2003), stress response (e.g., Hegarty & Vogel, 1993), and drinking behavior in rats (e.g., Hall, Humby, Wilkinson, & Robbins, 1997a). Hall et al. (1997a) investigated the effect of sucrose drinking behavior on housing conditions. They randomly assigned 64 Lister hooded male rats at the age of 21 days to 1 of 6 conditions. Rats experienced either an isolated (45 x 20 x 20 cm cage) or a social group with 4 rats per cage in a 56 x 38 x 18 cm cage in 1 of 3 experiments. The rats in Experiment 1 did not experience food or water deprivation while the rats in Experiment 2 experienced food and water deprivation. Researchers presented sucrose to the rats in Experiment 3 in either an ascending or a descending order. This study revealed that housing conditions by non-food-deprived rats and by food-deprived rats did not alter sucrose consumption. However, in Experiment 3 isolated rats had less frequent and longer drinking sessions than socialized rats. Isolated rats

may not change their behavioral tendencies as frequently as socialized rats. Socialized rats may shift their attention away from ethanol drinking more often than isolated rats, resulting in lower ethanol consumption.

Another interesting point made by Hall, Humby, Wilkinson and Robbins (1997b) was that socially isolated rats tend to prefer a novel situation over a familiar one as compared to socially housed rats. They randomly assigned 32 Lister hooded male rats in the same manner as in the previous study; however, Experiment 1 consisted of a novelty preference test under a red light while Experiment 2 took place under an identical white light. The red and white light bulbs simulate night and day lighting conditions, respectively. Hall et al. (1997b) noted that rats are more active at night which could lead to more exploratory behavior. The isolated rats could hear, see, and smell the other rats but could not physically touch them. Experimentation began around 56 days of age. The researchers assessed novel environment preference with a box divided into 3 parts. The smallest chamber was in the center of the box (10 x 10 x 74 cm), and it allowed access to 2 larger chambers of equal size (32 x 42 x 72 cm) through sliding doors on either side. The rats experienced habituation to only 1 of the 2 large chambers. The novelty test session began with a rat placed in the center chamber and both sliding doors removed. The researchers measured movement throughout all 3 parts of the box using inferred movement detectors. Isolated rats under inferred light conditions spent less time in a familiar environment and significantly more time in a novel environment than their socially housed counterparts. They did not find any significant differences under white light conditions. Lighting may influence novelty seeking behavior and, more importantly, isolated rats may experience less neophobia than socially housed rats. A further study into the effect of isolated rearing on neophobia suggested that isolated rats are more responsive to novelty

than socially housed rats only in approach situations such as latency to contact and eating novel foods (Hall et al., 1997c).

Physiological

Ethanol (e.g., Farnell & Ing, 2003) and social isolation (e.g., Hall, Wilkinson et al., 1998; Wongwitdecha & Marsden, 1996) are known to impact physiological functions such as decreasing the reinforcing properties of morphine. For example, Wongwitdecha and Marsden randomly divided an unknown number of male Lister rats at the age of 21 days into an isolated (41 x 26 x 20 cm) or social condition (4 rats in a 52 x 32 x 20 cm cage) for 6 weeks. They assessed morphine sensitivity with a place preference test which they conducted when the rats were least active. During the place preference test, researchers placed rats individually into an open arena (83 x 32 cm) for a 10 min assessment. Black lines on the floor of the arena divided it into 4 equal quadrants so that a rat's position within the arena could be determined and researchers added visual cues to the arena so that rats could orient themselves. Then they recorded the quadrants that the rats visited. The least visited quadrants became the treatment quadrants. After researchers determined a treatment quadrant, the rats experienced a daily injection of morphine (1 and 5 mg/kg) or saline. Ten min later researchers placed the rats in their treatment quadrant. Barriers prevented the rats from exploring outside their treatment quadrant. After a 15 min trial, they returned the rats to their home cage. During the day of testing, Wongwitdecha et al. did not give the rats an injection. They placed the rats in the center of the arena and allowed them to enter all quadrants for 10 min. They concluded that rats reared in isolation did not return to the morphine treatment quadrant, indicating that they were less sensitive to morphine than the socially housed rats. Wongwitdecha et al. stated that the inability to develop a place preference for morphine may

be due to alterations in the brain's opiate system in addition to other neurotransmitters like dopamine, serotonin, and noradrenaline.

Social isolation may alter numerous neurotransmitters such as dopamine and serotonin (e.g., Hall, Wilkinson et al., 1998). For example, Lapiz et al. (2003) found that isolated rats displayed behavior changes like alterations in conditioning responses and hyperactivity in response to novelty and amphetamine. They noted that these unusual behavioral patterns correspond to increases in presynaptic dopamine and serotonin in the nucleus accumbens. Interestingly, reduced central serotonin (5-HT) occurs in both isolated rats (Jones et al., 1990) and those exposed to ethanol (Melchior & Myers, 1976). Further, Matsumoto, Ojima and Watanabe (1997) indicated that social isolation may decrease the hypnotic activity of ethanol due to a decrease in the activity of a $GABA_A$, an inhibitory neurotransmitter chloride (Cl^-) channel. In addition, the stress of social isolation may significantly diminish the potentiality of a GABA chloride (Cl^-) influx. Interestingly, this stress does not diminish the ability of ethanol to enhance the potentiality of a GABA (Cl^-) influx in mice. This research indicates that many of the alterations in neurotransmitters that are associated with social isolation are also consistent with the alterations in neurotransmitters related to ethanol. Therefore, increases in ethanol consumption among isolated rats may relate to a similarity among the altered function of neurotransmitters such as dopamine and serotonin.

Thielen, McBride, Lumeng and Li (1993) suggest that isolated housing may alter some of the functional properties of the $GABA_A$ benzodiazepine receptors in alcohol preferring and alcohol nonpreferring rats. However, Rilke, May, Oehler and Wolffgramm (1995) do not agree that alterations in cortical benzodiazepine receptor density result from

isolation, but they do acknowledge alterations in brain chemistry resulting from isolation. Specifically, they identify an increase in the extracellular dopamine concentration in the striatum and the nucleus accumbens, a reduction in dopamine (D_2) receptor density and alterations in the structure and function of $GABA_A$ receptors.

Operant Conditioning

Much of the research thus far has focused on ethanol consumption in a free-access situation, a condition in which animals have unrestricted access to consume ethanol, by isolated versus socially housed rats (e.g., Deatherage, 1972); however, few researchers if any have investigated ethanol consumption in isolated and socially housed rats in a situation where they must work (lever press) for an oral presentation of ethanol. The operant self administration procedure is a reliable model for the reinforcing properties of ethanol (e.g., Roehrs & Samson, 1981). Rats do not readily consume ethanol (e.g., Roehrs & Samson). In order to establish a reliable lever pressing behavior for alcohol, many researchers use the well established sucrose fading procedure (e.g., Grover et al., 1991). Samson (1986) discovered that when water and ethanol (10%) are available in an operant chamber, rats will press the lever for ethanol. The sucrose fading procedure is a reliable method for the oral self-administration of ethanol. Rogowski, Kostowski, and Bienkowski (2002) examined the sucrose fading procedure to determine if a relationship exists between this procedure and free-choice ethanol drinking in Wistar rats. Rogowski et al. did not find a relationship between preference for sucrose and voluntary ethanol consumption. Sucrose self-administration predicted only the first phase of ethanol consumption by the sucrose fading procedure.

Summary

Recently, many studies have explored the relationship between alcohol consumption and living conditions (isolation vs. group housing) in the rat. Prior research using the rat model has shown that environmental conditions have a significant impact on alcohol consumption and anxiety in a free access situation. Rats have been widely used as models of environmental stress and ethanol consumption. This research has yielded interesting but at times contradictory results. For example, numerous studies have noted that isolation increases ethanol consumption whereas other studies report no difference or that socialization increases ethanol consumption. Many of these inconsistencies relate to differences in ethanol concentrations, the age of isolation onset, rat gender, and degree of social crowdedness, to name a few. While these variables seem to confound much of the research in this area, future investigations that utilize the same type of methods including subjects and equipment, for example, may not be as variable as the general research in this area because most of the variables of interest will be held constant (i.e., cage sizes, rat strain, and age of isolation).

Further research indicates that the role of the environment greatly impacts numerous physiological processes such as neurotransmitters like dopamine, serotonin, and noradrenaline. Further, isolated housing may alter some of the functions of $GABA_A$ benzodiazepine receptors in alcohol preferring and alcohol nonpreferring rats. These studies suggest that social isolation and ethanol may react in a similar way in the brain. This may explain the connection between isolation and ethanol consumption.

Lastly, the research seems to be somewhat unreliable due to confounding variables. Often researchers use numerous cage sizes for both isolated and social housing, while previously mentioned research indicates that cage size and crowding may affect consumption

levels. Further, researchers have used numerous rat species so that comparison between studies remains difficult. Further, some researchers conduct experiments during the rat's active night cycle while others are during the inactive day cycle.

I developed the following research questions and hypotheses based on prior research:

Research Question 1: Do housing conditions (isolated, socialized) affect ethanol consumption by rats in a two-bottle preference test?

Research Question 2: Do housing conditions (isolated, socialized) affect ethanol consumption by rats when they must work for ethanol or water in an operant situation?

Research Question 3: Does housing condition affect operant pressing for ethanol the same way it effects free access consumption of ethanol?

Hypothesis 1: Isolated and socialized rats will differ in ethanol consumption when exposed to a two-bottle preference test.

Hypothesis 2: Isolated and socialized rats will differ in ethanol consumption when they must work for ethanol or water in an operant situation.

Hypothesis 3: Isolated and social housing affect operant pressing for ethanol and free-access for ethanol differently.

CHAPTER 2

METHOD

Prior research using the rat model has shown that environmental conditions significantly impact alcohol consumption and anxiety in a free access situation (e.g., Wolffgramm, 1995). The purpose of this study is to investigate the effect of isolated vs. group housing on alcohol consumption when the animals must work for the alcohol in an operant chamber, as well as free access consumption.

Design

A mixed factorial design was the general design for this study. The primary independent variable was type of housing, isolated vs. socialized. I used a repeated measures design for days. The dependent variables were ethanol consumption for the two-bottle preference test and dipper deliveries for the operant situation. I assessed the two-bottle preference test situation using a ratio [ethanol (g) / (water (g) + ethanol (g)] of fluid consumption, and I assessed the operant situation using a ratio (dipper deliveries of ethanol / total dipper deliveries).

Subjects

I used 30 male Long Evans Blue Spruce rats (Harlan, Madison, WI) for this study. At weaning (20-24 days old), I randomly assigned these rats to 1 of 2 groups: isolated, or socialized housing. Initial (Isolated $M = 419.67$, $SD = 29.26$, $n = 15$; Social $M = 398.19$, $SD = 37.18$, $n = 15$; $t(28) = -1.76$, $p = .09$) and final (Isolated $M = 502.29$, $SD = 36.77$, $n = 15$; Social $M = 475.83$, $SD = 47.46$, $n = 14$) body weights (g) were similar for both groups of rats, $t(27) = -1.67$, $p = .11$). The Emporia State University Animal Care and Use Committee (ESU-ACUC-05-007) approved this research (see Appendix A), and I treated all rats in

accordance with the principles of 8.09 Human Care and Use of Animals in Research in the APA *Ethical Principles of Psychologists and Code of Conduct* (American Psychological Association, 2002).

Equipment

Each individual (24 x 17 x 20 cm) and social (24 x 17 x 43 cm) hanging stainless steel cage had a grid floor and front (Figure 1 Top). I constructed the operant cages from stainless steel hanging cages the same size and type that I used for each group's home cage; therefore, the dimensions of the operant cage were exactly the same as the home cages (Figure 1 Bottom). Each computerized operant cage contained 2 fixed response levers (MED-PC, ENV-110M) located on the right and left sides of the cage, 8.2 cm from the center and 4 cm above the floor and 2 (0.01 ml) liquid dippers (MED-PC, ENV-202M) located 2 cm above the grid floor and recessed into the front panel. In addition, each operant chamber contained a house light (MED-PC, ENV-315M, 100 mA) that illumintaed the chamber during each session. Also, each operant chamber contained a response light (MED-PC, ENV-221M, 100 mA) located directly above each lever. I programmed the response light to illuminate for 2 s when the rat reached the desired response rate indicating the activation of the corresponding liquid dipper. Responses on the right lever resulted in a dipper presentaiton to the right of that lever and responses on the left lever resulted in a dipper presentation to the right of that lever. Lever presses resulted in a 3 s liquid dipper (.01 ml) presentation. I programmed and operated the operant schedules and training sessions with MED-PC-IV software. I used 3 floor fans to circulate the air within the testing room and dampen any extraneous noise. I thoroughly cleaned each chamber with plain water after each session. Each operant session lasted 30 min, 1 hr, or 24 hrs as noted.

Figure 1. Top: Isolated (24 x 17 x 20 cm) and Social (24 x 17 x 43 cm) rat home cages. Bottom: Isolated (24 x 17 x 20 cm) and Social (24 x 17 x 43 cm) rat operant cages.

Procedures

Lab assistants handled rats freely from 24 to 31 days old. After 31 days of age, they handled all rats only when necessary. I provided food (Teklad 18% Protein Rodent Diet, Harlan, Madison, WI) and water (ball-sipper tubes) ad lib on the home cage unless otherwise specified. All rats remained on a 12/12 hr dark/light cycle. I marked (red, blue, green, or orange All-Weather Paintstik Livestock Marker) all rats' tails for identification. Prior to beginning the study, all rats experienced experimental housing for 2 months. Experimentation began at about 12 weeks of age. I conducted all training and testing in the dark during the rat's wake cycle unless otherwise noted. Experimentation required a total of 8 phases. Phases 1 and 8 consisted of two-bottle (water vs. ethanol) tests on the home cage. Phases 2 – 5 involved training rats to press a lever for ethanol reinforcement using a sucrose-fading procedure. Numerous studies have established the sucrose-fading procedure as a reliable method for oral self-ingestion of ethanol by rats and as a dependable model for establishing a lever pressing behavior for ethanol in the rat (e.g., Grover et al., 1991; Samson, 1986). Phases 6 and 7 consisted of choice operant lever pressing for water vs. ethanol.

More specifically, Phase 1 consisted of all rats receiving a continuous two-bottle preference test for 5 days. I provided tap water (90 ml) and ethanol (5% vol/vol) ad lib on the home cage. At the same time each day, I measured these solutions (g), replaced the bottles with fresh fluids, and alternated their positions. Phase 2 consisted of training the rats in an operant box to lever press for a 20% (wt/vol) sucrose solution for 1 hr/day for 15 days. I divided all 30 rats into two sets of 15 for the purpose of training. To counterbalance for possible lever preferences, I trained half of each group of the rats on the left lever/dipper and the other half of the rats on the right lever/dipper. I trained all rats individually, first one set

then the second set. During training the animals were water deprived with a 10 min supplement of water on the home cage after the session. I altered the water bottle positions each day to prevent a bottle position effect. I used a FR1 (one lever press resulted in a dipper delivery) for the first 7 days. For the next 2 days, I used a FR3 (three lever presses resulted in a dipper delivery) schedule and the remaining days consisted of a FR4 schedule. After all rats established a reliable lever pressing behavior, water was ad lib on the home cage for the remainder of the study. Phase 3 consisted of lever pressing for a 20% (wt/vol) sucrose solution for 30 min/day for 6 days. I alternated the sucrose solution daily from the dipper on one side to the dipper on the other side to train the rats to operate both dippers and to avoid a preference for a particular dipper. Phase 4 consisted of lever pressing for a 10% sucrose (wt/vol) and 5% ethanol (vol/vol) solution for 30 min/day for 6 days. Phase 5 consisted of lever pressing for a 5% ethanol (vol/vol) for 30 min/day for 2 days.

Phase 6 consisted of lever pressing for a 5% ethanol (vol/vol) vs. tap water in a concurrent situation where the rats selected which liquid to lever press for in a 30 min/day session for 2 days. I alternated lever/solution locations daily from the left to right lever to prevent a preference for a particular dipper. Phase 7 consisted of a single 24-hour session of lever pressing for a 5% ethanol (vol/vol) vs. tap water. During Phase 7, I tested each social group (3 rats) together in the same operant cage at the same time and video recorded each session for later review. Finally, Phase 8 consisted of a two-bottle preference test conducted in the same manner as Phase 1.

CHAPTER 3

RESULTS

Hypothesis 1

To test the hypothesis that isolated and socialized rats will differ in ethanol consumption when exposed to a two-bottle preference test, I first calculated means and standard deviations of ethanol and water consumption (g) across the 5 test days for Phase 1 (see Table 1) and Phase 8 (see Table 2). I also calculated means and standard deviations of consumption ratios [5% ethanol / (ethanol + water)] across the 5 test days for Phase 1 (see Figure 2 Top) and Phase 8 (see Figure 2 Bottom). I was unable to determine exactly how much liquid each socially housed rat consumed. Therefore, I divided the total consumption of each social cage by the total number of rats in that cage. Although researchers have used this method (e.g., Juárez & Vázquez-Cortés, 2003), this prevented me from determining actual within group variability for social rats. Next, I performed separate mixed factorial analyse of variances (ANOVAs) on the ratio of fluid consumption data for Phase 1 and Phase 8 to investigate possible differences in ethanol consumption among isolated and socialized rats. Finally, I compared the total mean ratios for Phase 1 and Phase 8 to determine if the effects recurred.

Phase 1. I performed a 2 Group (Isolated, Social) x 5 Days mixed factorial ANOVA on the ratio of fluid consumption data from the Phase 1 two-bottle preference test. Both the main effect of Group, $F(1, 28) = 76.73$, $p < .001$, $\eta^2 = .73$, and the main effect of Days, $F(4, 112) = 3.66$, $p = .01$, $\eta^2 = .16$, were significant. Most importantly, the interaction of Group x Days was significant, $F(4, 112) = 6.61$, $p < .001$, with a large effect size of $\eta^2 = .19$. A follow-up analysis (Tukey HSD) indicated that while isolated rats consistently consumed

Table 1

Phase 1: Fluid Consumption (g) Means and Standard Deviations by Isolated and Socialized Rats

	Isolated (n =15)		Social (n =15)	
Day	Water	Ethanol	Water	Ethanol
1	15.31 (9.20)	56.13 (37.35)	4.33 (1.90)	16.46 (1.12)
2	14.71 (10.06)	36.15 (18.61)	1.90 (.54)	19.34 (2.05)
3	15.57 (10.78)	23.62 (14.81)	.77 (.36)	15.83 (.89)
4	13.83 (7.91)	43.90 (23.55)	.50 (.12)	14.41 (.64)
5	11.41 (8.41)	60.85 (27.83)	1.70 (.69)	12.52 (1.90)

Table 2

Phase 8: Fluid Consumption (g) Means and Standard Deviations by Isolated and Socialized Rats

	Isolated		Social	
Day	Water	Ethanol	Water	Ethanol
1	4.57 (3.80)	46.09 (15.43)	.69 (.34)	16.96 (1.21)
2	5.67 (4.15)	52.91 (13.46)	.40 (.14)	18.36 (1.08)
3	6.78 (7.98)	47.29 (10.46)	.44 (.19)	16.96 (.99)
4	7.44 (7.22)	37.69 (14.80)	.73 (.30)	12.94 (.87)
5	8.88 (11.29)	35.95 (9.08)	.28 (.08)	14.44 (1.12)

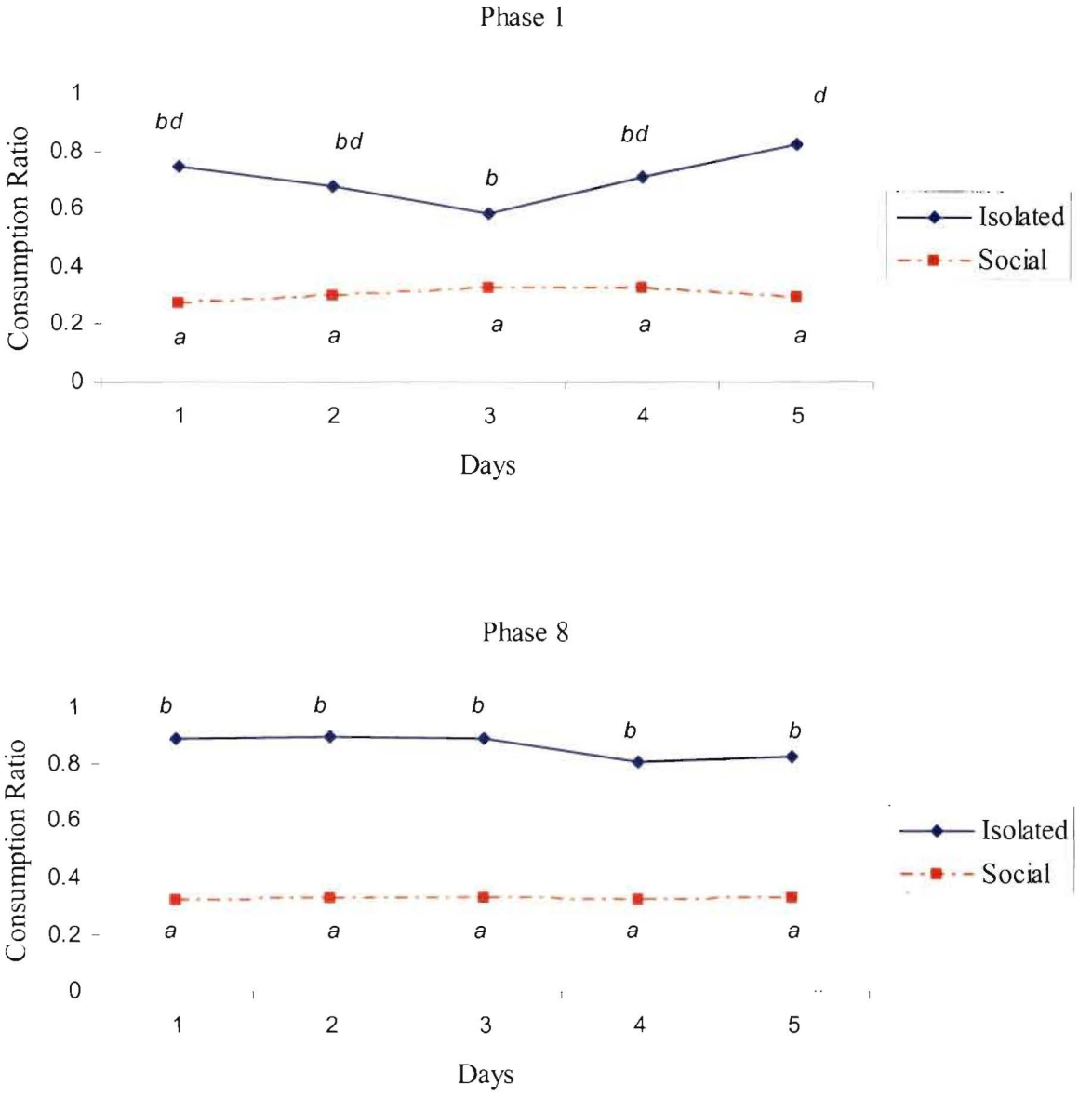

Figure 2. Top: Phase 1 consumption ratio (g) ethanol and water for isolated (n = 15) and socialized (n = 15) rats. Bottom: Phase 8 consumption ratio (g) ethanol and water for isolated (n = 15) and socialized (n = 15) rats. Symbols with different lowercase letters are significantly different, $p \leq .05$.

more ethanol and less water than socialized rats, only the isolated rats consumed significantly more ethanol on Day 5 than on Day 3.

Phase 8. I performed a 2 Group (Isolated, Social) x 5 Days mixed factorial ANOVA on the ratio of fluid consumption data from the final two-bottle preference test. The main effect of Group, $F(1, 28) = 544.01$, $p < .001$, was significant with a large effect size of $\eta^2 = .95$. Isolated rats had a significantly higher ethanol to total fluid ratio than social rats throughout Phase 8. Neither the main effect of Days, $F(4, 112) = 1.81$, $p = .13$, nor the interaction of Days x Group, $F(4, 112) = 1.50$, $p = .21$, was significant.

Phase 1 and Phase 8 comparison. I performed a 2 Group (Isolated, Social) x 2 Phase (Phase 1, Phase 8) mixed factorial ANOVA on the mean consumption ratios (see Figure 3) from the initial and final two-bottle preference tests to investigate whether the differences in ethanol consumption among isolated and socialized rats persisted across the study. Both the main effect of Group, $F(1, 28) = 235.62$, $p < .001$, $\eta^2 = .89$, and the main effect of Phase, $F(1, 28) = 19.81$, $p < .001$, $\eta^2 = .41$, were significant. Most importantly, the interaction of Group x Phase was significant, $F(1, 28) = 10.36$, $p < .01$, with a large effect size of $\eta^2 = .27$. In other words, the larger ethanol consumption by the isolated rats increased from Phase 1 to Phase 8 while the lower consumption of the social rats remained the same for the two phases.

Hypothesis 2

To test the hypothesis that isolated and socialized rats will differ in ethanol consumption when they must work for ethanol or water in an operant situation, I first calculated means and standard deviations of ethanol and water dipper deliveries across the

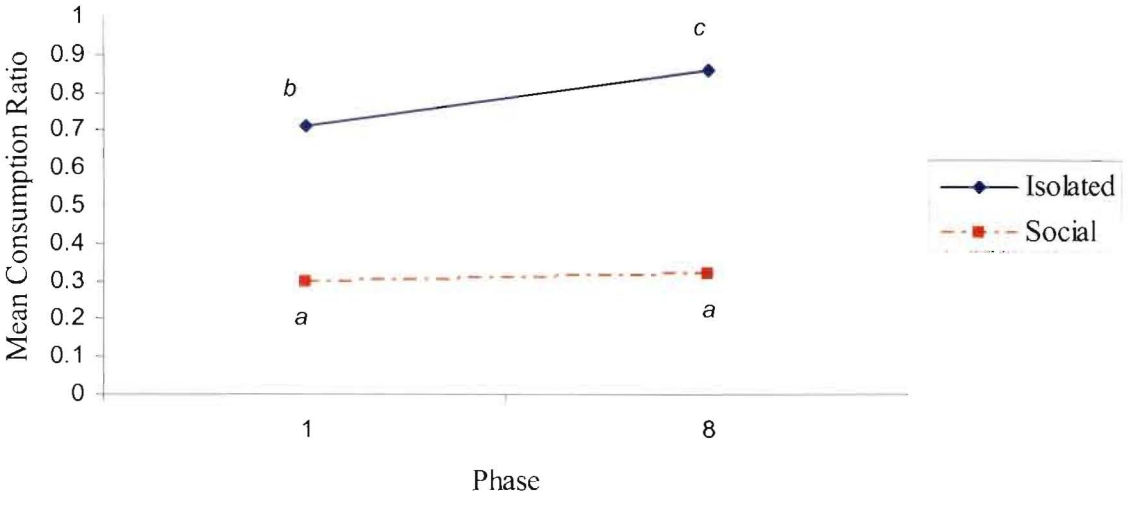

Figure 3. Total mean consumption ratio (g) ethanol and water for isolated (n = 15) and socialized (n = 15) rats during Phases 1 and 8. Symbols with different lowercase letters are significantly different, $p \leq .05$.

two test days for Phase 6 water and ethanol, and for the single 24-hour session for Phase 7 water and ethanol (see Table 3). I also calculated means and standard deviations of operant ratios (dipper deliveries of ethanol/ total dipper deliveries) for Phase 6 (see Figure 4 Top) and Phase 7 (see Figure 4 Bottom). Again, I was unable to determine exactly how many dipper deliveries each socially housed rat received and therefore divided the total dipper deliveries of each social cage by the total number of rats in that cage. Data from one isolated rat that was used in consumption ratio analyses was not included in operant analyses because he did not learn to press the lever for reinforcement during training phases 2 – 6. Next, I performed a two-way between subjects ANOVA on the operant ratio of dipper deliveries from the 2 one-hour sessions of 2-lever preference testing to investigate possible differences in ethanol reinforcement among isolated and socialized rats.

Phase 6. I performed a 2 Group (Isolated, Social) x 2 Days mixed factorial ANOVA on the operant ratio of dipper deliveries. The results indicate no statistically significant difference for the main effects of Group, $F(1, 28) = .03, p = .86$, or Days, $F(1, 28) = .15, p = .71$, and the interaction of Group x Days, $F(1, 28) = .04, p = .85$. Therefore, housing had no effect on reinforcement of lever pressing for ethanol and water. Additionally, because I tested all animals individually in the operant cages during this phase, I compared group variances and found that the Levene's Test for Equality of Variances indicated group variability was not significantly different ($p = .24$). This is important because it suggests that group variability may not have been different in the other phases where I tested social animals together and was unable to determine within-group variability.

Phase 7. I performed a one-factor between subjects ANOVA on the operant ratio of dipper deliveries from the 24-hour operant session to investigate possible differences in

Table 3

Phase 6 & 7: Dipper Deliveries Means and Standard Deviations for Isolated and Socialized Rats

	Isolated (n =14)		Social (n =15)	
	Water	Ethanol	Water	Ethanol
Phase 6				
Day 1	130.73 (140.11)	70.53 (78.52)	68.67 (49.13)	57.27 (54.74)
Day 2	104.00 (123.05)	82.00 (134.20)	44.60 (40.06)	39.47 (34.45)
Phase 7				
24 hrs	174.86 (180.38)	117.43 (107.40)	408.20 (46.98)	532.20 (142.36)

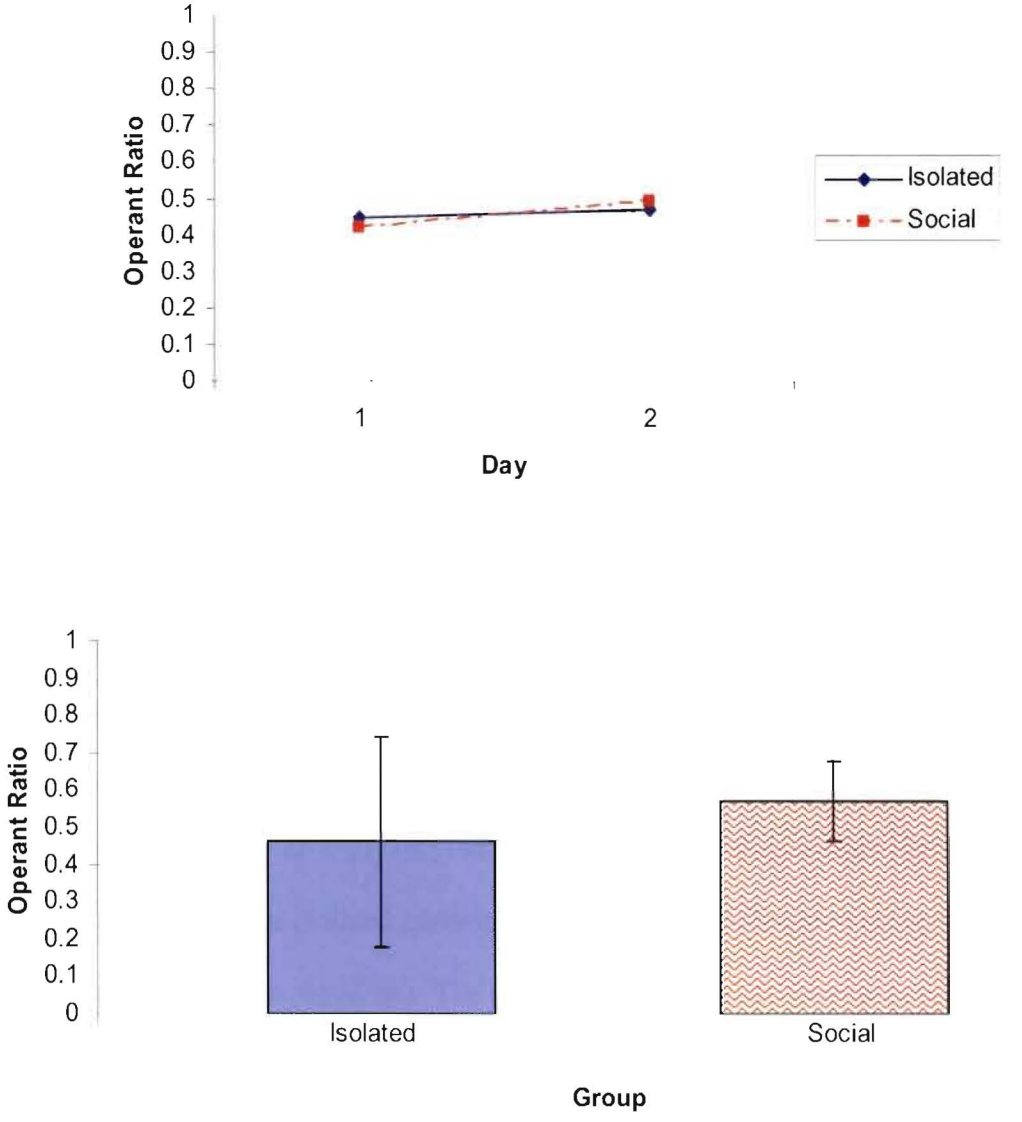

Figure 4. Top: Phase 6 (1 hr, individually tested) operant ratio for isolated (n = 14) and socialized (n = 15) rats for 2 days. Bottom: Phase 7 (24-hr, each social set tested together) operant ratio for isolated (n = 14) and socialized (n = 15) rats for a single 24-hour session. Error bars represent standard deviations.

ethanol reinforcement among isolated and socialized rats. The results indicate no statistically significant differences $F(1, 27) = 2.21, p = .15$ between isolated and socialized groups. Again, housing conditions appeared to have no effect on lever pressing for ethanol reinforcement.

Hypothesis 3

To test the hypothesis that isolated and social housing affect operant pressing for ethanol and free-access for ethanol differently, I performed a 2 Group (Isolated, Social) x 2 Ratio (Operant, Consumption) mixed factorial ANOVA on Phase 7 (24 hour choice operant for ethanol vs. water) and just Day 1 of Phase 8 (the first 24 hour two-bottle ethanol vs. water). The main effect of Group, $F(1, 27) = 20.35, p < .001, \eta^2 = .43$, and the main effect of Ratio, $F(1, 27) = 4.86, p = .04, \eta^2 = .15$, were significant. Most importantly, the interaction of Group x Ratio was significant, $F(1, 27) = 75.81, p < .001$, with a large effect size of $\eta^2 = .74$ (see Figure 5). A follow-up analysis (Tukey HSD) indicated that operant ratios were not significantly different for isolated and social rats, but the consumption ratios were significantly higher for isolated rats. The means and standard deviations ($n = 29$) for the ethanol consumption ratio for isolated (I) and socialized (S) groups during the first day of Phase 7 were as follows: $M_I = .46, SD_I = .28$; $M_S = .57, SD_S = .11$. The means and standard deviations ($n = 29$) for the dipper delivery ratio during Phase 8 were as follows: $M_I = .88, SD_I = .17$; $M_S = .32, SD_S = .01$.

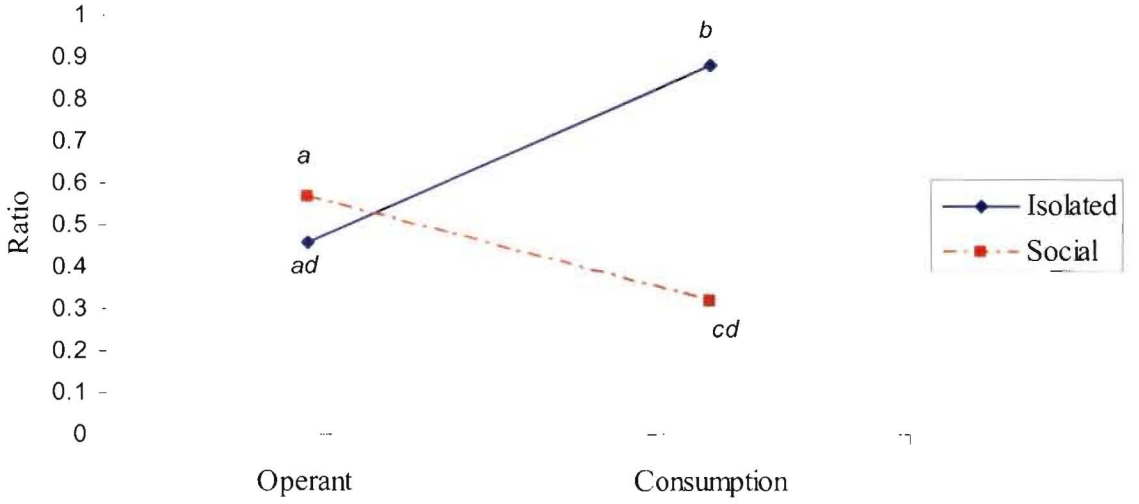

Figure 5. Comparison of mean operant and consumption ratios for the isolated (n = 14) and socialized (n = 15) rats in Phase 7 (operant) and the first 24-hrs of Phase 8 (two-bottle). Symbols with different lowercase letters are significantly different, $p \leq .05$.

CHAPTER 4

DISCUSSION

The purpose of my study was to examine the effects of housing conditions (Isolated, Social) on ethanol consumption when the animals have the choice to work for ethanol or water in an operant situation as well as in a free access condition. My most important findings were that housing conditions affected ethanol consumption differently. Isolated rats consumed more ethanol in a free-access situation than socialized rats both at initial exposure and after repeated exposure. Further, in an operant situation, where the animal must press a lever for ethanol, isolated and socialized rats did not differ in ethanol reinforcement.

Hypothesis 1 (isolated and socialized rats will differ in ethanol consumption when exposed to a two-bottle preference test) was supported. Hypothesis 2 (isolated and socialized rats will differ in ethanol consumption when they must work for ethanol or water in an operant situation) was not supported. Hypothesis 3 (isolated and social housing affect operant pressing for ethanol and free-access for ethanol differently) was supported.

Hypothesis 1

Although, a few studies describe no difference in ethanol consumption among isolated and socialized rats (e.g., Schenk, et al.), other research suggests that socialized rats are more likely to consume ethanol than isolated rats (e.g., Blanchard, Hori, Tom & Blanchard, 1987; Rockman, Borowski & Glavin, 1986). My isolated rats consumed significantly more ethanol than socialized rats during a two-bottle (water vs. ethanol) preference test, thereby supporting the more typical research finding of an increase in ethanol consumption by rats housed in isolation.

One explanation for isolated rats consuming more ethanol is that this drug may alleviate stress. Numerous studies suggest that stress caused by social isolation may be responsible for increased ethanol consumption among isolated rats (e.g., Ellison, 1981; Parker & Radlon, 1974; Rockman et al., 1988). For example, Parker and Radlon noted that isolated rats show higher incidence of enlarged adrenal glands and elevated cortisone levels (physiological indicators of stress) which increase an animal's ability to metabolize ethanol. Further, Patterson-Buckendahl et al. (2004) measured adrenal gene expression for enzymes of the catecholamine synthetic pathway in rats after 4-7 weeks of ethanol consumption. This pathway consists of proteins functioning together in the synthesis and regulation of dopamine and norepinephrine. Their results support previous research that suggests ethanol may enhance the ability to respond to acute or chronic stress.

My isolated rats consumed more ethanol in Phase 8 than in Phase 1. One explanation for these findings is that the isolated rats may have developed a greater tollerance to ethanol across Phases 2-7 than the social rats. With an increase in tolerance, isolated rats in Phase 8 would need to consume more ethanol than in Phase 1 to experience the same reinforcing properties. Another possibility is that the isolated rats may have experienced acute stress in Phase 1 and chronic stress in Phase 8. An additional explanation is that Phase 8 followed a sucrose/ethanol training phase where I paired ethanol with a sweet solution for operant training purposes. In contrast, the rats in Phase 1 were novel to the sucrose/ethanol solution. Rats will readily consume large amounts of sucrose (e.g., Samson, 1986); therefore, it is possible that the isolated rats in Phase 8 were more willing to consume ethanol than they were in Phase 1 due to the previous sucrose/ethanol pairing. However, according to Rogowski, Kostowski and Bienkowski (2002), a relationship between sucrose and ethanol

(8%) will develop during the sucrose fading procedure but the pairing is not robust and should fade away within a week. This means that alterations in ethanol consumption between Phase 1 and 8 are unlikely to be the result of sucrose exposure because the final two-bottle preference test (Phase 8) occurred more than a week after the rats were last exposed to sucrose. Additionally, Rogowski et al. also suggest that a time lag between sessions may not limit the brief relationship between exposure to sucrose and operant responding for ethanol. Therefore, I trained the rats in the operant situation after I removed the sucrose and before operant response testing began.

A further explanation relates to an increase in body weight. All rats gained weight during experimentation, and the larger sized rats had to consume more ethanol to experience the same effects of ethanol. Differences in ethanol consumption among isolated and socialized rats may result from body weights; however, I did not find a between-group difference in rat body weights that would explain the difference in ethanol consumption among the isolated and socialized rats.

Research on housing conditions and the oral self-administration of ethanol in rats has relied heavily on two-bottle preference tests where both ethanol and water are concurrently available in a free-access situation. These procedures allow the animals to freely consume both ethanol and water. The concentration strength of ethanol may be an important aspect of consumption for rats in isolated and social housing conditions. For example, Deatherage (1972) found that isolated rats consumed significantly more ethanol than social rats when the ethanol concentration was 20%, and he did not find any between group differences at 10%. While this study did not use a two-bottle preference test, Deatherage noted a clear consumption preference for the higher concentrations of ethanol. In support of Deatherage's

finding and contrary to mine, Hall, Huang et al., (1998) found that isolated rats consumed significantly more sucrose, saccharine, and ethanol in higher concentrations than socially reared rats, but that isolated rats did not consume significantly more ethanol at lower concentrations (2%, 4%). While most researchers do not identify the difference between low and high concentrations, Juarez and Vázquez-Cortés (2003) describe a low ethanol concentration as 1-6%. Most researchers in this area seem to refer to high ethanol consumption as 10% ethanol or more. Several studies used a two-bottle preference test and found that isolated rats consumed significantly more ethanol at only 10% (e.g., Roske, Baeger, Frenzel & Oehme, 1994; Schenk et al., 1990). Additionally, Parker and Radow (1974) found a significant difference in consumption among isolated and socialized rats at 25% ethanol. These findings imply that isolated rats are more likely to consume significant amounts of ethanol at high ethanol concentrations; however, my findings suggest that between group (Isolated, Social) differences may also exist at low ethanol (5%) concentrations. Few if any report that isolated rats consume significantly more ethanol at the 5% concentration or lower.

One explanation for why my results differed from previous findings regarding the concentration strength of ethanol may relate to the rat strain that I used. For example, numerous studies have reported significant differences in ethanol consumption among isolated and socialized rats with high ethnaol concentrations using the Wistar rat strain (e.g., Hall et al., 1998; Juarez, & Vázquez-Cortés, 2003; Roske, Baeger, Frenzel, & Oehme, 1994) or the Lister hooded rat strain (e.g., Wongwitdecha, & Marsden, 1996) However, this explanation is not consistent because several studies using the Long Evans rat strain have also reported significant differences only at high ethanol concentrations (e.g., Buckalew,

1979; Deatherage, 1972; Schenk, 1990). Another factor to consider may be the age of isolation. Schenk (1990) reported that rats isolated at weaning are more likely to consume more ethanol at higher concentrations than rats isolated at maturity. This research trend may explain why the rats in my study consumed a significant amount of ethanol at a low concentration. However, I established housing conditions at weaning which is consistent with the general research literature.

A further explanation may relate to differences in mean ethanol consumption. However, variations in reporting ethanol consumption make a direct comparison of the mean consumptions in this study with other studies difficult. Interestingly, the mean consumption ratios from my study appear to be lower than that Juarez and Vázquez-Cortés (2003) displayed in their Figure 2. This may be due to differences in the number of animals socially housed (8 rats per group), the age of ethanol exposure (as young as 25 – 35 days old), and the ethanol concentration (8%). Future research using my procedures needs to investigate several concentrations of ethanol and determine why these procedures result in group (isolated vs. social) differences at the 5% concentration. Clearly, prior research has shown that environmental conditions impact ethanol consumption in a free-access situation; however, few have studied the effects of housing on choice operant responding for ethanol vs. water.

Hypothesis 2

To establish reliable lever pressing for the oral self-administration of ethanol, many researchers use the sucrose fading procedure (e.g., Grover et al., 1991; Samson, 1986). I used this procedure to train both groups (Isolated, Social) prior to investigating Hypothesis 2 in Phases 6 and 7. I found that isolated and socialized rats did not differ in operant responding (lever pressing) when they worked for ethanol or water in Phases 6 and 7. Although few

operant sources are avaliable to compare with my findings, these data support the research trend for no difference in ethanol consumption among rats in socialized and isolated housing conditions using a two-bottle preference test (e.g., Schenk et al., 1990). The reinforcing properties of ethanol due to stress-related housing conditions are not significant when the animals must work for ethanol. Not all types of stress produce increases in ethanol consumption (e.g., Roske et al., 1994).

Exposure to the operant situation may affect the complex etiology surrounding housing stress in rats by either decreasing stress levels or by increasing the effects of ethanol resulting in lower ethanol consumption. For example, if the isolated rats' higher ethanol consumption in the two-bottle home cage free-access test was due to higher stress levels, then perhaps ethanol consumption of dipper delivered ethanol was not different because my social animals were just as stressed as the isolated rats in the operant situation. However, all animals actually consumed less ethanol during Phase 7 than during either two-bottle preference tests. This indicates that the social rats may not have been stressed or isolated rats were not as stressed as they were in the two-bottle preference test.

It is important to identify the actual ethanol consumption rate. Assuming that all rats consumed the solution delivered to them in the liquid dipper, 100 dipper deliveries are equivalent to 1 (g) of liquid. Therefore, the actual mean consumption for the operant situation is much lower than for the two-bottle preference test. One reason for this difference may be because all rats had access to water ad lib on their home cage during the operant sessions. In contrast, the two-bottle preference tests consisted of both solutions on the home cage at the same time. During Phase 6, the rats may have consumed most of their liquid for the day before entering the operant cage. Additionally, the decrease in mean consumption may relate

to the liquid dipper volume. Most research using the sucrose fading procedure or other similar techniques seems to use a .1 size dipper (e.g., Grover, et al.), whereas I used a .01 size dipper. Therefore, the rats in my study would have to work more to get the same amount of solution than the rats in other studies that use a larger sized dipper.

Another explanation for why I did not find a significant difference in ethanol consumption among the isolated and socialized groups during the operant situation may relate to differences in behavioral patterns among differently housed rats. For example, isolated rats may not change their behavioral tendencies as frequently as socialized rats (Hall et al., 1997a). Therefore, isolated animals may spend more time drinking because they do not divide their attention between drinking and interacting with other rats. Anecdotal evidence from video recordings of the operant sessions indicate that the social rats spent a large amount of time interacting. Occasionally, the social rats bumped into the levers enough to cause a dipper delivery. This may suggest that the social rats may have bumped the levers enough to suggest that they consumed as much ethanol or water as the isolated rats when they did not. However, evidence from Phase 6 suggests that this was not the case. The isolated rats did not consume significantly more ethanol than socialized rats during Phase 6 where I tested all rats individually, thereby supporting the idea that social rat exploitation of the liquid dipper and dipper bumping were not likely to affect my findings in Phase 7. Because I tested all three social rats together in the same operant box at the same time, group variability may not have differed in Phase 7 where I tested social animals together and was unable to determine within-group variability. Additionally, further video evidence of Phase 7 suggests that some social rats may have exploited the liquid dipper so that the rat that lever pressed for a reward was not necessarily the same rat that consumed the dipper delivery of

ethanol or water. This may also explain why I did not find a difference in dipper deliveries among isolated and socialized rats.

Additionally, size differences between the home cage and an operant box may contribute to an increase in ethanol consumption (e.g., Nagaraja & Jeganathan, 2003). Differences in cage sizes and operant box sizes may be another explanation for the previously mentioned inconsistencies in the literature. Research in this area has used various cage sizes. Few if any of the studies in this area use a similar size cage with a similar number of animals per cage. For example, Deatherage (1972) defined social housing as 66 x 26.7 x 18.4 cm cages with 6 rats per cage and isolated housing as a single rat in a 66 x 26.7 x 18.4 cm cage, Hall et al. (1997b) used 45 x 20 x 20 cm cages with 2 rats per cage and isolated housing as a single rat in a 20 x 20 x 20 cm cage, Schenk (1990) used 41 x 25 x 18 cm cages with 4 rats per cage and isolated housing as a single rat in a 20 x 25 x 18 cm cage, and Juarez (2003) used 49 x 37 x 19 cm cages with 8 rats per cage and isolated housing as a single rat in a 28 x 19 x 15 cm cage. Most of these studies used standard operant boxes which are approximately 30.5 x 21.4 x 21 cm. Brown and Grunberg (1995) relate ethanol consumption by rats to the spatial and population density associated with each animal. Cage sizes should not influence my findings because I constructed operant boxes for the present study from modified home cages. Therefore, differences in cage sizes and operant box sizes could not have influenced ethanol consumption for either group in my study; however, in other studies this could be a problem because the home cage and the operant box were different sizes. This area of research seems extremely limited and requires further study.

Hypothesis 3

In support of Hypothesis 3, isolated and social housing affected operant pressing for ethanol differently than it affected free-access consumption of ethanol. Limited to no research has directly investigated the relationship between these two oral ethanol self-administration methods with isolated and socialized rats. Rogowski et al. (2002) did use the sucrose fading procedure for operant ethanol self-administration and the two-bottle preference test but they did not have a preference condition in the operant box that was comparable to the two-bottle preference test. Also, researchers have investigated isolated and socialized rats by either the operant self-administration of ethanol (e.g., Vacca et al., 2002) or the two-bottle preference test (Deatherage, 1972). Perhaps one explanation for the lack of research comparing these methods is due to the difficulty setting up the boxes and the extensive amount of time necessary to test several rats in a 24-hr operant session. My findings suggest that differences in ethanol consumption among isolated and socialized rats exist only when I provided the animals with free-access to ethanol. When both groups of rats worked for ethanol, they did not consume significantly different amounts. This could mean that isolated rats experienced less stress while working for ethanol or water in the operant cage than when they experienced the two-bottle preference test, or I had a floor effect in the operant tests. Social stress resulting from subordination relates to significant increases in ethanol consumption in rats (e.g., Blanchard et al., 1987). Therefore, my data suggest that isolation stress may be greater than some aspects of social stress, like subordination.

Stress is an important factor in human ethanol consumption (e.g., Higley, Hasert, Suomi, & Linnoila, 1991). Higley et al. noted that many different forms of stress are associated with increases in ethanol consumption. Understanding how stress relates to

ethanol consumption can improve understanding the processes behind alcohol abuse. Therefore, I recommend a comparing between the oral self-administration of ethanol between situations where animals must work (lever press) for ethanol or do not work (free-access) for ethanol. Further, the investigation of a 24-hr session for ethanol preference is important to gain a better understanding of ethanol drinking behavior over time and to investigate ethanol consumption when rats experience an operant session without already consuming significant amounts of water from their home cage. Testing rats that are not water deprived in either an operant session or a two-bottle preference test is important because the animals are consuming liquid because they prefer it and not due to physiological need.

Conclusions

I have concluded from this study that housing conditions differentially affect ethanol consumption in different situations. More specifically, isolated rats consumed significantly more ethanol than socially housed rats when the animals had free access to the ethanol but not in an operant preference test. Future research may explain why isolated and socialized rats do not consume different amounts of ethanol when the animals are working for dipper deliveries of ethanol in an operant situation. My research seems to be the only study attempting to directly compare these two methods with these two housing conditions. For example, in order to gather data for the social groups during Phases 1, 7 and 8, I had to divide the consumption/responding rate by all of the animals in that cage. This procedure prevented me from determining within-group variability for the social cages. Also, I repeated the average consumption/responding rate for each social animal. This means that I used the average of the data for the social rats 3 times, once per each rat in the group. One solution may be to use more social cages because I could determine more accurate consumption and

operant responding rates. During the current study the consumption/responding rates seemed closer than they really are because variance decreases when the same data value occurs repetitively. By using 3 times the number of social groups that I used in this study, I could report each social group average only once rather than 3 times. In general future research should consider redesigning the operant cages, include more social groups, and further investigation of many possible confounding conditions such as: age of isolation, cage size, rat species differences, age of ethanol exposure, and environmental enrichment.

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Appendix

ESU-ACUC Approval Letter

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December 9, 2005

Wayland Howard
Department of Psychology and Special Education
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Dear Mr. Howard:

Your proposal for research entitled: *Housing Effects on Ethanol Consumption: Free Access and Operant Situations* has been approved by the E.S.U. Animal Care and Use Committee. Your protocol number is **ESU-ACUC-05-007** for the time period of November 28, 2005 through November 28, 2006. Please provide a written summary of the actual number of animals used and the actual disposition of those animals at the end of your research. As required by the U.S.D.A. A.W.A., the ESU ACUC will annually review all current ongoing teaching and research protocols involving animal use.

This protocol is in compliance with currently applicable standards for such studies as specified by Federal Regulations and E.S.U. policy. If any substantial changes are to be made to the research involving animal use, or if additional time is necessary, the ACUC should be notified before-hand. Please use this number if the animals are placed in any long-term animal housing.

If you have any questions, please contact me at your convenience.

Sincerely,

John Richard Schrock, Chair 2005-2006
ESU Animal Care and Use Committee

I, Waylon J. Howard, hereby submit this thesis to Emporia State University as partial fulfillment of the requirements for an advanced degree. I agree that the Library of the University may make it available for use in accordance with its regulations governing materials of this type. I further agree that quoting, photocopying, or other reproduction of this document is allowed for private study, scholarship (including teaching) and research purposes of a nonprofit nature. No copying which involves potential financial gain will be allowed without written permission of the author.

Waylon J. Howard
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Title of Thesis

Mary Cooper
Signature of Graduate Office Staff Member

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