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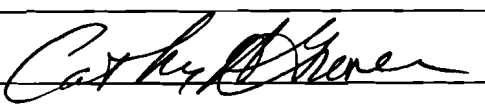
Abstract approved: 

This study investigated the effects of environment (enriched versus impoverished) and socialization (isolated versus socialized) on anxiety and alcohol consumption. Thirty-nine male Wistar rats (Harlan, Madison, WI) were randomly assigned to one of four housing-environment groups (isolated-enriched, isolated-impoverished, socialized-enriched, and socialized-impoverished). Following 97 days of environmental exposure, all rats were tested for anxiety using the elevated plus-maze (EPM). Following anxiety testing, rats were placed in individual hanging metal cages for 1 hr daily with food and increasing doses of alcohol drinking solution. At the end of the 20 days of alcohol consumption, all rats received 2 days of a two-bottle (water vs. 10% alcohol) preference test during the hour. Finally, all rats were again tested for anxiety on the elevated plus-maze. The most important findings were that housing and environment each affected alcohol consumption, and that alcohol reduced anxiety for all rats. All isolated rats drank significantly more alcohol and significantly less water than all socialized rats. All impoverished rats drank significantly more alcohol and significantly less water than all enriched rats Also, all rats, regardless of the type of housing and environment, spent less time on the closed arms of the elevated plus maze after alcohol consumption than they did before alcohol treatment.

THE EFFECTS OF ENVIRONMENT AND SOCIALIZATION ON ANXIETY AND ALCOHOL CONSUMPTION

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CHAPTER 1

INTRODUCTION

Of interest in recent years has been the role of the environment on a vast array of physiological and behavioral functions. Among these include the effects of environmental conditions on anxiety and alcohol consumption. Animals, specifically rats, have been widely used as models for anxiety (Colombo et al., 1995) and in research on alcohol consumption (e.g. Adams & Oldham, 1996; Fernandez et al., 2002; Hall, Huang, Fong, & Pert, 1998a; Hall, Huang, Fong, & Pert, 1998c; Lodge & Lawrence, 2003; Paivarinta, 1990; Rasmussen, Milton, Green, & Puchalski, 2001; Rilke, May, Oehler, & Wolffgramm, 1995; Rockman, Gibson, & Benarroch, 1989; Sandbak & Murison, 2001; Spear, 2000). Prior research using the rat model has shown that environmental conditions have a tremendous impact on, for example, exploration, neurological changes, maze performance, and sleep patterns. Further research has exhibited evidence of environmental effect on alcohol consumption and anxiety.

The first environmental factor discussed will be that of enriched versus impoverished environments. For the purpose of this study, enriched environments refer to caged housing consisting of various stimulus objects (e.g., pvc pipes, plastic bird toys, children's plastic building blocks). These objects consist of approximately 40 items that are rotated daily (Auvergne et al., 2002; Widman & Rosellini, 1990). Impoverished environments refer to simple caged housing that does not include stimulus objects.

The second environmental factor manipulated in this study is socialization versus isolation. Socialized environment is defined as housing arrangements of ten rats per cage, where the rats are only placed in individual cages for one hour daily.

Isolated environment is defined as individual housing arrangements consisting of one rat per cage. These rats have limited exposure to conspecific sounds and odor and no contact with other rats (Wolffgramm, 1990; Wongwitdecha & Marsden, 1996b). The purpose of this study was to examine the effects of enriched versus impoverished and socialization versus isolated environments on alcohol consumption and anxiety. Anxiety was measured using an elevated plus-maze (EPM). The EPM, considered a validated test for anxiety, is a plus-shaped maze approximately 50cm from the floor, with two open arms (without side or end walls) and two closed arms (opaque or transparent walls) that are 50cm long and 10cm wide (Sandback & Murison, 2001).

Review of the Literature

Enriched Environment

Physiological

Environmental stimuli, such as those found in enriched environments, affect aspects of physiology. For example, Auvergne et al. (2002) examined rats housed in enriched environments and found that susceptibility to developing epilepsy differed from those rats housed in non-enriched, isolated conditions. Auvergne et al. discussed that resistance to epileptogenesis may be related to an increased survival of new brain cells in rats housed in enriched environments compared to rats in isolated housing. Several have shown that enriched environments also have effects on neurological changes, such as increase in cell size of visual cortical tissue and thickening of the visual cortex (Bennet et al., 1964; Diamond et al., 1964, 1967; Mollgaard et al., 1971; as cited in Coyle & Singer, 1975). Rats reared in enriched environments have also reportedly shown increased total brain weights and total protein in the brain (Tagney, 1973) as well as increased cortical

thickness and weight, enzyme activity, and greater dendritic branching in the occipital cortex (Volkman & Greenough, 1972).

Rose, Davey, Love, and Dell (1987) investigated the effects of environmental enrichment on the postoperative recovery process in rats. Rose et al. performed extensive craniotomies on the rats and, following a 10-12 day recovery period, placed the rats in one of three housing conditions: standard (subjects housed in groups of four), enriched (subjects housed in groups of 15-18 and included various toys), and impoverished (individually housed). A series of behavioral and neurological tests, including the bracelets test, was performed 1 and 3 days before surgery, and again at 6 and 12 weeks after surgery. For the bracelets test, strips of self-adhesive paper, 0.5 cm in width, were firmly placed on the rats' front paws; order of removal and the time taken for removal were recorded. The purpose of the bracelets test was to examine the process of recovery of sensory loss in the rats. Degrees of stimuli neglect contralateral (opposite side) of neocortical lesions in humans have been documented, thus rats were presented as models for this phenomenon. Recovery from sensory loss was not enhanced, which is "consistent with the view that such environmental manipulations facilitate compensation rather than recovery" (p. 200).

Will, Deluzarche, and Kelche (1983) examined the effects of post-operative environments following hippocampal lesions in rats. Post surgery, rats were placed in one of three environmental conditions: impoverished (individually housed), social (housed in groups of 10), or enriched (housed in groups of 10 with various objects that were changed daily). Contrary to the findings of Rose et al. (1987), environmental enrichment did help attenuate post-operative symptoms in brain-damaged animals. However, as discussed,

Will et al.'s finding "does not exclude the possibility that there might also be an exacerbation of deleterious symptoms in the impoverished group" (p. 131).

The effects of enriched environment conditions on sleep patterns have also been studied. Tagney (1973) exposed rats to either enriched environment (housed in groups of six with various playthings that changed daily) or impoverished environment (housed in individual isolation cages). Electrodes were surgically implanted in the rats so that EEG, EMG, and EOG recordings could be taken in two 23-hour intervals. Tagney found that enriched rats had more total sleep (slow wave sleep time and REM sleep time). It may be worth noting that after impoverished rats were transferred to an enriched environment their REM sleep time increased by 7.2% and their slow wave sleep time increased by 15%.

Emotional and Behavioral

Enriched environments have reportedly reduced emotionality. For example, Denenberg, Garbanati, Sherman, Yutzey, and Kaplan (1978) used an open-field exploratory test, which measures emotional behavior and locomotor activity, to examine effects of rats that were raised in either simple laboratory cages or enriched environments, and were either handled or not handled. Once weaned, rats received right neocortical ablations, left neocortical ablations, sham operations, or no surgery. Denenberg et al. found that handled rats raised in enriched environments exhibited reduced emotionality and increased activity in an open-field exploratory test.

Further studies on environmental enrichment in rats that received right neocortical ablations, left neocortical ablations, sham operations, or no surgery (Garbanati et al., 1983) have also been used to study aggressive behaviors. Garbanati et al. found that

enrichment and handling additively and independently reduced emotional reactivity and the rate of muricide (mouse killing) in rats with intact brains.

Hodgson (1984) concluded that "environmentally enriched rats and mice have been shown to be more adaptive under several conditions" (p.17). According to Hodgson, swimming immobility is an adaptive behavior in rats that when forced to swim, rapid swimming behavior is inhibited. This allows for a passive, energy-conserving posture. Hodgson also concluded that environmental rearing conditions alter REM sleep, and that in turn affects swimming immobility behavior. Examination of enriched (group housed with a number of novel objects), impoverished (isolation housed without novel objects), and social control (group housed without novel objects) housing conditions on swimming immobility revealed that enriched rats showed greater adaptive immobility.

Performance

Environmental conditions have also shown significant effects on performance, such as problem solving and maze performance. For example, Sturgeon and Reid (1971) raised rats in individual (single housed cage in darkened portion of laboratory), together and enriched (group housed, large cage placed in secluded part of laboratory, with various playthings), or super together and enriched (group housed, large cage with open environment and various playthings that changed daily) environments from 21-81 days of age. Rats were then given a battery of tests in the Hebbs-Williams maze at 110 and 190 days of age. Rats raised in enriched environments performed superior to those rats raised in isolation and impoverished environments in the first test at 110 days. However, all subjects improved with continued testing and subjects raised in impoverished environmental conditions had significantly greater improvement than all other groups. It

was concluded that early experiences do have an effect on problem-solving abilities; however, these effects are only temporary, not permanent.

Alcohol Consumption

Findings from recent studies on the effects of environmental enrichment on ethanol consumption have yielded interesting results. Several have consistently found that environmental enrichment increases alcohol consumption (Adams & Oldham, 1996; Fernandez et al., 2002; Rockman, Hall, & Markert, 1988; Rockman et al., 1989). Rockman, Borowski, and Glavin (1986) investigated the effects of enriched environmental exposure on voluntary ethanol intake. In this study, rats at age 21 days were placed in either an enriched environment (43 rats per cage and including various toys) or individually in standard lab cages. Toys in the enriched environment were rotated every 72 hours and included items such as 3 glass bottles, 2 plastic toy trucks, 2 metal chains, 5 plastic bowls, 10 plastic hair curling rollers, 3 golf balls, 6 wooden 2" by 4" wooden planks, 4 ceramic vases, and 2 large seashells. Following a 90-day enrichment period, all rats were divided into ethanol exposed or water only groups, where they were housed individually for the ethanol exposure period and enriched groups were put back in enrichment cages for 8 hours each day. Ethanol exposed groups were exposed to increasing concentrations (changed every 8 days) of 3%, 5%, 7%, and 9%, respectively, before receiving a 10-day two-bottle (water versus 9% ethanol) test. It was concluded from this study that exposure to an enriched environment produces significantly increased voluntary ethanol consumption compared to those animals exposed to a non-enriched environment.

Rockman et al. (1989) reared 2 groups of rats initially in either an enriched environment for 90 days or isolated environment for 90 days. These groups were then further divided into 4 separate groups as follows: enriched (n = 9), enriched/isolated (n = 10), isolated (n = 9), and isolated/enriched (n = 6). In other words, half of the group that was raised in enriched environment was placed in isolated environment and the other half continued to live in enriched environment. Half of the group that was raised in isolated environment was placed in enriched environment and the other half continued to live in isolated environment. The enriched environment consisted of several toys (12 different sized sheet metal stove pipes, 2 Plexiglass cylinders, 2 running wheels, and metal table with attached ladder) that were changed every other day in order to create different tunnels, hills, and bridges. In order to measure ethanol consumption, animals in enriched and isolated/enriched groups were placed in individual cages for 4 hours daily. All groups received the same schedule of ethanol consumption, increasing concentrations of 3% to 9% respectively, on alternate days for a period of 8 days. At the end of the 8-day exposure, all animals received free choice of 9% ethanol and water for 16 days. This study concluded that, compared to all other groups, enrichment animals consumed significantly greater amounts of ethanol. It was further suggested that, "rearing in an enriched environment for 90 days and continued exposure following 111 days of age, are necessary to enhance voluntary ethanol consumption" (p. 487).

Fernandez et al. (2002) also found that enriched environment enhanced ethanol consumption in both Roman high-avoidance (RHA/Verh) and Roman low-avoidance (RLA/Verh) rats. The RHA/Verh rat strain represents low emotional/anxious and high novelty seekers and RLA/Verh rats represent high emotional/anxious and low novelty

seekers. In other words, RLA/Verh rats demonstrate a higher reactivity to stressful situations. In this study, experimental rat groups were housed in enriched environments (approximately 8 rats housed together with various playthings that changed every 2 days), and control rats were housed in pairs in macrolon cages (made of autoclavable polycarbonate with wire tops). After 6 months of environmental enrichment, the experimental groups were also housed in pairs in macrolon cages. Following a saccharin-water choice test and a novelty seeking in the hole-board test, rats were given a water-ethanol choice test for four consecutive days. It was concluded from this study that enriched environment rearing modifies the genetic patterns of substance-seeking behaviors. This means that the affects of enriched environment are also dependent on the line of rat. Enriched environment increased ethanol intake in RHA/Verh and RLA/Verh rats, however, the "RHA/Verh rats consumed more ethanol and displayed higher preference for it than RLA/Verh rats" (p. 230).

Rockman et al. (1988) studied the phenomenon of enhanced ethanol consumption of enrichment animals. Rats in this study were divided into four environmental-conditions groups. The first group was enriched environment, where 53 male rats were group housed in a pen with various toys that were changed every other day. Group 2 (n = 28) was housed as male-female partners; Group 3 (n = 29) was housed as male-male partners; and Group 4 (n = 27) was housed individually as male-alone. After a 90-day environmental exposure period, male rats were randomly subdivided into either ethanol exposed or water only groups. To test for alcohol consumption, rats were housed individually for 16 hours daily and enriched groups were given 8 hours daily in enriched environments. Rats were given a two-bottle choice between water and ethanol in

increasing concentrations (3%, 5%, 7% and 9% respectively) over a total of 24 days. Rockman et al. concluded that ethanol-consuming behavior of enriched animals was significantly higher than that of all other groups. It was also worth noting that because of controls used, handling, exposure to females, and placement in individual cages for the purpose of measuring ethanol exposure were not factors in the ethanol consumption of enriched rats.

Isolation

Physiological

Social isolation has also been shown to have an effect on a variety of physiological aspects. Isolation rearing induces neurochemical changes in reward nuclei of the brain, which is correlated to an enhanced response to psycho-stimulants (Lodge & Lawrence, 2003). Isolation reared rats have been reported to be less sensitive to cocaine (Wongwitdecha & Marsden, 1996a). Decreases in reinforcing properties of morphine result from social isolation (Wongwitdecha & Mardsen, 1996b). In this study, rats were housed for four weeks in either isolation (individually housed from weaning) or socialized housing (groups of four per cage). Sensitivity to morphine was tested using a place preference test. The place preference test is comprised of an open arena that is divided into four equal quadrants using black lines on the floor to mark each quadrant. Visual cues that help the rats to orient themselves were placed in surrounding areas of the arena and were held constant throughout the study. Rats were individually placed in the open arena for ten-minute intervals and time spent in each of the quadrants was recorded to determine treatment quadrants (quadrant with the least time spent became treatment quadrant). Rats were then injected with either saline or morphine (1 and 5 mg/kg) daily

and placed in their treatment quadrant (restricted from other quadrants by transparent Plexiglass barriers) ten minutes after their injection, for 15 minutes, and then returned to their home cage. On the final test day, rats were not injected and were placed in the center of the arena with access to all four quadrants. Time spent in each quadrant was measured over a ten-minute period to determine place preference. It was found that animals reared in isolation from weaning failed to exhibit place preference for morphine, concluding that they were less sensitive to morphine than those housed in social groups. Wongwitdecha and Marsden summarized that "isolation-reared rats demonstrate a dysfunction in opiate reward mechanisms in common with those of cocaine and other rewarding drugs. This dysfunction may reflect alterations not only in brain opiate mechanisms but also other transmitters such as dopamine, serotonin, and noradrenaline. The isolation reared rats may be a model to investigate developmental factors involved in predisposition to drug abuse in later life" (p. 534). In other words, the effects of early social environment play an important role in the subsequent behavioral expression of the effects of opiates.

Isolation rearing also has been shown to have effects on the responsitivity to the psychotropic drug diazepam. Wongwitdecha and Marsden (1996c) investigated social versus isolated rats and the effects of diazepam on social interaction behaviors. It was found that isolation reared rats were less sensitive to diazepam's anxiolytic effects than were socially reared rats. Sundstrom, Hall, Stellar, and Waugh (2002) also found that isolation-rearing down regulates dopamine D2 receptor function by implanting rats with monopolar stimulating electrodes in the lateral hypothalamus and assessing reward and operant motor functioning in social and isolation reared rats.

Prepulse inhibition (PPI) deficits in humans with schizophrenia have been the focus of numerous studies. PPI refers to when a startling stimulus is preceded by a non-startling stimulus, which in turn inhibits the startle response (Krebs-Thompson, Giracello, Solis, & Geyer, 2001). Isolation rearing in rats produces a deficit in sensorimotor gating, which refers to how organisms process their environment and surroundings, and PPI. Cilia, Reavill, Hagan, and Jones (2001) tested isolation and group housed rats and found that robust PPI deficits were produced by isolation rearing and are reversed with atypical anti-psychotics (i.e. olanzapine, clozapine, and risperidone).

Performance

Deficits in performance of rats raised in social isolation have been widely reported (e.g. Greenough, 1976; Greenough, Madden, & Fleischmann, 1972; Holson, 1986). For example, Jones, Marsden, and Robbins (1991) investigated several performance tasks of rats raised in social isolation. Results found that isolated rats exhibited a marked impairment in the acquisition of conditional visual discrimination. However, once a task was learned, performance between socially housed and isolation housed rats did not differ. Contrary to the reports of impaired learning due to isolation, several have found that isolation rearing enhances or has no effect on performance. For example, isolation reared rats have out-performed socially reared rats on the Morris water maze; place learning and reversal learning were enhanced (Wongwitdecha & Marsden, 1996a).

Emotional and Behavioral

Isolation rearing leads to various behavioral disturbances (Jones et al., 1991). Isolation-reared rats are more excitable; exhibit enhanced exploratory behaviors,

hyperactive in novel environments; and exhibit increased weight gain compared to social-reared rats (Jones, Robbins, & Marsden, 1989). According to Hall, Humby, Wilkinson, and Robbins (1997a), isolation-reared rats also exhibit an enhanced environmental neophobia (fear of anything novel) and a diminished food neophobia (fear of any novel food).

Hall, Huang, Fong, and Pert (1997b) mention that the isolation-reared rat serves as a model for depression. In their study, struggling behavior (viewed as an index of antidepressant behavior) was assessed using the forced swim test. Struggling, immobility, and swimming behaviors were assessed via videotape using blind observers. Both Wistar and Fawn Hooded rats were housed either in isolation or socially (two per cage). Fawn Hooded socials exhibited increased struggling behaviors and Fawn Hooded isolates exhibited increased swimming behavior. In other words, contrary to other findings, these animal models of depression did not exhibit depression-like effects (increased immobility or decreased swimming). Socially reared Fawn Hooded rats exhibited more struggling behavior than Wistar socials, suggesting that factors such as neurochemical differences in strain may be attributable to the effects found. In contrast, Yates, Panksepp, Ikemoto, and Nelson (1991) found increased immobility in mice that were exposed to a 15-minute forced swim test in those mice that were isolation-reared at age 17-21 days, but not in those mice that were isolation reared at age 26-30 days. It was further indicated that for the mouse, there is only a short period of time during early development where social isolation can promote despair (i.e., immobility in the forced swim test).

Of further interest are the findings that tickling (rapid finger and hand movements similar to human tickling) can be used to induce positive social affect in rodents (Burgdorf & Panksepp, 2001). Based on results demonstrated in conditioned place preference, on elevated operant behavior, and approach measures; tickling is viewed as positively reinforcing for rats. Rats were either social-reared or isolation-reared and tickling vocalizations (~50 KHz) were recorded. Burgdorf and Panksepp found that isolation housing increased tickle-induced vocalizations and approach speeds. In a prior study by Panksepp and Burgdorf (2000), isolation-reared animals vocalized more, in response to rewarding manual tickling, than those rats that were social-reared. It also is worth noting that when the rats' housing conditions were reversed, they gradually shift their vocalization tendencies. Isolation-reared animals also showed quicker acquisition of instrumental tasks for tickling. Ikemoto and Panksepp (1992) also indicated that isolation-reared rats reliably made more choices for social interaction over food reward compared to those rats that were social-reared.

Holson, Scallet, Ali, and Turner (1991) contend that isolation stress depends on an interaction of other factors, mainly human handling. While isolation from weaning did not produce enduring, chronic stress in rats physiologically, isolation stress syndrome can be seen in those rats raised in isolation without exposure to human handling. Holson et al. also concluded from their study that for socially reared rats, the fear demonstrated in open-field tests can also be eliminated by the presence of odor from a familiar cage-mate, which further supports the benefits of social versus isolated housing conditions.

Play Therapy

Of interest are the beneficial effects of play therapy in animals. Using rats with right frontal lesions as models of the hyperactivity found in humans diagnosed with ADHD, Panksepp, Burgdorf, Turner, and Gordon (2003) have concluded that chronic play with a conspecific during adolescence reduced both hyperactivity and excessive playfulness. In another study, rats that had been allowed rough and tumble play 30 minutes prior to sacrifice had significantly more activation in specific regions of the brain (i.e., inferior colliculus, dorsal periaquaductal gray, dorsal and ventral striatum, ventromedal hypothalamus) than those rats with similar histories that had received no play (Gordon, Kollack-Walker, Akil, & Panksepp, 2002).

Play in rats may function to establish stable social relations in rats (Panksepp, 1981). For example, research indicates that play behaviors are markedly increased by social isolation and reduced by social satiation (Panksepp, 1981; Panksepp & Beatty, 1980).

Consumption

Jones et al. (1989) found that isolation rearing impairs the development of the acquisition of schedule-induced polydipsia in rats. Schedule-induced polydipsia is defined as "the drinking of excessive amounts of water by food-deprived animals exposed to a schedule of intermittent food delivery" (p. 71). In this study, rats were reared in isolation or socially (5-6 rats per cage) for 10 weeks prior to being tested. Home cage drinking following water deprivation was significantly higher in isolation-reared rats. These effects on schedule-induced polydipsia resemble the results from studies on the exposure of pre-shock and to frustration on the development of schedule-induced

polydipsia. In other words, frustrating circumstances, such as the exposure to aversive stimuli such as isolation rearing and shock, impair the acquisition of polydipsia.

Consistent with prior findings that isolation-reared rats exhibit an increased incentive motivation, Hall, Humby, Wilkinson, and Robbins (1997c) discovered that isolation-reared rats drank significantly more sucrose solution than social-reared rats when the solution is presented in ascending order of concentration. Based on their evidence, the effects of positive contrast increased as a result of isolation rearing. Positive contrast refers to when the rewarding value of the reward exceeds the expectation and consumption is increased compared to the situation without the expectation. Hall et al.'s (1998c) study demonstrated that isolated rats consume more sucrose and saccharin solution at high concentrations than social housed rats, and voluntarily consumed more ethanol. Isolation-reared rats have an increased preference for ethanol. In this study, Hall et al. used both Fawn Hooded and Wistar rats and housed them either individually or socially (two per cage). After eight weeks of exposure to housing conditions, the concentration of ethanol presented to the rats was 2%, 4%, 8%, and 16% respectively. Isolation-reared rats consumed significantly greater amounts at higher concentrations than social-reared rats. This is in direct opposition of the many studies indicating social-reared rats consume more ethanol (e.g., Adams & Oldham, 1996).

Paivarinta (1990) stated, "The stimulatory effects of low alcohol doses are of great interest because of their role in human drinking and their possible relation to reinforcement from alcohol" (p. 401). Using mice as a model, Paivarinta suggested that mice reared in social isolation are more sensitive to ethanol's stimulatory effect on locomotor activity. Paivarinta's study set out to determine what effects social and isolated

housing had on the effects of the locomotor effects of low-dose ethanol. Mice were housed either in groups of 8-10 per cage or individually. Following 36-44 weeks of housing exposure, animals were injected with saline, 0.5g/kg ethanol, 1.0 g/kg ethanol, and 2g/kg ethanol randomly and were tested. Testing was in an apparatus that used movement sensitive electrical mattresses to detect vertical body movement. Results indicated that isolation reared mice exhibited high sensitivity to locomotor activating effect of small doses of ethanol (0.5g/kg & 1.0 g/kg).

In Wolffgramm's (1990) study, male Wistar rats were housed in individual cages, group caging (four rats per cage), or contact caging (partial social deprivation). The group-caged rats were separated for 24 hours each week and all rats received 5%, 10%, and 20% ethanol and water for a period of fourteen weeks. After 8-10 days, rats that were normally housed in groups that were separated consumed significantly more ethanol than all other groups; second highest being that of isolated rats. He concluded that consumption of alcohol is a stress reducing mechanism. Because of these reinforcing properties, the severe stress widely associated with social isolation leads to increased ethanol intake.

Contrary to these findings, as Schenk, Gorman, and Amit (1990) point out research on the effects of isolation rearing and ethanol intake produced "findings that isolation housing increases, decreases, or produces no effects on ethanol intake" (p. 321). The age at which animals experience social isolation is one explanation offered. Schenk et al. investigated housing conditions of rats housed from weaning and rats housed at maturity (age 65 days). There was no difference in ethanol consumption between group housed and isolated rats when housed at maturity. There was also no overall difference in

ethanol consumption of rats differentially housed at weaning; however, these isolates consumed higher amounts of ethanol when presented in higher concentrations.

Enhanced ethanol consumption has also been evidenced as an effect of environmental enrichment in Adams and Oldham's (1996) study. This study looked at social housing environment of male rats during their juvenile to early adult period to see if this had an effect on later ethanol consumption. For 16 weeks, rats were housed in isolation housing, group housing (eight rats in typical laboratory cage), or semi-natural housing (large enclosure housing eight rats, included burrows and tunnels). All groups were exposed to a two-bottle, water versus ethanol (10%) for eight weeks, beginning approximately two weeks after group housed and semi-natural housed rats were placed in individual cages. Males in semi-natural environments consumed greater amounts of ethanol than males in group-housed environments and males housed in isolated environments. Adams and Oldham speculated that possible variables associated with the increased ethanol consumption could be social differences from greater amount of physical space, stress, and fearfulness associated with semi-natural housing. This is similar to what Ellison (as cited in Adams & Oldham) demonstrated; heavy ethanol consumption correlates with low ranking individuals in semi-natural settings.

Rat Strains

A variety of rat strains have been used in previous studies. The most common include Sardinian, Maudsley Reactive, Roman High-Avoidance, Roman Low-Avoidance, Fawn-Hooded, Sprague-Dawley, Lister-hooded, and Wistar. Strains of rats are typically selected because of their genetics (characteristic traits and tendencies).

The Sardinian alcohol-preferring (sP) and Sardinian alcohol-non-preferring (snP) rats are typically selected because of their different alcohol seeking behavior. The Sardinian alcohol-preferring lines are also considered to be a genetic animal model of anxiety (Colombo et al., 1995). The Maudsley Reactive rat strains have been selected in prior studies because of their susceptibility to stress (e.g., Adams & Oldham, 1996). The two Roman sublines differ in many behavioral and neuroendocrine/neurochemical characteristics; which indicate that the Roman low avoidance (RLA/Verh) line represents higher emotionality, anxiety and reactivity to stressful situations compared to the Roman high avoidance (RHA/Verh) sublines (e.g., Fernandez et al., 2002). Fawn-Hooded rat lines (e.g., Sturgeon & Reid, 1971; Will, Deluzarche, & Kelche, 1983), Sprague-Dawley (e.g., Hodgson, 1984; Rasmussen et al., 1990; Widman & Rosellini, 2001), and Lister-hooded (e.g., Hall, Humby, Wilkinson, & Robbins, 1997b, 1997c) have been widely used in previous research, including various alcohol studies.

Wistar rats have also been widely used in research (e.g., Sandbak & Murison, 2001) and were selected for use in this study. A cursory search of Wistar rats and Elevated Plus-Maze in PsychInfo revealed 91 articles, suggesting the Wistar is an appropriate strain to be used with the Elevated Plus-Maze (e.g., Andrade, Tome, Santiago, Santos, & deAndrade, 2003; Escarabajal, Torre, & Flaherty, 2003; Marinelli, Quirion, & Gianoulakis, 2003; Silvestre, Pallares, Nadal, & Ferre, 2002). Wistars also have been used widely in alcohol research (e.g., Frye, Fincher, Grover, & Griffith, 1994; Gallate, Morley, Ambermoon, & McGregor, 2003).

Summary

Prior research indicates that the role of environment has a tremendous impact on a vast array of physiological and behavioral functions, such as exploration, neurological changes, maze performance, and sleep patterns. Environmental enrichment has yielded interesting physiological results, such as higher resistance to epilepsy, an increase in cell size of the visual cortical tissue, increased total brain weights, increased total protein in the brain, increased enzyme activity, enhanced compensation for sensory loss in post-operative recovery, and increased REM sleep time. Enriched environments have also been shown to reduce emotionality, increase open-field activity, enhance greater adaptive behaviors such as swimming immobility, and increase performance on problem solving and maze tasks.

It is also important to emphasize findings of the deleterious symptoms of exposure to impoverished environments. Isolation rearing has also yielded interesting results. Research has indicated that isolation reared rats are less sensitive to psychostimulants (e.g. cocaine, morphine, diazepam) and exhibit deficits in sensorimotor gating and prepulse inhibition. Isolation reared rats have also exhibited deficits in performance of tasks such as visual discrimination; however, isolation-reared rats have also outperformed social reared rats on tasks such as the Morris water maze. There have also been contradictory findings on isolation-reared rats. For example, several have found that isolation rearing leads to behavioral disturbances whereas others have found that results are dependent on other factors such as the neurochemical differences of various rat strains, human handling, and age at onset of environmental exposure. Research also indicates that social isolation increases play behaviors in rats.

Of particular interest to this study is the examination of the effects of socialization and enrichment on ethanol consumption and anxiety. Further research has exhibited contradictory results on the environmental effect on alcohol consumption and anxiety.

Several (e.g. Adams & Oldham, 1996; Rockman et al., 1989) have found that environmental enrichment increases alcohol consumption, whereas others (e.g. Hall et al., 1998c) have found that isolation-reared rats consumed greater amounts of ethanol than social-reared rats. Of further interest are the inconsistencies in previous definitions and methodologies of enriched, impoverished, and social environmental conditions. For example, several define enriched environments as including group housing, whereas others do not. It was the purpose of this study to examine the interactive effects of socialized versus isolated and enriched versus impoverished housing environments on anxiety and ethanol consumption.

Research Questions

Based on past research, the following questions were developed.

Research question 1: Do socialization and environment interactively affect ethanol consumption?

Research question 2: Do socialization and environment interactively affect anxiety?

Research question 3: Does ethanol reduce isolated-impoverished induced anxiety?

Hypotheses

The following hypotheses were developed.

Hypothesis 1: Isolated-impoverished rats will significantly consume more alcohol and socialized-enriched rats will have least alcohol consumption.

Hypothesis 2: Isolated-impoverished rats will significantly exhibit the greatest anxiety and socialized-enriched rats will exhibit the least anxiety.

Hypothesis 3: Anxiety will be reduced while animals are consuming alcohol because alcohol is a well-established anxiolytic and CNS depressant.

CHAPTER 2

METHOD

Subjects

Forty male Wistar rats (Harlan, Madison, WI) were used for this study. These rats were randomly assigned to one of four groups: isolated-enriched, isolated-impoverished, socialized-enriched, or socialized-impoverished. This research was approved by the Emporia State University Animal Care and Use Committee (ESU-ACUC-03-009, Appendix A).

Design

A 2 Socialization (Isolated, Group-housed) x 2 Environment (Enriched, Impoverished) completely randomized factorial design was used as the general design for this study. The independent variables were isolated environment, socialized environment, enriched environment, and impoverished environment, and where necessary repeated measures were acquired. The dependent variables were alcohol consumption and anxiety.

Apparatuses

The housing arenas used were constructed out of clear Plexiglass walls, 91.44 cm (3 ft) in length by 121.92 cm (4 ft) in width by 26.54 cm (2 ft) in height, and a metal covered wood floor with a wire mesh lid (Figure 1). Individually housed rats were in large propylene cages, 40.64 cm (16 in.) by 21.59 cm (8.5 in.) by 20.32 cm (8 in.). Approximately 1 cm of bedding was placed in the floor of all cages. Hanging stainless steel cages used for consumption testing were 17.78 cm (7 in.) in depth, 25.40 cm (10 in.) in length, and 20.32 cm (8 in.) in width, with grid floor and front.

Figure 1. Top: Social housing arena (91.44 cm x 121.92 cm x 26.54 cm) with enrichment toys and 10 rats. Bottom: Rats individually housed in large polypropylene cages (40.64 cm x 21.59 cm x 20.32 cm).

The Elevated Plus-Maze (EPM) used for this study is widely used as an anxiety paradigm and represents a test based on unconditioned responses to a potentially dangerous environment (Montgomery, 1955). The elevated plus maze consists of two closed arms and two open arms forming a cross, with a quadrangular center. The dimensions of the EPM (Figure 2) used in this study are as follows: height from floor 50 cm (20 in.), wall height 20 cm (8 in.), arms 50 cm (20 in.) by 10 cm (4 in.), and center 10 cm (4 in.) by 10 cm (4 in.). The degree of anxiety is assessed by recording time spent on open and closed arms and number of entries made in each. The concomitant behavioral, endocrinological, and physiological phenomena occurring in the open arms lend strong support for the face and construct validity of this test for measuring anxiety (Pellow, Chopin, File, & Briley, 1985). The EPM has relatively good predictive validity for anxiety, although false positives have been found (Rodgers & Cole, 1994).

Procedure

Environmental conditions. All animals were placed on a 12/12-hour light/dark cycle throughout the study. Temperature remained constant throughout the study (72°F, 22.22°C). Beginning at approximately 25 days-old, all rats were handled for two minutes daily for the first two weeks. Each rat was randomly assigned to one of four groups of environmental conditions. All rats were tail marked (red, blue, green, and/or black Sharpie® Permanent Marker) based on a coding system in order to differentiate between group-housed rats and to ensure consistency between all groups. For Group Isolated-Enriched (IE), rats were housed individually in large polypropylene cages with various plastic playthings (2 that were rotated daily out of a total of 40). Group Isolated-Impoverished (II) was housed individually in identical polypropylene cages, with no

playthings available. Group IE and Group II had cardboard dividers placed between each cage in order to prevent visualization of other rats. Group Socialized-Enriched (SE) was housed 10 rats per housing arena, with identical toys as Group IE (20 that are rotated daily out of a total of 40). Group Socialized-Impoverished (SI) was housed 10 rats per cage in an identical arena as Group SE, with no playthings available. The toy to animal ratio for Groups IE and SE was 2:1. All groups had food (Teklad 18% Protein Rodent Diet, Harlan Teklad, Madison, WI) and water available ad lib, while being exposed to environmental conditions for 96 days prior to testing.

Pre -alcohol anxiety test. At day 97, groups were tested for anxiety on the elevated plus-maze during their dark cycle with red lighting (GE Red Party Light 25 watt bulb). Testing (which was videoed) occurred twice weekly in 10-min increments per animal, (spread over 3 days each for a total of 6 days). Time spent on open and closed arms, but not in the center, was determined from the videos. Rats were placed back in their housing immediately following testing.

Alcohol consumption. Following the test week for anxiety, all food and water was removed from the rats' housing. Once daily, for one hour each day, all rats were placed in individual hanging metal cages where food (Teklad 18% Protein Rodent Diet, Harlan Teklad, Madison, WI) was available. Ethanol solutions (2%, 4%, 6%, 8%, & 10%) respectively, were administered in increasing doses (changed every four days) and were available during the one hour. Food and ethanol bottles were weighed (g) daily to measure consumption. At the end of the 20 days of ethanol consumption, all rats received

Figure 2. Top: Elevated plus-maze (EPM). Bottom: Placement of rat in center of EPM (darkness with red lighting).

2 days of a two-bottle (water vs. 10% ethanol solution) test for preference during their hour of food and solution consumption. The position of the tubes was changed daily to prevent the possibility of a position preference by the rats. Water and ethanol bottles were weighed (g) daily to measure consumption.

Post-alcohol anxiety test. After the two-bottle test was completed, all groups received a 10% ethanol only solution in the same manner as the 2%, 4%, 6%, 8%, and 10%. All groups were again tested for anxiety on the elevated plus-maze. Testing occurred in the same manner as the Pre-Alcohol Anxiety Test.

CHAPTER 3

RESULTS

Consumption

The means and standard deviations of body weight were calculated for each of the four groups of subjects. The means and standard deviations of food and fluid consumption were calculated for each of the four groups during the 27 days of consumption and 2 days of two-bottle testing. Appropriate factorial ANOVAs were conducted on body weight and food consumption. Factorial ANOVAs for each concentration level of ethanol (2%, 4%, 6%, 8%, & 10%) were conducted. For the two-bottle (water versus 10% ethanol) test, A 2 Housing (Isolated, Group-housed) x 2 Environment (Enriched, Impoverished) x 2 Bottle (Water, Alcohol) x 2 Day mixed factorial ANOVA was conducted.

Anxiety

The means and standard deviations of time spent on open and closed arms of the EPM were calculated for the Pre-Ethanol Anxiety Test and Post-Ethanol Anxiety Test. A 2 Housing (Isolated, Group-housed) x 2 Environment (Enriched, Impoverished) x 2 Session (Pre-alcohol Test, Post-alcohol Test) x 2 Arm (Open, Closed) mixed factorial ANOVA was conducted.

Alcohol Consumption

Body weights pre-alcohol (pre EPM1 & EPM2). The main effect of Environment was not significant, $F(1, 35) = .29, p = .59$. The main effect of housing was significant, $F(1, 35) = 21.61, p < .001, \eta^2 = .38$. Additionally, the main effect of session also was significant, $F(2,70) = 5{,}088.12, p < .001, \eta^2 = .99$. More importantly, the interaction of

Housing x Session was significant, $F(2,70) = 17.68, p < .001, \eta^2 = .34$. Subsequent *t* tests indicated that all rats weighed the same initially ($p > .05$), but isolated rats weighed significantly more than socially housed rats before both EPM tests ($ps < .05$). The mean body weights (g) and standard deviations (Table 1) for the socially housed (S) versus the isolated (I) rats for initial (IBW), pre-alcohol EPM test (EPM1), and post-alcohol EPM (EPM2) test are as follows: $M_{S\text{-}IBW} = 59.49$, $SD_{S\text{-}IBW} = 5.62$; $M_{I\text{-}IBW} = 59.71$, $SD_{I\text{-}IBW} = 5.97$; $M_{S\text{-}EPM1} = 429.34$, $SD_{S\text{-}EPM1} = 27.21$; $M_{I\text{-}EPM1} = 482.71$, $SD_{I\text{-}EPM1} = 33.59$; $M_{S\text{-}EPM2} = 426.50$, $SD_{S\text{-}EPM2} = 31.08$; $M_{I\text{-}EPM2} = 456.40$, $SD_{I\text{-}EPM2} = 23.67$. The Session x Environment ($F(2,70) = .25, p = .78$), Session x Housing x Environment ($F(2,70) = .24, p = .79$), or Housing x Toy ($F(1,35) = .04, p = .85$) interactions were not significant. In other words, group housing but not enrichment affected body weights. This difference may have been due to differences in physical activity.

Body weights 2 weeks pre-alcohol. The main effect of enrichment was not significant $F(1, 35) = .15, p = .70$, however, the main effects of Housing ($F(1, 35) = 29.90, p < .001, \eta^2 = .46$) and Session ($F(1,35) = 25.72, p < .001, \eta^2 = .42$) were significant. The interaction of Session x Housing x Environment ($F(1,35) = 5.56, p = .02, \eta^2 = .14$) was significant. The interaction of Housing x Session was not significant, $F(1,35) = .10, p = .75$. The mean body weights (g) and standard deviations for the socially housed (S) versus the isolated (I) rats for two weeks pre-alcohol (2BW) and one week pre-alcohol (BW) are as follows: $M_{S\text{-}2BW} = 429.34$, $SD_{S\text{-}2BW} = 27.21$; $M_{I\text{-}2BW} = 482.71$, $SD_{I\text{-}2BW} = 33.59$; $M_{S\text{-}BW} = 423.68$, $SD_{S\text{-}BW} = 28.21$; $M_{I\text{-}BW} = 476.05$, $SD_{I\text{-}BW} = 29.17$. Neither the interactions Session x Environment ($F(2,70) = .25, p = .78$) nor Housing x Environment ($F(1,35) = .04, p = .85$) were significant. In other words, group

Table 1

Summary of Pre-and Post-alcohol Body Weight Means and Standard Deviations For All Social and All Isolated Rats

Housing	*n*	*M*	*SD*
Social			
Initial Body Weight	20	59.49	5.62
Pre-alcohol (EPM1)	20	429.34	27.21
Post-alcohol (EPM2)	20	426.50	31.08
Isolated			
Initial Body Weight	19	59.71	5.97
Pre-alcohol (EPM1)	19	482.71	33.59
Post-alcohol (EPM2)	19	456.40	23.67

housing but not enrichment affected body weights; isolated rats weighed more than social rats 2 weeks and 1 week before alcohol testing, but the differences between the two decreased across the time span. This difference may have been due to differences in physical activity.

Body weights during alcohol exposure. The main effect of Environment ($F(1,35) = .05, p = .82$) was not significant, but the main effects of Housing ($F(1, 35) = 24.77, p < .001, \eta^2 = .41$) and Session ($F(5,175) = 53.85, p < .001, \eta^2 = .61$) were significant. More importantly, the interaction of Housing x Session was significant, $F(5,175) = 9.29, p < .001, \eta^2 = .21$. The mean body weights (g) and standard deviations (Table 2) for the socially housed (S) versus the isolated (I) rats during alcohol consumption are as follows: $M_{S\text{-}BW1} = 440.65$, $SD_{S\text{-}BW1} = 29.12$; $M_{I\text{-}BW1} = 499.26$, $SD_{I\text{-}BW1} = 35.21$; $M_{S\text{-}BW2} = 429.24$, $SD_{S\text{-}BW2} = 27.21$; $M_{I\text{-}BW2} = 482.71$, $SD_{I\text{-}BW2} = 33.59$; $M_{S\text{-}BW3} = 421.82$, $SD_{S\text{-}BW3} = 27.96$; $M_{I\text{-}BW3} = 470.71$, $SD_{I\text{-}BW3} = 28.52$; $M_{S\text{-}BW4} = 417.76$, $SD_{S\text{-}BW4} = 28.13$; $M_{I\text{-}BW4} = 459.28$, $SD_{I\text{-}BW4} = 26.90$; $M_{S\text{-}BW5} = 424.32$, $SD_{S\text{-}BW5} = 29.55$; $M_{I\text{-}BW5} = 459.55$, $SD_{I\text{-}BW5} = 25.44$; $M_{S\text{-}BW6} = 422.94$, $SD_{S\text{-}BW6} = 30.32$; $M_{I\text{-}BW6} = 456.02$, $SD_{I\text{-}BW6} = 24.73$. Neither the Session x Toy ($F(5,175) = .95, p = .45$) nor Session x Housing x Toy ($F(5,175) = .47, p = .80$) interactions were significant. Although socially housed rats consistently weighed less than isolated rats, both groups lost weight between the first and last week of alcohol exposure.

Water consumption (2 weeks pre-alcohol exposure). Neither the main effect of Environment ($F(1,35) = 1.38, p = .25$), Housing ($F(1,35) = 1.00, p = .32$), nor Session ($F(1,35) = 1.11, p = .30$), were significant. Neither the interactions Session x Environment ($F(1,35) = 1.11, p = .30$), Session x Housing x Environment ($F(1,35) =$

Table 2

Summary of Body Weights During Alcohol Consumption For All Social and All Isolated Rats

Housing	*n*	*M*	*SD*
Social			
Week 1	20	440.65	29.12
Week 2	20	429.24	27.21
Week 3	20	421.82	27.96
Week 4	20	417.76	28.13
Week 5	20	424.34	29.55
Week 6	20	422.94	30.32
Isolated			
Week 1	19	499.26	35.21
Week 2	19	482.71	33.59
Week 3	19	470.71	28.52
Week 4	19	459.28	26.90
Week 5	19	459.55	25.44
Week 6	19	459.02	24.73

1.06, p =.31), or Housing x Environment ($F(1,35) = 1.36, p = .25$) were significant. The mean water consumption (g) and standard deviations for the socially housed (S) versus the isolated (I) rats for one week (W2) and two weeks (W1) prior to alcohol consumption are as follows: $M_{S\text{-}W1} = 17.65$, $SD_{S\text{-}W1} = 4.18$; $M_{I\text{-}W1} = 30.22$, $SD_{I\text{-}W1} = 55.19$; $M_{S\text{-}W2} = 18.27$, $SD_{S\text{-}W2} = 2.32$; $M_{I\text{-}W2} = 17.30$, $SD_{I\text{-}W2} = 3.55$. This means that for the two weeks prior to alcohol exposure, socially housed and isolated rats drank the same amount of water.

Two-bottle test. I performed a 2 (Housing) x 2 (Environment) x 2 (Solution) x 2 (Day) mixed factorial ANOVA. The only significant main effects were Solution ($F(1, 35) = 40.40, p < .001, \eta^2 = .54$) and Days ($F(1,35) = 17.36, p < .001, \eta^2 = .33$). More importantly, the significant 2-way interactions were Solution x Housing ($F(1,35) = 7.74$, $p = .009, \eta^2 = .18$) and Solution x Environment ($F(1,35) = 7.21, p = .01, \eta^2 = .17$). The means (Figure 3) and standard deviations for alcohol (A) and water (W) consumption for isolated (I) and socialized (S) groups are as follows: $M_{I\text{-}A} = 14.93$, $SD_{I\text{-}A} = 14.07$, $M_{I\text{-}W} = 25.5$, $SD_{I\text{-}W} = 11.44$; $M_{S\text{-}A} = 8.99$, $SD_{S\text{-}A} = 6.93$, $M_{S\text{-}W} = 37.24$, $SD_{S\text{-}W} = 12.42$. This means that isolated rats drank more alcohol and less water than socialized rats. The means (Figure 4) and standard deviations for alcohol (A) and water (W) consumption for enriched (E) and impoverished (I) groups are as follows: $M_{E\text{-}A} = 8.51$, $SD_{E\text{-}A} = 6.95$, $M_{E\text{-}W} = 36.87$, $SD_{E\text{-}W} = 11.84$; $M_{I\text{-}A} = 15.09$, $SD_{I\text{-}A} = 13.62$, $M_{I\text{-}W} = 26.44$, $SD_{I\text{-}W} = 12.66$. This means that rats in the impoverished environment drank more alcohol and less water than enriched rats. Unfortunately, neither Environment x Housing x Solution ($F(1, 35) = .08, p = .79$) nor Environment x Housing x Solution x Days ($F(1,35) = .09, p = .77$) interaction were significant. The means and standard deviations of alcohol (A) and water (W)

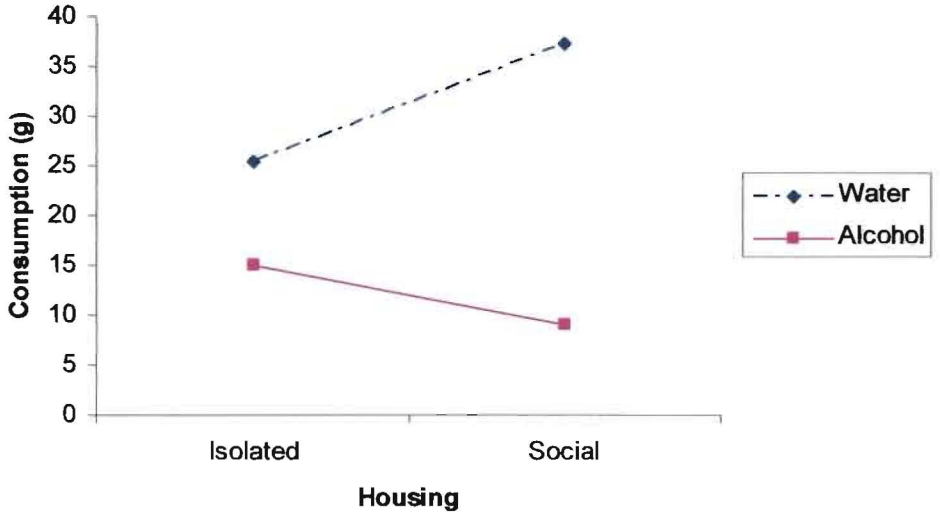

Figure 3. Mean (g) alcohol and water consumption for all isolated rats (n = 19) and all socialized rats (n = 20).

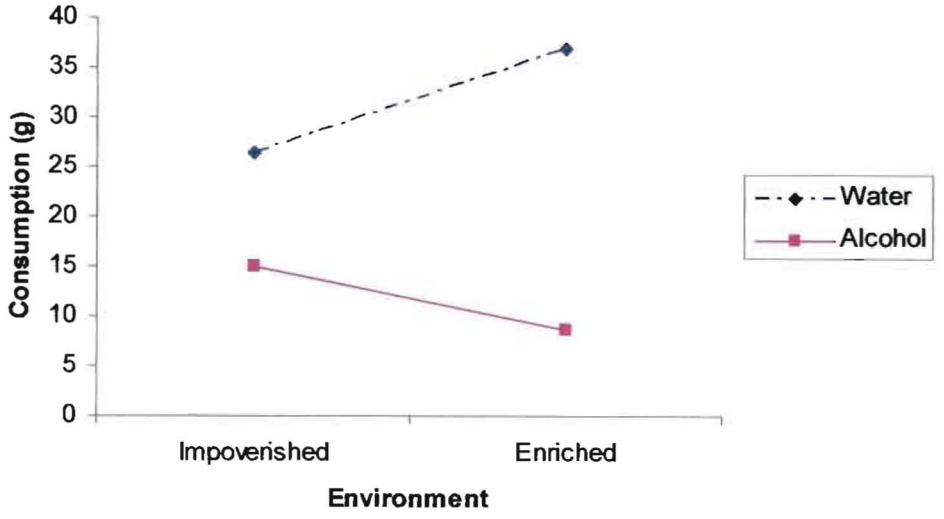

Figure 4. Mean (g) alcohol and water consumption for all impoverished rats ($n = 19$) and all enriched rats ($n = 20$).

consumption (g) for Groups IE, II, SE, and IE on Day 1 are as follows: $M_{IE\text{-}A} = 5.16$, $SD_{IE\text{-}A} = 4.17$, $M_{IE\text{-}W} = 15.97$, $SD_{IE\text{-}W} = 4.70$; $M_{II\text{-}A} = 7.29$, $SD_{II\text{-}A} = 6.23$, $M_{II\text{-}W} = 11.56$, $SD_{II\text{-}W} = 9.28$; $M_{SE\text{-}A} = 2.99$, $SD_{SE\text{-}A} = 1.50$, $M_{SE\text{-}W} = 26.59$, $SD_{SE\text{-}W} = 13.34$; $M_{SI\text{-}A} = 5.35$, $SD_{SI\text{-}A} = 6.42$; $M_{SI\text{-}W} = 17.20$, $SD_{SI\text{-}W} = 7.18$. The means and standard deviations of alcohol (A) and water (W) consumption (g) for Groups IE, II, SE, and IE on Day 2 are as follows: $M_{IE\text{-}A} = 4.40$, $SD_{IE\text{-}A} = 5.76$, $M_{IE\text{-}W} = 13.79$, $SD_{IE\text{-}W} = 5.67$; $M_{II\text{-}A} = 7.29$, $SD_{II\text{-}A} = 6.23$, $M_{II\text{-}W} = 10.11$, $SD_{II\text{-}W} = 4.94$; $M_{SE\text{-}A} = 4.58$, $SD_{SE\text{-}A} = 6.60$, $M_{SE\text{-}W} = 16.68$, $SD_{SE\text{-}W} = 4.78$; $M_{SI\text{-}A} = 5.05$, $SD_{SI\text{-}A} = 5.34$; $M_{SI\text{-}W} = 14.00$, $SD_{SI\text{-}W} = 7.79$. Importantly, housing and environment did not interact to affect alcohol consumption.

Elevated Plus-Maze

Pre- and post-EPM. There was a significant main effect of Arm, $F(1,35) = 82.21$, $p < .01$, $\eta^2 = .70$, whereas the main effect of Session, $F(1,35) = 1.58$, $p = .22$), was not significant. More importantly, the interactive effect of Session x Arm was significant $F(1,35) = 4.75$, $p = .04$, $\eta^2 = .12$. The mean time (s) spent (Figure 5) and standard deviations for pre (PRE) and post (POST) alcohol anxiety tests in the open (O) and closed (C) arms are as follows: $M_{PRE\text{-}O} = 79.69$, $SD_{PRE\text{-}O} = 64.69$; $M_{PRE\text{-}C} = 272.17$, $SD_{PRE\text{-}C} = 57.23$; $M_{POST\text{-}O} = 96.42$, $SD_{POST\text{-}O} = 70.62$; $M_{POST\text{-}C} = 232.37$, $SD_{POST\text{-}C} = 113.49$. Neither the interactive effects of Housing x Session ($F(1,35) = .67$, $p = .42$), Environment x Session ($F(1,35) = .14$, $p = .71$), nor Housing x Environment x Session ($F(1,35) = .12$, $p = .74$) were significant. There were also no significant interactive effects of Housing x Arm ($F(1,35) = .04$, $p = .85$), Environment x Arm ($F(1,35) = .40$, $p = .53$), Housing x Environment x Arm ($F(1,35) = .75$, $p = .39$). Also, neither Housing x Session x Arm ($F(1,35) = .02$, $p = .88$), Environment x Session x Arm ($F(1,35) = .30$, $p = .59$),

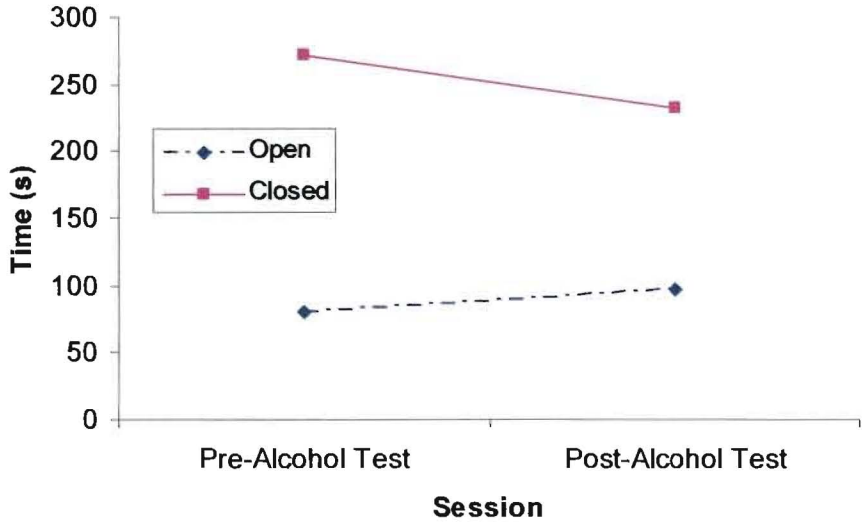

Figure 5. The mean number of seconds all rats spent on open and closed arms of the elevated plus-maze before and after consuming alcohol ($N = 39$).

nor Housing x Environment x Session x Arm ($F(1,35) = .03, p = .87$) were interactively significant. The main effect of Housing ($F(1,35) = .09, p = .77$) and the main effect of Environment ($F(1,35) = .11, p = .74$) were not significant. The interactive effect of Housing x Environment, $F(1,35) = .27, p = .61$ also was not significant. The means and standard deviations for isolated-impoverished (II), isolated-enriched (IE), socialized-impoverished (SI), and socialized-enriched (SE) for pre- (PRE) and post- (POST) alcohol anxiety tests on the open (O) and closed (C) arms are as follows: $M_{IEPRE\text{-}O} = 90.50$, $SD_{IEPRE\text{-}O} = 76.18$; $M_{IEPRE\text{-}C} = 259.35$, $SD_{IEPRE\text{-}C} = 58.96$; $M_{IIPRE\text{-}O} = 70.59$, $SD_{IIPRE\text{-}O} = 41.80$; $M_{IIPRE\text{-}C} = 274.73$, $SD_{IIPRE\text{-}C} = 53.95$; $M_{SEPRE\text{-}O} = 73.42$, $SD_{SEPRE\text{-}O} = 70.19$; $M_{SEPRE\text{-}C} = 280.05$, $SD_{SEPRE\text{-}C} = 54.35$; $M_{SIPRE\text{-}O} = 85.32$, $SD_{SIPRE\text{-}O} = 74.49$; $M_{SIPRE\text{-}C} = 273.29$, $SD_{SIPRE\text{-}C} = 68.07$; $M_{IEPOST\text{-}O} = 121.97$, $SD_{IEPOST\text{-}O} = 88.22$; $M_{IEPOST\text{-}C} = 219.30$, $SD_{IEPOST\text{-}C} = 106.90$; $M_{IIPOST\text{-}O} = 84.18$, $SD_{IIPOST\text{-}O} = 64.04$; $M_{IIPOST\text{-}C} = 253.91$, $SD_{IIPOST\text{-}C} = 62.60$; $M_{SEPOST\text{-}O} = 83.66$, $SD_{SEPOST\text{-}O} = 68.83$; $M_{SEPOST\text{-}C} = 219.31$, $SD_{SEPOST\text{-}C} = 112.98$; $M_{SIPOST\text{-}O} = 98.44$, $SD_{SIPOST\text{-}O} = 65.53$; $M_{SIPOST\text{-}C} = 235.66$, $SD_{SIPOST\text{-}C} = 163.24$. In summary, all rats spent more time on the closed arms for both pre- and post-alcohol anxiety tests. More importantly, all rats spent less time on the closed arms post-alcohol than pre-alcohol consumption. However, rather than spending more time on the open arms they may have spent more time in the center.

CHAPTER 4

DISCUSSION

The purpose of my study was to examine the effects of environment and socialization on anxiety and alcohol consumption. My most important findings were that housing and environment each affected alcohol consumption, and that alcohol reduced anxiety for all rats. All groups of rats, regardless of the type of housing and environment, spent less time on the closed arms of the elevated plus maze after alcohol treatment than they did before alcohol treatment.

Hypothesis 1 (isolated-impoverished rats will significantly consume more alcohol and socialized-enriched rats will have least alcohol consumption) was not supported. However, it is worth noting that all isolated rats consumed more alcohol than all socially housed rats during two-bottle testing. Also, all impoverished rats consumed more alcohol than enriched rats during the two-bottle testing. Hypothesis 2 (isolated-impoverished rats will significantly exhibit the greatest anxiety and socialized-enriched rats will exhibit the least anxiety) was not supported because there was no Housing by Environment interaction. Hypothesis 3 (anxiety will be reduced while animals are consuming alcohol because alcohol is a well established anxiolytic and CNS depressant) was supported.

Alcohol Consumption

Prior research has also demonstrated inconsistent findings regarding the effects of isolation. For example, isolation-rearing has been found to increase alcohol consumption (e.g., Hall, Humby, Wilkinson, & Robbins, 1997c), decrease alcohol consumption (e.g., Adams & Oldham, 1996), as well as "produce no effect on ethanol intake" (Schenk, Gorman, & Amit, 1990, p. 321). My results indicate that isolated rats drank significantly

more alcohol and significantly less water than socialized rats during a two-bottle (water versus alcohol) preference test. These results could be compatible with the suggestion that consumption of alcohol is a stress reducing mechanism and because of these reinforcing properties, the severe stress widely associated with social isolation leads to increased ethanol intake (Wolffgramm, 1990). Another explanation offered for isolated rats consuming more alcohol in my study may be because of differences in body weight. With an increased amount of body weight, there may be an increased amount of consumption in order to get the same amount of alcohol to body weight ratio. My isolated rats weighed significantly more than socially housed rats throughout alcohol exposure.

Considerable prior research has demonstrated that environmental enrichment increases alcohol consumption (e.g., Adams & Oldham, 1996; Fernandez et al., 2002; Rockman, Borowski, & Glavin, 1986; Rockman, Hall, & Markert, 1988; Rockman et al., 1989). Contrary to all literature cited above, I found that rats living in an impoverished environment consumed significantly more alcohol than rats living in an enriched environment. Clearly, this is worthy of further study.

Anxiety

All of the rats in my study spent significantly more time on the closed arms of the elevated plus-maze for both pre- and post-alcohol anxiety tests. In other words, given that greater time spent on the closed arms of the elevated plus maze is a widely accepted measure of anxiety (e.g., Colombo et al; 1995), all 4 groups of rats in my study (isolated-impoverished, isolated-enriched, socialized-impoverished, and socialized-enriched) exhibited significantly less anxiety after the alcohol treatment. The fact that my socially-enriched housed rats did not show less anxiety in the pre-alcohol EPM test than the other

groups of rats was surprising, and is in opposition to prior reports of reduced emotionality (e.g., Denenberg, Garbanati, Sherman, Yutzey, & Kaplan, 1978) and higher adaptability (Hodgson, 1984) of environmental enrichment. However, that my isolated rats did not spend more time on the closed arms than the socialized rats prior to alcohol may be consistent with the report that isolation-reared rats exhibit more excitability, hyperactivity in novel environments, and exhibit enhanced exploratory behaviors (Jones, Robbins, & Marsden, 1989).

One explanation for the non-significant behavioral differences in EPM behavior between groups may be due in part to the amount of handling all groups of rats were exposed to on a regular basis (cage cleaning, tail-marking, transportation to and from hanging metal cages, daily body weights). For example, other studies that found significant effects of isolation rearing using the EPM included minimal handling and no mention of tail-marking (e.g., Lodge & Lawrence, 2003). More specifically, Jones, Robbins, and Marsden's (1989) method included minimal handling, and only weighing their rats every 3-10 days, and Lodge and Lawrence (2003), who also found that isolation-reared rats spent less time on the open arms of the elevated plus-maze, did not report tail-marking, or more than minimal handling. Given that Holson, Scallet, Ali, and Turner (1991) reported that isolation stress depends on an interaction of other factors, mainly human handling, it reasonable to assume the consistent handling of all groups of rats in my study may have interfered with the effect of isolation in my study. According to their studies, while isolation from weaning does not produce enduring, chronic stress in rats physiologically, isolation stress syndrome can be seen in those rats raised in isolation without exposure to human handling. Also, Krebs-Thompson, Giracello, Solis,

and Geyer (2000) found, "regular handling of rats may interfere with the observation of the isolation rearing effect" (p. 221).

Another explanation for why my results differed from previous findings on the elevated plus-maze may be difference in time of day (day/light cycle) of testing between studies. Of the studies cited above, few reported whether they tested in light or dark cycle, therefore leading readers to assume testing occurred during the light cycle for convenience purposes. Wongwitdecha and Marsden (1996), for example, specifically stated testing on the elevated plus-maze occurred between 13.00 and 17.00 hr. However, for my study rats were tested in darkness during their dark cycle between 20.00 and 24.00 hr with the exception of one red light that provided the experimenters with just enough lighting to conduct the EPM test trials and allow night-video recording. Although Hall, Huang, Fong, and Pert (1998) tested on the elevated plus-maze in both low light and bright light conditions, "all testing was conducted during the light part of the cycle" (p. 204). Time of day and activity may play a role in the differences in behaviors exhibited on the elevated plus-maze.

Alcohol and Anxiety

In support of Hypothesis 3, all rats spent less time on the closed arms during post-alcohol than pre-alcohol consumption. This could possibly mean that all rats were significantly less anxious during the post-alcohol trials. Therefore my results could be consistent with the idea that alcohol serves as an anxiolytic and central nervous system depressant. In other words alcohol may have reduced anxiety, or anxious behavior, for all groups. However, although the elevated plus-maze is considered a validated test for

anxiety (Sandbak & Murison, 2001) it may be worth noting that it still may be difficult to determine if the behavior exhibited on the elevated plus-maze is solely anxiety related.

Conclusions

I have concluded from this study that environment and socialization do have an effect on behavior. More specifically, isolated-impoverished environment increased alcohol consumption. No differences were exhibited between groups (isolated-enriched, isolated-impoverished, socialized-enriched, socialized-impoverished) on the anxiety test using the elevated plus-maze. However, after consuming alcohol, all groups did spend less time on the closed arms (less anxiety-provoking) of the elevated plus-maze. I have also concluded that the interactive effects of environment and socialization are much more complex than the capacity of this study. Further research is needed in order to determine whether isolated housing conditions without the confounding of handling affect anxiety and alcohol consumption. Additionally, considering the EPM test may not have been sensitive enough to pick up anxiety differences, if they existed in this study, further research employing alternative procedures for measuring anxiety is warranted. Clearly, more research is needed to investigate the roles environment and socialization play on a vast array of effects, specifically anxiety and alcohol consumption.

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Appendix

ESU-ACUC Approval Letter

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December 9, 2003

M. L. Dawson
Department of Psychology and Special Education
Emporia State University

Dear Ms. Dawson:

Please be informed that the ESU Animal Care and Use Committee has received and reviewed your Application for Approval to Use Vertebrate Animals in Research and Training titled: *The Effects of Socialized Versus Isolated and Enriched Versus Impoverished Environments on Anxiety and Alcohol Consumption* (ESU-PROTOCOL-03-009).

The committee has determined that this protocol is in compliance with currently applicable standards for such studies as specified by Federal Regulations and ESU policy and therefore is approved. We request that you submit a notice of completion shortly after you have concluded the project (i.e., a letter stating that the project has been completed, how many animals were actually used, and the manner in which they were disposed of). Please be aware that if any changes are to be made to the study, the ACUC should be notified before hand, and if the project needs to be extended beyond the proposed ending date, you will need to submit a letter to the ESU ACUC requesting an extension.

Your approval number for this project is **ESU-ACUC-03-009.** Also, as a reminder, please label all cages housing animals used solely for this project with this protocol number.

Best wishes with your research. If you have any questions, please contact me at your convenience.

Sincerely,

John Richard Schrock, 2003-2004 Chair
Animal Care and Use Committee

ESU-ACUC-03-009 M.L. Dawson: *The Effects of Socialized Versus Isolated and Enriched Versus Impoverished Environments on Anxiety and Alcohol Consumption*

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