Long Term Effects of Benzylpiperazine and possible Amelioration by Environmental Enrichment

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Abbreviations

BZP Benzylpiperazine

MDMA 3,4-methylenedioxy-methamphetamine

PND Post Natal Day

TFMPP 3-Trifluoromethylphenylpiperazine

5HT Seretonin

Abstract

Adolescents have a proclivity for risk taking and novelty seeking which can lead to BZP use and this can be damaging to the maturing brain. BZP is commonly consumed within party pills alongside TFMPP for its amphetamine like qualities and has the potential to be addictive. Therefore, it is important to study long term effects of BZP of which anxiety has been previously shown. The aim of this study was to investigate long term effects of BZP on anxiety and memory and to determine if attenuation by environmental enrichment is possible (because enrichment has demonstrated this with other drugs). 120 PVG/c rats of equal sex were housed in either enriched or standard caging and exposed to either saline, 10mg/kg or 20mg/kg BZP PND 41. They were then tested in a Y maze, elevated plus maze, light dark box and open field after PND 60 and PND 100 with two days in between each test. The results of this study showed BZP may affect memory but not after PND 100, anxiety may also be present depending on the apparatus used and sex of the animal, however results were conflicting. Lastly enrichment showed some memory enhancing capabilities and anxiolytic properties which further varied across apparatus. In conclusion BZP may decrease memory and increase anxiety over time but due to conflicting results needs further investigation. Similarly, although enrichment improved memory performance and decreased anxiety for some measures it also showed opposite reactions in others therefore further investigation is also needed of enrichment.

1.0 Introduction

1.1 General Introduction

Adolescence is a period when risk-taking and novelty-seeking is a common occurrence (Casey, Getz, & Galvan, 2008). Although this is adaptive while teenager's transition from childhood till adulthood, it can also lead to partaking in drug use (Winters & Arria, 2011).. This proclivity to seek out these exciting experiences is due to changing brain structures, neurotransmitters, peer relationships and the strong effect of rewards at this stage of development (Winters & Arria, 2011) .These cause teenagers to be vulnerable to drug taking and can also lead to regular drug use. The changing brain additionally makes it vulnerable to any drug effects that might occur and theories of neuronal imprinting indicate these changes may not be apparent behaviourally until adulthood (Andersen & Navalta, 2004).

A drug that is commonly used by young people is 1-benzylpiperazine (BZP) which is most often consumed in the form of party pills alongside TFMPP. This drug affects neurotransmitters such as dopamine and 5HT and is initially absorbed by the liver (Antia, Lee, Kydd, Tingle, & Russell, 2009; Staack, Fritschi, & Maurer, 2002). Furthermore, it travels through dopaminergic pathways within the brain and activates similar areas to amphetamines (Bava & Tapert, 2010). In the short term, it can produce positive feelings such as euphoria, however it can also produce negative effects like nausea (https://www.drugfoundation.org.nz). Research has additionally shown that alongside similar properties to amphetamines it may also have an abuse potential similar to methamphetamine (Baumann et al., 2004). This suggests that if teenagers use BZP there is a possibility that they could use it regularly. If this drug is used frequently long term effects such as anxiety may become pronounced (Aitchison, 2015).

To reduce this anxiety and other effects that may occur such as memory problems, it is important to find ways to ameliorate BZP effects. Environmental enrichment which involves creating an enriched environment for captive animals has been shown to reduce anxiety and

ameliorate memory impairments of other drugs(Simpson & Kelly, 2011; Townsend, 1998). Therefore, it is important to see if this experimental manipulation could reduce memory or anxiety effects of long term BZP use.

Since anxiety is a problem experienced predominantly by women it is also important to include both sexes when investigating BZP effects and efforts to reduce them (Donner & Lowry, 2013) . Furthermore, although many studies don't include female animals, without including both sexes important sex-related effects may be obscured and results may not translate from animals to humans (Kokras & Dalla, 2014).

Lastly the aim of this experiment was to determine if BZP influences anxiety and memory in the long term when taken in adolescence. Environmental enrichment was also investigated as a possible method of ameliorating long term effects.

1.2 Adolescent Development and Brain Vulnerability

When considering ways of attenuating memory impairments or anxiogenic effects of BZP caused by use in adolescence it is important to demonstrate why BZP may be consumed during this age period and what effects drug taking can have on the vulnerable adolescent brain. Adolescence is a time when teenagers exhibit more risk taking and seek novel experiences. During this developmental period experimentation can begin with addictive drugs and patterns of long term drug abuse can emerge (Casey et al., 2008).

When 11 or 12 years old, synaptic pruning begins in the brain. This involves the elimination of synaptic connections that are infrequently used and the strengthening of those used a lot (Giedd, 2004). This process begins at the back of the brain within the limbic system and later moves forward to the front of the brain within the prefrontal cortex (Gogtay et al., 2004; E. Meririnne, M. Kajos, A. Kankaanpaa, & T. Seppala, 2006). The slower maturation of the prefrontal cortex, which helps to make informed decisions and regulate emotions may lead to increased risk taking and novelty-seeking (Lubman & Yücel, 2008) . This is because the

brain has not yet developed enough to enable appropriate decisions to be made about these behaviours (Lubman & Yücel, 2008). Furthermore, elevated activity in emotional processing areas of the brain begins can consequently lead to reactions that are affected predominantly by emotional context (Hare et al., 2008). Therefore, when faced with highly emotional situations or peer pressure, adolescents may be likely to perform risk-taking behaviours based on momentary feelings (Knowlton et al., 2010). Although this risk-taking can be adaptive as teenagers struggle to achieve independence from their parents, it can also lead to the consumption drugs such as BZP (Winters & Arria, 2011).

Risk-taking and novelty- seeking can lead from drug experimentation to drug abuse, due to many factors. If peers are deviant, the home environment is unstable or if predisposing genetic factors are present, drug taking is increasingly likely to occur and could lead to drug abuse (Winters & Arria, 2011). Additionally, the earlier experimentation occurs the higher the likelihood that a substance use disorder will develop later in life (Winters & Arria, 2011). Furthermore, the strength that a reward such as drug consumption has for adolescents is different from adults (Bava & Tapert, 2010). This means teenagers have a higher proclivity to try drugs due to the intensity of the reward anticipated (Galvan, 2010). This is seen in functional magnetic resonance imaging, where greater striatal activation has been shown in adolescents in anticipation of a reward and with the reception of a reward (Galvan, 2010; Van Leijenhorst et al., 2010). Furthermore, due to the prefrontal cortex still developing adolescents have a reduced ability to assess potential outcomes of rewards e.g. using drugs for the euphoria experienced without considering effects on the brain or potential additive risks (Bava & Tapert, 2010). Dopamine release during these rewarding events can further result in a cycle of reinforced behaviour (Bava & Tapert, 2010). This means that when drugs are consumed dopamine is released in reward pathways producing a positive feeling, which causes the teenager to be more likely to use that drug again. Therefore, if an adolescent is taking a drug

such as BZP the dopamine release they get from the drug could lead to seeking BZP again and possibly repeated drug use.

Furthermore, during this stage of brain development, changes in dopamine also play a significant role in maturation. Within the prefrontal cortex, dopaminergic connections increase to other parts of the brain allowing faster dopamine facilitation between back, front and middle areas of the brain (Tunbridge et al., 2007). It has also been suggested dopamine receptor densities increase during adolescence and D1 and D2 densities start to peak in the striatum (Wahlstrom, White, & Luciana, 2010) These changes in dopamine as well as other neurotransmitters (such as 5HT) occur in synchrony with changes in brain morphology and therefore can affect future behaviour, cognitions and other psychological processes (Paus, Giedd, & Keshavan, 2008). This is important for adolescents taking BZP party pills as this drug also affects the dopaminergic system and, as the brain is still developing, it is vulnerable to BZP effects.

Lastly, neuronal imprinting posits that drug exposure effects may be delayed and are only shown in adulthood meaning that some effects last longer than those produced by acute treatment with the drug (Andersen & Navalta, 2004). Low doses of stimulants other than BZP have shown an in increase depressive and anxious symptoms later in life (Bolaños, Barrot, Berton, Wallace-Black, & Nestler, 2003; Carlezon, Mague, & Andersen, 2003). This supports neuronal imprinting theory and suggests similar reactions may be demonstrated with BZP. However, most of these studies involved subjects in childhood and not adolescence (Andersen & Navalta, 2004). Therefore, neuronal imprinting maintains that the young brain is vulnerable to changes after stimulant consumption, but that these effects will not be seen till adulthood.

In conclusion, changing rates of maturation in different areas of the brain and teenagers' high sensitivity to rewards can cause them to seek out novel experiences and perform risk taking behaviours. This sensitivity to rewards as well as influences of deviant peers, unstable

homes and genetic factors may lead to drug-taking and substance abuse. Therefore, drugs may pose a risk of being abused by teenagers and thus could have long term effects on the brain that is undergoing significant structural and neurotransmitter changes. Neuronal imprinting suggests these effects may not be visible until adulthood.

1.3 Benzylpiperazine

Before we can consider the long-term effects of BZP on adolescents and how to prevent them it is important to consider BZP development, immediate effects and how it works within the body. BZP is a synthetic phenyl analogue of piperazine and in its freebase, form it is a pale yellow viscous liquid that reacts with air and light (Schep, Slaughter, Vale, Beasley, & Gee, 2011). Originally this substance was developed as a treatment for intestinal roundworm and threadworm infections by Wellcome Research Laboratories (Schep et al., 2011). Later in the 1970s it was investigated as a potential antidepressant since it was found to have similar effects to dexamphetamine in both subjective and objective reports. However, its potency was calculated to be only one tenth of that for dexamphetamine (Schep et al., 2011). It was later determined that BZP could be prone to abuse when administered to former addicts and consequently research into its antidepressant properties diminished (Campbell, Cline, Evans, Lloyd, & Peck, 1973).

In recent years, it has been used recreationally in designer party pills (usually in combination with trifluoromethylphenylpiperazine/ TFMPP) due to its amphetamine like effects. There has also been some suggestion that BZP is as a safer alternative to methamphetamine (Wilkins, Sweetsur, & Parker, 2014). BZP was considered so safe that it was legal to purchase party pills containing BZP at any age in New Zealand until 2005, after which it was restricted to people 18 years and over (Wilkins et al., 2014). After 2008 BZP-containing party pills were reclassified as a Class C drug by the New Zealand government

making it illegal to manufacture, import, export, supply, sell or consume (Cohen & Butler, 2011).

When investigating the short-term effects of BZP, it has been suggested that acute effects of BZP can be felt approximately 60 minutes after ingestion (Lin, Bangs, Lee, Kydd, & Russell, 2009). These effects can include a rise in blood pressure and heart rate which is caused by the additional increase in noradrenaline produced after BZP is consumed (Lin et al., 2009; Lin, Jan, Lee, et al., 2011; Schep et al., 2011). In humans, euphoria is commonly experienced as well as dysphoria once the drug effect begins to wear off (Lin et al., 2009; Lin, Jan, Kydd, & Russell, 2011). A decrease in fatigue has further been shown causing participants to stay awake for extended periods (Lin et al., 2009; Lin, Jan, Kydd, et al., 2011). Hunger can additionally be supressed, which resembles amphetamines that have been used as appetite suppressants (Kuo, 2005; Lin et al., 2009). Lastly, other effects can include increased energy and alertness, dehydration, stress, lack of emotional control, headaches (especially in combination with alcohol), nausea, hallucinations and in some cases convulsions (https://www.drugfoundation.org.nz). Therefore, varying positive and negative effects are witnessed acutely after BZP use.

After ingestion BZP is rapidly absorbed and then metabolised in the liver, with the main pathways including hydroxylation and N-dealkylation (Antia et al., 2009; Staack et al., 2002). Within the brain, one of major effects is to increase neuronal dopaminergic transmission (Staack, Fritschi, & Maurer, 2002). Like amphetamines, it is thought to release dopamine and inhibit dopamine reuptake as well as postsynaptic receptor activity (Oberlander, Euvrard, Dumont, & Boissier, 1979). *In vivo* BZP has shown an increase both dopamine and serotonin (5HT) activity similarly (E. Meririnne et al., 2006). As with many other drugs of abuse, this increase in dopamine is thought to underlie the rewarding properties of BZP (E. Meririnne et al., 2006). These *in vivo* results also suggests that BZP, especially in combination with TFMPP,

may act like MDMA (Baumann et al., 2004). Additionally, i*n vitro* dopamine response is mediated by dopamine transporters. However, a similar mechanism does not seem to characterise the effect of the drug on 5HT (Baumann et al., 2004). This result has also been seen with methamphetamine suggesting that BZP may work in a similar fashion to this drug (Baumann et al., 2004). Therefore, the mechanism by which BZP operates is similar to amphetamines and involves both dopamine and 5HT which indicates it has a similar addictive potential.

1.4 Addictive Potential of BZP and Long Term Effects

Before considering how any long-term adverse effects of BZP might be attenuated, it must first be demonstrated if people will be likely to take BZP regularly and if there are indeed any long-term effects of concern. Similarities in mechanisms of action between BZP and amphetamines suggest that BZP may have a similar dependence potential to these drugs. Similarities in the behavioural effects of recognised addictive stimulants and BZP also suggest that it may have some addictive potential. Fantegrossi, Winger, Woods, Woolverton, and Coop (2005) have demonstrated this with rhesus monkeys that self-administered two different BZP doses at rates just as high and higher than the baseline dose of cocaine. Additionally, subjects responded strongly to saline several hours after they self-administered BZP (Fantegrossi et al., 2005). The high self-administration responses with cocaine and the high responding after BZP was replaced with saline several hours later suggest that BZP was rewarding enough for monkeys to keep using it thereby suggesting an addictive potential. In another study by Brennan et al. (2007) lever responses previously maintained by cocaine continued to occur after the drug was replaced with BZP. BZP also showed high levels of responding on a drug lever in rats that had not previously been exposed to drugs. Furthermore, the time taken for this to occur was similar to MDMA and cocaine (K. A. Brennan et al., 2007). Therefore,

similarities between BZP and cocaine in self-administration and in behavioural effects also supported the premise that BZP has addictive potential.

Similar addictive potential has been shown in studies with methamphetamine and BZP. This has been shown in cross sensitisation in which chronic BZP administration leads to sensitisation to methamphetamine (Brennan, Johnstone, Fitzmaurice, Lea, & Schenk, 2007). Herbert and Hughes (2009) have further demonstrated that behavioural effects in an open field, light dark box and responsiveness to change Y maze were similar between BZP and methamphetamine. Therefore, due to its similar mechanisms of action, behavioural effects and cross sensitisation to amphetamines, BZP may have considerable addictive potential. Because of these characteristics, it is important determine any long-term effects of this drug especially as it is commonly used by adolescents.

Although there is limited research on the long-term effects of BZP use, some studies have indicated that anxiety may result from its repeated use during adolescence. This was highlighted in a study by Aitchison and Hughes, (2006) in which BZP was administered to rats for 10 days at the age equivalent of human adolescence. The results of their study showed that rats demonstrated higher anxiety than animals not given BZP (Aitchison & Hughes, 2006). Other studies in which BZP was given to adolescent rats have also demonstrated that anxiety is found later when rats reach adulthood (Aitchison, 2015).

In conclusion, if BZP is used during adolescence it has the potential to become addictive. This is evident from the research described in the above section. Consequently, if this drug is addictive it is important to investigate the possibility of any adverse long-term effects it may have. Although there is not much research supporting this possibility, past studies have nevertheless revealed that BZP taken regularly during adolescence can lead to higher anxiety in adulthood. Therefore, attempts to attenuate such anxiety (or any other adverse long-term effects that may become apparent after adolescent use) need to be considered.

1.5 Environmental Enrichment

To modify long term effects of BZP such as anxiety it's important to consider environmental enrichment. Environmental enrichment is the process of exposing laboratory animals to social or physical stimulation that is greater than they would get in standard caging (Rosenzweig & Bennett, 1996). Physical enrichment involves modifications of the cage that an animal is housed in and can include means for enabling exercise, play and exploration such as the provision of plastic tunnels, wooden objects to bite, swings, ropes, balls, wheels, ramps and other similar sized toys (Simpson & Kelly, 2011; Townsend, 1998).

Enrichment can lead to significant changes within the brain. In some studies, environmentally enriched rats have shown improved expression of the 5HT1 receptor in the hippocampus and frontal cortex (Brenes, Rodríguez, & Fornaguera, 2008; Rasmuson et al., 1998). In the hippocampus of adult rats, environmental enrichment has also been found to increase neurogenesis, which is the increased generation of neural cells within the brain (Lu et al., 2003; Van Praag, Kempermann, & Gage, 2000). Other structural changes that have been associated with environmental enrichment include improved brain plasticity (Rosenzweig & Bennett, 1996), an increase in dendritic growth (Leggio et al., 2005), heavier brain weight (Bennett, Rosenzweig, & Diamond, 1969), increased cortical thickness and synapse formation (Rosenzweig & Bennett, 1996).

Environmental enrichment can also have effects on neurotransmitters such as dopamine and 5HT which are both implicated in BZP use. Some studies have shown that after 12 weeks in environmentally enriched housing, D1 density in the prefrontal cortex was reduced compared to controls (Del Arco et al., 2007). In addition, dopamine uptake, has been shown to decrease in this type of housing (Jun Zhu, Apparsundaram, Bardo, & Dwoskin, 2005; J. Zhu, Green, Bardo, & Dwoskin, 2004). Therefore, environmental enrichment can lead to structural changes and neurochemical fluctuations.

The importance of environmental enrichment for changing behaviour was first seen in the 1940s when Donald Hebb took laboratory rats home where they were free to roam as house pets (Simpson & Kelly, 2011). This led to better learning and problem solving abilities than those housed in standard caging (Simpson & Kelly, 2011). For non-drug induced animals enrichment has been shown to increase spatial memory in both the water maze and T maze. In the water maze, enriched animals remembered the ladder's location better than controls housed in standard housing(C, D, & J, 1989; Wainwright, Lévesque, Krempulec, Bulman-Fleming, & McCutcheon, 1993). In the T maze, enriched animals also performed better on spatial memory tasks than non-enriched animals (Walsh & Cummins, 1975). In an experiment by Sampedro-Piquero, Zancada-Menendez, Begega, Rubio, and Arias (2013), general anxiety was also shown to be reduced by environmental enrichment. The showed that enrichment caused lower anxiety in the elevated zero maze reflected in shorter latencies to enter open areas and increased time spent in these areas. Improved spatial memory was also seen in the radial arm water maze (Sampedro-Piquero et al., 2013). These results support the view that environmental enrichment can affect both anxiety and spatial memory in animal subjects.

Lastly there have been no experiments reported in which environmental enrichment was used to counteract long term effects of BZP consumed in adolescence on later anxiety, or other adverse effects that might occur. There have however, been studies which have shown that enrichment can attenuate effects of other drugs (Hughes, 2013). In an experiment conducted by Hughes and Otto (2013), rats were treated with scopolamine and then later tested in an open field, y maze and light-dark box. Although scopolamine had an anxiogenic effect, this was attenuated in animals that were housed in enriched environments. In another experiment by Schneider, Turczak, and Przewłocki (2006) male rats exposed to valproic acid during gestation developed lower levels of subsequent anxiety when housed in enriched cages than rats from standard cages. This suggested that enrichment had attenuated the prenatal

anxiogenic effect of the drug. Results further showed that animals housed in enriched housing had lower anxiety than those in standard caging, suggesting enrichment attenuated anxiety. Therefore, since enrichment has been shown to attenuate the adverse effects of other drugs, it is possible it might also attenuate any adverse effects of BZP.

In conclusion, environmental enrichment is the process of providing housing that contains elements (such as "toys") that enable rats to explore and manipulate their environment. Such enrichment can also have effects on brain structures such as the hippocampus and the pre-frontal cortex, and can increase neurogenesis as well as brain plasticity. Changes in neurotransmitters such as dopamine and 5HT are also found when animals are housed in enriched environments. Furthermore, for both drug-treated and non-drug-exposed animals, environmental enrichment has been shown to improve memory and reduce anxiety as well as counteracting other adverse drug effects. Therefore, it is important to see if environmental enrichment can ameliorate any adverse effects of BZP.

1.6 Sex Differences

When considering long-term effects of BZP consumed during adolescence and possible attenuation by enrichment, it is important to determine if results can vary between sexes. This is especially useful since most animal models are intended to represent psychiatric disorders experienced by humans which can often be sex-dependent (Kokras & Dalla, 2014; Overstreet et al., 2003). It is also important because of sex differences in human anxiety, and because higher anxiety can be a long-term consequence of BZP use. Furthermore, lifetime prevalence of anxiety disorders in females is 60% higher than in males (Donner & Lowry, 2013; Wittchen et al., 2011). Similar onset course, severity and response to treatment of anxiety can also vary significantly for women (Donner & Lowry, 2013). However, despite such sex differences, most animal research into anxiety is predominantly completed with males (Beery & Zucker, 2011; Hughes, 2007).

Reasons why male rats are mainly used in research include the mistaken belief that female non-sexual behaviour is identical to that of males, but is more variable (Kokras & Dalla, 2014). Another reason for the use of males exclusively is the fact that female animals experience an oestrus cycle with accompanying hormonal changes. It is believed that this cycle could accordingly interfere with behavioural testing procedures (Jill B. Becker et al., 2005). These concerns about confounding outcomes are not completely unfounded. When female rats are in their oestrous cycle and exposed to amphetamine locomotion is increased (J. B. Becker & Cha, 1989) and when rats are administered cocaine they are more likely to be sensitive to effects of this drugs effect during this cycle (Festa & Quinones-Jenab, 2004; Lacy, Strickland, Feinstein, Robinson, & Smith, 2016). However, female's animals are not the only ones to be affected by hormones that could influence results. After consumption of ethanol aggression was mediated by testosterone in male mice (DeBold & Miczek, 1985), and social investigation has been shown to increase when caffeine is consumed by male rats (Holloway & Thor, 1984). Therefore, although concerns about using females in experiments can be justified in certain situations, males may also be subject to hormonal influences.

Despite aforementioned arguments against using females in experimental procedures there are reasons for their inclusion. This is because inclusion of both sexes can enhance animal model validity and assist in sex-related diagnosis, prevention and treatment of mental health disorders (Hughes, 2007). There is also the possibility that without the use of both sexes, important information based on sex will not be available (Blanchard, Griebel, & Blanchard, 1995). Additionally, without sex comparisons, animal research may not accurately reflect human responses to drugs or psychiatric illnesses (Hughes, 2007). This is because male-only research indicates that results may only apply to human men, and not to women (Kokras & Dalla, 2014). Therefore, it is clearly important to include both males and females in animal as well as human research.

In conclusion, a major long-term effect of BZP previously reported is increased anxiety. Since anxiety is commonly experienced by women, it is important that animal research reflects this. However, due to due to beliefs about close similarities between male and female behaviour and possible confounding effects of the female oestrus cycle in rats, only males are used in a majority of studies. Without the inclusion of females in psychopharmacological studies important information may be missed and results of studies may not accurately carry over to human females as well as males. Therefore, it is important to include both sexes when investigating relationships between the effects of drugs, such as BZP, and enrichment as both manipulations can be affected by sex.

2.0 Aim and Hypothesis

The aim of the research presented in this thesis was to determine if there were any long-term effects on anxiety and memory after repeatedly exposing male and female adolescent rats to BZP. It was predicted from the results of earlier research (described above) that adolescent BZP would increase later anxiety. Due to the comparative lack of similar research into effects on memory, the possibility of BZP-associated changes in this process was also investigated. The major aim of the research was to see if environmental enrichment could ameliorate any long-term effects of BZP. It was predicted that this enrichment would reduce any BZP-induced increases in anxiety, or any memory impairments that might also occur. The latter prediction was based on past evidence that enrichment can improve memory and anxiety in studies described previously.

3.0 Method

3.1 Subjects

The subjects in this experiment were 120 PVG/c hooded rats, that were breed at the University of Canterbury Psychology Animal Facility. Half of the rats in this experiment were male and the other half were female. This was arranged so that assessment of any sex differences in responsiveness to BZP or environmental enrichment could be analysed.

When the pups were 30 days old they were weaned and housed in an experiment room on a 12-hr light cycle from 8am onwards. Animals lived inside plastic cages with free access to water and food (rat pellets). These cages were 470 x 280 x 230 mm and the animals were housed in groups of 2 to 4 for the rest of the experiment. Half of the rats used in this experiment were randomly allocated to standard cages and the rest were in enriched cages that contained several toys. These toys included a selection of randomly chosen jars, metal lids, marbles, boxes, household utensils and tunnels. The objects were there to encourage the rats to explore by enabling them to manipulate, climb over or into, and generally investigate the toys providing perceptual enrichment. They were also replaced weekly with new randomly chosen objects to novelise their surroundings. Standard caging was the same as the enriched caging except for the toys which were only provided to the enriched group. This was arranged so that the impact of environment in BZP responsiveness could be assessed. All the subjects and procedures were approved by the Animal Ethics Committee of the University of Canterbury (see Appendix A).

Subjects were randomly allocated into saline and two different BZP groups with equal numbers in each caging condition. Then at PND 41 animals were given intraperitoneal injections with saline or BZP depending on what they were randomly assigned to, over 10 days. The age of drug exposure was selected to represent the equivalent age of adolescence in humans (Andersen, 2003). PND 60- 72 rats were all tested in each type of apparatus with a two-day

interval between each test and the order of testing varied for individual rats. Then, from 100-112 days after birth, all behavioural testing was repeated. These time periods were chosen to represent different stages of early and middle adulthood and therefore measure long term effects of BZP (Andersen, 2003).

3.2 Drug Dose

1-Benzylpiperazine was purchased from Sigma-Aldrich based in Missouri USA. For the 10mg/kg solution 0.25ml of BZP was mixed with 25ml of 0.9% isotonic saline and for the 20mg/kg dose 0.50ml of BZP was mixed with 25ml of saline. These doses were chosen to determine if strength of BZP showed dose effects and based on previous research where anxiolytic results were found with 10mg/kg dose (Aitchison & Hughes, 2006)

Subjects were intraperitoneally injected 1ml per kilogram based on their daily weights which were recorded over the dose treatment period. The same method was used when administering to the saline only group. Furthermore, intraperitoneal injection was used as a method of administration due to its accuracy and because it's relatively easy to administer and learn (Coria-Avila, Pfaus, Ménard, Gavrila, & Ismail, 2007).

3.3 Apparatus and Behavioural Procedures

Each subject was tested in a responsiveness to change Y maze, elevated plus maze, light dark box and open field test in a dimly lit experiment room. There were two days between each apparatus and subjects were randomly assigned to start on different apparatus as a counterbalancing measure. Furthermore a 3 second timer was used with headphones so that behaviours could be recorded at regular intervals.

3.4 Y Maze

In the responsiveness to change Y maze whether the animals could remember which arm of the apparatus was novel was used as a measure of spatial memory, through novel observations, entries and transitions (Conrad, Galea, Kuroda, & McEwen, 1996). This maze was placed on a 70-cm table and had a stem with two arms coming out from the stem at a 120° angle. Each of these two arms were 45 cm long, 10 cm wide and 14 cm high with a clear Perspex lid that covered the apparatus including the stem. Although the stem was 30 cm long it was cut through the middle by a wooden insert leaving a 15cm of the stem for the rats to be placed in. Furthermore, there were four metal inserts one painted white and the other three painted black which could be placed within the arm. The maze was lit from above with fluorescent lights.

The Y maze procedure involved an acquisition trial where a white insert was placed in one arm and a black insert in the other. A rat was then placed in the stem of the maze and allowed to freely roam the maze for 5 minutes timed with a stop watch. After that trial, the rat was returned to its cage and the apparatus were cleaned with a 20% Paraquat blue solution. Both arms were then replaced with two black arms and the rat was put back into the stem of the maze for a retention trial. During this retention trial behaviours were recorded for 3 minutes and attention was paid to time spent in the arm that had changed from white to black (novel arm).

Behaviours were recorded on testing sheets and entries of arms were only counted when subjects fully entered the arm with all paws. These behaviours included which arm (or the stem) rats were in, entries of the novel arm and total entries of both arms. This process was repeated for each rat and the novel arm was switched between subjects as a counterbalancing measure.

3.5 Elevated Plus Maze

The elevated plus maze was chosen as a measure of anxiety because it represents a conflict between wanting to explore the novel environment and the animals fear of heights (Rodgers & Dalvi, 1997). This is because it has been proposed that animals that are anxious will decrease time spent in the open arm of the apparatus (Rodgers & Dalvi, 1997). The maze had four 100 cm long and 50 cm wide arms. These arms extended from a 15cm long and 15 cm wide platform and these arms were 90° to each other. The open arms were facing each other and were 24 cm high on the ends and side walls which were constructed by Perspex. The closed arms had the same dimensions however they were made of painted black metal. The maze was lit by florescent lights and sat on a stand that was 1m high (a CCTV camera was also above the maze).

The maze procedure involved placing an animal in the centre of the apparatus facing one of the closed arms. For five minutes the animal could freely roam the maze and number of entries into either the closed or open arms were recorded when all four feet were inside. Additionally, every three seconds the location of the subject was recorded on a testing sheet, which was seen through CCTV. The animal was then replaced in its cage and apparatus cleaned with the Paraquat solution to stop olfactory smells interfering with behaviour.

3.6 Light Dark Box

The light dark box measured anxiety based on the premise that for rats there is a conflict between wanting to explore the novel environment and fear of the brightly lit room (Ennaceur & Chazot, 2016). Therefore, if animals are anxious they will decrease time spent in the light area and increase time taken to emerge into the light (Ennaceur & Chazot, 2016). The wooden light dark box was sitting on a 70-cm table and is split into a light area which is painted white

and is 30 cm long, 25 cm high and 25 cm wide. This area is also illuminated from above with a fluorescent light and covered with a clear Perspex lid. The light area is separated from the dark area by a wooden wall that has an opening big enough for the rat to go between parts of the box. It also has an insert that blocks this opening when needed. The dark area is painted black and has the same dimensions as the light, however it has a wooden lid allowing blocking the light.

The procedure for this apparatus involves placing the rat inside the dark component of the box for 30 seconds (measured using a stop watch) with the compartment slide in place. After this the slide is removed allowing the animal to freely move between compartments for 5 minutes. A stopwatch was then used to time how long it took for the rat to emerge (all four feet) into the light for the first time. This was used to determine the emergence latency of the animals. Then for every three seconds which compartment the animal was in was recorded on a testing sheet using the CCTV from the camera above the box. Afterwards the rat was returned and the box cleaned before the next rat was used.

3.7 Open Field

The open field was the last apparatus used to measure anxiety responses and is based on the premise that rodents show an aversion to unknown environments that are open and brightly lit (Seibenhener & Wooten, 2015). The wooden open field was comprised of a black wooden floor and walls that was 60 cm in both width and length and 30 cm high. There were also 16 numbered squares that were equally 15 cm long and wide with 4 across and 4 down. There was also a camera situated above the open field that allowed viewing of the subjects behaviour through CCTV.

In the open field rats were initially placed in the centre of the apparatus then for 5 minutes' rats could freely roam. Then on a record sheet every 3 seconds it was noted which

square rats were in (ambulation was calculated by the number of transitions between squares) and whether they were engaging in walking, rearing on hind legs, grooming or were immobile. At the end of the 5 minutes the subject was placed back in their cage and the number of faecal boluses left in the apparatus were counted. Lastly the open field was cleaned before the next rat was placed inside.

4.0 Results

For each behavioural task a 3 x 2 x2 (Dose x Sex x Cage Condition) factorial ANOVA was used. The total number of rats was 120, with equal numbers in each condition (i.e. 60 Male/60 Female; 60 Standard Housed/60 Enriched; 20 rats per dosage – control, 10mg/kg, 20mg/kg). When appropriate, post hoc comparisons were performed with Scheffé tests.

4.1 PND 60 Y Maze

Table 1 and 2 show means (±SEMs), and results of ANOVAs for main effects for the three Y-maze measures (% Novel entries, % Novel observations and Total entries). Results for significant dose main effects, plus two and three-way interactions are shown in Figures 1 through 4.

A BZP main effect for total entries was significant (see Table 1). Post hoc Scheffé tests showed that saline-treated subjects entered both arms of the Y maze significantly more often than 20mg/kg BZP subjects (see Figure 1). This did not occur with 10mg/kg BZP.

Table 1: 60 days mean (S.E.M) percentage of novel entries, percentage of novel observations and total entries of both arms for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests.

BZP Dose 60 Days	Saline	10mg/kg	20mg/kg	*F* (2, 108)	*P*
% Novel Entries	54.39 (1.00)	54.21(1.64)	54.19(2.85)	3.54	.996
% Novel Observations	48.91(2.29)	51.57(2.31)	49.15 (3.5)	0.34	.715
Total Entries	9.75(0.53)	8.68 (0.45)	7.22 (0.57)	7.12	.001

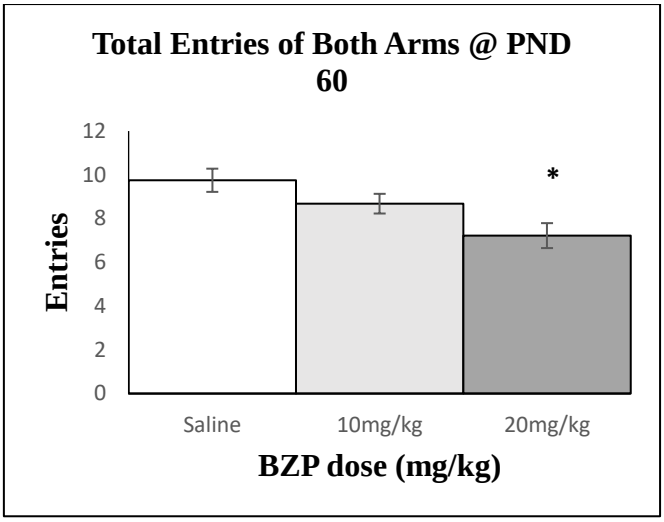

Figure 1: Main effects of dose in the total number of entries of both arms for control (n= 40 subjects), 10mg/kg (n=40 subjects) and 20mg/kg (n=40 subjects). Standard error means are represented by error bars and * = significantly different from saline.

As revealed by a significant dose x cage interaction (F (2,108) = 7.22, p = .001; see Figure 2) percent of entries into the novel arm with differing cage conditions depended on BZP dose. Those in enriched housing that received 10mg/kg of BZP entered the novel arm significantly more often than those in standard housing whereas for rats treated with 20mg/kg BZP, this relationship was reversed. Lastly 10mg/kg BZP subjects in standard caging made significantly fewer novel arm entries than those in the 20mg/kg dose group, this difference was not found between enriched BZP groups. Post hoc tests additionally showed that there was no difference in percentage of novel entries between standard and enriched rats in the saline dose group and that both BZP dose groups did not significantly differ from the saline group.

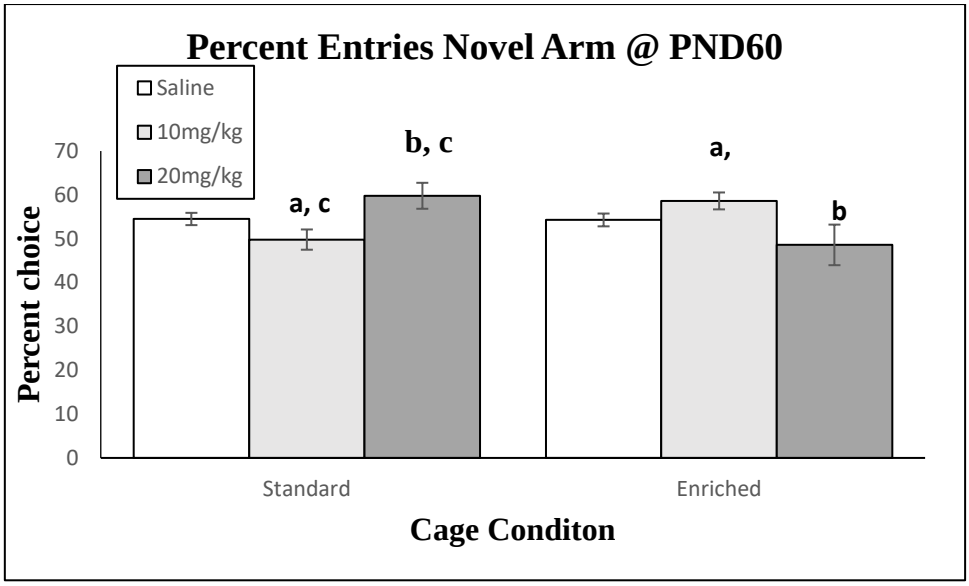

Figure 2: Dose x Cage interaction in percentage of novel entries for the Control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. Abc = groups with superscripts in common are significantly different.

For percent of time spent in the novel arm, there was a significant three-way interaction (F (2, 108) = 6.54, p = .002; see Figure 3). This is best accounted for by the response being decreased by the higher dose of BZP for females from standard cages, and increased by both doses for females from enriched cages. Males from either type of cage were not significantly affected by the drug. In addition, female enriched subjects in both BZP dose groups increased time in the novel arm of the apparatus than their counterparts in standard caging, and standard females in the saline dose group were observed significantly more often in the novel arm than those in the 20mg/kg group.

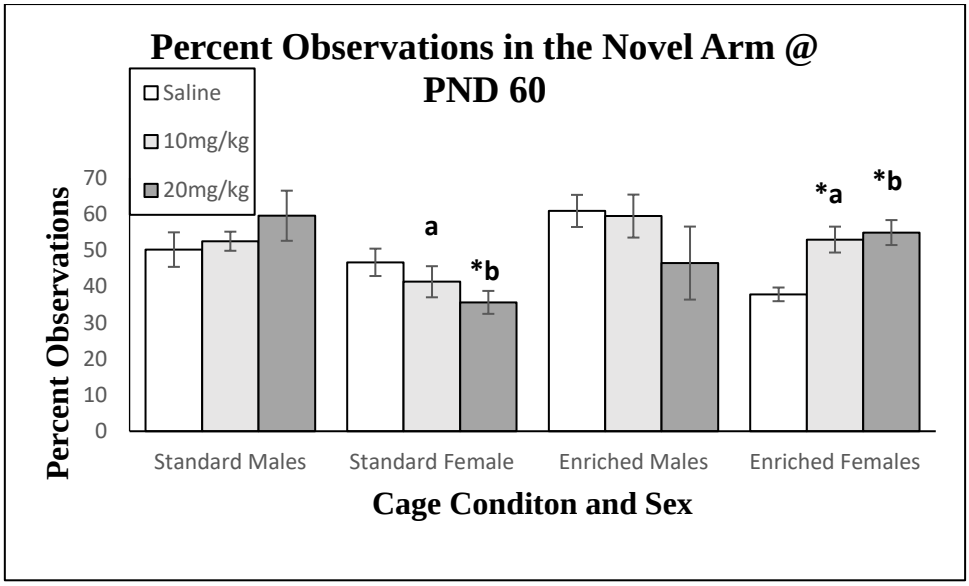

Figure 3: Dose x Sex x Cage interaction in the percentage of observation in the novel arm for control (n= 20 males half standard, half enriched and 20 females half standard and half enriched), 10mg/kg (distributed the same as control) and 20mg/kg (distributed the same way as the other two doses). Standard error means are represented by error bars. * = significantly different from saline and Abc = groups with superscripts in common are significantly different.

Within cage and sex conditions, rats housed in standard cages exhibited more total arm entries than enriched subjects (Cage Condition Main Effect for Total entries; Table 2) but no housing effects were observed for either entries to, or time observed in the novel arm. Furthermore, female rats were observed less often in the novel arm than males (Sex Main Effect for % Novel Observations; Table 2), but exhibited more total arm entries (Sex Main Effect for Total Entries; Table 2).

Lastly total arm entries varied by sex and housing (Sex x Cage Condition Interaction: F (1, 108) = 4.27, p = .041; Figure 4). Enriched males had significantly fewer total entries than enriched females and standard males. Therefore, enrichment effected male entries between cages and between sexes when housed in enriched environments.

Table 2: 60 days mean (S.E.M) percentage of novel entries, percentage of novel observations and total entries of both arms for enriched (n= 60), standard (n= 60), male (n=60) and female (n= 60) and results for *F* tests.

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
% Novel Entries	54.70 (1.42)	53.83 (1.79)	0.16	.689
% Novel Observation	47.66 (2.02)	52.10 (2.42)	2.30	.133
Total Entries	9.37 (0.42)	7.73 (0.43)	8.87	.003
Sex 60 Days	Male	Female	*F* (1, 108)	*P*
% Novel Entries	52.82 (1.90)	55.71 (1.25)	1.80	.183
% Novel Observations	54.86 (2.54)	44.90 (1.67)	11.55	.001
Total Entries	7.75 (0.47)	9.35 (0.38)	8.51	.004

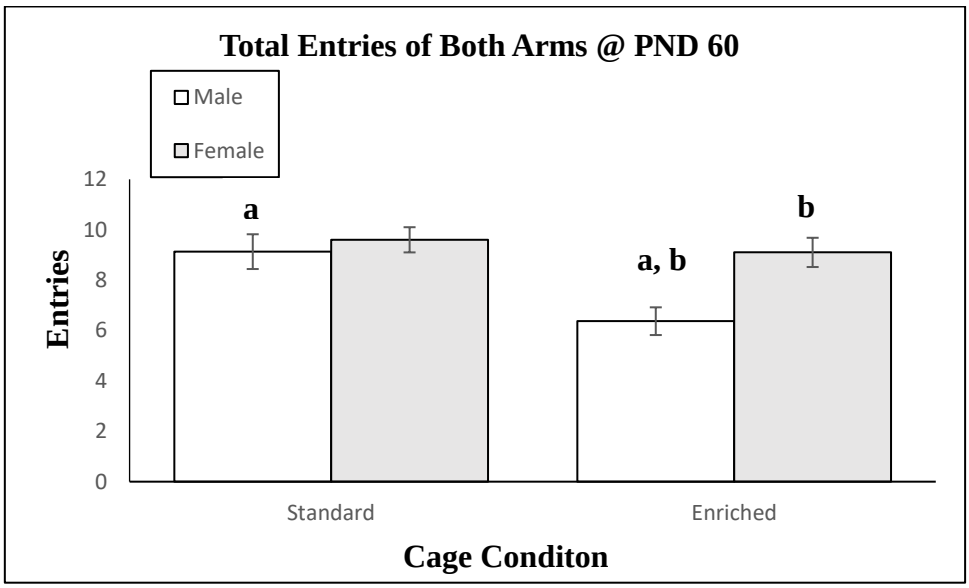

Figure 4: Sex x Cage interactions in the total number of entries of both arms of the maze for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars. * Abc = groups with superscripts in common are significantly different.

4.2 PND 100 Y Maze

Table 3 provides mean (±SEM), and ANOVA main effects of dose, cage and sex on the three Y-maze measures (% Novel entries, % Novel observations and Total entries). There were no longer any significant interactions at PND 100.

Dose main effects found at PND 60 were not present after PND 100 days and there were no longer any cage differences in total number of entries. In addition, there were no further sex differences in the observations of subjects in the novel arm. However, female rats continued to enter both arms of the Y maze more often than male subjects (Sex Main Effect for Total Entries; Table 3).

Table 3: 100 days mean (S.E.M) percentage of novel entries, percentage of novel observations and total entries of both arms for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40), standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests.

BZP Dose 100 Days	Saline	10mg/kg	20mg/kg	*F* (2, 108)	*P*
% Novel Entries	57.48 (1.48)	56.71(1.37)	60.28(2.33)	1.14	.322
% Novel Observations	56.45(1.63)	57.95(2.1)	57.6 (3.37)	0.10	.906
Total Entries	8(0.33)	7.1 (0.41)	6.8 (0.43)	2.58	.080

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
% Entries of Novel Arm	58.22 (1.48)	58.09 (1.44)	3.67	.952
% Observations in Novel Arm	56.61 (2.07)	55.07 (1.92)	2.46	.120
Total Entries of Both Arms	7.07 (0.31)	7.53 (0.34)	1.08	.301

Sex 100 Days	Male	Female	*F* (1, 108)	*P*
% Entries of Novel Arm	56.83 (1.45)	59.48 (1.45)	1.71	.194
% Observations in Novel Arm	55.20 (2.08)	59.47 (1.92)	2.17	.143
Total Entries of Both Arms	6.65 (0.31)	7.95 (0.33)	8.40	.005

4.3 PND 60 Elevated Plus Maze

Tables 4 and 5 provides means (±SEMs), and ANOVA main effects for three elevated plus maze measures (%Open Arm entries, %Open Arm observations and Closed entries). Significant interactions are shown in Figures 5 and 6.

All dose groups had an equal percentage of open arm entries and observations, as well as an equal number of closed arm entries. However, although there were no main effects of drug dose on percentage of observations in the open arm of the apparatus, effects of caging

depended on BZP dose (Dose x Cage Interaction: F (2,108) = 3.4, p = .037; see Figure 5). Saline-treated subjects and those in the 20mg/kg BZP group significantly increased time in the open arm of the elevated plus maze when housed in enriched environments. The 10mg/kg BZP dose group didn't show a difference between caging conditions. Time spent in the open arm was similar across drug groups and therefore no interactions was found between dose groups.

Table 4: 60 days mean (S.E.M) percent of open arm entries, percent of open arm observations and closed arm entries for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests.

BZP Dose 100 Days	Saline	10mg/kg	20mg/kg	*F* (2, 108)	*P*
% Open Arm Entries	50.71 (1.07)	50.5(0.94)	50.79(1.41)	0.02	.984
% Open Arm Observations	53.23(1.26)	52.24(1.15)	52.73 (1.50)	0.18	.839
Closed Arm Entries	9.98(0.41)	9.52 (0.32)	9.85 (0.42)	0.43	.652

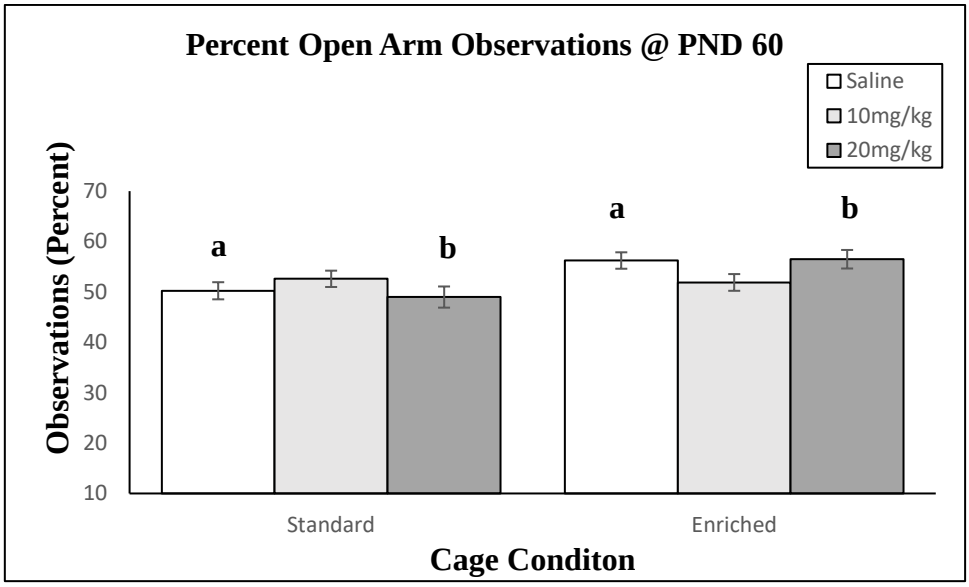

Figure 5: Dose x Cage interaction in the percentage of open arm observations for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

Within cage and sex conditions subjects housed in enriched environments increased time spent in the open arm than those in standard caging (Cage Main Effect for % Open Observations; Table 5), but showed no difference in percentage of open arm entries or number of closed arm entries. Additionally, female subjects were observed more often in the open arm

(Sex Main Effect for % Open Observations; Table 5), and the closed arm than males (Sex Main Effect for Closed Arm Entries; Table 5). Furthermore, closed arm entries varied by sex and housing (Sex x Cage Condition Interaction: F (1, 108) = 5.57, p = .020; Figure 6). Enriched males made significantly fewer closed arm entries than females and members of each sex entered the closed arms equally. There were no significant differences between cage conditions.

Table 5: 60 days mean (S.E.M) percent of open entries, percent of open observations and closed entries for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests.

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
% Open Arm Entries	49.52 (1.14)	51.82 (0.66)	2.96	.088
% Open Arm Observation	50.60 (1.05)	54.87 (1.01)	9.66	.002
Closed Arm Entries	9.77 (0.27)	9.80 (0.35)	0.01	.935

Sex 60 Days	Male	Female	*F* (1, 108)	*P*
% Open Arm Entries	50.13 (1.09)	51.2 (0.76)	0.64	.427
% Open Arm Observation	50.08 (1.10)	55.39 (9.10)	1.50	.000
Closed Arm Entries	8.87 (0.31)	10.7 (0.27)	20.03	.000

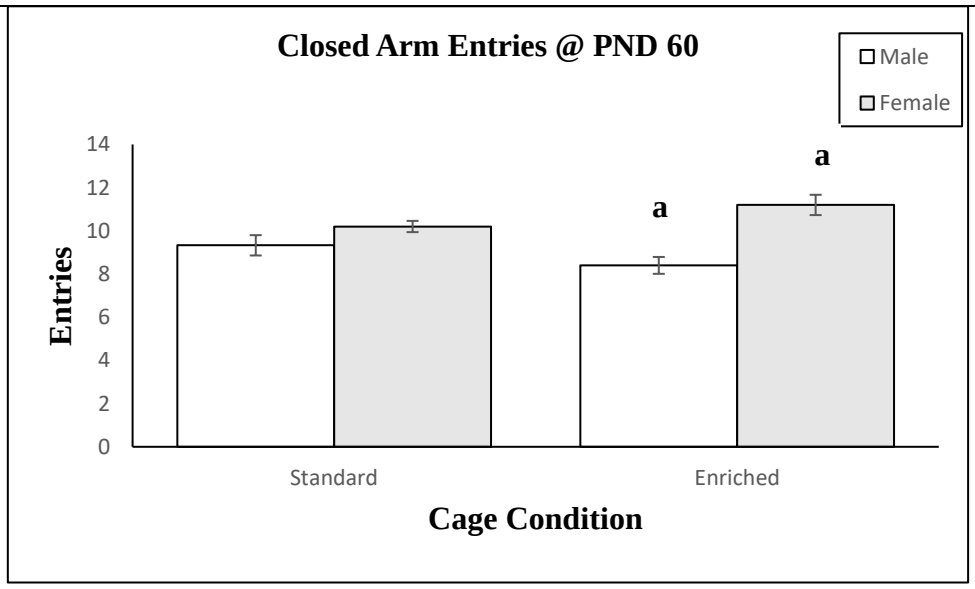

Figure 6: Sex x Cage type interactions in the number of closed arm entries for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

4.4 PND 100 Elevated Plus Maze

Table 6 and 7 provide means (±SEMs), and ANOVA main effects at PND 100 days for three elevated plus maze measures (% Open Arm entries, % Open Arm observations and Closed entries) and a significant interaction is demonstrated in Figure 7.

After 100 days, there were no main effects of dose (see Table 4). However, observations in the open arm of the apparatus at differing doses of BZP depended on housing condition (Dose x Cage Interaction: F (2,108) =5.13 p = .007; see Figure 6) i.e., response was decreased by both doses for standard-caged subjects, but not for those from enriched cages. Contrary to results at PND 60, saline-treated subjects in enriched housing spent decreased time in the open arm than standard caged subjects. Additionally, 20mg/kg BZP subjects were no longer affected by the cage conditions.

Table 6: 100 days mean (S.E.M) percent of open arm entries, percent of open arm observations and closed arm entries for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40), enriched (n= 60) and standard (n=60) and results for *F* tests.

BZP Dose 100 Days	Saline	10mg/kg	20mg/kg	*F* (2, 108)	*P*
% Open Arm Entries	51.17 (2.02)	48.08(1.18)	47.4(1.93)	1.36	.262
% Open Arm Observations	50.39(2.52)	46.03(1.81)	46.53 (2.31)	1.32	.272
Closed Arm Entries	7.32(0.45)	7.2 (0.37)	6.9 (0.45)	0.33	.719

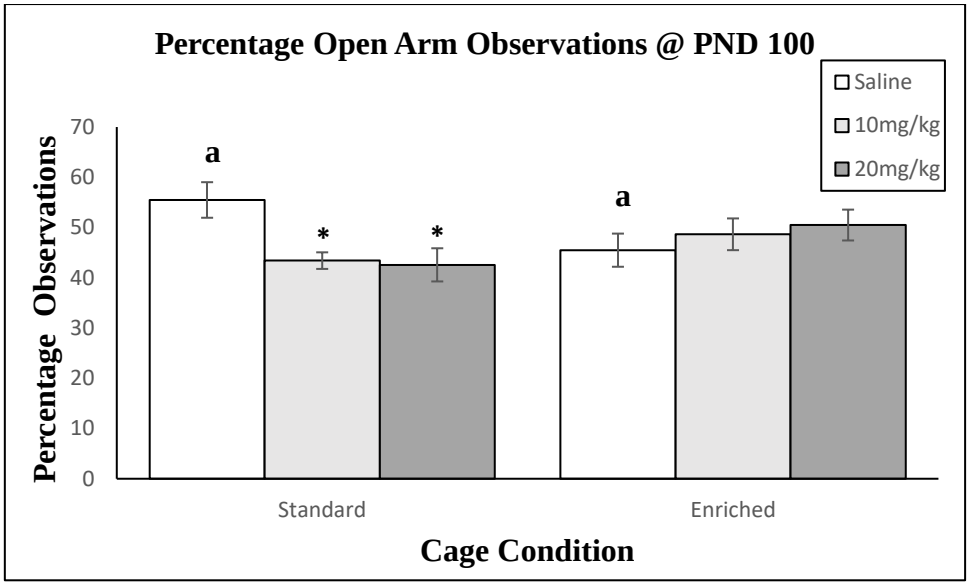

Figure 7: Dose x Cage interaction in the percentage of open arm observations for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from control and Abc= groups with superscripts in common are significantly different.

Cage condition no longer affected open arm entries, time spent in the open arm or closed arm entries (see Table 7). Females continued to be observed more often in the open arm (Sex Main Effect for % Open Observations; Table 7), and in the closed arm than males (Sex Main Effect for Closed Arm Entries; Table 7). The sex x cage interaction was no longer significant.

Table 7: 100 days mean (S.E.M) percentage of open arm entries, percentage of open arm observations and closed entries of both arms for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests.

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
% Open Arm Entries	47.17 (1.67)	50.6 (1.13)	2.98	.087
% Open Arm Observation	47.15 (1.84)	48.21 (1.82)	0.19	.667
Closed Arm Entries	7.08 (0.34)	7.20 (0.36)	0.07	.791
Sex 100 Days	Male	Female	*F* (1, 108)	*P*
% Open Arm Entries	47.6 (1.76)	50.17 (1.00)	1.67	.199
% Open Arm Observation	44.07 (2.10)	51.29 (1.37)	8.65	.004
Closed Arm Entries	5.83 (0.30)	8.45 (0.31)	35.58	.000

4.5 PND 60 Light Dark Box

Table 8 and 9 show means (±SEMs), and results of ANOVAs for each main effect for the four light dark box measures (Emergence Latency, Light entries, Light Dark transitions and Light observations). Results for significant two and three-way interactions are shown in Figures 8 through 12.

There were no significant main effects of BZP dose on any light dark box measure (see Table 6). However, time taken to emerge into the light side of the apparatus at differing doses of BZP depended on housing (Dose x Cage Interaction: F (2,108) = 4.52, p = .013; see Figure 8). This can be accounted for by the response being increased by both doses of BZP for enriched caged animals. Additionally, saline-treated subjects in enriched housing took significantly decreased time to emerge from the dark side of the apparatus than those in standard caging. There were no other significant differences.

Table 8: 60 days mean (S.E.M) emergence latency, entries of light, transitions and observations in light for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests.

BZP Dose	Saline	10mg/kg	20mg/kg	*F* (2,108)	*P*
Emergence Latency	8.57(1.46)	9.93(0.99)	8.75(1.32)	0.37	.691
Light Entries	9.9(0.28)	9.2(0.34)	9.6(0.36)	1.29	.281
Transitions	19.42(0.55)	18.1(0.67)	18.8(0.72)	1.21	.303
Light Observations	46.9(1.51)	44.12(1.48)	44.12(1.41)	1.35	.264

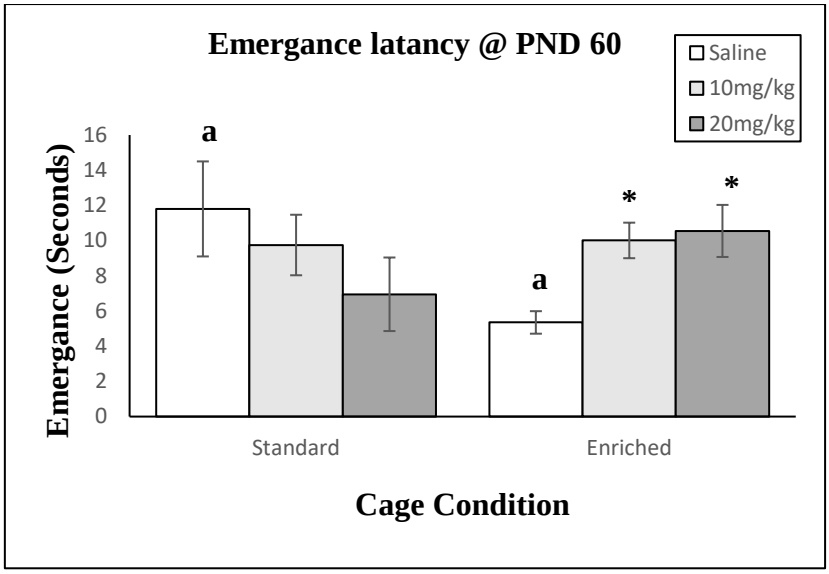

Figure 8: Dose x cage interaction in emergence latency for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

Entries of subjects into the light side of the apparatus at varying doses of BZP was also dependant on housing (Dose x Cage Interaction: F (2,108) = 5.14, p = .007; see Figure 9). Within the enriched cage condition both BZP groups entered the light fewer times than saline treated animals. There were no similar effects seen within the standard caging groups. Saline subjects in enriched housing entered the light side of the apparatus more often than those in standard housing. This was reversed for 20mg/kg BZP subjects and there was no relationship between housing for the 10mg/kg BZP group.

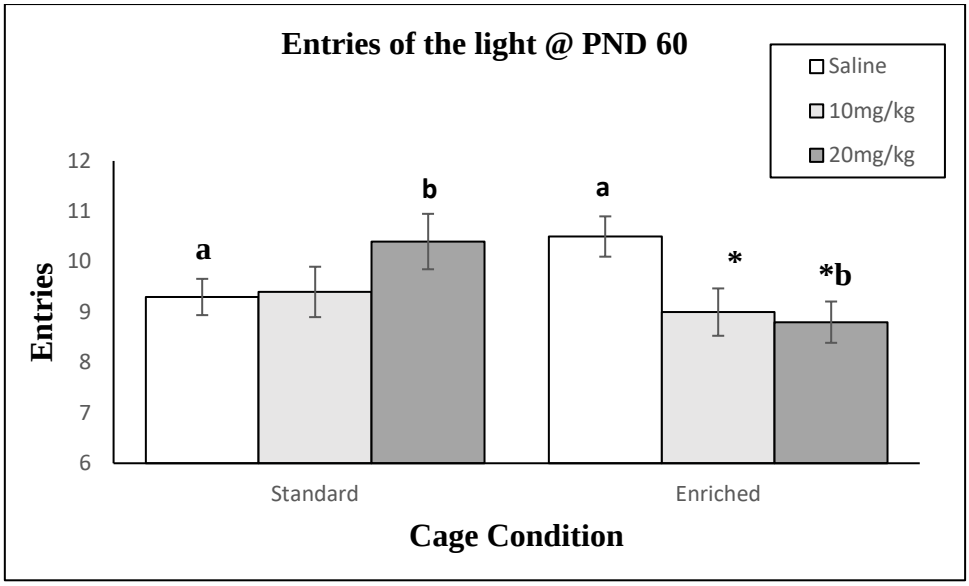

Figure 9: Dose x Cage interaction in entries of the light for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

Transitions of the subjects between the light and dark side of the apparatus at varying doses of BZP was dependant on housing (Dose x Cage Interaction: F (2,108) = 5.55, p = .005; see Figure 9). Enriched saline subjects transitioned between both sides of the apparatus significantly more often than 20mg/kg BZP subjects. No similar differences were found within standard cages or between enriched controls and 10mg/kg BZP animals. Like the previous measure saline treated subjects had higher transitions when living in enriched conditions, this was the opposite for 20mg/kg BZP subjects. There was no difference between cages for subjects exposed to 10mg/kg BZP.

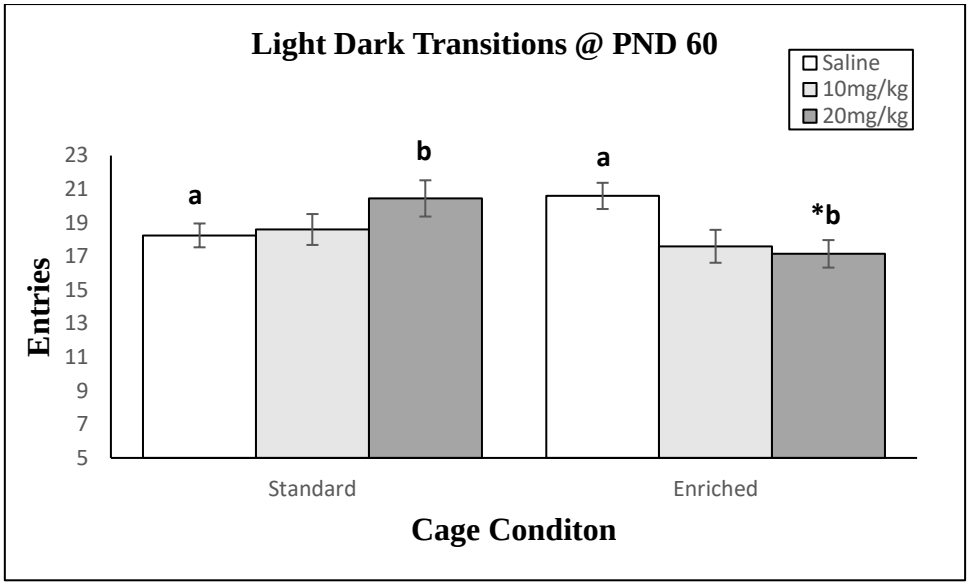

Figure 10: Dose x Cage interaction in transitions between the light and dark for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20) standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

For percent of time spent in the light, there was a significant three-way interaction (F (2, 108) = 4.95, p = .008; see Figure 11). This can be mainly accounted for by the response being decreased by the higher dose of BZP for females from enriched cages. Enriched 20mg/kg BZP females were also observed significantly less often in the light side of the apparatus than either the saline-treated or the 10mg/kg BZP group. This effect of drug dose was not apparent for females in standard cages, or for males in either type of cage. Additionally, enriched housing led to increased observations in the light side of the apparatus for females treated with saline and 10mg/kg BZP.

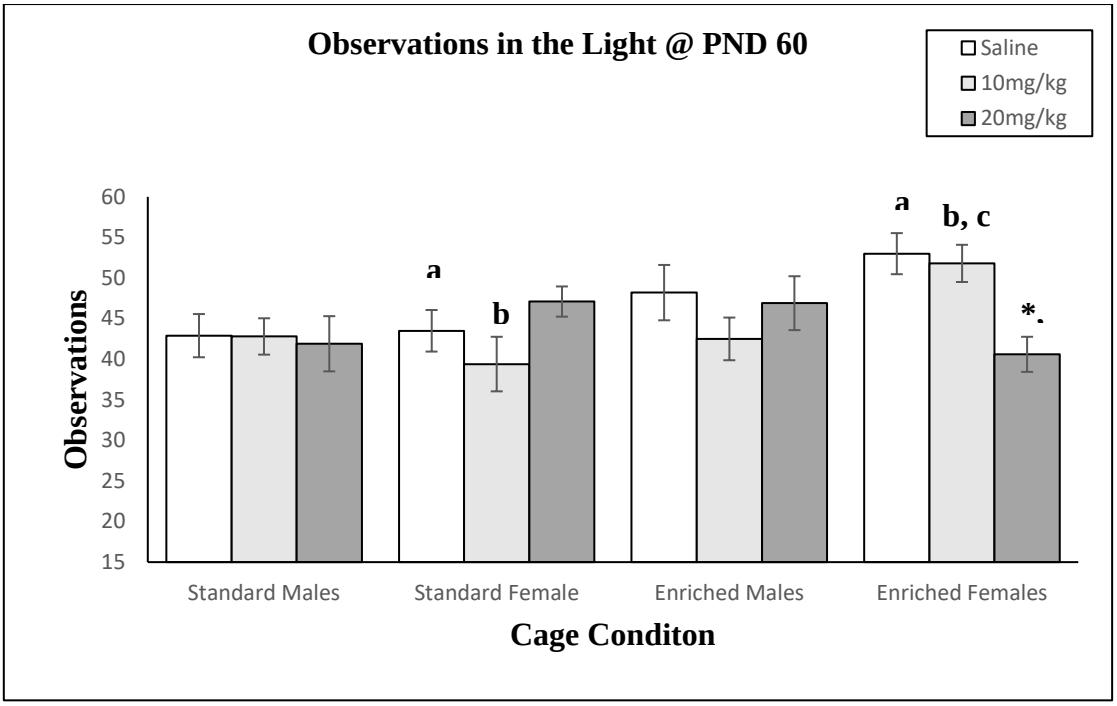

Figure 11: Dose x Sex x Cage interaction in emergence latency for control (n= 20 males half standard, half enriched and 20 females half standard and half enriched), 10mg/kg (distributed the same as control) and 20mg/kg (distributed the same way as the other two doses). Standard error means are represented by error bars. * = significantly different from saline and Abc = groups with superscripts in common are significantly different.

The time spent in the light side of the apparatus was significantly higher for enriched than standard-caged animals (Cage Condition Main Effect for Light Observations; Table 9). Additionally, females entered the light more often (Sex Main Effect for Light Entries; Table 9) and had significantly a higher amount of transitions than males (Sex Main Effect for Transitions; Table 9). There were no other significant main effects found for responses recorded in this apparatus. Lastly, sex differences in emergence latency depended on housing (Sex x Cage Condition Interaction: F (1, 108) = 4.64, p = .033; Figure 12). Females took less time to emerge than males only when housed in standard caging. Standard-caged females also emerged less often than enriched females – this did not characterise males.

Table :9 60 days mean (S.E.M) for emergence latency, entries of light, transitions and observations in light for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
Emergence Latency	9.50 (1.28)	8.67 (0.71)	0.36	.551
Entries of Light	9.70 (0.28)	9.43 (0.26)	0.56	.458
Transitions	19.10(0.54)	18.45 (0.53)	0.87	.353
Observations in Light	42.93 (1.10)	47.17 (1.23)	7.07	.009
Sex 60 Days	Male	Female	*F* (1, 108)	*P*
Emergence Latency	9.18 (0.96)	8.98 (1.11)	0.02	.886
Entries of Light	9.02 (0.25)	10.12 (0.27)	9.46	.003
Transitions	17.63 (0.48)	19.92 (0.54)	10.75	.001
Observations in Light	44.20 (1.21)	45.90 (1.19)	1.14	.288

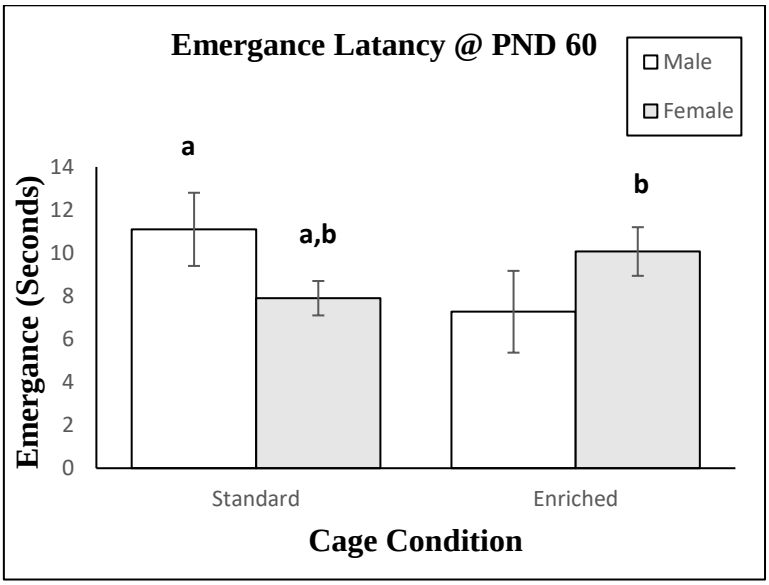

Figure 12: Sex x Cage interactions in emergance latency for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

4.6 PND 100 Light Dark Box

Tables 10 and 11 provide means (±SEMs), and ANOVA main effects at PND 100 for the four light dark box measures (Emergence Latency, Light entries, Light Dark transitions and Light Observations). Results for significant two way interactions are shown in Figures 13 through 17.

There were no dose main effects present after PND 100 (see Table 10) and no interactions involving BZP dose for emergence time, or for observations in the light. However, entries into the light at varying doses of BZP were still affected by housing (Dose x Cage Interaction: F (2,108) = 3.19, p = .045; see Figure 13). Of the results obtained at PND 60, the only effect that remained the same as previously was that 20mg/kg BZP subjects still entered the light less often than saline-treated animals. A new effect that appeared after 100 days was that enriched 10mg/kg subjects exhibited more entries into the light side than standard-housed animals.

Table 10: 100 days mean (S.E.M) emergence latency, entries of light, transitions and observations in light for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests.

BZP Dose	Saline	10mg/kg	20mg/kg	*F* (2,108)	*P*
Emergence Latency	12.65(3.23)	13.82(2.29)	17.25(4.75)	0.45	.641
Entries of Light	6.90(0.26)	6.07 (0.29)	6.40(0.37)	2.04	.135
Transitions	13.38(0.50)	11.77(0.67)	12.35(0.74)	1.95	.148
Observations in Light	47.28(1.85)	45.10(2.46)	44.72(2.14)	1.35	.264

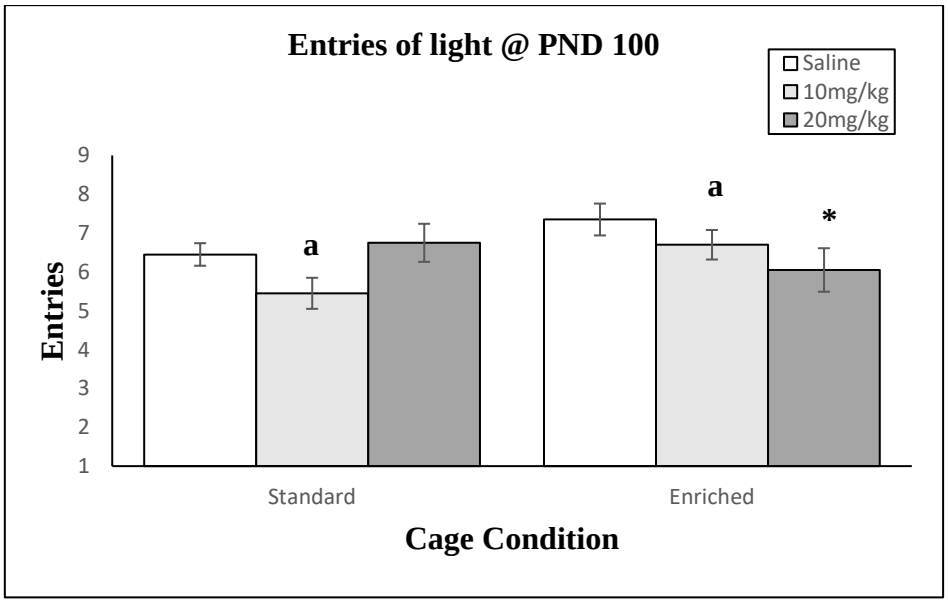

Figure 13: Dose x Cage interaction in entries of the light for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

This measure was also affected at different doses of BZP by sex (Dose x Sex Interaction: F (2,108) = 3.69, p = .028; see Figure 14) at PND 100. This could be accounted for by the response being decreased by both doses of BZP for males, but not for females. Furthermore, females treated with 20mg/kg of BZP entered the light more often than their male counterparts

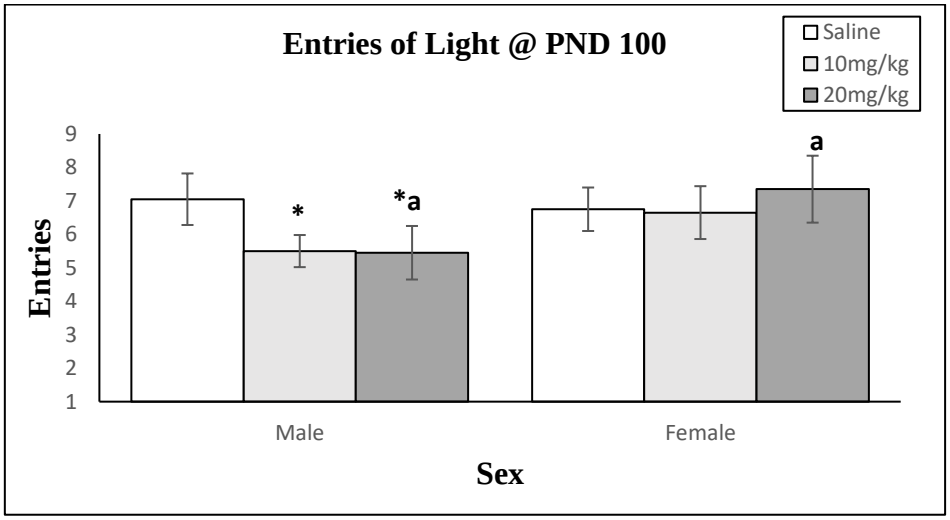

Figure 14: Dose x Sex interaction in entries of the light for the control (n= 20 male and 20 female), 10mg/kg (n= 20 male and 20 female) and 20mg/kg (n=20 male and 20 female). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

Light dark transitions continued to be affected by housing, but in a different way than before (Dose x Cage Interaction: F (2,108) = 3.12, p = .048; see Figure 15). After 100 days, none of the effects seen at PND 60 were present. However, a new effect was that 10mg/kg BZP enriched animals made exhibited more light dark transitions than their standard-caged counterparts.

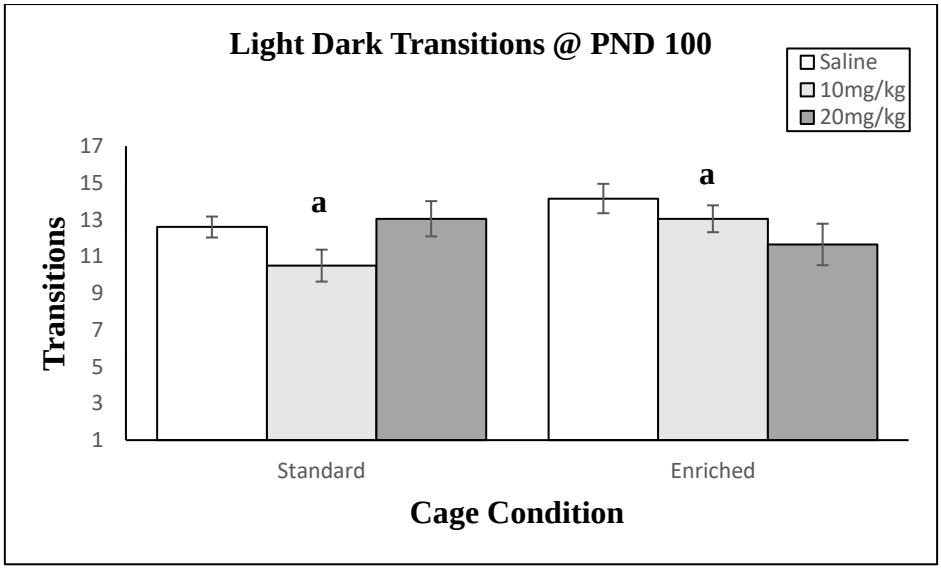

Figure 15: Dose x Cage interaction in light dark transitions for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. Abc= groups with superscripts in common are significantly different.

Unlike the earlier testing, light dark transitions at varying doses were also affected by sex (Dose x Sex Interaction: F (2,108) = 3.69, p = .028; see Figure 16). Both BZP male groups transitioned between the light and dark sides of the apparatus less often than saline-treated subjects. Post hoc testing also showed that 20mg/kg BZP females exhibited more light dark transitions than males.

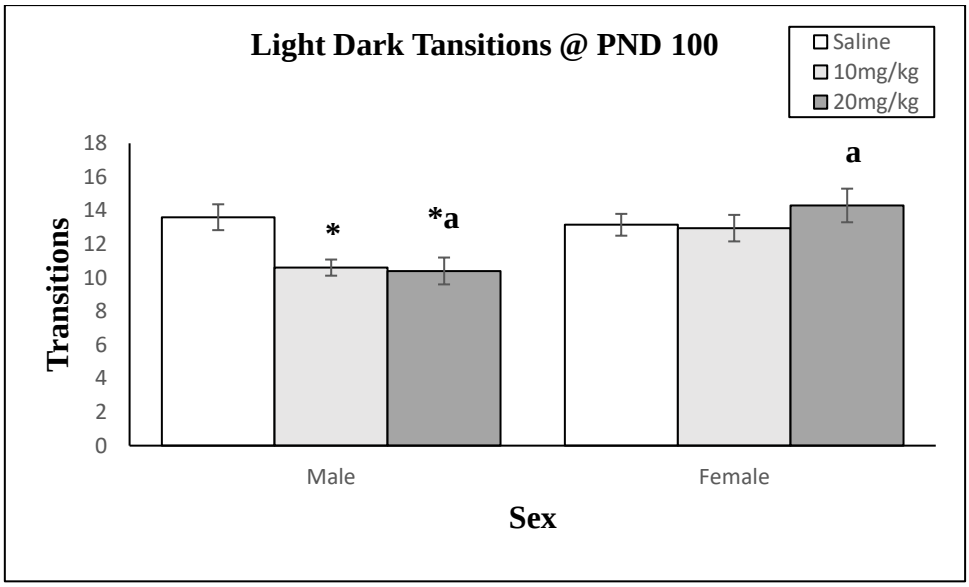

Figure 16: Dose x Sex interaction in light dark transitions for the control (n= 20 male and 20 female), 10mg/kg (n= 20 male and 20 female) and 20mg/kg (n=20 male and 20 female). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

For cage and sex conditions there was no longer a significant cage effect on light observations. Females continued to exhibit more entries of the light (Sex Main Effect for Light entries; Table 11) and transitions than males (Sex Main Effect for Transitions; Table 11). A new effect revealed after PND 100 was that females emerged into the light sooner than males. Lastly, as revealed in a significant sex x cage interaction (F (1, 108) = 4.27, p = .041), males from enriched cages increased time spent in the light side than those from standard cages (see Figure 17). This did not occur for females.

Table :11 100 days mean (S.E.M) for emergence latency, entries of light, transitions and observations in light for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
Emergence Latency	16.22 (3.52)	12.93 (2.11)	0.63	.429
Entries of Light	6.22 (0.24)	6.7 (0.27)	2.07	.153
Transitions	12.05 (0.49)	12.95 (0.53)	1.8	.183
Observations in Light	44.83 (1.8)	46.57 (1.72)	0.48	.490
Sex 100 Days	Male	Female	*F* (1, 108)	*P*
Emergence Latency	19.37 (3.87)	9.78 (1.09)	5.38	.022
Entries of Light	6.00(0.25)	6.92 (0.25)	7.44	.007
Transitions	11.53 (0.49)	13.47 (0.50)	8.30	.004
Observations in Light	45.88 (2.12)	45.52 (1.32)	0.02	.884

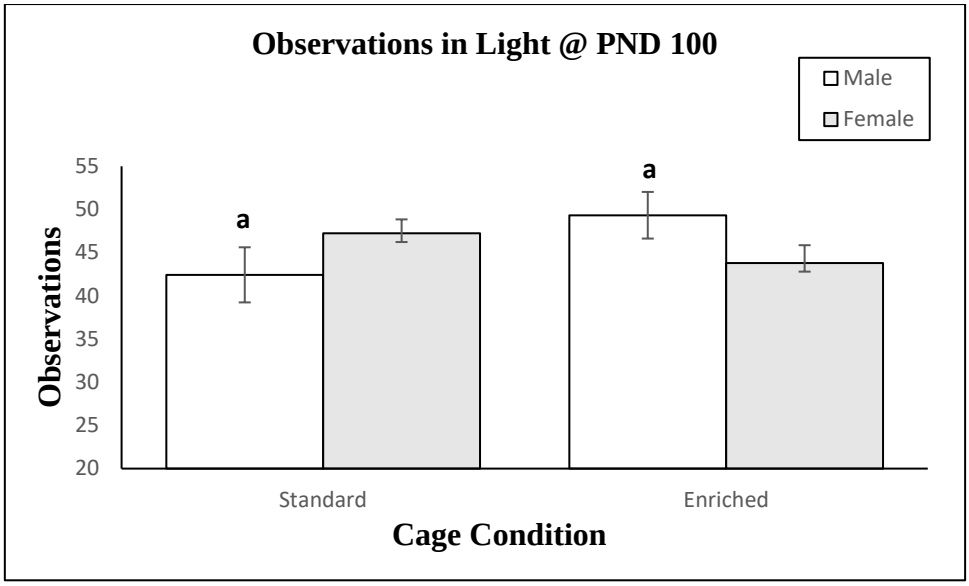

Figure 17: Sex x Cage interaction in observations in the light for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

4.7 PND 60 Open Field

Tables 12 and 13 show means (±SEMs), and results of ANOVAs for each main effect for the eight open field measures (Ambulation, Centre occupancy, Corner occupancy, Walking, Rearing, Grooming, Immobility and Faecal boluses). Results for significant dose main effects and significant two way interactions are shown in Figures 18 through 25.

According to ANOVA results, there was a significant BZP main effect for grooming observations and faecal boluses eliminated in the apparatus (see Table 12). Post hoc Scheffé tests showed that 10mg/kg BZP subjects groomed themselves less often than rats from both the saline and the higher BZP dose groups (see Figure 18). Additionally, rats treated with both BZP doses eliminated fewer faecal boluses than saline treated subjects (see Figure 19).

Table 12: 60 days mean (S.E.M) ambulation, centre occupancy, corner occupancy, walking, rearing, grooming, immobility and faecal boluses for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests.

BZP Dose	Saline	10mg/kg	20mg/kg	*F* (2,108)	*P*
Ambulation	63.20(1.58)	60.25(2.04)	63.95(1.79)	1.52	.224
Centre Occupancy	9.65(0.81)	9.32 (1.00)	10.07(1.3)	0.13	.879
Corner Occupancy	42.58(1.64)	44.05(1.93)	42.12(1.33)	0.37	.691
Walking	34.50(1.62)	34.95(1.62)	36.67(1.24)	0.70	.496
Rearing	42.70(1.35)	36.55(2.47)	40.85(1.56)	0.94	.395
Grooming	5.47(0.81)	2.8(0.41)	4.62(0.87)	3.73	.027
Immobility	16.92(1.86)	22.00(1.15)	17.12(1.63)	2.79	.066
Faecal Boluses	1.65(0.36)	0.85(0.26)	0.70(0.23)	3.40	.037

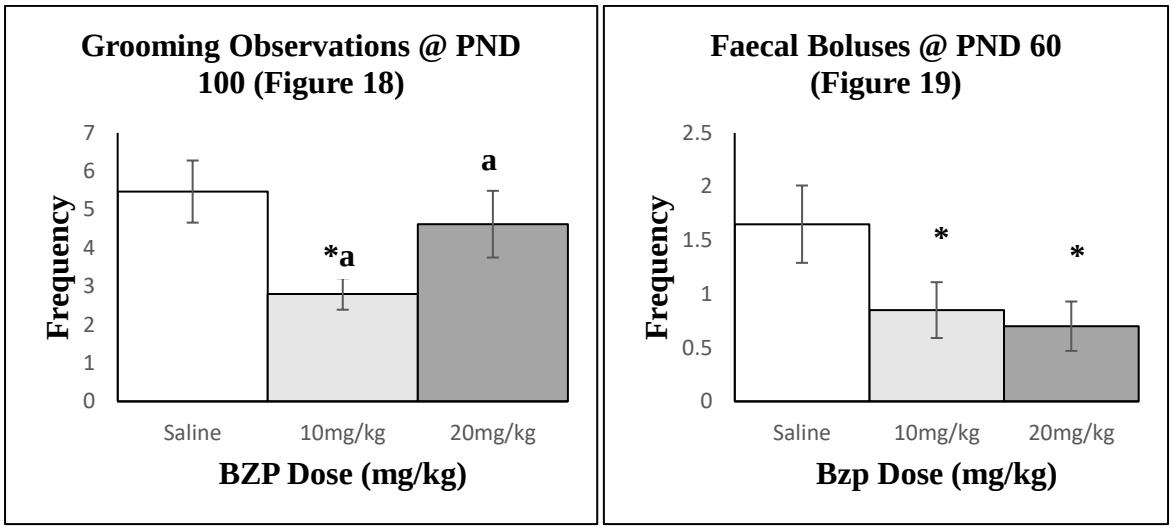

Figure 18 and 19: Main effects of dose in grooming observations (18) and faecal boluses (19) for control (n= 40 subjects), 10mg/kg (n=40 subjects) and 20mg/kg (n=40). Standard error means are represented by error bars and * = significantly different from saline and Abc = groups with superscripts in common are significantly different.

As shown by a significant dose x cage interaction (F (2,108) = 5.9, p = .003; see Figure 20), time spent walking in the apparatus with different doses of BZP depended on the cage condition i.e., both doses increased the response for rats housed in enriched cages, but not for those in standard cages. Furthermore, enrichment significantly decreased walking for saline treated animals.

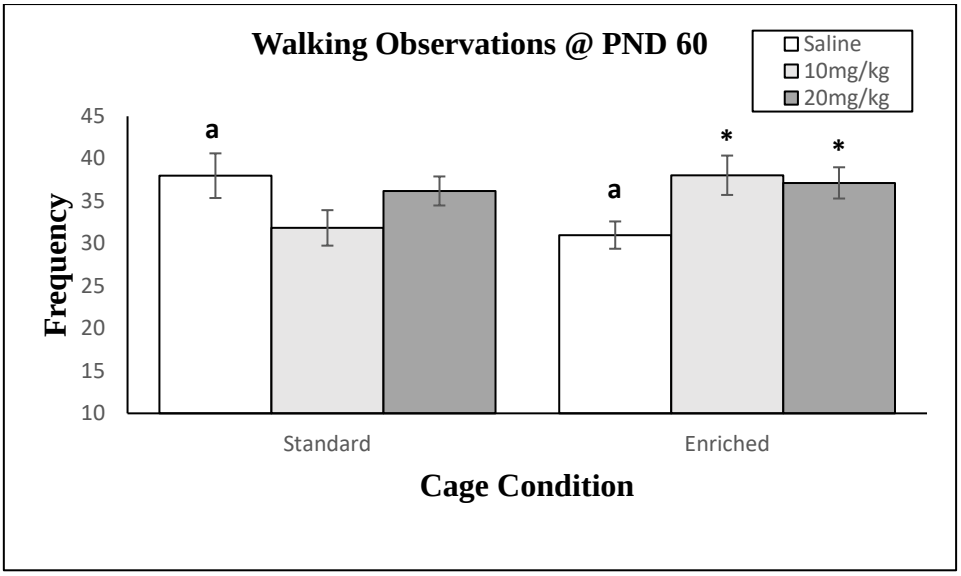

Figure 20: Dose x Cage interaction in walking observations for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

The time the animals spent walking in the apparatus was also dependent on sex (Dose x Sex Interaction: F (2,108) = 4.16, p = .018; see Figure 21). Males in the higher BZP group were observed walking more often than both the saline and lower BZP dose group. Similar differences were not found for female subjects. However, females treated with either saline or 10mg/kg BZP females walked more often than males.

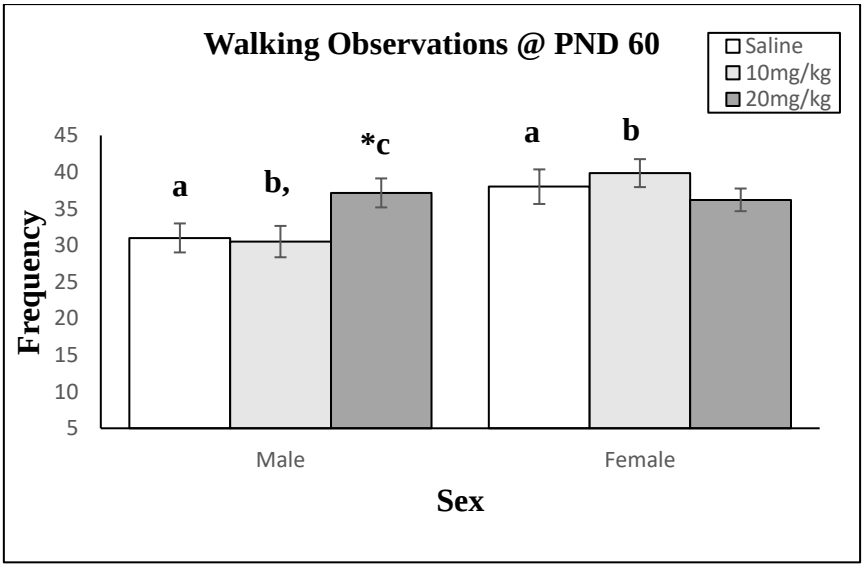

Figure 21: Dose x Sex interaction in walking observations for the control (n= 20 male and 20 female), 10mg/kg (n= 20 male and 20 female) and 20mg/kg (n=20 male and 20 female). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

The effects of BZP on rearing activity were also dependent on housing (Dose x Cage Interaction: F (2,108) = 6.95, p = .001; see Figure 22). Standard caged subjects treated with 10mg/kg BZP exhibited more rearing than saline-treated subjects. This was not found for the 20mg/kg BZP group. 10mg/kg BZP subjects in standard cages also exhibited more rearing than their enriched counterparts. This relationship was the opposite for saline-treated animals. In the 20mg/kg BZP group both cage conditions spent an equal amount of time rearing.

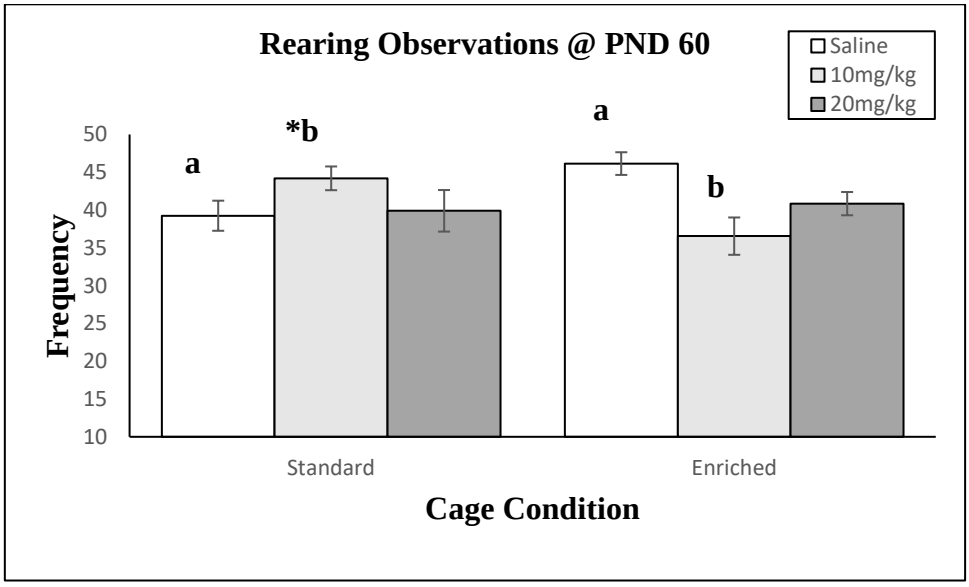

Figure 22: Dose x Cage interaction in rearing observations for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. Abc= groups with superscripts in common are significantly different.

The effects of BZP on immobility was dependant on sex (Dose x Sex Interaction: F (2,108) = 3.63, p = .029; see Figure 23). 10mg/kg males exhibited more immobility than both saline and 20mg/kg treated animals. Additionally, these males were immobile more often than their female counterparts. Females did not show any within-group differences, and no other significant sex differences across sexes were observed.

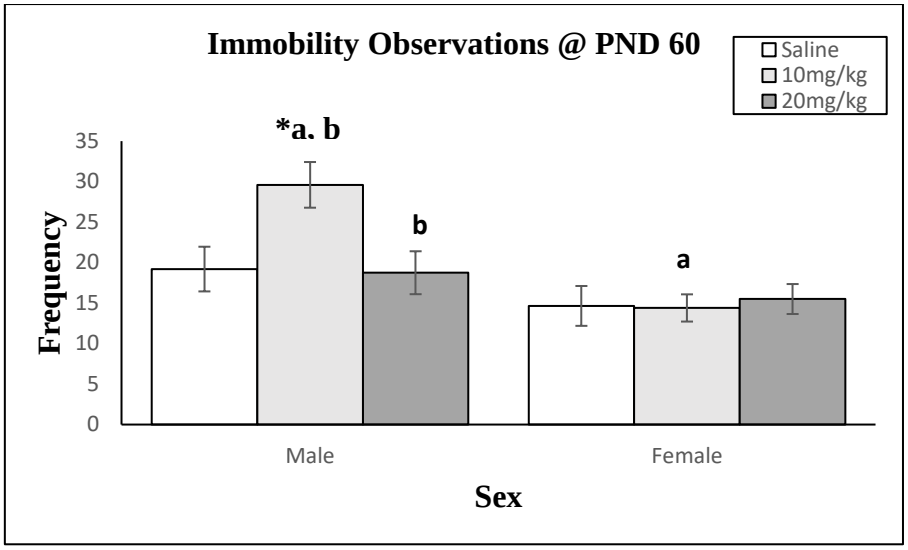

Figure 23: Dose x Sex interaction in immobility observations for the control (n= 20 male and 20 female), 10mg/kg (n= 20 male and 20 female) and 20mg/kg (n=20 male and 20 female). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

Lastly there were no significant main effects of cage on any measure (see Table 13). However, there were significant sex differences for four of the measures i.e., males were in the centre of the apparatus and were immobile more often than females (see Table 13). Females engaged more often in locomotor behaviour than males, as reflected in the sex differences for ambulation and walking. However, as indicated by a significant sex x cage interaction for ambulation (F (1, 108) = 4.37, p = .039), enrichment significantly increased time spent moving around the open field for females (see Figure 24). A significant sex x cage interaction for faecal boluses showed that males defecated significantly more often than females only when housed in enriched cages (F (1, 108) = 9.91, p = .005; Figure 25). The interaction also revealed that females (but not males) defecated more often when housed in standard caging.

Table :13 60 days mean (S.E.M) for ambulation, centre occupancy, corner occupancy, walking, rearing, grooming, immobility and faecal boluses for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
Ambulation	61.30 (1.36)	63.63 (1.59)	1.62	.206
Centre Occupancy	10.82 (1.06)	8.55 (0.56)	3.52	.063
Corner Occupancy	41.75 (1.32)	44.08 (1.36)	1.49	.225
Walking	35.35(1.28)	35.40 (1.18)	1.00	.975
Rearing	41.12 (1.26)	41.18 (1.19)	1.73	.967
Grooming	5.08 (0.72)	3.52(0.44)	3.68	.058
Immobility	17.92 (1.34)	19.45 (1.15)	0.60	.442
Faecal Boluses	1.15 (0.26)	0.98 (0.22)	0.27	.604
Sex 60 Days	Male	Female	*F* (1, 108)	*P*
Ambulation	57.12 (1.40)	67.82 (1.22)	34.06	.000
Centre Occupancy	10.98 (1.05)	8.38 (0.56)	4.64	.033
Corner Occupancy	42.62(1.64)	44.05 (1.43)	0.10	.754
Walking	32.73 (1.22)	38.02 (1.13)	11.19	.001
Rearing	39.78 (1.33)	42.52 (1.08)	2.91	.090
Grooming	4.15 (0.49)	4.45 (0.71)	0.13	.714
Immobility	22.52 (1.64)	14.85 (1.15)	14.89	.000
Faecal Boluses	1.37 (0.24)	0.77 (0.24)	3.52	.063

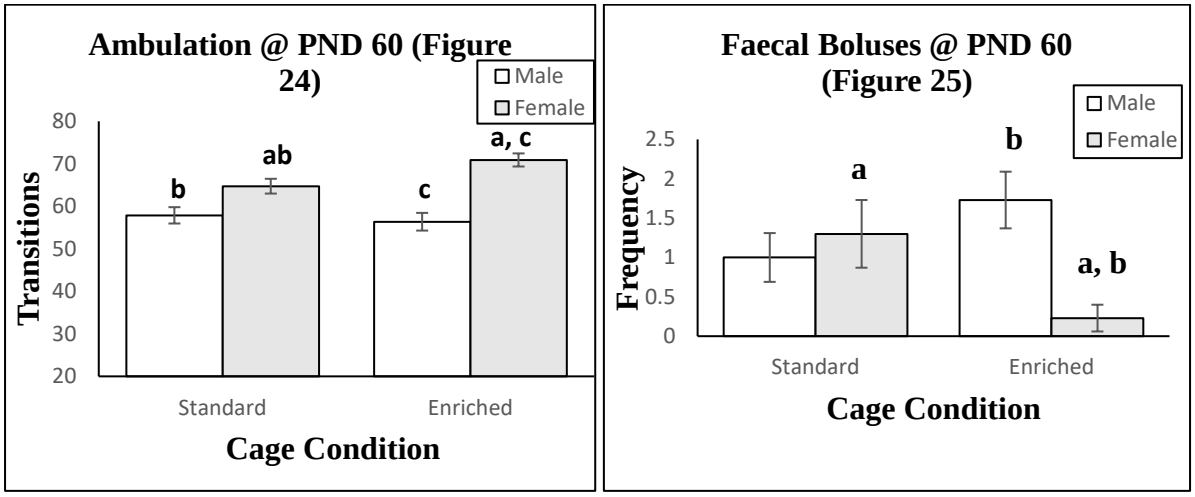

Figure 24 and 25: Sex x Cage interactions for ambulation (24) and faecal boluses (25) for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

4.8 PND 100 Open Field

Tables 14 and 15 show means (±SEMs), and results of ANOVAs for each main effect for the eight open field measures (Ambulation, Centre occupancy, Corner occupancy, Walking,

Rearing, Grooming, Immobility and Faecal boluses). Results for significant two way interactions are shown in Figures 26 through 28.

After 100 days, there were no longer any main effects of BZP dose on the time spent immobile or the number of faecal boluses left in the apparatus (see Table 14). However, cage condition still influenced walking for saline-treated subjects (Dose x Cage Interaction: $F(2,108) = 3.74$, $p = .027$; see Figure 26) namely, enriched saline-treated subjects continued to spend decreased time walking compared to standard.

Table 14: 100 days mean (S.E.M) ambulation, centre occupancy, corner occupancy, walking, rearing, grooming, immobility and faecal boluses for saline (n=40), 10mg/kg (n=40) and 20mg/kg(n=40) and results for *F* tests

BZP Dose	Saline	10mg/kg	20mg/kg	*F* (2,108)	*P*
Ambulation	54.28(2.46)	56.25(1.84)	54.97(2.21)	0.27	.767
Centre Occupancy	8.40(0.67)	9.45 (0.93)	9.07(0.77)	0.47	.627
Corner Occupancy	42.83(1.83)	42.42(1.52)	42.33(1.48)	0.80	.926
Walking	34.97(1.65)	36.00(1.33)	36.20(1.74)	0.23	.795
Rearing	41.00(1.61)	42.90(1.76)	39.45(1.90)	0.96	.387
Grooming	1.23(0.19)	1.42(0.25)	1.92(0.25)	2.69	.072
Immobility	22.67(2.39)	18.8(1.60)	22.35(2.77)	0.99	.374
Faecal Boluses	1.33(0.34)	0.32(0.20)	1.05(0.80)	1.05	.354

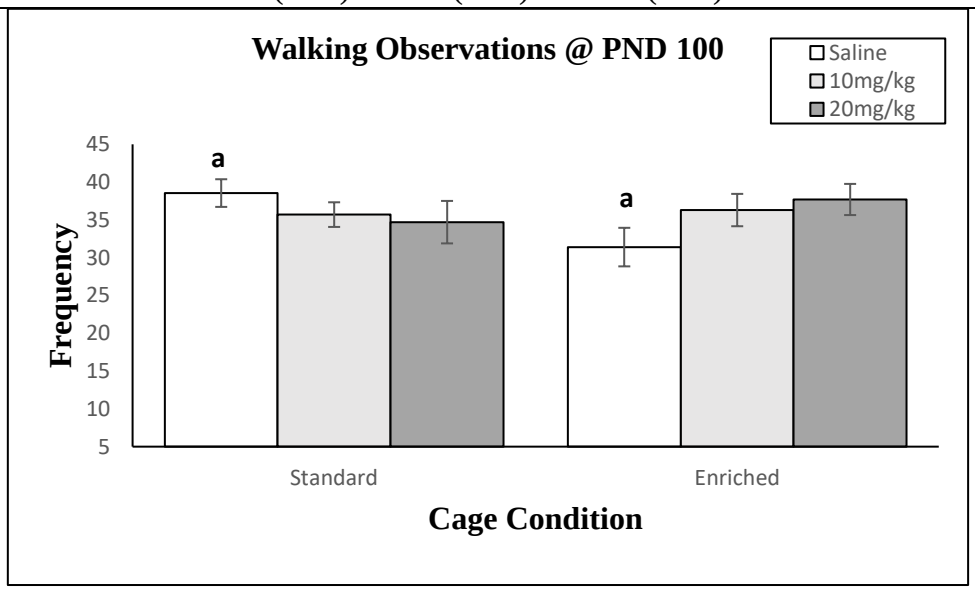

Figure 26: Dose x Cage interaction in walking observations for the control (n= 20 standard and 20 enriched), 10mg/kg (n= 20 standard and 20 enriched) and 20mg/kg (n=20 standard and 20 enriched). Standard error means are represented by error bars. Abc= groups with superscripts in common are significantly different.

Effects of BZP on walking continued to be dependent on sex at PND 100 (Dose x Sex Interaction: F (2,108) = 3.29, p = .041; see Figure 27) namely, males in the 20mg/kg BZP group continued to walk more often than those treated with saline. Additionally, saline and 10mg/kg BZP treated females continued to walk more often than males. There were no new interactions present after PND 100 and there was no longer a significant difference in walking between both BZP groups.

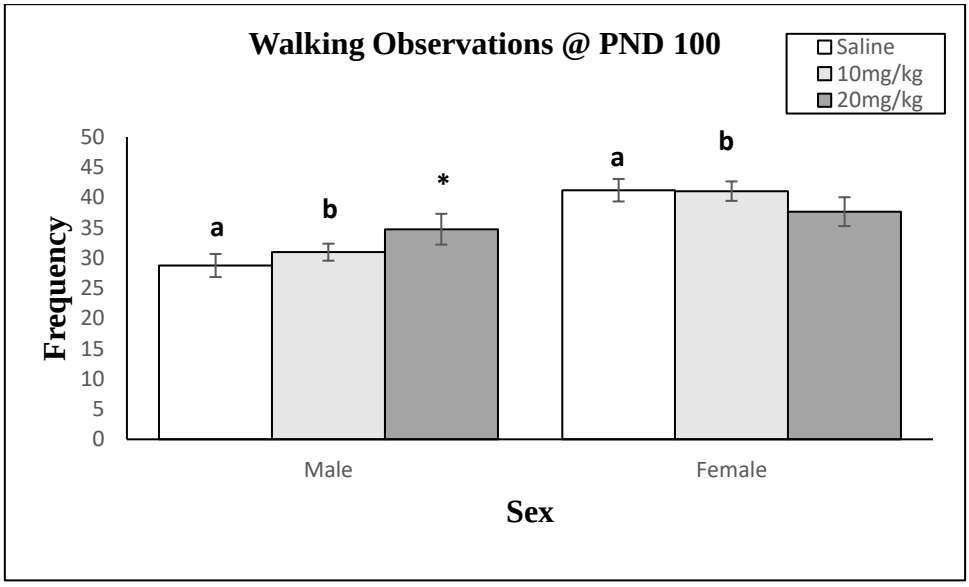

Figure 27: Dose x Sex interaction in walking observations for the control (n= 20 male and 20 female), 10mg/kg (n= 20 male and 20 female) and 20mg/kg (n=20 male and 20 female). Standard error means are represented by error bars. * = significantly different from saline and Abc= groups with superscripts in common are significantly different.

Cage Condition continued to have no main effects on any of the open field measures (see Table 15). However, at this age, there were significant sex differences for all measures except two (see Table 15). Males continued to have higher instances of immobility than females (Sex Main Effect for Immobility; Table 15). A new effect was that males were observed in the corners of the apparatus and eliminated faecal boluses more often than females (see Table 15). Females continued to score higher on ambulation and walk more often than males (Table 15). Contrary to results at PND 60, they also spent more time in the centre of the apparatus than males.

Lastly sex and cage no longer interacted to affect ambulation and faecal boluses. However, they both contributed to walking observations at PND 100 (Dose x Cage Interaction: F (2,108) = 4.21, p = .042; see Figure 28). Males in both types of housing exhibited less time walking than females. There were no significant main effects of housing on any measure.

Table :15 100 days mean (S.E.M) for ambulation, centre occupancy, corner occupancy, walking, rearing, grooming, immobility and faecal boluses for standard cages (n=60), enriched cages (n=60), male (n =60) and female (n=60) and results for *F* tests.

Cage Condition	Standard	Enriched	*F* (1, 108)	*P*
Ambulation	48.95 (1.55)	61.38 (1.62)	0.04	.835
Centre Occupancy	9.48 (0.74)	8.47 (0.53)	1.28	.26
Corner Occupancy	41.98 (1.55)	43.73 (1.33)	0.87	.353
Walking	36.32 (1.24)	35.13 (1.33)	0.56	.457
Rearing	39.88 (1.44)	42.35 (1.43)	1.46	.229
Grooming	1.62 (0.22)	1.43 (0.15)	0.52	.472
Immobility	22.47 (2.01)	20.08 (1.74)	0.92	.341
Faecal Boluses	0.55 (0.17)	1.25 (0.57)	1.44	.232
Sex 100 Days	Male	Female	*F* (1, 108)	*P*
Ambulation	48.95 (1.55)	61.38 (1.62)	30.81	.000
Centre Occupancy	7.78 (0.68)	10.17 (0.57)	7.05	.009
Corner Occupancy	47.35 (1.65)	38.37 (0.90)	22.95	.000
Walking	31.48 (1.14)	39.97 (1.14)	28.71	.000
Rearing	40.25 (1.59)	41.98 (1.27)	0.72	.397
Grooming	1.55 (0.20)	1.50 (0.18)	0.04	.844
Immobility	22.60 (1.96)	16.95 (1.64)	12.06	.001
Faecal Boluses	1.63 (0.57)	0.17 (0.10)	6.34	.013

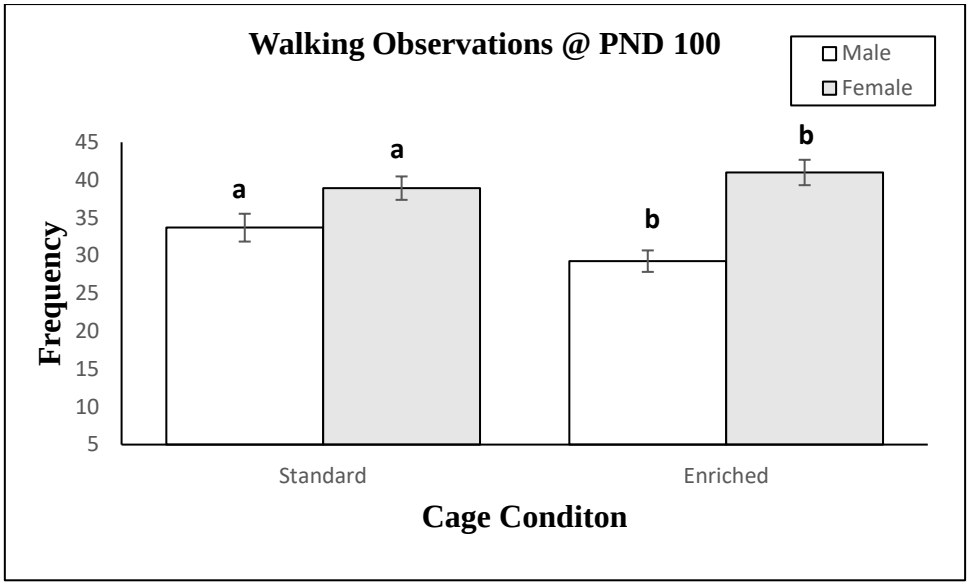

Figure 28: Sex x Cage interactions in walking observations for male (n= 30 standard and 30 enriched) and female (n= 30 standard and 30 enriched). Standard error means are represented by error bars and Abc= groups with superscripts in common are significantly different.

5.0 Discussion

In this study adolescent rats were treated with saline 10mg/kg or 20mg/kg of BZP. At PND 41 animals received intraperitoneal injections over 10 days representing adolescence. They were then tested in four different types of apparatus after PND 60 and PND 100.

5.1 Summary of Main Results

One of the main aims of this study was to discover whether BZP dose influenced spatial memory and if enrichment attenuated any of these effects if they did occur. After PND 60 20mg/kg BZP subjects entered both arms of the Y maze less often than after saline. Although this doesn't suggest memory deficiency it indicates that animals spent most of the time in the stem of the apparatus and were consequently, exhibiting higher anxiety (Hughes, 2003). A similar result was not found for the 10mg/kg group which suggests that dose strength of BZP influences its anxiogenic properties.

Although there are no other main effects of drug dose the effect of BZP can be seen within interactions for entries and observations in the novel Y-maze arm. Standard-caged 10mg/kg BZP subjects entered the novel arm less often than the higher BZP group, suggesting this group may have had poorer memory recall. However if BZP was the cause it would be expected that the 20mg/kg group would have showed a similar result. Therefore, this result could be explained by external factors not covered by this study. Results from observations in the novel arm do suggest that BZP may have influenced memory when sex is considered. Standard saline females were in the novel arm more often than the higher BZP group indicating that memory was impaired for this later group (Chambon, Wegener, Gravius, & Danysz, 2011). Conversely, enriched females treated with both BZP doses spent time in the novel arm more often than saline-treated subjects suggesting BZP improved spatial memory performance. However, because this was within the enriched group this may be explained by enrichment having a greater effect on memory for drug groups. Although this has not been previously shown it is important to consider. Additionally, the lack of results for males suggest that they may not be as susceptible as females to BZP's effects on memory.

Therefore after 60 days there are some suggestions that BZP may negatively affect memory consolidation in spatial memory tasks especially when exposed to higher doses of the drug and depending on sex. However, an increased amount of evidence is needed to support this due to conflicting results for the 10mg/kg group in entries of the novel arm. There are also results which suggest BZP may be anxiogenic which supports the hypothesis of this experiment. Furthermore, enrichment may have differing effects on memory in saline and BZP rats which needs to be considered further. After 100 days, any indications of BZP effecting memory were no longer present suggesting if these effects are an issue they may not continue long term.

Although results regarding BZP effect on memory were mixed, at PND 60 enrichment per se did influence BZP-treated animals, which was demonstrated in novel arm entries and observations. Within the 10mg/kg BZP group enrichment increased the number of novel arm entries and increased novel observations of the 10mg/kg BZP female groups. This indicates that enrichment may have improved memory for these groups. Furthermore, enrichment increased time spent in the novel arm for the female 20mg/kg BZP group suggesting that previously mentioned memory impairment seen in standard caging of BZP on memory were improved with the enriched housing (Patel, 2012). Lastly, 20mg/kg BZP subjects decreased novel arm entries when housed in enriched housing and male subjects in this BZP group also decreased time in the novel arm when housed similarly. This suggest that BZP may have impaired memory for which arm was novel. However, because females showed an increase in time spent in the novel arm due to enrichment, this could be because males engage in less locomotion than females and therefore remained longer in the stem of the maze and decrease time spent investigating the novel area (Meunier & Fishcer, 1985). The strength of this locomotor effect could also be the reason why 20mg/kg BZP subjects were shown to enter the novel arm less often. None of these results were apparent at PND 100 further suggesting that effects of the drug on memory and enrichments increase in memory for females and decrease in memory for males did not last long term

Therefore, enriched housing seems to have effect on memory performance caused by BZP in the Y Maze as predicted. However these results seemed to be reliant on the sex of the animal in some cases. Since not all the effects of enrichment seemed to be on BZP-related memory impairment, enrichment may also have attenuated other effects from standard caged animals such as anxiety. Lastly these effects seemed to diminish after an extended period.

A second major aim of this study was to determine if anxiety effects of BZP seen previously continue to be demonstrated in this experiment and if enrichment attenuates these

effects. At PND 60 no effects of BZP on anxiety effects occurred in any of the elevated plus measures. However, at PND 100, anxiety effects were seen in the number of open-arm observations. As predicted both standard-caged BZP groups spent less time in the open arm of the apparatus indicating higher anxiety. Therefore, anxiety may only be shown behaviourally after an extended period.

Enrichment did show an effect on both dose groups at PND 60, even though dose effects were not seen. As predicted for saline and the higher BZP dose group, enrichment increased time spent in the open arms suggesting that it had attenuated anxiety when housed in standard housing (Hughes, 2003). This shows that environmental enrichment did have an impact on certain groups. Unfortunately, PND 100 enrichment did not attenuate BZP effects seen in standard cages. Therefore, it can't be said that environmental enrichment attenuated BZP effects after 100 days in this model.

The light dark apparatus was also used to assess this aim. At PND 60 there were no main effects of dose, but this does not mean that dose did not have an impact on subjects. Enriched housing was shown to increase anxiety as reflected in effects on emergence latency, light entries, transitions and light observations. This was seen in longer emergence latencies for both BZP groups, fewer entries of the light for both BZP groups and fewer light dark transitions or light observations for the higher BZP group compared to saline-treated animals. At PND 100 emergence latency and light observations no longer showed this adverse effect of BZP. However, entries of the light still showed that 20mg/kg BZP animals entered the light less often thus suggesting higher anxiety was still present for this group. This was further apparent for males for which both BZP groups showed higher anxiety than the saline group on this measure. Likewise, both BZP groups continued to show fewer transitions when only considering male participants. However, results for males may have been due to their

predisposition for decreased locomotor activity alongside BZPs potential to decrease this activity (Herbert & Hughes, 2009; Meunier & Fishcer, 1985).

Therefore, BZP did affect anxiety at both ages supporting the hypothesis. However this was only for rats from enriched cages. The reason these dose differences were only seen for enriched-caged rats could have been due to enrichment enhancing anxiety effects. However it is difficult to suggest a reason for why this occurred. Furthermore, dose effects at PND 100 been revealed only in males is also difficult to explain. It may have been due to males ambulating less often than females (Elliott & Grunberg, 2005). Since both light entries and transitions represent ambulation as well as anxiety, males may have only showed an effect due to their proclivity to show less locomotion coupled with BZP effects on stereotyped behaviour (Herbert & Hughes, 2009; Meunier & Fishcer, 1985). Therefore, effects on anxiety are unlikely to persist into later adulthood unlike locomotor behaviour, which is not expected.

Enrichment affected anxiety with saline by decreasing emergence latency, increasing light entries, increasing transitions and, for females, increasing light observations. However, for BZP it had the opposite effect for entries of the light and light dark transitions and was only significant for the higher BZP group. Although this could indicate that enrichment increased anxiety it could also be explained by enrichment decreasing locomotion. Enrichment decreases activity because of faster acclimation to surroundings. Because of this coupled with BZP-related increases in stereotyped movement, effects on locomotion seem to be the best explanation for this result (Elliott & Grunberg, 2005; Herbert & Hughes, 2009). Enrichment did show an expected reduction of anxiety for the female 10mg/kg group as well as saline-treated individuals. Unfortunately, because BZP-treated rats from standard cages did not show an adverse effect compared to saline, this decrease could have been in base-line anxiety and not BZP-related anxiety. At PND 100, effects of enrichment previously seen were not present for either dose but there was an increase in light entries and transitions for the 10mg/kg group.

Again, this increase can't be attributed solely to BZP-induced anxiety. There is insufficient information as to why this was not seen for the other dose groups.

Therefore, while enrichment did seem to show an effect on anxiety, this may not have been a specific reduction in BZP-related anxiety. Additionally, in some measures of the apparatus enrichment effects may be relevant to changes in locomotor behaviour caused by a combination of BZP and enrichment or sex and enrichment.

The last type of apparatus used to investigate the aims of this study was the open field. There were no definitive anxiety effects attributed to BZP contrary to the hypothesis. For grooming, there were conflicting responses since 10mg/kg BZP produced fewer instances of grooming than either saline or 20mg/kg. This suggests this group was less anxious than the other two groups and it's difficult to explain why this occurred. It may be due to factors beyond this study such as other stimuli, for example decreased noise or other experimenters that were present in the room with the other doses not being present at the time 10mg/kg BZP group was tested within the experimentation room. Contrary to grooming measures within male animals 10mg/kg BZP increased immobility compared to other doses, which indicates increased anxiety. Due to these conflicting results for this group it is beyond the scope of this study to determine why these effects occurred. Furthermore, faecal boluses were reduced for both BZP groups contrary to what would be expected if BZP induced anxiety. This could be explained by BZP causing dehydration which means the body uses the fluid from the intestines making faecal boluses harder to pass (Thomas et al., 2008). Results from walking observations are also not best explained by anxiety. This is because the increased locomotor effects for both enriched BZP groups could be explained by BZP-increased energy, although this does not usually continue long past BZP administration (https://www.drugfoundation.org.nz). This is also supported by results which showed the higher BZP group had increased ambulation compared to saline treated animals and the 10mg/kg BZP group. At PND 100 the only dose results that

stayed the same were those for walking. This again suggested that locomotor effects may have persisted. However, no anxiety effects were seen at this age either

Therefore, these results suggested that BZP did not induce anxiety unlike previous findings and effects on locomotion may have been the only outcomes. Additionally, influences outside of the scope of this experiment affected the 10mg/kg BZP in different ways that need further investigation.

Lastly, saline-treated animals at PND 60 were affected by enrichment in walking and rearing as expected. It decreased walking a locomotion effect which is a common result of enrichment (Elliott & Grunberg, 2005). Furthermore, increases in rearing demonstrated a decrease in anxiety as rearing measures vertical exploration which can be inhibited by high anxiety (Walsh & Cummins, 1976). Rearing only affected the 10mg/kg BZP group by decreasing rearing. Again, this result is difficult to explain and since this is the same BZP group showing conflicting dose results more research is needed to determine why this occurred. Again at PND 100 the only measure to show a significant difference was walking. Enrichment again decreased locomotor effects for saline-treated individuals indicating enrichment still decreased locomotion, none of the other effects continued suggesting enrichment effects for other measures do not last long term. Therefore, within this apparatus enrichment did not show a significant effect on BZP anxiety, which is not what was predicted.

In conclusion, the main aims of this experiment were to see if memory was affected negatively by BZP and if anxiety was also effected by BZP as has been shown previously. BZP did show some effects on memory especially in the higher BZP group. However there were other conflicting results suggesting more research is needed and these effects did not seem to last long term. In terms of anxiety results did not fully support the hypothesis of the experiment. Some effects on anxiety was shown in the elevated plus maze but only for rats from enriched

housing and after animals were 100 days old. Conversely in the light dark box most anxiety effects were seen at PND 60 but only in enriched animals. Furthermore, no significant anxiety effects were seen in the open field, therefore with some support for the hypothesis and some against the hypothesis it is difficult to assign anxiety effects to BZP.

The other aim of the experiment was to see if enrichment attenuated any negative effects that might be present such as reduced memory or increased anxiety. Enrichment did show some memory enhancing effects but not only on measures that had shown a dose effect. This was similar for all the apparatuses suggesting enrichment may not attenuate memory impairment or anxiogenic effects of BZP but it may improve others such as locomotion or base anxiety. Many of these effects also diminished past PND 100 suggesting they did not last long term.

5.2 Secondary Effects

Although not all effects specifically related to the aim of the experiment it is still important to mention them as they have been shown to mediate main effects. As shown in the previous results, enrichment decreased ambulation in transitions of the Y maze but only at PND 60. Furthermore, most enrichment effects were on locomotion (Elliott & Grunberg, 2005). Differences between sexes showed in Y maze results. At both ages females transitioned more often thereby showing higher ambulation which has often been reported (Craft, Clark, Hart, & Pinckney, 2006). Females also showed fewer novel observations indicating more anxiety than males. This could explain why dose effects were mostly seen for females in this measure due to a greater effect of neophobia which can increase novelty-related emotional reactivity (Aitken, 1974).

In the elevated plus maze, cage enrichment increased open arm observations indicating decreased anxiety which supports previously mentioned results for saline and 20mg/kg BZP

groups. For sex, females were observed more often in the open and closed-arm which further suggests that they were more ambulatory than males at both ages. This was emphasised in the 60 day closed arm entries for the enriched group. It also supports previously mentioned results suggesting that enrichment decreases anxiety and can increase differences between dose groups.

For the light dark box the only main effect of cage type was that enriched animals were in the light more often which suggests enrichment decreased anxiety and thus supports previous mentioned results for rats treated with saline and 10mg/kg BZP. This effect was not apparent at PND 100 which also suggests these effects did not last into later adulthood. In this apparatus females once again showed higher instances of locomotion as reflected in entries of the light and total entries at both time periods. This was especially significant for rats in the 20mg/kg group. Females further showed lower anxiety than males which was shown in light observations at PND 60 and a faster emergence latency at PND 100.

Cage and sex also significantly interacted with each other suggesting that enrichment affected responses based on gender. Although emergence latency did not show any main effects of sex or cage, an interaction effect showed that enrichment increased emergence latency for females at PND 60 and in standard cages females emerged faster than males. This indicates that enrichment may have increased anxiety. However it may be better explained by the locomotion-reducing effects of enrichment (Elliott & Grunberg, 2005). Additionally, for PND 100 males' enrichment increased observations in the novel open arm suggesting for this group that anxiety was reduced. However this was not seen in other results.

Lastly, enrichment did not produce any main effects for the open field which supports the small enrichment effects seen in this apparatus. However, for sex as previously mentioned females displayed higher levels of locomotion than males in ambulation, walking especially

for saline treated animals and 10mg/kg BZP (this was seen in both cage conditions) and fewer instances of immobility especially for 10mg BZP. Furthermore, males occupied the centre more often than females suggesting lower anxiety compared with females which is contrary to the light dark box results. Conversely at PND 100 females occupied the centre more often than males suggesting that after 100 days the apparatus was no longer novel and therefore less anxiogenic. It is difficult to explain why males no longer occupied the centre more often and instead occupied the corners more often which suggests that males developed higher anxiety over time. This should be investigated in further study. Furthermore, females eliminated fewer faecal boil than males which indicates again that males developed higher anxiety over time. Therefore, females showed higher amounts of ambulation and over time males showed higher anxiety.

Even though cage type did not produce any main effects; interaction effects suggest for some measures sex may have been affected by enrichment. In ambulation measures, as previously mentioned males displayed less locomotion than females this was the same for both housing types. However, enrichment increased ambulation for females which is contrary to most enrichment effects on locomotor activity, though it has been seen before (Meunier & Fishcer, 1985). Another interaction between sex and enrichment was for faecal boluses at PND 60 even though this measure was not affected by sex or cage type. Females eliminated fewer faecal boluses when enriched and fewer compared to males in enriched conditions. This may have been due to enrichment creating a greater decrease in anxiety for females than males however this is still unable to be explained

In conclusion for each type of apparatus there were differences between males and females especially in locomotor measures. There were some effects of cage condition per se, however most differences were found when considering sex interactions.

5.3 Relationship to past research

There was no previous research on memory effects of BZP so results seen in this study are new. Anxiety effects however have been previously seen in an experiment by Aitchison and Hughes, (2006). In that experiment between PND 45-55 10mg/kg BZP was administered to animals. They were then tested in the Y Maze, light dark box, social interaction test and open field between PND 72-95. In the Y maze BZP animals spent decreased time in and entered the novel arm less often than controls as well as making fewer entries of both arms suggesting greater anxiety due to aversion to the novel area (Aitken, 1974). In the light dark box, they took longer to emerge and in the open field they ambulated and reared less often which have also proven to measures of increased anxiety (Aitken, 1974). The social interaction test also produced fewer interactions, which again suggests increased anxiety, as rats who have increased anxiety have fewer interactions in novel environments (File & Hyde, 1978; Rex, Voigt, Gustedt, Beckett, & Fink, 2004)

Compared to this experiment, results from this study were not as clear. This is because in the open field there were no clear anxiety effects. Compared to the light dark box results of this experiment seemed to indicate increased anxiety but only for enriched groups so the results were variable. Conversely results from the elevated plus and the Y maze showed similar anxiogenic effects of BZP.

There are many reasons why there may have been different anxiety effects between these studies. Firstly, in this experiment BZP was administered from 41 days old to 51 instead of 45- 55. Therefore BZP administered in mid adolescence compared to later adolescence seen preciously may have affected different brain areas (Broadwater & Spear, 2013). Furthermore, in the experiment from PND 72-95 was when subjects were tested and in this experiment two different periods of time were used. One was PND 60- 72 and was PND 100- 112 which were

used to represent early and mid-adulthood (Andersen, 2003). Because these two periods in which animals were tested were different from the previous experiment it may explain why conflicting results were found. Furthermore, enrichment was used in this experiment which was not used in the previous experiment this may have enhanced or decreased dose effects compared to saline.

There have been no previous experiments investigating enrichment effects on negative memory and anxiety effects of BZP however there has been experiments considering environmental enrichment effects on memory and anxiety for other drugs. In an experiment by Wainwright et al. (1993) animals prenatally exposed to ethanol were tested in the Morris water maze when they were 10 weeks old. Animals had previously been separated into groups receiving different types of enrichment and no enrichment. In this maze enrichment decreased time taken to find the hidden platform over testing days and they spent increased time in the target area in a probe trial compared to non-enriched, indicating enrichment decreased ethanol induced anxiety.

Results from this experiment were similar showing enrichment did improve spatial memory. Although this was not seen for all measures this may be because of the different drug and administration period used in that experiment. Furthermore, the experiment only investigated results after 10 weeks. In the current experiment, effects were investigated after a longer period (PND 100) as well as PND 60 results which is longer than what the aforementioned experiment investigated. Therefore, it is possible that enrichment may not continue to have memory enhancing effects after a PND 100. The type of apparatus was different as well so the Morris water maze may be better at showing enrichment effects on memory.

Lastly enrichment effects on BZP anxiety have not been previously investigated however enrichment effects on anxiety due to other drugs has been demonstrated previously. Hughes and Otto (2013) housed animals in enriched or standard caging for 4.5 months and before each of their trials in an open field, light dark box or Y maze 2mg/kg of scopolamine was injected into subjects. In the open field enrichment increased occupancy in the centre of the apparatus, and decreased time taken to emerge into the light of the light dark box. Increased entries into the light side of the apparatus was also observed. In the Y maze entries into and time spent in the novel arm increased for enriched subjects. These results therefore showed that enrichment reduced anxiety in this experiment.

Results in this experiment were significantly different as they showed both increased and decreased anxiety. This is because enrichment did not show significant effects in the open field, however it did show similar anxiety reducing effects for the elevated plus maze. In the light dark box although environmentally enriched subjects showed differences from standard housed subjects it's not clear whether the difference was due to an attenuation of BZP effects or other anxiety effects. The reason for these differences may be due to the longer exposure to enrichment before testing began and shorter period between drug administration and testing.

In conclusion results from this experiment did show some similarities to previous studies. However, differences that have occurred may be due to differences in administration periods, testing periods, exposure to BZP and apparatus used to measure anxiety and memory responses. Furthermore, the inclusion of enrichment and sex as well as two different time periods may have affected the results in this study.

5.4 Theoretical and practical implications

One of the theoretical implications of this study is that neuronal imprinting may only have validity in certain conditions. This is because for most of the apparatuses BZP effects

were seen after PND 60 but not in later adulthood. This is a problem since neuronal imprinting theory posits that effects of the drug should only be revealed later in adulthood (Andersen & Navalta, 2004). However, for the elevated plus maze BZP effects were only shown after PND 100 which supports neuronal imprinting theory. Therefore, this shows that neuronal imprinting may have some validity but only with the elevated plus maze. It is beyond the scope of this experiment to determine why this effect was only shown for this apparatus and needs investigating to be explained.

Although this experiment showed mixed results it still has implications for adolescents. This is because shorter term memory effects have been suggested from these results which suggest that spatial memory may be impaired. For humans, this is significant because we use spatial memory to remember where we have left an object or the layout of a house (Shrager, Bayley, Bontempi, Hopkins, & Squire, 2007). Therefore, this has implications for not remembering where things were put at both the home and at work or school. It also suggests that BZP may affect the hippocampus in the shorter term (Shrager et al., 2007).

The mixed anxiety results also have implications because it suggests BZP induced anxiety may reveal itself sooner in some people and later in others. Furthermore, because these results are mixed it suggests that anxiety may not always be an outcome of BZP use and is due to a combination of BZP as well as other factors. This is especially relevant as for humans using BZP in adolescence as a combination of factors such as sex and life experiences may lead to future anxiety not just BZP use alone (Donner & Lowry, 2013; Wittchen et al., 2011). However, because of the mixed results further research is still needed.

Lastly it has implications for enrichment research as it shows additional research is needed to determine how much enrichment is needed to attenuate decreased memory or heightened anxiety effects. It further implies that enrichment may not work as well for some

drugs as well as others and may inflate differences in effects which also needs to be investigated. Since environmental enrichment has attenuated some effects it is still important for teenagers. This is because if adolescents can perform cognitively enriching tasks or physical activity it could attenuate reduced memory or heightened anxiety effects caused by BZP. However, because enrichments effects were varied additional research is needed to determine whether this is something worth implementing.

5.5 Methodological Strengths

The first strength of this experiment was using rats as representations of human teenagers, because rats develop quicker than humans and therefore reach adulthood faster as well. This is important because it allows analysis of drug effects over a shorter period than would be used with humans. Furthermore, it allows controlled administration of a drug that would be illegal to administer in New Zealand. This is relevant because it would also be difficult to find subjects who were willing to admit using illicit substances regularly due to the illegality of taking the drug. Consequently, using rats instead of humans is an important strength of this research.

A second strength of this experiment was two different BZP doses were used. This is a strength because different doses of BZP may have produced different results, especially as some of the results of this research were only significant for certain doses of BZP. It also applies to humans because party pills that contain BZP can differ on the amount of BZP inside the pill (Cohen & Butler, 2011).

A third strength of this research was the testing of animals after PND 60 and PND 100. This was important because it showed that some BZP effects may only be present in early adulthood and may not continue into later adulthood. It also allowed analysis of enrichment effects over time as well. Therefore, using two different age ranges was important for

investigating long term effects. Furthermore, this was a strength because it allowed analysis of whether neuronal imprinting theory applied to BZP effects

A final strength of this research was using environmental enrichment to determine whether enrichment can mediate BZP effects. This was an important strength because it allowed determination if any memory or anxiety effects that occurred could be ameliorated by enrichment. It also showed that some BZP effects were increased by enrichment allowing for future study opportunities. It also had applications for humans because it meant that some effects that were effected by enrichment could be ameliorated in humans as well using cognitively enriching tools with teenagers.

In summary, there were many strengths to this experiment which allowed its results to be generalised to humans and thoroughly investigate long term BZP use. These included using an animal model of human responses to BZP, since this is commonly used and rats develop at a faster rate than humans. Furthermore, the application of two different BZP doses and age ranges allowed investigations of whether strength of dose plays a part and if effects continue into later adulthood. Lastly the use of enrichment procedures provided information about whether enrichment is a viable way of attenuating BZP effects and if it may emphasis differences between saline and BZP. These strengths provided further credibility to the results found in this experiment.

5.6 Limitations

There were some limitations of this study that are worth noting when considering the results produced. The first limitation was that the acute effects of the drug were not measured. This is an important limitation as information about BZP acute effects could have had relevance to the effects seen in this experiment. It could also be used to further research acute outcomes of BZP and whether environmental enrichment could have ameliorated these acute effects.

However, because this study was mostly focused on adulthood effects it is not a significant exclusion.

A second limitation was that a neurochemical analysis of the rat's brains was not performed after the experiment. This would have been important as it could have shown changes in dopamine which may have explained locomotor effects found in this experiment (Goodwin, Patel, Kenworthy, & Khoshbouei, 2010). Furthermore, differences between saline and BZP rats in 5-HT may have been found with these analyses which could have indicated anxiety (Padovan, 2013). This is important as changes in 5-HT and dopamine are caused by BZP consumption (Esa Meririnne, Miina Kajos, Aino Kankaanpaa, & Timo Seppala, 2006; Schep et al., 2011). Therefore, if a neurochemical analysis was performed it may have been easier to see BZP effects as represented by chemical changes. However, because behavioural measures were used any effects on anxiety and memory were still able to be studied so not having these analyses didn't subtract from this thesis.

A third limitation was that observer reliability tests were not used to ensure all observations the apparatus especially the open field were consistent. This is an important limitation because it is possible for an observer to be subconsciously seeing what they expect to see (Kaufman & Rosenthal, 2009). Furthermore, during the experiment results were recorded every 3 seconds and this fast pace recording may have led to mistakes in what was recorded (Kaufman & Rosenthal, 2009). Using another observer briefly to conduct inter-observer reliability examinations for a random portion of the data would have made sure that all results recorded were accurate (Kaufman & Rosenthal, 2009; Rosenthal, 1976). Therefore, it was a limitation to not include inter-observer testing to ensure results were accurate. However, having just one experimenter recording animal behaviour did allow consistency throughout the experiment, which could have been compromised if another experimenter was used.

Lastly another limitation is that there were multiple extraneous variables within this experiment. Firstly, during parts of the experiment the room in which animals were being tested was being shared with another experimenter. Although this was due to limited room capacity it could have distracted the animals due to persons other than the experimenter being in the room and the smell of the experimenter's other rats could have directed the subject attention elsewhere. Furthermore, due to sharing a room with other experimenters the time at which testing took place was varied throughout the day based on conflicting schedules. Therefore, sometimes rats were tested in the early afternoon and other times that were tested later at night. The change in timing of testing is an important issue as it can affect locomotion and anxiety responses (Kaya, Karakaş, & Coşkun, 2011; Keith et al., 2013; Popović et al., 2009). Therefore, extraneous variables related to the room in which animals were tested was a further limitation of this experiment. However, although these variables may have affected results they were not always present so they may not have had a significant impairment. Steps were also taken to minimise room noise from other experimenters and separate animals as far as possible to reduce these olfactory stimuli.

In summary although acute effects were not measured this experiment was focused on adulthood responses so exclusion of this measure was not of high importance. Secondly although neurochemical analysis would have been useful it did not reduce importance of behavioural effects that were measured. Furthermore, although inter-observer reliability testing would have been useful to ensure results were accurately recorded, the same experimenter was used throughout the study which would have produced consistent results. Lastly extraneous variables may have affected results however they were minimised so that results are as accurate as possible. Therefore, although there were limitations they were not sufficient to invalidate results.

5.7 Future Directions

For future research the limitations of this study need to be addressed. It would be advantageous to measure acute BZP effects to show the impact that different doses of BZP are having on the rats. Applying neurochemical techniques to look at differences between BZP treated and control animals to determine if the serotonergic signs of anxiety are present would also be needed in future research. This could also be used to look at neurochemical differences between enriched and standard caged animals. If this is used in future research it would also help explain conflicting results seen in this experiment. Using inter-observer measures to make sure results are being recorded accurately would also be advantageous as well as testing animals at the same time of day and testing animals in a room with fewer extraneous variables to allow consistency of results.

Future research should also investigate the conflicting results seen for the 10mg/kg BZP group and why these occurred. This is because within the open field there were conflicting anxiety results for this group so it is important to investigate why this occurred. Furthermore, many BZP anxiety effects revealed in this study were only found in enriched caging and not standard caging. This needs to be considered in future research to see if enrichment may enhance differences between saline and other drugs.

Results in the Y maze suggested that BZP may have a detrimental effect on memory in early adulthood. Therefore, investigation into whether these effects occur more prominently in other memory tasks such as the Morris water maze needs to be investigated. Anxiety effects although mixed depending on the apparatus need to be further investigated as there is still suggestion from these results that BZP is anxiogenic.

Lastly enrichment effects were also varied in this experiment but that may have been due to locomotor activity or extraneous variables. Therefore, further experiments should be

completed to see if enrichment does have an effect under different conditions and with fewer extraneous variables. It may also be advantageous to see if enrichment has an effect with only one dose type to make comparisons simpler.

Therefore, there are many future directions in which this research can be taken. Firstly, the limitations of this research can be improved upon to see if better results are obtained. Further research into whether memory effects seen in the Y maze at PND 60 are due to memory by using different apparatus would also be advantageous as well as investigating effects produced by 10mg/kg BZP. A continued investigation into the anxiety effects of BZP and possible amelioration also be considered in future research.

5.8 Conclusions

In conclusion, the aim of this experiment was to determine whether BZP use in adolescence has long term impact on memory and anxiety. It was also hypothesised that any effects that did occur would be ameliorated by environmental enrichment. This was hypothesised based on previous research which showed that adolescents are vulnerable to drug taking and that drug taking can influence the still developing brain (Lubman & Yücel, 2008). One of the drugs that adolescents could consume is BZP which is usually ingested in the form of party pills that are commonly taken by teenagers. Since research has shown that BZP may be addictive due to its chemical similarity to other amphetamines it is also possible adolescents may become addicted to this drug (Bava & Tapert, 2010). Therefore, it was important to investigate the adulthood effects of this drug especially since it has been posited that BZP may cause anxiety in adulthood BZP (Aitchison & Hughes, 2006) . Furthermore, enrichment has been shown to ameliorate decreased memory and heightened anxiety effects which is why environmental enrichment was chosen to try to attenuate similar effects cause by BZP(Wainwright et al., 1993; Hughes, 2013).

To investigate the aims of this experiment enriched and standard caged subjects were treated over 10 days with either saline, 10mg/kg or 20mg/kg BZP after PND 41. Then at PND 60 they were tested in four apparatus including the responsiveness to change Y maze, elevated plus maze, light dark box and open field. Approximately two days between testing in each apparatus was used so that animals didn't acclimate to the apparatuses. They were then tested at PND 100 and there behaviours were recorded at each time.

The results of this experiment were mixed with BZP showing a decrease in spatial memory capabilities in some measures and enrichment ameliorating some of these effects when sex was considered. In the elevated plus maze anxiety effects were shown but only after PND 100 and these effects were not ameliorated by enrichment, although enrichment did show some effects after PND 60. In the light dark box anxiety was shown at both time periods however this was only shown in enriched conditions which suggests that anxiety differences may be brought out by enrichment. Enrichment did show an effect in this apparatus but because BZP was not significantly different from saline in standard cages this effect may not have been on BZP induced anxiety. In the open field anxiety effects were not clearly seen and enrichment had greater effects on locomotion.

Therefore, results did show some support for anxiety induced by BZP, however this was only shown under certain conditions and in certain apparatus. Memory effects were also shown but only in the shorter term and not long term. Enrichment effects did attenuate some memory effects and some anxiety effects as expected but there were some conflicting results. Therefore, additional research is needed into these effects and whether with limitations removed memory, anxiety and enrichment effects become clearer.

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Appendix 1

UNIVERSITY OF CANTERBURY
Te Whare Wānanga o Waitaha
CHRISTCHURCH NEW ZEALAND

ANIMAL ETHICS COMMITTEE

Secretary, Rebecca Robinson
Telephone: +64 03 364 2987, Extn 45588
Email: animal-ethics@canterbury.ac.nz

Ref: 2016/13R

23 May 2016

Ellen Dixon
Psychology
UNIVERSITY OF CANTERBURY

Dear Ellen

I am pleased to inform you that the Animal Ethics Committee (AEC) has approved your application entitled: "Possible Amelioration by Environmental Enrichment of the Subsequent Effects of Exposure to BZP during Late Adolescence".

Approval has been granted:
(a) for the use of 120 PVGc hooded rats.
(b) for your research project to be undertaken from 23rd May 2016 to 31st January 2017. If you require an extension of this period please contact the AEC Secretary.

As part of AEC's new Code of Ethical Conduct all applicants receiving approval to work on animals are required to provide a final report at the completion of their project. The purpose is to provide the AEC with a record of your use of animals and what was achieved by your research project. We are very much interested in your findings and to learn what you have achieved. Following the completion date indicated above you are asked to provide this report using the new Final Report form which is available at the AEC web site (https://intranet.canterbury.ac.nz/research/ethics.shtml).

On an annual basis the University is legally required to provide to MPI statistical data on all animal manipulations undertaken in a calendar year. To assist us in collating this information you are also required to complete and return to the AEC Secretary the attached MPI Animal Manipulation Statistical form 30 days after the completion of this project, or once every three years, whichever comes first. If no animals have been manipulated in your project please provide a "Nil" return. Please also find enclosed a copy of the Animal Welfare (Records and Statistics) Regulations 1999 for your information, together with a list of Animal Type Codes and brief guideline notes for your assistance.

Yours sincerely

James Briskie

Associate Professor Jim Briskie
Chair
University of Canterbury Animal Ethics Committee