SET SHIFTING ABILITY OF RATS PERINATALLY EXPOSED TO BISPHENOL A AND A HIGH FAT DIET

BY

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THESIS

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ABSTRACT

Bisphenol-A (BPA) is an endocrine disruptor found ubiquitously in the environment. It has been shown to have a wide variety of effects on behavior and cognition as well as causing inflammation in the brain. Additionally, the traditional American diet is high fat, which also contributes to both physiological, behavioral and inflammation problems. Together, these two factors could exacerbate one another in the brain, which could lead to some cognitive impairment.

In order to test this hypothesis, pregnant female rats consumed 0, 40, or 400 μg/kg of BPA daily and either a control (CON; 18.5% kcal) or a high-fat diet (HF, 45% kcal) during gestation. The corresponding dose of BPA was pipetted into male and female pups' mouths from postnatal days (PND) 1-10 while the dams remained on their respective diets. After PND 10, dams were placed on separate chow diet which was maintained for the remainder of the offspring's' life. As adults (>PND 90), the perinatally exposed rats were tested on an extra-dimensional set-shifting task. A rotating plus maze with black/white and rough/smooth arms was used.

Final analysis indicates that there were no significant treatment differences for the number of trials needed to reach criterion during the initial discrimination. Additionally, there were no significant effects of treatment for accuracy or perseveration during the extra-dimensional shift. There were significant 3-way interactions, one between dose and diet, and the other between dose and diet and block. However, post hoc analyses revealed no significant

differences from control doses, only between doses of BPA. Finally, there was a significant sex difference between males and females in number of random errors made with females having more errors than males.

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CHAPTER ONE

INTRODUCTION

Bisphenol A (BPA) is a ubiquitous endocrine disrupter that has leached into our environment through the many different products that contain the compound. For example, BPA can be found in receipt paper, food receptacles such as cans and plastic containers, water bottles, and dental sealants (Brotons et al., 1995; Goodson et al., 2004; Liao and Kannan, 2013). Because BPA is weakly bound to the products it which it is contained, it can dissolve from our environment into our tissues (Brotons et al., 1995; Krishnan et al., 1993). The pervasive use of this chemical makes it virtually unavoidable and as a result, the majority of people in the United States have some detectable amount of BPA in their bodies (Calafat et al., 2005; Calafat et al., 2008).

Endocrine disruptors alter the normal functioning of hormones in the body and can lead to some long lasting physiological changes. Specifically, BPA is an estrogenic compound that mimics the role of estradiol. It binds to estrogen receptors at a lower affinity than estradiol, acting not only as an agonist at both ER-α receptors and ER-β receptors, but also as an antagonist at ER-α receptors (Krishnan et al., 1993; Steinmetz et al., 1997; Hiroi et al., 1999). As a result, it can alter estrogen related functioning in the body leading to increases in prolactin release and gene expression and decreases in plasma luteinizing hormone (LH), as well as negatively impacting the development of estrogen sensitive tissues such as the uterus and ovaries (Steinmetz et al., 1997; Rubin et al., 2001; Papaconstantinou et al., 2000; Berger et al., 2015). In addition, both human and animal studies have shown that BPA is an ovarian toxin that inhibits

the breakdown of germ nest cells (Peretz et al., 2014). It also impacts thyroid functioning by working as an antagonist against T_3 at the thyroid receptor resulting in inhibited transcription (Moriyama et al., 2002). Additionally, BPA causes oxidative stress, which can lead to possible inflammation in the brain (Bindhumol et al., 2003; Kabuto et al., 2004).

Though the physiological impacts of BPA are well documented, the effects on cognition are not as consistent (Peluso et al., 2014; Sadowski et al., 2014a; Kuwahara et al., 2013; Johnson et al., 2015; Kuwahara et al., 2014). Wang et al. (2014) found that male juvenile rats prenatally exposed to BPA committed more working and reference memory errors than did controls on the radial arm maze task. Additionally, Xu et al., (2010) found a significant difference in spatial memory in both juvenile and adult mice in the water maze, with BPA exposed animals taking longer to reach the escape platform. However, these results are not consistently replicated. Perinatal BPA at low doses (less than 5 mg/kg) has also been shown to have little to no effect on spatial memory in adulthood in the Morris Water Maze (MWM; Jones & Watson, 2012), the Barnes Maze (Ryan & Vandenbergh, 2006), and the 17 radial arm maze (Sadowski et al., 2014a) in rats, mice, and rats, respectively.

It is possible that the inconsistent results from the literature reflect the specificity of effects of BPA on only certain aspects of cognitive functioning that rely on the brain. Previous studies have focused on hippocampal, spatial memory, or working memory tasks (Kolb et al., 1983; Morris et al., 1982; Becker et al., 1980). However, previous research from our lab has shown that 400 μg/kg dose of BPA significantly increases the numbers of neurons and glia in Layers 5/6 of the prefrontal cortex (PFC) in adult male rats compared to controls, with the same

trend following in females (Sadowski et al. 2014b). Additionally, perinatal BPA has been found to alter the serotonin receptors in the PFC as well after gestation. By utilizing a task that more strongly test the function of the PFC, rather than the hippocampus, behavioral changes from BPA might become more apparent.

It is very likely that combination of environmental factors come together to cause cognitive deficits associated with BPA. Since one of the most common environmental attributes of western society is a high fat diet, the goal of this study was to explore potential effects of BPA in the background of a high fat diet as well as a normal diet. High fat (HF) diets are prevalent in American culture with the average fat content around 35% of a person's daily intake (Freedman et al., 2001). HF diets are proinflammatory in both the brain and the periphery (Ramos et al., 2003; Pistell et al., 2010). Maternal HF diet has been shown to increase anxiety like behavior in both rodents and nonhuman primates. In addition, a high fat diet has been found to alter the levels of tyrosine hydroxylase in both the ventral tegmental area (VTA) and the nucleus accumbens (NAc), and affect spatial memory (Sasaki et al., 2013; Sullivan et al., 2010; Naef et al., 2008; Page et al., 2014). Furthermore, a HF diet has been found to exacerbate physiological abnormalities caused by BPA (Wei et al., 2011; Wei et al., 2014; Stravkosky et al., 2015). Previous research has found that in conjunction with perinatal BPA, concurrent high fat diet exposure increases liver disease, pancreatic size, serum glucose, and predisposes offspring to metabolic syndrome (Wei et al., 2011; Ding et al., 2014; Wei et al., 2014).

Though some studies have investigated the effect of high fat diet and BPA on cognitive outcomes, no studies to our knowledge have investigated the combination. Therefore, we

exposed dams and their offspring to a combination of one of three BPA doses and either a HF or control (CON) diet during the perinatal period. We then tested the offspring in adulthood on an attentional set shift maze, a task that is usually measured based on performance of the prefrontal cortex (Stefani & Moghaddam, 2010).

To account for the possibility that the treatments (BPA, HF diet) could indirectly produce altered behavior by changing maternal care, maternal behaviors were also quantified for all of the rats during the early postnatal period. It has been shown that the amount of licking performed by the dam can impact certain developmental outcomes on the offspring (Francis et al. 1999; Champagne & Meaney, 2006). Moreover, both BPA and a HF diet have been known to impact maternal behavior separately (Palanza et al., 2002; Purcell et al., 2011).

CHAPTER TWO

MATERIALS AND METHODS

Breeding, Dosing, and Diet

Breeding

Sixty-eight male and female Long-Evans hooded rats were obtained from Harlan (Indianapolis, IN, USA) and used as breeders. Due to the number of behaviors being measured and resulting time needed for each behavior, rats were divided into five separate cohorts, distributed evenly throughout the course of a year. Given that BPA is prevalent in many animal care tools, precaution was used to minimize the resulting exposure in all aspects. Rats were housed in polysulfone cages. Water bottles containing reverse-osmosis water were either glass or polysulfone and food consisted of the Harlan 2020X diet, which has low, stables amounts of phytoestrogens. All animal procedures were in compliance with the guidelines for care and use of lab animals according to the National Institutes of Health (NIH) and were approved by the Institutional Animal Care and Use Committee (IACUC).

Animals were housed on a 12:12 light-dark cycle with lights on at 7AM. Breeding procedures took place one week following shipping. One male and one female were placed into a wire-mesh breeder cage until a sperm plug was detected or six days had elapsed, at which time females were rehoused with a new male until sperm plug was detected.

Dosing and Diet

To acclimate the females to the dosing procedures, they were given half of a cookie every day during breeding (Newman's Own Vanilla flavor, Westport CT, USA). Following detection of a sperm plug, animals were separated and single housed. For the first two days following the detection of a sperm plug, females were given cookies that had tocopherol-stripped corn oil (Sigma, St. Louis, MO, USA). From gestational day (G) 3 to parturition, females were given 0, 40, or 400 μg/kg BPA suspended in tocopherol-stripped corn oil that corresponded to their weight. Following parturition, pups were dosed with the same dose/weight received by their mother from postnatal day (PND) 1 through PND 10. At PND 2, litters were culled to a maximum of 10 pups. After PND 10, dosing ceased and 2 pups from each litter (1 male and 1 female) were sacrificed to examine inflammatory markers (results not reported in this paper). During this time, female breeders were put on either a control diet (CON, containing 15.8% kCal/fat, from Research Diets, Inc., New Brunswick, NJ) or a high fat diet (HFD, containing 45% kCal/fat, from Research Diets, Inc.) from G 0 until their offspring were at PND 10 at which point the dams were returned to the standard chow diet (Harlan 2020X).

Animals were weaned at PND 25 and then were double or triple housed with the same sex. Afterwards, all animals were assessed for play behavior between PND 26 through PND 40 (results not reported in this paper). One male and one female from each litter were selected for the attentional set shift maze task (n=116; for full breakdown see Table 1). At PND 90, animals went through a one-trial, five minute, elevated plus maze task prior to completing the task (results not reported in this paper).

Maternal Observations

Maternal behaviors were observed from PND 3 – PND 15. Behavioral observations were done using night vision goggles during the first 90 minutes of the dark cycle. The experimenter recorded the dam's behavior once every three minutes during that time period for a total of 30 observations per day per dam. Behaviors recorded were nursing (either with an arched or flat back), licking of the pups, retrieving the pups, nest building, active away from the nest (self-grooming, rearing, digging, or eating) and inactive away from the nest (laying down). Though all measures were taken, only the results from licking are reported in this paper.

Attentional Set Shift Maze

Apparatus

The task was adapted from the attentional set shift maze developed by Stefani & Moghaddam (2010). The maze consists of four arms, alternating black and white colors, with opposing arms of the same color having either a smooth or rough texture. Each arm was 40.6 cm long and 14 cm wide and 20.3 cm high. Walls were made out of plexiglass and the floor was made from painted wood. The textured floor was made from a flat interior textured paint. At the end of each arm was a 1.9 cm well that was 0.63 cm deep, which was deep enough to conceal any food reward that was placed at the end of the arm (Figure 1).

Maze Procedures

Preparation for this task included handling, habituation, and training. Animals were food restricted during both preparation and testing. Food restriction consisted of reducing the amount of food the animal was given, so that the animal would be between 85-90% of their *ad libitum* body weight during the training portion of the experiment. Following each day of habituation, training and testing, animals were returned to their cages after testing and fed approximately one hour later. The maze was cleaned with a 30% ethanol solution between each animal.

Handling/Habituation

Handling started on the first day of food restriction. Each animal was handled for a total of three minutes a day for five days prior to testing. After each bout of handling, animals were fed with 3-4 sucrose pellets that also served as the bait in the maze (Purified Rodent Tablets; 45 mg; TestDiet, St. Louis, MO). Habituation started two days following the last bout of handling. During habituation, rats had five minutes to find the food reward placed at the end of each of the arms. To start the habituation trial, rats were placed in the center of the maze. The trial ended and the rats were returned to their cages either when five minutes had elapsed or the rats had found all four pellets. The rats were fed approximately one hour after returning to their cage following the trial. This procedure was repeated twice over the course of two days. After two days of habituation trials, rats began the training.

Training Days

Training consisted of eight trials where a plexiglass block was slide in across from the starting arm (trunk arm) so that the maze became a T-maze with only two arm choices as the rat approached the center. Training trials started when the animal was placed at the end of the starting arm facing the two choice arms. Note that the starting arm was always in the same location in the room and the maze itself was rotated so that the texture and color of the starting arm changed, but not the location in space (Figure 2). Animals never started in the same arm twice in a row. The choice arms were either baited or unbaited with the probability 25% and 75%, respectively. Baited and unbaited trials were never separated by more than 5 trials to maintain the rats attention and the rat was removed from the maze if it took longer than a minute to make an arm choice. This was done in order to keep the rat motivated and to continue running the maze. At the end of eight trials, the task was over and animals were returned to their home cages. Arm choices, as well as random errors, were recorded. Random errors were considered if an animal climbed out of the maze or took longer than a minute to choose an arm. This process was repeated twice over two days. Following these two days, animals were tested.

Testing Days

Testing Day (TD) 1 was very similar to the training phase, but instead of randomly baiting the arms, either the white or the black arm was baited every trial. Black or white was consistent across all of TD1 for each animal. This first day was a test of working memory. As described previously, the arms opposite one another are always of opposite color and texture

meaning that the rats had to ignore the texture between the two arm choices and make a choice between either a white or black arm. The trials continued until the rat had made eight consecutively correct choices at which point it was taken out of the maze and returned to its home cage and fed one hour later. If rats failed to reach eight consecutive correct choices by the 100^{th} trial or the rat made eight consecutive random errors, the rat was excluded from final analysis. We interpreted eight consecutive random errors to indicate that the rat had a lack of motivation to complete the task. The total number of animals to reach criterion before 100 trials and without making consecutive random errors can be found in Table 2. Correct choices, incorrect choices and random errors were recorded for statistical analysis. The maze was cleaned with a 30 % ethanol solution between each animal.

TD2 followed the same protocols as TD1 except instead of either a black or white arm being baited; the rough or smooth arm was baited. This extra-dimensional shift, either rough or smooth, was kept consistent across TD2 for each animal. This meant that the animal had to ignore the previous day's rule and shift to the new rule in order to receive the food reward. This process lasted a total of 80 trials regardless of performance. Correct and incorrect choices, as well as the errors described during training, were recorded. Additionally, if the animal made eight consecutive random errors, they were excluded from final analysis. An updated total of the number of animals to reach criterion before 100 trials and without making 8 consecutive random errors can be found in Table 3. Trials were scored as correct, incorrect, or random error choices for statistical analysis. Following the 80^{th} trial, animals were removed and returned to their home cages.

The four measures that were examined for Testing Day 2 were accuracy, perseveration errors, omission errors, and random errors. Accuracy was defined as the number of correct trials per block. Perseveration errors were defined as an arm choice that was indicative of the TD1 choice (black or white), but not the TD2 choice (rough or smooth). An example of a perseveration error is that if the TD1 rule was black and the TD2 rule was rough, an arm choice of a black smooth arm was considered a perseveration error. An omission error was defined as an arm choice that was not indicative of either TD1 or TD2. For omission errors, an example would be if the TD1 choice was black and the TD2 choice was rough, an arm choice of white smooth would be considered an omission error. Random errors were when the rat took longer than a minute to choose an arm or they climbed out of the maze.

Statistical analysis

Day 1 testing trials to criterion were analyzed using a linear model that included average licking behaviors as a covariate, cohort (1-5), room (large or small), initial rule (black or white), sex (male or female), diet (HF or CON) and dose (0, 40, or 400 μg/kg) as factors, and interactions only between the last three factors.

Day 2 accuracy and perseveration errors were analyzed using a mixed effects linear model with licking, number of trials to criterion on day 1 as covariates, room and cohort as cofactors, and sex, dose and diet as treatment factors, interactions between all the treatment factors, and block as a repeated measure (within-subjects) factor. Day 2 omission and random errors were collapsed across all blocks; otherwise too many zeros violated the normality

assumption. In addition, total omission errors across blocks were raised to the power 0.5 to remove skewness of residuals.

CHAPTER THREE

RESULTS

Testing Day 1

Testing Day 1: Trials to Criterion

Neither BPA nor a HF diet affected the total number of trials to reach criterion. However, the initial rule used on TD1 (black or white color), had a large influence in trials to criterion. The rats took significantly longer to learn when the arm baited was white, than when the arm was black (Figure 3). We also observed a small effect of the room in which the testing occurred, where the rats took slightly longer to learn in the smaller room than the large. This was indicated by a significant effect of room ($F_{1,88} = 4.29$, $p = 0.04$) and initial rule (black or white; $F_{1,88} = 25.5$, $p < 0.0001$) in the linear model. No other covariates or factors or interactions were found to be significant.

Testing Day 2

Accuracy

We observed an increase in total number of trials correct across blocks indicating that the rats learned the task (Figure 4). However, there were no differences between groups treated with BPA or diet (HF or CON). This was indicated by a significant effect of block in the mixed

effects linear model ($F_{9,810} = 42.0$, $p < 0.0001$). A small effect of cohort was also detected ($F_{9,82} = 3.1$, $p = 0.02$). No other covariates, factors, or interactions were significant.

Perseveration Errors

Total number of perseveration errors decreased over the blocks indicating that the rats learned the task (Figure 5). However, there were no differences between groups treated with BPA or diet (HF or CON). This was indicated by a significant effect of block in the mixed effects linear model ($F_{9,810} = 37.3$, $p < 0.0001$). A small effect of cohort was also detected ($F_{9,82} = 3.5$, $p = 0.01$) and a slight positive relationship between maternal licking and perseveration errors ($F_{1,82} = 5.4$, $p = 0.02$). No other covariates, factors, or interactions were significant.

Omission Errors

Total number of omission errors across all the blocks did not differ between groups treated with BPA or diet (Figure 6; HF or CON). However, rats that took longer to learn the initial rule on TD1 displayed a significant increase in the number of omission errors on TD2 as indicated by the significant effect of the covariate, day 1 trials to criterion ($F_{1,81} = 9.1$, $p = 0.004$). We also observed a significant effect of the cofactor, initial rule on TD1. Rats that had black initially displayed fewer omission errors on day 2 ($F_{1,81} = 14.1$, $p = 0.0003$).

Random Errors

Total number of random errors across all the blocks did not differ between groups treated with BPA or diet (Figure 7; HF or CON). However, females made approximately 80% more errors than males ($F_{1,82} = 5.1$, $p = 0.03$). No other covariates, factors, or interactions were significant.

CHAPTER FOUR

DISCUSSION

Overview

This study provides more insight into the effects of BPA on cognition by examining behavior on a task that is more associated with the PFC rather than the hippocampus. Additionally, it investigates how concurrent exposure to a HF diet may affect cognition. No studies to date have examined cognitive flexibility in rats treated with BPA, HF diet, or a combination of both. There are several possible explanations for the lack of treatment effects in this study. It could have been due to the task being too hard, overall anxiety potentially masking the interaction between BPA and a HF diet, or the timing of exposure.

Floor Effect

It is possible that the task itself was too hard and there is a floor effect. Few animals reached perfect accuracy by the end of the task (n=17 or 16.5%), showing that there were few animals to reach the same criterion that was reached for TD1. This could either be too difficult, or at least, not sensitive enough to pick up the subtle differences between the treatment groups. It is possible that alterations to the prefrontal cortex may need to be greater in order to see significant differences between groups.

Anxiety Affecting Interactions

The random errors indicate show how an inherent sex difference may play a role in masking other effects of treatment. Females tended to have a higher number of random errors indicating a higher level of anxiety. It is also possible that the result of the initial choice for TD1 may indicate other anxiety behaviors. This increased anxiety could have interacted with how the females performed overall and masked any potential sex differences in other measures. This is especially important given that perinatal BPA and a HF diet, as well as the amount of maternal licking behavior, have been known to have effects on anxiety-like behavior following perinatal exposure (Starr-Phillips & Beery, 2014).

A study done by Jones and Watson (2012) found that while there were no significant differences from control following perinatal BPA exposure on the water maze, the sex difference that occurred at the control dose was abolished at the 5 μg/kg dose. They also found that females in the 500 μg/kg group had a higher number of fecal boli. In mice, they also found a sex reversal in BPA exposed males and females in the open field. BPA exposed males had behavior similar to control females and BPA exposed females were more similar to control males resulting in no significant sex difference across treatments (Palanza et al., 2008). On anxiety measures following a perinatal HF diet, female rats show decreased open arm entries and a higher number of fecal boli (Sasaki et al., 2013) and nonhuman primate showed increased anxiety in response to the presentation of a novel stimulus (Sullivan et al., 2010). Taken together, these studies show that there could have been an underlying difference in anxiety between males and females that might have impacted other performance measures such as accuracy or perseveration.

In the future, increasing the amount of pretraining time using the attentional set shift maze might help to reduce the observed anxiety in female rats by increasing the amount of exposure to the maze itself.

Timing of Exposure

Another possible reason there were no significant differences is due to the timing of the BPA exposure. There could have been compensatory adjustments in the brain that may account for the unobservable change in behavior between the perinatal period and adulthood. If this is the case, it suggests that the alterations that BPA makes may not have a long lasting impact on measures of cognition. Nonsignificant behavioral changes have been mirrored in several other behavior tasks following BPA exposure (Jones & Watson, 2012; Sadowski et al., 2014a). Both of the previously mentioned studies dosed their animals during the perinatal period and then tested their animals after PND 90. Some observable differences might have been seen if the animals were tested earlier. For example, previous studies also examining cognition have shown that animals perinatally exposed to BPA that were then tested in the juvenile period have significant behavioral changes (Wang et al., 2014; Xu et al., 2010). Additionally, studies with different windows of exposure such as adolescence or adulthood also show significant results, which could be attributed to their testing and exposure periods occurring closer together in time (Xu et al., 2011; Weinstein et al., 2013; Eilam-Stock et al., 2012). Taken together, these studies suggest that the developmental windows in which animals are exposed are not as important as the time period between when they are exposed and when they are tested. This follows the idea that BPA

may be having an effect, but behavioral and cognitive measures are not occurring in the appropriate time in which BPA would be having a measureable effect anymore.

It is also important to note that these studies are done during particular windows of exposure. Researchers are interested in the effect that BPA has during these sensitive time periods and as a result, they limit the amount of exposure following their protocols. However, normal BPA exposure happens throughout life, rather than during an isolated time period, and that there may be alterations occurring that the current research has not been able to address. Similar to BPA, a HF diet rarely seems to happen only in an isolated time point. Some studies have shown that when dams have been exposed to a HF diet during gestation and their offspring are then continued on either a HF or CON diet, those that continue on a HF diet tend to have worse behavioral and cognitive outcomes than their same litter counterparts (White et al., 2009). Future studies should look at a more consistent lifelong concurrent BPA and HF diet exposure and the resulting behavioral effects. If continuing on a HF diet after the perinatal period results in worse behavioral outcomes, it is possible that the continued exposure to BPA may also do the same.

CHAPTER FIVE

CONCLUSIONS

Overall, this study is novel in its approach to combining the effects of BPA and a HF diet. While most studies have examined these factors in isolation, their prevalence in society does not seem to suggest that exposure to either will happen in isolation. This study is important because it has taken into account the complex nature of exposure to endocrine disrupting chemicals and diets rich in fats. Prior studies have examined performance measures that are dependent on the hippocampus; this is the first task to utilize a prefrontal dependent task to assess cognitive measures. The final conclusion of this study is that there are no significant differences due to diet or dose of BPA when animals are exposed during the perinatal period. It is possible that alterations are occurring, but this study was not sensitive enough to pick up these changes in measures of cognition.

CHAPTER SIX

FIGURES AND TABLES

Table 1 – Breakdown of total n

	0 – CON	**0 – HFD**	**40 – CON**	**40 – HFD**	**400 – CON**	**400 – HFD**	*Total*
Male	8	10	10	10	10	10	*59*
Female	8	10	10	10	9	10	*58*
Total	*18*	*20*	*20*	*20*	*19*	*20*	***116***

Table 2 – Total breakdown of n after completion of Testing Day 1

	0 – CON	**0 – HFD**	**40 – CON**	**40 – HFD**	**400 – CON**	**400 – HFD**	*Total*
Male	9	10	8	9	10	9	*56*
Female	6	10	9	9	8	9	*52*
Total	*16*	*20*	*17*	*19*	*18*	*18*	***106***

Table 3 – Total breakdown of n after completion of Testing Day 2

	0 – CON	**0 – HFD**	**40 – CON**	**40 – HFD**	**400 – CON**	**400 – HFD**	*Total*
Male	8	10	8	9	9	9	*53*
Female	5	10	9	9	8	8	*50*
Total	*14*	*20*	*17*	*18*	*17*	*17*	***102***

Figure 1 – Layout of the Attentional Set Shift Maze

Figure 2 – Possible Layouts for the Attentional Set Shift Maze during Training, Testing Day 1, and Testing Day 2. The starting arm of the maze was always in the same position in the room, but the starting arm texture and color were varied because the maze was rotated between trials. The white/black line at each image represents the plexiglass block placed to create a T-maze.

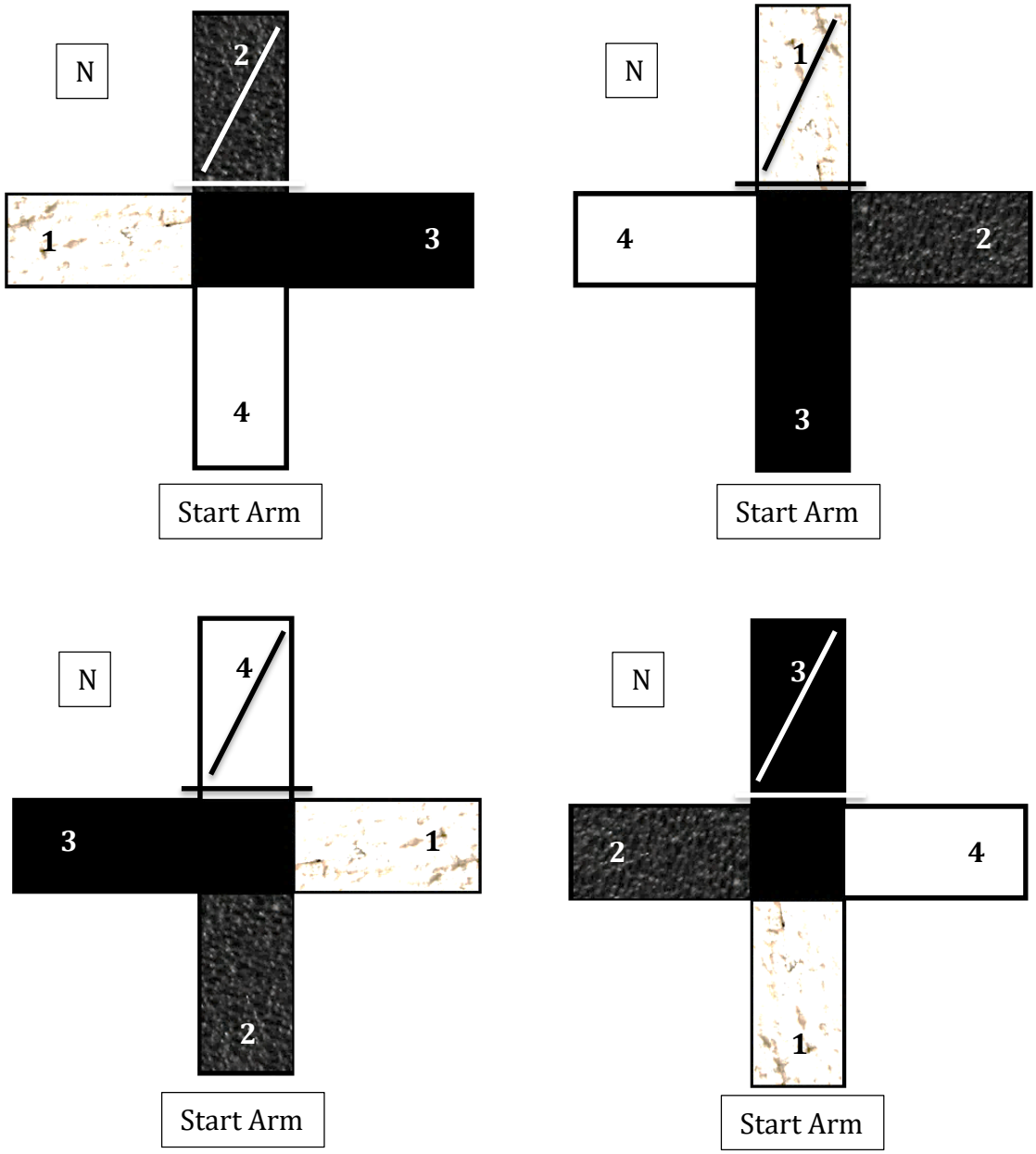


Figure 3 - Total number of trials needed to reach criterion. This graph is separated by dose and choice. Animals that were given the initial choice of black learned the initial rule quicker regardless of treatment. *** $p < 0.001$

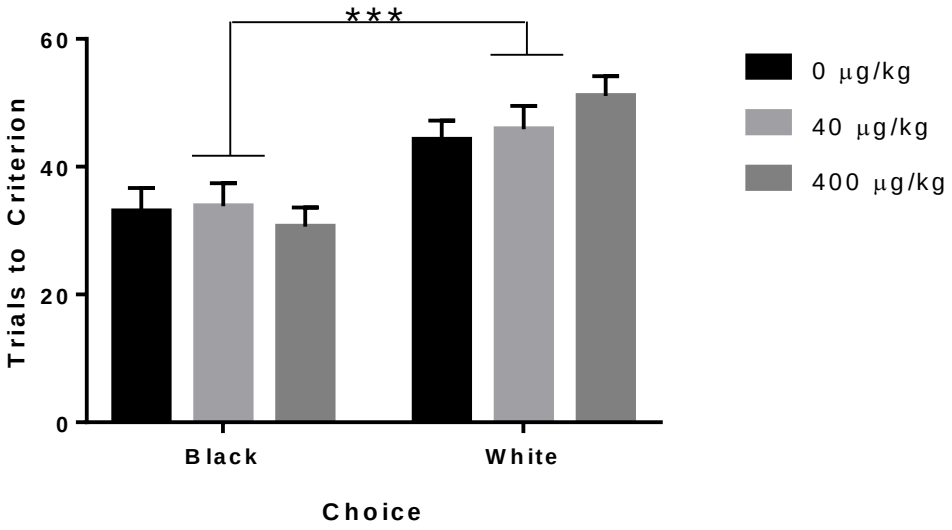


Figure 4 – Accuracy across blocks. Because there was no interaction of sex or diet on accuracy, the data were collapsed across diet and sex to show a more cohesive graph. This shows a significant block difference between all doses at block 1 and all doses and block 10 suggesting an improvement in performance regardless of treatment by means of an increased number of correct trials per block. *** $p < 0.001$

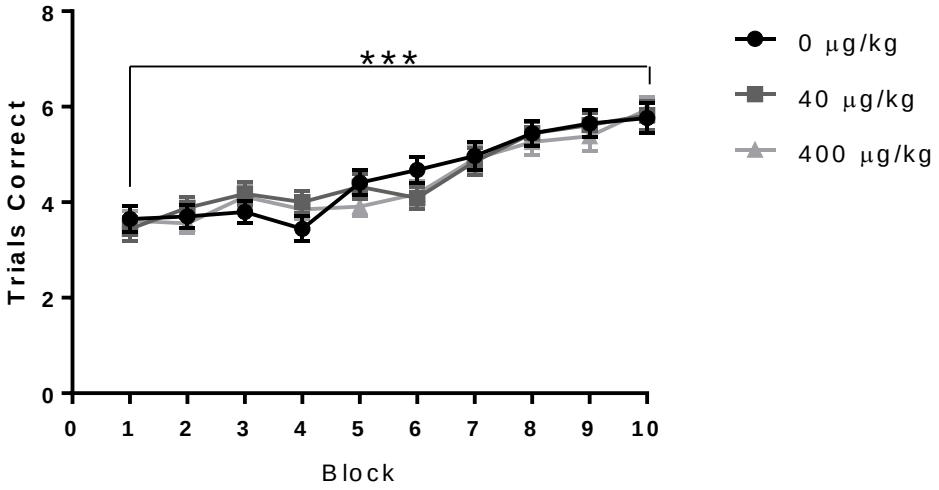


Figure 5 – Perseveration errors across blocks. There was no interaction of sex or diet on number of perseveration errors so the data were collapsed across dose to show a more cohesive graph. This shows a significant block difference between all doses at block 1 and all doses and block 10 suggesting an improvement in performance regardless of treatment by means of a decreased number of errors across blocks. *** $p < 0.001$

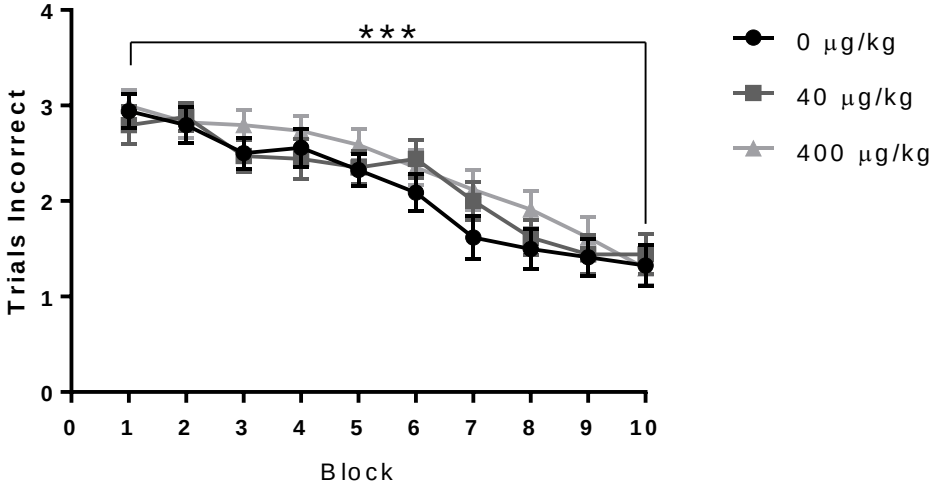


Figure 6 – Total omission errors. There was no sex difference, so the data are collapsed across sex. This graph illustrates the dose by diet difference. However, post hoc analyses revealed no significant differences between control dose and BPA doses, or between HF and CON diet.

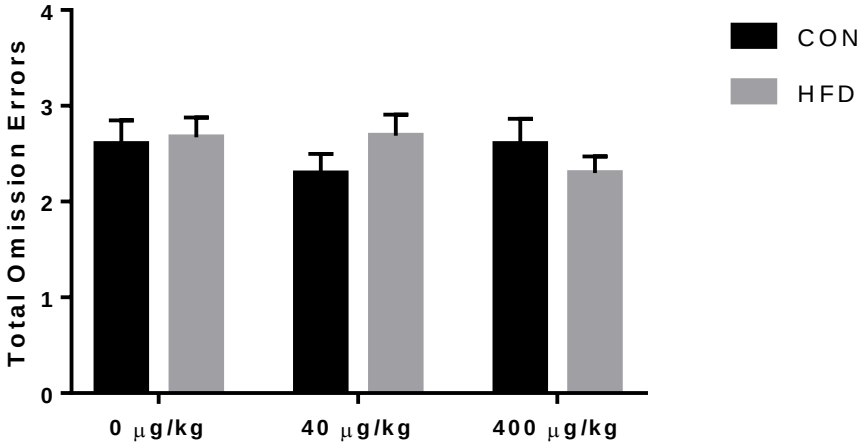
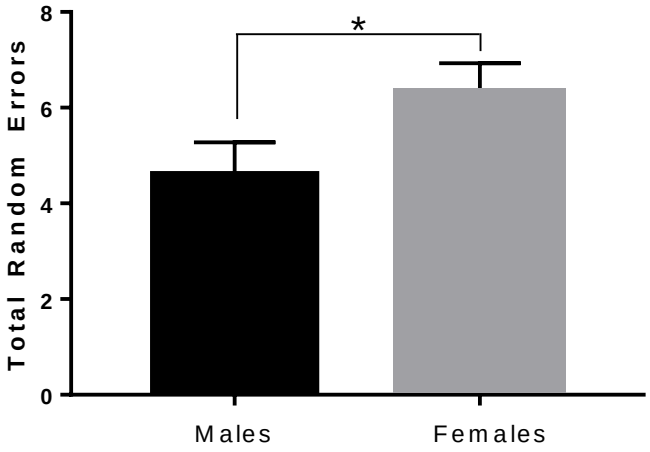


Figure 7 – Total Random Errors. There was no interaction of dose or diet on the total number of random errors, so the graph shown is collapsed across sex. This graph shows that males made significantly fewer random errors than females. $p < 0.05$



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