ORAL EXPOSURE OF BISPHENOL A DURING DEVELOPMENT AFFECTS
BEHAVIOR IN ADULTHOOD IN THE FEMALE PRAIRIE VOLE

(Microtus ochrogaster)

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Thesis

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ABSTRACT

Bisphenol A (BPA) is a known estrogenic mimic that is found in plastics and epoxy linings of canned food. Current production levels are estimated at 6 billion pounds per year and measurable levels have been found in human blood and urine. Previous studies have found significant effects of BPA dosages lower than those recommended by the Federal Drug Administration (FDA)(50µg/kg/day). These studies have shown a variety of effects from reproductive and brain physiology to behavioral effects. Most of these studies have been conducted in laboratory species, such as rats and mice, which, unlike human, display relatively low levels of social behavior. Therefore, the goal of this thesis is to examine the effects of BPA on social behavior in a human-relevant rodent model. This study utilized the socially monogamous, social prairie vole (Microtus ochrogaster) to analyze the behavioral effects of BPA. Pups received oral treatments with one of three doses of BPA, 5µg/kg, 50µg/kg, 50mg/kg or a vehicle control on neonatal days 8-14. Doses were chosen using FDA established safety levels for human consumption, which is set at 50µg/kg. As juveniles or adults they participated in 3 behavioral tests: an open field test, a novel social test, and a partner preference test. Results were sexually dimorphic and showed significant behavioral effects on females but not on males. Females exposed to 50mg/kg (high dose) showed significantly decreased activity and increased anxiety. Females exposed to 5µg/kg (low dose) showed increased levels of activity. Only control females demonstrated a statistically significant partner preference. These results support the hypothesis that BPA alters behaviors even below FDA estimated safety levels.
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CHAPTER I
INTRODUCTION

Human activity has drastically changed our world and how we interact with it. The exponential increase in industrial and technological growth over the last 100 years has had an enormous impact on the surrounding environment, which still to this day are not wholly understood. To try and assess our effects on ourselves and the rest of the biological world around us, 21 scientists from a variety of backgrounds, including endocrinologists, reproductive physiologists, and toxicologists, met in Racine, Washington, in 1991, to discuss the human effects on wildlife. One of the major results, was the coining of the term ‘endocrine disruption’, which hypothesized that certain chemicals could alter the production, processing, and transmission of hormones and thereby disrupt the normal functioning of the endocrine system (Vogel, 2009, Colborn & Clement, 1992). Although the term endocrine disruption was not used until 1991, it was based on more than 20 years of previous research in which apparently “normal” adult wildlife that were giving birth to offspring with a variety of different defects, suggesting whatever was causing the problems were affecting the “normal” developmental pathway (Colborn & Clement, 1992). The meeting led to a consensus statement titled “Chemically-Induced Alterations in Sexual and Functional Development: The Wildlife/Human Connection,” or the Wingspread Consensus Statement of 1991, in which
it stated that some chemicals, currently in the environment, had the potential to disrupt the endocrine system of humans and wildlife (Colborn & Clement, 1992).

The Wingspread Consensus identified several endocrine disruptors including dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), triazine herbicides, cadmium, lead, mercury, alkyl phenols (non-biodegradable detergents and anti-oxidants present in modified polystyrene and PVCs), soy products and more (Colborn & Clement, 1992). Not surprisingly, several of the scientists from the conference would become leaders in the field of endocrine disruption. One such person, Dr. Frederick vom Saal of the University of Missouri, whose laboratory had been studying the effects of in-utero exposure of natural hormones on development, became one of the pioneers in the field of endocrine disruption and his next publication looked at bisphenol A (BPA) and its effect on prostate weights on exposed mice (Nagel et al., 1997). This was one of the first studies to clearly demonstrate a potential role of BPA as an endocrine disruptor and suggest that it had a gonadal steroidal function as the regulation of prostate growth and development is testosterone dependent. These findings were also significant because the treatment levels used in the experiments were lower than 50mg/kg level established in 1995 by the Society of the Plastics Industry (Nagel et al., 1995). Since that time a significant empirical body of evidence and literature has been shown that BPA is a major endocrine disruptor with estrogenic effects. What perhaps should not have been at all surprising based upon the origin of BPA (see below).

BPA is a synthetic monomer with well-documented endocrine-disrupting properties (Welshons et al., 2003; Le et al., 2008; vom Saal et al., 2005; Patisaul & Polston, 2008) (Figure 1). Current production of BPA is estimated at more than 6 billion
pounds per year with expected continued growth in usage (Vogel, 2009). BPA was first synthesized by A.P. Dianin in 1891 while working with phenols (Vandenberg, 2009). With no significant use the compound was to “sit” on the shelf for the next 40 years. In the 1930s, when Charles Edwards Dodds was searching for a strong estrogenic compound that could be used to treat numerous female disorders associated with things such as menstruation, menopause, nausea during pregnancy, and for the prevention of miscarriages, as estrogen was already known to play a major role in female reproduction and to be a hormone that regulated the female reproductive system (Bell, 1995). While he concluded that there was an estrogenic effect in BPA, it was not as effective as the one which he ended up developing for that purpose diethylstilbestrol (DES), but that is another story and not relevant to the current introduction (Dodds & Lawson, 1936). As a result BPA, was never used for this purpose, instead going back onto the “shelf” for another 20 years. In the 1950s, the production of artificial polymers to replace natural products was becoming a major research focus of chemists in both the United States and Switzerland who were working independently to develop adhesives and strong protective coatings. Before that time, polymerized plastics were brittle, lacking the desired strength and flexibility. However, the compound on the shelf changed the reaction of BPA in combination with phosgene (COCl₂) produced a polycarbonate resin that was remarkably strong and could undergo large plastic deformations without cracking or breaking (Caliendo, 2012).

Shortly thereafter the first epoxy resin was made, which also used BPA, and as is often said the rest is history (Vogel, 2009). BPA can now be found not only in epoxy resins, but also in most polycarbonates, which are used in metal equipment, piping, food
can linings, dental sealants, automobiles, and electronics. By the late 1980s, BPA production had reached half a billion pounds in the U.S. alone (Vogel, 2009). Although BPA’s estrogenic properties were not wholly forgotten, due to its use in plastics and sealants it’s safety was assessed by its toxicity rather than its potential to function as steroidal ligand. The Food and Drug Administration (FDA) realized BPA was slowly leaking into foods and labeled it as an indirect food additive after the 1958 Federal Food, Drug and Cosmetics Act, which directed the FDA to regulate chemicals in food (FDA, 2013). The 1958 law required that companies obtain FDA approval for the use of chemicals that may contaminate food directly or indirectly during its production, processing, packaging, and distribution. Naturally, BPA was included in the list of indirectly added food contaminants through contact with food packaging. Research at the time showed low toxicity and rapid metabolism in animals, which led the FDA to assume BPA was safe to use to package foods (Vogel, 2009).

For the next few decades, BPA use continued to grow as it was used in more and more applications, such as, CDs, DVDs, and water bottles (Vandenburg et al., 2012). It was not until 1993 that BPA’s endocrine effects inadvertently came back into the spotlight. A laboratory at Stanford University was attempting to find endogenous estrogen in yeast. They thought they had successfully found an endogenous estrogen, especially when tested with estrogen-responsive breast cancer cells, but much to their disappointment it turned out that it was not an endogenous estrogen but instead that something from the equipment they were using was altering the effects. This finding, along with the meeting conclusions reported out of Wingspread Conference, lead scientists to begin to study and create the field of endocrine disruption. By the end of the
1990s several studies had challenged the presumption that BPA was only a weak estrogen.

Despite findings of potential dangers associated with BPA which may in part be due to the fact that estrogen regulates female reproductive activity at extremely low levels (picograms per mL in females), BPA use has continued and today can be found in everything from food and beverage can linings, to medical equipment and dental sealants (vom Saal et al., 2007). In 2009 the production of BPA was estimated at more than 6 billion pounds per year with expected continued growth (Vogel, 2009). Because of the pervasive and almost ubiquitous use of BPA and its consistent leaching it is estimated that about 95% of the adult population would have traceable amounts of BPA in their urine (Calafat et al 2005; Calafat et al., 2009). In reaction to several studies citing dangerous effects of BPA, the FDA requested that more research be conducted on the effects of BPA, with a focus on dosages at or below the Environmental Protection Agency (EPA) safety level for human consumption (50 µg/kg). Therefore the goal of this thesis was to examine the effects of BPA on the development and with focus that had primarily ignored, potential effects on the expression of social behavior.

Although BPA and estradiol (an estrogen) have drastically different chemical structures, current evidence shows that BPA acts as a SERM or selective estrogen receptor modulator (Welshons et al., 2006, Richter et al., 2007) (Figure 1). SERMs are not specifically agonists or antagonists but have various estrogen effects that are tissue dependent. They are chemically diverse and lack the common steroid structure of estrogens but their tertiary structure allows them to interact and bind to estrogen receptors (Riggs & Hartmann, 2003). Originally SERMs were used to define chemicals that were
specifically agonists or antagonists in different tissues, but now the classification has expanded to include chemicals like BPA that have varying effects on different tissues and species (Welshons et al., 2006).

![Estradiol and Bisphenol A](http://en.wikipedia.org/wiki/File:Estradiol.svg)

**Figure 1.** Figures representing the structures of estradiol and Bisphenol A (BPA) (Feldman & Krishnan, 1995; [http://en.wikipedia.org/wiki/File:Estradiol.svg](http://en.wikipedia.org/wiki/File:Estradiol.svg))

BPA, relative to estradiol, interacts differently within the ligand-binding domain of estrogen receptors (ERs) (Gould et al, 2003), with transcriptional coregulators (Routledge et al., 2000), and also demonstrates a different binding affinity for and regulation of both ERα and ERβ in target cells (Kuiper et al., 1997). More interestingly, BPA, similar to estradiol, can cause rapid responses in cells utilizing non-genomic signaling systems (Quesada et al., 2002).

As with most nuclear receptors, including estrogen receptors (ER), once the hormone is bound, the receptor regulates the rate of gene transcription through its association with coregulators. As previously stated, recent studies indicate that the interaction of BPA and estradiol with ERs is significantly different, and it is probable that
BPA bound ERs produce a unique ER conformation (Welshons et al., 2006). This can explain the differences seen in interactions with coregulators between BPA bound ERs and estradiol bound ERs (Kuiper et al., 1997). Specifically, the BPA-ERβ complex had over 500-fold greater effectiveness in recruiting the coactivator TIF2 than the BPA-ERα complex even though BPA displayed a 10-fold higher binding affinity for ERβ over ERα (Kuiper et al., 1997). This relationship between ER subtype and coregulators can be an important determinant of the sensitivity of the tissue to BPA and other SERMs. Even different cell types can have unique responses to BPA within the same tissue, which further confirms BPA’s status as a SERM and can often lead to confusing or misinterpreted results (Markey et al., 2001).

In addition to nuclear receptors there is growing evidence that estradiol effects can occur through membrane receptors (Judy & Welshons, 2010). These effects are much quicker and occur in conjunction with membrane bound receptor-mediated effects (Judy & Welshons, 2010). An important characteristic to note is that cell-signaling systems utilize a very high level of amplification from a very low concentration of compound to elicit large changes in cell function. In other words, a small amount of estradiol can have a large impact on cell function if it can interact with the cell signaling pathways located on the cell membrane.

BPA has been shown to activate some of these nongenomic response systems in rat pituitary tumor cells (Wozniak et al., 2005). BPA, even at a very small dose (1 picomolar), stimulated a rapid response (less than 30 seconds) of influx of calcium and prolactin release (within 1 minute). A similar effect has been observed with low doses of estradiol (Wozniak et al., 2005). In mouse pancreatic β-cells, the same dose of BPA and
estradiol caused phosphorylation of the transcription factor cAMP response associated with rapid induction of calcium influx (Quesada et al., 2002). In addition, similar results were also seen in human breast cancer cells at a low dose (0.1 nM for BPA) and significantly affected calcium influx within one and a half minutes (Walsh et al., 2005).

Several studies have shown that BPA affects brain development during the neonatal period, which can have behavioral effects in adulthood (Cushing & Kramer, 2005, Farabollini et al., 2002, Lonstein et al., 2002). Numerous studies have shown that early life exposure to low levels of BPA results in increased anxiety and diminished social and cognitive abilities (Bateman & Patisaul, 2008; Cao et al., 2012). Specifically, BPA itself has shown to affect behavior after receiving doses not only during the neonatal period but prenatally as well (Braun et al., 2009, Dessì-Fulgheri et al., 2002, Kubo et al., 2001). Elevated gestational urinary concentrations of BPA have been correlated with hyperactivity, anxiety, and executive functions in young human girls (Braun et al., 2011; Braun et al., 2009). Dosing mother Wistar rats during pregnancy and lactation with 1.5µg/kg/day led to decreased activity levels, avoidance memory, and a significantly smaller portion of the brain associated with stress and panic response (Kubo et al., 2001). Another study found that dosing prenatal and adult female mice lead them to nurse their young significantly less than controls (Palaza et al, 2002). These studies indicate a direct relationship between changes in the brain and behavioral modification, especially if exposed to BPA prior to the onset of puberty, which can be seen in exploratory and activity levels in an open field test and exploration in an elevated plus maze during adulthood (Palanza et al., 2002).
Other studies have found perinatal responses in both males and females. The males showed increased defensive behavior against intruders, increased sexual impairment (latency and frequency of intromissions), while females showed an increase in receptivity and sexual motivation (Farabollini et al., 2002). Overall, BPA has been proven to have significant low dose effects on rodents, including effects on the male reproductive tract, brain organization/development, and metabolism.

While rodents have been the primary mammalian model system from studying the effects of BPA there is some evidence that the effects occur in other mammals including primates. The Minipumps were used to dose African green monkeys (Chlorocebus aethiops sabaeus) with the EPA safety level (50µg/kg) per day treatment abolished synaptogenic responses to estradiol, which in females is known to affect cognition and mood, suggesting a potential behavioral effect of BPA in non-human primates as well as rodents (Leranth et al. 2008).

As previously discussed, BPA can have drastically different effects on different cell types within the same tissue, which may be differentially regulated by hormone profiles resulting not only in differential tissue response but also sexually dimorphic effects (Wolstenholme et al., 2011). Differences can be seen in many areas that lead to sexual dimorphism including brain development (Patisaul et al., 2007) and behavior (Wolstenholme et al., 2011). Results reported in recent studies seem to vary drastically depending on the species, the mode of BPA introduction (orally or injection), and the time of BPA introduction (prenatal, postnatal/prepubertal, or post-pubertal). The, as of yet, unpredictable biochemical mechanisms of BPA on the tissues, makes this chemical
difficult to assess its potential neuropsychiatric consequences in humans and other animals (Beronius et al., 2010; FAO/WHO, 2011).

Most of the BPA studies have been done on rats and mice. Although they are useful rodent models for studying *in vivo* effects on brain development and sexual function, it is difficult to analyze the impact of BPA on the expression of social behavior as polygamous rodents such as rats and mice display very different sociosexual behavior than humans. As a result, it would be more appropriate to use a more socially dependent animal model when analyzing behavioral effects. Obviously humans would represent the ideal study organism, but since this is not possible there are other options. Non-human primates could be used, but many of them have social organizations that also differ significantly from humans, they have long life expectancies, are difficult to keep in the laboratory, samples sizes are limited, and they are expensive to care for. In contrast the prairie vole (*Microtus ochrogaster*) is a small rodent that display many social behaviors in common with humans, including pair bonding, the formation of long-term male female-bonds, bi-parental care of offspring, and extended families which have resulted in them becoming the major human relevant rodent model system for studying social behavior (Carter et al., 2009; McGraw et al., 2011). Although there are studies that have examined the effects of BPA in females the majority of studies have focused on males, perhaps because of endocrine disruptors known effects on sperm production and the male reproductive system, such as the prostate. My thesis examines both males and females to determine if there are effects of BPA on the expression of social behavior in prairie voles is the effect the same in males and females or is it sexually dimorphic, as previous studies would predict? Compared with other many female mammals voles are somewhat unique
and provide unique opportunities for study, as they do not complete puberty until they are exposed to males and their associated chemical cues. Female prairie voles do not undergo a spontaneous estrous cycle; estrus is induced through exposure to a novel male for a significant period of time, about 24 hours (Carter et al., 1987). This allows us to work with females without having to control for the effects of the varying levels of female hormones often associated with their usage in scientific studies. The prairie vole presents the benefits of a social model with the ease of care associated with a rodent model. Prairie voles have a communal social system similar to that of humans, consisting of the formation of long-term heterosexual pair bonds, bi-parental care, and older offspring often stay to help raise younger siblings (Getz & Hoffman, 1986, Stalling, 1990). Prairie voles are housed in pairs because long-term social isolation causes depression-like symptoms (Grippo et al., 2007, McGraw & Young, 2010). Using a rodent model allows for use of the open field test, to test for anxiety and fear, and the novel social test, to test fear, anxiety, and social behavior with an unfamiliar prairie vole. The formation of pair bonds between voles, especially with the opposite sex, opens a unique opportunity to study social behavior in a controlled environment utilizing a partner preference test (Getz et al., 1981, Carter et al., 1995). This test, explained more in depth in the methods, gives the test vole a choice between two voles of the opposite sex, one in which it spent the last few hours with or a novel vole. Both males and females have been shown in the laboratory to prefer to spend more time with the “partner” to the novel “stranger” (Getz et al., 1981, Carter et al., 1995). This provides a significant advantage over typical laboratory species as prairie voles permit the assessment of the effects on a social species, with the benefits of a rodent model. This is
one of the primary reasons that the prairie vole has become a significant resource for these types of studies.

This study utilizes three different dosages: the 5µg/kg dose which is significantly lower than the EPA safety level, the 50µg/kg dose which is the dose at which the EPA claims is safe for daily human consumption, and a high dose at 50mg/kg which is well above the EPA recommended dosage. Past studies suggest that both the males and females, especially at the lower dosage should show significant differences from the control (Welshons et al, 2006; Welshons et al., 2003). Due to BPA’s classification as a SERM and its unpredictable nature between tissues, species, and sexes it makes it difficult to provide an accurate prediction for either sex in any one of the three behavioral experiments. I tested the hypothesis that postnatal exposure to estimated safe levels of orally administered BPA would alter the sociality, pair bonding, and exploratory behavior of prairie voles.

The only study that has tested this hypothesis previously, utilized female pine voles (*M. pinetorium*), which like prairie voles are socially monogamous, born to dams orally exposed to 2 mg/kg/day of the pesticide methoxychlor during gestation and lactation spent significantly decreased time in physical contact during the partner preference test, which indicates impaired sociality (Gray & Ostby, 1998; Gray et al., 1999; Engell et al., 2006).

In conclusion, I predicted that females would be more affected than males because a prairie vole male exposed to estrogen does not feminize its behavior (Carter et al., 1987). If an effect were seen in dosed males it would most likely be in areas that could show increased fear and anxiety which I tested in both the open field and novel
social tests (Morgan & Pfaff, 2002). I predicted that dosed females would be more affected overall especially during the partner preference but also including the fear and anxiety seen in the open field and novel social tests (Carter et al., 1987; Morgan & Pfaff, 2002).
CHAPTER II
MATERIALS & METHODS

The prairie voles used in this study were laboratory raised prairie voles that originally were obtained from the wild in Urbana, Illinois. The voles were kept on a 14:10 light-dark cycle and given Purina high fiber rabbit chow and water ad libitum in the AAALAC approved animal care facility at Northeast Ohio Medical University (NEOMED) in Rootstown, OH. Breeding pairs were kept together at all times, because of their documented monogamy and biparental care (Carter & Getz, 1993). A week after the pups were born, on postnatal day 8, they were toe clipped and sexed to allow for future identification. On postnatal day 21 they were weaned from their parents and housed in same sex cages with similarly aged prairie voles in 12x18x28 cm cages. Males and females were contained in separate rooms to prevent inducing estrous in the females. Housing and procedures were done in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were preapproved by the NEOMED and University of Akron Institutional Animal Care and Use Committee (IACUC) protocol #08-032 prior to conducting any aspects of this study.
Exposure

Beginning on postnatal day 8, once a day, between 4 and 5 hours after lights on, until postnatal day 14, the entire litter was removed from their parents and placed in a clean cage with bedding. On the first day pups were weighed, toe-clipped for identification and randomly assigned to one of four treatments. No more than one treatment per sex was assigned per litter, with each litter having at least one control. Each pup received one of three doses of BPA through their week of treatment: 5µg/kg, 50µg/kg, 50mg/kg, or a vehicle control. Doses were based on average weight of pups on Day 8 and dissolved in 2.7g of hydroxypropyl beta CD – Pharm grade in 10mL 0.9% NaCl. The pups received 25µl orally delivered using a micropipette. The solution is palatable and readily consumed by the pups (Palanza et al., 2002; Taylor et al., 2010). The pups were returned to the parents as a group immediately following treatment. Other than during treatment, the pups were not handled until they were weaned at day 21, at which time they were housed in same-sex pairs in 12x18x28 cm cages.

Behavioral Testing

To determine the effect of BPA on the expression of behavior, treated animals participated in three tests beginning one week after weaning at 28 days of age. Each test is designed to examine different behavioral responses and determine age related effects. As juveniles, treated prairie voles participated in an open field and novel social interaction test, while as adults they participated in a heterosexual partner preference test.
Open Field

A week after weaning (Day 28), the animals were placed into an open field test which was developed to ascertain anxiety and locomotor activities (Palanza et al., 2002a). Analysis of excessive time spent moving in general, but especially along the perimeter or in corners, indicates an increased level of anxiety and fear (Palanza et al., 2002a). In the wild most prairie voles stay to help raise younger offspring, but around this age some may leave the nest and join a nearby colony (Getz & Carter, 1996). Therefore exploratory behavior is necessary to find new colonies and forage for food and an increased level of anxiety and fear can be detrimental to finding food, a mate, or interacting with a mate once one is found. The grid provides three different areas to analyze the prairie vole’s behavior. Figure 2 below shows a color code and two-dimensional representation of the open field arena. A juvenile prairie vole should not spend all of its time along the walls in the red corners or the orange perimeter, but should spend a majority of the time in the blue area in the center of the arena. These grid locations help me to analyze the vole’s fear levels. Spending significant amounts of time in the corners or perimeter indicates increased levels of fear, while an increase in overall movement is indicative of increased anxiety (Palanza et al., 2002a; Valle, 1970).

Animals were placed in a 40cm² Plexiglas box with blackened walls for ten minutes. Tests were video recorded from above and TopScan (Clever Systems Inc.) software was used to analyze time spent in the center, perimeter (areas with walls), and corners. Utilizing a grid on the bottom of the test area we were also able to record distance traveled in each section as well as total distance traveled.
Figure 2. A two dimensional representation of an open field test. The different colors represent the three areas that were analyzed: center (blue), perimeter (orange and red), and corners (red).

Novel Social

On postnatal day 29 or 30, the animals were given a novel social test to identify any changes in social interactions between exposure groups. As described previously, prairie voles at this age are capable of leaving the nest to find food or to find a new colony/mate (Getz & Carter, 1996). This exploratory behavior is pertinent to survival of the species and if significantly decreased could be detrimental. Prairie voles are a highly social species and the purpose of the novel social test was to assess if BPA affected social behavior as a juvenile. A decrease in time spent with the other animal would indicate BPA’s interactions in the brain. Experimental setups such as this one are routinely used to observe aggression related behaviors, and although BPA increased aggression in other rodents, it is important to note that the purpose of this experiment was is to analyze the
willingness of juveniles to interact rather than to attack one another (Bales & Carter, 2003; Kawai et al., 2002).

The experimental animal was placed in a test arena that consisted of two 12x18x28cm Plexiglas cages connected with a Plexiglas tube. A novel vole of the same size, age, and sex, was tethered in one of the cages. The tether allowed it to move freely within its’ cage but it was unable to enter the other cage through the tube. The test animal was placed into the other cage for 30 minutes. Latency to enter the stimulus cage, frequency of movement between cages, number of contact bouts, duration of time spent in the stimulus animal cage, duration of exploratory behavior, and duration of time spent in side-by-side physical contact were analyzed using TopScan.

Figure 3. A two dimensional representation of a novel social test. The test animal is free to move from the test cage to the stimulus cage and back without restriction, but the stimulus animal is tethered in the stimulus cage. The arena setup allows us to monitor social behavior of the test animal in comparison to other treatment groups.
Partner Preference

As adults, between 60-90 days of age treated animals participated in a partner preference test. A partner preference test assesses the animal’s ability to form a pair bond. Prairie voles are socially monogamous and typically form long-term pair bonds with members of the opposite sex. The early stages of pair bond formation consists of developing a preference for specific individuals and the partner preference test has been used for more than 15 years to assess this process (Getz & Carter, 1996; Alcaro et al., 2008). The objective was to determine if BPA alters the formation of social preferences and/or affects the normal pattern of heterosexual social interactions (Jones & Miller, 2008).

Animals were placed in a partner preference test, which examined the BPA’s possible effect on social interaction and formation of a partner preference (Cushing & Carter, 2000). Experimental animals were housed in a 12x18x28cm cage with a sexually naïve animal of the opposite sex but approximately the same size and age for 3 hours. Females were not tested for their stage of estrus because female prairie voles do not undergo spontaneous estrus, and estrogen levels remain low unless a female is exposed to a male for an extended period of time (about 24 hours) (Carter et al., 1980; Carter et al., 1987b; Carter et al., 1988). Once the 3-hour co-habitation was complete, the test animal was placed into the partner preference arena, which consisted of a Y-shaped configuration of three 12x18x28cm Plexiglas cages connected by two Plexiglas tubes. Two of the cages are in parallel and house the stimulus animals and have no direct access to each other. They have direct access to a third cage (neutral), which is attached to each stimulus cage by a separate Plexiglas tube. In one of the cages, the stimulus animal that
had spent the last 3 hours in cohabitation with the test animal was tethered, which will
now be called the “partner.” In the other parallel cage a stranger of the opposite sex but
similar in size and age to the test animal, was tethered. The test animal was placed into
the neutral cage and its movements and actions were video recorded for three hours.
TopScan was used to analyze and record the duration of time spent in each cage and
duration spent side-by-side contact with either the partner or the stranger. By definition a
partner preference is formed when the test animal spends significantly more time in
physical contact with the partner than the stranger (Young & Wang, 2004; Carter et al.,
1980).
Figure 4. A two dimensional representation of a partner preference test. The test animal has the ability to move freely, while the stranger and partner animals are tethered within their respective cages. The arena allows for analysis of the test animal’s social behavior with both the partner and stranger.

Statistical Analysis

Analysis was done within sex because these behaviors are known to be sexually dimorphic (Carter et al., 1995; Carter & Getz, 1993; Carter et al., 1980; Carter et al., 1992). Statistical analysis was done using SPSS v 20.0.
Open Field

To analyze the duration the test animal spent in the three separate zones (center, perimeter, corners) a one-way between-groups analysis of variance (ANOVA) was used to determine if there was a treatment effect (P < 0.05). If there was a significant effect a Fisher’s LSD was used for post hoc pairwise comparisons (P < 0.05). Analysis of the bouts or the number of times the test animal entered a new zone was done by using a non-parametric Kruskal-Wallis to analyze significance between the groups as a whole and a Mann-Whitney U was used to determine the significance of the treatments against one another.

Novel Social

To analyze the duration the test animal spent in the two separate cages (non-social and stranger) a one-way between-groups analysis of variance (ANOVA). If there was a significant effect a Fisher’s LSD was used for post hoc pairwise comparisons (P < 0.05). Analysis of the bouts or the number of times the test animal entered a new cage was done by using a non-parametric Kruskal-Wallis to analyze significance between the groups as a whole and a Mann-Whitney U was used to determine the significance of the treatments against one another.

Partner Preference

A one-way between-groups analysis of variance (ANOVA) was used to analyze data collected on duration including time spent in one of the cages (partner, stranger, non-social) or time spent doing a certain activity exploratory contact or side-by-side contact.
If there was a significant effect a Fisher’s LSD was used for post hoc pairwise comparisons (P < 0.05). T-tests were used to assess if any of the treatment groups significantly preferred to either spend more time with the partner or the stranger as well as if they preferred to have exploratory contact or side-by-side contact with either the partner or the stranger.
CHAPTER III

RESULTS

Open Field

The results from the open field tests indicated that there were overall treatment effects of BPA and the effects were sexually dimorphic, occurring only in females, and varied depending on the variable being measured.
There was a significant overall treatment effect for the distance traveled along the perimeter ($F_{(3,75)}=3.527, p < 0.05$) (Figure 5). These differences were due to significant differences if the females treated with $5\mu$g/kg traveling significantly farther than both the control and the $50\mu$g/kg treated females ($p<0.05$). Error bars represent SEM.

There was also an overall treatment effect for total distance traveled across the treatments ($F_{(3,75)}=3.408, p < 0.05$) (Figure 6). The voles treated with the $5\mu$g/kg dose
traveled significantly farther than those treated with the 50mg/kg dose, but the distance traveled was not significantly different from the control or the 50µg/kg dosage.

Figure 6. The total distance (m) traveled by females and males by treatment. Females treated with 5µg/kg traveled significantly farther than the 50mg/kg treated females (p<0.05). There was no significant difference between the male treatment groups. Error bars represent SEM.

Analysis of the bouts (the number of times the test animal entered a specified area) revealed a significant difference across all treatment groups in all three areas: center ($\chi^2_{(3, 79)} = 10.981, p < 0.05$), corners ($\chi^2_{(3, 79)} = 10.302, p < 0.05$), and the perimeter ($\chi^2_{(3, 79)} = 12.699, p < 0.05$). For the center area, bouts at the 5µg/kg dosage was significantly higher than both the 50µg/kg and the 50mg/kg doses, but not the control (Figure 7).
Females treated with 50mg/kg had significantly fewer bouts of entering the corner or perimeters than control females (Figure 7). There was also an apparent dose effect with females treated with 5µg/kg entering the corners and the perimeter at a significantly higher rate than the higher BPA treated females, but they did not differ significantly from the control females (Figure 7).

![Figure 7](image.png)

**Figure 7.** The number of bouts or times the female enters a defined area during the open field test by treatment and area of the open field. Females treated with 5µg/kg entered the center, corner, and perimeter significantly more than both the 50µg/kg and 50mg/kg dosages (p<0.05). Females treated with 50mg/kg entered the corner and perimeter areas significantly less than the control and the 5µg/kg treatment (p<0.05). Error bars represent SEM.
There was no overall treatment effect for the amount of time spent in any of the specified areas: the center ($F_{(3, 75)} = 0.727, \text{ ns}$), the perimeter ($F_{(3, 75)} = 0.755, \text{ ns}$), and the corners ($F_{(3, 75)} = 1.509, \text{ ns}$) (Figure 8).

![Figure 8](image_url)

**Figure 8.** The duration of time the females spent in the defined areas of the open field test. There was no significant difference across treatment groups (ns). Error bars represent SEM.

**Males**

In males, there was no overall treatment effect for any of the parameters measured in the open field test. There was no significant effect seen in duration spent in any of the areas: center ($F_{(3, 52)} = 1.049, \text{ ns}$), corners ($F_{(3, 52)} = 0.221, \text{ ns}$), and perimeter ($F_{(3, 52)} = 0.536, \text{ ns}$). There was also no significant treatment effect for distances traveled in any of the areas: center ($F_{(3, 52)} = 0.482, \text{ ns}$), corners ($F_{(3, 52)} = 0.221, \text{ ns}$), perimeter ($F_{(3, 52)} = 0.952, \text{ ns}$),
and total distance ($F(3, 52)=0.654, \text{ ns}$) (Figure 6). Similarly, there was no significant treatment effect seen in the amount of bouts among the different areas: center ($\chi^2(3, 52)=2.266, \text{ ns}$), corner ($\chi^2(3, 52)=2.038, \text{ ns}$), and perimeter ($\chi^2(3, 52)=1.583, \text{ ns}$).

**Novel Social**

Significant treatment effects were observed only for females in the novel social test. As in the open field test, results were dose-dependent and sexually dimorphic. Females

There was a significant treatment effect for the number of exploratory contact bouts ($\chi^2(3, 76)=12.022, p < 0.05$). The differences were due to a significantly higher number of exploratory contact bouts by the 5µg/kg dosed females compared to all other treatments. All other comparisons of the number of bouts yielded no significant treatment effects: stranger cage ($\chi^2(3, 76)=6.632, \text{ ns}$), non-social cage ($\chi^2(3, 76)=6.527, \text{ ns}$), and side-by-side contact ($\chi^2(3, 76)=1.343, \text{ ns}$).
Figure 9. The number of bouts or times females engaged in either exploratory or side-by-side social contact during the novel social test by treatment. Females treated with 5µg/kg engaged in exploratory contact significantly more than all other treatment groups (p<0.05). There were no significant differences between the groups for side by side contact (ns). Error bars represent SEM.

Overall there was no significant treatment effect on the duration of time spent in either the stranger ($F_{(3,76)} = 0.749$, ns) or the non-social cage ($F_{(3,76)} = 0.560$, ns) nor was there an effect seen in the amount of time spent in exploratory ($F_{(3,76)} = 0.169$, ns) or side by side contact ($F_{(3,76)} = 2.440$, ns).
Figure 10. The amount of time females spent in social contact with the stranger, whether exploratory or side-by-side contact, by treatment. Analysis revealed there were no significant differences between treatment groups (ns). Error bars represent SEM.

Males

For the males, there was no significant treatment effect for any of the parameters measured in the novel social test. Duration of time spent in either cage, stranger ($F_{(3,50)}=0.503$, ns) or non-social ($F_{(3,50)}=1.109$, ns), or time spent in either type of contact, exploratory ($F_{(3,50)}=0.334$, ns) or side-by-side ($F_{(3,50)}=1.034$, ns), yielded no significant effect (Figure 11). The same is true for the number of bouts entering either cage, stranger ($\chi^2_{(3,50)}=4.881$, ns) or non-social ($\chi^2_{(3,50)}=3.935$, ns), and the number of bouts for both
types of contact, exploratory ($\chi^2_{(3, 50)}=3.081$, ns) or side-by-side ($\chi^2_{(3, 50)}=1.101$, ns), had no significant treatment effect (Figure 12).

Figure 11. The amount of time males spent in social contact with the stranger, either exploratory or side-by-side contact, across all treatment groups. Analysis revealed there were no significant differences between treatment groups (ns). Error bars represent SEM.
Figure 12. The number of bouts or times males engaged in either exploratory or side-by-side social contact during the novel social test by treatment. Analysis revealed there were no significant differences between treatment groups (ns). Error bars represent SEM.

Partner Preference

As the other two test results indicated that while there were treatment effects these effects were dose-dependent and limited to females.

Females

There was a significant preference for the partner for the amount of time spent in the partner cage by the 50µg/kg dose ($t_{16}=2.870$, $p < 0.05$) (Figure 13) and in side-by-side contact with the partner by the control ($t_{20}=2.165$, $p < 0.05$) (Figure 14).
Figure 13. The amount of time females spent in the partner or stranger cage by treatment. Females treated with 50μg/kg spent significantly more time with the partner (p>0.05). The other treatment groups showed no significant preference for either cage (ns). Error bars represent SEM.
Figure 14. The amount of time females spent in side-by-side contact with the partner or the stranger by treatment. Females treated with either the control were significantly more likely to spend more time in side-by-side contact with the partner rather than the stranger ($p > 0.05$). Error bars represent SEM.

There were no other significant preferences for duration spent with the partner or the stranger for the control ($t_{20}=0.808$, ns), 5µg/kg ($t_{16}=1.511$, ns), and the 50µg/kg ($t_{16}=1.219$, ns) (Figure 9). There was also no significant preference between the partner and stranger for duration of side-by-side contact for the 5µg/kg ($t_{16}=1.642$, ns), 50µg/kg ($t_{12}=1.971$, ns), and the 50mg/kg doses ($t_{16}=1.427$, ns) (Figure 14). Although there was no statistical difference at other dosages (e.g., 5µg/kg), the animals seemed to prefer the partner compared to the stranger. Lastly, there was no significant preference for
exploratory contact between the partner and the stranger for the control ($t_{20} = 0.865$, ns), 5µg/kg ($t_{16} = 1.189$, ns), 50µg/kg ($t_{12} = 1.788$, ns), and the 50mg/kg ($t_{16} = -0.049$, ns) (Figure 15).

![Bar graph showing time spent in exploratory contact]

**Figure 15.** The amount of time females spent in exploratory contact with the partner or the stranger by treatment. Females showed no significant preference for either the partner or the stranger independent of treatment (ns). Error bars represent SEM.

There were no significant treatment effects seen in any of parameters based on duration: non-social cage ($F_{(3,64)} = 0.953$, ns), partner cage ($F_{(3,64)} = 0.642$, ns), stranger cage ($F_{(3,64)} = 1.025$, ns), side-by-side contact with partner ($F_{(3,64)} = 0.314$, ns), exploratory contact with partner ($F_{(3,64)} = 1.175$, ns), side-by-side contact with stranger ($F_{(3,64)} = 0.350$, ns), exploratory contact with stranger ($F_{(3,64)} = 0.887$, ns), total social contact
(F(3,64)=0.424, ns), total exploratory contact (F(3,64)=0.457, ns), and total side-by-side contact (F(3,64)=0.403, ns).

Males

Analysis revealed that all parameters tested in the males were not significant. All categories measured by duration were not significant including time spent in the non-social cage (F(3,53)=0.360, ns), partner cage (F(3,53)=0.58, ns7), side-by-side contact with the partner (F(3,53)=0.972, ns), exploratory contact with the partner (F(3,53)=0.164, ns), side-by-side contact with the stranger (F(3,53)=0.720, ns), exploratory contact with the stranger (F(3,53)=1.335, ns), total social contact (F(3,53)=0.303), total exploratory contact (F(3,53)=0.332, ns), and total side-by-side contact (F(3,53)=0.478, ns).

There was no preference for either the partner or the stranger in any of the three parameters that were analyzed. There was no significant preference for duration spent with either the partner or the stranger for the control (t_{14}=0.661, ns), 5µg/kg (t_{15}=0.049, ns), 50µg/kg (t_{9}=1.190, ns), and the 50mg/kg (t_{15}=-1.005, ns). Similarly, there was also no significant preference between the partner and stranger for duration of side-by-side contact for the control (t_{14}=0.763, ns), 5µg/kg (t_{15}=0.158, ns), 50µg/kg (t_{9}=1.410, ns), and the 50mg/kg (t_{15}=-0.299, ns). Lastly, there was also no significant preference for exploratory contact between the partner and the stranger as well for the control (t_{15}=-0.585, ns), 5µg/kg (t_{15}=0.609, ns), 50µg/kg (t_{9}=0.609, ns), and the 50mg/kg (t_{15}=-1.768, ns).
CHAPTER IV
DISCUSSION

The results of my experiment supported the hypothesis that neonatal exposure to BPA can alter behavior and suggests that BPA is functioning as a SERM (Welshons et al., 2003; Welshons et al., 2006). The effects were dose dependent and sexually dimorphic. These findings were significant for two reasons. First the majority of the effects were associated with the lowest, FDA approved, level of BPA treatment. Second, the effects were only seen in females. Although not unheard of in other rodents tested, it is of interest that even the expected results for the males, such as partner preferences, were not significant (Poimenova et al., 2010; Jasarevic, 2011; Xu, 2011; Welshons et al., 2006).

The significant effects observed were found to be sexually dimorphic in a highly social species, of which the primary effects were observed in the females. The males showed no significant effects at any dosage for any of the parameters measured during the three behavioral tests. Current studies have confirmed that BPA can have sexually dimorphic effects, which ultimately leads to different behavioral effects (Howdeshell et al., 1999; Welshons et al., 2006). Male mice dosed with BPA caused them to be less “masculine” due to severely compromised spatial learning abilities and exploratory behaviors compared with control males (Jasarevic, 2011). The cause of this change in
behavior may be due to BPA’s effects on male testicular functions or the possible effects on brain chemistry (Tohei et al., 2001; Farabollini et al., 2002). As previously established, differential actions of gonadal steroids during the perinatal period plays a crucial role in permanently organizing sexual dimorphisms in behavior and its underlying neural substrates (Arnold and Gorski, 1984). Looking at my results, the BPA had no effect on males except that none of the males formed a partner preference with either the partner (as expected) or the stranger, including the control. This may have been caused by a short cohabitation period and it would be interesting to retest the males with the same protocols, but with an increased cohabitation period may elicit a response (Williams et al., 1992). It is worth mentioning that there is evidence of partner preference forming with a shorter cohabitation time, although not successfully in males (Cushing & Carter, 2000).

Several other factors may have influenced the observed lack of significant effects on males. There may be different sensitivity levels and periods of sensitivity between the sexes, but this has not been studied in prairie voles (Fitch & Denenberg, 1998). It is possible that the dose that would have affected the males was missed due to the range of dosages used. Using more dosages even over a shorter range may elicit a different behavioral response in male prairie voles. Other factors include complete insensitivity to BPA, timing of exposure, or duration of exposure. The timing of exposure was based on previous evidence of postnatal days 8-14 being a significant time for the organizational roles of steroids in male prairie vole social behavior (Kramer et al., 2009). A more likely possibility is that when prairie vole males, unlike rats, are given estrogen treatments during neonatal development it does not feminize their behavior (Carter et al., 1987).
Blocking aromatase activity yields similar results with no feminization of behavior in male prairie voles (Northcutt & Lonstein, 2008). This may imply that BPA is acting through similar molecular pathways as estrogen and therefore is not affecting male behavior in adulthood.

Lastly, the location and development of estrogen receptor (ER) alpha may have a significant impact as well (Cushing et al., 2004). It has been extensively established in the literature that BPA affects ER alpha, although these effects are not very well documented for prairie vole social behaviors (Gould et al., 1998; Routledge et al., 2001; Kuiper et al., 1997). The developmental period chosen, although pertinent to brain development, may have missed the time period at which ER alpha was being expressed (Kramer et al., 2009; Kuhnemann et al., 1994). This may explain why the females were so affected, because their ER alpha-receptors were being expressed at a higher rate causing a more definitive change in brain chemistry and behavioral response later in life.

As stated earlier, the brains from this study were collected and sent to Dr. Heather Patisaul and her laboratory at North Carolina State University in Raleigh, NC. Their focus was to look at oxytocin and vasopressin immunoreactive neurons in the paraventricular nucleus of the hypothalamus (Sullivan et al., unpublished). The analysis revealed that females exposed to the 50mg/kg dosage of BPA had more vasopressin immunoreactive neurons in the anterior paraventricular nucleus and fewer oxytocin immunoreactive neurons in the posterior paraventricular nucleus. Oxytocin and vasopressin have been linked to the mesolimbic dopamine pathways in several vole species over the years (Carter et al., 2009; Dick et al., 2009; Gabor et al., 2012; Williams et al., 1992; Insel, 2010). Recently the importance of these neuropeptides has been
recognized in humans in that intranasal oxytocin administration may be a potential therapy for autism and further manipulation of oxytocin and vasopressin may have therapeutic uses for depression (Meyer-Lindenburg et al., 2011; Guastella et al., 2010; Rotzinger et al., 2010).

The mesolimbic dopamine pathway plays key roles in several behavioral features including the reward pathway and other cognitive functions (Alcaro et al., 2008). The dopamine pathway is a key player in social behavior and the development of partner preferences in prairie voles (Wang & Aragona, 2004). The close relationship of sex steroids to the dopamine pathway may explain the results seen in the both the male and female partner preference results. The importance of dopamine in the formation of male prairie vole partner preference has been established, and if BPA interacts with this pathway, it may offer an explanation for why no partner preference was observed (Aragona et al., 2003). The only group in the study to show a partner preference was the control females. This shows a strong indication that BPA can lead to a rewiring of the brain, which can significantly affect behavior later in life. Neonatal manipulation of sex steroids such as estradiol and testosterone can affect prairie vole behavior later in life (Kramer et al., 2009; Lonstein et al., 2005; Roberts et al., 1997). Males castrated on the day of birth were unsuccessful in forming a pair bond even after vasopressin administration (Cushing et al., 2003). In early life manipulation of the number of neurons producing oxytocin and vasopressin in the paraventricular nucleus was associated with anxiety and changes in the normal male and female social behaviors (Ahern & Young, 2009; Martin et al., 2012; Yamamoto et al., 2004). As a result, changes
in the neuron density caused by endocrine disrupting chemicals such as BPA may alter the capacity to engage in normal social contact and pair bonding.

The females showed significant effects of the BPA dosages during all three of the behavioral tests performed. The lowest dosage, 5µg/kg, had more significant effects in both the open field and novel social tests, and is consistent with the many low-dose responses seen in the literature (Welshons et al., 2003; Welshons et al., 2006). Overall, BPA influenced behaviors associated with activity, sociality, and anxiety in female prairie voles. These results only further support the need not only for more research but also for a re-evaluation and re-characterization of BPA by the FDA (vom Saal et al., 2007).

In the open field test, females showed a variety of interesting effects from the BPA dosages. The open field test measures anxiety and fear by measuring the distance traveled especially in specific areas of the square arena. Results showed a significantly larger total distance traveled by the 5µg/kg dosage when compared to the 50mg/kg dosage. The significant increase in total distance traveled is indicative of increased anxiety (Palanza et al., 2002a; Valle, 1970). Increased anxiety in rodents, like in humans, leads to increased movement and pacing (Walsh & Cummins, 1976). The conclusion that the 5µg/kg (low) dose have increased anxiety responses was further supported by the significantly greater distance traveled along the perimeter compared to both the control and the 50mg/kg dosages. These findings correlate with findings in other rodent species such as mice and rats, where developmental exposure to BPA lead to increased anxiety and fear behaviors in females (Kubo et al, 2003; Bryce & Vandenburg, 2006; Patisaul & Bateman, 2008). For future study it would be informative to investigate the possible
significance of the observed decrease in anxiety in the 50mg/kg (highest) dose because it suggests a calming or anti-anxiety effect, though this observation is not supported by any other studies in the literature.

The novel social test yielded only one significant result. The 5µg/kg dosage had significantly more exploratory contact bouts than any other treatment including the control. Their average of 60 exploratory contact bouts far exceeded any of the other averages per dosage level. But it is interesting to note that none of the other parameters were significant, even time spent in the stranger arena. In other words, all treatments spent about the same amount of time in the stranger arena and in side-by-side contact with the novel male, but the 5µg/kg dosed females showed an increase in the number of times touching and sniffing the stranger. In the literature, the novel social test as preformed for this study is more often used to examine aggression rather than normal social behavior (Bales & Carter, 2003). In this case, I predicted correctly that the animals would not be aggressive to one another when placed into a novel environment. The goal of this study was not to look at the aggressive effects of BPA, but it is worth noting that some studies have found that BPA increases aggression (Kawai et al, 2002). No prairie voles, male or female, showed any signs of aggression during any interaction with the other animals in the novel social or partner preference tests.

Overall, the results from the open field and novel social tests support the findings in the open field test and the literature, which indicates that the 5µg/kg-dosed females are more anxious and hyperactive (Patisaul & Bateman, 2008, Ryan & Vandenbergh 2006, Poimenova et al., 2010; Ogi et al., 2013; Masuo & Ishido, 2011; Viberg & Lee. 2012). Similar findings have even been seen in human children, where gestational BPA
exposure was associated with higher scores for measures of anxiety and hyperactivity (Braun et al., 2011).

The partner preference test showed the only test group, including males, to have a significant preference for either the partner or the stranger was the 50µg/kg-dosed females. The 50µg/kg females preferred to spend time with partner to the stranger, which was not observed in the control. Other studies have utilized a longer cohabitation period, but it seems that the 50µg/kg dosed females have shown an increase in the speed to form the partner preference compared to controls and other dosages. Reasons for the lack of partner preference are difficult to determine but may indicate an unforeseen complication of parents that were exposed to BPA dosed pups over multiple litters (Young & Wang, 2004; Carter et al., 1980). The female control showed no preference to spend more time in the partner arena over the stranger arena; they did significantly prefer to have side-by-side contact with the partner to the stranger. As a result, even though the time spent between the partner and the stranger was about the same, when with the partner the test female preferred to be in side-by-side contact. In males it has been argued that mating is required for the formation of partner preferences, but this claim has not been made for females. If the effect of BPA on males is subtle and no mating occurred then a significant effect may not have been seen (Insel et al., 1995). This is an indication of partner preference and, an argument could be made that it is a better indication of a partner preference because it shows preference of contact with the partner rather than being in the same area or in close proximity to the partner.

The results further add to the enormity of evidence of the dangerous and potentially harmful effects of BPA, but it leads to more questions (Welshons et al 2003;
Welshons et al., 2003). Discovering the mechanism of how BPA directly affects the receptors, and specifically which receptors, would be an important advance, but one that seems currently out of reach. There are many studies that have shown molecular responses to low-level exposure to BPA including: calcium influx in rats and humans, and cAMP phosphorylation in mice (Quesada et al., 2002; Walsh et al., 2005; Wozniak et al., 2005). These findings show the potential systemic impact of low-level BPA exposure because calcium influx often precedes the release of hormones and neurotransmitters, which eventually can alter cell activity and metabolism through phosphorylated cAMP. The manipulation of both the release of hormones and the cellular metabolism provides an explanation for why current studies struggle to find BPA’s mechanism of action. With such a wide range of actions, this drug may be having a larger impact than anyone may have foreseen.

Recall that this study only dosed the animals for one week during development and although this week was chosen specifically for its importance in brain development, it begs the question that if these animals were dosed for most of their life would it have caused more damage (Cushing & Wynne-Edwards, 2006; Kramer et al., 2009; Northcutt & Lonstein, 2008; Roberts et al., 1997).

The results of this study showed that BPA may have significant effects at low dosages in a humanly relevant rodent social model. It confirmed many findings from other studies done in both mice and rats (Welshons et al., 2006). Although the effects in this study are fairly minimal the increased production of BPA, and therefore human exposure to BPA, could be linked to national increases in neurobehavioral problems such as autism and attention deficit hyperactivity disorder (ADHD) (vom Saal et al., 2007).
These connections have not been wholly proven, but even the results from this study alone indicate a very real and potentially harmful effect to neurological and behavioral development. When this study is taken in conjunction with other studies done in rodents, primates, and limited human trials it is appalling to note the continued abundance of this chemical in our everyday lives. Unfortunately, the EPA has stated as recently as March of 2012 that it will be keeping BPA in packaging and food products even with the large number of studies resulting in a variety of physiological and behavioral effects (Welsons et al., 2003, Carollo, 2012). They have stated that more evidence is needed to substantiate complete removal of the chemical from the U.S. food packaging market (Carollo, 2012).

Further studies should include a positive control to examine how a stronger estrogenic compound such as DES would affect the behavior and brain chemistry (Welshons et al., 2003), specifically in the male prairie voles. It would be interesting to also investigate the possible effects of the BPA dosages on the parents. If parenting is somehow affected by the BPA released through the pups’ urine it could affect the study drastically without the experimenter’s knowledge (Calafat et al., 2008).

In conclusion, this study provides more examples of how dosages of BPA below the EPA estimated safety level have significant and long-term effects on the expression of behavior. This study further supports the sexually dimorphic actions BPA and that it can have a significant impact in females (Ogi et al., 2013; Perera et al., 2012). The results of this study encourage caution when using this substance and further promote the re-evaluation of the negative effects it may be causing not only by the FDA but globally as well.


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