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The Effects of Arginine Vasopressin on Maternal Behavior and Aggression in *Peromyscus californicus* Mothers

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The Effects of Arginine Vasopressin on Maternal Behavior and Aggression
in *Peromyscus californicus* Mothers

by

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Abstract

Research studies since the 1950s have shown that a chemical within the brain called arginine vasopressin (AVP) is associated with the modulation of many different social behaviors in mammals. Some of these behaviors are related to parenting, such as parental care initiation, aggression, social recognition, depression and anxiety. Understanding the physiology behind AVP regulation could allow for the creation of new therapies for treating human social disorders, such as using an AVP receptor antagonist to attenuate anxiety. This project examines how neural injections of AVP and an AVP receptor antagonist affect both maternal care and aggression in female *Peromyscus californicus*. Though not yet statistically significant, the data suggest that administration of AVP lessens maternal behavior.

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INTRODUCTION

AVP

Arginine vasopressin (AVP) is a neuropeptide that is mainly synthesized in the hypothalamic magnocellular cells of the supraoptic nucleus (SON) and paraventricular nucleus (PVN). Axons from the SON and PVN project to the posterior pituitary where AVP is released into the bloodstream to maintain fluid balance and blood pressure (Caldwell et al., 2008). AVP is also synthesized and released by several populations of smaller, parvocellular neurons whose projections remain in the brain and are associated with the central effects of AVP. These parvocellular populations are located in the PVN, bed nucleus of the stria terminalis (BNST), medial amygdala (MeA), and suprachiasmatic nucleus (SCN) (Caldwell et al., 2008). These areas have a role in the regulation of social behavior. Particular parvocellular AVP neurons in the PVN project their axons to the portal capillary plexus of the anterior pituitary (Fig. 1) and induce secretion of anterior pituitary hormones such as adrenocorticotropic hormone (ACTH), implicating AVP in physiological stress-related responses (Koshimizu et al., 2012).

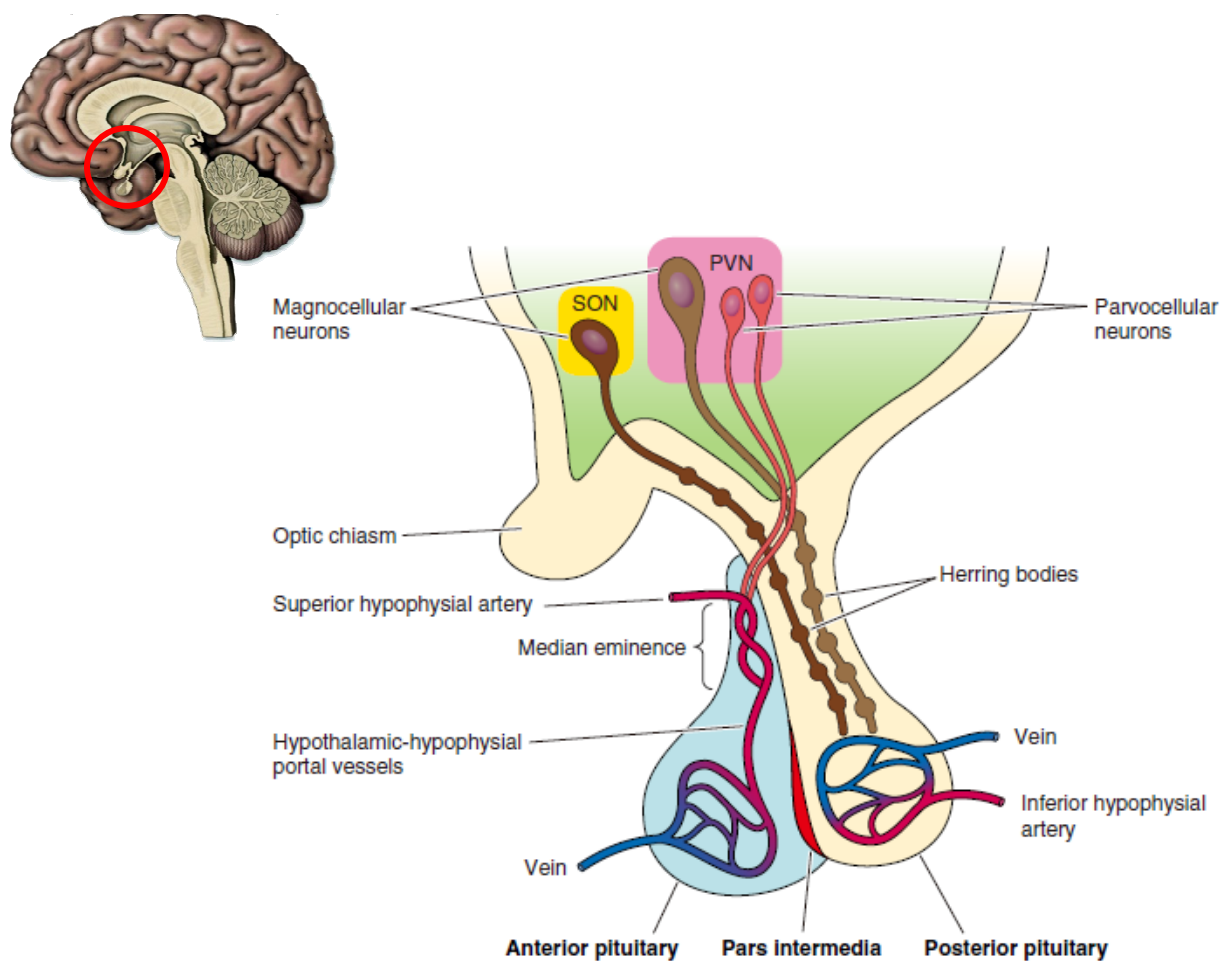


Figure 1. A simplified diagram of the SON and PVN cell groupings in the hypothalamus and how they project into both the anterior and posterior pituitary for regulation of peripheral physiological processes (Koshimizu et al., 2012) (brain image from Barbeau, 2004).

AVP is recognized by three distinct AVP receptors: V1a, V1b, and V2. These receptors are distributed throughout the body. Peripherally, the V1a receptor (V1aR) is notably found in the liver, kidney, and vascular walls. The V1b receptor (V1bR) is associated with the anterior pituitary (mediating ACTH secretion), the pancreas (linked to insulin secretion), and the adrenal glands (linked to catecholamine release). The V2 receptor (V2R) is found in the renal distal tubules and collecting ducts, and stimulates water absorption (Caldwell et al., 2008; Koshimizu et al., 2012; Stevenson & Caldwell, 2012). Centrally, only the V1aR and V1bR subtypes are expressed; V1aRs are located in a variety of places throughout the brain and V1bRs are prominent in more specific regions such as the anterior pituitary, the PVN, and the olfactory bulb, although the exact mapping of V1bRs has still yet to be discovered (Stevenson & Caldwell, 2012). Though most research has focused on V1a receptors' role in behavior, both central V1a and central V1b receptors have demonstrated importance in certain social behaviors (Bielsky et al., 2005). Research has shown that vasopressin acting upon these receptors regulates maternal and paternal affiliative behavior, aggression, social recognition, and anxiety in mice and rats (Bielsky et al., 2005; Caffrey et al., 2010; Stevenson & Caldwell, 2012). Despite the overlapping role of AVP in maternal and paternal behavior, there is substantial sexual dimorphism in AVP receptor expression. For example, male rats, prairie voles, meadow voles, and CD1 mice show more AVP-immunoreactive (AVP-ir) staining in the BNST and its projections to the lateral septum (LS) than do females, due to the fact that AVP V1a expression appears to be testosterone dependent (De Vries et al., 1986; Bester-Meredith et al., 2015). While this fact had previously led researchers to believe that vasopressin mainly modulated male behavior instead of female, recent research supports AVP's role in female rodent behavior (Bester-Meredith et al., 2015).

AVP modulates maternal care and aggression in rodents

Maternal care in mammals is essential to the survival of the offspring; mothers often display common behaviors such as nursing, huddling, grooming, and protecting their offspring to meet their offspring's basic needs. Maternal aggression is also an important aspect of maternal behavior, providing protection of the offspring from intruders and potential threats. In a study conducted with *Peromyscus leucopus* and *Peromyscus maniculatus* mice, pups were attacked by both male and female intruders 82 out of 84 times when the mother was absent; the intruders were conversely deterred in 83 out of 88 trials when the mother was present, demonstrating the importance of maternal aggression in rodent species (Wolff, 1985).

Before maternal behavior is expressed, females undergo a variety of hormonal and neurological changes that prime them for the care of their young (Bosch & Neumann, 2012). In one monogamous rodent species, *Microtus ochrogaster* (prairie vole), virgin females typically commit infanticide until maternal behavior is induced by parturition (Hayes & Vries, 2007). Reproductive state also has been shown to affect frequency of infanticide in *Mus musculus*: prepubertal females were the least likely to exhibit infanticide (39%), then adult virgin females (61%), and then pregnant females (>90%). Though the pregnant females were the most likely to commit infanticide, after parturition previously infanticidal female *M. musculus* cared for their own litters (McCarthy & Vom Saal, 1985).

In mothers, the AVP system is activated near parturition and in lactation; AVP mRNA expression within the PVN increases, and AVP release within distinct limbic brain regions has been observed during parturition (Bosch, 2011). Increased V1a-receptor binding was exhibited in lactating Wistar rats compared to virgin females and the full-range of maternal care in rats tended to occur with increased AVP release (Bosch et al., 2010). Administration of V1a-antagonist inhibited the recognition of pups in primiparous Sprague–Dawley rats, as shown by an increased latency of maternal care towards their pups (Nephew & Bridges, 2008^a). These experiments demonstrate the significant impact AVP has on maternal care in mammals.

AVP has been implicated, by a number of studies, in the regulation of maternal aggression in different rat species. There are some conflicting data in this area of research. A study done with Sprague-Dawley rats showed decreased maternal aggression towards a male intruder in both early and late-lactating mothers (primiparous and multiparous) treated with AVP (Nephew & Bridges, 2008^b, 2010). However, Bosch et al. (2010) discovered that V1aR-antagonist similarly decreased maternal aggression in Wistar rats, though in this experiment they used a female intruder. Bosch et al. (2012) also highlighted a correlation between high-anxiety and increased aggression in Wistar rats, and demonstrated that administration of AVP increased maternal aggression in rats bred for low anxiety. Additionally, functional magnetic resonance imaging (fMRI) showed that brain regions containing V1a receptors in female Long-Evans rats become active when dams respond aggressively to an unfamiliar male (Caffrey et al., 2010). The complex nature of maternal aggression contributes to these discrepancies, as AVP-regulated motivation for maternal aggression may be influenced by anxiety (mothers attack from anxiety rather than aggression), exposure to male intruders, and emotional state (Caffrey et al. 2010). Thus, more investigation is needed to discover the specific roles of centrally located AVP receptors in maternal aggression, anxiety, and emotion (Caffrey et al., 2010).

AVP and humans

Understanding the neuronal pathways in which AVP is expressed may lead to novel treatments for human social and behavioral disorders. For example, because V1bR knockout (KO) mice show attenuation in stress-related disorders such as depression and anxiety, modulation of emotional processes via the V1bR and its blockade may represent a novel possibility for the treatment of affective disorders (Koshimizu et al., 2012).

AVP is generally positively correlated with behaviors such as anxiety, aggression, and social recognition in both rodents and humans (Caldwell et al., 2008). Notably in rats, increased anxiety-related behavior is correlated with increased maternal care and aggression (Bosch, 2011, 2013). Augmented anxiety and maternal care could possibly be related to increased expression of AVP, lending AVP to further investigation for use as a possible pharmaceutical for post-partum depression (PPD); PPD is strongly associated with disturbances in the mother-infant relationship, often resulting in insensitive engagement towards one's child (Cooper et al., 1999).

However, overexpression of AVP is also linked to the inability to properly process social cues and form social bonds. In humans, elevated levels of AVP in the CSF and blood plasma are related to the severity of schizophrenia/anxiety disorders, possibly due to increased stress and hyperactivity of stress response systems (like the HPA axis), and mood disorders such as depression (Caldwell et al., 2008; Neumann & Landgraf, 2012; Rubin et al., 2013). AVP has been

shown to promote the secretion of ACTH which increases the endocrine stress response in humans (Meyer-Lindenberg et al., 2011). Whether or not this stress response would have positive or adverse social effects on parent-child social interaction is unclear; therefore, further investigation of AVP's effects on behavior is needed.

In both rats and humans, social contact has a large impact on the emotional and social development of the offspring (Bosch & Neumann, 2012). Child neglect is a serious, global problem. Children who are neglected (no parents or consistent caretaker) during the first 24-months of life exhibit dramatic impairment of social and neurological development; 12-year-old children who had experienced childhood neglect scored two standard deviations below the mean on intelligence and development quotients for their age group, and showed significant deficiency in brain tissue and electroencephalogram activity (Marshall, 2014). Early stress may cause alterations in the AVP system that interfere with the developing neuropeptide system and thereby promote the development of severe attachment disorders (Heinrichs et al., 2009). For example, borderline personality disorder (BPD) is associated with a remarkably high prevalence of severe childhood trauma and neglect; it has been associated with enhanced amygdala reactivity to negative scenes, negative facial expressions, and even to neutral faces (Heinrichs et al., 2009). This negative reactivity to neutral faces may be facilitated by overexpression of AVP, for even in healthy human males, intranasally administered AVP caused subjects to respond to neutral facial expressions in a magnitude similar to that of the placebo subjects' response to angry facial expressions (Thompson et al., 2004). This demonstrates that the AVP pathway may play a part in the abnormal social reactivity of neglected youth.

Therefore, it appears that AVP is an important neuropeptide for the development of maternal behavior, as well as the development of offspring's response to maternal behavior. Investigating the mechanisms underlying these parental cues will significantly aid our understanding of maternal care and the neurochemistry of parent-child bonding.

Peromyscus californicus as a model organism

Peromyscus californicus mice exhibit monogamous and biparental behavior; males spend an equal amount of time in contact with their young as the females in the 31 days before weaning (Gubernick & Alberts, 1987; Ribble, 1991). Only about 5% of mammalian species exhibit monogamy (Van Shaik, 1990), and it is this rare social trait that makes *P. californicus* a social analog for the study of AVP in humans, who exhibit a similar mating and pairing style.

Mating systems have been associated with substantial differences in neural pathways and behavioral responses to AVP. Winslow et al. (1993) found that in the monogamous prairie vole, *Microtus ochrogaster*, central vasopressin facilitated selective partner preference. Young et al. (1999) also demonstrated that central administration of vasopressin stimulated behavior such as paternal care, mate guarding, and pair bonding in monogamous male *M. ochrogaster*; however, similar treatment did not induce these behaviors in nonmonogamous vole species (*Microtus montanus*). It was revealed that these two types of voles had strikingly different distributions of V1a receptor binding in the brain, with higher levels of AVP-immunoreactive (AVP-ir) staining in nonmonogamous species (Young et al., 1999). In comparison, the receptor distributions of AVP in nonmonogamous and monogamous *Peromyscus* mice also showed significant difference in receptor distribution; however, in contrast to the voles, *Peromyscus*

demonstrated the opposite trend, with higher levels of AVP-ir staining in the monogamous species, *P. californicus* (Bester-Meredith et al., 1999). The neural capacity to form a selective social bond in adulthood involves neuropeptides like AVP, which modulate these species-typical behaviors (Hammock, 2015). Although there are ties between mating style and AVP expression, species variation makes it difficult to predict the dynamics of that relationship. However, since humans exhibit both a biparental and monogamous pairing styles, *P. californicus* is good candidate for study as a model organism in understanding human parental behavior at a rudimentary level.

Studies have shown that AVP regulates aggressive behavior in a variety rodent species. In *P. californicus*, intracerebroventricular (i.c.v.) injection of V1aR antagonist inhibits aggression in males during the resident intruder paradigm (Bester-Meredith et al., 2005), indicating V1aR vital for the initiation of aggression. V1b receptors also appear to be crucial for proper expression of aggression in certain social contexts, as V1bR KO mice do not initiate attacks as quickly or as often as wild-type controls during resident intruder tests (Caldwell et al., 2008). In the prairie vole *Microtus ochrogaster* – a species very similar to *P. californicus* in that it is monogamous, biparental, and is characterized by selective affiliation and aggression – i.c.v. infusion of AVP increases aggression in sexually naïve males (Winslow, 1993). Also, central expression of AVP in the LS of male Wistar rats is correlated with aggressive behavior in the resident intruder test (Veenema et al., 2010); similarly, central administration of AVP in the MeA increases maternal aggression in female Wistar rats, demonstrating that central AVP regulates aggressive behavior in males as well as female rats (Bosch & Neumann, 2010). In a male-male resident intruder paradigm, shorter attack latency and greater level of parental behavior are correlated with a greater expression of AVP V1a receptors in the BNST and the LS in *P. californicus* compared to its non-monogamous relative *P. leucopus* (Bester-Meredith et al., 1999). Also, higher levels of AVP in the BNST are correlated with higher levels of maternal behavior in female *P. californicus* and higher levels of aggression in male *P. californicus* mice (Bester-Meredith & Marler, 2001, 2011). AVP plays an important role in the modulation of aggressive behavior in both male and female *Peromyscus* mice, and the central effects of AVP on social behavior depend on both the distribution and the density of AVP V1a and V1b receptors in the brain.

Interestingly, however, disruption of V1a or V1b receptors does not result in a global disruption of aggressive behavior, indicating that they affect aggressive behavior in only specific social contexts (Caldwell et al., 2008; Stevenson & Caldwell, 2012). There are obviously a great deal of behavioral nuances that cannot be overlooked in these studies. However, we know that, at a fundamental level, AVP facilitates some aggressive behavior from these AVP administration and AVPR KO studies.

Female *P. californicus* mice also exhibit a peculiar tendency to commit infanticide on their own young, a behavior possibly related to AVP. Infanticide may occur due to inability to recognize pups, increased maternal anxiety, and/or increased maternal stress. Many studies demonstrate the importance of AVP in these behavioral characteristics, though in a male paradigm. Regarding social recognition, male V1aR KO mice show impaired social recognition, as V1aR-specific activation in the LS is critical for normal social recognition (Bielsky et al., 2004). Regarding anxiety, high densities of vasopressinergic fibers and V1a receptors are found in the

LS (Caldwell et al., 2008), and these receptors demonstrably play an important role in modulating anxiety; V1aR KO male mice display significantly less anxiety-related behavior than wild-type males in a variety of stressed situations (Bielsky et al., 2005; Egashira et al., 2007). Regarding stress response, the V1bR has been identified as an important regulator of the hypothalamic-pituitary-adrenal-axis (HPA) stimulating the release of ACTH. Although V1bR KO mice can express normal resting levels of ACTH and catecholamines, when facing different types of stressors they have a reduced level of ACTH release compared to wild-type controls (Stevenson & Caldwell, 2012), showing that V1bR may play a part in the anxiolytic effect we see in V1aR KOs. Though it is unclear what factors and neural networks are responsible for infanticidal behavior, this study may elucidate a direct role AVP has on maternal infanticide.

So far, there has not been much research on the effects of AVP on maternal behavior in a monogamous rodent species. Our research seeks to elucidate whether AVP has a positive or negative effect on maternal care. Based on related literature on AVP, I hypothesize that i.c.v. injection of AVP should increase the amount of maternal behavior in *P. californicus*.

METHODS

Animals

Sexually naïve female *Peromyscus californicus* were used in this study. The room was kept at 25°C under a reverse 14L:10D light cycle, with lights on at 05:00. Mice had access to water and food *ad libitum* (Purina 5001 mouse chow). Each female was housed with an unrelated male in a standard size (48.3 x 26.7 x 15.6 cm) cage with wire mesh lids and aspen shavings until pregnant. Pregnancy was confirmed by tracking the female's weight for significant and steady increase, as well as looking for the development of nipples and signs of nest building. When pregnancy was confirmed, cannulae were implanted bilaterally into the mother's lateral ventricles. Then, the mother was placed back in her own cage to recover and give birth. When parturition occurred, the female and her pups were placed in a polycarbonate cage for behavioral observation. During observation periods, animals were housed in clear Plexiglas observation chambers (30 x 29 x 30 cm) containing food, a water bottle, and aspen shavings. Age-matched, non-related female mice were used as intruders for the intruder tests. The mice were maintained in accordance with the recommendations of the National Institutes of Health Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1985).

Test compounds

AVP (Sigma-Aldrich, St. Louis, MO) and V1a antagonist (β -Mercapto- β , β -cyclopentamethylenepropiony¹,O-Me-Tyr²,Arg⁸)-Vasopressin (Sigma-Aldrich) was dissolved in the vehicle, an artificial extracellular fluid (147 mM NaCl, 1.3 mM CaCl₂, 0.9 mM MgCl₂, 2.5 mM KCl; pH 7.4). For i.c.v. administration, 3ng of AVP was dissolved in 2 μ l of vehicle, while 1ng of V1a antagonist was dissolved in 2 μ l of vehicle, as these doses of AVP (Parker and Lee, 2001) and V1a antagonist (Winslow et al., 1993) previously altered behavior in other rodent species (Bester-Meredith et al., 2005).

Cannulae placement

Cannulae were placed bilaterally into the lateral ventricles using the procedure of Guild and Dunn (1982) under isoflurane anesthesia when mice were approximately 1 week from giving birth. Cannulae were placed approximately 0.6 mm caudal from the Bregma and 1.8 mm lateral from the midline, reaching 3.4 mm below the skull. Animals were allowed to recover for one week prior to behavioral testing (Bester-Meredith et al., 2005).

Intercerebroventricular injections

Right after giving birth, each mother was randomly assigned one of three treatments for daily injection: AVP, AVP1aR antagonist, or artificial extracellular fluid (AECF). AECF served as a vehicle blank control. The mothers were injected twice a day, with two hours between each injection. The second injection was done immediately prior to behavioral videotaping (parental behavior or resident-intruder). Injections were done with 10 μ l Hamilton syringes, which dispensed the solution at 1 μ L/min. The AVP treatment group had a sample size of five (n = 5),

the V1aR-antagonist group had a sample size of three ($n = 3$), and the AECF group had a sample size of five ($n = 5$).

Parental behavior

The mice were videotaped for 30 minutes on days 2, 4, 6, and 8 for parental behavior measurements. Videos were analyzed by two separate viewers for the frequency of nursing, huddling, grooming, pup retrieving, and nest building, sampled every 10 seconds. Data from these two observers were averaged. Nursing was defined as attachment of pups to the mother's nipples; huddling was defined as physical contact with pups, excluding contact with the tail; grooming was defined as licking the body of the pup by a parent; pup retrievals were noted when pups were grasped just posterior to the forelegs, lifted up, turned sideways in the mother's mouth, and then transported, sometimes to the nest; and nest-building was defined as maternal manipulation of nesting material by collecting shavings, carrying shavings, or using the snout to form a bowl-like nest shape (Bester-Meredith & Marler, 2007).

Aggression/resident-intruder test

On day 5 after the start of the daily injections, the resident intruder test was carried out. After receiving her second injection, the mother was returned to her Plexiglas cage with her pups. During the intruder test, an age-matched, unrelated female was then placed in the Plexiglas cage with the resident. Video of the interaction was taken; the test ran until an attack occurred or until 10 minutes passed. Videos were then analyzed to measure attack latency; shorter attack latency signified increased aggression.

Statistical analysis

The data for nursing, huddling, grooming, attack latency, not in nest, pup retrieval, and nest building were analyzed for significance ($p < 0.05$) using the nonparametric, Kruskal-Wallis test. A repeated measures ANOVA was used to test for trend differences over time between the groups AVP and antagonist for nursing, huddling, and grooming. The infanticide data were analyzed for significance ($p < 0.05$) using a three-way ANOVA test.

RESULTS

A non-significant trend shows that AVP decreases the overall frequency of nursing, huddling, and grooming parental behaviors in the mothers; a repeated measures test for variance revealed that there is a change in nursing over time that differs between AVP and antagonist that is approaching significance ($F=6.588$, $p=0.062$). However, our current overall data are not statistically significant (Fig.1A, $H = 1.622$, $p = 0.444$; Fig.1B, $H = 1.534$, $p = 0.464$; Fig.1C, $H = 0.288$, $p = 0.866$).

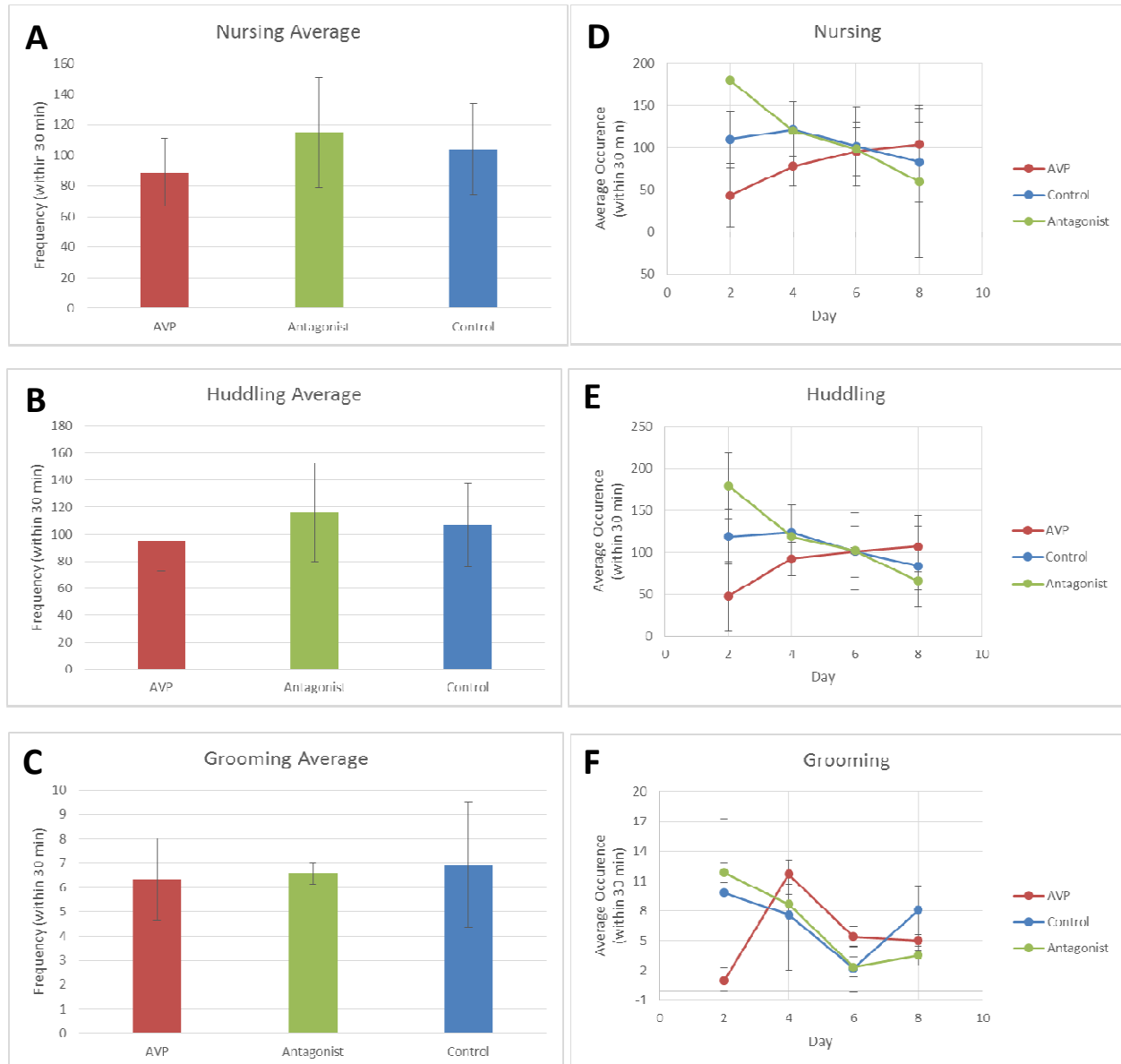


Figure 1. The average overall frequency of specific parental behaviors – nursing, huddling, and grooming – for each treatment (A-C), along with the average frequency of the parental behaviors during each day of observation (D-F). Error bars shown. No statistical significance (all p -values > 0.05)

Analysis of the daily averages for nursing, huddling, and grooming for each treatment day (days 2, 4, 6, 8) showed no significant difference among the three treatment types, as well (Fig1. D-F). In nursing and huddling, however, averages from Day 2 approached statistical significance (Fig.1D, day 2, $H = 5.761$, $p = 0.056$; Fig.1E, day 2, $H = 5.233$, $p = 0.073$).

Additionally, the groups did not differ in average attack latency, though AVP appeared to delay attack latency compared to antagonist and AECF (Fig. 2).

We noticed that the *Peromyscus* mothers in this study would commit infanticide quite often, so we analyzed the percentage of pups that were killed by their mothers across all three treatment groups (Fig. 3). However, AVP-treated mothers do not kill their pups more frequently than both the control and the antagonist ($F = 1.556$, $p = 0.254$).

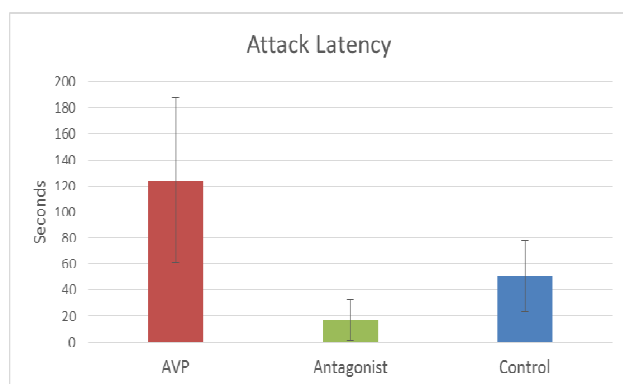


Figure 2. Average attack latency of mothers treated with AVP, V1aR-antagonist, and AECF. Kruskal-Wallis test yielded a significance value $p = 0.442$.

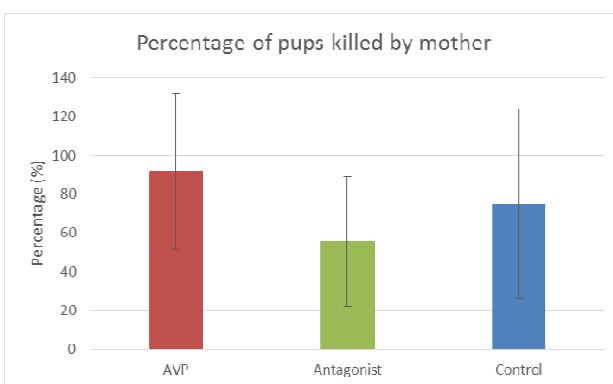


Figure 3. Percentage of pups killed by their mother before weaning in each treatment group. Three-way ANOVA test yielded a significance value $p = 0.254$.

Pup retrieval and nest building were relatively infrequent and not well-represented by our current sample size. It appears that AVP increases the frequency of pup retrieval and nest building (Fig. 4B, 4C), but AVP also appears to increase the amount of time a mother spends outside her nest (Fig. 4A). Statistical analysis using the Kruskal-Wallis test showed that our current data are not statistically significant (Fig.4A, $H = 1.688$, $p = 0.43$; Fig.4B, $H = 2.867$, $p = 0.238$; Fig.4C, $H = 2.571$, $p = 0.277$).

Analysis of the daily averages for frequency not in nest, pup retrieval, and nest building for each treatment day (days 2, 4, 6, 8) showed no significant difference among the three treatment types, as well (Fig4. D-F). In pup retrieval and nest building, however, averages from Day 4 approached or reached statistical significance (Fig.4E, day 4, $H = 3.467$, $p = 0.177$; Fig.4F, day 4, $H = 6.192$, $p = 0.045$).

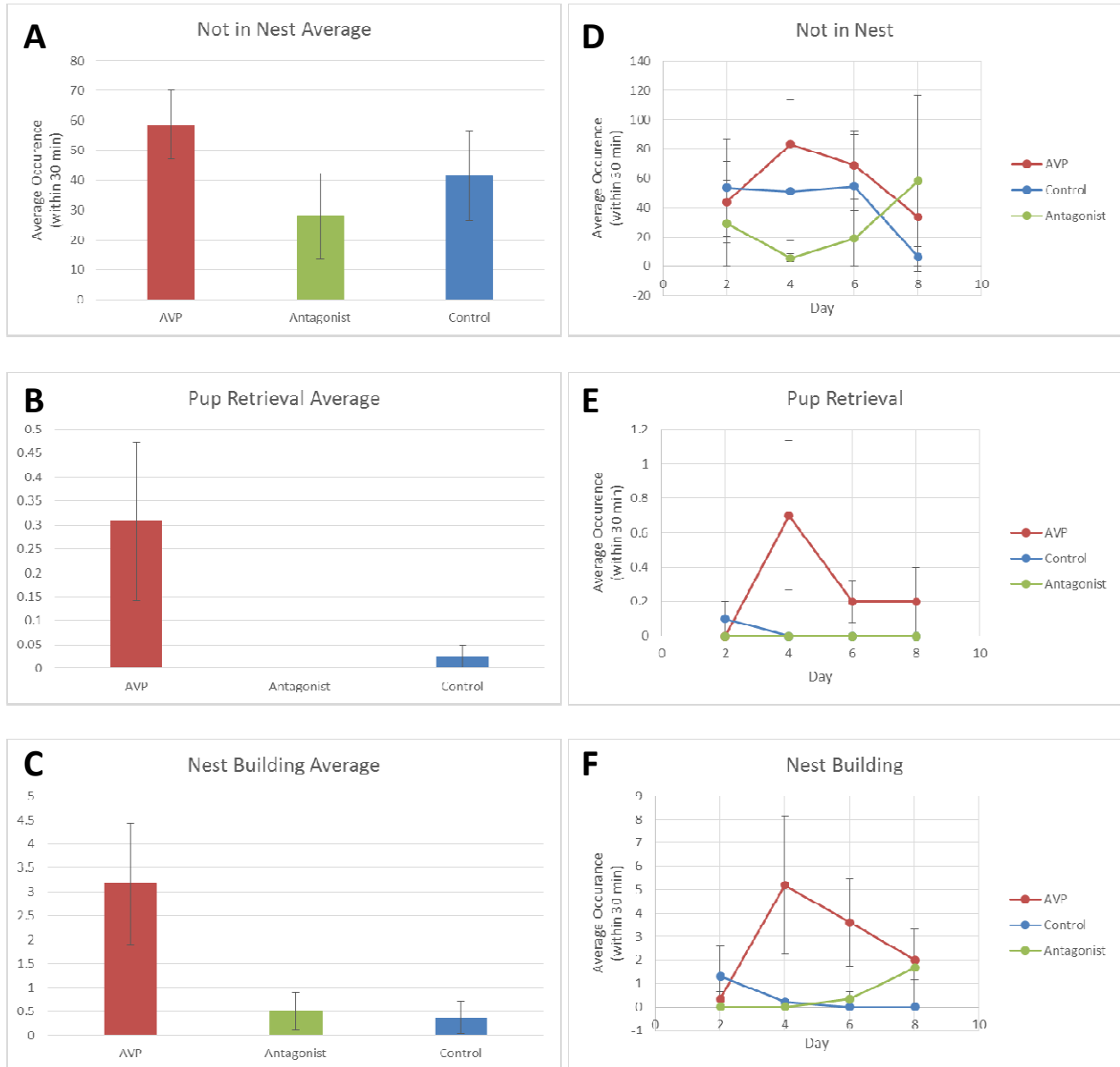


Figure 4. The average overall frequency of various behaviors – not in nest, pup retrieval, and nest building – for each treatment (A-C), along with the average frequency of the parental behaviors during each day of observation (D-F). Error bars shown. No statistical significance (all p-values > 0.05)

DISCUSSION

From this study, AVP appears to have a negative effect on overall maternal behavior and aggression that was not significant due to the small sample size. This is unexpected, however, as higher levels of AVP in the brain have been correlated with higher levels of maternal behavior in female *P. californicus* and higher levels of aggression in male *P. californicus* mice (Bester-Meredith & Marler, 2001, 2011). A recent study by Steinman et al. (2015) showed that the stress of social defeat both increased AVP staining in the caudal PVN of male and female *P. californicus*, and increased attack latency in female, but not male, *P. californicus* during resident-intruder tests. This demonstrates that there is sexual dimorphism in stress response, and that perhaps a different circuit may be used to control aggression in stressed males (Steinmann et al., 2015). Sex and social context differences may play a large role in behavioral outcomes, and should therefore not be overlooked.

Additionally, the data show patterns of behavior somewhat consistent with data found in the studies conducted by Nephew & Bridges. (2008^b, 2010) in which central infusions of AVP significantly reduced maternal aggression in female Sprague-Dawley rats on day 5 of lactation, whereas V1aR-antagonist infusions increased aggression on day 15 of lactation. Additionally, centrally administered AVP suppressed maternal grooming behavior on day 5 of lactation, while V1aR-antagonist infusions increased grooming behavior on day 15 of lactation (Nephew et al., 2010). Our data show that AVP initially decreases maternal behavior, while V1aR-antagonist increases maternal behavior (Fig. 1D, 1E, 1F). The longer-term effects seen on Day 8 of our study show that AVP increases maternal behavior above the control, while V1aR-antagonist decreases it (Fig. 1D, 1E, 1F), though these data points are far from statistical significance. These studies demonstrate that AVP can have acute effects on maternal behavior and aggression, though AVP networks may be more significantly involved in decreasing maternal behavior and aggression over longer periods of time.

Regarding infanticide, the data show that the rate of infanticide is high across all three treatments, killing at least 50% of the pups. Therefore, there may be other stressors influencing infanticide that are unrelated to the chemical treatment, e.g. the stress of the surgeries or injections themselves. In the future it would be ideal to breed a “no surgery” control group in order to observe the baseline level of infanticide in our experimental mice. The data reveal an apparent trend in which infanticide is heightened in the AVP treated mice, relative to the control, and infanticide is depressed in the antagonist treated mice relative to the control (Fig. 3). Perhaps anxiogenic properties of AVP cause mothers to become hostile toward their young. Further investigation of the mechanisms of this hostility is needed, however, since this increased aggression towards young is accompanied by an apparent decrease in aggression toward intruders (Fig. 2), which seems paradoxical.

Our sample size is small, with a total $n = 13$, and the amount of error is large in nearly all the data sets. Many factors could have played into the scattering of the data points within the same treatment group, such as natural behavioral variability. A study done in rats showed that maternal behavior declines in the late-postpartum (13-14 days after birth) due to changes in the needs of the developing pups (Pereira & Morrell, 2009); the general tendency for the maternal behavior averages in our data to show a decrease over time may be related to this.

Since the central effects of vasopressin cannot be tested in humans, animal models are necessary for the discovery of neuropeptide effects on behavior. Understanding how AVP affects maternal behavior, a social necessity for offspring survival, helps give us a basic understanding of how AVP might also act in the human brain to influence maternal behavior. There is only so much we can extrapolate from animal models to human application, however. Animals that model typical human behavior or human disorders are valuable in the insight they provide into human neurophysiology and neuroanatomy; nevertheless, species differences must be considered carefully (Stoez et al. 2013). Although we cannot make direct parallels between *P. californicus* and human maternal and affiliative behavior, we can deduce rudimentary functions of a given hormone on behavior. Our data show that AVP is involved in decreasing early maternal behavior and aggression in primiparous *P. californicus* mothers, so AVP may play a similar role in human mothers. It is worth investigating further, as there are not many studies that have investigated the effects of AVP on maternal behavior, and maternal behavior is indispensable for child development. AVP is related to many facets of behavior that affect motherhood, like maternal care, social recognition, anxiety, aggression, and possibly emotional state. Once we increase our sample size we may be able to gain statistical significance and allow the data to fully realize trends that may exist regarding the effects of AVP on maternal behavior.

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APPENDIX ON FAITH AND LEARNING

The Christian's Free Will and Implications of Behavioral Endocrinology

In studying the effects of hormones on animal behavior, one might ask the question: *how much* do neurochemical signals in the brain influence behavior – and what does this mean for humans? Though the behaviors of animals, such as mice, are nowhere near the richness and complexity of that of humans, we must consider the evolutionary history in which hormones developed to elicit a physiological response to an environmental stimulus. As behavioral endocrinology reveals chemicals in the brain that alter behavior, it brings into question the extent to which our decisions are predetermined by our biology. How much influence does an individual have over himself or herself when some behaviors are conditioned physiological responses to the environment, shaped by living contexts? Can we each be boiled down to a complex network of situational chemical reactions? In other words, does current research on the brain and behavior fundamentally undermine the notion of freewill?

“Freewill” is the premise that each human being has the ability to choose their thoughts and actions, and through those choices control their course in life. The concept of freewill is necessary in order to hold people accountable for their actions. In our justice system, people are punished for what they chose to do, and yet may also evade penalty by pleading insanity – i.e. a loss of freewill. For Christians, freewill is necessary to assign responsibility to humans for the inception of sin – our choice to rebel against Creator God. Freewill is the reason we can judge and evaluate one another by what we say and do, as each person can be roughly regarded as a product of their own volitions. But might it all be an illusion?

In psychology, neuroscience has been used to provide evidence that humans do not truly have free will, as conscious awareness of a decision happens after neuronal firing that causes the action. However, neuroscientist Peter Ulric Tse argues against this reigning paradigm and writes that the neuronal pathways that cause our choices are shaped and “rewired” by our conscious internal decisions. Tse’s book, *Criterial Causation Offers a Neural Basis for Free Will*, shows that our actions in the moment, though they occur spontaneously and not under our immediate volition, are not random – the neural circuitry that causes our actions is constantly, and consciously, being physically shaped by our own mental causation (Tse, 2014). By this logic, each person is their own modulator for their actions, and their actions are in response to their will, which is free. Our will involves entertaining different options and imagining their outcomes internally, physically reshaping connections between neurons.

Likewise, when we see different drugs increase a certain behavior it does not necessarily mean that the behavior is guaranteed to happen as a direct result of the chemical’s release into the brain. The beauty in studying the endocrinology of behavior is that you see how variable behavior is. On one hand, behavior *is* predictable, as certain actions are necessary for survival and social cohesion. But even in a seemingly simple animal such as a mouse, where factors such as age, environment, and treatment are all controlled for, the subjects may still act altogether different from one another. Although our mice were all administered the same treatment of arginine vasopressin, their behavior still managed to fall to each extreme of our test variables. Perhaps there was fault in our setup, procedures, or parameters. However, the complex nature of behavior – what gives an organism the mental desire to do something and how they choose

to act on that desire – gives individual behavior a constant undertone of unpredictability. Kelly & Goodson (2014) word it well: “behavior is not the product of a single peptide acting on a single receptor, but is involved in coordinated release to multiple brain regions, effectively modifying functional connectivity across numerous nodes of behavioral regulatory networks.” Neural networks are not straightforward!

From this spawns the philosophy that the presence of hormones in the brain simply increases the *propensity* for a certain behavior to be carried out, contributing to a likelihood, not a causation. It activates pathways in the brain which encourage a behavior, though the person can still choose not to act on their brain’s chemical signaling. Of course, it can be just as equally argued that the variations seen in behavior do not result from variation in choice, but rather the variation in other underlying causal factors such as genetics or environment; both have huge influence on behavior. However, these arguments can become rather cyclical, so we first need to take a step back for a second and discuss that which is the root of this entire discourse: the philosophy of “mind.”

The question of whether we have control over our brain’s decisions or whether our decisions are physically pre-determined is not something we will ever answer with pure science because it is, after all, a philosophical question – a philosophical question that is tackled in SPU’s UCOR 3000 course, at that (though, for UScholars we tackle it in Faith & Science II)! Dr. Stephen Layman, in his short essay called “Body and Soul”, explains three prevalent theories of mind: substance dualism, reductive physicalism, and non-reductive physicalism. Substance dualism is the view in which a non-physical “soul” intervenes on the physical mind in order to elicit thoughts and behavior. The justification for this view is that the body needs a soul to have freewill, and that it helps explain the human right to life and life after death. Plus, the Bible speaks of the soul (as in “Love the Lord your God with all your...soul”) and the soul is what bestows the image of a non-physical God on our physical beings. The substance dualism perspective is countered by that of physicalism, which states that current research points to the physicality of the brain and mind (e.g. stroke and lesion studies) that the mind most reasonably exists in physical terms alone. In other words, brain states and physical states are tied so closely together that any additional “substance” of soul is extraneous.

Reductive physicalism, one branch of physicalism, appeals to the empirical scientist in that everything about human thought and action can be reduced to physiological functions of the brain. Since the physical universe is beyond any individual’s control, the primary force of will is physical law; freewill is non-existent in this paradigm. The only danger in this thinking is that it assumes that we understand, or will eventually understand, brain states well enough to say that mental states are reducible to them, which may be assuming too much. Philosopher John Searle argues that “a description of the world under third-person physical terms, such as scientists provide, is not a complete description of the world. What is left out is precisely the subjective, first-person, conscious phenomena” (Layman, 2011). It is arguably not possible to reduce the workings of mental states down to specific brain states.

The other branch of physicalism is non-reductive physicalism, which also argues against the need for substance dualism, but asserts that mental states *can* differ from physical states; biological reality cannot solely be explained in physical terms. This perspective explains that though mental and physical states are closely tied, they are not so lashed together that one can

entirely predict (or cause) the other. There is some inherent mystery in the mind-brain experience that allows freewill to exist.

For people of the Christian faith, free will is an important issue. Without free will, God is the one accountable for our sin, disobedience, and evil. Who is to say whether or not someone who exhibits sinful behavior is simply acting that way due to a chemical imbalance they have no control over? Our wrongdoing against God brings judgment upon ourselves, due to our own decisions, but are we acquitted when we find out that physical determinants such as brain chemicals can alter our mind state or actions? Additionally, it seems unfair, even under the philosophy that behavior is only influenced, not absolutely caused, by chemicals in the brain, that certain people are more prone to certain sinful behaviors. This means that the playing field is not level when it comes to striving towards righteousness.

But, just as people are placed in different life-conditions, some having it worse than others; each person has their own burden to bear. It is simply a fact of life, even if it seems patently unfair. In fact, aspects of our freewill are not only biologically constrained by our genetics, but basic principles of theology show that our freedom is inevitably constrained. Psychologists David Myers and Malcolm Jeeves in their book "Psychology through the Eyes of Faith" discuss God's foreknowledge, God's sovereignty, and God's grace, which all imply divine determinism: in a way, these attributes say "God is God, and he is in control" which in turn seems to nullify the existence of human free will. Plus, since all things are God's, there is no difference between divine determinism and natural determinism, meaning that there is no will apart from God's will. We, thus, depend on forces "beyond our conscious knowledge," and appear to have no free will. So each of us is a piece in God's game, each of us is made and played by God?

Yes, if everything is predetermined by God and we are just participating in a story with the illusion of choice and free will, then God created sin to merely use it as a plot point – a major conflict with his existing divine goodness. It is a bit deceptive, as in this story, God made humans, told them not to sin, and then made them sinners to later come and save through the blood of Jesus Christ. In this paradigm we would have no responsibility for our actions, sin would be a hoax, and our rebellion is a ruse.

Could God have done this? An argument of the theologian John Henry Newman is: God is good, therefore the only way for evil to enter is through an external party that is not God, which is us (McGrath, 2012). If we were not responsible for sin, there would be no reason for repentance, and there would also be no justice in God's wrath toward our sin. Therefore, though theology of God's sovereignty may give argument to the nullification of our freewill, theology of God's goodness provides solid grounds for the absolute existence of human freewill. The sin issue is a freewill issue.

There is much about how the world works that we do not understand. Job, in the Bible, is presented with many plagues and sufferings, and God essentially explains to him "that's just how it is, Job!" Then Paul says to the Romans in Romans 8:28, "God works all things together for good for the ones who love God, for those who are called according to his purpose." In this dichotomy, we see that God is both mysteriously, yet actively, working in the world and in our lives. God commands the entire universe down to the smallest atom, working things out according to his will. Where is our will, in all of this? I believe that it is not beyond God to allow

us to have free will, for though it would make him vulnerable, God is love; love allows vulnerability and choice, and God allows us to have choice so that we can be like him, being love. Proverbs 16:9 says that “The heart of man plans his way, but the LORD establishes his steps.” At the center of each of our beings is a will that is free, but our will is in a universe ruled by God.

We are biological creatures in a physical universe ruled by powers beyond our own. Our minds and decisions can be influenced by chemicals and environmental cues. Our freewill seems to be constrained by so many different factors discovered in physiology, philosophy, and theology. However, some things about the human experience may not be able to be reduced to our biology, and some things about the nature of God may not be able to be reduced to logical proof. Though it is difficult to imagine, I believe that our will and God’s will can, and must, exist simultaneously.

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