DISCLAIMER:

This document does not meet the
current format guidelines of
the Graduate School at
The University of Texas at Austin.

It has been published for
informational use only.

The Thesis Committee for Andreas Sherron George
Certifies that this is the approved version of the following thesis:

Songs of Aggression: The Singing Mouse Model

APPROVED BY
SUPERVISING COMMITTEE:

Supervisor:

Steven M Phelps

David Crews

Songs of Aggression: The Singing Mouse Model

By

Andreas Sherron George, B.S.

Thesis

Presented to the Faculty of the Graduate School
of The University of Texas at Austin
in Partial Fulfillment
of the Requirements
for the Degree of

Master of Arts

The University of Texas at Austin
May 2015

Abstract

Songs of Aggression: The Singing Mouse Model

Andreas Sherron George, M.A.
The University of Texas at Austin, 2015

Supervisor: Steven M. Phelps

Social behavior is a vital part across vast taxa, from honey bees to elephants. This behavior is known to be modulated by nonapeptides acting on various nodes of the social behavior network (Newmann, 1999). Vasopressin and its evolutionary precursor vasotocin are both highly involved in the social brain. (De Vries and Panzica 2006; Goodson, 2005) Furthermore, it has been demonstrated that these systems are under the control of gonadal hormones (Bester-Meredith and Marler 2005). Here, we study the effects of hormones and experience on the social behaviors, specifically aggression, in the singing mouse *Scotinomys teguina*. These mice are known for their male stereotyped song used in territorial aggression as well as mate attraction. We gonadectomized the animals and coupled this with androgens at varying levels, or empty implants, to determine how gonadal hormones affect aggression and song. We discovered a significant increase in frequency of submission in animals following no hormone treatment, and a positive correlation with androgen level and physical aggression, as well as increase in propensity to sing. In a separate study, we also studied the singing response to social stimuli. We introduce the subjects to conspecific song, female bedding, and pink noise. We then recorded their responses following winning or losing encounters. We found animals increased their singing behavior in response to all stimuli following wins. Winning is known to increase aggression by allowing a transient increase in gonadal hormones. This study further bolsters the winner's effect. Taken together, these experiments demonstrate in dynamic social behavior in a novel species that is modulated by hormonal states as well as social encounters.

Table of Contents

List of Figures

Chapter 1: Neural Distribution of Vasopressin

INTRODUCTION

Social behavior is a critical component of animal behavior. Many of the interactions that define social behavior occur in reproductive contexts, including mate attraction, resource defense and parental care. We explore the use of Alston's singing mouse, Scotinomys teguina, as model for investigating the mechanisms male song – a social behavior that contributes to both mate attraction and male-male competition (Fernandez-Vargas et al. 2011; Pasch et al. 2013). In this chapter we specifically examine whether testosterone, which we have demonstrated can modulate male singing behavior, also modulates the expression of the neuropeptide vasopressin, a hormone important for a diversity of social behaviors in many taxa (Donaldson et al., 2008).

Given that much of social behavior are related to reproduction, it should come as no surprise that reproductive hormones often play a major role in regulating social behavior. Phoenix et al. (1959) explored the effects of hormones on an animal's development as well as its behavior. They coined the terms "organization" and "activation" as it relates to hormonal control. Organizational responses of a hormone refer to the permanent changes that occur when hormones are introduced early in development. Phoenix et al (1959) hypothesized these organizational changes allow an animal to respond in a sexually dimorphic fashion to hormones following maturation. The changes during development allow behaviors to be "activated" at the appropriate times. Activational responses are those transient changes observed in a mature individual when a hormone is introduced. The researchers found that testosterone propionate,

when administered to a neonate guinea pig, resulted in physiologically masculinized female. The females engaged in male specific behavior such as sexual mounting when testosterone was introduced later in life (Phoenix et al. 1959)

The authors suggest the central nervous system is masculinized following exposure to T during early development. This leads to sexual differentiation in the brain that can be seen in a wide variety of taxa (Arnold, 2009). Sexual dimorphism in the CNS became a major focus late 1970's and 80's (Raisman and Field, 1973; Nottebohm and Arnold, 1976; Bardin and Catterall, 1981; Nishizuka and Arai, 1981; Tobet et al., 1986), and a diversity of studies demonstrated the power of organizational effects as well as the wide spread differentiation in the brain. Many of the brain regions exhibiting sexual dimorphism proved to be critical for a range of social behaviors (Newman 1999).

In 1999, Newmann proposed the social behavior network. In this work, the author synthesizes a large body of work on diverse brain regions, illustrating that for many distinct social behaviors, there are functional data linking them to social behavior. The author focused on 6 brain regions that are extensively interconnected and were previously linked to social behavior: Lateral Septum (LS), Medial Amygdala (MeA), Ventromedial Hypothalamus (VMH), Anterior Hypothalamus (AH), Medial Preoptic Area (MPOA), and the Bed Nucleus of the Stria Terminalis (BNST). Interestingly, each of these regions also contains receptors for sex steroids (Fine et al, 1990) as well as receptors for various neurotransmitters. There is a high level of conservation in these regions across vertebrate taxa including amphibians (Sakata,

2003; Rhen and Crews, 1999), birds (Ball and Baltahazart, 2004), teleost fishes (Goodson, 2005), and summarized most recently in O'Connell and Hofmann (2012). Goodson's work explored the homologous network in teleosts and birds as well as the homologous AVT distributions. Although these regions are generally conserved, there is substantial interspecies variation in how areas of the social behavior network affect decision making strategies. Newman (1999) suggests that the connections between the BNST and the Medial Amygdala are important in many aspects of rodent social behavior, sexual function, olfactory investigation, resource holding and aggression. Newman (1999) also developed a strong and convenient framework by which we can study the interaction of hormones, brain areas and social behavior. This framework has been used to study the neural-endocrine control of aggression in anurans (Boyd and Moore, 1992), teleosts (Goodson and Bass, 2000; Lan and Hsu, 2011), reptiles (Ramsey and Crews, 2007), birds (Apfelbeck et al., 2011) and mammals (Oyegbile and Marler 2005).

Studies in small rodents in the *Peromyscus* family demonstrates the manipulative power of gonadal hormones on neuroanatomy, the transient nature of these changes, as well as species differences in the social behavior network. Marler conducted a series of comparative studies between *Peromyscus californicus* and *Peromyscus leucopus* to better understand the neuroendocrine regulation of their social behaviors (Fuxjager et al. 2009; Bester-Meredith and Marler, 2007; Oyegbile and Marler 2006; Oyegbile and Marler, 2005). . Marler and colleagues found that the more aggressive species were more sensitive to changes in gonadal

androgens (Bester-Meredith and Marler 2005). The BNST and MeA were highly involved in the production of aggression. Increased vasopressin expression in these areas is highly correlated with aggressive behavior in these and many other rodent species (Wang et al., 1993; Cervantes and Delville 2007).

Vasopressin is released differently both within (Bamshad et al., 1993) and across species (De Vries and Panzica, 2006) and is under gonadal control in many species (Goodson and Bass; 2000; Boyd, 1992). All nonapeptides have a common evolutionary ancestor which split with the rise of teleost to vasotocin (AVT) and isotocin (IT). In mammals, these have evolved to vasopressin (AVP) from AVT and oxytocin (OXT) from IT. AVP and OXT have been implicated in a wide variety of social behaviors in multiple nodes of Newman's social behavior network (Winslow et al., 1993; Ferguson et al., 2001; Landgraf and Neumann, 2004; Donaldson et al., 2008). While nonapeptides are present across a wide range of animals, their actions are highly diverse across taxa. AVT has been implicated in aggression and vocalizations of fish (Santangelo and Bass 2006) as well as birds (Manley et al. 1997) AVP modulates social communication as well as aggression in a wide variety of vertebrate taxa including humans (Scattoni et al., 2008; Thompson et al. 2006).

Considering the high level of conservation present in the social behavior network and with nonapeptides, it is no surprise AVP, and its homologs, are highly involved in control of social communication and aggression in small rodents as well. These patterns have been demonstrated in Syrian hamsters (*Mesocricetus auratus*; Delville et al., 2000) and the white

footed mouse (*Peromyscus leucopus*; Oyegbile and Marler, 2005). The vocalizing behavior of singing mice is profoundly influenced by androgens (Pasch et al. 2011a). Here, we examine the effects of androgens on neural AVP expression.

In the 1970's Emmet T. Hooper led a team to study a small, diurnal rodent native to the cloud forests of Central America (Hill and Hooper, 1971; Hooper, 1975; Carleton et al., 1975). *Scotinomys teguina* gets its common name; Alston's singing mouse, from the high frequency, audible songs they produce. These mice are closely related to the mice of the *Baiomys* genus that produce much shorter and simpler ultrasonic vocalizations (Miller et al. 2007). Scotinomys is also more distantly related to the *Peromyscus* genus. We hypothesized that androgen modulation of forebrain vasopressin, so important in social behaviors of other rodents (Bester-Meredith and Marler, 2000; Ferris and Delville, 1994), might also contribute to male song. The song seems to play a role in resource defense, especially present in separating the smaller *S. teguina* species from the larger *S. xerampelinus* species when the two live in sympatry. Furthermore, females show preferences for males with songs that are characteristic of males in good condition (Pasch, 2011b). The neuroendocrine control of singing in *Scotinomys teguina* is not understood. Because we previously demonstrated that androgens influence song rates, we here examined whether androgens also influence the neural distribution of vasopressin. The description of vasopressin staining regions of the *Scotinomys* brain also serves as a useful neuroanatomical characterization of this interesting species.

METHODS

Subjects

Subjects for this experiment were lab reared offspring of animals originally caught in Costa Rica and brought back to the lab. Animals are sib housed with 1 or 2 litter mates. Animals are given food, cat chow, and water *ad libitum*. They are housed in 12:12 dark: light cycle at 15.5-19^{o} C. Weekly enrichment included meal worms and misted moss to simulate the natural cloud forest environment. All phases of the experiment were performed during light hours under white light. Experiment animals were all sexually experienced. Animals were moved to individual chambers one month prior to beginning the experiment and were randomly assigned to 1 of 4 treatment groups. The following protocol is approved by Institutional Animal Care and Use Committee at the University of Florida (UF #200801939).

Surgery

Subjects were anesthetized using isoflurane (SurgiVet Isotec T

[3] Classic Isoflurane Vaporizer) and castrated using an incision through the scrotum. Each animal received one implant. Implants were 10mm silastic tubing filled with, 1mm of testosterone (approximately 15mg), 2mm testosterone (30mg), 1mm dihydrotestosterone or simply filled completely with saline solution. Silastic tubing was sourced from Dow Corning Corporation (1.47mm id x 1.96 od).Both testosterone (T) and dihydrotestosterone (DHT) were sourced Sigma, T1500 and A8380 respectively. Previous pilot studies revealed the appropriate amount of T that would replicate physiological levels. DHT was included in the study to explore the possibility of a role for aromatization of T in the possible behavioral differences observed. Implants were sealed with silicone adhesive and sterilized in ethylene oxide. All surgical tools were sterilized with glass beads (Germinator 500). Animals were closely monitored for 72 hours postsurgery to ensure a full recovery.

Behavioral Trials

Fourteen days pre-surgery, animal songs were recorded. One day following recording, subject male were moved from isolation, allowed to acclimate for 30 minutes and then exposed to a novel intruder in a resident intruder trial. The subject animals were the residents in all trials. Animals were allowed to interact for 10 minutes or until the winner was decided, which ever was shorter. Songs were also recorded 15 days post treatment and resident-intruder trials were performed with a novel intruder the day after the recordings. Details and data of these procedures discussed in Pasch et al. (2011a).

Tissue collection

Animals were euthanized 16 days post surgery in CO

$_2$ chambers. Trunk blood was collected for T immunoassays (Pasch et al. 2011a). Brains were also extracted and fixed in 4% paraformaldehyde then cryoprotected in 40% sucrose in paraformaldehyde. Brains from the subject animals were sectioned at 30µm in a cryostat. Sections were placed in 24 well plates containing PBS. Each brain was divided into 3 groups of serial sections. The first of the three was used in the current study. The remaining brain sections were stored long term in antifreeze at -80 Celsius.

Immunohistochemistry

The previously collected and separated (every 3rd) sections were washed in PBS for 10 minutes on a shaker plate. Sections were then pretreated in 0.3% hydrogen peroxide for 10 minutes and then rinsed with PBS. The sections were then incubated in 10% normal goat serum for 60 minutes to block nonspecific binding of the antibody. 0.3% Triton X was included here to maximize coverage of both antibodies. The sections were washed in PBS. Finally the sections were added to the primary antibody, Rabbit ant-[Arg^8]-Vasopressin Cat no. T-4563, Peninsula Laboratory LLC, and incubated overnight in 4^{o}C. The antibody was at a concentration of 1/10000.

The following day, the sections were rinsed in PBS for 15 minutes on a shaker plate. Sections were incubated in the secondary antibody at a 1/1000 dilution (Goat F (ab')

$_2$ Anti-rabbit Immunoglobin G Leinca Technologies Inc) in 4% NGS for 60 minutes. Sections were then rinsed in PBS for 10 minutes. Sections were then incubated another 60 minutes in a 0.5% Avidin-biotin complex. The staining was then visualized with a DAB kit (Vector SK 4100) with nickel enhancer. The reaction was stopped after 90 second by placing sections in PBS. The sections were mounted on glass slides, dried, and then dehydrated in 50%, 75%, 95%, and 100% Alcohol before a final dehydration in Citrasolv and then coverslipped using Permount. Pilot studies and controls lacking primary antibody ran in unison, ensured proper binding of the antibodies to the proteins.

Images

Following IHC procedures, brains were examined under an inverted microscope. Sections were exhaustively photographed for analysis. Stained cell bodies were counted on a 10x zoomed image. Sections were recounted at random to ensure consistent scoring. The Mouse Brain (Franklin and Paxinos, 2007) was used as a reference for consistent naming of neuroanatomical areas.

Statistical Analysis

Scores were compiled into a spreadsheet and imported into Statistics Package for the Social Sciences (SPSS) for analysis. We compared only 3 groups, T 1mm, DHT and Empty, since the T 2mm group was shown to increase circulating androgens far above physiological level. These 3 groups were compared at several locations in the brain, the AH, BNST, MeA, PVN, SON and SCN through Kruskal Wallis tests.

RESULTS

AVP-ir Staining

Bed Nucleus of Stria Terminalis

Just caudal of the anterior commissure, the Bed Nucleus of Stria Terminalis (BNST) contains a distinct population of cell bodies. These were typically small and yielded light staining. These stains were observed in the medial BNST medial and ventral to the IC (insular cortex) (Figure 1.1). There were also large darkly stained cell bodies in the more caudal and dorsal areas of the nucleus. These were observed lateral to the SM and medial and dorsal to the fornix (Figure 1.2). The BNST also contains populations of small AVP-ir fibers, the pathway of output from the amygdala.

Amygdala

Vasopressin containing cells were present on the medial and extended nuclei of the amygdala (MeA). The majority of MeA staining appeared on the medial edge of the nucleus dorsal and caudal to the lateral edge of the SON (Figure 1.3). Very few AVP-ir fibers were present in the MeA. The density of the fibers fades at the caudal ends of the amygdala. We also noted cells in the extended amygdala (EM) (Figure 1.4). This area is a group of AVP-ir extensions of the caudal BNST to the rostral MeA (Newmann 1999). The EM contained a small population of cell bodies and contained a significant populated with AVP-ir fibers.

Lateral and anterior hypothalamus

The lateral and anterior hypothalamus contains few AVP-ir cell bodies. The majority of these fibers are found in the peduncular lateral hypothalamus (PLH), dorsal and medial to the

SON (Figure 1.5). There is a light scattering of cells in the anterior hypothalamus (AH) just lateral of the midline central to the third ventricle. AVP-ir fibers are present here as well. The PLH is in line with fibers extended to and from the PVN and amygdala complex. The AH also contains few AVP-ir fibers extending from just above the optic chiasm to the AH.

Paraventricular Nucleus

The Paraventricular nucleus contained a very dense population of AVP-ir cell bodies as well as AVP-ir fibers. Beginning just after the anterior commisure, the number of stained cells increases as the PVN expands caudally. Cell bodies are less abundant at the caudal end of the PVN where the fornix falls ventral and caudal approaching the SON. There are 2 distinct populations of fibers extending from the PVN. The first set of fibers begins at the dorsal lateral edge of the PVN traveling laterally toward the thalamus and then mediodorsal to the SON (Figure 1.7). The second start at the ventral portions of the PVN, travels ventrolaterally through the AH, to optic chiasm (Figure 1.8). A subset of this population turns ventral just lateral to the fornix directly to the SON (Figure 1.9).

Suprachiasmatic Nucleus

The suprachiasmatic nucleus (SCN) contains a small population of AVP-ir cell bodies. These cells first appear at the caudal end of the BNST just as the fornix is in line dorsally with the third ventricle. The cells become more abundant caudally (Figure 1.10).

Supraoptic Nucleus

Perhaps the most abundant AVP-ir cells bodies as well as fibers are located in the supraoptic nucleus (SON). The SON AVP-ir cells cover a very long rostral to caudal range along the ventral edge of the brain. A dense population of cell bodies is present in the preoptic area (Figure 1.11). This population moves lateral and into the SON as it is moved caudal (Figure 1.13 and 1.14). The fibers projecting from the SON move dorsal and medial to the PLH, around the fornix onward to the PVN (Figure 1.13). Fibers spread further dorsolateral as they move caudal.

Androgen Effects

Androgen Concentrations

Concentrations circulating T differed significantly across groups following castration and implants. The concentrations all fell within the known physiological range for this species as found from wild caught animals (0.76-6.35 ng/ml) (Pasch et al. 2011a).

AVP-ir

Vasopressin immunoreactivity did not differ significantly across groups for all measured sections combined (α<0.05) [F (2, 21) = 0.58, p=0.57)] (Figure 1.15). Only the peduncular lateral hypothalamus (PLH) showed a significant difference across treatment groups [F (2, 21) = 3.55, p=0.047]. Tukey HSD revealed empty implant group (mean=77, SD=16) differed significantly (α<0.05) from the T implant group (mean=26.75, SE=9.46). Dihydrotestosterone (mean=44.67, SE=14.94) implant group did not differ significantly from T implant nor empty implant groups (Figure 1.16).

DISCUSSION

Neuropeptides in the arginine vasotocin family AVT (Goodson, 2005), and its mammalian homolog arginine vasopressin AVP, are known to play a major role in a host of social behaviors across many taxa including a great majority of rodent species; hamsters (Delville, 2000), voles (Phelps and Young, 03; De Vries et al., 1984), and mice (Bester-Meredith and Marler, 2007). We also know androgens and other hormones play a role in the control of these neuromodulators (Fuxjager et al., 2009). Here we investigate whether AVP expression in the singing mouse *Scotinomys teguina* follows the highly conserved patterns in the social behavior networks of other mammals and these regions are under the influence of androgens. This study will facilitate the use of this species as a model organism for the study of sociality across vertebrate taxa.

The neuroanatomical distribution of AVP-ir cells found in *Scotinomys teguina* is very similar with that of other rodents. Vasopressin-ir was found in the BNST, MeA, AH, three sites that are included in the social behavior network, as well as the SCN, PVN and SON. This species had a less pronounced MeA population than those reported for rodents of the *Peromyscus* family (Bester-Meredith and Marler, 2005). These patterns are not conserved in all small rodents. The golden hamster lacks BNST and MeA, vasopressin containing cells (Albers et al. 1991).

It is expected for AVP to be present in the MeA since it is known to receive projections from the olfactory bulbs. The olfactory bulbs pick up on sensory information, such

as pheromones, which are vital for social behavior and arousal. The lateral septum (LS) plays a vital role in memory. AVP injection studies have demonstrated AVP is required for the LS, which projects to the BNST, to modulate memory formation. Taken together, this suggests a role for AVP, acting in the BNST and MeA to have a major role in memory systems necessary to allow proper functioning in social situations in *Scotinomys teguina* (Dantzer et al., 1988).

The AVP is known to function in the control of the periphery playing a critical role in circadian rhythm from the SCN (Kalsbeek et al., 2010). With a very dense population of AVP-ir cell bodies and fibers, this puts vasopressin at a critical point for cyclical arousal states important for the control of timing in social behaviors such as mating seasons and thus aggression as well (van Balkom et al., 2003).

The paraventricular nucleus is densely populated with AVP-ir cells. AVP arising in the PVN transported to the posterior pituitary and then into the bloodstream to maintain osmotic equilibrium and blood pressure (Hirsch et al., 1993).

The AVP-ir patterns described above is widely conserved and known to be influenced by sex and hormones. The sexual differentiation in AVP expression is noted across all vertebrate taxa (De Vries and Panzica, 2006). Studies point to an androgen dependency of vasopressin, and a depletion of AVP-ir cell bodies and fibers in the BNST, MeA and related cell bodies. Males tend to have high numbers of AVP containing cell bodies (Goodson, 2005). Furthermore, it appears T spiked from winners' effect may also increase androgens, and thus,

AVP-ir in several nodes of the social behavior network (Neumann, 1999; Fuxjager et al. 2011). Given *S. teguina's* evolutionary relationship with several species on which AVP-ir differs with androgens, as well as *S. teguina's* AVP-ir which is very similar to many other rodents, we hypothesized that T and DHT implanted groups would show a higher expression of AVP-ir in many areas of the limbic system. Our results are discordant with many findings. This is interesting, especially since subjects in this experiment demonstrated across group variation in aggression as well as song dynamics. In songbirds, song parameters are under the influence of androgenic AVP circuits.

Two weeks is ample to cause differences in social behaviors as well as female preference due to castration in S. *teguina* (Pasch et al., 2011a). Since we know these behaviors are affected by androgens, it is possible there is a significant treatment difference that we were not able to demonstrate via our IHC procedures. Androgens manipulation is known to have a long lasting gradual effect on AVP expression. De Vries et al (1984) found AVP fiber density in the LS decreased constantly over a 15 week period. By limiting our study to two weeks, it is possible there was not adequate time to visualize the differences resulting from castrations. However, since we did find differences in singing along the same time frame, this does not seem likely to be a chief cause.

One alternative explanation is that colchicine treatment used in many other studies to dissemble actin and pool peptide in the cell bodies increased the ability to detect effects of androgens. Alternatively, it is possible that release of peptide confounds our ability to detect sex

steroid effects on production – something that could be addressed with in situ hybridization. The simplest interpretation of these data, however, is that androgen effects on singing behavior in *S. teguina* are not mediated by AVP. The significant difference found in the PLH is most likely a type I error. Several studies have demonstrated BNST and MeA AVP-ir to be more sensitive to androgen concentrations in monogamous species with significant paternal care (Wang et al., 1993; Bester-Meredith et al. 2005). *Scotinomys teguina* is a promiscuous species with little paternal care. It is possible these areas are not as sensitive to gonadal hormones as they are in other rodent species.

The previous study demonstrates the similarities between *Scotinomys teguina* and other small rodents in neural circuitry. AVP is highly expressed in the BNST and Medial Amygdala, two areas in which AVP-ir is modulated by androgens. The paraventricular nucleus also contained a dense population of AVP neurons. Another population originates in the SCN which travels into the pituitary. Although no significant differences were found between treatment groups, the similarities between our species and others suggest there should be a link between androgens and vasopressin expression. Future studies will explore the possibility of long term changes in vasopressin and vasopressin receptor due to reduced androgen levels. Double labeling studies can also shed light on whether or not androgens directly affect vasopressin receptors. V1aR was recently implicated in socially debilitating diseases such as depression and autism (Rood and De Vries, 2011). Vasopressin plays a vital role in all aspects of social behavior across vertebrate taxa. This study suggests the singing mouse is suited to

study how androgens modulate social behavior through a highly conserved and complex neural system, but a great remains to be elucidated.

Chapter 2: Singing Behavior in Social Encounters

INTRODUCTION

Individual differences are vital to the formation and maintenance of social structure across taxa. Elaborate displays of courtship and aggression are most often studied. These include the feathers of peacocks (Petrie et al., 1991), the dewlap of an anole lizard (Crews, 1980), the songs of birds (Goodson et al., 2004), or the calls of frogs (Kime et al., 2000). These demonstrations are primarily used to signal fitness, intimidating possible aggressors or attracting possible mates. Elaborate displays are beneficial because they allow both signaler and recipient to gauge their own fitness against that of a competitor without the risk of physical encounters. However, sometimes signaling does not match fitness and the signaler risks defeat (Viljugrein, 1997). Signalers must use prior experience and internal state (Fuxjager et al. 2011) to weigh the costs and benefits of elaborate displays when deciding whether or not to display. Losing forces the signaler to reevaluate its social status, decreasing signaling to avoid further damaging defeat whereas winning has the opposite effect (Peake et al. 2002,). In our current study, we manipulate social experience to determine how experience modulates vocalizing behavior in Alston's singing mouse *Scotinomys teguina*.

Social experience modulates signaling across many vocalizing taxa. Apfelbeck et al. (2012) demonstrated repeated simulated territorial intrusions (STI) at high intensity lead to an increase in vocalizations in black redstarts. Wingfield et al. (1990) proposed the 'challenge hypothesis' which states seasonal changes in testosterone led to behavioral changes seen during

the mating season of song birds. This increase in T leads to an increase in song production as well as aggression. More recently, Oyegbile and Marler (2005) examined the effects of successful aggressive experience in producing the "winner effect." Like the challenge hypothesis, this also suggested a role for testosterone in behavioral changes. Marler found that the California mouse, *Peromyscus Californicus,* increased its aggression following winning social encounters. A loser effect also exist, in which losers of social encounters decrease aggression and are more likely to submit when faced with an opponent. Although Marler's findings are robust and true across many taxa, there are limitations. The winner effect is likely environment specific (Gleason et al., 2009). Subjects largely retained their aggressive nature once returned to their own cages after having lost in another's cage. In crickets, Nelson and Nolen (1997) found that crickets (*Acheta domesticus*) retained their ability to attract mates and copulate even after losing social encounters. This demonstrates the winner effect is not necessarily the only component of social hierarchy.

The singing mouse is a small rodent species native to the cloud forest in Central America. There are several subspecies ranging in size from 10-15g in the wild. The singing mice are unique for their use of complex vocalizations between 18-40 Hz. Unlike ultrasonic vocalizations of other rodents, it is easily heard by humans without manipulations. The role of this signal is not fully understood, but, recent studies suggest a role in male-male aggression as well as mate attraction. The song is a short trill around 10 seconds long. Previous research has also found these songs change in structure based on T levels (Pasch, 2011a). Pasch (2011b),

found females approach a male song associated with higher physical condition. They easily identified individual differences in song structure, specifically an increase in trill rate and/or a lower dominant frequency. Pasch et al (2011) also found that manipulation of male T levels could produce songs that indicated better condition. In theory, winning encounters increase T (Oyegbile and Marler 2005), which increases signal quality, thus increasing the interest of females.

Field studies also suggest these mice are able to distinguish the slightly different songs across species. The subordinate *S. teguina*, counter-sings to the songs of the dominant *S. xerampelinus* when living in allopatry. However, in sympatry the subordinate species displays a fear response when encountering the song of the dominant *S. xerampelinus* (Pasch 2013). We attempt to further explore this finding that social experience modulates vocalizing behavior in this species.

We hypothesize winning social encounters actively increases the singing behavior of the mice. The initial step of the current study is a simple recording of responses to stimuli without social experience. We follow this with aggressive trials to simulate winning and losing. Then, we again recorded song responses to stimuli. Comparing the singing behavior of the winners and loser to each other and to their baseline will allow us to elucidate the role of social experience on the song use of *S. teguina*.

METHODS

Subjects for this experiment were lab reared offspring of animals originally caught in

Costa Rica and brought back to the lab. Animals are sib housed with 1 or 2 litter mates. Animals are given food, cat chow, and water *ad libitum*. They are housed in 12:12 dark: light cycle at 15.5-19^{o} C. All phase of the experiment were performed during light hours. The following protocol is approved by Institutional Animal Care and Use Committee at the University of Texas (AUP 008002).

Experiment animals were all naïve. They were separated from sibs and singly housed in a noise attenuating chamber and maintained on a 12:12 light cycle. Internal temperatures and humidity were constantly monitored. Animals were transferred to these chambers 3 days prior to experimental manipulations and remained housed here for the duration of the experiment.

On day 1, following 3 days of isolation, animals were recorded in their home cages under red light. Spontaneous song was recorded for 20 minutes followed by 3 stimuli, 1) soiled bedding from female cages, 2) a spontaneous song recorded in the lab from lab reared mice, 3) pink noise. The pink noise was developed by filtering the same song from stimulus 1, and retaining the same spectral envelope of the corresponding stimulus. We played stimuli at 50-dB SPL at 1 m from a Pioneer TS-250 speaker (flat frequency response 6–40 kHz) via an external soundcard (Edirol FA-66). We used an ACO Pacific microphone and preamplifier (Model 7016 and 4116) connected to a laptop running Raven Pro 1.3 via an external soundcard (Edirol FA-66) to record mouse songs at 30 cm. Microphones were calibrated prior to recording sessions with a Brüel and Kjær sound level meter (Type 2219) and calibrator (Type 4230). All songs at were sampled at 96 kHz and 24 bits (Pasch, 2011a).

On day 2, 4, 6 and 8 animals engaged in resident-intruder trials. Residents and intruders were age (+/- 4 months) and weight matched (+/- 2 grams) and divided into groups at random. Trials continued for 5 minutes or until a winner was determined. A winner was identified by 3 consecutive chases or attacks or inducing 3 consecutive submissive supine displays. All trials were recorded and then analyzed for aggressive and submissive behaviors. Following RI trials, subject animals were recorded again using the previously mentioned procedures.

Data was analyzed in JMP version 9. We performed GLM testing to compare effects of residency and treatment on song response (F,p). We then used Wilcoxon signed rank to compare group differences before and after behavioral trials (W,p). To analyze aggressive data, we used the Friedman test to analyze differences between groups and across trials. Graphs for all data were created using SPSS 20.1.

RESULTS

Behavior data showed differences between residents and intruders on a range of aggressive and submissive behaviors. There were also differences across these groups in their singing behaviors (α<0.05).

Aggressive behavior was different between the treatment groups as well as across trials. Friedman analysis revealed a strong effect between residents and intruders on aggressive behavior (p= 0.011). There is a trend to more aggressive behavior in later trials in the intruders (p= 0.065) (Figure 2.1). Intruders also display rearing behavior more frequently in later trials with the greatest frequency in the 4^{th} trial (Figure 2.2).

Singing behavior was not significantly different across groups before RI trials ($F_{3, 23}$= .999, p>0.05) (Figure 2.3). Residency status alone had a significant effect on total number of songs ($F_{3,23}$ =3.786, p=.0223). There was a significant difference between pre and post total songs with the residents but not intruders (W=47.500, 8.500; p=0.001, 0.576 respectively) (Figure 2.4). Residents increased their response songs to conspecific song following RI trials but not intruders (w= 18.5, 11; p=0.027, 0.360) (Figure 2.5). There were residency (p=0.002), treatment (p<0.001) and interaction (p<0.001) effects on song response to pink noise ($F_{3, 26}$=17.3418 p<0.001) (Figure 2.6). There was no difference between resident and intruders following RI trials (W=7.5, -3.00; p=0.066, 0.500). There were no effects of residency on song response to silence ($F_{3, 26}$= 0.1682, p= 0.168). No differences were found in residents nor intruders in response to silence following RI trials (W=10.5, 3.5; p=0.109, 0.437). Similar results were seen for spontaneous song. There were no effects of residency or treatments on spontaneous song ($F_{3, 26}$=0.620 p=0.608). No differences were found following RI trials in residents or intruders (W=16.50, 5.500; p=0.063, 0.533).

Regression analysis showed no relationship between propensity to sing and aggressiveness (R^2=0.019).

DISCUSSION

Acoustic communication plays a vital role across numerous vertebrae taxa including fish (Remage-Healey and Bass, 2006), anurans (Kime et al., 2000) songbirds (Goodson et al., 2004) and mammals (Asaba et al., 2014). Here, we demonstrate that social experience

modulates aggression and acoustic communication in *S. teguina*. Winners attack faster and more frequently than losers. Winners also sing more frequently than losers. Interestingly winners did not sing significantly more in response to female bedding or conspecific song than did losers, but they did increase song production in response to pink noise. This suggests the males listen to the overall spectral envelope of the song rather than individual notes. Subtle differences between residents and intruders became more conspicuous with experience suggesting a role of the winner's effect.

Residents clearly demonstrated dominance over intruders during the RI trials. Home advantage is a robust effect that is seen across a wide variety of vertebrate taxa (Fuxjager, 2011). Social experience also increased these effects. It is known that winning or losing aggressive encounters modifies physiology across vertebrate taxa. It increases aggression in rodents (Oyegbile and Marler, 2005) and increases singing in territorial song birds (Goodson et al., 2004). Dugatkin and Earley (2006) have also demonstrated how winning elevates one in social hierarchies. The challenge hypothesis suggests these changes may be due to a transient rise in androgens following winning. Residents were more likely to attack and did so much faster than intruders. These differences became more obvious with increasing experience. We did not observe a significant loser's effect. While this was initially surprising, it is worth noting that animals were tested in the home cage of the resident; in the studies by Marler and colleagues (Fuxjager et al. 2009), loser effects require losses within a male's home cage, a finding consistent with the apparent robustness of our males to losing fights when intruding.

Interestingly we found residents to groom less and intruders to display defensive rearing more often during the 4th RI round. Experimental limitations resulted in animals being exposed to the same individual in the 1st and 4th rounds. These behavioral changes could demonstrate recognition between the animals. Since hierarchy is typically formed through social experience, this may be a result of hierarchy already being formed between the two animals so the dominant male may have limited motivation to attack a weaker intruder. A similar decrease in motivation is seen in avian species. It is typical for a dominant bird to largely ignore the songs of a subordinate (Kroodsma, 1979).

There were no differences before RI trials within residents or intruders. However, residents increased their propensity to sing following social encounters. We calculated this by summing song responses to all stimuli, silence, pink noise and conspecific song. We found residents increased their singing behavior in response to conspecific song as well as to pink noise. Although noise elicited the greatest difference in response between resident and intruders, both groups counter-sang more frequently to conspecific song. This finding suggests singing mice continue to vocalize even after defeat; however, winners of social encounters may up regulate their responses to songs originating from a further distance. It is possible the mice interpret the noise stimuli as a degraded conspecific song. As sound travels, it degrades and loses information; the spectral envelope will remain intact at a greater distance than other parameters of the song. Unlike avian species, the singing mice were more likely to sing following social encounters. There is a general trend for residents to sing more often to the various stimuli

than intruders.

Previous field studies within this species and its sister species (Pasch et al., 2013) demonstrated *S. teguina* living in sympatry with the dominant species *S. xerampelinus* decrease singing in response to the songs of *S. xerampelinus*. It is known *S. xerampelinus* actively exclude *S.teguina* from their home ranges. It is possible *S. teguina* is learning to limit vocalizations in the presence of the dominant species to avoid being attacked. The current studies bolsters this hypothesis by demonstrating social experience will modulate vocal behavior.

We suggest that the *Scotinomys* genus is an excellent subject for studying a host of social behaviors, including the winners' effect, individual recognition, social defeat, female preference and intra-species competition.

Figure 1.
AVP-ir in the Posteriolateral BNST.

Figure 2.
AVP-ir in the BNST.

Figure 3.
AVP-ir cell bodies in the Medial Amygdala. Fibers extending from the SON toward the fornix.

Figure 4.
AVP-ir cell bodies and fibers in the extended amygdala.

Figure 5.
AVP-ir cell bodies and fibers in the peduncular lateral hypothalamus.

Figure 6.
AVP-ir cell bodies in the anterior hypothalamus. Fiber tracts from the SON to the AH.

Figure 7.
AVP-ir cell bodies in the PVN and fibers extending from the PVN.

Figure 8.
AVP-ir fibers extending between the PVN and SON.

Figure 9.
Dorsolateral AVP-ir fibers of the SON.

Figure 10.
AVP-ir cell bodies in the SCN.

Figure 11.
AVP-ir cell bodies in the preoptic area.

Figure 12.
AVP-ir cell bodies in the SON. AVP-ir fibers extending from AH.

Figure 13.
AVP-ir fiber tracts in the area of SON toward thalamus.

Figure 14.
AVP-ir cell bodies and fibers, caudal end of SON.

Figure 15.
Effects of androgens on the amount of vasopressin expressing cell bodies in the BNST, MeA, EM, and PLH in *S. teguina*. Data depicts means ± SE.

Figure 16.
Effects of androgens on vasopressin immunoreactivity in the Peduncular Lateral Hypothalamus Significance (α<0.05). Data depicts means ± SE.

Figure 2.1.
Aggression during resident-intruder trials. Data depicts means ± SE.

Figure 2.2.
Rearing during resident-intruder trials. Data depicts means ± SE.

Figure 2.3.
Singing during resident-intruder trials. Data depicts means ± SE.

Figure 2.4.
Singing behavior before and after social encounters. Data depicts means ± SE

Figure 2.5.
Singing responses to conspecific song before and after resident-intruder trials. Data depicts means ± SE.

Figure 2.6
Singing in response to pink noise following social experience. Data depicts means ± SE

References

Akre KL, Ryan MJ (2010) Proximity-dependent response to variably complex mating signals in tungara frogs (*Physalaemus pustulosus*). Ethology 116:1138-1145.

Albers HE, Rowland CM, Ferris CF (1991) Arginine-vasopressin immunoreactivity is not altered by photoperiod or gonadal-hormones in the Syrian-hamster (*Mesocricetus-auratus*). Brain Research 539:137-142.

Apfelbeck B, Goymann W (2011) Ignoring the challenge? Male black redstarts (*Phoenicurus ochruros*) do not increase testosterone levels during territorial conflicts but they do so in response to gonadotropin-releasing hormone. Proceedings of the Royal Society B-Biological Sciences 278:3233-3242.

Apfelbeck B, Kiefer S, Mortega KG, Goymann W, Kipper S (2012) Testosterone affects song modulation during simulated territorial intrusions in male black redstarts (*Phoenicurus ochruros*). Plos One 7:11.

Arnold AP (2009) The organizational-activational hypothesis as the foundation for a unified theory of sexual differentiation of all mammalian tissues. Hormones and Behavior 55:570-578.

Asaba A, Okabe S, Nagasawa M, Kato M, Koshida N, Osakada T, Mogi K, Kikusui T (2014) Developmental social environment imprints female preference for male song in mice. Plos One 9:7.

Ball GF, Balthazart J (2004) Hormonal regulation of brain circuits mediating male sexual behavior in birds. Physiology & Behavior 83:329-346.

Bamshad M, Novak MA, Devries GJ (1993) Sex and species-differences in the vasopressin innervations of sexually naïve and parental prairie voles, *Microtus ochrogaster* and meadow voles, *Microtus Pennsylvanicus*. Journal of Neuroendocrinology 5:247-255.

Bardin CW, Catterall JF (1981) Testosterone – a major determinant of extra-genital sexual dimorphism. Science 211:1285-1294.

Bester-Meredith JK, Marler CA (2000) Cross-fostering alters vasopressin-immunoreactive staining in Peromyscus mice. Society for Neuroscience Abstracts 26:Abstract No.-760.769.

Bester-Meredith JK, Marler CA (2007) Social experience during development and female offspring aggression in Peromyscus mice. Ethology 113:889-900.

Bester-Meredith JK, Martin PA, Marler CA (2005) Manipulations of vasopressin alter aggression differently across testing conditions in monogamous and nonmonogamous Peromyscus mice. Aggressive Behavior 31:189-199.

Boyd SK, Moore FL (1992) Sexually dimorphic concentrations of arginine vasotocin sensory regions of the amphibian brain. Brain Research 588:304-306.

Butchart SHM, Seddon N, Ekstrom JMM (1999) Yelling for sex: harem males compete for female access in bronze-winged jacanas. Animal Behavior 57:637-646.

Carleton MD, Hooper ET, Honacki J (1975) Karyotypes and accessory reproductive glands in rodent genus *Scotinomys*. Journal of Mammalogy 56:916-921.

Cervantes MC, Delville Y (2007) Individual differences in offensive aggression in golden hamsters: A model of reactive and impulsive aggression? Neuroscience 150:511-521.

Crews D (1980) Studies in squamate sexuality. Bioscience 30:835-838.

Dantzer R, Koob GF, Bluthe RM, Lemoal M (1988) Septal vasopressin modulates social memory in male-rats. Brain Research 457:143-147.

De Vries GJ, Panzica GC (2006) Sexual differentiation of central vasopressin and vasotocin systems in vertebrates: Different mechanisms, similar endpoints. Neuroscience 138:947-955.

Delville Y, De Vries GJ, Ferris CF (2000) Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. Brain Behavior and Evolution 55:53-76.

Devries GJ, Buijs RM, Vanleeuwen FW (1984) Sex differences in vasopressin and other neurotransmitter systems in the brain. Progress in Brain Research 61:185-203.

Donaldson ZR, Young LJ (2008) Oxytocin, Vasopressin, and the Neurogenetics of Sociality. Science 322:900-904.

Dugatkin LA (1997) Winner and loser effects and the structure of dominance hierarchies. Behavioral Ecology 8:583-587.

Dugatkin LA, Earley RL (2003) Group fusion: the impact of winner, loser, and bystander effects on hierarchy formation in large groups. Behavioral Ecology 14:367-373.

Ebensperger LA (2001) A review of the evolutionary causes of rodent group-living. Acta Theriologica 46:115-144.

Ferguson JN, Aldag JM, Insel TR, Young LJ (2001) Oxytocin in the medial amygdala is essential for social recognition in the mouse. Journal of Neuroscience 21:8278-8285.

Fernandez-Vargas M, Tang-Martinez Z, Phelps SM (2011) Singing, allogrooming, and allomarking behavior during inter- and intra-sexual encounters in the Neotropical short-tailed singing mouse (Scotinomys teguina). Behavior 148:945-965.

Ferris CF, Delville Y (1994) Vasopressin and serotonin interactions in the control of agonistic behavior. Psychoneuroendocrinology 19:593-601.

Fine ML, Keefer DA, Russelmergenthal H (1990) Autoradiographic localization of estrogen-concentrating cells in the brain and pituitary of the oyster toadfish. Brain Research 536:207-219.

Franklin KBJ, Paxinos G (2007). The mouse brain in stereotaxic coordinates. Amsterdam: Elsevier.

Fuxjager MJ, Oyegbile TO, Marler CA (2011a) Independent and Additive Contributions of Postvictory Testosterone and Social Experience to the Development of the Winner Effect. Endocrinology 152:3422-3429.

Fuxjager MJ, Montgomery JL, Marler CA (2011b) Species differences in the winner effect disappear in response to post-victory testosterone manipulations. Proceedings of the Royal Society B-Biological Sciences 278:3497-3503.

Fuxjager MJ, Mast G, Becker EA, Marler CA (2009) The 'home advantage' is necessary for a full winner effect and changes in post-encounter testosterone. Hormones and Behavior 56:214-219.

Gleason ED, Fuxjager MJ, Oyegbile TO, Marler CA (2009) Testosterone release and social context: When it occurs and why. Frontiers in Neuroendocrinology 30:460-469.

Goodson JL (2005) The vertebrate social behavior network: Evolutionary themes and variations. Hormones and Behavior 48:11-22.

Goodson JL, Bass AH (2000) Forebrain peptides modulate sexually polymorphic vocal circuitry. Nature 403:769-772.

Goodson JL, Lindberg L, Johnson P (2004) Effects of central vasotocin and mesotocin manipulations on social behavior in male and female zebra finches. Hormones and Behavior 45:136-143.

Goymann W, Wingfield JC (2004) Allostatic load, social status and stress hormones: the costs

of social status matter. Animal Behaviour 67:591-602.
Hill RW, Hooper ET (1971) Temperature regulation in mice of genus *Scotinomys*. Journal of Mammalogy 52:806-&.
Hooper ET (1975) Orbital region and size of eye in species of Scotinomys (*rodentia*). Journal of Mammalogy 56:667-671.
Kalsbeek A, Fliers E, Hofman MA, Swaab DF, Buijs RM (2010) Vasopressin and the output of the hypothalamic biological clock. Journal of Neuroendocrinology 22:362-372.
Kime NM, Turner WR, Ryan MJ (2000) The transmission of advertisement calls in Central American frogs. Behavioral Ecology 11:71-83.
Kroodsma DE (1979) Vocal dueling among male marsh wrens - evidence for ritualized expressions of dominance-subordinance. Auk 96:506-515.
Lan YT, Hsu YY (2011) Prior contest experience exerts a long-term influence on subsequent winner and loser effects. Frontiers in Zoology 8:12.
Landgraf R, Neumann ID (2004) Vasopressin and oxytocin release within the brain: a dynamic concept of multiple and variable modes of neuropeptide communication. Frontiers in Neuroendocrinology 25:150-176.
Maney DL, Goode CT, Wingfield JC (1997) Intraventricular infusion of arginine vasotocin induces singing in a female songbird. Journal of Neuroendocrinology 9:487-491.
Morgan MJ, Wilson CE, Crim LW (1999) The effect of stress on reproduction in Atlantic cod. Journal of Fish Biology 54:477-488.
Nelson CM, Nolen TG (1997) Courtship song, male agonistic encounters, and female mate choice in the house cricket, Acheta domesticus (Orthoptera: Gryllidae). Journal of Insect Behavior 10:557-570.
Newman SW (1999) The medial extended amygdala in male reproductive behavior - A node in the mammalian social behavior network. Advancing from the Ventral Striatum to the Extended Amygdala: Implications for Neuropsychiatry and Drug Abuse: in Honor of Lennart Heimer 877:242-257.
Nishizuka M, Arai Y (1981) Sexual dimorphism in synaptic organization in the amygdala and its dependence on neonatal hormone environment. Brain Research 212:31-38.
Nottebohm F, Arnold AP (1976) Sexual dimorphism in vocal control areas of songbird brain. Science 194:211-213.
Oyegbile TO, Marler CA (2005) Winning fights elevates testosterone levels in California mice and enhances future ability to win fights. Hormones and Behavior 48:259-267.
Oyegbile TO, Marler CA (2006) Weak winner effect in a less aggressive mammal: Correlations with corticosterone but not testosterone. Physiology & Behavior 89:171-179.

Pasch B, Bolker BM, Phelps SM (2013) Interspecific Dominance Via Vocal Interactions Mediates Altitudinal Zonation in Neotropical Singing Mice. American Naturalist 182:E161-E173.

Pasch B, George AS, Campbell P, Phelps SM (2011a) Androgen-dependent male vocal performance influences female preference in Neotropical singing mice. Animal Behaviour 82:177-183.

Pasch B, George AS, Hamlin HJ, Guillette LJ, Phelps SM (2011b) Androgens modulate song effort and aggression in Neotropical singing mice. Hormones and Behavior 59:90-97.

Peake TM, Terry AMR, McGregor PK, Dabelsteen T (2002) Do great tits assess rivals by combining direct experience with information gathered by eavesdropping? Proceedings of the Royal Society B-Biological Sciences 269:1925-1929.

Petrie M, Halliday T, Sanders C (1991) Peahens prefer peacocks with elaborate trains. Animal Behaviour 41:323-331.

Phelps SM, Young LJ (2003) Extraordinary diversity in vasopressin (V1a) receptor distributions among wild prairie voles (*Microtus ochrogaster*): Patterns of variation and covariation. Journal of Comparative Neurology 466:564-576.

Phoenix CH, Goy RW, Gerall AA, Young WC (1959) The organizing action of an androgen administered prenatally on the tissues mediating mating behavior in the guinea pig. Anatomical Record 133:323-323.

Portfors CV (2007) Types and functions of ultrasonic vocalizations in laboratory rats and mice. Journal of the American Association for Laboratory Animal Science 46:28-34.

Raisman G, Field PM (1973) Sexual dimorphism in neuropil of preoptic area of rat and its dependence on neonatal androgen. Brain Research 54:1-29.

Ramsey M, Crews D (2007) Steroid signaling system responds differently to temperature and hormone manipulation in the red-eared slider turtle (Trachemys scripta elegans), a reptile with temperature-dependent sex determination. Sexual Development 1:181-196.

Remage-Healey L, Bass AH (2005) Rapid elevations in both steroid hormones and vocal signaling during playback challenge: a field experiment in Gulf toadfish. Hormones and Behavior 47:297-305.

Remage-Healey L, Bass AH (2006) From social behavior to neural circuitry: Steroid hormones rapidly modulate advertisement calling via a vocal pattern generator. Hormones and Behavior 50:432-441.

Rhen T, Crews D (1999) Embryonic temperature and gonadal sex organize male-typical sexual and aggressive behavior in a lizard with temperature-dependent sex determination. Endocrinology 140:4501-4508.

Rood BD, De Vries GJ (2011) Vasopressin Innervation of the Mouse (*Mus musculus*) Brain and Spinal Cord. Journal of Comparative Neurology 519:2434-2474.

Sakata JT, Woolley SC, Gupta A, Crews D (2003) Differential effects of testosterone and progesterone on the activation and retention of courtship behavior in sexual and parthenogenetic whiptail lizards. Hormones and Behavior 43:523-530.

Santangelo N, Bass AH (2006) New insights into neuropeptide modulation of aggression: field studies of arginine vasotocin in a territorial tropical damselfish. Proceedings of the Royal Society B-Biological Sciences 273:3085-3092.

Scattoni ML, McFarlane HG, Zhodzishsky V, Caldwell HK, Young WS, Ricceri L, Crawley JN (2008) Reduced ultrasonic vocalizations in vasopressin 1b knockout mice. Behavioural Brain Research 187:371-378.

Thompson RR, George K, Walton JC, Orr SP, Benson J (2006) Sex-specific influences of vasopressin on human social communication. Proceedings of the National Academy of Sciences of the United States of America 103:7889-7894.

Tobet SA, Zahniser DJ, Baum MJ (1986) Sexual dimorphism in the preoptic anterior hypothalamic area of ferrets - effects of adult exposure to sex steroids. Brain Research 364:249-257.

van Balkom BWM, Hoffert JD, Chou CL, Knepper MA (2003) Proteomic analysis of long-term vasopressin response in inner medullary collecting duct of Brattleboro rat. Journal of the American Society of Nephrology 14:32A-32A.

Viljugrein H (1997) The cost of dishonesty. Proceedings of the Royal Society B-Biological Sciences 264:815-821.

Wagner WE, Reiser MG (2000) The importance of calling song and courtship song in female mate choice in the variable field cricket. Animal Behaviour 59:1219-1226.

Walton JC, Waxman B, Hoffbuhr K, Kennedy M, Beth E, Scangos J, Thompson RR (2010) Behavioral effects of hindbrain vasotocin in goldfish are seasonally variable but not sexually dimorphic. Neuropharmacology 58:126-134.

Wang ZX (1994) Testosterone effects on development of vasopressin messenger-rna expression in the bed nucleus of the stria terminalis and medial amygdaloid nucleus in male-rats. Developmental Brain Research 79:147-150.

Wang ZX, Bullock NA, Devries GJ (1993) Sexual-differentiation of vasopressin projections of the bed nucleus of the stria terminals and medial amygdaloid nucleus in rats. Endocrinology 132:2299-2306.

Wang ZX, Ferris CF, Devries GJ (1994) Role of septal vasopressin innervation in paternal behavior in prairie voles (*Microtus ochrogaster*). Proceedings of the National

Academy of Sciences of the United States of America 91:400-404.
Wingfield JC, Hegner RE, Dufty AM, Ball GF (1990) The challenge hypothesis - theoretical implications for patterns of testosterone secretion, mating systems, and breeding strategies. American Naturalist 136:829-846.
Winslow J, Insel TR (1991) Vasopressin modulates male squirrel-monkeys behavior during social separation. European Journal of Pharmacology 200:95-101.
Winslow JT, Hastings N, Carter CS, Harbaugh CR, Insel TR (1993) A role for central vasopressin in pair bonding in monogamous prairie voles. Nature 365:545-548.

Vita

Andreas Sherron George was born in St. Thomas USVI. He completed is HS diploma at Paxon School for Advanced studies in Jacksonville, FL. In the fall of 2010, Andreas entered the University of Florida in Gainesville, Fl. He completed a Bachelor of Science in Integrative Biology in the Spring of 2010. He entered graduate school at the University of Texas at Austin in the Fall of 2010.

Permanent email: 13ageorge@gmail.com
This thesis was typed by Andreas Sherron George.