The Effects of Testosterone on Emotional Processing in Male Rhesus Monkeys (Macaca Mulatta)

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THE EFFECTS OF TESTOSTERONE ON EMOTIONAL PROCESSING IN MALE RHESUS MONKEYS (*MACACA MULATTA*)

A Thesis Presented

by

HANNA M KING

Submitted to the Graduate School of the University of Massachusetts Amherst in partial fulfillment of the requirements for the degree of

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Neuroscience and Behavior Program
THE EFFECTS OF TESTOSTERONE ON EMOTIONAL PROCESSING IN MALE RHESUS MONKEYS (MACACA MULATTA)

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ABSTRACT

THE EFFECTS OF TESTOSTERONE ON EMOTIONAL PROCESSING IN MALE RHESUS MONKEYS (MACACA MULATTA)

SEPTEMBER 2010

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Directed by: Professor Agnès Lacreuse

The effects of testosterone (T) extend beyond reproductive behavior to the areas of cognitive and emotional functioning. While T effects on cognition have been extensively investigated, less is known about the role of T in the processing of emotional stimuli. Considering the role that T plays in aggressive behavior and dominance status, it is of particular interest to determine whether T modulates the processing of social threat. Due to their similarities to humans in brain organization, reproductive endocrinology and affective regulation, rhesus monkeys (macaca mulatta) provide an excellent model to investigate this relationship. In a within-subjects design, six male rhesus monkeys underwent treatment to suppress endogenous T and received either T or oil replacement. Tests of anxiety, attention and memory for social and non-social emotional stimuli, and risk-taking were administered to animals during both treatments. Data analyses indicate that T treatment resulted in faster response times, but had no effect on anxiety, attention or memory for emotional stimuli, or on risk-taking behavior. There are several limitations to this study that may account for the lack of effect of T and therefore, further investigation of the relationship between T and emotional processing is warranted.
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CHAPTER I

THE EFFECTS OF TESTOSTERONE ON EMOTIONAL PROCESSING IN MALE RHESUS MONKEYS (MACACA MULATTA)

Introduction

Although the role of steroid hormones in reproductive behavior has been studied at length, their role in cognition and emotion has received less attention to date. Specifically, while testosterone (T) has well-established effects on male sexual behavior, less is known about the role of T in cognitive and emotional processing. The presence of androgen receptors (AR) in various brain regions which play an important role in cognition and emotion, such as the amygdala, hypothalamus, bed nucleus of the stria terminalis (BNST) and hippocampus (Abdelgadir, Roselli, Choate & Resko, 1999; Brown et al., 1995; Michael, Clancy & Zumpe, 1995; Roselli, Klosterman & Resko, 2001; Simerly, Chang, Muramatsu & Swanson, 1990), suggests that T likely plays a role in cognitive and emotional functioning. Furthermore, there is evidence to suggest that T affects neural processing (in some cases resulting in behavioral changes) in these brain areas (Cooke, 2006; Delville, Mansour & Ferris, 1996; Derntl et al., 2008; Leranth, Petnehazy & MacLusky, 2002; Stanton, Wirth, Waugh & Schultheiss, 2009). The effects of T on cognition have been reviewed extensively (e.g. Beauchet, 2006; Janowsky, 2006; Warren, Serby & Roane, 2008) and therefore, I focus here on examining the influence of T on various aspects of emotional processing.

Testosterone, Aggression and Dominance

Many studies have found relationships between T and both aggressive and dominance behavior in animals and in humans. Dominance and aggressive behaviors
overlap quite often, but an important distinction between them is discussed by Mazur (1976). Aggressive behavior involves an organism intending to inflict physical harm on another, while dominance behavior involves an organism intending to achieve or maintain superior status over another. Therefore, dominance behavior may be aggressive, but need not be and vice versa (Mazur, 1976); however, it is reasonable that aggressive behavior directed toward conspecifics is often also dominance behavior. Although rodents tend to dominate aggressively, higher primates do so to a lesser degree (Mazur, 1973). For example, non-human primates may dominate with gestures such as stares, or lunges (Mazur, 1973), while dominance competitions in humans come in such forms as spelling bees, elections, academic jousting, criticism, and competitions for promotion (Mazur & Booth, 1998). Therefore, among higher primates, it is interesting to investigate not only the relationship between T and aggression, but also between T and social dominance.

A positive correlation between aggression and T levels has often been observed in animals and humans (see Archer, 2006; Mazur & Booth, 1998; Rubinow & Schmidt, 1996 for reviews). Such a positive relationship has also been reported between T levels and dominance behavior and status or rank in animals (Beehner, Phillips-Conroy & Whitten, 2005; Czoty, Gould & Nader, 2008; Muehlenbein, Watts, & Whitten, 2004; Muller & Wrangham, 2004; Parikh, Clement & Fernald, 2006; Rose, Holaday & Bernstein, 1971; Setchell, Smith, Wickings & Knapp, 2008; Winslow & Miczek, 1988), as well as in human males (Ehrenkranz et al., 1974). A significant, positive correlation between baseline T levels and trait dominance has been reported in human males (Carré, Putnam, McCormick, 2009) and furthermore, men with high baseline T levels seem to be
more sensitive to and/or aware of their status condition. For example, when men with high T levels were put into a “high-status” position, they performed better on two cognitive tasks and also showed less cardiac arousal compared to men with high T placed in the “low-status” condition (Newman, Sellers & Josephs, 2005).

In human females, baseline T levels have been positively correlated with stronger activation of subcortical structures involved in aggression, such as the amygdala and hypothalamus, in response to social threat (angry facial expressions) and T administration has resulted in more persistent activation of such areas compared to placebo (Hermans, Ramsey, & van Honk, 2008). Men have shown similar correlations between endogenous T levels and aggressive behavior (Berman, Gladue & Taylor, 1993; Gray, Jackson & McKinlay, 1991; Olewus, Mattisson, Schalling & Low, 1988). For example, higher T levels have been correlated with more violent crimes in both male and female inmates (Dabbs & Hargrove, 1997; Dabbs, Frady, Carr & Besch, 1987) and greater self-reported aggression in adolescent males (Olweus, Mattisson, Schalling & Low, 1980).

In some cases, intensity of aggressive behavior determines the degree to which T levels rise during an aggressive encounter in animals (Rilling, Winslow & Kilts, 2004; Ross, French & Patera, 2004). Similarly, a change in dominance status in animals has resulted in a change in T levels in many cases (Rose, Bernstein & Gordon, 1975; Rose, Gordon & Bernstein, 1972; Yodyingyuad, Eberhart & Keverne, 1982). Furthermore, competition outcome (which may be considered a change in dominance status) in human males often results in greater increase in T levels in winners compared to losers (Elias, 1981; Gladue, Boechler & McCaul, 1989; Mazur, Booth & Dabbs, 1992; Mazur & Lamb, 1980; McCaul, Gladue & Joppa, 1992). This evidence suggests that T levels are
dependent on both aggressive behavior and dominance rank; however, other studies suggest the reverse relationship: that aggression, dominance behavior and dominance rank are dependent on T levels.

Castration and T replacement has affected dominance behavior and rank in a variety of animals from rodents to non-human primates. Castration has been reported to result in increased subordinate behavior (Richards et al., 2009) and diminished dominance behavior as well as decline in dominance rank (Albert, Walsh, Gorzalka, Siemens & Louie, 1986; Green et al., 1972; Lee & Naranjo, 1974; Wilson & Vessey, 1968), while T replacement has resulted in improved dominance status (Clark & Birch, 1945). Furthermore, intact males and females who are administered T have exhibited more dominance behavior (Searcy & Wingfield, 1980) and also acquired and maintained higher rank than those who did not receive T administration (Bouissou, 1978; Veiga, Viñuela, Cordero, Aparicio & Polo, 2004). A rise in T levels from pre- to post-competition in human males also predicted their willingness to compete again (Carré & McCormick, 2008), suggesting that T is a factor in tendency to engage in dominance challenges in men as well as animals. In a rare case in which healthy males received either T treatment or placebo while participating in the Point Subtraction Aggression Paradigm (PSAP), participants showed significantly greater aggressive responding when receiving T treatment compared to placebo (Kouri, Lukas, Pope & Oliva, 1995).

There is sufficient evidence to suggest that dominance and aggressive behaviors cause changes in T, but also sufficient evidence to suggest that T levels affect dominance and aggression. Mazur (1985) suggested the Biosocial Model of Status in which testosterone and dominance are reciprocally related. In this case, a feedback loop is
mediating this relationship where T plays a role in determining dominance behavior, through which it indirectly mediates dominance rank, which in turn affects T levels. Considering such a reciprocal relationship, it is interesting to question which aspects of dominance and aggressive behavior T may be influencing. Evidence points to the role of T in decreasing anxiety, increasing attention to negative (threatening) social stimuli, and increasing risk-taking behavior. Anxiety, attention to threat, risk-taking and cognitive processing of threat are all likely components of aggressive and dominance behavior and therefore, need to be examined in relation to T levels.

**Testosterone and Anxiety**

Anxiety is likely involved in social dominance challenges, in that higher anxiety levels would likely result in an organism avoiding such uncertain and potentially dangerous situations, while lower anxiety levels would likely result in greater willingness to approach such a situation. Much evidence supports an anxiolytic role of T in animals as well as humans.

Compared to controls, male gonadectomized (GDX) rats exhibit heightened anxiety behaviors on multiple well-established rodent anxiety tests (Frye & Seliga, 2001; King, De Oliveira & Patel, 2005; Toufexis, Davis, Hammond & Davis, 2005). Furthermore, when GDX male rats are administered replacement T, their anxiety behavior is significantly reduced (Edinger & Frye, 2004; Fernández-Guasti & Martínez-Mota, 2005; Frye, Edinger & Sumida, 2008; Toufexis et al., 2005). There is also some indirect evidence of this anxiolytic effect of T in non-human primates. Intact male rhesus monkeys spend more time proximal to novel conspecifics compared to castrates, which may be a result of lower anxiety levels in the intact group (Richards et al., 2009).
Interestingly, an anxiolytic effect of T administration has also been observed in intact male animals. T reduced anxiety behavior in intact male rats, but only with seven-fold and not ten-fold increases in T levels (Bitran, Kellogg & Hilvers, 1993). In another case, T administration only reduced anxiety when doses were raised to 250 µg and had no effect at lower doses in intact male mice (Aikey, Nyby, Anmuth & James, 2002). This suggests that raising T to supraphysiological levels may be more anxiolytic than simply replacing T at normal physiological levels, but only at certain doses. At this point it is unclear what dose of T would result in greatest anxiety reduction.

Due to health risks mainly associated with prostate cancer, manipulating T levels in eugonadal human males is not recommended (Liverman & Blazer, 2004). Therefore, much research on the effects of T on human anxiety has been conducted with females. Administration of T to healthy, young adult females has resulted in reduction of fear-potentiated startle in a threat-of-shock paradigm and also reduced skin conductance responses to aversive photographs (Hermans, Putman, Baas, Koppeschaar & van Honk, 2006; Hermans et al., 2007). Moreover, T administration to highly anxious females resulted in attenuated affective startle modulation in response to acoustic probe (Hermans et al., 2007).

T has clear anxiety-reducing properties in both animals and in humans. If T is mediating approach toward social dominance challenge, it may be doing so, in part, through these anxiolytic actions; however, the optimal levels of T for maximum anxiety reduction remain to be determined, as do the effects of T on anxiety in healthy human males.
Testosterone and Attention to Threat

It would not be surprising if T also causes enhanced attention toward potential threats because attention to threat is an important aspect of achieving and/or maintaining one’s dominance position. A few studies have indicated that a relationship may exist between T levels and attention to negative social stimuli. We previously found that increasing T levels (following suppression of endogenous T) in young adult male rhesus monkeys increased the duration of watching time (per watching bout) of negative/threatening videos involving fights between unfamiliar conspecifics (Lacreuse et al., submitted). Similarly, in the emotional Stroop task, in which human participants were required to name the color of an emotional face as quickly as possible, both men and women with higher salivary T showed significantly greater selective attention (compared to those with lower T levels) to angry faces, as evidenced by greater interference (longer reaction time) in color naming on these trials (van Honk et al., 1999).

Additionally, men who showed greater preconscious selective attention to angry faces also showed greater T increase following their exposure to emotional faces compared to those who did not exhibit this attentional bias (van Honk et al., 2000). Higher morning T in both men and women has predicted greater attention to angry faces in a sub-threshold emotional Stroop task (Wirth & Schultheiss, 2007). A significant relationship between T and attention was not seen with consciously perceived stimuli in either of these studies, which suggests that T is acting on sub-cortical structures (as seen in Hermans et al., 2008) rather than on the cortex to produce a biological, rather than psychological response (van Honk, Schutter, Hermans & Putman, 2004).
This suggestion is interesting when considering that T reduces sensitivity for conscious recognition of facial threat. Women who received T administration took significantly longer to recognize faces being morphed from neutral to angry as expressing anger compared to women who received placebo (van Honk & Schutter, 2007). Therefore, the lack of T effect seen in studies using supraliminal stimuli may be due to T-induced impairment in the conscious recognition of emotion; however, evidence suggests that T is nonetheless affecting processing of social threat. When shown pictures of angry faces for a period of six seconds, female participants who received T treatment exhibited significantly increased cardiac acceleration compared to those with placebo treatment (van Honk et al., 2001). Considering the well-supported anxiolytic effects of T previously discussed, it is likely that this cardiac acceleration is indicative, as van Honk et al. (2001) interpret, of increased inclination toward dominance and aggressive behavior in response to social threat.

Attention is an essential component in the relationship between T and social status/threat in that increased attention may be indicative of greater inclination to challenge (due to either increased aggression or risk-taking), reduced anxiety in presence of threat, and/or increased cognitive processing of potential social threats.

**Testosterone and Emotional Memory**

If T is increasing attention to threatening stimuli, it is likely that this increased attention will result in increased memory for such stimuli. Emotional content tends to be better remembered than neutral content. For example, healthy adults exposed to an emotionally arousing story showed enhanced memory for the story compared to those exposed to an emotionally neutral story (Cahill & McGaugh, 1995). In a similar study,
subjects showed superior memory for emotional parts of stories compared to neutral parts (Cahill, Babinsky, Markowitsch & McGaugh, 1995). Additionally, Alzheimer’s patients show significantly less impairment in their memory for emotional stimuli compared to neutral (Moayeri, Cahill, Jin & Potkin, 2000). Emotional enhancement of memory has been correlated with amygdala and hippocampal activity (Sommer, Gläscher, Moritz & Büchel, 2008). If T is enhancing the experience of emotional material, it may also be improving memory for it. T has not been examined in relation to emotional working memory specifically; however, evidence suggests that T does play a role in non-emotional working memory tasks.

In male rats, castration has resulted in impaired working memory during acquisition of spatial maze tasks compared to controls (Danile, Winsauer & Moerschbaecher, 2003; Kritzer, McLaughlin, Smirlis & Robinson, 2001; Spritzer, Gill, Weinberg & Galea, 2008) and T replacement has reversed this impairment (Sandstrom, Kim & Wasserman, 2006). Furthermore, T-treated GDX male rats were less affected by an increasing delay on a delay-dependent working memory task than untreated castrates (Gibbs, 2005). Similar effects of T have been found in human males. Older men supplemented with T exhibited enhanced working memory to such a degree that their post-T treatment performance was approximately equivalent to that of younger men (Janowsky, Chavez & Orwoll, 2000). Despite this evidence supporting T’s role in working memory, there is a paucity of literature regarding the role T plays specifically in emotional working memory. However, a connection can be drawn between T and emotional memory by examining the amygdala which is both rich in ARs and plays a key role in emotion and emotional memory (Davis & Whelan, 2001).
Experimental evidence strongly points to the amygdala’s role in processing threat signals in non-human primates (Gothard, Battaglia, Erickson, Spitler & Amaral, 2007; Machado, Kazama & Bachevalier, 2009) and in humans (Satterthwaite et al., 2009). More specifically, evidence suggests that T affects emotionally induced amygdala activation. For example, in healthy young males, T levels were positively related to the degree of amygdala response to fearful faces (Derntl et al., 2008). In women, lower androgen levels were associated with decreased amygdala reactivity to angry and fearful faces and acute T administration resulted in increased amygdala reactivity to these faces (van Wingen et al., 2009).

Beyond its involvement in the processing of emotional stimuli, much evidence suggests that the amygdala also influences the processing of emotional memories (see McGaugh, Introini-Collison, Cahill, Kim & Liang, 1992). A case study of a patient with bilateral amygdaloid damage who lacked enhanced memory for emotional aspects of a story (unlike control subjects) demonstrates the importance of the amygdala in emotional memory (Cahill et al., 1995). While andrenergic, opioid peptidergic and GABAergic systems have been shown to modulate emotional memory storage through their actions in the amygdala (McGaugh, Introini-Collison, Cahill & Castellano, 1993), the role of T has not been examined. Given that T plays a role in working memory as well as amygdala reactivity to emotional stimuli and that the AR-rich amygdala is essential to emotional memory, it is likely that T has an effect on emotional memory through its action in the amygdala.
Testosterone and Risk-Taking

Approaching a dominance challenge or aggressive interaction is a risky decision in that engaging in such a situation may result in a drop in dominance status, injury, or death. Risk-taking is an essential factor in dominance and aggression and therefore it is interesting to investigate what effect T has on this behavior.

A positive correlation between T concentration and self-report sensation seeking has been described in females and males (Campbell et al., 2010; Kerschbaum, Ruemer, Weisshuhn & Klimesch, 2006) and a significant relationship between free T and self-reported non-aggressive risk-taking has been reported in adolescent boys (Vermeersch, T’Sjoen, Kaufman and Vincke (2008). On another self-report measure, men scoring high on disinhibition had higher T levels than those scoring low on this scale. Additionally, T levels were negatively correlated with self-control (Daitzman & Zuckerman, 1979). All of these self-report measures indicate that there is a positive relationship between T levels and risk-taking behavior, but behavioral studies provide more convincing evidence for such a relationship.

Male rats who were exposed to either chronic high doses of T or to one high dose both displayed increased punished drinking behavior in the Vogel conflict test (Bing et al., 1998). Although this test is traditionally thought to measure anxiety, increased punished drinking behavior has also been interpreted as heightened impulsivity (Bing et al., 1998) and may indicate greater inclination toward risk-taking. In human males, endogenous T levels have been positively correlated with risk-taking in an investment game (Apicella et al., 2008). Furthermore, females who were administered T made riskier choices on the Iowa gambling task, a task dependent on the amygdala and frontal lobe.
(Bechara, Damasio, Damasio & Lee, 1999), compared to those given placebo. The authors interpreted this shift toward risky decision-making as a result of reduced punishment sensitivity and increased reward sensitivity (van Honk et al., 2004). This change in reward and punishment sensitivity may also be a factor mediating risk-taking in situations involving social and dominance challenges.

**The Rhesus Monkey Model**

Studies involving various species suggest that T is affecting many aspects of emotional processing, including anxiety, attention, memory and risk-taking but no single study has utilized a complete battery to investigate this relationship in its entirety. Furthermore, much existing evidence has been collected in either rodent models or human females, leaving unanswered questions regarding the effects of T on emotional processing in healthy human males. As a result of health risks associated with T manipulation in men, an animal model is necessary for initial investigation of T effects on emotional processing in healthy males. Given that rhesus monkeys show similarities to humans in terms of brain organization, reproductive endocrinology, and affective regulation (see Kalin & Shelton, 2003 for a review; Suomi, 2007), the male rhesus monkey provides an ideal model to investigate this relationship.

To examine the influence of T on anxiety, attention to social threat, emotional memory and risk-taking behavior, we suppressed endogenous T by chemically-inducing hypogonadism and administering replacement T in six young adult male rhesus monkeys. Endogenous T levels were suppressed in order to 1) determine the effects of hypogonadism on emotional processing and 2) more precisely control the amount of T affecting the animals by eliminating fluctuations due to circadian rhythm, season,
dominance rank and aggressive interactions. Well-established tests of attention, emotional working memory, anxiety, risk-taking were administered to animals in a within-subjects, cross-over design. Furthermore, we examined the effect of T on attention and memory for both positive and negative social and non-social stimuli to determine whether T also influences processing of threatening, non-social stimuli or any type of positive stimuli.

**Methods**

**Subjects**

Six young adult male rhesus monkeys (*Macaca mulatta*), 7 years old participated in this study. Five of the six animals were surrogate-peer-reared and one was mother-reared. All animals were housed in the same room with constant 12:12h lighting conditions. Animals were not food or water deprived and were fed a consistent daily diet of monkey chow and fresh fruits and vegetables. They were treated humanely in accordance with the PHS policy on Humane Care and Uses of Laboratory Animals standards. This study was approved by the University of Massachusetts Amherst Institutional Animal Care and Use Committee.

**Design and Treatment**

This experiment consisted of four four-week treatment periods: Baseline, Lupron, Treatment I and Treatment II. Animals were tested on the same battery of cognitive and emotional tests during each of the treatment periods. The Baseline period involved testing, in the absence of any drug treatment. For the next four weeks (Lupron period), animals were administered leuprolide acetate (Depot Lupron), a long-acting GnRH-agonist that suppresses gonadal activity in humans (Bhasin et al., 2001) and nonhuman
primates (Wilson et al., 2004; Wilson et al., 2005). Intramuscular (IM) injections of Lupron were administered in doses of 200 µg/kg. This dose was selected based on a previous study (Lacreuse, Chiavetta, Shirai, Meyer & Grow, 2009) in which the same dose successfully suppressed endogenous T levels in male rhesus monkeys for one month. The purpose of the four week Lupron period was to ensure suppression of gonadal hormone activity before the onset of the treatment phases. Following the Lupron period, animals were randomly assigned to one of two treatment groups: Sequence 1 (Lupron + Testosterone Enanthate (TE), n=3) or Sequence 2 (Lupron + oil vehicle, n=3). The Lupron + TE group received one Lupron injection at the beginning of the treatment period as well as biweekly IM injections of TE (5 mg/kg). This dose and frequency of TE administration is roughly equivalent to regimens recommended for T therapy in hypogonadal men (Bhasin et al., 2006). This treatment results in a sharp rise in T levels followed by a progressive decline over subsequent weeks (Tyagi et al., 1999). The Lupron + oil vehicle group received one Lupron injection at the beginning of the treatment period as well as biweekly IM injections of oil vehicle. Following the Treatment I period, animals crossed over to the alternate treatment for the remaining four weeks of the study (Treatment II). The experimenters were blind to the treatment group assignments.

**Blood Samples and Assays**

Blood samples (~4 ml/sample) were drawn from a saphenous vein between 07h30 and 09h30 from anesthetized animals (with 6-10 mg/kg of IM ketamine or 3 mg/kg of IM Telazol) and were collected on day 5 of every week during the experiment. Serum was removed by centrifugation and frozen at -80°C. Serum samples were analyzed for total T
using commercially available radioimmunoassay (RIA) kits (Diagnostic System Laboratories, Inc., Webster, TX) and protocols in use in the laboratory of Dr. Jerrold Meyer at the University of Massachusetts.

**General Procedure**

Animals performed a total of four tasks, which were administered on days one through four each week, for the entire 16 weeks of the experiment. Each task was administered at approximately the same time each day. Two of the tasks, the delayed non-matching to sample (DNMS) and the dot-probe task were performed on days one through four of the week, each of the 16 weeks of the experiment. The gambling task was administered on day one of each testing week and the intruder paradigm was conducted every two weeks, on either day two, three or four of the week during each treatment period.

**Training** The DNMS and dot-probe tasks were performed on a computerized touchscreen system (with a 17-inch, color monitor) that was rolled in front of animals’ home cages. Animals had already been trained on the DNMS for a prior experiment and were able to successfully complete trials at 80% accuracy. Dot-probe training consisted of initially requiring animals to touch a small, yellow dot that appeared in random locations on the screen for a pellet reward. Following successful completion of this task, animals were trained on the dot-probe task as it was administered in this experiment, but with clip-art images, not designed to elicit any emotions, for 12 weeks. The gambling task was performed on a modified version of the Wisconsin General Testing Apparatus (WGTA) that affixed to the animal’s home cage. The task has been modeled after the Iowa Gambling Task, which is amygdala-dependent and used to measure risk-taking in
humans (Bechara et al., 1999). The WGTA consists of a small opaque box containing a tray with three wells and an opaque door which can be slid open or closed to allow or prevent visual and physical access to the wells. Animals were administered the gambling task once per week for ten weeks before the beginning of the experiment. The purpose of this gambling training was to establish that the red cover indicated a risky well and that the blue indicated a safe well. No training was required for the intruder paradigm.

**Dot-Probe Task** This task is designed to measure attention (Fig. 1). The dot-probe task was built using E-Prime software and provides automatic reward of flavored 90mg pellets (Test Diet, Inc.) for responses. This task was adapted from the dot-probe task used by Mather and Carstensen (2003) to measure attention to emotional stimuli in humans. In our modified version, animals were presented with a yellow fixation cross in the middle of a black screen. They were required to touch the fixation cross to begin the set of trials. Two images were then immediately presented, side by side and centered on the y-axis of the screen for the duration of 1,000 ms. One of the images displayed a neutral stimulus while the other displayed either a positive or negative stimulus. All images were presented in black and white to avoid the potential confound of color biases. Images comprised two categories: social and non-social, both of which consisted of neutral and emotional (positive and negative) images. Social images were composed of photos of unfamiliar conspecifics expressing either mouth threats (negative), lip smacks (positive) or no particular expression (neutral). Non-social images were composed of photos of jump boxes, capture gloves and syringes (negative), apples, bananas and grapes (positive), or shoes, cage locks and wall fixtures (neutral). A total of 144 images were included in this task: 48 neutral, 24 positive and 24 negative. Non-social images were
paired together, as were social images and neutral images were always paired with emotional images, but the specific pairing of images, within these restrictions, was randomized. Animals were presented with 32 trials per day and therefore were exposed to each image an average of twice per week. Following the 1,000 ms presentation of the images, a small, yellow dot appeared in place of one of the pictures and remained on the screen until the animal responded by touching the dot. Response time to touch the dot was recorded, as was the emotional valence and category of each picture presented in each trial. Faster response time to touch the dot indicated greater attention allocation to the picture preceding it.

**Delayed Non-Matching to Sample** This task was designed to measure working recognition memory (Fig. 2). The DNMS is a classic, trial-unique recognition memory task which assesses capacity to distinguish a familiar (previously seen) stimulus from a novel (previously unseen) stimulus (Mishkin & Delacour, 1975). The DNMS program is controlled by custom-designed Java-based software and provides automatic reward of flavored 90mg pellets for correct answers. This task involved the presentation of one image (from the same categories previously described for the dot-probe task) in the center of a black screen, which the animal was required to touch. Although the images were from the same categories as images used in the dot-probe task, none of the same images were used. One second after the animal touched the sample image, two images were presented side by side, centered on the y-axis of the screen, (one being the sample image and one a novel image). In order to obtain reward, the animal was required to choose the novel (non-matching) image. Animals were presented with 36 trials of the DNMS per testing day, resulting in daily exposure to 18 social and 18 non-social black
and white images. Social images and non-social images were always paired together and images of the same emotional valence were always paired together. Images were pseudorandomly paired and trials alternated between social and non-social images. A total of 288 images (144 pairs) were used in this task, resulting in animals being exposed to each image once per testing week. Order of image presentation was never repeated within a testing period, but the same order of presentation was repeated for each testing period. Accuracy was recorded, as well as emotional valence and category of pictures presented in each trial. Greater accuracy indicated better working memory. Additionally, we examined response time. Faster response times may also be an indicator of better memory in this task.

**Gambling Task** This task was designed to measure risk-taking and reward sensitivity. Only the lateral (right and left) wells were used in this task and were covered with either a red or blue, identically shaped cover to conceal the number of raisins present in the well. The red cover indicated the “risky” well in which .5 raisins appeared 75% of the time and 6 raisins appeared 25% of the time. The blue cover indicated the “safe” well in which 2 raisins appeared consistently. Thus, when the door was lifted open, animals were presented with a blue cover over either the right or left well and a red cover over the other. Animals were allowed to remove one of the covers to obtain the reward underneath. To begin, six raisins were left under the red cover until the animals chose the red cover for the first time. This initial high pay-off from the red-covered well is essential to the animal understanding that the red cover has potential for large reward. Following the initial choice of the red-covered well resulting in the high pay-off, 24 experimental trails were administered. Four separate lists were used for order and side (left or right) of
presentation of rewards; therefore, animals received different presentation each week of the treatment period, but all four lists were used in the same order for each treatment period, resulting in animals receiving identical exposure for each treatment. Number of red and blue well choices were recorded and higher number of red well choices indicated greater risk-taking behavior as well as heightened reward sensitivity.

**Intruder Paradigm** The intruder paradigm was designed to measure social anxiety. This test was modified from the classic intruder paradigm which has been used in many studies to induce anxiety in non-human primates (Kalin, 2003; Kalin & Shelton, 1989; Kalin, Shelton & Takahashi, 1991a; Kalin, Shelton & Turner, 1991b). In our modified version, the animal participating was separated from others in the housing room and placed in a Plexiglas cage in a nearby room for two minutes of habituation. Following this two minute habituation period, an unfamiliar human female (the intruder) entered the room, standing 2.5 meters from the cage, facing the animal. The intruder remained in the room for two minutes, staring directly at the animal and making eye contact as much as possible, but making no facial expressions, noises, or body movements. Following the two minute intruder exposure, animals were returned to their home cage. Animals’ behavior was video recorded during this task and analyzed for latency to look toward the intruder, movement toward or away from intruder, facial expressions, vocalizations, and any self-directed/anxiety-related behavior (e.g. scratching or yawning) and stereotyped behavior. See Figure 3 for behavioral ethogram that was used for analysis of affiliative, anxiety, and aggressive behaviors. Duration of circling and watching behaviors were also analyzed.
Statistical Analyses

**Testosterone Assays** T levels were analyzed using a repeated measures Analysis of Variance (ANOVA) with treatment phase as a within-subjects factor and sequence of treatment (TE or placebo first) as a between-subjects factor. Differences in T levels according to treatment were analyzed using paired samples T-tests.

**Dot-Probe and DNMS** Repeated measures ANOVAs, with treatment (Lupron + TE vs. Lupron + oil), image category (social/non-social) and emotional valence (negative/neutral/positive) as within-subjects factors and sequence of treatment as a between-subject factor, were used to analyze response time for each task and accuracy for the DNMS. Simple contrasts were used to compare effects of emotional valences in the DNMS. Associations between T levels and response times in the dot-probe task were examined using Pearson’s correlations.

**Intruder Paradigm** Behaviors were grouped into three categories: Anxious, Aggressive, and Affiliative (Fig. 3) for all analyses. The duration and proportion of time spent circling and watching the intruder were also analyzed. A repeated measures ANOVA, with the effect of treatment (Lupron + TE vs. Lupron + oil) as a within-subjects factor and sequence of treatment as between-subject factor, was used to analyze frequency of categorized behavior and duration of circling and watching behavior. Proportion of time spent circling and watching the intruder was analyzed using linear mixed effects logistic regressions.

**Gambling Task** The proportion of risky choices was compared between treatment groups using a linear mixed effects logistic regression. Binomials were used to assess side biases and risk preferences for each individual animal during each treatment period.
Anticipated Results

**Dot-Probe Task** We hypothesized that T would affect attention to negative, social stimuli. It was predicted that compared to placebo treated animals, animals treated with T would exhibit faster response time to the dot when it appeared in place of negative, social images, indicating greater attention allocation to these images. Furthermore, T was predicted to correlate positively with attention to negative, social stimuli. We did not anticipate that T would affect attention toward any other type of stimuli.

**Delayed Non-Matching to Sample** We hypothesized that T would affect working recognition memory for negative, social images. It was predicted that T administration would result in more accurate memory and possibly faster response time for negative, social images compared to placebo and that T would not affect memory for any other type of stimuli. T levels were also predicted to correlate positively with recognition accuracy for negative, social images.

**Gambling Task** We hypothesized that T would affect risk-taking behavior, as indicated by number of times the animal selects the red covered well, as opposed to the blue-covered well. It was predicted that animals would choose the red covered well (the risky choice) significantly more often while receiving T administration than while receiving placebo. T levels were also predicted to correlate positively with number of risky choices.

**Intruder Paradigm** We hypothesized that T would affect behavior toward the human intruder. It was predicted that while animals were treated with T, they would exhibit less anxiety and possibly greater aggression toward the intruder compared to
when they were treated with placebo. Furthermore, T levels were predicted to correlate negatively with frequency of anxious behaviors.

Results

Treatment

The precision of the T assay was within acceptable limits, with an intra-assay coefficient of variation (CV) of 11% and an inter-assay CV of 7.45%. Figures 4 depicts T levels a function of treatment phase. A repeated-measures ANOVA with treatment as a within-subjects factor and sequence of treatment (whether animals received TE or oil treatment first) as a between-subjects factor revealed no significant effect of sequence of treatment on T-levels, $F(1,4) = 0.54, p = 0.50$, and no interaction between treatment and sequence of treatment, $F(3,12) = 2.38, p = 0.12$. We observed a significant main effect of treatment, $F(3,12) = 29.56, p < 0.001$ on T levels. Paired T-tests indicated that mean T levels during the Lupron + TE phase were significantly higher than during the Lupron + oil phase, $t(5) = -8.41, p < 0.001$ and the baseline phase, $t(5) = -9.44, p < 0.001$. T levels during the Lupron + Oil phase were not significantly lower than T levels during baseline, $t(5) = -2.12, p = 0.09$.

Results from the RIAs revealed a total of 11 samples with T levels that were too high to fit the standard curve. Therefore, these 11 samples are not included in Figure 4; however, results from the remaining 85 samples included in Figure 4 clearly indicate that both the lupron and the T treatment are altering T levels as expected.

Dot- Probe Task

Prior to any analyses, all response times exceeding two standard deviations (4.11 sec.) above or below the mean were eliminated from the data set. This resulted in
elimination of 1,737 of the 10,753 total response times. The remaining data set was analyzed using a repeated measures ANOVA with side (left, right) as a within subjects factor. This analysis revealed no main effect of side, $F(1,5) = 0.89, p = 0.08$, indicating no side bias in the dot-probe task. Additionally, a repeated measures ANOVA with sequence of treatment as a between subjects factor indicated no effect of sequence on response times, $F(1,4) = 1.55, p = 0.28$ and no interaction between treatment and sequence, $F(1,4) = 1.89, p = 0.24$ were observed. Therefore, sequence of treatment was not included as a factor in our subsequent analyses. Mean responses times for each category of stimulus (social, non-social) and emotional valence (negative, neutral, positive) in each experimental condition (baseline, lupron, oil, testosterone) can be seen in Table 1.

Because animals received bi-weekly injections of either TE or oil during the two treatment months, a repeated measures ANOVA was run with treatment and week as within-subjects factors to determine whether or not performance during the injection weeks differed significantly from the non-injection weeks. We found no significant effect of week on response times, $F(3,15) = 0.539, p = 0.66$, and no interaction between week and treatment, $F(3,15) = 0.336, p = 0.80$.

**Baseline** Response times were examined using a repeated measures ANOVA with emotional valence and category as within-subjects factors. Mean response times at baseline did not differ significantly as a function of emotional valence, $F(2,10) = 2.60, p = 0.12$ or category, $F(1,5) = 0.23, p = 0.65$. Furthermore, there was no interaction between emotional valence and category at baseline, $F(2,10) = 0.02, p = 0.97$. 
Lupron  Response times during the lupron phase were examined using a repeated measures ANOVA with emotional valence and category as within-subjects factors. Mean response times did not differ significantly as a function of emotional valence, $F(2,10) = 0.32, p = 0.74$, or category, $F(1,5) = 0.20, p = 0.67$, and there was no interaction between emotional valence and category, $F(2,10) = 1.07, p = 0.38$.

Treatment  An omnibus ANOVA with treatment, emotional valence, and category as within subjects factors showed no significant effect of emotional valence, $F(2,10) = 0.70, p = 0.52$, or category, $F(1,5) = 1.433, p = 0.29$, on response times during the treatment months. There was a main effect of treatment, $F(1,5) = 8.96, p = 0.03$ (Fig.5), with response times being significantly faster when animals were on T ($M = 718.76, SD = 84.90$) compared to when they were on oil ($M = 764.82, SD = 96.62$). However, there was no interaction between treatment and emotional valence, $F(2,10) = 1.03, p = 0.39$, treatment and category, $F(1,5) = 0.05, p = 0.84$, or between emotional valence and category, $F(2,10) = 1.24, p = 0.33$.

Pearson’s correlations revealed no significant association between response times and T levels during T-treatment, $r = -0.046, p = 0.93$.

DNMS

Prior to any analyses, all response times exceeding two standard deviations (4.84 sec.) above or below the mean were eliminated from the data set. This resulted in elimination of 208 response times from a total of 13,391 values. A repeated measures ANOVA, with side (left, right) was run on the remaining data set for the DNMS. This analysis revealed no side bias for either accuracy, $F(1,5) = 0.02, p = 0.89$, or for response times, $F(1,5) = 0.87, p = 0.39$. Furthermore, a repeated measures ANOVA, with sequence
of treatment as a between subjects factor revealed no effect of sequence on response times, $F(1,4) = 3.16, p = 0.15$, or accuracy, $F(1,4) = 0.008, p = 0.93$, and no interaction between sequence of treatment and either accuracy, $F(1,4) = 1.37, p = 0.31$, or response times, $F(1,4) = 4.38, p = 0.11$. Therefore, sequence of treatment was omitted from subsequent analyses.

A repeated measures ANOVA was run with week and treatment as within-subjects factors to determine whether or not performance during injection weeks differed significantly from the non-injection weeks. We found no significant effect of week for either accuracy, $F(3,12) = 0.71, p = 0.56$, or for response times, $F(3,12) = 1.32, p = 0.31$. Furthermore, there was no interaction between treatment and week for accuracy, $F(3,12) = 0.19, p = 0.90$, or for response times, $F = 0.19, p = 0.90$, indicating that performance on the DNMS was not affected by whether or not an injection was received that week. The mean proportion of correct responses and mean response times for each stimulus category (social, non-social) and emotional valence (negative, neutral, positive) in each experimental condition can be seen in Tables 2 and 3, respectively.

**Baseline** A repeated measures ANOVA, with category and emotional valence as within-subjects factors, revealed a significant effect of category, $F(1,5) = 32.17, p = 0.002$, with accuracy being higher for non-social ($M = 0.79, SD = 0.13$) compared to social stimuli ($M = 0.67, SD = 0.14$) (Fig.6). Accuracy did not differ significantly by emotional valence, $F(2,10) = 1.96, p = 0.19$.

For response times, the main effect of emotional valence approached significance, $F(2,10) = 4.03, p = 0.052$, with simple contrasts revealing that response times to negative stimuli ($M = 1274.34, SD = 193.14$) were significantly faster than response times to
positive stimuli ($M = 1384.39, SD = 213.06$), $F(1,5) = 9.79, p = 0.03$, but there were no other significant differences in response times between emotional valences (Fig.7). There was no effect of category on response times, $F(1,5) = 1.19, p = 0.32$, and no interaction between category and emotional valence for accuracy, $F(2,10) = 1.27, p = 0.32$, or for response times, $F(2,10) = 1.22, p = 0.34$, was observed at baseline.

**Lupron** A repeated measures ANOVA, with category and emotional valence as within-subjects factors, revealed a significant effect of category, $F(1,5) = 37.94, p = 0.002$, with accuracy again being higher for non-social ($M = 0.82, SD = 0.13$) compared to social stimuli ($M = 0.71, SD = 0.11$; Fig.8). There was also a main effect of emotional valence on accuracy, $F(2,10) = 8.52, p = 0.007$, during the lupron phase (Fig.8). Simple contrasts revealed that accuracy was significantly better on negative ($M = 0.78, SD = 0.12$) compared to positive stimuli ($M = 0.73, SD = 0.12$), $F(1,5) = 40.69, p = 0.001$, and on neutral ($M = 0.78, SD = 0.12$) compared to positive stimuli, $F(1,5) = 8.79, p = 0.03$, but there was no difference between accuracy for negative and neutral stimuli, $F(1,5) = 0.01, p = 0.94$.

There was no effect of category on response times, $F(1,5) = 1.92, p = 0.23$. However, we did observe a main effect of emotional valence on response times, $F(2,10) = 8.65, p = 0.007$, with simple contrasts revealing that response times were significantly faster on negative ($M = 1240.23, SD = 236.73$) compared to positive stimuli ($M = 1276.40, SD = 244.08$), $F(1,5) = 7.116, p = 0.04$, and on neutral ($M = 1188.51, SD = 218.62$) compared to positive stimuli, $F(1,5) = 10.49, p = 0.02$ (Fig. 9). Furthermore, faster response times on neutral compared to negative stimuli approached significance, $F(1,5) = 6.17, p = 0.056$. No interaction between category and emotional valence was
observed for accuracy, \( F(2,10) = 3.42, p = 0.74 \), or for response times, \( F(2,10) = 1.18, p = 0.35 \).

**Treatment** An omnibus ANOVA, with treatment, category and emotional valence as within-subjects factors, revealed no significant effect of treatment on accuracy, \( F(1,5) = 0.25, p = 0.37 \), or on response times, \( F(1,5) = 1.18, p = 0.33 \), for the DNMS.

However, we did observe a main effect of emotional valence on accuracy during the two treatment months, \( F(2,10) = 8.52, p = 0.007 \), (Fig.10). Simple contrasts revealed that animals were significantly less accurate on positive (\( M = 0.72, SD = 0.12 \)) compared to negative stimuli (\( M = 0.78, SD = 0.12 \), \( F(1,5) = 40.69, p = 0.001 \), and compared to neutral stimuli (\( M = 0.78, SD = 0.12 \), \( F(1,5) = 8.79, p = 0.03 \), but there was no difference in accuracy between negative and neutral stimuli \( F(1,5) = 0.006, p = 0.94 \). We also observed a significant effect of category, \( F(1,5) = 37.94, p = 0.002 \), on accuracy (Fig.10), with higher accuracy on the non-social (\( M = 0.81, SD = 0.13 \)) compared to social stimuli (\( M = 0.71, SD = 0.11 \)).

There was a main effect of emotional valence on response times, \( F(2,10) = 8.28, p = 0.008 \), with simple contrasts revealing that response times were significantly slower for positive (\( M = 1075.74, SD = 1025.41 \)) compared to negative (\( M = 1050.60, SD = 1128.72 \)) stimuli, \( F(1,5) = 17.90, p = 0.008 \), and for positive (\( M = 1075.74, SD = 1025.41 \)) compared to neutral (\( M = 1032.03, SD = 1079.12 \)) stimuli during the treatment phase, \( F(1,5) = 11.74, p = 0.02 \) (Fig.11). However, there was no effect of category on response times, \( F(1,5) = 5.31, p = 0.07 \).

Interestingly, when categories were examined separately, the effect of emotional
valence on response times was only significant for the social category, $F(2,10) = 8.718, p = 0.006$, but not for the non-social category, $F(2,10) = 3.57, p = 0.68$. Simple contrasts indicated slower response times to positive ($M = 1131.33, SD = 118.88$) compared to negative ($M = 1077.06, SD = 124.04$), $F(1,5) = 22.75, p = 0.005$, and positive compared to neutral ($M = 1070.57, SD = 131.64$), $F(1,5) = 11.24, p = 0.02$, stimuli in the social category, but there was no difference in response times between emotional valences in the non-social category.

This significant interaction between category and emotional valence, $F(2,10) = 4.800, p = 0.04$, for response times was further investigated using additional simple contrasts. These analyses revealed that response times to social stimuli ($M = 1131.33, SD = 118.88$) were significantly slower than response times to non-social ($M = 1020.14, SD = 100.53$) stimuli for positive images only, $F(1,5) = 11.56, p = 0.02$ (Fig. 13).

In case there was a particular positive, social image to which animals were responding excessively slowly, thereby causing the mean response time to positive, social images to be deceptively high, we examined the response times to all individual positive, social images associated with response times between one and two standard deviations above the mean. The frequency with which each image occurred (as a match and as a non-match) for such trials was consistently between one and five times. Therefore, we determined that there was not a single positive, social image that was distorting the mean response time for this category and valence.

No interactions between treatment and category, $F(1,5) = 0.63, p = .46$, treatment and emotional valence, $F(2,10) = 0.58, p = 0.58$, or category and emotional valence, $F(2,10) = 3.42, p = 0.07$ were observed for accuracy. There were also no interactions
between treatment and category, $F(1,5) = 0.14$, $p = 0.73$, or between treatment and emotional valence, $F(2,10) = 0.29$, $p = 0.76$, for response times.

**Intruder Paradigm**

**Aggressive, Anxious, Affiliative Behavior** Frequencies of categorized behaviors across treatments are depicted in Table 4. Due to the low frequency with which aggressive and affiliative behaviors occurred throughout treatment, these categories were omitted from further analyses. A repeated measures ANOVA with treatment as a within-subjects factor and sequence of treatment as a between-subjects factor revealed no effect of T treatment, $F(1,4) = 3.94$, $p = 0.12$, or sequence of treatment, $F(1,4) = 0.08$, $p = 0.79$, on anxious behavior and no interaction between treatment and sequence of treatment, $F(1,4) = 0.67$, $p = 0.46$.

**Watching** Tables 5 and 6 respectively depict mean duration of watching bouts and mean proportion of time spent watching the intruder for baseline, lupron, oil, and testosterone phases.

A repeated measures ANOVA with treatment as a within-subjects factor and sequence of treatment as a between-subjects factor revealed no effect of sequence on mean duration of watching bouts, $F(1,4) = 0.18$, $p = 0.70$. Furthermore, there was no effect of treatment on mean duration of watching bouts, $F(1,4) = 0.51$, $p = 0.52$, and no interaction between treatment and sequence of treatment for mean duration of watching bouts, $F(1,4) = 0.00$, $p = 1.00$.

A mixed effects logistic regression was used to analyze the proportion of time spent watching the intruder. Only main effects of treatment and sequence of treatment were included, as model selection revealed that model fit was not significantly improved
by including the interaction between treatment and sequence of treatment. We observed no effect of either treatment, $z = -0.04$, $p = 0.97$, or sequence of treatment, $z = -0.15$, $p = 0.88$, on proportion of time spent watching the intruder.

**Circling/Pacing** Tables 5 and 6, respectively, depict mean duration of circling/pacing bouts and mean proportion of time spent circling/pacing for baseline, lupron, oil, and testosterone phases.

A repeated measures ANOVA with treatment as a within-subjects factor and sequence of treatment as a between-subjects factor revealed no effect of sequence of treatment, $F(1,4) = 1.56$, $p = 0.28$, or treatment on mean duration of circling/pacing bouts, $F(1,4) = 0.83$, $p = 0.41$. There was also no interaction between treatment and sequence of treatment, $F(1,4) = 0.57$, $p = 0.49$.

The proportion of time spent circling/pacing was analyzed using a linear mixed effects logistic regression. Model selection revealed that the best fit was obtained for a model which included only main effects of treatment and sequence of treatment; including the interaction between these two effects did not significantly improve model fit. There were neither effects of treatment, $z = -0.23$, $p = 0.82$, or of sequence of treatment, $z = -0.76$, $p = 0.44$.

**Gambling Task**

**Proportion of Risky Choices** Table 7 displays the mean proportion of choices from the risky well throughout the baseline, lupron, oil, and testosterone phases of the experiment. Binominal analyses of individual risk preferences throughout each phase of the experiment are depicted in Table 8.

Using a linear mixed effects logistic regression, we analyzed the effect of T on the
proportion of risky choices in the gambling task. Model selection revealed that the best fit was obtained for a model which included only main effects of sequence and treatment, without the interaction between these variables. There were no effects of treatment, \( z = -0.61, p = 0.54 \), or of sequence of treatment, \( z = -0.07, p = 0.94 \), on proportion of risky choices.

**Side Bias** Binomial tests revealed that five of the six animals in this experiment exhibited side biases throughout all four testing months. Three of the animals consistently preferred choices that were presented on the left side \( p < 0.001 \), while two others exhibited both left- and right-side biases at different points throughout the experiment, \( p < 0.05 \).

**Discussion**

The current study is the first to examine the effects of T on these specific aspects of emotional processing in non-human primates. Animals were tested with natural levels of testosterone, during a period of chemically-induced hypogonadism, and while receiving supplemental TE to examine the effects of exogenous T manipulations on emotional processing. Manipulations affected performance on the dot-probe task, with T resulting in faster response times compared to oil, but did not affect performance on any of the other three emotional tasks. These results are discussed below.

**Treatment**

Lupron and T treatment yielded expected differences in T levels, with Lupron + oil treatment resulting in T levels significantly lower than baseline, and Lupron + TE treatment resulting in T levels significantly higher than those seen during both baseline and Lupron + oil treatment periods.
Normal physiological T levels in male rhesus monkeys (depending on season) range from between 600-1200 ng/100ml (6-12 ng/ml) according to one report (Michael & Zumpe, 1978) and between and between 4.7-10.1 ng/ml according to others (Robinson, Scheffler, Eisele & Goy, 1975). Analyses for our study revealed that T levels were raised to a supraphysiological level during the majority of the T treatment period (averaging between 12.93 – 21.38 ng/ml), with two spikes in T (during injection weeks) which tapered off to lower levels during the non-injection weeks. These effects are comparable to those previously reported by others using a similar treatment regimen in male rhesus monkeys (Tyagi et al., 1999).

**Dot-Probe Task**

For the dot-probe task, it was predicted that T (compared to oil) treatment would result in faster response times (due to greater attention allocation) to the negative, social stimuli compared to all other stimuli. T was not predicted to have an effect on response times to stimuli of other valences or categories. The results of this experiment do not support our predictions. Animals responded significantly more rapidly when receiving T compared to when receiving oil treatment overall; however, T effects did not differ between social and non-social stimuli or between emotional valences.

Some evidence regarding T’s facilitatory effects on motor speed comes from men with lower salivary T levels who exhibit slower reaction time, compared to those with higher T levels (Müller, 1994). However, the majority of the literature suggests otherwise. No association has been found between T and faster movement execution in the choice reaction time task with sequential responses (CRT-SR) in women or men (Jennings, Janowsky & Orwoll, 1998). Moreover, we previously found no effect of T
manipulations on speed of motor movement in male rhesus monkeys performing the Life-saver task (Kurdziel, Otolo, Putcha & Lacruse, 2009). Similarly, testosterone manipulations in human males have been shown to have no effect on either reaction time or on movement time in the CRT-SR (Siegel et al., 2008). Thus, facilitated motor function may not adequately account for this finding.

An alternative interpretation of the faster response times seen during T treatment involves T’s potential effects on arousal, defined as “increased sensory alertness, motor activity and emotional reactivity,” (Pfaff, Frolich & Morgan, 2002). Higher T levels in humans have been associated with subjective reports of higher arousal (Dabbs, Strong & Milun, 1997). T has also resulted in increased cardiac acceleration during exposure to angry faces (van Honk et al., 2001), which may be interpreted as evidence of increased arousal. However, some studies suggest that T is inversely related to arousal. For example, the reduction in fear-potentiated startle, skin conductance, and affective startle modulation in women who received T treatment (Hermans et al., 2006; 2007) indicates that T is actually decreasing arousal levels. It is difficult to compare subjective reports of arousal to physiological measures when they come from studies utilizing such different methods of inquiry. Future studies should investigate the causal role of T in both subjective and physiological arousability.

T-induced changes in reward sensitivity also may have affected response times in the dot-probe task. It is quite possible that animals responded more quickly when receiving T compared to oil because they had a greater motivation to receive the food reward. Van Honk et al. (2004) showed that T enhances reward sensitivity in women. Furthermore, there is some evidence to suggest that anabolic-androgenic steroids
influence the sensitivity of brain reward systems (Clark, Lindenfeld, & Gibbons, 1996). Overall, there have been few studies to date that have further investigated the relationship between T and reward sensitivity. Our results may be interpreted as evidence for such a relationship and suggest that more studies in this area are warranted.

Regardless of these effects, the dot-probe task was administered specifically to investigate the effects of T on attention to emotional stimuli. In light of our previous findings that T increases watching time for videos depicting negative social interactions between unfamiliar conspecifics (Lacreuse et al., submitted), it is somewhat surprising that animals did not show increased attention to negative, social stimuli when receiving exogenous T on the dot-probe task. However, several factors may explain this finding.

First, animals may have required deeper cognitive processing or greater incentive in order to successfully make a distinction between the emotional facial expressions of the stimuli. Data from both behavioral (Parr & Heintz, 2009) and neurological (Gothard, Battaglia, Erickson, Spitler & Amaral, 2006) studies show that rhesus monkeys are capable of differentiating various emotional facial expressions. This ability is confirmed by our results from the DNMS (as discussed below) in which animals’ performance varied by the category and emotional valence of stimuli. The limited cognitive processing required by the dot-probe task may have contributed to the lack of category and/or valence effect observed here. Rhesus monkeys perceive direct stares as a threat and therefore may avoid gazing directly at an image of a conspecific (Hinde & Rowell, 1962), unless given adequate incentive to do so (as in the DNMS). Avoidance of direct gaze would result in attainment of limited information from the faces in the dot-probe task and may account for the lack of effect of emotional valence on response times. However, this
explanation does not account for the lack of category and valence effect seen with the non-social stimuli.

These results suggest that our previous findings (Lacreuse et al., submitted), concluding that longer watching time of negative videos reflected increased attention to negative, social stimuli, may need to be reinterpreted. The effect may have been a result of increased attention to agonistic \textit{interactions} or may have been specific to dynamic, rather than static stimuli. The negative videos (fight scenes) in our previous experiment included more individuals and involved much more physical action compared to the positive (grooming scenes) and neutral (sleeping scenes) videos. Due to these confounding factors and the results obtained in the present experiment, the possibility that T increased attention to active scenes and/or large group interactions, rather than specifically \textit{negative} social material, should be considered.

To our knowledge, this is the first time that the dot probe task has been used with non-human primates and it may not be an appropriate measure of attention in this form, which was developed for human subjects. It would be ideal to use eye-tracking techniques to measure looking time in this task or in other paradigms in which a variety of emotional pictures are presented and to specifically examine what facial features are attended to for each valence (Gothard et al., 2009). If T is reducing response times by increasing motivation for reward, training animals to continuously respond to the dot for a single reward following the completion of several trials may help to reduce this effect. Finally, considering the finding that T generally decreases response time in this experiment, further investigation into potential effects of T on motor speed is warranted.
The prediction that T administration would result in better memory for negative, social images, compared to other categories and valences, was not confirmed by this experiment. There was no effect of T treatment on accuracy or on response times for the DNMS. Considering the fact that T did not increase attention to negative, social stimuli in the dot-probe task, it is not entirely surprising that it also had no effect on memory for these specific stimuli in the DNMS. If the salience of negative, social images is not increased, then memory for such images will also likely not be increased.

Although other studies have shown that T generally enhances working memory, this evidence has come from rats (Gibbs, 2005; Sandstrom et al., 2006) and older men (Janowsky et al., 2000) and findings from these subject populations may not be generalizable to younger adult males. Estrogen has improved memory in aged female rhesus monkeys, but has had no effect in young females (Lacreuse, 2006; Hao et al., 2007) and T may have similar age-dependent effects on working memory in primates. Studies to investigate this potential age difference in T’s effects on cognition are currently underway.

Throughout the baseline, lupron, oil, and testosterone phases of the experiment, animals were consistently more accurate on the non-social stimuli compared to the social stimuli for the DNMS. In addition, there was an interaction between category and emotional valence, in that, during the two treatment months, response times to social stimuli were significantly slower than response times to non-social stimuli for positive images only.
Greater accuracy on non-social compared to social stimuli indicates that the non-social items were easier to distinguish. The non-social stimuli depicted items from multiple different categories such as various types of food, shoes, laboratory equipment, etc. and therefore the individual images were more visually distinct than social images which all portrayed conspecifics and differed primarily by facial expression. Using a similar task, others have reported that rhesus monkeys are unsuccessful at discriminating the faces of unfamiliar conspecifics due to their inability to identify similarities in facial appearance (Parr et al., 2008). Therefore, poorer performance on the social compared to the non-social trials would be expected.

It has recently been reported that, unlike humans and chimpanzees, rhesus monkeys lack expertise in face processing (Parr, Heintz & Pradhan, 2008). Specifically, their strategy for identifying rotated (Collishaw & Hole, 2002) and inverted rhesus monkey faces did not differ from their strategy for identifying faces of chimpanzees, houses (Parr & Heintz, 2008), human faces (Parr et al., 2008), capuchin faces, and automobiles (Parr, Winslow & Hopkins, 1999). The lack of inversion effect seen for rhesus monkeys processing faces of conspecifics indicates that they do not have a specialized face-processing mechanism and therefore have no advantage for processing conspecific faces over objects (Gothard, Erickson & Amaral, 2004). Although these findings are not entirely consistent throughout the literature, they are in general agreement with our results that rhesus monkeys have no significant advantage for processing faces over non-face stimuli.

During the two treatment months, animals were not only more accurate on non-social stimuli, but also responded significantly faster to positive non-social compared to
positive social stimuli. This indicates that they needed less time to perform accurately and/or had less difficulty processing the stimuli on positive, non-social trials compared to social trials. This is not surprising because all of the positive, non-social images depicted food items, toward which rhesus monkeys may have a natural bias, as it is relevant to their survival. Indeed, young adult rhesus monkeys have high motivation for food (Mattison et al., 2005) and show superiority for categorizing food images compared to images of animals (Fabre-Thorpe, Richard & Thorpe, 1998).

Accuracy scores for positive images (across both categories) were consistently lower compared to scores for neutral and negative images throughout most of the experiment (during the Lupron, oil, and testosterone phases). Furthermore, response times to positive stimuli were significantly slower than response times to negative and neutral stimuli during these phases, with the same trend for response times at baseline.

Interestingly, the significant difference in response times between the positive and the neutral and negative stimuli during the two treatment months was present only in the social category. As discussed above, animals had more difficulty distinguishing social images than non-social images and this effect appears to have been particularly true for the positive stimuli. Neurological evidence shows that rhesus monkeys exhibit valence-specific neurons and firing patterns when exposed to emotional faces of conspecifics. Specifically, response rates of amygdalar neurons to appeasing faces (lip-smacks) are marked by decreased firing rates, while response rates to negative faces (mouth threats) are marked by increased firing rates, and firing rates are equally likely to be increased or decreased in response to viewing neutral faces (Gothard et al., 2006). Variations in amygdalar engagement when viewing threatening (and to some extent neutral) compared
to positive faces may help to explain the superior performance on negative and neutral compared to positive images in our experiment.

Furthermore, behavioral evidence suggests that rhesus monkeys fixate on different facial features of conspecifics depending on what expression they are making; however, the specific variations in points of fixation have not been conclusively established. One study reported that when threatening faces are viewed, rhesus monkeys spend an equal amount of time fixating on the eyes and the mouth, but when neutral faces and lip-smacks are viewed, fixation tends to be focused mainly on the eyes (Gothard, et al., 2004). However, others have reported that the eyes are fixated upon more often in threatening and neutral portraits, whereas the eyes and mouth are fixated upon equally in lip-smacks (Nahm, Perret, Amaral & Albright, 1997). Despite the inconsistencies, this evidence suggests that animals’ poorer performance on the positive compared to the negative and neutral social stimuli may be a result of the features on which they fixate. Eye tracking would be a useful addition to this experiment in order to determine which features are fixated upon in which valences and if this may be affecting accuracy and/or response times in the DNMS.

Finally, the ability of animals to distinguish between stimuli categories and valences in the DNMS, but not in the dot-probe task must be addressed. The difference between the dot-probe task and DNMS may reflect the cognitive difficulty of the tasks. Unlike the dot-probe task, receiving a reward on the DNMS requires attention and memory for the presented stimuli. Therefore, this task incorporates deeper processing of the stimuli than does the dot-probe task, which merely requires touching the dot to obtain a reward and. This deeper processing likely accounts for the substantial difference
between the tasks regarding the impact of category and emotional valence on performance.

**Intruder Paradigm**

Our prediction that T would result in a lower frequency of anxious behaviors in the intruder paradigm was not confirmed by this experiment. The frequencies of aggressive and affiliative behaviors in the intruder paradigm were extremely low. This is not unexpected, however, as the intruder paradigm is not intended to elicit such types of behaviors. While the frequency of anxious behavior was higher, it was not affected by T treatment. Furthermore, there was no effect of T on either watching time or time spent circling/pacing in this paradigm.

The vast majority of literature from both rodents (Edinger & Frye, 2004; Fernández-Guasti & Martínez-Mota, 2005; Frye et al., 2008; Toufexis et al., 2005) and human subjects (Hermans et al., 2006; 2007) suggests that T does have anxiolytic properties. One possible reason for the lack of such evidence in this study is that we used a modified version of the original intruder paradigm. In the original intruder paradigm, the intruder spends two 10 minute segments in the room with the subject, the first presenting her/his profile and the second staring directly at the subject (Kalin & Shelton, 1989; Kalin et al., 1991a; 1991b). However, in the variation used in this experiment, the intruder entered only once and remained staring at the subject for a duration of two minutes. Therefore, the amount of time spent in the presence of the intruder in this experiment may not have been long enough to elicit an anxious response that was sufficiently large to show significant fluctuation in response to T.
Additionally, the original intruder paradigm was conducted with infant rhesus monkeys separated from their mothers, a population which may be more sensitive to anxiety-provoking situations than our 7-year-olds. Although others have used this paradigm to successfully elicit anxious behavior in somewhat older animals (19.3 ±13.2 months, Rogers et al., 2008), our animals are still substantially older, which may have contributed to the difference in their reactions to the intruders.

Furthermore, the behaviors exhibited by our animals in this experiment differed from those seen in the studies with the original paradigm. Most importantly, the behavioral category of “freezing,” has been shown to be particularly sensitive to the intruder paradigm. This behavior was rarely exhibited by our animals and therefore was included in the “anxious” behavior group in our ethogram. Vocalizations such as “coo” and “bark” were also important behaviors, occurring frequently, in the original intruder paradigm experiments, but our animals did not make such vocalizations.

Further differences between our study and the original intruder paradigm experiments are related to rearing conditions. The animals used in original intruder paradigm studies were all raised with their mothers (Kalin & Shelton, 1989; Kalin et al., 1991a; 1991b) whereas five of our animals were surrogate-peer reared and one was mother-reared. There is evidence to suggest that rearing conditions can alter stress, anxious, and fear-related factors in rhesus monkeys’ behavior and physiology (Davenport et al., 2003; Lutz, Davis, Ruggiero & Suomi, 2007; Nelson et al., 2009); however, such differences may diminish significantly after the first several months of life (Davenport et al., 2003). Nevertheless, variation in rearing conditions may have contributed to the
differences in exhibition of anxious behavior between our study and others using the Intruder Paradigm.

Finally, all of our intruders were human females. Although there is currently no literature to directly suggest this (Kalin & Shelton, 1989; Kalin et al., 1991a; 1991b do not report the sex of their intruders), it is possible that animals may have reacted with more anxiety to male intruders.

When considering our results in comparison to the majority of the literature, it should be noted that anxiety has been examined in relation to T levels mainly in rodent models (e.g. Edinger & Frye, 2004; Fernández-Guasti & Martínez-Mota, 2005; Frye et al., 2008; Toufexis et al., 2005) and in human females (e.g. Hermans et al., 2006; 2007) using very different paradigms from the one utilized in this experiment. Because of the drastic difference in levels of circulating sex hormones between males and females (Carter, 1993), it is somewhat precarious to generalize anxiolytic effects of T in women to men. Furthermore, compared to rodents, rhesus monkeys have more endocrine, brain organization, and affective similarities to humans and are therefore a superior model from which conclusions can be more accurately drawn regarding effects of T on anxiety in human males. To date, there has been only indirect evidence that T may be anxiolytic in male primates. Compared to castrates, intact rhesus monkeys spent more time proximal to novel conspecifics (Richards et al., 2009), which may be an indication of lower anxiety levels, but also may indicate increased curiosity or increased motivation to engage in social activity. Therefore, this (Richards et al., 2009) study does not provide conclusive evidence regarding T and anxiety in male primates.
The results of our experiment, showing no effect of T on anxiety in male rhesus monkeys, are an important addition to the literature in that this is the first direct examination of such a relationship. Future studies should continue to examine whether or not T has an effect on anxiety in male primates. Utilizing an equal number of male and female intruders and administering this paradigm as it was originally designed may yield more informative results.

**Gambling Task**

It was predicted that T would increase the frequency of risky choices in the gambling task. However, this prediction was not confirmed and we observed no effect of T on risk-taking behavior. This lack of effect is most likely not due to the methodology, as others have successfully used strikingly similar paradigms to examine risk-taking in non-human primates (Heilbronner, Rosati, Stevens, Hare & Hauser, 2008).

The most plausible reason for observing a lack of treatment effect in this task is due to five of the six animals exhibiting side biases. The use of such a strategy rendered the “risky” and “safe” aspects of this task irrelevant and with the majority of our sample using such a strategy, it is not surprising that we saw no effect of treatment on risk-taking. Following training to extinguish side biases in this sample, this task should be used in future experiments to examine how risky decision-making is affected by T levels in non-human primates.

An essential difference between this gambling task and the Iowa Gambling Task, on which we modeled it, is the absence of punishment in our version. In the Iowa Gambling Task, subjects have the potential to lose money based on their card selection (Bechara et al., 1999) and this is an important aspect of the task because it allows
examination of both reward and punishment sensitivity. Decision-making becomes riskier when there is potential to lose a portion of one’s reward. Therefore, this task could be improved by creating a computerized version in which images of reward tokens, representing the amount of food reward to be obtained, accumulated and/or disappeared from the screen as animals progressed throughout the task.

It should also be considered whether the effects of T on risk-taking may be more specific to either social or non-social risks. This gambling task examined economic risk-taking, but the other tasks in our study examined T in relation to social situations. Furthermore, because T is an important factor in dominance and aggression (Archer, 2006; ; Beehner et al., 2005; Czoty et al., 2008; Mazur & Booth, 1998; Muehlenbein et al., 2004; Muller & Wrangham, 2004; Parikh et al., 2006; Rose et al., 1971; Rubinow & Schmidt, 1996; Setchell et al., 2008; Winslow & Miczek, 1988), it may specifically enhance risk-taking in social situations. Paradigms in which T is manipulated in animals being introduced to new social groups and unfamiliar conspecifics (e.g. Bauman, Lavenex, Mason, Capitanio & Amaral, 2004; Fairbanks, McGuire & Kerber, 1978) would be ideal for such an investigation. It would be particularly interesting to examine the difference in T’s effects on social versus non-social (economic) risk-taking.

**General Discussion**

Overall, our predictions regarding T effects on attention and memory for emotional stimuli, anxiety, and risk-taking were not supported. There are several possible reasons for these results.

First, the vast majority of rodent studies, as well as many other studies utilizing non-human primates, use surgical castration as a means to eliminate endogenous T levels,
rather than pharmacological castration (e.g. Fernández-Guasti & Martínez-Mota, 2005; Frye et al., 2008; Gibbs, 2005; Richards et al., 2009; Sandstrom, Kim & Wasserman, 2006; Toufexis et al., 2005). In human males, bilateral orchidectomy consistently results in serum T levels below 50 ng/dl (0.5 ng/ml; see Novara, Galfano, Secco, Ficarra & Artibani, 2009 for a review); however, GnRH agonists, while lowering endogenous T levels to this threshold in most individuals (87.5-100%), do not always decrease T to this level (Novara et al., 2009), as was the case in our experiment. Mean T levels during the oil treatment period never dropped below 1.5 ng/ml. Using Lupron to suppress T may be a more appropriate method to use when applying findings to T replacement in elderly men. Hypogonadism in men is defined by bioavailable T levels being below 3.8 nmlol/L (1.1 ng/ml; Matousek & Sherwin, 2010) and total T below 325 ng/dl (3.25 ng/ml; Harman, Metter, Tobin, Pearson & Blackman et al., 2001) Since these levels are higher than the 0.5ng/ml levels seen with surgical castration, Lupron (lowering T levels to a mean level of 2.37 ng/ml during the Oil phase of our study) may provide a more accurate model of hypogonadism.

There is also a potential drawback to using pharmacological castration. Lupron is a GnRH analog which causes over-stimulation of the pituitary gland, leading to initial excessive production of follicle stimulating hormone (FSH) and lutenizing hormone (LH) and therefore sex hormones, followed by subsequent desensitization of the pituitary gonadorophs, and suppression of endogenous gonadotropins and sex hormones (see Schally et al., 2001 for a review). There is limited evidence to suggest that FSH and LH are related to affect and expression of affect (Houser, 1979; Persky, Zuckerman & Curtis, 1968) and also that LH may influence memory (Hyde et al., 2010). Although this
evidence is only correlational, the elimination of FSH and LH through the use of Lupron in our animals may have been a confounding factor in this study.

Another point to consider when comparing the results of this study with what is currently available in the literature is the duration of T treatment. Unlike this experiment, the majority of studies examining the effects of T in human females administer acute doses of T (e.g. Hermans et al., 2006; 2007; 2008; van Honk et al., 2001; van Honk & Schutter, 2007), the effects of which last for only hours (Tuiten et al., 2009). One study in rodents found that short-term T treatment (one week) resulted in anxiolysis, but with longer-term (2 week) treatment, the anxiolytic effects of T were no longer present (Bitran et al., 1993). Thus, it may be that more pronounced effects of T are seen with acute, rather than chronic treatment. However, some evidence suggests the opposite: that T only has anxiolytic effects with chronic (but not acute) treatment (Fernandez-Guasti & Martinez-Mota, 2005). From the available literature, it seems that chronic and acute effects of T are potentially different; however, more studies in this area are needed to determine exactly what differences result from varying lengths of treatment. There is some evidence to suggest that chronic treatment with AAS results in an up-regulation of androgen receptors in muscle (Sheffield-Moore et al., 1999). Although research in this area is lacking, up-regulation of neural androgen receptors with chronic T treatment may partially account for differences between chronic and acute effects. When T binds to androgen receptors, it causes altered gene transcription (Meyer & Quenzer, 2005), and therefore, effects of acute T administration are likely a result of a different mechanism.

In addition to the length of time the animals received treatment, the T dose is also a factor to consider in this study. The dose and the frequency with which it was given
may not have been adequate to affect emotional processing. As we are among the first to investigate the effects of T on emotional processing in male non-human primates, we did not have a standard dose and frequency available for this subject population. Our treatment regimen resulted in T levels being raised to supraphysiological levels and we may have seen greater effects with administration of smaller T doses. Several studies have suggested a curvilinear relationship between T levels and cognition (Matousek & Sherwin, 2010; Moffat & Hampson, 1996; Müller, Aleman, Grobbee, de Haan & van der Schouw, 2005) and the same relationship may exist between T and emotional processing. As research in this area continues to be conducted, dose and frequency of T administration should be refined.

Finally, an obvious limitation of this study is the small sample size. Six subjects does not provide strong statistical power and the results from the gambling task were not useful due most of our animals having a side bias. Future studies should utilize a larger number of animals.

Overall, the results of this study indicate that T has no effect on attention to or memory for emotional stimuli or on social anxiety in the male rhesus monkey. Conclusions cannot be drawn regarding the effect of T on risk-taking behavior due to the presence of a side bias in the majority of our animals on the gambling task. Much additional research is needed to elucidate the effects of T on emotional processing in male primates and the results from these experiments provide a foundation on which to build future research.
Table 1. Dot-Probe Task: Response Times
Mean response times (ms) across treatments on the dot-probe task for emotional stimuli in both the social and non-social categories.

<table>
<thead>
<tr>
<th>Valence</th>
<th>Category</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
</tr>
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<td></td>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
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<tr>
<td>Negative</td>
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<td>313.46</td>
<td>775.10</td>
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<tr>
<td></td>
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<td>1100.36</td>
<td>350.77</td>
<td>819.82</td>
<td>189.08</td>
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Table 2. DNMS: Proportion of Accurate Responses
Proportion of accurate responses across treatments on the DNMS for emotional stimuli in both the social and non-social categories

<table>
<thead>
<tr>
<th>Valence</th>
<th>Category</th>
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<td></td>
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<td>SD</td>
<td>M</td>
<td>SD</td>
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<td>Neutral</td>
<td>Social</td>
<td>0.68</td>
<td>0.14</td>
<td>0.72</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Non-social</td>
<td>0.81</td>
<td>0.14</td>
<td>0.84</td>
<td>0.13</td>
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<tr>
<td>Positive</td>
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<td>0.14</td>
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<td>0.78</td>
<td>0.15</td>
<td>0.79</td>
<td>0.13</td>
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Table 3. DNMS: Response Times
Mean response times across treatments on the DNMS for emotional stimuli in both the social and non-social categories

<table>
<thead>
<tr>
<th>Valence</th>
<th>Category</th>
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<th>SD</th>
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<tr>
<td>Negative</td>
<td>Social</td>
<td>1227.70</td>
<td>180.98</td>
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<td>220.89</td>
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<td></td>
<td>Non-social</td>
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<td>1000.23</td>
<td>152.67</td>
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<tr>
<td></td>
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<td>1328.09</td>
<td>248.21</td>
<td>1207.90</td>
<td>247.80</td>
<td>1045.30</td>
<td>99.07</td>
<td>994.98</td>
<td>104.36</td>
</tr>
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</table>

Table 4. Intruder Paradigm: Frequency of Behaviors
Mean frequency of behaviors per category across treatments in the intruder paradigm

<table>
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<tr>
<th>Behavior Category</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
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<tr>
<td></td>
<td>M</td>
<td>SD</td>
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<td>SD</td>
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<tr>
<td>Anxious</td>
<td>11.83</td>
<td>11.54</td>
<td>3.17</td>
<td>1.70</td>
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<tr>
<td>Aggressive</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Affiliative</td>
<td>2.82</td>
<td>4.22</td>
<td>0.17</td>
<td>0.40</td>
</tr>
</tbody>
</table>

Table 5. Intruder Paradigm: Duration of Watching and Circling/Pacing Bouts
Mean duration of circling/pacing and watching bouts (sec) across treatments in the intruder paradigm

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Circling/Pacing</td>
<td>6.87</td>
<td>10.67</td>
<td>8.16</td>
<td>12.98</td>
</tr>
<tr>
<td>Watching</td>
<td>10.07</td>
<td>7.92</td>
<td>6.34</td>
<td>2.84</td>
</tr>
</tbody>
</table>
Table 6. Intruder Paradigm: Proportion of Time Spent Watching and Circling/Pacing
Mean proportion of time spent circling/pacing and watching the intruder across treatments in the intruder paradigm

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Circling/Pacing</td>
<td>0.16</td>
<td>0.33</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>Watching</td>
<td>0.41</td>
<td>0.24</td>
<td>0.28</td>
<td>0.18</td>
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</tbody>
</table>

Table 7. Gambling Task: Proportion of Risky Choices
Mean proportion of “risky” choices in the gambling task.

<table>
<thead>
<tr>
<th>Behavior</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td>SD</td>
</tr>
<tr>
<td>Risky Choice</td>
<td>0.42</td>
<td>0.11</td>
<td>0.44</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table 8. Gambling Task: Individual Risk Preferences
Binomial analysis of risk preference for each animal, across all four treatments

<table>
<thead>
<tr>
<th>Subject</th>
<th>Choice</th>
<th>Baseline</th>
<th>Lupron</th>
<th>Lupron + Oil</th>
<th>Lupron + TE</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>N01</td>
<td>Safe</td>
<td>56</td>
<td>49</td>
<td>47</td>
<td>48</td>
<td>200</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>40</td>
<td>47</td>
<td>49</td>
<td>48</td>
<td>184</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>ZA1</td>
<td>Safe</td>
<td>48</td>
<td>49</td>
<td>48</td>
<td>48</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>48</td>
<td>47</td>
<td>48</td>
<td>48</td>
<td>191</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>ZA2</td>
<td>Safe</td>
<td>60</td>
<td>58</td>
<td>53</td>
<td>55</td>
<td>226</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>36</td>
<td>38</td>
<td>43</td>
<td>41</td>
<td>158</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>p&lt;0.02</td>
<td>p&lt;0.05</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
<tr>
<td>ZA31</td>
<td>Safe</td>
<td>48</td>
<td>57</td>
<td>61</td>
<td>63</td>
<td>229</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>48</td>
<td>39</td>
<td>35</td>
<td>33</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>Total</td>
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<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>n.s.</td>
<td>p&lt;0.10</td>
<td>p&lt;0.01</td>
<td>p&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>ZA54</td>
<td>Safe</td>
<td>75</td>
<td>71</td>
<td>69</td>
<td>76</td>
<td>291</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>21</td>
<td>25</td>
<td>27</td>
<td>20</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ZA63</td>
<td>Safe</td>
<td>49</td>
<td>41</td>
<td>43</td>
<td>42</td>
<td>175</td>
</tr>
<tr>
<td></td>
<td>Risky</td>
<td>47</td>
<td>55</td>
<td>53</td>
<td>54</td>
<td>209</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>96</td>
<td>384</td>
</tr>
<tr>
<td></td>
<td>Binomial</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td>n.s.</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1. Dot-Probe Task: In the Dot-Probe task, the animal must respond by touching the fixation cross on the screen and is then presented with one neutral and one emotional (positive or negative) photograph for one second, followed by a dot appearing in place of one of the photos. Animals must respond by touching the dot in order to obtain a reward. Faster response times to touch the dot indicate greater attention allocation to the picture preceding it (in this case, the negative picture).
Figure 2. Delayed Non-Matching to Sample: The Delayed Non-Matching to Sample task requires animals to respond to the sample image by touching it on the screen. Following a delay of one second, both the sample image and a new (non-matching) image are presented and the animal must touch the non-matching image in order to obtain reward. This task tests memory accuracy for emotional (negative in this example) versus neutral stimuli.
### Ethogram of Behaviors

All behaviors are quantified by frequency except for circling/pacing and watching behaviors, which are quantified by duration. Watching behavior indicates attention toward intruder and is not classified as anxious, aggressive or affiliative.

<table>
<thead>
<tr>
<th>Category</th>
<th>Behavior</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anxious</td>
<td>Self-Directed Behavior</td>
<td>scratches, grooms, holds any part of the body</td>
</tr>
<tr>
<td></td>
<td>Locomotor Stereotypies</td>
<td>activities (rock/sway/jump) repeated 3X or more</td>
</tr>
<tr>
<td></td>
<td>Teeth Gnashing</td>
<td>chewing motion without food in mouth</td>
</tr>
<tr>
<td></td>
<td>Freezing</td>
<td>motionless and crouched for $\geq 3$ seconds</td>
</tr>
<tr>
<td></td>
<td>Yawn</td>
<td>opens mouth wide baring upper teeth</td>
</tr>
<tr>
<td></td>
<td>Circling/Pacing</td>
<td>circling in the cage $\geq 3$ times</td>
</tr>
<tr>
<td>Aggressive</td>
<td>Cage Shake</td>
<td>vigorously shakes cage for $&gt;1$ second</td>
</tr>
<tr>
<td></td>
<td>Mouth-Threat/Lunge</td>
<td>opens mouth slightly or head/body lunge</td>
</tr>
<tr>
<td>Affiliative</td>
<td>Lip Smack</td>
<td>purses and alternatively closes and opens lips</td>
</tr>
<tr>
<td></td>
<td>Presentation</td>
<td>presents hindquarters with tail up</td>
</tr>
<tr>
<td>Other</td>
<td>Watching</td>
<td>looks directly at the intruder</td>
</tr>
</tbody>
</table>

**Figure 3.** Ethogram of Behaviors: All behaviors are quantified by frequency except for circling/pacing and watching behaviors, which are quantified by duration. Watching behavior indicates attention toward intruder and is not classified as anxious, aggressive or affiliative.
Figure 4. T Levels Across Treatments: Mean testosterone levels (ng/ml) ± SEM for all six animals across all four treatments: Baseline (B1-B4), Lupron (L1-L4), Oil (O1-O4), and Testosterone (T1-T4).
Figure 5. Dot-Probe Task - Oil and TE: Mean response times ± SEM as a function of treatment
* p < 0.05
Figure 6. DNMS – Baseline Accuracy: Mean proportion correct ± SEM as a function of category and emotional valence
**p < 0.01

Figure 7. DNMS – Baseline Response Times: Mean response times ± SEM as a function of category and emotional valence
*p < 0.05
**Figure 8.** DNMS Lupron – Accuracy: Mean proportion correct ± SEM as a function of category and emotional valence

**p < 0.01**

**Figure 9.** DNMS Lupron – Response Times: Mean response times ± SEM as a function of category and emotional valence

**p < 0.01**
**Figure 10.** DNMS Treatment – Accuracy: Mean proportion correct ± SEM as a function of category and emotional valence

***p < 0.01

**Figure 11.** DNMS Treatment – Response Times: Mean response times ± SEM as a function of category and emotional valence

* p < 0.05

** p < 0.01


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