Study Title: The Influence of Testosterone on Experimental Pain Perception

NCT Number: NCT 05781685

Document Date: 08/26/2017

UNM IRB PROTOCOL

TITLE:	The Influence of Testosterone on Experimental Pain Perception and Social Psychological Functioning
VERSION DATE:	08/26/17
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FUNDING AGENCY:	N/A

BACKGROUND/SCIENTIFIC RATIONALE

Scientists and health providers are currently limited in their knowledge of all of the biopsychosocial processes that constitute a pain experience. Pain perception is influenced by, and modulated through, processes that involve tissue stress and damage (e.g., physical disease and injury), peripheral and central nociception (i.e., afferent input and brain processing), mental thoughts (e.g., memories), emotions, and social settings (e.g., Gatchel, Peng, Peters, Fuchs, & Turk, 2007; McCaffery & Pasero, 1999). Although significant progress has been made in investigating many of these processes (e.g., opioid reception), we still do not fully understand how certain aspects of pain experiences operate, including the role of endogenous steroids on the behavioral demonstration of pain. It is well-established that biological sex modulates pain. As compared to males, females report greater prevalence, frequency, and duration of clinical pain and pain-related distress (Unruh, 1996; Berkley, 1997; Fillingim, Edwards, & Powell, 2000; Riley, Robinson, Wise, Myers, & Fillingim, 1998; Ruau, Liu, Clark, Angst, & Butte 2012). Experimental studies show that women are more likely to report lower pain threshold and tolerance, and higher pain intensity associated with various types of noxious stimuli (e.g., ischemic, pressure, electrical, and thermal; Berkley, 1997; Fillingim, Edwards, & Powell, 2000; Riley, Robinson, Wise, Myers, & Fillingim, 1998; Fillingim, & Maixner, 1996; Shinal & Fillingim, 2007; Vigil & Coulombe, 2011). The magnitude of these effects varies from moderate to large depending on sample size, nature of the stimulus, and whether pain sensitivity is indexed by threshold or tolerance (Berkley, 1997Riley, Robinson, Wise, Myers, & Fillingim, 1998; Shinal & Fillingim, 2007, Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009). It is generally assumed that gonadal sex hormones contribute to greater clinical and experimental pain experiences in women as compared to men (Fillingim, King, Ribeiro-Dasilva, Rahim-Williams, & Riley, 2009; Kuba & Quinones-Jenab, 2005; Riley, Robinson, Wise, Myers, & Fillingim, 1998; Ruau, Liu, Clark, Angst, & Butte, 2011; Vigil & Coulombe, 2011). Among the potential endocrine substrates responsible for gender differences in pain sensitivity is testosterone, an androgen produced primarily in the testis in males. Testosterone has been linked to several behaviors that prepare, motivate, and otherwise promote exposure to environmental dangers that result in pain experiences in vivo. Previous research with animal models and humans has found that individuals with higher levels of testosterone tend to be on average more aggressive and perform riskier behaviors than individuals with lower levels of testosterone (Beeman, 1947; Albert, Walsh, Gorzalka, Siemens, & Louie, 1986; Hume, & Wynne-Edwards, 2005; Stenstrom, Saad, Nepomuceno, &Mendenhall, 2011; Vermeersch, T'Sjoen, Kaufman, & Vincke, 2008; Booth, Johnson, & Granger, 1998). Studies looking at competition have found that humans that succeed in a competitive tasks (e.g., wrestling,tennis, stock-trading) had higher levels of testosterone and lower levels of the stress hormone cortisol than the losers of these tasks (Coats & Herbert, 2008; Mazur & Lamb, 1980; Elias, 1981). Dominant males in several primate groups have been found to have higher testosterone and lower cortisol than submissive males (Rose, Holaday, & Bernstein, 1971; Muller & Wrangham, 2004). Therefore, it has been assumed that testosterone has been linked to prototypical male behaviors such as aggression, risk taking behaviors, competition, and dominance.

Experimental studies in females has found that women with higher basal levels of testosterone show higher levels of what are generally considered protypical male behaviors, and repeated experiments have replicated similar effects. For example, a study looking at dominance in females found that females with high levels of testosterone attributed more dominant characteristics to themselves than women with lower levels of testosterone (Grant & France, 2001). Female twins with a male twin brother show higher mean levels of aggression than same-sex, female twins, and this has been attributed to testosterone exposure in the womb (Cohen-Bendahan, Buitelaar, van Goozen, Orlebeke, & Cohen-Kettenis, 2005). Under controlled laboratory conditions, females that are given low doses of testosterone show reductions in empathetic behaviors (Hermans, Putman, & van Honk, 2006; van Honk, Schutter, Bos, Kruijt, Lentjes, & Baron-Cohen, 2013; Montoya, Terburg, Bos, Will, Buskens, Raub, van Honk, 2013). A logical extension of the above described work as it relates to sex differences in pain perception is to use a controlled laboratory experiment to examine how ephemeral and thus safe, short-lasting testosterone induction interacts with naturally circulating basal levels of testosterone to influence external pain sensitivity as part of a broad spectrum of changes in social behaviors associated with fluctuating testosterone levels.

OBJECTIVES/AIMS/HYPOTHESES

Our hypotheses are that: a) Females with high levels of circulating salivary (basal) Testosterone levels will experience reduced pain tolerance (hypoanalgesia) in a ischemic pressure pain task (primary outcome) ; b); Testosterone will alter the participants basal levels of estradiol and progesterone (secondary outcome) c): Further, exploratory outcomes will test whether testosterone will increase risk-taking behaviors, aggression, perception of health, attractiveness, disgust response, and sexual attitudes and beliefs.

STUDY DESIGN AND PROCEDURES

I. *Study Design*

The first part of the experiment will: a) examine basal levels of testosterone in females b) measure baseline pain tolerance) experimentally manipulate ephemeral (short-lasting) testosterone levels in females. The second part of the experiment takes place 4 hours later and consists of a pain task and behavioral measures/tasks. A double-blind protocol will be used so neither the researchers nor participants will know whether the participants were in the testosterone group or the placebo group until after the experimental procedures have been completed.

II. *Study Procedures*

Main Procedure

The proposed study will consist of 2 experimental sessions where each participant will spend at most 5 hours per session. Prospective participants who meet our initial inclusionary criteria will come to the lab for a pre-lab session (See Appendix: Flow Chart of Experimental Procedures that Will Occur Over 4 Days for Each Participant). This session will be used to explain to the participants about the risks and benefits of participating in this study and to make sure they understand the time commitment. Once the prospective participants have read and stated they understand what is required of them, they will be allowed to sign the consent form and schedule their first lab visit. All lab visits will be determined based on the participant's menstrual cycle (See Appendix: Menstrual Cycle Scale). All female participants will visit the lab during their luteal phase of their menstrual cycle. The second lab visit will be 3 days after the first to ensure that the female participants are still in their luteal phase during the second lab visit. This will ensure that the changes in behavior and in pain are not the result of variations of within the menstrual cycle. At their first lab visit, participants will be pre-screened for pregnancy, tree nut allergies, and any use of drugs (e.g., Insulin, Blood-Thinners, Corticosteroids, Oxyphenbutazone, Triamcinolone) that may negatively interact with testosterone. After the first session, female participants will continue to be screened for pregnancy via a pregnancy test. Females who do test positive for pregnancy will be excused from the experiment and will be referred to the UNM Student Health and Counseling Center (SHAC) where they can seek further care. All participants will be inquired about any new medication use before the start of each session. Because exercise, sexual behaviors, and competitive activities may increase testosterone, all participants will be instructed to avoid these activities on the days that they participate in the experiment. Participants will also be asked to report the time they woke up in the morning to control for fluctuations in testosterone over the course of the day.

Participants will be randomly assigned to either a placebo group or a testosterone group before arrival. The experimenters will be blinded to this assignment. Randomization of the placebo and experimental drugs will be controlled by the research pharmacist.

Participants will provide an initial salivary sample to establish baseline testosterone levels. Once they have given this sample, the participants will be given either the placebo or testosterone. They will then provide saliva samples 15 mins following the intervention. This will establish an intervention check. Baseline saliva sample before and after each intervention will be provided. Participants will remain in the lab for the duration of

this 5-hour session. They will be allowed to watch Netflix and listen to music as they wait. However, the content they can view or listen to will be limited to G or PG ratings in order to avoid fluctuation in their testosterone level. Participants will also be instructed to turn their cell phones off to ensure that their mood is not significantly altered. At 4 hours, participants will complete the post administration pain task. This pain task, like the behavioral measures, will only be administered once after 4 hours has passed. This is to prevent contamination and practice effect, which can occur when a behavioral task or measurement is administered multiple times during a session. The experimental sessions will take place 3 days apart. Participants will be reminded about their informed consent before their second session to ensure that they understand their continued rights as a research participant across the 2 experimental sessions. Participants will be awarded 3 research credits for each session for a total of 6 research credits. Participants will also be paid $5 after completing each session for a total of $10 for their participation.

Saliva Samples

Each participant will be given a saliva oral swab for saliva collection. Participants will be instructed to lightly chew on the cotton swab for 1 min. At the end of this time, participants will be instructed to place the swab in a test tube where it will be stored in our lab freezer. These saliva samples will be labeled with the participant's subject number. Three hormones will be assessed from the participant's saliva: testosterone, estradiol, and progesterone. An enzyme-linked immunosorbent assay (ELISA) diagnostic tool will be used to assess the specific level of each of these hormones at Hominoid Reproductive Ecology Laboratory located on UNM Main Campus. Measurement of testosterone using salivary samples has been found to be a reliable methods of assessing hormonal levels of individuals and has been used for diagnoses of endocrine dysfunctions in both males and females (Shirtcliff, Granger, & Likos, 2002; Morely, Perry, Dollbaum, & Kells, 2006; Cardoso, Contreras, Tumilasci, Elbert, Aguirre, Aquilano, & Arregger, 2011; Karrer-Voegeli, Rey, Reymond, Meuwly, Gaillard, & Gomez, 2009). Three hormones will be assessed in these analyses: testosterone, estradiol, and progesterone. Once these samples have been processed, they will be disposed of by the lab.

Testosterone Administration

The drug will be attained from the UNM Research Pharmacy that will prepare both the placebo and the drug for the purposes of this study (See Appendix: Letter from Research Pharmacist). The researchers will be blinded to this study. On the day of the experiment, the medical doctor on staff will write an individual prescription for the subjects participating in the study that day. The researchers conducting this study will then present the prescriptions to the UNM Research Laboratory and pick up the drug for the purposes of this study. Each subject will have an individual syringe prepared for them in which they will self-administer with directions from the researchers of this study. When the drug/placebo is in the lab it will be secured in a locked cabinet in our lab facilities; however, only testosterone/placebos being used for that days session will be stored at our lab . Only authorized lab personnel and/or medical personnel will have access to the drug at any time and a log will be kept indicating the inventory of the drug and when the drug has been removed from the cabinet and used. In the case that an unauthorized removal of the drug occurs (i.e. theft) or if there is indication that the log has been fabricated, the IRB will be notified immediately.

Participant Safety. Previous research has established that the current protocol will increase circulating levels of testosterone in the body by a factor of 10 within 15 mins of administration with baseline levels of testosterone returning to normal in 1.5 hrs after administration. As noted, however, behavioral effects of .5 mg of Testosterone reach their maximum levels 3-4 hours after administration (Tuten, van Honk, Koppeschaar, Bernaards, Thijssen, & Verbauten, 2000). Several studies using this same procedure for administration for Testosterone have found it to be a safe and effective means of administration for women with no ill effects or complications being reported by the authors as described in the form in the Appendix called "Table of Past Studies" (van Honk, Schutter, Bos, Kruijt, Lentjes, & Baron-Cohen, 2011; Montoya, Terburg, Bos, Will, Busken,

Raub, & van Honk, 2013; Hermans, Putman, & van Honk, 2006). We consulted a medical doctor about the dosage we intend on administering and they confirmed that the amount we will administer is safe, miniscule, and should not cause any long term issues with the participants (See Appendix: Letter from Medical Doctor). Subjects will be notified of the side-effects of more potent, repeated hormonal medication therapy which include: stomach or bowel complaints, acne/skin irritation, sleeplessness, chills, headache, anxiety, mental depression, excitation, confusion, dizziness, muscle pain, prolonged abnormal erection, inflamed testies, bladder, sexual dysfunction, prostatic growth, high blood pressure, blood disorders, shortness of breath, fluid retention, breast enlargement, and changes in liver function. However, the amount of testosterone we will be administrating is very low when compared to a typical dosage used for androgen replacement therapies (e.g., 40 mg to 300 mg) and will only be administered once. Further, previous studies show that potential complications associated with this drug are very minimal (see Table of Past Studies in Appendix). This same method was used in a previously approved protocol at UNM (IRB Approval Code: 902016-3). None of our participants reported any complications or side-effects from receiving the testosterone. Known medications that negatively interact with this form of Testosterone are: barbiturates, antidiabetic drugs, cyclosporine, Insulin, and anti-coagulants. Subjects will provide information on the medications they are currently taking in order to avoid these potentially harmful interactions.

Ischemic Discomfort Task

The ischemic discomfort task will require applying a sphygmomanometer (blood pressure cuff) 5 cm below their elbow. The participants will raise their arm vertically in the air and hold it there for 1 minute to desanguinate the limb. The cuff is then inflated to 200 mmHg over the course of 20 seconds. The subjects are then asked to lower their arm and perform handgrip exercise for a 30 second period. This procedure was previously found to be a safe and effective means to produce pain (Johnson & Tabasam, 2003; Ring et al., 2007). Participants will report their pain using the visual analogue scale (VAS) every 30 seconds. The pain task will end after 6 minutes (participants will not be aware of this); all participants will be told that they can discontinue the task at any point they desire. The main primary outcome will measure pain tolerance, which will be measured by the length of time the participant is able to tolerate the pain task in seconds. The longer the participant in the task, the higher their pain tolerance will be.

Disgust

Tybur, Lieberman, Kurzban, and DeScioli (2013) proposed three domains of disgust: moral, pathogen, and sexual. Overall, women are more sensitive to disgust, but it was specifically found that women are more sensitive to sexual disgust (Tybur, Bryan, Lieberman, Hooper, & Merriman, 2011: Tybur et al., 2009; Tybur et al., 2013). Disgust sensitivity has not been explored using testosterone manipulation methods, however, due to the large sex difference found in the literature, it is probable that testosterone might manipulate disgust perceptions. To measure disgust, we plan on using the Three Domain Disgust Videos (TDDV; Del Giudice, in preparation). The videos can be found here http://tinyurl.com/nl995ew. These 20 soundless stimuli arouse disgust in the domains of moral, sexual, and pathogen disgust. Five of these videos serve as controls. A confirmatory factor analysis provided evidence that the TDDV is comparable to the Three Domain Disgust Scale (TDDS), a measure often used in the literature to measure the three domains of disgust (Del Giudice, in preparation). We also plan on administering the TDDS along with the TDDV.

Balloon Analogue Risk Task (BART)

The BART is a computerized measure of risk taking behavior developed by Lejuez et al. (2002). The BART models real-world risk behavior through the conceptual frame of balancing the potential for reward versus loss. In the task, the participant is presented with a balloon and offered the chance to earn money by pumping the balloon

up by clicking a button. Each click causes the balloon to incrementally inflate and money to be added to a counter up until some threshold, at which point the balloon is over inflated and explodes.

Thus, each pump confers greater risk, but also greater potential reward. If the participant chooses to cash-out prior to the balloon exploding then they collect the money earned for that trail, but if balloon explodes earnings for that trial are lost. Participants are not informed about the balloons breakpoints; the absence of this information allows for testing both participants' initial responses to the task and changes in responding as they gain experience with the task contingencies.

Reimbursement is based on the number of pumps accrued without the balloon exploding. Subjects will likely complete 30 trials and will be reimbursed for 5 cents per pump. This leads to an estimated reimbursement of $10 per subject (expected range $5 and $20).

Behavioral Measures

As reviewed above, testosterone has been known to influence aggression, risk taking behavior, and empathy in human subjects. It is our intention to measure all three of these aspects to see if the behavioral effects of testosterone are manifesting at 3.5 hours as previous studies have established (See Appendix: Table of Past Studies). The behavioral tasks will consist of the Buss-Perry Aggression Questionnaire (Buss & Perry 1992; Bryant & Smith, 2001), which measures 4 domains of aggression that include: physical aggression, verbal aggression, anger, and hostility. Risk-taking behaviors will be measured using the Domain-Specific Risk-Attitude Scale (Weber, Blais, & Betz, 2002) that measures risk taking behavior over 5 domains that include: financial, health, ethical, recreational, and social risk-taking behaviors. The Body-Esteem Scale (Franzoi & Shields, 1984) is a 35 items scale used to measure how the participants feel about certain aspects of their bodies. The Self-Perceived Mating Success Scale (Landolt, Lalumière, & Quinsey, 1995) is a 12 item scale used to measure a participants self-perceived mating success. The SF-36 Health Survey (Ware & Sherbourne, 1992) is a 36 items survey and measures self-perceived physical and mental health. It is widely used in medical setting to assess patient's physical and mental health. Finally sexual attitudes and beliefs will be assessed using a scale developed by Gangestad, Simpson, cousins, Garver-Apgar, and Christensen (2004). Participants will be have to complete these behavioral tasks once in a single session. Once to establish a baseline and second to measure the effect of the drug/placebo (See Appendix: Flow Chart of Experimental Procedures). The order of the questions will be randomized each time. This is to ensure that the participants are not answering a question as they had previously answered it See Appendix: Behavioral Measures).

Feasibility

It is important to note, that the testosterone administration being proposed here is exactly the same to a previous study approved by this IRB (UNM Approval Code #902016-3). During the course of that previous study, none of the participants reported any ill effects from taking testosterone. With our previous experience, we feel that we can execute this protocol with minimum risk to the participants. The exact same researchers that worked on the previous study will also work on current study. These individuals will bring their experience and knowledge from the pilot study to this current study.

Compared to that previous study, this study is scaled down. Our Pilot study had 4 sessions consisting of 5 saliva samples per session. The current study, will only have 2 sessions with 2 saliva samples per session. The participants in the pilot study could receive the drug intervention (i.e. testosterone) twice during the course of the experiment. In the current study, they will only receive the drug intervention once. Therefore, the amount of risk to the participants is significantly reduced.

The pain component of this study is new, but our team has used this pain procedure in the past. In fact, we have published several papers using the ischemic pain task (Vigil & Coulombe, 2011; Vigil & Alcock, 2014; Vigil Strenth, Trujillo, & Gangestad, 2014; Vigil, Rowell, & Lutz, 2014). Again, we feel confident that we can complete this protocol successfully with little risk to the participants.

I. *Target Population and Inclusion/Exclusion Criteria*

College-aged females that are over the age of 18 and younger than 25 will be used for this study.

Exclusion Criteria:

All participants will be shown a list of exclusion criteria for this study on the Sona website. Before given the consent form the experimenter will go through this list to make sure the subjects do not meet any of the exclusion criteria. All women will be given a pregnancy test at each experimental session to ensure that the drug will not negatively impact an unborn child. If women are pregnant, they will be brought aside in the research lab and told they are pregnant and be given information on the Student Health and Counseling Center (SHAC) here on campus and excluded from the experiment. The following criteria will exclude a participant from the study:

Anyone that has a tree nut allergy.
Females that are pregnant or may feel that they may be pregnant or breast-feeding.
Anyone taking supplements that may influence their testosterone level.
Anyone taking any kind of steroid that may increase his or her testosterone level.
Anyone that smokes tobacco or uses smokeless tobacco.
Anyone that reports any condition associated with nerve damage.
Anyone that is using hormonal contraceptive.

Each participant will complete a Testosterone Pre-Screen form (see Appendix). This form will ask each participant individually whether they are taking any drugs or have any conditions that might be exacerbated by testosterone. This form will be completed before they participate sign the consent form and each time they arrive in the lab for an experimental session.

II. *Participant Enrollment*

Twenty Female Participants will be enrolled in this study.

III. *Recruitment and Screening Procedures*

Participants will be recruited via the UNM Psychology SONA system.

IV. *Informed Consent Process*

The participants have several opportunities to decide whether to continue or to terminate their involvement. Solicitations will operate through the SONA system, in which prospective participants are fully informed of the study protocol. All participants will attend a pre-lab session where only the consent form will be discussed. The experimenter will go through the consent form and make sure that the participant understands all that is required of them before they sign the consent form. Students will thus be allowed up to two weeks prior to a scheduled start date to decide whether they would like to participate in this study. I am also freely available to answer questions and concerns. Prior to their involvement in the study (upon arrival to the study location), participants are expected to provide written consent for the overall study (See Consent Form).

V. *Data Collection Procedures*

Described above in the Main Experiment Procedure. Two saliva samples will be collected during each of the 2 sessions that each subject attends:

1. Pre-Experiment saliva
2. Post Testosterone administration at 15 mins

Participants will also complete the pain task 4 hours following administration of T. Following the pain task, the behavioral measures will be administered at each experimental session to assess their state of aggression, risk-taking behavior, disgust, self-perceived health, self-perceived attraction, and sexual attitudes and beliefs. These surveys will be stored on an electronic survey site (Opinio) at UNM. We estimate that in all the experiment should take 5 hours to complete.

This same procedure will be repeated 3 days after the first experimental session.

VI. *Study Timelines*

The study will take approximately 12 months to complete starting from data collection and ending with the manuscript write up. It will take approximately 3 to 4 months to recruit and collect the data, and an additional month to 2 to 3 months to analyze the data and to process the biological samples, an additional month is needed to interpret the results, and finally and additional 3 to 4 months to prepare the manuscript write up. .

VII. *Study Location(s)*

The study will be conducted in the Pain Lab (Room B56) at Logan Hall on UNM Campus

VIII. *Participant Compensation*

Participants will be given 6 class credits (2 credits for 1st experimental session and 4 credits for the 2nd experimental session) for participation in this study and participants will be given $5 for participating in each session of this study. Additionally, when participants preform the BART task they can earn between $5 to $20 per session. In total, participants can receive between $10 and $25 for each session they complete. In each session, participants will receive both the class credit and monetary compensation. The participants will be paid in cash. The class credit and the monetary compensation will be pro-rated based on the completion of each experimental session. If a participant fails to appear for an experimental session they will not be awarded credit or money for not showing up. If a participant decides to terminate the experiment during an experimental session they will be awarded credit and money for that session, but no further compensation will be provided if they do not complete the following experimental sessions. If participants terminate the session before they complete the BART, they will only be awarded the class credit for the session and the $5.

If the participants are injured or become sick as a result of this study, the University of New Mexico Health Sciences Center (UNM HSC) will provide them with emergency treatment, at the patient's cost. The University makes no commitment to provide free medical care or money for injuries to participants in this study.

IX. *Study Resources*

The ideal research team consists of a tenured, Associate Professor and expert pain researcher with over 50 published scientific articles in the areas of psychology and pain perception, Jacob Vigil, and a Master's level graduate student with experience in running multiple types of social psychological and pain experiments, Chance Strenth. The researchers have protected research facilities and lab space for conducting the proposed experiments.

EXPECTED RISKS/BENEFITS

I. *Potential Risks*

The potential risks associated with the induction of 0.5mg Testosterone are not documented. Typical Testosterone Replacement therapies use a dosage that ranges from 10 mg to 200 mg (Bassil, Alkaade, & Morely, 2009). As such, the dosage used in the current study is miniscule in comparison. However, as with typical testosterone replacement therapies, the general risks include: increased aggression and increased libido, liver dysfunction, sleep apnea, edema may increase or worsen, gynecomastia, exacerbation of breast cancer, and finally skin irritation (Bassil, Alkaade, & Morely, 2009). However, it should be noted that these

side effects of testosterone only manifest after prolonged exposure to testosterone. Again, because we are only administrating two doses of 0.5mg Testosterone over a 4 day period, we feel that these potential risks will be minimal. The Table of Past Studies, which we have included in the Appendix , contains 6 studies where a similar experimental procedure was used. None of those studies reported any adverse problems to the participants and no participant dropped out of any of those studies due to complications of taking the T. Therefore, we believe that this experimental procedure is safe.

In our previous pilot study (UNM Approval Code #902016-3), an error occurred where a participant received 3 doses of testosterone instead of 2. This error occurred at the research pharmacy where the drug/placebo was prepared and randomized. We believe this incident was due to random human error during the course of the preparation of the placebo. This incident was reported to the IRB as soon as it was discovered. The participant in question did not report any adverse effects due to receiving 3 doses and in the current consent form under potential risks, the new participants will be warned that a potential error of this sort could occur. It is important to note, that this study will only have 2 sessions, not 4. During the course of the pilot study, no participant reported adverse effects of receiving 2 doses of testosterone. It is very unlikely that this error will occur again, but even if it did the participants should not be adversely affected by receiving two doses of testosterone.

The pain task is designed to experimentally induce mild to severe pain over a short period of time. The pain is manually controlled and will be stopped upon the participants' wishes. To avoid potential medical complications, only people without circulation problems, neuropathy, and alcoholism are permitted to participate in the pain task.

II. *Benefits*

The participants will not gain a direct benefit from this study. However, the knowledge gained from this study will contribute to a better understanding of how hormones influence pain in normal individuals. This could eventually lead to better treatments for pain and better means of diagnosing pain.

III. *Privacy of Participants*

Privacy will be protected by securing consent forms and storing in a secure location separate from the participants data in the lab. All other material will be labeled with subject number as oppose to subject's name. All biological samples will also be labeled with subject's number. The Vigil Pain Lab is located in the basement of Logan Hall. Logan Hall has any number of ongoing experiments taking place from day to day. The basement of Logan Hall and the actual location of the Pain lab or relatively secluded. Therefore, participants can enter the pain lab with little worry that they will be identified as participating in this specific study. All research assistants have completed the CITI certification course and are sensitive to privacy issues and will therefore take necessary steps to ensure that the privacy of the participant is protected.

IV. *Unanticipated Problems/Adverse Events*

We will terminate the study in the event that a participant claims they have experienced significant psychological or physical distress lasting more than 1 hour in duration after study participation. The PI will also notify the IRB of any unanticipated problems within 24 hours.

V. *Participant Complaints*

Upon completion of the study, participants will be given a debriefing form, which will include study contact information. If the participants wish to gain more knowledge on the research they participated in, they may contact the PI of the study. When concerning complaints, the PI will consider each complaint very carefully and address them in a timely manner. However, if the complaint appears to be of a serious nature, the IRB will be contacted within 24 hours and the PI will consider halting the study.

STUDY DATA

I. *Data Management Procedures and Confidentiality*

Paper surveys will be used to collect basic demographic information. The paper surveys will be entered into an excel spreadsheet once data collection is completed. Data from the biological samples will be provided by the lab performing the analysis. The data from all of these sources will be combined into a single spreadsheet from which the data will be analyzed. All the paper-pencil data will be stored in a private area (i. e. locked storage cabinets within a locked lab) and the electronic data will be secured under password protection on secure lab computers/hard drives. All paper surveys, electronic files, and biological samples will be labeled using the participants subject number to ensure privacy and confidentiality.

Pregnancy tests will be used to assess pregnancy status of each of the female participants in order to exclude females that may be pregnant. In order to protect the confidentiality of each of the female participants, no identifying information will be written on any of the pregnancy tests. Upon completing the pregnancy test, if the test is negative, the pregnancy test will be disposed of and the subjects will be allowed to continue. If the participants test positive, they will be excluded from the study. No written record or reason for exclusion will be recorded and the positive pregnancy test with no identifying information on it will be disposed of and the research assistant will provide information on SHAC as described above.

After 5 years, all paper documents will be destroyed. All electronic data will be archived at the UNM Zimmerman Library. All data will be submitted to the library to be archived by archival specialist and made available to the public upon request. All data will be formatted according to the guidelines established by the Rocky Mountain Online Archive (RMOA).

II. *Data Analysis/Statistical Considerations*

Data analysis will be conducted using Multilevel Modeling to examine any potential differences across the experimental conditions. The pilot study used 12 participants. A power analysis was not carried out on the pilot study data due to the complication of conducting a power analysis using multilevel modeling. However, a prior power analysis conducted using the effect size reported in Chen, Decety, Huang, Chen, and Cheng (2016) used similar methods. Using these reported effects a sample size of 15 was adequate to find a significant effect with a power of 0.80 and an alpha of 0.05. The findings in the power analysis and the results from the pilot study, highly suggest that 15 participants will be sufficient in capturing significant results.

Participant Withdrawal

Subjects who decide to not participate in the study or who are otherwise ineligible for participation in the experimental protocol will be given the option to write a 5 page essay for the equivalent of 6 hours of research credit (for instructors who accept such credit).

Due to the expense and the complication of this study, if participants choose to withdraw, the researchers will process the biological samples and psychological data that were collected from the participants. This data will be used in subsequent analyses for this study. However, upon withdrawal if the participant would not like their biological or psychological data to be used for this study: they will be instructed to inform either the research assistants or the Principle Investigator for this study and their data will be excluded and their biological samples and psychological data will be destroyed.

PRIOR APPROVALS/REVIEWED AT OTHER IRBS

n/a

REFERENCES

Albert, D. J., Walsh, M. L., Gorzalka, B. B., Siemens, Y., & Louie, H. (1986). Testosterone removal in rats results in a decrease in social aggression and a loss of social dominance. *Physiology & behavior*, *36*(3), 401-407.

Beeman, E. A. (1947). The effect of male hormone on aggressive behavior in mice. *Physiological Zoology*, 373-405.

Berkley, K. J. (1997). Sex differences in pain. *Behavioral and Brain Sciences*, *20*(03), 371-380.

Birnie, K. A., Noel, M., Chambers, C. T., von Baeyer, C. L., & Fernandez, C. V. (2010). The cold pressor task: Is it an ethically acceptable pain research method in children?. *Journal of pediatric psychology*, jsq092.

Bassil, N., Alkaade, S., & Morley, J. E. (2009). The benefits and risks of testosterone replacement therapy: a review. *Therapeutics and Clinical Risk Management*, *5*, 427.

Booth, A., Johnson, D. R., & Granger, D. A. (1999). Testosterone and men's depression: the role of social behavior. *Journal of Health and Social Behavior*, 130-140.

Bryant, F. B., & Smith, B. D. (2001). Refining the architecture of aggression: A measurement model for the Buss–Perry Aggression Questionnaire. Journal of Research in Personality, 35(2), 138-167.

Buss, A. H., & Perry, M. (1992). The aggression questionnaire. Journal of personality and social psychology, 63(3), 452.

Cohen-Bendahan, C. C., Buitelaar, J. K., Van Goozen, S. H., Orlebeke, J. F., & Cohen-Kettenis, P. T. (2005). Is there an effect of prenatal testosterone on aggression and other behavioral traits? A study comparing same-sex and opposite-sex twin girls. *Hormones and Behavior*, *47*(2), 230-237.

Chen, C., Decety, J., Huang, P. C., Chen, C. Y., & Cheng, Y. (2016). Testosterone administration in females modulates moral judgment and patterns of brain activation and functional connectivity. Human brain mapping, 37(10), 3417-3430.

Donnellan, M. B., Oswald, F. L., Baird, B. M., & Lucas, R. E. (2006). The mini-IPIP scales: tiny-yet-effective measures of the Big Five factors of personality. Psychological assessment, 18(2), 192.

Elias, M. (1981). Serum cortisol, testosterone, and testosterone-binding globulin responses to competitive fighting in human males. *Aggressive Behavior*.

Ekman, P., & Friesen, W. V. (1977). Facial action coding system.

Fillingim, R. B., Edwards, R. R., & Powell, T. (2000). Sex-dependent effects of reported familial pain history on recent pain complaints and experimental pain responses. *Pain*, *86*(1), 87-94.

Fillingim, R. B., King, C. D., Ribeiro-Dasilva, M. C., Rahim-Williams, B., & Riley, J. L. (2009). Sex, gender, and pain: a review of recent clinical and experimental findings. *The Journal of Pain*, *10*(5), 447-485.

Fillingim, R. B., & Maixner, W. (1996, February). Gender differences in the responses to noxious stimuli. In *Pain forum* (Vol. 4, No. 4, pp. 209-221). Churchill Livingstone.

Gatchel, R. J., Peng, Y. B., Peters, M. L., Fuchs, P. N., & Turk, D. C. (2007). The biopsychosocial approach to chronic pain: scientific advances and future directions. *Psychological bulletin*, *133*(4), 581.

Hermans, E. J., Putman, P., & Van Honk, J. (2006). Testosterone administration reduces empathetic behavior: A facial mimicry study. *Psychoneuroendocrinology*, *31*(7), 859-866.

Hume, J. M., & Wynne-Edwards, K. E. (2005). Castration reduces male testosterone, estradiol, and territorial aggression, but not paternal behavior in biparental dwarf hamsters (Phodopus campbelli). *Hormones and Behavior*, *48*(3), 303-310.

Johnson, M. I., & Tabasam, G. (2003). An investigation into the analgesic effects of interferental currents and transcutaneous electrical nerve stimulation on experimentally induced ischemic pain in otherwise pain-free volunteers. Physical Therapy, 83(3), 208.

Jones, D. N., & Paulhus, D. L. (2014). Introducing the short dark triad (SD3) a brief measure of dark personality traits. Assessment, 21(1), 28-41.

Kemper, C. J., & Schwerdtfeger, A. (2009). Comparing indirect methods of digit ratio (2D: 4D) measurement. American Journal of Human Biology, 21(2), 188-191.

Kuba, T., & Quinones-Jenab, V. (2005). The role of female gonadal hormones in behavioral sex differences in persistent and chronic pain: clinical versus preclinical studies. *Brain research bulletin*, *66*(3), 179-188.

Lippa, R. A. (2006). Finger lengths, 2D: 4D ratios, and their relation to gender-related personality traits and the Big Five. Biological Psychology, 71(1), 116-121.

Penke, L., & Asendorpf, J. B. (2008). Beyond global sociosexual orientations: a more differentiated look at sociosexuality and its effects on courtship and romantic relationships. Journal of personality and social psychology, 95(5), 1113.

Manning, J. T., & Fink, B. (2008). Digit ratio (2D: 4D), dominance, reproductive success, asymmetry, and sociosexuality in the BBC Internet Study. American Journal of Human Biology, 20(4), 451-461.

Mazur, A., & Lamb, T. A. (1980). Testosterone, status, and mood in human males. *Hormones and Behavior*, *14*(3), 236-246.

McCaffery, M. and Pasero, C. (1999). *Pain—Clinical Manual*. St. Louis: Mosby.

Montoya, E. R., Terburg, D., Bos, P. A., Will, G. J., Buskens, V., Raub, W., & van Honk, J. (2013). Testosterone administration modulates moral judgments depending on second-to-fourth digit ratio. *Psychoneuroendocrinology*, *38*(8), 1362-1369.

Muller, M. N., & Wrangham, R. W. (2004). Dominance, aggression and testosterone in wild chimpanzees: a test of the 'challenge hypothesis'. *Animal Behaviour*, *67*(1), 113-123.

Riley III, J. L., Robinson, M. E., Wise, E. A., Myers, C. D., & Fillingim, R. B. (1998). Sex differences in the perception of noxious experimental stimuli: a meta-analysis. *Pain*, *74*(2), 181-187.

Ring, C., France, C. R., al'Absi, M., Beesley, L., Edwards, L., McIntyre, D., ... & Martin, U. (2007). Effects of opioid blockade with naltrexone and distraction on cold and ischemic pain in hypertension. Journal of behavioral medicine, 30(1), 59.

Rose, R. M., Holaday, J. W., & Bernstein, I. S. (1971). Plasma testosterone, dominance rank and aggressive behaviour in male rhesus monkeys. *Nature*.

Ruau, D., Liu, L. Y., Clark, J. D., Angst, M. S., & Butte, A. J. (2012). Sex differences in reported pain across 11,000 patients captured in electronic medical records. *The Journal of Pain*, *13*(3), 228-234.

Shinal, R. M., & Fillingim, R. B. (2007). Overview of orofacial pain: epidemiology and gender differences in orofacial pain. *Dental Clinics of North America*, *51*(1), 1-18.

Stenstrom, E., Saad, G., Nepomuceno, M. V., & Mendenhall, Z. (2011). Testosterone and domain-specific risk: Digit ratios (2D: 4D and rel2) as predictors of recreational, financial, and social risk-taking behaviors. *Personality and Individual Differences*, *51*(4), 412-416.

Tuiten, A., Van Honk, J., Koppeschaar, H., Bernaards, C., Thijssen, J., & Verbaten, R. (2000). Time course of effects of testosterone administration on sexual arousal in women. *Archives of General Psychiatry*, *57*(2), 149-153.

Tybur, J. M., Lieberman, D. L., & Griskevicius, V. G. (2009). Microbes, mating, and morality: Individual differences in three functional domains of disgust. Journal of Personality and Social Psychology, 97(1), 103-122

Unruh, A. M. (1996). Gender variations in clinical pain experience. *Pain*, *65*(2), 123-167.

Vermeersch, H., T'sjoen, G., Kaufman, J. M., & Vincke, J. (2008). The role of testosterone in aggressive and non-aggressive risk-taking in adolescent boys. *Hormones and Behavior*, *53*(3), 463-471.

Vigil, J. M. (2009). A socio-relational framework of sex differences in the expression of emotion. *Behavioral and Brain Sciences*, *32*, 375-428.

Vigil, J. M., & Coulombe, P. (2011). Biological sex and social setting affects pain intensity and observational coding of other people's pain behaviors. *Pain*, *152*(9), 2125-2130.

Van Honk, J., Schutter, D. J., Bos, P. A., Kruijt, A. W., Lentjes, E. G., & Baron-Cohen, S. (2011). Testosterone administration impairs cognitive empathy in women depending on second-to-fourth digit ratio. *Proceedings of the National Academy of Sciences*, *108*(8), 3448-3452.

von Baeyer, C. L., Piira, T., Chambers, C. T., Trapanotto, M., & Zeltzer, L. K. (2005). Guidelines for the cold pressor task as an experimental pain stimulus for use with children. *The Journal of Pain*, *6*(4), 218-227.

APPENDICES

Appendix

I. Table of Past Studies

II. Flow Chart of Experimental Procedures.

III. Letter from Research Pharmacist

IV. Experimenter Generated Questionnaire

   a. Demographic Questionnaire

b. Pre-Lab Testosterone Related Behaviors Survey

V. Non-Experimental Generated Questionnaires

a. Buss-Perry Aggression Scale

b. Domain-Specific Risk-Attitude Scale

c. The Body-Esteem Scale (Franzoi & Shields, 1984)

d. The Self-Perceived Mating Success Scale

e. Sexual Attitudes and Beliefs Scale

f. Menstrual Cycle Scale

g. Three-Domain Disgust Scale

VI. Debriefing Form

VII. SONA Recruitment Form

VIII. Testosterone Pre-Screen Form