Testosterone Reactivity and Neural Activation in the MID task

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Testosterone Reactivity and Neural Activation in the MID task

A Thesis

Submitted to the Graduate Faculty of the
University of New Orleans
in partial fulfillment of the
requirements for the degree of

Master of Science
in
Psychology

by
Yoojin Lee
B.A. University of Minnesota, Twin Cities
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Abstract

The purpose of the project was to determine if testosterone reactivity and neural changes could be observed in response to a reward-seeking competitive task, respectively, and whether testosterone was related to neural activation. Forty-nine undergraduate students were recruited playing the Monetary Incentive Delay (MID). We found that a subset of participants (N=20) showed testosterone reactivity to the task ($p < .05$). During the EEG analyses, cue had a main effect on FRN amplitude in a trend level ($p = .084$): The large incentive cue triggered smaller (less negative) FRN amplitude than the small incentive cue did ($p < .05$), especially during the second reward seeking block ($A'$) ($p = .065$) and especially within males ($p < .05$). Testosterone level and reactivity were not further associated with FRN amplitude ($p > .1$). Taken together, results show both testosterone and FRN amplitude may be sensitive to a complex reward-seeking and competition.

*Keywords*: testosterone, neuroendocrinology, reward seeking, monetary incentive delay task, electroencephalography
Introduction

Testosterone is well known as a male dominant sex hormone; however, recently, researchers have suggested that testosterone work as a social hormone beyond its traditional role as a male sex hormone. Furthermore, this hormone may be responsive to salient social challenges, including those that involve competition (Bos, Panksepp, Bluthe, & van Honk, 2012; Zilioli & Watson, 2013) and reward seeking behavior (Campbell et al., 2010; van Honk et al., 2004). The process of social competition is where the winning individual is rewarded by tangible gains (e.g., financial rewards or awards) as well as intangible gains (e.g., gain in social status and prestige). Plus, reward seeking behavior is an intention of organisms to maximize the tangible gains which would eventually increase their possibility in survival (Carre, Putnam, & McCormick, 2009). The competition and reward seeking behavior are thus expected to be manifested by a change in the testosterone level of individuals (i.e., reactivity). Perhaps more importantly, this reactivity is not expected to occur primarily in the gonads, but rather to be initiated by the brain in an acute manner. Electroencephalography (EEG) and event-related potential (ERP) of EEG are capable to capture a neuronal change during the competition and reward seeking. We thought that measuring testosterone reactivity and its link to neuronal activation during a reward seeking and a social competition would lend insight into the role of testosterone as a social hormone which undergoes centrally-mediated changes to help the individual achieve social status and goals in certain contexts.
Testosterone Overview

What is Testosterone?

Testosterone is a steroid hormone from the androgen family. Cholesterol, the master chemical of all steroid hormones, synthesizes Prognenolone with hydroxylation at the side-chain at C20 and C22 positions by cytochrome P450scC (Cholesterol desmolase), and the rate of synthesization is regulated by anterior pituitary hormones, such as Adrenocorticotropic hormone (ACTH), Follicle stimulating hormone (FSH), and Luteinizing hormone (LH). Prognenolone can be converted to Testosterone through two different pathways. First, Prognenolone is transferred to Progesterone by 3-beta-hydroxysteroid dehydrogenase (3\(\beta\)-HSD). Progesterone is changed to 17-hydroxy progesterone, which turns to Androstenedione, one of the androgens. Due to the oxidation of 17-beta-hydroxysteroid dehydrogenase (17\(\beta\)-HSD), Androstenedione is altered to Testosterone. Second, Prognenolone is also converted to 17-hydroxy-pregnenolone by 17\(\alpha\)-hydroxylase (CYP17A1). 17-hydroxy-pregnenolone is then converted to dehydroepiandrosterone (DHEA). DHEA, the androgen hormone, turns to Androstenediol due to the oxidation of 17\(\beta\)-HSD, which is the precursor of Testosterone. Much of this metabolic pathway is mediated by enzymatic conversions within male and female gonads, but the brain initiates the hormonal cascade to cause these enzymatic changes (see below).

Testosterone is referred to as a male-dominant sex hormone. Testosterone is mainly produced by the testicles in males, although to a much lesser extent, within the ovaries in females, and the adrenal gland, especially zona reticularis, in both sexes. Even though both males and females secrete Testosterone, males show seven to ten folds of the basal Testosterone levels, relative to females (Coates & Herbert, 2008; Eisenegger, Haushofer, and Fehr, 2011), at least
after puberty. Additionally, testosterone levels in females are sensitive to their menstrual cycles. The peak level of testosterone in females is after ovulation, shortly after the surge of LH triggers ovulation and the elevation in LH likewise causes a rise in testosterone release.

Regardless of when or where, after secretion, testosterone is carried to target tissues through the blood stream while bound to Sex hormone binding globulin (SHBG). As long as it is bound to SHBG, Testosterone cannot make physiological changes in organisms (Hiipakka & Liao, 1998; McPhaul & Young, 2001). Once free or unbound Testosterone is transported into the target cells, it activates either androgen or estrogen receptors. Testosterone and 5α-dihydrotestosterone (DHT) can stimulate androgen receptors, and the receptor complex brings up the sequential change in DNA through the change of gene transcription (Breiner, Romalo, & Schweikert, 1986). A small amount of Testosterone can also be aromatized into estradiol through the enzyme aromatase, and subsequently activates estrogen receptors. This illustrates that testosterone has direct and indirect effects on gene expression. Besides the genealogical changes, testosterone can have non-genomic effects, which convey a much quicker physiological effect to organisms (Heinlein & Chang, 2002; Michels & Hoppe, 2008).

In humans, testosterone synthesis is the end of a hormonal cascade of the Hypothalamic-pituitary-gonadal (HPG) axis (Swerdloff, Wang, & Bhasin, 1992). This is notable because it implies that testosterone release is initially controlled by the brain. The HPG cascade is initiated as a regulatory system composed of positive feed forward and negative feedback. Researchers are increasingly paying attention to feedback of testosterone back to the brain rather than the initiation of the HPG cascade. That is, after testosterone is secreted, the human body takes a homeostatic hormonal adjustment in terms of testosterone levels. When testosterone is abundant in the body, testosterone acts like a break in the car, inhibiting the metabolite secretion from
hypothalamus and pituitary. Testosterone’s ability to inhibit itself is called negative feedback of HPG axis (Soma, Francis, Wingfield, & Fernald, 1996). Given that testosterone’s release is initiated in the brain, and the HPG cascade is fast acting, we now focus on understanding why this hormone’s release is able to be modified quickly following neural signals.

Sapolsky (1998) argued that a hormone is more likely to change in response to the social context than to change the social context itself. However, a social status context that involves salient social cues will lead to a change of testosterone level within individuals as well. The acute change of testosterone level is called as the testosterone reactivity. Traditionally, testosterone level has been known as a determining agent for masculine behaviors (Higley et al., 1996; Marti-Carbonell, Darbra, Garau, & Balada, 1992); however, some recent papers shed light on the change of testosterone level as a worthwhile avenue to consider in understanding human behavior more broadly and, as Sapolsky (1998) hinted, this broader view can be supported by primarily considering the social context; this can be accomplished by examining testosterone reactivity. Chichinadze and her colleagues (2012) argued that testosterone reactivity can be observed if males expect their victory and the possible satisfaction from the victory in an anticipated period of the competition and particularly while they experience this episode during a positive mood like pleasure, which also can occur from the testosterone increase in blood. Put differently, testosterone reactivity will be shown if individuals expect their social status increased and have a high expectation of success in the competition, and feels good when this increase really happens. This finding suggests that testosterone reactivity might relate to a broad array of social factors, which is salient to individuals in a specific context (Granger, Shirtcliff, Booth, Kivlighan, & Schwartz, 2004). Apicella and colleagues (2014) recently found that testosterone reactivity was related to the risk taking of individuals in a financial competition.
regardless of a result of the previous competition. After winning a competitive activity, testosterone reactivity in males was tied closest with risk-taking behavior more so than the previous triumph or failure in the context of a financial competition. These findings suggest that additional research is warranted in the area of testosterone reactivity in order to shed light on a broad array of social contexts to which testosterone changes and to better understand this hormone’s relationship to human behavior.

*Testosterone on Purpose: Testosterone and a social behavior*

Above, we examined testosterone’s release, especially acutely or reactively, as the end product of the HPG axis. This was intended to put forward the idea that testosterone release might be important within salient social contexts in order to help influence neural functioning and, ultimately, behavior. Next, we consider what types of behaviors have been linked with testosterone in order to justify examination of specific social contexts with testosterone release. Sexually biased quantity of testosterone secretion allowed researchers to think that since males are more aggressive than females in general (Giammanco, Tabacchi, Giammanco, Di Majo, & La Guardia, 2005), testosterone might be an agent for the aggression. Some animal studies supported the idea. For example, by using Silastic capsule containing testosterone, DeBold and Miczek (1985) found that castrated male rats with a low dose testosterone acted less aggressive than castrated rats with a high dose testosterone capsule. In humans, hypogonadal boys showed an increase of physical aggression after testosterone treatment (Finkelstein, 1997). Further, prison studies indicated that male prisoners who display high salivary testosterone levels served longer time in prison, and they were more likely to commit severe violence than low testosterone level prisoners (Dabbs, Frady, Carr, & Besch, 1987) and to violate prison rules (Dabbs, Jurkovic, & Frady, 1991). Thus, some studies associate testosterone with aggression.
Nonetheless, other studies have doubted the one-to-one relationship between testosterone and aggression. An important guiding theory, for example, links testosterone with dominance (A. Mazur & Booth, 1998) and suggest testosterone is only linked with aggression in social contexts that call for an aggressive response. This perspective is important for pointing toward social context in testosterone regulation. Steklis and colleagues (1985) further illustrate the importance of social context in their study of vervet monkeys. They defined a degree of the same sex aggression as an indication of dominancy. The results showed that even though dominant male monkeys secreted more testosterone than the subordinate ones, the difference between the two groups was not significant. This was mainly due to a fact that the subordinate ones failed to show meaningful testosterone reactivity to their aggression behaviors. Other animal research maintained that loss of aggression in alpha male rats after the castration was because of their loss of social status (Albert, Walsh, Gorzalka, Siemens, & Louie, 1986). These studies further supports that the relationship between testosterone and a behavior manifestation such as aggression might be modulated by social components, including dominance and competition.

Researchers put forward a challenge hypothesis to better understand the social context of testosterone release. Building from the role of testosterone as an androgenic hormone, the challenge hypothesis indicates that testosterone level is tied to aggression or dominance because these behaviors benefited organisms in reproduction and these behaviors are physiologically promoted in social contexts if their expression incurs benefits to the organism (Ferree, Wikelski, & Anderson, 2004; Ros, Dieleman, & Groothuis, 2002). Further, findings from chimpanzees (Muehlenbein, Watts, & Whitten, 2004), Ethiopian wolves (van Kesteren et al., 2012), and song sparrows (Wingfield, 1994) suggested that intra-sexual competition was strong enough to convey a testosterone increase, regardless of a reproductive purpose and instead generalize more broadly
to many contexts in which the organism is challenged and desires to gain a sense of winning. Bos and colleagues (2012) reviewed the challenge hypothesis and asserted that testosterone was strongly related to a social competition in humans even without a clear reproductive purpose. Based on these findings, we expected that a social competition with the same gender competitor would lead to a testosterone increase within individuals.

Gaining a sense of winning is not only achieved through competition or reproduction, but may be achieved through many social contexts in which something valuable is gained. Beyond survival, competition in a human society happens as an attempt to achieve or maintain social dominance which can maximize well-being in the long term. To sustain this level of well-being, individuals may seek a potential reward such as a monetary value. Note that a form of dominance in the competition is displayed not only as physical but also in a psychological or economical way (Eisenegger, Haushofer, & Fehr, 2011), so the reward seeking behavior would be observed from not only a physical competition (Edwards & Casto, 2013) but also from the standpoint of a financial competition (Coates & Herbert, 2008). These result allowed us to think that the reward seeking in the financial competition would lead to a testosterone increase within individuals.

Testosterone Reactivity is hypothesized to occur during the MID task

Focusing on why this task is expected to trigger testosterone reactivity, the following section describes why a computer based reward task, the monetary incentive delay (MID) task, was utilized in our study (Knutson, Adams, Fong, & Hommer, 2001). The MID in relation to neural activation is described much later. A modified version of MID was selected because it contains elements that have been shown be related to testosterone although the MID has never
been related to testosterone (to our knowledge). In addition, modifications to the task were made to enhance the relevance of the task for testosterone and testosterone reactivity. Thus, this section is based largely on theoretical extrapolations from our understanding of testosterone.

In brief, within the MID task, participants were informed that they would be compensated for their performance on MID, which implicates that monetary values earned during MID would be converted to the real-life monetary incentives. This was done because testosterone is linked specifically to tangible rewards that matter to the individual. We cannot measure the relationship between the individual perceptual amount of reward and a degree of the reward seeking in terms of a testosterone level since the perceptual amount of reward (i.e., cue) changed within 500ms, which was too fast to observe the testosterone responsivity. However, we hypothesized that the reward seeking property of the MID task would lead to the testosterone responsivity across the entire task.

Unlike the original version of MID task, three sessions of the modified MID, including the first reward seeking block (A), the competition block (B), and the second reward seeking block (A’) block were conducted in our study. The modified version was utilized since the competition block (B) contained a social competition, which testosterone appears to be responsive to. The competition property included MID task was never used before, so our hypothesis regarding competition was exploratory. We hypothesize that the competition block (B) would show the highest degree of testosterone responsivity among the blocks since the block accumulate the testosterone responsivity due to the reward seeking and competition. These hypotheses were examined by using testosterone assaying and event related potential (ERP), especially feedback related negativity.
ERP

Theories of FRN

Next, we return attention to the brain and how neural functioning operates in salient social contexts, specifically the MID task. The current study examined activation values for one event-related potential (ERP), the feedback related negativity (FRN). ERPs are comprised of averaged EEG generated by cortical regions. More specifically, similar trials were averaged together to provide a relatively pure measure of the cortical activation underlying the construct of interest. The FRN is time-locked to when participants receive feedback information, either positive or negative. FRN is the negative deflection of neural amplitude observed about 200 and 400ms after feedback appears.

Previous research has attempted to understand the role of the FRN. Some researchers argued that FRN was the result of the reward prediction and its error, called the reward prediction error. Li and his colleagues (2009) found that FRN disappeared when participants made a false choice or when they lost points in the context of a gambling task. Based on the result, they maintained that FRN would happen if participants assumed their answer would be correct and if they expected a positive feedback or reward from their performance from the task. Talmi and colleagues (2013) reviewed this hypothesis, saying that since the strength of FRN amplitude related to the reward seeking expectation, it might be linked to the dopaminergic pathway, known as the reward pathway, and that the probabilistic reward seeking behavior led to a prediction error, which manifested as neural signals originated from midbrain dopaminergic projections to the anterior cingulate cortex. Due to the nature of EEG, we could not detect the direct communication between those regions, but rather measured post synaptic potentials displaying the anterior cingulate cortex dendritic activation, which might be invigorated by the
dopamine pathway. Given that the MID task is thought to evoke reward seeking behavior (Knutson, Fong, Adams, Varner, & Hommer, 2001), and that participants were provided feedback information at the end of each trial, it may be that individual FRN amplitudes are responsive to gain and loss information. Consequently, we hypothesized that the magnitude of FRN amplitudes would change across different conditions, including blocks and cues in the MID task.

*FRN during the MID task*

Knutson and colleagues developed the MID task to measure neural activation evoked by monetary rewards and punishments (Knutson, Westdorp, Kaiser, & Hommer, 2000), and found that ventral striatal and prefrontal activation was related to the reward seeking behavior. The follow-up studies revealed that anticipation of the reward was related to the ventral striatum whereas the outcome of reward was associated with ventromedial prefrontal cortex (Knutson, Fong, et al., 2001; Knutson, Fong, Bennett, Adams, & Hommer, 2003). These results suggest unique neural contributions for reward anticipation and reward outcome evaluation. Since feedback related negativity is more closely related to the outcome of reward, rather than the anticipation of reward, FRN activation might originate form ventromedial prefrontal cortex (VMPFC) activation. Furthermore, given that the MID task measures reward seeking behavior, we expected that FRN amplitudes, measured at mediofrontal electrode sites, would be activate while participants played the MID task.

To the best of our knowledge, FRN amplitude had never been utilized in testing for a competition-related idea; however, some previous literature attempted to find the relationship between the social competition and its relation to the electrophysiological change in humans. de
Bruijn and colleagues (2008) introduced the social competition property in the go/no-go task, by asking participants to respond to the cue faster than their competitors did. They found that P3, the positive deflection of neural amplitude observed after 300ms after cue appeared, was associated with an inhibition of participants to the cue during the competition. Zeng and colleagues (2013) asked participants to play a bidding task against either their competitors or computer. They found that participants revealed larger late positive complex responses both with and without a reward when they played against the human competitors, relative to their late positive complex response which was observed when they played against the computer. These findings showed evidence that the social competition was associated with the late latency of ERPs. Although this literature focused on the P3, it allows us to think that FRN could be associated with the social competition as well given the enhanced rewards experienced within competitive winning.

The present study used a block design (A, B, A’) in which reward-seeking is present across all three blocks, but the B-block additionally has competition. Another difference between the blocks is that the A’ rewards are experienced late in the task. This design is in contrast to the A rewards due to the absence of novelty. Theoretically, FRN has a little association with a novelty property of event (Cooper, Duke, Pickering, & Smillie, 2014; Luu, Tucker, Derryberry, Reed, & Poulsen, 2003), so there should not be habituation to reward across the task. Still, the FRN may be linked with learning during the event: Previous researches revealed that FRN amplitude was strongly bound to the learning experience of individuals under the positive expectation for reward (Arbel, Goforth, & Donchin, 2013; Ichikawa, Siegle, Dombrovski, & Ohira, 2010), indicating that FRN might be related to the previous experience with a reward.
Based on these results, we hypothesized that FRN amplitude strength would be positively related to the previous experience with a reward, especially during and after competition.

Moreover, the FRN amplitude difference due to a different size of cue in the MID task was worthwhile to consider. Even though previous research hinted a monetary value might be related to a degree of the neural activation in the prefrontal neural area (Knutson et al., 2003), it was unsuccessful in describing the relationship between a size of reward and a degree of neural activation. Some recent researchers had attempted to find the relationship between a size of the potential reward and a degree of the brain activation, especially FRN amplitude. San Martin (2010) and his colleagues asked participants to play a game by simply pressing the button to a randomly assigned trial, including a win or lose trial. After the competition of each trial, participants were notified their potential incentive (i.e., feedback). The amount of incentive for the feedback varied in each trial. The results indicated for the winning trials, participants revealed smaller (less negative) FRN amplitude, relative to the losing condition. It was also revealed that a higher incentive cue revealed smaller (less negative) FRN amplitude than a smaller incentive cue did, implying that the relationship between an amount of the monetary value and a degree of FRN amplitude was negative. The other study showed participants a monetary cue at first, including 5, 20, and 50 cents, and asked them to find a box where a coin was hidden among six boxes (Bellebaum, Polezzi, & Daum, 2010). Researchers recorded participants’ FRN amplitude by using an fMRI while participants were playing the game. This study demonstrated that a higher potential monetary incentive cue led to greater (more negative) FRN amplitude than a lower potential monetary incentive cue when participants failed to find a correct box. There was a caveat that with a success in choosing a right box, participants revealed smaller (less negative) FRN amplitude to a higher monetary incentive cue, relative FRN
amplitude to a lower potential monetary incentive value; but, the analysis with a successive choice of box condition was not valid due to the poor statistical power. These findings implied that the monetary reward modulated the neural activation at the frontal region of brain during the MID task. Based on these results, we expected that an amount of the monetary value (i.e. cue) would be related to FRN amplitude in the MID task.

The gender effect on the FRN amplitude has not yet been examined yet; however, previous research has analyzed data to understand the relationship between gender and its relation to the electrophysiological change in humans. Jin and colleagues (2013) conducted the task to see how participants reacted to the cue, in terms of the ERP strength change. They provided cues based on their predictability, including predictable and non-predictable pictures, and the emotional property, including negative and neutral pictures. Overall the results showed that females revealed larger P2 amplitude, the positive electrical potential that peaks around 200ms after the onset of cue, relative to males, especially with the unpredictable pictures. The study was not directly related to the FRN or the MID task, but it gave us a chance to think of a gender effect on FRN in the MID task because our study consisted of the feedback score and culminated scores which could not be predictable until all the task was done. Thus, we expected that gender would modulate the FRN amplitude in the MID task.

**FRN, Testosterone, and the MID task**

To the best of our knowledge, no known research has been examined yet to see the direct association between FRN and testosterone. FRN is selected for the target neural signal for study since both testosterone and FRN amplitude are responsive to the monetary reward (Op de Macks...
et al., 2011; Zhou, Yu, & Zhou, 2010) and the competition (Eisenegger et al., 2011). Because the MID task included both monetary reward and competition properties, we hypothesized that during the MID task, participants having significantly increased testosterone levels due to the reward seeking would show the strengthened FRN amplitude, especially at the competition block (B), and that an amount of the potential incentive and gender would modulate the relationship in the MID task.

Hypotheses

First, we hypothesized that testosterone level would be reactive to the MID task due to the reward and competition components of the task. This was because all of our participants who won in the lab induced competition and took the monetary reward during the task. Specifically, we hypothesized that testosterone levels would increase during the MID task, and then decrease (or recover) after the task. Plus, we expected that testosterone levels on the task day would not significantly differ from testosterone levels on the basal day because we expected the acute testosterone change to be time-delimited. Second, we hypothesized that FRN amplitude would change across time in the modulation of the potential incentive amount (i.e. cue) and the type of blocks. The reward seeking and competition parts of the MID task would make individuals reactive to the cues and blocks, respectively. The reactivity would be manifested as the increased strength of FRN amplitude. Specifically, FRN amplitude would be greater (more negative) during the competition block (B) with a large cue, relative to the other conditions. These analyses account for gender given suspected gender differences in testosterone levels and potentially reactivity. Third, we hypothesized that individual difference in testosterone levels would be positively related to the strength of FRN amplitude. This hypothesis was based on the observation that both individual testosterone levels and FRN amplitude were related to the
reward seeking and competition and a degree of the potential incentive (i.e. cue) and, if so, might relate to reward and competition through a shared neurobiological mechanism. Specifically, we hypothesized that individuals in a high testosterone group would reveal stronger FRN amplitude, relative to ones in a low testosterone group. Fourth, testosterone reactivity would be related to the neural activation of FRN amplitude. This was examined by using responders, showing more than 15% increase of testosterone levels from the baseline (T1) to either the reward seeking task only (T3) or the reward seeking task after competition (T4). Even though previous research utilized the responders only as an interested subset for study (e.g., a responder-nonresponder strategy which is common with other hormones); (Buchanan, Tranel, & Adolphs, 2006; Dedovic et al., 2005; Pruessner et al., 2010), we used the testosterone reactivity and enter it as an independent variable. We thought that responders were more reactive to the reward seeking and competition in the MID task, relative to non-responders, and that FRN amplitude would be positively associated with the reward seeking and competition. Consequently, we hypothesized that the responders would show the stronger FRN amplitude during the competition block (B), relative to the non-responders.

Methods

Participants

Within the study, forty nine participants, including twenty males and twenty nine females, were recruited from undergraduate psychology classes at the University of New Orleans. Participants were considered only if they had no noticeable health problems by screening them over the phone the day before participants visited. Both genders were recruited and accepted. Participation was voluntary, and participants were allowed to exit the study at any step if they felt discomfort. Among forty nine participants, one participant was excluded from hormone
analyses due to insufficient saliva collections. Seven participants were excluded since their data were either missing or lost due to the insufficient trials (i.e., no data survived artifacting), by using the Net Station. Only seventeen males and twenty four females were analyzed for EEG analyses due to other participants having insufficient artifact free trials (N=41) whereas one participant was excluded for the hormone analyses due to the missing variable (N=48). All aspects of this research protocol are approved by the Institutional Review Board at the University of New Orleans.

![Figure 1: A schematic design of the study](image)

**Procedure**

Individuals recruited for this research were expected to participate for two days (Figure 1). The first day of participation was on the experiment day when participants visited; the second day of involvement was a comparison day, called the basal day. Before the lab visit, participants were screened if they had any health condition, which might affect hormonal activity.

The procedure consisted primarily of a series of saliva samples taken before and after the
MID task. Sample (T1), indicating a baseline and pre-task anticipation. The experimenter then measured pain threshold, digit length, and BMI (height/weight) which are important measures within the parent project but are not central measures for the proposed research. Then, participants played a practice block with the experimenter: At first, they played fifteen trials with experimenters; they then played by themselves fifteen trials. Participants were allowed to proceed to the task if their performance accuracy of the practice block reached up to 66 percentile of accuracy. Experimenters collected the second saliva sample (T2) right after the practice block so that this sample would indicate task anticipation. In the first reward seeking block (A) of the MID task, participants were required to play alone. After completion of the block A, participants provided a third saliva sample, and proceeded to the competition block (B). The third saliva sample collection (T3) indicates testosterone responsivity to the reward seeking task only. Participants were informed that they were going to play against the confederate over the internet. They were informed that the winner of the block B would keep their points and the losers of the block B would lose their points. The block B and the second reward seeking block (A’) were conducted afterwards. At the completion of block A’, the experimenter collected a fourth saliva sample (T4), indicating testosterone responsivity to a reward and competition. Participants then reported another pain threshold and filled out a questionnaire packet. The last saliva sample on the task day (T5) was collected to measure recovery of testosterone after the task, which helped to determine the timing of the peak in testosterone reactivity. The collection of the last saliva sample was conducted right before they went home.

The MID task

Each of the three MID task sessions consisted of 210 trials, yielding a total of 630 trials. During each trial, participants saw one of seven cue shapes (cue) for 250ms, fixate on a crosshair
waiting for the target (delay), and then responded to a solid white square (target) with a button press within 300ms (Figure 2).

Figure 2: A schematic design of the MID task trial
Feedback (feedback) showed up after target disappears, notifying participants whether they had won or lost money during that trial and their cumulative total score for about 1650ms. Time taken for the delay, between 1000 to 1300ms, was titrated for a dynamic adjustment of the task difficulty, based on the individual performance: If participants aced several trials, the difficulty of task increased by shortening the delay time so that participants did not have enough time to anticipate the target.

The different types of cue included gain (a circle), lose (a square), and neutral (a triangle), and a horizontal line on the cue indicated the amount of either a penalty or reward. For example, a circle with three horizontal lines indicated a large gain, a circle with two horizontal lines indicated a medium gain, and a circle with one horizontal line indicated a small gain; on the other hand, a square with one horizontal line indicated a small loss, a square with two horizontal lines indicated a medium loss, and a square with three horizontal lines indicated a large loss. Triangle indicated neither gain nor loss.

Participants could win or prevent losing money by pressing the button during target presentation. As described above, cues consisted of squares, circles, and triangles. Squares indicated loss trials and circles indicated gain trials. Within each square or circle horizontal lines were presented. Each line of cue indicated an amount of monetary value: +20 (a circle with three horizontal lines), +10 (a circle with two horizontal lines), +5 (a circle with one horizontal line), -5 (a square with one horizontal line), -10 (a square with two horizontal lines), and -20(a square with three horizontal lines). Triangle cues implied no monetary outcome. On the first reward seeking block (A) and the second reward seeking block (A’) sessions, only one feedback appeared for their own wins or loses whereas on the competition block (B) session, two feedback appeared for their own and competitors’ wins or loses. Feedback about monetary incentives is
advantageous because testosterone is expected to respond to the reward-seeking property of the MID task. For the current project, all participants competed with themselves during the first and second reward seeking blocks (A & A’) and with their competitors during the competition block (B), and eventually won the competition.

Measures

Figure 3: Sensor layout for the Electrical Geodesics Inc. (EGI) 128-channel hydrocel sensor net
EEG/ERP measures: Noninvasive measure of neuronal activity assessed the temporal change of brain activation during MID on the experiment day. EEG was recorded using a 128-channel Geodesic Sensor Net, using an EGI amplifier and software, including Net Station™ (Electrical Geodesic, Inc.). Data acquisition was started once all impedance values for all channels were below 50 kΩ. Data were filtered using a FIR bandpass filter with a lowpass frequency of 30 Hz and a highpass frequency of .3 Hz. To best capture eye blinks, eye blink artifact thresholds were set to 140 µV. Furthermore, signal activation changes across the entire trial of 90 µV were marked as bad and removed after visual inspection. All ERP were baseline corrected to 200ms after the stimulus of interest, the feedback. At data acquisition, all channels were referenced to CZ and then later referenced to the average reference. After the data acquisition, FRN amplitudes were averaged, which were originated from the mediofrontal region (electrode 15 and 16), the left anterior frontal region (electrode 18 and 19), and the right anterior frontal region (electrode 4 and 10) of brain (Figure 1).

Saliva collection: Saliva samples were collected in individual tubes. Participants were asked if they needed water immediately after they provided informed consent. Experimenters waited at least five minutes after water consumption before collecting saliva. All saliva collection times were capped at ten minutes to minimize participant burden and maximize quality of samples. As described above, on an experiment day, participants were asked to provide five saliva samples. The first sample (T1) was collected when participants visited, and the second sample (T2) was collected right before playing the first reward seeking block (A). After the completion of the first reward seeking block (A), the third sample (T3) was collected. The fourth sample (T4) was collected after the completion of both the competition block (B) and the second reward seeking block (A’). The last saliva collection (T5) was conducted right before
the compensation given to participant.

Basal Box. After the completion of tasks on the test day, each participant was given the basal box, including an ice pack, a daily diary, and two empty vials. Instructions for the basal box were provided to participants so they could collect their own samples on the basal day. Collection times corresponded with the first and last saliva collections on the experiment day in order to measure pre-task anticipation and individual differences in recovery, respectively. The basal box was returned to the Stress Physiology in Teens (SPIT) lab on ice and then frozen immediately.

**Daily Diary:** At the time of each saliva collection, a daily diary was processed which examines about exercise, eating and sleep habits, and mood/emotion at the time of each sample.

**Testosterone Assay.** Saliva assaying was conducted in WET lab at the University of New Orleans using a commercially available enzyme immunoassay from Salimetrics. After the sample collection, all samples had been stored in the ultra-freezer below -80 °C until an assay was conducted. On the assay day, samples and enzyme immunoassay kits were set outside of the freezer to come to room temperature and thawed before one and half hours prior to the assaying. Additional thawing was conducted if needed. Then, researchers mixed all samples by using the vortex mixer so that our samples could have their own uniform viscosity in each tube. Samples were centrifuged at 1500 rpm for 10 min prior to assay so that only the clear top phase was pipetted. Research technicians pipetted adequate amount of saliva, an assay diluent, and an assay conjugate into wells on the micotitre plate. With the competitive binding, the labelled analytes bound to an antibody, and then the rest of analytes which did not bind to an antibody were manifested with color. The color change (i.e., optical density) was measured by using the
EPOCH plate reader. After the data exported by using Gen 5™, it was saved and stored with an Excel file format and then later exported to SPSS for statistical analysis. Intra-assay coefficient of variance (CV) was below 7% and inter-assay CV was below 15%. Testosterone was logarithmic transformed (“LnTesto”) to normalize the data. We defined responders who revealed more than 15% of testosterone level increase from the baseline (T1) to either the reward seeking task only (T3) or the reward seeking task after competition (T4) since less than 15% testosterone level increase could be due to chance variation given the assay’s level of precision and variation (Granger et al., 2004).

Statistical Analysis

Our first hypothesis was that testosterone level would be reactive to the MID task given the components of reward and competition in the task. To see a testosterone level change in individuals during MID task, a repeated measures ANOVA was conducted with time as the main predictor and a testosterone level as the outcome. Gender was examined as a potential covariate. We then distinguished responders from non-responders and conducted an analysis to see if responders would show testosterone reactivity whereas non-responders would not do so. A follow-up repeated ANOVA with time as the main predictor and testosterone level as the outcome was conducted. A chi-square test of independence was conducted to see if there were more responders than would be expected by chance. Although we focused on reactivity during the task, it was possible that the entire laboratory day represented a social context of reward and novelty and competition. To test this, we compared the experiment to basal day. A 2 (basal vs. experiment day) x 2 (time of day; arrival time and departure time) repeated measures factorials ANOVA were conducted. The saliva samples at the baseline and the recovery from the task periods on the basal day were taken by participants with the exact same time when experimenters
collected saliva samples of participant at the baseline and the recovery from the task periods on the task day.

Next, we hypothesized that FRN amplitude would change across incentive values (i.e. cue size) and motivational blocks. To test a FRN amplitude change and its relation to cues and blocks in individuals during MID task, a 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: male and female) repeated measures ANOVA was conducted with gender as the between subject factor and the amplitude of FRN waves as the outcomes. Gender was added in the model since the previous study assisted that gender could modulate the strength of FRN amplitude (Jin et al., 2013). We decomposed variables and conducted a series of repeated measures ANOVA analyses with gender and blocks for each block and a pair of repeated ANOVA analyses with cues and blocks for each gender. Tukey HSD post-hoc tests were conducted as follow-up analysis.

We also expected that individual differences in testosterone levels would be positively related to the strength of FRN amplitude. A 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: male and female) repeated measures ANCOVA was conducted with gender, cues, and blocks as the main predictors; the power of FRN waves as the outcomes; and, the standardized testosterone levels were entered as covariates. Gender was entered as the between group variable to see its effect on FRN amplitude. Moreover, we aggregated log linear testosterone levels to calculate the averaged individual testosterone level. Since testosterone levels differ between genders, we calculated a gender specific standardized score from the gender specific average testosterone level, also called z-score, so that we could acknowledge the association without a gender effect. We also examined whether a testosterone by gender interaction was necessary. Post hoc probing of the interaction among variable was proceeded as
Finally, we expected that the responders would show the stronger FRN amplitude during the competition block, relative to the non-responders. To test the hypothesis, a 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: males and females) x 2 (Testosterone reactivity: responders and non-responders) repeated measures ANOVA was conducted with gender, cues, blocks, and a response variable as the main predictors; and, the power of FRN waves as the outcomes. Gender and the testosterone reactivity were entered as the between group variables. Again, gender was entered to see its modulation effect on FRN amplitude. Tukey HSD post-hoc tests were conducted as follow-up analyses.

**Results**

*Testosterone level changes across time*

First of all, we hypothesized that testosterone level would increase during the MID task and then decrease (or recover) after the task. To examine whether testosterone level changes across time throughout the experiment, a repeated measures ANOVA was conducted (N=48) with gender as a covariate. Testosterone measurements in five different time points were entered as dependent variables; gender was entered as a between group variable. The assumption of sphericity was violated \( X^2(9) = 29.81, p < .001 \), so the degree of freedom was corrected by using Greenhouse-Geisser. Testosterone levels changed significantly across time during the experiment \[ F (2.872, 132.127) = 7.23, p < .05 \]. A Tukey HSD post-hoc tests confirmed that testosterone levels significantly higher at the reward-seeking task only (T3), relative to the reward-seeking task after competition (T4) \[ MD = .09, SE = .04, p < .05 \], and the recovery from the task (T5) measurement of testosterone level was significantly lower than the baseline testosterone level (T1) \[ MD = .15, SE = .05, p < .05 \]. Moreover, testosterone levels had a peak at
The reward seeking task only period \([M = 4.43, SD = .58]\). These findings did not simplistically match with our hypothesis that testosterone level would increase during the MID task, and then decrease (or recover) after the task given that the magnitude of decline after the task was larger than the reactivity rise. Nonetheless, the findings do show the highest values were achieved at T3 as hypothesized.

**Figure 4: Testosterone responsivity of responders**

<table>
<thead>
<tr>
<th></th>
<th>All participants (N=48, \text{M/SE})</th>
<th>Males (N=19, \text{M/SE})</th>
<th>Females (N=29, \text{M/SE})</th>
<th>Responders (N=20, \text{M/SE})</th>
<th>Non-responders (N=28, \text{M/SE})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (T1)</td>
<td>4.40/ .58</td>
<td>4.95/ .30</td>
<td>4.04/ .40</td>
<td>4.33/ .63</td>
<td>4.44/ .54</td>
</tr>
<tr>
<td>Anticipation for the task (T2)</td>
<td>4.40/ .61</td>
<td>5.01/ .34</td>
<td>4.00/ .38</td>
<td>4.47/ .70</td>
<td>4.35/ .55</td>
</tr>
<tr>
<td>Reward-seeking task only (T3)</td>
<td>4.43/ .58</td>
<td>4.99/ .34</td>
<td>4.07/ .39</td>
<td>4.59/ .65</td>
<td>4.32/ .51</td>
</tr>
</tbody>
</table>
Table 1: Demographic testosterone responsivity of participants

<table>
<thead>
<tr>
<th>Reward-seeking task after competition (T4)</th>
<th>4.35/.65</th>
<th>4.97/.30</th>
<th>3.94/.47</th>
<th>4.46/.73</th>
<th>4.26/.59</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recovery from the task (T5)</td>
<td>4.25/.63</td>
<td>4.83/.34</td>
<td>3.86/.46</td>
<td>4.36/.69</td>
<td>4.17/.59</td>
</tr>
</tbody>
</table>

Testosterone levels were significantly different between genders \( F (1, 46) = 86.48, p < .001 \), showing males had higher overall testosterone levels than females. Gender did not show an interaction with testosterone change across times \( F (2.872, 132.127) = 1.01, p > .1 \), suggesting that males and females did not show different testosterone change across time during the MID task.

Reactivity tasks are known to work only on a subset of participants (Dedovic et al., 2009). Individuals can be classified as responders and non-responders. Responders were defined as participants having a 15% or more testosterone level increase by the reward seeking task only time point or due to the reward seeking task after competition, relative to the baseline testosterone level at the task day. Responders were defined as either time point given the fact that individual differences in peak responder times are well known with hormones and visual inspection of each individual’s reactivity profile confirmed that some participants achieved peak testosterone levels at the reward seeking task only (i.e., T3) whereas others peaked during the reward seeking task after competition (i.e., T4) on the task day. There were twenty responders observed out of forty eight participants (42%). Mostly, responders showed non-exclusive testosterone reactivity: Eleven of the responders achieved peak testosterone levels at T3, indicating responsivity due to the reward seeking, whereas nine of them peaked during T4 on the task day, implying responsivity due to the competition. Repeated measures ANOVA were
conducted (N=48). Testosterone measurements in five different time points were entered as dependent variables, and a response variable, showing whether participants responded to the task significantly or not, was entered as a between individual variable. The result showed that testosterone levels changed considerably across the session \([F (2.617, 120.403) = 8.74, p < .001]\), and the testosterone change was significantly different depending on whether the participant was classified as a responder or non-responder \([F (2.617, 120.403) = 9.66, p < .001]\). A Tukey HSD post-hoc tests then examined testosterone change across times based on responder group (Figure 4). For responders, testosterone levels significantly increased from the baseline (T1) to the anticipation (T2) \((MD = .13, SE = .04, p < .01)\), the baseline to the reward seeking task only (T3) \((MD = .26, SE=.03, p < .001)\), and the anticipation to the reward seeking task only \((MD = .12, SE = .05, p < .05)\) and decreased from the reward seeking task only to the recovery (T5) \((MD = -.23, SE=.07, p < .01)\). The decrease from the reward seeking task only (T3) to the reward seeking task after competition (T4) was not significant, suggestive of prolonged response to the task from T3 to T4. For non-responders, however, testosterone levels at the anticipation \((MD = -.10, SE=.03, p < .01)\), the reward-seeking task only \((MD = -.13, SE=.03, p < .001)\), the reward seeking task after competition \((MD = -.18, SE=.04, p < .001)\), and the recovery \((MD = -.28, SE=.06, p < .001)\) were significantly lower than the baseline. These findings indicated that responders were reactive to the MID task, especially during the reward-seeking and the after competition time points whereas non-responders were not.

Given the role of testosterone as a sex steroid, we were concerned that responders were only one gender, males. To examine this, we conducted the crosstab analysis of gender by responder (N=48). There were twenty responders, including nine males and eleven females, and
twenty eight non-responders, including ten males and eighteen females \(\chi^2(1) = .42, p > .1\), showing that responders were not biased toward one gender.

The analyses above concerned testosterone change across the task, but it was possible that testosterone was unique on that day compared to a non-challenge day. The non-challenge day included two saliva samples corresponding to the baseline testosterone level (T1) and the recovery from the task (T5) on the challenge day. It was also possible that testosterone reactivity led to the hyper testosterone levels on the experiment day, relative to the basal day. To clarify whether testosterone levels on the task day were abnormally different from them on the non-challenge day and whether responders would have distinct testosterone levels on the experiment day versus the basal day, but non-responders would not, we conducted a 2 (basal vs. experiment day) x 2 (time of day; arrival time and departure time) repeated measures ANOVA. Saliva collection times were entered as independent variables: On the task day, the baseline testosterone level was entered as pre and the recovery from the task was entered as post whereas the baseline testosterone level was entered as pre and the anticipation to the task (T2) was entered as post on the basal day. Gender and testosterone reactivity were entered as between subject factors.

The result showed that day had a significant main effect on testosterone \(F(1, 38) = 6.63, p < .05\), but failed to show its interaction with testosterone reactivity \(F(1, 38) = .15, p > .1\), gender \(F(1, 38) = .01, p > .1\), and both of them \(F(1, 38) = .04, p > .1\). Pairwise comparisons revealed that testosterone to be somewhat higher on the task day than the basal day (M = 4.41, SD = .06 for task day and M = 4.27, SD = .07 for basal day; MD = .14, SE = .05, p < .05). As a follow-up test, Paired samples T test (N=42) was conducted. The result of test showed that pre testosterone level on the experiment day (M = 4.39, SD = .57) was significantly higher than that on the basal day (M = 4.39, SD = .57; t(41) = 2.77, p < .01) whereas the post testosterone level
on the experiment day ($M = 4.24$, $SD = .58$) was higher than that on the basal day in a trend level ($M = 4.12$, $SD = .61$; $t(41) = 1.98$, $p = .054$), indicating that testosterone levels on the experiment day were somewhat elevated, relative to testosterone levels on the basal day. Nonetheless, pre testosterone levels on the basal and experiment day were significantly correlated with each other ($r = .76$, $p < .001$), and post testosterone levels on the basal and experiment day were significantly correlated as well ($r = .80$, $p < .001$), suggestive of stability in testosterone levels across days. Because we found that no interaction between day and testosterone reactivity, day was not a reason leading to the hyper testosterone levels on the experiment day, compared to the basal day.

In sum, we found that a subset of participants, termed responders, supported the hypothesis that testosterone would be reactive to the MID task. Testosterone reactivity of responders was observed from both males and females. Moreover, testosterone levels on the experiment day were somewhat elevated, relative to that on the basal day, and this elevation in testosterone level on the experiment day was not driven by responders.

*The strength of FRN amplitude across blocks and cues*

We hypothesized that FRN amplitude would change across time in the modulation of the potential incentive amount (i.e., cue) and the type of blocks. To test the hypothesis, a 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: male and female) repeated measures ANOVA was conducted with gender as the between subject factor and FRN amplitudes as the dependent measure. The repeated-measures factors were cues and blocks. Gender was added in the model to see whether it interacted with the strength of FRN amplitude. Note that FRN is the negative amplitude, located from 200 to 300ms from the cue (Cooper et al.,
2014), and in a FRN analysis if participant shows more negative FRN scores during the task, he or she reveals greater FRN amplitude than other participants.

The result of study revealed that the sphericity was assumed for cues \(X^2(2) = .22, p > .1\), blocks \(X^2(2) = 2.42, p > .1\), and the interaction of them \(X^2(9) = 7.71, p > .1\), so we did not use the Greenhouse-Geisser scores. The cue variable showed a main effect on the FRN amplitude in a trend level \(F (2, 78) = 2.69, p = .074\). The pairwise comparison analysis revealed that the FRN amplitude due to the small cue (\(M = -4.61, SD = .58\)) was significantly greater (more negative) than that due to the large cue (\(M = -4.03, SD = .46; MD = .58, p < .05\)). The FRN amplitude due to the medium cue (\(M = -4.27, SD = .49\)) was slightly greater (more negative) than the FRN amplitude due to the large cue with the non-significant difference (MD = -.24, SE = .25, p > .1). The FRN amplitude due to the small cue was slightly greater (more negative) than the FRN amplitude due to the medium cue, but the difference was not significant (MD = -.34, SE = .26, p > .1) (Figure 5).

Figure 5: FRN amplitude during the MID task

<table>
<thead>
<tr>
<th>Small Cue</th>
<th>Medium Cue</th>
<th>Large Cue</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRN amplitude (μV)</td>
<td>FRN amplitude (μV)</td>
<td>FRN amplitude (μV)</td>
</tr>
<tr>
<td>3.00</td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>-4.10</td>
<td>-4.10</td>
<td>-4.10</td>
</tr>
<tr>
<td>-3.80</td>
<td>-3.80</td>
<td>-3.80</td>
</tr>
<tr>
<td>-4.20</td>
<td>-4.20</td>
<td>-4.20</td>
</tr>
<tr>
<td>-4.60</td>
<td>-4.60</td>
<td>-4.60</td>
</tr>
<tr>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
</tbody>
</table>

\(p < .05\)
We failed to find a direct main effect of blocks \([F (2, 78) = 2.37, p > .1]\) and its interactions with cues \([F (4, 156) = 1.50, p > .1]\) on the FRN amplitude. Gender also did not show an interaction with blocks \([F (2, 78) = .31, p > .1]\) or cues \([F (2, 78) = .28, p > .1]\) on the FRN amplitude. However, there was a trend for a 3-way interaction among cues, blocks, and gender \([F (4, 156) = 2.23, p = .068]\) on the FRN amplitude.

To decompose the trend for a Gender x Cue x Block effect on the FRN amplitude, we conducted a pair of 3(Cues: large, medium, and small cues) x 3(Blocks: A, B, and A’) repeated measures ANOVA, separately for each gender. Within females, there was not a significant main effect of cues \([F (2, 46) = 1.48, p > .1]\), blocks \([F (2, 46) = 1.34, p > .1]\), or the interaction of cue by block \([F (4, 92) = .80, p > .1]\) on the FRN amplitude. Conversely, within males, there was not a main effect of cues \([F (2, 32) = 1.34, p > .1]\) nor blocks \([F (2, 32) = 1.29, p > .1]\) on the FRN amplitude, but males showed the interaction between cues and blocks at a trend level \([F (4, 64) = 2.21, p = .078]\).

To further understand the cue by block trend within males, a series of repeated measures ANOVA were conducted separately for each block in males (N=17), where the cue variable was the repeated measures factor and FRN amplitude was the dependent measure. The result showed that cues failed to show the main effect on FRN amplitude at the first reward seeking block (A) \([F (2, 32) = .59, p > .1]\) and the competition block (B) \([F (2, 32) = .48, p > .1]\). Interestingly, at the second reward seeking block (A’), the cue variable had a main effect on the FRN amplitude in males \([F (2, 32) = 5.38, p < .05]\). Pairwise comparison analyses revealed that a FRN amplitude of males due to the large cue \((M = -2.91, SE = .78)\) was significantly smaller (less negative), relative to that the medium \((M = -4.81, SE = .88; MD = 1.90, SE = .64, p < .01)\) and small \((M = -4.61, SE = 1.02; MD = 1.70, SE = .58, p < .05)\) cues in the second reward seeking block (A’).
FRN amplitude due to the medium cue was slightly greater (more negative) than that due to the small cue (MD = -.20, SE = .69, p > .1), but the difference was not significant (Figure 6).

** A: The first reward seeking block; B: The competition block; A’: The second reward seeking block; Large: Large Cue; Medium: Medium Cue; and, Small: Small Cue

** Figure 6: Gender effect on the FRN amplitude during the second reward seeking block (A’) in the MID task

In sum, we expected that FRN amplitude would change across time in the modulation of a cue size and a block type. Even though we failed to find a main effect of block on FRN amplitude, we found cue had a main effect on FRN amplitude: The small cue led to the greater (more negative) FRN amplitude, relative to the large cue. The pattern was only valid in males at the second reward seeking block (A’).
Individual Testosterone levels and the strength of FRN amplitude

We then tested the hypothesis that individual difference in testosterone levels would be positively related to the strength of FRN amplitude. A 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: males and females) repeated measures ANOVA was conducted (N=41) with between subject factors being gender and standardized testosterone levels (i.e., Z-scores of individual testosterone level), and the power of FRN waves as the outcome. The repeated measures factors were cues and blocks. We built a model where interactions of gender or testosterone were examined with the within subjects factors (i.e., cue or block) to see if they exerted a modulation effect on FRN amplitude. For simplicity, only effects with the testosterone level are described in order to reduce redundancy with prior description of gender, cue size, and block effects. The result showed that the standardized individual testosterone level score failed to show its effect in its interactions with cues [F (2, 74) = .24, p > .1], blocks [F (2, 74) = .13, p > 1], and the interaction of cues and blocks [F (4, 148) = .22, p > .1]. The result failed to support our hypothesis by showing that individual testosterone level was not related to FRN amplitude in relations with a cue size, a block type, and an interaction of them.

We also ran a model in which we explored whether a testosterone by gender interaction was necessary. The result showed that the interaction between gender and standardized individual testosterone level score failed to show its effect in its interactions with cues [F (2, 74) = .76, p > .1], blocks [F (2, 74) = .16, p > 1], and the interaction of cues and blocks [F (4, 148) = 1.75, p > .1]. Due to the insignificancies, the further analysis was not examined.
Testosterone reactivity and the strength of FRN amplitude

We were then interested in testing the hypothesis that the FRN amplitude would be related to testosterone reactivity during the task. This was examined with a 3 (Cues: large, medium, and small cues) x 3 (Blocks: A, B, and A’) x 2 (Gender: males and females) x 2 (Testosterone reactivity: responders and non-responders) repeated measures ANOVA where the dependent variable was FRN amplitude strength and the repeated measures factors were cues and blocks, and between groups factors included gender and testosterone reactivity group. Again, gender was added to see its modulation effect on FRN amplitude. The sample size was N=41 given the need for data on both testosterone reactivity and ERP data. For simplicity, only effects with testosterone reactivity are described in order to reduce redundancy with prior description of gender, cue size, and block effects.

The results revealed that testosterone reactivity failed to show its effect in its interactions with cues [F (2, 74) = .80, p > .1], blocks [F (2, 74) = .39, p > .1], and the interaction of cues and blocks [F (4, 148) = 1.62, p > .1]. Also, testosterone reactivity did not show three-way interactions with gender by cues [F (2, 74) = .99, p > .1], or gender by blocks [F (2, 74) = 2.10, p > .1]. Finally, there was not a four-way interaction of testosterone reactivity by gender by cues by blocks [F (4, 148) = 1.51, p > .1]. These result failed to indicate that testosterone reactivity related to the FRN amplitude.

Discussion

To start, we hypothesized that testosterone level would increase during the MID task, and then decrease (or recover) after the task. Our finding did not simplistically support our hypothesis. While we did find that testosterone was highest at T3 and declined (or recovered)
thereafter, testosterone reactivity was not observed within all participants. Instead, testosterone reactivity was observed within a subset of 42% of the respondents who showed greater than 15% increase in testosterone during the task. The non-responders, who evinced less than 15% increase of testosterone levels due to the task, actually showed consistent declines in testosterone during the task. This method of categorizing participants as responders and non-responders has been utilized in previous work (Dedovic et al., 2009). The present study also found categorizing had utility as it allowed us to ask specific research questions about individuals who responded to the task. This distinction may be important because if we included non-sensitive responders to the task without caution, as we would have risked losing statistical power and validity of the study. Moreover, this distinction of responders vs non-responders is realistic given that it does not assume that all participants would react to the task since testosterone responsivity is affected by tons of different reasons. Twenty responders showed the significant testosterone level increase at the reward seeking task only (T3) and the reward-seeking task after competition, relative the testosterone level to the baseline. From these findings, we can say that our hypothesis aligned with testosterone trajectory within responders. Specifically, responders demonstrated a peak testosterone level due to the reward-seeking task only (T3), which suggests the reward-seeking property of MID task may be useful to eliciting testosterone responsivity. Furthermore, although some individuals showed a peak testosterone level after competition, there was not a further testosterone increase by the reward-seeking task after competition (T4). This suggests that additional testosterone release does not require competition beyond the reactivity introduced by reward only. Prior literature on competition tasks indicate testosterone reactivity (Carre, Campbell, Lozoya, Goetz, & Welker, 2013; Chichinadze et al., 2012; Higley et al., 1996; Marti-Carbonell et al., 1992; Zilioli & Watson, 2013), but the present study suggests that if competition
and reward are disentangled, it is the reward component which testosterone responds and the further introduction of competition and winning may be unnecessary to stimulate the release of this hormone. This idea fits with our observation that testosterone was somewhat more elevated on the task day, relative to that on the basal day, suggesting that testosterone elevations experienced in general in response to receiving rewards may occur compared to other normal days. At a broad level, our findings fit with previous literature maintaining that testosterone is only reactive to winning if the win/game was rewarding to the individual (Carre, Gilchrist, Morrissey, & McCormick, 2010; A. Mazur & Booth, 1998).

Revealing that testosterone is tied most closely to reward fits with the emerging biological literature that emphasizes testosterone as a social hormone which serves an adaptive purpose to achieve a reward. Research with Syrian hamsters supports the concept that testosterone might be bound as a social reward. For example, Bell and Sisk (Bell & Sisk, 2013) found that sexually unexperienced adult males formed a place-preference for where they received a reward (in this case reproduction), and cues about that reward (i.e., vaginal secretion of female hamsters). They found that gonadectomized adult and adolescent male hamsters needed testosterone in order to form the conditioned place preference. Not surprisingly given dopamine’s role with reward-seeking, they revealed that the reward-seeking behavior may have been dopaminergic given that haloperidol, the antagonist of the dopaminergic reaction, disrupted the formulation of that preference. Further, hints towards dopaminergic involvement in testosterone release are revealed in Zebra fish. Teles and colleagues (2013) found that Dopaminergic activity of Zebra fish was significantly higher in winners, relative to losers, during their interaction with a mirror image. The findings of the study implied that winners showed more dopaminergic reaction, which suggests evidence of social reward. Our study fits with this
research by showing that testosterone levels were reactive to a social reward, in this case monetary gain, within a subset of responders. More broadly, we recommend considering that testosterone could serve an adaptive purpose to help an individual achieve their goal by activating the reward system in humans.

As expected, we found significant testosterone level difference among genders. This finding is critical since it confirms that males secrete more testosterone than females (Coates & Herbert, 2008; Eisenegger, Haushofer, and Fehr, 2011). Without this validation, we cannot guarantee if our finding is salient and capable of being generalized to real life situations. That said, we did not find that acute testosterone change across time (i.e., testosterone reactivity) was different across genders suggesting that both males and females responded in a similar way to the reward and competition properties of the MID task.

Prior literature is mixed regarding testosterone reactivity. Some researchers found that only males showed significant testosterone increase (i.e., testosterone reactivity) in the competition such as the lifting competition (Le Panse et al., 2012) and the financial competition (Apicella et al., 2014). The testosterone reactivity was also observed in studies dealing with status related aggression (Carre et al., 2013) and a social cognition associated with facial expression (Ackermann et al., 2012) in males only. However, similar to the present study, some prior work found testosterone reactivity in female soccer players (Oliveira, Gouveia, & Oliveira, 2009), and a wide array of testosterone administration studies which find powerful acute testosterone changes within females (Bos et al., 2012; Bos, Terburg, & van Honk, 2010).

To explain further, there has been tentative suggesting of a gender difference in testosterone reactivity within responders such that males revealed greater reactivity across the
session whereas females showed significantly more specific peak in testosterone to reward. The finding implies that testosterone is responsive to reward for some populations, which may include both males and females, but testosterone may be more broadly responsive within males, by showing a testosterone level increase as early as the task anticipation (T2) and the level persisting as late as to the competition (T4). The broad testosterone reactivity might be due to an individual salience of males for a competition property of the MID task, meaning that males might be more concerned the competition part of task, relative to females, which led to the relatively prolonged testosterone reactivity in males. In the video game contest study, researchers found that males and females showed different testosterone reactivity after the video game competition with the same-sex competitors (Allan Mazur, Susman, & Edelbrock, 1997). Females showed a decreased testosterone after competition whereas males showed an increased testosterone in prior to competition. The discrepancy in testosterone reactivity between males and females is most likely due to the individual affinity to the video game competition. Because females cared less for the video game type competition, when compared to males, the decreased testosterone of females might be a result of the non-salient cue. Future research is needed to discover individual characteristics leading to the testosterone reactivity.

Second, we hypothesized that neuronal activation would be enhanced by the MID task, specifically that the size of cue and block would modulate FRN amplitude. We expected that the large cue would trigger greater (more negative) FRN amplitude than the medium and small cues, and greater FRN amplitude (more negative) was expected during the competition block (B), relative to other blocks. The result showed that blocks did not affect FRN amplitude. Rather, our participants showed the meaningful FRN amplitude difference across three cues in the MID task during the second reward seeking block (A’). The large incentive cue triggered smaller (less
negative) FRN amplitude than the medium and small incentive cues did. Our finding supported the previous research, showing smaller FRN amplitude (less negative) in response to the high potential incentive cue, relative to the FRN amplitude due to the low potential incentive cue (San Martin et al., 2010). Bellebaum and his colleagues (Bellebaum et al., 2010) found the similar pattern with the reward condition although they lacked statistical power. Taken together, a picture emerges in which the FRN amplitude appears smallest (less negative) in conditions of greatest reward (i.e., large cues and within the reward seeking block).

It remains unclear as to why the large potential incentive cue triggered smaller (less negative) FRN amplitude, relative to the other cues, specifically during the second reward seeking block (A’). There is a caveat that the pattern might be due to the learning effect insofar as repetition of the same task might trigger the enlarged FRN amplitude at the second reward seeking block (A’). Put another way, it is possible that the FRN needs time to show up, with enough rewards accumulating in order to heighten the FRN. The previous findings suggested that positive feedback elicited FRN when compared to negative feedback, which related to the long-term learning outcomes (Arbel et al., 2013). Since we showed the feedback score to participants, based on their performance, we are limited in our analysis to know how positive and negative feedbacks are associated with the FRN amplitude strength and whether they have a persistent effect through time. From our understanding, it is not yet known if the FRN amplitude is strengthened, weakened, or maintained after a repetition of the same task. Bismark and colleagues (Bismark, Hajcak, Whitworth, & Allen, 2013) examined FRN amplitude strength in relation to positive or negative feedback. They revealed that time for an expectation of outcome was required in order to achieve significant FRN amplitude. Without enough time for participants to construct their own expectation for the result of task, FRN was not observed. This
supports our suggestion for a learning effect in that FRN amplitude was augmented across time. Future FRN related research should allow participants to have enough time to be capable in expecting the reward of the task so that they may react to the feedback in a more meaningful way. Future research is needed as well to see if the mere repetition of several trials of the task was the reason for the enlarged FRN amplitude.

We also found gender by cue by block interaction that indicated that only males showed the significant effect of the potential incentive amount on FRN amplitude. A recent article studied the effect of gender on the sensitivity of FRN amplitude (Grose-Fifer, Migliaccio, & Zottoli, 2014). With the gambling task, researchers manipulated the degree of winning and found that males were sensitive to the size of winning, but females did not display the same magnitude of sensitivity. The gender difference in cognition throughout the reward seeking process would lead to the behavioral change in individuals. Even though a FRN amplitude was not dealt, a recent fMRI study supported the idea. With a cold pressor task, males showed the increased neural activation, especially dorsal striatum and anterior insula, relative to females (Lighthall et al., 2012). In the behavior observation part of the study revealed that males with the cold pressor task made decision faster and collected more reward than females did in the monetary reward task. In a control condition, where the cold pressor task was not conducted, the electrophysiological and behavioral gender differences were not observed. From these findings, the researchers maintained that stress was the key factor leading to the gender difference in the neural signal. These two studies confirmed that gender can lead to the cognitive difference in individuals and even behavioral changes in the reward-seeking. Moreover, the changes that participants make throughout the reward-seeking task can be modulated by stress. From this
point of view, future research dealing with the reward-seeking property of individuals should concern an effect of gender and stress, especially in a financial competition like the MID task.

Lastly, we hypothesized that high testosterone individuals and testosterone responders would show greater (more negative) FRN during the competition block (B), relative to other blocks. Our finding showed that FRN amplitude had no interaction with both the standardized individual testosterone score and the testosterone reactivity. We based our hypothesis on the premise that testosterone level and its reactivity would make the task more evident, especially during the competition. This explanation dovetails nicely with the testosterone literature on the competition effect although it is specific to the FRN. Gonzalez-Bono and his colleagues (Gonzalez-Bono, Salvador, Serrano, & Ricarte, 1999) maintained that the testosterone level change might not be directed related to the outcome of competition, but rather to the salience of competition for individuals, making an individual more into the competition.

Recent neuro electrophysiology papers maintained that FRN amplitude originated from not only the reward seeking but also saliency of the task (Hauser et al., 2014; Talmi et al., 2013). There are several conceptual reasons for the null results. First, these null results might be due to the failure of bringing up saliency of the task. Our lab-induced competition did not involve participants facing their competitors during the competition block (B) which may have reduced salience of the competition. It would be interesting if we can determine whether the competition in person makes any difference in FRN amplitude and testosterone, relative to the competition through internet. Second, null results could be due to the latency of the FRN in relation to the timing of testosterone reactivity. That is, the FRN appeared to be delayed, observed most readily during the last block. Similarly, peak testosterone levels were also observed late and it may have required more time for top-down neural activation from the FRN to be manifested in peripherally
released testosterone levels. Given the novelty of the present study, it was not possible to know the time course of both FRN and testosterone changes to best synchronize these measures. Finally, our study design focused on FRN amplitude and simultaneous testosterone reactivity, as they are both related to reward (Cohen, Elger, & Ranganath, 2007; Eisenegger et al., 2011); however, their link to reward-seeking may be indirect and mediated through other reward-related neurocircuitry which our ERP measures did not capture. Future research is needed to clarify whether the relation of FRN amplitude strength and testosterone.

**Limitations**

This study has some limitations in understanding a role of FRN amplitude in the reward seeking task. We forwarded a caveat that the FRN amplitude strength difference between the first and second reward seeking blocks would be due to the learning. In that sense, we cannot explain the FRN amplitude strength at the first reward seeking block (A) when the learning never happened yet. Further, our study cannot answer for the individual difference in the amount of FRN reactivity (Cooper et al., 2014; Smillie, Cooper, & Pickering, 2011) since we conducted analysis with the averaged FRN amplitude in the frontal region of brain. Some previous FRN related researcher mentioned the FRN amplitude as an error related negativity (Huang & Yu, 2014; Talmi et al., 2013; van der Helden, Boksem, & Blom, 2010). We cannot see if the FRN is originated from the error made by participants or not because we only exported the feedback related, not the cue related amplitude of participants. We plan to answer these questions in the future analysis.

In regards to the study design, it is questionable why we did not collect the saliva sample right after the competition but after the second reward seeking block (A’). The design might be
incapable to capture the temporal change of testosterone level right after the competition. We designed the study to not only determine whether FRN amplitude of the competition block (B) differs from that of other blocks but also whether FRN amplitude differ between the exactly same reward seeking task before and after the competition. However, we cannot make a clear distinction if testosterone level at T4, the reward seeking task after competition, secreted due to the reward, the competition, or both of them. Future research is needed to resolve limitations.

**Conclusion**

The current study has provided evidence that bio and neurophysiology factors are induced due to the reward seeking in the MID task. We found an individual difference in testosterone reactivity (responders and non-responders). We also found that a size of cue, time required for learning, and gender contribute to FRN amplitude. Based on these findings, neuroscience-related future researchers should consider a potential monetary incentive, which directly affects the neural amplitude, especially in the reward seeking task.

*Figure 7: A theoretical construct of testosterone response and neural activation to the social competition*
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