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Short-term testosterone manipulations do not affect cognition or motor function but differentially modulate emotions in young and older male rhesus monkeys

Brian Kelly^{1,2,3}, Vanessa Maguire-Herring², Christian M. Rose¹, Heather E. Gore¹, Stephen Ferrigno¹, Melinda A. Novak^{1,2}, and Agnès Lacreuse^{1,*}

¹Department of Psychology, University of Massachusetts, Amherst, MA 01003

²Harvard Medical School, New England Primate Research Center, Southborough, MA 01772

³Behavioral Sciences, Fitchburg State University, Fitchburg MA 01420

Abstract

Human aging is characterized by declines in cognition and fine motor function as well as improved emotional regulation. In men, declining levels of testosterone (T) with age have been implicated in the development of these age-related changes. However, studies examining the effects of T replacement on cognition, emotion and fine motor function in older men have not provided consistent results. Rhesus monkeys (*Macaca mulatta*) are excellent models for human cognitive aging and may provide novel insights on this issue. We tested 10 aged intact male rhesus monkeys (mean age = 19, range 15–25) on a battery of cognitive, motor and emotional tasks at baseline and under low or high T experimental conditions. Their performance was compared to that of 6 young males previously tested in the same paradigm (Lacreuse et al., 2009; Lacreuse et al., 2010). Following a 4-week baseline testing period, monkeys were treated with a gonadotropin releasing hormone agonist (Depot Lupron, 200 µg/kg) to suppress endogenous T and were tested on the task battery under a 4-week high T condition (injection of Lupron + T enanthate, 20 mg/kg, n = 8) or 4-week low T condition (injection of Lupron + oil vehicle, n = 8) before crossing over to the opposite treatment. The cognitive tasks consisted of the Delayed Non-Matching-to-Sample (DNMS), the Delayed Response (DR), and the Delayed Recognition Span Test (spatial-DRST). The emotional tasks included an object Approach-Avoidance task and a task in which monkeys were played videos of unfamiliar conspecifics in different emotional context (Social Playbacks). The fine motor task was the Lifesaver task that required monkeys to remove a Lifesaver candy from rods of different complexity.

T manipulations did not significantly affect visual recognition memory, working memory, reference memory or fine motor function at any age. In the Approach-Avoidance task, older

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*Correspondence to: Agnès Lacreuse, Department of Psychology, 135 Hicks Way, University of Massachusetts, Amherst, MA 01003, Phone: 413-545-2183, Fax: 413-545-0996, alacreuse@psych.umass.edu.

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monkeys, but not younger monkeys, spent more time in proximity of novel objects in the high T condition relative to the low T condition. In both age groups, high T increased watching time of threatening social stimuli in the Social Playbacks. These results suggest that T affects some aspects of emotional processing but has no effect on fine motor function or cognition in young or older male macaques. It is possible that the duration of T treatment was not long enough to affect cognition or fine motor function or that T levels were too high to improve these outcomes. An alternative explanation for the discrepancies of our findings with some of the cognitive and emotional effects of T reported in rodents and humans may be the use of a chemical castration, which reduced circulating gonadotropins in addition to T. Further studies will investigate whether the luteinizing hormone LH mediates the effects of T on brain function in male primates.

Keywords

Aging; androgens; leuprolide; macaque; memory; primate

INTRODUCTION

Human cognitive aging is characterized by declines in working memory, executive function, long-term memory and speed of processing (Park and Schwartz, 2000). These declines are substantial, develop as early as the 30s and affect everyone (Salthouse, 2010). Deficits in fine motor function also develop with age (Seidler et al., 2010; Smith et al., 1999), and can lead to serious impairments in quality of life and activities of daily living (Desrosiers et al., 1999). In contrast to the age-related declines in cognitive and motor function, emotional regulation has consistently been shown to significantly improve with age in humans (Carstensen et al., 2003; Carstensen et al., 2011).

With the aging population growing at an unprecedented rate worldwide (Kinsella and Wan, 2009), it is imperative to design interventions that would lessen age-related declines in cognitive and motor function. Because both estrogens and androgens affect cognition and motor function (Hampson, 2002) and decline with age in men (Harman et al., 2001) and women (Burger, 1996), hormone replacement therapy (HRT) has long been considered as such an intervention. Most of the work on this issue has focused on women's health and has provided inconsistent and still highly debated results regarding the potential benefits of estrogen therapy on cognitive aging (Maki and Henderson, 2012; Sherwin and Henry, 2008). Fewer data are available regarding the effects of T on cognition in older men, but they also show mixed results. Whereas positive effects of T have been reported for spatial and verbal memory (Cherrier et al., 2001), spatial ability (Janowsky et al., 1994) and working memory (Janowsky et al., 2000), other studies found detrimental effects of T on verbal memory (Maki et al., 2007) or no effect of T on cognition (Emmelot-Vonk et al., 2008; Young et al., 2010). With regards to motor function, very limited data are available, with one recent report finding no effect of T on sequential movement in young or older adults (Siegel et al., 2008). Finally, growing evidence indicates that T increases amygdala reactivity (van Wingen et al., 2011) and has a robust influence on socioemotional processing, at least in young adults (Bos et al., 2012; Eisenegger et al., 2011). Surprisingly little is known about the effects of T on

emotions in older adults, although antidepressant effects have been noted (Zarrouf et al., 2009).

Well-controlled investigations in appropriate animal models are clearly needed to shed a new light on the association between T and brain functioning in males. Studies in male rats have shown that T affects brain regions important for cognitive function: T increases the number of dendritic spines in the CA1 field of the hippocampus (Leranth et al., 2003) as well as the prefrontal cortex (Hajszan et al., 2007). In addition, T has a myriad of neurotrophic and neuroprotective effects, which have been implicated in a decreased risk of developing Alzheimer's disease (Pike et al., 2008; Pike et al., 2006).

Cognitive studies in male rats have generally found that T administration was able to restore memory impairments induced by gonadectomy in a wide range of working memory tasks such as the Morris water maze (Sandstrom et al., 2006; Spritzer et al., 2011), radial arm maze (Gibbs and Johnson, 2008; Spritzer et al., 2008), Y maze (Hawley et al., 2013) or delayed matching-to-position task (Gibbs, 2005). The effects of T are complex, however, and appear dose-, duration- and task-dependent (Spritzer et al., 2011). For example, Spritzer et al (2011) indicated that T differentially affected working memory and reference memory depending on the test used, the dose used and the timing of T administration. There is a paucity of data on the effects of T on cognition in aged male rodents. In one study, T administered to supraphysiological levels increased working memory in aged male rats (Bimonte-Nelson et al., 2003). An earlier study, however, failed to find any beneficial effects of T on spatial learning in aged male rats tested in the Morris water maze (Goudsmit et al., 1990). It has generally been found that the cognitive effects of T in male rodents are not mediated by sensorimotor effects (Kritzer et al., 2001).

Besides its cognitive effects, T has been shown to modulate the reactivity of the hypothalamic–pituitary–adrenal axis (Bingham et al., 2011; Handa et al., 1994) and to have anxiolytic effects in a number of studies in male rodents (Aikey et al., 2002; Bitran et al., 1993; Edinger and Frye, 2004; Fernandez-Guasti and Martinez-Mota, 2005; Toufexis et al., 2005), cattle (Boissy and Bouissou, 1994) and ewes (Bouissou and Vandenheede, 1996). However, whether the anxiolytic properties of T are maintained in aged animals is not known.

Male rhesus monkeys are particularly well suited for investigating the effects of T on cognition, motor function and emotions because they share many characteristics with humans, including in the organization of the brain and neuroendocrine systems (Sorwell and Urbanski, 2013). They exhibit age-related declines in cognition (Baxter, 2001; Herndon and Lacreuse, 2002; Herndon et al., 1997), fine motor function (Lacreuse et al., 2005; Zhang et al., 2000), and T levels (Sorwell and Urbanski, 2013) that closely resemble those observed in humans. In addition, rhesus monkeys are highly social primates that share a vast repertoire of socioemotional behaviors with humans (Kalin and Shelton, 2003). However, there is a paucity of data on age-related changes in socioemotional behaviors. In a longitudinal study spanning the ages of 6 to 20 years, (Suomi et al., 1996) reported that older rhesus monkeys retained the temperamental characteristics that they exhibited in early adulthood, but that the distribution of specific activities (e.g., agonism) changed across time.

In a related macaque species (*M. fascicularis*), Veenema et al., (1997) found in contrast that older females withdrew from social interactions and showed increased arousal (as shown by increased frequencies of yawning, scratching, and body shaking), perhaps due to the inability to deal with complex social interactions. Thus, it remains unclear how emotions change with age in macaques and what influence, if any, T may exert on this pattern.

The goal of the present study was to examine the effects of low and high T conditions on cognitive, motor and emotional behavior in male rhesus monkeys. We tested 10 aged males in a battery of tasks assessing these functions and compared the results to those of 6 younger male rhesus monkeys tested in the same paradigms in prior studies from our laboratory (Lacreuse et al., 2009; Lacreuse et al., 2010). Based on the literature referenced above, we predicted that T would improve selective aspects of cognition, would have little or no effect on motor function, and would bias emotional processing towards negative stimuli.

METHODS

Subjects

Ten aged adult male rhesus monkeys (mean age = 19 years, range = 15–25 years) participated in the study. Their performance on a battery of cognitive, emotional and fine motor tasks was compared to that of 6 younger male rhesus monkeys (mean age = 5.8 years, range = 5–6 years), whose cognitive (Lacreuse et al., 2009) and emotional data (Lacreuse et al., 2010) have been published previously (see Table 1). Data on the older group were collected over 2009–2011. Data in the young monkeys were collected during 2007–2009. Eleven monkeys, including 5 older monkeys and 6 young monkeys, were housed at the University of Massachusetts. The remaining 5 older monkeys were housed at the New England Primate Research Center of Harvard University. All monkeys were housed indoors in stainless steel primate cages at constant 12:12h lighting conditions. The monkeys were not food or water deprived and received their normal ration of monkey chow and fresh fruits every day. The monkeys were humanely treated in accordance with the standards of the PHS policy on Humane Care and Use of Laboratory Animals. The study was approved by the Institutional Animal Care and Use Committees of the University of Massachusetts and Harvard University.

Experimental Design

The cross-over experiment involved four 4-week periods (Fig. 1). During the baseline phase, monkeys were tested on the battery of cognitive, emotional and motor tasks in the absence of any drug treatment. After the initial baseline period of 4 weeks, monkeys were injected intramuscularly (I.M.) with leuprolide acetate (Lupron Depot, Abbott laboratories, North Chicago, IL; 200 µg/kg) to induce chemical gonadectomy. Initially, Lupron induces a sharp increase in T levels within 3 days of treatment before suppressing gonadotropins and T within 4 weeks. Monkeys were not tested on the battery during this 4-week phase in order to ensure that all testing was completed under maximum T suppression. At the onset of the third period, half of the monkeys (n=8) were treated with lupron + testosterone enanthate (TE, Watson Pharma, Inc., Corona, CA; 20 mg/kg) and the other half (n=8) with lupron + oil vehicle. In male rhesus monkeys, a single intramuscular TE injection at this dose induces

supraphysiological levels of T that progressively decline to baseline levels by the end of 4 weeks (Tyagi et al, 1999). Each group crossed-over to the alternate treatment for the final 4 weeks of the experiment. Importantly, the experimenters were blind to the order in which monkeys received each treatment.

Cognitive Tests

All cognitive testing was conducted in a modified, custom Wisconsin General Testing Apparatus (WGTA) that attached to the home cage of the participating monkey. The modified WGTA consisted of an opaque box (29.2 cm L × 33.0 cm W × 25.4 cm H) containing a test tray (29.2 cm L × 15.2 cm W × 1.3 cm H) with three or more food wells. Wells were baited with raisins, cereals or mini-marshmallows and covered with stimulus objects. The trays were concealed from view by an opaque panel between trials. Prior to the onset of the study, the monkeys were familiarized to the apparatus and were trained on each cognitive task until a specific learning criterion in the following order: Delayed Non-Match-to-Sample (DNMS), Delayed Recognition Span Task (Spatial-DRST) and Delayed Response (DR). Specifically, monkeys were trained on the DNMS (10 s delay) until 90% of correct responses over 5 consecutive sessions, performed 10 sessions of the spatial-DRST (100 trials) and were trained on the DR (1 s delay) until 90% of correct responses over 5 consecutive sessions. All of the procedures and apparatus were duplicated between the two facilities and personnel from both places were directly trained by the PI (A.L.). Each cognitive testing paradigm, as well as subsequent testing apparatus (emotional and motor testing), are described below. Monkeys performed all cognitive tasks in a 3-week period. Monkeys were randomly assigned to one of three task orders, with each monkey keeping the same task order throughout the experiment.

DNMS Task

The DNMS measures visual recognition memory, which is mediated by the medial temporal lobes (Zola-Morgan and Squire, 1985). The task was administered using a pool of approximately 500 “junk” objects to which the monkeys had limited previous exposure. To begin the test, the opaque door on the WGTA was lowered in order to conceal the testing tray as the experimenter placed a reward in the center well under a sample object. Subsequently, the door was opened and the monkey responded by displacing the object and retrieving the reward. The opaque door was then closed for a delay period of 10, 60, 120, 300, or 600 seconds, during which the sample object was moved to one of the side wells and a novel object was placed over the other side well, concealing a food reward. After the specified delay period, the opaque door was opened and the monkey had to displace the novel object to find the reward. The left or right location of the novel object and the delay order on each trial was randomly determined prior to the testing period. Monkeys were tested for 20 trials per day, and each delay period was presented 4 times within each testing session. The DNMS testing phase lasted for 4 consecutive days, after which the proportion of correct trials was calculated and used for analysis.

Spatial-DRST

The Spatial-DRST is a commonly used method for assessing hippocampally mediated spatial memory (Beason-Held et al., 1999). The goal of the task is to identify a stimulus

when it appears in a novel location among an increasing number of identical stimuli on a matrix. For the current study, the testing tray consisted of 18 wells arranged in a 3×6 array. The response stimuli were identical blue water bottle caps. In the first trial, a small food reward was placed in one of the wells, out of view of the monkey. The experimenter raised the opaque panel and the monkey had to displace the cap and retrieve the reward. On subsequent trials, the opaque panel was again lowered for a 1s interval during which the previous cap was replaced and the experimenter baited an additional well and covered the reward with an identical cap. The monkey had to displace the cap in the new location to find the reward. After each correct answer, a new bottle cap was added to the tray until a maximum of 9 correct choices were made (i.e., the monkey correctly identified the novel location on 9 consecutive trials). If the monkey made an error (i.e., selected the incorrect location), the trial was stopped and a new sequence started. After each trial, the memory span for that trial was recorded (i.e., the number of correct selections before and error was made). Each sequence administered was either a novel or repeated sequence of baited locations. For novel sequences, a randomly selected array of locations were presented to the monkey. The repeated sequence consisted of a specific sequence of locations that was presented 2–3 times within each experimental session of 10 sequences. As the monkey could not predict where the next correct location would be, the novel sequences measured capacities of spatial working memory. However, the repeated sequences measured reference memory, since the sequence of correct locations could be memorized. The Spatial-DRST task was presented on 4 consecutive days during each testing phase. For each session, overall memory span (averaged number of caps successfully chosen before making a mistake), the memory span obtained for novel sequences (novel span), the memory span obtained for repeated sequences (repeated span) and the maximal span obtained per session were recorded.

DR Task

The DR measures working memory, mediated by the dorsolateral prefrontal cortex (Goldman et al., 1971). For each trial the monkey observed, through a clear panel, as the experimenter placed a reward into the left or right well on the standard testing tray and covered both wells with identical opaque plaques. The opaque panel was then closed for a delay period of 5, 10, 15, 30, or 60 seconds. After the delay the panels were opened and the monkey had to displace the correct plaque to retrieve the reward. The reward locations were determined randomly. Monkeys were tested for 20 trials per day, and each delay period was presented 4 times within each testing session. Monkeys were tested on the task for 4 consecutive days during each testing phase. The number of correct trials was recorded for later analysis.

Emotional Tasks

Approach–Avoidance Task—The Approach-Avoidance task is administered to assess anxiety (Betha et al., 2004). During the test, monkeys were moved from their home cages to a two-compartment Plexiglas enclosure ($1.5 \times 0.71 \times 0.68$ m) in an adjacent room. The enclosure could be divided into two compartments by an opaque screen, prohibiting the monkey from observing the changes made to the environment on one side of the pen. Outside of the view of the monkey, an object (familiar, novel, or aversive) was placed into

the left compartment of the pen and baited with half a banana. Familiar objects served as control stimuli and were enrichment toys to which the monkeys had previously been exposed. Novel objects were also enrichment toys of similar size that the monkeys had never seen. Aversive objects were novel objects selected to elicit fear in the participating monkey. These objects included a plastic snake, a capture glove, and various masks with large eyes. Prior to the start of the experiment, each monkey was given four familiarization trials without an object present to become accustomed to the transport procedure and the novel enclosure.

At the start of each trial, the monkey was placed into the right compartment of the enclosure; the opaque screen prevented the monkey from seeing into or entering the left compartment. The enclosure was already baited with one of the three types of objects. After the monkey was transported into the enclosure, the opaque screen was removed. A digital video camera (Panasonic PV-GS300) was used to record the monkeys' behaviors during each 5 min trial and the camera was turned on as soon as the opaque screen was removed. The experimenter left the room and the monkey remained in the enclosure with the object for the duration of the trial (5 min). After five minutes, the monkey was transferred back to his home cage. Monkeys were tested 3 times during one week, and were exposed to one object from each of the 3 categories. Thus there were a total of 9 Approach–Avoidance trials per monkey across the testing phases (3 objects $\times$ 3 testing phases). Object-type exposure order was counterbalanced across testing phases. Latency to approach and time spent in contact with the object was recorded from the video footage of each trial.

Social Playbacks

For this task, monkeys were transported into a single compartment of the Plexiglas enclosure used for the Approach-Avoidance Task. While in the enclosure, the monkeys were exposed to 3 min long video clips that were played on a 43.2 cm video screen placed 20 cm in front of the compartment. Three types of video clips were presented. Positive videos consisted of rhesus monkeys engaging in grooming behaviors; negative videos consisted of fighting and aggressive interactions between rhesus monkeys; neutral videos consisted of sleeping rhesus monkeys. The monkeys in all clips were unfamiliar to the monkeys being tested. The videos clips were generously provided by Sarah Partan (School of Cognitive Science, Hampshire College, MA) and were edited from footage of free ranging rhesus monkeys in a natural habitat at Cayo Santiago, Puerto Rico. Each trial was recorded by a Panasonic PV-GS300 digital video camera placed 1 m away from the compartments and oriented to capture the entire monkey's body within the enclosure. Monkeys were tested 3 times during one week, and were exposed to one video from each of the 3 categories on each day. Thus there were a total of 9 video trials per monkey across the testing phases (3 video types $\times$ 3 testing phases). Each trial utilized a novel video and the order of video-type presentation was counterbalanced across testing phases.

The older monkeys were tested on the approach-avoidance task and social playbacks on weeks 1 and 2 of each testing phase. The younger monkeys completed each task twice (weeks 1–3 for approach-avoidance and 2–4 for objects), but only data from weeks 1 and 2

were analyzed for comparison with the older monkeys. The data of the younger monkeys on either task were reported previously (Lacreuse et al, 2010).

Lifesaver Test

The Lifesaver test was designed after the automated movement assessment panel described by (Gash et al., 1999). The apparatus consisted of a clear vertical Lexan panel and a flat board that could be affixed to the open door of the monkey's home cage. The monkey could insert a hand through one of two openings on the left and right sides of the panel to retrieve a LifeSaver candy from a metal rod attached to the left or right sides of the flat board. A clear door could slide over the openings to prevent access to a specific side. Three metal rod shapes of increasing difficulty, consisting of a Straight rod, a DoubleS shaped rod, and a Question Mark shaped rod, were used (Fig. 8a).

A trial started when the experimenter placed a Lifesaver over one of the metal rods and opened one of the sliding doors to let the monkey access one metal rod with the corresponding hand. A second experimenter operated a digital video camera to record the manual movements of the monkey while retrieving the candy from the rod. Monkeys were exposed to each of the 3 rods twice (once with each hand) for a total of six trials per experimental session. For each session, the rod type and position of the rod were presented in a randomized order. Monkeys were tested twice a week for four consecutive weeks within a testing phase, for a total of 24 sessions of 6 trials each across the test phases. The latency to remove the candy was measured from the digital video recordings, as described in Lacreuse and Herndon, (2003).

Blood Samples

Blood samples were collected at weekly intervals for analysis of testosterone levels. Blood samples (~4.0 ml/sample) were collected once a week (i.e., on Fridays) during the entire experiment (16 blood draws per monkey). Blood samples were obtained from a saphenous vein between 08:00 and 09:00 under ketamine anesthetization (6.0–10.0 mg/kg I.M.). Serum was removed by centrifugation and frozen. Serum samples were analyzed for total T using commercially available RIA kits and protocols in use in the laboratory of Dr. Jerrold Meyer at the University of Massachusetts. For this study, the RIA kit used was purchased from Siemens Healthcare Diagnostics (TKTT kit, Siemens Healthcare Diagnostics Inc., NJ). The RIA kit previously used for the younger monkeys (Diagnostic System Laboratories, Inc., Webster TX) was discontinued and therefore could not be used in the analysis of T for the current samples.

Statistical Analysis

The hormonal, cognitive, motor and emotional data were analyzed using mixed repeated measures analyses of variance (ANOVA), with Phase (Baseline, Oil, TE) as within-subject factors and Age (young, old) and Sequence of Treatment (1, 2) as between-subject factors. For the DR and DNMS, Delay was added as a within-subject factor. Planned contrasts examined differences between the Oil and TE phase and the TE and Baseline phase.

For the emotional data, Object Type (familiar, novel, aversive) for the Approach Avoidance task, and Video Type (positive, negative, neutral) for the Social Playbacks were added as within-subject factors in the ANOVAs. For the Lifesaver test, Shape (Straight, DoubleS, Question mark) and Session (1–8) were the within-subject factors. Effect size estimates (eta squared η^2) are provided for the main results. According to Cohen (1988), $\eta^2 = 0.02$ is considered a small effect, $\eta^2 = 0.13$ a medium effect and $\eta^2 = 0.26$ a large effect.

RESULTS

T Levels

T levels for the 10 aged monkeys can be seen in Fig. 2. Due to the use of different RIA kits between the two age groups, we were not able to compare T levels between young and older monkeys. However, inspection of Fig.2 does not show any obvious differences between the two groups. The ANOVA indicated a significant effect of Phase on T levels ($F(3, 24) = 14.61$, $p < .001$; $\eta^2 = 0.62$) with no effect of Sequence ($F(1,8) = 0.39$, ns; $\eta^2 = 0.04$) or Phase by Sequence interaction ($F(3,24) = 0.76$, $\eta^2 = 0.03$). Planned contrasts indicated that T levels achieved during the TE phase were significantly higher than T levels observed during the oil phase ($F(1,8) = 19.84$, $p < .01$; $\eta^2 = 0.68$). T levels in the oil phase were significantly lower than baseline T levels ($F(1,8) = 16.80$, $p < .01$; $\eta^2 = 0.64$).

Cognitive Data

Delayed Non Matching to Sample (DNMS)—On average, monkeys obtained $78\% \pm 1.5$ correct trials in the DNMS, with younger monkeys achieving $81\% \pm 2.3$ and older monkeys $76\% \pm 1.8$. This difference did not reach significance ($F(1,12) = 2.84$, ns; $\eta^2 = 0.16$). There was a significant effect of Delay ($F(4,48) = 25.69$, $p < .001$; $\eta^2 = 0.31$), with performance decreasing for longer delays, but the interaction between Delay and Age was not significant ($F(4, 48) = 2.16$, ns; $\eta^2 = 0.05$; Fig. 3). The main effect of Phase was not significant ($F(2,24) = 1.06$, ns; $\eta^2 = 0.07$) and did not interact with the effect of Delay ($F(8, 96) = 1.0$, ns; $\eta^2 = 0.06$). Planned contrasts indicated that performance during T treatment was not significantly different from performance during oil treatment ($F(1,12) = .14$, ns; $\eta^2 = 0.08$). The effect of Sequence of Treatment was not significant ($F(1,12) = 1.20$, ns; $\eta^2 = 0.07$) and did not interact with any other variables.

Spatial DRST—A significant effect of age was observed in this task ($F(1,12) = 8.49$, $p < .02$; $\eta^2 = 0.34$), with younger monkeys achieving an overall span of $2.54 \pm .12$ and older monkeys a span of $2.1 \pm .09$ (Fig. 4). The effect of age was also observed for novel sequences $2.35 \pm .13$ vs. $1.92 \pm .1$; $F(1,12) = 6.9$, $p < .02$; $\eta^2 = 0.30$) and repeated sequences $3.02 \pm .15$ vs. $2.6 \pm .12$; $F(1,12) = 4.85$, $p < .05$; $\eta^2 = 0.24$). The maximal span was also significantly different between younger ($4.74 \pm .31$) and older monkeys ($2.89 \pm .24$; $F(1,12) = 21.8$, $p < .001$; $\eta^2 = 0.56$).

The main effect of Phase was significant for all the measures (total span ($F(2,24) = 16.48$, $p < .001$; $\eta^2 = 0.48$); novel span ($F(2,24) = 10.6$, $p < .001$; $\eta^2 = 0.43$); repeated span ($F(2,24) = 5.15$, $p < .02$; $\eta^2 = 0.22$); maximal span ($F(2,24) = 6.4$, $p < .01$; $\eta^2 = 0.26$). However, this effect was due to a practice effect between the baseline and treatment phases. Planned

contrasts revealed indeed that performance during T treatment was not significantly different from performance during oil treatment for total span ($F(1,12) = .25$, ns), novel span ($F(1,12) = .944$, ns), repeated span ($F(1,12) = .056$, ns), or maximum span ($F(1,12) = .003$, ns). The main effect of Sequence was not significant for any measure: mean span ($F(1,12) = .62$, ns; $\eta^2 = 0.025$), novel span ($F(1,12) = .08$, ns; $\eta^2 = 0.004$), repeated span ($F(1,12) = .870$, ns; $\eta^2 = 0.04$), or max span ($F(1,12) = .78$, ns; $\eta^2 = 0.02$). The interaction between Age and Phase was significant for the maximum span ($F(2,24) = 4.2$, $p < .05$; $\eta^2 = 0.10$). No other interactions were significant.

Delayed Response (DR)—On average, monkeys obtained $62\% \pm 1.4$ correct trials in the DR, with younger monkeys achieving $64\% \pm 2.3$ and older monkeys $61\% \pm 1.8$. This difference was not significant ($F(1,12) = 1.23$, ns; $\eta^2 = 0.09$). There was a significant effect of Delay ($F(4,48) = 13.65$, $p < .001$; $\eta^2 = 0.44$), with performance decreasing with longer delays (Fig. 5). The main effect of Phase was not significant ($F(2,24) = .17$, ns; $\eta^2 = 0.009$). Planned contrasts indicated that performance during T treatment was not significantly different from performance during oil treatment ($F(1,12) = .37$, ns; $\eta^2 = 0.1$). The main effect of Sequence of Treatment was not significant ($F(1,12) = .031$, ns; $\eta^2 = 0.002$). There were no significant interactions.

Emotional Data

One older monkey failed to habituate to the procedures and was not tested in the emotional tasks (Table 1). Thus, the sample for the emotional data consisted of 15 monkeys, 9 old monkeys (mean age = 19.2 y.o) and the 6 young monkeys.

Approach Avoidance Task—Older monkeys were significantly faster (mean = 90 sec) than the younger monkeys (mean = 184 s) to touch the banana ($F(1,11) = 5.74$, $p < .05$; $\eta^2 = 0.27$). The effect of Object Type was significant for all measures (all $ps < .001$), indicating that monkeys crossed into the object compartment more quickly when the object was familiar than when it was novel or aversive (Fig. 6). There was no effect of Phase for any measures (all $ps > .05$). However, the interaction between Phase and Age was of marginal significance for the proportion of time spent with the object ($F(2,22) = 3.17$, $p < .07$; $\eta^2 = 0.16$). Planned contrasts indicated that the proportion of time spent with an object was greater during T treatment than oil treatment for the older animals only ($F(1,11) = 5.86$, $p < .05$; $\eta^2 = 0.12$). In addition, a significant interaction between Type and Phase ($F(4,44) = 4.44$, $p < .01$; $\eta^2 = 0.24$) for the proportion of time spent with an object, suggested that the effect of Phase varied according to the Object Type. To examine this interaction in further detail, we conducted an ANOVA for each object separately. For novel objects, the effect of Phase on the proportion of time spent in proximity to the object was significant ($F(2,22) = 7.42$, $p < .01$; $\eta^2 = 0.29$). This indicated that monkeys spent more time with a novel object when treated with T than with oil ($F(1,11) = 7.51$, $p < .02$; $\eta^2 = 0.24$). In addition, this effect depended on age as indicated by a marginal Age $\times$ Phase interaction ($F(2,22) = 3.44$, $p = .05$; $\eta^2 = 0.13$). These results indicated that older monkeys spent more time with a novel object when treated with T (about 55%) compared to oil (about 27%; $F(1, 7) = 19.08$, $p < .01$; $\eta^2 = 0.70$). Such an effect was absent in younger animals (Fig 6).

Social Playbacks—As can be seen in Fig. 7, there was a marginal effect of age in this task for the total watching time, with younger monkeys watching the videos for a longer time than older monkeys ($F(1, 11) = 4.49$, $p = .058$; $\eta^2 = 0.11$). There was also a significant effect of Video Type on total watching time ($F(2,22) = 33.05$, $p < .001$; $\eta^2 = 0.57$), indicating that monkeys watched negative videos longer than other types of videos. However, this effect interacted with Age ($F(2,22) = 12.26$, $p < .001$; $\eta^2 = 0.21$). This interaction indicated that the effect of Age was only significant for the negative videos. The effect of Phase was also significant ($F(2,22) = 7.56$, $p < .01$; $\eta^2 = 0.33$), as well as the interaction between Video Type and Phase ($F(1,22) = 3.52$, $p < .02$; $\eta^2 = 0.20$). The Phase effect indicated that total watching time was greater during T treatment relative to baseline ($F(1,11) = 9.61$, $p < .01$; $\eta^2 = 0.38$) and to a lesser extent the oil condition ($F(1,11) = 3.67$, $p < .10$; $\eta^2 = 0.22$). This effect depended on the Type of video, however. For negative videos, total watching time was significantly greater during T treatment than during oil treatment ($F(1,11) = 5.69$, $p < .05$; $\eta^2 = 0.29$) or baseline ($F(1,11) = 7.02$, $p < .05$; $\eta^2 = 0.34$). For positive videos, the effect of Phase reflected greater watching time during T treatment than during baseline ($F(1,11) = 7.92$, $p < .02$; $\eta^2 = 0.31$) but there was no difference between the T and oil phases ($F(1,11) = 0.62$, ns; $\eta^2 = 0.04$). Total watching time for neutral objects did not vary as a function of Phase.

Motor Data

Because of missing fingers, lack of motivation to perform the task or both, five older monkeys failed to master the motor task or had too many missing data to be included into the analyses. The total sample for the motor data consisted of 5 older monkeys (mean age = 19.8 y.o) and the 6 young monkeys (Table 1).

First, we examined whether hand use had a significant effect on the latency to remove the candy. An ANOVA with Shape, Age and Hand was conducted that revealed no effect of Hand, Age $\times$ Hand or Hand $\times$ Shape interaction. Therefore, Hand was excluded from subsequent analyses. Analyses of performance at baseline showed that the latency to remove the candy varied significantly as a function of Session ($F(7,35) = 3.69$, $p < .01$; $\eta^2 = 0.41$) and Shape ($F(2, 10) = 17.39$, $p < .001$; $\eta^2 = 0.76$), but not as a function of Age ($F(1,5) = 0.44$, ns; $\eta^2 = 0.08$; Fig. 8b). None of the interactions was significant. In addition, the latency to remove the candy increased from the Straight shape (mean = 0.99 ± 0.19) to the DoubleS shape (mean = 3.45 ± 0.65) and Question Mark shape (mean = 5.29 ± 0.96).

The analysis of the Treatment Phase indicated that Sequence of Treatment was not significant ($F(1,7) = .003$, ns). There was no effect of Treatment ($F(1,7) = .017$, ns; $\eta^2 = 0.01$) or Age ($F(1,7) = 0.03$, ns; $\eta^2 = 0.0003$) on the latency to remove the candy (Fig. 8c). The effect of Shape was significant ($F(2,10) = 18.39$, $p < .001$; $\eta^2 = 0.69$), indicating that faster removal for the Straight shape (mean = 1.01 ± 0.10) compared to the Question Mark (mean = 3.52 ± 0.67) and DoubleS (mean = 3.18 ± 0.60) shapes. None of the interactions was significant.

DISCUSSION

In this study, 10 aged male rhesus monkeys were tested on a battery of cognitive, emotional and fine motor tasks at baseline and under high T and low T conditions induced by treatment with a GnRH agonist and add-back of T or oil vehicle. Their performance was compared to that of 6 young adult males previously tested in the same paradigm in our laboratory.

Baseline T levels

The comparison of baseline T levels in young and older monkeys was problematic in this study because different assays for the two groups were used due to the discontinuation of one assay kit. Nevertheless, the results obtained from these different assays did not reveal any clear difference in T levels between young and older monkeys. This is not entirely surprising: first, our sample of older monkeys may not have been old enough to show a significant decline in T levels. Second, baseline T levels were based on 4 weekly blood samples which were collected in the morning. Given that T shows a clear circadian rhythm and is released in a pulsatile manner (Sitzmann et al., 2014), serial blood samples may be necessary to accurately capture age-related differences in T levels in male rhesus monkeys (Sorwell and Urbanski, 2013; Urbanski and Sorwell, 2012).

Cognitive performance

With respect to age effects on cognitive performance, no significant difference was found between young and older monkeys in the DNMS or DR, though performance in both tasks decreased with increasing delays. The lack of age-related decline in these two tasks was likely due to the inclusion of middle-aged monkeys (15–19 years old) in the older group, along with the poor performance of the young adult males in the DR task. In contrast, a detrimental effect of age was found for all measures of the spatial-DRST, confirming that spatial working memory and reference memory as assessed by this task are particularly sensitive to age effects (Herndon et al., 1997). Importantly, no effect of T manipulations was found for any cognitive tasks, whether or not they were sensitive to age differences. These data do not support our main hypothesis of a beneficial effect of T on visual recognition memory, working memory, or spatial memory. In the fine motor task, shape difficulty was associated with longer latencies to remove the candy, but there was no effect of age or T treatment. The lack of age differences was unexpected, as we previously found slower motor function in aged relative to younger male rhesus monkeys in the Lifesaver task (Lacreuse et al., 2005). However, the males tested in the previous study were on average older (23 years old) than the males included in the present experiment. Thus, we cannot rule out that an effect of T would have been observed in older monkeys exhibiting motor impairments.

Overall, our findings are consistent with recent studies in young or aged men reporting no effect of exogenous T on cognition (Bhasin et al., 2001; Emmelot-Vonk et al., 2008; Haren et al., 2005; Lu et al., 2006; Young et al., 2010) or motor function (Siegel et al., 2008). They contradict the results of other studies showing positive effects of T on selective cognitive domains in young (Cherrier et al., 2001) or older men (Janowsky et al., 2000; Janowsky et al., 1994) and young (Hawley et al., 2013; Sandstrom et al., 2006; Spritzer et al., 2011) and older male rats (Bimonte-Nelson et al., 2003). The source of the discrepancies is not entirely

clear. As mentioned above, one possibility for the lack of T effects in aged animals is that our sample of older monkeys may not have been old enough to be sensitive to T manipulations. The lack of age-related impairment in the DNMS, DR and Lifesaver test is in agreement with such an interpretation. However, even the task for which older monkeys were impaired (i.e., spatial-DRST) was insensitive to T, indicating that the presence of an age-related cognitive impairment in a particular task is not sufficient for T to modulate performance. Of note, we cannot rule out that the duration of exogenous T manipulations (1 month duration) may not have been long enough to yield significant changes in cognitive and motor function. However, data from this (Lacreuse et al., 2012) and other labs (Wolf et al., 2000) have shown that even very short durations of T manipulations (7 days and 5 days, respectively) can modulate cognitive performance under specific conditions. Similarly, it is also possible that lower T levels than those achieved in this study may have resulted in a different pattern of result, since a few studies in men have suggested that T has a curvilinear effect on cognitive function, with too low or too high levels of T impairing cognition (Moffat and Hampson, 1996; Cherrier et al., 2007; Matousek and Sherwin, 2010).

An alternative explanation may be related to the use of a chemical castration with a GnRH agonist, which shuts down gonadotropins in addition to T secretion. In contrast, low levels of T, as would be experienced by surgically castrated animal models and naturally hypogonadal men, are associated with a compensatory elevation of LH and FSH levels. It has been suggested that LH may have direct effects on cognition. LH crosses the blood brain barrier (Lukacs et al., 1995) and LH receptors are found in several brain regions involved in cognition, including the hippocampus and cerebral cortex (Barron et al., 2006; Lei et al., 1993). Recent studies suggest that high levels of circulating LH can be detrimental to the hippocampus and spatial memory in rodents (Berry et al., 2008; Bryan et al., 2010; Casadesus et al., 2007; McConnell et al., 2012; Ziegler and Thornton, 2010) and to memory recall in men (Hyde et al., 2010). Thus, it is possible that the reduction in circulating LH levels following Lupron treatment compensated for the loss of T in our study. In support of this hypothesis, it is interesting to note that the two human studies that have used Lupron to lower T levels in men found no effect of T treatment on cognition in either young (Bhasin et al., 2001; Young et al., 2010) or older men (Young et al., 2010). Future studies in male macaques should investigate whether the effects of T on cognition are indeed mediated by changes in circulating LH levels.

In contrast to the lack of T manipulations in the cognitive or motor tasks, exogenous T affected some emotional measures, suggesting the effects of T on cognition and emotion are mediated by different neural pathways. In the approach-avoidance task, both groups of monkeys showed a robust effect of Object-Type on the latencies to approach the object and to touch the banana, with longer latencies observed for novel and aversive objects than familiar objects. An effect of T was observed in older monkeys only, which spent more time in proximity to a novel object during the high T condition (i.e., Lupron + TE) compared to the low T condition (i.e., Lupron + oil). These data are generally consistent with the hypothesis that T decreases anxiety, but provide important new information: the anxiolytic effect of T was restricted to novel objects, with no effect of T observed for aversive objects. One interpretation of these findings is high T decreases anxiety in mildly stressful contexts (i.e., novel object exposure), but has no effect on fearful behavior (i.e., aversive object

exposure). Second, the beneficial effect of T on anxiety was age-dependent, occurring only in the older monkey group. This finding suggests that younger monkeys may have been more fearful than older monkeys. The fact that they took significantly longer than older males to touch the reward in all 3 object conditions supports this hypothesis. These age differences in fearful behavior could be mediated by age-related changes in amygdala reactivity to aversive stimuli (Nashiro et al., 2012; St Jacques et al., 2009).

For social playbacks, a marginal effect of age was found, with longer watching times observed in younger than older monkeys. Negative videos were watched significantly longer than the other video types in both age groups. These data do not provide support for a shift from a negative bias to a positive bias with age (Socioemotional Selectivity Theory; Carstensen et al., 2003), as aged male monkeys did not demonstrate a shift in attention from the negative to the positive social scenes in the social playback task for any of the treatment phases. Although more studies need to be conducted to confirm these results, it is possible that the positivity effect is a phenomenon unique to humans, caused by a psychological process associated with the knowledge of a limited lifespan (Carstensen, 2006; Carstensen et al., 2003; Carstensen et al., 2011). Interestingly, for both young and aged monkeys, watching times were longer during T treatment than oil treatment when negative videos were considered. These data support the hypothesis that T affects socioemotional processing by increasing attention and/or vigilance to negative social stimuli (Eisenegger et al., 2011).

In conclusion, the findings from the present study do not support the hypothesis that exogenous T improves cognition or fine motor function in young or aged male rhesus monkeys, but are consistent with the hypothesis that T modulates emotional states, whereby T increases attention to negative social stimuli in both young and aged male rhesus monkeys, and decreases anxiety to novel objects in aged monkeys. We cannot rule out that longer durations of treatment, lower T levels, the use of surgical castration rather than chemical castration, or the testing of monkeys of more advanced age would have resulted in more positive effects on the cognitive and motor outcomes.

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Highlights

- Testosterone manipulations do not affect cognition or motor function but modulate emotions
- High testosterone increases watching time of negative social scenes in young and old monkeys
- High testosterone decreases anxiety to novel objects in older monkeys only

Figure 1.
Experimental design.

Figure 2. Averaged T levels ($\pm$ SEM) in weekly serum samples in 6 young (upper) and 10 aged (lower) male rhesus monkeys. For illustration purposes, the data are plotted as a single sequence of treatment. Arrows mark injections (day 5 of each week). B1-4: Baseline; L1-4: Lupron; O1-4: Oil; T1-4: Testosterone

Figure 3.
Proportion of correct trials in the Delayed non Matching-to-Sample as a function of Phase and Delay, in young (left) and old (right) monkeys.

Figure 4. Memory spans as a function of Phase and Delay, in young (left) and old (right) monkeys.

Figure 5.
Proportion of correct trials as a function of Phase and Delay, in young (left) and old (right) monkeys.

Figure 6.

Latency to cross into object compartment, latency to touch banana and proportion of time spent with object as a function of Phase and Object Type in young (left) and older (right) monkeys. The older monkeys spend more time with a novel object when treated with T compared to oil * $p < .05$.

Figure 7. Total watching time (s) as a function of Phase and Video Type in young (left) and older (right) monkeys. Monkeys watched negative videos longer when treated with T relative to oil or baseline. * $p < .05$.

Figure 8.

(a) Photographs of a young male removing a lifesaver candy from the Straight, DoubleS and Question Mark shapes; (b) Latency (s) to remove the candy as a function of Age, Session and Shape at baseline; (c) Latency (s) to remove the candy as a function of Shape and Treatment in young (left) and old (right) monkeys.

Table 1

Age (years), housing location and missing data of the 16 male rhesus monkeys

Subjects	Age (years)	Housing location	Missing Data
<i>Older group</i>			
1	15	NEPRC	Motor data
2	15	NEPRC	N/A
3	17	NEPRC	Emotional data
4	17	UMass	Motor data
5	18	UMass	Motor data
6	20	NEPRC	Motor data
7	20	NEPRC	N/A
8	21	UMass	Motor data
9	22	UMass	N/A
10	25	UMass	N/A
<i>Younger group</i>			
1	5	UMass	N/A
2	6	UMass	N/A
3	6	UMass	N/A
4	6	UMass	N/A
5	6	UMass	N/A
6	6	UMass	N/A