Published in final edited form as:

Anim Cogn. 2016 May ; 19(3): 619–630. doi:10.1007/s10071-016-0966-5.

Reversal learning in gonadectomized marmosets with and without hormone replacement: are males more sensitive to punishment?

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Abstract

This study examined sex differences in executive function in middle-aged gonadectomized marmosets (*Callithrix jacchus*) with or without hormonal replacement. We tested ten castrated male (mean age 5.5 years) marmosets treated with testosterone cypionate (T, $n = 5$) or vehicle ($n = 5$) on Reversal Learning, which contributes to cognitive flexibility, and the Delayed Response task, measuring working memory. Their performance was compared to that of 11 ovariectomized females (mean age = 3.7 years) treated with Silastic capsules filled with 17- β estradiol (E2, $n = 6$) or empty capsules ($n = 5$), previously tested on the same tasks (Lacreuse et al. in *J Neuroendocrinol* 26:296–309, 2014. doi:10.1111/jne. 12147). Behavioral observations were conducted daily. Females exhibited more locomotor behaviors than males. Males and females did not differ in the number of trials taken to reach criterion on the reversals, but males had significantly longer response latencies, regardless of hormone replacement. They also had a greater number of refusals than females. Additionally, both control and T-treated males, but not females, had slower responses on incorrect trials, suggesting that males were making errors due to distraction, lack of motivation or uncertainty. Furthermore, although both males and females had slower responding following an incorrect compared to a correct trial, the sex difference in response latencies was disproportionately large following an incorrect trial. No sex difference was found in the Delayed Response task. Overall, slower response latencies in males than females during Reversal Learning, especially during and following an incorrect trial, may reflect greater sensitivity to punishment (omission of reward) and greater performance monitoring in males, compared to females. Because these differences occurred in gonadectomized animals and regardless of hormone replacement, they may be organized early in life.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Keywords

Cognitive flexibility; Estrogen; Executive function; Response inhibition; Testosterone; Working memory

Introduction

In humans, sex differences in the performance of particular cognitive tasks are well documented. Men tend to outperform women on many spatial tasks, such as mental rotation, in which participants must match a sample object to its rotated pair (Voyer et al. 1995) or navigational tasks, such as way-finding and navigating three-dimensional maps (Sandstrom et al. 1998). There is also growing evidence for sex differences in certain prefrontal (PFC)-dependent tasks. For example, the Iowa gambling task (IGT), in which participants must choose cards from the advantageous decks in order to amass a maximal number of points, tends to yield a male advantage (Evans and Hampson 2015a; van den Bos et al. 2013). Conversely, women tend to outperform men on other spatial tasks (memory for object location), as well as tasks of verbal fluency, verbal memory (Hampson 2002) and episodic memory (Herlitz 1997). The neural basis for these differences remains unclear, but many sex differences have been identified in brain anatomy (Goldstein et al. 2001) and function (Sacher et al. 2013) and there is some evidence that men and women recruit different brain regions (Gron et al. 2000; Shaywitz et al. 1995) and/or utilize different strategies when performing some of these tasks (Overman et al. 2006). It is important to note that there are some disagreements on the magnitude and extent of cognitive sex differences in humans. For example, several meta-analyses (Hyde 2005, 2014) concluded that, with the exception of 3D mental rotation, sex differences in cognition are small or trivial. Secondly, clear evidence has been provided that human cognitive sex differences are influenced by living conditions and educational levels. For example, recent data (Weber et al. 2014) demonstrate that cognitive sex differences have changed with societal improvements in Europe, leading to increased sex differences in some cognitive abilities (episodic memory), and reduced (numeracy) or elimination of sex differences in other cognitive abilities (category fluency); finally, a recent analysis of a large dataset of human brains MRIs confirmed the existence of sex differences in brain structure, but reported that most brains are composed of a unique mosaic of features, some more common in males, some more common in females and some common in both sexes (Joel et al. 2015). Notwithstanding these important points, there is compelling evidence for pervasive and robust sex differences in brain and cognition in humans (Cahill 2006, 2014; McCarthy 2012).

Most sex differences in phenotype derive from the sexual differentiation of the gonads and sex-specific patterns of hormonal secretion (Arnold 2009). Gonadal hormones, in particular testosterone and estradiol, have both organizational (influences early in development) and activational effects (influences in adulthood) on the brain (McCarthy 2012) and cognitive abilities (Hampson 2002). Thus, manipulations of gonadal hormones at different periods of the lifetime produce different phenotypes. In general, testosterone exposure early in life (and also estradiol exposure in rodents) masculinizes the brain and increases spatial abilities in both females and males (Berenbaum and Beltz 2011); the results are more mixed for the

activational effects of sex hormones on cognitive function, but there is ample evidence that both testosterone in men and estradiol in women affect a range of cognitive behaviors throughout adulthood (Hampson 2002; Hogervorst 2013; Van Goozen et al. 1995).

Because studies in humans are constrained by ethical issues and many confounding factors, studies in animal models are necessary to clarify the effects of gonadal hormones on cognitive abilities. Considerable evidence has been accumulated that testosterone and estradiol modulate learning and memory in rodents, with evidence for both organizational (Williams and Meck 1991) and activational effects (Galea et al. 2008; Luine and Frankfurt 2015). There are fewer studies in nonhuman primates, but they also support organizational (Hagger and Bachevalier 1991) and activational influences on cognitive abilities (Lacreuse et al. 2015; Lacreuse and Simoni 2012). Yet, evidence for sex differences in cognition in adult nonhuman primates is very limited, in part due to the difficulty of including both males and females in already very costly studies. One study in adult rhesus monkeys demonstrated that males outperformed females in a task of spatial working memory, the spatial delayed recognition span test (Lacreuse et al. 2005b). Another study in the same species found a female advantage in a spatial search task that could be solved by using visual or spatial cues (Herman and Wallen 2007). Both studies used spatial tasks likely to engage hippocampal (Beason-Held et al. 1999) processes. In a prior study investigating sex differences in young and old adult rhesus monkeys tested on battery of cognitive tasks including the spatial and object Reversals, no sex difference was found in either reversal (Lacreuse et al. 1999). However, this study was conducted in sexually intact monkeys and did not control for potential effects of gonadal hormones. There is convincing evidence that early in development, performance on PFC-dependent tasks is under the influence of sex hormone exposure (Clark and Goldman-Rakic 1989; Goldman et al. 1974). However, monkeys over the age of 1.5 years have not been tested in these paradigms, leaving unanswered whether sex hormones influence PFC-dependent tasks in adult nonhuman primates, as observed in adult humans.

The present study used middle-aged common marmosets (*Callithrix jacchus*) to investigate sex differences and their modulation by sex hormones in two tasks of executive function, a Reversal Learning task, which assesses the ability to flexibly change response selections guided by reward contingencies, and a Delayed Response task, which assesses working memory. Marmosets are well suited to study cognitive abilities: they have a large brain relative to their small size (300–500 g), with brain networks showing many similarities with those of humans (Belcher et al. 2013), and they are able to perform complex cognitive tasks (Spinelli et al. 2004). Reversal Learning, which involves learning and reversal of stimulus–reinforcement associations and cognitive control, has been shown to critically involve the orbital and dorsal PFC in both humans (Hornak et al. 2004) and marmosets (Clarke et al. 2008). A few studies have reported a male advantage in certain Reversal Learning tasks in humans (Evans and Hampson 2015a). The Delayed Response task is dependent on the dorsolateral PFC and involves maintaining information in short-term memory for various periods of time (Goldman and Rosvold 1970). Although sex differences have not been reported for this specific task, other tasks of spatial and verbal working memory have been found to yield a female advantage in humans (Duff and Hampson 2001).

The objectives of the present study were twofold: (1) to determine whether sex differences were present in these two tasks of executive function in gonadectomized (GDX) nonhuman primates with low circulating hormone levels (2) to examine the influence of sex-specific gonadal treatment on cognitive performance.

Materials and methods

Subjects

This experiment was not initially designed to test sex differences in cognitive abilities, but to examine the effects of sex-specific gonadal hormone treatments on cognition. The females, housed with castrated males, were tested in a prior experiment examining the effects of estradiol on cognitive function, as reported in Lacreuse et al. (2014). Following completion of this experiment, we took advantage of the availability of their male partners to (1) test the males on the same battery of tasks, using identical procedures in an identical setting; (2) examine whether testosterone replacement would affect performance on these tasks; and (3) compare the performance of the two sex groups. Ten castrated male (mean age 5.5 years, 3.53–8.64) common marmosets were used in this experiment. Their performance was compared to that of 11 ovariectomized (OVX) females (mean age 3.7 years, 2.4–4.82) tested on the same tasks, about 1 year prior (Lacreuse et al. 2014). The marmosets were housed in male/female pairs at the University of Massachusetts, Amherst. All animals were maintained under a 12:12 light/dark cycle (lights on at 7:30 AM) at an ambient temperature of 80 F with a relative humidity of 50 %. The pairs were housed in steel mesh cages ($101 \times 76.2 \times 78.7$) equipped with perches, hammock, nest boxes and branches to encourage species typical behaviors. All marmosets were GDX prior to the start of the study (see Table 1). Marmosets were fed a diet of Mazuri Callitrichid High Fiber Diet 5M16 (Purina Mills, St. Louis, MO, USA) and supplemented with fruits, seeds, nuts and mealworms. Fruits and nuts were provided twice a day (between 8–9 AM and 2–3 PM), Mazuri was provided once a day (between 2–3 PM), and water was available ad libitum. Animals were given enrichment opportunities each afternoon in the form of foraging tubes and baskets and other toys, in accordance with the guidelines published in the Guide for Care and Use of Laboratory Animals. The UMass Institutional Animal Care and Use Committee approved this study.

Procedures

Hormone replacement—Males were randomly assigned to one of two treatment groups: five males received weekly injections (i.e., Monday mornings) of testosterone cypionate (T group, 1.4 mg/kg) mixed with cottonseed oil, and five animals received injections of oil vehicle (male control group). All males were acclimated to the injection procedure. The female group consisted in six OVX females implanted with Silastic capsules (Dow Corning, Midland, MI, USA; inner diameter 0.058 inches; outer diameter 0.077 inches, length 11 mm) containing 17- β estradiol (E2; E2 group), and five females implanted with empty capsules (female control group). The capsules delivered a dose of E2 achieving levels experienced during the mid-follicular of the cycle (Lacreuse et al. 2014).

General cognitive testing procedure—During cognitive testing, a transport box ($34.1 \times 20.65 \times 30.8$) with a mesh front panel, through which the animal could reach its hands to

participate in testing, was attached to the front of the animal's home cage. The animals entered the box voluntarily for testing. A custom-made, modified Wisconsin General Testing Apparatus (WGTA) was placed in front of the transport box. The modified WGTA was an opaque box ($43.2 \times 42.3 \times 44.5$ cm) containing a testing tray ($40.65 \times 11.5 \times 1.25$ cm) with two food wells (diameter 2.5 cm) and an opaque door that could be opened and closed by the experimenter between trials. Rewards could be placed in the wells and then covered with stimulus objects for cognitive testing. Animals were given a maximum of 2 min to respond on each trial. If no response was made within the time limit, the trial was terminated.

Reversal learning task—All animals were given the Reversal Learning task using the WGTA box. The task begins with a simple discrimination, in which animals learn to select one of two objects (a white sphere or a black star) randomly placed on the left or right side of the testing board to receive a reward (dried mini marshmallow). Monkeys were administered ten trials per day. Once the animals were reliably choosing the target object (90 % accuracy over two test days), the rewarded object switched. The monkey had to select the previously unrewarded object to receive a reward. A total of three reversals were administered. We analyzed the total number of trials and errors to reach the 90 % accuracy criterion on each reversal, as well as the response latencies on each trial. Response latency was defined as the amount of time the animal took to touch an object once the experimenter opened the WGTA door during the choice phase. The monkey was allowed 2 min for responding on each trial. In addition, we also calculated the number of perseverative errors (when the number of errors is significantly above chance: 7–10), the number of chance errors (when the number of errors are at chance levels: 4–6) and the number of learning errors (when the number of errors is significantly below chance: 0–3) for each of the ten trial sessions, as described in Lai et al. (1995).

Delayed response (DR)—All animals were first trained and then tested on the DR task using the WGTA box. In this task, the animal is shown the location of a reward placed randomly in the right- or left-hand well through a clear plexi-glass screen. The wells are covered with identical tokens, and the door is closed for a specific delay. When the doors are reopened the animal must displace the token that covers the reward. In order to ensure that all animals would be able to perform the task with various delays, we first trained the animals on each successive delay (0, 1, 3, 6, 10 s) until a criterion of 90 % correct over two test days (ten trials/day). Once they reached criterion on a particular delay, they moved on to the next delay until they mastered the 10-s delay. When the animals reached criteria on all delays, they were given ten sessions, ten trials per day, of mixed delays randomly presented within a session (two trials/delay). The percent of correct trials as well as response latencies were recorded. The monkeys were allowed 2 min for responding on each trial.

Behavioral observations—Behavioral observations were performed daily between 3:00 and 5:00 PM by individuals to whom the marmosets had been previously acclimated. All observers were trained and tested until they achieved 90 % inter-rater reliability over three testing sessions. Observers used a modified frequency scoring system to record the occurrence of 25 behaviors in a focal animal, at 15-s intervals for 5 min. Behaviors included

measures of feeding, sociality and aggression, adapted from an extensive ethogram developed specifically for the common marmoset (Stevenson and Poole 1976; Table 2).

Urine collection and assays—On Thursday mornings (i.e., 3 days after injection), urine samples were collected from all animals, using a method described in Saltzman et al. (2004). Animals entered the transport box at 7:30 AM, i.e., a few minutes after the lights turned on, and remained there until they urinated or until 30 min had elapsed. Urine was pipetted into 1.5-ml vials, spun for 5 min and then frozen at -20°C . Urinary T assays were performed by the Endocrine BioServices Assay Laboratory at the University of Omaha, NE, USA. Urine collection was performed similarly in females, but estrogen assays were based on blood samples, as reported in Lacreuse et al. (2014). Plasma assays were performed by the Assays services of the Wisconsin National Primate Research Center, University of Wisconsin-Madison, Madison WI, USA.

Statistical analyses

Because the females were significantly younger than the males [$t(13.7) = 3.03$, $P = .009$, df adjusted for unequal variance], age was used as a covariate in all statistics.

Hormonal data—Male data were analyzed using independent samples t tests with an adjustment for unequal variance in t values between the groups.

Behavioral data—Because there was no effect of hormonal treatment on the focal behaviors for females or males, the treated and untreated animals were combined in each sex group. One-way ANOVAs controlling for age were performed comparing males and females on two behaviors of interest, vocalization and locomotion, and two groupings of behaviors, sociality (including initiate social contact and initiate social play) and aggression (including displace, aggress and genital display). A Bonferroni correction was used to compensate for multiple comparisons.

Reversal learning—Females learned the initial discrimination prior to ovariectomy, while males acquired the initial discrimination when already castrated and given T or oil vehicle. Therefore, the initial discrimination data were analyzed separately from the reversal trials. The number of trials to reach criterion on reversals 1–3, and the number of perseverative errors and the response latencies were analyzed using a mixed repeated measures ANOVA, with hormonal treatment and sex as the between-subjects variable, reversal number as the within-subjects variable and age as a covariate. In addition, for response latencies, the effects of the current and preceding trial outcome (correct or incorrect) were also examined. In order to create a more normal distribution of response latencies, data points that were two standard deviations from the mean for both sexes were removed from the data set.

Delayed response—Females were trained on the DR task prior to ovariectomy, while the males were trained after GDX and treatment, therefore the sex difference analysis was limited to the testing phase, when both groups of animals were GDX and received hormonal treatment or placebo. Percent correct and response latencies were analyzed using separate two-way ANOVA, with sex and hormone treatment as the between-subjects factors, delay as

a within-subject factor and age as a covariate. Because too few errors were made on this task, the effect of trial outcome (correct or incorrect) on response latencies was not calculated.

Results

Hormonal data

The average number of urine samples collected from the male animals was 13 (SD 4.9), with a range of 7 samples (1 animal) to 18 samples (4 animals). T-treated monkeys had significantly higher levels of urinary T than animals receiving vehicle [$t(4.52) = 3.27$, $P = .02$], adjusted for unequal variance; Fig. 1). E2-treated females showed significantly higher levels of plasma estrogen levels than control animals [$t(9) = 6.60$, $P < .001$], as previously reported (Lacreuse et al. 2014). Age did not differ significantly between treatment groups in either the females [$t(9) = .023$, $P = .98$] or the males [$t(8) = .012$, $P = .99$].

Behavior

Females engaged in locomotor activity significantly more often than males [$F(1, 18) = 12.23$, $P = .003$]. Females and males did not differ in sociality, aggression or vocalization (all P s $> .01$, Bonferroni-corrected P value; Fig. 2).

Reversal learning performance

Initial acquisition—Performance on the initial discrimination was analyzed separately from the reversals, because the females acquired this stage prior to ovariectomy, while males performed the initial discrimination post-castration and hormonal replacement. Because there was no effect of T treatment in males [Reversal $\times$ Treatment, $F(3, 24) = .64$, $P = .6$], the treated and untreated male groups were combined and compared to the group of intact females, controlling for differences in age. There was no difference between the two groups in the number of trials needed to acquire the first object discrimination [$F(1, 18) = .52$, $P = .48$].

Reversals—Our main focus was to examine the Reversals, where both the males and females were GDX and administered sex hormones or placebo. A mixed-measures ANOVA controlling for age showed that there was no effect of Sex [$F(1, 18) = .3$, $P = .59$], Reversal [$F(2, 36) = .82$, $P = .45$], or Sex $\times$ Reversal interaction [$F(2, 36) = .68$, $P = .51$] on the number of trials to reach criterion. There was an effect of sex on the number of trials in which the animal did not respond within the 2-min time limit [$F(1, 18) = 5.82$, $P = .027$], with males failing to respond on significantly more trials than females (4.2 vs .04 trials, respectively). E2 tended to impair Reversal Learning in females (previously reported in Lacreuse et al. 2014), but there was no effect of T on performance in males when comparing the two treatment groups [$F(1, 8) = .51$, $P = .50$; Fig. 3). When combining the two treatment groups, T was significantly correlated with performance on the third reversal [$r(8) = .66$, $P = .036$], with increased T associated with a greater number of trials to reach criterion (poorer performance). Correlations between T and performance on the other reversals were not significant (all P s $> .05$).

When we examined response latencies, we found that males ($M = 7.2 \pm .68$ SEM) had significantly longer latencies than females ($M = 4.47 \pm .65$ SEM), independent of hormone replacement status [$F(1, 16) = 6.97, P = .02$; Fig. 4]. We found no correlation between circulating T and performance on the task when both groups of males were combined (all P s $> .05$). We further analyzed response latencies by examining whether or not the animal was correct or incorrect on the trial. Males took significantly longer to respond on trials when they were incorrect ($M = 7.67 \pm .79$ SEM) than on trials when they were correct ($M = 6.72 \pm 1.08$ SEM), while female response times were not significantly affected by trial outcome (correct: $M = 4.49 \pm .60$ SEM, incorrect: $M = 4.45 \pm .65$ SEM); Outcome $\times$ Sex: $F(1, 16) = 6.98, P = .02$; Fig. 5a).

Additionally, while all animals were significantly slower if they had been incorrect on the previous trial [$F(1, 17) = 12.01, P = .003$], there was a significant Sex $\times$ Previous Trial Outcome interaction, indicating a sex difference in response latencies when the previous trial was incorrect, as opposed to correct [$F(1, 17) = 9.78, P = .006$; correct: $t(19) = 1.97, P = .062$, incorrect: $t(19) = 2.36, P = .03$; Fig. 5b). E2 treatment had an effect on perseverative responding in females, with E2-treated females having a increased number of perseverative responses with each reversal, and control females having reduced perseverative responding with each reversal (Lacreuse et al. 2014). T treatment did not affect perseverative responding in males [$F(2, 16) = .93, P = .42$].

Delayed Response performance

Treatment [$F(1, 17) = 1.40, P = .25$], Sex [$F(1, 17) = .21, P = .66$] and their interaction [$F(1, 17) = .36, P = .56$] did not significantly affect the percent of correct responses on the DR (Fig. 6). There were no significant correlations between T and performance (all P s $> .05$). Similarly, no effect of Treatment [$F(1, 17) = .94, P = .35$], Sex [$F(1, 17) = .01, P = .91$] or their interaction [$F(1, 17) = .16, P = .69$] was found for response latencies, and there were no significant correlations between T and response latencies (all P s $> .05$). No refusal occurred on the DR.

Discussion

We tested females and males GDX marmosets, half of which were replaced with E2 (females) or T (males), on two tasks of executive function: Reversal Learning, which assesses the ability to reverse associations between stimuli and reward or punishment, and DR, which targets working memory. In addition, we collected behavioral data daily. We found significant sex differences in Reversal Learning and locomotor behavior that were independent of hormonal treatment. In Reversal Learning, males showed significantly slower responding than females, in the absence of sex differences in the number of trials to acquire each reversal. Males were also slower than females following an incorrect response on the previous trial or when committing an error on the current trial. They also had greater refusals within the 2-min limit than the females. Furthermore, males exhibited fewer locomotor behaviors than females. T replacement in males did not affect behavior or performance on either task; in contrast, E2 replacement in females impaired Reversal

Learning with a similar trend for the DR, as reported previously (Lacreuse et al. 2014). We discuss these findings below.

Sex differences in reversal response latencies: motor, motivational or performance monitoring?

The only significant sex difference in the performance of cognitive tasks in this study was observed for response latencies and number of refusals in Reversal Learning. Sex differences were absent in the numbers of trials to acquire the reversals, as well as in response latencies and accuracy on the DR task. This is in contrast to a few humans studies, which reported a male advantage for Reversal Learning in children (Overman 2004) and adults (Evans and Hampson 2015a, b) and a female advantage in working memory (Duff and Hampson 2001). Interestingly, Duff and Hampson (2001) also found a female advantage in the time to complete the task. In the classic speed–accuracy trade-off dilemma, it is held that fast decisions tend to be risky (more likely to be incorrect), while slower decisions tend to be safer (more likely to be correct; Bogacz et al. 2010). In the present study, males' slower responses were not associated with better performance when compared to females. One possibility is that this task was in fact more difficult for males, but in the absence of a stringent time limit for completion (2 min allowed for responding), they were able to perform more slowly to achieve accuracy levels comparable as those of females. Time constraints have been shown to influence sex differences in cognitive performance in humans. For example, in a mental rotation task, which yields a male advantage, women tend to perform just as well as men when the time limit is eliminated (Voyer 2011).

Alternatively, slower responses in males might have been the result of a sex difference in age-related motor slowing, as a few males were in the older age range. Increased locomotion in females relative to males during our behavioral sampling would be consistent with this hypothesis. Previous research from this laboratory has shown that old male rhesus macaques perform more slowly than old females on a task of fine motor control (Lacreuse et al. 2005a). Whether marmosets also show age-related fine motor deficits is currently unknown, but will be tested in upcoming longitudinal studies. Nevertheless, the lack of sex differences in DR response latencies suggests that the slow responses exhibited by males in the reversal task were likely due to factors other than a general age-related motor slowing.

The analysis of response latencies as a function of errors provides additional cues. Males were slower on trials for which they were incorrect, which could reflect more susceptibility to distraction. In this scenario, distracted males would take longer to make their choice, which would more likely lead to an error, due to either memory failure or lack of attention. At least one other study reported slower responses in male than female marmosets in a task requiring the monkeys to open a container in order to extract a raisin (Yamamoto et al. 2004). In another study testing the ability of male marmosets to perceive biological motion, as assessed by the discrimination of visual patterns presented on a touch screen, males were found to be less attentive than females and unable to discriminate biological motion (Brown et al. 2010). Interestingly, Schubiger et al. (2015) recently interpreted these findings as motivational differences, rather than cognitive differences. The authors found that male marmosets are generally more emotionally aroused by an experimenter, which is associated

with decreased participation and attention to cognitive tasks, without affecting performance. Further, they noted that these differences could be due to females' higher interest to work for food and males' heightened vigilance to the environment. Consistent with these findings, we also found that males exhibited more refusals than females in the Reversal task. In the future, it will be important to compare male and female performance on a progressive ratio task, in which animals have to press a button an increasing number of times to receive a reward, as a means to objectively quantify their motivation to work for a reward. It should also be interesting to determine whether males are more attentive to the environment than females. Devoting attentional resources to social monitoring has been shown to impair executive control in baboons (Huguët et al. 2014). Because the DR did not show sex differences however, a hypothesis solely based on motivational/attentional differences cannot fully account for our results.

A complementary interpretation is that males were more sensitive to punishment (omission of reward) than females and exerted more inhibitory control as a result, probably through enhanced performance monitoring. Such differences could explain females' tendency for fast responding regardless of the trial outcome, whereas males proceeded more cautiously for trials on which they ultimately made an error. Although uncertainty monitoring has not been studied in marmosets, there is clear evidence that Old World monkeys such as macaques and to a lesser extent New World monkeys such as capuchins, monitor and respond to uncertainty (Beran et al. 2014). In addition, although both sexes were sensitive to errors in the preceding trial, as shown by post-error slowing, males were disproportionately slower than females on these trials, providing further support for enhanced sensitivity to punishment and/or greater performance monitoring.

While differences in response latencies were observed between males and females in Reversal Learning, no sex difference was found in the DR. One important difference between these two PFC-dependent tasks is that Reversal Learning requires learning stimulus–response contingencies through valenced feedback (learning through reward and omission of reward), while the DR task requires keeping the location of a reward in short-term memory (following task acquisition). These different task demands further support the idea that sex differences in response latencies reflect sex differences in processing feedback, with males being more sensitive to punishment and exerting greater inhibition control than females. This heightened sensitivity to punishment in males could also explain their higher number of refusals during learning. Interestingly, regardless of the cause of these sex differences, they occurred independently of circulating sex hormones.

Effects of hormone replacement in each sex

Several studies have implicated circulating T in the ability to perform PFC-dependent task like the IGT in humans (Evans and Hampson 2015b; Reavis and Overman 2001; Stanton et al. 2011; van Honk et al. 2004), with T benefiting performance. In our study, T replacement did not affect performance on the Reversal Learning and DR tasks, unlike E2 in females (Lacreuse et al. 2014). The large variation in circulating T levels that was achieved by our treatment regimen may have obscured treatment group differences, as higher T was associated with poorer performance on the third reversal when combining the two treatment

groups. The possibility that T is detrimental to Reversal Learning in male marmosets will have to be confirmed in larger studies. We note that previous studies in young adult and older rhesus monkeys failed to demonstrate an effect of T replacement on cognitive or motor performance (Kelly et al. 2014). In addition, studies examining the effects of T on cognition in older men have also provided mixed results (for review see, Hogervorst 2013) and the only study examining the effect of T in human motor function reported no effect of treatment in young or older men (Siegel et al. 2008). Importantly, E2 or T did not affect response latencies in the reversals. Thus, the presence of a sex difference in response latencies in adult GDX animals regardless of sex-specific hormone replacement suggests that these differences may be organized early in development. Interestingly, sex differences in Reversal Learning have been found in infant rhesus monkeys before the age of 15 months, but in the opposite direction. Goldman et al. (1974) reported that 75-day-old male monkeys outperform age-matched females in object reversal tasks. Later work clearly showed that this sex difference was modulated by T, as perinatally masculinized infant females performed as well as normal males and better than normal females (Clark and Goldman-Rakic 1989). Further evidence showed that T accelerated the maturation of the OFC, as these lesions impaired the performance of males and masculinized females without effect on normal females until later in life (Clark and Goldman-Rakic 1989). It is possible that these early influences of T on OFC development have long-term consequences for executive functioning later in adulthood (i.e., more cautious responding in males). As studies in nonhuman primates, including our own, have often disregarded response time measures (e.g., Lacreuse et al. 1999), it is possible that sex differences in response speed were overlooked in prior studies in rhesus monkeys.

Conclusion

In conclusion, we found that GDX males exhibited slower response latencies than females in Reversal Learning, especially during an incorrect trial and following an error in the preceding trial. They also had reduced participation in the task (i.e., greater refusals) compared to the females. In addition, these effects were specific of the Reversal Learning task, with no sex difference exhibited in the DR, and were independent of circulating sex hormones. Overall, these results suggest that in tasks requiring the learning and reversal of stimulus–reinforcement associations, male marmosets may be more sensitive to punishment than females, leading to greater performance monitoring and inhibitory control and reduced participation in the task. Furthermore, these differences may be organized early in development.

Acknowledgments

This research was supported in part by NIH grant # MH091492 to Agnès Lacreuse and a graduate student grant from the UMass Center for Research on Families (CRF) to Matthew LaClair. We are very grateful to Jeemin Chang for his assistance with many aspects of this project. We also thank Karen Bui, Raymond Guigni, Alexander McFarland, Jocelyn Mejia, Molly Morgan, Katharine Newman, Christine O'Brien and Courtney Tolliday for their help with data collection. We thank the UMass Veterinary Staff, Animal Care Staff and Shop staff for their expert assistance. We also thank the CRF for statistical consult.

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Fig. 1. Urinary testosterone levels (µg/mg creatinine) in control and T-treated GDX male marmosets. T levels were significantly higher in the T-treated group than control group ($P < .05$)

Fig. 2. Average number of observations as a function of behavioral category in male and female GDX marmosets. Females exhibited more locomotor activity than males ($P < .005$). Error bars indicate SEM

Fig. 3.

Average trials to criterion on the initial discrimination and three reversals in control and E2-treated OVX females and control and T-treated GDX males. Males and females performed similarly. *Error bars* indicate SEM

Fig. 4.

Average response latencies on all reversals for control and E2-treated OVX females and control and T-treated GDX males. Males had longer latencies than females, independent of hormone replacement. *Error bars* indicate SEM

Fig. 5.

Average response latencies for current correct and incorrect trials (**a**) and (**b**) when the previous trial was correct or incorrect in female and male GDX marmosets. Males, but not females, took longer to respond on current incorrect versus correct trials ($P < .05$); males were slower than females following an incorrect trial ($P < .05$). *Error bars* indicate SEM

Fig. 6.
Average percent correct on the Delayed Response test in control and E2-treated OVX female and control and T-treated GDX males. No sex difference was detected

Table 1

Age at castration and age at test (years) for all marmosets

Animal #	Sex	Age at castration	Age at test
1	Male	3.22	5.00
2	Male	2.04	3.62
3	Male	1.80	3.53
4	Male	6.91	8.64
5	Male	3.04	4.61
6	Male	5.48	7.21
7	Male	4.05	5.78
8	Male	3.64	5.36
9	Male	5.56	7.28
10	Male	2.62	4.19
11	Female	3.80	3.9
12	Female	3.30	3.40
13	Female	2.97	3.07
14	Female	3.22	3.32
15	Female	4.30	4.40
16	Female	2.89	2.99
17	Female	4.72	4.82
18	Female	2.30	2.4
19	Female	3.15	3.25
20	Female	3.13	3.23
21	Female	4.3	4.4

Table 2**Marmoset behavioral ethogram**

Behavior	Definition
Vocalization	Any sound made from mouth, including chirps, whistles and chittering
Aggress	Grapple with another marmoset, involving biting, clawing, wrestling and chasing
Displace	Takeover of position of another animal
Locomotion	More than one step in a directed plane
Visual explore	Sitting passively for more than 3 s, not scored in conjunction with any other behavior
Headcock	Turning of the head in inspection of an observer, animal or object
Genital display	Raise tail to expose genitals
Scentmark	Rub or drag anogenital, suprapubic, or sternal region along substrate, object or partner
Scratch	Vigorous rubbing of a body part
Tuft-flick	Rapid back-and-forth movement of ear tufts
Tactile oral	Sniff, bite, chew, handle or otherwise manipulate inanimate object, excluding food items and water bottle, for at least 1 s
Eat	Consumption of food
Drink	Licking or sucking on water bottle
Social contact	Passive close contact with another marmoset, within an arm's length, with both animals remaining stationary and in passive contact for at least 3 s
Sniff/nuzzle	Orient face against or toward partner, excluding anogenital region
Mount	Climb on partner's back from behind and grip partner around waist and legs may be accompanied by pelvic thrusting
Social play	Social interactions involving non-aggressive physical contact with other individuals; high activity
Self play	Repetitive movements toward objects or fixtures in cage may include spinning, swinging and hanging
Social groom	Use hands and/or mouth to pick through fur and/or mouth of partner, excluding anogenital region
Self groom	Licking, picking or spreading of one's own hair or skin
Other	Sneezing, coughing, piloerection or any other behavior not identified