The effects of gonadal hormones on learning and memory in male mammals: A review

Stuart T. LEONARD*, Peter J. WINSAUER

Department of Pharmacology and Experimental Therapeutics, Louisiana State University Health Sciences Center, 1901 Perdido Street, New Orleans, LA 70112-1393

Abstract The primary role of the gonadal steroid hormones in mammals is to regulate reproduction and related behaviors; however, both androgens and estrogens are also integrally involved in mediating higher brain function and processes including cognition, neural development, and neural plasticity. In particular, a number of studies show that estradiol modulates dendritic spine growth and synapse density (synaptic plasticity) in the hippocampus of females, and that increased estradiol levels are generally associated with improvements on a variety of learning and memory tasks. While the majority of research has focused on the beneficial effects of estradiol in females, much less attention has been given to testosterone and its effects on learning and memory in males. Similar to estradiol titers in females, testosterone titers in males decline with age, albeit more gradually, and this decline has been correlated with impairment of certain cognitive tasks. Moreover, studies involving both humans and animals indicate that testosterone and its metabolites can augment responding on certain behavioral tasks, depending on the subject’s current hormonal state, the response required, and the stimuli involved (e.g., those involving spatial or nonspatial stimuli). While the exact mechanisms by which testosterone exerts its effects on learning and memory are not fully understood, recent findings suggest that testosterone modulates learning and memory in males through an interaction with the cholinergic system. The overall objective of this review is to discuss studies investigating the role of the gonadal hormones in mediating learning and memory processes in male mammals [Current Zoology 57 (4): 543–558, 2011].

Keywords Testosterone, Learning and memory, Cholinergic system, Mammals, Cognition, Androgens

1 Introduction

In general, males acquire behavioral tasks involving spatial and working memory more readily than females. This finding is well documented in humans (see Andreano and Cahill, 2009) as well as in rodents (see van Haaren et al., 1990) responding on a variety of maze tasks including the Morris water maze (Galea et al., 1994; Kavaliers et al., 1996; Kavaliers et al., 1998; Perrot-Sinal et al., 1998; Cimadevilla et al., 1999; Blokland et al., 2006; Saucier et al., 2006), the Tolman sunburst maze (Dawson, 1972; Gaulin and Fitzgerald, 1986), the symmetrical maze (Davenport et al., 1970; Dawson et al., 1975; Dawson et al., 1977; Gaulin and Fitzgerald, 1986), the Barnes maze (Barrett et al., 2009), and several versions of the radial-arm maze (Einon, 1980; Mishima et al., 1986; Harrell and Parsons, 1988; Seymour et al., 1996; Gibbs and Johnson, 2008). Male nonhuman primates such as young rhesus macaques Macaca mulatta are also known to exhibit better spatial abilities on the working memory component of a spatial delayed recognition span test compared to young female macaques (Lacreuse et al., 1999; Lacreuse et al., 2005). Such differences in spatial abilities between males and females have led to the notion that certain cognitive processes are under the influence of the gonadal hormones. Indeed, the gonadal hormones play an integral role in sexual differentiation of the brain during development in mammals (see Arnold and Gorski, 1984; Morris et al., 2004), and there is evidence in the rodent literature that sex differences in spatial ability are due in part to both the organizational and activational effects of the gonadal hormones (e.g., Beatty, 1979; Arnold and Breedlove, 1985; Williams and Meck, 1991; Galea et al., 1996; Isgor and Sengelaub, 1998).

To date, most investigations examining the involvement of gonadal hormones in cognitive processes have focused on estradiol in females, largely due to the reported deficits in cognitive function that can occur in women after menopause (see Sherwin, 1997; Sherwin,
2 Mediation of Hippocampal Synaptic Plasticity by Testosterone

The hippocampus is integrally involved in mediating various learning and memory processes, particularly those involving spatial and working memory (for review see Squire, 1992; Lynch, 2004). Both estrogen and androgen receptors are located on neurons throughout the hippocampal formation (Pfaff and Keiner, 1973; Stumpf and Sar, 1978; Sar et al., 1990; Simerly et al., 1990; Clancy et al., 1992; Kerr et al., 1995; Shughrue and Merchenthaler, 2000; Tabori et al., 2005; Sarkey et al., 2008) giving rise to the notion that facilitation of learning and memory by the gonadal hormones is through modulation of the structure and function of the hippocampus. Indeed, the gonadal hormones do affect the density and growth of dendritic spines and synapses in the pyramidal cells of the hippocampus, and the growth and modification of these structures appear to be integral in the formation and maintenance of learning and memory processes (Geinisman et al., 2001; Kasai et al., 2003; Lang et al., 2004). In particular, the capacity of estradiol to maintain and increase dendritic spine density and spine synapses on pyramidal cells in the prefrontal cortex and the CA1 (but not the CA3) region of the hippocampus in female rats and nonhuman primates is well established (e.g., Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992; Leranth et al., 2002; Hao et al., 2003; Tang et al., 2004). Similar to estradiol, testosterone also maintains normal dendritic spine synapse density on CA1 pyramidal cells in the hippocampus of male rodents. In male mice, spine density formation in the CA1 hippocampal region peaks at puberty (35–45 days old); however, this increase is prevented if mice are gonadectomized prior to puberty (Meyer et al., 1978). Apical dendritic spine density in the CA1 region is also reduced in male rats gonadectomized during early adulthood compared to gonadally intact males (Lewis et al., 1995). Photoperiodic changes in testosterone titers are correlated with variation in hippocampal spine density as well. In seasonally breeding rodents, testosterone titers are elevated during the breeding season and under long-day photoperiod (LP) conditions, whereas during the non-breeding season and under short-day photoperiod (SP) conditions testosterone titers are at their nadir. In deer mice Peromyscus maniculatus, adult males housed under SP conditions have reduced apical spine density in the CA1 hippocampal region (stratum lacunosum-moleculare) and increased basilar spine density in the CA3 region (stratum oriens) compared to males housed under LP conditions (Pyter et al., 2005). Gonadectomy also reduced the volumetric density of spine synapses (number of spine synapses per μm³) in the CA1 region of the hippocampus by 40%–50% in both adult rats (Kovacs et al., 2003; Leranth et al., 2003) and St. Kitts vervet monkeys (Chlorocebus aethiops sabaenus; Leranth et al., 2004). In the former study, administration of either 500 μg/day of testosterone or its nonaromatizable metabolite, 5α-dihydrotestosterone (DHT), for two days restored the number of spine synapses in GX males; exogenous estradiol (10 μg/day) administration did not (Kovacs et al., 2003; Leranth et al., 2003). Thus, testosterone in males can modulate certain structural components of the hippocampus in a manner similar to estradiol in females through both organizational and activational mechanisms. Moreover, it appears that these effects are mediated through testosterone’s action at androgen receptors (either directly and/or through conversion to DHT) rather than at estrogen receptors via aromatization to estradiol.

3 Effects of Androgens on Non-operant Cognitive Tasks

Although varied, there is increasing evidence that testosterone can mediate performance on cognitive tasks in adult males, particularly those that assess spatial and working memory. For instance, in a spontaneous novel-
object recognition test that measures a form of non-spatial working memory (Ennaceur and Delacour, 1988), both GX male rats implanted with a slow-release testosterone pellet (3–4 ng/ml of blood/day) and control (sham-operated) males spent more time exploring a novel object than the familiar object during test trials (Aubele et al., 2008). The greater amount of time spent exploring the novel object is thought to reflect the strength of the memory for the object presented during the sample trial (i.e., the familiar object). In contrast, GX males that did not receive hormone replacement spent an equal amount of time investigating both objects during the test trials. The fact that GX male rats did not discriminate between the novel and familiar object suggests that testosterone facilitates retention of the familiar object. In a similar study, which utilized a modified object-recognition test, GX male rats spent less time in contact with a novel object than did gonadally intact males (Ceccarelli et al., 2001), a finding that further underscores the role of testosterone in facilitating non-spatial working memory.

Testosterone also influences chemosensorily-mediated social memory of novel odors in male golden hamsters Mesocricetus auratus and rats. Over a set of three consecutive test trials, the amount of time both gonadally intact and GX male golden hamsters investigated the odor of a female conspecific was reduced, a result indicative of habituation to the odor (Havens and Rose, 1992). Upon exposure to the odor of a novel female on the fourth trial, intact males spent a greater amount of time investigating the novel odor than they did investigating the odor of the first female in which they were exposed to during the third trial. In contrast, male hamsters that were gonadectomized for three weeks prior to testing displayed poor retention of the familiar female’s odor but males tested three months after gonadectomy did not. Interestingly, when male golden hamsters were habituated to a non-sex related odor (pine shavings), both GX and intact juveniles displayed preferences for the familiar odor over a novel odor (cedar shavings; Cornwell-Jones and Kovanic, 1981). Gonadectomized adult male hamsters also preferred the familiar odor over the novel odor, whereas adult intact males and adult GX males implanted with a 16 mm (but not 4 mm) testosterone capsule showed no preference for either odor. In a third study, adult male rats were first exposed to either a male juvenile conspecific or its urine, and then re-exposed 10 min later to the same conspecific, its urine, or the urine from a novel conspecific. Upon re-exposure to the stimuli, gonadally intact males spent more time investigating the urine from a novel conspecific than the familiar conspecific or its urine, whereas GX males spent equal amounts of time investigating the two urine samples (Sawyer et al., 1984). Together, these studies demonstrate testosterone’s involvement in maintaining memory for opposite- and same-sex conspecific odors, and increasing the exploration of areas containing novel odors, both of which could be advantageous from an ecological standpoint. For instance, exploring novel environments and investigating novel female odors could increase a male’s reproductive success.

The influence of testosterone on spatial working memory in rodents is often assessed with various maze tasks, and gonadectomy generally disrupts performance of these tasks. For instance, GX male rats responding in a traditional 8-arm radial maze required a greater number of trials to meet the initial learning criterion (Harrell et al., 1990), and consistently made more arm-choice errors during acquisition of the task than intact male rats (Daniel, Winsauer, and Moerschbaecher, 2003; Spritzer, Gill, Weinberg, and Galea, 2008; Spritzer, Daviau, Coneeny, Engelman, Prince, et al., 2011). Similarly, GX male rats responding in a modified version of the 12-arm radial maze made more working memory errors than gonadally intact males throughout training (Gibbs and Johnson, 2008), and emitted a greater number of errors during the acquisition of a cross-maze task compared to intact male subjects (Lagunas et al., 2011). In another spatial memory task, the Morris water maze, GX male rats required more time to find the hidden platform (latency period) relative to those that received saline, an indication of compromised spatial memory (Khalil et al., 2005).

Testosterone also mediates delay-dependent memory (spatial working memory assessed after increasing delays) in male rats as indicated by impairments in spatial memory retention after a 60-min (but not a 10-min) delay interval in GX male rats responding on a delayed-matching-to-place (DMTP) version of the water maze task (Sandstrom et al., 2006). Likewise, GX male mice had lower retention scores compared to intact controls following a 60-min but not a 1-min retention interval on a similar version of the DMTP task (Benice and Raber, 2009). Consistent with these findings, gonadectomy impaired delay-dependent memory of adult male rats after a set of intertrial delays ranging from 30–90 seconds on a DMTP version of the T-maze task (Gibbs, 2005), and impaired acquisition of a delayed-alternation task in a T-maze in adult male rats.
relative to intact rats (Kritzer et al., 2001). Thus, a reduction in testosterone titers as a result of gonadectomy can disrupt various spatial working memory processes in male rodents demonstrating that such processes are dependent on the presence of circulating titers of testosterone. These findings parallel the effects of ovariectomy on spatial working memory in female rodents in that the subsequent loss of estrogens after ovariectomy often negatively affects the performance of tasks that are dependent on spatial working memory (e.g., O’Neal et al., 1996; Daniel et al., 1997; Luine et al., 1998; Fader et al., 1999; Wilson et al., 1999; Sandstrom and Williams, 2001).

With one exception (i.e., Gibbs and Johnson, 2008), exogenous testosterone replacement reversed the cognitive impairment(s) resulting from gonadectomy in the studies mentioned above (i.e., Havens and Rose, 1992; Kritzer et al., 2001; Gibbs, 2005; Sandstrom et al., 2006; Aubele et al., 2008; Spritzer et al., 2011). Moreover, exogenous testosterone administered to gonadally intact male rats can also enhance working memory. Some studies have shown, in particular, that testosterone can reverse age-dependent deficits in memory. For instance, chronic administration of testosterone (50 mg pellet) to intact, aged (22 month-old) male rats improved learning and working memory as assessed in a 12-arm water-escape radial maze (Bimonte-Nelson et al., 2003). Chronic, systemic administration of testosterone (25 mg pellet) also improved the acquisition and retention of a foot-shock avoidance task in older (12 month-old) gonadally intact male SAMP8/TaJf (‘senescence-accelerated mouse, P8 strain’) mice (Flood et al., 1995).

Testosterone can also improve the memory of gonadally intact, non-senescence male rats and mice. When administered to intact male rats prior to training, both 20 and 30 mg of testosterone facilitated short-term (10 min) memory, whereas only 30 mg facilitated long-term (24 hr) memory retention in the one-trial passive-avoidance task (Vazquez-Pereyra et al., 1995); a 20-mg dose facilitated extinction of this task as demonstrated in an earlier study (Rivas-Arancibia and Vazquez-Pereyra, 1994). Likewise, in gonadally intact male mice, post-training administration of either testosterone or androstenedione (350 pmol) intracerebroventricularly (icv) improved retention (i.e., fewer trials to reach criterion) in a foot-shock avoidance task compared to mice administered vehicle (Flood et al., 1992). Using a conditioned place-preference paradigm, Alexander and colleagues (1994) showed that intact male rats preferred the side paired with administration of 800 and 1200 µg/kg (but not 400 µg/kg) of testosterone over the side paired with saline. However, endogenous testosterone titers were elevated to supraphysiological levels by all three of these doses of testosterone, suggesting that the reinforcing effects of testosterone occur only above baseline titers. This may also be the case in the other studies in which testosterone was administered to gonadally intact males; thus, the results of these studies need to be interpreted with caution.

Photoperiodically-induced variation in endogenous circulating testosterone titers also provides evidence, albeit indirect, for the role of testosterone in mediating learning and memory in males. Studies involving two species of Peromyscus suggest a positive correlation between photoperiodically-induced variation in endogenous circulating testosterone titers and spatial memory. For instance, in two separate populations of deer mice, adult males housed under a LP condition (high testosterone titers) displayed greater acquisition of the Morris water maze task relative to males housed under a SP condition (low testosterone titers; Galea et al., 1994). Young (3-3.5 month) LP deer mice also displayed greater acquisition and memory retention in the Morris water maze relative to their SP male counterparts (Perrot-Sinal et al., 1998). Similar seasonal variation in long-term spatial learning and memory was exhibited by adult male white-footed mice P. leucopus. Short-photoperiod male mice responding in a Morris water maze task displayed longer latencies and path lengths to reach a hidden platform after seven and eight blocks of trials (Pyter et al., 2005). Likewise, SP males had longer latencies and path lengths on the third block of trials during training, and spent more time in the previously reinforced maze quadrant during a reversal learning trial.

Both LP and SP white-footed mice were also tested for seasonal variation in cued and contextual conditioning memory (nonspatial) using a passive-avoidance task. Short-photoperiod males exhibited shorter crossover latencies after conditioning than LP males; however, there is no indication if these data were significantly different (Pyter et al., 2005). In a follow-up study, Pyter and colleagues (2006) demonstrated that an LP equivalent dose of testosterone administered to SP GX white-footed mice attenuated these spatial memory deficits. Interestingly, no relationship between circulating plasma testosterone levels and spatial memory was shown in adult male meadow voles Microtus pennsylvanicus responding in the Morris water maze (Galea et al., 1995) even
though meadow voles are also seasonal breeders (Dark et al., 1983; Kerbeshian et al., 1994). Overall, these findings suggest that photoperiodic-induced variations in spatial learning and memory are positively correlated with changes in testosterone titers. Unfortunately, these studies only provide indirect evidence for this hypothesis; therefore, more work is warranted in order to confirm a direct correlation between photoperiodic variation in testosterone levels and cognition.

In contrast to the beneficial effects of testosterone, other studies report that testosterone either impaired or had no effect on learning and memory in males. For instance, not only did chronic administration of exogenous testosterone (2.5 mm capsule) not reverse age-dependent deficits in spatial learning or memory retention in older (31 mo) intact male rats responding on the Morris water maze task, but testosterone impaired the retention (but not learning) of spatial information in young (4.5 mo) and middle-aged (20 mo) males (Goudsmit et al., 1990). Intrahippocampal administration of testosterone also impaired spatial learning and retention in intact male adult rats responding in the Morris water maze (Naghdi et al., 2001). Although the lowest dose of testosterone tested (20 μg) did decrease path length to the hidden platform on the first day, the two highest doses (40 and 80 μg) actually increased the path length on the third day; the highest dose also decreased time spent in the target quadrant across all days (Naghdi et al., 2001). In this same study, administration of either 5 or 10 μg of flutamide, an androgen receptor antagonist, decreased time spent in the target quadrant and increased path length on days 2 and 3 (Naghdi et al., 2001). When testosterone was injected directly into the basolateral nucleus of the amygdala, only the highest dose tested (120 μg) increased the escape latency and path length to the hidden platform (Naghdi et al., 2003). This same dose of testosterone also decreased the number of entrances to, and the time spent in, the target quadrant, whereas no dose of flutamide tested (2, 5, 10, 20, 40 μg/0.5 μl) affected spatial learning or memory.

Systemic administration of exogenous testosterone (5 mg/kg) to intact male rats also impaired both the acquisition and retention of a conditioned active-avoidance task (Fedotova, 1999).

A lack of difference in the acquisition or retention of reference memory (memory for information consistent across trials or sessions) between GX and intact males has been reported in rats responding on both the visible and hidden platform versions of the Morris water maze task (Sandstrom et al., 2006; Spritzer et al., 2008; Benice and Raber, 2009), the 8-arm radial maze task (Spritzer et al., 2011), and a modified version of the 12-arm radial maze (Gibbs and Johnson, 2008). Acquisition of a DMTP T-maze task (Gibbs, 2005), an active avoidance task (Scouten et al., 1975), and a passive avoidance task (Benice and Raber, 2009) was also comparable between gonadally intact and GX male rats. The performance of LP white-footed male mice in a passive-avoidance task was unaffected by gonadectomy as well (Pyter et al., 2006). Also, 4-month old SAMP8/Tajf male mice showed no impairments in the acquisition or retention of a foot-shock avoidance task after gonadectomy (Flood et al., 1995), and GX C57BL/6J male mice did not differ from intact mice responding on a novel object recognition task (Benice and Raber, 2009). Furthermore, chronic administration of testosterone (50 mg pellet) to intact, aged (22 month-old) male rats did not improve reference memory as assessed in a 12-arm water-escape radial maze (Bimonte-Nelson et al., 2003), and had no effect on the acquisition or retention of a conditioned passive-avoidance task in intact adult male rats (5 mg/kg; Fedotova, 1999). Likewise, chronic administration of testosterone to adult, gonadally intact males did not alter delayed nonmatching-to-sample (DNMTS) behavior (7.5 mg/kg; Smith et al., 1996) or behavior in the radial-arm maze (Knoth et al., 1993; Gibbs and Johnson, 2008).

Overall, the current literature indicates that testosterone plays a significant role in modulating spatial and working memory in male rodents. However, the effects of testosterone on these non-operant tasks vary in both direction and magnitude across studies. Such inconsistencies may be explained, at least in part, by differences in animal strain, dosing regimen (i.e., acute vs. chronic treatment), the dose of hormone administered, or the age of the animal subjects. In addition, the nature of the task employed may be important as testosterone appears to have differential effects on working and reference memory tasks much like estrogen does in females. Because of the limited amount of data in males, further work is necessary to better understand the basis of such variation in the reported effects of testosterone on learning and memory.

4 Effects of Androgens on Nonspatial Operant Tasks

The results from the small number of studies that utilized operant or schedule-controlled behavior in determining the effects of testosterone on learning and
memory in males are as varied as those studies investigating spatial working memory. For instance, in a study by Milner (1976), GX male rats were impaired in their ability to acquire the Sidman avoidance task; however, McCord et al. (1979) found no differences in the ability of GX males, intact males, and GX males receiving testosterone replacement (10 mg/day for 8 days) to acquire the same task despite the supraphysiological levels of circulating testosterone with this dosing regimen. Similarly, Kritzer and colleagues (2007) reported that in male rats, gonadectomy hindered the acquisition of responding under a differential-reinforcement-of-low-rate schedule (DRL) compared to intact males and GX males receiving testosterone replacement (3–4 ng/ml of blood). However, in an earlier study, Beatty (1973) found no differences between GX males and intact male rats responding on the same task. Although these two studies differed with regard to the DRL schedule, DRL-12 compared to DRL-20, it is unlikely that this is the source of discrepancy in the findings. Gonadectomy also decreased the response rate of male rats during the acquisition of different random-ratio schedules of reinforcement (Heinsbroek et al., 1987) and the accuracy of responding during the acquisition of a delayed two-lever alternation task compared to intact males (van Hest et al., 1988). The shorter crossover latencies in a step-through inhibitory avoidance procedure by GX male rats compared to gonadally intact and testosterone treated (10 mm capsule) males suggests that testosterone may also be important in mediating avoidance conditioning (Frye and Seliga, 2001; Frye et al., 2004; Edinger and Frye, 2007).

In other studies, testosterone had little or no effect in males responding on non-spatial tasks. For instance, in a two-lever attention task that required males to discriminate between the presence or absence of a visual signal, gonadectomy did not disrupt this discrimination (Johnson and Burk, 2006). In the same study, however, attentional processing was disrupted when exogenous testosterone was administered to gonadally intact males. More specifically, 0.5 mg/kg of testosterone decreased accuracy (percent correct rejections) on “non-signal” trials independent of the presence of a distractor, and 0.1 mg/kg of testosterone decreased the latency to retrieve the reinforcer (photocell latency). The 0.5-mg/kg dose of testosterone also decreased the photocell latency, but the difference from control was not statistically significant. The authors suggested that these latter findings were due to the supraphysiological levels of testosterone that occurred as a result of testosterone administration to gonadally intact males. Neither gonadectomy nor testosterone replacement in GX male rats affected acquisition of a configurial association negative patterning task (Gibbs, 2005) or response rates in an operant task sensitive to perseveration and response stereotypy (van Hest et al., 1989). Similarly, the effects of gonadectomy on responding under a light-dark discrimination, a matching-to-position procedure, and a nonmatching-to-position procedure were not significantly different from intact males, and the effects on responding under a response-alternation procedure and a progressive-ratio schedule only approached significance in a recent study by Kritzer et al. (2007). Such an overall lack of consistent effects across operant procedures suggests a type of task-specific sensitivity for the effects of testosterone after gonadectomy and/or that the establishment of strong stimulus control under these operant tasks could mitigate the influence of testosterone.

5 Testosterone or Its Metabolites?

Adding to the difficulty in ascertaining the mechanisms by which testosterone affects learning and memory is that testosterone is readily metabolized by the 5α-reductase enzyme to DHT, which also binds to androgen receptors (see Andriole, Bruchovsky, Chung, Matsumoto, Rittmaster, et al., 2004), and is reported to have higher affinity (Wilbert et al., 1983) and efficacy at androgen receptors than testosterone (Deslypere et al., 1992). Thus, there is the possibility that testosterone could affect cognitive functioning not only directly but also indirectly via conversion to an active metabolite such as DHT. The few studies that have investigated DHT’s effects on learning and memory show that its effects vary and depend on the task employed. For example, DHT (50 mg pellet/60 day release) was ineffective at improving the spatial or reference memory of aged male rats in a water-escape version of the 8-arm radial water maze (Bimonte-Nelson et al., 2003) or at enhancing responding in a cued and contextual fear-conditioning procedure in young, GX male rats (1 mg/kg DHT; Edinger et al., 2004). Also, when a visual distractor was present, administration of 0.5 mg/kg of DHT (but not 0.1 mg/kg) decreased accuracy on no-signal trials in intact males discriminating between a visual signal and no signal in a two-lever attention task (Johnson and Burk, 2006).

On the contrary, other studies have shown a more positive effect of DHT on learning and memory. Chronic DHT administration (2.5 cm capsule) attenuated working memory deficits in GX male mice as as-
sessed in the DMTP water maze task, but only when tested after a 24-hour retention interval (Benice and Raber, 2009). Gonadally intact male mice receiving post-training DHT administration (iv; 350 pmol) also had greater retention (i.e., fewer trials to reach criterion) in a foot-shock avoidance task than mice administered vehicle (Flood et al., 1992). Likewise, DHT administered either systemically (1 mg/kg; 10 mm capsule) or intrahippocampally (1200 ng) into the dorsal hippocampus was nearly or just as effective as testosterone and 3α-androstenediol (a metabolite of DHT) at attenuating gonadectomy-induced impairments in crossover latencies in a inhibitory avoidance task (Edinger and Frye, 2004; Edinger et al., 2004; Frye, Edinger et al., 2004; Edinger and Frye, 2007). Interestingly, 3α-androstenediol (1 mg/kg) increased the cross-over latency in young adrenalectomized, GX male rats responding in a shuttlebox avoidance task (Frye and McCormick, 2000), and was more effective than testosterone or DHT in establishing a conditioned place preference (see Rosellini et al., 2001; Frye et al., 2002).

Testosterone may also mediate learning and memory through aromatization to estradiol by the aromatase enzyme (Naftolin, 1994). The effect of estradiol on learning and memory in males has been reported to range from enhancement to impairment of responding on certain tasks. For instance, estradiol attenuated the acquisition deficits shown after gonadectomy in male rats responding under a DRL schedule (slow-release pellet; Kritzer, Brewer, Montalmaant, Davenport, and Robinson, 2007) and enhanced acquisition of a delayed matching-to-position task (5 mm capsule; Gibbs, 2005). Also, administration of estradiol to young, GX male rats and intact aged males modestly improved responding in an 8-arm radial maze task, but only after delays of 1–3 hours were instituted (5 mm capsule; Luine and Rodriguez, 1994). Similarly, 0.32 μg of estradiol and 2 mg/kg of diarylpropionitrile, a selective estrogen receptor β agonist, improved the acquisition of GX males responding on a cross-maze task (Lagunas et al., 2011). Intrahippocampal administration of 1 μg estradiol post-training has also been shown to decrease the latency of gonadally intact male rats to find a submerged platform in the Morris water maze when tested after a delay of 24-hr, but not 2-hr (Packard et al., 1996). When estradiol (0.2–1.2 mg) was administered systemically to intact male rats responding in a one-trial passive-avoidance procedure prior to the training session, only 0.4 mg of estradiol facilitated short-memory (10 min) and only the highest dose (1.2 mg) facilitated long-term (24 hr) retention (Vazquez-Pereyra et al., 1995); 0.8 mg facilitated extinction of this task (Rivas-Arancibia and Vazquez-Pereyra, 1994).

In contrast, estradiol (5 mm capsule) had no effect on delay-dependent working memory in GX male rats responding in the delayed matching-to-position task (Gibbs, 2005). Estradiol replacement (20 μg/0.05 ml) also had no effect on spatial discrimination in GX wild type and estrogen receptor α knockout male mice responding in the Morris water maze (Fuggeret al., 1998). Likewise, estradiol (slow-release pellet; 25 pg estradiol/ml blood) failed to attenuate acquisition deficits produced by gonadectomy in a delayed-alternation task in a T maze (Kritzer et al., 2001), and the administration of estradiol to intact male rats did not improve male-appropriate responding over two trials in the Tolman sunburst maze (Dawson, 1972). Lastly, in a novel object recognition test, both GX males and GX males receiving estradiol replacement (25 pg/ml of blood/day) spent an equal amount of time investigating both the novel and familiar objects unlike intact males and GX males with testosterone replacement that spent more time exploring the novel object (Aubele et al., 2008).

6 Gonadal Hormones and the Cholinergic System

The neurotransmitter, acetylcholine (ACh), has been implicated in the modulation of various learning and memory processes by a large number of studies (for review see Everitt and Robbins, 1997; Gold, 2003; Hasselmo, 2006). In general, increased ACh release in areas of the brain such as the hippocampus and prefrontal cortex is associated with enhanced responding under various learning and memory tasks (e.g. Orsetti et al., 1996; Stancampiano et al., 1999; Fadda et al., 2000; Hironaka et al., 2001; Arnold et al., 2002; McIntyre, Pal et al., 2002), whereas decreased synaptic levels of ACh are associated with reduced responding (e.g. Leanza et al., 1996; Shenet al., 1996; Vnek et al., 1996; McDonald et al., 1997; Lehmann et al., 2002). In spite of a paucity of data, there is evidence, although sometimes contradictory, that testosterone in males interacts with the cholinergic system to mediate learning and memory. Similar to the effects of estrogen on learning and memory in females (e.g. Luine, 1985; Gibbset al., 1994; Singhet al., 1994; Gibbset al., 1997), the effects of testosterone in males are likely mediated largely through potentiation of cholinergic neurotransmission in the septo-hippocampal pathway and other related
brain areas (see Mitsushima et al., 2009; Mitsushima, 2010). Support for this interaction comes primarily from studies that measured changes in the levels of two common markers of cholinergic activity and function, the enzymes choline acetyltransferase (ChAT), which synthesizes ACh from acetyl-CoA and choline, and acetylcholinesterase (AChE), which metabolizes ACh into acetate and choline. Data from such studies indicate that ACh release is positively correlated with ChAT activity levels and negatively correlated with those of AChE.

Regarding the relationship between ChAT and testosterone, James and Kanungo (1978) demonstrated an age-dependent effect of gonadectomy on ChAT activity in three groups of male rats ranging in age from 8 to 80 weeks. In each group studied, gonadectomy significantly reduced the specific activity of ChAT in the cerebral hemisphere, whereas administration of either 100 or 1000 μg/kg of testosterone to young (8 week) GX male rats elicited an increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70% increase in ChAT activity; only the 100-μg dose was effective in adult (40 week) and old (80 week) males. Administration of 127.3 μg/kg of testosterone to sexually mature GX male rats for 7 days also resulted in a 70%

In addition to demonstrating that gonadectomy reduced ChAT activity levels in the cerebral hemisphere of male rats, James and Kanungo (1978) showed that gonadectomy also altered AChE activity levels in an age-dependent manner. That is, the specific activity of AChE was reduced after gonadectomy in the cerebral hemisphere of young (8 week) and adult (40 week) males, but not old (80 week) males. The lack of reduction in AChE activity in the group of old males after gonadectomy was likely a result of the already low levels of AChE due to aging. After administration of a single dose of 0.1 mg/kg of testosterone, AChE activity levels were increased in the group of old males, but decreased in the young males; both 0.1 and 1 mg/kg of testosterone increased AChE activity levels in the group of adult males (James and Kanungo, 1978). Likewise, AChE activity levels were reduced in the sexually dimorphic area of the MPOA-anterior hypothalamus of adult, male Mongolian gerbils Meriones unguiculatus after gonadectomy (Commins and Yahr, 1984), and exogenous testosterone replacement (three 10 mm capsules) restored these AChE activity levels to baseline. Findings by Libertun and colleagues (1973) also indicate that the specific activity of AChE is reduced in the preoptic suprachiasmatic area, but not the cerebral cortex or arcuate-mammillary area, of male rats that were gonadectomized at birth. Based on these findings, testosterone may be increasing cholinergic function by enhancing ACh release and thereby producing a compensatory increase in AChE activity. However, we recently demonstrated that GX male rats had higher activity levels of AChE than gonadally intact males in the striatum and hippocampus, two brain regions integral for learning and memory (Leonard et al., 2010). Activity levels in the prefrontal cortex were also higher in GX males than in gonadally intact males, but this result only approached statistical significance. Thus, a loss of testosterone after gonadectomy can decrease AChE activity.
levels in certain areas of the brain, while increasing its activity in others areas, a finding that mirrors the effects of testosterone on ChAT levels.

7 The Interactive Effects of Testosterone and the Cholinergic System on Behavior

The literature regarding the interaction of testosterone with the cholinergic system on complex behavior is very limited and often difficult to interpret. Besides demonstrating that gonadectomy decreases arm-choice accuracy in male rats responding on an eight-arm radial maze task, data from our laboratory show that gonadectomy increases the disruptive effects of two cholinergic antagonists in males responding on this task (Daniel et al., 2003). More specifically, compared with intact males, GX males were more sensitive to the dose-dependent error-increasing effects produced by scopolamine, a muscarinic antagonist, and mecamylamine, a nicotinic antagonist; however, the rate of arm entry was not disrupted by either cholinergic antagonist. This study, which involved explicit memory of spatial orientation and stimuli, suggests that testosterone tonically increases cholinergic function and that the loss of testosterone through gonadectomy potentiated the disruptive effects of two cholinergic antagonists on spatial working memory.

This notion, however, directly contrasts with recent data from our laboratory on the interactive effects of testosterone and scopolamine in male rats responding under an operant learning procedure involving a repeated-acquisition technique (Leonard et al., 2007). The baseline for this study was a multiple schedule comprised of both repeated-acquisition and performance components (Moerschbaecher et al., 1979). During the acquisition components, subjects acquired a different predetermined sequence of responses (of fixed length) each session. During the performance components, the same subjects were reinforced for responding on an invariant sequence of responses, and responding in this component served as a behavioral control for the non-specific effects of either drug or hormone administration. In this study, gonadectomy alone did not disrupt rate or accuracy of responding of adult male rats in either component. However, males were less sensitive to the scopolamine-induced (0.1–0.32 mg/kg) disruptions of response rate and accuracy after gonadectomy than after receiving chronic testosterone replacement (30 mm capsule). In addition to showing these changes in sensitivity to scopolamine using a within-subject design, we have shown that GX males are less sensitive to scopolamine than gonadally intact males (Leonard et al., 2007). These results suggest that testosterone replacement can enhance scopolamine-induced behavioral effects in GX male rats responding under a multiple schedule of repeated acquisition and performance, a finding that conflicts with results previously found for males responding on a spatial task (i.e., Daniel et al., 2003). Furthermore, these findings for males responding under the multiple schedule suggest that testosterone may decrease the activity of the cholinergic system during nonspatial tasks and thereby work in concert with the antagonism produced by scopolamine. This interpretation would also be consistent with findings from Johnson and colleagues (2006) who found that scopolamine (0.1 and 0.2 mg/kg) was more disruptive in intact male rats than GX males responding in a two-lever attention task that required discrimination of visual signals and non-signals.

The likelihood that the interactive effects of testosterone and scopolamine in males were mediated by testosterone’s conversion to one of its two main metabolites (DHT or estradiol) was also investigated by our laboratory. Using a within-subjects design, we demonstrated that adult male GX rats responding under a multiple schedule were less sensitive to the rate-decreasing and error-increasing effects of scopolamine (0.1–1 mg/kg) than gonadally intact males and GX males with DHT replacement (30 mm capsule; Leonard, Moerschbaecher, and Winsauer, 2008). In contrast, scopolamine-induced disruptions of both rate and accuracy were the same, or larger, in GX males that received chronic estradiol replacement (5 mm capsule) than in intact males. Thus, testosterone’s interactive effects with the cholinergic system may not be mediated by androgen receptors as DHT was less effective than testosterone at enhancing scopolamine’s rate-decreasing and error-increasing effects. Instead, testosterone’s interactive effects with the cholinergic system on learning in male rats may be mediated, in part, by estrogen receptors following the aromatization of testosterone to estradiol, and is dependent on the type of task (i.e., spatial vs. nonspatial). Estradiol’s interaction with the cholinergic system in males is further supported by data involving the Morris water maze, which demonstrate that the memory enhancing effect of intrahippocampally administered estradiol can be blocked by the systemic administration of a subeffective dose of scopolamine (Pack-
ard et al., 1996).

If testosterone is decreasing cholinergic activity during certain behavioral tasks in the CNS of male rats, then increasing levels of ACh should be disruptive to those behaviors. In general, low doses of donepezil, an AChE inhibitor that blocks the synaptic degradation of ACh through inhibition of AChE, enhance memory and improve responding on spatial and working memory tasks in compromised male rodents (e.g., aged, pharmacologically-impaired, or lesioned subjects), whereas larger doses are ineffective or produce impairments (for review see van der Staay and Bouger, 2005; Wise et al., 2007; Yoo et al., 2007; Yuede et al., 2007). Chronic administration of donepezil (0.5 mg/kg for three weeks, then 0.2 mg/kg for one week) in uncompromised male rats has also been shown to enhance acquisition and performance on several spatial and nonspatial tasks including a Morris water maze task, serial learning task, and radial arm maze task (Cutuli et al., 2008). Doses of 0.3 and 1 mg/kg of donepezil were also reported to decrease the number of errors emitted by male rats responding in a two-phase radial-arm maze procedure (Wise et al., 2007), whereas a similar dose range of donepezil (0.03–1 mg/kg) did not have an effect on males responding in a delayed matching-to-position task (Poorheidari et al., 1998). Hence, although donepezil has been shown to improve responding in both compromised and uncompromised animals, its effects may also be task- and dose-dependent, as it does not uniformly improve all learning and memory tasks (e.g., spatial vs. operant).

In a recent study from our laboratory involving intact and hormonally-manipulated male rats, low doses of donepezil (0.56 mg/kg–1.8 mg/kg) had little or no effect in several hormonally-manipulated groups of males compared with the respective control injections of saline (Leonard et al., 2010). As the doses of donepezil increased (3.2–5.6 mg/kg), however, dose-dependent rate-decreasing and error-increasing effects occurred in both the acquisition and performance components of a multiple schedule, suggesting a threshold above which an increase in ACh is disruptive to behavior. These disruptive effects on responding under a repeated-acquisition procedure were also consistent with the effects of other cholinesterase inhibitors in both adult male rats (Howard and Pollard, 1983) and nonhuman primates (i.e., M. mulatta and M. fascicularis; Anger and Setzer, 1979; Frederick et al., 1995). More important, the higher doses of donepezil (3.2 and 5.6 mg/kg) were more disruptive in GX male rats than in intact or GX males that received chronic testosterone replacement (Leonard et al., 2010), which supports the notion that testosterone may be tonically inhibiting cholinergic activity in some behavioral tasks. Interestingly, GX males that received chronic estradiol replacement had a higher baseline (daily) rate of errors in the acquisition component, and displayed greater sensitivity to the rate-decreasing and error-increasing effects of donepezil in both components of the multiple schedule task than gonadally intact males. Thus, testosterone attenuated the disruptive effects of donepezil, possibly by dampening the increase in ACh that results from donepezil’s inhibition of AChE activity. In addition, estradiol enhanced the disruptive effects of donepezil, possibly by increasing ACh to levels above basal levels, at least in males.

8 Conclusions

The exact mechanisms by which the gonadal hormones mediate cognitive processes in males are still not fully understood. However, there is ample evidence that testosterone directly, and indirectly through its metabolites DHT and estradiol, modulates various learning and memory tasks likely through interaction with the cholinergic system. Similar to the effects of estradiol in females, the effects of testosterone in males are not comparable across all cognitive tasks and can differ among studies utilizing the same task. It is likely that testosterone differentially affects behavior depending on the subject’s current hormonal state, the response required, and the stimuli involved (e.g., those involving spatial or nonspatial stimuli). In addition, certain behavioral tasks may be more difficult to disrupt after strong stimulus control has been established.

These findings are clinically significant in that hormone replacement has been touted as a potential means for abrogating the effects of age and age-related hormonal decline, and hormone deprivation therapy has become a primary means of treating a variety of cancers including those of the breast (see Howel et al., 2004; Brown and Davidson, 2006) and prostate (see Miamoto et al., 2004; Loblaw et al., 2007). Furthermore, recent studies indicate that androgens can influence the pathogenesis of Alzheimer’s disease in males and that low circulating testosterone titers appear to be correlated with an increased risk of these types of neurodegenerative diseases (for review see Rosario and Pike, 2008; Drummond et al., 2009). Despite data indicating the involvement of the gonadal hormones in these conditions and diseases, caution with chronic androgen supplementation is recommended until there is a greater
understanding of its overall effects on cognition and behavior. Such an approach would seem warranted as research on the effects of the androgens indicates that testosterone may not be uniformly beneficial to all complex behavioral processes requiring learning and memory. Lastly, future research should also begin to address more fully the role of testosterone in mediating learning and memory in females especially because of the increasing clinical use of testosterone to stimulate libido in post-menopausal women, and the fact that relatively little is known regarding the effects of testosterone on cognition in women.

References


Andreano JM, Cahill L, 2009. Sex influences on the neurobiology of learning and memory. Learn. Mem. 16: 248–266.


Frye et al., 2001. Testosterone increases analgesia.


Edinger et al., 2004. Testosterone’s analgesic, anxiolytic, and cognitive-enhancing effects may be due in part to actions of its 5alpha-reduced metabolites in the hippocampus. Behav. Neurosci. 118: 1352–1364.

Edinger et al., 2007. Androgens’ effects to enhance learning may be mediated in part through actions at estrogen receptor-beta in the hippocampus. Neurobiol. Learn. Mem. 87: 78–85.


Frye et al., Seliga, 2001. Testosterone increases analgesia,


Leonard ST, Moorschaebecjer JM, Winsauer PJ, 2008. Estradiol replacement in gonadectomized male rats alters scopol-


Tabori NE, Stewart LS, Znamensky V, Romeo RD, Alves SE et al., 2005. Ultrastructural evidence that androgen receptors are located at extranuclear sites in the rat hippocampal formation. Neuroscience 130: 151–163.


