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Testosterone and photoperiod interact to affect spatial learning and memory in adult male white-footed mice (*Peromyscus leucopus*)

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Abstract

Gonadal hormones affect spatial learning and memory in mammals and circulating gonadal hormone concentrations fluctuate by season. Most nontropical rodents are spring/summer breeders and males display higher testosterone concentrations during the breeding season compared with the nonbreeding season (fall/winter). Seasonal patterns of testosterone concentration (as well as many other seasonal modifications of physiology, morphology, and behaviour) are induced by manipulation of photoperiod (day length; i.e. short or long days) in the laboratory. Coincident with reducing testosterone concentration, short days also impair spatial learning and memory performance in male white-footed mice (*Peromyscus leucopus*) compared with long days. We hypothesized that short-day-induced reduction of testosterone concentrations inhibits spatial learning and memory performance compared with long days. Adult male white-footed mice were maintained in long (16 h light/day) or short (8 h light/day) days for 14 weeks following sham-castration, castration plus saline implant, or castration plus testosterone implant treatment. Spatial learning and memory was assessed using a water maze, and photoperiod-evoked changes in gene expression of sex steroid receptors within the hippocampus were also examined. Castrated, short-day mice with testosterone replacement displayed enhanced water maze performance compared with other short-day mice, but no differences among testosterone treatments were observed in long-day mice. Photoperiod did not affect hippocampal androgen, oestrogen α, or oestrogen β receptor gene expression. These results suggest that photoperiod modulates the effects of testosterone on spatial learning performance by mechanisms indirect of the hippocampus.

Introduction

Sex differences in rodent spatial learning and memory favours males (Galea *et al*., 1995; Galea *et al*., 1996). These sex differences are usually specific to polygynous as compared to monogamous species, and are therefore hypothesized to benefit the territorial navigation and mate searching characteristic of polygynous males (Sherry *et al*., 1992). Gonadal hormones may mediate sex differences in learning and memory. Testosterone injections facilitate water maze performance in male rats (Vazquez-Pereyra *et al*., 1995); testosterone implants enhance spatial memory performance in castrated male zebra finches (*Poephila gattata*; Oberlander *et al*., 2004). However, no differences in water maze learning performance are observed in male meadow voles (*Microtus pennsylvanicus*) with high vs. low testosterone concentrations (Galea *et al*., 1995). In comparison with activational effects, stronger evidence for organizational effects of testosterone on adult spatial learning and memory exists (Dawson *et al*., 1975; Isgor & Sengelaub, 1998; Goto *et al*., 2005). For example, individual meadow voles (both male and female) from male-biased litters (i.e. high *in utero* testosterone exposure) perform better at the spatial water maze task than voles from female-biased litters (Galea *et al*., 1994b).

In addition to sex differences in spatial learning and memory, seasonal differences in learning and memory performance may also exist. Seasonal changes in morphology, physiology, and behaviour allow animals to survive environmental changes and to anticipate appropriate environmental conditions. For example, most mammals use day length (photoperiod) to time mating such that the birth of offspring coincides with mild temperatures and abundant food (i.e. spring/summer; Bronson, 1985; Bronson & Heideman, 1994). Nontropical rodents usually breed and give birth within the same spring/summer and suspend reproductive activities during the winter (Prendergast *et al*., 2002). In the laboratory, photoperiod manipulation (i.e. short vs. long days) alone induces reproductive and other biological alterations. Short days inhibit testosterone production and gonadal size in male rodents. Regarding learning and memory behaviours, grey squirrels (*Sciurus carolinensis*) and black-capped chickadees (*Parus atricapillus*) display seasonal differences in spatially dependent food-caching behaviour (Thompson & Thompson, 1980; Smulders *et al*., 1995). In two mouse species (*Peromyscus maniculatus* and *P. leucopus*), long-day males (in reproductive condition with high testosterone concentrations) perform better at the spatial water maze task and have larger hippocampi than short-day males (in the nonreproductive condition with low testosterone concentrations; Galea *et al*., 1994a; Perrot-Sinal *et al*., 1998; Pyter *et al*., 2005c). Enhanced spatial learning and memory in breeding male rodents compared with nonbreeding males is consistent with the hypothesis that spatial learning and memory is advantageous for territorial navigation and mate searching (Jacobs, 1996). However the possibility that seasonal changes in testosterone may mediate these

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seasonal differences in spatial learning and memory has not been directly tested.

If testosterone mediates the photoperiodic differences in spatial learning and memory, then castration should impair spatial learning and memory performance in long-day mice and testosterone replacement should enhance performance in short-day mice. We also tested whether photoperiod alters gene expression of sex steroid receptors within the hippocampus that mediates the putative photoperiod-provoked modulation of testosterone on learning and memory.

Materials and methods

Animals

Seventy-one adult (> 55 days of age) male white-footed mice (*Peromyscus leucopus*) from our breeding colony were used in these experiments. Siblings were pseudo-randomly distributed among all groups. Animals were housed individually in polypropylene cages (27.8 × 7.5 × 13 cm) with a constant temperature and humidity of 21 ± 5 °C and 50 ± 5%, respectively, and *ad libitum* access to food (Harlan Teklad 8640 rodent diet, Indianapolis, IN, USA) and filtered tap water. Mice were either housed in long-day conditions [LD; 16 h light/day; lights illuminated at 23:00 h Eastern Standard Time (EST)], or in reversed short-day conditions (SD; 8 h light/day; lights illuminated at 07:00 h). All studies were conducted with approval of the Ohio State Institutional Animal Care and Use Committee and were conducted in compliance with all US federal animal welfare requirements. Mice were anesthetized for surgery with isoflurane vapours (Abbott Laboratories, North Chicago, IL, USA). For tissue collection, mice were decapitated.

Experiment 1. Effects of photoperiod and testosterone manipulation on spatial learning and memory

Forty-seven mice were used in this experiment. Mice from both photoperiod treatments were divided into three surgical groups: sham-castration (SHAM; LD $n = 9$; SD $n = 7$), bilateral castration plus empty implant (CAST; LD $n = 8$; SD $n = 8$), or bilateral castration plus testosterone proprionate (Sigma-Aldrich, St. Louis, MO, USA) implant (CAST + T; LD $n = 7$; SD $n = 8$). Implants were soaked in saline 2 h prior to implantation and thus empty implants filled with saline. Implants were designed to mimic long-day-typical testosterone concentrations as previously described (Demas & Nelson, 1998). Mice were allowed approximately one week recovery prior to onset of photoperiod treatment. All mice were exposed to their respective photoperiod conditions for a total of 14 weeks; water maze training occurred during the final two weeks. After the termination of behavioural testing, mice were decapitated and castration was verified. For SHAM mice, testes were removed and weighed.

Water maze

The water maze was used to test long-term spatial learning and memory (Morris, 1984). Testing occurred during the end of the light phase (between 12:00 and 15:00 h EST). The maze consisted of a white tank (1.3 m diameter) filled with 27 °C water to a depth of 47.5 cm. The water was made opaque with white nontoxic tempera paint. The maze was divided into four equal quadrants and release points were designated at each quadrant as N, E, S, and W. Fixed extra-maze cues in the form of large black geometric shapes surrounded the tank. A tracking video camera was suspended from the ceiling above the pool and 2020 PLUS tracking software (HVS Image, Buckingham, UK) was used. Mice were handled using a small fishing net to avoid the stress of direct handling. On day 1, mice were allowed to swim freely for 60 s without a platform to acclimate to the pool. On days 2–5, a platform (9 cm diameter) was hidden 0.5 cm below the water surface in one quadrant. Mice were given two consecutive blocks of trials per day. Each block consisted of three 60-s trials during which the mice were trained to locate the hidden platform from random release points around the pool to 'escape' from the water. Upon reaching the platform or after 60 s, mice were placed on the platform for 10 s and then returned to the home cage. The inter-trial intervals were ∼15 s during which the pool was skimmed of debris. Latency to reach the platform, the distance of the mouse's path, and swim speed were recorded by the system for each trial to assess acquisition of the spatial task. After the last trial of each block, mice received a piece of tissue paper in their cage to expedite drying. On day 6, the platform was removed and a 60-s probe trial was run to examine retention of spatial memory. The per cent time spent in each quadrant (including the quadrant in which the platform had been) was recorded. To evaluate reversal learning, the platform was repositioned in a different quadrant and retraining to the new location on days 7–8 (a total of four blocks of trials) was completed as previously described. A second probe trial followed reversal training on day 9. On day 10, a single 60-s visible platform trial was run to determine general visual acuity of the mice in this paradigm. The visible platform (9 cm diameter) was raised 0.5 cm above water level and was encircled with a black rim. Latency to reach the platform was recorded. One mouse (LD CAST) seized when placed in the water and was removed from the study.

Experiment 2. Effects of photoperiod on oestrogen and androgen receptor gene expression in the hippocampus

Twenty-four mice were used in this experiment. All mice were exposed to their respective photoperiod conditions for either 7 ($n = 6$/photoperiod) or 14 ($n = 6$/photoperiod) weeks. These time points were chosen based on the time course of gonadal regression in response to short days (complete by ∼14 weeks) and half-way through gonadal regression (7 weeks) to examine potential photoperiod-evoked changes in brain gene expression (Pyter *et al*., 2005a). At 7 or 14 weeks, mice were decapitated, brains were removed, and the hippocampus was dissected and stored in RNALater solution (Qiagen, Valencia, CA, USA) at −70 °C until RNA processing. In addition to directly binding to androgen receptors (AR), androgens can be aromatized to oestrogens within the brain and thereby affect brain function (i.e. behaviour) via oestrogen receptors α and β (ERα and ERβ; Roselli & Resko, 1993). Thus, we tested potential photoperiodic differences of AR, ERα, and ERβ expression in the hippocampus that may underlie putative differences in water maze performance.

RNA extraction

Total RNA was extracted from ≥30 mg of individual hippocampi using a homogenizer (Ultra-Turrax T8, IKA Works, Inc., Wilmington, NC, USA) with an RNeasy Mini Kit according to manufacturer's protocol (Qiagen). Extracted RNA was suspended in 30 μL RNase-free water and RNA concentration was determined using a spectrophotometer (SmartSpec™ 3000, Bio-Rad, Hercules, CA, USA). All RNA samples were stored at −70 °C until further analysis. cDNA was created via reverse transcription of 2 μg of RNA from each sample with MMLV Reverse Transcriptase enzyme (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol.

Gene sequencing

To design primers and a probe for quantitative PCR (qPCR) with high specificity for this species, a portion of each gene of interest was

sequenced. To sequence portions of these genes, semiquantitative PCR was conducted on 1 μL of pooled *Peromyscus* brain cDNA with Taq DNA Polymerase enzyme (Invitrogen) according to the manufacturer's protocol in a thermocycler for 40 cycles (Bio-Rad). Degenerate primers were designed based on conserved regions among multiple species with known gene sequences (GenBank) using PrimerExpress software (Applied Biosystems). PCR gene product amplification was visualized on 2% TAE-agarose gels containing ethidium bromide using a CCD camera. To verify amplification of the correct gene, PCR products were purified (Centricon-100, Millipore, Billerica, MA, USA) and sequenced at the Plant-Genomics Centre at Ohio State University. The resulting amplicon sequences that were >90% homologous to the *Mus* gene of interest were assumed to be the correct *P. leucopus* gene of interest.

qPCR

After confirmation of gene products, primers and probes for quantitative PCR were designed using PrimerExpress. Primers and probes were synthesized as follows, with probes labelled with 6-FAM and MGB (nonfluorescent quencher) at the 5′ and 3′ ends, respectively:

*ER*α forward 5′-GAACAGCCCCGCCTTGT-3′,
*ER*α reverse 5′-GCATCCAGCAAGGCACTGA-3′,
*ER*α probe 5′-TGACAGCTGACCAGATG-3′;
*ER*β forward 5′-GCTGATGTGGCGCTCGAT-3′,
*ER*β reverse 5′-CCCTCATCCCTGTCCAGAAC-3′,
*ER*β probe 5′-ACCACCCTGGCAAGCTCATCTTT-3′;
AR forward 5′-GTGGTGTGTGCTGGACATGAC-3′,
AR reverse 5′-GGCTAGATAACAGGGCAGCAA-3′,
AR probe 5′-ACAACCAACCTGACTCC-3′.

A TaqMan 18S Ribosomal RNA primer and probe set (labelled with VIC; Applied Biosystems) was used to as the control gene for relative quantification. Amplification was performed on an ABI 7000 Sequencing Detection System by using Taqman® Universal PCR Master Mix. The universal two-step RT-PCR cycling conditions used were 50 °C for 2 min, 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative gene expression of individual samples run pseudo-randomly in duplicate was calculated by comparison to a relative standard curve consisting of serial dilutions of pooled *P. leucopus* hypothalamic cDNA (1 : 1, 1 : 10, 1 : 100, 1 : 1000, 1 : 10 000) followed by normalization to *18S rRNA* gene expression.

Statistical analyses

Repeated measures ANOVAs were used to compare water maze performance over days with photoperiod and surgery as variables. Within days, pairwise comparisons were planned *a priori* in the analysis models and were conducted using two-tailed Student's *t*-tests (Keppel & Wickens, 2004). Student's *t*-tests were also used for other behavioural and physiological comparisons. Data with unequal variances were compared using nonparametric Mann–Whitney tests to compare photoperiod differences. All comparisons were considered statistically significant when $P < 0.05$. StatView software was used for all analyses (v. 5.0.1, Cary, NC, USA).

Results

Experiment 1

Short days decreased testes mass (both relative and absolute) compared with long days in SHAM mice (absolute, LD 377.5 ± 27.6 mg; SD 167.8 ± 36.5 mg; relative, LD 17.3 ± 0.9 mg/g body mass; SD 8.6 ± 1.9 mg/g body mass; $t_{13} = 4.6$ and 4.7, respectively, $P < 0.05$ in both cases).

The latency to reach the hidden platform and swim route length decreased over blocks of trials in all groups (Fig. 1; $F_{7,203} = 37.719$ and $F_{7,196} = 25.263$, $P < 0.05$) whereas, swim speed did not change (Fig. 1G–I; $P > 0.05$). Repeated measures tests revealed significant effects of photoperiod on the latency to reach the original hidden platform ($F_{7,203} = 2.303$, $P < 0.05$), but not pathlength ($F_{7,196} = 1.743$, $P = 0.1$) nor swim speed ($F_{7,196} = 0.245$, $P = 0.9$). *Posthoc* analyses revealed that in SHAM mice, short days increased the latency to reach the hidden platform compared with long days on blocks 3 and 5 of the hidden platform training (Fig. 1A; $t_{13} = -1.8$ and -1.7, $P < 0.05$). In long-day mice, the latency to reach the hidden platform did not differ among surgical treatments in any of the blocks of trials (Fig. 1B; $P > 0.05$). In short days, however, CAST + T mice decreased the latency to reach the platform compared with CAST mice on block 5 ($F_{2,20} = 3.4$), compared with SHAM mice on block 3 ($F_{2,20} = 2.8$), and compared with both CAST and SHAM mice on block 7 (Fig. 1C; $F_{2,20} = 4.1$) ($P < 0.05$ in all cases). No significant differences in path length were observed in SHAM mice (Fig. 1D; $P > 0.05$). In long days, CAST + T mice reduced the path length compared with SHAM mice on block 4 (Fig. 1E; $F_{2,13} = 2.9$, $P < 0.05$). Similarly, in short days, CAST + T mice reduced the path length compared with CAST mice on blocks 5–8 (Fig. 1F) ($F_{2,20} = 2.7$, 1.7, 3.2, 2.5, $P < 0.05$ in all cases). Swim speed did not differ among any of the groups (Fig. 1G–I; $P > 0.05$). Mice from all groups persisted in spending more time in the quadrant from which the platform had been removed from the original hidden platform trials (Quadrant 1) compared with other quadrants (Fig. 2; $F_{3,117} = 45.2$, $P < 0.05$ in all cases). No differences in the per cent time spent in Quadrant 1 were observed among groups (Fig. 1J–L; $P > 0.05$).

Throughout reversal training to a hidden platform, latency to reach the hidden platform decreased over blocks of trials in all groups (Fig. 2A–C; $F_{3,117} = 12.402$, $P < 0.05$). Repeated measures tests revealed that significant interactions existed between photoperiod and surgery for the latency to reach the reversal platform ($F_{6,117} = 2.302$, $P = 0.03$) and pathlength ($F_{6,108} = 2.659$, $P = 0.02$), but not swim speed ($F_{6,111} = 0.741$, $P = 0.6$). However, surgery alone affected reversal swim speed ($F_{6,111} = 2.362$, $P = 0.03$). *Posthoc* analyses revealed that in SHAM mice, short-day mice took longer to reach the hidden platform on block 1 of reversal training (Fig. 2A; $t_7 = 2.1$, $P < 0.05$). In long-day mice, testosterone manipulations did not affect the latency to reach the platform (Fig. 2B; $P > 0.05$). However, in short-day mice, CAST + T decreased the latency to reach the platform compared with CAST mice on block 2 ($F_{2,20} = 5.4$), compared with SHAM mice on block 1 ($F_{2,20} = 2.2$), and compared with both CAST and SHAM mice on blocks 3 and 4 (Fig. 2C; $F_{2,20} = 6.4$, 5.2, $P < 0.05$ in both cases). The path length to reach the platform decreased over blocks of reversal trials in all mice (Fig. 2D–F; $F_{6,111} = 2.4$, $P < 0.05$). In SHAM mice and within long-day mice, no differences in path length were observed during reversal training (Fig. 2D–E; $P > 0.05$). However, in short-day mice, CAST + T mice swam a more direct route to the platform compared with CAST mice on blocks 2 and 4 ($F_{2,20} = 5.8$, 5.1) and compared with both CAST and SHAM mice on block 3 (Fig. 2F; $F_{2,20} = 4.5$, $P < 0.05$ in all cases). Swim speed did not differ among any of the groups (Fig. 2G–I; $P > 0.05$). No differences in the amount of time spent among pool quadrants following the reversal probe trial were observed (Fig. 2J–L) ($P > 0.05$). Also, no differences in the latency to reach the visible platform during the visible platform trial were observed among groups (data not shown; $P > 0.05$).

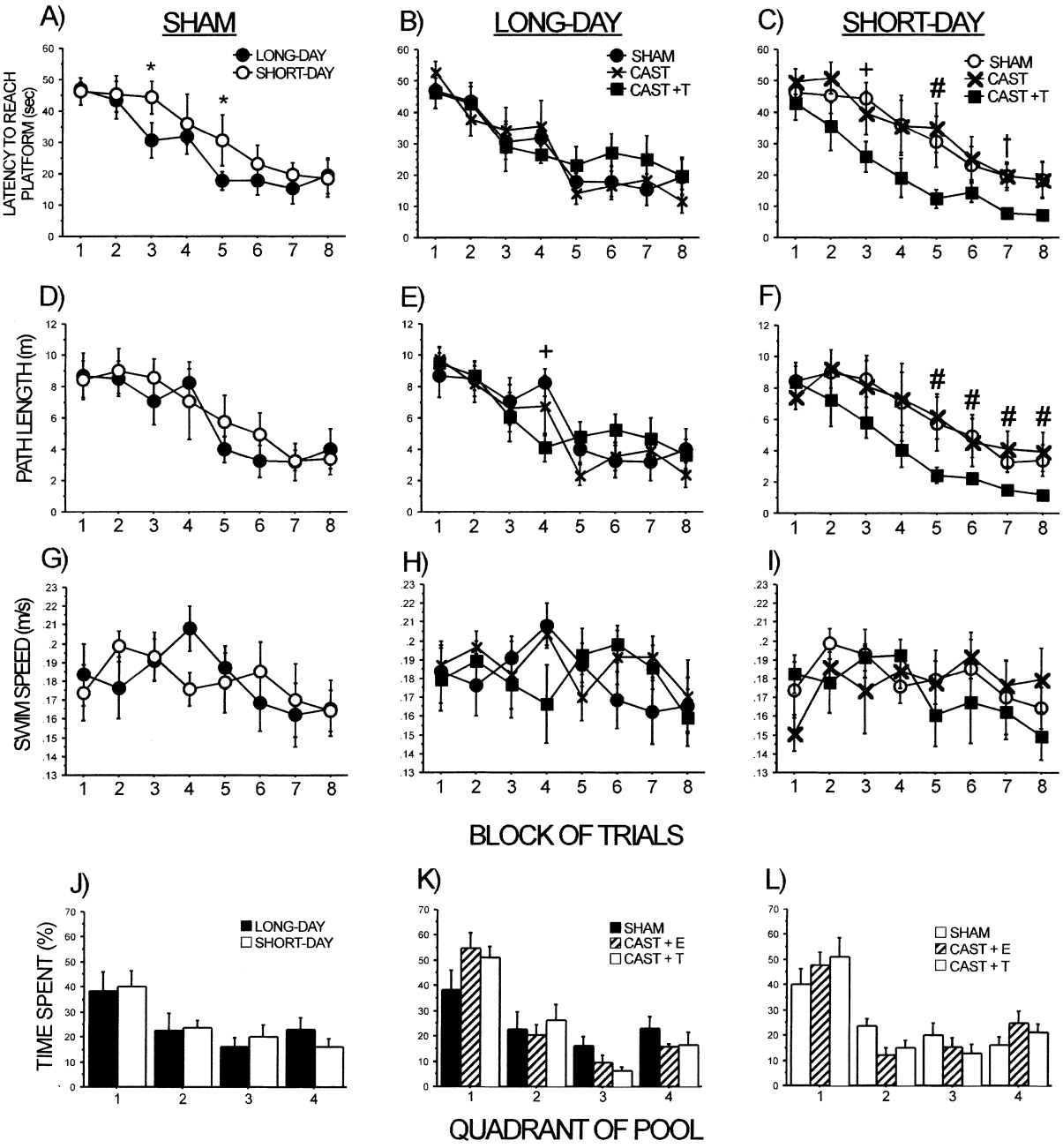

FIG. 1. Photoperiod and testosterone affect spatial learning and memory (via water maze). Latency to reach the original hidden platform in SHAM (A), long-day (B), and short-day (C) mice. Path length swam to reach the original hidden platform in SHAM (D), long-day (E), short-day (F) mice. Swim speed during original hidden platform trials in SHAM (G), long-day (H), and short-day (I) mice. Per cent time spent in each quadrant of the pool during memory probe trial for SHAM (J), long-day (K), and short-day (L) mice. Each block equals three trials. $^{*}P < 0.05$ between photoperiod; $^{+}P < 0.05$ between CAST + T and SHAM mice; $^{\#}P < 0.05$ between CAST + T and CAST mice; $^{\dagger}P < 0.05$ between CAST + T and both SHAM and CAST mice.

Experiment 2

AR mRNA expression was greater than ERα and ERβ in the hippocampus of all groups ($P < 0.05$; Fig. 3). However, no significant photoperiodic differences in AR, ERα, or ERβ expression were observed after 7 or 14 weeks of photoperiod ($P > 0.05$; Fig. 3).

Discussion

This study provides evidence for photoperiodic modulation of hormonal effects on behaviour. Specifically, removal of long-day typical concentrations of testosterone did not alter spatial learning and memory performance in long-day mice, but supplementation of testosterone in short-day mice significantly improved spatial learning and memory. The photoperiodic differences in behavioural responses to testosterone were not manifested in the amount of androgen or oestrogen receptor gene expression in the hippocampus. Thus, the mechanism by which the hippocampal function is more sensitive to testosterone in short-day mice is likely indirect.

Short-day CAST + T mice out-performed short-day SHAM and CAST mice in both original hidden platform learning trials and reversal learning trials. The water maze performance of short-day CAST + T

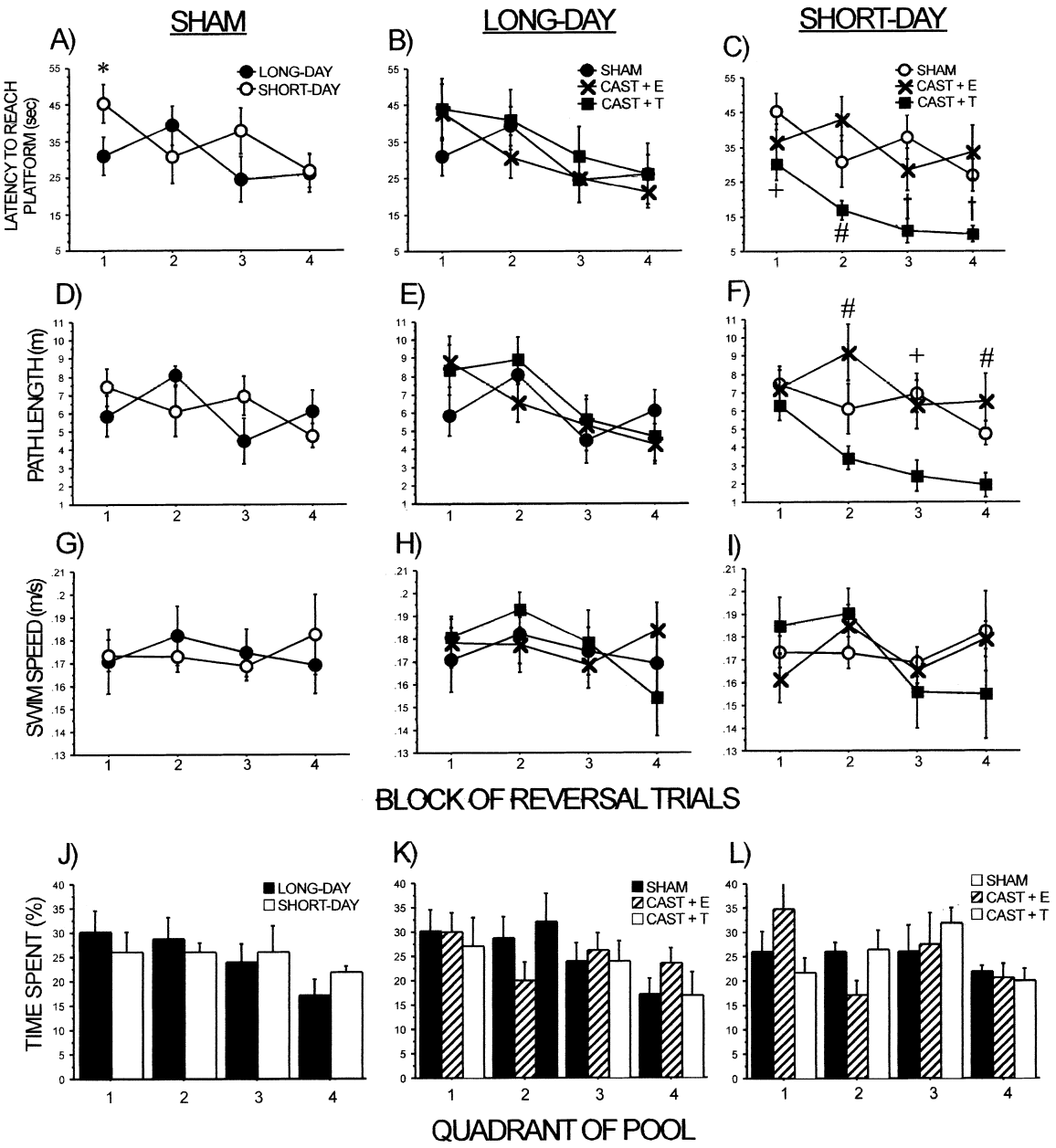

FIG. 2. Photoperiod and testosterone affect reversal spatial learning and memory (via water maze). Latency to reach the reversed hidden platform in SHAM (A), long-day (B), and short-day (C) mice. Path length swam to reach the reversed hidden platform in SHAM (D), long-day (E), short-day (F) mice. Swim speed during reversal hidden platform trials in SHAM (G), long-day (H), and short-day (I) mice. Per cent time spent in each quadrant of the pool during reversal memory probe trial for SHAM (J), long-day (K), and short-day (L) mice. Each block equals three trials. $^{*}P < 0.05$ between photoperiod; $^{+}P < 0.05$ between CAST + T and SHAM mice; $^{\#}P < 0.05$ between CAST + T and CAST mice; $^{\dagger}P < 0.05$ between CAST + T and both SHAM and CAST mice (see 2C).

mice was enhanced beyond the performance of long-day mice. Facilitation of learning and memory performance by testosterone has also been demonstrated in rodents and humans that are reproductively nonresponsive to photoperiod (Vazquez-Pereyra *et al*., 1995; Lessov-Schlaggar *et al*., 2005; but see Gouchie & Kimura, 1991). Castration of short-day mice did not alter spatial learning and memory. This finding was predictable because, similar to castration, gonadal regression in short days results in low to undetectable testosterone concentrations (Pyter *et al*., 2005b). Thus, in contrast to long days, short days appear to increase the sensitivity of spatial learning and memory brain circuitry to the effects of testosterone. Similarly, spatial learning and memory in female photoperiod-responsive rodents may be dependent upon photoperiodic modulation of oestrogen (Galea *et al*., 1995).

Our results corroborate previous studies in which long-day intact male rodents display enhanced spatial learning and memory performance relative to short-day SHAM rodents (Galea *et al*., 1995; Perrot-Sinal *et al*., 1998; Pyter *et al*., 2005c). These differences in learning and memory performance in SHAM mice may be mediated by structural changes in hippocampal spine density (Pyter *et al*., 2005c). Although previous studies in photoperiodic rodents have attempted to correlate testosterone concentrations with learning and memory performance (Perrot-Sinal *et al*., 1998), this is the first study to directly test the effects of testosterone on photoperiod-induced learning and memory differences.

In contrast to the short-day mice, our results suggest that the activational effects of long-day typical concentrations of testosterone are

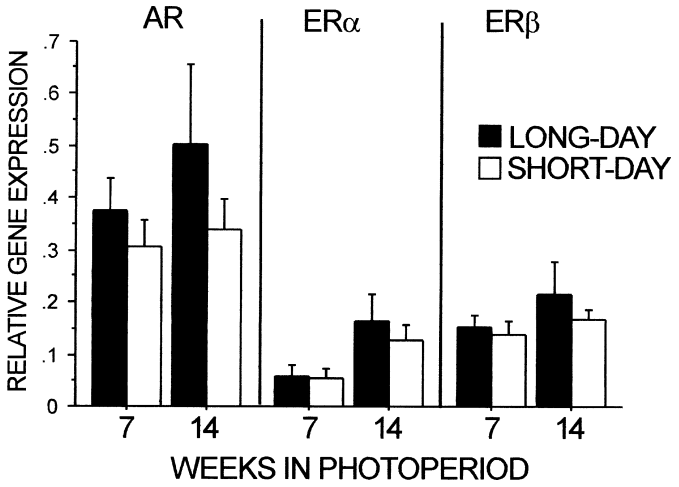

FIG. 3. Effects of 7 or 14 weeks of photoperiod on relative androgen receptor (AR), oestrogen receptor α (ERα), and oestrogen receptor β (ERβ) gene expression in the hippocampus as measured by quantitative PCR.

not necessary for long-day typical spatial learning performance. Regardless of their testosterone manipulations, all long-day mice performed similarly to SHAM long-day mice. These results concur with those reported in a previous study in which variation in testosterone concentrations of long-day male voles did not affect water maze performance (Galea *et al*., 1995). The unaffected learning performance in long-day mice that were castrated with testosterone replacement in the present study was predictable given that the testosterone treatment was designed to attain testosterone concentrations similar to intact long-day mice (Demas & Nelson, 1998). Presumably, the observed differences in learning were not confounded by potential differences in activity levels because no differences in swim speed were observed among groups. In sum, long photoperiods appear to enhance spatial learning and memory performance compared with short photoperiods regardless of circulating testosterone concentrations.

We also tested whether the potential mechanism by which testosterone sensitivity may differ between photoperiods and affects spatial learning relates to the expression of androgen or oestrogen receptors in the hippocampus. In addition to directly binding to androgen receptors, androgens can be aromatized to oestrogens within the brain and thereby affect brain function (i.e. behaviour) via oestrogen receptors (Roselli & Resko, 1993), although in adult male mammals hippocampal aromatase activity is typically low (Roselli & Resko, 1993). Androgen and oestrogen receptors are located within the rodent hippocampus and entorhinal cortex (Loy *et al*., 1988; Xiao & Jordan, 2002). Testosterone antagonists administered directly into the hippocampus impair spatial learning and memory in rats (Naghdi *et al*., 2001) and androgen insensitive rats display impaired spatial learning and memory (Jones & Watson, 2005). However, in the present study no differences in androgen or either subtype of oestrogen receptor gene expression were detected in the hippocampus. However, hippocampal samples from mice that have undergone photoperiod and testosterone manipulation are necessary to determine whether photoperiod and testosterone treatment interacted to affect hippocampal steroid receptor expression. Previous studies that have examined photoperiodic regulation of AR, ERα, or ERβ (excluding studies prior to technical differentiation between ERα and ERβ) in the brain did not examine the hippocampus (Mangels *et al*., 1998; Tetel *et al*., 2004; Trainor & Nelson, 2005). However, photoperiod and testosterone treatment interact to affect steroid receptor expression in regions of the brain outside of the hippocampus (Wood & Newman, 1993; Bittman *et al*., 2003) and brain morphology (Cooke *et al*., 2002). Although we detected no differences in steroid receptor mRNA expression, photoperiod could affect local steroid production in the hippocampus (Mukai *et al*., 2005). Thus, it is possible that photoperiod directly affects hippocampal hormone production leading to differences in hippocampal function (i.e. learning and memory). It is also possible that the differential interaction between testosterone and photoperiod on hippocampal function described in the present study is not mediated directly via the hippocampus.

Testosterone inhibits release of gonadotropin releasing hormone (GnRH) at the level of the hypothalamus and gonadotropins at the level of the pituitary. In photoperiod-responsive rodents, short days increase hypothalamic sensitivity to testosterone negative feedback of the hypothalamic-pituitary-gonadal (HPG) axis, whereas long days do not (Turek, 1977; Ellis & Turek, 1979, 1980). The increased sensitivity to HPG negative feedback allows the reduced short-day testosterone concentrations to suppress GnRH and gonadotropin release. This increase in testosterone sensitivity in the HPG axis of short-day mice may indirectly mediate the observed differences in hippocampal function in the present study. For example, increased hypothalamic sensitivity to testosterone specific to short-day brains may affect synaptic communication to the hippocampus via the fimbrio and thereby modulate hippocampal function (i.e. learning performance) described in the present study. In support of this hypothesis, lesions or electrical stimulation of the fimbrial input to the hippocampus alters learning and memory behaviour in rodents (Jarrard *et al*., 1984; Weiler *et al*., 1998).

In sum, short days appear to increase the sensitivity of hippocampal function (i.e. spatial learning and memory) to testosterone. These changes are not due to photoperiodic differences in hippocampal steroid receptor expression. In the field, winter mice may not typically be exposed to testosterone concentrations characteristic of long-day mice, but see Prendergast *et al*. (2001). However, the increased testosterone sensitivity of the short-day hypothalamus may result in the observed enhanced spatial learning performance because of hypothalamic-hippocampal communication. Finally, our study also suggests that seasonal regulation of hormone concentrations may impact cognitive functions.

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Abbreviations

AR, androgen receptors; CAST, castration plus empty implant; CAST + T, bilateral castration plus testosterone proprionate; ERα, oestrogen receptors α; ERβ, oestrogen receptors β; and LD, long days; SD, short days.

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