EJN fens EUROPEAN JOURNAL OF NEUROSCIENCE

European Journal of Neuroscience, pp. 1–10, 2010 doi:10.1111/j.1460-9568.2010.07528.x

Photoperiod interacts with food restriction in performance in the Barnes maze in female California mice

Michael Q. Steinman, Katie K. Crean and Brian C. Trainor
Department of Psychology, University of California, 1 Shields Avenue, Davis, CA 95616, USA

Keywords: food deprivation, hippocampus, learning and memory, photoperiodism, spatial cognition, synapsin I

Abstract

Food restriction has been reported to have positive effects on cognition. This study examines how another environmental factor, daylength, can alter the impact of food restriction on the brain and behavior. Female California mice (*Peromyscus californicus*), housed on either long days (16 h of light and 8 h of darkness) or short days (8 h of light and 16 h of darkness), were restricted to 80% of their normal baseline food intake or provided with food *ad libitum*. Testing in a Barnes maze revealed that the effects of food restriction depended on photoperiod, and that these effects differed for acquisition vs. reversal learning. During acquisition testing, food restriction increased latency to finding the target hole in short-day mice but not in long-day mice. In reversal testing, food restriction decreased latency to finding the target hole in long-day mice but not in short-day mice. Latency to finding the hole was positively and independently correlated with both errors and time spent freezing, suggesting that changes in both spatial learning and anxiety-like behavior contributed to performance. Short days increased hippocampal expression of the synaptic protein, synapsin I, which was reversed by food restriction. Short days also reduced plasma corticosterone levels, but diet had no effect. There was no effect of diet or photoperiod on hippocampal expression of the glial marker, glial fibrillary acidic protein. The present findings suggest that, in female California mice, the differential effects of food restriction on acquisition and reversal learning are photoperiod-dependent. These results justify further testing of the relationship between food restriction and hippocampal synapsin I in the context of spatial learning.

Introduction

There is growing evidence that food restriction has important effects on the brain that affect cognition. Twelve months of food restriction improved the performance of aged mice in a radial maze (Idrobo *et al.*, 1986), and life-long restriction to 60% of baseline food intake improved performance of aged rats in the Morris water maze test (Stewart *et al.*, 1989). Intriguingly, the effects of restricted diets are not limited to aged mice, as food-restricted (FR) mice across a wide age range showed more rapid improvement and reduced variability during water maze testing than *ad libitum* (AL) mice (Stewart *et al.*, 1989). Another study found that food restriction regimens in which male mice were maintained on 80 or 65% of baseline food intake for 6 months improved learning but not memory when tested in a Y-maze (Wu *et al.*, 2003). These studies suggest that a calorie-restricted diet may be beneficial for spatial learning. Similar findings were obtained in a recent study on elderly women, which showed that restriction to 70% of normal baseline caloric intake improved verbal memory (Witte *et al.*, 2009).

Most food restriction studies reduce caloric intake by providing a certain percentage of the baseline AL diet. It has been argued that, in the context of aging, food restriction is hermetic (Masoro, 1998), functioning like a pharmacological agent, which may have beneficial effects at low doses but is toxic at higher doses (Boxenbaum *et al.*, 1988). The hypothesis that food restriction is an environmental factor with pharmaceutical characteristics has prompted researchers to investigate the molecular and cellular mechanisms that exert the observed beneficial effects, so that interventions can be developed that exhibit more specific modes of action (Ingram *et al.*, 2004; Anderson *et al.*, 2009). An alternative strategy is to investigate the mechanisms affected by food restriction and how they are affected by other salient environmental stimuli. In many species, food availability is seasonally variable, and daylength (photoperiod) is used to anticipate seasonal changes in environmental conditions (Bronson, 1985; Nelson *et al.*, 1995a).

Photoperiod, like food availability, is an environmental factor that can impact on spatial learning. Indeed, photoperiodic changes in spatial cognition are well established in mice of the genus *Peromyscus*. Short days (SDs) augment spatial learning in female deer mice (*Peromyscus maniculatus*) (Galea *et al.*, 1994), and have been shown to have the opposite effect in male deer mice and white-footed mice (*Peromyscus leucopus*) (Galea *et al.*, 1994; Pyter *et al.*, 2005, 2006, 2007). Photoperiodic effects on spatial learning and memory are also evident in avian species. Black-capped chickadees (*Poecile atricapillus*) engage in high levels of seed caching in the autumn, and this is dependent on spatial memory. Laboratory studies have shown increased caching activity when chickadees are transferred from long days (LDs) to SDs (Krebs *et al.*, 1995), and decreased caching when they are moved from SDs to LDs (MacDougall-Shackleton *et al.*,

Correspondence: M. Q. Steinman, as above.
E-mail: mqsteinman@ucdavis.edu

Received 15 May 2010, revised 3 October 2010, accepted 19 October 2010

2003). Therefore, it is reasonable to hypothesize that photoperiod and food availability would interact in their effects on spatial learning.

The California mouse (*Peromyscus californicus*) can reproduce all year round, but demonstrates peak breeding around December and May (Ribble, 1992). It has been reported that this species may integrate food and photoperiod cues to regulate its reproductive timing (Nelson *et al.*, 1995b), and, as such, it may serve as a suitable model of seasonal photoperiod × diet interactions. In order to assess whether such interactions apply to spatial learning and learning, we assigned female California mice maintained in either winter-like short days or summer-like LDs to either an FR diet or an AL diet. We then tested spatial learning by use of a Barnes maze. There is growing awareness that females are understudied in neuroscience research (Zucker & Beery, 2010) and that there are important biological sex differences that affect both brain and behavior (Cahill, 2006). Accordingly, the vast majority of studies on the effects of food restriction on spatial memory employ males, and there is some evidence that food restriction may not benefit female spatial memory to the same extent as male spatial memory (Wu *et al.*, 2003). The present experimental setup simultaneously explored the effects of food restriction and photoperiod on spatial learning, allowing both factors to be assessed in females.

In addition to examining behavioral parameters, we also sought to determine mechanistically how photoperiod and food restriction affect spatial learning. We assayed plasma corticosterone levels, because corticosterone can affect spatial memory, enhancing it (Pyter *et al.*, 2007; Conboy & Sandi, 2010) or diminishing it (Schwabe *et al.*, 2010). We also examined the proteins synapsin I and glial fibrillary acidic protein (GFAP) in the hippocampus, an important neural locus of spatial memory (Handelmann & Olton, 1981). Synapsin I is localized to the inner membrane of synaptic vesicles, and is involved in both the regulation of neurotransmitter release and synaptic formation (Greengard *et al.*, 1993; Chin *et al.*, 1995). GFAP is a glial-specific cytoskeletal protein that is frequently upregulated in response to spatial memory-impairing hippocampal damage (de la Torre *et al.*, 1992; Eng & Ghirnikar, 1994). We used these approaches to gain insights into how reduced food availability influences spatial learning and whether these effects are photoperiod-dependent.

Materials and methods

Animals

Fifty-eight female California mice (*P. californicus*) at least 90 days of age were single-housed in polypropylene cages and randomly assigned to reside under photoschedules mimicking either SDs (8 h of light and 16 h of darkness) or LDs (16 h of light and 8 h of darkness) (lights off at 14:00 h Pacific standard time in long days and short days). Mice were further randomly subdivided into dietary groups that were restricted to 80% of their individual baseline daily food intake (FR) or that had access to food AL (2016 rodent diet; Harland Teklab, Indianapolis, IN, USA). Baseline daily food intake for each FR mouse was determined by averaging the weights of food consumed each day over a 1-week-period. Mice were maintained for 8 weeks under LD-AL (n = 19), SD-AL (n = 15), LD-FR (n = 11) or SD-FR (n = 13) conditions, and a subset of mice [LD-AL (n = 10), SD-AL (n = 7), LD-FR (n = 6) and SD-FR (n = 8)] were tested in a Barnes maze, starting at 6.5 weeks into the experiment. Approximately 24 h following the final Barnes maze trial, mice were anesthetized with isoflurane gas (Minirad, Bethlehem, PA, USA) and killed by decapitation. Brains were quickly removed and fixed in 5% acrolein (Sigma, St Louis MO, USA) in 0.1 M phosphate-buffered saline (PBS) overnight at 4 °C. On the next day, brains were placed in 25% sucrose (Fisher, Pittsburgh, PA, USA) in PBS, and on the day after that, they were frozen on dry ice. Brains were subsequently stored at −40 °C until being processed for immunohistochemical analysis. Two mice were killed as previously described, and the brains were rapidly removed and placed in a brain matrix, so that 2-mm-thick hippocampal sections could be obtained (Trainor *et al.*, 2010). Sections were placed on a freezing plate, and 1-mm hippocampal punch samples were taken, flash frozen on dry ice and stored at −40 °C until being homogenized for western blotting. At the time of killing, stage of estrous cycle was determined by vaginal lavage. Some FR mice appeared to have stopped cycling, and had closed their reproductive tracts, so that they were inaccessible to lavage. These mice were presumed to be in diestrus, because vaginal closure following introitus is indicative of suppressed cycling (Whitsett & Miller, 1982; Wube *et al.*, 2008). All procedures were approved by the UC Davis Institutional Animal Care and Use Committee, and followed the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Barnes maze

Spatial learning was assessed with a Barnes maze, which is generally considered to be a less stressful test of spatial learning and memory than the Morris water maze (Barnes, 1979; Harrison *et al.*, 2009). The Barnes maze was composed of a white circular board (85 cm in diameter) containing 16 equally spaced holes (5 cm in diameter) that were situated 1.3 cm from the maze's edge. As the experiment relies on subjects being motivated to escape from an open, well-lit platform, testing was conducted during the light phase of the light cycle, between 07:00 and 12:00 h Pacific standard time. For each trial, all treatment groups were run in the maze during a single testing period, and the order in which mice were tested was randomized daily. The test involved positioning a cage with fresh bedding under one of the holes on the maze (the target hole) and then placing a mouse in the center of the maze. The Barnes maze has been previously used to assess spatial learning with California mice (Bredy *et al.*, 2004). Each mouse was randomly assigned a target hole and was given 5 min to enter. If the mouse did not enter the target hole, the experimenter guided the mouse into the target hole, and the mouse was allowed to remain in the cage for 1 min. Day 1 was the habituation phase, in which mice were run on the maze for the usual 5 min in order to introduce them to the assigned target hole. The same target hole for any given mouse was used on days 2–5, which constituted the acquisition phase of testing. Days 6–9 constituted the reversal phase, wherein the previous target hole was moved 180° across the maze. The latency to entering the correct hole, the number of times that a mouse made an error by poking its head in an incorrect hole and the total amount of time for which the mouse was froze on the maze (starting on day 2) were recorded using Stopwatch+ (Center for Behavioral Neuroscience, Atlanta, GA, USA). Time spent freezing was recorded as an estimate of anxiety-like behavior (Crawley, 2007). On day 10, the mice were tested in a 30-s probe trial, in which the target cage was not placed under any of the holes. The number of head pokes into incorrect holes and the reversal target hole were recorded.

Immunohistochemistry

Brain tissue was sliced on a microtome at 40 μm. Afterwards, thawed sections were stored at −20 °C in cryoprotectant (50% v/v phosphate

buffer, 30% w/v sucrose, 1% w/v polyvinylpyrrolidone and 30% v/v ethylene glycol) until staining. Chromogenic immunostaining for synapsin I (n = 5 per group) and GFAP (n = 6 for LD-AL mice and SD-FR; n = 5 for SD-AL and LD-FD mice) was performed on every fourth section, beginning with the rostral hippocampus and proceeding caudally approximately 640 μm for synapsin I-stained slices and 800 μm for GFAP-stained slices. All treatment groups were run simultaneously for a given antibody, negating any potential for batch differences. Tissue was washed three times in PBS for 5 min per wash, and then incubated for 10 min in 0.1 M sodium borohydride in PBS as an antigen retrieval step. Tissue was subsequently blocked in 10% normal goat serum in PBS containing 0.3% hydrogen peroxide to quench endogenous peroxidases. Sections were incubated overnight at 4 °C in primary antibody solution consisting of either rabbit anti-synapsin I (ab8, 1 : 500; Abcam, Cambridge, MA, USA) or rabbit anti-GFAP (ab7779, 1 : 750; Abcam) diluted in PBS with 0.5% Triton X (TX) and 2% normal goat serum. On the following morning, tissue was washed three times with PBS, and was incubated for 2 h at room temperature in a secondary antibody solution consisting of PBS-TX, 2% normal goat serum, and either biotin-conjugated goat anti-rabbit antibody (BA-1000, 1 : 500; Vector Laboratories, Burlingame, CA, USA) or horseradish peroxidase-conjugated goat anti-rabbit antibody (PI-1000, 1 : 350; Vector Laboratories) for synapsin I and GFAP sections, respectively. Synapsin I sections were washed three times in PBS, and then incubated for 30 min in avidin–biotin complex (ABC Elite kit; Vector Laboratories). All sections were washed three times in PBS before undergoing development in nickel-enhanced diaminobenzidine (Vector Laboratories) for 2 min. Following development, tissue was rinsed for 5 min in deionized water. Stained sections were mounted onto Superfrost plus slides (Fisher), dehydrated in 100% ethanol, cleared for 3 min in Histoclear (National Diagnostics, Atlanta, GA, USA), and coverslipped with Permount (Fisher). Some sections were processed with primary antibody omitted to serve as negative controls.

Image analysis

Immunostained sections were photographed through a Zeiss Axioimager (Carl Zeiss Meditec, Dublin, CA, USA) equipped with an Axiocam MRC camera (Carl Zeiss Meditec). During all quantification procedures, the observer was blind to treatment. Three to four synapsin I-stained sections were analyzed per brain. Photomicrographs were taken at ×20 magnification, all on the same day and under identical lighting conditions. The mean optical density (OD) of immunoreactive synapsin I puncta was quantified with IMAGE J (NIH, Bethesda, MD, USA) calibrated to a calibrated gray scale transmission step tablet (#T2115C; Stauffer, Mishawaka, IN, USA). Images were converted to black and white, and eight circles, each with an area of 0.002 mm^2, were placed side-by-side in the stratum lucidum on one side of the hippocampus. Background was accounted for by measuring the mean OD in the corpus callosum of each section, using a rectangle that covered an area of 0.05 mm^2, and then subtracting this density from the average of the mean OD for the eight circles.

Four to five GFAP-immunostained sections were analyzed per brain. With the use of IMAGE J, a square box covering an area of 0.14 mm^2 was placed in a consistent portion of CA1 and CA3, and a square box with an area of 0.08 mm^2 was positioned consistently in the dentate gyrus (DG). Percentage staining of immunoreactive astrocytes within a box was quantified with the threshold feature of IMAGE J. The threshold was set manually by determining the level at which most fibers were accounted for without including areas that did not contain fibers.

Western blotting

Western blotting was used to confirm the specificity of antibodies. Hippocampal punch samples were homogenized in lysis buffer (20 mM HEPES, 0.4 M NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA, 0.1 mM phenylmethanesulfonyl fluoride, and 20% v/v glycerol). Sample in lysis buffer was then diluted 1 : 2 in Laemmli buffer (Sigma) and denatured at 100 °C for 3 min, after which proteins were separated by electrophoresis on a 10% sodium dodecylsulfate–polyacrylamide gel. Proteins were subsequently transferred to a polyvinylidene fluoride membrane (pore size 0.45 μm; Invitrogen, Carlsbad, CA, USA). The membrane was then blocked for 1 h in 5% skimmed milk (Oxoid, Basingstoke, UK) in Tris-buffered saline (TBS) (55 mM Tris, 150 mM NaCl, pH 7.4) with 0.1% TX. After blocking, the membrane was washed twice for 5 min each in TBS-TX before being incubated on an orbital shaker overnight at 4 °C in rabbit anti-synapsin I antibody diluted 1 : 1000 in TBS-TX with 2% normal goat serum. On the following day, the membrane was washed three times for 5 min each in TBS-TX, and then incubated for 2 h at room temperature in horseradish peroxidase-conjugated goat anti-rabbit antibody (PI-1000; Vector Laboratories) diluted 1 : 2000 in TBS-TX with 2% normal goat serum. The membrane was then washed three times for 5 min each in TBS-TX, and developed for 1 min with the Immun-Star WesternC kit (Bio-Rad Laboratories, Hercules, CA, USA). The membrane was viewed and photographed with a Chem-iDoc XRS+ molecular Imager with IMAGE LAB software (Bio-Rad Laboratories). On the following day, the membrane was incubated in stripping buffer [2% w/v sodium dodecylsulfate, 0.7% 2-mercaptoethanol, 62.5 mM Tris (pH 6.7)] for 30 min at 50 °C. The membrane was washed twice for 10 min each in TBS-TX and then reblocked in 5% milk in TBS-TX at room temperature for 1 h. After being washed twice for 5 min each in TBS-TX, the membrane was incubated overnight at 4 °C in rabbit anti-GFAP antibody diluted 1 : 1000 in TBS-TX with 2% normal goat serum. On the next morning, the membrane was washed, incubated in secondary antibody, and developed as described for synapsin I. The molecular masses of bands were determined by comparing their positions on the membrane with a protein ladder (Precision Plus Protein WesternC Standard; Bio-Rad Laboratories). When the membrane was incubated in anti-synapsin I antibody, a band of approximately 75 kDa (Fig. 1) was detected, an appropriate size for the synapsin 1a isoform (Nicol *et al.*, 1997). A different membrane was stripped after immunoblotting, and incubated in anti-GFAP antibody, which revealed a band of approximately 50 kDa (DeArmond *et al.*, 1986).

Corticosterone radioimmunoassay

Trunk blood was collected at the time of killing, and was stored at −4 °C until being centrifuged at 16.1 ***g*** for 12 min at −4 °C. Plasma was removed by pipette and stored at −40 °C until use. Duplicated samples were assayed in order to establish intra-assay variation, the mean of which was 2.3%. A single ^{125}I radioimmunoassay kit (ICN Biomedicals, Costa Mesa, CA, USA) was used to evaluate cortico-

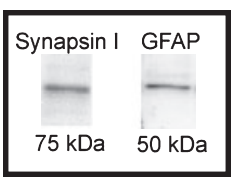

FIG. 1. Immunoblots showing the presence of bands at about 75 kDa for synapsin I and 50 kDa for GFAP.

sterone levels from plasma samples for each treatment [LD-AL (n = 19), SD-AL (n = 15), LD-FR (n = 11), and SD-FR (n = 13)]. This kit has a cross-reactivity of under 0.5% and has a lower detection limit of 5 ng/mL. Plasma was suspended at a dilution of 1 : 2000 in steroid diluent (MP Biomedicals, Solon, OH, USA) to compensate for the fact the kit was developed for *Mus and Rattus*, which tend to have significantly lower corticosterone levels than those of *Peromyscus* (Glasper and Devries, 2005).

Estradiol (E2) enzyme immunoassay

Plasma E2 concentrations were determined with an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI, USA). This assay has a sensitivity of 6.6 pg/mL, and the intra-assay coefficient of variation was 12.0%. Data were log transformed for statistical analysis. Statistical analysis was performed on samples from mice in diestrus only, because we obtained insufficient numbers of mice in proestrus and estrus.

Statistics

Latencies, errors and freezing behavior from the Barnes maze were analyzed with repeated measures ANOVA, testing for effects of diet and photoperiod as well as the interaction. Because different neurobiological processes may be involved in reversal learning, we analyzed data for the acquisition and reversal phases separately (Shuai *et al.*, 2010). We also ran independent t-tests for each day of the Barnes maze (Fig. 2). Synapsin I, GFAP and corticosterone levels were analyzed by two-way ANOVA, testing for effects of diet, photoperiod, and photoperiod × diet interaction. Non-parametric Spearman correlations were used to determine associations between various performance measures in the maze and between hippocampal proteins and corticosterone. We used partial correlations to determine whether variance in freezing behavior and errors overlapped with respect to predicting latency. For the probe trial, we used Fisher's exact test to test whether treatments influenced the likelihood of mice making at least one head poke in the correct hole from the reversal phase.

Results

Barnes maze

During the acquisition phase of the Barnes maze, there was a significant photoperiod × diet interaction (Fig. 2A and B; repeated measures ANOVA, $F_{1,27}$ = 5.96, P = 0.021). There were no significant main effects of photoperiod or diet (all P-values > 0.96). In SDs, food restriction increased latency to finding the target hole on day 4 (Fig. 2A; P = 0.023) and day 5 (Fig. 2A; P = 0.017) during the acquisition phase. In contrast, LD-FR mice had a shorter latency to finding the target hole on day 2 than LD-AL mice (Fig. 2B, P = 0.046). Repeated measure ANOVA indicated that only SD-AL mice showed a significant reduction in latency to entering the target hole over the course of the acquisition phase (Fig. 2A; Table 1). There was a non-significant trend for reduced latency in SD-AL mice as compared with LD-AL mice (repeated measures ANOVA, $F_{1,15}$ = 3.86, P = 0.068). Neither errors nor freezing time changed for any group over the course of testing, although a trend for a reduced number of

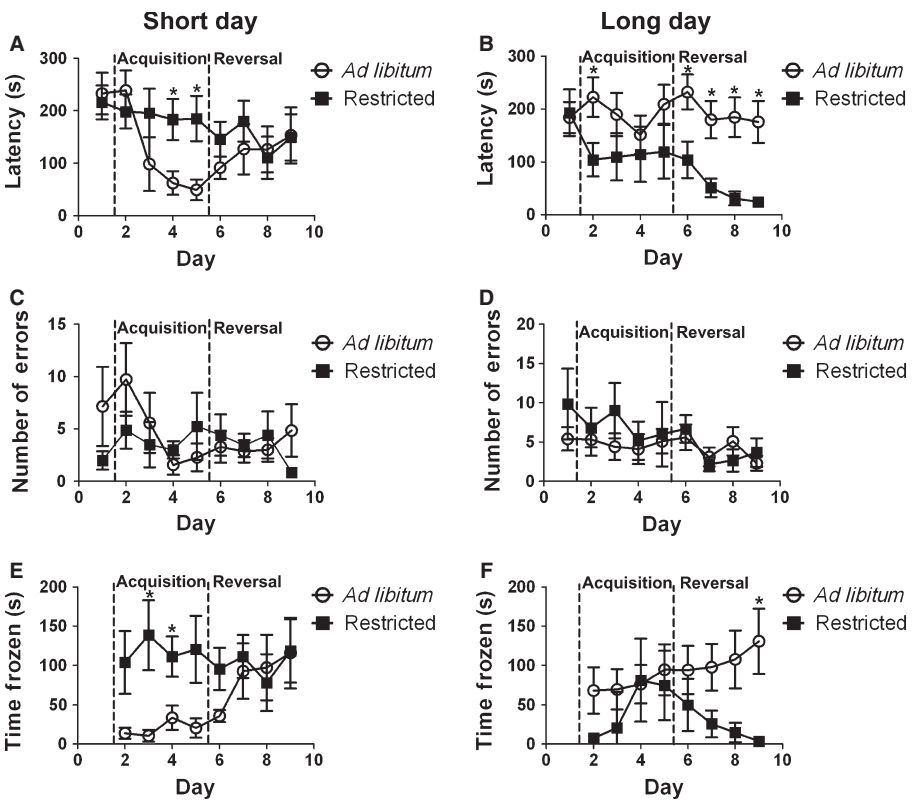

FIG. 2. (A and B) Graphs depicting latency to finding the target hole on each day of Barnes maze testing in FR and AL mice maintained under SDs (A) or LDs (B). (C and D) Graphs displaying number of errors made on each day of Barnes maze testing in FR and AL mice maintained under SDs (C) or LDs (D). (E and F) Graphs of time spent freezing by SD (E) and LD (F) mice. Day 1 is the habituation phase. Days 2–5 constitute the acquisition phase. Days 6–9 constitute the reversal phase. *Effect of food restriction, P < 0.05.

TABLE 1. Repeated measures ANOVA statistics for within group differences in latency, number of errors and freezing time during the acquisition phase and the reversal phase

Acquisition	*F*	*P*	Reversal	*F*	*P*
Latency					
SD-AL	$F_{3,18} = 9.0$	0.001*	SD-AL	$F_{3,18} = 0.4$	0.77
SD-FR	$F_{3,21} = 0.1$	0.97	SD-FR	$F_{3,21} = 0.7$	0.58
LD-AL	$F_{3,27} = 1.3$	0.30	LD-AL	$F_{3,27} = 1.0$	0.41
LD-FR	$F_{3,15} = 0.1$	0.97	LD-FR	$F_{3,15} = 4.4$	0.02*
Errors					
SD-AL	$F_{3,18} = 3.0$	0.06	SD-AL	$F_{3,18} = 0.4$	0.75
SD-FR	$F_{3,21} = 0.3$	0.83	SD-FR	$F_{3,21} = 1.0$	0.41
LD-AL	$F_{3,27} = 0.2$	0.90	LD-AL	$F_{3,27} = 0.2$	0.10
LD-FR	$F_{3,15} = 0.4$	0.76	LD-FR	$F_{3,15} = 1.7$	0.21
Freezing					
SD-AL	$F_{3,18} = 1.1$	0.37	SD-AL	$F_{3,18} = 1.2$	0.33
SD-FR	$F_{3,21} = 0.4$	0.75	SD-FR	$F_{3,21} = 0.4$	0.76
LD-AL	$F_{3,27} = 0.6$	0.62	LD-AL	$F_{3,27} = 0.7$	0.59
LD-FR	$F_{3,15} = 2.1$	0.15	LD-FR	$F_{3,15} = 1.8$	0.20

Latency decreased over time in short-day *ad libitum* (SD-AL) mice during acquisition and in long-day feed restricted mice (LD-FR) during reversal. Neither errors nor freezing changed over time for any group. Short-day feed restricted (SD-FR); long-day *ad libitum* (LD-AL). *$P \leq 0.02$.

errors approached significance for SD-AL mice (Table 1; $F_{3,18} = 3.0$, $P = 0.057$).

A significant photoperiod × diet interaction was also observed during the reversal phase of the experiment (Fig. 2A and B; $F_{1,27} = 9.65$, $P = 0.004$), and differed from that seen in the acquisition phase. Food restriction reduced latency in LD mice on each day of reversal testing (Fig. 2B; all *P*-values <0.025). In contrast, food restriction had no effect on latency on any day of reversal testing for SD mice (all *P*-values > 0.2). Latency to finding the hole progressively decreased only in LD-FR mice (Table 1; $F_{4,15} = 4.44$, $P = 0.02$). LD-FR mice also demonstrated shorter latencies than SD-FR mice ($F_{1,12} = 9.19$, $P = 0.011$). There was no change in number of errors or freezing with progressive trials (Table 1).

Repeated measures ANOVA indicated that the number of errors made in the Barnes maze was affected neither by diet nor photoperiod, nor by interactions between these treatments, in either phase of testing (Fig. 2C and D; all *P*-values > 0.4). There were, however, consistent significant positive correlations between latency and errors, which were observed on day 3 ($r = 0.41$, $P = 0.022$), day 4 ($r = 0.41$, $P = 0.021$), day 5 (Fig. 3A; $r = 0.55$, $P = 0.001$), day 7 (Fig. 3B; $r = 0.49$, $P = 0.005$), and day 8 ($r = 0.37$, $P = 0.042$). There was a non-significant trend for a correlation on day 6 ($r = 0.35$, $P = 0.051$).

Analyses of total freezing time revealed a significant photoperiod × diet interaction during the acquisition phase (Fig. 2E and F; $F_{1,27} = 6.02$, $P = 0.021$), with SD-AL mice freezing significantly less than SD-FR mice ($F_{1,13} = 7.48$, $P = 0.017$). During the reversal phase, there were no treatment effects or interactions for freezing time (all *P*-values > 0.05). Freezing showed a significant positive association with latency on each day of acquisition and reversal testing (days 5 and 7, shown in Fig. 3C and D; all *P*-values ≤ 0.005). There were, however, no significant correlations between freezing time and errors (Fig. 3E and F; all *P*-values > 0.1), suggesting that even though some animals froze more, they still explored the maze and examined incorrect holes. Partial correlations confirmed that the frequent positive correlations between latency and errors were present when freezing was controlled for on each day (Supporting Information Table S1; all *P*-values ≤ 0.004), except for day 2 (Supporting Information Table S1; $P = 0.270$), and that the positive correlations between freezing and latency were maintained when errors were controlled for (Supporting Information Table S1; all *P*-values ≤ 0.002).

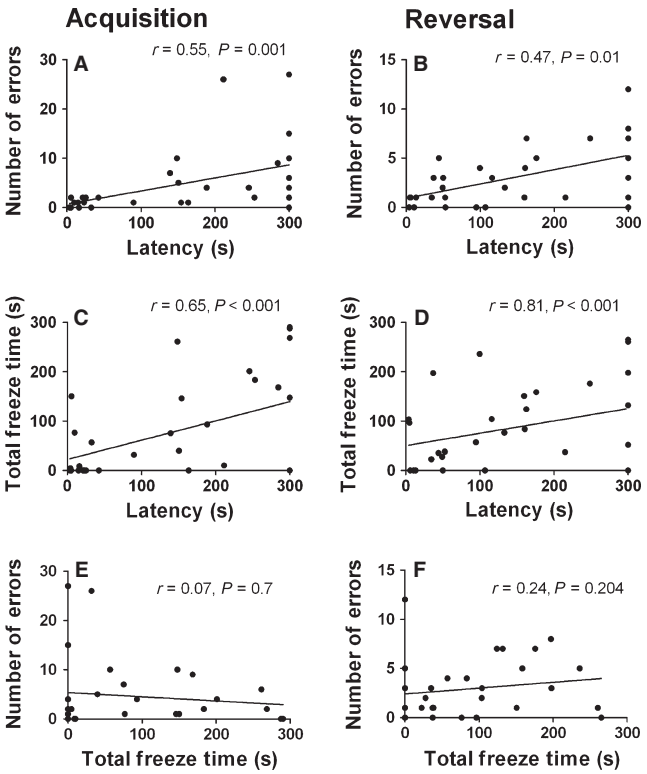

FIG. 3. Correlations between performance parameters in the Barnes maze. Correlations between latency, errors and freezing on test days 5 (A, C and E) and 7 (B, D and F). No significant correlations exist between number of errors made and time spent freezing on test days 5 (E) and 7 (F).

TABLE 2. Behavioral data from day 10 probe trial. Number of head pokes into the incorrect hole (errors), and freezing time

	Short day *ad lib*	Short day restricted	Long day *ad lib*	Long day restricted
# of Errors	3.1 ± 1.8	0.8 ± 0.9	0.6 ± 0.5	6 ± 2.3*
Freezing (s)	10 ± 5	18 ± 4	13 ± 3	6 ± 5

*$P < 0.02$ when means are compared within photoperiod.

In the probe trial, there was a significant interaction ($F_{1,27} = 9.8$, $P = 0.004$) for the total number of errors, with LD-FR mice making more errors than LD-AL mice, whereas diet had no effect in SDs. There were no main effects on errors (Table 2; all *P*-values > 0.2). There were also no main effects or interactions of diet or photoperiod for freezing time (Table 2; all *P*-values > 0.06). During the probe trial, LD-FR mice were more likely to visit the correct hole than SD-FR mice (Table 3; Fisher's exact test, $P = 0.026$), whereas photoperiod had no effect in AL mice (Fisher's exact test, $P > 0.99$).

Corticosterone radioimmunoassay

There was a main effect of photoperiod (Fig. 4; $F_{1,57} = 13.92$, $P < 0.001$), with SDs reducing plasma corticosterone as compared with LDs. There was neither an effect of diet nor any interaction (all *P*-values > 0.1).

TABLE 3. Frequency table displaying the number of mice in each treatment group that entered the correct hole at least once during the probe trial. When feed restricted, long-day mice were more likely to head poke this hole than short-day mice (two-sided Fisher's exact test, $P = 0.026$)

	Entered ≥ 1 time	Long day	Short day
Ad Lib	Yes	7	5
	No	3	2
Restricted	Yes	1	7
	No	5	1

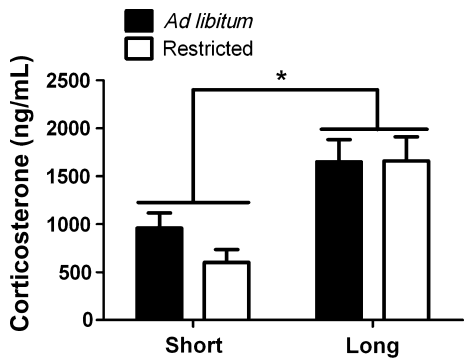

FIG. 4. Plasma corticosterone levels are reduced in SD mice as compared with LD mice. *Effect of photoperiod, $P < 0.001$.

E2 immunoassay

There was no effect of photoperiod or diet on plasma E2 concentrations in diestrus mice, and nor was there an interaction (Table 4; all *P*-values > 0.15).

Synapsin I immunoreactivity

Synapsin I expression was conspicuous throughout the stratum lucidum (Fig. 5) of the hippocampus, as previously described (Morioka *et al.*, 1997; Iwata *et al.*, 2006). Within this hippocampal structure, the synapsin I mean OD was significantly altered by both photoperiod ($F_{1,16} = 7.75$, $P = 0.013$) and diet ($F_{1,16} = 8.8$, $P = 0.009$). In AL mice, synapsin I protein was significantly upregulated by SDs (Fig. 6A; $P = 0.012$); however, food restriction abolished this SD-induced increase in synapsin I immunoreactivity (Fig. 6A; $P = 0.001$). In LD mice, food restriction had no effect on synapsin I expression within the stratum lucidum ($P > 0.05$). The synapsin I mean OD correlated negatively with corticosterone levels (Fig. 6B; $r = -0.45$, $P = 0.048$).

GFAP immunoreactivity

Hippocampal GFAP expression was assessed by analyzing percentage staining (Fig. 7). Within the CA1, CA3 and DG, no main effects or interactions were indentified (Table 5; all *P*-values > 0.1). Nevertheless, an interesting interaction emerged when GFAP immunoreactivity was correlated with corticosterone levels, as there were significant negative correlations between GFAP expression and corticosterone concentration for all hippocampal regions in AL mice [CA1 (Fig. 8A; $r = -0.658$, $P = 0.028$), CA3 (Fig. 8C; $r = -0.73$, $P = 0.012$) and DG (Fig. 8E; $r = -0.73$, $P = 0.010$)], whereas such correlations were non-existent in FR mice [Fig. 8B; CA1 ($r = 0.17$, $P = 0.610$), CA3 (Fig. 8D, $r = 0.43$, $P = 0.183$) and DG (Fig. 8F; $r = 0.39$, $P = 0.232$)].

Discussion

We hypothesized that photoperiod and diet would exert interacting effects on spatial learning in female California mice. Barnes maze testing showed that food restriction increased latency to finding the target hole during acquisition testing in SD mice but shortened latency for one trial in LD mice. Furthermore, food restriction had no effect on performance during reversal learning in SD mice, but improved performance in LD mice. During the acquisition phase, only SD-AL mice showed overall improvement in finding the target hole with progressive trials, whereas in the reversal phase, only LD-FR mice showed improvement. Our data suggest that differences in perfor-

TABLE 4. The effects of photoperiod and diet on plasma estradiol levels (pg/mL) in female mice. Due to a low *n*, statistical analysis was not run for mice in proestrus and estrus. Three of the LD-FR and six of SD-FR mice were inaccessible to lavage and presumed to be in diestrus

	Short day *ad lib*		Short day restricted		Long day *ad lib*		Long day restricted	
Estrous stage	E2 (pg/mL)	*n*	E2 (pg/mL)	*n*	E2 (pg/mL)	*n*	E2 (pg/mL)	*n*
Diestrus	18 ± 3.3	7	21.4 ± 4.5	8	21.3 ± 3.0	7	27.1 ± 3.8	6
Proetrus	16.8	1	N/A	0	13.2	1	N/A	0
Estrus	N/A	0	N/A	0	18.8 ± 5.3	4	10.5	1

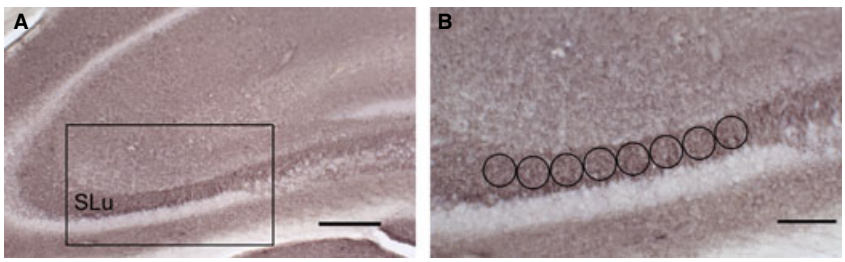

FIG. 5. (A) Representative microphotograph depicting synapsin I immunostaining in the hippocampus. (B) Higher magnification of the boxed area in A, with circles demonstrating the portion of the stratum lucidum (SLu) in which synapsin I mean OD was quantified. Scale bars – 200 μm in A and 100 μm in B.

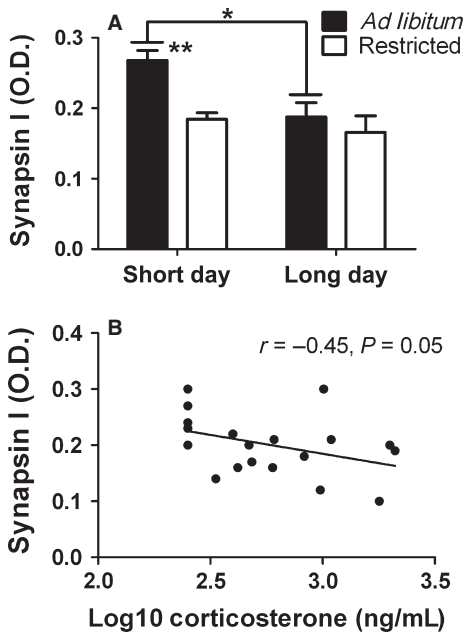

FIG. 6. (A) Effects of food restriction and photoperiod on synapsin I mean OD in the stratum lucidum of the hippocampus. SDs increase synapsin I expression in AL mice, but not in FR mice. (B) Synapsin I immunostaining OD in the stratum lucidum is negatively correlated with plasma corticosterone concentrations. *Effect of photoperiod, $P = 0.012$; **effect of food restriction, $P = 0.001$.

mance in the Barnes maze are affected by changes in both spatial memory (estimated by errors) and anxiety-like behavior (estimated by freezing). In AL mice, acquisition learning was generally improved under SDs as compared with LDs, consistent with previous data (Galea *et al.*, 1994). Our results demonstrate that the effects of food restriction on spatial learning are not uniform, but depend on photoperiod.

Reversal vs. acquisition learning

Reversal learning has been used as a model of adaptive forgetting, facilitating the replacement of older memories. This process appears to rely on molecular mechanisms and neuroanatomical structures that are distinct from those used to acquire memories (Thompson *et al.*, 1981; Shuai *et al.*, 2010). Previous studies in rats housed under photoschedules of 12 h of light and 12 h of darkness have reported that FR diets (Gyger *et al.*, 1992) improve reversal but not acquisition leaning. Our data suggest that this effect depends on the environment, as we observed that food restriction improved reversal learning when California mice were housed under LDs, but not when they were housed under SDs. This is consistent with results from the probe trial, which demonstrated that, under food restriction, LD mice were more likely to head poke the correct hole than were SD mice.

Contributions of spatial strategies and anxiety to Barnes maze performance

The latency to finding the target hole in the Barnes maze can be affected by spatial learning and other processes, such as activity or anxiety-like behavior. Our analyses suggest that both spatial learning and anxiety-like behavior contributed to the latency data. Latency was positively correlated with errors, suggesting that individuals with long latencies had difficulty in remembering the location of the target hole. In the probe trial, LD-FR mice were more likely than SD-FR mice to head poke the correct hole, further suggesting a role for spatial learning. However, latency was also positively correlated with freezing. This suggests that individuals with long latencies were also showing increases in anxiety-like behavior. Partial correlations demonstrated that latency was correlated with both errors and freezing independently. Similarly, errors and freezing were never correlated with each other. These data indicate that freezing and errors make independent contributions to variability in the latency to finding the correct hole. There was a significant photoperiod × diet interaction for freezing time during the acquisition phase that paralleled the interaction seen for latency, but no interaction during the reversal phase. Previous studies show that SDs increased anxiety-like behavior in an elevated plus maze in both collared lemmings (*Dicrostonyx groenlandicus*) (Weil *et al.*, 2007) and Siberian hamsters (*Phodopus sungorus*) (Prendergast & Nelson, 2005). In contrast, the effects of food restriction on anxiety-like behavior have been inconsistent (Jahng *et al.*, 2007; Yamamoto *et al.*, 2009), further supporting the hypothesis that the effects of food restriction on behavior may depend on the environment.

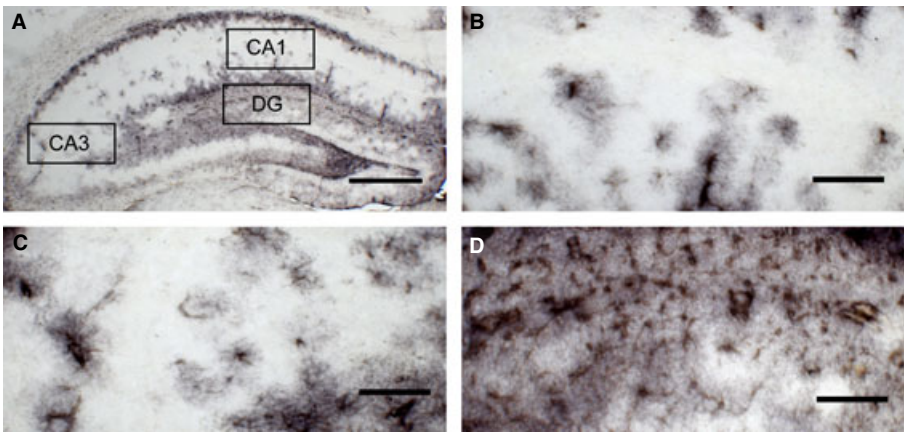

FIG. 7. (A) Representative microphotograph of GFAP immunostaining in the hippocampus. (B–D) Higher-magnification images of the boxed areas in A are shown for CA1 (B), CA3 (C) and the dentate gyrus (DG) (D). Scale bars – 500 μm in A and 100 μm in B–D.

TABLE 5. GFAP-immunoreactivity within the CA1, CA3 and dentate gyrus (DG) of the hippocampus. Short-day *ad libitum* and long-day restricted (n = 5); short-day restricted and long-day *ad libitum* (n = 6)

% Stain	Short day *ad lib*	Short day restricted	Long day *ad lib*	Long day restricted
CA1	22 ± 9	18 ± 6	17 ± 6	15 ± 6
CA3	33 ± 9	24 ± 7	22 ± 8	20 ± 9
DG	54 ± 3	51 ± 5	45 ± 4	49 ± 6

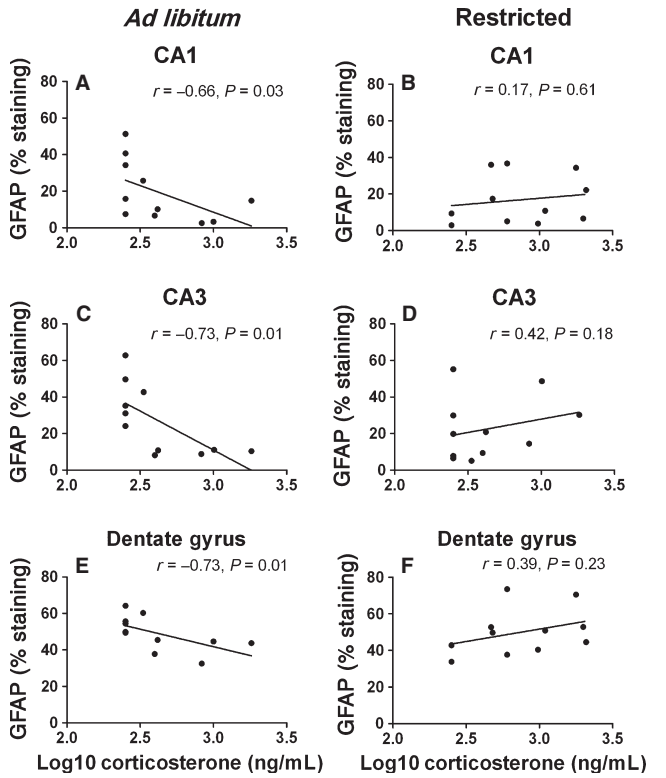

FIG. 8. Graph demonstrating the significant negative correlations between plasma corticosterone levels and GFAP immunoreactivity in CA1 (A), CA3 (C) and the DG (E) of the hippocampus of AL mice. Food restriction to 80% of baseline daily intake eliminated the significant correlations between corticosterone and GFAP percentage staining (B, D and F).

Although the number of errors did not vary with treatment, a previous study with male and female California mice also found stronger changes in latency than in number of errors (Bredy *et al.*, 2004). Freezing behavior was not reported in that study. Although we did not measure path length, it is possible that changes in search strategy may have also affected performance. Increased glucocorticoids induce a switch from a spatial strategy to a stimulus–response strategy in C57BL/6J mice (Schwabe *et al.*, 2010). In our study, it is possible that, in some cases, a spatial strategy was not the primary strategy used, and this will need to be addressed in future studies. Although LD mice had been in the light phase of the light cycle for longer than SD mice, it is unlikely that this significantly altered maze performance. Circadian timing of testing does not impact on spatial learning in a Morris water maze, or performance in various anxiety-based tests (Beeler *et al.*, 2006), and nor does it affect locomotion in an open field test or habituation to a novel environment (Valentinuzzi *et al.*, 2000).

Effects of photoperiod and food restriction on the hippocampus

Previous studies have established a link between synapsin I expression and spatial memory formation (Gomez-Pinilla *et al.*, 2001; John *et al.*, 2009), so we measured synapsin I immunoreactivity. We focused on the stratum lucidum of the CA3, because this structure is a putative neural locus of spatial memory (Sudhof *et al.*, 1989; Villacres *et al.*, 1998; Holahan *et al.*, 2006). Under AL conditions, SD mice had shorter latencies than LD mice during the acquisition phase, and also had increased synapsin I activity in the stratum lucidum. In contrast, food restriction blocked the augmentation of synapsin I expression by SDs, and this could be linked to the longer latencies observed in SD-FR mice. This, taken with the finding that food restriction improved spatial memory in LD mice without affecting synapsin I expression, suggests that synapsin I in the stratum lucidum is more important for acquisition than for reversal spatial memory. Studies in Kunming mice found that a moderately restricted diet regimen (80% of AL) increased brain levels of synapsin I, but that more severe food restriction (20–40% AL) decreased synapsin I expression (Deng *et al.*, 2009). Therefore, SDs may make California mice more susceptible to food restriction-induced downregulation of synapsin I. Further work is necessary to determine whether synapsin I directly influences spatial memory.

Effects of photoperiod and food restriction on steroid hormones

Studies examining the effects of ovarian steroids on spatial learning and memory ability in female rodents have yielded inconsistent results (Heikkinen *et al.*, 2004; Ping *et al.*, 2008; Hammond *et al.*, 2009; Luine & Rodriguez, 1994; Singh *et al.*, 1994). We did not detect any treatment differences for E2, suggesting that its levels do not underlie the behavioral differences observed here. The lack of a photoperiodic effect during diestrus in the present study contrasts with previous work from our laboratory, in which plasma was collected during the lights-off period (Silva *et al.*, 2010). Furthermore, it is likely that the number of mice in proestrus and estrus was too small for estrous cycle-dependent changes in E2 levels to emerge. California mice spend the majority of the cycle in diestrus (Gubernick, 1988), but food restriction (Tropp & Markus, 2001) probably impacted on the distribution of stages as well, by impairing cyclicity. Mice were not staged during maze testing, because lavage itself alters behavior (Davis & Marler, 2003; Silva *et al.*, 2010).

Corticosterone is known to suppress GFAP transcription and expression in the hippocampus (Laping *et al.*, 1994). The present findings in AL mice support this view, as there were significant negative correlations between corticosterone levels and GFAP immunoreactivity in all examined hippocampal regions. Food restriction eliminated these correlations, appearing to alter the relationship between corticosterone and GFAP.

Conclusions

Our data from female California mice indicate that the specific effects of food restriction on performance in the Barnes maze are sensitive to photoperiod. It appears that the beneficial effects of food restriction on spatial learning are biased towards reversal learning and are evident only in LD mice. In contrast, food restriction impairs acquisition learning during short photoperiods. The negative effects of food restriction in SDs are associated with reduced expression of synapsin I within the hippocampus. Future studies should further test the involvement of enhanced hippocampal synapsin I expression in spatial acquisition memory of SD mice. It is also likely that treatment-induced

changes in anxiety-like behavior affected latency to finding the target hole. Taken as a whole, this study suggests that the effects of food restriction on learning are context-dependent and influenced by the environment. Food restriction can have different effects on acquisition and reversal learning, and these effects may depend on salient environmental cues such as photoperiod.

Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Matrix showing correlation coefficients and *P*-values for partial correlations of errors vs. latency, controlling for freezing, and freezing vs. latency, controlling for errors.

Please note: As a service to our authors and readers, this journal provides supporting information supplied by the authors. Such materials are peer-reviewed and may be re-organized for online delivery, but are not copy-edited or typeset by Wiley-Blackwell. Technical support issues arising from supporting information (other than missing files) should be addressed to the authors.

Acknowledgements

The authors would like to thank Jennifer Cai, Jennifer New and Andrea Silva for technical help, Cindy Clayton for assisting with animal care, Doug Bean for assistance in constructing the Barnes maze, and Andy Yonelinas for manuscript advice. This work was supported by a Sigma Xi Grant-in-Aid to M. Q. Steinman and NIH R01 MH085069-01 to B. C. Trainor.

Abbreviations

AL, *ad libitum;* DG, dentate gyrus; E2, estradiol; FR, food-restricted; GFAP, glial acidic fibrillary protein; LD, long day; OD, optical density; PBS, phosphate-buffered saline; SD, short day; TBS, Tris-buffered saline; TX, Triton X.

References

Anderson, R.M., Shanmuganayagam, D. & Weindruch, R. (2009) Caloric restriction and aging: studies in mice and monkeys. *Toxicol. Pathol.*, **37**, 47–51.

Barnes, C.A. (1979) Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. *J. Comp. Physiol. Psychol.*, **93**, 74–104.

Beeler, J.A., Prendergast, B. & Zhuang, X.X. (2006) Low amplitude entrainment of mice and the impact of circadian phase on behavior tests. *Physiol. Behav.*, **87**, 870–880.

Boxenbaum, H., Neafsey, P.J. & Fournier, D.J. (1988) Hormesis, Gompertz functions, and risk assessment. *Drug Metab. Rev.*, **19**, 195–229.

Bredy, T.W., Lee, A.W., Meaney, M.J. & Brown, R.E. (2004) Effect of neonatal handling and paternal care on offspring cognitive development in the monogamous California mouse (*Peromyscus californicus*). *Horm. Behav.*, **46**, 30–38.

Bronson, F.H. (1985) Mammalian reproduction: an ecological perspective. *Biol. Reprod.*, **32**, 1–26.

Cahill, L. (2006) Why sex matters for neuroscience. *Nat. Rev. Neurosci.*, **7**, 477–484.

Chin, L.S., Li, L., Ferreira, A., Kosik, K.S. & Greengard, P. (1995) Impairment of axonal development and of synaptogenesis in hippocampal neurons of synapsin I-deficient mice. *Proc. Natl. Acad. Sci. USA*, **92**, 9230–9234.

Conboy, L. & Sandi, C. (2010) Stress at learning facilitates memory formation by regulating AMPA receptor trafficking through a glucocorticoid action. *Neuropsychopharmacology*, **35**, 674–685.

Crawley, J.N. (2007) *What's Wrong with My Mouse? Behavioral Phenotyping of Transgenic and Knockout Mice*. John Wiley & Sons, NJ.

Davis, E.S. & Marler, C.A. (2003) The progesterone challenge: steroid hormone changes following a simulated territorial intrusion in female Peromyscus californicus. *Horm. Behav.*, **44**, 185–198.

DeArmond, S.J., Lee, Y.L., Kretzschmar, H.A. & Eng, L.F. (1986) Turnover of glial filaments in mouse spinal cord. *J. Neurochem.*, **47**, 1749–1753.

Deng, L., Wu, Z.N. & Han, P.Z. (2009) Effects of different levels of food restriction on passive-avoidance memory and the expression of synapsin I in young mice. *Int. J. Neurosci.*, **119**, 291–304.

Eng, L.F. & Ghirnikar, R.S. (1994) GFAP and astrogliosis. *Brain Pathol. (Zurich)*, **4**, 229–237.

Galea, L.A., Kavaliers, M., Ossenkopp, K.P., Innes, D. & Hargreaves, E.L. (1994) Sexually dimorphic spatial learning varies seasonally in two populations of deer mice. *Brain Res.*, **635**, 18–26.

Glasper, E.R. & Devries, A.C. (2005) Social structure influences effects of pair-housing on wound healing. *Brain Behav. Immun.*, **19**, 61–68.

Gomez-Pinilla, F., So, V. & Kesslak, J.P. (2001) Spatial learning induces neurotrophin receptor and synapsin I in the hippocampus. *Brain Res.*, **904**, 13–19.

Greengard, P., Valtorta, F., Czernik, A.J. & Benfenati, F. (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science*, **259**, 780–785.

Gubernick, D.J. (1988) Reproduction in the California mouse, *Peromyscus californicus*. *J. Mammal.*, **69**, 857–860.

Gyger, M., Kolly, D. & Guigoz, Y. (1992) Aging, modulation of food intake and spatial memory: a longitudinal study. *Arch. Gerontol. Geriatr.*, **15**(Suppl 1), 185–195.

Hammond, R., Mauk, R., Ninaci, D., Nelson, D. & Gibbs, R.B. (2009) Chronic treatment with estrogen receptor agonists restores acquisition of a spatial learning task in young ovariectomized rats. *Horm. Behav.*, **56**, 309–314.

Handelmann, G.E. & Olton, D.S. (1981) Spatial memory following damage to hippocampal CA3 pyramidal cells with kainic acid: impairment and recovery with preoperative training. *Brain Res.*, **217**, 41–58.

Harrison, F.E., Hosseini, A.H. & McDonald, M.P. (2009) Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. *Behav. Brain Res.*, **198**, 247–251.

Heikkinen, T., Puolivali, J. & Tanila, H. (2004) Effects of long-term ovariectomy and estrogen treatment on maze learning in aged mice. *Exp. Gerontol.*, **39**, 1277–1283.

Holahan, M.R., Rekart, J.L., Sandoval, J. & Routtenberg, A. (2006) Spatial learning induces presynaptic structural remodeling in the hippocampal mossy fiber system of two rat strains. *Hippocampus*, **16**, 560–570.

Idrobo, F., Nandy, K., Mostofsky, D.I., Blatt, L. & Nandy, L. (1986) Dietary restriction effects on radial maze learning and lipofuscin pigment deposition in the hippocampus and frontal cortex. *Arch. Gerontol. Geriatr.*, **6**, 355–362.

Ingram, D.K., Anson, R.M., de Cabo, R., Mamczarz, J., Zhu, M., Mattison, J., Lane, M.A. & Roth, G.S. (2004) Development of calorie restriction mimetics as a prolongevity strategy. *Ann. N Y Acad. Sci.*, **1019**, 412–423.

Iwata, M., Shirayama, Y., Ishida, H. & Kawahara, R. (2006) Hippocampal synapsin I, growth-associated protein-43, and microtubule-associated protein-2 immunoreactivity in learned helplessness rats and antidepressant-treated rats. *Neuroscience*, **141**, 1301–1313.

Jahng, J.W., Kim, J.G., Kim, H.J., Kim, B.T., Kang, D.W. & Lee, J.H. (2007) Chronic food restriction in young rats results in depression- and anxiety-like behaviors with decreased expression of serotonin reuptake transporter. *Brain Res.*, **1150**, 100–107.

John, J.P., Sunyer, B., Hoger, H., Pollak, A. & Lubec, G. (2009) Hippocampal synapsin isoform levels are linked to spatial memory enhancement by SGS742. *Hippocampus*, **19**, 731–738.

Krebs, J.R., Clayton, N.S., Hampton, R.R. & Shettleworth, S.J. (1995) Effects of photoperiod on food-storing and the hippocampus in birds. *Neuroreport*, **6**, 1701–1704.

Laping, N.J., Nichols, N.R., Day, J.R., Johnson, S.A. & Finch, C.E. (1994) Transcriptional control of glial fibrillary acidic protein and glutamine synthetase in vivo shows opposite responses to corticosterone in the hippocampus. *Endocrinology*, **135**, 1928–1933.

Luine, V. & Rodriguez, M. (1994) Effects of estradiol on radial arm maze performance of young and aged rats. *Behav. Neural Biol.*, **62**, 230–236.

MacDougall-Shackleton, S.A., Sherry, D.F., Clark, A.P., Pinkus, R. & Hernandez, A.M. (2003) Photoperiodic regulation of food storing and hippocampus volume in black-capped chickadees, *Poecile atricapillus*. *Anim. Behav.*, **65**, 805–812.

Masoro, E.J. (1998) Hormesis and the antiaging action of dietary restriction. *Exp. Gerontol.*, **33**, 61–66.

Morioka, M., Nagahiro, S., Fukunaga, K., Miyamoto, E. & Ushio, Y. (1997) Calcineurin in the adult rat hippocampus: different distribution in CA1 and CA3 subfields. *Neuroscience*, **78**, 673–684.

Nelson, R.J., Demas, G.E., Klein, S.L. & Kriegsfeld, L.J. (1995a) The influence of season, photoperiod, and pineal melatonin on immune function. *J. Pineal Res.*, **19**, 149–165.

Nelson, R.J., Gubernick, D.J. & Blom, J.M. (1995b) Influence of photoperiod, green food, and water availability on reproduction in male California mice (*Peromyscus californicus*). *Physiol. Behav.*, **57**, 1175–1180.

Nicol, S., Rahman, D. & Baines, A.J. (1997) Ca-$^{2+}$-dependent interaction with calmodulin is conserved in the synapsin family: identification of a high-affinity site. *Biochemistry*, **36**, 11487–11495.

Ping, S.E., Trieu, J., Wlodek, M.E. & Barrett, G.L. (2008) Effects of estrogen on basal forebrain cholinergic neurons and spatial learning. *J. Neurosci. Res.*, **86**, 1588–1598.

Prendergast, B.J. & Nelson, R.J. (2005) Affective responses to changes in day length in Siberian hamsters (*Phodopus sungorus*). *Psychoneuroendocrinology*, **30**, 438–452.

Pyter, L.M., Reader, B.F. & Nelson, R.J. (2005) Short photoperiods impair spatial learning and alter hippocampal dendritic morphology in adult male white-footed mice (*Peromyscus leucopus*). *J. Neurosci.*, **25**, 4521–4526.

Pyter, L.M., Trainor, B.C. & Nelson, R.J. (2006) Testosterone and photoperiod interact to affect spatial learning and memory in adult male white-footed mice (*Peromyscus leucopus*). *Eur. J. Neurosci.*, **23**, 3056–3062.

Pyter, L.M., Adelson, J.D. & Nelson, R.J. (2007) Short days increase hypothalamic-pituitary-adrenal axis responsiveness. *Endocrinology*, **148**, 3402–3409.

Ribble, D.O. (1992) Lifetime reproductive success and its correlates in the monogamous rodent *Peromyscus californicus*. *J. Anim. Ecol.*, **61**, 457–468.

Schwabe, L., Schachinger, H., de Kloet, E.R. & Oitzl, M.S. (2010) Corticosteroids operate as a switch between memory systems. *J. Cogn. Neurosci.*, **22**, 1362–1372.

Shuai, Y., Lu, B., Hu, Y., Wang, L., Sun, K. & Zhong, Y. (2010) Forgetting is regulated through Rac activity in Drosophila. *Cell*, **140**, 579–589.

Silva, A.L., Fry, W.H., Sweeney, C. & Trainor, B.C. (2010) Effects of photoperiod and experience on aggressive behavior in female California mice. *Behav. Brain Res.*, **208**, 528–534.

Singh, M., Meyer, E.M., Millard, W.J. & Simpkins, J.W. (1994) Ovarian steroid deprivation results in a reversible learning impairment and compromised cholinergic function in female Sprague–Dawley rats. *Brain Res.*, **644**, 305–312.

Stewart, J., Mitchell, J. & Kalant, N. (1989) The effects of life-long food restriction on spatial memory in young and aged Fischer 344 rats measured in the eight-arm radial and the Morris water mazes. *Neurobiol. Aging*, **10**, 669–675.

Sudhof, T.C., Czernik, A.J., Kao, H.T., Takei, K., Johnston, P.A., Horiuchi, A., Kanazir, S.D., Wagner, M.A., Perin, M.S., De Camilli, P. & Greengard, P. (1989) Synapsins: mosaics of shared and individual domains in a family of synaptic vesicle phosphoproteins. *Science*, **245**, 1474–1480.

Thompson, R., Kao, L. & Yang, S. (1981) Rapid forgetting of individual spatial reversal problems in rats with parafascicular lesions. *Behav. Neural Biol.*, **33**, 1–16.

de la Torre, J.C., Fortin, T., Park, G.A., Butler, K.S., Kozlowski, P., Pappas, B.A., de Socarraz, H., Saunders, J.K. & Richard, M.T. (1992) Chronic cerebrovascular insufficiency induces dementia-like deficits in aged rats. *Brain Res.*, **582**, 186–195.

Trainor, B.C., Crean, K.K., Fry, W.H.D. & Sweeney, C. (2010) Activation of extracellular signal-regulated kinases in social behavior circuits during resident–intruder aggression tests. *Neuroscience*, **165**, 325–336.

Tropp, J. & Markus, E.J. (2001) Effects of mild food deprivation on the estrous cycle of rats. *Physiol. Behav.*, **73**, 553–559.

Valentinuzzi, V.S., Buxton, O.M., Chang, A.M., Scarbrough, K., Ferrari, E.A., Takahashi, J.S. & Turek, F.W. (2000) Locomotor response to an open field during C57BL/6J active and inactive phases: differences dependent on conditions of illumination. *Physiol. Behav.*, **69**, 269–275.

Villacres, E.C., Wong, S.T., Chavkin, C. & Storm, D.R. (1998) Type I adenylyl cyclase mutant mice have impaired mossy fiber long-term potentiation. *J. Neurosci.*, **18**, 3186–3194.

Weil, Z.M., Bowers, S.L. & Nelson, R.J. (2007) Photoperiod alters affective responses in collared lemmings. *Behav. Brain Res.*, **179**, 305–309.

Whitsett, J.M. & Miller, L.L. (1982) Photoperiod and reproduction in female deer mice. *Biol. Reprod.*, **26**, 296–304.

Witte, A.V., Fobker, M., Gellner, R., Knecht, S. & Floel, A. (2009) Caloric restriction improves memory in elderly humans. *Proc. Natl. Acad. Sci. USA*, **106**, 1255–1260.

Wu, A., Sun, X. & Liu, Y. (2003) Effects of caloric restriction on cognition and behavior in developing mice. *Neurosci. Lett.*, **339**, 166–168.

Wube, T., Fares, F. & Haim, A. (2008) A differential response in the reproductive system and energy balance of spiny mice Acomys populations to vasopressin treatment. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **151**, 499–504.

Yamamoto, Y., Tanahashi, T., Kawai, T., Chikahisa, S., Katsuura, S., Nishida, K., Teshima-Kondo, S., Sei, H. & Rokutan, K. (2009) Changes in behavior and gene expression induced by caloric restriction in C57BL/6 mice. *Physiol. Genomics*, **39**, 227–235.

Zucker, I. & Beery, A.K. (2010) Males still dominate animal studies. *Nature*, **465**, 690.