Short day lengths alter stress and depressive-like responses, and hippocampal morphology in Siberian hamsters

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ABSTRACT

Many psychological disorders comprise a seasonal component. For instance, seasonal affective disorder (SAD) is characterized by depression during autumn and winter. Because hippocampal atrophy may underlie the symptoms of depression and depressive-like behaviors, one goal of this study was to determine whether short days also induce structural changes in the hippocampus using photoperiod responsive rodents — Siberian hamsters. Exposure to short days increases depressive-like responses (increased immobility in the forced swim test) in hamsters. Male hamsters were housed in either short (LD 8:16) or long days (LD 16:8) for 10 weeks and tested in the forced swim test. Brains were removed and processed for Golgi impregnation. HPA axis function may account for photoperiod-related changes in depressive-like responses. Thus, stress reactivity was assessed in another cohort of photoperiod-manipulated animals. Short days reduced soma size and dendritic complexity in the CA1 region. Photoperiod did not induce gross changes in stress reactivity, but an acute stressor disrupted the typical nocturnal peak in cortisol concentrations. These data reveal that immobility induced by exposure to short days is correlated with reduced CA1 cell complexity (and perhaps connectivity). This study is the first to investigate hippocampal changes in the context of short-day induced immobility and may be relevant for understanding psychological disorders with a seasonal component.

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Introduction

In seasonally-changing environments, small mammals must alter physiology and behavior in order to coordinate endogenous processes with ambient conditions. Winter is a particularly challenging time to survive and reproduce. In order to reliably coordinate physiological processes with environmental conditions, many rodents monitor day length, a precise and relatively noise-free cue. Day length (photoperiod) information is encoded physiologically through the duration of nighttime melatonin secretion, which is inversely proportional to day length (Reiter, 1993). In addition to coordinating reproductive processes, day length alters many nonreproductive behavioral and physiological changes. Seasonally-breeding rodents, for instance, undergo changes in brain morphology dependent on photoperiod. Wild-caught rodents have reduced skull size (Pucek, 1963), whole brain weights and hippocampal weight in winter (Yaskin, 1984). Exposure to short days in the laboratory reduces whole brain and hippocampal volume in white-footed mice (Peromyscus leucopus) (Perrot-Sinal et al., 1998; Pyter et al., 2005) suggesting that changes in brain volume in the wild may reflect changes induced by decreased day length. The brain requires a disproportionate amount of energy compared to other tissues, consequently slight reductions in brain volume may be adaptive because they help conserve energy in harsh environments. Seasonally-breeding rodents can be used in the laboratory to isolate the effects of day length on brain morphology; research in this area may reveal mechanisms of how day length alters the brain (and subsequently mental processes) in humans.

SAD is one disorder with a strong seasonal component, and is a subtype of major depressive disorder that encompasses similar symptoms to major depression (e.g., depressed mood, anhedonia, guilt), but some atypical symptoms as well (e.g., hypersomnia, hyperphagia). These symptoms remit in the summer and some individuals may become hypomanic. SAD has been associated with an inability to inhibit morning melatonin secretion in the winter (Lewy et al., 1999; Lewy and Sack, 1988; Rosenthal et al., 1984; Wehr et al., 2001) suggesting that shortened day length, and in turn extended melatonin secretion, may induce symptoms in vulnerable individuals. Currently, there is no animal model of SAD but several species exhibit greater depressive-like responses after exposure to short days. The forced swim test (FST) is a well-validated behavioral measure used to...
screen for antidepressant effects of pharmaceuticals in mice and rats (Porsolt et al., 1977, 1978). Although it has not yet been validated for use with hamsters, immobility in the FST is widely interpreted as a measure of ‘depressive-like behavior’ in many out-bred rodents. For instance, short days increase immobility in the FST in Siberian hamsters (Phodopus sungorus) (Prendergast and Nelson, 2005; Pyter and Nelson, 2006), Nile Grass rats (Ashkenazy-Frolinger et al., 2010), fat sand rats (Ashkenazy et al., 2009b), and Wistar rats (Prendergast and Kay, 2008). The FST has also been pharmacologically validated in fat sand rats (Krivisky et al., 2011). Little is known, however, regarding how the brain responds to reduced day length in these species, although constant darkness induces a depressive-like phenotype and alters monoamine and cytokine signaling in the brain (Gonzalez and Aston-Jones, 2008; Monje et al., 2011).

Current research in major depressive disorder and animal models of depression suggests that hippocampal atrophy is associated with much of the neuroendocrine phenomena (such as blunted circadian rhythm of cortisol and impaired negative feedback) and psychological symptoms of depression. For instance, the hippocampus atrophies in major depression and this is correlated with the duration of illness (Sheline et al., 1996, 1999). Chronic stressors are employed in many animal models of depression; this manipulation increases immobility in the forced swim test, as well as compromises hippocampal neurogenesis and CA3 dendritic integrity (Galea et al., 1997; Magarinos and McEwen, 1995; Watanabe et al., 1992). Selective serotonin reuptake inhibitors (SSRIs) stimulate hippocampal regeneration through neurogenesis and synaptic growth (Bessa et al., 2009; Pitterger and Duman, 2008; Wang et al., 2008). The latency in recovery of depressed individuals after beginning SSRl treatment may be related to the time course during which SSRIs alter creation, proliferation, survival, and connectivity of neurons within the hippocampus.

The hippocampus is rich in glucocorticoid and mineralocorticoid receptors (McEwen et al., 1968) and glucocorticoids have been strongly implicated in mood disorders in humans (McEwen, 2005). The hypothalamic–pituitary–adrenal (HPA) axis is often dysregulated in individuals diagnosed with depression (Young et al., 2000). Additionally, individuals with Cushing disease, whose adrenal glands produce an excess of cortisol, present with decreased hippocampal volume (Starkman et al., 1992), and depressed affect (Condren and Thakore, 2001). Further, reducing excessive glucocorticoids in patients with Cushing disease reverses hippocampal atrophy (Starkman et al., 1999). Chronic stressors induce dendritic retraction in the CA3 region of the hippocampus (Galea et al., 1997; Watanabe et al., 1992) and reduce hippocampal neurogenesis (Gould and Tanapat, 1999) in rats and high corticosteroids are often used to mimic these disruptions and induce depressive-like responses in animal models (Brummelte et al., 2006; Brummelte and Galea, 2010; Kalynchuk et al., 2004; Marks et al., 2009). These changes are reversible, however, as administration of antidepressants (such as tricyclics and SSRIs) restores neurogenesis (Malberg et al., 2000; Santarelli et al., 2003) and the integrity of hippocampal dendrites (Bessa et al., 2009; Wang et al., 2008). It is currently unknown how short-days regulate negative feedback in hamsters.

The goal of this study was to determine whether short days alter hippocampal neuronal morphology in conjunction with depressive-like responses in Siberian hamsters. We used this species because they reliably respond to short days with elevated immobility in the forced swim test (Prendergast and Nelson, 2005; Pyter and Nelson, 2006), which may represent a depressive-like response to short days. Subsequent to behavioral testing, we used the Golgi impregnation method to investigate neuronal morphology. Additionally, because of the strong association between depression (and animal models of depression) and glucocorticoids, we sought to determine whether short day lengths disrupt HPA axis reactivity and the activity of hamsters to return to cortisol concentrations similar to baseline 1 h after a stressor.

### Materials and methods

#### Animals

Siberian hamsters (P. sungorus) used in these studies were bred in our colony at the Ohio State University from a wild-bred stock obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were weaned during the light phase at 21 ± 2 d of age and immediately placed into either short photoperiod (8:16 LD) or maintained in their natal, long photoperiod (16:8 LD) conditions. Lights-off occurred at 1500 Eastern Standard Time (EST). Hamsters were housed in their respective photoperiods for 10 weeks prior to testing. Animal rooms were held at constant temperature and relative humidity (21 °C ± 2 °C and 50% ± 10%, respectively). All hamsters were singly housed in polycarbonate cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corncob bedding and had ad libitum access to food (Harlan Teklad Rodent Diet 8640; Indianapolis, IN, USA) and filtered tap water. All procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and comply with guidelines established by the National Institutes of Health published in Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (U.S.), 1996). No short-day hamsters in either experiment met the criterion for photoperiod nonresponsive (paired tests mass within 2 standard deviations of the mean of long day hamsters).

#### Experiment 1

##### Behavioral testing

After 10 weeks of photoperiod exposure, hamsters (n = 8 in LD; 9 in SD) were moved to a testing room and allowed to habituate for 30 min at the beginning of the dark phase. To assess depressive-like responses (Porsolt et al., 1977), hamsters were placed individually in room-temperature water – 17 cm deep within an opaque, cylindrical tank (24 cm diameter, 53 cm height). Behavior was recorded on video for 5 min under dim red light and scored by an observer unaware of experimental treatment assignments with The Observer software (XT 8.0; Noldus, Leesburg, VA, USA) to quantify latency to immobility, total number of times immobility was attained, and total time spent immobile. Immobility was scored when hamsters engage in movements only necessary to keep the head above water for at least 1 s. Increased immobility is interpreted as an elevated depressive-like response (Porsolt et al., 1977, 1978). One group of hamsters (n = 4 in LD; 6 in SD) remained undisturbed to control for the potential effect of behavioral testing on hippocampal morphology.

##### Tissue collection and processing

Forty hours after behavioral testing, hamsters were anesthetized with isoflurane vapors and body mass and pelage score (Duncan and Goldman, 1984a, b) were assessed. Then, hamsters were rapidly decapitated, trunk blood was collected, and brains were removed and processed for Golgi impregnation using the FD Rapid GolgiStain™ Kit (FD NeuroTechnologies Inc., Ellicott City, MD, USA) according to the manufacturer’s instructions. Tissue was collected between 0800 and 1000 h EST. Testes, epididymides, gonadal fat pads, and seminal vesicles were also removed at this time and weighed to assess reproductive responsiveness to photoperiod. Trunk blood samples (approximately 1 ml) were collected at necropsy using heparinized 200 μl tubes and stored in 1.5 ml polypropylene microcentrifuge tubes on ice until centrifugation at 6000 rpm (3.3 g) for 30 min. Plasma was immediately drawn from the samples and stored in 1.5 ml micro-centrifuge tubes at –80 °C until radioimmunoassay.
Histology and microscopy

Brains were sliced at 80 μm, counterstained with cresyl violet (Sigma) and otherwise processed according to the manufacturer’s instructions within the GolgiStain™ Kit (FD NeuroTechnologies Inc.). Brains were assessed for hippocampal cell morphology in three subfields in the dorsal hippocampus: dentate gyrus (DG), CA1, and CA3. Tracings were conducted by an experimenter blind to treatment groups. Sections were visualized using a Nikon E800 brightfield microscope and entire neurons were traced using NeuronXcida software (MicroBrightField, Burlington, VT, USA) at a magnification of 20×. Six representative neurons were selected per area, per animal for tracing. Selected neurons had to meet 3 criteria prior to tracing: (1) neurons had to be fully impregnated, (2) dendrites could not be truncated, and (3) for the DG neurons had to be granule cells with somas lying within the granule cell layer and [for the CA1 and CA3 regions], neurons had to be pyramidal cells with somas lying within the pyramidal cell layer. Whole cell traces were analyzed using the accompanying NeuroExplorer software (MicroBrightField, Burlington, VT). Cell body size and perimeter and dendritic length were calculated. To assess dendritic arborization patterns, Sholl analyses were also conducted. Because the Sholl analyses revealed that short days reduced dendritic complexity of CA1 pyramidal cells, we then counted branch points of apical and basal arbor of each trace to determine whether this effect was limited to dendrites in either orientation.

For spine density analyses, six neurons (in granule cell layer or pyramidal cell layer, depending on area) were again selected per area, per animal. Dendritic segments were traced at 100× in Neurulucida. For DG granule cells, four 20-μm dendritic segments were selected for counting if they were beyond at least one branch point. For pyramidal cells in the CA1 and CA3 regions, cells were selected if they had both apical and basal arbors; both of which were counted for each cell. For basal dendrites in both regions, four 20-μm dendritic segments beyond at least one branch point were included in the stratum oriens. For apical dendrites in both regions, four 20-micron dendritic segments beyond at least one branch point were included in the stratum radiatum. Spines were traced regardless of attributes (i.e., we counted filopodia, as well as mature spines) as long as they made a continuous connection with the dendritic shaft. Spine density (spines per 1 μm) was calculated for each trace in NeuroExplorer software and then averaged per cell and per area.

Experiment 2

Restraint and blood collection

After 10 weeks in photoperiod, hamsters in the stress group (n = 8–9 per group) were anesthetized with isoflurane vapors and underwent a retro-orbital sinus bleed at 0800 EST. Thereafter, hamsters were placed in restraint tubes for 1 h and bled again upon removal from restraint. This restraint process is a potent psychological stressor that reliably produces stress responses in hamsters. Specifically, hamsters were placed in restraint tubes for 1 h and bled again upon removal from restraint. Retro-orbital sinus bleed at 0800 EST. Thereafter, hamsters were placed in restraint tubes for 1 h and bled again upon removal from restraint. Sections were conducted by an experimenter blind to treatment groups. Sections were visualized using a Nikon E800 brightfield microscope and entire neurons were traced using NeuronXcida software (MicroBrightField, Burlington, VT, USA) at a magnification of 20×. Six representative neurons were selected per area, per animal for tracing. Selected neurons had to meet 3 criteria prior to tracing: (1) neurons had to be fully impregnated, (2) dendrites could not be truncated, and (3) for the DG neurons had to be granule cells with somas lying within the granule cell layer and [for the CA1 and CA3 regions], neurons had to be pyramidal cells with somas lying within the pyramidal cell layer. Whole cell traces were analyzed using the accompanying NeuroExplorer software (MicroBrightField, Burlington, VT). Cell body size and perimeter and dendritic length were calculated. To assess dendritic arborization patterns, Sholl analyses were also conducted. Because the Sholl analyses revealed that short days reduced dendritic complexity of CA1 pyramidal cells, we then counted branch points of apical and basal arbor of each trace to determine whether this effect was limited to dendrites in either orientation.

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Cortisol radioimmunoassay

All plasma samples from experiments 1 and 2 were measured in duplicate in a single assay. Cortisol, the primary circulating glucocorticoid in Siberian hamsters, was measured using a double antibody 125I–radioimmunoassay kit (Diagnostic Systems Laboratories, Inc., TX, USA) following the manufacturer’s instructions. This assay kit has been previously validated for use in this species (Reburn and Wynne-Edwards, 1999). The intra-assay coefficient of variation was <10%.

Statistical analysis

Two-by-two ANOVAs were first conducted with photoperiod and behavioral testing as independent factors with cortisol concentrations and hippocampal measures as dependent variables. If behavioral testing did not significantly alter the dependent measure, then groups were collapsed and analyzed by photoperiod only. Measures of immobility were log transformed because they were not normally distributed and analyzed by one-tailed Student’s t-tests with photoperiod as the independent variable. We predicted that short days would increase immobility based on previous studies (Prendergast and Nelson, 2005; Pyter and Nelson, 2006), which allows for one-tailed t-tests. Reproductive and somatic (except pelage score) measures and cortisol concentrations were also analyzed by two-tailed Student’s t-tests with photoperiod as the independent variable. Pelage scores were analyzed using a Mann–Whitney U test. Data from Sholl analyses were analyzed using repeated measures ANOVAs. Pearson’s product moment correlations were conducted between significant hippocampal measures and measures of immobility. Correlations were also performed between body mass and immobility. In experiment 2, somatic and reproductive measures were analyzed using 2×2 ANOVAs with photoperiod and stress exposure as independent factors. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor. Cortisol concentrations at baseline, post-restraint, and 1 h post-restraint were analyzed using a repeated measures ANOVA with photoperiod as the independent factor and time of collection as the repeated factor.

**Fig. 1.** Timeline for experiment 2 in relation to light cycles for long- and short-day hamsters (LD and SD, respectively). First two arrows indicate blood collection and restraint procedure. First, hamsters were bled from the retro-orbital sinus (0800 EST), placed in restraint tubes, bled again after restraint 1 h later (0900), returned to colony, then bled again 1 h later (1000 EST) to assess negative feedback. Third arrow indicates final blood draw taken to assess cortisol concentrations at the onset of the active phase.
Cary, NC, USA). All mean differences were considered statistically significant if \( p \leq 0.05 \).

**Results**

**Behavioral measures**

Photoperiod significantly altered depressive-like responses. Short days increased time spent floating \((t_{18} = 2.358; p < 0.05; \text{Fig. 2A})\) and float bouts \((t_{18} = 2.017; p < 0.05; \text{Fig. 2B})\). Short days also reduced the latency to float \((t_{18} = 3.457; p < 0.05; \text{Fig. 2C})\).

**Reproductive and somatic measures**

Short days significantly reduced body mass \((t_{28} = 3.838; p < 0.01)\), paired testes mass \((t_{28} = 23.395; p < 0.01)\), paired fat pad mass \((t_{28} = 7.193; p < 0.01)\), and paired epididymides mass \((t_{28} = 7.778; p < 0.01)\) compared with long days. Short days also provoked significantly lighter pelage color \((p < 0.01)\).

**Hippocampal measures**

Short days significantly reduced soma area of pyramidal cells in the CA1 region \((F_{1,21} = 4.392; p < 0.05; \text{Fig. 3A})\). Photoperiod did not alter soma area of granule cells in the dentate gyrus \((t_{24} = 0.792; p > 0.05; \text{Fig. 3C})\) or pyramidal cells in the CA3 region \((t_{25} = 0.782; p > 0.05; \text{Fig. 3B})\). Photoperiod did not alter soma perimeter or dendritic length in the CA1, CA3, or dentate gyrus \((p > 0.05\text{ in all cases})\). Photoperiod and distance from cell body significantly interacted to alter number of intersections in the CA1 region \((F_{2,80} = 1.68; p > 0.01; \text{Fig. 3B})\) such that short days significantly reduced intersections proximal to the cell body. Short days did not alter branch points of apical \((t_{25} = 1.146; p > 0.05)\) or basilar \((t_{25} = 0.642; p > 0.05)\) dendrites in the CA1 region. Short days did not alter number of intersections in the CA3 region or DG \((p > 0.05\text{ in both ANOVAs}; \text{Figs. 3D and F})\). Short days significantly increased spine density within the dentate gyrus \((t_{25} = 2.357; p < 0.05; \text{Fig. 4B})\). Photoperiod did not alter spine density on CA3 apical \((t_{25} = 0.799; p > 0.05)\) or basilar dendrites \((t_{25} = 0.276; p > 0.05)\). Photoperiod did not alter spine densities on CA1 apical \((t_{24} = 0.17; p > 0.05)\) or basilar dendrites \((t_{24} = 0.05; p > 0.05)\).

**Correlations**

Total CA1 intersections negatively correlated with time immobile \(r = -0.691; p < 0.01; \text{Fig. 5A})\) and both immobility bouts \((r = -0.542; p < 0.05; \text{Fig. 5B})\). Correlations significantly increased latency to immobility \((r = 0.616; p < 0.01; \text{Fig. 5C})\). Soma area in the CA1 was positively correlated with CA1 intersections \((r = 0.552; p < 0.05)\), but not with behavioral measures in the forced swim test \((p > 0.05\text{ in all cases})\). CA1 soma area was also significantly correlated with spines in the dentate gyrus \((r = -0.529; p < 0.05)\). There were no significant correlations between body mass and measures of immobility in the FST \(r = 0.191, p = 0.45\) body mass and bouts immobile: \(r = 0.023, p = 0.92\); body mass and latency to immobility: \(r = 0.057, p = 0.82\).

**Experiment 2**

**Reproductive and somatic measures**

Short days significantly reduced body mass \((F_{1,24} = 16.549; p < 0.0005)\), but an acute stressor did not alter body mass assessed 40 h later \((F_{1,24} = 0.707; p > 0.05)\). Short days reduced paired epididymides mass \((F_{1,25} = 600.677; p < 0.0001); \text{paired fat pad mass} \((F_{1,26} = 53.862; p < 0.0001); \text{paired testes mass} \((F_{1,26} = 519.085; p < 0.0001); \text{and paired seminal vesicle mass} \((F_{1,26} = 34.473; p < 0.0001).\) Stress did not affect any reproductive measure \((p > 0.05\text{ in all ANOVAs})\).

**Cortisol concentrations**

Restraint significantly altered cortisol concentrations \((F_{2,20} = 174.978; p < 0.0001)\) such that cortisol concentrations post-restraint were significantly higher than both baseline and 1 h post-restraint samples \((t_{15} = 11.97; p < 0.0001; t_{16} = 16.156; p < 0.0001, \text{respectively}\). Cortisol concentrations assessed 1 h after restraint were also lower than baseline cortisol \((t_{15} = 3.258; p < 0.01).\) Photoperiod, however, did not significantly interact with time of collection \((F_{2,20} = 2.402; p > 0.05)\). At baseline, short days significantly increased cortisol concentrations \((t_{13} = 3.015; p = 0.01)\), but not at the other two time points \((p > 0.05\text{ in both cases}; \text{Fig. 6A})\). Time of day and photoperiod did not interact to alter cortisol concentrations \((F_{1,23} = 0.373; p > 0.05)\) but both time of day and photoperiod had significant main effects \((F_{1,23} = 4.685; p < 0.05; F_{1,23} = 6.617; p < 0.05, \text{respectively}\) such that short days and onset of dark phase both increased cortisol concentrations among the no-stress hamsters. Prior stress, however, reversed the photoperiod difference in cortisol concentrations: stressed, short-day hamsters had significantly lower cortisol concentrations in the dark phase compared with long-day hamsters \((t_{13} = 9.009; p < 0.01)\) and compared with unstressed hamsters \((t_{13} = 2.11; p = 0.05; \text{Fig. 6B})\).

**Discussion**

We demonstrate that short photoperiod, an environmental cue that predicts the onset of winter, increases depressive-like responses, reduces soma size and dendritic complexity in the CA1 region, and increases spine density in the DG. Moreover, CA1 dendritic complexity significantly correlated with behavioral measures in the forced swim test.
test, which suggests that short-day-induced changes in the hippocampus may be behaviorally relevant. This study may be important for understanding how day length influences neural structure and symptoms in people with psychological disorders that fluctuate seasonally. Several other studies have investigated the effect of short days on other species, including diurnal and nonseasonally-breeding rodents with similar behavioral results. Many species increase depressive-like responses when exposed to short days (Ashkenazy et al., 2009a,b; Ashkenazy-Frolinger et al., 2010; Prendergast and Kay, 2008) but studies have not investigated structural changes in the brain.

The hippocampus is important in regulating both cognitive and emotional processes and stress responses (Fanselow and Dong, 2010). Prior research has established that hippocampal morphology fluctuates across biological time. For instance, estrogens coordinate changes in hippocampal spine density over the estrous cycle (Woolley et al., 1990). Hippocampal dendritic complexity and spines also change during hibernation (Magarinos et al., 2006; Popov et al., 1992, 2007). These structural changes are related to changes in behaviors such as activity, learning and memory, and depressive-like responses (Magarinos et al., 2006; Popov et al., 1992, 2007; Pyter et al., 2005, 2007). In our study, exposure to short days significantly reduced cell complexity and soma size in the CA1 region. The total number of intersecting dendrites was also significantly correlated with each measure in the forced swim test. The best documented function of the CA1 region is learning and memory. However, remodeling of CA1 synapses is also related to expression of depressive-like responses (Hajszan et al., 2009, 2010). In these prior studies, CA1 spines were negatively correlated with escape latency in a learned helplessness task, suggesting that the CA1 is involved in depressive-like responses. In Siberian hamsters, exposure to dim light at night also reduced spine density in the CA1 region, sucrose consumption, and increased time spent immobile in the forced swim test (Bedrosian et al., 2011). Moreover, spine density in this study was significantly correlated with both measures, further supporting the relationship between in CA1 region and depressive-like responses (Bedrosian et al., 2011). We did not report differences in spine density in the CA1 region however, our assessment was limited to dendritic segments relatively proximal to the cell body (but >50 μm away).
Whether photoperiod alters terminal dendritic spine density in this species remains to be determined, but photoperiod does alter spine density at terminal tips of dendrites in white-footed mice (P. leucopus) (Pyter et al., 2005). Short days also reduced soma size in the CA1, and this was positively correlated with total number of intersections in the CA1. Humans with major depression have reduced in soma size in the CA1, CA2, and CA3 regions (Stockmeier et al., 2004), which may be one

Fig. 4. Scatter plots with simple regression lines depicting the relationship between CA1 dendritic complexity (i.e., total intersecting dendrites) and behavioral measures in the forced swim test. CA1 intersections negatively correlated with A) time spent immobile, B) immobility bouts, and C) positively correlated with the latency to immobility.

Fig. 5. Representative micrographs of dendritic segments of granule cells in the dentate gyrus from A) a long-day hamster and B) a short-day hamster. Scale bar represents 10 μm. C) Mean ± SEM spines per micron of granule cells within the dentate gyrus. Short days significantly increased spine density in the dentate gyrus; *p < 0.05.

Fig. 6. Mean ± SEM cortisol concentrations (ng/ml). A) Restraint stress significantly increased cortisol concentrations compared with baseline and 1 h post-restraint. Cortisol concentrations 1 h post-restraint were lower than at initial baseline. Photoperiod did not alter reactivity to the acute stressor, nor did photoperiod alter the ability of hamsters to reduce cortisol to concentrations similar to baseline. However, short days increased baseline cortisol concentrations, *p < 0.05 within baseline measurement, letters denote differences in concentrations at each timepoint. B) Data in first two bars are replotted from figure A. Regardless of time of collection, (dark versus light phase) short days increased cortisol concentrations (*p < 0.05). Among no-stress hamsters, cortisol concentrations were significantly higher in the dark phase compared with concentrations at the light phase (**p < 0.05). However, an acute stressor 40 h prior reversed the typical pattern of cortisol concentrations, such that the stressor reduced cortisol in short-day hamsters, but increased cortisol in long day, †p ≤ 0.05; comparison within photoperiod, between stress groups.

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component that contributes to hippocampal volume reduction in some depressed individuals.

In contrast to our predictions, short days increased spine density in the dentate gyrus (DG). Spine density in the DG was not significantly correlated with any behaviors, but was negatively correlated with CA1 soma size. The functional implications of an increase in DG spine density is not clear, but reduced spine density does not necessarily represent impaired hippocampal function. Although reduced spine density is typically associated with increased depressive-like behaviors, function of spines may be dissociable from pure numbers of spines (van Spronsen and Hoogenraad, 2010). Further, high spine density should not always be interpreted as beneficial for the organism as increased cortical spine density has been associated with autism spectrum disorders (Hutsler and Zhang, 2010). We also did not assess spine type in this study. Distribution and density of spine types (e.g., mushroom, thin, and stubby spines) along dendritic segments may also alter electrophysiological properties of cells, and subsequently behaviors, as different spine types have distinct functional and biochemical properties (Bourne and Harris, 2008). Because dendritic spines in the DG negatively correlated with CA1 soma size, it is possible that these changes represent two distinct but closely related processes. Region-specific changes in spine density and dendritic morphology may be interpreted as restructuring of a complete, integrated circuit.

It should be noted that because the nature of this research is descriptive, proximate mechanisms whereby day length alters brain morphology are as yet unknown. Short days may regulate dendritic complexity and spines in myriad ways and future research should address how the hormonal milieu in different environmental contexts reorganizes the hippocampal network. One potential hormone of interest is cortisol, and although we did not directly test if cortisol regulates photoperiod-induced changes in hippocampal morphology, we did assess HPA axis function in short and long days. The HPA axis is tightly regulated as prolonged secretion of its associated hormones can be maladaptive. Reduction of glucocorticoid secretion after cessation of a stressor is referred to as fast negative feedback and regulated primarily by hippocampal glucocorticoid and mineralocorticoid receptors (Jacobson and Sapolsky, 1991; Sapolsky et al., 1984, 1991). In the present study, photoperiod did not alter cortisol concentrations directly after an acute 1 h stressor, nor 1 h after the stressor was removed. This suggests that short-day hamsters have similar HPA axis reactivity to long-day hamsters. This is contrary to a prior study (Bilbo et al., 2002) wherein short days induced higher cortisol concentrations after restraint stress. Methodological differences, such as age of hamsters upon photoperiod assignment and paired housing conditions may explain this disparity. At baseline, short-day hamsters had higher baseline cortisol concentrations compared with long-day hamsters.

As expected, cortisol concentrations were higher at the beginning of the dark phase compared with cortisol concentrations in samples taken at the light phase. However, a prior acute stressor reversed the typical photoperiod-related differences in cortisol concentrations. Specifically, forty hours after the stressor, short-day hamsters displayed significantly reduced cortisol concentrations compared with both stressed, long-day hamsters and unstressed, short-day hamsters. This suggests that an acute stressor produces long-term changes (compared with the time course typically assessed with negative feedback) in the HPA axis that are photoperiod-related. It is well documented that chronic stressors lead to disruptions in physiological and behavioral processes but despite effective fast feedback control over the HPA axis, short-term, or acute, stressors can also lead to long standing changes in behavior and brain function. For instance, short-term foot shock can lead to long term behavioral changes and changes within the brain that last approximately 28 days (van Dijken et al., 1993). Additionally, social defeat can blunt circadian rhythms in body temperature, heart rate and locomotor activity (Meerlo et al., 1997, 2002; Tomatzky and Miczek, 1993).

Dysregulated patterns of cortisol secretion have been consistently associated with affective disorders (Pregelj, 2008; Young, 2004). Many individuals with SAD may have a circadian phase delay in the offset of melatonin secretion, but circadian patterns in cortisol secretion appear to be similar between those with and without SAD (Danilenko and Putlivo, 1993). In patients with SAD, morning light treatment leads to a slight advance in the nadir of cortisol and a delay in cortisol secretion was correlated with symptom severity (Thalen et al., 1997). Additionally, individuals diagnosed with SAD show typical cortisol responses to dexamethasone suppression test suggesting that they do not have dysregulated negative feedback (James et al., 1986).

Stress responses in Siberian hamsters may more closely mimic those of individuals with SAD, rather than those with major depressive disorder. Blood samples after the acute stressor were not collected at the onset of the light phase so it is not obvious whether stress dysregulated the full diurnal rhythm in this study. Additional research should address how day length alters hamsters’ ability to adapt to acute and chronic stressors and the behavioral consequences of long-term adjustments in circadian rhythmicity in cortisol secretion.

There are several limitations that should be noted when interpreting these data. First, hamsters undergo substantial physiological and behavioral responses to photoperiod that are vastly different from human responses to day length. For example, androgens decrease to nearly undetectable concentrations when hamsters are exposed to short days and androgens can alter immobility in the FST and hippocampal morphology. Thus, future research should determine if replacement of testosterone reverses short-day induced immobility in the FST and hippocampal complexity. Additionally, pharmacological validation of the FST with Siberian hamsters has not been formally conducted; thus immobility may not represent a depressive-like phenotype, but result from motoric or energetic capacity. Some evidence does not support this hypothesis, as limb strength does not differ in short compared with long days (Prendergast and Nelson, 2005). Further, when given access to a running wheel, short-day hamsters run more than long-day hamsters (Bilbo and Nelson, 2004). Finally, we conducted Pearson’s correlations between body mass and immobility measures in the FST and found no relationship. Together, this suggests that immobility in the FST is not merely the result of energetic capacity. Rather, immobility may represent a more centrally governed behavior to prevent expenditure of energy.

An additional consideration is that behavior and hormone concentrations may be different due to photoperiod-related differences in circadian organization. This is especially pertinent when considering the first blood draws and stress procedure (see Fig. 1 for timeline). Although they were phase-locked to the onset of the dark phase (and thus the natural circadian peak in cortisol), the first blood samples were collected during different circadian times relative to the onset of the light phase for short- versus long-day hamsters. Future studies should investigate whether such differences persist at the same time points. For instance, a follow-up study could examine whether immobility or cortisol concentrations remain higher for short day hamsters if assessed at the middle of the dark phase for both short- and long-day hamsters.

In sum, these results may yield new insights into the study of day length, brain, and behavior and add to our understanding regarding environmental factors that influence hippocampal plasticity and depressive-like responses. However, additional studies are necessary to determine the hormonal mechanisms by which day length alters the brain and behavior.

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