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Research report

Photoperiod alters fear responses and basolateral amygdala neuronal spine density in white-footed mice (*Peromyscus leucopus*)

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HIGHLIGHTS

- Shorts day lengths enhance fear memory in white-footed mice (*Peromyscus leucopus*).
- Short days increase spine density in the basolateral amygdala.
- Short days do not alter dendritic spines or arborization in the infralimbic PFC.

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ABSTRACT

Photoperiodism is a biological phenomenon in which environmental day length is monitored to ascertain time of year to engage in seasonally appropriate adaptations. This trait is common among organisms living outside of the tropics. White-footed mice (*Peromyscus leucopus*) are small photoperiodic rodents which display a suite of adaptive responses to short day lengths, including reduced hippocampal volume, impairments in hippocampal-mediated memory, and enhanced hypothalamic-pituitary-adrenal axis reactivity. Because these photoperiodic changes in brain and behavior mirror some of the etiology of post-traumatic stress disorder (PTSD), we hypothesized that photoperiod may also alter fear memory and neuronal morphology within the hippocampus–basolateral amygdala–prefrontal cortex fear circuit. Ten weeks of exposure to short days increased fear memory in an auditory-cued fear conditioning test. Short days also increased dendritic spine density of the neurons of the basolateral amygdala, without affecting morphology of pyramidal neurons within the infralimbic region of the medial prefrontal cortex. Taken together, photoperiodic phenotypic changes in brain morphology and physiology induced by a single environmental factor, exposure to short day lengths, affect responses to fearful stimuli in white-footed mice. These results have potential implications for understanding seasonal changes in fear responsiveness, as well as for expanding translational animal models for studying gene–environment interactions underlying psychiatric diseases, such as PTSD.

1. Introduction

Photoperiodism is the biological ability to measure environmental day length (photoperiod) to ascertain the time of year and engage in seasonally appropriate adaptations of physiology and behavior. These seasonal changes in physiology and behavior can be induced in a laboratory setting by simply exposing animals to different static day lengths [1]. White-footed mice (*Peromyscus leucopus*) display a suite of changes in physiology and behavior induced by exposure to short days, including changes in behavior, brain volume and functional connectivity, and enhanced HPA axis reactivity [2–4]. In recent years, photoperiodic rodent models are finding more utility as models of human pathologies involving alterations in brain structure and function [5,6].

Among human post-traumatic stress disorder (PTSD) patients, reduced hippocampal volume is associated with susceptibility to PTSD, but not severity of symptoms [7], amygdala activity is associated with symptom severity [8], and PTSD patients have enhanced HPA axis negative feedback [7]. Additionally, the medial prefrontal cortex (mPFC) is hyporesponsive to fear-related stimuli in patients suffering from PTSD [9,10]. Translational rodent studies have identified these three brain regions as critical components of the neural circuit underlying associative fear memory. The basolateral amygdala (BLA), hippocampus, and mPFC all play critical roles in fear memory: complex modulation of reciprocal connections among the mPFC, hippocampus, and basolateral amygdala (BLA) are integral to associative fear memory [7,11–13].

Although standard laboratory rodents (*Rattus norvegicus* and *Mus musculus*) have been widely used to model PTSD and other human psychiatric disorders, to fully understand combined

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factors underlying the development, maintenance, and treatment of these diseases, more diverse animal models are needed [5]. Toward this end, photoperiod-induced changes in the brain of white-footed mice mirror several components of the etiology of PTSD. For example, in common with the human PTSD condition, short day exposed white-footed mice have reduced hippocampal volume [4] and increased HPA axis feedback [3]. To our knowledge, photoperiod-mediated changes in fear memory and responses in this, and other, photoperiodic rodent species remain largely undescribed. A preliminary study in our lab indicated that, unlike short-day induced impairments in hippocampal-mediated spatial learning and memory, white-footed mice exposed to short days may have enhanced non-spatial fear memory in the passive avoidance test [4].

Based on the commonality of photoperiodic changes in white-footed mice with the etiology of PTSD described above, and on preliminary data demonstrating short day enhancement of fear memory, we hypothesized that exposure to short days would enhance fear memory and alter neuronal morphology in brain regions implicated in associative fear memory. To test our hypothesis, we exposed male white-footed mice to either short or long day lengths for ten weeks to induce maximal photoperiodic responses, tested them in an auditory fear conditioning test, and examined the neuronal morphology in the BLA and the infralimbic region of the mPFC (IL) using Golgi-Cox staining.

2. Materials and methods

2.1. Animals

Nineteen adult (>55 d of age) male *P. leucopus*, from our breeding colony maintained at the Ohio State University, were randomly assigned to either long (LD; 16L:8D, $n = 10$) or short day lengths (SD; 8L:16D, $n = 9$) for 10 weeks to establish photoperiod-induced changes prior to behavioral testing [2,4]. Mice were housed in standard polycarbonate cages (32 cm × 18 cm × 14 cm), maintained at constant temperature (21 ± 4 °C) and relative humidity (50 ± 5%), provided ad libitum access to filtered tap water and food (Harlan Teklad 8640, Indianapolis, IN, USA), and received care from the Ohio State University Laboratory Animal Resource staff for the duration of the study. All procedures proposed were approved by the Ohio State University Institutional Animal Care and Use Committee and are in compliance with guidelines established by the National Institutes of Health [14].

2.2. Behavioral test

2.2.1. Auditory fear conditioning

To assess tone-conditioned fear acquisition and retention, 19 ($n = 10$ LD; $n = 9$ SD) mice were assessed using the Near-IR Video Fear Conditioning System (Med Associates Inc., St. Albans, VT, USA). For acquisition of the tone-conditioned fear, during the light phase mice were brought directly from their vivarium rooms and were placed in the test chamber illuminated with white light for a 2 min habituation period with 68 dB white noise. Mice were then exposed to a series of 8 conditional stimuli (80 dB tone, CS) for 6 s with the last 2 s paired with a 0.75 mA foot shock (unconditioned stimulus, US). Mice remained in the chamber for an additional 60 s after the last CS/US pairing before being returned to their home cages. Freezing behavior was recorded by the software for the 2 min baseline, during the first 4 s of each tone, during the 30 s interval between CS presentations, and for the 60 s after the final CS. To assess contextual fear retention, 24 h after the acquisition session, mice were placed in the original unmodified chamber and freezing behavior to the unmodified chamber was recorded for180 s, and mice were returned to their home cages. Four hours after the contextual fear retention test, mice were tested for retention of the CS–US pairing with the following modifications to alter context. Mice were transported from their vivarium rooms via sound- and light-attenuating boxes to a staging area. From there, mice were brought into the testing room lit with dim red light and placed into the chambers. To avoid context-dependent freezing, the chamber was modified via the addition of a smooth plastic floor, a semi-circular unlit testing chamber, lights were extinguished, and a gauze pad with a drop of vanilla extract was placed in the chamber to present the CS in a novel environment. Mice were then tested for retention of the CS/US pairing by using the procedure described above for the acquisition trial above without receiving the foot shock (US).

2.3. Sample collection and histology

Twenty hours after the completion of auditory fear conditioning, under deep isoflurane anesthesia, mice were exsanguinated via the retro-orbital sinus, plasma was collected from the blood samples as previously described [4], and samples were stored at −80 °C for corticosterone assay. Immediately after blood collection, mice were rapidly decapitated and brains were processed to study neuronal morphology (after [4,15]) using a commercially available Golgi-Cox impregnation kit (FD NeuroTechnologies, Ellicott City, MD, USA) according to the manufacturer's instructions. Briefly, after impregnation, brains were cut into 100 μm coronal sections and thaw mounted on to gelatin-coated slides. Slides were then developed, counterstained with cresyl violet acetate, dehydrated, cleared with xylenes, and coverslipped with Permount (Fisher).

2.3.1. Dendritic arborization analysis

Pyramidal neurons ($n = 4$–6 for each mouse) in the infralimbic cortex (IL), identified by its cytoarchitecture and neuroanatomical position medial to the forceps minor and cingulum between 1.3 and 1.9 mm anterior to bregma [16], were traced at 400× and quantified using neuronal tracing software (Neurolucida, Microbrightfield, VT, USA). Neurons were traced only if they met the following criteria: (1) completely and uniformly impregnated with Golgi stain, (2) all dendrites were intact and visible, and (3) not obscured by other stained neurons (after [4]). The basolateral amygdala (BLA), identified by its location bounded by the branched arms of the external capsule between 0.8 and 2.0 mm posterior to bregma [16], did not contain sufficient numbers of neurons that met the above criteria for analysis. Representative values for each parameter measured by the software (see Section 3) from each animal were calculated by averaging values from all neurons traced. Representative values calculated for each animal were then used for further analysis.

2.3.2. Dendritic spine density analysis

Dendritic spines of the neurons were traced at 1000× using Neurolucida software (Microbrightfield, VT, USA). Within the BLA, for each animal average spine density was calculated by selecting six neurons, and an unbranched, unbroken, and consistently stained dendritic segment at least 50 μm away from the soma from each neuron was quantified. Any protrusion originating from the dendritic shaft was classified as a spine, and all spines along a continuous 80 μm segment were counted for spine density analysis (after [17]). For the IL, average spine densities were quantified for each animal from both basilar and apical dendrites, selected as above. For each neuron a total of 80 μm of basilar and 80 μm apical dendrite were quantified for spine density. For each brain region, the average spine density calculated from each animal was then used for further comparative analysis.

2.3.3. Corticosterone assay

Frozen plasma samples were thawed on ice and assayed for corticosterone using a commercially available double antibody RIA kit according to manufacturer's instructions (Cat# 07120102; MP Biomedicals, Costa Mesa, CA, USA). The intra-assay coefficient of variation was 7.9%.

2.3.4. Statistics

Repeated measures ANOVA were used to compare auditory fear responses over time and dendritic arborization measures (Sholl analysis). Student's t tests were used for comparisons between photoperiods for physiological data, spine density analysis, and for follow up testing of specific time points within trials for auditory fear testing after a main effect was identified by ANOVA. Data with unequal variance were log transformed prior to analysis. All analyses were performed using SPSS software (v19; IBM, NY, USA) and differences were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Physiological measures

Exposure to short day lengths did not affect body mass ($t_{(17)} = 0.185$, $p > 0.05$) or inguinal fat pad mass ($t_{(17)} = 0.036$, $p > 0.05$). Compared to LD-exposed counterparts, exposure to SD reduced masses of all reproductive tissues assessed (paired testes, $t_{(15)} = 4.934$, $p < 0.001$; epididymides, $t_{(17)} = 2.872$, $p < 0.05$; seminal vesicles, $t_{(17)} = 2.655$, $p < 0.05$ Fig. 1, left). Short day exposure did not affect basal corticosterone concentrations at the terminal bleed ($t_{(16)} = 0.904$, $p > 0.05$; Fig. 1, right).

3.2. Behavioral measures

3.2.1. Auditory-cued fear conditioning

LD and SD mice did not differ in their freezing responses during acquisition of the CS–US pairing across trials (repeated measures ANOVA: $F_{(1,15)} = 0.056$, $p > 0.05$: Fig. 2A). Comparing baseline to post CS–US presentation, both SD and LD mice increased freezing behavior across acquisition (LD, $t_{(8)} = -2.714$, $p < 0.05$; SD $t_{(7)} = -3.457$,