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TIMING OF LIGHT PULSES AND PHOTOPERIOD ON THE DIURNAL RHYTHM OF HIPPOCAMPAL NEURONAL MORPHOLOGY OF SIBERIAN HAMSTERS

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Abstract—Rapid remodeling of neurons provides the brain with flexibility to adjust to environmental fluctuations. In Siberian hamsters, hippocampal dendritic morphology fluctuates across the day. To reveal the regulatory mechanism of diurnal remodeling of hippocampal neurons, we investigated the effects of light signals applied under different photoperiodic conditions on dendritic morphology. A 4-h dark pulse during the morning of long days (LD) increased basilar dendritic length, as well as complexity of basilar dendrites of neurons in the CA1. A light pulse during the late night in short days (SD) reduced basilar dendrite branching and increased primary apical dendrites of CA1 neurons. Spine density of dentate gyrus (DG) dendrites was increased by a dark pulse in LD and spine density of CA1 basilar dendrites was decreased by a light pulse in SD. These results indicate that light signals induce rapid remodeling of dendritic morphology in a hippocampal subregion-specific manner. A light pulse in SD decreased hippocampal expression of *fetal liver kinase 1* (*Flk1*), a receptor for vascular endothelial growth factor (VEGF), raising the possibility that VEGF–FLK1 signaling might be involved in the rapid decrease of branching or spine density of CA1 basilar dendrites by light.

Key words: diurnal rhythm, hippocampus, neuroplasticity, neurotrophic factor.

INTRODUCTION

Although long-term stability of the neuronal system is important for proper brain function, rapid remodeling of neurons is also necessary for the brain to cope with the changing environment. One of the most plastic sites in adult mammalian brains is the hippocampus, which plays crucial roles in learning, memory formation, and cognitive behaviors (Breedlove and Jordan, 2001). In response to various external and internal conditions, such as stressors (Watanabe et al., 1992; Chen et al., 2008; Magariños et al., 2011), hibernation (Popov et al., 1992; Magariños et al., 2006), reproductive status (Gould et al., 1990; Woolley et al., 1990), and photoperiod (Pyter et al., 2005; Workman et al., 2011; Walton et al., 2013), hippocampal neurons undergo structural alterations in dendrite generation, growth, branching, and spine formation.

Hippocampal dendritic patterning and spine density undergo diurnal fluctuations that are influenced by photoperiod in Siberian hamsters (Ikeno et al., 2013). A diurnal rhythm in neuronal morphology within the infralimbic cortex, an area implicated in cognitive function, has also been reported (Perez-Cruz et al., 2009). These findings suggest that rapid regulation of neuronal architecture contributes to synchronizing neuronal function to external daily cycles. However, the regulation of diurnal rhythms of dendritic alterations is not fully understood.

Because daily environmental fluctuations are derived from light–dark conditions, most diurnal rhythms are acutely affected by photic signals through phase shifting of the circadian clock, an endogenous mechanism generating internal circadian rhythms, or through the direct masking of clock outputs (Ko and Takahashi, 2006). Clock-regulated synthesis and release of the pineal hormone melatonin are inhibited by light, and melatonin acts as temporal cues for the circadian clocks located in various tissues (Pevet and Challet, 2011). Melatonin also plays an important role in the photoperiodic response, because the duration of melatonin secretion represents the length of night, which varies seasonally (Pevet and Challet, 2011). In seasonal mammals including Siberian hamsters, which undergo regression of reproductive organs in short days, suppression of nighttime melatonin secretion by light in short days triggers reproductive activation by inducing the long-day pattern of the hypothalamic genes important for the gonadotropin regulation (Barrett and Bolborea, 2012). Recent evidence has suggested that melatonin has a neuroprotective function and stimulates dendritogenesis and spinogenesis (González-Burgos et al., 2007; Ramirez-Rodriguez et al., 2011; Domínguez-Alonso et al., 2012) via its

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Abbreviations: BDNF, brain-derived neurotrophic factor; DG, dentate gyrus; Flk1, fetal liver kinase 1; GnRH, gonadotropin-releasing hormone; LD, long-day conditions; SD, short-day conditions; TrkB, tropomyosin receptor kinase B; VEGF, vascular endothelial growth factor; ZT, zeitgeber time.

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actions as free radical scavenger and antioxidant (Reiter, 1998) and via its modulatory action on cytoskeleton organization (Benítez-King, 2006). Melatonin also increases neurotrophic factors in cultured neurons and in the hippocampus (Imbesi et al., 2008; Soumier et al., 2009). Neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF), have been implicated in neuroplasticity by signaling through their receptors, tropomyosin receptor kinase B (TrkB) and fetal liver kinase 1 (FLK1), respectively (McAllister, 2001; Carmeliet and Ruiz de Almodovar, 2013). Taken together, these studies raise the possibility that remodeling of hippocampal dendrites depends on external photic signals, which are mediated by nocturnal secretion of melatonin.

In the present study, we investigated the acute effects of a dark pulse during the early morning of long days and a light pulse during the late night of short days on dendritic morphology of hippocampal neurons to reveal the mechanism underlying the diurnal rhythm of neuronal morphology in male Siberian hamsters. We also investigated mRNA expression of neurotrophic factors, *Bdnf* and *Vegf*, and their receptors, *trkB* and *Flk1*, in the hippocampus to investigate the possibility that the dendritic remodeling was mediated by neurotrophic factor signaling.

EXPERIMENTAL PROCEDURES

Animals

Siberian hamsters (*Phodopus sungorus*) used in this study were bred in our colony at The Ohio State University. Male hamsters were weaned during the light phase at 21–24 d of age and immediately placed into either short-day conditions (SD: 8-h light–16-h dark) or maintained in long-day conditions (LD: 16-h light–8-h dark; lights off at 15:00 h EST in all cases) and at a constant temperature of 21 ± 2 °C and relative humidity of 50 ± 10%. Hamsters were individually housed in polypropylene cages (30 × 15 × 14 cm) 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 Instituted of Health published in *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources (U.S.), 2011).

Experimental design

The experimental design is depicted in Fig. 1. Hamsters were housed in their respective photoperiods for 5 weeks (N = 10 in LD; N = 11 in SD). During the light phase of the experimental day, five animals of the LD group were transferred to SD to expose them to dark at the early light phase (a dark pulse). At ZT (zeitgeber time) 4 (4 h after light on) in LD and corresponding time in LD with a dark pulse, animals were killed and tissues were collected. Six animals of the SD group were transferred to LD to expose them to light at the late dark phase (a light pulse). At ZT20 in SD and SD with a light pulse, animals were killed and tissues were collected.

Tissue collection and processing

Hamsters were anesthetized with isoflurane vapors and rapidly decapitated. Testes were removed and weighed. Brains were removed and cut along the anterior–posterior axis by using a razor blade. Right and left hemispheres were chosen randomly and half of the brain was placed in RNA later (Ambion, TX, USA) and held at 4 °C to maintain mRNA integrity for gene expression analysis, and the other half was processed for Golgi impregnation using the FD Rapid GolgiStain Kit (FD NeuroTechnologies Inc., MD, USA) according to the manufacturer's instructions.

Analysis of neuronal morphology

Brains were sliced at 100 µm on a cryostat and counterstained with cresyl violet (Sigma–Aldrich, MO, USA). Hippocampal cell morphology was assessed in the CA1 and dentate gyrus (DG) fields in the dorsal hippocampus. Sections were visualized using a Nikon E800 brightfield microscope and intact neurons were reconstructed using Neurolucida software (MicroBrightField, VT, USA) with a 20× objective. Six representative neurons were traced per area, from each animal. The criteria for neuronal selection were: (1) neurons had to be fully impregnated, (2) dendrites could

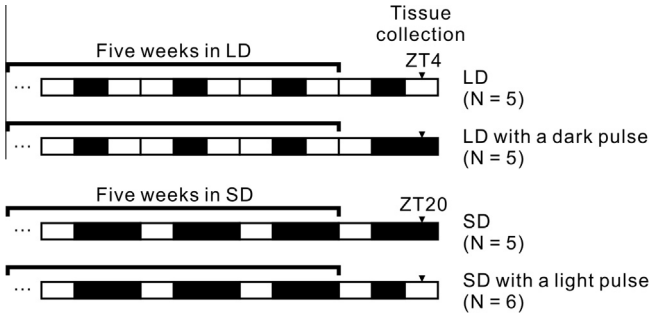

Fig. 1. Experimental design. The white and black bars show the light and dark phases, respectively. Hamsters housed in long-day conditions (LD) for 5 weeks were transferred to short-day conditions (SD) to expose them to dark pulse during the early light phase (a dark pulse), or those housed in SD for 5 weeks were transferred to LD to expose them to a light pulse during the late dark phase (a light pulse). At zeitgeber time 4 (ZT4) in LD or ZT20 in SD and corresponding times in LD with a dark pulse and SD with a light pulse, animals were killed and tissues were collected.