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DIM NIGHTTIME ILLUMINATION ALTERS PHOTOPERIODIC RESPONSES OF HAMSTERS THROUGH THE INTERGENICULATE LEAFLET AND OTHER PHOTIC PATHWAYS

J. A. EVANS,[a1]* S. N. CARTER,[b] D. A. FREEMAN[b] AND M. R. GORMAN[a,c]

[a]*Department of Psychology, University of California, San Diego, La Jolla, CA, USA*

[b]*Department of Biological Sciences, University of Memphis, Memphis, TN, USA*

[c]*Center for Chronobiology, University of California, San Diego, La Jolla, CA, USA*

Abstract—In mammals, light entrains the central pacemaker within the suprachiasmatic nucleus (SCN) through both a direct neuronal projection from the retina and an indirect projection from the intergeniculate leaflet (IGL) of the thalamus. Although light comparable in intensity to moonlight is minimally effective at resetting the phase of the circadian clock, dimly lit and completely dark nights are nevertheless perceived differentially by the circadian system, even when nighttime illumination is below putative thresholds for phase resetting. Under a variety of experimental paradigms, dim nighttime illumination exerts effects that may be characterized as enhancing the plasticity of circadian entrainment. For example, relative to completely dark nights, dimly lit nights accelerate development of photoperiodic responses of Siberian hamsters transferred from summer to winter day lengths. Here we assess the neural pathways underlying this response by testing whether IGL lesions eliminate the effects of dim nighttime illumination under short day lengths. Consistent with previous work, dimly lit nights facilitated the expansion of activity duration under short day lengths. Ablation of the IGL, moreover, did not influence photoperiodic responses in animals held under completely dark nights. However, among animals that were provided dimly lit nights, IGL lesions prevented the short-day typical expansion of activity duration as well as the seasonally appropriate gonadal regression and reduction in body weight. Thus, the present data indicate that the IGL plays a central role in mediating the facilitative effects of dim nighttime illumination under short day lengths, but in the absence of the IGL, dim light at night influences photoperiodic responses through residual photic pathways.

Key words: circadian, intergeniculate leaflet, dim nighttime illumination, short day photoperiod, Siberian hamster.

The mammalian circadian pacemaker within the suprachiasmatic nucleus (SCN) is entrained to the 24 h environment primarily by light. The circadian system's best-characterized responses to light (e.g. phase resetting and melatonin suppression) rely on mechanisms that are functionally and anatomically distinct from those of the image-forming visual system. Specifically, the former responses are characterized by higher intensity thresholds, a unique action spectrum with peak sensitivity to short wavelength light, and the capacity for photon integration over several hours (Brainard et al., 1982; Takahashi et al., 1984; Nelson and Takahashi, 1991a,b). These classic circadian responses to light are mediated in large part by intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain the photopigment melanopsin (Berson, 2003; Gooley et al., 2003). In addition to intrinsic photosensitivity, ipRGCs relay signals from rods and cones, which can influence both ipRGC and SCN function (Belenky et al., 2003; Hattar et al., 2003; Bullough et al., 2005; Güler et al., 2008).

Photic entrainment in mammals is mediated exclusively by input from the retina and is conveyed to the SCN by the retino-hypothalamic (RHT) and geniculo-hypothalamic tracts (GHT) (Meijer and Schwartz, 2003). The RHT is formed by axon collaterals of ipRGCs (Morin et al., 2003; Hattar et al., 2006), and this tract is both necessary and sufficient for circadian photoentrainment (Johnson et al., 1988a). The GHT, in contrast, arises from the intergeniculate leaflet (IGL) within the lateral geniculate nucleus of the thalamus, a structure that also receives ipRGC input (Harrington and Rusak, 1989; Morin et al., 2003; Hattar et al., 2006). Although the IGL is not required for photoentrainment to standard light:dark cycles, it nevertheless modulates a variety of circadian responses to light (Harrington and Rusak, 1986; Pickard et al., 1987; Edelstein and Amir, 1999; Redlin et al., 1999; Morin and Pace, 2002). In particular, IGL lesions influence circadian entrainment under seasonally changing and skeleton photoperiods, suggesting that this structure is important for entrainment under conditions that would be experienced by animals in nature (Harrington and Rusak, 1986; Pickard et al., 1987; Pickard, 1989; Shinohara et al., 1993a; Edelstein and Amir, 1999; Freeman et al., 2004). The IGL also mediates nonphotic inputs to the SCN (Johnson et al., 1988b; Janik and Mrosovsky, 1994; Wickland and Turek, 1994).

Light below the intensity of moonlight (~0.3 lx at full moon; Biberman et al., 1966; Thorington, 1980; Brainard et al., 1984) has been demonstrated to be only minimally effective at resetting circadian phase or suppressing mel-

[1] Present address: Neuroscience Institute, Morehouse School of Medicine, 720 Westview Drive South West, Atlanta, GA 30310, USA.
*Corresponding author. Tel: +1-404-752-1561.
E-mail address: jevans@msm.edu (J. A. Evans).

Abbreviations: IGL, intergeniculate leaflet; ipRGCs, intrinsically photosensitive retinal ganglion cells; LSM, least squared means; NPY-ir, NPY immunoreactivity; RHT, retino-hypothalamic; SC, scotophase condition; SCN, suprachiasmatic nucleus; α, activity duration.

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atonin secretion (Brainard et al., 1982; Nelson and Takahashi, 1991a,b). Nevertheless, across a wide array of circadian entrainment paradigms, dim nighttime illumination below this intensity (0.004–0.1 lx) alters entrainment of activity rhythms when compared with entrainment under identical LD cycles with complete darkness at night (Gorman et al., 2006; Evans et al., 2009). For example, after transfer from long day to short day photoperiods, Siberian hamsters exposed to dimly lit nights display accelerated photoperiodic responses (i.e. expansion of nocturnal activity duration (α), gonadal regression, and weight loss; Gorman and Elliott, 2004). Additional effects of dim nighttime illumination in hamsters include enhanced re-entrainment to simulated jetlag protocols (Evans et al., 2009; Frank et al., 2010), elevated incidence of bifurcated rhythm entrainment under 24 h light:dark:light:dark (LDLD) cycles (Gorman et al., 2003; Gorman and Elliott, 2004; Evans et al., 2005), and an increase in the upper limit of entrainment to non 24-h days (Gorman et al., 2005). Taking the larger corpus of published results, the collective effects of dim light are consistent with the hypothesis that dim nighttime illumination enhances circadian plasticity with respect to both changes in phase and in waveform. The potent and pervasive effects of dim light appear to differ both qualitatively and quantitatively from classical circadian responses to brighter light (Evans et al., 2007), raising the possibility that dim light responses are mediated by physiological mechanisms categorically distinct from those underlying phase shifting and melatonin suppression.

The present study is the first to assess the photic pathway that transmits dim nighttime illumination to the central pacemaker by determining whether its influence persists following lesions of the IGL. If the IGL is the primary conduit of this signal, then IGL-lesioned animals housed under dimly lit nights should respond like animals held under completely dark nights. If the IGL plays no role in the facilitative effects of dim light, then ablation of the IGL would not compromise the dim light effect. The results demonstrate that the IGL mediates the facilitative effects of dim nighttime illumination on expansion of activity duration in short photoperiods. Moreover, the data suggest that other photic pathways are capable of transmitting dim nighttime illumination to the central pacemaker with results opposite of those under the intact condition.

EXPERIMENTAL PROCEDURES

Breeding and initial husbandry

Male Siberian hamsters (*Phodopus sungorus*) were selected from a colony established at University of California, San Diego since 1994 and maintained under a 24 h light:dark cycle with 14 h light and 10 h darkness (LD 14:10, lights on: 0600 PST, lights off: 2000 PST; photophase: ~100 lx, scotophase: 0 lx). After weaning, hamsters were group-housed inside polypropylene cages (48×27×20 cm^3) on open racks. Ambient temperature was maintained at 22±2 °C with *ad libitum* access to water and food (Purina Rodent Chow #5001, St Louis, MO, USA). Experimental procedures were approved by the Institutional Animal Care and Use Committee, University of California, San Diego and were conducted in compliance with all the rules and regulations of this committee.

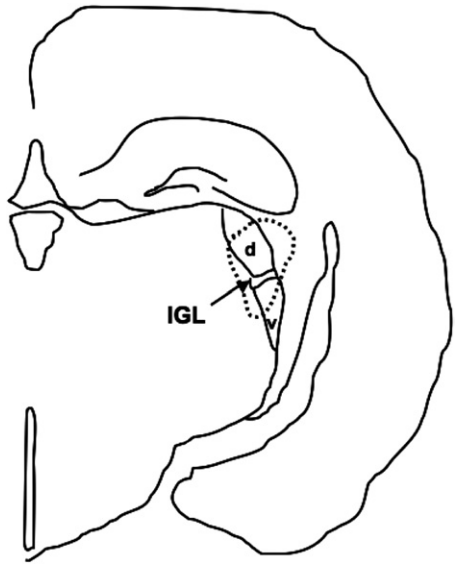

Fig. 1. Line drawing illustrating the lesion of a representative hamster with a complete ablation of the IGL (area of lesion indicated by dotted line). d, dorsal lateral geniculate nuclei; v, ventral lateral geniculate nuclei; IGL, Intergeniculate leaflet.

Surgical procedures

At 30 days of age, animals were positioned in a stereotaxic apparatus (Leica Microsystems, Bannockburn, IL, USA) under deep sodium pentobarbital anesthesia (80 mg/kg, i.p.). Bilateral radio frequency lesions (1 mA, 15 s) were produced with a high voltage stimulus isolator (A360D; WPI, Berlin, Germany) controlled by a Pro4 timer (WPI, Berlin, Germany) using coordinates determined in preliminary studies using age-matched animals (AP: −2.2 mm, ML: ±2.75 mm from bregma, and DV: −4.2 mm below dura; skull level). Successful lesions typically damaged at least some portions of the surrounding dorsal and ventral lateral geniculate nuclei in addition to the IGL (Fig. 1). For sham lesions, the electrode was lowered to the same coordinates for the same amount of time but no current was passed. For both IGL- and sham-lesioned hamsters, the microelectrode was withdrawn after an additional 4 s, and the head was cleaned, sutured and salved with nitrofurazone before animals were injected i.p. with buprenorphine (0.05 mg/kg) and returned to a clean cage. Hamsters remained group-housed for at least 4 weeks post-operatively.

Short day photoperiod entrainment

IGL- and sham-lesioned hamsters were weighed and transferred to individual cages housed within experimental chambers (photophase intensity: 500 lx provided by broad-spectrum, cool white fluorescent bulbs). Each cage was equipped with a passive infrared motion detector (PIR, Coral Plus, Visonic, Bloomfield, CT, USA) positioned ~16 cm above the cage floor for continuous monitoring of locomotor activity rhythms. Hamsters were maintained under LD 14:10 for 1 week to assess baseline entrainment (Fig. 2). The lighting cycle was then changed to a short day photoperiod (LD10:14; lights on 0800 PST) with either dimly lit (DIM-IGLx, DIM-Intact) or completely dark nights (DARK-IGLx, DARK-Intact). Dim nighttime illumination (0.03±0.002 lx, mean intensity equivalent to 5.4×10^{-9} W/cm^2 and 1.5×10^{10} photons/cm^2sec) was provided by narrowband, green light-emitting diodes (LEDs, 0.03 W, λ=560±23 nm). DIM and DARK groups did not differ in photophase light intensity ($P>0.5$). Animals remained under the short day photoperiod with one of these two different scotophase conditions for 8 weeks.