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INTERGENICULATE LEAFLET: CONTRIBUTIONS TO PHOTIC AND NON-PHOTIC RESPONSIVENESS OF THE HAMSTER CIRCADIAN SYSTEM

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Abstract—The circadian visual system is able to integrate light energy over time, enabling phase response and Fos induction in the suprachiasmatic nucleus to increase in proportion to the total energy of the photic stimulus. In the present studies, the contribution of the intergeniculate leaflet to light energy integration by the hamster circadian rhythm system was evaluated. Fos protein is induced in intergeniculate leaflet neurons at much lower irradiance levels than seen in suprachiasmatic nucleus neurons. Bilateral *N*-methyl-D-aspartate lesions of the intergeniculate leaflet decreased phase response of the circadian locomotor rhythm to high irradiance and, in animals exposed to long duration light stimuli, reduced Fos induction in the suprachiasmatic nucleus. Normal photon integration, as indicated by attenuated rhythm phase shifts and Fos induction in suprachiasmatic nucleus cells in response to the energy in light stimuli, does not occur in the absence of the intergeniculate leaflet and is likely to be a property of the circadian rhythm system, rather than solely of the suprachiasmatic nucleus. Anatomical analysis showed that virtually no intergeniculate leaflet neurons projecting to the suprachiasmatic nucleus contain Fos induced by either light or locomotion in a novel wheel. However, cells projecting to the pretectum were found to contain novel-wheel induced Fos. The intergeniculate leaflet is implicated in the normal assessment of light by the circadian rhythm system, but the circuitry by which either photic or non-photic information gains access to the suprachiasmatic nucleus may be more complex than previously thought.

Key words: circadian rhythm, photon integration, suprachiasmatic nucleus, intergeniculate leaflet, Fos, pretectum.

The master generator of mammalian circadian rhythms is the suprachiasmatic nucleus (SCN) (Klein et al., 1991). Information about light, the primary stimulus synchronizing circadian rhythms with the environment, reaches the SCN through two major pathways. One is a direct projection from the retina, the retinohypothalamic tract (RHT) (Johnson et al., 1988b; Moore and Lenn, 1972; Muscat et al., 2003; Morin et al., 2003). The other is an indirect pathway through the intergeniculate leaflet (IGL) of the lateral geniculate complex and its projection, the geniculohypothalamic tract (GHT), to the SCN (Pickard, 1982; Pickard et al., 1987; Morin et al., 1992). The IGL is a long retinorecipient zone located between the dorsal lateral (DLG) and the ventral lateral geniculate (VLG) nuclei (Hickey and Spear, 1976), with widespread connections with the basal forebrain, diencephalon and midbrain including nuclei of the pretectum (PT) (Morin and Blanchard, 1997, 1998a, 1999, 2005; Mikkelsen and Moller, 1990; Moore et al., 1996; Vrang et al., 2003; Horowitz et al., 2004, 2005).

The IGL is an integral component of the neural circuitry mediating the effects of light on circadian rhythmicity (Pickard et al., 1987; Morin and Pace, 2002; Harrington and Rusak, 1986, 1988, 1989). It receives bilateral, direct retinal innervation (Morin et al., 1992, 2003; Pickard, 1985; Muscat et al., 2003). IGL neurons are sensitive to changes in general illumination intensity (Harrington and Rusak, 1989) and convey photic information to the SCN (Zhang and Rusak, 1989) where the GHT terminal field covers nearly the entire nucleus (Morin and Blanchard, 2001). The IGL contributes to phasic light effects and constant light-induced circadian period lengthening (Pickard et al., 1987; Harrington and Rusak, 1986; Morin and Pace, 2002). However, the exact role of the IGL in photic entrainment and the extent to which it may modulate the responsiveness of the circadian system to variations in light intensity are uncertain.

Light-induced Fos in the SCN and rhythm phase shift magnitude are proportional to the energy in the photic stimulus (Muscat and Morin, 2005; Nelson and Takahashi, 1991b, 1999; Dkhissi-Benyahya et al., 2000) and existence of a "photon counter" within the circadian visual system that integrates stimulus energy has been suggested. The location of the mechanism is unknown, although evidence of its function is expressed at a cellular level in the SCN (Dkhissi-Benyahya et al., 2000; Muscat and Morin, 2005). Contributors to light energy integration may include components of the circadian visual system such as the SCN, retina and IGL.

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Abbreviations: ANOVA, analysis of variance; CT-B, cholera toxin B subunit; DD, constant darkness; DLG, dorsal lateral geniculate nucleus; GHT, geniculohypothalamic tract; IGL, intergeniculate leaflet; IR, immunoreactive/immunoreactivity; NMDA, *N*-methyl-D-aspartate; NPY, neuropeptide-Y; Pa, paraventricular hypothalamus; PBS, phosphate-buffered saline; PT, pretectal nucleus; SCN, suprachiasmatic nucleus; subPa, subparaventricular hypothalamus.

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The present analysis evaluates the IGL-GHT as a modulator of circadian rhythm system response to light and whether it contributes to energy integration. It also evaluates photic or non-photic stimulus induction of Fos protein in IGL neurons and compares the results to similar studies (Janik et al., 1995; Peters et al., 1996; Janik and Mrosovsky, 1992) in which Fos was induced by light or a non-photic stimulus in neuropeptide-Y- (NPY) containing neurons in the IGL. This knowledge is essential for understanding the neural system through which photic and non-photic cues act to alter circadian rhythm phase.

EXPERIMENTAL PROCEDURES

Subjects

Adult, outbred male golden hamsters (*Mesocricetus auratus*) weighing 90–100 g (approximately 41–45 days old) were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA). The animals were individually housed in clear plastic cages with corncob bedding under a 14-h light/10-h dark photoperiod for 3 weeks to habituate to the laboratory conditions. At the end of this period, each animal was placed in a cage containing a 16.5 cm diameter stainless steel running wheel. Each wheel rotation closed a switch that was monitored and counted by a computer in 1-min bins. This information was further reduced to 5-min bins and double plotted in typical raster format. Running records were collected beginning 3 weeks prior to surgery. Food and water were continuously available. All procedures were approved by the Institutional Animal Care and Use Committee, Stony Brook University, which follows U.S. National Institutes of Health guidelines and governmental regulations for minimizing the number of animals used and their suffering.

Neurochemical IGL lesions

Individual animals entrained to a LD 14:10 photoperiod were randomly assigned to a sham-operated control group or a lesion group and injected s.c. with 0.02 ml of atropine sulfate solution (54 mg/ml; Phoenix Pharmaceutical, St. Joseph, MO, USA). Thirty minutes later, each animal was deeply anesthetized with pentobarbital (100 mg/kg body weight; Nembutal Sodium Solution, Abbott Laboratories, North Chicago, IL, USA), placed in a stereotaxic apparatus and, using previously described surgical procedures and stereotaxic coordinates (Morin and Pace, 2002; Johnson et al., 1988c), given a single 0.6 μl injection of the neurotoxin, *N*-methyl-D-aspartate (NMDA; Sigma, St. Louis, MO, USA; 14.6 mg in 500 μl of 0.2 M 0.9% saline), or saline into the IGL on the right side of the brain. Upon recovering from the anesthetic, each animal was returned to its home cage. Six to seven days later, the injection procedures sustained by each animal were repeated on the left side.

Retrograde tract tracing

Intact hamsters were anesthetized with pentobarbital (100 mg/kg body weight), placed in a stereotaxic apparatus, and given an injection of cholera toxin B subunit (CT-B; product 104; List Biological Laboratories, Inc., Campbell, CA, USA; 1% in distilled water) by iontophoresis using a Stoelting Precision Current Source and glass micropipette (tip diameter, about 20 μm). In 15 animals, the CT-B was injected unilaterally into the SCN (6 μA pulsed 7 s on and 7 s off for about 6–10 min). In eight animals, a unilateral CT-B injection was aimed at the PT (5 μA pulsed 7 s on and 7 s off for about 5 min). In another eight animals, the CT-B was unilaterally aimed at the paraventricular (Pa) and subparaventricular hypothalamus (subPa; 5 μA pulsed 7 s on and 7 s off for about 5 min). Stereotaxic coordinates for the SCN and PT were those published previously (Morin and Blanchard, 2001). Stereotaxic coordinates for the Pa/subPa were: anterior-posterior, +0.06: medial-lateral, ±0.15; and dorsal-ventral, −0.73 mm.

Light delivery system

The procedures for providing light stimuli have been previously described in detail (Muscat and Morin, 2005). Briefly, each animal was removed from its home cage to a polycarbonate cylinder with corncob bedding, which was placed in a ventilated, light-tight stimulus chamber that allowed multiple animals in individual cylinders to be stimulated simultaneously. Location of animals in the chamber was unrelated to treatment group. Illumination was provided by a computer-controlled Kodak carousel projector fitted with an IR/UV cut filter to eliminate wavelengths below 380 nm and above 680 nm. Irradiance, the radiant power arriving at a surface per unit area, was controlled via slides made of neutral density filters and stimulus duration was timed by the computer.

Light measurements, specifically the irradiance (μW/cm^2) and total energy (J/m^2, the energy per unit area during a given exposure), were obtained with a radiometer/photometer (Tektronix J16, Richardson, TX, USA) and an optometer (Model # P-9170, Gigahertz-Optik, Newburyport, MA, USA) with the sensor for each meter placed in a cylinder within the animal chamber during measurement. Light measurements were taken several times and at various locations within the chamber. All regions of the chamber received light of approximately the same irradiance and total energy. The maximum irradiance output that the projector system could deliver was 57.0 μW/cm^2 with no limit on duration. Studies evaluating response to irradiance used six stimuli: 57.0, 4.3, 0.51, 0.05, 0.0057, and 0.001 μW/cm^2. Studies evaluating response to duration used five stimuli: 0.25, 1.5, 5.0, 15.0 and 150.0 min.

Procedures

Experiment 1: phase response and Fos induction in the SCN and IGL as a function of light irradiance. Intact hamsters stably entrained to a LD 14:10 photoperiod were placed in constant darkness (DD). On day 7 of DD, each animal was removed from its home cage, transferred to a cylinder and placed in the stimulus chamber of the photostimulation apparatus. At CT 19, each animal received one of the six irradiances for 5 min (N=8–10 per irradiance). About one hour after the end of the pulse, each animal was returned to its home cage where it remained in DD for an additional 2 weeks at which time the magnitude of phase shift was measured. In order to maximize efficient use of the animals, a repeated measures procedure was used. After phase shift measurements were made, the animals were transferred back to LD 14:10 for a three-week re-entrainment interval. The animals were then returned to DD in preparation for the light pulse on day 7 of DD. This procedure was repeated until each animal received five different light pulse irradiances. No animal received the same irradiance more than once and the order in which each pulse irradiance was received was randomized.

Eighteen hamsters stably entrained to a LD 14:10 photoperiod were placed in DD. On day 7 of DD, animals were placed in stimulus cylinders and transferred to the photostimulation chamber as previously described. At CT 19, each animal received one of the six irradiances for 5 min (N=4 per irradiance). Animals were killed 90 min after the onset of the light pulse and the brains were taken for the counting of Fos-immunoreactive (Fos-IR) cells in the SCN and IGL (see Histology).

Experiment 2: bilateral IGL ablation and phase response to light duration or irradiance. After 2–3 weeks of post-surgical recovery, stably entrained animals with bilateral NMDA and sham lesions were placed in DD. On day 7 of DD, equal numbers of lesion and control animals were individually placed in stimulus