Neuroscience 355 (2017) 225–237

NORMAL BEHAVIORAL RESPONSES TO LIGHT AND DARKNESS AND THE PUPILLARY LIGHT REFLEX ARE DEPENDENT UPON THE OLIVARY PRETECTAL NUCLEUS IN THE DIURNAL NILE GRASS RAT

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Abstract—The olivary pretectal nucleus (OPT) is a midbrain structure that receives reciprocal bilateral retinal projections, is involved in the pupillary light reflex, and connects reciprocally with the intergeniculate leaflet (IGL), a retinorecipient brain region that mediates behavioral responses to light pulses (i.e., masking) in diurnal Nile grass rats. Here, we lesioned the OPT and evaluated behavioral responses in grass rats to various lighting conditions, as well as their anxiety-like responses to light exposure. While control grass rats remained diurnal, grass rats with OPT lesions exhibited a more night-active pattern under 12 h:12 h light–dark (LD) conditions. However, when placed in constant darkness, OPT-lesioned grass rats became more active during their subjective day, suggesting that an exaggerated masking response to light may be responsible for the effect of OPT lesions on locomotor activity in LD. To test this hypothesis, we presented dark and light pulses to controls and grass rats with OPT lesions; controls increased their activity in response to *light,* whereas those with OPT lesions significantly increased activity in response to *darkness*. Further, when placed in a 7-h ultradian LD cycle, animals with OPT lesions were more active during darkness than controls. OPT lesions also abolished the pupillary light reflex, but did not affect anxiety-like behaviors. Finally, in animals with OPT lesions, light did not induce Fos expression in the ventrolateral geniculate nucleus, as it did in controls. Altogether, these results suggest that masking responses to light and darkness are dependent upon nuclei within the subcortical visual shell in grass rats.

Key words: olivary pretectal nucleus, circadian, masking, light, diurnality, behavior.

INTRODUCTION

Light influences behavior and physiology in mammals by entraining circadian rhythms (Daan and Pittendrigh, 1976a,b), as well as through acute inhibition or stimulation of the animals' activity, a process called masking (Redlin, 2001). Masking responses enable animals to respond in adaptive ways to changes in illumination that may not be anticipated by the circadian system. Although there has been substantial progress elucidating the mechanisms responsible for the workings of the circadian system in nocturnal species, less is known about the mechanisms that support the diurnal profile of activity of many mammalian species (Smale et al., 2003). We recently showed that the intergeniculate leaflet (IGL), a direct retinorecipient region in the thalamus, is critical for the display of normal activity patterns of diurnal Nile grass rats (*Arvicanthis niloticus*) (Gall et al., 2013). Specifically, IGL lesions reversed the activity patterns of these animals, such that they became nocturnal through both circadian mechanisms and masking. The IGL receives direct inputs from melanopsin-containing retinal ganglion cells that are intrinsically photosensitive (ipRGCs; (Chen et al., 2011)) and encode retinal luminance levels (Allen et al., 2014). The IGL has reciprocal connections with the olivary pretectal nucleus (OPT) (Klooster et al., 1995; Moore et al., 2000), which also receives inputs from ipRGCs (Hattar et al., 2006). Thus, we hypothesized that together with the IGL, the OPT is part of a neural system that in diurnal mammals shapes the distribution of activity across the light–dark (LD) cycle, as well as responses to pulses of light and darkness.

There is ample evidence that OPT neurons function as luminance detectors and that the OPT mediates the pupillary light reflex (Trejo and Cicerone, 1984; Gamlin et al., 1995; Gamlin and Clarke, 1995; Szkudlarek et al., 2012). In laboratory rats, it also plays a role in the triggering of rapid eye movement (REM) sleep in response to the shift from light to darkness (Miller et al., 1998, 1999), thus suggesting a more comprehensive role for the OPT in mediating responses to changes in illumination. Other than its role in mediating the pupillary light reflex (Gamlin et al., 1995), very little is known about the role of the OPT in diurnal species. We recently showed that it exhibits light-induced Fos expression in

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Abbreviations: APT, anterior pretectal nucleus; CT, circadian time; DLG, dorsolateral geniculate nucleus; IGL, intergeniculate leaflet; OPT, olivary pretectal nucleus; PPT, posterior pretectal nucleus; VLG, ventrolateral geniculate nucleus; ZT, zeitgeber time.

http://dx.doi.org/10.1016/j.neuroscience.2017.05.002
0306-4522/

intact grass rats (Gall et al., 2014), and that this response is reversed by IGL lesions that also reverse the behavioral responses of grass rats to light pulses (Gall et al., 2014). These results suggest that reciprocal connections between the IGL and OPT may play a key role in modulating the direction of behavioral responses to light in grass rats.

Here, we evaluated the contribution of the OPT to entrainment and masking responses to light in the diurnal grass rat. We first tested the hypothesis that in diurnal grass rats, the OPT is involved in regulating the daily distribution of activity, as well as masking responses to different exposures to light. To that end, we monitored general activity in controls and in animals with bilateral OPT lesions under standard 12:12 LD conditions, under constant darkness (DD), under an ultradian LD cycle, and following 2-h dark and light pulses during the subjective day and night, respectively. We then tested the hypothesis that the OPT is involved in the pupillary light reflex in grass rats, as it is the case in other species (Trejo and Cicerone, 1984; Clarke and Ikeda, 1985a,b; Young and Lund, 1994). If OPT lesions indeed abolish the pupillary light reflex in grass rats, then excessive light exposure due to a dilated pupil may be anxiogenic to these animals, and result in behavioral inhibition when the lights are on. Therefore, we assessed the extent to which OPT lesions affect anxiety-like behaviors using an open field test and a light–dark preference box. Finally, using light-induced Fos expression as a marker for neural activation, we examined the pathways through which the OPT might influence masking effects of light in grass rats. Altogether, our results suggest that masking responses to light and darkness and the pupillary light reflex are dependent upon the OPT in a diurnal species.

EXPERIMENTAL PROCEDURES

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee of Michigan State University. All efforts were made to minimize the number of animals used in these experiments.

Subjects

A total of 32 adult female grass rats (*Arvicanthis niloticus)* from a breeding colony maintained at Michigan State University were used in the experiments in this study. Virgin female grass rats do not exhibit an estrous cycle when singly housed (McElhinny, 1996). We used female grass rats in this study in order to standardize the sex, and also so that we could make comparisons to our previous lesion studies in grass rats, which used only females (Gall et al., 2013, 2014, 2016). All animals were singly housed in plexiglass cages (34 × 28 × 17 cm); food (PMI Nutrition Prolab RMH 2000, Brentwood, MO, USA) and water were available *ad libitum*. In order to monitor general locomotor activity in the grass rats, infrared motion detectors (IRs; Visonic, Tel Aviv, Israel) were placed on top of each cage. These IRs allowed us to collect behavioral general locomotor activity data every 5 min using the VitalView Program (Mini-Mitter, Bend, OR, USA). Animals were maintained in a 12:12 LD cycle with lights on at Zeitgeber time 0 (ZT 0); (light intensities were always either 300 lux during the light phase or less than 5 lux during the dark phase). General activity was monitored in 12:12 LD for at least 2 weeks prior to surgery.

Surgery

Animals were anesthetized using isoflurane anesthesia (maintained at 1.5–2.5%), and were placed in a stereotaxic apparatus (Stoelting Co., Wood Dale, Illinois, USA). The animals were injected with an anesthetic (Lidocaine, Elkins-Sin, Inc., Cherry Hill, NJ; 0.2 cc, s.c.), and artificial tears (Butler Company, Columbus, OH) were applied over the eyes. After shaving the animals' scalps, an incision was made and two small holes were drilled in the skull. An insulated tungsten microelectrode (A-M systems, Model 5770, 500 μm Diameter, Sequim, WA, USA) was used to make bilateral electrolytic lesions in experimental animals to destroy tissue within the OPT using the following coordinates, with the tooth bar set at 0: AP: −0.12 cm from bregma, ML: ±0.05 cm from midline, and DV: −0.28 cm ventral to the meningeal surface. A lesion-making device was used (Stoelting, Model 58040, Chicago, IL, USA) to deliver 2.0 mA of DC current for 15 s. The incision was closed with autoclips, and antiseptic ointment (Nolvasan, Fort Dodge, IA; 1% chlorhexidine acetate in 10% sterile alcohol base) was applied. In sham animals, the same procedure was performed, except current was not applied. All animals received an injection of sterile saline (Abbott, s.c.; 1.0 mL) and ketoprofen (Fort Dodge Animal Health; s.c.; 0.2 mL dose) immediately following surgery. 24 and 48 h after surgery, all animals were given meloxicam (Boehringer Ingelheim Vetmedica Inc., St. Joseph, MO, USA; 20 μL) infused in a piece of apple (Castillo-Ruiz and Nunez, 2011). Animals recovered in their home cages for at least 1 h on a warming pad, and were then moved back to the recording room where their activity was continually monitored under standard 12:12 LD conditions. After 7–10 days post-surgery, the autoclips were removed. 24 grass rats received bilateral electrolytic lesions aimed at the OPT, and 20 of them survived (83.3% survival rate). Eight grass rats received sham surgery as controls (100% survival rate).

Light treatment procedure

General activity of all animals was recorded as animals were exposed to the following sequence of lighting conditions: (1) 12:12 LD for at least 4 weeks after surgery, (2) DD for 2 weeks, (3) 12:12 LD for approximately 4 weeks (at which point all animals were entrained). (4) At this point, two–hour masking pulses were administered in 3-day cycles, as described previously (Shuboni et al., 2012): day 1 = maintenance day (12:12 LD); day 2 = baseline day (12:12 LD); and day 3 = pulse day (2-h light pulse in the dark phase of a 12:12 LD cycle given at ZT 14, 18, or 22, or a 2-h dark pulse in the light phase of a 12:12 LD cycle given at ZT 2, 6, or 10). All animals received light pulses first and then