9-5-2014

Intergeniculate Leaflet Lesions Result in Differential Activation of Brain Regions Following the Presentation of Photic Stimuli in Nile Grass Rats

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Published in: *Neuroscience Letters*, Volume 579, September 5, 2014, pages 101-105.

Published in final edited form as:
Neurosci Lett. 2014 September 5; 579: 101–105. doi:10.1016/j.neulet.2014.07.016.

Intergeniculate leaflet lesions result in differential activation of brain regions following the presentation of photic stimuli in nile grass rats

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Abstract

The intergeniculate leaflet (IGL) plays an important role in the entrainment of circadian rhythms and the mediation of acute behavioral responses to light (i.e., masking). Recently, we reported that IGL lesions in diurnal grass rats result in a reversal in masking responses to light as compared to controls. Here, we used Fos as a marker of neural activation to examine the mechanisms by which the IGL may influence this masking effect of light in grass rats. Specifically, we examined the patterns of Fos activation in retinorecipient areas and in brain regions that receive IGL inputs following 1-h light pulses given during the early night in IGL-lesioned and sham-operated grass rats. Three patterns emerged: (1) IGL lesions had no effect on the Fos response to light, (2) IGL lesions resulted in a reversal in Fos responses to light, and (3) IGL lesions resulted in a lack of a Fos response to light. Of specific interest were the suprachiasmatic nucleus (SCN) and the olivary pretectal nucleus (OPT), both of which are retinorecipient and connect reciprocally with the IGL. Light-induced Fos expression in the SCN was unaffected by IGL lesions, whereas the OPT exhibited a significant reduction in Fos expression following a light pulse in animals with IGL lesions. Altogether, our results suggest that the OPT, but not the SCN, exhibits a reversal in Fos responses to light following IGL lesions that reverse masking responses in diurnal grass rats. Our results suggest that interconnections between the IGL and downstream brain areas (e.g., OPT) may play a role in determining the direction of the behavioral response to light.

Keywords

intergeniculate leaflet; Fos; masking; grass rat; diurnality

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The authors declare no competing financial interests.

Introduction

Light and darkness modulate behavior and physiology not only by entraining circadian rhythms, but also by acutely inhibiting or stimulating activity, a process called masking [1]. In diurnal animals, light generally stimulates activity, whereas light generally inhibits activity in nocturnal animals [2,3]. The neural substrates involved in masking responses to light are relatively unknown, especially in diurnal animals.

The intergeniculate leaflet (IGL), which receives direct retinal input [4], has been implicated in the mediation of masking responses in nocturnal rodents [5]. We recently showed that light pulses result in a significant reduction in activity levels following IGL lesions in diurnal grass rats (*Arvicanthis niloticus*), whereas control grass rats exhibit a significant increase in activity levels [6]. Therefore, destroying the IGL in these diurnal animals alters their masking patterns to resemble those of nocturnal animals. The mechanisms by which this reversal in behavior occurs following IGL lesions have not yet been examined.

Here, we used Fos expression to examine whether the IGL influences responses to light in retinorecipient regions in the brain, or in regions that are involved in sleep and arousal. We focused particularly on the suprachiasmatic nucleus (SCN) and olivary pretectal nucleus (OPT) since both areas are retinorecipient [7,8], connect reciprocally with the IGL [4,9], play a functional role in sleep-wake processes [10,11], and have been linked to masking [12,13], although the role of the SCN in the mediation of masking responses to light remains controversial [14]. Further, since light is capable of inducing sleep in nocturnal animals [2] and arousal in diurnal ones [15], we also measured Fos expression in brain regions that receive inputs from the IGL and are involved in the control of sleep or wakefulness, including the ventrolateral preoptic nucleus (VLPO), ventral subparaventricular zone (vSPVZ), dorsomedial hypothalamus (DMH), locus coeruleus (LC), and dorsal raphe (DR) [16].

Based on our earlier observations [6], we predicted that the SCN's response to light would be unaffected by the IGL lesions, since the effects of light on the period of activity rhythms was unaffected by IGL lesions. In addition, we predicted that one or more brain areas outside the SCN would exhibit a reversal in Fos expression following light pulses given to IGL-lesioned animals. Such a finding would suggest a mechanism by which the IGL might promote a diurnal pattern of masking behavior in grass rats.

Materials and Methods

All experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and were approved by the Institutional Animal Care and Use Committee of Michigan State University. All efforts were made to minimize the number of animals used.

Subjects

Twenty-eight singly housed adult female grass rats (n = 20 complete IGL-lesioned subjects, *n* = 8 shams) were used. These 28 animals were the same used in a previous report, which

describes the behavioral effects of IGL lesions that also extended beyond the IGL (see [6] for surgical and histological details). Female grass rats do not exhibit estrous cycles in the laboratory [17], and do not differ from males in masking responses to light [3].

Experimental Procedures

Light treatment procedure—As described previously [6], at least 10 weeks after surgery, following the placement of animals in constant dark (DD) and constant light (LL), animals were re-entrained to a 12:12 LD cycle, and then subjected to a series of dark and light pulses given in 12:12 LD (lights on = ZT 0) while behavior was monitored. Finally, approximately half of the animals (n = 9 lesions, n = 4 shams) received a 1-h light pulse (300 lux of white light) at ZT14 during the dark phase of a 12:12 LD cycle, and were sacrificed at ZT15. The other half (n = 11 lesions, n = 4 shams) were also sacrificed at ZT15, but without receiving a light pulse (LP).

c-Fos Immunohistochemistry

Following transcardial perfusion, brains were removed and sectioned as described previously [6]. Labeling of Fos-immunoreactive (Fos-ir) cells followed protocols previously established in the grass rat brain [18]. Sections were incubated in Fos antibody raised in rabbit (1:25,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and processed with avidin-biotin-immunoperoxidase using DAB (3,3'-diaminobenzidine) as the chromogen enhanced with nickel sulfate. Sections were mounted on gelatin-coated glass slides, dehydrated, and coverslipped with dibutyl phthalate xylene (DPX; Sigma-Aldrich, St. Louis, MO, USA).

Cell counting

For quantifying Fos expression, observers blind to experimental condition selected two sections containing each brain region of interest, including the SCN, VLPO, OPT, vSPVZ, DMH, LC, and DR. Sections were examined under a light microscope (Leitz, Laborlux S, Wetzlar, Germany) equipped with a drawing tube to produce bilateral maps of Fos positive cells. Counting boxes were used to delineate the VLPO (190 μm × 190 μm; [19]), vSPVZ (215 μm × 160 μm; [20]), LC (400 μm × 700 μm; [18]), and DR (150 μm × 650 μm; [18]). The SCN, OPT, and DMH were outlined using thionin counterstained tissue. For the OPT, Fos-positive cells were counted separately for the shell and core, but since the counts were not significantly different between the two areas, data for the OPT are reported as the total of shell plus core. The number of Fos-positive cells for each region were counted bilaterally and divided by 2 to obtain an average of unilateral Fos-ir counts. The Fos-ir counts from the OPT of two brains were excluded from the analysis due to the region being partially lesioned bilaterally.

Statistical analysis

A two-way ANOVA was used to analyze the data with a 2 × 2 factorial design [surgical condition (sham vs. IGL lesion) × lighting condition (darkness vs. light pulse)]. Significant interactions were followed by evaluation of simple main effects using independent sample t-

tests. For all analyses, differences were significant when $p < 0.05$. All means are presented with their standard errors.

Results

IGL lesions did not affect Fos responses to light in the SCN or VLPO

For the SCN (Figure 1), a two-way ANOVA found a significant main effect of lighting condition ($F_{1,24} = 63.4$, $p < .0001$), but not of surgical condition ($F_{1,24} = .04$, $p = .837$), and no interaction between the two variables ($F_{1,24} = .003$, $p = .959$). For the VLPO (Table 1), a two-way ANOVA found a significant main effect of lighting condition ($F_{1,24} = 45.1$, $p < .0001$), and of surgical condition ($F_{1,24} = 4.5$, $p = .045$), but no interaction between the two variables ($F_{1,24} = 1.5$, $p = .229$).

IGL lesions resulted in a reversal in Fos responses to light in the OPT

For the OPT (Figure 2), a two-way ANOVA found a significant interaction between lighting and surgical condition ($F_{1,22} = 79.0$, $p < .0001$). Analysis of simple main effects of lighting condition revealed that Fos-ir was significantly increased following a light pulse in shams ($t_6 = 8.0$, $p < .0001$), but significantly decreased in lesioned animals ($t_{16} = -3.3$, $p = .004$). Analysis of simple main effects of surgical condition showed that following a light pulse, IGL-lesioned animals exhibited significantly decreased Fos-ir as compared to shams ($t_{10} = 5.8$, $p < .0001$). For the control night, IGL-lesioned animals exhibited significantly increased Fos-ir as compared to shams ($t_{12} = -6.8$, $p < .0001$).

IGL lesions resulted in a lack of Fos responses to light in the vSPVZ, DMH, LC, and DR

In the vSPVZ, DMH, LC, and DR (Table 1), two-way ANOVAs found significant interactions between lighting and surgical condition ($F_{1,24}$s > 4.3, *ps* < .05). Analysis of simple main effects of lighting condition revealed that Fos-ir was significantly increased following a light pulse in all four areas for shams (t_6s > 4.3, *ps* < .005), but not for lesioned animals (t_{18}s < 1.7, *ps* > .096). In the vSPVZ and LC, the simple main effects of surgical condition on Fos-ir did not reach statistical significance following a light pulse (t_{11}s < 1.9, *ps* > .089) or for the control night (t_{13}s < 1.9, *ps* > .084). However, in the DMH and DR, the simple main effect of surgical condition was significant for the light pulse condition; IGL-lesioned animals exhibited significantly less Fos-ir as compared to shams (t_{11}s > 2.6, *ps* < .024). For the control night, no significant differences were found in Fos-ir for IGL-lesioned animals as compared to shams (t_{13}s < 1.3, *ps* > .218).

Discussion

IGL lesions in grass rats result in a redistribution of activity across the circadian cycle and a striking reversal in the direction of behavioral masking responses to light pulses presented at ZT14 as compared to controls [6]. The current study used Fos expression to investigate how selected brain regions change their responses to light after IGL lesions, and to use that information to identify possible pathways through which the IGL might influence masking effects of light in grass rats. Our results demonstrate that the OPT, vSPVZ, DMH, DR, and LC, but not the SCN or VLPO, change their responses to light following IGL lesions.

Interestingly, the OPT exhibited a reversal in Fos induction following light pulses in IGL-lesioned grass rats, raising the possibility that interconnections between the IGL and OPT play a role in determining the direction of the behavioral response to light.

Considering the key role that the SCN plays in the circadian regulation of behavior, and given its possible, but controversial, involvement in mediating behavioral masking responses to light [12,14], it may seem surprising that Fos-ir in the SCN was unaffected by light pulses given to grass rats with IGL lesions, even though circadian activity is significantly affected by these lesions. However, as mentioned above, IGL lesions in the same animals did not affect how constant light and darkness influenced the period of free running activity rhythms in grass rats [6], thus suggesting normal SCN responses to light after IGL lesions. Further, there is no consensus about the necessity for a functional SCN for the mediation of masking responses, since following complete SCN lesions, hamsters can show masking responses to light [14]. Taken together, our past [6] and current data indicate that IGL lesions can have profound effects on masking responses and on the distribution of daily activity without apparent changes in how the SCN responds to light.

Although our results show that Fos expression in the SCN in response to light is unaffected by IGL lesions, we cannot rule out that other aspects of light transduction by the SCN were not affected by the lesions. Importantly, light stimulation during the subjective day increases the number of action potentials recorded from SCN neurons in nocturnal rats *in vivo* [21] and induces clock gene expression in the adrenal gland [22], without inducing Fos or clock-gene expression in the SCN [22,23]. Therefore, the SCN may be capable of transducing photic input in a phase-independent manner without altering gene expression or the phase and period of its oscillator. Thus, the lack of change in light-induced Fos expression observed in grass rats following IGL lesions is not sufficient to conclude that IGL lesions are incapable of affecting photic transduction by SCN neurons.

The OPT is retinorecipient [8], reciprocally connected to the IGL [4,9], and exhibits a Fos response to light [13,24]. The OPT mediates pupillary reflexes to light and is involved in the generation of eye movements [25], but may also be involved in sleep and circadian behavior [10,26,27]. Here, the phase-dependent increase in activity at night, along with the reversal in the direction of masking responses of grass rats after IGL lesions [6], was matched by a reversal in Fos expression in the OPT of these animals, thus suggesting that normal behavioral and neural responses to light by this diurnal species require an intact IGL-OPT circuit.

Other observations also point to the OPT as a candidate for mediating responses to light in a chronotype-specific fashion. For example, for grass rats that become night-active when given a running wheel, the OPT does not exhibit light-induced Fos at ZT14, whereas it does so in day-active wheel-runners [24]. Also, light induces Fos in the OPT of intact grass rats, but decreases it in intact mice [28]; this result stands in contrast to findings in laboratory rats [13], in which 2-h light pulses given at ZT19 induce increased Fos-ir in the OPT. This difference in lab rats could be due to a difference in the time of day in which animals were light pulsed, or could indicate a species difference. Taken together, data obtained from night-active grass rats, nocturnal mice, and IGL-lesioned grass rats with enhanced nocturnal

activity, show that the induction of Fos-ir by light within the OPT at ZT14 is either reversed or absent in night-active compared to day-active animals. Therefore, the OPT is a possible candidate for mediating the differences observed in masking between night-active and day-active phenotypes at ZT14, both within and between species.

The VLPO is a forebrain structure that is retinorecipient [7], but its role in masking is virtually unknown. We reported recently that light presented at ZT14 results in a significant increase in Fos-ir in the VLPO in grass rats [28]. This appears to be a paradoxical response, given that the VLPO is a sleep-active region in both diurnal and nocturnal species [19,29]. Galanin-containing cells of the VLPO appear to be responsible for inducing sleep in nocturnal animals [30]. One untested possibility is that light stimulates a different subset of cells within the VLPO in grass rats, perhaps a population of local inhibitory neurons that synapses on the galanin-containing cells. As shown here, lesions of the IGL do not affect light-induced Fos responsiveness in the VLPO, even though the masking response is reversed in these animals. Since IGL-lesioned grass rats behave more like nocturnal animals in terms of masking and circadian behavior [6], we predict that the same cells that respond to light in nocturnal species respond to light in IGL- lesioned grass rats.

The vSPVZ [23,31,32], DMH [31], LC [33], and DR [27,34] exhibit a Fos response to light, with orexin being necessary for this response in the DR of grass rats [34]. As shown here, these areas responded to light in control grass rats, but not in animals with IGL lesions. Thus, connections between the IGL and these areas may be vital to their ability to respond to light. It is possible that these sites receive information about light directly from the IGL or via pathways affected by IGL lesions (e.g., orexinergic pathways). Different IGL neurons express neuropeptide Y (NPY) or enkephalin (Enk) [35]; we are currently testing the hypothesis that one or both of these cell types project to downstream areas (e.g., OPT) to modulate neural and behavioral responses to light.

Conclusions

Grass rats with complete IGL lesions behave more like nocturnal animals in terms of masking and circadian behavior [6]. Concurrent with a reversal in behavior are changes in Fos expression following light pulses within the vSPVZ, DMH, LC, DR, and OPT, but not within the SCN or VLPO. Importantly, only the OPT exhibited a reversal in its response to light following IGL lesions. Altogether, interconnections between the IGL and downstream brain areas such as the OPT may play a role in determining the direction of the behavioral response to light that differs across day-active and night-active individuals.

Acknowledgments

We thank Dorela Shuboni, Jennifer Langel, Ken Bennett, Megan Luck, and Thomas Groves for technical assistance. Supported by a National Science Foundation (NSF) grant (IOS-1051919, to L.S., A.A.N., and L.Y.) and A.J.G. was supported by a National Institutes of Health (NIH) Ruth L. Kirschstein National Research Service Award (NRSA) from the National Institute of Neurological Disorders and Stroke (NINDS) (F32 NS083360-01).

Abbreviations

DR	dorsal raphe
DMH	dorsomedial hypothalamus
IGL	intergeniculate leaflet
LP	light pulse
LC	locus coeruleus
OPT	olivary pretectal nucleus
SCN	suprachiasmatic nucleus
vSPVZ	ventral subparaventricular zone
VLPO	ventrolateral preoptic nucleus
ZT	Zeitgeber time

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Highlights

- Light-induced Fos expression in the SCN was unaffected by IGL lesions.
- IGL lesions resulted in a reversal in Fos responses to light in the OPT.
- Interconnections between the IGL and OPT may mediate masking in grass rats.

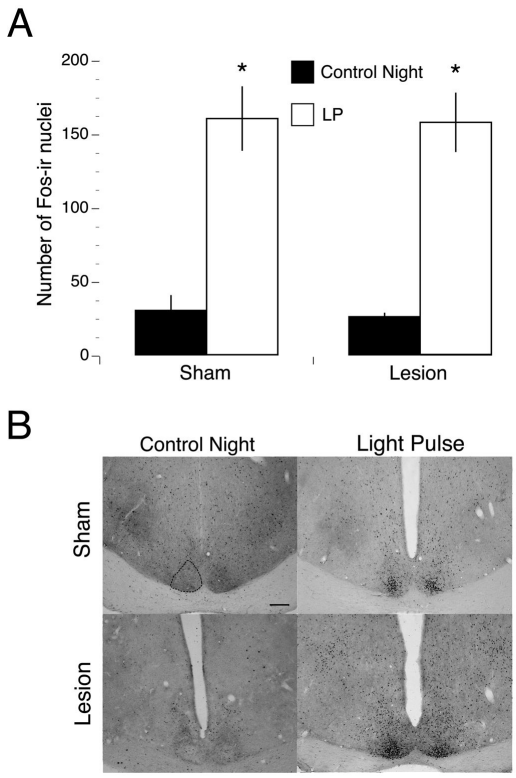

Figure 1. IGL lesions did not affect the Fos response to light in the SCN

(A) Mean number of Fos positive cells in the SCN in animals sacrificed on a control night (dark) as compared to those sacrificed following a LP. * Significantly different from control night. (B) Representative photomicrographs of Fos induction in the SCN in shams and IGL-lesioned grass rats on a control night vs. following a LP. Sampling areas are outlined in the top left photomicrograph. Scale bar = 100μm. Abbreviations: IGL: intergeniculate leaflet; SCN: suprachiasmatic nucleus; VLPO: ventrolateral preoptic nucleus; LP: light pulse.

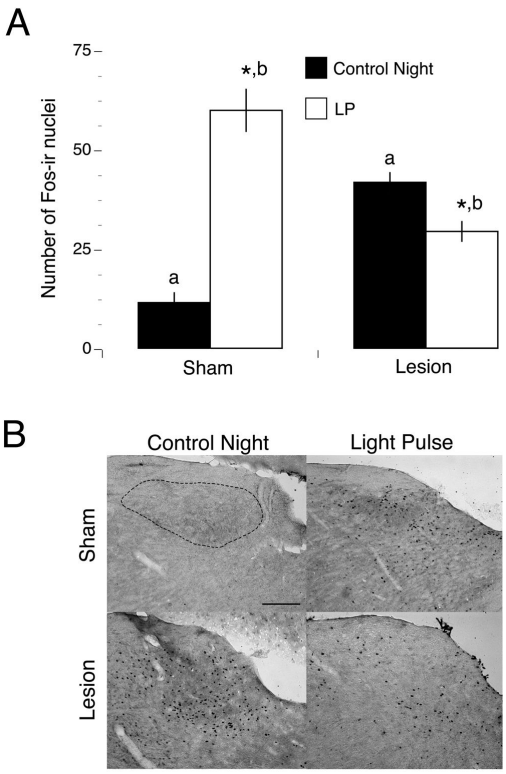

Figure 2. IGL lesions resulted in a reversal in Fos responses to light in the OPT

(A) Mean number of Fos positive cells in the OPT in animals sacrificed on a control night (dark) vs. those sacrificed following a LP. * Significantly different from control night. [a] Significant difference between sham and lesion Fos-ir values on the control night. [b] Significant difference between sham and lesion Fos-ir values following a LP. (B) Representative photomicrographs of Fos induction in the OPT in shams and IGL-lesioned grass rats on a control night vs. following a LP. Sampling areas are outlined in the top left photomicrograph. Scale bar = 100μm. Abbreviations: IGL: intergeniculate leaflet; OPT: olivary pretectal nucleus; LP: light pulse.

Table 1

Mean number of Fos positive cells in the VLPO, vSPVZ, DMH, LC, and DR.

	Sham		Lesion	
	Control Night	**Light Pulse**	**Control Night**	**Light Pulse**
VLPO	11.0(3.6)	**29.6 (5.3)**	9.0 (0.9)	**21.8 (1.6)**
vSPVZ	23.1 (4.7)	**57.2 (3.6)**	41.1 (5.4)	41.8 (8.5)
DMH	95.3 (26.8)	**264.6 (29.4)** [a]	142.8 (19.7)	167.9 (20.9)[a]
LC	20.6 (4.4)	**67.1 (3.8)**	39.8 (7.8)	45.7 (7.3)
DR	27.1 (4.3)	**58.3 (3.0)** [a]	39.5 (7.3)	24.1 (3.5)[a]

Bold values indicate significant difference from control night.

Abbreviations: VLPO: ventrolateral preoptic nucleus; vSPVZ: ventral subparaventricular zone; DMH: dorsomedial hypothalamus; LC: locus coeruleus; DR: dorsal raphe.

[a] Significant difference between sham and lesion Fos-ir values following a LP.