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Acute effects of light on the brain and behavior of diurnal *Arvicanthis niloticus* and nocturnal *Mus musculus*

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HIGHLIGHTS

- Light induced changes in brains of diurnal grass rats and nocturnal mice.
- cFos responses of the two species differed in several retinorecipient regions.
- cFos was stimulated in sleep/arousal-related regions in grass rats but not mice.

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ABSTRACT

Photic cues influence daily patterns of activity via two complementary mechanisms: (1) entraining the internal circadian clock and (2) directly increasing or decreasing activity, a phenomenon referred to as "masking". The direction of this masking response is dependent on the temporal niche an organism occupies, as nocturnal animals often decrease activity when exposed to light, while the opposite response is more likely to be seen in diurnal animals. Little is known about the neural mechanisms underlying these differences. Here, we examined the masking effects of light on behavior and the activation of several brain regions by that light, in diurnal *Arvicanthis niloticus* (Nile grass rats) and nocturnal *Mus musculus* (mice). Each species displayed the expected behavioral response to a 1 h pulse of light presented 2 h after lights-off, with the diurnal grass rats and nocturnal mice increasing and decreasing their activity, respectively. In grass rats light induced an increase in cFOS in all retinorecipient areas examined, which included the suprachiasmatic nucleus (SCN), the ventral subparaventricular zone (vSPZ), intergeniculate leaflet (IGL), lateral habenula (LH), olivary pretectal nucleus (OPT) and the dorsal lateral geniculate (DLG). In mice, light led to an increase in cFOS in one of these regions (SCN), no change in others (vSPZ, IGL and LH) and a decrease in two (OPT and DLG). In addition, light increased cFOS expression in three arousal-related brain regions (the lateral hypothalamus, dorsal raphe, and locus coeruleus) and in one sleep-promoting region (the ventrolateral preoptic area) in grass rats. In mice, light had no effect on cFOS in these four regions. Taken together, these results highlight several brain regions whose responses to light suggest that they may play a role in masking, and that the possibility that they contribute to species-specific patterns of behavioral responses to light should be explored in future.

1. Introduction

Light is a powerful environmental cue that can have a major impact on daily patterns of behavior and physiology by entraining the endogenous circadian pacemaker and through more acute mechanisms that lead to a process referred to sometimes as "masking" [1]. Disentangling the influences of these two processes can be difficult in natural conditions because in environments with rhythmic alteration of light and darkness masking and circadian mechanisms complement each other to coordinate an animal's patterns of adaptation to the day/night cycle [2]. Early circadian biologists devised experimental protocols to measure the influences of these two systems on behavior, but in doing so they generally dismissed masking as an important biological process in its own right [3]. This is evident in the original definition of the term, "…certain (sometimes overlooked) experimental conditions can *obscure* the real Zeitgeber-mechanism. We may call them masking

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conditions" [4]. Masking, however, can reflect adaptive mechanisms that contribute to regulation of the daily patterning of activity, rather than processes that simply obscure the influences of the circadian system.

Masking is a complex process (e.g. [5–8]) and is typically quite different in day- and night-active animals, as light is more likely to increase activity in the former (a process referred to as positive masking) and decrease it in the latter (a process referred to as negative masking [9]). The patterns of response to photic cues can also change across the day in different ways (e.g. [1,10]). Many experiments have documented the suppression of activity by light in nocturnal mice [11–13], rats [14], and hamsters [15]. In recent years, several studies have described masking in diurnal rodents, such as Nile grass rats [10,16], degus [6–8,17], Mongolian gerbils [18] and golden spiny mice [19]. In a recent study we directly compared the behavior of nocturnal mice and diurnal Nile grass rats exposed to the same light stimuli presented at the same times of day and found that the animals responded in opposite ways: the light that suppressed activity of mice increased it in grass rats [10].

Although several experimental approaches have been used to study the neural substrates of masking responses, relatively little is known about them. One approach has been to examine the effects of lesions of retinorecipient regions of the brain on acute behavioral responses to photic stimuli. The suprachiasmatic nucleus (SCN) has been implicated in masking through such studies, though its role has been somewhat controversial [5,20,21] and effects that have been seen could be due to damage to cells in the surrounding region (the ventral subparaventricular zone, vSPZ) [21] or damage to retinal fibers that go through the region of the lesions but do not terminate in the SCN [22]. Other areas that have been implicated in masking through lesion studies include the intergeniculate leaflet (IGL) [23,24], the dorsal lateral geniculate nucleus (DLG) [25], visual cortex [26], and the olivary pretectal nucleus (OPN) [27,28]. Consideration of the effects of lesions of different retinorecipient areas of the brain led Redlin [2] to propose that multiple areas mediate masking of activity by light in nocturnal species.

A second approach to exploration of neural substrates of masking has focused on the recently discovered melanopsin-containing intrinsically photo-responsive retinal ganglion cells (ipRGCs) and the brain regions to which they project. In mice, the masking response is absent when these cells are absent or reduced [29–31]. ipRGCs project to many areas of the brain [32], one or more of which is likely to be functionally linked to masking.

Finally, several studies of nocturnal rodents have used the immediate early gene cFOS to characterize responsiveness to light of cells in regions to which the ipRGCs project. Results from these studies have revealed considerable differences across regions, species, and strains, as summarized in Table 1. For example, in two strains of mice exposure to 1 h of light of the same intensity in the same lab elicited different cFOS responses within the IGL [36,38]. There are very few studies that have examined light-induced cFOS activation in diurnal species and most of these have focused exclusively on the SCN [57–60]. Only two have looked outside the SCN in a diurnal species, and these have revealed light-induced increases in cFOS in the peri-SCN region of Nile grass rats [59] and the IGL of degus [58]. In diurnal animals, nothing is known about patterns of responsiveness to photic stimuli in other brain regions that receive input from ipRGCs.

In the current study we examined cFOS in several brain regions of animals exposed to light that triggered an increase in activity of grass rats and a decrease in mice. First, we looked at areas that receive direct input from the retina, including the SCN, vSPZ, IGL, lateral hypothalamus (LH), olivary pretectal area (OPT) and the DLG. These areas might produce acute effects on general activity via pathways extending to structures that regulate sleep/wake state [5], as light can rapidly trigger sleep in nocturnal mammals (e.g. mice [61,62]) and heighten arousal/alertness in diurnal ones (e.g. humans [63]). We therefore also examined responses to light in the ventrolateral preoptic area (VLPO), a sleep-promoting region that receives retinal input (32), as well as in three brain regions that stimulate arousal, the lateral hypothalamus (LH), dorsal raphe (DR), and locus coeruleus (LC). Although the latter areas receive little or no direct visual input they could respond to light via indirect projections from the retina.

2. Methods

2.1. Animals

Adult female grass rats (n = 8) were obtained from the breeding colony at Michigan State University and adult male CD1 mice (n = 10) were obtained from Charles River Laboratory (Wilmington, MA, USA). Female grass rats are anestrous in our standard laboratory conditions [64], and masking responses to light are the same in male and female grass rats [10]. All animals were maintained on a 12:12 light–dark (LD) cycle with 300 lux of white light during the light phase and <1 lux of red light during the dark phase. All animals were singly housed in Plexiglas cages (34×28×17 cm) equipped with an enrichment device (PVC, length: 8 cm, diameter: 6 cm); food (PMI Nutrition Prolab RMH 2000, Brentwood, MO) and water were available *ad libitum*. The Institutional Animal Care and Use Committee of Michigan State University approved all experimental procedures.

2.2. Experimental procedures

All animals were kept on a 12:12 LD cycle unless otherwise noted; zeitgeber time (ZT) 0 refers to the time of lights-on. Activity levels were monitored via infrared motion detectors (IRs, Visonic Tel Aviv, Israel), and all counts from them were recorded with the VitalView Program (Minimitter, Bend, OR, USA). After two weeks animals were assigned to either a control group (grass rat: n = 4, mice: n = 5) or a group that was exposed to a one-hour light pulse (LP; grass rats: n = 4, mice: n = 5) beginning at ZT 14. All of these animals were perfused (see above) at the end of the light pulse (i.e. ZT 15).

2.3. Immunocytochemistry (ICC)

One series of sections from each animal (i.e. every third section) was processed with immunohistochemistry to visualize the distribution of cells containing the protein cFOS. The protocol for grass rats followed the same steps outlined for the CTβ reaction. In brief, sections were incubated in (i) 5% normal donkey serum (Jackson ImmunoResearch, West Grow, PA, USA), (ii) a primary rabbit anti-cFOS antibody (1:50,000, Santa Cruz Biochemistry, Santa Cruz, CA, USA), (iii) biotinylated donkey anti-rabbit antibody (1:200, Jackson ImmunoResearch) and (iv) the ABC complex (Vector Laboratory). From this point the procedure differs from that used for the CTβ. Sections were rinsed in a 0.14 M acetate buffer (pH 7.2), and then reacted in a mixture of DAB (0.5 mg/mL) and nickel sulfate in 0.14 M acetate buffer (pH 7.2) with 3% hydrogen peroxide. Sections were then mounted, dehydrated, and coverslipped with dibutyl phthalate xylene (Sigma-Aldrich).

The procedure for processing tissue from the mice followed similar steps except that: (1) the tissue was rinsed in 0.1% PBT (PBS with .01% Triton X-100) rather than PBS, (2) the concentration of the primary antibody, rabbit anti-cFOS, was higher (1:20,000), and (3) the tissue was incubated overnight in the ABC solution. Finally, the DAB reaction was carried out in a Trizma buffer. A second series of brain sections from each animal was stained with cresyl violet and used to delineate regions of interest for analysis.

2.4. Data analysis

To analyze the behavioral data, Vital View files were converted into actograms via ClockLab (Actimetrics, Wilmette, IL, USA) and raw data were transferred into Microsoft Excel. The actograms provided visual confirmation of masking behavior during the light pulse, while Excel allowed for quantitative assessment of the data. To statistically compare