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ATTENUATED OREXINERGIC SIGNALING UNDERLIES DEPRESSION-LIKE RESPONSES INDUCED BY DAYTIME LIGHT DEFICIENCY

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Abstract—Light has profound effects on mood, as exemplified by seasonal affective disorder (SAD) and the beneficial effects of bright light therapy. However, the underlying neural pathways through which light regulates mood are not well understood. Our previous work has developed the diurnal grass rat, *Arvicanthis niloticus*, as an animal model of SAD (Leach et al., 2013a,b). By utilizing a 12:12-h dim light: dark (DLD) paradigm that simulates the lower light intensity of winter, we showed that the animals housed in DLD exhibited increased depression-like behaviors in the forced swim test (FST) and sweet solution preference (SSP) compared to animals housed in bright light during the day (BLD). The objective of the present study was to test the hypothesis that light affects mood by acting on the brain orexinergic system in the diurnal grass rat model of SAD. First, orexin A immunoreactivity (OXA-ir) was examined in DLD and BLD grass rats. Results revealed a reduction in the number of OXA-ir neurons in the hypothalamus and attenuated OXA-ir fiber density in the dorsal raphe nucleus of animals in the DLD compared to those in the BLD group. Then, the animals in BLD were treated systemically with SB-334867, a selective orexin 1 receptor (OX1R) antagonist, which led to a depressive phenotype characterized by increased immobility in the FST and a decrease in SSP compared to vehicle-treated controls. Results suggest that attenuated orexinergic signaling is associated with increased depression-like behaviors in grass rats, and support the hypothesis that the orexinergic system mediates the effects of light on mood.

Key words: orexin, seasonal affective disorder, diurnal grass rats, SB-334867.

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Abbreviations: 5-HT, serotonin; BLD, bright light:dark; BNST, bed nucleus of stria terminalis; DLD, dim light:dark; DMH, dorsomedial hypothalamus; DRN, dorsal raphe nucleus; FST, forced swim test; ir, immunoreactivity; ICC, immunocytochemistry; LH, lateral hypothalamus; mPFC, medial prefrontal cortex; OX, orexin; OX1R, orexin 1 receptor; PFA, perifornical area; SAD, seasonal affective disorder; SSP, sweet solution preference; VTA, ventral tegmental area.

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INTRODUCTION

Environmental lighting conditions have a profound effect on mood, which is best exemplified in seasonal affective disorder (SAD). SAD is a major depressive disorder, in which affected individuals experience regularly recurring episodes of depression and anxiety each fall and winter, when there is less sunlight (Rosenthal et al., 1984). Symptoms associated with SAD remit in spring and summer when the ambient light gets brighter, and can be alleviated by bright-light exposure in winter (Rosenthal et al., 1984; Lewy et al., 1987). Although these phenomena have been characterized over decades, the mechanisms underlying the light-dependent changes in affective state have not been fully elucidated (Levitan, 2007).

To explore the neural substrates involved in SAD, we have utilized the Nile grass rat, *Arvicanthis niloticus*, a diurnal equatorial rodent species (McElhinny et al., 1997; Blanchong et al., 1999). Depression-like behaviors have been consistently observed by our group and others in diurnal grass rats housed in winter-like lighting conditions involving short day-length (Ashkenazy-Frolinger et al., 2009; Leach et al., 2013b) or low light intensity during the day (Leach et al., 2013a). For humans, due to the use of artificial lights, the duration of daily light exposure we experience across seasons does not fluctuate as much as the quality/intensity of the light (Hebert et al., 1998). Therefore, the changes in light intensity over the seasons may be a more salient determinant than changes in light duration for regulating mood in humans. By manipulating light intensity during the day, which is more etiologically relevant to humans, we have found increased depressive behaviors in grass rats housed in 12-h dim-light/12-h dark (DLD) compared to those housed in bright-light/dark (BLD) (Leach et al., 2013a). The reliable depression-like behavior under winter-like lighting conditions strongly supports the face validity of the diurnal grass rat as a model of SAD.

Using the grass rat model of SAD, the present study explored the hypothesis that light affects mood-related behaviors by acting on the brain's orexinergic (OXergic) system. The neuropeptide orexin (OX), also known as hypocretin, has been implicated in many important physiological functions including wakefulness, energy homeostasis, reward and mood regulation (Tsujino and Sakurai, 2009). In laboratory rats, OXergic neurons receive indirect retinal input (Deurveilher and Semba, 2005). Similar pathways are likely conserved in the diurnal grass rats. Although direct retinal innervation of

OXergic neurons remains to be confirmed, in both laboratory rats and grass rats, there are direct retinal projections to the lateral hypothalamus (LH) where most OXergic cells are found (Johnson et al., 1988; Leak and Moore, 1997; Gaillard et al., 2013). Critically important for modulating mood and anxiety, OXergic cells project very heavily to the prefrontal cortex, limbic structures including the amygdala and bed nucleus of stria terminalis (BNST), and monoaminergic systems in both nocturnal laboratory rats and diurnal grass rats (Peyron et al., 1998; Nixon and Smale, 2007). Furthermore, the OX receptors have been found in these regions in laboratory rats (Gotter et al., 2012). Recently, we have found that in grass rats, a light pulse stimulates immediate-early gene activity in OXergic cells and cell in the dorsal raphe nucleus (DRN), and that blocking OXergic signaling with a selective OX receptor 1 (OX1R) antagonist SB-334867 inhibits light-induced activation of neurons in the DRN (Adidharma et al., 2012). Based on these results, we hypothesize that OXergic system mediates the effects of light on neural pathways that ultimately regulate mood and anxiety. To evaluate this hypothesis further, the present study used the grass rat SAD model to determine (1) whether the level of OX abundance, measured by immunoreactivity (ir), is affected by lighting conditions and associated with depression-like behaviors elicited by light deficiency (DLD), and (2) if there is a causal link between OX receptor antagonism and depression-like behaviors. Results provide insights into the role of OXergic signaling in light-dependent fluctuations in affective state relevant to SAD.

EXPERIMENTAL PROCEDURES

Animals and housing conditions

Adult male grass rats (*A. niloticus*) were obtained from our breeding colony established with animals originating from sub-Saharan Africa. The colony was maintained/bred as previously described (McElhinny et al., 1997; Leach et al., 2013a,b). These equatorial animals were housed in a 12-h light:12-h dark (LD) cycle with food (Prolab 2000 #5P06, PMI Nutrition LLC, MO, USA) and water available *ad libitum.* The time of lights-on was defined as Zeitgeber time (ZT) 0. All procedures were conducted in accordance with the Michigan State University IACUC.

Experiment 1: Effects of daytime light intensity on orexin A immunoreactivity (OXA ir)

Brains (n = 6/group) used in the experiment were obtained from animals in a previous study, in which male grass rats were singly housed in either bright light:dark (BLD, 1000 lux/1 lux) or dim light:dark (DLD, 50 lux/1 lux) condition for 4 weeks prior to the assessment of depression-like behaviors (Leach et al., 2013a). Following the behavioral tests, the animals were left undisturbed under the same illumination conditions for 5 days before being sacrificed at the middle of the light phase (ZT6) for brain analysis as previously described (Leach et al., 2013a). Brains were fixed with 4% paraformaldehyde, cryoprotected, and sectioned at 40 μm using a cryostat (Leica, IL).

Immunocytochemistry (ICC). ICC for orexin A (OXA) was carried out using methodology described in previous studies (Yan et al., 2010; Adidharma et al., 2012). Every third section was incubated with an antiserum against OXA (1:20,000, s-19, Santa Cruz Biotechnology, Inc, CA) and processed with the avidin–biotin–immunoperoxidase technique using DAB as the chromogen. The orexin-containing cell bodies and fibers were stained brown. Following the ICC, sections were mounted on slides, dehydrated with alcohol, cleared with xylene, and coverslipped with Permount (Fisher Scientific, NJ, USA).

Quantitative analysis of ICC results. For quantification, images of the brain sections were captured using a CCD video camera (CX9000, MBF bioscience, VM, USA) attached to a light microscope (Nikon Instruments Inc., NY, USA). The camera and microscope settings were identical for every image. All the images were analyzed by investigators who were unaware of the experimental conditions of the animals. The number of OXA-ir cells was counted in serial sections of the hypothalamic region from its rostral to caudal extent (Fig. 1). The number of OXA-ir cells was further analyzed subregionally in the LH and perifornical area/dorsomedial hypothalamic region (PFA/DMH) using a vertical line across the fornix (~0.6 mm from the third ventricle) to separate the two subregions, as done previously in laboratory rats (Harris et al., 2005). The density of OXA-ir fibers/terminals was also analyzed in the DRN from four levels across its rostro-caudal extent (Janusonis and Fite, 2001). The sections from levels 1 to 3 where the serotonin (5-HT) neurons are clustered along the midline were grouped as rostral, while those from level 4 where the clusters of the 5-HT neurons are lateralized were defined as the middle DRN as done in our previous study (Leach et al., 2013a). The density of fibers/terminals was quantified using NIH Image J as previously described (Adidharma et al., 2012; Leach et al., 2013a). The size of each area of interest being measured was kept consistent across the sections/animals. A threshold that distinguished the immunoreactive staining from the background was also set consistently for each area. The percentage of pixels above the threshold in the area of interest was measured and averaged across the sections from the same region. The average percentage represented the density of staining per animal. The number of OXA-ir cells was analyzed using a two-way ANOVA. In the DRN, a previous study revealed regional effects in 5-HT-ir when BLD and DLD animals were compared, such that a reduction of 5-HT-ir in the DLD group was only observed in the middle but not in the rostral DRN (Leach et al., 2013a). Therefore, in the present study, the density of OXA-ir was analyzed within each subregion separately using unpaired t-tests.

Experiment 2: Effects of OX1 receptor antagonism with SB-334867 on depression-like behavior

Animals were housed in the same manner as those in experiment 1. After 4 weeks of being housed under BLD conditions, animals were tested for depression-like