**Decreased daytime illumination leads to anxiety-like behaviors and HPA axis dysregulation
in the diurnal grass rat (*Arvicanthis niloticus*)**

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5 18 **ABSTRACT**
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8 19 The impact of ambient light on mood and anxiety is best exemplified in seasonal affective
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11 20 disorder, in which patients experience depression and anxiety in winter when there is less light in
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14 21 the environment. However, the brain mechanisms underlying light-dependent changes in
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18 22 affective state remain unclear. Our previous work revealed increased depression-like behaviors in
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21 23 the diurnal Nile grass rat (*Arvicanthis niloticus*) housed in a dim light-dark (dim-LD) cycle as
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24 24 compared to the controls housed in a bright light-dark (bright-LD) condition. As depression is
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27 25 often comorbid with anxiety and is associated with dysregulation of the body's stress response
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30 26 system, the present study examined the anxiety-like behaviors as well as indicators of the
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33 27 hypothalamic-pituitary-adrenal (HPA) axis functioning in the grass rats. Animals housed in dim-
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37 28 LD showed increased anxiety-like behaviors compared to bright-LD controls, as revealed by
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40 29 fewer entries and less time spent at the center in the open field test and more marbles buried
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43 30 during the marble-burying test. Following the marble-burying test, dim-LD animals showed
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46 31 higher plasma corticosterone (CORT) levels and hippocampal Fos expression. Although the daily
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50 32 CORT rhythm was comparable between bright-LD and dim-LD groups, the day/night variation of
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53 33 *corticotropin-releasing hormone* mRNA expression in the paraventricular nucleus was
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56 34 diminished in dim-LD animals. In addition, *glucocorticoid receptor* and *mineralocorticoid*
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59 35 *receptor* mRNA expression were higher in the hippocampus of dim-LD animals. The results
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36 suggest that in diurnal species, reduced daytime illumination can lead to increased anxiety-like
37 behaviors and altered HPA axis functioning, providing insights into the link between decreased
38 environmental illumination and negative emotion.

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40 **Key words:** anxiety; corticosterone; corticotropin-releasing hormone; hippocampus;
41 hypothalamic-pituitary-adrenal axis; seasonal affective disorder.

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5 43   **Abbreviations**
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8 44   ANOVA, analysis of variance; bright-LD, bright light-dark cycles; CORT, corticosterone; CRH,
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12 45   corticotropin-releasing hormone; dim-LD, dim light-dark cycles; GR, glucocorticoid receptor;
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15 46   HPA, hypothalamic-pituitary-adrenal; MR, mineralocorticoid receptor; PVN, paraventricular
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18 47   nucleus; SAD, seasonal affective disorder; ZT, zeitgeber time.
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1. Introduction

Beyond its essential role in vision, light has profound effects on our brain functions and behaviors [1]. Ambient light can influence mood and emotions, which is best exemplified in seasonal affective disorder (SAD). SAD is a type of major depressive disorder, with patients suffering from recurring episodes of depression in fall and winter, followed by full remission of symptoms in spring and summer [2]. In addition to depressed mood, most of SAD patients also experience elevated anxiety and greater stress level [2–4]. The seasonal patterns of this affective disorder and the effectiveness of bright-light therapy in alleviating the depression and anxiety symptoms suggest that the mood and emotional fluctuation is caused by changes in the ambient lighting conditions across seasons. Although day length is the most salient signal for seasonal changes, humans in modern societies do not usually experience seasonal changes in day length due to the use of artificial lighting [5]. Thus, the seasonal changes in the quality of light (i.e. the intensity) may play a more important role in the etiology of SAD than the duration of light exposure (i.e., day length) [5–7]. Indeed, low ambient illumination levels have been found to be associated with negative mood in SAD patients [8] and in general population [9,10]. It has also been reported that diminished perception of ambient light is strongly associated with the degree of depressive symptoms, further supporting the link between light intensity and mood [11,12]. However, the brain mechanisms underlying light intensity-dependent changes in affective state

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8 69 Our previous work has developed a diurnal rodent model, the diurnal Nile grass rat
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10 70 (*Arvicanthis niloticus*) to study the neural mechanisms through which the intensity of the
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14 71 environmental lighting condition modulates affective state [13–16]. Depression-like behaviors
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17 72 have been observed in grass rats following daytime low light intensity [13,16]. However, the
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21 73 results on anxiety-like behaviors are less consistent. Our recent work observed enhanced
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24 74 thigmotaxis during the forced swim test in grass rats housed in a condition involving relatively
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27 75 dim light during the day (dim-LD) compared to the control animals housed in relatively bright
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31 76 light during the day (bright-LD) [13]. The results seem to suggest elevated anxiety, as
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34 77 thigmotaxis in a water maze has been shown in laboratory rats to positively correlate with their
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37 78 trait anxiety and susceptibility to stressors [17–19]. While in a previous study no significant
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40 79 difference was observed between the bright-LD and dim-LD groups in two common tests of
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43 80 rodent anxiety, i.e., open field and light/dark box tests [16], it is likely that any effects might have
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46 81 been masked by the acute stress response associated with the tests in our unhandled animals. To
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50 82 further evaluate the anxiety-like behaviors in the dim-LD condition and to address this seeming
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53 83 inconsistency in our previous work, the present study utilized modified open field and marble-
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56 84 burying tests. The modified open field test introduced pre-test handling sessions to have the
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59 85 animals acclimated to the experimenter, while the marble burying test was conducted in a cage
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that was the same as the subjects' home cage in terms of size and bedding, both aiming to reduce any acute stress induced by the experimental procedures.

In addition to their behavioral responses, we also examined the effect of housing in decreased illumination on the hypothalamic-pituitary-adrenal (HPA) axis functioning in the grass rat. A body of evidence suggests connections between dysregulation of the HPA axis and affective behaviors [20,21]. For example, hyperactivity of the HPA axis [22–25], enlarged adrenal glands [26], altered daily rhythm of corticoids secretion [27], and impairments in the HPA negative feedback mechanism [28,29] have been implicated in depressive disorders. Abnormal HPA axis functioning is also reported in anxiety disorders [30,31]. In the present study, the acute stress response was assessed following the marble-burying test (a novel mild stressor) by analyzing plasma corticosterone (CORT) and expression of the Fos protein product of the immediate early gene *c-fos* in the paraventricular nucleus (PVN) and hippocampus [32–35]. In addition, to assess the basal function of the HPA axis, we examined the daily rhythms of plasma CORT, corticotropin-releasing hormone (*CRH*) mRNA expression in the PVN, and expression of glucocorticoid receptors (*GR*) and mineralocorticoid receptors (*MR*) mRNA in both the PVN and hippocampus. The results from the present study demonstrate that decreased daytime illumination leads to anxiety-like behaviors and altered HPA axis function in the diurnal grass rat.

2. Materials and methods

2.1. Animals

Male Nile grass rats (*Arvicanthis niloticus*) used in this study were originally captured and imported from sub-Saharan Africa and bred in our colony at the Michigan State University [36]. The animals were maintained in 12 h light-12 h dark conditions, which is typical of their natural equatorial habitat. Grass rats had *ad libitum* access to food (Prolab 2000 #5P06, PMI Nutrition LLC, MO, USA) and water. For use in the experiments, young adults (~ 4-months old) were singly housed in either 12 h bright light (1000 lux)-12 h dark (1 lux) conditions (bright-LD) or 12 h dim light (50 lux)-12 h dark (1 lux) conditions (dim-LD) as previously described [13,16]. All procedures were approved by the Michigan State University Animal Use and Care Committee.

2.2. Open field test

Animals (n = 8/condition) were housed in either bright-LD or dim-LD lighting condition for two weeks. The animals were gently handled for 10 min each day for seven days before testing to acclimate to the experimenter. On the test day, animals were brought in their home cages to the testing room and were left undisturbed for an hour between zeitgeber time 5 (ZT5, 5 h after lights on) and ZT9. After the hour acclimation phase, each animal was placed in the center of a white Plexiglas arena (80 × 80 × 47 cm, 100 lux at the arena floor level) and allowed to explore

the arena for 10 min. Their behaviors were recorded and scored by Ethovision XT (v. 8.5, Noldus Information Technology, Wageningen, Netherlands). Center-point detection tracking was used in order to determine where the animal was located in the arena. The center of the arena was defined as an area with the size of 60×60 cm. One animal in the dim-LD group had both open-field variables, i.e., the number of center entries and center time, at two standard deviations above the group means so was excluded from the analysis. The arena was wiped down with 70% ethanol between each animal.

2.3. Marble burying test

A second cohort of grass rats ($n = 8$ /condition) was housed in either bright-LD or dim-LD condition for four weeks. Animals were individually housed in a Plexiglas cage ($47 \times 25 \times 20$ cm) and were left undisturbed prior to the marble-burying test. During the test, the animals were transferred to a fresh cage of the same size that contained 20 glass marbles (1.5 cm in diameter, arranged in a 4×5 grid) on top of the 5-cm thick bedding. The testing cages were placed back to where the home cage was located, thus the light intensities during the test were identical to the animals' housing conditions, i.e., 1000 lux for bright-LD animals and 50 lux for dim-LD animals. After a 30-min test, the animals were returned to their home cage and the number of marbles buried (to $2/3$ their depth) with bedding was counted [37]. The test was performed between ZT4

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5 140 and ZT5. One dim-LD animal that escaped from the testing cage was excluded from behavior,
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8 141 CORT, and Fos analyses. Sixty minutes after completion of the test, animals were anesthetized
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11 142 with pentobarbital (20 mg/kg). Blood samples were collected from the left ventricle for CORT
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14 143 analyses (see details below) prior to transcardial perfusion with saline followed by fixative (4%
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18 144 paraformaldehyde in 0.1 M phosphate buffer). Brains were removed and later processed for
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27 147 ***2.4. Daily rhythm of HPA axis activity***

30 148 The last cohort of animals (bright-LD, n = 14; dim-LD, n = 15) was housed in each lighting
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33 149 condition for four weeks. Body weight was monitored in half of the animals from each group
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37 150 during the last week of their lighting conditions (n = 7/condition). The animals were anesthetized
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43 152 CORT analyses. Adrenal glands were removed and weighed. Brains were removed and placed in
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46 153 RNAlater solution (Ambion, TX, USA) and stored at 4°C until gene analyses.
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53 155 ***2.5. Plasma CORT analysis***

56 156 Blood was collected in tubes containing 100 µl of heparin (100 USP, Sagent, Schaumburg, IL,
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5 158 and stored at -20°C until analysis. Plasma was assayed for CORT using Coat-a-Count
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12 160 Center for Population and Animals Health at the Michigan State University. All standards and
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15 161 samples were run in duplicate. The intra-assay coefficient of variation was 3.3%. The highest and
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27 165 ***2.6. Immunohistochemistry***

30 166 Brains were sectioned at 40 μm using a cryostat, and subjected to immunohistochemistry for
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37 168 against the Fos protein (1:1000, sc-52, Santa Cruz Biotechnology, Inc., CA, USA) and processed
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40 169 with avidin-biotin-immunoperoxidase technique using DAB enhanced with 4% nickel sulfate as
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50 172 quantification, images were captured using a CCD video camera (CX9000, MBF bioscience,
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53 173 Williston, VT, USA) attached to a light microscope (Zeiss, Gottingen, Germany) with a 10 $\times$
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56 174 objective lens. Numbers of Fos-immunoreactive (Fos-ir) nuclei were counted in a standardized
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14 179 in the dentate gyrus and calculated as cell counts per $10^3 \mu\text{m}^2$, and the values from left and right
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24 182 ***2.7. RNA extraction and quantitative real-time PCR***

27 183 Brains were sliced at 300 μm using a cryostat. The entire hippocampus in the right hemisphere
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30 184 was also dissected out from the brain slices ($n = 5/\text{time point/condition}$). The PVN region was
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37 186 Sciences, PA, USA) ($n = 7-8/\text{time point/condition}$). Compared to the hippocampus that can be
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43 188 could contribute to variability in the results. Therefore, more samples were used for the PVN
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46 189 analysis. Total RNA was isolated using Trizol (Life Technologies, CA, USA). DNA in RNA
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50 190 samples was digested with deoxyribonuclease I, Amplification Grade (Life Technologies).
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53 191 cDNAs were synthesized using High Capacity cDNA Reverse Transcription Kit (Applied
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56 192 Biosystems, CA, USA). For real-time PCR analysis, 1% of the cDNA was used at a final
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primer sets for *CRH*, *GR*, *MR*, and *Gapdh*, which was used as a reference gene for all comparisons, using an ABI 7000 Real-Time PCR System (Life Technologies). Each reaction was performed in duplicate. Forward and reverse primer sequences used were 5'-GAT CCG CAT GGG TGA AGA AT-3' and 5'-AGC AGC GGG ACT TCT GTT GA-3', respectively, for *CRH*, 5'-ACC AAC GGA GGC AGT GTG AA-3' and 5'-TCC CGC CAA AGG AGAAAG CA-3', respectively, for *GR*, 5'-GCG TTT CCG GTG CTA TTC CG-3' and 5'-ATG GAC TCA GCT ACC GG GC-3', respectively, for *MR*, and 5'-ATC CAC TGG TGC TGC CAA G-3' and 5'-CCG TTC AGC TCT GGG ATG AC-3', respectively, for *Gapdh*. Primers were designed based on the corresponding sequences in laboratory mice and rats. In all the reactions, the generation of only a single expected amplicon was confirmed by melting analysis. Quantification of cDNAs was performed by the standard curve method.

2.8. Statistical analysis

The statistical analyses were conducted using the statistical program, *R* [38]. All data are presented as means $\pm$ SEMs. CORT concentrations and gene expression data were analyzed with two-way analysis of variance (ANOVA; time $\times$ lighting conditions). The Tukey's honest significant difference (HSD) test was used to further evaluate group differences. Tissue weights, behavioral data, and Fos-ir cell counts were analyzed with student's *t*-tests. Differences were

considered statistically significant when $P < 0.05$. Animals with any measure higher or lower than two standard deviations of the group mean were considered as outliers and excluded from the analyses. The numbers of animals used for the analyses are described in each figure.

3. Results

3.1. Decreased illumination elicited anxiety-like behaviors as well as enhanced responses of *CORT* and hippocampal *Fos* expression to a mild stressor

The anxiety-related exploratory activity in the open field arena was compared between bright-LD (Fig. 1A) and dim-LD animals (Fig. 1B). The number of entries into the center of the arena was significantly lower in the dim-LD group compared to the bright-LD group (Fig. 1C, $t_{(13)} = 3.73$, $P < 0.01$). In addition, the dim-LD group spent significantly less time in the center than the bright-LD group (Fig. 1D, $t_{(13)} = 4.13$, $P < 0.01$).

In the marble burying test, dim-LD animals buried significantly more marbles than bright-LD animals (Fig. 2A, $t_{(13)} = 2.23$, $P = 0.043$). Moreover, at 60 min after completion of the test, dim-LD animals had significantly higher plasma *CORT* levels compared to bright-LD animals (Fig. 2B, $t_{(12)} = 2.23$, $P = 0.046$).

The expression of *Fos* in the PVN and hippocampus was also examined following the exposure to marbles, which was a mild novel stressor. In the PVN, the number of *Fos*-ir nuclei was not

different between bright-LD and dim-LD groups (Fig. 3A,B, $t_{(13)} = 0.05$, $P = 0.96$). Similarly, there was no significant difference in the number of Fos-ir nuclei in the CA1 subfield of the hippocampus between bright-LD and dim-LD groups (Fig. 3C,D, $t_{(13)} = 1.52$, $P = 0.15$). However, the number of Fos-ir nuclei was significantly higher in the dim-LD group compared to the bright-LD group in the CA3 (Fig. 3C,D, $t_{(13)} = 2.43$, $P = 0.030$) and the dentate gyrus (Fig. 3C,D, $t_{(13)} = 4.12$, $P < 0.01$).

3.2. Daily rhythm in plasma CORT concentration and CRH mRNA expression in the PVN

Grass rats housed in both bright-LD and dim-LD conditions showed a clear daily rhythm in plasma CORT concentrations, which was high at the beginning of the day and low at the beginning of the night (Fig. 4A). The two-way ANOVA revealed a significant effects of time ($F_{(1,24)} = 17.4$, $P < 0.01$), but no effect of light intensity ($F_{(1,24)} = 0.69$, $P = 0.41$) or interaction between the factors ($F_{(1,24)} = 0.34$, $P = 0.56$). There were no significant differences between the bright-LD and dim-LD groups in their adrenal gland weights (Fig. 4B $t_{(26)} = 0.08$, $P = 0.94$) or in their body weights (Fig. 4C, $t_{(12)} = 0.17$, $P = 0.87$). However, the day/night profile of *CRH* mRNA expression was altered by decreased daytime illumination (Fig. 4D). Although the two-way ANOVA revealed no main effects of time ($F_{(1,24)} = 0.02$, $P = 0.96$) or light intensity ($F_{(1,24)} = 0.72$, $P = 0.40$), there was a significant interaction between time and lighting conditions ($F_{(1,24)} =$

5.64, $P = 0.026$) in *CRH* mRNA expression in the PVN.

3.3. *GR and MR mRNA expression in the PVN and hippocampus*

In the PVN, there were no significant effects of time, lighting conditions or interaction between time and lighting on the expression of *GR* (two-way ANOVA, $P_s > 0.05$) or *MR* mRNA (two-way ANOVA, $P_s > 0.05$) (Fig. 5A). In the hippocampus, there was also no effect of time ($F_{(1,16)} = 0.22$, $P > 0.65$) on *GR* expression, but there was a significant effect of lighting conditions ($F_{(1,16)} = 12.25$, $P < 0.01$) and a significant interaction between the two factors ($F_{(1,16)} = 12.01$, $P < 0.01$) (Fig. 5B). *GR* expression was higher in the dim-LD condition than in the bright-LD condition at the beginning of the day ($F_{(1,8)} = 22.71$, $P < 0.01$). Hippocampal *MR* expression was also higher in the dim-LD condition (Fig. 5B, effect of lighting conditions: $F_{(1,16)} = 5.99$, $P = 0.026$; effect of time $F_{(1,16)} = 0.15$, $P = 0.7$; interaction: $F_{(1,16)} = 0.0$, $P = 0.96$).

4. Discussion

The results of the present study revealed that grass rats experiencing a reduction in daytime light intensity (i.e., the dim-LD condition) showed increased anxiety-like behaviors and several indicators of HPA axis dysregulation. The anxiety-like behaviors of grass rats in dim-LD and bright-LD conditions were reevaluated in the present study by a modified open field test, which

revealed fewer incidents of center entry and less time spent in the center of the arena in dim-LD

animals compared to the bright-LD controls. Although a previous study from our group did not

find significant differences between the bright-LD and dim-LD animals during an open field test

[16], the discrepancy in these outcomes is likely due to the pre-test handling session introduced in

the present study. It has been shown in other rodent models that regular handling before the test

decreases stress-related responses produced by the experimental procedure [39,40]. Indeed,

anecdotally we noted that the pre-test handling in the present study resulted in noticeable

differences in the behaviors of grass rats, such that the animals became calmer and less jittery

during the test. Therefore, the reduced stress response likely unmasked the potential differences

in anxiety-like behaviors associated with ambient lighting conditions.

It should be noted that both groups were tested in the open field where the light intensity was

100 lux, which was dimmer than what animals were housed in for the bright-LD condition but

brighter than what animals were housed in for the dim-LD condition. The light intensity during

testing has been shown to affect anxiety-like behaviors [41,42], thus we kept it consistent

between the two groups. However, a caveat is that the dim-LD animals experienced a shift for

testing from dim to brighter light, which might have contributed to the increased anxiety-like

behaviors. This seems unlikely though, because in their natural habitat, the grass rats come out

from their underground burrow to forage during the day under bright sunlight; anxiety induced by

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5 284 brighter light would presumably be against their diurnal nature. Furthermore, we found that the
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8 285 dim-LD animals also showed more anxiety-like behavior in the marble-burying test [43-45],
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21 289 Consistent with their elevated anxiety behaviors, the dim-LD group also had a higher plasma
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27 291 measured at an hour after the marble burying test. An acute stressor (the presence of marbles in
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30 292 this case) typically induces a surge of CORT secretion within 15 to 30 minutes, which is
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34 293 suppressed by the action of CORT negative feedback [43,44]. The mechanism of negative
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37 294 feedback allows hormone levels to quickly return to basal levels, which prevents hyperactivation
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40 295 of the HPA axis. The CORT level of bright-LD animals was comparable to that reported in
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43 296 juvenile grass rats exposed to the novel environment [45]. However, the finding that housing in
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49 298 stressor may suggest dysregulation of the HPA negative feedback in these animals. In addition to
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53 299 CORT secretion, it has been well demonstrated that exposure to acute stress induces Fos
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56 300 expression in stress-related brain regions including the PVN and the hippocampus [32–35]. Fos
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59 301 expression is likely mediated by CORT's stimulation of GR in these sites [46]. In the present
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study, while stress-induced Fos expression was not different between the bright-LD and dim-LD groups in in the PVN, it was higher in the hippocampus of dim-LD animals, consistent with the higher CORT level. Our findings are consistent with previous studies, in which a higher level of stress-induced Fos expression in the hippocampus is often associated with increased CORT levels [47,48]. Collectively, our data suggest that following daytime light deficiency, stress responses were enhanced at multiple levels ranging from the behavior, hormone secretion, to hippocampal gene expression in grass rats housed in the dim-LD condition.

Dysregulation of the HPA axis is a common feature in depression and anxiety disorders [22–25]. Altered daily rhythms of corticoids, enlarged adrenal glands, and changes in *CRH* mRNA or peptide expression have been observed in some cases of affective disorders [26,27,49,50]. In the diurnal grass rats, a clear daily rhythm in plasma CORT levels was observed in both bright-LD and dim-LD groups, with high levels at the beginning of the active phase (ZT2), which is similar to the CORT daily pattern reported in other diurnal rodents such as degus [51]. The basal CORT levels, the weight of the adrenal glands, and body weight in the dim-LD animals were not different from the bright-LD controls, though, suggesting that the dim-LD paradigm does not induce chronic stress, which would have been expected to affect the basal CORT level, the adrenal gland weight, and body weight [52–54]. The intact CORT rhythms in dim-LD group are consistent with the findings in SAD patients, in which cortisol rhythms were not different from

control subjects [55–58]. In spite of the intact CORT rhythm, we found that lighting conditions altered the daily variation pattern of *CRH* mRNA expression in the PVN, suggesting that decreased daytime illumination impairs proper regulation of *CRH* mRNA expression. Indeed, altered *CRH* expression in the PVN has been reported in animal models which show increased anxiety- or depression-like behaviors as well as disrupted stress responses [59,60]. Therefore, the dysregulation of *CRH* expression is likely related to anxiety-like behaviors induced by low light intensity.

CORT exerts its effect through two types of receptors, the low-affinity GR and high-affinity MR, which are expressed in a variety of brain regions [61]. In the PVN of nocturnal rats, GR is co-localized with CRH [62] and plays a role in regulation of *CRH* expression [63]. It was also shown in mice that disruption of GR function in the PVN results in HPA axis hyperactivity [64]. In the grass rats, we found that the expression of *GR* or *MR* mRNA in the PVN was comparable between the bright-LD and dim-LD groups, suggesting that the altered HPA responses in the dim-LD condition was likely regulated by brain regions outside the PVN.

The hippocampus is an attractive candidate to play this role [65–67]. Intra-hippocampal injections of GR and MR antagonists alter HPA axis activity, indicating an importance of hippocampal CORT receptors for regulating the HPA axis [68]. Hippocampal *GR* expression is higher in mice showing elevated anxiety-like behaviors [59], and activation of GR in the dentate

gyrus of the hippocampus leads to elevated anxiety- and depression-like behaviors and stress responses [69]. In the present study, we found that both *GR* and *MR* expressions were elevated in the hippocampus of dim-LD animals, suggesting that light deficiency alters hippocampal *CORT* signaling. Our results are consistent with the reports in Siberian hamsters, in which elevated *GR* expression in the hippocampus, altered HPA axis activity and increased anxiety-like behaviors are induced by short day length [70–72]. Although the hippocampal modulation of the HPA axis is not fully understood [66], several lines of evidence suggest that the hippocampus has an inhibitory action on the HPA axis and suppresses stress-induced *CORT* secretion [73–75]. In addition, it was demonstrated in nocturnal rats that lesions of the ventral subiculum, which is a major target of hippocampal projections, increased daytime *CRH* mRNA expression in the PVN without affecting daily *CORT* rhythms, and enhanced stress-induced *CORT* secretion [76]. Thus, our results raise a possibility that increased *GR* and *MR* expression in the hippocampus is associated with HPA axis dysregulation (rather than enhanced sensitivity to *CORT* and stronger negative feedback) and anxiety-like behaviors induced by daytime light deficiency. Considering that *GR* is involved in *Fos* expression after stress exposure [46], up-regulation of *GR* in the hippocampus but not in the PVN in dim-LD animals may be responsible for the observation that elevated stress-induced *Fos* expression by daytime light deficiency was hippocampus-specific.

In summary, grass rats that experienced a chronic deficiency in daytime illumination showed

increased anxiety-like behaviors, enhanced stress response and altered expression of genes involved in the HPA axis and CORT signaling. The results demonstrate that ambient lighting condition can influence anxiety and stress in a diurnal species, and contribute to our understanding of the neural mechanisms underlying the effects of light on mood and anxiety.

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Figure Legends

Fig. 1. Behavioral responses during the open field test. (A, B) Representative tracks of animals housed in the bright-LD (A) and dim-LD (B) conditions. The center/perimeter boundaries are shown in grey lines. (C) Total number of center entries. (D) Total time spent in center. Results are shown as mean $\pm$ SEM. Numbers on each column indicate the sample size. $**P < 0.01$.

Fig. 2. Marble burying test. (A) Total numbers of buried marbles during the test. (B) Plasma CORT concentrations at 60 minutes following the marble burying test. Results are shown as mean $\pm$ SEM. Numbers on each column indicate the sample size. $*P < 0.05$.

Fig. 3. Fos expression following the marble burying test. (A) Representative photographs of Fos-ir in the PVN. OT, optic tract; 3V, third ventricle. (B) Numbers of Fos-ir nuclei in the PVN. (C) Representative photographs of Fos-ir nuclei in hippocampal subfields i.e., the CA1, CA3, and dentate gyrus. (D) Numbers of Fos-ir nuclei in the hippocampal subfields. Results are shown as mean $\pm$ SEM. Numbers on each column indicate the sample size. $*P < 0.05$, $**P < 0.01$.

Fig. 4. The basal HPA axis activity in bright-LD and dim-LD conditions. (A) Plasma CORT concentrations at ZT2 and ZT14. Two-way ANOVA revealed a significant effect of time ($P < 0.01$). (B) Relative *CRH* mRNA levels in the PVN at ZT2 and ZT14. Two-way ANOVA revealed a significant interaction between time and lighting conditions ($P < 0.01$). There was no significant difference in paired adrenal gland weight (C) or body weight (D) between animals housed in bright- and dim-LD. Results are shown as mean $\pm$ SEM. Numbers on each column indicate the sample size.

Fig. 5. CORT receptors expression in bright-LD and dim-LD conditions. Relative *GR* and *MR* mRNA levels were examined at ZT2 and ZT14 in the PVN (A) and hippocampus (B). In the hippocampus, two-way ANOVA revealed a significant effect of lighting conditions ($P < 0.01$) and a significant interaction between light and time ($P < 0.01$) in *GR* expression, as well as a significant effect of lighting conditions ($P = 0.03$) in *MR* expression. Results are shown as mean $\pm$ SEM. Numbers on each column indicate the sample size. * $P < 0.05$; ** $P < 0.01$.

Fig.1

Fig.2

Fig.3

Fig.4

A. PVN

B. Hippocampus

Fig.5