Light rescues circadian behavior and brain dopamine abnormalities in diurnal rodents exposed to a winter-like photoperiod

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Abstract

Seasonal affective disorder (SAD), beyond mood changes, is characterized by alterations in daily rhythms of behavior and physiology. The pathophysiological conditions of SAD involve changes in day length and its first-line treatment is bright light therapy. Animal models using nocturnal rodents have been studied to elucidate the neurobiological mechanisms of depression, but might be ill suited to study the therapeutic effects of light in SAD since they exhibit light-aversive responses. Here *Arvicanthis ansorgei*, a diurnal rodent, was used to determine behavioral, molecular and brain dopamine changes in response to exposure to a Winter-like photoperiod consisting of a light-dark cycle with 8h of light, under diminished light intensity, and 16h of darkness. Furthermore, we evaluated whether timed-daily bright light exposure has an effect on behavior and brain physiology of Winter-like exposed animals. *Arvicanthis* under a Winter-like condition showed alterations in the synchronization of the locomotor activity rhythm to the light-dark cycle. Moreover, alterations in day-night activity of dopaminergic neurotransmission were revealed in the nucleus accumbens and the dorsal striatum, and in the day-night clock gene expression in the suprachiasmatic nucleus. Interestingly, whereas dopamine disturbances were reversed in animals exposed to daily light at early or late day, altered phase of the daily rhythm of locomotion was reverted only in animals exposed to light at the late day. Moreover, *Per2* gene expression in the SCN was also affected by light exposure at late day in Winter-like exposed animals. These findings suggest that light induces effects on behavior by mechanisms that rely on both circadian and rhythm-independent pathways influencing the dopaminergic circuitry. This last point might be crucial for understanding the mechanisms of non-pharmacological treatment in SAD.

Introduction

Seasonal Affective Disorder (SAD) is a subset of depressive symptoms which correlate with seasonal fluctuations in environmental light (Rosenthal et al. 1984). SAD symptomatology features changes in circadian rhythmicity (e.g. sleep, feeding and activity) which are modulated by light. Indeed, Bright Light Therapy (BLT) is a useful treatment of SAD and other psychiatric disorders (Rosenthal et al. 1984; Lieveverse et al. 2011; Terman and Terman 2005). BLT efficacy is dependent on variables like light intensity, wavelength, duration and timing of exposure (Wirz-Justice et al. 1993; LeGates et al. 2014). However, little is known about the brain changes that anticipate or are secondary to the light effects on mood.

The monoaminergic hypothesis, which proposes that mood disorders result through neurotransmitter systems imbalances, has led to various therapies acting mostly on the serotonergic (5-HT) system (Neumeister et al. 2001; Stahl et al. 2013). While serotonergic targets in the frontal cortex are critical therapeutic mediators, other reward-mediating areas like the nucleus accumbens (NAcc) and ventral tegmental area (VTA), important in dopaminergic (DA) transmission, are promising therapeutic targets that might have been overlooked (Russo and Nestler 2013). DAergic transmission from the VTA to NAcc mediates behavioral responses to natural and non-natural rewards, motivation and motor functions (Berridge and Kringelbach 2013). In major depression, lower motivation and motor activity, and DAergic changes are classical symptoms (Russo and Nestler 2013; Opmeer et al. 2010). Hence, DA transmission might also be altered in SAD patients.

DA activity shows circadian and seasonal variations, which could be a result of the influence of solar time on the circadian clock in the suprachiasmatic nucleus (SCN) (Hood et al. 2010; Mendoza and Challet 2014; Verwey et al. 2016; Eisenberg et al. 2010; Diehl et al. 1994). Some studies have reported a possible influence of photoperiodic conditions and light in the brain DAergic system, which can be correlated with emotional states (Neumeister et al. 2001; Tsai et al. 2011; Cawley et al. 2013). However, DAergic changes in SAD and the effects of light on DA brain content remain to be elucidated. Previously, animal models in this field relied on nocturnal species such as rats and mice which have aversive responses to light (Tavolaro et al. 2015; Deibel et al. 2014). Diurnal species like the grass rat *Arvicanthis niloticus* display acute behavioral responses to light (Shuboni et al. 2012). They, together with other diurnal rodents, have proven useful to investigate depression-like behaviors under Winter-like lighting conditions (Deats et al. 2015; Krivisky et al. 2011; Leach et al. 2013a; Leach et al. 2013b; Ashkenazy-Frolinger et al. 2010).

Here, a series of experiments were designed to elucidate behavioral and central changes induced by Winter-like photoperiods in a diurnal rodent, as well as the possible rescue effects of bright light at two different times at day (early day vs. late day). Grass rats (*Arvicanthis ansorgei*) were exposed to Winter-like photoperiodic conditions, consisting of shorter light phases (8h) with lower light intensity around the 24h. The effects of these conditions were studied on rhythmic behavior, DA content in the dorsal striatum (caudate-putamen; CP) and NAcc and clock gene expression in the SCN. Furthermore, the effects of daily light pulses at the early vs. late day on animals exposed to Winter-like photoperiod were also studied in the aforementioned conditions. Our data reveal that a Winter-like condition in the diurnal rodent *Arvicanthis ansorgei* alters daily rhythms of locomotion, DA content in the forebrain and clock gene expression in the SCN. More important, whereas DAergic alterations were rescued when animals are exposed to daily light at either early or late day, circadian phase was only recovered in animals exposed to light at the late day.

Materials and methods

Animals and housing

This study was conducted using Sudanian unstriped grass rats (*Arvicanthis ansorgei*) born and reared in animal facilities in Strasbourg (Chronobiotron UMS 3415). Animals were housed in

regulated temperature ($23\pm 1^{\circ}\text{C}$) in individual cages under a 12/12h light-dark (LD) cycle and provided *ad libitum* access to food (Ref. 105, SAFE, 89290, Augy, France) and tap water, unless otherwise indicated. Animal handling was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23, revised 1985) and the French Department of Agriculture (License no. 67-378 to J.M.).

Experimental design

Male adult *Arvicantis*, weighing 191.1 ± 5.3 g (5 months of age) at the beginning of the experiment, were acclimatized in single cages under a 12/12h LD cycle (light at 150 lux at cage level, dark at <5 lux dim red light), lights on at 7:00h (defined as Zeitgeber Time [ZT0]), lights off at 19:00h for 3 weeks. Daily locomotor activity rhythms were measured throughout the whole experiment.

Thereafter, animals were exposed to a Winter-like photoperiod condition with a LD8/16h with reduced light intensity during day phase (45 lux), lights on at 9:00h (redefined ZT0) and lights off at 17:00h (ZT8) for 6 weeks. Light intensity was measured by handheld luxometer at the base of the animal cage. Then animals were divided in two groups: a Winter-like + Light exposure at ZT0 group (early day, $n=12$) and a Winter-like + Light exposure at ZT7 (late day, $n=12$) group. The bright light exposed animals received a 45 minutes light pulse of 150 lux starting at ZT0 (early day) or ZT7 (late day) and an additional 15 minute pulse of 1900 lux at cage level, finishing with lights off at ZT1 (1 hour after lights on) or ZT8 (last hour of the light phase), respectively, for 4 weeks. The 15 min light pulse was administered with an overhead lamp housing two cool white fluorescent bulbs (TL-D36W/965; Philips, Somerset, NJ) mounted on a custom-made aluminum frame whose height could be adjusted to vary light intensity.

Locomotor activity recordings

Infrared captors placed on the top of each cage logged locomotor activity counts which were stored in a computer using CAMS (Circadian Activity Monitoring System, Lyon France) software. Actograms and average waveforms were constructed using ClockLab software (Actimetrics, Evanston, IL, USA). Quantified general activity parameters included 24h mean activity profiles for animals under each photoperiod and the phase angle of entrainment of locomotor activity rhythms, defined as the time of activity onset relative to the time of lights-on. To evaluate differences in the phase angle of entrainment (activity onsets and offsets) between different conditions (LD12/12h vs. Winter-like vs. Winter-like + Light), we compared the mean of the activity onsets and offsets per each experimental condition. Moreover, we determine the robustness of behavioral rhythms analyzing the amplitude of rhythm under each experimental condition using the fast Fourier transform, which estimates the relative power of 24hr period rhythms in comparison with other periodicities in the time-series (ClockLab software, Actimetrics, Evanston, IL, USA).

Biochemical analysis

Brains were recovered and frozen in methylbutane at -30°C , and stored at -80°C for posterior analysis. Samples from the dorsal striatum (caudate-putamen, CP) and NAcc were punched out from 1-mm thick brain slices under a cryostat using a 2.5 mm diameter aluminum tube and placed in Eppendorf tubes, stored at -80°C and analyzed within 4 weeks of collection. A mobile phase consisting of 50 mM Citric acid, 40 mM Na_2HPO_4 , 0.8 mM EDTA, 0.3 mM Octanesulfonic Acid and 1% Methanol, dissolved in ultrapure water $0.22\text{ }\mu\text{m}$ was filtered, degassed. Tissue samples were individually homogenized in 150 μL of mobile phase by sonication. Samples were kept at 4°C during preparation and before sampling via autosampler (Triathlon, Spark, Emmen, the Netherlands). DA and its metabolite 3,4-dihydroxyphenylacetic acid (DOPAC) were detected with a HPLC system using an amperometric electrochemical detector (Decade Antec, Leyden, the Netherlands). The electrochemical flowcell VT-03 was an Ag/AgCl (with saturated KCl) versus glassy carbon

electrode configuration. Potential between working and auxiliary electrode was set at 0.8V for detection. The temperature for the oven including cell detection and column was maintained at 30°C. The system featured a high pressure pump (LC-10AD, Shimadzu, Kyoto, Japan). Each 20 µl sample was injected on 750-mm length, 4.6-mm internal diameter, 3-µm C18 columns (Ultrasphere ODS, Beckman, Fullerton, CA, USA). Isocratic separation flow rate was kept at 0.5-ml/min. Every analysis lasted 60 min. DA and DOPAC (Sigma Aldrich) standard solutions were prepared in 10 mM HCl at 4°C and protected from light. Appropriate dilutions in the mobile phase were used to obtain a 3-point curve with good correlation rate for each standard. Retention times for our chromatographic conditions were 7.5 min for DA and 10.9 min for DOPAC. Values were calculated using Azur acquisition software v4.5 (Datalys, St Martin d'Hères, France). Results were given as pg/mg of total protein after protein dosage with the Bradford method. Furthermore, we analyzed the DOPAC/DA ratio commonly used as an index of DA turnover (Bagchi 1998).

In situ hybridization

After recovery of samples containing the dorsal striatum and NAcc for HPLC analysis, caudal part of brains was sectioned at the level of the SCN. Coronal 18 µm SCN slices were obtained with a cryostat. *In situ* hybridization of *Per2*, *Bmal1* and Vasopressin (*Avp*) mRNA expression was performed using riboprobes of *Per2* (kindly provided by Dr. H. Okamura, Kyoto University, Japan), *Bmal1* and *Avp* (kindly provided by Dr. H. Dardente, Tours University, France). Antisense RNA probes were generated with an *in vitro* transcription kit (Maxiscript; Ambion, Austin, TX, USA). Hybridization was carried out as described previously (Mendoza et al. 2012). Slices and radioactive standards were exposed to an autoradiographic film (Biomax MS-1 Kodak, Sigma-Aldrich, St Louis, MO, USA). Quantitative analysis of the autoradiograms was performed using the ImageJ software (W. Rasband, National Institutes of Health, Bethesda, MD, USA). The optical density of the whole SCN was measured and relative optical density was determined by subtracting the background intensity (measured in the anterior hypothalamic area) from the signal for each animal. Left and right SCN sided relative optical densities were averaged.

Statistical analysis

Data were compared using either one-way or two-way ANOVA for independent and repeated measures using SigmaPlot 13.0 software (Systat Software, Inc.). When significant main effects were obtained ($p < 0.05$), differences between groups at each condition were tested using either LSD Fisher post-hoc tests.

Results

Effects of daily-light exposure at the early or late day on daily rhythms of behavior of *Arvicanthis* exposed to Winter-like conditions

Arvicanthis exposed to control LD12/12h photoperiod display stable activity rhythms with locomotion predominantly during day time (Fig. 1A, 1C), and with an activity onset anticipating lights on (Figs. 1B and 1D, 1.23 ± 0.09 h before ZT0). Once exposed to a Winter-like photoperiod LD8/16h, *Arvicanthis* displayed an activity phase advance (Figs. 1A-D 2.08 ± 0.16 h before ZT0) characterized by earlier onset compared to the control LD12/12h condition (Fig. 1D, $F_{(2,35)}=12.81$; $p < 0.001$). This change in phase was also observed in activity offsets (Figs. 1A-D -0.47 ± 0.20 h after lights off, $F_{(2,35)}=12.5$; $p < 0.001$). No effects of Winter-like conditions on the amplitude of behavioral rhythms were observed (Fig. 1C, $F_{(2,35)}=1.05$; $p=0.3$).

Then, we evaluated the effects of 1h daily bright light exposure at the early day (ZT0) on daily rhythms behavior in animals exposed to Winter-like photoperiods. The phase advance induced by LD8/16h photoperiod was not delayed in animals exposed to 1h of light from ZT0 to ZT1 (Figs. 1A-D; Post-hoc $p=0.09$).

In *Arvicanthis* from the Winter-like + Light ZT7 group daily rhythms of locomotion under control LD12/12h photoperiod display stable and diurnal activity rhythms (Figs. 2A and 2C), and with an activity onset anticipating lights on (Figs. 2B and 2D, $1.46 \pm 0.1\text{h}$ before ZT0). Similarly to *Arvicanthis* from the previous group (Fig. 1), the exposure to a Winter-like photoperiod LD8/16h lead in an activity phase advance characterized by earlier onset (Figs. 2A-D, $2.01 \pm 0.12\text{h}$ before ZT0) and offset ($-0.40 \pm 0.24\text{h}$ after lights off) compared to the control LD12/12h condition (Fig. 2D, onsets, $F_{(2,35)}=2.92$; $p<0.001$; offsets, $F_{(2,35)}=23.21$; $p<0.001$). The amplitude of behavioral rhythms was not affected by the Winter-like conditions (Fig. 2C, $p=0.840$).

However, when Winter-like exposed *Arvicanthis* received daily light pulses at the late day (from ZT7 to ZT8), activity onsets and offsets were delayed and rescued in comparison to the Winter-like condition (Fig. 2A-D; *Post-hoc* $p<0.001$). Moreover, the amplitude of the daily locomotor activity rhythms was significantly increased in animals exposed to daily light at ZT7 (Fig. 2C; *Post-hoc* $p=0.003$).

Daily-light exposure effects on brain DAergic activity of Winter-like exposed animals

We asked whether a Winter-like photoperiod alters the day-night difference in the DA content and turnover as described by DOPAC/DA ratios measured by HPLC. For NAcc, a significant group effect for DA content ($F_{(3,37)}=12.58$; $p<0.001$) and its metabolite DOPAC ($F_{(3,37)}=5.59$; $p=0.004$) was revealed, showing higher levels of both molecules in Winter-exposed animals at night time (ZT19), in comparison to control LD12/12h *Arvicanthis* (Table 1). When we analyzed the DOPAC/DA ratio, the ANOVA indicates a significant effect for the time factor ($F_{(1,37)}=7.67$; $p=0.01$), showing a downregulation on DA turnover by the Winter-like condition (Fig. 3A). In the CP there was a significant difference for the group condition for both DA ($F_{(3,36)}=12.58$; $p=0.006$) and DOPAC content ($F_{(3,36)}=3.01$; $p=0.04$), with higher levels in the Winter-like group than in control LD12/12h animals at night (ZT19) (Table 1). The DOPAC/DA ratio in the CP showed a significant difference for the factor of time ($F_{(1,36)}=6.58$; $p=0.01$) revealing a day-time difference with significantly higher DA turnover at ZT19 than ZT7 for the control group, and with no day-night difference under Winter-like conditions (Fig. 3B).

The effects of light on DA metabolism showed significant changes on the content of DA and DOPAC in the NAcc at night time (ZT19) (*Post-hoc* $p<0.05$), in comparison to Winter-like *Arvicanthis* (Table 1). When we analyzed the DOPAC/DA ratio, the ANOVA indicates a significant effect for the time factor ($F_{(1,37)}=7.67$; $p=0.01$) showing a recovery of the day-night DA turnover by light at late day (ZT7; *Post-hoc* $p=0.005$), but not at early day (ZT0), despite the statistical analyses was at the limit of significance ($p=0.08$; Fig. 3A). In the CP there was also an effect of light on DA and DOPAC content, similarly to results in the NAcc (Table 1; *Post-hoc* $p<0.05$). The DOPAC/DA ratio in the CP showed a significant difference for the factor of time ($F_{(1,36)}=6.58$; $p=0.01$) revealing a day-night difference in control conditions (LD12/12h) but suppressed under Winter-like conditions, and which showed a tendency to be rescued in *Arvicanthis* receiving daily light stimulation at both ZT0 or ZT7, but the post-hoc analyses was not significant ($p=0.08$; Fig. 3B).

Daily-light exposure effects on clock gene expression in the SCN of Winter-like exposed animals

Day-night expression of *Per2*, but not *Bmal1*, gene in the SCN was down regulated by Winter-light condition in comparison to LD12/12h conditions (Figs. 4A and 4B; $F_{(3,29)}=4.2$; $p=0.017$). Day-night expression of *Avp*, a clock output gene, in the SCN was not affected in Winter-like animals in comparison to control LD12/12h *Arvicanthis* (Figs. 4A and 4B; $F_{(3,28)}=0.69$; $p=0.56$).

Then, we analyzed the effects of 1h daily bright light exposure at the early (ZT0) or late day (ZT7) on clock gene expression in the SCN of animals exposed to Winter-like photoperiods.

Day-night expression of the *Per2* gene in the SCN, down regulated in Winter-light exposed animals, was not significantly affected by light exposure at ZT0 or ZT7 ($F_{(3,29)}=0.98$; $p=0.41$) despite an increase of expression at daytime (ZT7, Figs. 4A and 4B). However, the ANOVA indicated a significant difference in the interaction of housing conditions (LD12/12h vs. Winter-like vs. Winter-like + Light) and time factors (day vs. night), in which the expression of *Per2* in the SCN at day was different between LD12/12h control animals, Winter-like and Winter-like + Light exposed animals at ZT0, but not at ZT7 (Fig. 4B; $F_{(3,29)}=4.2$; $p=0.017$). This effect might suggest a mild but significant rescue of *Per2* expression by light exposure at ZT7 in Winter-like exposed animals. Finally, the *Bmal1* and *Avp* day-night expression in the SCN was not affected by light exposure at ZT0 or ZT7 in Winter-like exposed *Arvicanthis* (Figs. 4A and 4B; *Bmal1*, $F_{(3,27)}=0.29$; $p=0.82$; *Avp*, $F_{(3,28)}=0.69$; $p=0.56$).

Discussion

In the present study we observed that when diurnal rodents *Arvicanthis ansorgei* are exposed to a Winter-like photoperiod, consisting of a LD8/16h cycle with low light levels (45 lux) during the day, animals show altered synchronization to the LD cycle, day-night brain DA content and clock gene expression in the SCN. Importantly, these effects in circadian behavior, DA content and SCN *Per2* gene expression were reverted when animals were treated with a daily light exposure late in the day. However, when light exposure was applied at the early day DA changes, but not the daily rhythm of locomotion, were rescued. These results indicate that daily light exposure can rescue altered behavior and physiology induced by a Winter-like photoperiod in a time-dependent manner.

In depression the daily activity rhythm is often disturbed (Raoux et al. 1994; Volkens et al. 2002). Moreover, the amount of activity is reduced in depressed patients, including those suffering from SAD (Raoux et al. 1994; Teicher et al. 1997). The phase-shift hypothesis in SAD accounts, patients show rest-activity cycles and other physiological rhythms (e.g. body temperature, melatonin) that are phase-delayed (Dahl et al. 1993; Teicher et al. 1997; Terman et al. 1988). Here we observed that *Arvicanthis ansorgei* show shifted locomotor activity rhythms when exposed to a Winter-like photoperiod, but the phase-shift found was an advance rather than a delay. This suggests that Winter-like photoperiod affects circadian rhythms of behavior by producing phase-shifts that are not stringent upon direction (advance or delay). Previously, other diurnal rodents showed similar changes in the phase angle of entrainment, desperate behavior (evaluated by FST) and sucrose consumption when exposed to short photoperiods (Ashkenazy-Frolinger et al. 2010; Deats et al. 2015; Krivisky et al. 2011; Leach et al. 2013b; Leach et al. 2013a). Importantly, in one study, *Arvicanthis niloticus* showed reduced saccharine preference and desperate behavior in the FST solely when exposed to dimmer day light intensity (50lux) despite unchanged day length (LD12/12h) (Leach et al. 2013a). SAD patients tend to exhibit hypersomnia, weight gain, increased appetite and evening carbohydrate craving (Berman et al. 1993; Lewy et al. 2006). Sucrose intake in *Arvicanthis ansorgei* increases when animals are exposed to the Winter-like photoperiod (data not shown) in agreement with eating attitudes in SAD patients.

Other central clinical features of SAD are low mood, lack of drive and concentration, and decreased interest, with reduced activity levels, which leads to attenuation of the amplitude of the daily rhythm of activity (Lam and Levitan 2000). Whereas desperate-behavior observed in previous studies using diurnal rodents clearly indicates the effects of photoperiod conditions in mood (Ashkenazy-Frolinger et al. 2010; Krivisky et al. 2011), more studies using other behavioral test or models are still necessary.

Alterations of clock gene expression have been implicated in the appearance of psychiatric pathologies including depression (Albrecht 2017). In humans, seasonal (winter) depression has been associated with the clock genes *Per2*, *Bmal1* and *Npas2* (Partonen et al. 2007). Here, we found that day-night expression of *Per2*, but not *Bmal1*, in the SCN is affected in

Arvicanthis exposed to Winter-like conditions. In mice showing depressive-like behaviors *Per2* expression is also decreased in the SCN (Logan et al. 2015). Nevertheless, *Per2* mutant mice are resistant to depressive-like behavior (evaluated in the FST) due to higher brain DA release (Hampp et al. 2008). However, because in *Arvicanthis* exposed to Winter-like conditions there was a significant phase-advance of daily rhythms it is possible that the down regulation at daytime is the consequence of a phase-shift rather than an inhibitory effect of photoperiod on *Per2* gene expression. Indeed, in mice with depressive-like behavior the daily rhythm of *Per2* expression in the NAcc and SCN is phase-shifted (Logan et al. 2015). It will be interesting to evaluate the daily profile of *Per2* gene expression in the SCN of Winter-like exposed *Arvicanthis*.

The monoaminergic hypothesis has implicated various neurotransmitter systems in the pathophysiology of depression, including the serotonergic system (5-HT) (Russo and Nestler 2013; Neumeister et al. 2001). Brain 5-HT concentrations and activity of the serotonin transporter (5-HTT) vary with seasons; In addition, 5-HTT turnover rate is affected in the forebrain of SAD patients (Willeit et al. 2008). These results are in line with findings in diurnal rodents on the effect of short-photoperiods in 5-HT neurons in the raphe nuclei (Leach et al. 2013b). Moreover, though Selective Serotonin Reuptake Inhibitors (SSRIs) have been used as first-line therapy for depression. One SSRI, escitalopram, has been found to suppress the mean firing rate of VTA DA neurons through enhanced activation of 5-HT_{2C} receptors likely on GABA neurons (Dremencov et al. 2009). This finding, with data on catecholamine changes in patients treated with bright light, yields new insight into the role of DA in SAD (Cawley et al. 2013). Here, we examined the DAergic content and turnover in two forebrain reward system structures, the CP and NAcc, in *Arvicanthis ansorgei* exposed to Winter-like photoperiods. DAergic afferents to both areas arise from the midbrain VTA to modulate motor coordination, reward, motivation and emotion-related behaviors (Berridge and Kringelbach 2013). These and other reward system structures possess circadian activity in several forms (Mendoza and Challet 2014). Here, higher DA turnover (DOPAC/DA ratio) was found at night (ZT19) than day, a day-night difference that was blunted in Winter-like conditions. Since the DOPAC/DA ratio reflects DA activity, the reduction of this ratio in Winter-like animals indicates a decrease in DA metabolism. However, due to the rhythmic activity of DAergic system (Mendoza and Challet 2014), it is possible that the reduced levels of the DOPAC/DA ratio in animals exposed to Winter-like conditions reflects a phase-change of DA turnover induced by the photoperiodic conditions. Further experiments using *in vivo* microdialysis to measure DA release could give important information about the effects of Winter-like conditions in DAergic activity in *Arvicanthis ansorgei*.

Similarly, in *Arvicanthis niloticus*, the number of hypothalamic DA neurons of animals housed in short photoperiod is downregulated (Deats et al. 2015). Long term light deprivation in rats has been found to damage the monoaminergic system, including DA, 5-HT and NA, and induce depression-like behavior (Gonzalez and Aston-Jones 2008). All together, these findings suggest the DA system can be affected by changes in the daytime length and light intensity (Abilio et al. 1999). In accordance with animal data, several studies reported changes in the DAergic activity secondary to SAD or even due to seasons in healthy people (Eisenberg et al. 2010; Hartikainen et al. 1991). All together, these studies indicate that lighting conditions affect the DAergic system.

Biological timing is strongly affected in SAD and light is the most powerful synchronizer of the biological clock in the SCN, suggesting why light therapy has become as one of the most prolific treatments for SAD. The beneficial effect of bright light in SAD has been observed in the phase and levels of locomotor activity and physiology in patients (Burgess et al. 2004; LeGates et al. 2014; Wirz-Justice et al. 1993). Daily light exposure in *Arvicanthis*, either at the early day (ZT0) or late day (ZT7), has a tendency to reduce sucrose intake (data not

shown), holding up the predictive validity of light effects in behavior. To note, light has been proposed to treat night eating disorders in which people reduce food intake at night, mood and sleep disturbances when treated with bright light (McCune and Lundgren 2015). However, in the present study the efficacy of light to delay the onset of locomotor activity and to restore the phase of the behavioral rhythm was significant only in animals exposed to light at the late day (ZT7).

In humans light therapy is imposed mainly at the early morning (Lewy et al. 2006). The phase response curve (PRC) to light in humans using a single light pulse, and using the midpoint of the melatonin rhythm as a phase marker, indicates that light causes phase delays when it occurs in the late night, and phase advances in the early morning (St Hilaire et al. 2012; Ruger et al. 2013). In SAD light at the early morning is the recommended treatment since its antidepressant effects which corrects the delayed phase of circadian rhythms caused by winter conditions (Lewy et al. 1998).

In *Arvicanthis ansorgei* phase delays occur when light exposure is applied at the beginning of the night, whereas light exposure at the early day leads in phase advances of behavioral rhythms (Caldelas et al. 2003). Thus, in the present study, the phase-advanced behavioral rhythm in *Arvicanthis* under a Winter-like condition is delayed when daily light occurred at the late, but not early day. Moreover, day-night *Per2* gene expression in the SCN was affected in animals exposed to light at ZT7. *Per2* expression in the SCN of *Arvicanthis ansorgei* rises at day time in animals exposed to a 12/12 LD cycle (Caldelas et al. 2003). Moreover, light exposure at the early subjective night induces *Per2* expression in the SCN of *Arvicanthis* (Caldelas et al. 2003). Here, in *Arvicanthis* exposed to 1h light pulses at ZT7 induced a slight, but significant, rescue of the day-night expression of *Per2* in the SCN of *Arvicanthis* under Winter-like conditions accompanying the resetting (phase delays) of the rhythm of locomotor activity. Interestingly, in humans phase is not relevant for the beneficial effects of light in mood (Wirz-Justice et al. 1993). Hence, even if light at the early morning (ZT0) does not restore the phase of locomotor activity in *Arvicanthis*, mood-related behavior could be affected by light independently of the time of exposure. This suggests that rhythm alterations (phase change or amplitude) induced by Winter-like exposure might not be totally part of the pathology in SAD. In *Arvicanthis*, the exposure to daily-light at ZT0 induces an increase of locomotor activity. Exercise or physical activity have reward and antidepressant properties affecting the DAergic system (Brene et al. 2007). Therefore, despite no effects in phase resetting by light at ZT0, the improved DA tone in animals might depend on the locomotor activity induced by light. Importantly, it has been reported that both light and physical activity in the day reduce depressive symptoms in patients with winter depression and non-seasonal depression (Pinchasov et al. 2000). Together, these features make *Arvicanthis* an adequate diurnal animal model to study the light impact in physiology and behavior, circumventing the light aversion found in traditional nocturnal rodent models. Despite its efficacy, the neural mechanism by which light ameliorates mood remains to be clarified.

Light, beyond the SCN clock, also targets other brain structures implicated in mood and depression. For instance, the effects of light exposure on DAergic structures has been shown both in humans (Diehl et al. 1994) and animals (Abilio et al. 1999). Moreover, an experiment in which DA synthesis was reduced in women with mild seasonal mood changes affects mood and reversed with bright light therapy, suggested that DA changes induced by light may be partly necessary for the beneficial effects of light in mood (Cawley et al. 2013).

In the present experiments, daily light exposure resolved DA turnover disturbances elicited by Winter-like conditions as expected; it increased the DOPAC/DA ratio in the NAcc and CP which suggests an increased DA metabolism and release. DA tone is implicated in a variety of circadian rhythms such as feeding, sleep-wake, locomotion and motivation, which are

disrupted in SAD (Lam and Levitan, 2000; Lewy et al. 2006). Thus, light effects on mood might be dependent on regulating mechanisms on the DA activity (Neumeister et al. 2001). Current models of mood regulation by light account for two main pathways, an indirect one mediated by circadian rhythms and a direct route that does not disrupt sleep or cause circadian changes (LeGates et al. 2014). The circadian clock is connected to the reward system via several circuits, eg., the SCN is connected to the VTA via the medial preoptic nucleus of the hypothalamus or the lateral hypothalamic orexinergic system, and to the striatum via the paraventricular thalamic nuclei (Luo and Aston-Jones 2009; Moga et al. 1995; Moorman and Aston-Jones 2010). And very recently, a study demonstrated the existence of a direct functional projection from the DAergic VTA to the SCN clock (Grippo et al. 2017). A previous work revealed that light exposure in early subjective day increases c-Fos expression in orexin neurons in *Arvicanthis niloticus* (Adidharma et al. 2012), suggesting a role of orexin in mediating the effects of light on mood. In the same study, however, c-Fos expression in the SCN was not induced by light exposure, indicating light modulation by SCN-independent pathways.

The lateral habenula (LHb) in the mid-posterior thalamus could account for direct effects of light on mood (Lazzerini Ospri et al. 2017). It receives projections from retinal ganglion cells and is involved in reward processing for relevant control of monoaminergic system in the VTA (for DA) and raphe nuclei (for 5-HT) (Hattar et al. 2006; Baker and Mizumori 2017). Moreover, the LHb possess oscillating properties which coordinate with the SCN to control circadian rest/activity rhythms (Mendoza 2017; Salaberry and Mendoza 2015), and short brief light pulses at the early night in *Arvicanthis niloticus*, but not in mice, increase c-Fos expression in the LHb (Shuboni et al. 2015). As intermediary structures it is necessary to elucidate the respective contribution of the direct or indirect pathway of the modulation of mood by light. Nonetheless, this diurnal animal model might provide adequate means to elucidate this matter.

In conclusion, this study has brought light to the potential of the diurnal *Arvicanthis ansorgei* to reveal the effects of light in behavior and brain physiology (i.e., DAergic system).

Several questions, regarding particular manifestations of SAD and its treatment by light, demand further probing. Their elucidation will overflow into the understanding of the neurobiological and circadian mechanisms of light effects in behavior (e.g., mood, eating disorders).

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: All procedures performed in studies involving animals were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23, revised 1985) and the French Department of Agriculture (License no. 67-378 to J.M.).

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Table 1. Day-night (ZT7 vs. ZT19) dopaminergic changes in the dorsal striatum (CP) and nucleus accumbens (NAcc) of animals under control (LD12/12h), Winter-like (LD8/16h) and Winter-like + Light conditions, with light exposure at ZT0 (early day) or ZT7 (late day). Values represent the mean values $\pm$ SEM.

	Control (LD12/12h)	Winter-like (LD8/16h)	Winter-like + Light (ZT0)	Winter-like + Light (ZT7)
NAcc	<i>Day/Night</i>	<i>Day/Night</i>	<i>Day/Night</i>	<i>Day/Night</i>
Dopamine (pg/mg)	17.8 $\pm$ 4.9 ^a 10.7 $\pm$ 1.9 ^a	67.6 $\pm$ 14 ^b 99.6 $\pm$ 39.1 ^b	36.5 $\pm$ 6.8 ^a 15.9 $\pm$ 3.8 ^a	76.2 $\pm$ 6.9 ^b 27.6 $\pm$ 9.1 ^{a*}
DOPAC (pg/mg)	26 $\pm$ 3.6 ^a 33.6 $\pm$ 9.8 ^a	52.1 $\pm$ 2.4 ^a 57.7 $\pm$ 8.8 ^b	27.9 $\pm$ 5.3 ^a 28.4 $\pm$ 7.8 ^a	43.7 $\pm$ 5 ^a 46.1 $\pm$ 7.8 ^b
CP	<i>Day/Night</i>	<i>Day/Night</i>	<i>Day/Night</i>	<i>Day/Night</i>
Dopamine (pg/mg)	59.3 $\pm$ 12 ^a 20.9 $\pm$ 3.5 ^a	112.3 $\pm$ 11.6 ^a 190.5 $\pm$ 42.5 ^b	66.2 $\pm$ 12.5 ^a 38.8 $\pm$ 15.6 ^a	93.3 $\pm$ 16 ^a 73.2 $\pm$ 46.2 ^a
DOPAC (pg/mg)	37.4 $\pm$ 11.6 ^a 35.9 $\pm$ 8.8 ^a	48.8 $\pm$ 7.1 ^a 71.3 $\pm$ 15 ^b	29.9 $\pm$ 4.7 ^a 30.3 $\pm$ 11.4 ^a	40.1 $\pm$ 5.2 ^a 37.6 $\pm$ 8.1 ^a
Means lacking common letters are significantly different (between groups post hoc test, $P < 0.05$).				
* $P < 0.05$, between ZT's				

Figure 1. Behavioral rhythms of *Arvicanthis ansorgei* exposed to Winter-like photoperiod and exposed to daily light at the early day (ZT0). **(A)** Representative actograms of the rhythm of locomotor activity of animals during the whole experiment. *Arvicanthis* kept under LD12/12h conditions (12 hours of light at 150 lux and 12 hours of darkness, < 5 lux [gray shaded area of graph]) were later switched to Winter-like LD8/16h photoperiod (8 hours of light at 45 lux, and 16 hours of darkness, <5 lux), and finally animals were exposed to 1h daily light pulses at lights on from ZT0 to ZT1 (vertical yellow bar in actograms). **(B)** Activity onsets averaged across each day during the whole experiment; LD12/12h, Winter-like condition (dotted line) and Winter-like + Light (shaded yellow area). **(C) Left:** Daily activity profiles of animals during different experimental conditions (LD12/12h vs. Winter-like vs. Winter-like + Light). Top bars represent the LD cycle in control conditions (black and white bars) and during Winter-like (black and grey bars) and Winter-like + Light exposure (yellow bar). **Right:** Amplitude of locomotor activity rhythms during different experimental conditions. **(D)** Daily average onsets (hours before lights on) and offsets (hours after lights off) of activity showing a phase-advance under Winter-like photoperiodic conditions which was not affected by daily light exposure at ZT0. Data are shown as the mean $\pm$ SEM. Means lacking common letters are significantly different (between groups LSD post hoc test, $p < 0.05$).

Figure 2. Behavioral rhythms of *Arvicanthis ansorgei* exposed to Winter-like photoperiod and exposed to daily light at the late day (ZT7). **(A)** Representative actograms of the rhythm of locomotor activity of animals during the whole experiment. *Arvicanthis* kept under LD12/12h conditions (12 hours of light at 150 lux and 12 hours of darkness, <5 lux [gray shaded area of graph]) then to Winter-like LD8/16h photoperiod (8 hours of light at 45 lux, and 16 hours of darkness, <5 lux), and exposed to 1h daily light pulses right before lights off, from ZT7 to ZT8 (vertical yellow bars). **(B)** Activity onsets averaged across each day during the whole experiment; LD12/12h, Winter-like condition (dotted line) and Winter-like + Light (shaded yellow area). **(C) Left:** Daily activity profiles of animals during different experimental conditions (LD12/12h vs. Winter-like vs. Winter-like + Light). Top bars represent the LD cycle in control conditions (black and white bars) and during Winter-like (black and grey bars) and Winter-like + Light exposure (yellow bar). **Right:** Amplitude of locomotor activity rhythms during different experimental conditions. **(D)** Daily average onsets (hours before lights on) and offsets (hours after lights off) of activity showing a phase-advance under Winter-like photoperiodic conditions in comparison to the LD12/12h control condition. Light exposure at ZT7 delays significantly activity onsets and offsets of locomotor activity rhythms. Data are shown as the mean $\pm$ SEM. Means lacking common letters are significantly different (between groups LSD post hoc test, $p < 0.05$).

Figure 3. Effects of Winter-like photoperiod and light exposure on brain dopamine (DA). DA turnover (ratio of metabolite 3,4-dihydroxyphenylacetic acid DOPAC/DA) in brain tissue samples of Nucleus Accumbens (NAcc) (A) and Dorsal Striatum (Caudate putamen, CP) (B). Samples were obtained at Day (Zeitgeber Time, ZT7) and night (ZT19). There is a loss of the day-night variations of DA turnover when animals are exposed to Winter-like photoperiods compared to control LD12/12h conditions in both NAcc and CP. Light exposure at early day (ZT0) or late day (ZT7) increase DA turnover at night rescuing rhythmic brain DA in the NAcc although at ZT0 the *p* value was not significant (*p*=0.08). In the CP there was also a tendency to increase DA turnover at night by light, but the post-hoc analyses was not significant (*p*=0.08). Data are shown as the mean $\pm$ SEM. **p*<0.05 (ZT7 vs. ZT19) (LSD Fisher post hoc comparison).

Figure 4. Daily-light effects on the day-night expression of clock genes in the SCN of Winter-like exposed animals. **(A)** Representative images of *in situ* hybridization and **(B)** day-night expression of *Per2*, *Bmal1* and *Avp* in the SCN of *Arvicanthis* under different experimental conditions. Data are means $\pm$ SEM. * $p < 0.05$ LSD Fisher post hoc comparison; *Per2* expression at ZT7 from LD12/12h group is significant different to the Winter-like and Winter-like + Light ZT0 group.