Direct sunlight exposure reduces hair cortisol levels in rhesus monkeys (*Macaca mulatta*)

DEAR EDITOR,

Major depressive disorder (MDD), commonly known as depression, is a mental disease characterized by a core symptom of low mood. It lasts at least two weeks (Badamasi et al., 2019; Wang et al., 2019) and is frequently accompanied by low self-esteem, loss of interest in routinely enjoyable activities, low energy, and unexplained pain (Huey et al., 2018; Park et al., 2012; Post & Warden, 2018; Rice et al., 2019; Xiao et al., 2018). Approximately 2%−8% of adults with MDD commit suicide (Richards & O'Hara, 2014; Strakowski & Nelson, 2015), and around half of suicidal individuals suffer depression or other mood disorders (Bachmann, 2018).

According to the criteria of the American Psychiatric Association's Diagnostic & Statistical Manual, Fourth Edition (DSM-IV), seasonal affective disorder (SAD) is a type of depression (Wirz-Justice, 2018) and can occur with major depressive episodes (Birtwistle & Martin, 1999). SAD sufferers exhibit various associated symptoms, such as feelings of hopelessness and worthlessness, suicidal thoughts, loss of interest in activities, and withdrawal from social interactions (Austen & Wilson, 2001; Eagles, 2004). SAD is typically associated with winter depression, and symptoms normally improve upon the arrival of summer (Austen & Wilson, 2001). Seasonal mood variations are also believed to be associated with light. Specifically, SAD is reported to be correlated with duration of sunlight exposure (Lam et al., 2006). In spring and summer, sunlight exposure is longer and daytime hours exceed nighttime hours. In winter, sunlight exposure becomes shorter and evenings arrive earlier. Thus, SAD is noticeably more frequent at latitudes in the Arctic region, including Alaska (N64°00′) where the rate of SAD is as high as 9.2% (Booker & Hellekson, 1992; Kegel et al., 2009). Cloud coverage may contribute to the negative effects of SAD (Modell et al., 2005).

There is growing evidence that many SAD patients exhibit delays in circadian rhythm and that bright light therapy may improve symptoms by correcting these delays (Avery et al., 2001a; Lam & Levitan, 2000).

Although bright light is known to be an effective therapy for depression (Avery et al., 2001b; Howland, 2009), the underlying mechanism has remained elusive. Etiological hypotheses have focused on alterations in biological cycles due to seasonal light variations. For example, previous research on starry flounder showed that prolonged light exposure was associated with a reduction in melatonin secretion (Bögner et al., 2018). Melatonin plays a vital role in the regulation of biological rhythms (Ardura et al., 2003; Emet et al., 2016). As a result, the quality of sleep in SAD sufferers declines and they are more likely to get nervous and stressed. Although light has been shown to relieve depression by impacting the circadian rhythms of cortisol secretion (Avery et al., 1997; Koller et al., 1994; Rutten et al., 2019), other studies have failed to detect any correlation between light and cortisol rhythm (Bögner et al., 2018; Schmidt et al., 2018). Thus, these conflicting results indicate that other aspects of cortisol variation may be involved.

The cumulative level of cortisol is considered to be an important biochemical factor in the pathogenesis and treatment of depression (Caparros-Gonzalez et al., 2017; Keller et al., 2017). The rate of depression is higher among patients with hypercortisolism (Maripuu et al., 2014), and the cumulative level of cortisol is abnormal in 40%−60% of depression sufferers (Booij et al., 2015). More importantly, most anti-depressants work through their effects on cortisol receptors and the alteration in cumulative levels of cortisol (Carvalho & Pariante, 2008; Holsboer, 2000; Mason & Pariante, 2006). Based on these assumptions, light therapy may improve depression by altering the cumulative levels of cortisol, with levels possibly correlated with light duration. Thus, in the current study, we investigated the correlation

between light duration and cumulative levels of hair cortisol in rhesus macaques (*Macaca mulatta*).

As the final product of the hypothalamic-pituitary-adrenal (HPA) axis, cortisol is an important stress hormone and can reflect and regulate stress system function. Compared with other analytical methods, including serum and saliva cortisol, hair cortisol levels are not affected by circadian rhythm, food intake, or acute stress (Li et al., 2009; Yamada et al., 2007). Furthermore, the cortisol in hair is freely combined and thus biologically active and relatively stable. It can reflect cumulative cortisol levels in an organism over a 3–5-month period and is thus suitable for measuring the long-term cumulative levels of cortisol (Davenport et al., 2006; Kalra et al., 2007).

To examine the effects of sunlight exposure duration on HPA axis function, hair samples were collected twice in 7-year-old monkeys. The position of monkeys was recorded on the second day after hair sampling to exclude potential interference from sampling and to investigate the duration of sunlight exposure. A total of 20 and 22 age-matched adult male rhesus macaques were assigned to a short sunlight exposure group (n=8, 4, 4, 4 within each cage) and long sunlight exposure group (n=7, 4, 4, 4, 3 within each cage), respectively. All animals resided within their groups with fixed counterparts for more than six months and their living environments were deemed stable. This study was approved by the Biological Research Ethics Committee of the Kunming Institute of Zoology, Chinese Academy of Sciences. All animal procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The adult males lived with their cage mates either in small (n≤4) or large social groups (n=7). They were raised in indoor (2.61 m×2.46 m×2.58 m) and outdoor (2.67 m×2.66 m×2.67 m) colonies. Each group of rhesus macaques was able to enter and exit the cage at will, i.e., they could freely move between indoor and outdoor colonies. Monkeys in the outdoor colonies were exposed to sunlight during the daytime. Commercial monkey biscuits were provided twice daily with tap water *ad libitum*, and fruit and vegetables were offered once daily.

The cages of the short sunlight exposure group were oriented eastwards, whereas the cages of the long sunlight exposure group were oriented southwards, thus the durations of sunlight exposure differed between the two groups. The east- and south-oriented cages received direct sunlight simultaneously. At sunset, the east-oriented cages were cast under a large shadow, whereas the south-oriented cages continued to have direct sunlight for an additional hour. As a result, east-oriented cage mates represented the short sunlight exposure group, whereas the south-oriented cage mates represented the long sunlight exposure group.

The monkeys were approximately seven years old (7± 1 years). Hair samples were collected between 1330h and 1500h. Each animal was captured by an experienced technician using a net and subsequently taken out of the colony for hair sampling. After manual restraint, the back hair

was clipped with scissors and then stored in a small pouch of aluminum foil. The hair cortisol extraction procedure was based on our previous study (Feng et al., 2011). For removing surface contaminants, hair samples were rinsed twice in 5 mL of isopropanol (3 min each), dried at 35 °C for 8 h, and then pulverized using a Retsch ball mill (Retsch MM400, Germany) at 26 Hz for 2.5 min. Approximately 200 mg of powdered hair was weighed and incubated at a slow rotation for 24 h in 4 mL of methanol at room temperature. After centrifugation at 8 000 g for 5 min at 4 °C, 2 mL of supernatant was transferred into a centrifuge tube and dried under a stream of nitrogen gas. Finally, the extract was reconstituted with 0.25 mL of phosphate buffer solution (PBS) and assayed for cortisol levels using a commercial kit (CORTISOL RIA KIT REF IM1841, Beckman, USA).

Dedicated observers were stationed in front of the outdoor cages to record the position of the caged monkeys and to calculate the duration of direct sunlight exposure. Observations were conducted for 2 h daily, including 1 h in the morning and 1 h in the afternoon. If a monkey spent time in the outdoor colony, then it was considered to have received direct sunlight. In contrast, those in the indoor colony received no direct sunlight. To avoid disturbing the animals, observers stayed at least 5 m away from the cage during recording. Specific measurement procedures were followed. Using Monkey-A as an example, when it stayed in an outdoor colony, the precise timepoint was recorded (e.g., 1010h). If it moved to an indoor colony, the precise timepoint was also recorded (e. g., 1015h). The interval between the two timepoints was marked (e.g., 5 min) as one episodic event of direct sunlight exposure. Multiple episodes of locomotion occurred within the 1 h of recording. All recorded durations of direct sunlight exposure in the morning were added for total morning duration of direct sunlight exposure (e.g., 5+7+3+2+ 17=34 min). Afternoon durations of direct sunlight exposure were collected similarly. The total duration of direct sunlight exposure was calculated by summing all durations.

The long sunlight exposed monkeys in the south-oriented cages had significantly lower cumulative levels of cortisol than the short sunlight exposed counterparts in the east-oriented cages (Mann-Whitney test, n=42, U=123, P=0.014) (Figure 1A). After removing two outliers, the same pattern was still found (unpaired t-test, n=40, t=2.388, P=0.022) (Figure 1B).

Results showed that the long sunlight exposed monkeys had significantly lower cumulative levels of cortisol than their short sunlight exposed counterparts. To the best of our knowledge, this is the first study demonstrating that the effects of sunlight exposure on the cumulative cortisol levels in monkeys are stable and reproducible. Furthermore, this study has pioneered the use of hair analysis to prove that longer sunlight exposure can lower the level of freely combined, biologically active cortisol in rhesus monkeys. Thus, stable-living rhesus monkeys could serve as an excellent animal model for examining sunlight exposure in humans and elucidating the mechanism of SAD for more effective interventions. Our results agree with previous findings on the effects of indoor artificial light on monkeys (Qin et al., 2015).
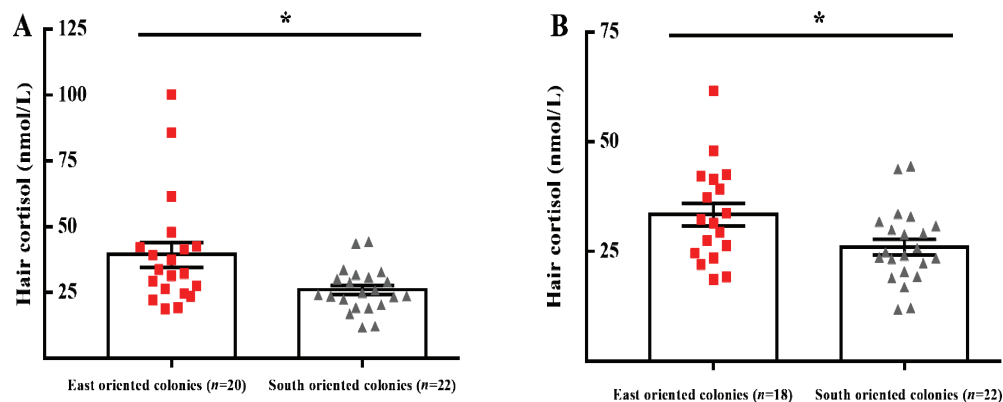
Figure 1 Comparison of cumulative hair cortisol levels in long and short sunlight exposed monkeys
A: Specific concentrations of hair cortisol in each monkey in short (east-oriented cages, n=20, red squares) and long sunlight exposed monkeys (south-oriented cages, n=22, gray triangles). Data are means±*SEM* (standard error of the mean). *: P<0.05. B: Specific concentrations of hair cortisol in long (n=22) and short sunlight exposed monkeys (n=18) after removing two outliers.

Sunlight has a relatively wide spectrum, and thus the impact of direct natural sunlight exposure is more enduring and stable. Although other research has shown the opposite results (Tian et al., 2019), we speculate that different subjects or species and methods of cortisol extraction may account for these inconsistencies.

One possible reason for the long-lasting hypercortisolism in the short sunlight exposed monkeys could be excessive activation of the HPA axis induced by short duration of direct sunlight exposure, a common stressor for daily living. The development of psychiatric disorders such as SAD may include an onset of hypercortisolism phase due to a lack of sunlight exposure. In contrast, due to the lack of a short sunlight exposure stressor, the long sunlight exposed monkeys exhibited a lower level of hair cortisol. It could be speculated that a lower level of cumulative cortisol may have a positive effect on SAD patients.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

X. L. F., X. T. H., X. F. R., and H. L. C. designed the study. Y. W. and Z. F. H. captured the animals and helped collect samples. X.N. and X.Y.B. collected hair samples. J.L. and J.F.Z. recording the position of caged monkeys. X.F. R. extracted and assayed cortisol from hair samples. H. L.C calculated the duration of direct sunlight exposure. X.F.R. and H.L.C. analyzed the data. X. F. R. wrote the manuscript with input from all other authors. X. L. F., X. T. H., and H.L.C. revised the manuscript. All authors read and approved the final version of the manuscript.

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