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Short communication

Serotonin suppresses food anticipatory activity and synchronizes the food-entrainable oscillator during time-restricted feeding

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HIGHLIGHTS

- Depletion of serotonin under LD leads to increased activity.
- Under LD and RF conditions, serotonin suppresses the FEO and leads to reduced FAA.
- In DD, serotonin depletion leads to high activity with free running of the FAA.
- Serotonin leads to SCN-mediated activity inhibition and FEO entrainment/activity.

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ABSTRACT

The serotonergic and circadian systems are intertwined as serotonin modulates the response of the central brain suprachiasmatic nuclei (SCN) clock to light. Time-restricted feeding (RF) is characterized by increased food anticipatory activity (FAA) and controlled by the food-entrainable oscillator (FEO) rather than the SCN. Our objective was to test whether serotonin affects the FEO. Mice were treated with the selective serotonin reuptake inhibitor (SSRI) fluvoxamine (FLX) or the tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA) and locomotor activity under *ad libitum* feeding, RF and different lighting conditions was monitored. Under AL, FLX administration did not affect 24-h locomotor activity, while mice treated with PCPA exhibited increased activity. RF-FLX-treated mice showed less FAA 2 h before food availability (ZT2–ZT4) compared to RF- or RF-PCPA-fed mice. Under DD, RF-PCPA-treated mice displayed increased activity, as was seen under LD conditions. Surprisingly, RF-PCPA-treated mice showed free running in the FAA component. These results emphasize the role of serotonin in SCN-mediated activity inhibition and FEO entrainment and activity.

1. Introduction

Mammals have developed an endogenous circadian clock located in the brain suprachiasmatic nuclei (SCN) of the anterior hypothalamus that responds to the environmental light–dark cycle. The SCN receives light information from the retina and transmits synchronization cues *via* neuronal connections or circulating humoral factors to peripheral clocks, such as the liver, heart and lungs, regulating cellular and physiological functions [1].

Serotonin is a monoaminergic neurotransmitter synthesized initially by tryptophan hydroxylase (TPH) in the brain raphe nuclei with activities that modulate central functions, such as food intake, sleep, anxiety, sexual behavior and mood [2]. The serotonergic and circadian systems are intertwined, as the SCN receives direct serotonergic innervation from the median raphe nuclei, and, in turn, makes polysynaptic output back to these mid-brain serotonergic nuclei [3]. It has been shown that depletion of brain serotonin by 5,7-dihydroxytryptamine (DHT) modifies hamster circadian rhythms and the response to light [4,5]. SCN neurons express several serotonin receptors [6]. Upon binding, serotonin modulates the response of the circadian system to light as well as the response to non-photic stimuli [7,8].

Restricted feeding allows food to be available *ad libitum* at the same time every day for only a few hours [9,10]. Animals adjust to the feeding schedule within a few days and present food anticipatory activity (FAA) before food introduction every

Abbreviations: AL, *ad libitum*; DD, dark–dark conditions; FAA, food anticipatory activity; FEO, food-entraiable oscillator; FLX, fluvoxamine; LD, light–dark conditions; PCPA, parachlorophenylalanine; RF, restricted feeding; SCN, suprachiasmatic nuclei; SSRI, selective serotonin reuptake inhibitor; TPH, tryptophan hydroxylase.

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day [11,12]. Generally, RF affects circadian rhythms in peripheral tissues without affecting the SCN pacemaker [13,14], causing uncoupling between the central clock and the periphery. RF drives rhythms regardless of the lighting conditions and even in arrhythmic and clock mutant mice and animals with lesioned SCN [15,16]. Although lesions in various brain regions have been performed to locate this food-entrainable oscillator (FEO), its exact location is still unknown [17]. Currently, a limited number of studies have investigated serotonergic regulation of the FEO. We tested whether the serotonin selective inhibitor (SSRI) fluvoxamine (FLX) and the tryptophan hydroxylase inhibitor parachlorophenylalanine (PCPA) affect the FEO by monitoring mouse locomotor activity under *ad libitum* feeding, RF and different lighting condition.

2. Materials and methods

2.1. Animals and experimental design

Eight-week-old C57BL/6 male mice (Harlan Laboratories, Jerusalem, Israel) were housed in a temperature- and humidity-controlled facility (23–24 °C, 60% humidity). Mice were entrained to 12 h light and 12 h darkness (LD) for 10 days with food available *ad libitum*. After 10 days, mice were fed *ad libitum* or time-restricted feeding (RF) and each group was divided into 3 subgroups that were treated with water (control), fluvoxamine (FLX, Cayman chemical company, Ann Arbor, MI, USA) (9 mg/kg/day) or parachlorophenylalanine (PCPA, Sigma, Rehovot, Israel) (300 mg/kg/day) *ad libitum* for 3 weeks. The RF group was given food between *zeitgeber* time (ZT) 4 and ZT8 (ZT0 is the time of lights on). After 3 weeks, lights were turned off and mice were kept under total darkness (DD) for 10 days with the treatments. In another experiment, after 10 days of entrainment to LD, mice were fed RF with three different treatments water (control), FLX (9 mg/kg/day) or PCPA (300 mg/kg/day) for 2 weeks under DD conditions. Daily food intake and body weight were monitored once weekly throughout the experiment. FLX and PCPA were provided in the drinking water.

2.2. Animal locomotor activity

General cage activity was monitored continuously at 6-min intervals using a custom-made system composed of infrared detectors placed above each cage. Cage locomotor activity was recorded continuously under LD conditions, after which mice were released into DD and activity recording was continued. Double-plotted actograms were generated using Actogram software (kindly provided by Roberto Refinetti, Circadian Rhythm Laboratory, University of South Carolina. Walterboro, SC, USA). Period lengths of circadian activity rhythms in DD (*tau*) were calculated individually by χ^2 analyses using Tau software (kindly provided by Roberto Refinetti). Onset of activity was calculated by ChronoShop v1.02 software (kindly provided by Roelof A. Hut, Chronobiology Unit, University of Groningen, The Netherlands). Regression lines representing free running activity were calculated using the slope of the onset of activity every day.

2.3. Statistical analyses

All results are expressed as means ± SE. For comparison between control and fluvoxamine-treated mice or control and PCPA-treated mice, t-test was used. Tukey's honestly significant difference (HSD) was performed as a single-step multiple comparison procedure for the evaluation of significant differences among groups. For all analyses, the significance level was set at $p < 0.05$. Statistical analysis was performed with JMP (version 7) software (SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

3.1. Effect of serotonin on drinking

Water consumption was measured and was found to be not significantly different among the *ad libitum* groups ($p \geq 0.05$, Tukey's HSD). Under AL, mice consumed 0.21 ± 0.01 mg/day fluvoxamine (FLX, an SSRI) and 7.92 ± 0.70 mg/day parachlorophenylalanine (PCPA, a TPH inhibitor). Water consumption was significantly higher ($p \leq 0.05$, Tukey's HSD) in the RF group compared to the RF groups treated with FLX and PCPA. Under RF, mice consumed 0.27 ± 0.004 mg/day FLX and 10.27 ± 0.85 mg/day PCPA. PCPA dosage was not significantly different between the AL and RF groups. FLX dosage was slightly (0.21 *vs.* 0.27), but significantly ($p \leq 0.05$, Tukey's HSD) higher in the RF group. Thus, the only group that had significantly higher water intake was the RF group and the FLX and PCPA treatments were comparable within each treatment.

3.2. Effect of serotonin on locomotor activity under LD

To establish the connection between serotonin and the food-entrainable oscillator (FEO), we checked the activity of mice under *ad libitum* (AL) or 4-h time-restricted feeding (RF) each with 3 different treatments: water (control), FLX, and PCPA (Fig. 1A). Under AL, FLX administration did not affect 24-h locomotor activity, while mice treated with PCPA exhibited increased locomotor activity compared to the control and FLX groups ($p < 0.05$, Tukey's HSD) (Fig. 1A and B). As expected, locomotor activity was changed in the timed groups (RF, RF-FLX and RF-PCPA), i.e., before the time of food availability, mice displayed food anticipatory activity (FAA) (Fig. 1A and C). It is noteworthy that RF-FLX-treated mice had reduced locomotor activity during the light phase compared to RF- or RF-PCPA-treated mice ($p < 0.05$, Tukey's HSD), whereas RF-PCPA-treated mice exhibited the highest total locomotor activity ($p < 0.05$, Tukey's HSD) (Fig. 1D). RF-FLX-treated mice showed less FAA 2 h before food availability (ZT2–ZT4) compared to RF- or RF-PCPA-fed mice ($p < 0.05$, Tukey's HSD) (Fig. 1E). When switched to DD and *ad libitum* feeding with FLX or PCPA treatment, groups were not significantly different in their endogenous period (data not shown). These results are congruent with previous studies that showed that PCPA treatment leads to hyperactivity [18]. In parallel, FLX treatment led to decreased FAA under LD conditions. It was reported that repeated treatment with fluvoxamine attenuated the hyperactivity, which is exclusively dependent on the substantial reduction in the post-feeding hyperactivity, but not FAA [19]. However, our results are supported by other studies that showed that 5-HT2R antagonists and 5-HT2CR knockout led to increased FAA [20,21]. In addition, fluoxetine, an SSRI, reduced FAA in the 'activity-based anorexia' model [22], in which rodents subjected to restricted food access and housed with running wheels develop paradoxical hyperactivity, hypophagia, and extreme weight loss resulting in death [23]. Although PCPA treatment led to increased FAA compared to FLX treatment, its effect was not significantly different from that of RF under LD. These results suggest that the RF regimen might lead to reduced levels of serotonin due to the refeeding after 20-h fasting, as has been suggested [24], matching the levels achieved with PCPA treatment. Taken together, these results demonstrate that in the absence of serotonin locomotor activity is increased during the dark phase. This surmise is supported by the findings that depletion of brain serotonin by 5,7-dihydroxytryptamine (DHT) modifies the response to light [4]. In addition, serotonin affects the FEO to reduce FAA.