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Photoperiodic responses of depression-like behavior, the brain serotonergic system, and peripheral metabolism in laboratory mice

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Summary Seasonal affective disorder (SAD) is characterized by depression during specific seasons, generally winter. The pathophysiological mechanisms underlying SAD remain elusive due to a limited number of animal models with high availability and validity. Here we show that laboratory C57BL/6J mice display photoperiodic changes in depression-like behavior and brain serotonin content. C57BL/6J mice maintained under short-day conditions, as compared to those under long-day conditions, demonstrated prolonged immobility times in the forced swimming test with lower brain levels of serotonin and its precursor L-tryptophan. Furthermore, photoperiod altered multiple parameters reflective of peripheral metabolism, including the ratio of plasma L-tryptophan to the sum of other large neutral amino acids that compete for transport across the blood–brain barrier, responses of circulating glucose and insulin to glucose load, sucrose intake under restricted feeding condition, and sensitivity of the brain serotonergic system to peripherally administered glucose. These data suggest that the mechanisms underlying SAD involve the brain–peripheral tissue network, and C57BL/6J mice can serve as a powerful tool for investigating the link between seasons and mood.

1. Introduction

Seasonal changes in photoperiod regulate mood-related behavior and physiology in mammals, including humans (Rosenthal et al., 1984; Einat et al., 2006; Prendergast and Nelson, 2005). Seasonal affective disorder (SAD) is a mood disorder marked by depression, hypersomnia, hyperphagia, and carbohydrate craving during specific seasons, generally winter (Rosenthal et al., 1984). Numerous studies have been performed on the clinical and epidemiological aspects of SAD (Rosenthal et al., 1984; Lam and Levitan, 2000; Magnusson, 2000). Several animals have been proposed as animal models of SAD, including diurnal rodents (fat sand

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rats: Einat et al., 2006; grass rats: Leach et al., 2013) and Siberian hamsters (Prendergast and Nelson, 2005). However, basic research of SAD using animals has been hampered due to limited animal models with high availability and fixed genetic background such as laboratory-based, inbred strains.

Laboratory mice have been considered to be inappropriate for investigating photoperiodic phenotype, since most laboratory mouse strains, including C57BL/6J mice, cannot produce detectable levels of melatonin, a hormonal mediator of photoperiodic information. This is due to a truncation in arylalkylamine *N*-acetyltransferase, a rate-limiting enzyme in melatonin synthesis (Ebihara et al., 1986; Roseboom et al., 1998), and results in these mice losing the seasonality in their reproduction. Recently, however, we found that melatonin injections had little impact on corticosterone rhythms in Fischer 344 rats (Otsuka et al., 2012). Furthermore, laboratory C57BL/6J mice maintained under short-day conditions exhibited amplified plasma corticosterone rhythms, while no significant rhythmicity was detected in mice under long-day conditions (Otsuka et al., 2012). These data suggest that corticosterone secretion is mediated by photoperiod via melatonin-independent pathways, and stress-associated functions in C57BL/6J mice respond to photoperiod via these pathways.

In this study, we addressed whether C57BL/6J mice exhibit photoperiodic responses in mood-related behaviors, and found that depression-like behavior was increased under short-day conditions. We further clarified the photoperiodic changes in levels of brain serotonin (5-HT), a key neurotransmitter involved in the pathophysiology of SAD (Carlsson et al., 1980; Lambert et al., 2002; Neumeister et al., 2001), and related physiological and behavioral outputs in these mice, including a serotonin precursor L-tryptophan content in the brain, composition of free amino acids in plasma, glucose intolerance, intake of sucrose solutions, and the response of serotonin synthesis to glucose. Based on these data, we propose C57BL/6J mice as a useful animal model for SAD with an emphasis on the central-peripheral network.

2. Methods

2.1. Animals

Male 4-week-old C57BL/6J, CBA/N, and ICR mice were obtained from Japan SLC (Shizuoka, Japan). Mice were housed in a group of three or four animals. After acclimation for 1—2 weeks, they were exposed to short-day conditions (8 h of light, 16 h of darkness [8L16D], 50 lx) or long-day conditions (16L8D, 50 lx) for 3 weeks before the behavioral tests. Behavioral analysis was also performed using different light intensities (5 and 100 lx) and light:dark ratios (4L20D and 20L4D). We administered a glucose tolerance test and sucrose intake test under the short-day condition of 8L16D with 5 lx and the long-day condition of 16L8D with 100 or 50 lx. The animal boxes were placed in a room at a temperature of 25 ± 1 °C. Water and a standard diet for laboratory rodents (MF, Oriental Yeast, Tokyo, Japan) were available ad libitum. All animal experiments were conducted in accordance with the Guidelines for Animal Experiments of the Faculty of Agriculture at Kyushu University, as well as the Law (No. 105) and Notification (No. 6) of the Japanese Government.

2.2. Behavioral tests

To examine the photoperiod responses in mood-related behaviors in mice, we administered three behavioral tests — the open field test (OFT), elevated plus maze test (EPMT), and forced swimming test (FST) — during light and dark periods, under long- and short-day conditions. The OFT and EPMT are widely used to evaluate exploration, anxiety-like behaviors, and locomotor activity in novel environments, and thus used to detect potential anxiolytic properties (Karl et al., 2003). FST is used to evaluate depression-like behavior (behavioral despair), a measurement of the duration of immobility after an initial period of vigorous activity when mice are forced to swim in an inescapable situation. The immobility in the FST is reduced by various antidepressants (Porsolt et al., 1977). A priori power analysis indicated that a minimum of 8 animals per group was needed to achieve 80% power to detect a 40% difference ($p < 0.05$) in the FST. We used more than 8 animals per group in the behavioral tests ($n = 11$—12 for the experiments using C57BL/6J; $n = 8$—12 for the experiments of strain differences, and $n = 8$—9 for the experiments with modified light intensity and light:dark ratios).

Each test was performed sequentially, each 2 days apart. Tests were performed during either light or dark periods, i.e., Zeitgeber time (ZT: ZT0 represents light onset) 1—2 or 17—18 under the long-day condition, and ZT1—2 or 9—10 under the short-day condition, respectively. Tests during the light period were performed under white light (50 lx), whereas tests during the dark phase were performed under dim red light (2 lx). Mice were further maintained under the long- or short-day conditions for 3—4 days after the FST, and then euthanized with isoflurane gas at ZT2, 6, 10, 14, 18, and 22 ($n = 3$—4). The brain and plasma samples were collected for analysis. All behavioral analyses were conducted in a blind manner.

The OFT was performed using an apparatus consisting of a black square base (40 cm × 40 cm) with walls 40 cm high. At the beginning of the test, a mouse was placed in the center of the apparatus and then allowed to move freely for 5 min. Open field behavior was analyzed with ANY-maze Software (Stoelting Co, IL, USA) by dividing the field into 25 squares (5 × 5 grid). The number of grid lines crossed and the time spent in the central 9 grids were measured.

The EPMT apparatus consisted of a plus-shaped maze elevated 60 cm above the floor. Two opposing arms (30 cm × 5 cm) were enclosed by acrylic walls (25 cm high) while the other two open arms had a small acrylic edge (5 mm high) to prevent animal falls. A mouse was placed in the central square and allowed to explore the maze freely for 5 min. The number of entries into the open arms and the time spent there were scored with ANY-maze Software (Stoelting Co, IL, USA).

For the FST, mice were individually placed into plastic cylinders (27 cm high, 17 cm diameter) containing 14.5 cm high water, maintained at 25 ± 1 °C, for 7 min. Immobility time was recorded during the last 5 min. Mice were considered to be immobile when they floated in an upright position and made only small movements to keep their heads above water.

2.3. Sucrose/saccharin intake test

For the long-term test, individually housed mice were given two bottles, one containing tap water and the other