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Research report

Short photoperiod condition increases susceptibility to stress in adolescent male rats

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HIGHLIGHTS

- Set-up a winter depression model in adolescent rat.
- Short photoperiod condition disturbs the diurnal rhythm of corticosterone, melatonin and NPY.
- The mechanism explains adolescent individuals prone to depression in winter.

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ABSTRACT

The seasonality of depressive symptoms is prevalent in children and adolescents. However, the mechanisms that underlie such susceptibility to seasonal influences on mood disorders are unclear. We examined the effects of a short photoperiod condition on the susceptibility to subchronic unpredictable mild stress (SCUS) and rhythmic alterations of plasma corticosterone (CORT), melatonin, and neuropeptide Y (NPY) in adolescent male rats. Compared with the 12 h/12 h light/dark photoperiod control (CON) rats, the 8 h/16 h photoperiod SCUS rats exhibited significant anhedonia, a core symptom of human depression, together with a blunted diurnal rhythm and elevation of 24 h CORT, melatonin, and NPY levels. The 8 h/16 h photoperiod condition also blunted the rhythmicity of CORT, caused a phase inversion of melatonin, and caused a phase delay of NPY compared with 12 h/12 h CON rats. Such abnormalities of plasma CORT, NPY, and melatonin might cause adolescent individuals to present higher stress reactivity and greater vulnerability to stress over their lifetimes. The present study provides evidence of the susceptibility to the seasonality of stress-related disorders in adolescence.

1. Introduction

Previous studies reported seasonal variations in the expression of psychiatric phenomena, especially mood and anxiety symptoms [1]. Depressive symptoms in children and adolescents are prevalent and considered a key risk factor for later psychiatric problems [2]. However, explanations for these relationships between seasonal changes and the exacerbation of psychopathology in children and adolescents remain unclear.

The prevalence of the seasonal onset of depressive symptoms was retrospectively reported to range from 3.3% to 4.2% among American youths [3,4]. Previous epidemiological surveys found effects of seasonal variations on mood and behavior in adolescence, and mood-related problems were more severe and more overt during the fall and winter than during the spring and summer [5–7]. Even in places that experience mild winters, people who do not suffer from clinical depression appear to suffer declines in mood and energy [8]. Seasonal effects on psychopathology can also be present in healthy populations during the winter. These epidemiological surveys revealed the phenomenon of seasonal effects on

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mood-related problems, especially in adolescence. However, the neurobiological mechanisms in humans remain unclear because of the ethical and technical challenges associated with studying such a phenomenon.

Previous studies reported that a short photoperiod induced a depression-like phenotype in the forced swim test (FST) [9,10]. In collared lemmings (*Dicrostonyx groenlandicus*), short and intermediate photoperiods increased anxiety-like responses in females and elicited more behavioral despair in males [11]. Some diurnal rodents, such as fat sand rats (*Psammomys obesus*; males), Nile grass rats (*Arvicanthis niloticus*; males), and nocturnal rodents (*Rattus norvegicus* [Wistar rats]), presented numerous traits that were modulated by the specific photoperiod, including negative affective responses [12–14]. Sprague-Dawley rats are photoresponsive, and changes in the photoperiod can influence their reproductive functions [15]. However, whether mood states in Sprague-Dawley rats are photoresponsive has not been previously studied, and the role of a short photoperiod in the susceptibility to stress in adolescence is unknown.

Generally, previous animal studies on the photoperiodicity of mood have focused on the effects of pure photoperiod conditions in adult animals. In real life, individuals commonly undergo many kinds of stress. Our hypothesis was that seasonal effects may make individuals more sensitive to stress. In the present study, we used a short photoperiod condition (8 h/16 h light/dark cycle) to simulate the short days of winter and subchronic unpredictable mild stress (SCUS; 16 days) to simulate complex, low levels of stress that adolescence might experience in real life. We explored the effects of a short photoperiod condition on the susceptibility to stress and alterations in the circadian rhythms of plasma corticosterone (CORT), melatonin, and neuropeptide Y (NPY) levels in adolescent rats.

2. Materials and methods

2.1. Animals and housing

Forty-eight male Sprague-Dawley rats were purchased from the Center of Laboratory Animal Science, Peking University Health Science Center. The rats were 4 weeks of age at the time of arrival in the laboratory. They were given food and water ad libitum and allowed to acclimate to the laboratory environment for 1 week before the experiments began.

The rats were group-housed in cages (4 rats/cage) with sterile wood shavings as bedding. The rats were divided into four groups: two CON groups (12 h/12 h light/dark cycle, lights on 8:00 PM, lights off 8:00 AM [denoted 12:12CON group]; 8 h/16 h light dark cycle, lights on 8:00 PM, lights off 4:00 AM [denoted 8:16CON group]) and two SCUS groups (12 h/12 h light/dark cycle, lights on 8:00 PM, lights off 8:00 AM [denoted 12:12 SCUS group]; 8 h/16 h light dark cycle, lights on 8:00 PM, lights off 4:00 AM [denoted 8:16 SCUS group]). The groups were exposed to their respective photoperiods for 3 weeks. The lighting conditions were controlled independently of each other, thus allowing simultaneously different photoperiods for each group. The experimental cages were contained within lockers, with six cages per locker. The lockers were light-tight and ventilated. Lighting was controlled by clocklab2 computer software. The rats in one cage could see other rats in the neighboring cages within the same locker. Six fans were located on the back wall of each locker for ventilation. Three weeks were sufficient for testing for physiological acclimation [16] and the synchronization of circadian rhythms [17]. The experimental procedures were performed in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals, and the experiments were approved by the University Animal Use Committee.

2.2. Subchronic unpredictable mild stress procedure

The unpredictable mild stress procedure consisted of a variable sequence of 12 stressors as described previously [18,19]. The stressors included the following: restraint (1 h), rotation (130 rotations per minute [rpm] for 2 h), forced swim (10 °C for 5 min), cage tilt (overnight), wet sawdust (24 h), cold (4 °C for 1 h), crowding (overnight), reverse light/dark cycle, food or water deprivation (24 h), and tail clamp with long tail clamps (3 min). The stressors were applied during the light phase, with the exception of the reverse light/dark cycle and food or water deprivation (24 h). The rats were randomly exposed to two stressors every day for 16 days (for details, see Figs. 1 and 2a).

2.3. Behavioral tests

Each behavioral test was performed sequentially, 1 day apart, as described previously [18,19]. The circadian time of the behavioral tests is described below. The rats were further maintained under the same photoperiod conditions for 1 day after the FST and then euthanized. All of the behavioral analyses were performed in a blinded manner (for details, see Fig. 1a). Plasma samples were collected from another rats who subjected the same experimental protocol but not involved in behavioral detection.

2.3.1. Open field test

The open field test (OFT) was performed to assess exploratory locomotor activity. The test was conducted in a dark room during the light period (ZT0–ZT4). The apparatus consisted of a gray box that was open on top. The dimensions of the box were 100 cm × 100 cm × 40 cm, and the floor was divided into 25 equal squares (20 cm × 20 cm). The arena was illuminated with a 40 W lamp in the center, 60 cm above the floor. Each rat was gently placed in the center square of the floor of the arena, and behavior was videotaped for 5 min. The level of activity is expressed as the total number of squares crossed (all four paws inside the square) and was used to monitor motor ability in an unfamiliar environment as a rat model of depression. After each rat was tested, the box was thoroughly cleaned to remove odor cues. This model of depression in rats mimics some aspects of human depression, including lower levels of activity, and lack of interest.

2.3.2. Forced swim test

The FST was conducted during the dark period under dim red light (ZT12–ZT16 for the 12:12 groups and ZT9–ZT12 for the 8:16 groups). Plastic cylinders (50 cm height, 24 cm diameter) contained tap water (25 ± 1 °C) to a depth of 35 cm (the animals were unable to touch the cylinder's bottom with their hindpaws and tail). The FST consisted of two sessions on two consecutive days (15 min pretest and 5 min test). The rats were individually placed in the cylinders for 15 min on the first day and then placed again in the cylinders 24 h later. The total duration of immobility (immobility time) was measured during a 5-min test. The behaviors of each animal were simultaneously monitored by a video camera. Immobility was defined as the absence of any active movements other than those necessary to keep the head and nose above the water (e.g., when the rats floated in a vertical position). After each session, the rats were removed from the water, dried with a towel, and placed in a warm enclosure. The cylinders were then cleaned and refilled.

2.3.3. Sucrose preference test

The sucrose preference test (SPT) was conducted during the dark period under dim red light (ZT12–ZT16 for the 12:12 groups and ZT9–ZT12 for the 8:16 groups). The rats were habituated to a 1% sucrose solution for 48 h before the test day, during which water