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Research report

Melatonin treatment during early life interacts with restraint to alter neuronal morphology and provoke depressive-like responses

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HIGHLIGHTS

- Prenatal restraint and postnatal melatonin increased depressive-like responses.
- Prenatal restraint increased the number of fecal boli during the forced swim test.
- Prenatal restraint reduced CA1 dendritic branching.
- Perinatal melatonin protected hamsters from this restraint-induced reduction.

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ABSTRACT

Stressors during early life induce anxiety- and depressive-like responses in adult rodents. Siberian hamsters (*Phodopus sungorus*) exposed to short days post-weaning also increase adult anxiety- and depressive-like behaviors. To test the hypothesis that melatonin and exposure to stressors early in life interact to alter adult affective responses, we administered melatonin either during the perinatal (gestational day 7 to postnatal day 14) or postnatal (day 15–56) periods and also exposed a subset of dams to restraint during gestation (1 h–2×/day for 4 days). During the final week of injections, depressive-like behaviors were assessed using the sucrose anhedonia and forced swim tests. Hamsters exposed to prenatal restraint and treated with melatonin only during the postnatal period increased depressive-like responses in the forced swim test relative to all other groups. Offspring from restrained dams increased the number of fecal boli produced during the forced swim test, an anxiety-like response. In the present study, prenatal restraint reduced CA1 dendritic branching overall and perinatal melatonin protected hamsters from this restraint-induced reduction. These results suggest that the photoperiodic conditions coincident with birth and early life stressors are important in the development of adult affective responses.

1. Introduction

Experiences early in life can have lasting effects into adulthood. Specifically, early life stress contributes to the development of affective disorders in adulthood and can alter adult cortisol concentrations [1,2]. Children of depressed mothers experience increased overall health problems, poor social functioning as adults and are themselves more likely to be depressed [3]. Because the effects of early life stressors on adult affect and behavior remain unspecified, several animal models have been developed to understand this developmental process [4,5]. For example, male rats exposed to one week of a prenatal stressor increased anxiety and depressive-like behavior as adults [2,5].

In addition to altering adult behavior early life experiences also alter how individuals respond to stress as adults. Men with adult depression and increased early life stress respond to a social stress test with increased inflammatory responses [6]. Similarly, depressed men who experienced abuse during childhood increase hypothalamic-pituitary-adrenal (HPA) axis activity [7]. Given the role of the HPA axis in the response to stress and the development of depression, early life stress likely increases the risk of developing depression particularly with concurrent adult stress [8].

The HPA axis can also be independently altered by day length, an important environmental factor related to seasonal affective disorder (SAD) [9]. SAD patients report increased depressed mood after a laboratory stressor than nondepressed individuals; whereas, depressed mood following a stressor does not differ between individuals with nonseasonal depression and nondepressed individuals [10]. Similarly, elevated cortisol concentrations by short days are

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further increased in response to an acute stressor in Siberian hamsters [11,12]. These data suggest changes in day length similar to early life stress could predispose individuals to the development of depression by altering the function of the HPA axis.

Siberian hamsters (*Phodopus sungorus*) are photoperiodic, seasonally-breeding rodents that adjust their phenotype when exposed to short, winter-like day lengths and are a good model to study the influence of altered day lengths on behavior. In short days, hamsters increase depressive-like responses [13]. Also, short days reduce neuronal soma size and dendritic complexity in the CA1 region of the hippocampus [11]. Reduced CA1 complexity is correlated with increased depressive-like behavior in hamsters [11]. Exposure to dim light at night decreases CA1 apical spine density that occur concomitantly with increases in depressive-like behavior; following return to dark nights both return to normal levels [14]. Behavioral and hippocampal morphological changes appear to be linked, particularly in the CA1 region. These short-day induced behavioral and morphological changes mimic the seasonal pattern of depression in people with SAD.

In common with photoperiod-induced adjustments during adulthood, Siberian hamsters are highly responsive to day length early in life. Specifically, perinatal exposure to short days increases the depressive-like response to short days after weaning and thus alters the developmental trajectory and establishes enduring depressive-like responses [13]. In humans, season of birth can influence the incidence and severity of depression and anxiety in adulthood [15,16]. People born during spring and summer have a higher global seasonality score (indicating seasonal changes in sleep, mood, social activity, weight, energy and appetite) than people born during the autumn and winter [17]. More individuals with SAD are born during the spring and summer and fewer in the fall and winter than would be expected, highlighting the potential importance of transitioning between long and short day lengths in the development of SAD [15]. Thus, day lengths experienced during early sensitive developmental periods can alter adult affective responses.

SAD occurs during the prolonged nights of fall and winter and is associated with both relatively long durations of melatonin secretion and lack of morning inhibition of melatonin in susceptible individuals [18–20]. In humans, morning bright light therapy decreases depression and advances the evening onset of melatonin production and shuts off melatonin in the morning within two hours of light exposure [19,21]. Successful treatment of SAD with bright light therapy, suggests that prolonged melatonin signals after waking contribute to depressive symptoms [18,19]. Individuals with SAD display prolonged periods of elevated melatonin during the winter relative to the summer, similar to changes observed in animals that use photoperiod to time reproduction [18,22,23]. Nondepressed individuals did not display seasonal differences in circulating melatonin concentrations, suggesting a role for melatonin in SAD etiology [18]. Administration of exogenous melatonin to diurnal rats before lights off, which presumably sums with endogenous melatonin to provide a signal that is interpreted as a short day, induced depressive-like behaviors [24]. There is also seasonal variation in intensity of day time lighting and diurnal rats exposed to dim intensity light during the day have increased depressive-like behaviors [25]. Taken together, these data provide support for a role of day length and extended duration of melatonin, in SAD.

SAD does not affect everyone despite seasonal changes in day length and melatonin secretion. Therefore, we investigated the combined effect of prolonged melatonin secretion and stressors, that can exacerbate otherwise innocuous stimuli, early in development on adult depressive-like behaviors. We tested the hypotheses that (1) long duration melatonin early in life would have enduring effects on adult behavior and neuronal morphology and (2) that prenatal restraint would alter the relationship among melatonin, adult neuronal morphology, and behavior.

Table 1
Sample size of all treatment groups.

Perinatal injection	Post-parturition injection	Prenatal restraint exposure (N)	No prenatal restraint exposure (N)
Saline	Saline	7	12
Saline	Melatonin	7	8
Melatonin	Saline	9	12
Melatonin	Melatonin	9	11

2. Methods

2.1. Animals

Thirty-eight female hamsters from our colony at The Ohio State University were mated to produce 77 male Siberian hamsters used in this study. Following weaning the hamsters were group-housed in propylene cages (dimensions: 27.8 × 7.5 × 13 cm) at an ambient temperature of 22 ± 2 °C, relative humidity of 50% ± 10%, and provided food (Harlan-Teklad #8640, Indianapolis, IN) and filtered tap water *ad libitum*. Hamsters were housed in a 16:8 light dark cycle. Starting the first day of behavioral testing, hamsters were housed individually; this change in housing might have produced stress differentially among groups that influenced subsequent behavioral testing. This species was chosen because they are responsive to photoperiod and exhibit robust seasonal changes in body mass, reproductive tissues, brain, and behavior [13,26].

2.2. Prenatal restraint

Some dams ($n = 8$/injection) underwent restraint in ventilated Plexiglas tubes. Restraint lasted for 1 h and was conducted twice a day at random intervals [27] on gestational days 14–17 during their inactive (light) period. Other than injections, the remaining dams were not handled.

2.3. Injections

Dams were either injected subcutaneously (SC) with 0.1 ml 1% ethanol-saline containing 20 μg melatonin or vehicle (0.1 ml 1% EtOH-saline SC), at zeitgeber time (ZT) 8 starting on gestational day 7 until 15 days post parturition, hereafter called perinatal injection [28]. ZT is a standard of time based on the period of a zeitgeber, ZT 12 in nocturnal animals is defined as the time when the lights go off. Melatonin injections have the ability to communicate photoperiodic information in hamsters starting *in utero* [29]. The transfer of melatonin signaling continues after birth via maternal milk [30]. Melatonin receptors in Siberian hamsters are first present about gestational day 10; thus, maternal injections began on gestational day seven in the present study [31]. Melatonin injections summate with the endogenous nightly increase to mimic the endogenous short day pattern.

At 15 days of age hamsters from all perinatal conditions were randomly assigned to receive either melatonin (10 μg SC in 1% EtOH-saline) or vehicle (0.1 ml 1% EtOH-saline SC) injections at ZT 8 for 6 weeks, hereafter referred to as postnatal injection. This experiment generated 8 groups (Table 1). The endogenous melatonin rhythm matures at 15 days of age when the pineal gland first becomes innervated by the superior cervical ganglion and endogenous melatonin rhythms start to form [32]; thus, we used this age as the time to switch hamsters among conditions. A dose of 10–25 μg