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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

of melatonin SC in 1% EtOH-saline during the afternoon produces regression of gonadal tissues [33].

### 2.4. Mass and pelage

Hamster pups were weighed and pelage scored each week starting at postnatal day 15. Pelage was scored on a 1–5 scale with 1 = white, winter pelage and 5 = dark, summer pelage. We did not individually track mass and pelage until the third week of the first cohort of hamsters; therefore, these animals do not have weights and pelage scores for post-parturition day 15 to week 3.

### 2.5. Sucrose anhedonia

Following the six weeks of injections hamsters were given two modified water bottles for four consecutive days. The bottles were weighed every day at ZT 8 during the light phase to quantify the volume of liquid consumed daily as described previously [34].

### 2.6. Forced swim test

Forty-eight hours after cessation of the sucrose test hamsters were assessed for depressive-like behaviors on the forced swim test as described previously [34]. The video was scored on Observer software (Noldus Corp. Leesburg, VA) by a condition-blind observer for: latency to first float, the number of floating bouts, total time floating, total time swimming, and total time climbing.

### 2.7. Tissue collection and processing

Two days following the forced swim test hamsters were euthanized before ZT 8 by rapid decapitation under isoflurane anesthesia. At necropsy reproductive tissues, testes, epididymides, seminal vesicles, and fat pads, were collected and weighed to assess response to short-days. Spleen tissue was also collected and weighed. Brain tissue was collected, cut in half sagitally and placed in RNAlater (Applied Biosystems, Foster City, CA) for RNA extraction and gene expression analysis and the other half was stained with a Rapid GolgiStain Kit (FD NeuroTechnologies). The hemisphere of brain used for Golgi-Cox staining or RNA later (Applied Biosystems, Foster City, CA) was pseudo-randomly selected such that each group contained both right and left hemispheres.

Golgi-Cox stained brains were stored and processed as described previously [34]. Six representative CA1 pyramidal neurons were selected per animals that met the following criteria: neurons were clearly stained, lacked truncated dendrites, and were not obscured by neighboring neurons. Neurons and dendrites were traced at 20× (0.5). Dendritic spines were traced at 100× (1.40) in 6 apical and 6 basilar, randomly selected, representative dendritic segments of at least 20 μm in length and at least 50 μm from the cell body, using Neurolucida 8 software (MicroBrightField, Williston, VT, USA) for PC and a Zeiss Imager, M2 microscope. Neurons, dendrites and spine density were analyzed using Neurolucida Explorer software (MicroBrightField, Williston, VT, USA).

### 2.8. Statistical analysis

Main effects of perinatal restraint (no restraint, restraint), perinatal injections (saline, melatonin), postnatal injections (saline, melatonin), and interactions between the three variables were assessed. Post gestational day 15, weaning, and final pelage and body mass and sucrose anhedonia were analyzed as a $2 \times 2 \times 2$ univariate ANOVA. Forced swim test behavior was analyzed as a $2 \times 2 \times 2$ multivariate ANOVA, variables with unequal variance or not normally distributed were log transformed to run statistical analysis. Following log-transformation time floating and latency to float still had unequal variances, however, given the number of groups it was still run as part of the multivariate ANOVA. Tissue masses were analyzed as a $2 \times 2 \times 2$ multivariate ANOVA with final mass as a covariate, tissues from two animals were lost during transport and were therefore omitted from analysis. Statistics were performed using SPSS 19 for Windows (IBM, Armonk,

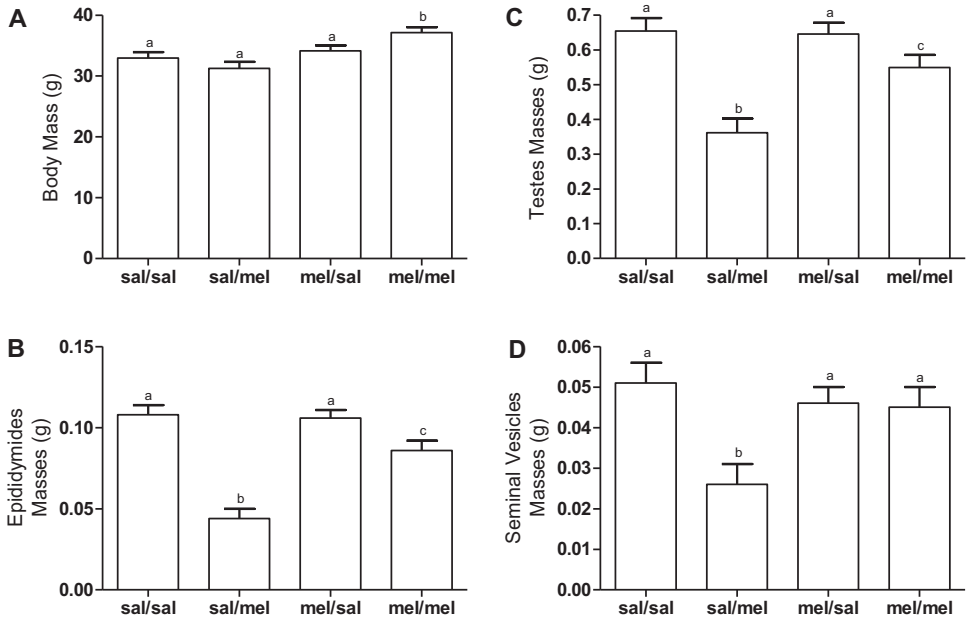


**Fig. 1.** Final body mass and tissue masses following melatonin or saline administration. Mean (±Standard Error of the Mean (SEM)) final body mass was increased by perinatal melatonin; this was driven by hamsters that received melatonin throughout the experiment (A), Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 11$. Epididymides masses were reduced by only melatonin injections, decreases were most pronounced in saline then melatonin injected hamsters (B), the same pattern was observed in testes masses (C), and seminal vesicles masses were only reduced in hamster that only received postnatal melatonin (D). For all tissue masses: Sal/Sal UR $n = 12$; Sal/Sal R $n = 6$; Sal/Mel UR $n = 7$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$. Because there was no effect of restraint data were shown collapsed for unrestraint and restrained. Different letters mark statistical significance at $p < 0.05$.

New York). One hamster had abnormal organ masses and was removed from all analyses. Mean differences were considered statistically significant when $p$ was $\leq 0.05$.

## 3. Results

### 3.1. Masses

Body mass did not differ at postnatal day 15 or at weaning ($p > 0.05$, Sal/Sal UR $n = 9$; Sal/Sal R $n = 6$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 6$; Mel/Mel UR $n = 9$, Mel/Mel R $n = 7$, Mel/Sal UR $n = 11$; Mel/Sal R $n = 8$). Final body mass was increased by perinatal melatonin ($F_{1,57} = 13.700$, $p < 0.01$, Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 11$; Fig. 1A); this was due to an interaction between perinatal and postnatal injection type as body mass was increased in hamsters receiving melatonin throughout the experiment compared to hamsters receiving only postnatal melatonin ($F_{1,57} = 8.977$, $p < 0.01$; Fig. 1A). Hamsters treated with melatonin throughout the experiment had increased final body mass compared to all other groups ($p < 0.05$). Final body mass did not differ between pups from restrained dams and unrestrained dams ($p > 0.05$).

Melatonin administration did not affect spleen mass ($p > 0.05$, for all tissue masses Sal/Sal UR $n = 12$; Sal/Sal R $n = 6$; Sal/Mel UR $n = 7$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$). Epididymides mass was decreased by postnatal injection with melatonin compared to saline; this decrease was most pronounced in hamsters receiving perinatal saline injections ($F_{1,61} = 13.405$, $p < 0.01$; Fig. 1B). Testes mass was decreased by postnatal injection with melatonin compared to saline, this decrease was most pronounced in hamsters receiving perinatal saline injections ($F_{1,61} = 5.084$, $p < 0.05$; Fig. 1C). Hamsters that received perinatal and postnatal melatonin decreased epididymides and testes mass compared to saline/saline or melatonin/saline injected hamsters ($p < 0.05$). Hamsters that received perinatal saline and postnatal melatonin decreased epididymides and testes masses compared to all other groups ($p < 0.05$). Seminal vesicles mass was decreased by perinatal saline and postnatal melatonin injection, but not in hamsters injected with melatonin throughout the experiment ($F_{1,61} = 4.871$, $p < 0.05$; Fig. 1D); perinatal saline and postnatal melatonin decreased seminal vesicles mass compared to all other groups ($p < 0.05$). None of the tissue masses differed between pups from restrained dams and unrestrained dams ($p > 0.05$); therefore, restrained and unrestrained data were collapsed in Fig. 1.

### 3.2. Pelage

Pelage was lightened by perinatal melatonin injections, an indicator of a short-day phenotype ($F_{1,57} = 5.919$, $p < 0.05$, Sal/Sal UR $n = 10$; Sal/Sal R $n = 6$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 6$; Mel/Mel UR $n = 9$, Mel/Mel R $n = 7$, Mel/Sal UR $n = 11$; Mel/Sal R $n = 8$; Fig. 2A). At the final time point, postnatal injection with melatonin lightened pelage ($F_{1,67} = 5.246$, $p < 0.05$, Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$; Fig. 2B). Because we did not record individual pelage during the first two weeks of the first cohort 10 hamsters did not have a postnatal day 15 pelage score and were omitted from analysis.

### 3.3. Sucrose anhedonia

Percentage of sucrose consumed did not differ among groups ($p > 0.05$, data not shown).

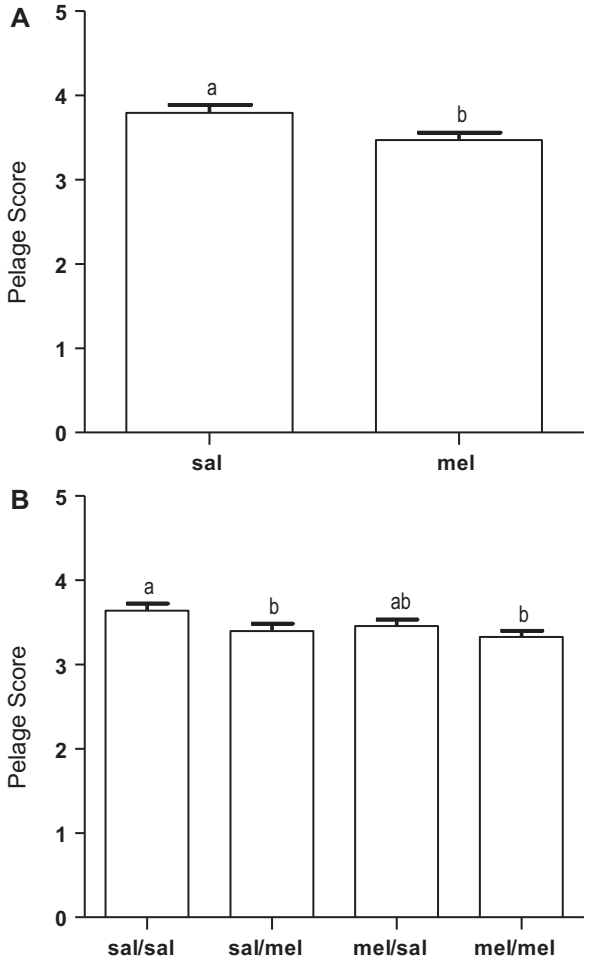


**Fig. 2.** Pelage score before and after switching melatonin and saline administration. Mean (±SEM) pelage score from 1 to 5, 1 = light coat "winter" phenotype and 5 = dark coat "summer" phenotype. Pelage at post-gestation day 15, before switching to second type of injection, was decreased by melatonin (A), Sal/Sal UR $n = 10$; Sal/Sal R $n = 6$; Sal/Mel UR n= 8; Sal/Mel R $n = 6$; Mel/Mel UR $n = 9$, Mel/Mel R $n = 7$, Mel/Sal UR $n = 11$; Mel/Sal R $n = 8$. Final pelage score was decreased by perinatal and postnatal melatonin injections (B), Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$. Because there was no effect of restraint data were shown collapsed for unrestraint and restrained. Different letters mark significance at $p < 0.05$.

### 3.4. Forced swim test

Exposure to a prenatal restraint increased the number of fecal boli released during the forced swim test compared to hamsters not exposed to a prenatal restraint ($F_{1,65} = 4.596$, $p < 0.05$, Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 6$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$; Fig. 3A). Exposure to prenatal restraint decreased latency to first float compared to hamsters not exposed to prenatal restraint ($F_{1,65} = 7.455$, $p < 0.01$; Fig. 3B). Prenatal restraint and perinatal injection interacted to affect latency to first floating bout ($F_{1,65} = 5.793$, $p < 0.05$; Fig. 3B). This was driven by a decreased latency to float in hamsters exposed to prenatal restraint that received perinatal saline and postnatal melatonin ($p < 0.05$; Fig. 3C). Two hamsters were outliers based on Z score (±2SEM) for latency to float and were removed from all forced swim test analyses. Time floating did not differ among groups ($p > 0.05$).

Prenatal restraint and perinatal injection interacted to affect time climbing ($F_{1,65} = 4.450$, $p < 0.05$; Fig. 3D). Prenatal restraint also interacted with postnatal injection to alter time climbing

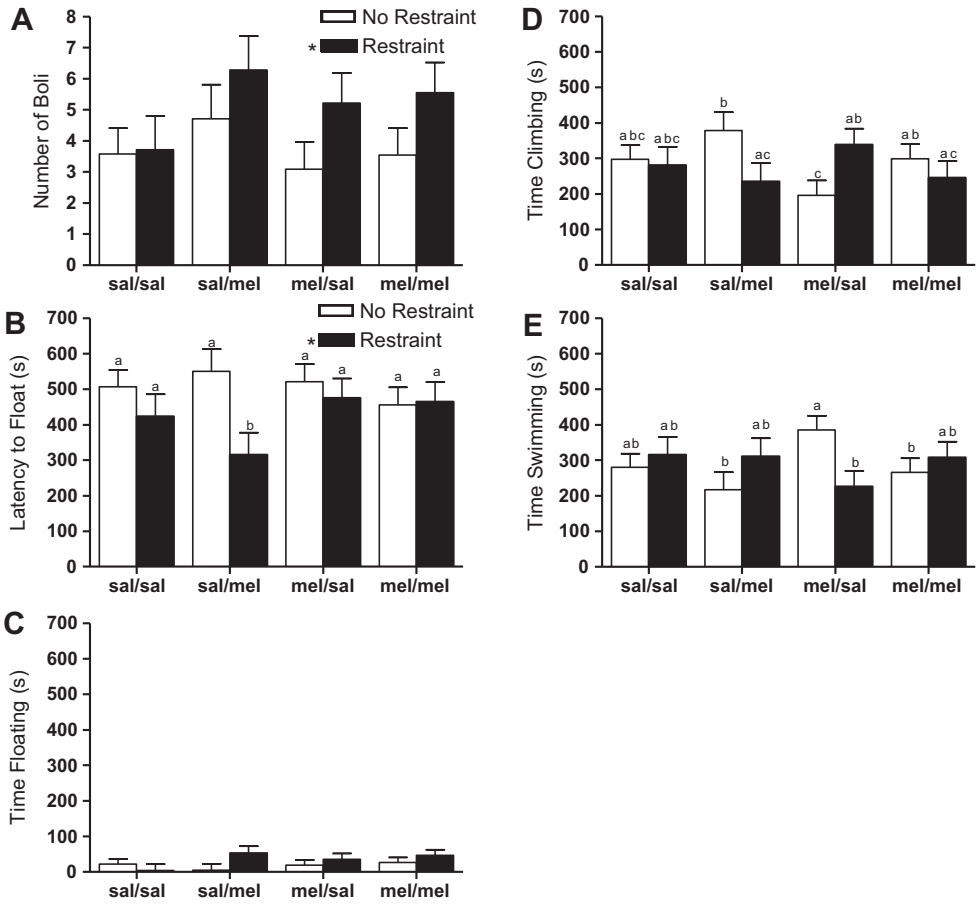


**Fig. 3.** Depressive- and anxiety-like behavior in the forced swim test. Mean (±SEM) number of fecal boli during FST (A), restraint increased anxiety-like behavior, * significance at $p < 0.05$. Prenatal restraint decreased latency to first floating bout and prenatal restraint and perinatal injection interacted to affect latency to first floating bout, this was driven by hamsters injected with postnatal melatonin (B), time spent floating (C), Prenatal restraint and perinatal injection interacted to affect time climbing and prenatal restraint also interacted with postnatal injection to alter time climbing, so that hamsters that switched between melatonin and saline injections were differentially affected by restraint (D), and a similar pattern was observed with time swimming (E). For all forced swim test measures: Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 6$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$. Different letters mark significance at $p < 0.05$.

($F_{1,65} = 6.055$, $p < 0.05$; Fig. 3D). Hamsters exposed to prenatal restraint that received perinatal saline and postnatal melatonin decreased time climbing compared to hamsters that received perinatal saline and postnatal melatonin but not exposed to prenatal restraint ($p < 0.05$; Fig. 3D). Hamsters exposed to prenatal restraint that received perinatal melatonin and postnatal saline increased time climbing compared to hamsters that received perinatal melatonin and postnatal saline but not exposed to prenatal restraint ($p < 0.05$; Fig. 3D).

Prenatal restraint and postnatal injection interacted to alter time swimming ($F_{1,65} = 4.188$, $p < 0.05$; Fig. 3E). This interaction was driven by decreased time swimming in hamsters that were exposed to prenatal restraint and received perinatal melatonin and postnatal saline ($p < 0.05$; Fig. 3E).

### 3.5. Hippocampal cell morphology

Prenatal restraint decreased apical dendritic branching in Sholl analysis of CA1 neurons compared to hamsters from dams that were unrestrained ($F_{68,5160} = 1.397$, $p < 0.01$, Sal/Sal UR $n = 12$; Sal/Sal R $n = 5$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 7$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 7$, Mel/Sal UR $n = 11$; Mel/Sal R $n = 7$; Fig. 4A). Perinatal melatonin prevented restraint-induced decreases in apical branching in Sholl analysis compared to saline injections ($F_{86,5160} = 1.38$, $p < 0.05$; Fig. 4B and D). Perinatal melatonin also prevented the prenatal restraint-induced decrease in apical dendritic length ($F_{1,60} = 4.746$, $p < 0.05$; Fig. 4C). For Golgi analysis 5 animals did not have adequate staining to trace neuronal morphology. An additional 2 animals, both hamsters were exposed to prenatal restraint and one received prenatal and postnatal saline and the other received prenatal melatonin and postnatal saline, were outliers based on $Z$ score (±2SEM from mean) and were removed from the final analysis.

## 4. Discussion

Day length coordinates seasonal adjustments in physiology and behavior in both free-living and laboratory animals. Development of SAD is also linked to changes in day length; in some cases, increased melatonin duration accounts for affective changes [19]. 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. Treatment with postnatal melatonin produced a short-day phenotype, indicated by decreased gonadal mass. Hamsters that were exposed to prenatal restraint and treated with melatonin during the postnatal period displayed increased depressive-like responses in the forced swim test. Exposure to prenatal restraint or

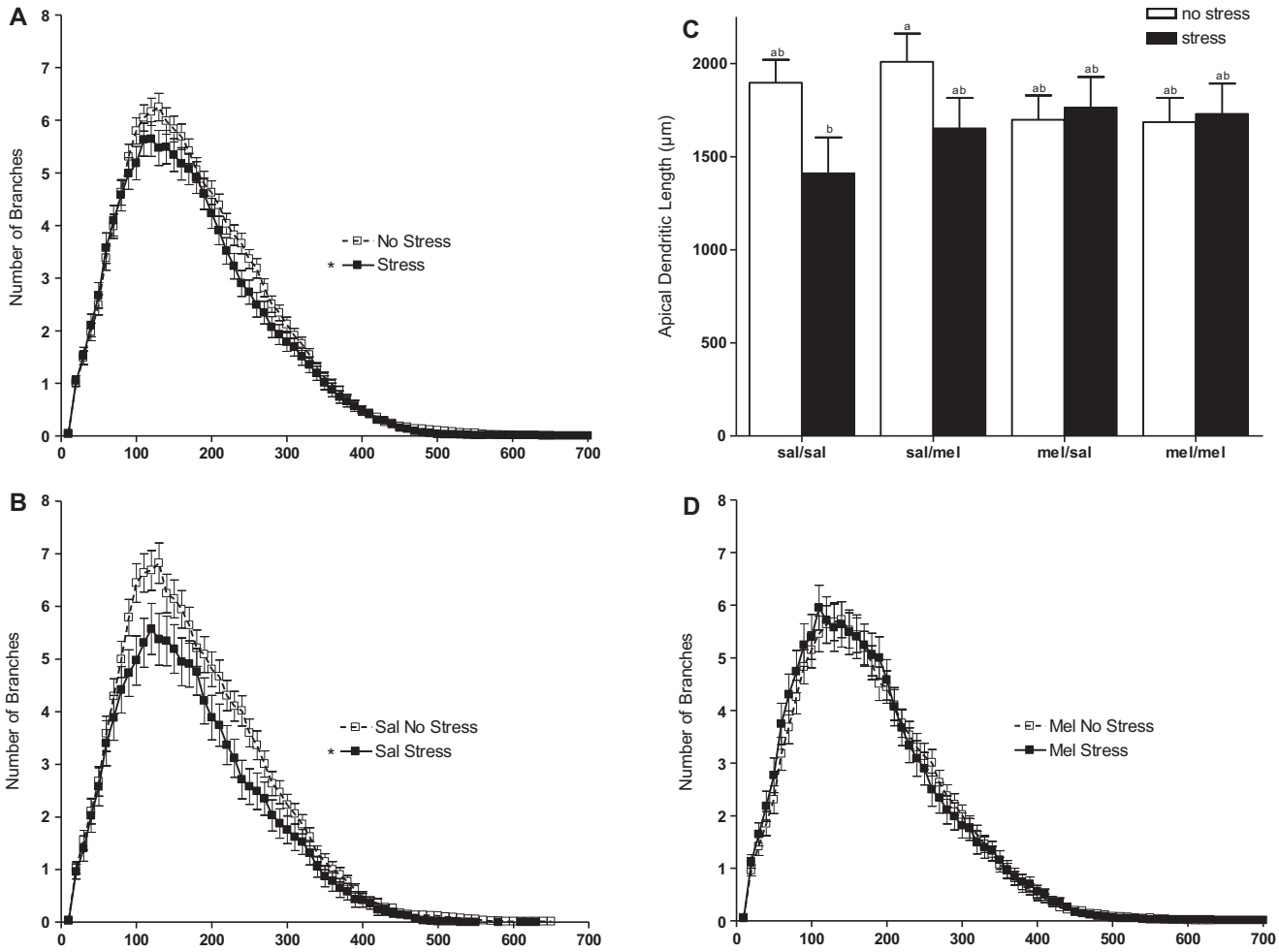


**Fig. 4.** Restraint-induced changes in apical dendritic complexity and length in the CA1 region of the hippocampus assessed by Golgi-Cox method, Sal/Sal UR $n = 12$; Sal/Sal R $n = 7$; Sal/Mel UR $n = 8$; Sal/Mel R $n = 6$; Mel/Mel UR $n = 11$, Mel/Mel R $n = 9$, Mel/Sal UR $n = 12$; Mel/Sal R $n = 9$. Sholl analysis of early life restraint exposure or no exposure to restraint, prenatal restraint decreased apical dendritic branching (A), Sholl perinatal saline and restraint exposure or no exposure to restraint, restraint decreased apical dendritic branching ($F_{86}$B), Sholl perinatal melatonin and restraint or no restraint (D). Perinatal melatonin prevented the prenatal restraint-induced decrease in total apical dendritic length (C), different letters or Astersik (*) mark significance at $p < 0.05$.

postnatal melatonin increased adult anxiety-like behavior. In the CA1 field of the hippocampus, prenatal restraint reduced dendritic branching overall, but perinatal melatonin protected hamsters from this restraint-induced reduction in dendritic branching. These results suggest a combined role for early life restraint and melatonin in adult affective responses and hippocampal morphology.

Siberian hamsters have a stereotypical response to short day lengths including: reduced body mass, gonadal regression, and lightened coat color [13,35,36]. Melatonin injections that extend the duration of nightly melatonin exposure provides a short day signal and induces these phenotypic changes [33]. Hamsters that received melatonin throughout the entire experiment increased body mass, contrary to the expected reduction with a short day phenotype. Postnatal melatonin decreased testicular and epididymal mass. Pups responded differently to gestational melatonin implants in different photoperiods, suggesting that melatonin may not be the only means of transferring photoperiod information from mother to pups [29]. Another explanation for the lack of short day phenotype in hamsters treated both before and after parturition with melatonin is perception of photoperiod *in utero* and postnatally may be different. Additionally, a long melatonin signal *in utero* and following birth typically does not occur in nature and might contribute to the mixed results observed. Alternatively, the reduced concentration of injected melatonin during the postnatal period may have altered photoperiodic response. Both, perinatal and postnatal melatonin administration produced lighter coats, a characteristic common to short day hamsters [35]. Together, these data suggest that prolonged exposure to long-duration melatonin signals induced the complete short day phenotype only if received after a perceived long day.

Short days in adulthood increase depressive-like behaviors in Siberian hamsters as well as diurnal fat sand rats [37]; however, the specific role for early-life melatonin remains unspecified. Elevated floating and decreased latencies to float in the forced swim test are interpreted as increased depressive-like behavior that can be elicited by dim daytime lighting in rats [25]. Exposure to prenatal restraint and melatonin during the postnatal period decreased latency to float. Transfer from a short to long melatonin duration, a manipulation that simulates the summer to autumn transition, combined with prenatal restraint increased depressive-like behavior. This corresponds to some season of birth literature that more people with SAD are born during spring and summer [15]. Individuals born during spring and summer transition to short days earlier in life than individuals born during autumn or winter, this early-life transition might contribute to the development of depressive

behavior as observed in our study [15]. The combination of prenatal restraint and melatonin treatment only during early life, a manipulation that mimics a winter to spring transition, decreased depressive-like behavior. Depressive-like behavior did not increase among hamsters that received melatonin or saline throughout the experiment, all together implicating changing day length and season of birth in the development of depressive-like behavior [38].

Exposure to prenatal maternal restraint reduced apical dendritic branching in the CA1 region of the hippocampus. Restraint decreases dendritic length in the CA3 and inhibits neurogenesis in most species including humans, tree shrews, and rats [39]. In the current study, perinatal melatonin prevented the restraint-induced reduction in apical dendritic branching. Perinatal melatonin treatment reduced total branching among unrestrained hamsters compared to saline-treated animals. It remains unspecified whether melatonin prevents restraint induced decreases in apical dendritic branching. Dendritic branching is slightly reduced in melatonin-treated hamsters independent of prenatal restraint; however, restraint did not further reduce dendritic branching in melatonin treated animals. Dendritic complexity and soma size in the CA1 region of the hippocampus is reduced in short day hamsters [11]. Short day exposure in adult white-footed mice (*Peromyscus leucopus*) decreased hippocampal volume, impaired spatial memory, and decreased apical spine density in the CA1 region of the hippocampus [40]. White-footed mice exhibit similar seasonal adjustments as Siberian hamsters; those results in combination with the current data support the argument that the duration of melatonin secretion regulates dendritic morphology.

In conclusion, our results reinforce the connection among seasonality, melatonin, early life stressors, and adult affect. Our data indicate that melatonin coordinates changes in body morphology in response to short days. In combination with prenatal restraint, transitioning from short to long duration melatonin increased depressive-like behavior and decreased apical dendritic length and branching. This transition from short to long duration melatonin is a manipulation that recapitulates late summer birth followed by shorter days of fall; people born during the comparable season of the year have higher rates of SAD [15]. These data suggest that photoperiodic conditions during the time of birth combined with early life stressors is important in the development of adult affective responses.

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