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MELATONIN CORRECTS DEPRESSIVE-LIKE BEHAVIOUR AND ANXIETY IN MALE AND FEMALE OFFSPRING RATS IN A MODEL OF PRENATAL MELATONIN DEFICIT

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Abstract

Melatonin deficiency caused by a prolonged light regime leads to a disturbance in the circadian rhythmicity of a number of physiological and biochemical parameters, including disruption of the synchronizing role of the hormone on endocrine and metabolic functions in the body. The aim of the present study was to explore the sex-dependent consequences of prenatal exposure to constant light (CL) on behavioural responses of rat adult offspring. In addition, the effect of melatonin supplementation on pregnant rats exposed to CL was evaluated on emotional disturbance due to melatonin deficiency. Chronic exposure to CL during pregnancy induced impaired emotional responses in male and female adult offspring, including decreased visits to aversive central zone in the open field test and elevated plus maze test and reduced consumption of sweet solutions. The elevated anxiety and anhedonia was corrected in a generation of prenatal melatonin-deficient rats of the two sexes. Our results suggest that prenatal rat exposure to CL provokes long-term behavioural impairments in male and female adult offspring. The beneficial role of melatonin supplementation on these disturbed responses suggests that our prenatal model induces melatonin deficiency that causes sustainable changes in offspring. Further data are

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needed to ascertain the mechanism underlying the prenatal-induced deleterious effect of melatonin deficiency and possible therapeutic options.

Key words: sex difference, anxiety, depression, rat, prenatal stress, corticosterone

Introduction. Depression is a remarkably destructive syndrome characterized by significant and lasting depressed mood, anhedonia, insomnia, nightmares, hallucinations, and suicidal tendencies [1]. Uncovering its pathogenesis could give valuable clues to its treatment since most antidepressant medications used at an all-time high have not demonstrated a consistent advantage over placebo pills [2]. Melatonin (N-acetyl-5-methoxytryptamine), synthesized from L-tryptophan, is a pineal gland neurohormone, intensively investigated as to its role in the biological regulation of circadian rhythms, sleep, reproduction, endocrine functions, and neuroprotection [3]. It seems that melatonin interplays with, and on some occasions favourably influences, the most critical pathogenic units described until now in the development of depressive syndrome. Furthermore, evidence exists that melatonin shifts during the intrauterine development time could affect the hypothalamus, pituitary gland, or other CNS (Central Nervous System) areas and aid in future pathologic conditions manifestation [4]. On the other hand, gender differences have also been described for melatonin influence [5]. During intrauterine development, the pineal gland of the fetus does not synthesize melatonin, and the necessary amount is obtained from the mother's organism [6]. Melatonin deficiency caused by a prolonged light regime leads to a disturbance in the circadian rhythmicity of a number of physiological and biochemical parameters, including disruption of the synchronizing role of the hormone on endocrine and metabolic functions in the body, dysfunction of the myocardium, vessels and kidneys [7]. Data on melatonin deficiency through prolonged exposure of pregnant mothers and the subsequent physiological and biochemical changes in their offspring are very scarce. Exposure to prolonged light during the prenatal period was found to lead to changes in the diurnal oxidations of malondialdehyde and lactate dehydrogenase in the testes of male rat offspring and was accompanied by an anxiogenic effect and changes in copulatory behaviour. Administration of melatonin during pregnancy corrects these disorders in the offspring [8].

Given the significant relationships between depression and melatonin, the prenatal importance of the hormone, as well as the gender differences in its effects, we set our experimental programme with the intention of evaluating whether:

- The prenatal disturbance in the circadian rhythm of melatonin secretion in pregnant mothers would cause depression-like behaviour in their pups;
- Any gender differences would be observed;
- Melatonin supplementation of pregnant mothers with prolonged light exposure would have a beneficial effect on the depressive-like behaviour of the offspring.

Materials and methods. ***Subjects.*** Sexually mature male and female Wistar rats were raised undisturbed in plastic cages ($n = 3{-}4$) under standard conditions (To $21 \pm 1\,^{\circ}$C, 50–60% humidity). After getting pregnant, confirmed with the presence of a plug, the female rats were divided into groups according to the lighting regime (Light/Dark and Light/Light) and the way of treatment as follows: Control pregnant rats LD-veh group, under artificial lighting regime, 08:00–20:00 light /dark and treated with vehicle; LL-veh group, under 24 h Light/Light regime and treated with vehicle and LL-mel group, under Light/Light regime and treated with melatonin. According to previous literature and our studies, the subcutaneous (sc) injection of pregnant rats was conducted 2 h before the subjective dark phase [10]. Melatonin/vehicle was administered at 10 mg/kg from gestational day 0 (G0) to G21. The matched groups (LD-veh and LL-veh) were treated the same way with the vehicle.

After postnatal day 21, same-sex offspring were distributed in groups according to the lighting regime and prenatal treatment. Each control and the experimental group consisted of at least eight rats containing pups from 4 or 5 litters

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Open Field Test (OF). The tested rat was placed in the central quadrant of a grey polystyrene box (100 × 100 × 60 cm). The time spent in the central zone for 5 min was measured. Animals from each group were placed once for the test session.

Elevated Plus Maze Test (EPM). EPM test was performed in apparatus with two open (50 × 10 cm) and two closed (50 × 10 × 50 cm) arms which were perpendicular to each other and separated by a central zone (10 × 10 cm). The tested rat was placed at the central zone of the maze facing the open arms. The time (in seconds) spent in the open arms, for 5 min was measured. Animals from each group were placed once for the test session.

Sucrose Preference Test (SPT). Each tested rat was placed in an individual cage and adapted to drink from two identical, graduated, and plastic bottles with tap water (100 ml) for a week. During the pre-test performed for two days, the water in one of the bottles was replaced by 1% sucrose taste preference. The preference to the sweet solution was assessed in rats placed in individual cages with access to two bottles of 100 ml filled with 1% sucrose and tap water, respectively, as was reported previously [9]. Preference for the sweet solution was calculated as a percentage of sucrose consumed to the total liquid consumed during the light and dark phase.

Statistical analysis. Data were shown as mean ± SEM. Behavioural results were analyzed using a one-way ANOVA. Statistically signiffcant differences were accepted at $p \leq 0.05$.

Results. The open field (OF) and elevated plus-maze (EPM) tests are used to measure anxiety. Reduced time spent in the central zone in the OF or in the open arms in the EPM was considered a marker of increased anxiety. In the OF test, the time spent in the central, aversive zone was reduced in the LL-veh male and female group, respectively ($p < 0.0001$ and $p = 0.0237$, respectively, compared to LD-veh group) and effect was more pronounced in males (Fig. 1). The time spent in the aversive zone (open arms) in the EPM test was reduced in the male

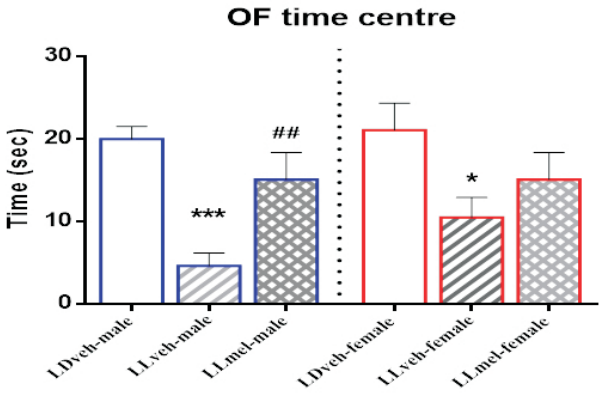


Fig. 1. Effect of chronic treatment with melatonin (mel) on time (s) spent in the centre of the open field test. Data is presented as mean ± S.E.M. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to LD-veh group. LD-veh, control group (light/dark) treated with vehicle ($n = 8$), LL-veh, model group (light/light) treated with vehicle, LL-mel, group (light/light) treated with melatonin

($p < 0.001$ compared to LD-veh group) and female ($p < 0.001$ compared to LD-veh group) generation of melatonin-deficient rats (Fig. 2). Melatonin increased time spent in the open arms, thereby decreasing anxiety level in LL-mel-male group ($p = 0.006$), whereas its beneficial effect was not significant in females. Melatonin deficiency in male offspring caused a phase-dependent diminished preference for sweet solutions during the Dark ($p = 0.002$ compared to male LD-veh group) (Fig. 3). More pronounced anhedonia was detected in female LL-veh offspring group both during the Light ($p = 0.0013$ compared to female LD-veh group) and the Dark phases ($p = 0.001$ compared to female LD-veh group), which group did not demonstrate diurnal variations in sucrose consumption. Melatonin treatment reduced depressive-like responses in male (Dark: $p = 0.0022$) and female (Light: $p = 0.0002$; Dark: $p = 0.0003$) offspring with melatonin deficiency.

Discussion. Numerous experimental and clinical studies have shown that intense psychological and emotional experiences during pregnancy can have consequences of varying degrees of severity on the normal course of labour and birth, on the one hand, and the psychosomatic development of the offspring, on the

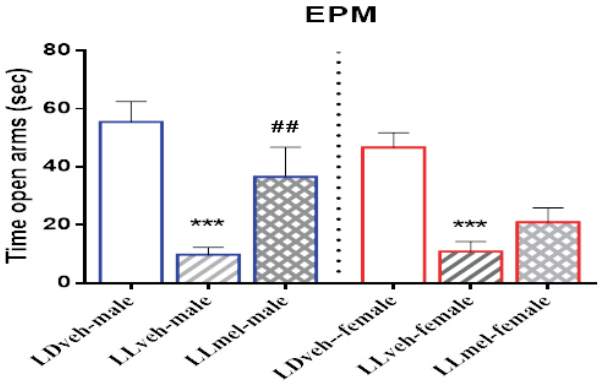


Fig. 2. Effect of chronic treatment with melatonin (mel) on time (s) spent in the open arms of the elevated plus maze test. Data is presented as mean ± S.E.M. $^{**}p < 0.01$; $^{***}p < 0.001$; $^{\#}p = 0.006$. Abbreviations in legends as in Fig. 1

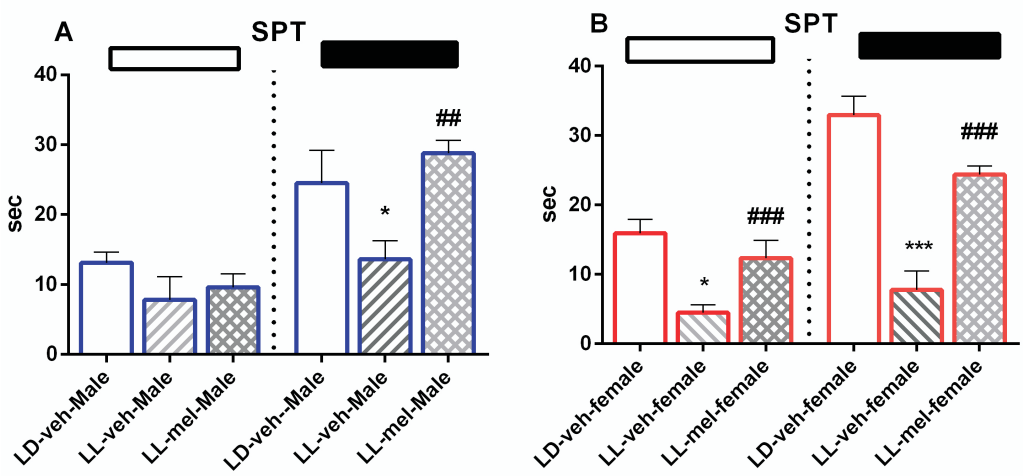


Fig. 3. Effect of chronic treatment with melatonin on preference to sucrose solution (%) in the sucrose preference test: (A) male group and (B) female group. Data is presented as mean ± S.E.M. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to LD-veh group

other [10,11]. The pineal gland is the primary source of circulating plasma melatonin, and its circadian secretion modulates the circadian dynamics of a number of physiological functions [12]. Melatonin deficiency caused by a prolonged light regime leads to a disturbance in the circadian rhythm of a number of physiological and biochemical parameters, including disruption of the synchronizing role of the hormone on endocrine and metabolic functions in the body, dysfunction of the myocardium, vessels, and kidneys [6]. Melatonin has been suggested to have an essential role in regulating the fetal circadian rhythm, as it is one of

the few hormones whose molecule crosses the placental barrier unchanged [13,14]. During intrauterine development, the pineal gland of the fetus does not synthesize melatonin, and the necessary amount is obtained from the mother's organism [8]. The maternal programme regulates the development of the circadian system during the fetal and neonatal periods [6]. Chronic exposure to constant light provokes anhedonia and increases anxiety in a generation of prenatal melatonin-deficient male and female Wistar rats. Our results agree with a previous report showing anhedonia in adult rats exposed to constant light for an extended period [15]. An experimental setup with constant light exposure of pregnant mothers for the entire pregnancy period led to melatonin deficiency caused by suppression of hormonal secretion during the dark period. In the present study, we report that prenatal melatonin treatment attenuated anhedonia in both sexes of prenatal melatonin-deficient offspring. Exposure to constant light for a long time leads to an increase in anxiety [7,11]. Anxiety level was impaired both in male and female prenatally stressed offspring. Prenatal melatonin supplementation corrected behavioural deficits associated with emotional disturbance (anxiety and anhedonia). Based on the results obtained, we can conclude that prenatal treatment with melatonin is a beneficial tool for restoring the behavioural impairment of male and female offspring in a model of prenatal constant light exposure.

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