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Melatonin receptor (MT1) knockout mice display depression-like behaviors and deficits in sensorimotor gating

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Abstract

Although critical for transducing seasonal information, melatonin has also been implicated in several physiological systems, as well as the regulation of behavioral and cognitive processes. Therefore, we investigated the neurobehavioral effects of mice missing the type 1 melatonin receptor (MT1). Male and female MT1 knockout (MT1−/−) and wild-type (WT) mice were tested in the acoustic startle/prepulse inhibition (PPI), open field and Porsolt forced swim tests. Male and female MT1−/− mice displayed dramatically impaired prepulse inhibition in the acoustic startle response. Female WT mice were more active in the open field than WT males. However, male and female MT1−/− mice did not differ in total locomotor activity. WT animals spent significantly more time in the center of the arena (a behavioral outcome associated with reduced anxiety-like behavior) than MT1−/− mice. Also, the sex difference between male and female WT mice in the amount of time spent in the center versus periphery was not observed among MT1−/− mice. Both male and female MT1−/− mice significantly increased the time spent immobile in the forced swim test, an indication of depressed-like behavior. The lifetime lack of MT1 signaling contributes to behavioral abnormalities including impairments in sensorimotor gating and increases in depressive-like behaviors. Taken together, MT1 receptor signaling may be important for normal brain and behavioral function.

Keywords: Melatonin receptor; Knockout mice; Behavior; Prepulse inhibition; Porsolt; Depression

1. Introduction

Melatonin, secreted in rhythmic fashion from the pineal gland has been implicated in several physiological systems. Traditionally, melatonin has been studied in the context of the regulation of biological timekeeping systems including circadian rhythms [31] and reproductive patterns in seasonally breeding animals [23]. Recently, however, the role of melatonin in modulating other biological processes, such as behavior, immune function and aging has received increased attention [14]. Melatonin signaling occurs via three distinct, G-protein-coupled receptors, the high affinity MT1 (Mel_{1a}) and MT2 (Mel_{1b}) [26,28], as well as a more recently discovered low affinity MT3 receptor [5]. The differential roles of these receptor subtypes, however, have been difficult to study because receptor-specific pharmacological agents are unavailable.

Melatonin has been implicated in several behavioral processes including pain perception, anxiety- and depression-like behaviors, as well as general arousal [12,13,15]. As a pharmacological agent, melatonin enhances the anxiolytic properties of diazepam when given acutely [10], and alters behavior in the forced swim test when administered chronically [4].

Melatonin has been implicated in seasonal affective disorder (SAD) [20]. This phenomenon can be modeled with the use of some seasonally breeding animals that exhibit markedly different behavioral phenotypes based on day length and its neuroendocrine mediator, melatonin [23,24]. In particular, several indicators of affect and arousal are regulated by day length. Although laboratory strains of mice are not

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reproductively responsive to day length, melatonin affects other, non-reproductive physiological and behavioral processes in this species (e.g. ref. [15]). Therefore, we sought to test the question of whether a life-long deficit of MT1 receptors would alter adult affective behavior.

2. Materials and methods

2.1. Animals

Melatonin receptor 1a (MT1) knockout mice were bred in our facilities at the Ohio State University. MT1−/− breeder mice were generously provided by Drs. Steven Reppert and David Weaver to establish a breeding colony in our laboratory. Similarly aged (13–15 months) wild-type (WT) (C57BL/6; Charles River Laboratories, Wilmington, MA, USA) (*Mus musculus*) mice served as controls. Wild-type mice were gradually exposed to other animals in cages separated by wire mesh, then group housed for several weeks prior to testing. All animals were group-housed in polycarbonate cages (27.8 cm × 7.5 cm × 13 cm) in colony rooms held under constant temperature (21 ± 4 °C) and relative humidity (50 ± 10%) and provided ad libitum access to food (Harlan Teklad 8640 Rodent Diet, Indianapolis, IN, USA) and filtered tap water. The colony rooms were maintained on a 14-h light:10-h dark cycle; lights on at 0100 h EST. All behavioral testing was conducted during the early portion of the dark period (i.e. 15:30–19:00 EST). The Ohio State University Institutional Lab Animal Care and Use Committee approved all animal protocols in accordance with National Institutes of Health guidelines.

2.2. Acoustic startle/prepulse inhibition (PPI)

Startle reactivity was measured using a single startle chamber (SR-Lab, San Diego Instruments, San Diego, CA, USA). Startle chambers consisted of a clear non-restrictive plexiglas cylinder 8.2 cm in diameter resting on a 12.5 cm × 3 cm × 25.5 cm plexiglas frame inside a ventilated chamber. A high-frequency loudspeaker inside the chamber (30 cm above the platform) produced both a continuous background noise of 65 dB and the various acoustic stimuli. Vibrations of the plexiglas cylinder caused by the whole-body startle response of the mice were transduced into analog signals by a piezoelectric unit attached to the platform. The signals were then digitized and stored by the computer. Sixty-five readings were taken at 1-ms intervals, starting at stimulus onset and the average amplitude was used to determine the acoustic startle response. All prepulse inhibition test sessions consisted of startle trials (pulse alone), prepulse trials (prepulse + pulse) and no-stimulus trials (no-stim). The pulse alone trial consisted of a 40 ms 120 dB pulse of broad-band noise. PPI was measured by prepulse + pulse trials that consisted of a 20 ms noise prepulse, 100 ms delay, then a 40 ms 120 dB startle pulse. The acoustic prepulse intensities were 73 and 81 dB. The test session began and ended with five presentations of the pulse alone trial; in between, each acoustic or no-stimulus trial type was presented 10 times in pseudorandom order. There was an average of 15 s (range 12–30 s) between trials. The mice were placed into the startle chambers immediately upon entering the behavior room and a 65 dB background noise level was presented for a 10 min acclimation period and continued throughout the test session. Percent of PPI was determined by dividing the startle response from each prepulse trial by the mean startle from pulse only trials. The prepulse inhibition protocol was based on published accounts (e.g. ref. [6]).

2.3. Porsolt swim test

For each session, a mouse was placed into a circular tank (35.5 cm in diameter) filled to approximately 15 cm with 26 °C water and scored in real time for latency to first float and time spent floating. Floating was operationally defined as remaining immobile in the water without struggling or actively swimming.

2.4. Open field

The test chamber was enclosed in a sound and light attenuating cabinet and consisted of a 60 cm^3 clear plexiglas arena lined with corncob bedding. The arena was surrounded by a series of infrared lights that tracked the movement of the mouse in three dimensions. The test chamber was rinsed thoroughly with a 70% ethanol solution and the bedding changed between each test. Each test session was 30 min in duration. The results were generated online by the PAS software package (San Diego Instruments, San Diego, CA, USA). The total locomotor activity (number of beam breaks), percentage of activity in the periphery versus the center of the arena and the total number of rears served as the dependent measures.

2.5. Data analyses

Open field and forced swim data were analyzed with a two-factor (sex × genotype) analysis of variance (ANOVA). Following a significant difference, multiple comparisons were conducted with one-way ANOVAs. Prepulse inhibition data were analyzed with a three-factor repeated measures ANOVA with sex and genotype as between subject variables and prepulse amplitude as a within subject variable. Following a significant interaction in the repeated measures ANOVA a *t*-test was performed for genotype at each prepulse intensity. All differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Acoustic startle/prepulse inhibition

MT1−/− mice exhibited considerable deficits in sensorimotor gating as measured by the prepulse inhibition paradigm. Sex had no effect on prepulse inhibition and was therefore not included in subsequent analyses. The repeated measures ANOVA revealed a main effect of genotype ($F(1, 58) = 5.082$, $p = 0.028$; Fig. 1) and of prepulse intensity ($F(2, 58) = 21.948$, $p < 0.0001$) as well as an interaction between the two terms ($F(2, 58) = 7.061$, $p = 0.002$); the MT1−/− animals displayed significant deficits at each of the two high prepulse intensities ($p < 0.05$ in each case).

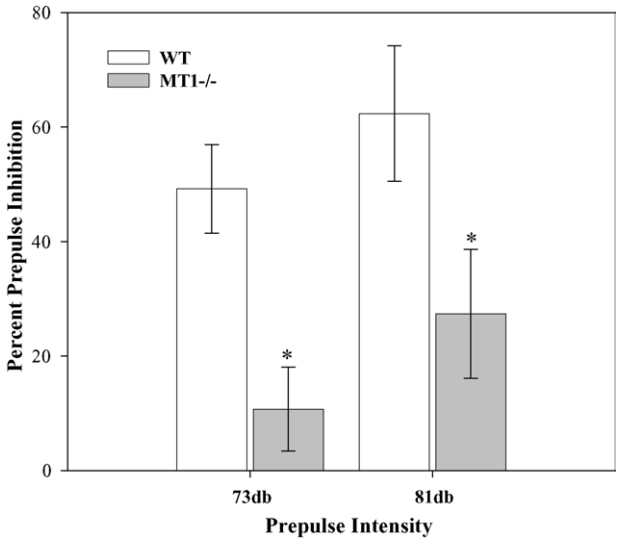

Fig. 1. MT1 transgenic mice had significant deficits in prepulse inhibition (PPI). Data are presented as mean percentage prepulse inhibition (±S.E.M.). Both MT1−/− and WT mice were better able to inhibit their startle response at high stimulus intensities ($p < 0.0001$). At each of the two highest prepulse intensities (73 and 81 dB), however, WT mice were significantly better ($p < 0.0001$) at inhibiting their startle response than MT1−/− mice. Sex of the animal did not modulate performance of PPI; thus, data are displayed collapsed across sex. $^{*}p < 0.05$ is significantly lower than WT animal at same prepulse intensity.

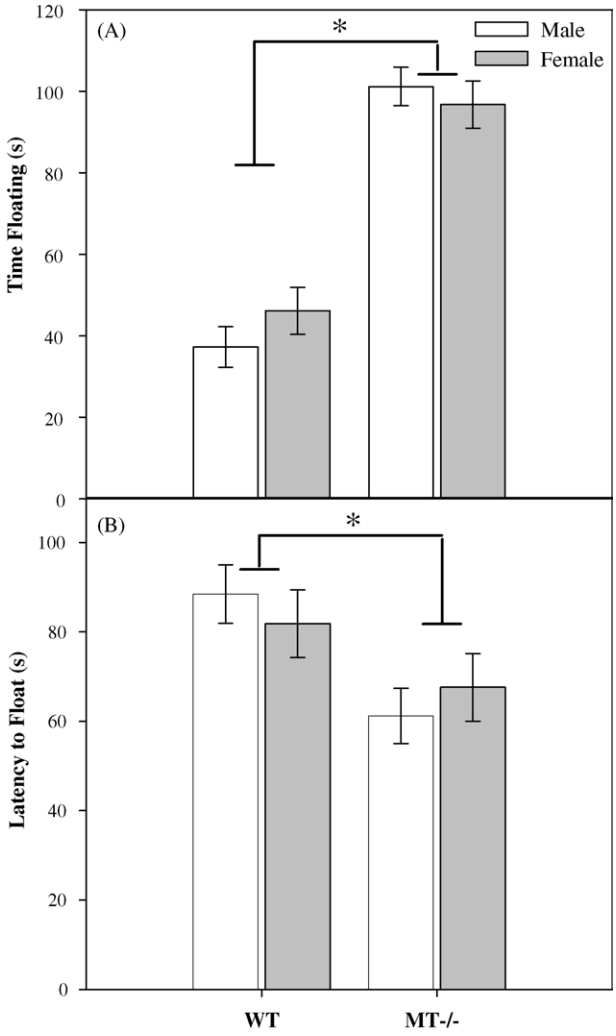

Fig. 2. (a) MT−/− animals had a shorter latency to float than WT mice ($p < 0.01$). (b) Wild-type animals spent significantly less time immobile ($p < 0.0001$) in the Porsolt forced swim test than MT1−/− mice. There were no significant sex differences in either group ($p > 0.05$). Data are presented as means (±S.E.M.). $^{*}p < 0.05$ is significant difference between WT and MT1−/− mice.

3.2. Porsolt forced swim test

Wild-type mice had a significantly longer latency to float than did MT1−/− animals ($F(1, 57) = 8.793$, $p < 0.01$); this effect was not modulated by sex. Similarly, both male and female MT1−/−mice spent significantly more time floating as compared to the WT mice ($F(1, 57) = 115.096$, $p < 0.0001$; Fig. 2) and there was no interaction with sex ($p > 0.05$).

3.3. Open field

WT mice were significantly more active in the open field than were the MT1−/− animals ($F(1, 78) = 9.738$, $p < 0.01$; Fig. 3a). This effect was mediated by an interaction between genotype and sex ($F(1, 78) = 10.136$, $p < 0.0001$) such that the WT females were significantly more active than all other groups. The MT1−/− mice did not exhibit a sex difference in locomotor activity. There was no significant difference between genotypes

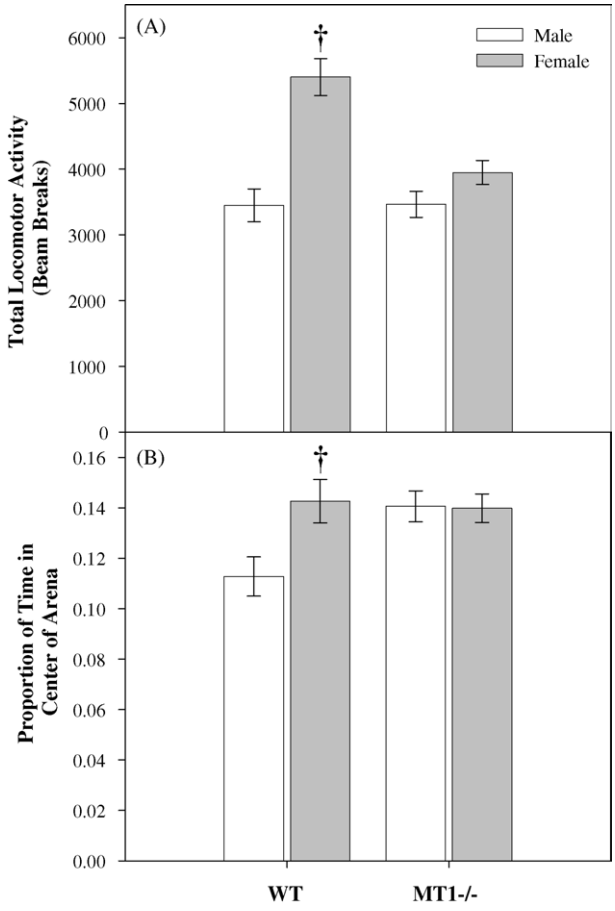

Fig. 3. (a) WT mice were more active than MT1−/− mice. This effect was due to the increased activity of female WT mice as compared to WT males ($p < 0.001$). This sex difference was not observed among MT1−/− mice. $^{\dagger}p < 0.05$ is significantly different from WT males. (b) MT1−/− mice spent slightly more time in the center of the arena. A sex difference was apparent in the WT, but not the MT1−/− mice. $^{\dagger}p < 0.05$ is significantly different from WT males. Data are presented as means (±S.E.M.).

on time spent in the center of the arena ($p > 0.05$; Fig. 3b). However, there was a significant effect of sex ($F(1, 79) = 4.122$, $p < 0.05$) and an interaction between the two variables ($F(1, 79) = 4.566$, $p < 0.05$). WT males spent less time in the center of the arena than did WT females ($F(1, 27) = 49.475$, $p < 0.01$), but there was no difference between the sexes in the MT1−/− mice ($p > 0.05$).

4. Discussion

Mice lacking the MT1 receptor displayed alterations in behavior relative to WT animals. Specifically, the transgenic mice displayed striking deficits in sensorimotor gating, an increase in depressive-like behaviors and sex-dependent alterations in open field behavior.

It is important to note that the C57BL/6 mice, in common with several other strains of *Mus* do not demonstrate robust melatonin rhythmicity or reliably demonstrable pineal melatonin content due to an apparent genetic defect in the biosynthetic pathway [9,27] although the receptor system remains intact. However, both in vitro and in vivo stimulation of the C57 pineal by

short day lengths or norepinephrine induces melatonin synthesis albeit at significantly reduced levels as compared to melatonin-proficient strains [30]. Additionally, there is mounting evidence that melatonin is produced in extra-pineal tissues including the retina, gut and bone marrow [25] that could affect physiology and behavior.

Prepulse inhibition, in particular, and sensorimotor gating in general, have been used to investigate human mental disorders with an attentional component, such as schizophrenia [3]. The significant deficits in this mouse model suggest that further investigation into the role of melatonin in the pathogenesis and etiology of such disorders is needed. The evidence for the relationship between melatonin and schizophrenia in human models is fairly weak and contradictory; however, some studies have reported blunted nighttime melatonin concentrations in a subset of affected individuals [16]. There is also evidence for a seasonal component to disease incidence [18] and season of birth as a risk factor for schizophrenia [33]. Melatonin secretion is directly related to night length and as such varies across seasons. The proximate mediators that alter PPI in the absence of MT1 receptors remain unspecified. PPI deficits observed in MT1−/− animals may be due to an accelerated aging process, as other studies have reported that PPI responsiveness declines with age [26].

The link between pineal melatonin and affective behaviors has primarily received attention in association with seasonal affective disorder. In general, greater duration of melatonin secretion has been associated with seasonal depression. Humans with SAD, often show lengthened or free-running melatonin rhythms [21,32]. The acute administration of melatonin mimics many symptoms of SAD including lethargy and attentional deficits. Further, melatonin administration to SAD patients increases the severity of the disease [20]. Siberian hamsters (*Phodopus sungorus*) housed in short day lengths (long duration melatonin secretion) display increased depressive- and anxiety-like behaviors compared to those housed in long days [22]. Antidepressant effects of melatonin have been reported in the forced swim test [17] and melatonin has synergistic effects when co-administered with monoamine oxidase inhibitors and tricyclic antidepressants [19]. In our model, animals deficient in MT1 signaling spent more time floating in the Porsolt test. These data are consistent with a loss of the antidepressant effects of melatonin.

In this model of melatonin receptor deficiency, behavioral sex differences that were apparent in the WT mice were not present in the transgenic animals. Specifically, MT1−/− mice did not display sex differences in locomotor performance observed in WT mice both for total activity and of time in the center of the arena. Behavioral sex differences on the elevated plus maze have been previously reported in rats [11]; these sex differences in performance have been attributed, in part, to circulating gonadal steroids [37]. Although females tend to be less anxious than males in the elevated plus maze, other tests of anxiety-like behavior have yielded opposite results [11].

Melatonin may influence behavior through its interaction with the central neurotransmitter systems. Melatonin alters GABAergic transmission [8]. For instance, acute melatonin administration increased GABA turnover in the hypothalamus, cerebral cortex and cerebellum [29]. Further, many of the effects of melatonin administration including its sedative [34], anxiolytic [36] and anticonvulsive [7] properties mimic those of GABAergic activity [8]. Melatonin also interacts with the serotonergic system and acts as a 5-HT2c receptor antagonist [2]. Both of these systems are sexually dimorphic and are altered by ovarian steroids [1,35]. Finally, the effects of chronic melatonin administration in the forced swim test are sexually dimorphic [4]. Taken together, it seems reasonable to suggest that MT1 may play a role in hypothalamic–pituitary–gonadal functioning and that the interaction between these neuroendocrine and neurotransmitter systems may modulate behavior. Investigation of other behaviors linked to reproductive functioning seems warranted.

The behavioral alterations observed in the present study suggest an important role for MT1 in normal neurological functioning. Melatonin receptor knockout mice exhibit sensorimotor deficits and behavioral depression. Future studies should attempt to determine the proximate mediators of these alterations and other behavioral abnormalities that may exist at different developmental stages.

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