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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).

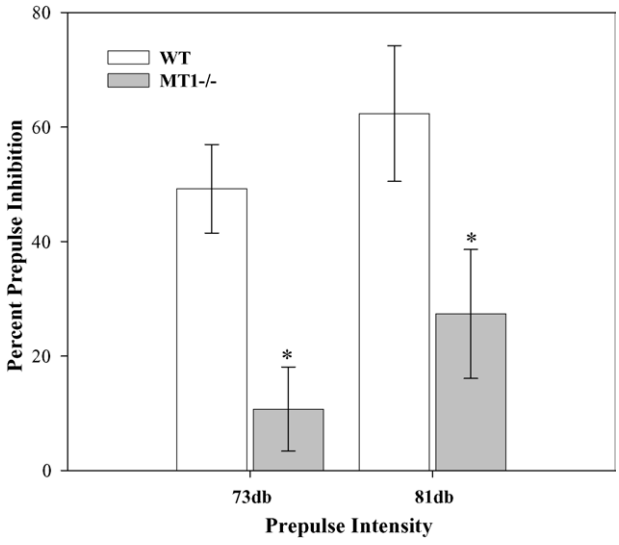

Fig. 1. MT1 transgenic mice had significant deficits in prepulse inhibition (PPI). Data are presented as mean percentage prepulse inhibition (±S.E.M.). Both MT1−/− and WT mice were better able to inhibit their startle response at high stimulus intensities ($p < 0.0001$). At each of the two highest prepulse 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.