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Melatonin enhances sexual behavior in the male rat

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Abstract

Anecdotal reports suggest that melatonin enhances libido in men. However, controlled trials remain to be published for any species. Accordingly, adult male rats were chronically treated for 12 weeks with melatonin via the drinking water. On the 13th week, all males were tested in the presence of sexually receptive females on measures of sexual behavior. Moreover, because of the established inverse relationship between male sexual behavior and serotonergic type 2A (5-HT_{2A}) receptor activity, "wet-dog shakes" (WDS), a 5-HT_{2A} receptor mediated behavior, were measured concurrently. All aspects of sexual activity were significantly facilitated in males treated with melatonin. In addition, there was a consistent, progressive reduction in the frequency of WDS, suggestive of a temporal decrement in serotonergic receptor activity and supportive of previous indications that melatonin possesses 5-HT_{2A} antagonistic properties. These results provide the first empirical evidence for a facilitatory role of melatonin in sexual behavior, and suggest that its mechanism of action may involve the 5-HT_{2A} receptor.

Keywords: Melatonin; Sexual behavior; 5-HT_{2A} receptors; Rats; Serotonin

1. Introduction

Melatonin has been postulated to be involved in a number of behavioral and physiological processes, and controlled clinical trials have established its therapeutic efficacy for specific sleep abnormalities [1]. Whereas melatonin's role in reproductive physiology is reasonably established [2], its potential function in reproductive behavior has received only scant attention. Melatonin fluctuations resulting from seasonal variations in light duration have been shown to control pubertal development in several species [3,4]. Although data linking melatonin to pubertal development in the human remain equivocal [5], deficiencies in melatonin may be related to impaired sexual functioning in human males [6,7]. Abnormally low levels of melatonin have been reported in men with psychogenic impotence [6,7], and it has been speculated that dietary melatonin supplementation might reverse some aspects of diseases associated with low levels of melatonin [7]. In addition, there exists an age-related decline in plasma melatonin levels [8] coinciding with a steady reduction in the frequency of sexual activity, sexual motivation, and potency [9,10]. Furthermore, despite the absence of randomized control trials, a number of individuals taking melatonin for sleep difficulties have claimed libido-enhancing properties of the treatment [11].

In addition to its own receptors in the mammalian brain, melatonin interacts directly with receptors from other neurotransmitter systems, or their second messenger responses. Central mechanisms of action for melatonin have been widely studied, and in particular, interactions of melatonin with the serotonergic system have been noted [12]. For example, Eison et al. [13] used radioligand binding, phosphoinositide hydrolysis, and observations of the serotonergic type 2A (5-HT_{2A}) receptor-mediated behavior "wet-dog shakes" (WDS) to infer a 5-HT_{2A} antagonism by melatonin. WDS resemble a rotational shudder of the head, neck, and trunk, and correlate positively with increases in serotonergic activity [14], and have been considered a reliable behavioral assay of 5-HT_{2A} receptor activity in vivo [15]. Further support for a melatonin–serotonin receptor interaction originates from receptor ligand-binding studies that have revealed 5-HT_{2A} receptors in the pineal gland [16]. In addition, melatonin pretreatment has been found to attenuate the 5-HT_{2A} agonist, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM)–induced effects on sleep–wakefulness regulation in the rat [17]. Taken together, these studies suggest that melatonin might possess 5-HT_{2A} antagonistic properties.

In the male rat, systemic administration of relatively selective 5-HT_{2A} antagonists, such as 1-(2,5-dimethoxy-4-iodephenyl)-2-aminopropane (DOI), have been shown to inhibit sexual behavior in the male [18]. A variety of relatively selective 5-HT_{2A} antagonists reverse the DOI-

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induced inhibition of sexual activity [19]. Furthermore, a strong inverse correlation exists between male sexual behavior and WDS, such that activation of the 5-HT_{2A} receptor produces an increase in WDS, and a concomitant decrease in sexual behavior [20]. As a result, spontaneously occurring WDS have been frequently employed as a noninvasive index of 5-HT_{2A} activity during sexual behavior.

Given anecdotal reports of melatonin's sexual behavior-enhancing effects in humans, and the available evidence indicating melatonin's role in sexual physiology, it seems reasonable to predict that melatonin may influence sexual behavior in the rat. Moreover, reports of melatonin's 5-HT_{2A} antagonistic properties will be investigated using wet-dog shakes as a behavioral assay of 5-HT_{2A} receptor activity.

2. Method

2.1. Subjects

Thirty Long–Evans male rats (Charles River Canada Inc., Quebec) were obtained at 8 weeks of age. They were screened for sexual proficiency, and those displaying consistently vigorous sexual activity were selected for the study. Twenty-two males were chosen, and housed in groups of two or three until they reached 9 months of age (500–700 g). In addition, 20 sexually experienced female Long–Evans rats were used to elicit copulatory activity in males. Females had been previously ovariectomized at 3 months of age under a combination of ketamine HCl (75 mg/kg) and xylazine (7 mg/kg) using standard surgical procedures. Females were housed in groups of three or four.

All rats were housed in standard, triple wire mesh cages, in a housing environment maintained at 21 ± 1°C. Purina Rat Chow and tap water were provided ad lib, and colony lights were set on a 12/12 h light cycle with the lights off at 0900 h.

2.2. Hormone administration

Melatonin (Sigma Chemical Co., Chicago) was dissolved in dimethyl sulfoxide (DMSO). Melatonin was administered via the drinking water in a manner previously described, and shown to enhance survival rates in aging mice [21]. Briefly, 4 mg melatonin was dissolved in 0.1 mL DMSO, and the solution was mixed vigorously in 1 L tap water. Rats in the control condition received 0.1 mL DMSO dissolved 1 L tap water. Each triple cage was supplied with two water bottles, each with a 500-mL capacity. Females were injected with 10 μg estradiol benzoate 48 h prior, and 500 μg progesterone 4 h prior to testing. These hormones were dissolved in 0.1 mL peanut oil.

2.3. Procedure

Measures of sexual behavior included: mount, intromission, and ejaculation frequencies, and latencies, and the postejaculatory interval (duration of time in seconds between the first ejaculation and the first intromission of the following copulatory bout). Copulatory efficiency, defined as the proportion of mounts resulting in vaginal penetration relative to the total number of mounts (with and without intromission), was also scored. In addition, the frequencies of WDS were tallied for a 30-min observation period. Males were given a 5-min habituation time before presentation of sexually receptive females. An initial baseline sexual behavior test was performed, after which similarly proficient animals were matched based on their ejaculation and wet-dog shake frequencies, and then randomly assigned to either the melatonin ($n = 12$) or the control ($n = 13$) condition. At this point, males selected for the melatonin condition began to receive melatonin via the drinking water, and control males received the vehicle. All males were tested at weekly intervals for an additional 12 weeks on the same measures. Water bottles were cleaned weekly, and fresh solutions were prepared every 2 days. The average fluid consumption per rat was 0.75 L/week, equivalent to a weekly melatonin intake of approximately 3 mg/rat.

A factorial analysis of variance, with treatment as a between-subject variable and repeated weekly tests as a within-subject variable, resulting in 13 within-subject levels, was performed on all the data. Significance levels were preset at $p < 0.05$.

3. Results

Four males died over the course of the experiment, and were, therefore, excluded from statistical analyses. There was no relationship between treatment condition and mortality. Furthermore, males that were unable to achieve ejaculation were dropped from analyses of mount frequency, intromission frequency, and copulatory efficiency. Missing latency scores were set to the maximum (1800 s).

An Independent samples *t*-test was performed on baseline measures between animals assigned to the melatonin and to the control groups. There were no statistically significant differences between the groups prior to melatonin administration on any measure, $p > 0.05$.

Statistical analysis revealed a significant main effect of week, $F(12, 228) = 2.23$, $p = 0.011$, and a significant main effect of melatonin, $F(1, 19) = 4.455$, $p = 0.048$, on ejaculation frequency. As shown in Table 1, males given melatonin had an increased ejaculation frequency. There was no significant interaction between week and melatonin, $p > 0.05$. The mount frequency, or number of mounts required for an ejaculation was significantly reduced in males treated with melatonin, $F(1, 16) = 4.943$, $p = 0.041$. There was no significant main effect of week on mount frequency, $F(12, 192) = 1.197$, $p = 0.288$, nor was there a significant interaction. Intromission frequency was not found to be significantly altered following melatonin treatment or time, $p > 0.05$.

Mount latencies were shortened in males treated with melatonin, 6.626, $p = 0.019$; however, there was no signifi-

Table 1
Effects of chronic melatonin treatment on frequencies of mounts, intromissions, and ejaculations, latencies of mounts (ML), intromissions (IL), and ejaculations (EL) in seconds, postejaculatory interval (PEI) in seconds, copulatory efficiency, and frequency of wet-dog shakes (WDS) in male rats

	Baseline	Weeks 1–4	Weeks 5–8	Weeks 9–12
Mounts*				
CONTROL	8.4 ± 2.5	9.1 ± 3.1	6.6 ± 2.2	8.5 ± 2.3
MELATONIN	10.8 ± 1.4	4.1 ± 1.0	5.0 ± 1.6	5.6 ± 1.7
Intromissions				
CONTROL	9.4 ± 1.5	11.6 ± 1.2	10.8 ± 1.7	10.0 ± 1.1
MELATONIN	13.7 ± 1.2	13.4 ± 1.1	13.1 ± 1.1	10.9 ± 1.3
Ejaculations*				
CONTROL	2.6 ± 0.2	2.7 ± 0.3	2.9 ± 0.3	2.8 ± 0.3
MELATONIN	2.6 ± 0.3	3.6 ± 0.2	3.3 ± 0.3	3.2 ± 0.3
ML*				
CONTROL	208.7 ± 181.3	122.0 ± 73.3	99.1 ± 57.9	92.0 ± 49.1
MELATONIN	22.2 ± 4.7	8.7 ± 1.8	19.2 ± 7.5	18.1 ± 7.7
IL*				
CONTROL	38.8 ± 14.3	132.7 ± 75.6	117.8 ± 65.9	117.9 ± 59.3
MELATONIN	49.8 ± 12.8	12.4 ± 3	45.8 ± 22.1	43.2 ± 16.4
EL				
CONTROL	458.2 ± 84.1	458.8 ± 108.3	346.4 ± 89.6	447.7 ± 103.3
MELATONIN	510.1 ± 80.9	279.15 ± 42.3	313.0 ± 54.4	332.9 ± 58.8
PEI*				
CONTROL	336.8 ± 25.1	386.7 ± 76.0	298.9 ± 11.4	316.5 ± 22.2
MELATONIN	353.6 ± 42.5	255.0 ± 14.3	278.2 ± 27.6	269.6 ± 19.0
WDS*				
CONTROL	1.9 ± 0.7	2.4 ± 0.7	2.4 ± 0.8	2.0 ± 0.7
MELATONIN	2.6 ± 0.7	1.5 ± 0.6	1.2 ± 0.5	1.0 ± 0.5
Copulatory Efficiency*				
CONTROL	0.57 ± 0.07	0.63 ± 0.06	0.66 ± 0.07	0.59 ± 0.07
MELATONIN	0.56 ± 0.03	0.79 ± 0.04	0.77 ± 0.05	0.69 ± 0.06

Values represent means ± SEMs.
*Denotes that melatonin-treated rats differed significantly from control rats, $p < 0.05$.

cant main effect of week, $F(12, 228) = 0.936$, $p = 0.512$, or interaction between week and melatonin on this measure, $p > 0.05$. Intromission latencies were found to be shorter in melatonin-treated males, $F(1, 19) = 4.794$, $p = 0.041$. There was no significant main effect of week, nor was there a significant interaction on this measure, $p > 0.05$. Ejaculation latency was not significantly influenced following melatonin treatment or time, $p > 0.05$. There was a significant effect of week, $F(12, 156) = 2{,}169$, $p = 0.016$, and a main effect of melatonin, $F(1, 13) = 5.718$, $p = 0.033$, on the postejactulatory interval. Inspection of the data reveal that males treated with melatonin showed a progressive decrease in their postejaculatory intervals. The apparent interaction between week and melatonin did not reach statistical significance, $p > 0.05$.

Statistical analyses revealed a significant main effect of melatonin, $F(1, 16) = 9.380$, $p = 0.007$, as well as a significant main effect of week, $F(12, 192) = 2.822$, $p = 0.001$, on copulatory efficiency. As shown in Table 1, melatonin increased copulatory efficiency. There was no significant week by melatonin interaction, $p > 0.05$.

A significant interaction between week and melatonin treatment was found for WDS, $F(12, 228) = 1.881$, $p = 0.038$. Inspection of Table 1 reveals that melatonin-treated males showed a progressive decline in the frequency of WDS, whereas WDS in control males did not change over time.

4. Discussion

To date, this is the first empirical report of a facilitatory role for melatonin in sexual behavior. Acute treatment with melatonin was recently shown to attenuate the corticosterone-induced inhibition of sexual behavior in the male rat, but had no facilitatory effect on its own [22]. This suggests that melatonin must be administered chronically to facilitate sexual activity. By contrast, an earlier report implied that reproductive behavior might be inhibited in male rats chronically given high levels of melatonin (8.0 mg/kg/day) for 30 days [23]. This suggestion was based entirely on evidence that the pregnancy rate for dams paired with melatonin treated males was significantly lower than that for dams paired with control males [23]. However, because behavioral observations were absent, it is not possible to determine whether melatonin inhibited reproductive physiology rather than reproductive behavior. Furthermore, it should be noted that melatonin levels given in that study were equivalent to a 70-kg human consuming 560 mg of melatonin–almost 190 times the typical dose used as a sleep aid.

As mentioned previously, WDS are considered to be a robust behavioral assay of 5-HT_{2A} receptor activity [15], and have been used to differentiate males of varying levels of sexual proficiency [20]. The progressive decline in WDS after melatonin treatment suggests a decrease in 5-HT_{2A} re-

ceptor sensitivity [13]. Given the negative correlation between 5-HT_{2A} activity and male rat copulatory behavior [18–20,24], this may account for the increase in sexual behavior after melatonin treatment.

The increase in sexual behavior after chronic melatonin treatment cannot be explained by a general facilitation of motor activity. In fact, it has been found that rats treated with melatonin exhibited less spontaneous movements, and spent more time in a frozen posture [25]. In the present study, melatonin increased ejaculations yet did not increase mounts or intromissions. Therefore, the increase in sexual behavior cannot be attributed to a nonspecific increase in motor behaviors. Taken together, the results demonstrate that melatonin enhances sexual performance, and that these results may be explained, in part, by a 5-HT_{2A} antagonism.

The potential implications of these findings are clear. Given the facilitatory effect on sexually proficient rats, it seems reasonable to speculate that melatonin might exert even greater improvements in less proficient animals. It is worth noting that the rat has become widely used as a model for human copulatory functioning, as behaviors representative of human libido and potency have been elucidated in the rat [26], and there are physiological similarities in sexual functioning between these species [27]. For instance, rat penile reflexes following central and pharmacological manipulation have been utilized to understand erectile functioning in humans with spinal injuries [27]. Moreover, agents that have been found to enhance erection in rats have successfully been used as therapeutic treatments for human erectile dysfunction [27]. Given that behaviors representative of both motivation and potency were enhanced after melatonin treatment in the rat, these results may be applicable to human sexual libido and potency, and provide support for anecdotal reports of an enhancing effect of melatonin on human male sexual functioning. Also, information is required about the toxicity and efficacy of melatonin following chronic administration.

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