

Melatonin protects against the effects of chronic stress on sexual behaviour in male rats

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The effects of chronic mild stress (CMS) on both sexual behaviour and wet dog shakes (WDS), a serotonergic type 2A (5-HT_{2A}) receptor-mediated behaviour, were explored in the male rat. In addition, the possible attenuation of these effects by chronic treatment with melatonin, a putative 5-HT_{2A} antagonist, was examined. The CMS procedure resulted in a significant increase in WDS and an overall decrease in all aspects of sexual behaviour. Concurrent melatonin administration attenuated the CMS-induced effects on sexual behaviour,

but not the effects on either spontaneous WDS or WDS in response to the 5-HT_{2A} agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane, suggesting a mechanism of action other than exclusive 5-HT_{2A} antagonism. These results are the first to demonstrate that melatonin significantly protects against the detrimental effects of a chronic stressor on sexual behaviour. *NeuroReport* 12:3465–3469 © 2001 Lippincott Williams & Wilkins.

Key words: Chronic mild stress; 5-HT_{2A} receptors; Melatonin; Serotonin; Sexual behaviour

INTRODUCTION

The chronic mild stress (CMS) procedure, in which rats are repeatedly exposed to a variety of mild stressors, is associated with behavioural and biochemical sequelae that are commonly associated with anhedonia [1]. CMS has been shown to decrease responsiveness to rewards, suppress exploratory behaviours in the open field test, and to cause a general decrease in locomotor and sexual activity [2]. The pineal hormone melatonin attenuates certain behavioural and neuroendocrine consequences of a prolonged stressful experience. In particular, chronic treatment with melatonin potently, and dose-dependently, attenuates the effects of a 21-day CMS procedure on sucrose intake and behavioural locomotion in the male rat [3].

The nature of melatonin's putative anti-stress effects may be linked to its interactions with the serotonergic (5-HT) system. Recent interest has focused on melatonin's activity at the serotonergic type 2A (5-HT_{2A}) receptor, and the influence of melatonin treatment on 5-HT_{2A} receptor mediated behaviours, such as wet dog shakes (WDS), have been investigated. WDS have been described as a paroxysmal shudder of the neck and trunk in rats [4], and have been shown to be a valid behavioural assay of 5-HT_{2A} receptor activity, such that 5-HT_{2A} activity and WDS are positively correlated [5]. Treatment with the 5-HT_{2A} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), significantly increases the frequency of WDS [6,7], and melatonin pretreatment attenuates this facilitation [8]. Moreover, biochemical analyses suggest that this beha-

vioural effect of melatonin is mediated via a reduction in 5-HT_{2A} receptor activity rather than altered central 5-HT_{2A} receptor density [8]. The demonstration that melatonin reduces the concentration-dependent 5-HT_{2A} receptor-mediated phosphoinositide hydrolysis response to 5-HT_{2A} agonists [8] indicates that melatonin exerts a putative 5-HT_{2A} receptor antagonism.

Evidence for melatonin's role as a 5-HT_{2A} receptor antagonist has been supported in behavioural studies implementing corticosterone treatment. Chronic administration of corticosterone, which produces physiological consequences parallel to those of stress, results in an upregulation of 5-HT_{2A} receptor binding [9], and elicits an increase in the frequency of WDS [10]. It has been speculated that the actions of corticosterone on WDS are due to increased activity at the 5-HT_{2A} receptor [9,10]. Acute administration of melatonin blocks the facilitatory effect of corticosterone on WDS [11]. Furthermore, chronic administration of melatonin alone has been shown to produce a consistent, progressive reduction in spontaneous WDS behaviour [12]. Taken together, these studies indicate that melatonin may act upon the 5-HT_{2A} system by exerting antagonistic effects [12], and that it has the potential for attenuating 5-HT_{2A} receptor-induced behavioural disruption.

Numerous studies have documented a relationship between 5-HT_{2A} receptor activity and male rat sexual behaviour. Research employing 5-HT_{2A} receptor agonists [6,7] and antagonists [13] has revealed an inverse relationship

between 5-HT_{2A} receptor activity and male rat sexual behaviour, and a positive relationship between 5-HT_{2A} receptor activity and WDS. Moreover, the spontaneous frequency of WDS has been shown to differentiate male rats of varying copulatory proficiency [6], and is thought to be mediated by 5-HT_{2A} receptor activity. Chronic stress regimens [14], as well as chronic corticosterone administration [11], have been shown to inhibit sexual behaviour in male rats while consistently leading to an increased display of WDS. Thus, the consequences of stress and corticosterone on sexual behaviour and WDS appear to be strongly linked to increases in 5-HT_{2A} receptor activity. Support for this stems from research which has shown that as with corticosterone, the administration of a chronic stressor also results in an upregulation of 5-HT_{2A} receptor binding [15]. Furthermore, the degree of change in 5-HT_{2A} receptor activity was found to be proportional to the degree of hypothalamo-pituitary-adrenal axis activation and corticosterone secretion [15], suggesting that the effects of the stressor were mediated by corticosterone.

Given that melatonin was able to block the effects of CMS on sucrose preference and locomotor activity [3], and the demonstration that melatonin attenuates the corticosterone-induced inhibition of sexual behaviour [11], it seems reasonable to predict that the CMS procedure alone should result in inhibited sexual behaviour, and that melatonin should attenuate this effect of CMS. If the effects of CMS on sexual behaviour are mediated by 5-HT_{2A} receptor activity, it is also reasonable to predict that there would be a concurrent increase in the display of WDS behaviour. Furthermore, if the mechanism of action of melatonin is indeed via the 5-HT_{2A} receptor, then melatonin should attenuate the effects of CMS on WDS. Assuming these behavioural changes are 5-HT_{2A} receptor-mediated, one might expect a potentiation of these effects after co-administration of a 5-HT_{2A} receptor agonist, such as DOI. The aim of the present study is to investigate the effects of CMS, both alone and in combination with melatonin treatment, on sexual behaviour and WDS. The possibility of 5-HT_{2A} receptor involvement in the mediation of these effects will be further explored with the use of DOI administration.

MATERIALS AND METHODS

Materials: Melatonin (Sigma Chemical Co., Chicago, USA) was dissolved in a solution containing equal parts propylene glycol and 0.9% saline (10 mg/ml). The control solution consisted of equal parts propylene glycol and saline. (\pm)1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Research Biochemicals International, Natick, MA) was dissolved in 0.9% saline (1.25 mg/ml). Estradiol benzoate (10 μ g; Sigma) and progesterone (500 μ g; Sigma), were each dissolved in 0.1 ml peanut oil.

Animals: Fifty-one Long-Evans male rats (Charles River Canada Inc., Quebec) were obtained at 3 weeks of age. Prior to testing, males were screened for copulatory proficiency, and of these, 40 were shown to consistently display vigorous sexual activity, i.e. ejaculation within 15 min, and therefore were included in the study. Rats were 13 weeks of age at the onset of the study, and weighed 450–550 g. Rats were weighed on days 0, 7, 14, and 21 of the CMS

procedure. Female rats used as stimuli for male sexual behaviour were previously bilaterally ovariectomized at 3 months of age using standard surgical procedures while anesthetized with ketamine HCl (75 mg/kg) and xylazine (7 mg/kg) obtained from the Animal Care Centre, University of British Columbia, Vancouver, Canada. Standards of surgical care were in accordance with the requirements of the Canadian Council on Animal Care, and all animals recovered successfully from the surgery. Rats were housed in same-sex groups of three, in standard wire mesh cages, and were allowed free access to Purina Rat Chow and water. Colony conditions were maintained at $21 \pm 1^\circ\text{C}$, and lights were programmed on a reverse 12:12 h light cycle (lights off at 09:00 h).

Stress/injection procedure: Male subjects were randomly assigned to one of four treatment groups, with 10 animals/group: (1) no stress-vehicle; (2) no stress-melatonin; (3) stress-vehicle; and (4) stress-melatonin. The stressor regimen was administered for 21 consecutive days, and was modeled after that described by Kopp and colleagues [3]. In brief, it involved exposure to 2–3 mild stressors each day at 09:00 h, 12:00 h and 16:00 h. Stressors lasted for 1 h and included tube confinement, food and/or water deprivation, random cage rotation, damp bedding environment, and crowding/white noise, stroboscopic illumination stress. Immediately following exposure to the first stressor, animals in the stress and control conditions received an injection of either melatonin (10 mg/kg, s.c.) or vehicle. On testing days, males received the stressor and melatonin immediately following behavioural testing.

Behavioural testing procedure: Females were injected s.c. with 10 μ g estradiol benzoate 2 days before testing, and with 500 μ g progesterone 4 h before testing. Males were tested in Plexiglas chambers (30 \times 30 \times 45 cm in height) covered with contact bedding.

Sexual behaviour testing occurred at three time points for 30 min: after 7, 14, and 21 days of stress and melatonin administration. On each test day, males were given 5 min to habituate to the chambers before being presented with a receptive female. Measures of sexual behaviour included mount, intromission, and ejaculation frequencies and latencies, and the post-ejaculatory interval. In addition, the frequency of WDS was tallied during the 30 min observation period. All testing was conducted during the middle third of the dark cycle by trained observers blind to the experimental conditions of the animals.

Two hours following sexual behaviour testing on day 21, each animal was given an i.p. injection of the 5-HT_{2A} agonist, DOI, and was tested for the frequency of WDS for 10 min. Given that previous findings indicate that the frequency of spontaneously occurring WDS is quite low in copulating male rats [6], DOI was given in order to amplify any effects potentially mediated by 5-HT_{2A} receptor activity. Following testing, all animals were returned to their natural housing conditions.

Statistical analyses: *A priori* tests using Scheffé's method of planned contrasts were used in the current analyses. *A priori* hypotheses formed the basis for the following contrasts: (1) the CMS condition would significantly differ

from all other conditions combined, with CMS producing a significant inhibition on copulatory measures, (2) CMS and CMS-melatonin conditions would significantly differ from each other, with melatonin attenuating the effects of CMS, and (3) melatonin and control conditions would not significantly differ from each other. In addition, a repeated measures ANOVA was used for analyses of weight, with time as the within-subjects factor and stress and melatonin as between-subjects factors. In all cases, $\alpha = 0.05$ was used.

RESULTS

Data for ejaculation latency, ejaculation frequency, and spontaneous WDS are presented in Fig. 1, Fig. 2 and Fig. 3, respectively. Although other measures of sexual behaviour reached statistical significance, these measures were selected for presentation as they are the most commonly reported measures of sexual behaviour in the rat.

Day 7: After one week of exposure to the CMS paradigm and chronic melatonin administration there were no statistically significant effects in the direction predicted for

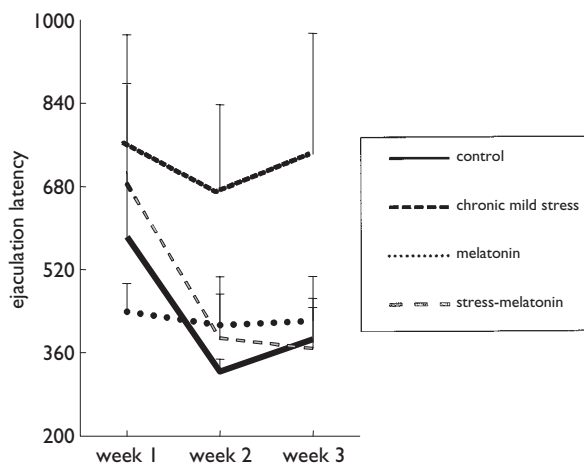


Fig. 1. Effects of chronic mild stress, melatonin and their combination on mean \pm s.e.m. ejaculation latency scores (s).

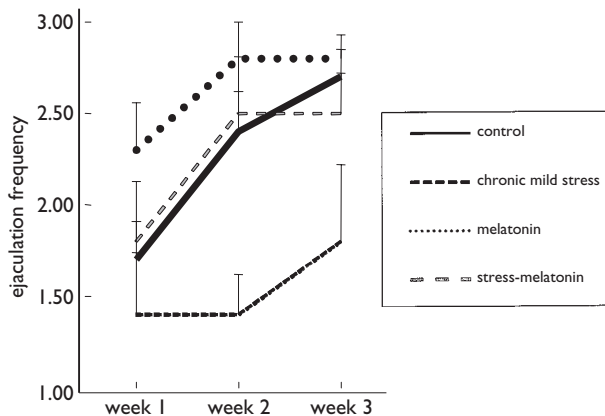


Fig. 2. Effects of chronic mild stress, melatonin and their combination on mean \pm s.e.m. ejaculation frequency scores (per 30 min).

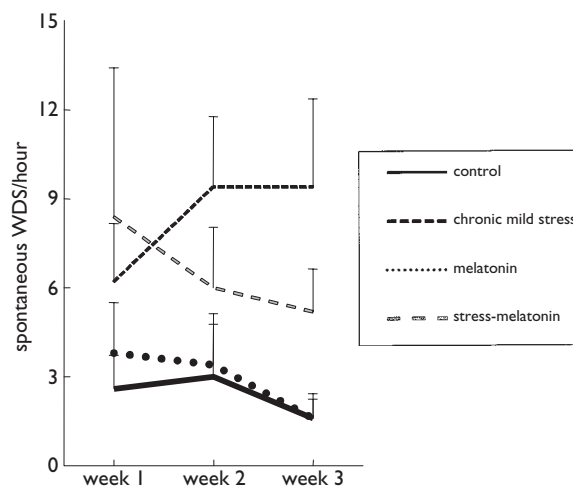


Fig. 3. Effects of chronic mild stress, melatonin and their combination on mean \pm s.e.m. number of spontaneous WDS/h.

CMS, melatonin, or CMS and melatonin combined on any measure of sexual behaviour or WDS (all $p > 0.05$).

Day 14: Data analysis performed after the second week of CMS and/or melatonin exposure revealed significant effects on nearly every measure of sexual behaviour in the predicted direction. For mount latency, males exposed to CMS showed significantly longer mount latencies compared to the other groups ($p < 0.001$) and treatment with melatonin significantly attenuated the stress-induced increase in this measure ($p = 0.01$). There was no effect of melatonin treatment alone on mount latency ($p > 0.05$). Analysis of intromission latency revealed a similar pattern in that CMS males showed significantly longer latencies to intromit ($p < 0.001$) and this effect was significantly attenuated by melatonin pretreatment ($p = 0.02$). There was no effect of melatonin treatment alone on this measure ($p > 0.05$). Ejaculation latency was significantly longer in CMS-exposed males ($p = 0.02$). Although not quite reaching statistical significance, there was a trend for melatonin to attenuate the stress-induced increase in ejaculation latency scores ($p = 0.07$). Melatonin treatment alone had no significant effect on ejaculation latency ($p > 0.05$; Fig. 1). The post-ejaculatory interval was significantly lengthened in stressed rats compared to all other groups ($p = 0.02$), and melatonin significantly prevented this effect ($p = 0.05$). There was no effect of melatonin treatment alone on post-ejaculatory interval ($p > 0.05$).

Stressed males exhibited ejaculation frequencies substantially below all other groups ($p < 0.001$). This detrimental effect of CMS was significantly attenuated with melatonin treatment ($p = 0.003$). There was no effect of melatonin treatment alone on the frequency of ejaculation ($p > 0.05$; Fig. 2). Mount and intromission frequencies were not significantly affected by chronic stress or chronic melatonin exposure (all $p > 0.05$).

Wet dog shakes were significantly increased in male rats who had undergone CMS ($p = 0.03$). Although melatonin co-administration appeared to prevent this increase, this was not statistically significant ($p = 0.24$). Melatonin alone also had no effect on WDS ($p > 0.05$; Fig. 3).

Day 21: Data analysis performed after the third week revealed effects consistent with those observed during the second week. Mount latency was significantly longer in males exposed to a chronic stressor compared to other males, and although not quite reaching statistical significance, melatonin treatment tended to attenuate this effect ($p=0.08$). Melatonin alone did not affect mount latencies ($p>0.05$). Analyses of intromission latencies demonstrated a similar pattern with CMS producing significantly longer intromission latencies ($p=0.02$). Melatonin was unable to completely attenuate this effect of stress ($p=0.12$) and melatonin treatment alone had no effect on intromission latency ($p>0.05$). CMS induced significantly longer ejaculation latencies ($p=0.03$), which tended to be attenuated by melatonin treatment ($p=0.06$). Again, melatonin alone had no effect on ejaculation latency ($p>0.05$; Fig. 1). The pattern for post-ejaculatory behaviour was similar to that seen after 14 days: stress significantly increased the post-ejaculatory interval ($p=0.002$) and melatonin pretreatment significantly blocked this effect ($p=0.01$). Melatonin alone did not influence this measure ($p>0.05$).

Ejaculation frequencies remained significantly lower in stress-treated males than in all other groups ($p=0.01$). Melatonin treatment of CMS males tended towards attenuating this effect ($p=0.06$). There was a non-significant trend towards melatonin treatment alone increasing ejaculation frequencies ($p=0.08$; Fig. 2). There were no significant effects of stress or melatonin on intromission frequency ($p>0.05$). For mount frequency, an interesting pattern emerged that was not apparent during earlier weeks of testing. Melatonin treatment alone significantly reduced the number of mounts required for an ejaculation ($p=0.015$). Neither CMS alone nor CMS with melatonin, affected mount frequency ($p>0.05$). There were no significant effects of stress or melatonin on intromission frequency ($p>0.05$).

Exactly paralleling the effects seen after day 14, stressed males showed significantly higher WDS than all other groups ($p=0.002$). Melatonin treatment exhibited a trend to attenuate this effect, but it did not reach statistical significance ($p=0.09$). Melatonin alone had no effect on WDS ($p>0.05$; Fig. 3).

Analysis of weights: Repeated measures analyses of weight change over the 3 weeks of testing revealed a significant week \times CMS interaction ($p<0.001$) and a significant main effect of week ($p=0.01$) on weight. All stressed males significantly lost weight over the course of the experiment, while non-stressed males progressively gained weight throughout. Melatonin treatment had no effect on weight changes ($p>0.05$).

Analysis of DOI-induced WDS: DOI treatment increased the frequency of WDS compared to the frequency of spontaneous WDS. Planned comparisons revealed that stressed males showed significantly more DOI-induced WDS than non-stressed males ($p=0.041$; mean \pm s.e., 20.4 ± 4.58 WDS/h) but that this effect was not attenuated by melatonin treatment ($p>0.05$; 20.4 ± 5.31 WDS/h). DOI-induced WDS in animals treated with melatonin alone (6.6 ± 2.44 WDS/h) did not differ from DOI-induced WDS in the control condition (5.4 ± 2.60 WDS/h).

DISCUSSION

The current findings demonstrate that a paradigm of chronic mild stress administration to male rats impairs copulatory behaviour after 2 weeks. The stressor also resulted in a significant facilitation in the display of WDS behaviour that coincided with the time course for the effects on sexual behaviour. In males who had received melatonin treatment concomitant with CMS, the stressor was ineffective in reducing copulatory behaviour, suggesting a protective role for melatonin. These are the first published data to demonstrate that melatonin significantly protects against the inhibitory influence of a stressor, chronic or acute, on sexual behaviour.

The CMS procedure in the current study resulted in impaired copulatory behaviour after 2 weeks, but not after 1 week of administration. The CMS paradigm, rather than a shorter duration stressor of greater severity, was chosen in order to better parallel the human phenomenon in which chronic confrontation with mild stressors has deleterious effects [3]. Originally described in the context of assessing antidepressant efficacy [16], the CMS paradigm involves chronic exposure to a variety of mild psychosocial stressors throughout the day. The procedure has been shown to blunt responsivity to reward such as sucrose preference and increase submissive behaviour in the resident-intruder test [3]. The inhibition of copulatory measures is consistent with a CMS-induced reduction in reward sensitivity, and concurs with other evidence that has shown impaired sexual responding following chronic isolation stress [17] and chronic random cage rotation [14] in male rats. However, the demonstration that CMS has a direct facilitatory effect on WDS provides evidence for an alternative mechanism, one mediated by serotonergic activity. Others have shown that chronic stress results in increased 5-HT_{2A} receptor binding [15] and various behavioural changes [2] in male rats.

The present data are supported by other evidence that shows an anti-stress effect of melatonin on decreased sucrose preference and locomotor inactivity [3], gastric lesions [18], and on adrenocortical secretory responses [19]. Given that acute injections of melatonin can attenuate the effects of chronic corticosterone treatment on rat sexual behaviour [11], a possible mechanism of explaining this effect is implicated. More specifically, it may be posited that melatonin is partially attenuating the effects of CMS through 5-HT_{2A} antagonism. This suggestion is consistent with the overall trend that, by the third week of the procedure, melatonin appeared to attenuate the CMS-induced increase in WDS. However, this effect did not achieve statistical significance. Previous work supports a melatonin-serotonin interaction with evidence that 5-HT_{2A} receptors are found on the melatonin-secreting pineal gland [20], and that melatonin decreases 5-HT_{2A} neurotransmission by inhibiting 5-HT_{2A} receptor-mediated phosphoinositide hydrolysis in a noncompetitive manner [8]. Behavioural evidence shows that melatonin attenuates DOI-induced WDS [8,11] and DOI-induced hypophagia and HPA-axis activation [21]. However, as melatonin was unable to attenuate the facilitation of WDS by administration of DOI in the present study, it is unlikely that 5-HT_{2A} receptor antagonism by melatonin accounts for the effects of CMS on sexual behaviour.

Notably, the effects of melatonin alone on sexual behaviour and WDS were not significant throughout the three testing periods, with the exception of a significant decrease in the frequency of mounts required for an ejaculation during the third week of testing. These data are in contrast to those of Brotto and Gorzalka who found a significant facilitatory effect of melatonin administration alone on nearly every parameter of sexual behaviour and WDS when administered chronically through the drinking water [12]. These discrepant findings may be a function of dose differences between the former and the current study (0.43 mg/day and 10 mg/day respectively). It is possible that administration at physiological levels in a manner that continually makes the hormone available (i.e., via the drinking water) exerts different effects to those produced when administered once daily at higher supraphysiological levels. The demonstration of a facilitatory effect of melatonin on sexual behaviour after the third week of testing (i.e., a decrease in mount frequency), may represent actions of melatonin that occur independent of 5-HT_{2A} receptor activity. Drago and colleagues have reported a detrimental effect on female rat sexual behaviour with high doses of melatonin [22]. This sex difference is likely attributable to the fact that increased 5-HT_{2A} receptor activity in female rats is shown to increase sexual behaviour, opposite to that shown with male rat sexual behaviour [14]. Therefore, if melatonin is at least partially exerting its effects through 5-HT_{2A} antagonism, it follows that melatonin would have the effect of facilitating male, while inhibiting female rat sexual behaviour. The opposite effects of melatonin on male and female rat sexual behaviour require further study and clarification.

The effects of CMS on 5-HT_{2A} receptor-mediated WDS and sexual behaviour were not consistently attenuated by concomitant melatonin treatment. Moreover, the significant weight loss evident in chronically stressed males was also not prevented by melatonin treatment. This suggests that melatonin's attenuation of the negative effects of CMS on sexual behaviour occurred in a selective manner, rather than reversing any general CMS-produced debilitation such as weight loss. In the case of WDS behaviour, this explanation may also be plausible given that in the absence of 5-HT_{2A} agonist activation, melatonin weakly, but not significantly prevented the effects of CMS on WDS. Given that WDS were used as a behavioural assay of serotonergic activity [6] and melatonin has been purported to exert serotonergic antagonism [8,11], it was predicted that if the effects of melatonin on CMS-induced sexual behaviour were mediated via this mechanism, similar effects would be observed on WDS behaviour. The data suggest that non-serotonergic mechanisms contribute to the effects of CMS and melatonin on sexual behaviour. For example, dopamine has a facilitatory role in male sexual behaviour [23], and dopaminergic activity increases in response to melatonin treatment [24]. Moreover, the ease of melatonin entry into all cells and directly into genomic activity [24] make it difficult to assign one specific mode of action to its behavioural effects. Previous research has shown that the

inhibitory effect of corticosterone on sexual behaviour, and its reversal by melatonin, are mediated by 5-HT_{2A} activity [11]. The present data suggest that exclusive 5-HT_{2A} mechanisms do not account for the effects of chronic stress on sexual behaviour, even though they are melatonin-reversible. Future studies will investigate the role of other serotonin receptor subtypes, as well as non-serotonergic mechanisms.

CONCLUSION

Chronic mild stress disrupted sexual behaviour in male rats and increased the display of WDS behaviour. This finding agrees with and extends previous findings that 5-HT_{2A} receptor activity is positively correlated with WDS and negatively correlated with male rat sexual behaviour. It is likely that these effects are associated with stress-induced corticosterone secretion. The finding that melatonin completely attenuated the deleterious effects of CMS on sexual behaviour is an important novel finding with potential implications. The precise mechanism of action of melatonin in this regard is unknown; however, it may be partially explained by altered 5-HT_{2A} receptor activity. Future studies should further investigate the therapeutic effects of melatonin on disrupted behaviour, and explore its precise mode of action.

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