HHS Public Access

Author manuscript

Physiol Behav. Author manuscript; available in PMC 2016 January 04.

Published in final edited form as:

Physiol Behav. 2014 June 10; 132: 79–86. doi:10.1016/j.physbeh.2014.04.049.

Genetic Deletion of the MT_1 or MT_2 Melatonin Receptors Abrogates Methamphetamine-Induced Reward in C3H/HeN Mice

Shannon J. Clough[a], **Anthony J. Hutchinson**[a], **Randall L. Hudson**[b], and **Margarita L. Dubocovich**[a]

[a]Department of Pharmacology & Toxicology, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214

[b]Department of Physiology and Biophysics, School of Medicine and Biomedical Sciences, University at Buffalo, Buffalo, NY 14214

Abstract

The drug of abuse methamphetamine (METH) is known for its ability to enhance reward responses. The rewarding properties of psychostimulants have been shown to vary across time of day in mice. The goal of this study was to determine the role of the MT_1 and MT_2 melatonin receptors in METH-induced reward, as measured by the conditioned place preference (CPP) paradigm during the light and dark phases. C3H/HeN wild-type mice were trained for METH-induced CPP at either ZT 6–8 (ZT: Zeitgeber time; ZT 0 = lights on), when endogenous melatonin levels are low, or ZT 19–21, when melatonin levels are high. These time points also correspond to the high and low points for expression of the circadian gene *Period1*, respectively. The locomotor response to METH (1.2 mg/kg, ip) treatment was of similar magnitude at both times, however only C3H/HeN mice conditioned to METH at ZT 6–8 developed a place preference. C3H/HeN mice with a genetic deletion of either the MT_1 (MT_1KO) or MT_2 (MT_2KO) receptor tested at ZT 6–8 or ZT 19–21 did not develop a place preference for METH, though both showed a similar increase in locomotor activity following METH treatment when compared to wild-type mice. We conclude that in our mouse model METH-induced conditioned place preference is dependent on time of day and the presence of the MT_1 or MT_2 receptors, suggesting a role for melatonin in METH-induced reward.

Address correspondence to: Margarita L. Dubocovich, Ph.D., Department of Pharmacology & Toxicology, School of Medicine and Biomedical Sciences, 3435 Main Street (Farber Hall 102), University at Buffalo (SUNY), Buffalo, NY 14214, Phone: 1-716-829-3048, Fax: 1-716-829-2801, mdubo@buffalo.edu.

Author's contributions

All authors contributed to the conception and design, acquisition, and/or analysis and/or interpretation of data (SJC, AJH, RLH, MLD). SJC conducted all the in vivo experiments, analyzed and interpreted the data in consultation with MLD and all authors. RLH built the chambers and tested the equipment as necessary. SJC drafted the manuscript, which was edited by MLD, and subsequently by all authors. The final version of the manuscript was approved before submission by all authors.

Conflict of interest

The authors declare that DA 021870 to MLD funded this work. The authors declare that over the last three years MLD was a consultant for and received compensation from Takeda Pharmaceutical Northamerica Inc.

Keywords

melatonin; melatonin receptors; C3H/HeN mouse; conditioned place preference; MT_1 or MT_2 knockout mice

1. Introduction

The circadian timing system plays an important role in the expression of psychostimulant induced reward and sensitization [1]. Interestingly, overdose-related emergency room admissions [2] and the concentrations of illicit substances in wastewater [3] have been found to follow a circadian rhythm, suggesting a diurnal variation in drug abuse exists in humans. Similarly, in rodents the expression of conditioned place preference (CPP) for cocaine has been shown to vary according to the time of day the drug is administered [4]. Furthermore, the diurnal variations observed in cocaine-induced CPP are absent in pinealectomized C3H/HeN mice [5], suggesting the involvement of a circadian oscillator, molecule and/or signaling pathway in psychostimulant induced CPP. Clock genes, including *Period 1* (*Per 1*) and CLOCK, have been linked to CPP [4] and drug-induced sensitization [6, 7]. Melatonin, a molecule secreted from the pineal gland following a circadian rhythm with high levels at night and low levels during the day [8], is a potential modulator of psychostimulant-induced CPP. In nocturnal animals the circadian rhythm of melatonin production is entrained by light, with levels of this molecule being elevated during the active phase [9]. In the C3H/HeN mouse pineal and circulating melatonin levels reach their trough between Zeitgeber time (ZT) (ZT0 = lights on) 6–8 and peak between ZT 19–21 [10].

Melatonin exerts its effects through action on two G protein-coupled receptors, termed MT_1 and MT_2 [11]. Recent studies in our laboratory have linked deletion of both the MT_1 and MT_2 receptors to a complete abrogation of methamphetamine (METH)-induced locomotor sensitization, suggesting a role for melatonin in sensitizing responses [12]. Melatonin receptors are located in various brain regions including areas of the reward pathway such as the nucleus accumbens (MT_1), ventral tegmental area (MT_1) [13], substantia nigra reticulata (MT_2) [14] and hippocampus (MT_2) [15, 16]. Melatonin also inhibits depolarization induced dopamine release in the retina [17, 18] and several brain regions including the ventral hippocampus and hypothalamus [19]. Moreover the peak of the circadian gene *Per 1* occurs anti-phase to the peak of melatonin [10, 20], and exogenous melatonin down regulates PER1 through the MT_1 receptor [21–23]. *Per1* expression has been shown to be required for the expression of CPP [4]. Together this evidence suggests a critical role for melatonin as a modulator of reward and reinforcement behaviors. Melatonin, through modulation of receptor function and/or in combination with other circadian molecules (ex. Clock genes such as *Per 1*) [20], may interact to affect the ability of METH to differentially affect CPP across the 24h light/dark cycle [4, 5, 24].

The goal of this study was first to determine the role of the melatonin receptors (MT_1 and MT_2) in METH-induced CPP and to assess potential diurnal variations in this behavior. The CPP paradigm works by pairing a given set of environmental stimuli with the reinforcing properties garnered from a given substance, then allowing for an animal to choose, in a drug free state, which environment they would prefer to spend time in based on the cues present

[25]. This paradigm has been used to assess the reinforcing properties of the highly abused psychostimulant METH [26, 27]. METH exerts its effects through action in the mesolimbic dopamine pathway [28], specifically by reversing the flow of dopamine through the dopamine transporter [29]. This is in contrast to cocaine, which increases dopamine in the synaptic cleft by blocking the dopamine transporter [30]. The release of dopamine is central to the acquisition of reward-seeking behaviors and reward-based learning [31].

Here, we demonstrated that METH-induced place preference in C3H/HeN mice was dependent on the presence of the MT_1 and MT_2 melatonin receptors. METH induced a statistically significant place preference in wild-type mice when administered during the light period (ZT 6–8), however this preference was abrogated during the dark period (ZT 19–21), when melatonin levels are high.

2. Materials and Methods

2.1 Animals and Husbandry

C3H/HeN (138 males) mice were bred and maintained in the Laboratory Animal Facility at the University at Buffalo. Wild-type C3H/HeN mice and C3H/HeN mice homozygous for receptor deletions of the MT_1 melatonin receptor (MT_1KO) were generated in our former laboratory at Northwestern University as previously described [12, 32]. C57Bl/6J MT_1KO mice (donated by Dr. Steven Reppert; Massachusetts General Hospital, Boston, MA, USA) [33, 34] were backcrossed for seven generations with C3H/HeN mice (Harlan, Indianapolis, IN, USA). Female C3H/HeN mice, homozygous for the MT_2 melatonin receptor deletion (MT_2KO) and congenic (8 backcrossings) with C3H/HeN mice were also donated by Dr. Steven Reppert [35]. MT_1KO and MT_2KO mice were bred to male C3H/HeN mice to generate heterozygotes and, subsequently, the respective homozygous mouse lines. Both MT_1KO and MT_2KO mice were congenic with the C3H/HeN strain expressing the *rd* (retina degeneration) mutation on the rod photoreceptor cGMP phosphodiesterase gene and the wild-type allele at the *N*-acetyltransferase (AA-NAT) gene [36]. In order to ensure identical phenotypes among strains, detailed analysis of several behavioral domains using a 24-hour automated video analysis system. We found no differences between the wild-type, MT_1KO, and MT_2KO mice in any of the exploratory, locomotor, ingestive, or sleep-related behaviors (Dubocovich lab, unpublished data).

Mice were maintained in humidity and temperature-controlled (22±1°C) rooms with food (Harlan Teklad 2018sx) and water provided *ad libitum*. All animal procedures were approved by the University at Buffalo Institutional Animal Care and Use Committee and adhered to the National Institutes of Health guidelines.

Male mice were group-housed (3–5 per cage) at weaning and maintained in a 14h: 10h light/dark cycle, with 150 to 200 lux light illumination at the level of the cage. Animals were housed in standard polycarbonate cages (30 × 19 cm) with corncob bedding. At 4–7 weeks of age mice were switched to a 12h: 12h light/dark cycle within ventilated and light-tight cabinets 10–14 days prior to experiment onset. The 12h: 12h light/dark cycle is traditionally used in rodent research paradigms [37]. Additionally, departures toward longer or shorter day lengths were found to increase neophobic behaviors in C3H/He mice [38]. Mice were

weighed every day of the experiment to ensure METH treatment did not result in a loss of greater than 15% of body weight.

2.2 Drug Preparation

(+)-Methamphetamine hydrochloride (Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline and administered at a dose of 1.2 mg/kg via intraperitoneal (ip) injections in a volume of 0.01 ml/g. This dose was chosen based on previous results showing a significant place preference to METH occurred with doses of 1 mg/kg and 2 mg/kg [39]. Furthermore, a dose of 1.2 mg/kg (ip) produced robust sensitization in wild-type C3H/HeN mice in experiments conducted in our laboratory [12]. Mice were randomly assigned to groups receiving either vehicle (VEH: 0.9% saline, ip) or METH (1.2 mg/kg, ip).

2.3 Video Tracking System and Apparatus

Mouse location and distance traveled in the CPP apparatus were monitored by TopScan (CleverSys Inc, Reston, VA, USA) video tracking software. The apparatus consisted of four overhead Sony video cameras, which were set up to observe 8 testing chambers, with each camera providing a top-view image of two chambers. Each chamber was situated over a light panel capable of emitting both white (daytime studies; ZT 6–8) and infrared light (nighttime studies; ZT 19–21). Each test chamber contained three compartments, 2 choice compartments and one neutral center compartment (Figure 1). Each choice compartment measured 15×15×25 cm with a distinct floor texture and wall color. One choice compartment had black walls with a fine metal mesh floor, and the other had white walls with a coarse metal mesh floor. The neutral center compartment measured 10×15×25cm and had grey walls with a smooth floor. The choice compartments were separated from the center compartment by guillotine doors.

2.4 Experimental Design

Experiments were conducted at two time points representing the peak and trough of melatonin production in C3H/HeN mice (WT: Masana et al., 2000 [10]; MT_1KO: M. L. Dubocovich, unpublished data; MT_1KO and MT_2KO: C. von Gall, personal communication). Daytime experiments, representing the trough of melatonin production, were performed from Zeitgeber time (ZT) 6–8 (ZT 0 = lights on). Nighttime experiments, representing the peak of melatonin production, were conducted from ZT 19–21. For all experiments mice were moved into the testing room one hour prior to test onset.

The CPP experimental design was a modified version of the Brown et al. [40] protocol as described in Figure 1. Mice were handled and received saline injections (0.2 ml saline, ip) during the three days prior to the initiation of the experimental protocol in order to acclimate mice to the experimental conditions.

The first day of the experiment (Day 1), known as *habituation*, was used to familiarize the mice to the test chambers, during this session mice were given 20 min of free time within the chamber. On Day 2 mice were once again given 20 min unlimited access to the chamber and a Pre-test (*Pre-CPP*) baseline was established to determine the initial compartment preference. Mice that spent greater than 80% (980s) of the test in any given compartment

were excluded from the remainder of the experiment. Of the total 40 C3H/HeN wild-type mice tested at both ZT 6–8 and ZT 19–21, one was excluded based on this criterion. Similarly, 4 of 51 MT_1KO mice and 9 of 47 MT_2KO were also excluded. Additionally, two C3H/HeN wild-type mice were excluded due to a problem with the video analysis.

Day 3 through Day 8 served as the *conditioning period*. Each conditioning session lasted 60 min during which mice were confined to one compartment. The data used to determine compartment biasing are shown in Table 1. On days 3, 5, and 7 half of the mice received METH (1.2 mg/kg, ip) and half of the mice received vehicle (VEH: 0.9% saline, ip). On these days mice were confined to their initially non-preferred compartment based on the *Pre-CPP* data (Day 2). On Days 4, 6, and 8 all mice received VEH and were confined to their initially preferred compartment. The 60 min session represents a commonly used conditioning time for mice treated with METH [41–44].

On Day 9, termed *Post-CPP*, mice were once again given unlimited access to the chamber for a 20 min test session. The place preference score was calculated by subtracting the Post-CPP time spent in the initially preferred compartment from the Post-CPP time spent in the opposite compartment. Preference scores of significantly greater than zero were considered to indicate reinforcement, whereas scores approximately equal to zero were considered neutral. The stimulant effect of METH was monitored by measuring distance traveled (m) on all days of the experiment.

2.5 Statistical Analysis

2.5.1 CPP—Place preference was characterized by duration spent in the choice compartments during the *Post-CPP* phase. Data for the whole 20-min post-test were analyzed by Students t-test. Time course data were analyzed and then grouped in 5-min bins. Time point averages for each experimental condition were expressed as Mean ± SEM. Time course studies were analyzed by two-way mixed design analysis of variance (ANOVA) having the between groups factor of treatment group (VEH or METH) and time. The Bonferroni correction was used for post hoc comparisons. Student t-tests and ANOVAs were conducted using GraphPad Prism v. 6.01 (GraphPad Software Inc., LaJolla, CA). A power analysis was conducted to ensure all groups had sufficient n values to resolve a significant difference at a minimum of 80% power using the effect size observed in wild-type mice at ZT 6–8 as a reference. The power analysis was conducted using GraphPad Statmate v. 2.0 (GraphPad Software Inc., LaJolla, CA). For all analyses $p < 0.05$ was considered statistically significant.

2.5.2 Locomotor Activity—Locomotor activity was expressed as distance traveled (m). Unconditioned locomotor activity was analyzed by repeated measures analysis of variance followed by Bonferroni post-hoc tests. The locomotor effect of METH was assessed by comparing the distances traveled after METH treatments on Days 3, 5, and 7 to the corresponding distances traveled after VEH treatment on Days 4, 6, and 8.

3. Results

We utilized a three-chamber CPP paradigm to assess METH-induced reward seeking behavior in C3H/HeN mice, as shown in Figure 1. Several precautions were taken in order to provide a rigorous level of control over the experimental conditions. Assessments were systematically conducted between ZT 6–8 and ZT 19–21, corresponding to the peak and trough of melatonin respectively [10]. Comparisons were made among genotype and between two times of day using a METH dose of 1.2mg/kg, which produces robust sensitization in the C3H/HeN mice [12]. These controls allow for accurate conclusions on the effect of melatonin receptor genotype and time of day at a single dose of METH.

3.1 Conditioned Place Preference During the Light Phase (ZT 6–8)

Wild-type mice receiving VEH (n = 13) during the conditioning phase displayed no significant differences in time spent in either choice compartment across the 5-min bins (Figure 2A) or the whole 20-min test session (Figure 2D). Conversely, wild-type mice receiving METH during the conditioning phase spent significantly more time in the compartment paired with METH vs. VEH across every 5-min bin (Figure 2B; $F [1, 60] = 50.41$, $p<0.01$, n = 11) as well as the whole test session (Figure 2E; $p<0.0001$). Preference scores, calculated by subtracting the time spent in the initially preferred compartment from the time spent in the initially non-preferred compartment, were higher in the METH group compared to VEH at each of the 5-min bins (Figure 2C; $F [1, 63] = 34.11$, $p<0.01$) and the whole test session (Figure 2F; $p<0.0001$).

We next assessed the role of the MT_1 and MT_2 melatonin receptors in METH-induced place conditioning during the light phase (ZT 6–8), utilizing mice with a targeted genetic deletion of either receptor. No differences in the time spent in either compartment for the MT_1KO mice treated with VEH (Figure 2G; n = 13) or METH (Figure 2H; n = 17) were observed when compartment duration was examined over the whole 20-min test session. This pattern was similar to that observed in MT_2KO mice, as mice treated with VEH (Figure 2J; n = 8) or METH (Figure 2K; n = 11) did not show any statistically significant differences in time spent in either compartment. Preference scores for MT_1KO and MT_2KO mice (Figure 2I & J) showed no differences between VEH and METH groups.

3.2 Conditioned Place Preference During the Dark Phase (ZT 19–21)

We subsequently examined METH induced CPP during the dark phase (ZT 19–21) when melatonin levels are at their peak [10] and *Per 1* levels are at their trough [20]. Wild-type mice receiving VEH during the conditioning phase displayed no significant differences in time spent in either compartment (Figure 3A & B; n = 7). Similarly, mice receiving METH during the conditioning phase spent approximately the same amount of time in the compartment paired with METH as the compartment paired with VEH (Figure 3C & D; n = 7). Preference scores for both groups were approximately equal to zero, indicating that neither group displayed a significant preference (Figure 3E & F). MT_1KO displayed no differences in the time spent in either compartment when treated with VEH (Figure 3G; n = 8) or METH (Figure 3H; n = 9). This pattern was also observed in MT_2KO mice, as mice treated with VEH (Figure 3J; n = 8) or METH (Figure 3K; n = 10) showed no significant

differences in time spent in either compartment. Preference scores for MT_1KO and MT_2KO mice (Figure 3I & J) also exhibited no differences between VEH and METH groups.

3.3 Locomotor Activity

The locomotor stimulant effect of METH was assessed by within-group comparisons between distances traveled on METH and VEH treatment days. METH elevated locomotor activity above VEH at both ZT 6–8 (Figure 4A: $p<0.05$, $n = 11$) and ZT 19–21 (Figure 4D: $p<0.05$, $n = 7$) in wild-type mice, MT_1KO (Figure 4B: ZT 6–8, $p<0.05$, $n = 17$; Figure 4E: ZT 19–21, $p<0.05$, $n = 9$) and MT_2KO mice (Figure 4C: ZT 6–8, $p<0.05$, $n = 11$; Figure 4F: ZT 19–21, $p<0.05$, $n = 10$). A small but statistically significant difference in METH-induced locomotor activity was observed on Day 7 when compared with Day 3 in WT and MT_1KO mice, but not in MT_2KO mice when testing occurred at ZT 6–8. This elevation of locomotor activity was not observed in any genotype at ZT 19–21.

4. Discussion

This study elucidates a role for the melatonin receptors and time of day in modulating the reinforcing properties of the drug of abuse METH. The results demonstrate a diurnal variation in METH-induced CPP in C3H/HeN mice, with the effect being maximal during the light period (ZT 6–8) and absent during the dark period (ZT 19–21). Together these findings suggest a potential role in METH-induced reward for endogenous circadian oscillators and/or signaling molecules following a diurnal pattern. This could include melatonin and its receptors [10] and/or clock genes such as PER1, whose rhythmicity is dependent upon the presence of pineal melatonin [20].

4.1 Conditioned Place Preference

The present study is the first demonstration of METH-induced place preference in C3H/HeN mice, though this strain has previously been used to examine preference for nicotine [45] and cocaine [5, 46]. The magnitude of preference observed in our study is similar to that reported by Brown et al., 2010 [40], which used a similar protocol to examine cocaine-induced CPP. In the wild-type mice METH induced a strong place preference during the light phase (ZT 6–8) when melatonin levels are low, however place preference was abrogated when melatonin levels are known to be elevated (ZT 19–21) [10, 47, 48]. This diurnal variation in place preference is similar to what has previously been observed in mice undergoing conditioning with cocaine [4, 5]. Interestingly this diurnal variation of preference for cocaine was eliminated in C3H/HeN mice by pinealectomy [5], suggesting pineal products such as melatonin may be essential to the rhythmic expression of CPP.

One possible explanation for the diurnal variation observed could in part be due to the system being preferentially primed for natural rewards. For example the place preference observed in response to sexual performance in male rats follows an opposite pattern to that observed in the current study, with the highest levels of preference observed at ZT 17 and the lowest levels observed at ZT 5 [24]. In the same study preference for amphetamine reward, displayed peaks at ZT 5, 17 and 23 and reached its lowest point at ZT 11. As the peaks for natural and drug reward corresponded to increased levels in tyrosine hydroxylase

in different parts of the mesolimbic dopamine system this suggests the system could be shifting its sensitivity in a circadian fashion [24]. With this in mind it is possible our studies may have occurred at a time when the system would be more receptive to natural rewards than a drug reward.

These studies also demonstrate an important role for the melatonin receptors in METH-induced CPP. Deletion of either the MT_1 or MT_2 receptor at ZT 6–8 abolished place preference. It is interesting to note that this lack of place preference is not due to a global loss of responsiveness to METH in the knockout mice, as METH was able to induce significant locomotor responses in all genotypes tested. At ZT 6–8 METH increased locomotor activity in all genotypes while at ZT 19–21 activity was not significantly enhanced until Day 7. Activity during both light and dark phases followed a similar pattern of increase. The trend in all groups showed locomotor activity increasing across trial days, with significant locomotor sensitization observed in WT and MT_1KO mice at ZT 6–8. This is consistent with our previous finding demonstrating a diurnal variation in METH–induced locomotor sensitization in C3H/HeN mice [12]. Thus we conclude that the effects of melatonin receptor deletion and time of day are specific to METH-induced reinforcing behaviors, as neither of these factors significantly altered the locomotor stimulant properties of METH at the dose tested. CPP to nicotine [49], cocaine [50] and amphetamine [24] have been shown to be dose-dependent [51] However, in this study we elected to run systematic, controlled experiments utilizing a single dose of METH (1.2 mg/kg) in order to test the hypothesis that METH-induced CPP is dependent upon melatonin receptor expression and time of day. We chose the 1.2 mg/kg dose because it induced sensitization without stereotypy in C3H/HeN mice [12]. Interestingly, the locomotor response to METH was unchanged in the MT_1 and MT_2KO mice compared to WT, making it unlikely that the differential effects of METH across genotypes and time of day were due to differences in mouse phenotype and/or drug metabolism. Accordingly it has been suggested there is a positive link between the rewarding and stimulating effects of drugs of abuse [52, 53]. These results imply a role for the melatonin receptors in METH-induced reward; more specifically the receptors appear to be necessary for the reinforcing properties of the drug to be expressed at the dose tested.

Genetic deletion of either the MT_1 or MT_2 melatonin receptors impaired the ability of METH to induce a place preference during the light phase, suggesting the melatonin receptors may be activated during this period. Melatonin receptor activation, even when the levels of melatonin are low [10], could result from tight, irreversible binding of picomolar concentration of nocturnal endogenous melatonin to a population of receptors in a high affinity state [54, 55] or by the presence of constitutively active receptors [56, 57]. The lack of METH-induced CPP during the dark period when melatonin levels are high could be due to the presence of melatonin receptor desensitization [58, 59]. Brain regions involved in METH-induced reward are located close to the third ventricle, where melatonin has been shown reach concentrations at least 10-fold higher than in the circulating blood in several species, including sheep [60, 61], goats [62], and calves [63]. With the caveat that the melatonin concentration in rodent cerebrospinal fluid has not been measured, it is conceivable that high melatonin levels in the mouse brain relative to the circulation could

result in desensitization of the melatonin receptors in brain areas involved in reward, especially at night when melatonin levels are already increased.

The induction of place preference in wild-type mice may be accounted for by the formation of MT_1/MT_2 heterodimers [64]. Recently the presence of MT_1/MT_2 heteromers has been demonstrated in vivo in the retina [65], as disruption of the MT_1/MT_2 heteromers results in a lack of circadian rhythm of the a-wave and b-wave of the scotopic electroretinogram [65, 66]. These heteromers are capable of providing a different pharmacological response pattern to that of either the MT_1 or MT_2 receptor individually. Provided the MT_1 and MT_2 receptors are localized to the same cell population, these heteromer-mediated signaling pathways may be responsible for the expression of CPP. Deletion of either receptor as in the MT_1KO or MT_2KO would disrupt the formation of herteromers thus resulting in a lack of place preference. Further studies are needed to determine the specific role of the heteromers in METH-induced CPP.

Another potential candidate for the nocturnal suppression of CPP relative to the light phase is the circadian gene *Per 1*, expression of which follows a circadian rhythm with a peak occurring around ZT 5 and its lowest levels occurring between ZT 17 – 21 in the nucleus accumbens and caudate putamen of C3H/HeN mice [20]. PER1 has been linked to the actions of psychostimulants by the observations that *Per1* knockout mice are unable to express CPP [4] and that acute METH treatment up regulates PER1 protein levels in mouse caudate-putamen [6]. PER1 is potently regulated by melatonin, as shown by the complete abolishment of PER1 rhythmicity in pinealectomized C3H/HeN mice [20] and the acute down regulation of PER1 through the MT_1 receptor in melatonin-treated mouse striatal neuron cultures [21] and the pars tuberalis [22, 23] of C3H/HeN mice. The lack of preference observed at ZT 19 – 21 in our studies coincides with the timing for reduced levels of PER1. Future studies may determine that METH-induced CPP may be negatively correlated with PER1 expression.

4.2 Conclusions

This study demonstrated for the first time that the MT_1 and MT_2 melatonin receptors are necessary for the expression of METH-induced CPP in the C3H/HeN mice during the light phase. The lack of METH-induced place preference at night in the wild-type mice could potentially result from melatonin receptor desensitization, receptor heterodimerization and/or circadian variations in clock gene expression, particularly PER1. Locomotor responses to METH during the condition phase were similar in all genotypes suggesting the loss of place preference observed at night is not due to a global loss of responsiveness to METH. The link between melatonin receptors and clock gene expression (sensitivity) with circadian variations in METH-induced reward may help elucidate the mechanism(s) by which this drug of abuse induces reward in humans.

Acknowledgments

The authors would like to thank Iwona Stepien for expert technical assistance with animal management and equipment maintenance. We are indebted to Dr. Jun-Xu Li for his expert guidance on experimental design, perspectives on data interpretation and critical feedback on earlier versions of the manuscript.

References

1. McClung CA. Circadian rhythms, the mesolimbic dopaminergic circuit, and drug addiction. Scientific World Journal. 2007; 7:194–202. [PubMed: 17982593]
2. Raymond R, Warren M, Morris R, Leikin J. Periodicity of presentations of drugs of abuse and overdose in an emergency department. J Toxicol Clin Toxicol. 1992; 30(3):467–78. [PubMed: 1512819]
3. Brewer AJ, Ort C, Banta-Green CJ, Berset JD, Field JA. Normalized Diurnal and Between-Day Trends in Illicit and Legal Drug Loads that Account for Changes in Population. Environ Sci Technol. 2012; 46(15):8305–14. [PubMed: 22804833]
4. Abarca C, Albrecht U, Spanagel R. Cocaine sensitization and reward are under the influence of circadian genes and rhythm. Proc Natl Acad Sci U S A. 2002; 99(13):9026–30. [PubMed: 12084940]
5. Kurtuncu M, Arslan AD, Akhisaroglu M, Manev H, Uz T. Involvement of the pineal gland in diurnal cocaine reward in mice. Eur J Pharmacol. 2004; 489(3):203–5. [PubMed: 15087244]
6. Nikaido T, Akiyama M, Moriya T, Shibata S. Sensitized increase of period gene expression in the mouse caudate/putamen caused by repeated injection of methamphetamine. Mol Pharmacol. 2001; 59(4):894–900. [PubMed: 11259635]
7. Falcon E, McClung CA. A role for the circadian genes in drug addiction. Neuropharmacology. 2009; 56(Suppl 1):91–6. [PubMed: 18644396]
8. Reiter RJ. Melatonin: the chemical expression of darkness. Mol Cell Endocrinol. 1991; 79(1–3):C153–8. [PubMed: 1936532]
9. Turek FW, Gillette MU. Melatonin, sleep, and circadian rhythms: rationale for development of specific melatonin agonists. Sleep Medicine. 2004; 5(6):523–32. [PubMed: 15511698]
10. Masana MI, Benloucif S, Dubocovich ML. Circadian rhythm of mt1 melatonin receptor expression in the suprachiasmatic nucleus of the C3H/HeN mouse. J Pineal Res. 2000; 28(3):185–92. [PubMed: 10739306]
11. Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. Pharmacol Rev. 2010; 62(3):343–80. [PubMed: 20605968]
12. Hutchinson AJ, Hudson RL, Dubocovich ML. Genetic deletion of MT1 and MT2 melatonin receptors differentially abrogates the development and expression of methamphetamine-induced locomotor sensitization during the day and the night in C3H/HeN mice. J Pineal Res. 2012; 53(4): 399–409. [PubMed: 22672659]
13. Uz T, Arslan AD, Kurtuncu M, Imbesi M, Akhisaroglu M, Dwivedi Y, Pandey GN, Manev H. The regional and cellular expression profile of the melatonin receptor MT1 in the central dopaminergic system. Brain Res Mol Brain Res. 2005; 136(1–2):45–53. [PubMed: 15893586]
14. Ochoa-Sanchez R, Comai S, Lacoste B, Bambico FR, Dominguez-Lopez S, Spadoni G, Rivara S, Bedini A, Angeloni D, Fraschini F, Mor M, Tarzia G, Descarries L, Gobbi G. Promotion of non-rapid eye movement sleep and activation of reticular thalamic neurons by a novel MT2 melatonin receptor ligand. J Neurosci. 2011; 31(50):18439–52. [PubMed: 22171046]
15. Wang LM, Suthana NA, Chaudhury D, Weaver DR, Colwell CS. Melatonin inhibits hippocampal long-term potentiation. Eur J Neurosci. 2005; 22(9):2231–7. [PubMed: 16262661]
16. Savaskan E, Ayoub MA, Ravid R, Angeloni D, Fraschini F, Meier F, Eckert A, Muller-Spahn F, Jockers R. Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. J Pineal Res. 2005; 38(1):10–6. [PubMed: 15617532]
17. Dubocovich ML. Melatonin is a potent modulator of dopamine release in the retina. Nature. 1983; 306(5945):782–4. [PubMed: 6656879]
18. Dubocovich ML, Masana MI, Iacob S, Sauri DM. Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. Naunyn Schmiedebergs Arch Pharmacol. 1997; 355(3):365–75. [PubMed: 9089668]

19. Zisapel N, Egozi Y, Laudon M. Inhibition of dopamine release by melatonin: regional distribution in the rat brain. Brain Res. 1982; 246(1):161–3. [PubMed: 7127086]
20. Uz T, Akhisaroglu M, Ahmed R, Manev H. The Pineal Gland is Critical for Circadian Period1 Expression in the Striatum and for Circadian Cocaine Sensitization in Mice. Neuropsychopharmacology. 2003; 28(12):2117–23. [PubMed: 12865893]
21. Imbesi M, Arslan AD, Yildiz S, Sharma R, Gavin D, Tun N, Manev H, Uz T. The melatonin receptor MT1 is required for the differential regulatory actions of melatonin on neuronal 'clock' gene expression in striatal neurons in vitro. J Pineal Res. 2009; 46(1):87–94. [PubMed: 18798788]
22. von Gall C, Weaver DR, Moek J, Jilg A, Stehle JH, Korf HW. Melatonin Plays a Crucial Role in the Regulation of Rhythmic Clock Gene Expression in the Mouse Pars Tuberalis. Ann N Y Acad Sci. 2005; 1040(1):508–11. [PubMed: 15891103]
23. von Gall C, Garabette ML, Kell CA, Frenzel S, Dehghani F, Schumm-Draeger PM, Weaver DR, Korf HW, Hastings MH, Stehle JH. Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. Nat Neurosci. 2002; 5(3):234–8. [PubMed: 11836530]
24. Webb IC, Baltazar RM, Wang X, Pitchers KK, Coolen LM, Lehman MN. Diurnal variations in natural and drug reward, mesolimbic tyrosine hydroxylase, and clock gene expression in the male rat. J Biol Rhythms. 2009; 24(6):465–76. [PubMed: 19926806]
25. Tzschentke TM. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. Prog Neurobiol. 1998; 56(6):613–72. [PubMed: 9871940]
26. Zakharova E, Leoni G, Kichko I, Izenwasser S. Differential effects of methamphetamine and cocaine on conditioned place preference and locomotor activity in adult and adolescent male rats. Behav Brain Res. 2009; 198(1):45–50. [PubMed: 18996417]
27. Kuo CS, Chai SC, Chen HH. Mediodorsal nucleus of the thalamus is critical for the expression of memory of methamphetamine-produced conditioned place preference in rats. Neuroscience. 2011; 178:138–46. [PubMed: 21256933]
28. Mortensen OV, Amara SG. Dynamic regulation of the dopamine transporter. Eur J Pharmacol. 2003; 479(1–3):159–70. [PubMed: 14612147]
29. Sulzer D, Sonders MS, Poulsen NW, Galli A. Mechanisms of neurotransmitter release by amphetamines: a review. Prog Neurobiol. 2005; 75(6):406–33. [PubMed: 15955613]
30. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature. 1996; 379(6566): 606–12. [PubMed: 8628395]
31. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev. 1998; 28(3):309–69. [PubMed: 9858756]
32. Sumaya IC, Masana MI, Dubocovich ML. The antidepressant-like effect of the melatonin receptor ligand luzindole in mice during forced swimming requires expression of MT2 but not MT1 melatonin receptors. J Pineal Res. 2005; 39(2):170–7. [PubMed: 16098095]
33. Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. Neuron. 1997; 19(1):91–102. [PubMed: 9247266]
34. Dubocovich ML, Hudson RL, Sumaya IC, Masana MI, Manna E. Effect of MT1 melatonin receptor deletion on melatonin-mediated phase shift of circadian rhythms in the C57BL/6 mouse. J Pineal Res. 2005; 39(2):113–20. [PubMed: 16098087]
35. Jin X, von Gall C, Pieschl RL, Gribkoff VK, Stehle JH, Reppert SM, Weaver DR. Targeted disruption of the mouse Mel1b melatonin receptor. Mol Cell Biol. 2003; 23(3):1054–60. [PubMed: 12529409]
36. Pittler SJ, Baehr W. Identification of a nonsense mutation in the rod photoreceptor cGMP phosphodiesterase beta-subunit gene of the rd mouse. Proc Natl Acad Sci. 1991; 88(19):8322–6. [PubMed: 1656438]
37. Faith, R.; Huerkamp, M. Environmental considerations for research animals. In: Hessler, J.; Lehner, N., editors. Planning and designing research animal facilities. Elsevier; London: 2009. p. 59-83.

38. Kopp C, Vogel E, Rettori M, Delagrange P, Renard P, Lesieur D, Misslin R. Regulation of emotional behaviour by day length in mice: implication of melatonin. Behav Pharmacol. 1999; 10:747–52. [PubMed: 10780290]
39. Tzschentke TM. REVIEW ON CPP: Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. Addict Biol. 2007; 12(3–4):227–462. [PubMed: 17678505]
40. Brown RM, Short JL, Lawrence AJ. Identification of brain nuclei implicated in cocaine-primed reinstatement of conditioned place preference: a behaviour dissociable from sensitization. PLoS ONE. 2010; 5(12):e15889. [PubMed: 21209913]
41. Qi J, Yang J-Y, Wang F, Zhao Y-N, Song M, Wu C-F. Effects of oxytocin on methamphetamine-induced conditioned place preference and the possible role of glutamatergic neurotransmission in the medial prefrontal cortex of mice in reinstatement. Neuropharmacology. 2009; 56(5):856–65. [PubMed: 19371575]
42. Kamei J, Ohsawa M. Effects of diabetes on methamphetamine-induced place preference in mice. Eur J Pharmacol. 1996; 318(2–3):251–6. [PubMed: 9016912]
43. Itzhak Y, Martin JL, Ali SF. Methamphetamine-induced dopaminergic neurotoxicity in mice: Long-lasting sensitization to the locomotor stimulation and desensitization to the rewarding effects of methamphetamine. Prog Neuropsychopharmacol Biol Psychiatry. 2002; 26(6):1177–83. [PubMed: 12452543]
44. Shimosato K, Ohkuma S. Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine. Pharmacol Biochem Behav. 2000; 66(2):285–92. [PubMed: 10880680]
45. Agatsuma S, Lee M, Zhu H, Chen K, Shih JC, Seif I, Hiroi N. Monoamine oxidase A knockout mice exhibit impaired nicotine preference but normal responses to novel stimuli. Hum Mol Genet. 2006; 15(18):2721–31. [PubMed: 16893910]
46. Eisener-Dorman AF, Grabowski-Boase L, Tarantino LM. Cocaine locomotor activation, sensitization and place preference in six inbred strains of mice. Behav Brain Funct. 2011; 7(1):29. [PubMed: 21806802]
47. Goto M, Oshima I, Tomita T, Ebihara S. Melatonin content of the pineal gland in different mouse strains. J Pineal Res. 1989; 7(2):195–204. [PubMed: 2769571]
48. Vivien-Roels B, Malan A, Rettori M-C, Delagrange P, Jeanniot J-P, Pévet P. Daily variations in pineal melatonin concentrations in inbred and outbred mice. J Biol Rhythms. 1998; 13(5):403–9. [PubMed: 9783231]
49. Jackson KJ, Marks MJ, Vann RE, Chen X, Gamage TF, Warner JA, Damaj MI. Role of α5 Nicotinic Acetylcholine Receptors in Pharmacological and Behavioral Effects of Nicotine in Mice. J Pharmacol Exp Ther. 2010; 334(1):137–46. [PubMed: 20400469]
50. Hall FS, Drgonova J, Goeb M, Uhl GR. Reduced Behavioral Effects of Cocaine in Heterozygous Brain-Derived Neurotrophic Factor (BDNF) Knockout Mice. Neuropsychopharmacology. 2003; 28(8):1485–90. [PubMed: 12784114]
51. Uhl GR, Drgonova J, Hall FS. Curious cases: Altered dose–response relationships in addiction genetics. Pharmacol Ther. 2014; 141(3):335–46. [PubMed: 24189489]
52. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Brain Res Rev. 1993; 18(3):247–91. [PubMed: 8401595]
53. Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. Psychol Rev. 1987; 94(4): 469–92. [PubMed: 3317472]
54. Witt-Enderby PA, Dubocovich ML. Characterization and regulation of the human ML1A melatonin receptor stably expressed in Chinese hamster ovary cells. Mol Pharmacol. 1996; 50(1): 166–74. [PubMed: 8700109]
55. Browning C, Beresford I, Fraser N, Giles H. Pharmacological characterization of human recombinant melatonin mt(1) and MT(2) receptors. Br J Pharmacol. 2000; 129(5):877–86. [PubMed: 10696085]
56. Ersahin C, Masana MI, Dubocovich ML. Constitutively active melatonin MT(1) receptors in male rat caudal arteries. Eur J Pharmacol. 2002; 439(1–3):171–2. [PubMed: 11937107]

57. Soares JM Jr, Masana MI, Ersahin C, Dubocovich ML. Functional melatonin receptors in rat ovaries at various stages of the estrous cycle. J Pharmacol Exp Ther. 2003; 306(2):694–702. [PubMed: 12721330]

58. Gerdin MJ, Masana MI, Ren D, Miller RJ, Dubocovich ML. Short-term exposure to melatonin differentially affects the functional sensitivity and trafficking of the hMT1 and hMT2 melatonin receptors. J Pharmacol Exp Ther. 2003; 304(3):931–9. [PubMed: 12604667]

59. Gerdin MJ, Masana MI, Rivera-Bermudez MA, Hudson RL, Earnest DJ, Gillette MU, Dubocovich ML. Melatonin desensitizes endogenous MT2 melatonin receptors in the rat suprachiasmatic nucleus: relevance for defining the periods of sensitivity of the mammalian circadian clock to melatonin. FASEB J. 2004; 18(14):1646–56. [PubMed: 15522910]

60. Skinner DC, Malpaux B. High Melatonin Concentrations in Third Ventricular Cerebrospinal Fluid Are Not due to Galen Vein Blood Recirculating through the Choroid Plexus. Endocrinology. 1999; 140(10):4399–405. [PubMed: 10499491]

61. Shaw PF, Kennaway DJ, Seamark RF. Evidence of high concentrations of melatonin in lateral ventricular cerebrospinal fluid of sheep. J Pineal Res. 1989; 6(3):201–8. [PubMed: 2709303]

62. Kanematsu N, Mori Y, Hayashi S, Hoshino K. Presence of a distinct 24-hour melatonin rhythm in the ventricular cerebrospinal fluid of the goat. J Pineal Res. 1989; 7(2):143–52. [PubMed: 2769567]

63. Hedlund L, Lischko M, Rollag M, Niswender G. Melatonin: daily cycle in plasma and cerebrospinal fluid of calves. Science. 1977; 195(4279):686–7. [PubMed: 841305]

64. Jockers R, Maurice P, Boutin JA, Delagrange P. Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? Br J Pharmacol. 2008; 154(6):1182–95. [PubMed: 18493248]

65. Baba K, Benleulmi-Chaachoua A, Journe AS, Kamal M, Guillaume JL, Dussaud S, Gbahou F, Yettou K, Liu C, Contreras-Alcantara S, Jockers R, Tosini G. Heteromeric MT1/MT2 Melatonin Receptors Modulate Photoreceptor Function. Sci Signal. 2013; 6(296):ra89. [PubMed: 24106342]

66. Baba K, Pozdeyev N, Mazzoni F, Contreras-Alcantara S, Liu C, Kasamatsu M, Martinez-Merlos T, Strettoi E, Iuvone PM, Tosini G. Melatonin modulates visual function and cell viability in the mouse retina via the MT1 melatonin receptor. Proc Natl Acad Sci. 2009; 106(35):15043–8. [PubMed: 19706469]

Highlights

- Methamphetamine-induced CPP in mice during the light but not the dark period.
- Deletion of the MT_1 or MT_2 melatonin receptors blocked methamphetamine-induced CPP.
- MT_1 and MT_2 melatonin receptors are necessary for methamphetamine to induce CPP.

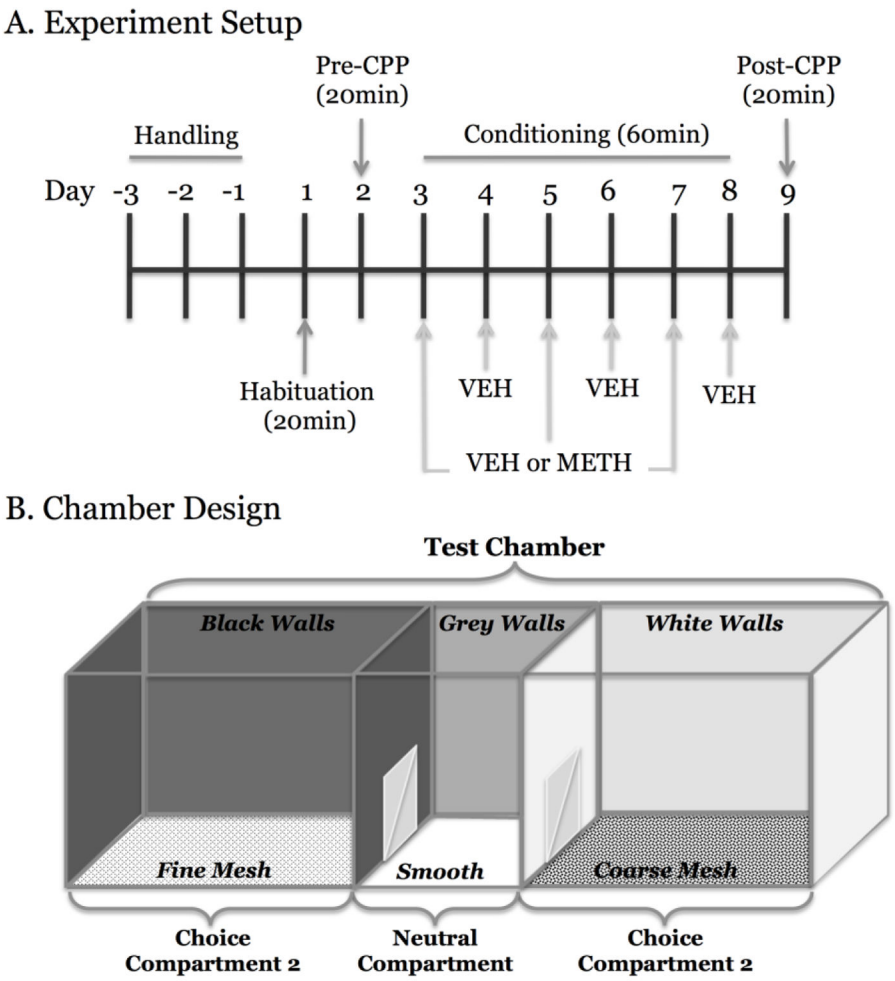

Figure 1. Experimental Design

Mice were subjected to 3 days of handling during which they were weighed and restrained long enough to inject 0.2ml saline, ip. Day 1 (*Habituation*) and Day 2 (*Pre-CPP*) consisted of free access to the entire test chamber for 20 min in order to eliminate novelty. Time spent in each choice compartment during the *Pre-CPP* was used to determine the initial compartment preference. Days 3–8 consisted of 60 min conditioning sessions during which mice were confined to one choice compartment and either METH (1.2mg/kg, ip) or VEH (0.9% saline, ip) was administered as indicated. Day 9 (*Post-CPP*) consisted of free access to the entire test chamber for a 20 min test session to determine final compartment preference.

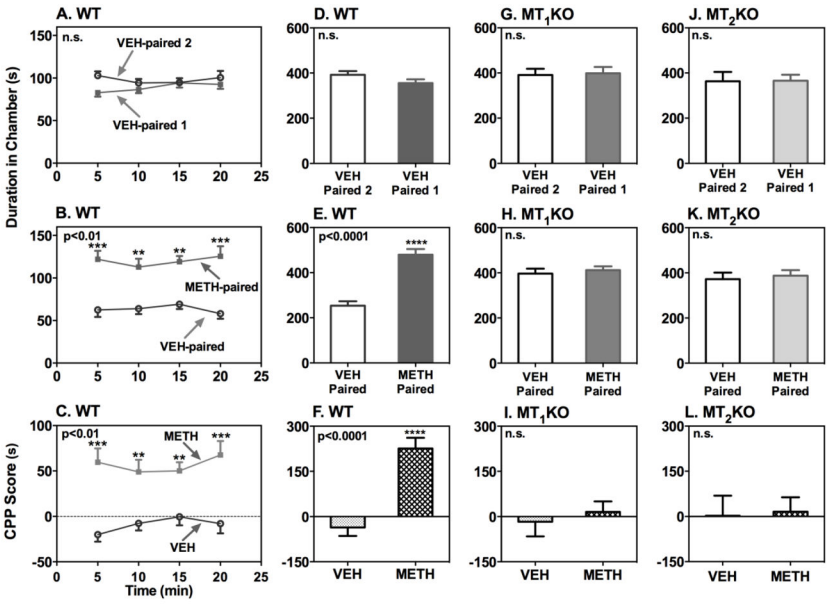

Figure 2. Effect of MT_1 or MT_2 Melatonin Receptor Deletion on METH-Induced Place Preference at ZT 6–8 (light phase)

Panels A – C: Time spent in each compartment during Post-CPP was measured in 5-min bins for the wild-type mice following treatment with VEH (A: n = 12) and METH (B: n = 11). CPP scores were calculated for each 5-min bin by subtracting time spent in the initially preferred compartment from time spent in the initially non-preferred compartment (C). Data represent mean ± S.E.M. of time (s) spent in each compartment during each 5 min bin. An overall effect of treatment was assessed using Two-Way ANOVA, with a main effect of treatment indicated by the p value in the upper left corner. ** $p < 0.01$; *** $p < 0.001$ when compared with VEH treated (Bonferoni post- test).

Panels D – L: Total duration of time spent in each compartment during the whole (20 min) Post-CPP following treatment with VEH or METH respectively was measured for wild-type (D: n = 12 or E: n = 11), MT_1KO (G: n = 13 or H: n = 17) and MT_2KO (J: n = 8 or K: n = 11) mice. Bars represent mean ± S.E.M. of time (s) spent in either compartment. **** $p< 0.0001$ when compared with duration spent in VEH-paired compartment (Student's t-test). CPP scores were calculated by subtracting time spent in the initially preferred compartment from time spent in the initially non-preferred compartment for wild-type (F), MT_1KO (I) and MT_2KO (L) mice for the whole 20 min test session. **** $p<0.0001$ when compared with VEH (Student's t-test). s: second; n.s.: non-significant.

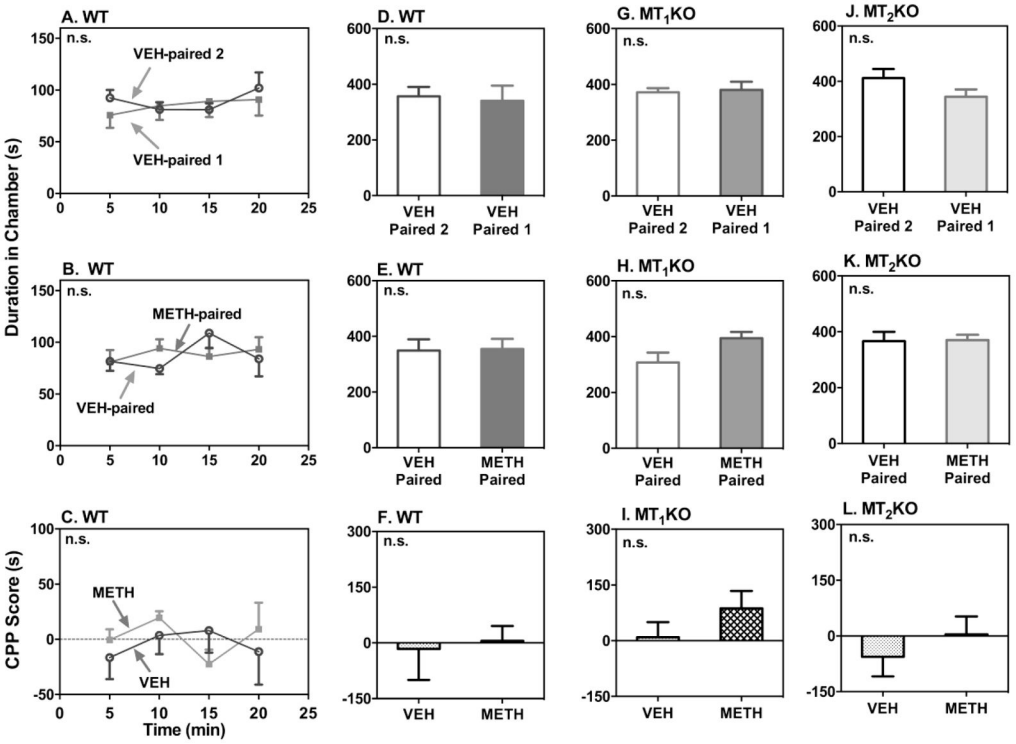

Figure 3. Effect of MT_1 or MT_2 Melatonin Receptor Deletion on METH-Induced Place Preference at ZT 19–21 (dark phase)

Panels A – C: Time spent in each compartment during Post-CPP was measured in 5-min bins for the wild-type mice following treatment with VEH (A: n = 7) and METH (B: n = 7). CPP scores were calculated for each 5-min bin by subtracting time spent in the initially preferred compartment from time spent in the initially non-preferred compartment (C). Data represent mean ± S.E.M. of time (s) spent in each compartment during each 5 min bin. An overall effect of treatment was assessed using Two-Way ANOVA, with a main effect of treatment indicated by the p value in the upper left corner.

Panels D – L: Total duration of time spent in each compartment during the whole (20 min) Post-CPP following treatment with VEH or METH respectively was measured for wild-type (D: n = 7 or E: n = 7), MT_1KO (G: n = 8 or H: n = 9) and MT_2KO (J: n = 8 or K: n = 10) mice. Bars represent mean ± S.E.M. of time (s) spent in either compartment. Compartment durations were compared using Student's t-test.

CPP scores were calculated by subtracting time spent in the initially preferred compartment from time spent in the initially non-preferred compartment for wild-type (F), MT_1KO (I) and MT_2KO (L) mice for the whole 20 min test session. CPP scores were compared using Student's t-test. s: second; n.s.: non-significant.

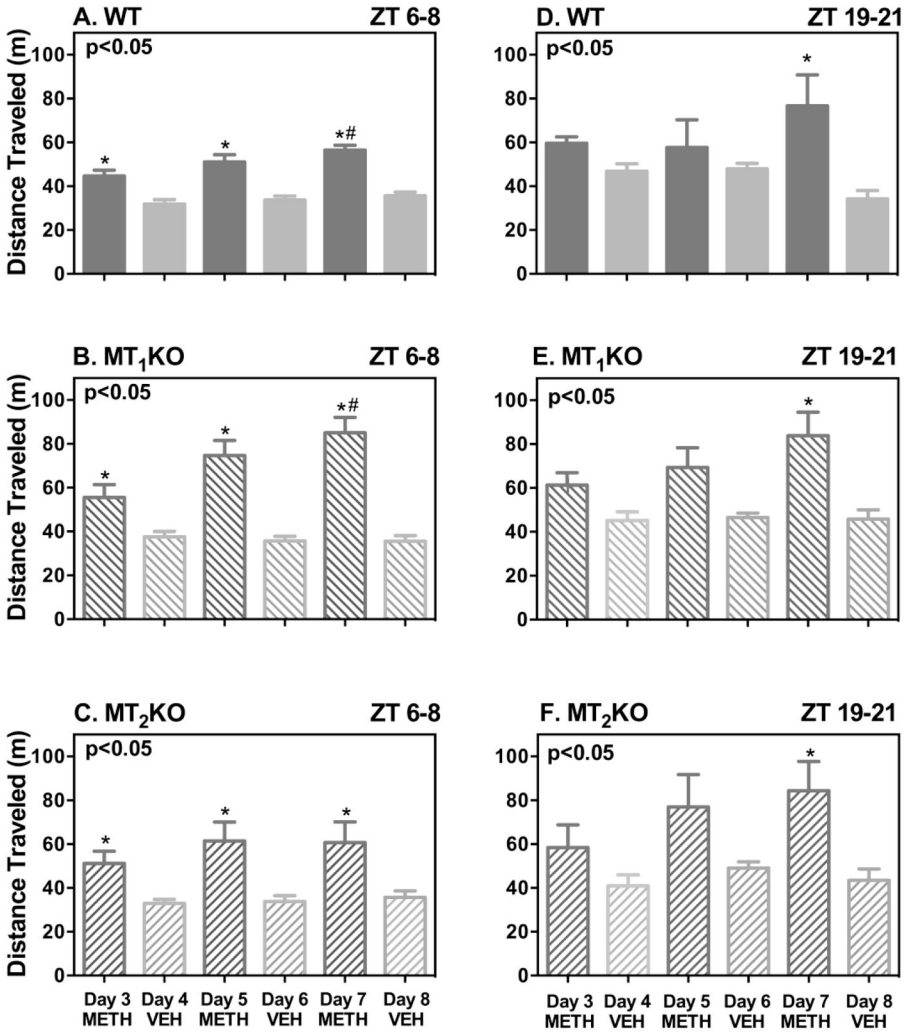

Figure 4. Distance Traveled During Conditioning Days

Distance traveled was measured for the METH treated group during each conditioning day, with Days 3, 5, and 7 representing the days of METH treatment (1.2 mg/kg, ip) and Days 4, 6, and 8 representing days of VEH treatment (0.9% saline, ip). Effect of treatment was compared using One-Way ANOVA. Bars represent the mean ± S.E.M. of distance traveled expressed in meters (m) at ZT 6–8 (light period) for the wild-type (A: n = 11), MT_1KO (B: n = 17) and MT_2KO (C: n = 11) mice as well as at ZT 19–21 (dark period) for the wild-type (D: n = 7), MT_1KO (E: n = 9) and MT_2KO (F: n = 10) mice. *p<0.05 when comparing METH treatment days with the preceding VEH treatment days; # p<0.05 when comparing Day 7 (METH treatment) to Day 3 (METH treatment).

Table 1

Time Spent in Choice Compartments During Pre-CPP

Genotype	ZT	n	Compartment 1 Seconds	Compartment 2 Seconds	*p*
WT	6 – 8	23	284.7 ± 30.4 s	393.5 ± 40.9 s	< 0.05
	19 – 21	14	251.2 ± 25.6 s	412.4 ± 60.8 s	n.s.
MT1KO	6 – 8	30	335.0 ± 30.6 s	411.7 ± 25.3 s	n.s.
	19 – 21	17	318.0 ± 18.7 s	413.7 ± 23.4 s	< 0.05
MT2KO	6 – 8	19	248.4 ± 27.8 s	430.7 ± 55.7 s	n.s.
	19 – 21	17	202.0 ± 16.2 s	509.6 ± 41.1 s	< 0.05

Note: Data were compared by paired t-test.