MDMA induces *Per1*, *Per2* and *c-fos* gene expression in rat suprachiasmatic nuclei

Rowan P. Ogeil · David J. Kennaway · Mark D. Salkeld · Shantha M. W. Rajaratnam · Jillian H. Broadbear

Received: 8 June 2011 / Accepted: 9 October 2011 / Published online: 26 October 2011 © Springer-Verlag 2011

Abstract

**Rationale** 3,4-Methylenedioxymethamphetamine (MDMA, ‘ecstasy’) is a psychoactive drug that has marked effects on the serotonergic system. Serotonergic agonists are known to interact with the circadian pacemaker located in the suprachiasmatic nuclei (SCN).

**Objectives** Given changes reported in the behavioral activity rhythm following MDMA treatment, the effects of MDMA on core clock gene (*Per1*, *Per2*) and *c-fos* expression were evaluated.

**Methods** Male Long-Evans rats (*n* = 72) were injected once with MDMA (5 mg/kg i.p.) or saline either at the middle of their ‘rest’ phase (Zeitgeber Time: ZT6) or the middle of their ‘active’ phase (Zeitgeber Time: ZT16) and killed at 30, 60, or 120 min posttreatment for gene expression analysis in the SCN using PCR. Behavioral rhythms of a separate group of rats (*n* = 20) were measured following treatment at ZT16 while they were held in constant darkness for 10 days posttreatment.

**Results** At ZT6, *c-fos* mRNA was significantly induced 120 min post-MDMA treatment but there were no significant changes in *Per1* or *Per2* mRNA expression. At ZT16, there were significant inductions of *c-fos* mRNA (30 and 60 min) and *Per1* and *Per2* mRNA (both 60 min) post-MDMA treatment. However, no differences in behavioral activity patterns were noted following MDMA treatment at ZT16.

**Conclusions** These data provide evidence that MDMA has time of day dependent actions on SCN functioning, as evident from its induction of core clock genes that are important for generating and maintaining circadian rhythmicity.

**Keywords** MDMA · Circadian · Clock genes · Rat · Activity

Introduction

Many organisms have evolved to synchronize with a 24-hour day in order to coordinate their internal biological processes with external environmental cues (Reppert and Weaver 2002; reviewed in Weaver (1998)). Circadian rhythmicity is genetically determined and endogenously generated (Moore 2003) by the central circadian pacemaker located in the hypothalamic suprachiasmatic nuclei (SCN) (Dijk and Czeisler 1995; Moore and Eichler 1972; Stephan and Zucker 1972). Circadian timing is accomplished via a number of hierarchical oscillators (Reppert and Weaver 2002) controlled by a series of cellular positive and negative feedback loops involving the clock gene corepressors *Clock, Bmal1, Per1,2* and *Cry1,2* (reviewed in Reppert and Weaver (2001)).

Light is the most potent synchronizer of the SCN, with exposure during the subjective night causing phase shifts linked to the induction of *Per1* and *Per2* (Albrecht 2002; Reppert and Weaver 2001). Nonphotic cues including melatonin, induced wheel-running and 5-HT agonists also modulate the SCN (reviewed in Challet (2007), Kennaway et al. (1996), and Mistlberger (1991)). The role of serotonin (5-HT) in modulating the circadian pacemaker is complex. Serotonin agonists can reset the central pacemaker, differ-
ing in their effects according to when they are administered (reviewed in Kennaway (2004), Varcoe and Kennaway (2008)). For example, Kohler et al. (1999) reported that rats treated with quipazine (a nonselective 5-HT agonist), during the subjective night (CT 22) had phase advanced behavioral rhythms and significant increases in c-fos induction in the ventrolateral SCN. Rats treated earlier in the night (circadian time: CT 14) were phase delayed, while those treated during the subjective day (CT 6) demonstrated no behavioral phase shift or induction of c-fos compared with controls. Other research has also examined differences in the time course of phase responses to serotonergic agonists (Cuesta et al. 2009; Cutrera et al. 1996; Horikawa and Shibata 2004) with inconsistencies demonstrated. For example, Edgar et al. (1993) found that quipazine and 8-OH-DPAT phase advanced activity and drinking rhythms in rats when administered during the subjective day (CT 6), but not during the night (CT 18). These studies demonstrate the complex role played by 5-HT at multiple 5-HT receptor subtypes in its interaction with the circadian system (Graff et al. 2007; Kennaway and Moyer 1998).

MDMA during the active phase.

These changes in circadian parameters following treatment with MDMA at ZT6 may be secondary to an overall lengthening of the active phase, or alternatively, may suggest that MDMA has actions directly at the SCN (Ogeil et al. 2010). The first aim of the current study was to investigate the expression of Per1, Per2 and c-fos mRNA in the rat SCN following administration of MDMA at ZT6 and ZT16. The second aim was to determine whether any changes in the expression of Per1, Per2 and c-fos mRNA following treatment at ZT16 were associated with changes in the circadian rhythm of activity.

Methods

Gene expression study

Animals

Male Long–Evans rats (~200 g, n=72) sourced from Monash University Animal Services (Clayton, Australia) were group-housed for acclimatization for 1 week prior to being transferred to individual wire/acrylic cages (250×300×250 mm) fitted with a running wheel. They were given ad libitum access to food and water. The laboratory lighting comprised two overhead lights containing incandescent 60 W globes, two light banks on the wall each containing five incandescent 25 W globes, and two lamps containing red globes (15 W) behind a LEE light filter (Number 211, Lightmoves, Melbourne, Australia). The latter were continuously illuminated to facilitate routine husbandry and maintenance. During dark periods, the light level recorded in the laboratory was less than 0.1 lx at cage level. All experimental procedures were approved by the School of Psychology Animal Ethics Committee at Monash University. Procedures were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th Edition).

Treatment and sample preparation

After 1 week of individual housing, rats were randomized to receive either MDMA (5 mg/kg) or saline (vehicle) delivered by intraperitoneal injection (i.p., 0.5 ml/kg) using a 30-gauge needle at either 6 (ZT6) or 16 (ZT16) hours after lights on. Rats were subsequently killed by decapitation 30, 60 or 120 min posttreatment (n=6 rats/group) following a brief exposure to isoflurane. Brains were removed and immediately stored in RNAlater® (Ambion, Texas, USA) for 24 h at 4°C, and then kept at −20°C. For processing, a 1-mm coronal section containing the SCN was prepared from each brain using a Vibroslice仪 (Campden Instruments, London, UK) placed on a slide and frozen on
dry ice for approximately 2 min. SCN from both hemispheres were subsequently punched out using a modified 22-gauge needle and expelled into 100 μl of Ambion RNAqueous lysis buffer (Ambion, Texas, USA). Brain sections were subsequently examined under a dissecting microscope to confirm that both SCN had been completely punched out. Samples were then stored at −20°C. In nine cases, the SCN could not be recovered due to damage caused by torsion of the optic nerve during brain removal.

RNA extraction and quantitation

Ambion RNAqueous® micro kits (Ambion, Texas, USA) were used to extract RNA from the SCN samples, which was further processed as previously described (Varcoe et al. 2003; Varcoe and Kennaway 2008). After lysis of the SCN tissue, the lysate/ethanol mixture was loaded onto a microfilter cartridge and centrifuged. The microfilter was washed with Ambion RNAqueous Wash Solution 1 and centrifuged and washed twice with Ambion RNAqueous Wash Solution 2. The cartridge was centrifuged to dry the filter and preheated (75°C) elution solution was added, kept for 1 min at room temperature and centrifuged to elute the RNA. This wash step was repeated, resulting in a final sample volume of 25 μl. Potential residual DNA was digested using an Ambion DNA-free kit (Ambion, Texas, USA) according to the manufacturer’s instructions.

Reverse transcription

RNA was reverse transcribed using an Invitrogen Superscript III kit (Invitrogen, California, USA) with a mastermix containing random hexamers (GeneWorks, Adelaide, Australia), 5′-triphosphates (Amersham Pharmacia Biotech, New Jersey, USA) and molecular biology grade water. Following incubation at 65°C for 5 min and subsequent cooling, a second mastermix containing DTT, forward reaction buffer and SuperScript III enzyme was added. The samples were heated for 5 min at 25°C followed by 60 min at 50°C. The Superscript III enzyme was denatured by heating for 15 min at 70°C and samples stored at −20°C.

Primers

Primers (see Table 1) were designed with the ABI Prism Primer Express program (Applied Biosystems, Foster City, CA). All primers were designed around the following optimal characteristics: optimal primer melting temperature of 59°C, primer GC content between 20% and 80% with a 3′ GC clamp of zero residues, optimal primer length of 20, optimal amplicon length of 100 and spanning exons (Per1 and c-fos). Preliminary experiments confirmed that the amplification efficiencies for the target genes and β-actin were close to the expected 100% efficiency. Because of the extremely small amount of tissue obtained from the punches, all of the extracted RNA was reverse-transcribed and DNase-treated with freshly prepared and tested reagents.

Polymerase chain reaction (PCR)

An Applied Biosystems 7500 Real Time PCR System (Applied Biosystems, California, USA) was used to amplify the cDNA using SYBR green. The samples were amplified in 1 cycle of 50°C for 2 min, 95°C for 10 min, followed by 40 cycles of 95°C for 10 s and 60°C for 1 min. An arbitrary threshold of fluorescence was set within the exponential phase of amplification. The cycle at which the amplification of the gene of interest exceeded the threshold was designated as the cycle threshold (Ct). The expression of each gene of interest (Per1, Per2, c-fos) was normalized against the housekeeper gene β-actin and expressed relative to a calibrator sample according to the formula 2−ΔΔCt (Livak and Schmittgen 2001). The term ΔΔCt is defined as: [Ct of gene of interest (unknown sample) − Ct of β-actin (unknown sample)] − [Ct of gene of interest (calibrator sample) − Ct of β-actin (calibrator sample)].

The calibrator or reference group normally used in this type of analysis is either an untreated control or a sample at time course zero. For the purposes of the current analyses,

### Table 1 Primers used for β-actin, c-fos, Per1 and Per2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession number</th>
<th>Primer</th>
<th>Nucleotide numbers</th>
<th>Primer sequence 5’ to 3’</th>
<th>Tm</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-actin</td>
<td>NM031144</td>
<td>Forward</td>
<td>325 to 344</td>
<td>CCTCTGAAACCTAAGGCGCA</td>
<td>58°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>414 to 395</td>
<td>AGCCCTGGATGGCCTACGTA</td>
<td>57°C</td>
</tr>
<tr>
<td>Per1</td>
<td>NM_001034125</td>
<td>Forward</td>
<td>783 to 800</td>
<td>GCGTTGCAAACGCGGATG</td>
<td>59°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>883 to 862</td>
<td>GCACAGCGAGATGCTAGTGA</td>
<td>58°C</td>
</tr>
<tr>
<td>Per2</td>
<td>AB016532</td>
<td>Forward</td>
<td>3,042 to 3,064</td>
<td>AGCAGTCCCCCTACAGCTAACT</td>
<td>59°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>3,170 to 3,153</td>
<td>CGGATGGCAGCACGATG</td>
<td>59°C</td>
</tr>
<tr>
<td>c-fos</td>
<td>X06769</td>
<td>Forward</td>
<td>205 to 228</td>
<td>GGGACAGCCTTTCCTACTACCAT</td>
<td>60°C</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse</td>
<td>285 to 265</td>
<td>CGAAGAAATGCTGTGTTGA</td>
<td>58°C</td>
</tr>
</tbody>
</table>
the untreated saline group that were killed 30 min posttreatment at ZT6 served as the reference group. This group value was set to 1, and hence all data are expressed as the fold change in comparison to 1.

Behavioral study following treatment with MDMA at ZT16

Male Long–Evans rats (*n*=20) were acclimatized and housed under the same conditions described for the gene expression experiment. Running wheel revolutions were detected using a magnetic switch and compiled using Clocklab® acquisition software (Coulbourn Instruments, Pennsylvania, USA). Prior to treatment, rats were matched on their baseline activity and their cage location relative to the light intensity and assigned to receive either MDMA (5 mg/kg) or saline (vehicle). Treatments were delivered as per the gene expression experiment at ZT16 and after this time rats were held under dim light (<0.1 lx) so that the effects of treatment on their free-running rhythms could be studied for the subsequent 10 days. Behavioral effects of MDMA administration at ZT6 have been reported previously by our group (Ogeil et al. 2010).

Data analyses

To determine whether changes in gene expression were altered by the administration of MDMA, data were analyzed using a 2 (treatment: MDMA, saline)×3 (sampling time: 30, 60, 120 min) Analysis of Variance (ANOVA), Tukey’s HSD for significant main effects, and simple contrasts for interaction effects. Eta-squared (*η²*) is reported as an estimate of the amount of variance explained by the analysis. In the behavioral study, independent measure *t*-tests were used to compare changes between MDMA-treated and saline-treated rats. SPSS v19 (IBM, SPSS, Chicago, IL) was used to conduct statistical analyses.

Results

Gene expression changes following treatment

Data on the change in gene expression of *Per1*, *Per2*, and *c-fos* following treatment with MDMA or saline at either ZT16 or ZT6 are illustrated in Fig. 1.

Figure 1 shows that following MDMA administration at ZT16, *c-fos* mRNA was increased (~2-fold) 30 and 60 min posttreatment, while both *Per1* and *Per2* mRNA were increased by ~1.8-fold at 60 min posttreatment. In contrast, MDMA administration at ZT6 increased *c-fos* mRNA ~1.6-fold 120 min post treatment, but there was little change in *Per1* or *Per2* mRNA expression.

Activity changes following treatment at ZT16

There was no significant treatment × time interaction for either *Per1* mRNA expression: *F*(2,26)=0.08, *p*>0.05, *η²*=0.006, or *Per2* mRNA expression after treatment at ZT6: *F*(2,26)=1.34, *p*>0.05, *η²*=0.093. Similarly, for changes in *c-fos* mRNA expression, there was no significant treatment × time interaction: *F*(2,26)=0.53, *p*>0.05, *η²*=0.039. However, there was a significant main effect of treatment for change in *c-fos* mRNA expression: *F*(1,26)=10.07, *p*=0.01, *η²*=0.279, with MDMA-treated rats having significantly higher expression compared to saline treated rats. In addition, there was a significant main effect of time: *F*(2,26)=3.77, *p*<0.05, *η²*=0.225, with *c-fos* expression differing between 60 and 120-min post treatment.

There were no significant main effects of either time or treatment for either *Per1* or *Per2*. There were, however, significant treatment × time interactions for *Per1* expression: *F*(2,22)=3.51, *p*<0.05, *η²*=0.242, with increased expression for the MDMA-treated rats at 60 min post treatment. Similarly, for *Per2* mRNA expression, there was a significant treatment × time interaction: *F*(2,22)=3.77, *p*<0.05, *η²*=0.255, with a significant increase in expression for the MDMA-treated rats at 60 min. With respect to changes in *c-fos* expression, there was a significant treatment × time interaction: *F*(2,22)=3.55, *p*<0.05, *η²*=0.244, with a significant increase in expression for the MDMA-treated rats at 60 min. With respect to changes in *Per1* mRNA expression, there was a significant main effect of treatment for change in *c-fos* mRNA expression: *F*(1,22)=55.34, *p*<0.001, *η²*=0.716, with MDMA-treated rats having significantly higher expression compared to saline treated rats and across time: *F*(2,22)=21.970, *p*<0.0001, *η²*=0.666.

Actograms were generated from running wheel data and sent to two independent raters who were blind to the experimental treatments. Each rater marked a line of best fit through activity onsets and offsets (Agostino et al. 2007) for the 10 days prior to and the 10 days post treatment. From these records, changes in circadian parameters for each animal were calculated, specifically (i) the change in activity onsets and offsets (difference in time of activity onset or offset between the day prior to treatment and 1 day post treatment), (ii) the change in alpha (duration of the active phase 10 days prior to treatment compared to 10 days post treatment), and (iii) tau (or circadian period, the cycle length of the activity rhythm, assessed using activity onset or offset for 10 days post treatment). Evaluation of activity
counts on the treatment day in comparison with 1 week of baseline activity for each rat showed a tendency towards increased activity in the MDMA-treated rats (n=11; M=197.54, SD=163.03) compared to the saline-treated rats (n=9; M=54.72, SD=175.91, t(18)=1.88, p=0.08), suggesting that MDMA was behaviorally active. However, there were no significant differences between treatment groups for any of the circadian measures. Means, standard deviations and t-statistics for each of these measures are presented in Table 2.

Discussion

In the present study, MDMA administered at ZT6 induced no changes in Per1 or Per2 mRNA expression but increased the expression of the immediate early gene c-fos. However, MDMA administered at ZT16 increased c-fos mRNA (30 and 60 min) and Per1 and Per2 mRNA expression at 60 min post treatment. MDMA administration at ZT16 failed to alter any aspect of wheel running behavior of the rats.
5-HT2C agonists on SCN function in rats, Varcoe et al. (2003) found significant increases in Per1, Per2 and c-fos mRNA at 30 and 120 min post treatment. Varcoe et al. (2003) concluded that serotonin (through 5-HT2C receptors) plays an integral role in the transmission of light information to the SCN early in the night. The observation that treatment with MDMA at ZT16 affected the expression of Per1 and Per2 genes in the current study suggests that MDMA disrupted the role normally played by the 5-HT system in the regulation of the circadian system.

There was a dissociation between the effects of MDMA on gene expression and behavior, reported here and in our previous study (Ogeil et al. 2010). Paradoxically, despite the changes in gene expression at ZT16, no significant changes were found in the current study for the timing of wheel running activity rhythm following MDMA in contrast to those reported after treatment at ZT6 (Ogeil et al. 2010). In the hamster, Colbron et al. (2002) reported that MDMA treatment prior to a light pulse at ZT14 attenuated the normal phase shift seen in response to the light pulse. Given the complexity of the actions of 5-HT agonists on the circadian system, it is feasible that treatment of nocturnal mammals with MDMA at ZT16 produces minimal effects on activity because the resulting increase in 5-HT release coincides with their ‘active’ phase when 5-HT levels are near their peak (Cuesta et al. 2009). The functional effect of serotonergic agonists including MDMA administered at this time may occur through an interaction with photic stimuli that on its own would normally provide the impetus for a phase shift (Cuesta et al. 2009). In contrast, administration of MDMA at ZT6, in the middle of the ‘rest’ phase, may have affected activity regulation because levels of 5-HT at this time are low (Cuesta et al. 2009).

In an interesting parallel to the present study, Cuesta et al. (2009) investigated the administration of the SSRI, fluoxetine, which like MDMA acutely increases extracellular levels of 5-HT. They reported changes in activity rhythms at CT6 but not at CT22 as well as an upregulation of Per1 and Per2 in the SCN at CT22 when a photic stimulus was presented but not at CT6. Notably, Cuesta et al. (2009) found no significant changes in circadian activity when fluoxetine was administered alone at CT22; however, when its administration was paired with a light pulse, fluoxetine attenuated the normal shifting response. Despite the testing period in the present study being earlier in the subjective night, it seems likely that MDMA, via a 5-HT mechanism, modulates the circadian system in multiple ways depending on its time of administration.

We previously reported changes in some circadian parameters following treatment with MDMA at ZT6 including a lengthening of active phase, a change in activity offsets and in circadian period. We suggested that these changes in activity offset and period were either secondary to the change in active phase or may represent a more direct effect involving multiple interactions of MDMA with the circadian system. Indeed, the present study showed that there was a delayed increase of expression of the immediate early gene c-fos occurring 120 min following treatment at ZT6, suggesting that MDMA-induced changes in gene expression within the SCN may be delayed during the ‘rest’ phase. These findings suggest that the effect of MDMA on gene expression in the SCN may be indirect, perhaps occurring via the raphe nuclei (Mistlberger et al. 2000). As the raphe provide dense serotonergic connections to the SCN (Stephan et al. 1981), which release 5-HT after a bout of forced wheel-running or

### Table 2 Changes in circadian activity parameters following treatment at ZT16

<table>
<thead>
<tr>
<th>Measure</th>
<th>Mean</th>
<th>SD</th>
<th>t-Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference in activity onsets, h</td>
<td>−0.10</td>
<td>0.21</td>
<td>0.774, p&gt;0.05</td>
</tr>
<tr>
<td>Saline</td>
<td>0.01</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>0.27</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Difference in activity offsets, h</td>
<td>0.33</td>
<td>0.33</td>
<td>0.332, p&gt;0.05</td>
</tr>
<tr>
<td>Saline</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>0.27</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Difference in duration of active phase (alpha), h</td>
<td>0.04</td>
<td>0.04</td>
<td>0.344, p&gt;0.05</td>
</tr>
<tr>
<td>Saline</td>
<td>24.07</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>MDMA</td>
<td>24.08</td>
<td>0.04</td>
<td></td>
</tr>
</tbody>
</table>

Zeitgeber Time 16 (ZT16) = 16 h after lights on
sleep deprivation (Dudley et al. 1999; Mistlberger et al. 2000), the changes in activity rhythms following MDMA treatment that were previously reported (Ogeil et al. 2010) may have arisen by this indirect action. In support for an indirect effect, Colbron et al. (2002) reported that pretreatment with MDMA prior to administration of 8-OH-DPAT at ZT8 inhibited the normal phase-shifting response seen following 8-OH-DPAT. However, since their study involved the use of hamsters, further studies in rats are needed as there may be species differences in their responses to these stimuli (Mrosovsky 1996).

The actions of MDMA on the circadian system, whether through a disruption to the normal expression of core clock-controlled genes or through an interaction with light, have potential implications for ecstasy users. Given that ecstasy users report problems with their sleep (Jones et al. 2008) and that restless mood occurs concurrently with these sleep disturbances for up to 6 days post ecstasy use (Huxster 2006), it is possible that changes to the circadian system are mediating these effects. Alterations in the circadian system have also been associated with affective disorders (Kennaway 2010), with clock gene polymorphisms perhaps contributing to increased vulnerability to mood disorders (Johansson et al. 2003; Wirz-Justice 2006). Indeed, Wirz-Justice (2003, 2006) has postulated that mood state is partially regulated by the timing of sleep and circadian rhythmicity, since clock gene polymorphisms influence certain features of mood disorders including their age of onset, the symptoms likely to be experienced and a person’s response to treatment. Since mood changes have been extensively studied post ecstasy use, with users reporting depressed mood during days subsequent to ecstasy use (Liechti and Vollenweider 2000; Verheyden et al. 2002), the interaction between mood changes and changes in the circadian system warrants further research.

**Limitations and future directions**

The present study demonstrated the complexity of MDMA’s actions on the circadian system. Its effects on Per gene expression and behavior were shown to be time of day dependent. One limitation of the present study was that other clock genes that may also be affected by MDMA were not investigated. Indeed, Cuesta et al. (2009), in their examination of the effects of fluoxetine, suggested a role for RorST, which is important in the transcription of Bmal1 (Dardente and Cermakian 2007). Despite the changes in behavior reported when MDMA was administered at ZT6 (Ogeil et al. 2010), the present study did not find a MDMA-induced change in Per gene expression at this time. However, the present data would suggest an indirect effect of MDMA at this time given that c-fos expression was increased 120 min following treatment. When MDMA is administered at ZT6, its effect on the circadian system may be delayed and/or sustained, which may reflect chronobiological differences in MDMA’s pharmacokinetics and pharmacodynamics. The specifics of different 5-HT receptors involved in these effects and the nature of this apparently delayed action of MDMA is worthy of examination in future studies.

**Conclusions**

The present study demonstrated MDMA-induced changes in the expression of c-fos, Per1 and Per2 mRNA following administration of MDMA at ZT16. In addition, the current study showed that the time course for c-fos induction was delayed at ZT6 compared to ZT16, and possibly underlies the dissociation between the genetic and behavioral findings at these times. The results of the current study suggest that like other drugs that enhance 5-HT neurotransmission, MDMA has multiple actions on the circadian system. This finding may have ramifications for ecstasy users given the potential contribution of circadian disruption to the frequently reported negative effects of ecstasy use on sleep and mood.

**Acknowledgements** We thank Dr. Tamara Varcoe, A/Prof. Jennifer Redman, Prof. Grahame Coleman, Dr. Zane Andrews, Dr. Sarah Spencer, Ms. Cheryl Roberts, Ms Hania Czerwinska and Ms. Terry Lane for their guidance and support. Rowan Ogeil was the recipient of an Australian Postgraduate award and a Faculty of Medicine, Nursing and Health Sciences Postgraduate Excellence Award from Monash University.

**Author disclosure** This research was conducted without specific funding from the public, commercial or not-for-profit sectors. All authors declare that they have no conflicts of interest.

**References**


Oberlander R, Nichols DE (1990) (+)-N-methyl-1-(1,3-benzodioxol-5-yl)-2-butanamine as a discriminative stimulus in studies of 3,4-methylenedioxymethamphetamine-like behavioral activity. J Pharmacol Exp Ther 255:1098–1106
Stephan FK, Zucker I (1972) Circadian rhythms in drinking behavior and locomotor activity of rats are eliminated by hypothalamic lesions. Proc Natl Acad Sci USA 69:1583–1586