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Effect of amphetamine on the clock gene expression in rat striatum

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HIGHLIGHTS

- Chronic D-amphetamine altered the daily pattern of clock genes expressions in the rat striatum.
- *Bmal1* was shifted from a diurnal to a nocturnal pattern by D-amphetamine treatments.
- D-Amphetamine altered the *Rev-erbα* rhythm expression in the striatum was shown for the first time.

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ABSTRACT

Drug addicts have severe disruptions in many physiological and behavioral rhythms, such as the sleep/wake cycle. Interestingly, amphetamine, a psychostimulant, is able to alter many circadian patterns, which are independent of the master biological clock located in the suprachiasmatic nucleus. To increase our understanding of the circadian regulation of amphetamine on clock gene expression, rats received subcutaneous injections of D-amphetamine and the clock gene mRNA levels were analyzed using real-time PCR to obtain a daily profile. In the striatum, acute injection of D-amphetamine did not alter Period (*Per*)*1*, *Per2* and Reverse erythroblastosis virus α (*Rev-erbα*) expressions. Chronic administration shifted the phase of *Per1* and *Per2* expressions from a nocturnal to diurnal pattern and advance shifted the peak *of Rev-erbα* in D-amphetamine-treated animals. In contrast, the rhythm of Brain and muscle Arnt-like protein-1 (*Bmal1*) was shifted from a diurnal to a nocturnal pattern by both acute and chronic treatments. These results demonstrated that chronic D-amphetamine treatment altered the expression of clock genes in the striatum. This might further influence the expression of related gene within the striatum and lead to behavioral and physiological changes which are associated to drug addiction.

1. Introduction

The circadian timing system in mammals is controlled by a master biological clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The SCN is entrained by an input from the light/dark cycle to control various rhythms in the peripheral physiology and behavior. The circadian rhythm within the SCN is generated by a cell-autonomous transcriptional/translational feedback loop composed of a set of clock gene families, including the circadian locomotor cycle kaput (*Clock*), Brain and muscle Arnt-like protein-1 (*Bmal1*), Period (*Per1–3*), the Cryptochrome (*Cry1–2*), Reverse erythroblastosis virus α (*Rev-erbα*), Retinoic acid receptor-related orphan receptor (*Ror*) genes and their corresponding proteins [24]. Furthermore, many posttranscriptional and posttranslational modification processes help to sustain and stabilize the accuracy of the circadian oscillation based on the 24-h solar cycle [8]. The expression of clock genes is also observed outside the SCN with circadian rhythms. Although there is a similarity in the molecular clock mechanism, the role of these clock genes in the non-SCN structures is still unclear.

The mammalian circadian rhythms are able to be entrained by nonphotic stimuli, such as social cues, sleep deprivation, feeding and pharmacological treatments, which can also be SCN-dependent or -independent entrainments. Several studies have shown that psychostimulants, amphetamines, severely disrupt drinking and eating habits and also affect the rhythm of corticosteroid levels and body temperature [11]. Methamphetamine can restore free-running locomotor rhythms in arrhythmic animals, which cause by SCN lesion [10,12], constant bright light suppression [33] or genetic mutations [22]. Thus, methamphetamine-induced activity rhythms did not directly depend on the SCN circadian clock, but

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Table 1
Assay details of all studied genes.

Interrogated sequence			Translated protein	Exon boundary	Amplicon length
Gene name	Assay No.	Ref. sequence			
Per1	Rn01496753_g1	NM_001034125.1	NP_001029297.1	12–13	142
Per2	Rn01427704_m1	NM_031678.1	NP_113866.1	22–23	100
Bmal1	Mm01269616_m1	NM_007489.3	NP_031515.1	9–10	100
Rev-erbα	Rn01460659_g1	NM_145775.2	NP_665718.2	2–3	123
Actb	PN 4352931E	NM_031144.2	NP_112406.1	4–5	91

little is known about the location and mechanism underlying this oscillation. Recent studies have suggested that clock genes play an important role in drug reward and addiction [5,26]. The selected mutation of clock gene was able to disrupt the sleep–wake cycle or deregulated many circadian rhythms of hormones that can contribute to psychiatric disorder [2,32]. Moreover, the association studies indicated that single nucleotide polymorphisms in several clock genes such as *Cry1*, *Cry2*, *Per2*, *Npas2* and *Clock* significantly associated with psychiatric disorder [17,27,28]. This suggests an important role of clock gene in the pathophysiology of drug addiction. Previous studies have reported that methamphetamine was unable to alter the expression of circadian genes in the master clock of the SCN [14,20]. The alteration of molecular clock component patterns may involve in the circadian rhythmicity by changing the behavior caused by amphetamine addiction. In order to increase our understanding of the circadian regulation of amphetamine on circadian gene expression, we examined the effects of acute and chronic amphetamine injection on clock gene expression in the striatum.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with experimental protocols approved by the Laboratory Animal Care and Use Committee of Mahidol University (MU-ACUC Review, Protocol No. 161). Adult male Wistar rats (National Laboratory Animal Center of Mahidol University, Thailand) were housed under 12 h light (200 lux)/12 h dark (2 lux dim red light) conditions for at least two weeks. The lights-on is denoted as zeitgeber time (ZT) 0, and the temperature was 22 ± 2 °C. Animals were supplied with access to water and food ad libitum.

2.2. Drug administration

Rats were divided into two groups. Each group consisted of twelve rats. The animals were injected subcutaneously with saline or D-amphetamine (5 mg/kg) dissolved in sterile saline once daily at ZT5 for 6 consecutive days. Additional group received saline for 5 days and then on ZT5 at day 6, D-amphetamine was injected subcutaneously to examine an acute effect of D-amphetamine. The drug doses and the time of the injection (i.e., daytime) were chosen based on previous behavioral studies and gene expression in the striatum [14,23]. Three rats per each time-point and four time-points were used. The animals were sacrificed at ZT 21 on day 6 and ZT 3, 9 and 15 on day 7. The brains were immediately removed, and the striatum was dissected under a stereo dissecting microscope at 4 °C. The tissues were stored at −80 °C until RNA extraction.

2.3. RNA extraction and reverse transcription

Total RNAs from the striatum were extracted using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) according to the manufacturer's protocol. The RNA concentrations were determined using UV spectrophotometry, and the RNA quality was assessed by electrophoresis on a 1% agarose gel. Total RNA (2 μg) was reverse transcribed using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems, Foster City, CA, USA) with random hexamer primers according to the manufacturer's instructions.

2.4. Real-time PCR

The specific primers and TaqMan probes used to analyze the mRNA levels of the 4 clock genes, *Per1*, *Per2*, *Bmal1* and *Rev-erbα* (Table 1), were ordered from Applied Biosystems (Foster City, CA, USA). In this study, rat beta-actin (*Actb*), an endogenous control gene, was used to normalize the differences in sample RNA content. Real-time PCR was performed according to the protocol described previously [36]. Briefly, PCR reactions were set up in 96-well reaction plates and were run on an ABI 7500 real-time PCR system (Applied Biosystems). All studied gene reactions were performed in separate tubes, and all samples were run in triplicate to ensure the accuracy of the data. Data were expressed as relative values with respect to the mRNA amount at ZT 3 of control group for *Per1* and *Per2* and at ZT15 for *Bmal1* and *Rev-erbα* genes. The relative mRNA expression was achieved with SDS software, version 1.4 (Applied Biosystems), by performing the comparative Ct method.

2.5. Statistical analysis

Statistical analysis was done using STATISTICA software (StatSoft, Tulsa, OK, USA). Daily profiles of the clock genes are presented as means from at least three animals ± SEM per time point. The data were analyzed by one- or two-way analysis of variance (ANOVA) and, subsequently, by Tukey's post hoc tests. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Circadian clock gene expression in the rat striatum

In the control, mRNA levels of the four clock genes, *Per1*, *Per2*, *Bmal1* and *Rev-erbα*, displayed different daily variations in the rat striatum (Fig. 1). One-way ANOVA revealed a significant effect of time on the mRNA levels of *Per1* ($p < 0.001$), *Per2* ($p < 0.01$), *Bmal1* ($p < 0.0001$) and *Rev-erbα* ($p < 0.00001$). *Per1* mRNA showed a nocturnal pattern of expression with its highest values at ZT15 (significantly higher than ZT3, $p < 0.001$). *Per2* mRNA levels increased at night, with a maximal value observed 3 h after the dark onset (ZT15, significantly higher than those at ZT3 and ZT9, $p < 0.01$). *Bmal1* mRNA levels were significantly elevated at the beginning of the light onset (ZT3, significantly higher than those at ZT9 and ZT15, $p < 0.001$). *Rev-erbα* mRNA levels increased and peaked during the daytime at ZT9 (significantly higher than those other time points, $p < 0.001$).