Short Communication

Effect of Quetiapine on *Per1*, *Per2*, and *Bmal1* Clock Gene Expression in the Mouse Amygdala and HippocampusShunpei Moriya^{1,2}, Yu Tahara¹, Hiroyuki Sasaki¹, Yutaro Hamaguchi¹, Daisuke Kuriki¹, Ryosuke Ishikawa¹, Jun Ishigooka², and Shigenobu Shibata^{1,*}¹Laboratory of Physiology and Pharmacology, School of Advanced Science and Engineering, Waseda University, Shinjuku-ku, Tokyo 162-8480, Japan²Department of Psychiatry, Tokyo Women's Medical University, School of Medicine, Shinjuku-ku, Tokyo 162-8666, Japan

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Abstract. Circadian rhythms are related to various psychiatric disorders. Recently, antipsychotics, including quetiapine (QTP), have been accepted as potential therapeutic agents for the treatment of depression, but its mechanism remains poorly understood. In this study, we examined clock gene fluctuation patterns in QTP-treated mice. QTP significantly increased *Per2* mRNA at ZT12 and *Per1* and *Per2* expression at ZT18 in the amygdala. There were significant differences between the control and QTP groups in the cross-time effects of *Per2* mRNA expression in the amygdala. Our findings suggest that QTP possibly acts on the circadian system, which then induces changes in mood symptoms.

Keywords: quetiapine, clock gene, depression

In mammals, a circadian rhythm refers to an approximately 24-h internal oscillation that controls physiological and behavioral rhythms. Circadian oscillators are located in the suprachiasmatic nucleus (SCN), which is referred to as the central clock, and in the peripheral organs, referred to as the peripheral clock (1). Moore and Eichler discovered that destruction of the SCN in rats led to the loss of sleep-wake cycles and corticosterone rhythms. After that, the SCN was determined to be the central location of the clock system in mammals. Circadian rhythms are regulated by endogenous circadian clock genes (1), such as *Per1*, *Per2*, *Clock*, *Bmal1*; and their expression is regulated by transcriptional–translational feedback loops.

Circadian rhythms are related to various disorders. Patients with major depressive disorder (MDD) exhibit disruptions in biological circadian rhythms (2). However, there has been no direct evidence linking deficits in clock gene expression with MDD.

MDD is often treated with first-line antidepressants (AD), such as selective serotonin-reuptake inhibitors and

serotonin-noradrenaline–reuptake inhibitors. The problem with these drugs, however, is that they do not always produce sufficient effects (3). This lack of response to AD despite an adequate dose and duration is called treatment-resistant depression (4). In 2007, the World Federation of Societies of Biological Psychiatry Task Force recommended lithium as the first alternative medication choice in treatment-resistant depression management (3). More recently, antipsychotics have received attention as alternative agents for augmenting depression therapy (5). Quetiapine (QTP), an atypical antipsychotic, was approved by the US Food and Drug Administration as a treatment for both manic and depressive phases of bipolar disorder (6) and as a drug for adjunctive therapy in MDD (7). QTP monotherapy is not currently approved for the treatment of MDD, but several studies have demonstrated a clinical efficacy for MDD patients (8). Yet QTP's mechanism of effect in depression remains poorly understood. As described above, there is no direct evidence linking deficits in clock gene expression with MDD. Furthermore, no studies have explored whether the mechanism of QTP action is related to clock gene expression. Therefore, in this study we examined clock gene fluctuation patterns in QTP-treated mice in order to investigate whether the mechanism of

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QTP action is related to clock gene expression.

Six-week-old male C57BL/6JKwI mice were obtained from Tokyo Laboratory Animal Science Co., Ltd. (Tokyo). Animals were maintained in the laboratory for 1 week prior to the start of the experiment in order to adapt to laboratory conditions, which included a 12/12-h light/dark cycle, a $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ temperature, humidity of $60\% \pm 5\%$, and food and water ad libitum. In the 24-h cycle, Zeitgeber time 0 (ZT0) was designated as lights-on, and ZT12 was lights-off. All procedures were carried out with permission (2013-A058, 2013-A071) from the Committee for Animal Experimentation of the School of Science and Engineering at Waseda University.

Mice were divided into two groups: QTP and control (Con). Quetiapine fumarate (Wako Pure Chemical Industries, Ltd., Osaka) was dissolved in distilled water with 1% dimethyl sulfoxide (DMSO) and administered intraperitoneally (i.p.) at 0.1 ml/10 g body weight. Animals in the QTP group were injected with 10 mg/kg QTP once a day at ZT6 for 12 days, while animals in the Con group were injected with vehicle (1% DMSO in distilled water) once a day at ZT6 for 12 days. In clinical treatment, both QTP and fluoxetine have been used at 75 mg/day; and in animal studies, 10 mg/kg per day is the usual dose used in fluoxetine experiments. Thus we decided to use 10 mg/kg QTP for the present experiment.

Each experimental group (QTP, Con) contained 9 mice per time point (ZT0, ZT6, ZT12, ZT18; total $n = 72$). Mice were sacrificed at ZT6, ZT12, ZT18, or ZT0 in this order after 24 h from the last injection at ZT6. Mice were anesthetized with ether and their brains were removed. Brains were bilaterally dissected with 2-mm width using a brain matrix (#0530, Bioresearch Center Co., Nagoya). Respective samples of the amygdala and hippocampus were taken with scissors (mean weight: 6.9 mg for amygdala, 16.5 mg for hippocampus).

Total RNA was extracted using TRIzol Reagent (Ambion, Oakland, CA, USA). Specific primer pairs (see Table 1) were designed using Primer3 Input (version

0.4.0). Real-time RT-PCR was executed under the following conditions: cDNA Synthesis at 42°C for 15 min, followed by 95°C for 2 min, PCR amplification for 40 cycles with denaturation at 95°C for 5 s, and annealing and extension at 60°C for 20 s (9). The relative levels of the target gene PCR product were normalized to those of 18S ribosomal RNA. The data were analyzed by the delta-delta C_t method. A melting curve analysis was performed to check nonspecific products, as to which there were no non-specific product amplifications.

Data are expressed as the mean $\pm$ S.E.M. values (Fig. 1). Significance differences between Con and QTP groups at each ZT were analyzed by Student's t -test. Additionally, a two-way analysis of variance (ANOVA) was performed to examine the statistical significance of the cross-time effect of QTP administration and clock gene mRNA expression using StatView software (SAS Institute, Cary, NC, USA).

Injection of QTP for 12 days did not affect body weight (22.8 ± 1.1 g, $n = 36$ for Con; 22.3 ± 1.0 g, $n = 36$, for QTP, no significance), suggesting good health condition in QTP-treated mouse group. Repeated injections of QTP significantly increased *Per2* and *Bmal1* mRNA at ZT12 and *Per1* and *Per2* expression at ZT18, in the amygdala (Student's t -test, $P < 0.01$) (Fig. 1). QTP treatment increased *Per1* and *Per2* expression at ZT6, and *Bmal1* at ZT18, in the hippocampus (Student's t -test, $P < 0.01$) (Fig. 1). There were significant differences in *Per2* mRNA expressions for ZT $\times$ QTP in the amygdala, but no significant differences in *Per1* or *Bmal1* expression in the amygdala.

In this study, we found a significant association between clock gene expression and QTP treatment in mice. To our knowledge, this is the first study to examine clock gene expression in the amygdala and hippocampus of QTP-treated mice. *Per1* and *Per2* mRNA expression was significantly elevated in the amygdala at ZT18 and at ZT12/ZT18, while *Bmal1* mRNA expression was significantly elevated in the amygdala at ZT12 and in the hippocampus at ZT18. In addition, there were significant differences in the cross-time effects of *Per2* mRNA expression in the amygdala between the Con and QTP groups.

QTP is known as an atypical antipsychotic that has D_2 anti-dopaminergic properties but low striatal D_2 -receptor occupancy (10). QTP has various receptor binding profiles and, because of this, is called a multi-acting receptor-targeted antipsychotic that elicits various positive and negative clinical effects (e.g., antipsychotic, antidepressant, sedative, anticholinergic, causes weight gain). Recently, antipsychotics, including QTP, have been considered as alternative augmentation agents in depression therapy (5). QTP is approved by the US

Table 1. Sequence primers for real-time PCR

Gene	Primer sequence
<i>rRNA</i>	5'-ggggagatgatggtgcaaagc-3'
	5'-tgtcaatcctgtccgtgtcc-3'
<i>Per1</i>	5'-caagtggcaatgagtccaacg-3'
	5'-cgaagtgttgagctcccgaagtg-3'
<i>Per2</i>	5'-tgtgtgcttacacgggtgtccta-3'
	5'-acgtttggtttgcgcattgga-3'
<i>Bmal1</i>	5'-ccacctcagagccattgataca-3'
	5'-gagcagggttagtccactttgtct-3'

Fig. 1. Time-course-dependent quetiapine (10 mg/kg, i.p.)-induced changes in clock gene expression in the amygdala and hippocampus. Twenty-four hours or more after the last injection of vehicle or quetiapine, total RNA was extracted from the amygdala and hippocampus, and mRNA gene expression at each sampling time was measured by real time RT-PCR. Values are expressed as the mean $\pm$ S.E.M. The relative unit of the target gene PCR product was normalized to that of 18S ribosomal RNA. ** $P < 0.01$ (vs. control).

Food and Drug Administration as an agent for both manic and depressive phases of bipolar disorder (6) and as an agent for adjunctive therapy in MDD (7). MDD is the world's most common mental illness (11), and MDD patients present with disruptions in biological circadian rhythms (2). However, there is no direct evidence with regard to dysregulation of clock gene expression and MDD.

Uz et al. (12) reported that repeated fluoxetine treatment increased *Per2* mRNA expression in several key brain regions. These results suggest that clock genes and the mechanisms regulating these genes may participate in the long-term changes and antidepressive effects of selective serotonin reuptake inhibitors. Our present results suggest that QTP is linked to clock gene fluctuations and concomitant antidepressive effects. QTP monotherapy is not yet approved for the treatment of MDD, but several studies have demonstrated a clinical efficacy for MDD patients (8). Together, these reports positively support the results we present here.

The antidepressant effects of QTP remain poorly understood, but a few theories have proposed a potential mechanism of action. Blier et al. (13) proposed that QTP increases noradrenergic neurotransmission via α -2

blockade and resulted in antidepressant action. Jensen et al. (14) suggested that a QTP metabolite, *N*-desalkyl-quetiapine, is a potent noradrenaline reuptake inhibitor and a partial 5-HT_{1A} agonist. Despite this evidence, QTP's role as an antidepressant is still poorly understood. As described above, there is no direct evidence in regard to dysregulation of clock gene expression and MDD. Recently, Li et al. (15) reported that the cyclic patterns of expression in several clock genes, *Bmal1*, *Per1-2-3*, *Rev-erbBr*, *Dec1*, and *Dec2*, in high-quality postmortem human samples were much weaker in the brains of MDD patients. This report supports that hypothesis that fluctuations in clock gene mRNA expression may be dependent on the degree of mood symptoms, and QTP may act on mood by varying clock gene mRNA expression. It is well known that depressive patients have diurnal variations in their symptoms. In this study, variations in clock gene mRNA expression induced by QTP administration were evident in mice during the active phases of the 24-h day. Thus, these findings indicate that the effects of QTP may occur during the daytime, suggesting that QTP acts on the circadian system and positively alters mood.

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Conflicts of Interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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