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A Circadian Genomic Signature Common to Ketamine and Sleep Deprivation in the Anterior Cingulate Cortex

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ABSTRACT

BACKGROUND: Conventional antidepressants usually require several weeks to achieve a full clinical response in patients with major depressive disorder, an illness associated with dysregulated circadian rhythms and a high incidence of suicidality. Two rapid-acting antidepressant strategies, low-dose ketamine (KT) and sleep deprivation (SD) therapies, dramatically reduce depressive symptoms within 24 hours in a subset of major depressive disorder patients. However, it is unknown whether they exert their actions through shared regulatory mechanisms. To address this question, we performed comparative transcriptomics analyses to identify candidate genes and relevant pathways common to KT and SD.

METHODS: We used the forced swim test, a standardized behavioral approach to measure antidepressant-like activity of KT and SD. We investigated gene expression changes using high-density microarrays and pathway analyses (Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, Gene Set Enrichment Analysis) in KT- and SD-treated mice compared with saline-treated control male mice.

RESULTS: We show that KT and SD elicit common transcriptional responses implicating distinct elements of the circadian clock and processes involved in neuronal plasticity. There is an overlap of 64 genes whose expression is common in KT and SD. Specifically, there is downregulation of clock genes including *Ciart, Per2, Npas4, Dbp,* and *Rorb* in both KT- and SD-treated mice.

CONCLUSIONS: We demonstrate a potential involvement of the circadian clock in rapid antidepressant responses. These findings could open new research avenues to help design chronopharmacological strategies to treat major depressive disorder.

Keywords: Anterior cingulate cortex, Circadian clock, Depression, Ketamine, Sleep deprivation, Transcriptome

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Major depressive disorder (MDD) is one of the most serious and common psychiatric disorders in the United States. According to the latest statistics, the National Institute of Mental Health estimated that 15.7 million adults over the age of 18 years in the United States had at least one major depressive episode, representing 6.7% of all U.S. adults (1). The two most rapid-acting antidepressant strategies, low-dose ketamine (KT) and sleep deprivation (SD) therapies, motivated a large number of studies into their mechanisms of action. In contrast to conventional antidepressants that can take weeks for full clinical response, 40% to 60% of patients with depression improve within hours of treatment (2,3). Importantly, both KT and SD decrease suicidality (4–10).

Circadian rhythms are intimately linked to the sleep-wake cycle (11). A subgroup of MDD patients has altered circadian processes including sleep, mood, temperature, and hormone secretions, all of which are regulated by circadian clock genes (12). Findings show a significant correlation between symptom severity and the degree of desynchronization. Moreover, many rhythms normalize as symptoms remit (13–16).

SD therapy usually involves keeping patients awake for approximately 36 hours. We proposed that by altering the sleep-wake cycle, the abnormal circadian clock genes that control rhythms could be reset (17,18). Although relapse can occur following recovery sleep, improvement can be sustained for weeks by circadian-related treatments. These include slowly advancing bedtimes (sleep-phase advance) and exposure to morning bright light (19).

Circadian studies of clock genes in mice show that in response to sleep deprivation, a subset of circadian clock genes (e.g., *Per1*, *Per2*) appear to behave as immediate early genes and are transcriptionally responsive within hours of treatment (20–23). It was also shown that depriving animals of sleep suppresses approximately 80% of rhythmic genes in the mouse (22,24).

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Compared with SD, KT's action on the circadian clock genes is less clear. In our earlier research in neuronal cell culture (NG108-15), we demonstrated KT's effect in repressing circadian expression of a group of genes essential to maintaining circadian rhythmicity. We found dose-dependent reductions in the amplitude of circadian transcription including *Bmal1*, *Per2*, and *Cry1* genes (25).

We propose that SD and KT may share common mechanisms of action that converge on circadian-related processes that act to accelerate antidepressant response (12). In this study, we performed comparative transcriptome analyses in SD- and KT-treated mice to identify candidate genes and pathways common to both treatments.

The first direct evidence for the dysregulation of the clock genes in the MDD brain comes from a microarray study. Control brains showed robust circadian rhythms across six brain areas that were dramatically altered in matched MDD patients. Of the brain regions studied, the anterior cingulate cortex (ACC) showed the most significant disruption in clock gene rhythms (26). The ACC is a major component of an extended neural network thought to regulate mood, and a growing body of data implicates the ACC as a key brain region associated with depression (27). Functional brain imaging studies show that increased activation of the ACC significantly correlates with improvement to a wide range of interventions including low-dose KT (28) and SD therapies (29).

Given the similarities in the rapid antidepressive effects elicited by KT and SD therapies, we hypothesized that these treatments may act through common molecular pathways in the ACC. To address this question, we analyzed the whole transcriptome in the ACC in groups of mice subjected to KT or SD treatment and compared them with a control group. We show that KT and SD antidepressant treatments activate common pathways and neuronal functions including synaptic plasticity, neurogenesis, and, notably, the circadian clock.

METHODS AND MATERIALS

Animals

Animals and protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee of the University of California, Irvine. Six-month-old male mice from the C57BL6/J strain were obtained from Jackson Laboratories (Bar Harbor, ME).

Two groups of 4 mice each were habituated for 1 week in standard cages. A third group ($n = 4$) was habituated to a circular cage containing a rotatory bar that was stationary during habituation. All groups were housed with ad libitum food access and water at 24°C to 25°C room temperature. After habituation, mice were either sleep deprived (SD) or injected with ketamine (KT) or saline (control mice).

Sleep Deprivation

Mice housed in the circular cage were kept awake for 12 hours (zeitgeber time [ZT] 0 to ZT 12) by a slowly rotating bar (1.5 revolutions/min), suspended 1 cm above the floor and bedding within the mouse cage (30). During the SD period, ad libitum food and water were provided.

Ketamine

KT-treated mice were injected intraperitoneally with a KT solution of 3 mg/kg at ZT 5.

Control Mice

Control mice were injected intraperitoneally with saline solution in a volume of 100 μL/0.03 kg at ZT 5.

Forced Swim Test

We conducted the forced swim test (FST) 7 hours post-injection (ZT 12) of KT or saline. SD-treated mice were subjected to the FST at ZT 12 (following SD). FST was conducted according to a standardized protocol (31). Mice were placed in a cylindrical container (height 30 cm, diameter 20 cm) filled with tap water at a temperature of 25°C. Measurements were recorded in dark conditions using an infrared recording system for a period of 8 minutes. The immobility time was measured as 1-minute bins to identify subtle behavioral differences between the different experimental groups. At the end of the FST, the animals were gently dried and euthanized.

ACC Microdissection and RNA Isolation

Immediately after euthanization, brains were extracted, frozen in dry ice, and stored at −80°C. The microdissection of the ACC (bregma 1.34 to −0.5 mm) (Figure 1C) has been previously described (32). Total RNA was extracted from each sample using TRIzol (Invitrogen, Carlsbad, CA) following manufacturer's instructions and scaled 1:10. Total RNA was resuspended in 30 μL of RNase-free water and quantified using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE). Complementary DNA (cDNA) was synthesized from 50 ng of RNA using the cDNA Synthesis kit iScript (Bio-Rad, Hercules, CA). The obtained cDNA was then diluted 1:10 and 2 μL were used as the template for reverse transcriptase polymerase chain reaction (PCR) amplification using SYBR Green (Bio-Rad) as the fluorogenic intercalating dye and the CFX96 Real-Time System (Bio-Rad). The housekeeping gene β-actin was used as a control. The remaining RNA was used for microarray experiments. The primers used for reverse transcriptase PCR amplification are presented in Supplemental Table S3.

Microarray Analysis

The remaining TRIzol-extracted RNA was diluted to 100 μL final volume of RNase-free water and cleaned using the Qiagen RNeasy Mini purification kit (Redwood, CA), following the manufacturer's protocol. Eluted RNA was quantified with a NanoDrop spectrophotometer. The quality of the RNA was assessed on an Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA). Probe synthesis and chip hybridization were performed at the Genomic High-Throughput Facility at the University of California, Irvine. Briefly, 100 ng of total RNA per sample was used as a template to obtain cDNA with the GeneChip cDNA synthesis Kit (Affymetrix, Santa Clara, CA). Mouse Gene ST arrays 2.0 (Affymetrix) were used to determine the transcriptome expression levels in the three groups of mice. The arrays were scanned in the GeneChip Scanner 3000