Archival Report

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Chronic Stress Induces Brain Region-Specific Alterations of Molecular Rhythms that Correlate with Depression-like Behavior in Mice

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ABSTRACT

BACKGROUND: Emerging evidence implicates circadian abnormalities as a component of the pathophysiology of major depressive disorder (MDD). The suprachiasmatic nucleus (SCN) of the hypothalamus coordinates rhythms throughout the brain and body. On a cellular level, rhythms are generated by transcriptional, translational, and posttranslational feedback loops of core circadian genes and proteins. In patients with MDD, recent evidence suggests reduced amplitude of molecular rhythms in extra-SCN brain regions. We investigated whether unpredictable chronic mild stress (UCMS), an animal model that induces a depression-like physiological and behavioral phenotype, induces circadian disruptions similar to those seen with MDD.

METHODS: Activity and temperature rhythms were recorded in C57BL/6J mice before, during, and after exposure to UCMS, and brain tissue explants were collected from Period2 luciferase mice following UCMS to assess cellular rhythmicity.

RESULTS: UCMS significantly decreased circadian amplitude of activity and body temperature in mice, similar to findings in MDD patients, and these changes directly correlated with depression-related behavior. While amplitude of molecular rhythms in the SCN was decreased following UCMS, surprisingly, rhythms in the nucleus accumbens (NAc) were amplified with no changes seen in the prefrontal cortex or amygdala. These molecular rhythm changes in the SCN and the NAc also directly correlated with mood-related behavior.

CONCLUSIONS: These studies found that circadian rhythm abnormalities directly correlate with depression-related behavior following UCMS and suggest a desynchronization of rhythms in the brain with an independent enhancement of rhythms in the NAc.

Keywords: Amplitude, Chronic stress, Circadian, Depression, LumiCycle, Period2

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Disruptions in circadian rhythms have been implicated in a variety of psychiatric disorders including major depressive disorder (MDD) (1). Core symptoms of MDD include low mood and anhedonia, with patients experiencing a variety of other symptoms, many of which are thought to stem from disruptions in the circadian system. For instance, MDD subjects display changes in the sleep/wake cycles and altered daily activity patterns (2,3). In addition, they frequently display altered rhythms in body temperature, hormones, cortisol, and certain neurotransmitters (2,4). Furthermore, treatments modulating the circadian cycle, such as light therapy and agomelatine z(a melatonin receptor agonist) are effective antidepressant treatments (5,6) and appear to require an intact master circadian pacemaker (7), suggesting that certain MDD symptoms may be related to circadian disruption.

Circadian function is controlled by the master clock in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (8). Circadian rhythms in the SCN and other brain regions are generated by a cycle of gene expression in individual cells that form transcriptional-translational feedback loops (9). The SCN also coordinates subordinate oscillators throughout the brain and periphery (e.g., rhythms in other brain regions and organ systems). Increasing evidence in rodents suggests that region-specific oscillations in limbic regions are instrumental regulators of emotional and motivational behaviors due to their integration of information from the SCN and homeostatic cues from hormonal signals (10). For example, acute and chronic stress, major precipitating factors of MDD, likely affect rhythms in suboscillators, without affecting phase and period of the circadian pacemaker (11). In addition, rhythms of the core circadian gene Period 2 *(Per2)* are selectively altered in emotion-related brain regions (e.g., amygdala) following disruption of circulating corticosterone (12,13). Further support for a role of molecular clocks in MDD was recently shown in a study reporting significant alterations in the diurnal variation of expression of core circadian genes in extra-SCN brain regions, including the amygdala, prefrontal cortex, hippocampus, and nucleus accumbens (NAc), of human postmortem subjects with MDD (14). In patients with bipolar disorder, skin fibroblasts transfected with *Per2* luciferase reporter constructs

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(*Per2::luc*) displayed longer circadian periods compared with healthy control subjects, and lithium (a common treatment for bipolar disorder) resynchronized and enhanced the amplitude of the dampened molecular rhythms in these cells (15). Collectively, these pioneering studies in humans suggest circadian disruptions could precipitate or underlie the psychopathology of mood disorders, and these alterations or disruptions to rhythms may lie in the SCN or other brain regions.

While there is emerging evidence in MDD for disruption of circadian rhythms in the brain, the direct effect of chronic stress on molecular, physiological, and behavioral circadian rhythms is unclear. Furthermore, it is unknown which brain regions are primarily affected by circadian disruptions in MDD and what specific circadian parameters are altered by chronic stress in mood-regulating brain regions. Here, we exposed mice to unpredictable chronic mild stress (UCMS), a well-established rodent model of a depression-like syndrome, to first characterize the effects of chronic stress on physiological and molecular rhythms in mice. We used *Per2::luc* reporter mice to noninvasively and continuously monitor the effects of UCMS on brain region-specific molecular rhythms. By identifying specific brain regions where circadian disruptions are most prominent, we can then target these regions in future studies to fully investigate the role of circadian mechanisms underlying depression.

METHODS AND MATERIALS

Animals

Adult male C57BL/6J (B6) mice were used for the telemetry and gene expression studies (Jackson Laboratory, Bar Harbor, Maine). *Per2::luc* mice were maintained on a C57BL/6J background. Mice were housed under a standard light-dark cycle (lights on at 0800 hours and lights off at 2000 hours) during the entire experiment. Experiments were conducted in compliance with the National Institutes of Health laboratory animal care guidelines and protocols approved by the Institutional Animal Care and Use Committee at the University of Pittsburgh.

Unpredictable Chronic Mild Stress

Mice (telemetry or LumiCycle and gene expression cohorts) were subjected to 4 weeks of UCMS, as previously described (16) (Supplement 1). Fur ratings were as follows [see (16)]: 1 = generally well-groomed; 2 = slightly fluffy with spiky patches; 3 = most of body fluffy with coat discoloration; eye conjunctivae slightly red; and 4 = fluffy, stained, dirty coat with bald patches; eye conjunctivae red.

Behavioral Assays

Behavioral assays were conducted during the day (Zeitgeber time [ZT] 4–10 corresponding to ZT0 (or 24) lights on and ZT12 lights off) using the elevated plus maze (EPM), open field (OF), dark/light box, novelty suppressed feeding, and forced swim test (FST) (Supplement 1).

Telemetry Recording

Telemetry transmitters (TA-F20; Data Sciences International, St. Paul, Minnesota) were implanted in the abdomen of B6 mice (control mice: $n = 7$ and UCMS: $n = 16$). Control mice were implanted with nonfunctioning dummy transmitters. Following recovery of 2 weeks from surgery, locomotor activity and body temperature were recorded for the duration of the experiment (10-minute bins across 24 hours per day). Rhythms were recorded before (2 weeks), during (6 weeks), and after (3 weeks) UCMS (Supplementary Figure S1).

LumiCycle Recordings

Following UCMS, *Per2::luc* mice underwent behavioral testing (Supplementary Figure S1) and then were sacrificed to extract explants of brain regions implicated in MDD: medial prefrontal cortex, central amygdala (CeA), basolateral amygdala (BLA), NAc, ventral tegmental area (VTA), and SCN. Individual samples were cultured on a culture membrane in a 35 mm dish (PICMORG50; Millipore, Billerica, Massachusetts), as previously described (17). Circadian rhythms were assessed for 4 days by continuous recording of bioluminescence of *Per2::luc* reporter activity using the LumiCycle 32 (Actimetrics, Wilmette, Illinois).

Quantitative Real-Time Reverse Transcriptase-Polymerase Chain Reaction

Following UCMS, male B6 mice (control mice: $n = 36$ and UCMS: $n = 36$) underwent behavioral testing (Supplementary Figure S1) and mice were sacrificed at six ZTs across the day. RNA was isolated and converted to complementary DNA (cDNA), followed by gene expression analysis for *Per2* normalized to the housekeeping gene *Gapdh* (Supplement 1).

Data Analysis

Due to the masking effects of light in standard light-dark cycle housing conditions, period and phase are not reported here for locomotor activity and body temperature rhythms because under entrained conditions these become unreliable measures of rhythm. Therefore, we used circadian amplitude as the primary marker of disruption for activity and temperature rhythms. The amplitudes of activity and temperature rhythms were calculated as the spectral power at the corresponding peak tau or period of the rhythm as calculated from chi-square periodogram analyses (ClockLab Software, Actimetrics). Circadian activity and temperature waveforms were constructed for the last week of each experimental period and also over the entire duration of the control or UCMS paradigms. These were analyzed using repeated-measures analysis of variance (ANOVA) (baseline, UCMS, recovery) followed by Tukey's post hoc tests corrected for multiple comparisons. *Per2::luc* rhythms in brain region explants were detrended and assessed for period, amplitude, and phase (Supplement 1). Fur rating and body weights were analyzed using one-way ANOVA followed by Dunnett's multiple comparisons test. Locomotor activity and body temperature rhythms were analyzed using repeated-measures ANOVA followed by Dunnett's post hoc tests. Behavioral tests were analyzed using independent samples *t* tests. Gene expression was analyzed using CircWave v.1.4 to test for significant rhythmicity using harmonic regression comprised of both sine and cosine waveforms (constrained period of 24 hours and $\alpha = .05$) and two-way ANOVA with the main factors of group and time and