

Motor-Activity Markers of Circadian Timekeeping Are Related to Ketamine's Rapid Antidepressant Properties

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ABSTRACT

BACKGROUND: The rapid clinical antidepressant effects of the glutamatergic modulator ketamine may be due to its ability to restore synaptic plasticity and related effects on sleep-wake and circadian systems. Preclinical studies indicate that ketamine alters expression of circadian clock-associated molecules, and clinical studies of ketamine on plasticity-related biomarkers further suggest an association with sleep slow waves and sleep homeostasis.

METHODS: Wrist-activity monitors were used to examine the pharmacologic and rapid antidepressant effects of ketamine on markers of circadian timekeeping (amplitude and timing) in mood disorders. Circadian amplitude and timing of activity at baseline, postinfusion day 1 (D1), and day 3 (D3) were measured in 51 patients with major depressive disorder or bipolar disorder.

RESULTS: Compared with either placebo or baseline, a mood-independent decrease of the central circadian value (mesor) was present on D1 after ketamine treatment. Mood-associated circadian effects between rapid (D1) responders and nonresponders were found at baseline, D1, and D3. At baseline, a phase-advanced activity pattern and lower mesor distinguished subsequent responders from nonresponders. On D1, ketamine nonresponders had a lower mesor and a blunted 24-hour amplitude relative to baseline. On D3, patients with a persisting clinical response exhibited a higher amplitude and mesor compared with nonresponders.

CONCLUSIONS: The findings are the first to demonstrate an association between ketamine's clinical antidepressant effects and circadian timekeeping. The results suggest that traitlike circadian activity patterns indicate rapid mood response to ketamine, and that mediators of continuing ketamine-induced mood changes include altered timing and amplitude of the circadian system.

Keywords: Clock genes, Neuroplasticity, Sleep deprivation, Slow wave sleep, Wrist activity

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Sleep deprivation (SD) and ketamine treatment both rapidly relieve symptoms in patients with major depressive disorder (MDD). The fact that both interventions affect sleep homeostasis and circadian processes suggests that the circadian and sleep-wake systems and their interactions are associated with rapid mood effects. Understanding the separate and interacting effects of these processes may provide useful clues for developing novel rapid antidepressant therapies for mood disorders.

Preclinical studies have noted that both SD and ketamine affect central circadian clock-associated molecules (1). Further, clinical ketamine studies suggest that this agent affects sleep, slow waves, and synaptic plasticity (2–4). In healthy subjects, sleep quality interacts with the circadian system to affect the temporal organization of the human transcriptome (5). Taken together, these studies suggest that interventions that restore and normalize sleep quality—such as ketamine—could correct interactions between disrupted sleep

and circadian systems to enhance temporal organization of the circadian sleep-wake system and ultimately improve mood and behavioral health.

Ketamine exerts its initial rapid antidepressant properties via a prolonged change in glutamatergic signaling resulting in increased synaptic strength and plasticity. Changes in glutamatergic transmission affect downstream structural changes in dendritic spines and local synaptic protein synthesis (6), including transport and release of brain-derived neurotrophic factor (BDNF) (7). BDNF secretion, activation of tropomyosin receptor kinase B, and downstream trafficking lead to further dendritic structural complexity, spine and BDNF synthesis, and synaptic plasticity (7,8). Ultimately, changes in critical local neuronal circuits that converge via enhanced synaptic plasticity and neuronal synchronization would hypothetically produce rapid antidepressant effects, particularly in areas involved in mood and behavior (8,9). Notably, ketamine-induced changes in BDNF levels correlate

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with both sleep slow waves (SWSs) and mood changes, as well as with improved sleep quality in individuals with treatment-resistant depression (2,4). Numerous interactions between sleep homeostatic and circadian systems are possible, such as ketamine's effects on clock genes to influence circadian timing and on BDNF and SWSs to affect sleep quality.

Wrist activity is a useful indirect measure of central circadian timekeeping (10), which may be an important transdiagnostic measure of circadian function. To advance translational research, the National Institute of Mental Health (NIMH) Research Domain Criteria (<https://www.nimh.nih.gov/research-priorities/rdoc/index.shtml>) includes circadian rhythms as a core construct within the Arousal and Regulatory System domain, and actigraphy-monitored rest/activity rhythms as a paradigm to evaluate this construct. Because changes in activity levels have been linked to circadian rhythm disorders (11), seasonal affective disorder (12), and bipolar disorder (BD) (13–15), as well as current (10,16), subsyndromal (17), and euthymic (18) MDD, the relationship between activity and specific diagnoses is likely complex. For example, activity patterns are phase-delayed and blunted in seasonal affective disorder (12) as well as blunted in both at-risk bipolar spectrum disorder (19) and in persons with elevated manic-depressive symptoms (as assessed by the Young Mania Rating Scale) (20). Furthermore, an evening circadian chronotype, regardless of delayed sleep phase syndrome, conveys risk for anxiety, depressive, or substance use disorders (21).

Indeed, dysregulated circadian timekeeping measures (amplitude, phase, day vs. night levels) often contribute to mood symptom severity. Correlations between clinical ratings and nighttime activity in MDD (22), as well as daytime activity in melancholic depression (23), indicate that day-night patterns of activity vary with symptom severity (24). For instance, severity of depression has been associated with amplitude and timing, particularly low amplitude and/or delayed daily peak activity (25), or circadian rhythm misalignment (26,27). In addition, specific markers of circadian timekeeping are often associated with effective antidepressant intervention. For example, activity patterns are phase advanced after SD (28) and bright light (12) therapies, and altered 24-hour amplitude is often associated with increased day and decreased night activity following treatment interventions (12,29–32).

In addition, clock gene variants are often associated with diurnal preference [reviewed in (33)] and have been explored in the pathogenesis of mood disorders (34). Preclinical and clinical evidence suggests that both *CLOCK* (35,36) and *Per* (37,38) mutations are associated with mood disorders. The altered motor activity patterns present with these mutations (36,39) are consistent with the possibility that mood-related temporal variations of activity are associated with genetic variants of clock-related molecules. Furthermore, disrupted patterns of activity are linked to clock-gene mutations associated with mood disorders (37,40–42). The observations that circadian clock genes also interact to affect sleep homeostasis (43–45), and that mistimed sleep affects circadian regulation of the human transcriptome (5,46), suggests that ketamine's effects on sleep timing and clock gene expression might ultimately improve the underlying molecular disorganization of the circadian system.

The present study is the first to investigate the effects of a single ketamine infusion on circadian rhythm expression and clinical response in treatment-resistant mood disorders (both MDD and BD). Specifically, we analyzed the clinical evidence for ketamine's effects on 24-hour activity patterns over the course of five days in individuals with mood disorders. The specific objectives of the study were to 1) identify ketamine's circadian timekeeping effects relative to placebo treatment and 2) assess whether ketamine's rapid antidepressant effects (e.g., its effects on mood and relapse parameters) were associated with altered patterns of circadian timekeeping.

METHODS AND MATERIALS

This study was conducted using data drawn from different investigations (under protocol 04-M-0222) exploring ketamine's antidepressant mechanism of action in patients with treatment-resistant mood disorders (2,47,48). The studies were conducted at the NIMH Clinical Research Center Mood Disorders Research Unit in Bethesda, MD, and were approved by the Combined Neuroscience Institutional Review Board of the National Institutes of Health; specific details have been previously reported (2). One study investigated the clinical effects of ketamine in MDD patients who subsequently received riluzole (another glutamatergic modulator) in an effort to extend ketamine's antidepressant effects (47), and a second study investigated ketamine's antidepressant effects in MDD and BD patients, some of whom received maintenance mood stabilizers. A continuing study is examining ketamine's effects in MDD and BD in a placebo-controlled, crossover study.

Participants

Fifty-one subjects (29 women, 22 men), 20 to 65 years old (mean age \pm SEM = 42.6 \pm 11.8 years) with confirmed clinical diagnoses of either MDD (n = 30) or BD (n = 21) were pooled in the analysis. All subjects had a Montgomery-Åsberg Depression Rating Scale (MADRS) score of ≥ 20 and were experiencing a current major depressive episode lasting at least 4 weeks. In addition, all subjects had previously not responded to at least one adequate antidepressant trial [as assessed by the Antidepressant Treatment History Form, modified (49)]. BD patients were required to not have responded to a prospective open trial of a mood stabilizer while at the NIMH (either lithium or valproate for at least 4 weeks at therapeutic levels; serum lithium, 0.6–1.2 mEq/L; or valproic acid, 50–125 μ g/mL). Exclusion criteria included the presence of psychotic features, a DSM-IV diagnosis of drug or alcohol abuse or dependence in the last 3 months, or the presence of an unstable, serious, medical illness. Women could not be pregnant or nursing. Participants were free of all psychotropic medications for 2 to 5 weeks before the assessment, with the exception of mood stabilizers among some BD patients (14 of 21 patients were receiving lithium, 5 of 21 patients were receiving valproic acid, and 2 of 21 patients were drug free). Cigarette use was permitted during the clinical trial, but alcohol use was not. Participants were not allowed to nap

during the 3 days before and after the infusion procedure and were encouraged not to nap on all days of the protocol. All subjects provided written informed consent before entry into the study and were assigned a clinical research advocate from the NIMH Human Subjects Protection Unit to monitor the consent process and research participation throughout the study.

Experimental Design

The 51 participants wore an Actiwatch (Model AW64; Mini-Mitter Co. Inc., Bend, OR) for 3 to 4 days before and for 5 days after a scheduled ketamine or placebo infusion (ketamine $n = 51$; placebo $n = 38$). The watch was removed during selected procedures and bathing. A diary was used to track watch removal and replacement. Ketamine infusion was conducted as previously described (50). Briefly, at about 10:00 PM, depressed patients received a single intravenous infusion of 0.5 mg/kg ketamine hydrochloride or placebo that lasted about 40 minutes.

Depressive symptoms were assessed via MADRS ratings conducted at baseline (60 minutes before ketamine infusion), 230 minutes postinfusion (D0), 1 day postinfusion (D1), and 3 days postinfusion (D3). At all time points, change in depressive symptoms was expressed as change in score from baseline.

Patients exhibiting a 50% reduction in MADRS scores on either D0 (230 minutes postinfusion) or at 9:00 AM on D1 or D3 were classified as D1 (within 24 hours) or D3 (through 72 hours) ketamine responders, respectively. Patients showing a less than 50% improvement on D0, D1, or D3 were classified as D1 and D3 nonresponders, respectively.

Data Collection and Analysis

Twenty-four-hour wrist-activity patterns were analyzed for differences in amplitude, phase, and mesor (the central 24-hour value of a fitted sinusoidal curve) at baseline (2 days preinfusion), D1, and D3 (where D0 = infusion); ketamine- and mood-dependent effects were examined. The Supplement contains a detailed description of the data collection methods used and the statistical analyses conducted.

RESULTS

Baseline Pooled Patient Groups

Overall, the 24-hour baseline activity pattern was characterized by low nighttime values (mean <25 counts/min) that began to rise between 5:00 AM and 6:00 AM. Values continued to rise to peak values (200–300 counts/min) at about 2:00 PM, then decreased until the lowest levels were observed the next night at about 3:00 AM (Figure 1C-a; Supplemental Figure S2, left panel). The specific parameter estimates for baseline, ketamine versus placebo, and responders versus nonresponders are summarized in Tables 1, 2, and 3.

Ketamine Versus Placebo

Ketamine infusion decreased hourly activity levels and significantly decreased the estimated mesor relative to baseline and to placebo on D1 (Figure 1B; Supplemental Figure S2), with no differences on baseline days (Supplemental Figure S1, left panel). The overall fit of the D1 24-hour activity pattern showed

significant overall effects for ketamine ($p = .0005$; Table 1). Relative to baseline, ketamine decreased the mesor of the circadian pattern ($F_{1,240} = 12.41$, $p < .001$; Table 1) with a trend ($F_{1,240} = 3.02$, $p = .0823$) to phase-advance the activity pattern on D1. Compared with D1 placebo, D1 ketamine produced a trend to decrease the mesor ($F_{1,1974} = 4.62$, $p = .0317$). Placebo infusion had no significant D1 effects on the circadian pattern relative to baseline days.

Ketamine D1: Responders Versus Nonresponders

The overall 24-hour pattern of D1 activity in the ketamine responders group differed significantly ($F_{3,1188} = 3.53$, $p < .0144$) relative to nonresponders on D1, with activity higher in the early part of the day (12:00 midnight–6:00 AM) and lower in the afternoon (12:00 noon–6:00 PM). Specifically, the timing of activity was phase-advanced in responders versus nonresponders ($F_{1,1188} = 8.42$, $p < .0038$; Figure 1C, center panel; Table 2), although the timing did not differ from baseline. Relative to baseline, D1 nonresponders, but not D1 responders, showed a decrease in the mesor ($F_{1,1188} = 9.843$, $p = .0017$) and a trend toward decrease in the amplitude ($F_{1,1399} = 4.57$, $p = .0328$; Table 2) of the 24-hour activity pattern. Similar results were obtained when excluding ketamine open-label subjects ($n = 12$) and including MDD-only subjects (for further analyses, see the Supplement).

Ketamine D1: Responders Versus Placebo

Activity timing was advanced ($F_{1,1281} = 5.357$, $p < .0208$) and the 24-hour mesor was lower ($F_{1,1281} = 6.238$, $p = .012$) in D1 ketamine responders versus D1 for those who received placebo (for further analyses, see the Supplement).

Ketamine D3: Responders Versus Nonresponders

Morning and afternoon activity levels were higher on D3 in ketamine responders (patients with a 50% reduction in MADRS scores on D3 compared with D3 nonresponders). The overall 24-hour activity pattern of D3 responders differed significantly from nonresponders ($F_{3,1111} = 3.230$, $p = .0218$), with the mesor ($F_{1,1111} = 5.411$, $p = .0202$) and amplitude ($F_{1,1111} = 3.890$, $p = .0488$) each contributing to the D3 group difference in activity patterns (Figure 1C, right panel). Increased mesor and amplitude parameters distinguished ketamine D3 responders from the placebo-treated group on D3 (for further analyses, see the Supplement).

Baseline Activity Indicators of Rapid D1 Clinical Response

Baseline 24-hour patterns of activity indicated the subsequent D1 clinical response of responders and nonresponders to ketamine infusion ($F_{3,1210} = 5.373$, $p = .0011$; Table 3). Specifically, at baseline (i.e., before ketamine infusion), next-day responders to ketamine had less activity relative to nonresponders between 12:00 noon and 11:00 PM (Figure 1C, left panel), an effect consistent with a lower mesor ($F_{1,1210} = 7.575$, $p = .006$) of the fitted curve. In addition, the baseline timing (acrophase) of the 24-hour pattern was significantly earlier in ketamine responders than nonresponders ($F_{1,1210} = 5.516$, $p = .019$; Figure 1A and C, left panel).

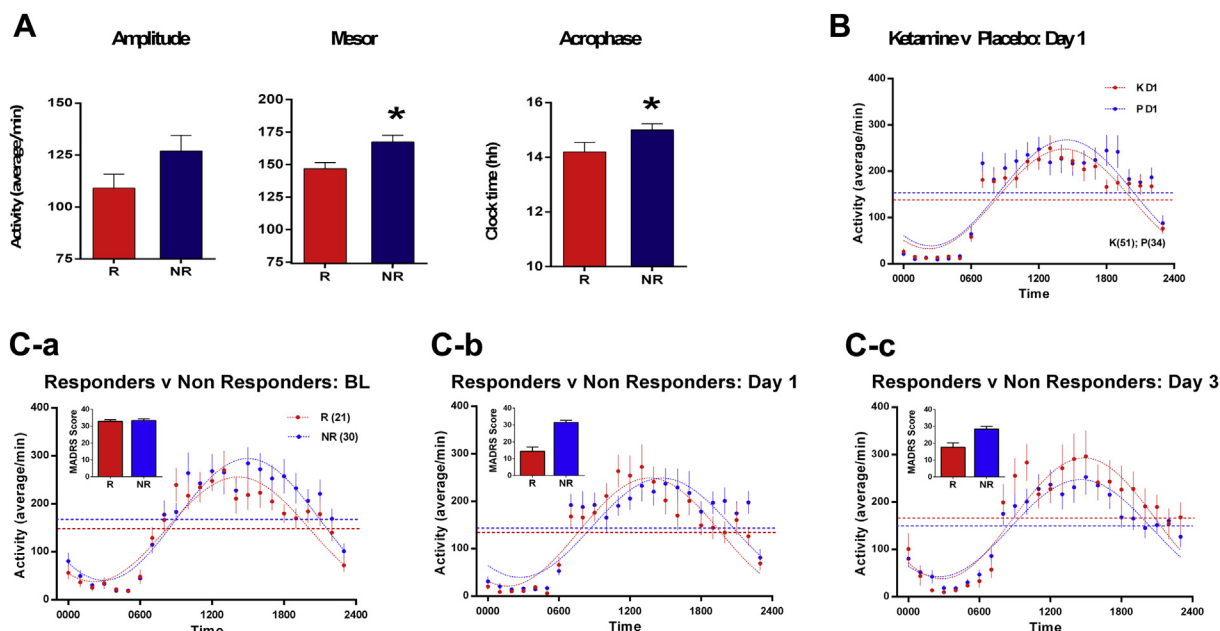


Figure 1. (A) Baseline circadian parameter estimates of amplitude, time of peak phase (acrophase), and mesor in ketamine responders (R) and nonresponders (NR). Phase-advanced 24-hour activity and decreased central value (mesor) were associated with rapid antidepressant response to ketamine. (B) Ketamine's effects on the 24-hour pattern of wrist activity for ketamine-treated patients on day 1 (K D1) after infusion compared with placebo (P D1). Ketamine decreased the mesor without changing the amplitude or phase of activity. The mesor of the 24-hour activity pattern was lower for ketamine vs. placebo treatment ($p = .0317$). (C) Baseline (BL), D1, and day 3 (D3) patterns of wrist activity for ketamine-treated patients who responded ($>50\%$ decrease in Montgomery–Åsberg Depression Rating Scale [MADRS] scores) within 1 day of ketamine infusion compared with patterns of nonresponders. Raw MADRS scores for each cohort are shown as bar chart inserts for each day. Left panel: at baseline before infusion, subsequent D1 responders are compared with D1 nonresponders. During baseline, the mesor ($p = .006$) and the phase ($p = .019$) of the baseline 24-hour activity patterns are different in D1 responders vs. nonresponders. Middle panel: responders compared with nonresponders on D1 after ketamine infusion. On D1, the phase of the 24-hour pattern of activity in ketamine responders differed from nonresponders ($p = .0038$). Right panel: responders who maintained the 50% decrease in MADRS score on D3 compared with patients who did not meet response criteria on D3. On D3, the mesor ($p = .0202$) and amplitude ($p = .0488$) of the 24-hour pattern significantly differed between responders and nonresponders. Filled circles correspond to mean activity counts per minute in hourly bins \pm SEM. The dotted sinusoidal curves correspond to the best fit line to the 24-hour data for each group. The dotted horizontal line (mesor) corresponds to the estimated 24-hour average (mesor) of the curve fits to each group. Group sizes are as follows: baseline and D1 (responders = 21, nonresponders = 30) and D3 (responders = 13, nonresponders = 35).

MADRS Ratings for Baseline, D1, and D3 in Ketamine Responders Versus Nonresponders

No difference was noted between baseline MADRS ratings of prospective ketamine responders and nonresponders (mean \pm SEM = 32.8 ± 0.98 vs. 33.4 ± 0.98 , respectively). In contrast, D1 responders had significantly lower MADRS ratings than nonresponders (11.9 ± 1.99 vs. 30.0 ± 1.51 ; $t_{49} = 7.3$, $p < .0001$), and D3 responders had lower MADRS ratings than did nonresponders (15.8 ± 2.42 vs. 27.5 ± 1.72 , respectively; $t_{46} = 4.05$, $p < .0001$; Figure 1C, bar-graph inserts). AM activity (midnight to 06:00 AM) and MADRS scores were positively correlated on D1 ($p < .05$, Pearson correlation), but not on D3. PM activity (12:00 noon to 6:00 PM) and MADRS scores were not correlated on D1, but rather showed significantly negative correlations on D3 ($p < .001$). Correlations between change in MADRS score (baseline minus D1 and D3) and corresponding amplitude, mesor, and acrophase (D1 and D3 minus baseline) change scores were significant on D3 for amplitude ($p = .016$). Trends towards significance were also seen for D1 amplitude ($p = .32$) and D3 mesor ($p = .018$; see Table 4 and Supplement).

DISCUSSION

The present study found that ketamine had distinct mood-dependent effects on wrist-activity markers of circadian timekeeping. In those who responded to ketamine, treatment was associated with advanced timing on both baseline and D1 as well as increased amplitude on D3. In nonresponders, ketamine treatment was associated with decreased amplitude on D1 and D3 (Table 2, Figure 1C). Independent of mood, ketamine had only small effects on circadian timekeeping parameters on D1: a trend to phase advance timing, but no effects on amplitude. The current findings, which are specific to individuals with treatment-resistant depression, represent the first clinical evidence linking the circadian system to ketamine's rapid antidepressant effects. The fact that clinical response was related to baseline circadian differences and that altered circadian timekeeping on D1 and D3 was mood dependent suggests that underlying circadian rhythm-related mechanisms predict and contribute to ketamine-mediated mood effects.

Taken together, the evidence suggests that activity markers of circadian timekeeping may be useful for identifying both the

Table 1. Comparison of Baseline Wrist-Activity Levels on D1 Following Ketamine or Placebo

Contrast		Individual Parameters			Combined	
		Mesor (Counts)	Amplitude (Counts)	Acrophase (Hour:min)	<i>p</i>	<i>F</i>
Ketamine: Baseline vs. D1 (<i>n</i> = 51)	Baseline	158.9 ± 3.7	118.8 ± 5.24	14:40 ± 0:09	.0005 ^a	5.940 (3,2404)
	D1	140.4 ± 3.69 ^b	107.5 ± 5.23	14:14 ± 0:11		
Placebo: Baseline vs. D1	Baseline (<i>n</i> = 38)	159.8 ± 3.95	117.6 ± 5.59	14:50 ± 0:11	.3917	1.000 (3,1690)
	D1 (<i>n</i> = 34)	153.4 ± 4.84	114.4 ± 6.82	14:26 ± 0:14		
Ketamine vs. Placebo: D1	Ketamine infusion (<i>n</i> = 51)	140.4 ± 3.69	107.5 ± 5.23	14:14 ± 0:11	.123	1.928 (3,1974)
	Placebo infusion (<i>n</i> = 34)	153.4 ± 4.84	114.4 ± 6.82	14:26 ± 0:14		

Values are mean ± SEM.

D1, day 1.

^a*p* < .001 (overall curve fit difference).

^b*p* < .001 (independent parameter test).

underlying mechanisms of ketamine's rapid antidepressant effects and target populations of patients likely to benefit from this agent. Further, because ketamine's rapid effects on mood are linked to markers of circadian timekeeping (i.e., phase and amplitude), the fact that these markers have previously been shown to be altered by circadian clock gene–related variants suggests a possible connection to clock gene machinery (33,36). Alternatively, the measured effects on these markers might be influenced by external factors, such as light or behavior.

Baseline Differences Between Responders and Nonresponders

In this study, baseline differences in the estimated circadian parameter values distinguished ketamine responders from nonresponders. Relative to nonresponders, responders showed phase-advanced circadian timing (~50 minutes), a lower mesor (~88%), and decreased amplitude (~86%) at baseline (Table 3). Baseline differences in the sleep-wake patterns of ketamine responders versus nonresponders have been described (2,3), but the possible contribution of circadian-related circuits to clinical response has not.

The baseline phase differences between ketamine responders and nonresponders suggest a functional difference between circadian circuits controlling the timing of activity rhythms that subsequently influence mood response to

ketamine. Thus, the phase-delayed and elevated activity pattern seen during pretreatment in nonresponders may provide important clues to the mechanisms underlying ketamine's rapid antidepressant effects (see below). *Clock* gene variants are associated with increased motor activity (33,36), analogous to baseline increased amplitude in nonresponders. The fact that increased circadian clock amplitude is associated with greater redundancy of clock gene variants and greater resistance to phase change after a change of external lighting cues (51) may be relevant to the treatment-resistant status of the ketamine nonresponders in this study. Further research with a larger sample is needed to understand the relationship between circadian biomarkers, clock gene variants, and ketamine's rapid antidepressant effects.

Rapid Antidepressant Effects in Responders and Nonresponders

The relationship between ketamine's effects on activity levels and mood is complex, involving both central circadian clock–controlled (52,53), and treatment-associated (24) mood effects on motor activity. In this study, D1 and D3 activity patterns appear to have been driven by ketamine's effects on underlying circadian mechanisms rather than by links between mood and activity level. For example, on D1, the responder and nonresponder groups did not differ in activity levels or amplitude, and no correlation between mood change and PM

Table 2. D1 Patterns of Wrist Activity in D1 Ketamine Responders and Nonresponders

Contrast		Individual Parameters			Combined	
		Mesor (Counts)	Amplitude (<i>n</i>)	Acrophase (Hour:min)	<i>p</i>	<i>F</i>
D1: Responders vs. Nonresponders	Responders (<i>n</i> = 21)	135.2 ± 5.06	113.9 ± 7.16	13:38 ± 0:14	.0144 ^a	3.533 (3,1188)
	Nonresponders (<i>n</i> = 30)	144.2 ± 5.18	104.6 ± 7.33	14:43 ± 0:16 ^b		
Responders: Baseline vs. D1 (<i>n</i> = 21)	Baseline	146.9 ± 4.77	109.1 ± 6.74	14:09 ± 0:14	.139	1.835 (3,999)
	D1	135.2 ± 5.06	113.9 ± 7.16	13:38 ± 0:14		
Nonresponders: Baseline vs. D1 (<i>n</i> = 30)	Baseline	167.4 ± 5.30	127 ± 7.51	14:59 ± 0:14	.0019 ^c	5.004 (3,1399)
	D1	144.2 ± 5.18 ^b	104.6 ± 7.33 ^d	14:43 ± 0:16		

Values are mean ± SEM.

D1, day 1.

^a*p* < .05 (overall curve fit difference).

^b*p* < .02 (independent parameter test).

^c*p* < .01 (overall curve fit difference).

^d*p* < .05 (independent parameter test).

Table 3. Baseline 24-Hour Patterns of Wrist Activity in Subsequent D1 Ketamine Responders Versus Nonresponders

Contrast		Individual Parameters			Combined	
		Mesor (Counts)	Amplitude (n)	Acrophase (Hour:min)	p	F
Baseline Activity:	Responders (n = 21)	146.9 ± 4.77	109.1 ± 6.74	14:09 ± 0:14	.0011 ^a	5.373 (3,1210)
Responders vs. Nonresponders	Nonresponders (n = 30)	167.4 ± 5.30 ^b	127 ± 7.51	14:59 ± 0:14 ^c		

Values are mean ± SEM.

D1, day 1.

^ap < .05 (overall curve fit difference).

^bp < .01 (independent parameter test).

^cp < .05 (independent parameter test).

activity level was observed despite significant differences in mood (MADRS scores declined by >50% in responders; Figure 1C, center panel and bar-graph inset). The fact that responders had an earlier acrophase of 24-hour activity at baseline and D1 (Table 3) suggests that the phase-advanced activity-rest pattern observed here was associated with potential rapid response, similar to SD and light therapies (28).

Responder and nonresponder groups had different timing and amplitude parameters (Figure 1, A and C) at baseline, D1, and D3, further suggesting biological differences in the organization of their circadian system, consistent with different sleep-wake effects for these groups with regard to ketamine's effects on slow wave sleep and BDNF (2,3). Delayed circadian phase, which was present in the nonresponders, was previously linked to elevated glutamatergic levels in emerging depression (54), suggesting that these levels may persist in ketamine nonresponders.

CLOCK gene variants alter circadian amplitude and phase (55,56), biomarkers also associated with morning-evening variation in chronotypes (57), suggesting that such gene mutations might mediate rapid antidepressant response to ketamine (Table 3), and that their markers (amplitude and phase) may also be useful for predicting ketamine response per se.

Interacting Sleep and Circadian Processes Affect Mood Change

The observation that circadian amplitude progressively increased from D1 to D3 in responders indicates a strengthening of the circadian system consistent with progressively greater interaction between sleep homeostatic and circadian processes across D0, D1, and D3. This interaction may involve a cascade of events initiated by plasticity-associated molecules [e.g., BDNF (2,4,58)] as well as improved sleep quality.

Table 4. Correlations Between Treatment Response and Individual Circadian Parameter Estimates

Contrast	Circadian Parameter ^a		
	Amplitude	Mesor	Acrophase
MADRS BL Minus Day 1	.3012 ^{b,c}	.1855	.1364
MADRS BL Minus Day 3	.3483 ^d	.3430 ^e	-.0027

BL, baseline; MADRS, Montgomery-Åsberg Depression Rating Scale.

^aDay 1 and day 3 minus baseline.

^bPearson correlation coefficient.

^cp < .1.

^dp < .05.

^ep < .06.

Accordingly, D1 MADRS scores correlated with decreased activity counts and increased SWSs the first night post-infusion. In contrast, markers of circadian timekeeping (amplitude and acrophase) were not affected on D1.

Clinical ketamine studies show that this agent affects sleep, SWSs, and synaptic plasticity (2–4), while preclinical SD studies and ketamine interventions have identified their effects on central circadian clock-associated molecules (1,59). It has been proposed that circadian and sleep-related processes interact to mediate the rapid and continued features of mood response through a progressive temporal reorganization of the human transcriptome (5) that involves properly synchronizing sleep with phase of the circadian clock to positively affect the human transcriptome (5,46). Circadian clock genes affect sleep homeostasis (43–45), suggesting that ketamine's effects on sleep timing and/or clock gene expression may improve the underlying molecular organization of the circadian system. Both ketamine and SD rapidly (within 24 hours) improve mood, alter cellular/molecular events associated with plasticity and sleep homeostasis, and alter clock gene-associated molecules. In contrast, while selective serotonin reuptake inhibitors and light therapy rapidly alter clock gene-associated molecules, the beneficial mood effects are more delayed (days to weeks). This suggests that ketamine's plasticity and sleep-associated effects are more important than clock-gene effects as initiating factors for rapid mood benefits, and that the later interaction of these early plasticity changes with circadian clock-associated effects could be linked to the extended mood benefits (Figure 2).

Conceptual Organization and Localization of Ketamine's Circadian Timekeeping Effects

Several conceptual levels may be considered in discussing postinfusion reorganization of circadian timekeeping. First, ketamine may alter timekeeping of the central clock itself by acting on clock gene-related molecules within the central clock, on transmitters, or on cellular coupling within the central clock. The fact that ketamine alters ectopically expressed *CLOCK:BMAL1*-mediated expression as well as transcription of *Bmal1*, *Per2*, and *Cry1* (1)—which are also present within the suprachiasmatic nucleus—is consistent with central clock effects. If central clock genes were altered by ketamine, markers and output rhythms generated by the central clock (activity, 24-hour body temperature, melatonin, cortisol, and gene expression) might be similarly affected. This is therefore a testable hypothesis in future experiments.

Ketamine Alters Circadian Timekeeping Markers

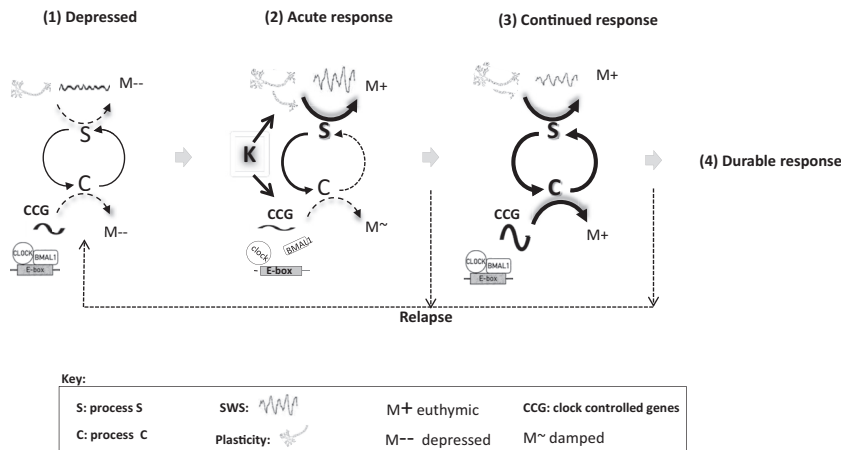


Figure 2. A model of rapid mood response to ketamine (K) is shown that incorporates the temporal interactions of sleep, plasticity, and clock-associated genes. In this schematic, ketamine's rapid antidepressant effects and individuals' subsequent relapse are related to the interaction of sleep homeostasis (process S) and circadian (process C) processes. While depressed (1), sleep loss, stress, environmental, and genetic factors are associated with diminished sleep homeostatic (S) and circadian (C) mechanisms, weakening S-C interactions, and promoting depressed mood (M--). (2) Acutely (four to 24 hours) after ketamine infusion, ketamine rapidly increases plasticity and improves mood (M+), as well as increases sleep slow waves (SWS) and sleep quality. Simultaneously, ketamine acts on clock-controlled gene (CCG)-associated molecules to alter timing and diminish circadian output, thus weakening the S-C interaction and lessening the circadian mood component. (3) During

a continued antidepressant response, the interaction between S and C is strengthened as increased S acts on C, thus facilitating and re-establishing a more functional S-C interaction, greater temporal organization of the transcriptome, continued mood improvement (M+), and potentially contributing to a durable response (4). Alternatively, a weakened S-C interaction is associated with relapse.

However, when its effects were examined independent of mood in the current analysis, ketamine did not alter amplitude or phase on D1. This suggests that ketamine, when administered at about 10:00 AM, did not alter central clock function when effects were examined independent of mood change.

A second possibility is that ketamine might affect entrainment circuits that synchronize the central clock to external lighting cycles. Interestingly, *N*-methyl-D-aspartate antagonism alters glutamate-mediated light input to the central clock as well as *Per* expression (60,61), which would be consistent with ketamine-altered light input to the central clock. However, the fact that ketamine has a half-life of 2.5 to 3 hours, and was infused midmorning when the phase-shifting effects of light are negligible, argues against a direct effect of ketamine on light input per se. While the presence of psychoactive ketamine metabolites with more extended presence (62,63) might implicate these metabolites in light-input pathways, to our knowledge their circadian system properties have not been evaluated.

A third possibility is that ketamine affects mood-related circuitry in a way critical to both its rapid and/or enduring mood effects. Ketamine, acting outside of the central clock, might thus affect noncentral clock gene expression (such as *PER2* and *BMAL1*) within reward circuits of the ventral tegmental area and ventral striatum (37,38). This interpretation would require ketamine to act on abnormal clock gene patterns within regions and circuits implicated in both mood and motor activity (64), thus linking ketamine's effects to the timing and amplitude of activity. It has been suggested that *Clock* (65) and *Per2* (37,38) may affect monoamine oxidase A transcription, dopamine levels (37,38), and mood (39). Ketamine is known to enhance dopamine turnover (66), which is closely linked to known antidepressant mechanisms of action. Thus, while the current results do not allow a distinction between ketamine acting at the central clock versus a noncentral site, the results support the possibility that it could act on clock genes within circuits also implicated in mood and activity.

Study Limitations and Strengths

This study is associated with several limitations. First, due to clinical considerations, only one measure of circadian clock output was used; future studies would benefit by selecting more specific markers of the central clock, such as 24-hour melatonin, cortisol, and core body temperature, as well as measurement of light exposure, which is critical to external synchronization. In addition, the convenience marker used here, wrist activity, does not necessarily mark the expression of the underlying clock, although it was optimized for studying sleep and circadian rhythms (11) by using multiple days (67,68). The current study required us to compare single days to examine rapid mood changes. Second, while the sample size of the pooled subject groups was large for a study of this kind, if increased, it could enable researchers to distinguish between MDD and BD cohorts and/or the effects of mood stabilizers. As discussed earlier, the cohort sizes were small, and cohort results should thus be regarded as preliminary. Third, the study lacked a healthy control group needed for comparing pharmacological, phase, and amplitude markers of circadian timekeeping with the patient cohorts. This study is currently in progress.

The study is also associated with several strengths. First, the overall results are based on populations in which significant clinical depression is a core symptom, which strengthens the finding as it relates to treatment-resistant depression and significant clinical depression per se. This strength informs a broader interpretation of the biological underpinnings of ketamine response. Second, the study had several controls, including a baseline, placebo arm, and responder versus nonresponder contrasts for ketamine infusion.

Taken together, the results indicate that wrist-activity markers are linked to drug and mood-dependent effects of ketamine. As initiatives such as Research Domain Criteria purport to understand the neural basis of human functioning, rapid-acting treatments such as ketamine can provide key insights into the relationship among circadian rhythms, activity, and mood response.

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CAZ is listed as a co-inventor on a patent for the use of (2*R*,6*R*)-hydroxynorketamine, (S)-dehydronorketamine, and other stereoisomeric dehydro- and hydroxylated metabolites of (*R*,*S*)-ketamine metabolites in the treatment of depression and neuropathic pain. CAZ is listed as co-inventor on a patent application for the use of (2*R*,6*R*)-hydroxynorketamine and (2*S*,6*S*)-hydroxynorketamine in the treatment of depression, anxiety, anhedonia, suicidal ideation, and posttraumatic stress disorders; he has assigned his patent rights to the U.S. government but will share a percentage of any royalties that may be received by the government. All other authors report no biomedical financial interests or potential conflicts of interest.

ClinicalTrials.gov; Investigation of the Rapid (Next Day) Antidepressant Effects of an NMDA Antagonist; <https://clinicaltrials.gov/ct2/show/NCT00088699>; NCT00088699.

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