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

Supplemental Information

Supplemental Methods

Data Collection and Analysis

Activity counts were collected in one-minute bins. The individual time series were edited for intervals of watch removal and outlier values corresponding to exercise. One-minute averages were then calculated for each hour.

The 24-hour activity pattern differences (amplitude, timing) were compared on D1 for: a) ketamine versus placebo treatment effects, and b) responder vs non-responder group differences (GraphPad Prism v 6.04). In addition, secondary analyses were conducted to a) evaluate baseline indicators of later ketamine response; b) compare double-blind infusion effects with open-label effects in patients who received ketamine and placebo (as part of a double-blind protocol evaluating the capacity of a second intervention (riluzole) to prolong ketamine response) (1); and c) to evaluate diagnostic (MDD versus BD) contributions to circadian activity patterns.

Statistics

Circadian (24-hour) patterns of wrist-activity were compared for ketamine vs placebo on D1 and D3, and between baseline and treatment on D1 (the first day after infusion). The 24-hour activity patterns were also compared in ketamine responders vs non-responders for group differences at baseline, D1, and D3.

The best-fit 24-hour curves for the above treatment contrasts (baseline versus treatment) were characterized using a least-squares sine wave fit to activity scores. Specifically, mesor (the central sinusoidal value), amplitude, and phase estimates of wrist activity were derived using a minimal least squares algorithm to fit the 24-hour time series to a sinusoidal curve of the form:

$[y(t) = M + A\sin(2\pi f t + P)]$ where M = *mesor (a centrality estimate of the sinusoid)*, A = *amplitude*, f = *frequency*, and P = *phase*.

Frequency was constrained to be 6.28 (24 hours). Best-fit parameters for mesor, amplitude, and phase were derived for the contrasts described above (e.g., D1 ketamine versus D1 placebo). However, amplitude and phase (timing) were the primary focus of this analysis due to their utility as markers of central circadian timekeeping. For convenience, the clock time of the peak activity (acrophase) is reported in the results as a phase marker. The study objectives described in the main manuscript were addressed by examining group and treatment differences between 24-hour activity curves by comparing an F-test "loss of fit" when applying shared

versus independently-derived parameter estimates to the group data; Bonferroni correction for multiple curve comparisons was used with a corrected alpha criterion significance level of p<.0125, two-tailed. Exploratory analysis of variance with repeated measures was used to compare activity levels of ketamine- and placebo-treated groups on treatment D0. Significance was evaluated at $p < .05$, two-tailed. Pearson correlation coefficients were calculated to examine relationships between MADRS scores and activity counts using Bonferroni correction.

Supplemental Results

Ketamine versus Placebo

Ketamine infusion decreased hourly activity levels and significantly decreased the estimated mesor relative to baseline and to placebo on D1 (Fig.1B; Supplemental Fig.S2) with no differences on baseline days (Supplemental Fig.S1, left panel). On D0, ketamine and placebo interventions were associated with an eight-hour interval of reduced activity with no difference between interventions from 12:00-23:00 hours (Supplemental Fig.S1, right panel). The overall fit of the D1 24-hour activity pattern showed significant overall effects for ketamine (p=.0005, Table1). Post-hoc analysis indicated that this effect was significant (p<.0128) when patients receiving open-label ketamine as part of a riluzole study (n=12) were omitted from the analysis.

Ketamine D1: Responders versus Non-Responders

Post-hoc analysis suggested that the D1 phase-advance of responders versus non-responders was significant ($p<.0163$) when patients receiving open-label ketamine (n=12) were omitted from the analysis. Relative to their baseline, D1 non-responders, but not responders, showed a decrease in the mesor ($p=.0017$; $F=9.843$; $df=1, 1188$), and a trend toward decrease in the amplitude ($p=.0328$; $F=4.57$; $df=1,1399$; Table2) of the 24-hour activity pattern. Similar activity patterns were observed in the subset comprising only MDD patients (Supplemental TableS1), indicating MDD specificity and independence from the effects of mood stabilizers (used within the BD cohort).

Placebo Contrasts Between Responders and Non-Responders

Post-hoc comparison of the ketamine responders to the placebo group (n=38) on D1 were consistent, with some differences noted above between ketamine responders and non-responders. Activity timing in responders was phase advanced ($p<.0208$; $F=5.357$; $df=1, 1281$), and the 24-hour mesor was lower ($p=.012$; $F=6.238$; $df=1, 1281$) in D1 responders versus D1 for those who received placebo. However, the activity mesor in non-responders was also significantly decreased relative to placebo, consistent with a ketamine mood-independent effect on the mesor.

Ketamine D3: Responders versus Non-Responders

Placebo Contrasts: Post-hoc analysis indicated that elevated mesor and amplitude parameters ($p<.04$; $F=3.234$; $df=2, 1065$) distinguished ketamine D3 responders from the placebo-treated group on D3. There were no D3 differences between ketamine non-responders and placebo non-responders.

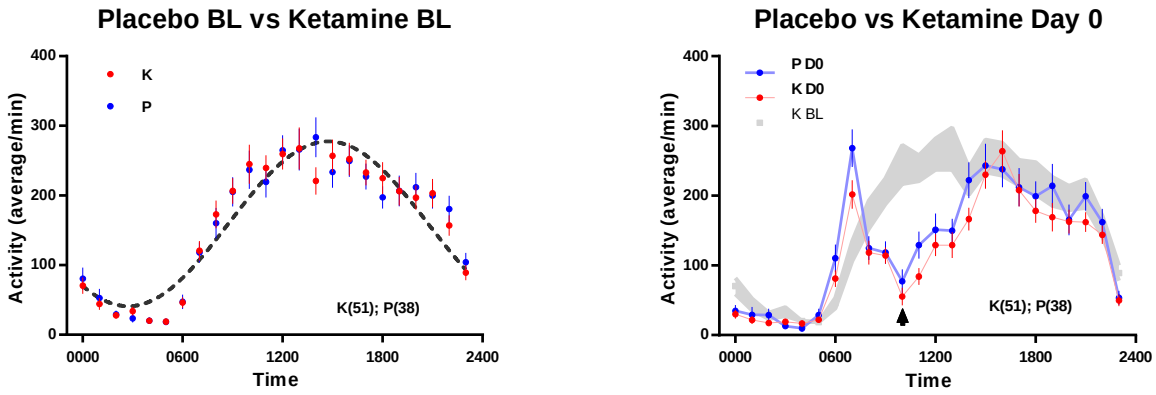
Baseline Activity Indicators of a Rapid D1 Clinical Response

Baseline 24-hour patterns of activity indicated the subsequent D1 clinical response of responders and non-responders to ketamine infusion (Table3; $p=.0011$; $F=5.373$; $df=3, 1210$). Exploratory analysis indicated these effects were significant ($p<.0231$) when patients receiving open-label ketamine (n=12) were omitted from the analysis.

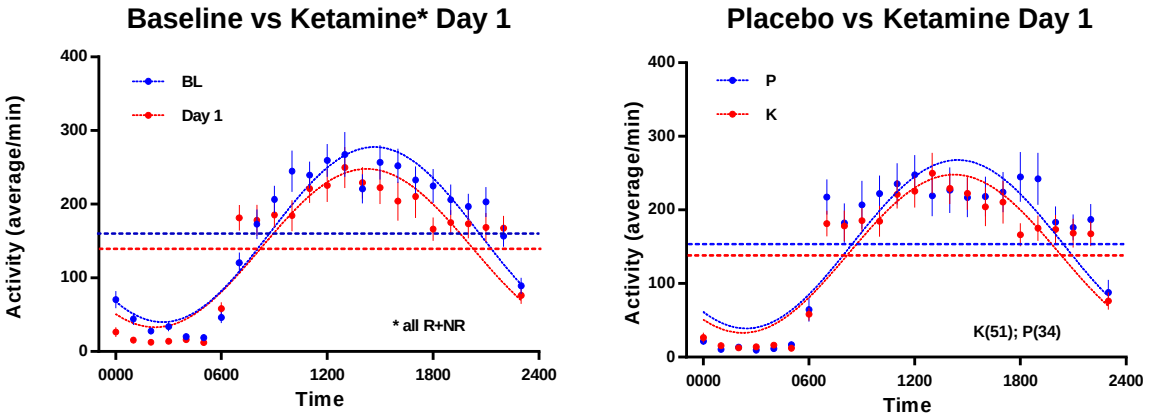
MADRS ratings for Baseline, D1, and D3 in Ketamine Responders versus Non-Responders

In order to examine the relationship between mood and activity levels, post-hoc Pearson correlation coefficients were calculated between change in MADRS score and change in two measures of wrist activity (morning activity (midnight-06:00; the interval of lowest 24-hour activity) and afternoon activity (12:00-18:00; the interval of peak 24-hour activity)) on D1 and D3 (baseline D1 and baseline D3). Morning activity and MADRS scores were positively

correlated on D1 ($p<.05$), but not on D3. Afternoon activity and MADRS scores were not correlated on D1, but showed significantly negative correlations on D3 ($p<.001$). This suggests that on D1, but not D3, improved mood was associated with decreased night activity (consistent with improved sleep quality); in contrast, on D3—but not D1—improved mood was associated with higher daytime activity. Consistent with the relationship noted above between mood and activity levels on D3 (after Bonferroni correction using an alpha criterion significance level of $p<.0167$), a significant correlation was found between mood and amplitude change scores on D3 with a trend on D1. Correlations between change in MADRS score (baseline minus D1 and D3) and the corresponding amplitude, mesor, and acrophase (D1 and D3 minus baseline) change scores were significant on D3 for amplitude (uncorrected $p=.016$); trends were also present for D1 amplitude (uncorrected $p=.032$) and D3 mesor (uncorrected $p=.018$) (see Table4).



Supplemental Figure S1: Ketamine's (K) effects on the 24-hour pattern of wrist activity are shown for ketamine treatment at baseline (BL; one day before ketamine treatment; left panel) versus placebo (P) treatment on the day of ketamine treatment (right panel). At baseline there was no difference between the 24-hour patterns of placebo and ketamine activity (left panel). Both placebo and ketamine infusions (indicated by arrow at 10:00 on D0) were associated with an eight-hour infusion- and recovery-related decrease in the level of activity (right panel; ketamine and placebo are shown relative to the pre-ketamine baseline mean ± SEM (shaded area)). On D0 there was no difference between ketamine and placebo treatments from 12:00-00:00; ($F_{1,94}$ = 0.79, p=.37). Filled circles correspond to mean activity counts/minute in hourly bins ± SEM. The dotted sinusoidal curve corresponds to the best fit line to the 24-hour data for both placebo and ketamine baseline days.



Supplemental Figure S2: Ketamine's effects on the 24-hour pattern of wrist activity are shown for ketamine-treated patients one day after infusion (D1) versus their prior baseline (BL) (left panel), and compared with D1 placebo treatment (right). On D1, the central value (mesor) of the 24-hour activity pattern was lower for ketamine versus baseline (p=.0004, left panel), and there was a trend for ketamine to be lower than placebo treatment (p=.0317, right panel). There was also a trend (p=.0823) for ketamine to advance the phase of wrist activity relative to prior baseline days (left panel). Filled circles correspond to mean activity counts/minute in hourly bins ± 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.

Supplemental Table S1: Comparisons of 24-hour patterns of wrist activity in patients with mood disorders who did and did not respond to ketamine infusion on Day 1 (D1).

CONTRAST		Individual Parameters			Overall	
		Mesor (counts)	Amplitude (counts)	Acrophase (hh:mm)	*P*	F
D1: Responder vs Non-Responder	Responders (n = 13)	141 ± 6.99	125.7 ± 9.90	13:23 ± 18.0	.0087**	3.915 (3,696)
	Non-Responders (n = 17)	160.2 ± 6.34†	124.8 ± 8.97	14:30 ± 18.0††		
Responders: Baseline vs D1 (n=13)	Baseline	149.7 ± 6.32	113.3 ± 8.94	13:25 ± 18.1	0.6273	0.5816 (3,616)
	D1	141 ± 6.99	125.7 ± 9.90	13:23 ± 18.0		
Non-Responders: Baseline vs D1 (n = 17)	Baseline	185.7 ± 7.36	153.8 ± 10.43	14:52 ± 15.5	0.0072**	4.044 (3,786)
	D1	160.2 ± 6.33 ††	124.8 ± 8.97†	14:30 ± 18.0		
Mean Estimate ± SEM *For overall curve fit difference: * p < .05 ** p < .01 *** p < .001* *For independent parameter tests: † p < .05 †† p < .01 ††† p < .001*						

Supplemental Reference

1. Ibrahim L, Diazgranados N, Franco-Chaves J, Brutsche N, Henter ID, Kronstein P, et al. (2012): Course of improvement in depressive symptoms to a single intravenous infusion of ketamine vs add-on riluzole: results from a 4-week, double-blind, placebo-controlled study. *Neuropsychopharmacology* 37:1526-1533.