Treatment Enhances Ultradian Rhythms of CSF Monoamine Metabolites in Patients with Major Depressive Episodes

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Unipolar and bipolar depressions show abnormal behavioral manifestations of ultradian (less than 24 h) rhythms, but abnormal rhythms of the central neurotransmitters thought to be important for depression pathophysiology (eg dopamine (DA) and serotonin (5-HT)) have not been shown in this time frame. Since antidepressant treatments normalize disrupted rhythms in depression (eg rapid-eye-movement sleep and hormonal rhythms), we hypothesized that depression-related changes in ultradian oscillations of DA and 5-HT might be revealed during antidepressant treatment. Cerebrospinal fluid (CSF) samples collected q10 min for 24 h in 13 patients experiencing major depressive episodes (MDE) before and after treatment for 5 weeks with sertraline or bupropion were assayed for levels of homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA), and their ratio was calculated. Data were analyzed in the frequency domain using Fourier transforms and multivariate permutation testing. Antidepressant treatments were associated with decreased variance for 5-HIAA, increased variance for HVA, and markedly increased variance for the HVA : 5-HIAA ratio ($p < 0.05$, $p < 0.02$, and $p < 0.003$, respectively). With treatment, the correlations between 5-HIAA and HVA weakened ($p = 0.06$). Power spectral density (PSD—the Fourier magnitude squared) of the 5-HIAA signals at periods of 1.75 and 3.7 h (both $p < 0.05$) decreased, while circadian cycling of HVA levels ($p < 0.05$) and of the ratio ($p < 0.005$) increased after treatment. The PSD of the full-length HVA : 5-HIAA ratio series after treatment increased in rapid variability (20–103 min periods, $p < 0.05$). Spectrographic windowing demonstrated a focal span of enhanced HVA : 5-HIAA ratio variability following antidepressant treatment, in an approximately 84-min period through the evening ($p < 0.05$). Periodic neurotransmitter relationships in depressed patients were altered by treatment in this analysis of a small data set. This may represent a baseline abnormality in the regulation of periodic functions involved in the depression pathophysiology, but it could also be due to an unrelated antidepressant effect. Further studies including comparisons with healthy subject data are in progress.
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INTRODUCTION

Diminished variability in thought, behavior, and mood are distinguishing features of major depression that are reliably detected in serial measures or ratings over periods of time shorter than 24 h. Healthy individuals experience variable moods over time and attribute them to elective thoughts or events in the environment, while in depression the mood changes are felt to be 'uninfluenceable' (Wefelmeyer and Kuhs, 1996). Studies using high-frequency sampling to investigate neurobiological variability in depression are needed. Cross-sectional data across a population at a single time point may not reliably capture mood since depressed people often smile for a moment just as happy people sometimes frown. Time series information from EEG lacks selective neurotransmitter information and is limited to cortical activation. Most studies of neurochemical changes in depression have utilized only single time values as the primary outcome measures, for example, levels of cerebrospinal fluid (CSF) metabolites, neuroendocrine outputs as area-under-the-curve (AUC), PET uptake or ligand binding, or average of activities in fMRI. Geracioti *et al* (1992, 1997, 1998) have previously shown the safety and utility of high-frequency CSF sampling to study dynamic neurochemical changes in depression and other disorders.

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Altered circadian periodicities in mood behaviors (Kraepelin, 1913) were recognized before central neurochemical measurements were possible. Disrupted biological rhythms have been hypothesized to underlie affective dysregulation (Healy, 1987; Ehlers et al, 1988). Phase-advanced temperature profiles and onsets of rapid-eye-movement (REM) sleep relative to sleep schedules in depression prompted treatment trials using a shift of the sleep schedule forward by 6 h to correct the shift in temperature and REM rhythms (Wehr et al, 1979). Alternatively, biological rhythm disruptions and depression after psychosocial losses have also been linked to changes in social zeitgebers resulting from such losses (Ehlers et al, 1988).

Neurophysiologic ultradian rhythms are diminished in depression. Ultradian oscillatory patterns in electrical (EEG) activity (Polich, 1997) change with mood (Cazard et al, 1992; Hayashi et al, 1994). EEG asymmetry across hemispheres (Klein and Armitage, 1979; Shannahoff-Khalsa, 1993) and intra-and interhemispheric coherence losses (Armitage et al, 1999) demonstrate dysfunctional oscillatory activity in the cortex in depression (Armitage et al, 2000). EEG dynamical complexity (nonlinear forecasting and entropy) shows lower entropy in depression and an association between poor recovery of these measures during treatment and progression and recurrences of the illness (Pezard et al, 1996).

Circadian mood rhythms (Carpenter et al, 1986; Wirz-Justice, 2000) and the predictions of treatment outcome based on pretreatment diurnal patterns (Carpenter et al, 1986; Haug and Wirz-Justice, 1993) suggest that rhythmic variability is important for healthy emotional function. Evidence suggests potential mechanisms and effects of rhythmic variability, for example, tryptophan hydroxylase function shows dynamic variability in serotonin (5-HT) synthesis (Knapp and Mandell, 1983), and in depression hypothalamic peptides modulate EEG complexity during sleep (Ehlers and Kupfer, 1987). Peripheral 5-HT function, as reflected in platelet activity, shows circadian disturbances in depression (Oxenkrug, 1979) and schizophrenia (Rao et al, 1994). Circadian neurochemical changes have been related to pacemaker effects of the suprachiasmatic nucleus (Bunney and Bunney, 2000).

However, the existence of a true basic rest–activity cycle (Kleitman, 1982) or an ultradian neurochemical oscillator remains controversial because ultradian rhythms in neurochemical activity remains under-reported and poorly understood. Recently, measures from samples taken every 10 min for 24 h from 12 healthy subjects showed variability of both the serotonin precursor (tryptophan, TRP) and its major metabolite (5-HIAA) in lumbar CSF that significantly exceeded variability of the assay used (both $p < 0.0001$; 25.4 vs 7.9% and 32 vs 7.0%, respectively). A strong circadian variability accounted for much of the TRP variance but was not a significant component of the 5-HIAA variance (Kennedy et al, 2002). Ultradian rhythms accounted for much of the remaining variance, with prominent 12 h, 3 h, 90 min, and 53 min wavelengths in the 5-HIAA time series (accounting for 69% of the variance), and with 12 and 3 h wavelengths in HVA levels (accounting for 73% of the variance) in healthy subjects (H Gwirtsman, personal communication). Thus, intensive and prolonged sampling of healthy human lumbar CSF has shown neurochemical

ultradian rhythms, but changes in these rhythms have not previously been shown in depression.

Serial sampling studies have examined levels of the individual neurotransmitter metabolites. The HVA : 5-HIAA ratio has been used previously in single sample studies to indicate relative transmitter turnover rates. Antidepressant treatments increased this ratio (DeBellis et al, 1993), largely the result of diminished 5-HIAA levels in the denominator, but differently among various treatments (Risby et al, 1987) and, in pretreatment measures, predicting clinical outcome (Carpenter et al, 2004). This ratio has been shown to be remarkably constant and unaffected by the stress of the lumbar puncture (Geracioti et al, 1998; Hill et al, 1999). In a time series, changes in the ratio may reflect the independence of the dopamine (DA) and 5-HT systems. Low local variability in the ratio (eg, a flat profile) would suggest strong positive interactions or an external mechanism that coregulates or synchronizes the systems. Increased variability in the ratio would be anticipated with greater independence or with negative interactions. Time-dependent ultradian changes in the ratio differ from simple correlations, which only give an average 'independence' over the entire signal.

Preclinical evidence of mechanisms that could cause diminished neurobiologic rhythms in depression is abundant. Covariability of monoaminergic function varies throughout the 24 h cycle. Strong intercorrelations of dorsal raphe 5-HT and locus coeruleus norepinephrine concentrations during the day were diminished at night (Agren et al, 1986). Expression of the 5-HT transporter fluctuates over time and affects citalopram binding, sleep behaviors, brain 5-HT levels, 5-HT$_{1A}$ receptors, and 5-HIAA levels (Fabre et al 2000). Both citalopram binding at the serotonin transporter (Kulikov et al, 1997) and 5-HT$_{2C}$ receptor RNA expression (Holmes et al, 1997) increase significantly during morning hours. Monoamine function varies diurnally in rodent hypothalamus slices (Blier et al, 1989) and 5-HIAA rhythms vary regionally in rodent microdialysis studies (Nakayama, 2002).

Specific receptor subtypes control circadian events. The 5-HT$_{1A}$ partial agonist, gepirone, induces phase-advances in circadian activity rhythms (Van Reeth et al, 1999). The 5-HT$_7$ receptor also may mediate phase-advances in the suprachiasmatic nucleus in the rat and is downregulated in response to fluoxetine. Activators of 5-HT$_7$ include a variety of antidepressants (Lovenberg et al, 1993; Mullins et al, 1999). Periodic cell function determinants, possibly including cyclin-dependant kinases, may point to novel treatments for psychiatric disorders (Chergui et al, 2004). 'Healthy' oscillatory function would appear to be important since neurotrophic activations are enhanced by oscillations in transmitter levels (Nishi et al, 2000).

Clinical studies have identified altered circadian neurochemical rhythms in depression, with diminished variability of plasma amino-acid monoamine precursors (Birkmayer and Linauer, 1970) and plasma monoamine metabolites (Riederer et al, 1974). Periodicity of hypocretin levels is diminished in depression (Prospero-Garcia et al, 1999; Salomon et al, 2003). MAOI effects on rest–activity cycles are controlled in part by light schedules (Duncan et al, 1998). In view of these findings, lost efficacy with chronic antidepressant use (Cassano and Fava, 2004) could be due

to recurrent maladaptive network attractors (boundary-limited looping patterns of activity), creating an overly constrained ultradian cycle.

Antidepressant treatments restore normal cycles of mood (Jouvent et al, 1998), REM sleep (Thase et al, 1998), EEG θ frequencies (Knott et al, 1996), rest–activity cycles (Raoux et al, 1994; Duncan et al, 1998), and hormonal rhythms (see review, Duncan, 1986). To test a hypothesis that ultradian neurochemical rhythms would be affected by antidepressants, CSF samples at 10-min intervals for 24 h were collected from depressed patients and examined for changes in spectral (oscillatory frequency) features before and after antidepressant treatment.

Frequency domain analyses of neurochemical time series data should help to refine and reconcile models of neurochemical dysfunction in depression, for example, the catecholamine deficit (Schildkraut, 1965) or the phase-advance (Wehr et al, 1980) models. The Fourier transformation displays the relative strengths of various frequencies in a set of coefficients for specific wavelengths, analogous to marking signal strengths along an FM radio dial. Validity of the transformed data is established by its mathematical uniqueness, the independence (orthogonality) of each frequency coefficient, and the faithful reproduction of the original signal by the inverse transform with minimal gains or losses of information. The power spectral density (PSD), which is the square of the Fourier transform magnitude, is more often implemented. Spectrograms are serial PSDs from shorter segments of the sample that are lined up consecutively by time period along the time axis. Paired Fourier or PSD coefficients in the spectrogram can be compared statistically with multivariate permutation testing. We used these methods to test the hypothesis that power in a specific bandwidth or limited number of bandwidths would change after treatment in patients with major depressive episodes in a within-subjects design. The data suggest that changes in periodic patterns in neurochemical output may be important markers of neural network dysfunction in major depression.

MATERIALS AND METHODS

Patients

All studies were approved by the Vanderbilt IRB. After extensive individual discussions to obtain written informed consent, subjects were admitted to the Vanderbilt General Clinical Research Center (GCRC). A total of 15 depressed patients (10 female, five male, ages 25–60 years, mean 37.7 years) met all criteria below and DSM-IV (APA, 1994) criteria for Major Depressive Episode (Major Depressive Disorder or Bipolar Disorder, Depressed). Diagnoses of major depression were established by SCID-P (First et al, 1996a). Severity of depression was quantified by mood ratings (Hamilton Rating Scale for Depression, HRSD, from the Yale Depression Inventory, Mazure et al, 1986). Both were confirmed in a clinical interview by a boarded psychiatrist (RMS). Exclusions for severe personality disorders were assured by SCID-II (First et al, 1996b). Medical history review, physical exam, ECG, and laboratory evaluations showed that all subjects were free of other significant abnormalities. Patients were free of psychotropic

medications and substance abuse for 2 and 6 months, respectively, and were free of all other medications for at least 1 month, including OTC analgesics. Potentially suicidal patients were excluded to avoid delaying treatment, because of invasive study risks, and because of potentially inappropriate motivation for participation. Adequate volitional and cognitive capacity was required. Data from 13 patients, none of whom had a lifetime history of major substance abuse, were analyzed (Data from one male was excluded because of a catheter occlusion. One female was excluded because she was maintained on lithium continuously and her data were extreme outliers, to be reported elsewhere.).

Procedure

Diet for 72 h before and 24 h during collections was caffeine-free and monoamine-balanced. Patients were supine overnight before catheter placement. Lights out was from 2230 to 0600 hours, interrupted briefly by flashlight-only entries for sample retrieval every 30 min and tympanic temperature at 0200 hours. Tympanic temperature was also determined every 2 h while awake. Daytime napping was discouraged.

CSF for monoamine metabolite assays was obtained by lumbar catheterization for 24 h. A 20 g catheter from the L4-5 interspace was connected to a peristaltic pump for continuous collection of 1-ml CSF samples every 10 min in a refrigerated fraction collector ($\leqslant 5^{\circ}$C). Samples were frozen on dry ice within 30 min of collection and later stored at -70°C. High pressure liquid chromatography (HPLC) with electrochemical detection (Schmidt et al, 1990) was used to determine concentrations of tryptophan (the amino-acid precursor of 5-HT, TRP), homovanillic acid (the DA metabolite, HVA), 5-hydroxyindoleacetic acid (the 5-HT metabolite, 5-HIAA), and the metabolite ratio (HVA:5-HIAA). Studies measuring the HVA:5-HIAA ratio have been reported previously (Engstrom et al, 1999; Risby et al, 1987) and were also determined in these sets.

The CSF collection procedure was repeated after treatment with antidepressants, continuing antidepressant administration throughout the second CSF collection at week 5 and then through week 8 to the treatment end point. Except moderate to severe headache after half of the catheterizations, procedures were well tolerated. Blood patches were performed for nine of the 30 studies and all headaches resolved. There were no serious adverse events or long-term sequellae.

Mood Outcome Measures

Mood response to treatment was defined a priori as a 50% decline in 17-item HRSD. HRSD scores at screening, twice daily during each admission, and weekly at out patient visits were summarized, with response status determined at week 8.

Antidepressant Treatment

Two medications with divergent transporter selectivities were used to test whether each would equally affect neurochemical variance. If the primary hypothesis that variance in neurochemical function is an attribute of health

Table 1 Mean Area-Under-the-Curve (AUC) of Power Spectral Densities (PSD) for Full Spectra from Full 24-h Data Set

	Pretreatment mean	Pretreatment SD	Post-treatment mean	Post-treatment SD	t	p
5-HIAA*	7.74 e7	6.92 e7	4.29 e7	2.39 e7	**−2.217**	0.047
TRP	1.01 e10	8.48 e9	1.32 e10	1.16 e10	0.804	0.437
HVA*	3.63 e8	2.09 e8	6.57 e8	5.04 e8	**2.701**	0.019
HVA/5-HIAA**	2.02 e4	1.36 e4	6.92 e4	4.28 e4	**3.891**	0.002

*$p < 0.05$; **$p < 0.005$, paired t-tests, two-tailed.
Spectral power from 24 h of CSF samples with 10 min intersample interval, $n = 13$. Decline in 5-HIAA spectral power density reflects decreased variance after treatment, and increased HVA and ratio spectral power density (HVA : 5-HIAA) reflect increased variance with treatment.
Values in bold are for $p < 0.05$.

Table 2 Change in Frequency Response with Treatment—t-Values for Area-Under-the Curve (AUC) in Five Bandwidths from 24-h Serial Sampling Data (Samples Every 10 min, 144 Samples per Collection)

Band	PSD coeffs.	Period length (time per cycle)	Label	5-HIAA	TRP	HVA	Ratio
1	1–7	Infinite → 4 h	24 h	−1.384	0.575	2.447*	3.560**
2	7–8	4 → 3.4 h	3.7 h	−2.547*	0.954	−0.241	1.098
3	8–14	3.4 → 1.85 h	2.5 h	−1.460	0.803	0.250	1.979
4	14-15	1.85 → 1.7 h	1.75 h	−2.406*	1.58	0.767	1.958
5	16-72	1.7 → 0.3 h	0.75 h	−1.246	1.282	1.884	2.843*

*$p < 0.05$; **$p < 0.005$.
Calculated t-values for area-under-the curve (AUC) of five bandwidths of the power spectral density (PSD) from 24-h serial sampling for monoamine metabolite levels (samples every 10 min, 144 samples per collection, no windowing). Change with treatment shows significantly enhanced power in the slowest frequencies (24-h to 4-h periods) for HVA and the HVA : 5-HIAA ratio, but in moderate frequencies (4- to 3.5-h periods and 111- to 103 min periods) for the 5-HIAA fluctuations alone.

holds true, then *any* effective antidepressant treatment would be expected to enhance neurochemical variance. A single medication (sertraline) was given to the first five patients, leaving open the question of whether a singular *vs* multiple drug mechanisms would be critical to enhancements of variance. The randomization procedure (with enrollment sequence double blind to future assignment) was added to the protocol at that point, adding four more patients on sertraline and four on bupropion to the total set. Open-label sertraline treatment (50 mg/day week one, then 100 mg daily) or bupropion treatment (150 mg slow-release/ day week 1, then 150 mg BID) began 3 days after the first CSF collection.

Analysis

Time series data were examined initially for treatment-associated changes in means, standard deviation, and overall variance. Frequency domain exploration used the Fourier transform of the full-length data set (Table 1). The Fourier coefficients were then averaged across individuals and post- minus pretreatment differences were calculated. Frequency coefficients of the PSD from the 24-h time series were derived without log expansion, and five frequency bandwidths (Table 2) were assessed using an AUC in order to compare groups of adjacent coefficients, accommodating slight individual differences in peak frequency components.

Spectrogram analyses allow testing for isolated periodicity within shorter time spans than the full 24-h data set. The data were windowed in time in order to uncover specific times of day when these changes in periodicity

characteristics might be most prominent. Spectrograms used data windows of 52 points = 8.67 h, a 51-point overlap, with PSDs calculated after local linear-detrending, hamming-windowing of each window segment, and zero-padding to 64 points (10.7 h). A wide range of window lengths was tested to assure that similar final results would be obtained and that the particular window choice was not spurious. Trapezoidal areas-under-the-curve (AUC) created 10 evenly spaced PSD bandwidths for spectrogram analysis (Table 3).

Missing data were less than 3% of the total and were manually interpolated prior to analysis. Cohen's effect size (*d*) was determined and is reported for the main findings (Cohen, 1992). Pretreatment and post-treatment data were compared using multivariate permutation testing. Multivariate permutation paired t-tests (MPT, Blair and Karniski, 1994; Blackford, 2002) were performed on the difference spectrogram in both frequency and time simultaneously while controlling for family-wise error. All analyses were performed using the open source-code package, *R* (R ver. 1.8.1, 2003). All statistics are two-tailed with $\alpha = 0.05$.

RESULTS

Overview

Following antidepressant treatment, both the variance and overall spectral power of the HVA : 5-HIAA ratio increased. Spectrograms also changed after treatment, showing increased periodicity that was maximal at a cycle length of about 84 min and lasted about 2.8 h in the evening. This

Table 3 Bandwidths and Corresponding Coefficients for Spectrograms

Bandwidth	Coefficient number	Period length
1	1:2	Infinity–10.7 h
2	2:3	10.7–5.3 h
3	3:5	5.3–2.7 h
4	5:7	2.7–1.7 h
5	7:10	1.7–1.2 h
6	10:13	1.2 h–53 min
7	13:17	53–40 min
8	17:21	40–32 min
9	21:26	32–27 min
10	26:31	27–21 min

Spectral power from 24 h of CSF samples with 10 min intersample interval, $n = 13$. Decline in 5-HIAA spectral power density reflects decreased variance after treatment, and increased HVA and ratio spectral power density (HVA : 5-HIAA) reflect increased variance with treatment.
Distribution of PSD coefficients across 10 bandwidths used for spectrogram statistical analysis (see Figure 2). Note that coefficient density was preserved across bandwidths by disregarding the last two coefficients (32 and 33), effectively shortening the period range by only 0.5 min/cycle, ending instead at 20.5 min/cycle.

robust change was seen across all discernable subject subsets (gender, age, severity, treatment response, and antidepressant treatment group) in this set of nongeriatric adult depressed patients, and was observed in a continuous cluster of significant points through an uninterrupted block of time.

Medication Treatment

Overall, antidepressant treatments yielded a mood response in 62% of the subjects (sertraline 6/9 treated, bupropion 2/4 treated), who also met criteria for remission. Severity at baseline may have partly predicted remission but was not statistically significant (responders' baseline HRSD $x = 18.3 \pm 4.6$; nonresponders' baseline HRSD $x = 20.3 \pm 3.1$). No statistically significant relationship between mood response and change in spectral power was detected in this small group.

Both drug groups, both response groups, and both gender groups displayed similarly enhanced variance of the HVA : 5-HIAA ratio in the ca. 84 min (1.2–1.7 h) frequency bandwidth. The data set is clearly underpowered to detect any subtle difference in these groupings, and no clear trends were found. Some subset analyses are given in Supplementary material available online, demonstrating the acceptability of merging treatment and response groups for analysis.

Metabolite Level Means, Correlations, and Variances

Means of each sample series were compared ($n = 13$ pairs). CSF 5-HIAA levels dropped by 29% with treatment, from 119.7 ± 28.6 nM/l pretreatment to 85.1 ± 27.7 nM/l posttreatment (means of 1872 samples; 24-h collections, 144 samples from 13 patients; as paired t-tests $n = 13$, $t(12) = 3.32$, $p = 0.006$). For comparison purposes, healthy

control CSF 5-HIAA levels collected in previous studies under similar conditions were higher than the depressed baseline and much higher than the post-treatment measures (136% of depressed values, 163.0 ± 50.2 nM/l, $n = 12 \times 144$ samples).

Mean HVA levels in the depressed sample were virtually unchanged from pretreatment (280.1 ± 100.4) to post-treatment (283.1 ± 78.5). Mean ratios of HVA : 5-HIAA changed proportionately with the change in the denominator values, from 2.34 ± 0.55 pretreatment to 3.46 ± 0.99 after treatment (paired $t(12) = -4.23$, $p = 0.001$). The measurements appear to be valid, as they agreed in direction and magnitude with changes in mean metabolite levels from single-tap, larger population studies of antidepressant treatment effects.

Correlations between 5-HIAA and HVA post-treatment ($r = 0.39$) were weaker than pretreatment ($r = 0.70$), suggesting a possible increase in independence of the two neurotransmitter systems with treatment (Pearson–Filon z score (Raghunathan et al, 1996), ZPF = 1.56, $p = 0.06$). Given this putative increase in independence between these systems with treatment, the time series data were examined for changes in variance and sources of the variance. Statistical variance (averaging individual variance across subjects) of 5-HIAA, a measure replicated by the AUC of the PSDs (Table 1), decreased by 44% overall with treatment, while data from the same assays showed increased variances of HVA and of the HVA : 5-HIAA ratio by 81 and 243%, respectively. Variance of simultaneously measured CSF tryptophan (TRP, the precursor of serotonin) was unchanged, providing a negative control (Table 1) and
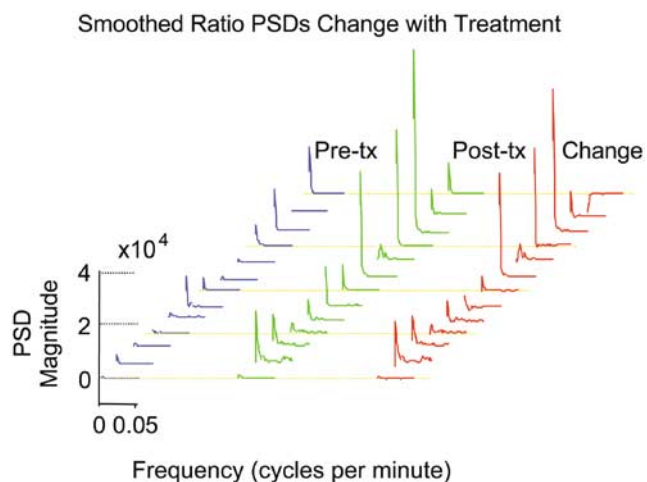
Figure 1 24-h PSD of HVA : 5-HIAA ratio. Change with treatment in PSDs of the CSF HVA : 5-HIAA ratio from 24-h serial data (sampled every 10 min), showing marked within-subject changes for 13 depressed patients, corresponding to the means summarized in Table 2. The horizontal axis (log (frequency); cycles/min), runs from one cycle per 24 h (near the origin) to one cycle per 20 min ($= 0.05$ cycles per min). The halfway point is at about 100 min/cycle. Vertical axis reflects PSD power with local smoothing for clarity. The three series of individual plots for each patient are pretreatment, post-treatment, and their difference (post- minus pre-), respectively, from left to right, with each data from patient aligned in sequence. Power is consistently elevated at low frequencies, shown near the origin of each PSD, with lower power at high frequencies (20 min periods) at the far right for each curve. The PSD is the square of Fourier transform coefficients. Identical axis scaling was used for every plot.

supporting an assertion that the sample collection method and HPLC methods were not likely sources of artefact that could explain the variance changes in other measures.

Fourier Analysis and Spectrograms

Spectral analysis (Table 2) of the full-length series of 5-HIAA levels following treatment showed significantly reduced power in low frequencies (long periods, 3.75 h/cycle) and midrange frequencies (medium periods, 1.75 h/cycle) compared with pretreatment values. In contrast, the HVA signal (still full length) showed increased power predominantly at the lowest detectable frequency (24 h/cycle). Spectral power of the HVA : 5-HIAA ratio (Figure 1) increased in both the lowest (24 h/cycle) and high (0.75 h/

cycle, or 45 min/cycle) frequencies (again, calculated over the entire sample).

Spectrograms, serially examining 8.7 h data windows through the day (and zero-padded to give a first (non-DC) coefficient at 10.7 h periods) were divided into 10 frequency bandwidths to cluster nearby frequencies in a single analysis (Table 3, Figure 2). The ratio data showed changes in only the fifth bandwidth (1.2–1.7 h/cycle, centered at 84 min/cycle) after correction for multiple comparisons. Treatment was associated with significantly increased spectral power of the HVA : 5-HIAA ratio in the evening hours, with peak power at 2100 hours for this bandwidth (17 adjacent windows totaling 2.8 h, significant t-values from 3.54 to 4.00; effect size, $d \geqslant 2.04$; $p < 0.05$ by MPT, Figure 2c). The centers of this block of windows spanned the evening from 1910 to 2150 hours with window
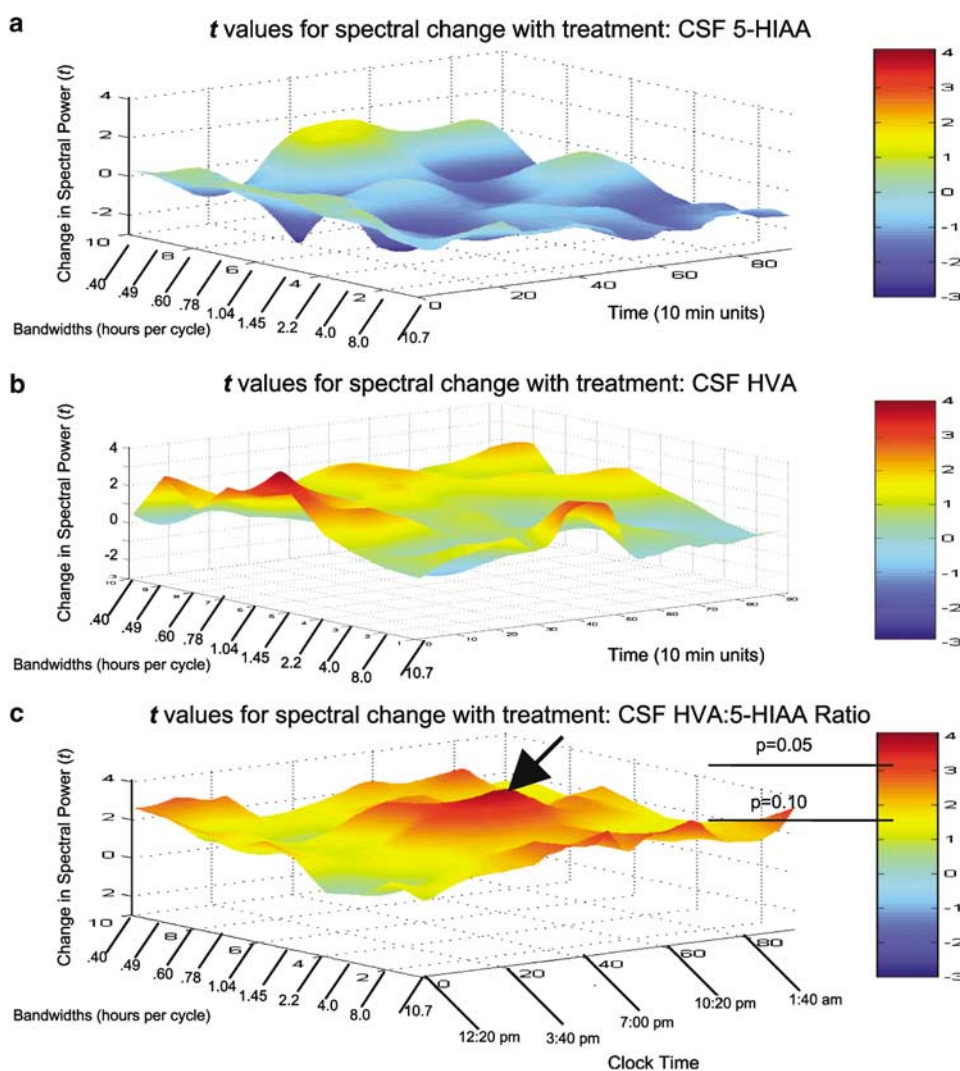
Figure 2 Spectrograms of change in CSF monoamine metabolite oscillations. Treatment-associated t-values for changes in spectral density (PSD) using 520-min windows through the 24-h period after 5 weeks treatment in 13 patients to form spectrograms. The spectrogram displays PSDs from overlapping windows along the time axis. Time axis labels are window centers, with clock times for all plots as marked on the bottom plot. Frequency axis bandwidths run from 10.7 h per cycle (band 1) to 20 min per cycle (band 10), see Table 3. (a) 5-HIAA: Strongest change was a decline in PSD power at midrange frequencies in the early evening. (NS). (b) HVA: Strongest change was a rise in PSD power at midrange frequencies (bandwidth 6) between noon and 1300 hours (trend significance for 3 points, df = 12, with t-values 3.96–4.07; effect size, $d \geqslant 2.29$; $p < 0.10$). (c) Significantly increased HVA : 5-HIAA ratio oscillatory power associated with antidepressant treatment. Arrow marks a peak at 84-min periods at 2100 hours, significant elevation across 17 adjacent windows, lasting 2.8 h (significant t-values from 3.54 to 4.00; effect size $d \geqslant 2.04$; $p < 0.05$ by MPT).

margins extending from 1450 to 0210 hours. This change in the ratio spectrum was not simply due to changes in either of the 5-HIAA or HVA mean spectra since neither showed significant changes in the spectrogram function with treatment (Figures 2a and b). The MPT method corrects for the number of comparisons conducted in the single test.

DISCUSSION

To our knowledge, this is the first report to show ultradian rhythms in the ratio of HVA:5-HIAA or changes in those rhythms with treatment for depression. Antidepressant treatment was associated with increased spectral power of the HVA:5-HIAA ratio for frequencies in a bandwidth around 84 min, and lasting 2.8 h in the evening. Oscillatory changes were less consistent in the individual monoamine measures (HVA or 5-HIAA) than in their ratio, possibly because the ratio analysis detected true changes in interactions between the systems.

Enhanced variability in the HVA:5-HIAA ratio after treatment suggests an increase in the functional independence of these aminergic systems whether by increased negative interactions, decreased positive interaction, or diminished external synchronization. This raises questions regarding mood regulation and etiology of depression. In the current literature, no single transmitter or transmitter-related mechanism has been conclusively linked to depression, but greater antidepressant efficacy has been observed with agents that affect multiple neurotransmitter mechanisms (for a review, see Tran et al, 2003), or in strategies that employ more frequent medication changes (Trivedi et al, 2004). Two different antidepressant classes were equally potent for memory and affective perceptions in healthy volunteers (Harmer et al, 2004). While seemingly counter-intuitive, ceasing treatments that have lost efficacy often results in temporary mood improvement. Taken together, these observations suggest that selectively altering the function of one component of an interactive network of transmitter systems may serve to alleviate maladaptively 'locked' patterns that interfere with healthy function.

Locked patterns are also observed behaviorally throughout the depression diagnostic subtype spectrum. Depression subtypes show excessively fixed symptoms at extremes of function (for example, overactive or inhibited appetite). Melancholic severity and elevated suicidality (Grunebaum et al, 2004) have been related to defective second messenger systems coupled to the-serotonin-2a receptor (Akin et al, 2004), a mechanism that profoundly alters physiologic variability. Melancholia disrupts anterior cingulate function (Rogers et al, 2004), a correlate of mental slowness and cognitive deficits that also represent a lack of functional variability. Patients with atypical depression endure fixed anger and extreme sadness, while those with seasonal affective disorder who fixed low mood and poor energy. It may be that invariant interactions between transmitter systems fix pathogenic network behaviors, in part by constraining stress responses. Regulators that control variability of system interactions, such as clock genes or other metabolic switching mechanisms, may present a future target for depression treatments.

Previous studies examining similar CSF time series data did not report changes in oscillatory function, most likely because of shorter and less frequent sampling. Healthy subjects, sampled every 30 min for 6 h, did not show oscillations in the HVA:5-HIAA ratio (Geracioti et al, 1998). In depression, an uncoupling of 5-HT and norepinephrine was suggested based on the absence of a negative linear relationship that was present in healthy subjects after 6 h sampling (Geracioti et al, 1997). More intensive and longer collections would be helpful.

The literature provides no similar comparison data sets, wherein one could assess subgroups (gender, drug mechanism, or treatment response) in patients undergoing CSF measurements with a prolonged time series. However, in one study, variance in CSF metabolites was affected by the presence or absence of premenstrual symptoms. Single CSF samples taken during luteal and follicular phases were compared in 'PMS' and control patients, and showed less intrasubject variability in CSF levels of HVA and 5-HIAA in the 'PMS' group (Eriksson et al, 1994). Accurate last menstrual period data and assessments of late luteal symptom changes are not available for the current population sample, and women who were postmenopausal or posthysterectomy were included, thus limiting the number available for direct comparison to the Eriksson study. Studies currently under way are limited to subjects under age 50 years, in part in order to address this issue.

The findings reported here are not meant to suggest a singular, exclusive effect of antidepressants on any single transmitter system or dynamical measure. Collection and analysis conditions were poised to detect rhythmic periods between 20 min and 24 h, with the longest period dependent on window length in the spectrogram (about 10 h in our analysis). A limited number of substances have been assayed. Moreover, it is likely that there are regionally specific rhythms that are obscured or weakened by the global nature of lumbar sampling. Caveats remain that the present ratio analysis cannot clearly distinguish between, for example, enhanced variability in HVA and dampening of variability in 5-HIAA, and that the changes observed with antidepressant treatment might be due to artefacts of medication that are unrelated to mood pathophysiology.

Finding periodicity changes in the HVA:5-HIAA ratio but not in the single metabolite levels could be related to factors other than true system interactions that are discussed above. Combining data from two antidepressant treatments (sertraline and bupropion) with different treatment mechanisms, or a masking of signal noise in the HVA:5-HIAA ratio that obscures a signal in the individual metabolite series might also explain the isolation of the finding in the ratio data. The absence of enhancement later in the evening and through the night might have been influenced by less than ideal sleep conditions, given the frequent room entries for sample retrieval and vital sign monitoring. The consistent absence of the 90-min oscillations in the evening during the pretreatment collection may herald an abnormal loss of variability in depression. It should be noted that the use of clustered frequency coefficients in the analysis can be justified since strict rhythms of nocturnal REM cycles are not expected; in EEG data, 95% of the distribution would require REM onset periodicity to range from 50 to 130 min (Bennington, 1999).

Heuristically, a rhythm-driven conceptualization of mood dysfunction is evident in clinical practice, where depressed patients frequently complain of their 'stuckness'—a symptom that is not part of our routine diagnostic criteria. Physiologically, blunted neuroendocrine responses may be a reflection of 'stuckness', not just of the endocrine output but also of the monoaminergic inputs. In this view, neural network rhythms would need to be healthy in order to maintain healthy network functional output. Fluctuations in transmitter activity may confer health and flexibility in neural network function, and this may be a critical aspect of neurochemical dysfunction in depression. To find genes that control ultradian rhythms, strengths of these signals will need to be measured with time series analysis in the frequency domain. Neurochemical systems are unlikely to project perfect circles onto maps of their orbits. Complex attractors represent imperfect oscillators within bounded orbits, modeling periodic functions common in nature. Oscillatory cycling (Hobson *et al*, 1975) and chaotic attractors (McCarley and Massaquoi, 1986) modeled REM on the basis of REM-on and REM-off neuronal activities. The argument regarding the existence of ultradian oscillators is far from closed, as it is becoming increasingly difficult to refute the likelihood of a genetic regulation of sleep physiology and its ultradian markers. The recognition of regulators of neurochemical ultradian mood behavior may not be far behind.

In conclusion, these data present evidence from a small number of patients of an antidepressant effect on ultradian rhythms in central neurochemical time series measures in human depression. Enhancements of transmitter system independence in near REM-cycle frequencies may be a hallmark of treatment effects. Further study may uncover genetic timers that control ultradian regulatory variability for mood circuitry, even if strictly precise rhythms are not evident. The relationship between depression and a periodic variability in transmitter system independence merits further study.

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