Supporting Information

Dallmann et al. 10.1073/pnas.1114410109

SI Methods

Subjects. Volunteers were recruited through advertisements in the Basel and Alsace areas. Before entering the study, each participant completed a general medical questionnaire, along with the Short-Form-36 Quality of Life Questionnaire, Horne–Östberg questionnaire, Munich Chronotype questionnaire, Pittsburgh Sleep Quality Index, Epworth Sleepiness Scale, IQ questionnaire, Hamilton Depression Rating Scale and Atypical Depression Supplement, and Insomnia Severity Index questionnaire (1–6). Volunteers were excluded who met any of the following exclusion criteria: smoking, medication or drug consumption, shift work within the last 3 mo, transmeridian flight during the month before the study, sleep efficiency <80%, >10 periodic leg movements per hour, or an apnea-hypopnea index >10.

At 3 wk before the start of constant routine study in the laboratory, participants recorded individual sleep/wake times by actigraphy and in sleep diaries. Habitual sleep times were determined after 2 wk, and participants were instructed to sleep according to the habitual times during the week before coming to the laboratory. Compliance was monitored by actigraphy and a sleep diary during this week. Demographic data and significant sleep parameters are presented in Table S1. Further details on study design and participants can be found in ref. 7.

Detection of Rhythmic Metabolites. After normalization, JTK-cycle analysis was performed with the two replicate time series for plasma and saliva, as has been described previously for data from gene expression arrays (8, 9). The nonparametric algorithm used by JTK-cycle analysis is a combination of the Jonckheere–Terpstra test for monotonic ordering and Kendall's τ test for association of measured quantities and is optimized for detection of rhythmicity. Based on visual inspection of each metabolite curve by a trained investigator, the α level of rhythmicity in this program was set at $P < 0.1$. Next, the significance of rhythmicity for each compound was determined using a modified version of the permutation analysis described previously (10). The data were randomly shuffled in time, resulting in a new time series for each sample. A discrete Fourier transform (DFT) was then used to transform the time series into the frequency domain. The DFT of a time series $x_1, x_2, \ldots, x_N$ is called a periodogram and is defined as

$$S(e^{jw}) = \frac{1}{2\pi N}\left|\sum_{n=1}^{N} x_n e^{-jwn}\right|^2.$$

The output of the periodogram summarizes the strength, number, and frequency of sinusoidal components in the time series. A significant sinusoidal component present in the time series is indicated by a peak in the periodogram. The periodogram was calculated for 1,000 random permutations of the data for a given sample, and the DFT of the experimentally observed time series was computed. The value at the peak in the periodogram (restricted to a circadian range) obtained from the observed time series was compared with the value found at that frequency in the 1,000 permutated spectra. The number of permuted values that were larger than the value obtained from the original time series was then computed and divided by 1,000, resulting in a P value. Such a permutation test has been used previously for circadian metabolomics (11) and has several advantages. For a dataset of limited dimensionality, such a permutation test is more appropriate than false discovery rate (FDR)-based statistics because it makes no assumptions regarding the data, including distribution or independence. FDR-based methodology assumes a sufficiently large number of P values to comprise a normal distribution (12), which is not possible with the 281 metabolites in plasma or the 178 metabolites in saliva that we identified (each $P < 0.001$, D'Agostino–Pearson omnibus normality test). Furthermore, test statistics for which P values are derived from parametric statistics assume independence of tests, which also is not the case in the present study. In fact, an argument can be made that different metabolites that share a single biochemical pathway should be dependent on one another to a certain degree, especially if they are under clock control. Finally, in our permutation analysis, the experimentally observed values of each metabolite are compared with a probability distribution generated by the data, rather than with a theoretical distribution; thus, the FDR for each metabolite is equal to the P value obtained with the permutation test (13).

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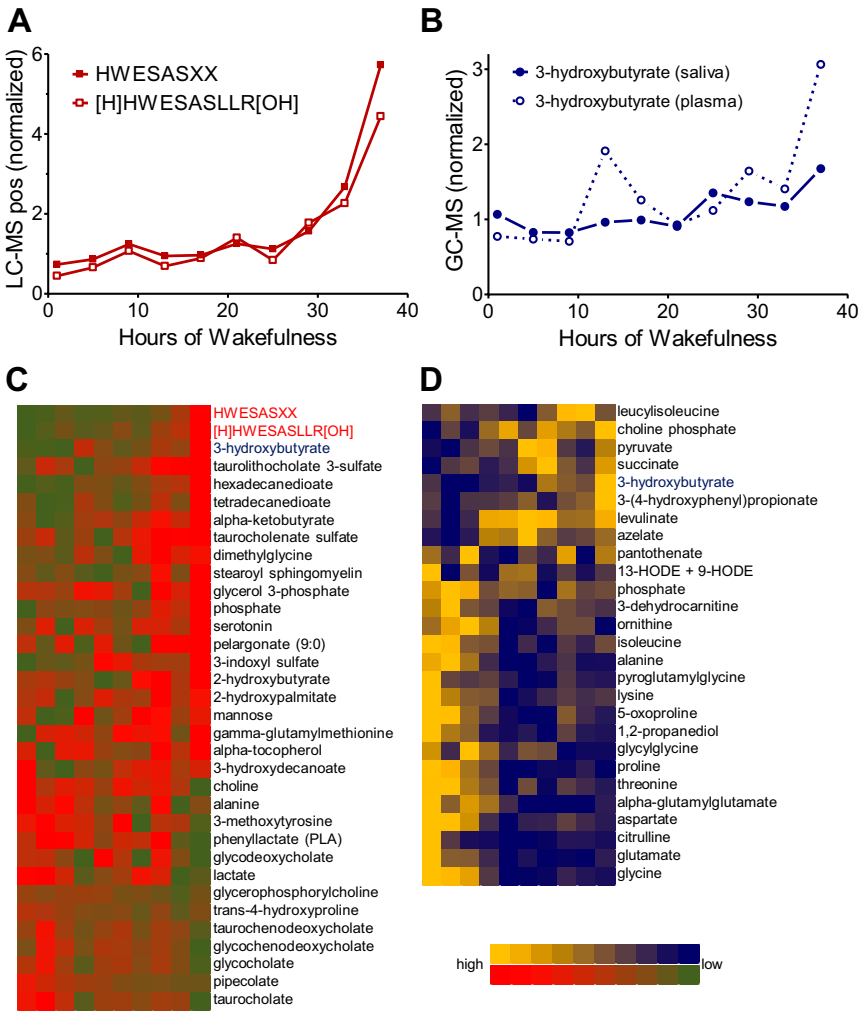

Fig. S1. Montotonically increasing or decreasing metabolites in plasma and saliva. (*A* and *B*) Examples of profiles of substances previously implicated in sleep–wake regulation. (*C* and *D*) Heat map of all such substances. High levels of metabolites are shown in red (plasma) and yellow (saliva), and low levels are shown in green (plasma) and blue (saliva). Colored labels indicate exemplified metabolites in *A* and *B*.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOCX)
Table S3 (DOCX)
Table S4 (DOCX)
Table S5 (DOCX)
Table S6 (DOCX)