PNAS
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Supplementary Information for:

A Large-scale Study Reveals 24 hour Operational Rhythms in Hospital Treatment

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Datasets S1 to S2

www.pnas.org/cgi/doi/10.1073/pnas.1909557116

SI Methods

Study design

We performed an observational cohort study of 1,546 patients receiving hydralazine, a commonly used antihypertensive in the ICU, at a tertiary pediatric hospital from Jan 2010 to Dec 2017. All patients receiving hydralazine while admitted to the hospital were included. Many were admitted multiple times (average of 5 per patient) over this 7 year period. Although hydralazine treatment was the basis for patient inclusion, our analysis also included 11 other drugs commonly administered to these patients. Treatments were administered in ICUs or general floors. Orders for treatment were categorized as “once”, “as needed”, or “scheduled”. Demographic data, medical history, BP, and order and administration times for the 12 different drug therapies were recorded.

Statistical analyses —- tests for uniformity and periodic pattern of orders and doses

For each drug, we tested if 24 h patterns in ordering or administration deviated from a uniform distribution (Kuiper’s test). To avoid bias from scheduled doses (“take every 8 h”, for example), we limited initial analyses to the first dose after each order unless otherwise specified.

To test for 24 h rhythms in treatment, we applied three independent detection methods, cosinor analysis, JTK_CYCLE, and RAIN. For each drug, orders and first-doses were discretized into 2-hour time bins. The amplitude, phase, and overall significance determined from each rhythmic model are presented in SI Appendix, Dataset 1. Essential model parameters are detailed below. Figures throughout display output from cosinor analysis, including cosinor curves, p values and relative amplitudes (rAmp) —a measure of the magnitude of rhythm, normalized to the fit mean.

Model parameters

Cosinor analysis: run from the Cosinor 2 package in R. Period = 24 h.

JTK_CYCLE: run from the MetaCycle package in R. Min and max period = 24 h.

RAIN: run from the RAIN package in Bioconductor in R. Period = 24, deltat = 2, method = independent.

Test for daily variation in dosing BP

To analyze BPs associated with hydralazine administration, we identified the BP measure just prior to each dose, referred to as the dosing BP (d-BP). Only d-BPs that were less than 1 h prior to dose were considered. d-BPs in the top and bottom 3rd percentile were excluded.

Test for daily variation in response to hydralazine

We analyzed inpatient responses to hydralazine as a function of dosing time. From 10,999 total doses, we limited analyses to those that were (1) IV-administered, (2) not within 4 h of another hydralazine dose, and (3) flanked by BP measures, with at least one measure < 0.5 h before and one < 2 h after dosing. For each of these 8,842 doses, response was defined as the percent change between dosing BP (just before dose) and mean BP over the 3 h following each dose. We eliminated outlier responses, defined as the upper and lower 3rd percentiles, leaving 7,953 doses (72% of total) for time-of-day analysis. To control for the impact of dosage on response, we stratified doses by concentration (mg/kg body weight) into 4 dosage subgroups, each with at least 300 doses. To test for variation in response over 24 h, we applied a linear model (ANOVA) with dosing BP as a covariate to control for the effect of pretreatment BP. We ran a post-hoc Tukey's test to estimate the mean difference in response, 95th percentile confidence levels, and multiple hypothesis adjusted P-values for all possible pairwise comparisons between time bins.

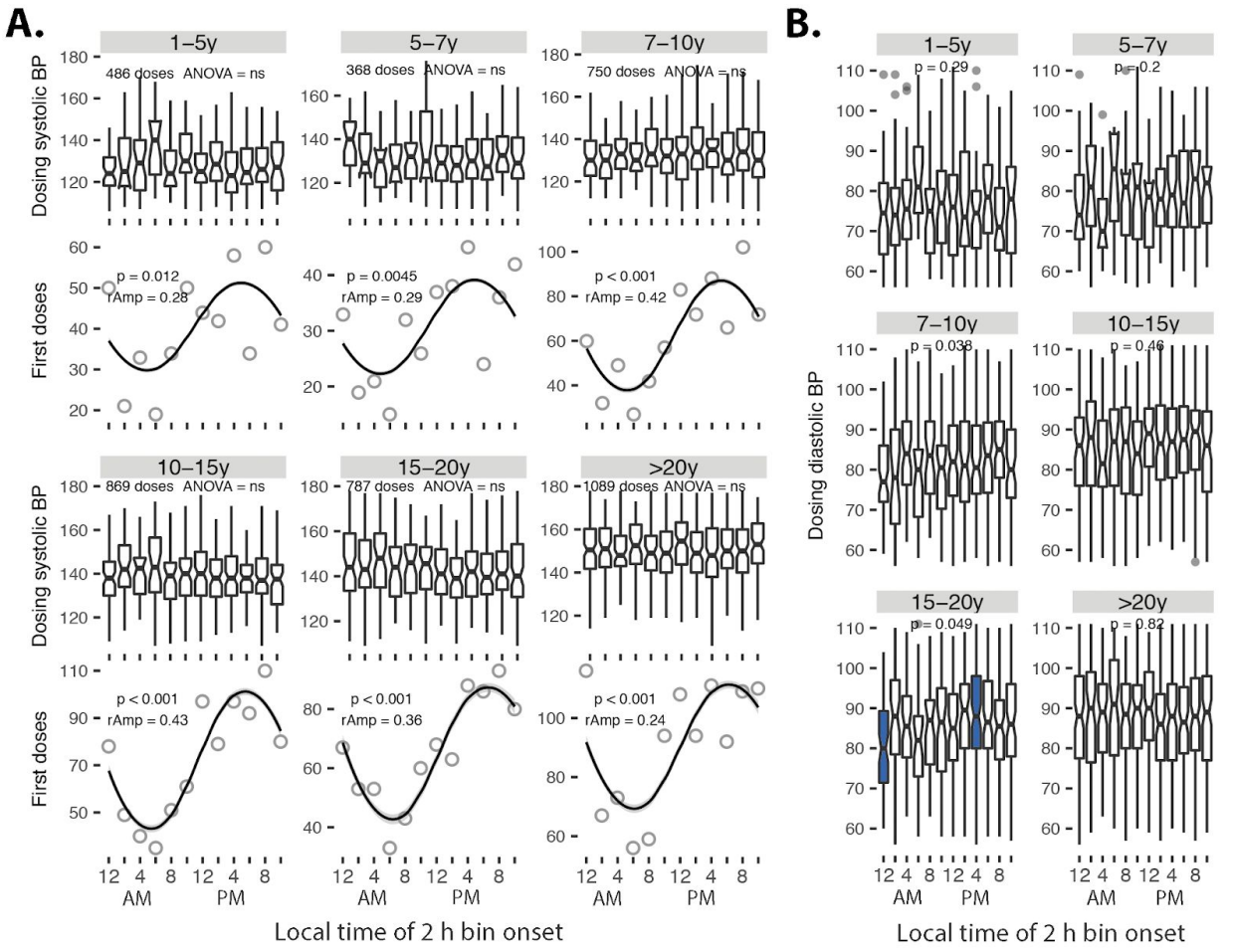

Fig. S1. 24 h profile of hydralazine dosing time compared to dosing BP. (A) Top panels: Systolic BP immediately prior to hydralazine dosing (d-SBP). d-SBPs were assigned to time bins according to time of hydralazine administration. Within each age group, no differences in d-SBP were detected between time bins (one-way ANOVA, P > .05). Boxplot center lines indicate median d-SBP, whiskers the upper/lower 25th percentiles. Outliers, defined as 1.5x the interquartile range, are not shown. Bottom panels: The number of hydralazine first-doses in each time bin modeled by a cosinor waveform with a 24 h period. P value (p) and relative amplitude (rAmp) indicated. **(B)** Diastolic BP immediately prior to hydralazine dosing (d-DBP). Multivariate analysis detected marginally significant time-of-day differences in two of the six age

groups (one-way ANOVA, p = ~0.05). Post-hoc pairwise comparisons between 2 h time bins detected only one difference (indicated in blue) (Tukey's test, adjusted $p < 0.05$) (*SI Appendix, Dataset S2*).

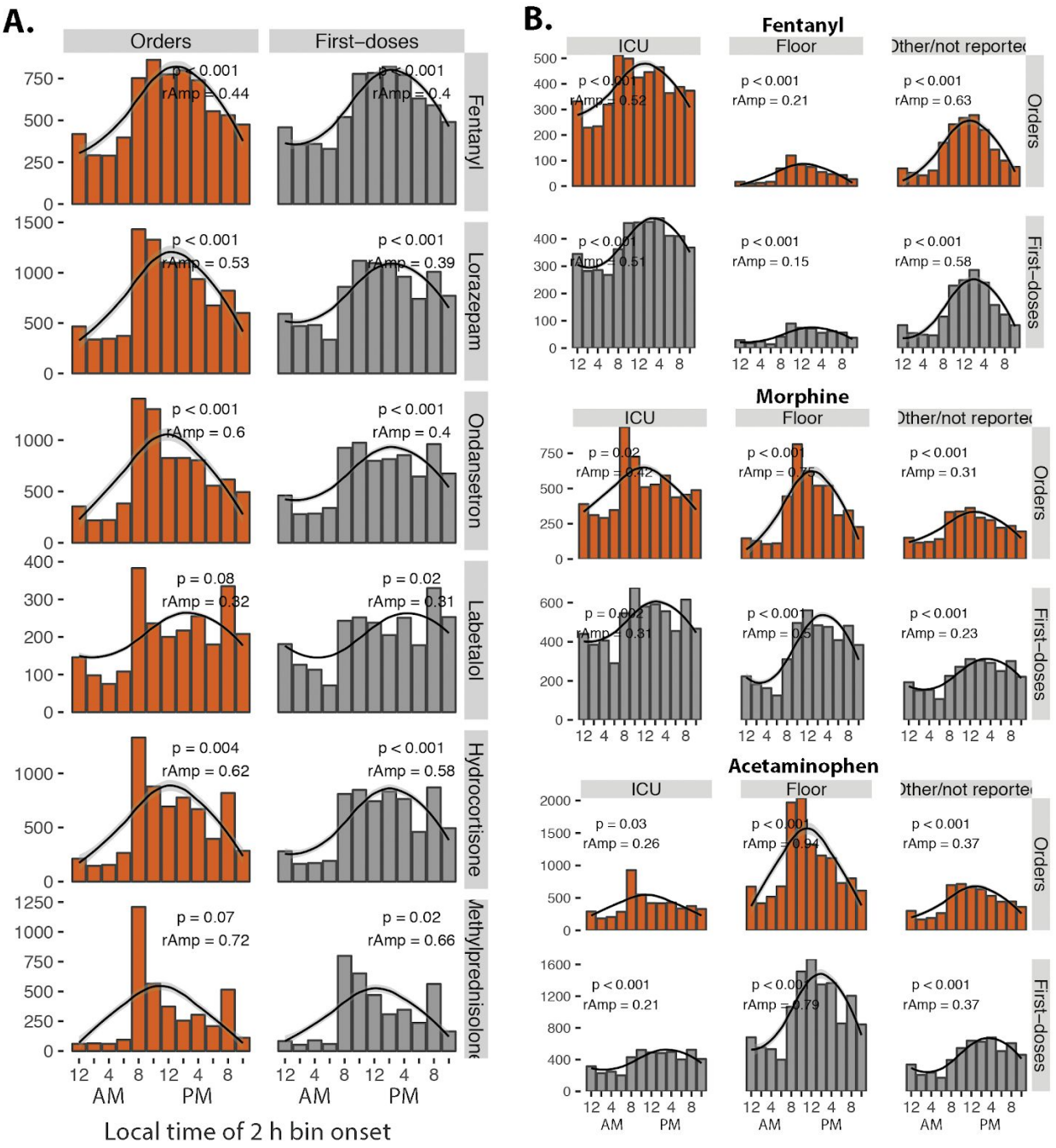

Fig. S2. Rhythms in orders and first-doses across drug classes and hospital units. (A) Orders and first-doses of different drug classes were all non-uniformly distributed over 24 h, the majority with detectable 24 h rhythms (cosinor analysis, P < 0.05, see also *SI Appendix, Dataset*

1). Note that one single model did not provide the best fit for all drug profiles in this study. Whereas sine wave based methods (cosinor analysis, JTK_CYCLE) fit particularly well to profiles with a smooth rise and fall, RAIN (an umbrella function that can detect waveforms that deviate from sine waves) provided a better fit for several of the spiky, or multi-peak, profiles. (B) Daily rhythms in orders and first doses were independent of the hospital treatment unit.

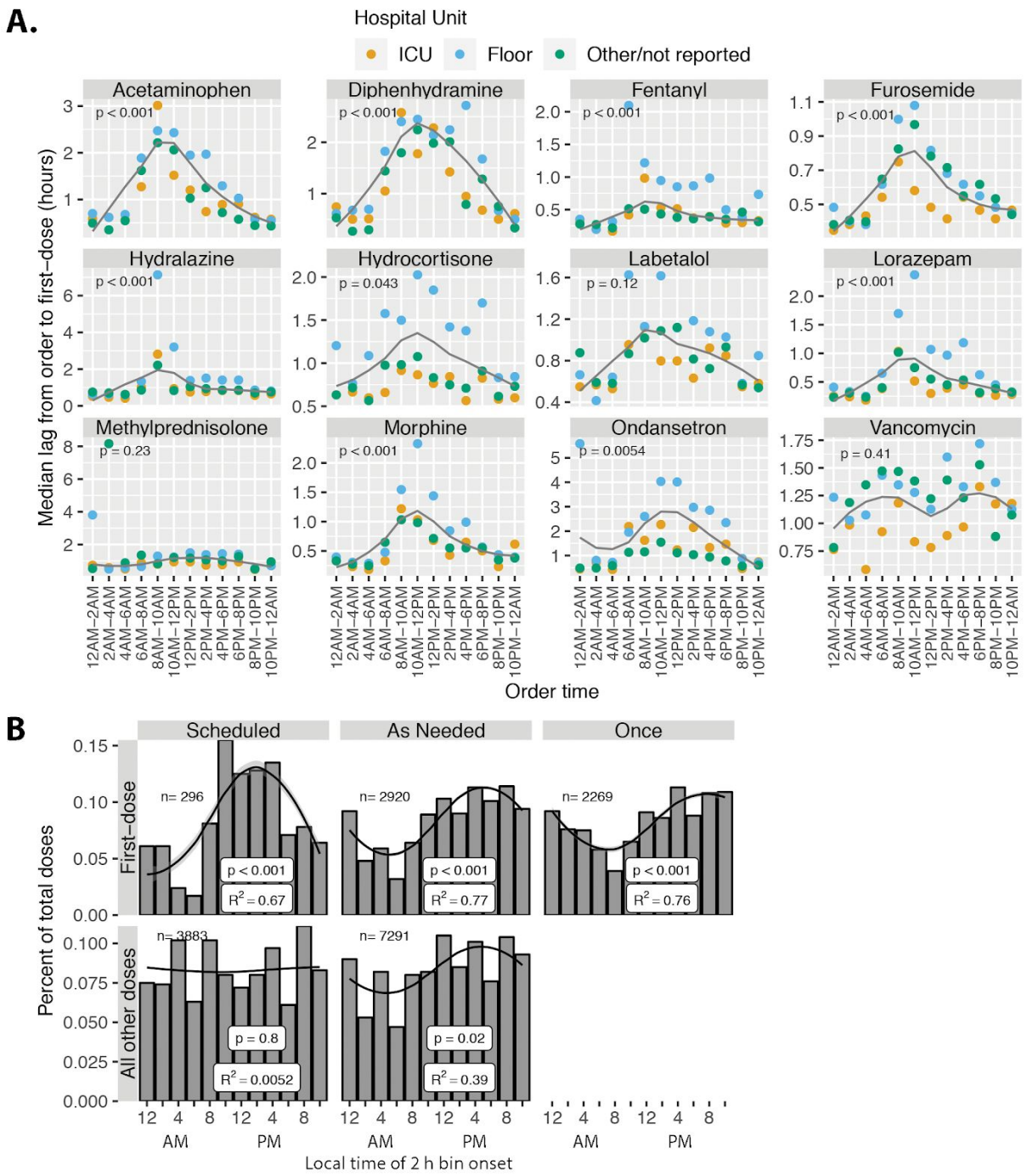

Fig. S3. (A) The time lag from order to first-dose depended on the time the order was placed (one-way ANOVA, *p value* < 0.05, 9/12 drugs). Each point indicates the median time lag for a given drug, order time, and hospital unit. For ANOVA only considered order time, not treatment

unit. The black curve shows a smooth fit across the median time lags for each 2 h order time bin. **(B)** Distribution of hydralazine doses for “once”, “as needed”, and “scheduled” orders. First-doses were rhythmic regardless of order type. “All other doses” were weakly rhythmic for “as needed” orders, and not rhythmic for “scheduled” orders (p value and coefficient of determination (R^2) from cosinor regression analysis).

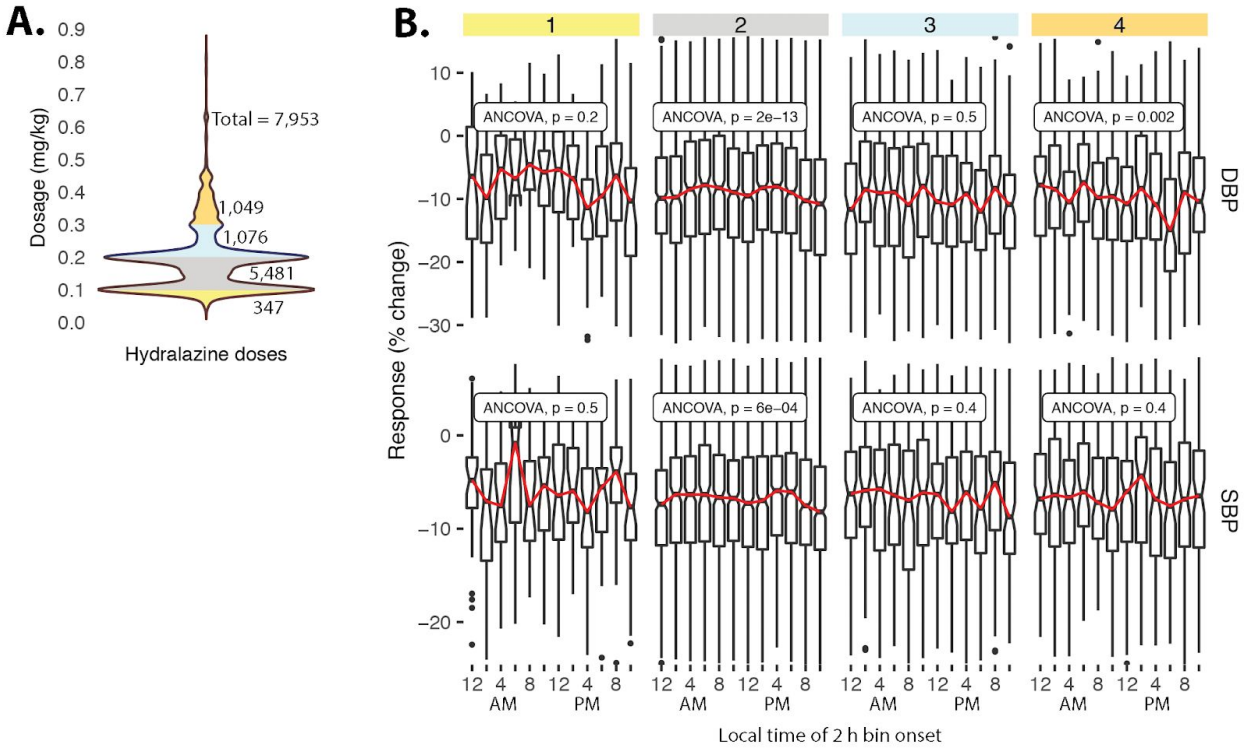

Fig. S4. Clinical response to hydralazine by time of administration. (A) Numbers of hydralazine doses ranging from 0–0.1, 0.1–0.2, 0.2–0.3, and > 0.3 mg/kg body weight. Percent change in diastolic (DBP) and systolic (SBP) blood pressure as a function of dosing time. Percent change was computed as the difference between dosing BP (just before dose) and mean BP over the 3 h following each dose. Boxplot center lines indicate median response, whiskers the upper and lower 25th percentiles. Outliers, defined as more than 1.5x the interquartile range, are shown as black circles. For visual aid, a line was fit through the medians.

Dataset S1. Detection of 24 h rhythms in orders and first-doses. Results from two independent tests, RAIN and JTK_CYCLE, for periodic patterns in data. For each drug, orders and first-doses were discretized into 2-hour time bins. Peak phase and overall significance were computed for both models specifying a period of 24 h.

Dataset S2. Detection of 24 h rhythms in BP and response to treatment.