Endogenous circadian rhythm in human motor activity uncoupled from circadian influences on cardiac dynamics

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The endogenous circadian pacemaker influences key physiologic functions, such as body temperature and heart rate, and is normally synchronized with the sleep/wake cycle. Epidemiological studies demonstrate a 24-h pattern in adverse cardiovascular events with a peak at ≈10 a.m. It is unknown whether this pattern in cardiac risk is caused by a day/night pattern of behaviors, including activity level and/or influences from the internal circadian pacemaker. We recently found that a scaling index of cardiac vulnerability has an endogenous circadian peak at the circadian phase corresponding to ≈10 a.m., which conceivably could contribute to the morning peak in cardiac risk. Here, we test whether this endogenous circadian influence on cardiac dynamics is caused by circadian-mediated changes in motor activity or whether activity and heart rate dynamics are decoupled across the circadian cycle. We analyze high-frequency recordings of motion from young healthy subjects during two complementary protocols that decouple the sleep/wake cycle from the circadian cycle while controlling scheduled behaviors. We find that static activity properties (mean and standard deviation) exhibit significant circadian rhythms with a peak at the circadian phase corresponding to 5–9 p.m. (≈9 h later than the peak in the scale-invariant index of heartbeat fluctuations). In contrast, dynamic characteristics of the temporal scale-invariant organization of activity fluctuations (long-range correlations) do not exhibit a circadian rhythm. These findings suggest that endogenous circadian-mediated activity variations are not responsible for the endogenous circadian rhythm in the scale-invariant structure of heartbeat fluctuations and likely do not contribute to the increase in cardiac risk at ≈10 a.m.

cardiac vulnerability | circadian pacemaker | locomotor activity | scale invariance

Epidemiological studies demonstrate that myocardial infarction (1–4), stroke (5, 6), and sudden cardiac death (7) have a 24-h daily pattern with a broad peak at 9–11 a.m. This 24-h pattern is widely assumed to be due to day/night patterns in behaviors that affect cardiovascular variables, such as autonomic balance, blood pressure, and platelet aggregability, in vulnerable individuals (8). However, endogenous influences from the circadian pacemaker [suprachiasmatic nuclei of the hypothalamus (SCN)], independent from external behavioral effects, may also contribute to this daily pattern of adverse cardiovascular events. These circadian influences could occur via hormonal effects, direct neuronal links between the SCN and the sympathetic system (9) and through circadian modulation of the sympathovagal balance (10). Recently, we demonstrated (11) that dynamical scale-invariant features of heartbeat fluctuations [related to underlying mechanisms of cardiac control (12–17)], exhibit a significant endogenous circadian rhythm, independent from extrinsic scheduled behaviors and the sleep/wake cycle. These dynamical features of heartbeat fluctuations move closer to the features observed under pathologic conditions (13, 16, 18) at the endogenous circadian phase corresponding to 9–11 a.m. (11). These findings raise two plausible hypotheses for the endogenous pathways of circadian influence on cardiac dynamics: that the SCN directly influences cardiac regulation or that the SCN affects the intrinsic regulation of physical activity, which in turn influences cardiac dynamics (Fig. 1).

The output of integrated, multiple-component physiologic systems under neural regulation, such as activity and heart rate, often exhibit complex continuous fluctuations, even under healthy resting conditions and in the absence of external perturbations (14, 15, 19–22). Static properties (e.g., mean and standard deviation) as well as dynamic scale-invariant properties of these variables (e.g., long-range power-law correlations) relate to cardiac vulnerability. For instance, static measures of heartbeat fluctuations change with pathologic conditions (23–25); e.g., reduced vagal tone in congestive heart failure leads to much lower average interbeat interval (16, 18, 26). Furthermore, increased cardiac vulnerability is characterized by a smaller standard deviation of heartbeat fluctuations in pathologic conditions of impaired cardiac responsiveness (26, 27).

In terms of dynamic measures, studies have revealed that heartbeat fluctuations in healthy subjects possess a self-similar temporal structure related to the underlying cardiac control mechanism, which is characterized by long-range power-law correlations over a broad range of time scales (12–14). These dynamic scale-invariant features change with sleep/wake states (20, 21, 28, 29), sympathetic and parasympathetic blockade (30), and exercise (31, 32) and under pathologic conditions, such as congestive heart failure (13, 16, 18). Moreover, the scaling exponent associated with these power-law correlations is a robust marker sensitive to predicting mortality in patients with heart failure (26).

Scale-invariant dynamic patterns also have been recently found in the fluctuations of human motor activity, such as forearm motion and gait (22, 33, 34), with long-range power-law correlations on time scales of seconds to hours that are insensitive to changes in mean activity level and to fluctuations caused by random and scheduled extrinsic factors (22). Furthermore, this scale-invariant dynamic measure changes under pathologic conditions (35). These combined results suggest that scale-invariant dynamic changes in activity in humans are regulated by an intrinsic activity control mechanism.

Average motor activity clearly affects average heart rate, but it is not known how the dynamic scale-invariant measures of these two

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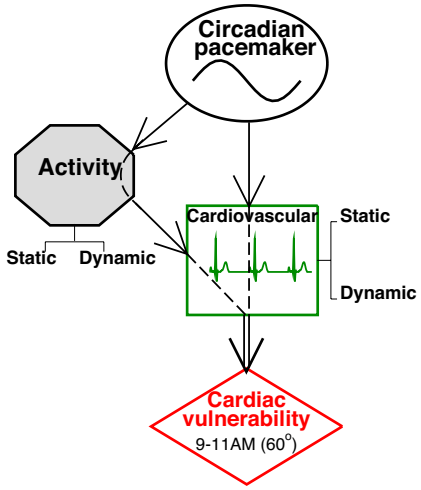

Fig. 1. Schematic diagram of two potential hypotheses for separate pathways of intrinsic circadian influence on the mechanism of cardiac control, which ultimately may lead to increased cardiac risk. (*i*) Direct circadian influence: Static and/or dynamic measures of heartbeat fluctuations have an intrinsic circadian rhythm that may contribute to the epidemiologically observed increase in cardiac vulnerability at 60° circadian phase (relative to CBT minimum at 0°). (*ii*) Indirect activity-mediated circadian influence on cardiac control: Static and dynamic measures of motor activity fluctuations exhibit an intrinsic circadian rhythm, which in turn may influence cardiac regulation leading to increased cardiac risk at particular circadian phases. Our results shown in Figs. 2 and 3 do not support the second hypothesis and suggest that the endogenous circadian variability in physical activity does not contribute to increased cardiac risk at 9–11 a.m. However, the temporal fractal organization of heartbeat fluctuations, quantified by the scale-invariant dynamic index α (Fig. 3), changes significantly under the direct influence of the circadian pacemaker with a pronounced peak at ≈60° circadian phase, suggesting that the endogenous circadian pacemaker may contribute to the increased cardiac vulnerability observed at this circadian phase (1, 11).

physiologic variables are related. For instance, are the dynamic changes in heartbeat fluctuations across the circadian cycle caused by dynamic changes in activity regulation across the circadian cycle (Fig. 1). In this study, we investigate activity and heartbeat data simultaneously recorded in healthy individuals across all circadian phases and determine whether circadian influences on static or dynamic features of heart rate regulation are uncoupled from the circadian influences on activity regulation by using two complementary protocols: (*i*) a forced desynchrony protocol (36–38) that decouples the sleep/wake cycle from the endogenous circadian cycle while controlling for (and averaging out) scheduled events and extrinsic behavioral influences; (*ii*) a constant-routine protocol, when the average and variance of activity levels are minimized in an attempt to uncouple the endogenous circadian influences on cardiac dynamics from activity variations. Because sudden onset of adverse cardiovascular events often occurs in ostensibly healthy, asymptomatic people (39, 40), the study of healthy subjects may provide information concerning circadian or activity-related mechanisms in cardiac vulnerability. Specifically, we examine whether circadian-mediated changes in the statistical indices of activity data exhibit a peak at the endogenous circadian phase corresponding to ≈10 a.m. [i.e., 60° circadian phase, with 0° defined as the core body temperature (CBT) minimum]. If endogenous activity fluctuations do exhibit circadian rhythms with a peak at 60°, it raises the possibility that such changes may be involved in the peak in cardiac vulnerability observed at this phase (Fig. 1) (1–3). To discern the separate intrinsic pathways of SCN influence on the mechanisms of cardiac control, i.e., the direct influence from the indirect activity-mediated influence, we compare how indices of activity and cardiac dynamics change with circadian phase.

Results

The group-averaged results for the static measures of activity and heartbeat RR intervals from wakefulness in the forced desynchrony protocol are presented in Fig. 2 *A–D*. The subjects exhibit a significant endogenous circadian rhythm in mean activity with a large amplitude equivalent to 60% of the average 24-h mean activity (Fig. 2*A*). This pronounced rhythm occurs despite constrains on the activity imposed by the scheduled events as well as being confined to a laboratory suite. The minimum of the mean activity level is at ≈0° circadian phase, corresponding to the endogenous circadian temperature minimum (which normally occurs during sleep in most individuals, although in this protocol only the scheduled wake episodes were analyzed). We find a broad peak in mean activity at 180–240° (corresponding to the habitual hours of 5–9 p.m.). We also find a significant circadian rhythm in the group average of the standard deviation of activity levels during wakefulness with a minimum and a maximum at the same circadian phases as we find for the mean activity levels (Fig. 2*B*).

Mean data for the average and standard deviation of RR interval recordings are presented for comparison with activity recordings in Fig. 2 *C* and *D*. The results in Fig. 2 *A–D* show a strong correlation between the circadian rhythms in the static measures of heart rate and those of activity fluctuations, with minima in the activity variables corresponding to the maxima in the static measures of the RR intervals. Thus, the minimum in the circadian rhythm for the mean interbeat interval (i.e., highest heart rate) coincides with the circadian maximum in the mean activity level (Fig. 2, compare *A* with *C*). Similarly, the circadian maximum in mean heartbeat interval (i.e., lowest heart rate) coincides with the circadian minimum in the mean activity level at 330–30°, corresponding to the habitual sleep period (Fig. 2, compare *A* with *C*). The circadian minimum in the heartbeat interval standard deviation (i.e., lowest heart rate variability) at ≈240° coincides with the circadian peak in the standard deviation of activity fluctuations (Fig. 2, compare *B* with *D*), whereas the circadian maximum in the heartbeat standard deviation at ≈0–20° circadian phase coincides with the minimum in the activity standard deviation (Fig. 2, compare *B* with *D*). Thus, during the forced desynchrony protocol that allows spontaneous activities, the static measures of activity and heart rate appear to be coupled.

The contrasting results from the constant-routine protocol are presented in Fig. 2 *E–H*. As expected, due to the design of the constant routine that greatly constrains activity, we find no significant circadian rhythms in the mean and standard deviation of activity (Fig. 2 *E* and *F*). Thus, the strong circadian rhythm in these static measures of activity (Fig. 2 *A* and *B*) can be volitionally or experimentally constrained. However, we find very similar and significant circadian rhythms in both the average RR interval and the standard deviation of RR intervals during the constant routine (Fig. 2 *G* and *H*), as occurred under the forced desynchrony protocol (compare with Fig. 2 *C* and *D*). Thus, although the static measures of activity and heart rate appear to be coupled across the circadian cycle, constraining mean activity does not affect the circadian rhythm of the RR intervals, suggesting that the circadian rhythm in the mean and standard deviation of RR intervals may not be simply a consequence of circadian changes in the mean and standard deviation of activity.

To determine how the circadian pacemaker influences dynamic control of motor activity, we examine the temporal organization in the fluctuations of activity values over a broad range of time scales (Fig. 3). We apply the detrended fluctuation analysis (DFA) method to quantify long-range temporal correlations in activity fluctuations after accounting for nonstationarities in data by subtracting underlying polynomial trends in the average activity level (41, 42). The scaling behavior in activity fluctuations as assessed by the DFA method is characterized by a scaling exponent $\alpha = 0.9$ (Fig. 3*A*), much greater than $\alpha = 0.5$ for white noise, indicating

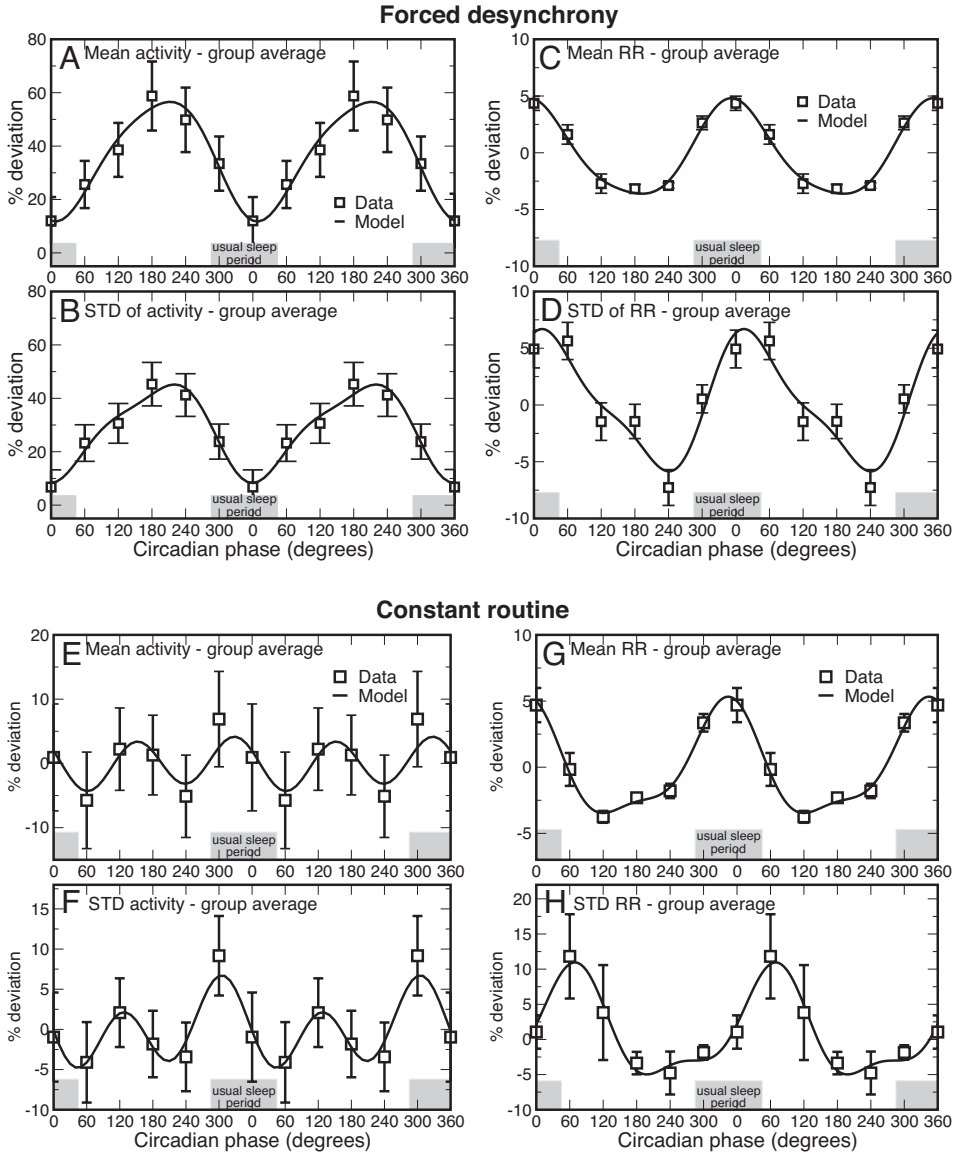

Fig. 2. Endogenous circadian rhythms in static measures of activity and heartbeat fluctuations. (*A* and *B*) Statistically significant circadian rhythms are observed during forced desynchrony in the mean activity levels ($P = 6.2 \times 10^{-4}$ obtained from the cosinor analysis) (*A*) and the standard deviation of activity fluctuations ($P = 8.5 \times 10^{-5}$) (*B*), with a maximum at 180–240° and a minimum at ≈0° circadian phase. Group-averaged data are shown as symbols (error bars represent standard error), and the cosinor analysis fits are shown as solid lines. The results are double-plotted to better visualize rhythmicity. The habitual sleep period when living outside of the laboratory is indicated by gray shaded boxes. The percent deviation in *A* takes only positive values because the mean activity is calculated over both wake and sleep periods, although this analysis includes data only from wakefulness when activity is usually higher. (*C* and *D*) Statistically significant circadian rhythms also are observed during forced desynchrony in the mean value ($P = 3.62 \times 10^{-10}$) (*C*) and the standard deviation ($P = 6.25 \times 10^{-5}$) (*D*) of heartbeat intervals RR, with a minimum at 180–240° and a peak during the habitual sleep period at ≈0° circadian phase (corresponding to minimum CBT). The mean heart rate data in *C* have previously been published (11) and are presented for comparison with activity data. Both activity and heartbeat data were analyzed during wakeful periods in the forced desynchrony protocol. (*E*–*H*) No significant circadian rhythms were observed during constant routine in the mean activity level (*E*) and the standard deviation (*F*) of activity fluctuations, whereas the circadian rhythms in the mean RR interval ($P = 1.6 \times 10^{-9}$) (*G*) and the standard deviation of heartbeat intervals ($P = 0.01$) (*H*) persist.

strong positive correlations and the presence of a robust scale-invariant organization embedded in activity fluctuations across a broad range of time scales. In Fig. 3*A*, we show the scaling behavior of activity fluctuations for a single subject at three different circadian phases corresponding to 9 a.m., 1 p.m., and 5 p.m. obtained during the forced desynchrony protocol. We observe a stable value for the slope of the scaling function $F(n)$ characterized by a scaling exponent $\alpha = 0.9$ for all three circadian phases, indicating that the scale-invariant/fractal temporal structure in activity fluctuations does not significantly change with circadian phase. Similarly for the group, we find no significant circadian rhythm in the average scaling exponent of activity fluctuations during the forced desynchrony protocol ($P = 0.91$) (Fig. 3*B*), although α varies somewhat between different 4-h bins.

This finding for activity fluctuations is in contrast to the significant circadian pattern in the scaling exponent α for the heartbeat interval fluctuations previously published in ref. 11 (Fig. 3*D*), indicating a strong circadian influence on cardiac dynamics. Moreover, the maximum value of α [i.e., a deviation that brings the scale-invariant features of cardiac dynamics closer to those observed under pathologic conditions (13, 16, 18)] occurs at between 60° and 90°, which corresponds to the window 9–11 a.m., where epidemiological studies have reported highest cardiac risk (1–4).

Results of the dynamic measures of activity and RR interval data from the constant-routine protocol are shown in Fig. 3 *E* and *F*. There is no significant circadian rhythm in the scaling exponent α of activity (Fig. 3*E*), whereas a strong circadian rhythm in α of RR intervals persists (Fig. 3*F*), with a similar circadian profile as observed for the forced desynchrony protocol (Fig. 3, compare *D* with *F*).

Discussion

Our investigations demonstrate the presence of a large-amplitude circadian influence upon the static measures (mean and standard deviation) of spontaneous physical activity. Specifically, during the forced desynchrony protocol we find a pronounced peak in the mean activity level and in the standard deviation of activity fluctuations at the circadian phase interval 180–240° (corresponding to the habitual afternoon and evening hours of 5–9 p.m.) and a minimum at ≈0° (corresponding to the lowest CBT, ≈5 a.m.) (Fig. 2 *A* and *B*). In the forced-desynchrony protocol, subjects repeated the same sleep/wake and behavior schedule (i.e., timing of meals and shower, etc.) in all wake periods so that statistically the same

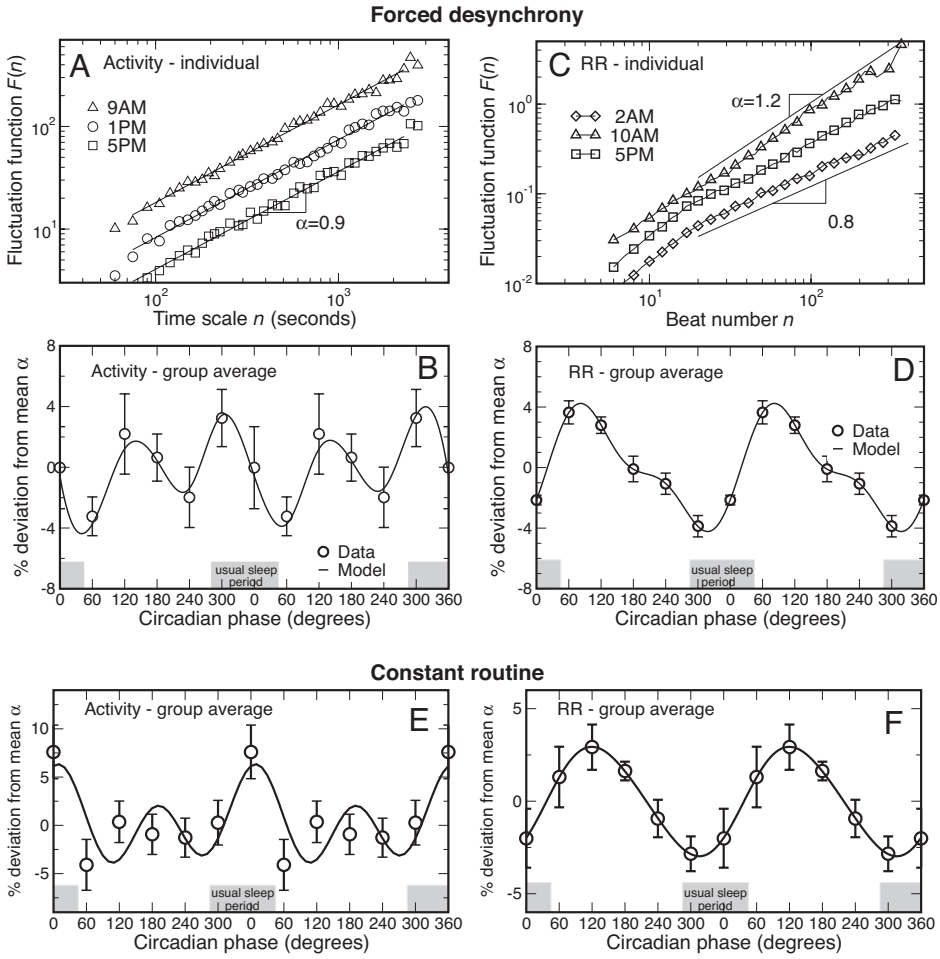

Fig. 3. Scale-invariant dynamic index of activity and heartbeat fluctuations as a function of the circadian phase. (*A* and *C*) Long-range power-law correlations during forced desynchrony in human motor activity fluctuations (*A*) and heartbeat fluctuations (*C*) as quantified by the DFA method (47–49) for one representative subject. Scaling curves $F(n)$ represent the DFA results for different data segments across different circadian phases during wake periods. The values for the exponent α are obtained by fitting $F(n)$ for activity fluctuations in the time scale range $60 < n < 2{,}600$ sec (*A*) and for heartbeat fluctuations in the range $20 < n < 400$ beats (*C*). For clarity, $F(n)$ curves are vertically offset. (*C* and *D*) The results for the exponent α of heartbeat data during forced desynchrony have previously been published (11) and are presented for comparison with activity data. (*B–F*) Cosinor analysis for the group-averaged scaling exponent α during the forced desynchrony (*B* and *D*) and constant-routine (*E* and *F*) protocols for activity fluctuations (*B* and *E*) and heartbeat fluctuations (*D* and *F*). Group-averaged data are shown as symbols (error bars represent standard error), and the cosinor analysis fits are shown as solid lines. (*B* and *D–F*) A significant circadian rhythm in the deviation of the α value is observed only for the heartbeat RR intervals during the forced-desynchrony protocol ($P = 0.01$) (*D*), with a pronounced peak at ≈60° corresponding to the 9–11 a.m. window of increased cardiac risk, and during the constant-routine protocol ($P = 0.004$) (*F*) but not for activity data (*B* and *E*). The habitual sleep period when living outside the laboratory is indicated by gray shaded boxes.

behaviors were evenly distributed across all circadian phases, thus allowing the statistical separation of scheduled behaviors across the wake periods from the influences of the endogenous circadian cycle. Although scheduled behaviors were evenly balanced across the circadian cycle, spontaneous activity was permitted with some mild constraints. With this technique, our findings demonstrate an endogenous circadian variation in mean spontaneous physical activity in humans. Moreover, this variation occurred despite constraints imposed by the laboratory schedule and laboratory environment, including no formal exercise and no unscheduled sleep, suggesting that the endogenous circadian rhythm is a very robust physiological drive. Although day/night activity patterns have been demonstrated in other mammalian species, and even though this mean activity pattern is often used as a circadian phase marker (e.g., ref. 43), generally behaviors, including the sleep/wake cycle, have not been decoupled from the endogenous circadian cycle in such prior animal studies.

We also observe a statistically significant endogenous circadian variation for both mean and standard deviation of the heart rate. The circadian rhythm in the heart rate static measures parallels the rhythm in activity, i.e., the circadian maximum and minimum phases for mean heart rate (inversely proportional to the heartbeat interval) correspond to the circadian peaks and troughs in mean activity level (Fig. 2 *A* and *C*). These findings suggest that the endogenous circadian system alters activity and heart rate regulation in ways that would be appropriate for the expected habitual behavior at that time of the day when living outside the laboratory, namely a lower heart rate when habitual sleep usually occurs. Notably, the lower heart rate at 330–30° is accompanied by an increased probability for large interbeat fluctuations, i.e., a peak in the standard deviation (Fig. 2*D*). Such circadian-mediated decrease of the mean heart rate coupled with an increase of the standard deviation between 3 and 7 a.m. suggests a reduced sympathovagal balance that may be cardioprotective. The apparent synchrony between the rhythms in the static measures of activity and heart rate, characterized by matching minima and maxima (Fig. 2 *A–D*), may reflect a physiological coupling between activity and cardiac control. Notably, it can be seen in Fig. 2 that there was no peak in any of the activity or RR interval static measures around the time of increased cardiac vulnerability (≈9–11 a.m.) (11). That is, the peak in static measures of activity (and heart rate) occur at 5–9 p.m., i.e., ≈9 h later, and there is a very gradual increase in the static characteristics of activity during the morning hours (Fig. 2 *A–D*). Thus, the circadian variation in the static measures of heart rate and activity fluctuations are unlikely to be a contributor to the increased cardiac risk in the time window of 9–11 a.m. (Fig. 1).

In the constant routine in which activity is minimal and constant and, thus, there is no circadian rhythm in the static measurements of activity data (Fig. 2 *E* and *F*), surprisingly we find that the significant circadian rhythm of heart rate persists (Fig. 2 *G* and *H*) and can therefore be uncoupled (be independent) from endogenous circadian rhythms of activity.

In contrast to the static measures of activity fluctuations, our investigations show no significant circadian rhythm in the scale-invariant dynamic index α of activity in either the forced desynchrony or constant-routine protocol (Fig. 3 *A*, *B*, and *E*). This finding suggests that a key measure of activity dynamics, which is related to the intrinsic nonlinear multiscale mechanism of activity

regulation (22), is not influenced by the circadian system. Although there is no significant 24-h rhythm, a significant 12-h rhythm ($P = 0.01$) is apparent in the dynamic index α of activity data but not in the heartbeat interval data (Fig. 3), providing further evidence of decoupling between activity and heart rate. Our findings in the forced desynchrony protocol of pronounced circadian rhythms in the static measures and absence of endogenous circadian modulation in the dynamic scale-invariant measure of activity fluctuations suggest that separate physiologic pathways may be involved in the circadian influence on these different elements of activity regulation.

In contrast to activity fluctuations, the scale-invariant temporal organization of heartbeat fluctuations, as quantified by the dynamic index α, exhibits a significant 24-h circadian pattern (Fig. 3*D*), characterized by a pronounced peak at 60–90°, bringing α closer to the values observed for subjects with congestive heart failure (13) around that time. Notably, this circadian period corresponds to the window 9–11 a.m. of highest cardiac risk (1). Furthermore, the minimum value of α occurs at 300–360°, corresponding to the habitual sleep period outside the laboratory (Fig. 3*D*), bringing the index α closer to the values observed for subjects during sleep (20). Because there is no significant circadian rhythm in the scaling index α of motor activity fluctuations, our results from both the forced desynchrony protocol (Fig. 3*B*) and constant routine (Fig. 3*E*) indicate that circadian influences on dynamic measures of activity are unlikely to contribute to the peak in α observed for heartbeat fluctuations at 9–11 a.m. and are therefore unlikely to contribute to increased cardiac vulnerability.

In summary, our results demonstrate the presence of an endogenous circadian rhythm in the average level and the standard deviation of human motor activity fluctuations, indicating that the circadian pacemaker affects the intrinsic regulation of physical activity. In contrast, the dynamic measure α, which quantifies the scale-invariant temporal structure in activity fluctuations, does not exhibit a significant circadian rhythm. The endogenous circadian variability in the static measures of activity is usually synchronized to the circadian changes in static measures of heart rate, yet the results from the constant-routine protocol demonstrate that the circadian rhythm in static and dynamics measures of the human heart rate can be decoupled from the rhythms in activity. Overall, our data from both forced desynchrony and constant-routine protocols provide no evidence for an activity-mediated circadian influence on either static or dynamic measures of cardiac control (indirect pathway on Fig. 1). Thus, central circadian influences on activity are unlikely to contribute to the observed increase of cardiac risk at given circadian phases. Rather, a direct endogenous circadian influence on cardiac neuroautonomic regulation, which affects the scale-invariant/fractal temporal organization of heartbeat fluctuations over a broad range of time scales, appears more likely to play a role for the peak in adverse cardiac events at ≈10 a.m. Provided a similar circadian rhythm in the dynamic scaling index α for RR intervals also is observed for subjects with cardiac disease, it would potentially contribute to increased risk, because a shift in the scaling exponent α of RR intervals to higher values closer to 1.5 has been robustly linked to pathologic conditions and higher mortality rate (13, 18, 26).

We note, that external behavioral factors, such as exercise, which are independent from the intrinsic circadian influence reported here and which were curtailed in this laboratory experiment, may also be an independent contributing factor to increased cardiac risk when living outside the laboratory environment. Moreover, if such specific activities have a day/night frequency distribution of occurrence, these behaviors could be implicated in the day/night pattern of adverse cardiovascular events. At the same time, our findings in the forced desynchrony of average activity level and standard deviation of activity fluctuations being endogenously driven by the circadian system to higher values in the interval 180–240° (corresponding to 5–9 p.m., the habitual afternoon and evening hours), may have implications when choosing the best time for physical exercise. It is not known to what degree the endogenous circadian rhythm of activity contributes to the day/night activity patterns when living in unconstrained conditions. It is conceivable that preferred times to be more active would coincide with the endogenous circadian rhythm of activity and would lead to a greater amplitude in the day/night pattern of activity in unconstrained conditions. Such a finding would have implications for the optimal time to perform work or volitional exercise and deserves further study.

Finally, our observations raise the possibility that the circadian system operates through a complex feedback mechanism (14, 17), which intrinsically coordinates activity regulation to reduce cardiac stress at particular circadian phases, e.g., by endogenously lowering the mean activity level and the standard deviation of activity fluctuations in the morning hours during the 9–11 a.m. window of elevated sympathetic response (10) and highest cardiac risk (1–4). This putative feedback mechanism of the circadian system may have a cardioprotective role. In contrast, maintaining the scale-invariant/fractal temporal structure in activity fluctuations unchanged across circadian phases may be evolutionary advantageous, as motor control response has to remain optimal over a broad range of time scales (frequencies) throughout the circadian cycle.

Data Collection and Methods

Subjects. We studied six healthy subjects (four male, two female) with a mean age of 25 years (range, 21–32 years) during a forced desynchrony protocol and nine healthy subjects (seven male, two female) with a mean age of 28 years (range, 21–36 years) during a constant-routine protocol. All subjects had no medical disorders other than mild asthma, as assessed by history, physical examination, overnight polysomnography, psychological examination, pulmonary function tests, a 12-lead ECG, and routine blood and urine chemistry.

Forced Desynchrony Protocol. We collected physiologic data throughout a 10-day ''forced desynchrony protocol,'' with subjects living in an individual suite conducting controlled daily behaviors (36–38). There were two initial baseline acclimatization days with 8-h sleep opportunities and 16 h of wakefulness. After a 48-h baseline, sleep periods were delayed by 4 h every day such that subjects were living on recurring 28-h ''days,'' with 9 h and 20 min of sleep opportunity and 18 h and 40 min of scheduled wakefulness. This 28-h recurring sleep/wake schedule was repeated for seven cycles [supporting information (SI) Fig. 4] in the absence of known zeitgebers, such as bright light, so that the body clock oscillated at its inherent rate. Light was kept constant and dim at <8 lux to avoid resetting the body clock, and the subjects had no external cues regarding the time of day. Room temperature was 23°C. Subjects repeated the same behavior schedule in all wake periods so that, statistically, the same scheduled behaviors, including the sleep/wake cycle, occurred evenly across all circadian phases by the end of the protocol. Thus, all scheduled activities become desynchronized from the endogenous circadian pacemaker (36–38), which allows separation of behavioral effects (sleep/wake cycle as well as scheduled activities) from circadian effects. During the periods of wakefulness, spontaneous activity was still possible although somewhat constrained, being limited to walking around the suite, sitting, and resting.

Constant-Routine Protocol. To assess intrinsic activity controllers (i.e., circadian or other neural centers) independent of scheduled and random external influences, activity recordings were made in the laboratory throughout a 38-h constant routine. Subjects were asked to remain awake and seated semirecumbent on a bed (45° torso elevation) in a constant environment with a room temperature of 23°C and dim (<8 lux) indoor light. The dietary intake consisted of a measured portion of food and drink every 2 hours containing ≈100 mEq of potassium and 150 mEq of sodium every 24 h and consisting of 25% fat, 25% protein, and 50% carbohydrates. Fluid intake was constant at 3.5 liters/day evenly distributed and consumed at 2-h intervals. These highly controlled and constant experimental conditions result in reduced average and variance of activity levels. Thus, all scheduled activities remained the same across the entire circadian cycle.

Measurements. As a marker of the endogenous circadian pacemaker, CBT, was recorded throughout the protocols by using a rectal temperature sensor (YSI 20463; Yellow Springs Instruments) with values stored to a computer once per minute. For an assessment of human motor activity, subjects wore a wristwatch-sized *Actiwatch* recorder (MiniMitter) that unobtrusively measured changes in

forearm acceleration in any plane (sensitive to $0.01 \times g$). Each data point recorded in the device's internal memory represents the value of changes in acceleration sampled at 32 Hz and integrated over a 15-s epoch length (44). For an assessment of the cardiac interbeat interval, a chest lead ECG was recorded on an ambulatory recording device (Vitaport; Temec Instruments) at 256 Hz throughout the forced desynchrony and constant routine protocols. Cardiac interbeat intervals were obtained from the ECG by using a QRS wave detector based on the Aristotle algorithm (45). Data on RR intervals from the forced desynchrony protocol have previously been published (11).

Estimation of Circadian Phases. CBT was used as the marker of the circadian phase (36, 46). Each subject's phase and period of the CBT circadian rhythm was estimated by nonlinear least-squares regression (38), and a circadian phase was assigned to hourly averages of activity and heartbeat data relative to the time of the minimum CBT (CBT minimum = 0° circadian phase corresponding to ≈5 a.m. in these subjects).

DFA. We used the DFA to estimate correlations in the activity and heartbeat interval fluctuations (47). Compared with traditional correlation analyses, such as autocorrelation, power-spectrum analysis, and Hurst analysis, the advantage of the DFA method is that it can accurately quantify the correlation property of signals masked by polynomial trends (41, 42). Details on the DFA method are presented in *SI Data Collection and Methods*.

Analysis of Circadian Rhythmicity in Activity and Cardiac Dynamics. We analyzed and compared activity and heartbeat data recorded only during the periods of wakefulness in the forced desynchrony and constant-routine protocols. We separated activity data during the wakeful periods into nonoverlapping segments of the same size, and, for each segment, we calculated the values of the DFA scaling exponent α (a dynamic scale-invariant measure of activity fluctuations), and the mean and the standard deviation of activity levels (static measures). We used different segment sizes for the different measures we estimated, i.e., 1-h segments for the mean and the standard deviation of activity levels and 4-h segments for the DFA scaling exponent α. We chose 4-h as the segment size for the DFA scaling exponent because the DFA method requires ≈1,000 data points for an accurate estimate of the long-range power-law correlations and the scaling exponent α (each 4-h segment contains 960 data points) (41, 42). For each 4-h data segment, we estimated the scaling exponent α over the same range of time scales, from 1 to 40 min. For each subject, we analyzed ≈124 h for wake periods throughout the forced desynchrony protocol (SI Fig. 4). Next, we assigned a circadian phase (determined from the regression analysis of CBT) for each DFA exponent value obtained from 4-h activity data segments as well as for each mean and standard deviation value obtained from 1-h activity data segments. Because the activity level is much lower (large percentage of zero values in the actigraphy recordings) and the motor control mechanism is quite different during sleep, we analyzed data only during wake periods in the forced desynchrony protocol. Similar recordings and analyses of RR interval data from the same individuals in the forced desynchrony protocol have previously been published (11). Details regarding the cosinor analysis, our data binning procedure across circadian phases, and data statistics for each bin are presented in *SI Data Collection and Methods*.

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