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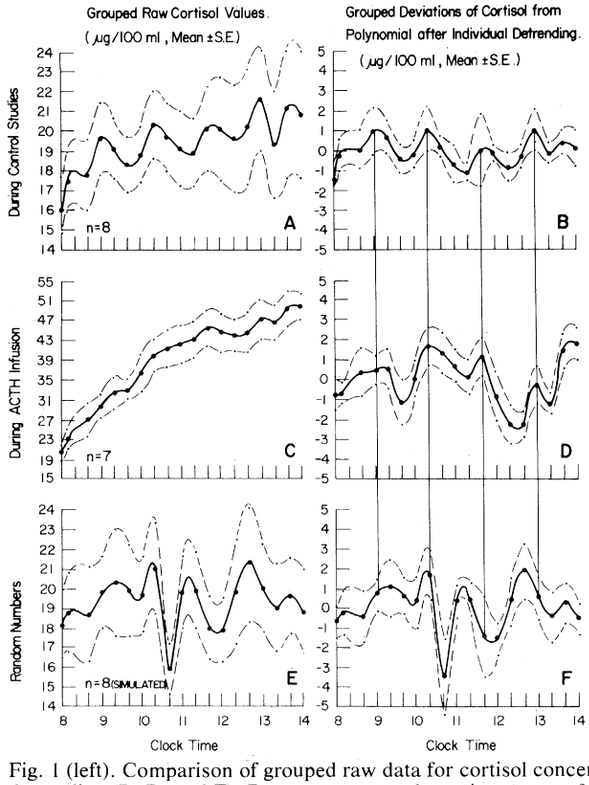
Synchronized Ultradian Cortisol Rhythms in Monkeys: Persistence During Corticotropin Infusion

Abstract. *A highly synchronized ultradian cortisol rhythm with a predominant periodicity of 85 to 90 minutes was observed in eight isolated monkeys; this rhythm may be harmonically related to the circadian rhythm. The persistence of this synchronized rhythm during supramaximal infusions of adrenocorticotropin not only suggests that feedback is not causative but also challenges the classic concept that bursts of cortisol secretion are dependent upon an immediately preceding release of adrenocorticotropin.*

Biological ultradian rhythms are those with a frequency greater than one cycle in 20 hours (*1*). These rhythms are described by Halberg as being wobbling frequencies that are difficult to evaluate by the inspection of raw data (*1*). Such a description especially holds for rapid changes in adrenal corticosteroid concentrations over time. These have been reported to fluctuate variably in both man and monkeys (*2*), and time-series analyses of these fast-frequency adrenocortical fluctuations have uniformly shown broad variance spectra (*3*) consistent with this irregularity. Consequently, the pulsatile patterns of cortisol release have been referred to as "episodic" rather than rhythmic (*1–3*).

In recent work designed to minimize variance of cortisol concentrations both within and among subjects by rigorous environmental control, we reported rapid, cyclic fluctuations of cortisol concentrations in individual monkeys that are superimposed upon a circadian rhythm (*4, 5*). We now report (i) that these fluctuations over time do not disappear when data from individually isolated monkeys are grouped, indicating phasic consistency or synchrony of this ultradian rhythm among animals, (ii) that the variance spectra calculated from these raw data are narrow with a predominant periodicity of 85 to 90 minutes, and (iii) that this ultradian rhythm is not disrupted by the infusion of large doses of adrenocorticotropin (ACTH). Furthermore, evidence is presented that suggests that ultradian cortisol rhythms are harmonically related to the circadian rhythm.

Eight adult male monkeys (*Macaca mulatta*) were adapted to living in pri-



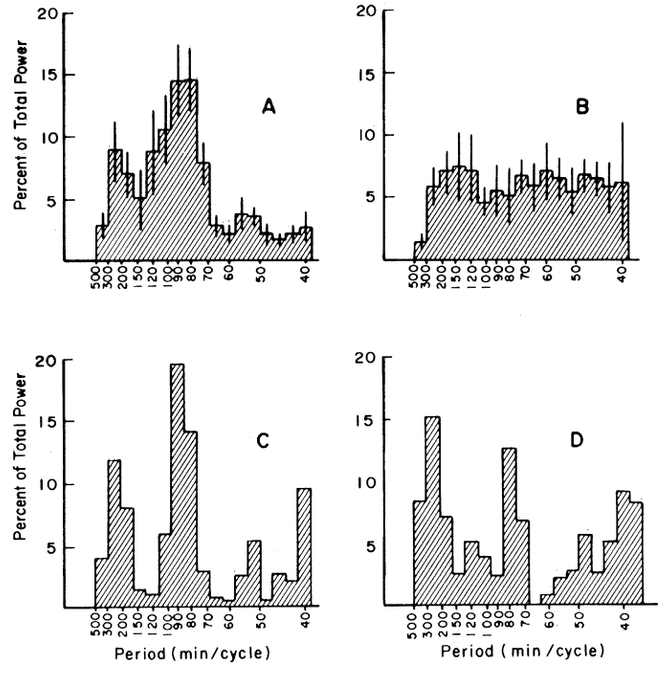


Fig. 1 (left). Comparison of grouped raw data for cortisol concentrations (A, C, and E) with the same data subsequent to individual polynomial detrending (B, D, and F). Data are expressed as micrograms of cortisol per 100 ml of plasma. Dashed lines indicate standard error of the mean computed at each 20-minute interval. Note the different scale on the ordinate in (C). Vertical lines (right) accentuate the exact synchrony between data for control and ACTH-stimulated animals. Random numbers are asynchronous. Fig. 2 (right). Power spectra depicting predominant periods of ultradian cortisol oscillations. (A) Composite of individually detrended control power spectra (mean ± standard error; $n = 8$). (B) Composite of individually detrended random number power spectra (mean ± standard error; $n = 8$). (C) Power spectrum of detrended, grouped control data ($n = 8$). (D) Power spectrum of detrended, grouped cortisol data during ACTH infusion ($n = 7$). The data in (D) reveal the same rhythmic components as seen in (A) and (C). Random number periodograms (B) are arrhythmic. These high-resolution power spectra were computed with essentially a 100 percent lag.

mate chairs. They were housed in individual sound-attenuating booths and were surgically prepared with permanently implanted catheters in the inferior vena cava 2 to 3 weeks before we began the studies. Lights were turned on at 0957 hours and off at 2145 hours; the monkeys were given food and water for 3 to 4 hours at 1400 hours. Apart from these interactions, the animals were rarely disturbed. Cortisol concentrations were measured by a competitive protein-binding assay (*6*) from venous blood samples collected through a cannula extending outside the booth every 20 minutes for 6 hours beginning at 0800 hours.

Two studies, separated by at least 2 weeks, were performed to determine (i) the spontaneous and (ii) the ACTH-stimulated cortisol secretory patterns. During the ACTH experiments, corticotropin (Parke-Davis) was constantly infused during the 6-hour study at 10 unit/hour with additional rapid injections of 10 units being given on the hour. Details of these procedures appear elsewhere (*4*); spontaneous cortisol patterns for individual monkeys are depicted on the cover.

Figure 1A shows that the cyclic fluctuations in plasma cortisol concentrations which we previously noted in individual subjects are not lost when data from these individually isolated animals are grouped. To reduce the variance spread of these raw data and to allow comparison with ACTH-stimulated values, we separately detrended each subject's 6-hour cortisol concentrations by calculating deviations from a second-order polynomial. This manipulation removes upward or downward trends over time and essentially places each subject's data on a common base, that is, change from expected concentration. Subsequent grouping of cortisol values treated in this manner (Fig. 1B) reveals a reduced, relatively constant variance over time when compared with averaged raw data (Fig. 1A). To see such a highly synchronized rhythm means that individual differences in phase among monkeys must be relatively minor. This temporal synchrony indicates entrainment by an environmental factor.

To define quantitatively the ultradian band, time-series analysis (*7*) was applied to detrended data. The major period of 85 to 90 minutes was described by data from individual subject's power spectra (Fig. 2A), as well as from grouped data (Fig. 2C), and thus is not an artifact of averaging. Since the ordinate in Fig. 2 is expressed as the percentage of total power, the percentage of total variance accounted for by each period of interest can be directly estimated; for example, the 80- and 90-minute periods each account for approximately 15 percent of total variance in Fig 2A.

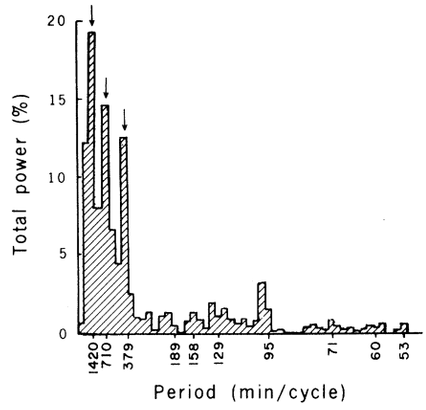


Fig. 3. Power spectrum obtained from undetrended cortisol data collected at 20-minute intervals for 24 hours. Arrows highlight observed frequencies at approximate harmonic intervals. This spectrum, as well as components near 120 minutes and 70 minutes, was typical for both monkeys studied.

In the second study, ACTH infused at a total rate of 20 units/hour produced the expected increase in absolute cortisol concentrations (approximately 2.5-fold over the 6-hour study, Fig. 1C). When the individual animal's data were detrended and then averaged, the fluctuations seen in the absolute cortisol concentrations were greatly accentuated and revealed cyclical data (Fig. 1D) whose peaks and troughs coincided *exactly* with those of basal cortisol values (compare B and D in Fig. 1). This phasic consistency of the persistent, rhythmic oscillations with and without ACTH administration demonstrates the temporal synchronization across both studies. In addition, the striking similarity of the power spectral displays derived from the data for control and ACTH-stimulated animals is further evidence that the same predominant ultradian rhythms were observed in both studies (compare C and D in Fig. 2).

These oscillations are not sinusoidal but are characterized by a greater ascending slope than descending slope (B and D in Fig. 1). Pharmacokinetic estimates of cortisol secretion and clearance rates from these grouped data are in agreement with published values obtained from individual monkeys (*2, 5*).

To test the possibility that these rhythms had occurred by chance, which can happen even with a short series of random numbers (*8*), we generated random number data (*9*) within the 95 percent confidence limits described by raw cortisol values for each monkey at simulated 20-minute intervals. This serves as an extreme test of the significance of the biological data as well as the analytical methods employed. Figure 2B shows that the fluctuations in Fig. 1, E and F, are random; peaks and troughs in Fig. 1F correlate poorly with those in B and D.

Synchronizers which entrain or reinforce ultradian fluctuations have not been defined (*10*). Because of the striking synchrony of ultradian rhythms among monkeys, we wondered whether our regimen of 12 hours of light and 12 hours of darkness was somehow responsible for the generation of this 85-minute rhythm. Reexamination of Fig. 2, A and C, supported this idea; in addition to the major peak at 85 minutes, other variance peaks were found at 200 minutes and 50 minutes. These peaks are temporally close to the true third, fourth, and fifth harmonics of the 24-hour (1440 minutes) day (180, 90, and 45 minutes, respectively). If our artificially induced circadian rhythm had indeed produced harmonics that were manifested as an ultradian rhythm, one would also expect variance peaks at the lower-frequency harmonics of 24 hours. To test this, we collected plasma samples for cortisol determinations at 20-minute intervals for 24 hours from two monkeys and subjected each animal's data to a time-series analysis without prior detrending. Figure 3 shows peaks that are temporally close to the true first and second harmonics (720 and 360 minutes, respectively). All these variance peaks are temporally close to but not exactly at their theoretical harmonic; this slight imprecision is acceptable for the same reasons Halberg (*1*) suggested that daily rhythms need only approximate 24 hours (that is, *circa*-dian).

This report indicates that obvious ultradian cortisol rhythms with a periodicity of 85 to 90 minutes do occur in separately maintained, environmentally impoverished monkeys. If such fluctuations were due to either to cortisol feedback on brain or pituitary sites responsible for ACTH release or to periodic ACTH secretion (*11*), one would expect loss of rhythmicity when ACTH is added in supraphysiological amounts. Since the rhythm persists during such an ACTH infusion, we interpret this as evidence that fast-frequency oscillations in corticosteroid output can be independent of immediately preceding periodic ACTH input. Recently, Meier has shown a similar independence of circadian rhythms in teleost fish and rats (*12*).

We do not know the source of this ul-

tradian rhythm but assume that there is a central neural mediator because of the striking synchrony of this fast-frequency rhythm across animals; the rhythms may stem from the light-dark circadian cycle, since the 90-minute period is a harmonic of 24 hours. In prior work monitoring the clearance of [^{14}C]cortisol from peripheral blood in this animal model, we demonstrated that rapid changes in distribution, binding, and metabolism were not important factors in generating this rhythm (*5*). We now add findings which argue against the classic concept (*11*) that the adrenal cortex is integrally and inseparably linked to immediately preceding hormonal events occurring in the hypothalamopituitary axis.

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Alpha Blocking: Absence in Visuobehavioral Deprivation

Abstract. *Subjects with congenital deficits that allowed only diffuse light perception through one eye were examined for blocking of the alpha rhythm. With only the deprived eye open the electroencephalogram is dominated by alpha rhythm that is not blocked by photic stimulation, even though the stimuli evoke a response from occipital cortex.*

In his pioneering work on cerebral electrical potentials, Berger (*1*) described the alpha rhythm of the electroencephalogram (EEG). Alpha rhythm was one of the first EEG parameters to be linked with behavioral states. Its presence is associated with a meditative, quiescent state whereas its absence (cortical desynchronization) is associated with focused attention and arousal (*2*).

Blocking of the alpha rhythm by photic stimulation is useful both clinically and in the research laboratory (*3*). Alpha blocking can occur under two conditions. If a person is resting quietly with his eyes closed and is producing alpha waves, if he opens his eyes the alpha rhythm will be blocked and EEG desynchrony will be induced; or, if a person is relaxed with the eyes open and generating alpha rhythm, a flash of light will similarly block the alpha rhythm.

The exact nature of the alpha blocking process is not known. Alpha blocking may result from activation of the geniculostriate pathway alone. On the other hand, for blocking to occur perhaps this afferent volley must not only excite primary visual mechanisms but also engage the arousal and attentional processes of the brain which depend upon extrasensory neural pathways. These two explanations for alpha blocking may be studied in individuals born with a deficit of one eye that allows only diffuse light perception. The lack of form perception in these persons limits the use of the deprived eye for behavior; such persons rely on the "good" eye for visual function.

In such individuals the diffuse input from the deprived eye presumably maintains the retinocortical pathways so that stimulation of the deprived eye will evoke a response from the occipital cortex (*4*). However, if a subject grows up seeing only diffuse light, the activation of the nonspecific pathways of the brain (*5*, *6*) responsible for the generation of cortical desynchronization (*7*) is disrupted. I now report that photic stimulation of the deprived eye does not block the alpha rhythm, even though it evokes a response from the occiptal cortex.

The data for this study were collected from three human subjects (*8*). Subject A is a 19-year-old female. She had a congenital cataract of the right eye which was removed 7 months before the testing. Prior to the cataract removal she could count fingers at a distance of 7.5 cm; at the time of testing she could count fingers at a distance of 2.5 m. Her left eye is normal. Subject B is a 5-year-old boy with whitish cellular debris located centrally in the anterior vitreous of the left eye due to birth trauma. Only light perception is possible with his left eye; his right eye is normal. Subject C is a 20-year-old male with a congenital cataract of the right eye. His deficit is uniform throughout the visual field and allows him to detect hand movements. His left eye is normal with a corrective lens. The three subjects stated that they did not use their "bad" eye for vision nor could they ever recall doing so.

The EEG was recorded from the subjects with an electrode placed on the scalp contralateral to the deprived eye and 1 cm lateral to the midline over the occipital protuberance of the skull. The reference electrode was placed on the ear ipsilateral to the recording electrode. The subjects lay on a bed and looked up at a stimulus panel (milk-white plexiglass covering 35° of visual angle) illuminated from behind by a Grass photic stimulator at a rate approximating one flash every 5 seconds. The EEG was recorded with a Grass polygraph (low-frequency cutoff, 1 hertz; high-frequency cutoff, 70 hertz) and stored on magnetic tape. With one eye patched, a series of stimuli were presented until 50 artifact-free responses were accumulated. The evoked response

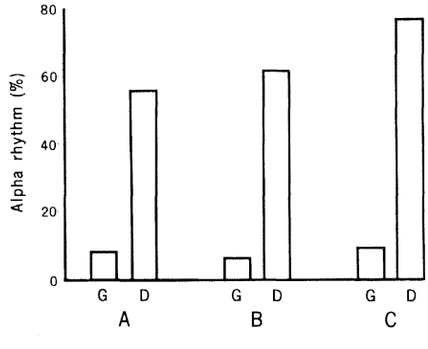

Fig. 1. Histogram showing the percentage of the EEG recording time that consisted of alpha rhythm, for the three subjects, A, B, and C with either the G (good) or D (deprived) eye open.