Temporal Precision in Circadian Systems: A Reliable Neuronal Clock from Unreliable Components?

Abstract. *Mutual coupling among oscillators of an ensemble has been proposed to explain the precision of some circadian rhythms. Reciprocal triggering is one of the most familiar forms of mutual coupling in nervous systems, but it can at best produce only modest improvement in temporal precision. Nevertheless, models with an elementary elaboration of such coupling show that circadian precision could be derived from oscillators that are intrinsically "sloppy"; sufficient conditions are that output of the individual components be summed and that mutual triggering be mediated by a nonlinear phenomenon, such as a threshold.*

Circadian rhythms of higher vertebrates exhibit remarkable temporal precision. Cycle-to-cycle variability in period of the free-running rhythm of a bird or a rodent under constant conditions is often on the order of 3 to 5 minutes (standard deviation), that is, 1 part in 300 to 1 part in 500. Such reproducibility contrasts with the behavior of most other unsynchronized biological rhythms. In systems as diverse as single spontaneously firing neurons (*1*), the human menstrual cycle, the chirping of crickets, or the heartbeat of sleeping subjects (*2*), even when the rhythms seem to be quite regular, cycle-to-cycle variation is typically on the order of 1 part in 10 to 1 part in 50. Precision greater than circadian performances has been reported only from one highly specialized biological rhythm: the high-frequency electric-organ discharge patterns of certain gymnotoid and gymnarchid fish. Cycle-to-cycle variability of as little as 1 part in 8000 has been observed in the discharge patterns over intervals of a few seconds (*3*), and hour-to-hour and day-to-day reproducibility of mean frequency is also impressive (commonly only 1 or 2 percent).

The cycle-to-cycle reliability of the high-frequency discharges of electric fish is clearly superior to that of circadian systems, but in view of the time scale involved, circadian precision remains an astonishing phenomenon. It is conceivable that intense natural selection has led to the evolution of extremely precise oscillators in the form of specialized single cells in the nervous or hormonal system, but such an ad hoc explanation has apparently never been invoked for circadian rhythms. The only alternative proposed (*4*, *5*) is that coupling within a large ensemble of circadian oscillators, presumably single cells, might produce whole-animal precision far greater than that of any single cellular pacemaker. This hypothesis is intuitively appealing since mutual coupling suggests the possibility that errors in the performance of the single elements could "average out." Beyond such qualitative reasoning, the ergodic theorems of Von Neumann (*6*) and Birkhoff (*6*) have been invoked. That body of theory demonstrates that the "time average" for a single element over a sufficiently long time can be replaced by an "ensemble average" over many similar elements (*6*). This means that the average instantaneous frequency of a set of equivalent oscillators that are stationary and independent is equivalent to the average frequency of a single such oscillator over time. Noting that some kinds of mutual coupling among oscillators with different average frequencies can reduce variability in the instantaneous frequency distribution of the ensemble, both Barlow and Winfree (*4*) have concluded from ergodic theory that coupling among circadian oscillators should also reduce variability of frequency over time and lead to more precise system output.

A counterexample, however, demonstrates that this particular application of ergodic theory can be misleading. Consider a set of oscillators in which the elements differ in intrinsic frequency and in which rhythmic group performance is triggered in each cycle by that element which discharges first. Such reciprocal excitatory coupling is seen in the several distinct potential pacemaker tissues in the vertebrate heart and also occurs in many other multiunit structures of nervous systems (*5*). The result is that all elements achieve the same average frequency, but if stochastic variation is small, the cycle-to-cycle reproducibility of period would be no greater than that of the single pacemaker with the highest intrinsic frequency.

As this example demonstrates, achieving homogeneity of average frequency across an ensemble through coupling is not directly comparable with improving cycle-to-cycle precision of the system. Coupling invalidates the assumption of independence that underlies ergodic theory. Nevertheless, in the type of system considered here, one might suspect that coupling could be beneficial if the stochastic variability of the higher frequency elements were large enough so that the role of "leadership" would often be exchanged among members of the group.

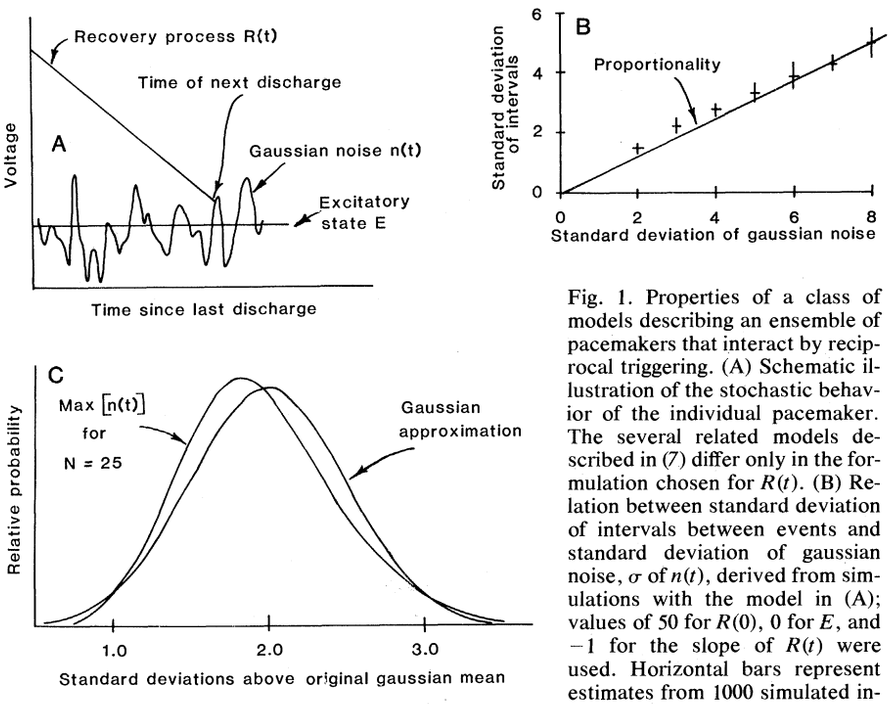

Fig. 1. Properties of a class of models describing an ensemble of pacemakers that interact by reciprocal triggering. (A) Schematic illustration of the stochastic behavior of the individual pacemaker. The several related models described in (*7*) differ only in the formulation chosen for $R(t)$. (B) Relation between standard deviation of intervals between events and standard deviation of gaussian noise, σ of $n(t)$, derived from simulations with the model in (A); values of 50 for $R(0)$, 0 for E, and -1 for the slope of $R(t)$ were used. Horizontal bars represent estimates from 1000 simulated intervals; vertical lines indicate range of values from ten subsamples of 100 intervals each. The most accurate line to the data would have slightly lower slope than that dictated by direct proportionality between variables. (C) Comparison of the theoretical distribution of max$[n(t)]$ for $N = 25$ with a gaussian approximation which has a mean of 2σ and standard deviation of $\sigma/2$.

0036-8075/80/0926-1542$00.50/0

What would happen, for example, if an idealized array of pacemakers, interacting by such triggering, all had identical intrinsic frequency, and each element had equal opportunity in each cycle to trigger the rest? To examine this question, an explicit model is needed for the behavior of the individual pacemaker. For this purpose, an elementary model of relaxation-oscillator dynamics, often proposed to describe stochastic variation in the behavior of spontaneously firing neurons (*7*), can be invoked.

In these models a gradual recovery of sensitivity, $R(t)$, which is analogous to a progressive decrease in threshold, begins from a fixed, high level immediately after the neuron has fired. A long-term average value of excitatory state, E, is dictated by the neuron's milieu, including possible tonic input; and the momentary value of excitatory state is subject to a stochastic noise process, $n(t)$, which is represented by a one-per-unit-time additive element chosen at random from a gaussian distribution (mean of zero and standard deviation of σ). According to such models (Fig. 1A), the oscillator, in isolation, discharges as soon as

$$E + n(t) > R(t) \qquad (1)$$

Such a system is simulated by asking, in each successive time unit after the last nerve impulse, whether this inequality is fulfilled. Models of this sort are, of course, gross oversimplifications of the many complex processes that underlie neuronal rhythmicity. Their virtue lies in the demonstration that, when attention is focused only on major events (spikes), the models are adequate to describe several complex temporal properties of the discharge patterns under steady-state conditions, even with varying levels of tonic stimulation (*1*). With given values of E and $R(t)$, the distribution of interspike intervals is determined solely by the gaussian distribution of noise. Simulations have shown that if the slope of $R(t)$ is roughly commensurate with σ, the standard deviation of intervals between events is approximately proportional to the standard deviation of the noise process (Fig. 1B).

Consider now an ensemble, consisting of 25 such pacemakers, all identical, in which the first member to discharge immediately triggers all others. Intrinsic identity of the pacemakers is taken here to mean identity in the values of $R(t)$, E, and σ. The ensemble will be triggered as soon as any element has fulfilled the inequality shown in Eq. 1, that is, when

$$E + \max[n(t)] > R(t) \qquad (2)$$

where $\max[n(t)]$ is the largest (positive) gaussian variate in a sample of 25 values from a distribution with mean of zero and standard deviation of σ. The probability-density function of $\max[n(t)]$ for any value of N (the number of elements in the system) can be readily derived from the gaussian distribution (*8*). These curves are slightly skewed, but with N equal to 25, the probability-density function can be satisfactorily approximated by a new gaussian distribution with a mean of about 2σ and a standard deviation of 0.5σ (Fig. 1C). This approximation permits an ensemble model, based on inequality (Eq. 2), to be treated as equivalent to the single-element model of inequality (Eq. 1) simply by adding 2σ units to E, and by specifying that the relevant gaussian distribution has half as large a standard deviation as that of the single elements in the set. Increasing the value of E means that the ensemble would have a somewhat shorter average cycle length than each element in isolation; reduction of σ to $\sigma/2$ means that the 25-element array would have a cycle-to-cycle variability about half that of any constituent oscillator (Fig. 1B).

This result looks promising. If 25 elements, so coupled, can be twice as precise as a single unit, adding more elements should lead to further improvement. Investigation of that possibility, however, proves to be disappointing. Comparable calculations demonstrate that nearly 10^6 elements are required to improve precision by another factor of 2, beyond that obtainable with 25 elements. Modest improvement in precision can be achieved by coupling a few identical elements in this way, but further improvement requires a disproportionate increase in the size of the ensemble (*9*). The identity of components assumed here represents the optimal case; if, more realistically, the elements of the ensemble are permitted to diverge from each other in intrinsic frequency, the improvements in performance calculated here for identical pacemakers will deteriorate, asymptotically approaching the variability of the single highest frequency oscillator.

Now consider a slight elaboration on this sort of coupling. Assume that resetting of the ensemble is evoked not by the first element which discharges spontane-

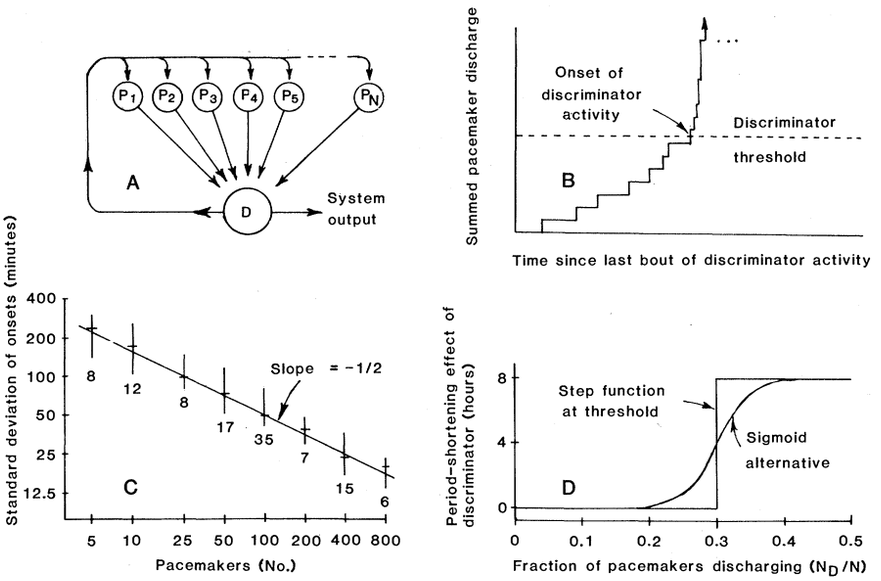

Fig. 2. Properties of a modified model for an ensemble of coupled pacemakers. (A) Schematic illustration of connectivity envisioned between pacemakers ($P_1, P_2, \ldots P_N$) and discriminator D. (B) Schematic illustration of the behavior envisioned. Each pacemaker, during its discharge phase, provides unitary input to the discriminator; when the summed discharges exceeds threshold, discriminator activity is evoked, leading to an explosive triggering and a resetting of other nondischarging pacemakers. (C) Relation between temporal variability in onset of discriminator activity and number of pacemakers in the ensemble, derived from simulations with a model of the sort shown in (A) and (B). Horizontal bars represent median estimates for standard deviation of period; vertical bars represent the range of estimates from the number of simulations, indicated beneath each bar. Because this is a double-logarithmic plot, a line with slope of $-1/2$ corresponds to an equation of the form $Y = cX^{-1/2}$. In these simulations, the overall mean period of the pacemakers, when uncoupled, was 25.5 hours, with inter-pacemaker standard deviation of about 4.9 hours; standard deviation of stochastic intra-pacemaker variability was about 3.8 hours; threshold was $3N/10$. (D) Sigmoid alternative for the relation between pacemaker input to the discriminator and its output, measured as the magnitude of period shortening experienced by nondischarging pacemakers. The step function corresponds to behavior illustrated in (B).

ously, but by the ith element, where i is an appreciable fraction of the total number of pacemakers present. For example, one might assume that each element, once triggered, produces a sustained discharge and that the sum of these discharges from all active elements is the stimulus that, if of sufficient magnitude, S_i, can trigger and reset all other nondischarging elements. Figure 2A presents a hypothetical structural arrangement by which such coupling might be mediated; Fig. 2B is a schematic illustration of the behavior envisioned. As soon as the summed discharge of the (spontaneously triggered) individual rhythmic elements exceeds some threshold value, each nondischarging element receives excitatory input from the integrating component D (discriminator), thereby greatly increasing the probability that the nondischarging elements will also be activated. A simple way in which such coupling can be formally specified is to invoke a model similar to that in Fig. 1A for each pacemaker and to postulate that suprathreshold output of the discriminator increases the value of E for all pacemakers.

Extensive Monte Carlo simulation with such models has shown that this sort of mutual coupling is capable of greatly improving precision in system output from an ensemble of "sloppy" oscillators, that is, elements which differ markedly from each other in their intrinsic average frequencies and which are individually unreliable on a cycle-to-cycle basis (*10*). With a given set of parameter values, the standard deviation of cycle length between successive onsets of discriminator activity has been found to be proportional to $N^{-1/2}$, where N is the number of elements in the ensemble (Fig. 2C). This empirical result can be rationalized in terms of Poisson processes (*11*). The result holds for many models with different sets of parameters (for example, threshold values between $N/5$ and $N/2$) and applies also to a variety of alternative formulations (for example, replacing threshold of the discriminator with a continuous, nonlinear relationship between summed pacemaker discharge and discriminator output, as shown in Fig. 2D).

Other parameters of such a model, which affect precision in output, are the variability of mean period length in the array of elements (inter-pacemaker intrinsic variability) and the cycle-to-cycle variability in period of a single element in isolation (intra-pacemaker stochastic variability). When 1.66 and 1.88 hours were used for the standard deviations of these distributions—values commensurate with the inter- and intracellular circadian variability (1.9 and 2.1 hours, respectively), which were derived from the only available experimental data, from the alga *Acetabularia* (*12*)—and N was set at 100 pacemakers, replicate 20-cycle simulations had values for standard deviation of period in the range from 12 to 21 minutes. Invoking the proportionality between standard deviation and $N^{-1/2}$, we find that an ensemble of about 1600 elements with these properties—a not implausible number—would be as precise as the best of circadian records (standard deviation equal to 3 to 5 minutes). If one assumes a more homogeneous population of more reliable elements, the required number of oscillators would be reduced.

These calculations assume that threshold of the discriminator (Fig. 2B) is invariant with time; more realistically, stochastic variation in threshold must eventually set an upper limit on the precision attainable by increasing the size of the array. With the ensemble of 1600 pacemakers, it can be shown (*10*, pp. 77–79 and 113) that low-frequency fluctuations in threshold (those with periods of many hours) with standard deviation less than about 3 percent of the mean will have little effect on system precision; high-frequency fluctuations of even larger magnitude are also unimportant. If the discrete step function for threshold is replaced by a continuous, nonlinear interaction (Fig. 2D), low-frequency stochastic variation of as much as 25 percent in the output-input relation (effect of the discriminator for a given value of summed pacemaker discharge) can be present before such effects limit precision in a 1600-element ensemble (*10*, pp. 77–79 and 113).

These calculations demonstrate that mutual coupling among stochastic relaxation-oscillator elements could account for circadian precision without requiring outrageous assumptions about the properties of the cellular oscillators, their numbers, or their coupling. If one makes the supplementary assumption that discriminator threshold (or its equivalent in Fig. 2D) varies with light intensity (light increases threshold for nocturnal animals and decreases it for diurnal animals), then models of this type can also account in considerable detail for a large body of experimental data on the complex effects of lighting regimes on the circadian rhythms of birds and rodents. The simulations that demonstrate these correspondences (*10*, pp. 82–104 and 120–198) have also shown that the coupling envisioned here confers on the models a number of properties that are intuitively unexpected for an ensemble of stationary relaxation oscillators. For example, a single stimulus can either accelerate or delay the ensemble rhythm, depending on its timing, although the individual oscillator can only be accelerated by the discriminator. Transients that persist for several cycles can arise after certain types of perturbation, although no single component has this property. The free-running period of the system is plastic and can be molded by its past history; a long-term "memory" is stored dynamically in the phase relations among the elements, although no component alone can "remember" beyond its single cycle (*10*, pp. 82–104 and 120–198). Hence, the replication of similar elements in such a composite pacemaker could serve not only to enhance temporal precision but also to confer on the organism adaptive capabilities which are not inherent in the single cellular oscillators.

J. T. ENRIGHT

Scripps Institution of Oceanography, University of California, La Jolla 92093

References and Notes

1. J. T. Enright, *J. Theor. Biol.* **16**, 54 (1967), and references cited therein.
2. J. Buck and E. Buck, *Science* **159**, 1319 (1968).
3. T. H. Bullock, R. H. Hamstra, H. Scheich, *J. Comp. Physiol.* **77**, 1 (1972); T. H. Bullock, K. Behrend, W. Heiligenberg, *ibid.* **103**, 97 (1975).
4. J. S. Barlow, *Cold Spring Harbor Symp. Quant. Biol.* **25**, 54 (1961); A. T. Winfree, *J. Theor. Biol.* **16**, 15 (1967).
5. Similar statements about this expected consequence of mutual coupling have also been advanced in a noncircadian context [T. H. Bullock and G. A. Horridge, *Structure and Function in the Nervous System of Invertebrates* (Freeman, San Francisco, 1965), vol. 1, p. 502; G. A. Horridge, *J. Exp. Biol.* **36**, 72 (1959)].
6. Nihon Sūgakkai, *Encyclopedic Dictionary of Mathematics* (MIT Press, Cambridge, Mass., 1977), p. 486. This volume contains a summary of these theorems.
7. S. Hagiwara, *Jpn. J. Physiol.* **4**, 234 (1954); A. J. Buller, *J. Physiol. (London)* **179**, 402 (1965); W. H. Calvin and C. F. Stevens, *Proc. 18th Annu. Conf. Eng. Med. Biol.* (1965), p. 118; L. J. Viernstein and R. G. Grossman, in *Information Theory, 4th London Symposium*, C. Cherry, Ed. (Butterworths, London, 1961), p. 252; T. F. Weiss, *Mass. Inst. Technol. Res. Lab. Electron. Tech. Rep. No. 418* (1964), p. 1; A. A. Verveen and H. E. Derksen, *Kybernetik* **2**, 152 (1965); C. D. Geisler and J. M. Goldberg, *Biophys. J.* **6**, 53 (1965). For a comparison of these various models, see (*1*).
8. Any percentile, $P_{X'}$, of the probability-density function can be calculated as gauss^{-1} $[(X/100)^{1/N}]$, where N is the sample size and gauss^{-1} is the inverse gaussian function. See also E. J. Gumble, *The Statistics of Extremes* (Columbia Univ. Press, New York, 1958).
9. Calculations based on this sort of reciprocally triggered system, but with more elementary models than that in Fig. 1A, have been described by M. J. Murray [*J. Comp. Physiol.* **117**, 63 (1977)]. Murray also found that such coupling among identical oscillators can improve ensemble precision. Only networks of 12 elements or less, however, were investigated; the extent to which the benefits of coupling saturate with further increase in number of oscillators was not examined.
10. J. T. Enright, *The Timing of Sleep and Wakefulness: On the Substructure and Dynamics of the Circadian Pacemakers Underlying the Wake-Sleep Cycle* (Springer-Verlag, New York, 1980). A FORTRAN program for such simulations is listed on p. 45.
11. J. Thorson, in (*10*), p. 81. After submitting this report, similar proportionality between standard

deviation and $N^{-1/2}$ has been empirically demonstrated in coupled aggregates of in vitro cultured heart cells, with $1 < N < 100$ [J. R. Clay and R. L. DeHaan, *Biophys. J.* **28**, 377 (1979)]. The model proposed there for pacemaker behavior is quite similar to that in Fig. 1A, but the interpretation proposed for the $N^{-1/2}$ result is derived from empirical data relating amplitude of the noise in membrane voltage with size and number of the heart cells in the aggregate.

12. M. W. Karakashian and H. G. Schweiger, *Exp. Cell Res.* **97**, 366 (1976).

13. This work was supported by NSF grants GB 2469, GB 5471, GB 23949, GB 3750, and PCM-7719949. I thank A. Winfree, W. Heiligenberg, J. Corwin, C. Baker, J. Thorson, and T. H. Bullock for their helpful comments on the manuscript.

10 December 1979; revised 22 April 1980

Mood and Behavioral Effects of Physostigmine on Humans Are Accompanied by Elevations in Plasma β-Endorphin and Cortisol

Abstract. *Administration of physostigmine to normal volunteers produced significant elevations in plasma cortisol and β-endorphin immunoreactivity as well as alterations in mood, cognition, and behavior. These observations might be explained by a cholinergically mediated stress syndrome. However, peak elevations in plasma β-endorphin immunoreactivity (but not in plasma cortisol) were significantly correlated with physostigmine-induced increases in depression ratings. These results suggest that a cholinergically mediated β-endorphin pathway may be involved in the observed affective changes.*

The intravenous administration of physostigmine leads to decreases in speech and spontaneous behavior, slowed thoughts, sedation, and occasionally nausea (*1*). Individuals with a past history of affective disorder frequently experience a brief recurrence of depressive symptomatology when given physostigmine (*2*). The drug also has antimanic properties (*3*). Although physostigmine is known to be a cholinesterase inhibitor, with both nicotinic and muscarinic cholinomimetic properties, other aspects of its biological effects on humans have been little explored. Preliminary evidence has suggested that, in some individuals, elevations in cortisol accompany physostigmine administration (*4*, *5*).

Elevations in plasma cortisol levels have been observed in animals and humans under stress and in some psychiatric states, such as depression (*6*). The preliminary reports of elevated plasma cortisol after physostigmine administration raise the possibility that plasma β-endorphin might also be elevated, since cortisol is regulated by adrenocorticotropic hormone (ACTH), and since evidence from animal studies suggests that ACTH and β-endorphin are released together from the anterior pituitary (*7*). Concurrent elevations in ACTH and β-endorphin occur in rats stressed by limb fracture or foot shock; such elevations do not occur in hypophysectomized rats (*7*). Similarly, the synthetic glucocorticoids dexamethasone and prednisolone, which suppress pituitary ACTH release in animals and humans, also suppress, in a dose-dependent manner, the secretion of β-endorphin from normal pituitary gland cultures and from the mouse pituitary cell line AtT-20 (*8*).

We gave physostigmine to normal volunteers in a random-assignment, double-blind crossover study to evaluate whether the behavioral and psychological changes produced by the drug are associated with observable biological (especially neuroendocrine) changes. Nine volunteers, who were screened for the absence of psychiatric illness, were treated with methscopolamine (1.0 mg, intramuscularly) and 20 minutes later with physostigmine (22 μg/kg) or saline intravenously over a 10-minute period. Subjects rated themselves and were rated by a trained observer before and at intervals after the infusions. Blood samples were taken repeatedly for several neuroendocrine measures, including plasma cortisol and β-endorphin immunoreactivity (*9*).

There were significant increases in plasma cortisol ($P < .001$, one-tailed t-test) (Fig. 1) and β-endorphin immunoreactivity ($P < .005$) (Fig. 2) after administration of physostigmine, but not after saline. There was no significant correlation between the increases in plasma β-endorphin and cortisol—surprising, since current evidence supports a common ACTH-β-endorphin pituitary regulatory system. This may reflect recent findings of hypothalamic (*10*) or peripheral (*11*) sources of β-endorphin that are independent of the pituitary regulatory system. Alternatively, since all nine of our subjects had large increases in plasma cortisol while only six had increases in plasma β-endorphin, pituitary ACTH release may be more sensitive to cholinergic stimulation than pituitary β-endorphin release.

In all our subjects, increases in plasma cortisol were 1.9-fold or greater, in contrast to the 17 individuals studied by Davis and Davis (*5*), only six of whom manifested plasma cortisol elevations after intravenous doses of physostigmine (1 to 2 mg). The six also experienced nausea or vomiting, and Davis and Davis (*5*) interpreted the cortisol increase as the sign of a nonspecific stress syndrome. Only three of our subjects developed nausea or vomiting, and there was no significant correlation between self-ratings of nausea or the occurrence of vomiting and changes in plasma cortisol or β-endorphin immunoreactivity.

Our first interpretation of these data, nonetheless, was that the higher frequency of cortisol increases was due to the use of a 10-minute rather than 60-minute physostigmine infusion period, and that the elevations in concentration of the two hormones in the plasma might be re-

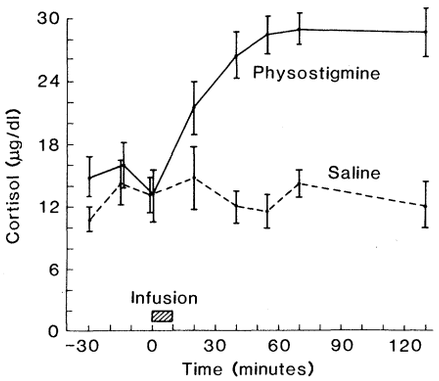

Fig. 1. Changes in plasma cortisol levels (means ± standard errors) after administration of physostigmine or saline in nine subjects. Blood samples were drawn 30, 15, and 0 minutes before the 10-minute infusions and 20, 40, 55, 70, and 130 minutes after beginning them. Paired t-tests were performed to test for significant differences between means.

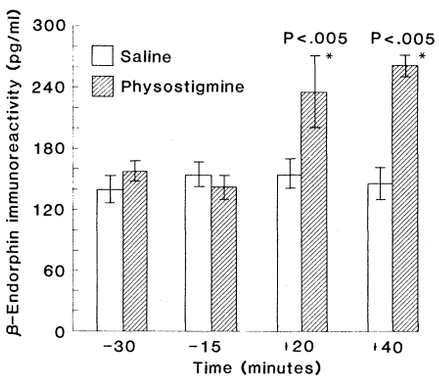

Fig. 2. Changes in plasma β-endorphin immunoreactivity (means ± standard errors) after administration of physostigmine or saline in nine subjects. Blood samples were drawn 30 and 15 minutes before the infusions and 20 and 40 minutes later. Paired t-tests were performed to test for significant differences between means.

0036-8075/80/0926-1545$00.50/0