Table 1. Mucosal uptake and mucosal transfer of Fe$^{59}$SO$_4$ (50 $\mu$g containing 2.0 × 10$^6$ disintegrations per minute) from isolated intestinal loops. A, Mucosal uptake; B, remaining in mucosa; A−B, mucosal transfer.

| Group | Rats (No.) | A | B (% ± S.D.) | A − B | B/A (%) |
|---|---|---|---|---|---|
| Control | 20 | 64.8 ± 11.4 | 27.8 ± 9.3 | 37.0 ± 10.9 | 42 |
| Iron-deficient | 10 | 82.9 ± 5.8 | 27.3 ± 5.1 | 55.6 ± 7.1 | 33 |
| Iron-loaded | 14 | 51.6 ± 11.5 | 35.9 ± 12.6 | 15.7 ± 8.2 | 69 |
| Cycloheximide | 18 | 28.8 ± 14.1 | 17.0 ± 6.8 | 11.8 ± 8.2 | 59 |

of iron was compared in control and cycloheximide-treated rats. Isolated intestinal loops were removed 15, 30, or 60 minutes after injection of radioiron, and the mucosal uptake and mucosal transfer were determined as described above. The amount of iron absorbed was calculated on the basis of the mucosal transfer and the dose of radioiron, 50 $\mu$g (Fig. 1). In control animals iron absorption progressively increased; 23.5 $\mu$g was absorbed in 60 minutes. In contrast, in cycloheximide-treated rats there was a minimal increase in iron absorption over a 60-minute period with only 7.4 $\mu$g being absorbed.

The absorption of glucose from isolated intestinal loops was compared in control and cycloheximide-treated rats. After an intraduodenal injection of glucose (1.5 g per kilogram of body weight) the increase in blood glucose was similar in both groups. The maximum values for blood glucose were 216 ± 50 (1 S.D.) and 210 ± 42 mg per 100 ml, in control and cycloheximide-treated animals respectively. The normal absorption of glucose and the minimum changes in the intestinal mucosa detected by light and electron microscopy suggest that the impaired iron absorption associated with the administration of cycloheximide is not a nonspecific effect due to tissue damage. In this connection, it is pertinent that rats treated wtih acetoxycycloheximide absorbed triglycerides of fatty acids of medium-chain length at nearly the same rate as controls (*6*).

Several studies have indicated that iron is present in the intestinal mucosa in two forms: (i) nonprotein-bound iron which is transported rapidly across the mucosa and (ii) iron which is slowly transported across the mucosa (*2, 3, 4*). Two groups of investigators (*4, 7*) have suggested that the slowly transported iron has been incorporated into mucosal ferritin. The nature of the rapidly transported iron has not been clearly defined.

The mechanisms regulating iron absorption remain poorly understood. It has been proposed that the intestinal mucosa receives a "signal" and proportionate amounts of iron are either rapidly transported across the mucosa or incorporated into ferritin with a resultant delay in transport (*4*).

Our data are in accord with those which show that iron absorption is increased by a diet deficient in iron (*4, 8*) and that the mucosal uptake and transfer of iron is significantly impaired by iron loading (*2, 4*). A similar defect in the mucosal uptake and transfer of iron was produced by treatment with cycloheximide. The mechanism of the impaired iron absorption resulting from these treatments is unknown. Our data suggest that a specific carrier substance transfers iron in the mucosa. Such a substance could be a protein, a polypeptide, or any specific amino acid which forms complexes with iron (*3*). Iron loading might saturate this carrier or repress its synthesis. It is possible that an agent such as cycloheximide impairs synthesis of the carrier. Furthermore, a carrier substance might be important in the entry of iron into the mucosal cell as well as the transport of iron from the cell. This hypothesis could account not only for the decreased mucosal transfer, but also for the decreased mucosal uptake of iron noted after treatment with iron or cycloheximide.

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### References and Notes

1. J. G. Manis and D. Schacter, *Amer. J. Physiol.* **203**, 73 (1962).
2. M. S. Wheby, L. G. Jones, W. H. Crosby, *J. Clin. Invest.* **43**, 1433 (1964).
3. E. B. Brown and M. L. Rother, *J. Lab. Clin. Med.* **62**, 357 (1963).
4. R. W. Charlton, P. Jacobs, J. D. Torrance, T. H. Bothwell, *J. Clin. Invest.* **44**, 543 (1965).
5. C. W. Young, P. F. Robinson, B. Sacktor, *Biochem. Pharmacol.* **12**, 855 (1963).
6. N. J. Greenberger, J. B. Rodgers, K. J. Isselbacher, *J. Clin. Invest.* **45**, 217 (1966).
7. R. S. Hartman, M. E. Conrad, Jr., R. F. Hartman, R. J. T. Joy, W. H. Crosby, *Blood* **22**, 397 (1963).
8. S. Pollack, R. M. Kaufman, W. H. Crosby, *Science* **144**, 1015 (1964).

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## Simultaneous Changes in Visual Separation Threshold and Voltage of Cortical Alpha Rhythm

Abstract. *An automated psychophysical method allows a human subject to track his own separation threshold for two flashes delivered in sequence without interfering with the simultaneous recording of the alpha rhythm of his electroencephalogram. A systematic rise in this threshold is observed when the alpha voltage declines from its normal alert level. This suggests that an increase in the minimum resolvable temporal sequence may contribute to the development of the negative time errors associated with drowsiness and sleep.*

A previous study (*1*) demonstrated that as the alpha voltage, shown in an electroencephalogram (EEG), declines from a general alert level there is an increasing tendency to underestimate the passage of time, that is, to make negative time errors. A mechanical timepiece making negative time errors would ordinarily be described as "losing time," a fault indicating that there is something wrong with either the mechanism generating its basic temporal units or the mechanism summing these units, or both. The present experiment was designed to explore the first of these two possibilities in the case of human perception of change during drowsiness, namely, that there may be some distortion in the perception of the smallest units of change. The question asked is not how effectively is time spanned but rather how effectively is time resolved? Specifically, are the fluctuations in the *minimum resolvable sequence* of photic stimuli systematically related to variations in the alpha voltage shown in the EEG?

If the second of two flashes in sequence follows too soon after the first, the subject reports only one flash instead of two. By progressively lengthening the interval between the two stimuli (the interflash interval, or IFI) a value is found at which the subject first reports seeing two flashes (the separation threshold); conversely, by progressively decreasing the IFI a value is found at which the subject first reports seeing only one flash (the fusion threshold). The existence of the separation threshold demonstrates that there is a limit to the temporal resolving power of the perceptual system. An orderly rise in this threshold during drowsiness would be consistent, therefore, with the hypothesis that the negative time errors observed during drowsiness are attributable, in part at least, to a loss in the temporal resolving power of the human "clock," or, in other words, to a coarsening of the subjective units of temporal perception.

A method, which is essentially an automated version of the psychophysical method of limits patterned after an audiometric technique introduced by Békésy (*2*) and similar to Blough's method (*3*) for studying visual thresholds in pigeons, devised for the present experiment allows the subject to track his own separation and fusion thresholds alternately by simply tapping one key when he observes just one flash (although there are always objectively two discrete photic stimuli) and by tapping a second key with the other hand when he perceives the stimulus-pair as "two." The subject's key responses are fed back into the flash-generating system to influence subsequent IFI's as follows: a report of "one" causes the next IFI to be lengthened by a discrete amount, whereas a report of "two" produces a shortening of the next IFI. Automatic recording of the activities of the servomechanism producing the variations in the IFI provides a convenient graphic account of the subject's oscillations between separation and fusion thresholds.

Electroencephalographic potentials from a transoccipital linkage between needle electrodes attached to the scalp are subjected to automatic frequency analysis of the type described by Meister *et al.* (*4*). The present, somewhat modified, apparatus passes the EEG signal through various bandpass filters; the output of each filter is then independently integrated, converted from analog to discrete form (*5*), and then recorded both by printing counters and as cumulative charts (*6*). A subject's alpha frequency is empirically defined according to the bandpass filter that shows the highest output when the subject is known to be fully alert. Four filters (passive LC-type) with equal energy characteristics and a constant $Q$ of 7.5 cover the usual alpha frequency range. Throughout the present report a subject's "alpha voltage" will refer to the output of one of these filters selected in the manner described above.

The six subjects described in the present report were male university students, ranging in age from 20 to 25 years, who were selected for (i) the quality of the alpha rhythm in their brain waves, (ii) their ability to produce stable threshold-tracking data when alert, and (iii) their ability to relax to the point of extreme drowsiness or light sleep in the experimental situation. The EEG's of all prospective subjects were examined prior to experimental testing. Only subjects with well-defined alpha activity were accepted for further study. Although there is no reason to expect that otherwise-normal subjects from whom the experimenter fails to obtain a striking record of alpha activity would be unable to perform the tracking operation as well as subjects with good alpha rhythms, it is impractical to attempt to observe the decline of a variable if the variable is poorly defined under presumably optimal conditions. Of eight subjects with satisfactory EEG's, one was eliminated because he was unable to track the stimulus consistently even when fully alert, and a second subject, though a superb tracker, failed to give a usable record because he never became even mildly drowsy except when drugged.

While being tested, each subject was isolated in an air-conditioned, light-proof, sound-attenuated chamber. He lay in a supine position on a comfortable bed with the flash lamp holder mounted directly over his head and with an electrically shielded telegraph key under each hand. His instructions were to keep his eyelids closed at all times and to tap the appropriate key according to the number of flashes observed as soon as possible after each flash pair. The flash generator (*7*) produces two flashes, each of 10 μsec duration, separated by a variable delay (15 to 150 msec); instantaneous peak intensity of the flash is approximately 50,000 peak candles at the surface of the eyelids. In the present arrangement the light falls equally on both eyes and at an intensity sufficient to penetrate the eyelids and allow the separation-fusion discriminations to be made without undue attentive effort and without excessive visual discomfort. For the purpose of isolating the subject from any audible flash-tube sounds, the flash-tube holder was encased in a specially constructed housing and the subject was required to wear a set of earphones through which he received a masking white noise from an audiogenerator. The time elapsing between the first flash of one flash-pair and the first flash of the next pair was fixed at 1.5 seconds, a value selected by a number of preliminary subjects as providing a comfortable pace. The exact arrangement was as follows: an electronic timer delivered a pulse every 1.5 seconds which first changed the IFI setting (either adding or subtracting approximately 1 msec) and then triggered a pair of flashes.

The subject's key response influ-

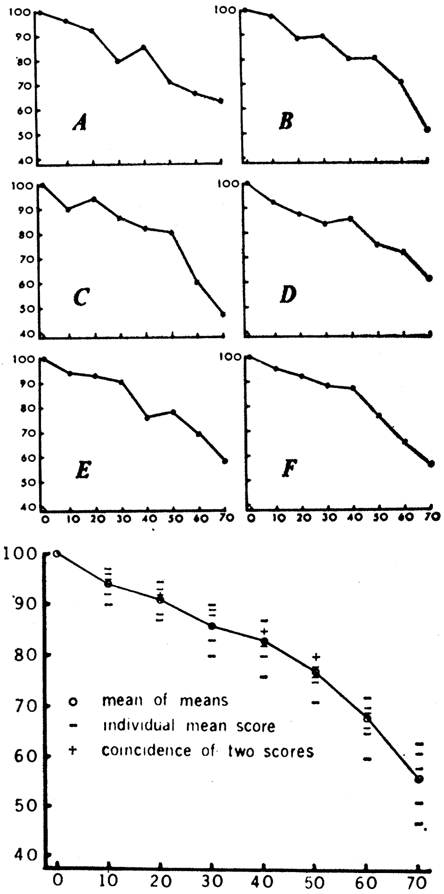

Fig. 1. Increase in threshold for discrimination of two flashes (in milliseconds; abscissa) as an alpha voltage falls to various percentages of the alert level (ordinate). (Top) Mean scores for six individual subjects (*A–F*). (Bottom) Means of the means.

enced only the direction of change in the subsequent IFI, a pair of flashes being delivered every 1.5 seconds whether the subject responded or not. In the absence of a response the servomechanism continued to step in the same direction. Only a change in response (from right key to left key, or vice versa) would cause a reversal in the direction of alteration of IFI size. To control for hand-preference effects, the meaning of right and left keys was arbitrarily assigned to each subject, three of the subjects being instructed to use the right key to indicate "one" and the other three subjects being instructed to use the right key to indicate "two flashes." The controlling circuitry was altered according to whichever instructions were given, so that the records for all subjects were collected in similar form. There was no evidence that handedness biased the results in any way.

Each subject was tested and retested until he produced three sessions on three different days containing at least one drowsy episode in each session. The records for these days were exhaustively analyzed (not selectively sampled) in the following manner. Each separation threshold report (a response initiating a change in direction of the servomotor) was assigned a voltage score based on the average rate of voltage integration during the 60 seconds prior to the response. (A shorter voltage integration epoch of, say, 10 seconds can be used, but greater variability will be observed in the results; instantaneous voltage does not predict this type of performance as well as an average voltage derived from a longer sample. Some compromise must be made in order to avoid the variability that attends voltage measurements based on epochs of too short duration and to avoid serious loss of resolution of changes associated with real time if the epochs are too long.) The voltage scores expressed as percentages of the subject's alert voltage were then classified according to the number of milliseconds the corresponding threshold measurement lay above the subject's normal alert threshold, that is, according to the number of milliseconds of increase in the delay between flashes required to reach the separation threshold. This stabilization of the voltage scale in percentage units of the alert alpha voltage and the stabilization of the threshold measurements in units of IFI increase above the alert separation threshold facilitates the comparison and collation of the data from the various subjects.

Thresholds could not be assessed in the present arrangement beyond the range provided for in the mechanism. If a subject tracked to either limit and ceased to deliver any key taps for 60 seconds or more, it was assumed that he was "asleep" in the sense that he had lost effective contact with the testing situation, and therefore scoring was discontinued until he had returned to active tracking for 5 minutes or until he had resumed oscillatory tracking in the alert threshold range. This exclusion was necessary to avoid artifacts in the data attributable chiefly to inertia in the tracking system; if, for example, the subject recovered abruptly from a "lapse" in responding it would take some time before the servo-record actually reflected the subject's discriminative capacity.

The analyses are summarized in Fig. 1, which contains the data both for individual subjects and for the subjects collectively. Each of the individual records shows essentially the same relations found in the composite record: as alpha voltage declines from the normal alert level there is a rise in the separation threshold. What is of considerable interest is the fact that relatively small losses of alpha voltage are associated with rather sizeable increments in separation thresholds.

The present results are not attributable to motor failure to respond because all values are restricted to the occurrence of actual responses. In addition, the subjects were usually making key responses (in one direction only) for some minutes after they had tracked to the limit of the servomechanism. The primary loss, then, was not of motor capacity but rather of discriminative capacity.

These findings, taken in conjunction with the previous study on time estimation *(1)*, indicate that great caution is required in controlling for the level of alertness in the measurement of psychological thresholds and in the assessment of performance, especially in cases where the procedures employed in the study tend to encourage drowsiness in subjects because of isolation, immobilization, and boredom. The absence of adequate controls on level of alertness renders the interpretation of such studies extremely problematical.

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### References and Notes

1. J. E. Anliker, *Science* **140**, 1307 (1963).
2. G. v. Békésy, *Acta Oto-laryingol.* **35**, 411 (1947).
3. D. S. Blough, *Science* **121**, 703 (1955).
4. M. Meister, R. S. Schwab, E. Petersen, A. Grass, *Electroencephalog. Clin. Neurophysiol.* **11**, 594 (1959).
5. Analog-to-discrete data conversion: a circuit provides for the momentary operation of a relay whenever an arbitrary preselected level of voltage integration is reached. The operation of the relay provides an output pulse and also resets the integrator to zero voltage.
6. Harvard cumulative recorder, Ralph Gerbrands Co., Arlington, Mass.
7. Photo-stimulator, model PS 2, Grass Instrument Co., Quincy, Mass.

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# Inhibitory and Facilitatory Effect of Two Related Peptides on Extinction of Avoidance Behavior

Abstract. *The polypeptide chain which constitutes the first ten amino acids of the ACTH molecule inhibits extinction of a shuttle-box avoidance response. If the phenylalanine molecule in the 7th position of this peptide is replaced by its dextrorotatory form, extinction is facilitated.*

During studies on the inhibitory effects of adrenocorticotrophic hormone (ACTH) and related peptides on extinction of an avoidance response *(1)*, we found that the polypeptide containing the first ten amino acids of ACTH, but in which the phenylalanine in the 7th position had been replaced by the dextrorotatory form [ACTH 1-10 (7-D-Phe)], exhibited an opposite effect.

Twenty-nine albino rats (male, 110 to 130 g) were conditioned in a shuttle-box divided into two equal compartments by a 5-cm barrier to avoid an electric shock *(2)*. The floor was made of a grid through which an electric