about 70 to 80 percent of the population exposed to vinblastine sulfate at both concentrations, whereas none or only a few were observed in the control culture. As seen in Fig. 1, the structures are distinct. Both longitudinal and what appear to be cross sections can be observed. Depending on the sectioning plane, these structures often seemed to be composed of alternating units, about 160 Å in diameter, suggesting a helical configuration. A single helix is about 600 Å in width but varies in length. The pitch is on the average 180 Å. According to these data the structures are similar, if not identical, to those naturally occurring in *E. coli*. In the assumed cross sections, the helices appear to contain four closely packed units. The majority of cells in which ribosomal helices were observed were dividing, and the structures were perpendicular to the plane of division or occasionally oriented parallel to the membrane (Fig. 2). Frequently they appeared to be attached directly to the membrane. No distinct ribosomal helices were seen in control cells (Fig. 3), but "pairing" of ribosomes was often noticed near the division plane. The vinblastine sulfate–treated specimen contained many more dividing cells than the control specimen, suggesting either a partial arrest of division or an increased degree of synchrony in the culture. Growth curves indicated that vinblastine sulfate reduces the growth rate of *E. coli sud 24*.

We also subjected strain K-12 to the same vinblastine sulfate treatment as that of *sud 24*, but omitted sucrose from the medium and fixation reagents. Neither increase in the number of helices nor a decrease in growth rate was observed. We concluded that the difference in response to vinblastine sulfate of K-12 and *sud 24* may be a matter of permeability. Since *sud 24* is an osmotically fragile mutant, this strain might allow the passage of vinblastine sulfate into the cell, whereas K-12 may be impermeable to the alkaloid. In order to test this theory, we applied the method of Leive (*12*), of increasing cell permeability by removal of lipopolysaccharides from the cell wall through a brief exposure to ethylenediaminetetraacetate. Thus treated, cells were transferred to fresh medium C with and without vinblastine sulfate ($5.4 \times 10^{-4} M$) and incubated as stated previously. Samples for electron microscopy were taken at various time intervals and processed as above.

After 2 hours of growth, vinblastine sulfate induced a great number of ribosomal helices in K-12 cells similar to the *sud 24* cultures. We concluded that, normally, *E. coli* is impermeable to vinblastine sulfate, but alteration of the permeability barriers either by ethylenediaminetetraacetate or mutation permits the uptake of vinblastine sulfate.

This system can be used profitably for at least two different types of studies. First, the ribosomal helices may be studied for formation, structure, and function. Since the helical arrangement of polysomes appears to be a phenomenon pertaining to eucaryotic as well as to procaryotic cells, information in regard to these structures and their relationship to DNA and cell membranes would aid in defining the concepts which exist about the function of ribosomes in protein synthesis. Furthermore, it may be possible to gain information on the structure of ribosomes, as it exists in vivo. This approach permits the observation of unaltered cytoplasmic units which are generally distorted by the methods used for their isolation. Although we have not tried other genera or *E. coli* strains, it seems probable that this method could be adapted to a number of different organisms. Secondly, this method could be used to investigate the mode of action of vinblastine sulfate on cells, generally an easier procedure in procaryotic cells than in the structurally complicated eucaryotic cell systems.

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References and Notes

1. O. Behnke, *Exp. Cell Res.* **30**, 597 (1963); C. H. Waddington and M. M. Perry, *ibid.*, p. 599; E. Shelton and E. L. Kuff, *J. Mol. Biol.* **22**, 23 (1966); P. Weiss and N. B. Grover, *Proc. Nat. Acad. Sci. U.S.* **59**, 763 (1968).
2. P. Echlin, *J. Cell Biol.* **24**, 150 (1965); V. I. Bruskov and N. A. Kiselev, *J. Mol. Biol.* **38**, 443 (1968); W. A. Jensen, *J. Cell Biol.* **36**, 403 (1968); F. B. P. Wooding, *J. Ultrastruct. Res.* **24**, 157 (1968).
3. C. H. Domermuth, M. H. Nielsen, E. A. Freundt, A. Birch-Andersen, *J. Bacteriol.* **88**, 727 (1964); J. Maniloff, H. J. Morrovitz, R. J. Barrnett, *ibid.* **90**, 193 (1965); H. Slayter, Y. Kiho, C. E. Hall, A. Rich, *J. Cell Biol.* **37**, 583 (1968); F. M. Schwartz and N. D. Zinder, *Virology* **34**, 352 (1968).
4. D. C. Barker, *Z. Zellforsch. Mikroskop. Anat.* **58**, 641 (1963); R. S. Morgan, H. S. Slayter, D. L. Weller, *J. Cell Biol.* **36**, 45 (1968); H. Voelz, *J. Ultrastruct. Res.* **25**, 29 (1968).
5. E. W. Kingsbury and H. Voelz, *J. Bacteriol.* **95**, 1478 (1968).
6. I. S. Johnson, H. F. Wright, G. H. Svoboda, J. Vlantis, *Cancer Res.* **20**, 1016 (1960).
7. A. Krishan, D. Hsu, P. Hutchins, *J. Cell Biol.* **39**, 211 (1968); J. H. Cutts, *Cancer Res.* **21**, 168 (1961).
8. K. G. Bensch and S. E. Malawista, *Nature* **218**, 1176 (1968).
9. G. Mangiarotti, D. Apirion, D. Schlessinger, *Science* **153**, 892 (1966).
10. G. Mangiarotti and D. Schlessinger, *J. Mol. Biol.* **20**, 123 (1966).
11. R. Ryter and E. Kellenberger, *Z. Naturforsch.* **13b**, 597 (1958).
12. L. Leive, *Biophys. Biochem. Res. Commun.* **21**, 290 (1965).
13. This work was supported by grants CA-05170 and AI-06600 from the U.S. Public Health Service. We thank the Lilly Research Laboratories for a complimentary sample of vinblastine sulfate.
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7 August 1969

Evoked Potentials: Modifications by Classical Conditioning

Abstract. *Visual evoked potentials to a positive discriminative stimulus change systematically during sensory conditioning and extinction. Changes due to conditioning are manifested in the increased amplitude of the late component of the evoked response. This effect is attenuated during extinction and reappears after reconditioning.*

Many changes in brain evoked potentials obtained during conditioning have been demonstrated in animals (*1*). Comparisons of wave forms obtained during "correct" and "incorrect" behavioral responses suggest that differences occur primarily in the late components. Inspection of simultaneously computed wave forms suggests a covariation between late components and the poentials evoked in mesencephalic reticular formation and centralis lateralis (*2*). During the conditioning procedure, changes in the significance of the conditioned stimulus are accompanied by modifications of the later activity of the evoked potentials.

We now report the results of a study in which we explored the relation between classical conditioning and the late components of the visual evoked potentials (VEP) in humans. All data were derived from monopolar scalp recordings of 16 college students. The active electrode was located on the midline 2.5 cm above the inion; the combination of the two ear lobes formed the reference electrode. Evoked potentials were recorded by means of a Grass Model 7 P5A wide-band AC EEG amplifier, whose low-frequency cutoff filter was set at 0.15 hz. The driver amplifier high-frequency cutoff filter was set at 75 hz, and the gain was set at 50 hz. Amplifier time-constant setting was 0.1. The averaged evoked potentials were computed with the Mnemotron (CAT 1000) and written out on a Moseley (7590 CMR) XY plotter.

The subject was seated in an acoustically shielded enclosure, so that he or she was looking directly into a viewing hood which was flush against the one-way mirror of the enclosure. On the other side of the glass window a Grass PS-2 photo stimulator was mounted and set at No. 8 intensity. The stimuli were presented in front of the photo stimulator located 50 cm from the subject's eyes, and subtended the central 25° of the visual field.

The two visual stimuli were black arrows mounted on a transparent slide, one arrow pointing upward, the other downward. Each arrow was 9 cm long and 2 cm wide and was placed in the center of a round slide 13 cm in diameter. The slides were placed in a random access projector which used the photo stimulator as its light source.

Since a discriminative procedure was used, one arrow served as the positive conditioned stimulus (CS+) and the other as the negative conditioned stimulus (CS−); the stimuli were presented individually and were counterbalanced across subjects. During the initial base line (B_1), both stimuli were presented randomly for 50 presentations per stimulus, with a 2- to 4-second interval between stimuli. Habituation of the evoked potentials to both stimuli was established by repeating the same procedure for base line 2 (B_2) and base line 3 (B_3).

Table 1. Means for amplitude C ($N = 16$) in microvolts for CS+ and CS− during various phases of conditioning.

Item	B_3	A_1	E_1	E_2	A_2
	Positive conditioned stimulus				
Mean	14.20	16.89	14.33	13.12	14.46
S.D.	5.86	7.25	5.91	6.16	6.03
	Negative conditioned stimulus				
Mean	13.75	13.85	12.29	12.69	12.23
S.D.	5.22	7.38	5.67	6.50	5.93

The unconditioned stimulus (UCS) was a train of clicks delivered through a loudspeaker. The onset of the 20-hz train of clicks began with the onset of the 10-μsec CS+ and lasted for a period of 500 msec. The click was a stimulus of 0.1-msec duration and an intensity of 90 db with reference to 0.0002 dyne/cm^2.

During the conditioning procedure (Acquisition, A_1) the CS+ was reinforced on 50 percent of the trials, and the CS− was never reinforced. Altogether, 150 stimuli were presented randomly during A_1. The CS− was presented 50 times, the reinforced CS+ 50 times, and the nonreinforced CS+ 50 times. Subsequent to A_1, an extinction procedure was used during which time both CS+ and CS− were again presented at random without UCS for 50 presentations per stimulus. This procedure was repeated twice (E_1, E_2). To study the effectiveness of the conditioning paradigm we used a reacquisition block (A_2).

The characteristics of the visual evoked potential were measured in accordance with described methods (*3, 4*). Statistical comparisons of the data obtained were always done between the CS− and the nonreinforced CS+.

Figure 1 shows the visual evoked potential of a typical subject through the five blocks of trials. Major changes in the later components of the wave form take place during conditioning and extinction. These changes are most marked in amplitude C (negative peak at 155 to 160 msec).

Prior to conditioning, amplitude C of the evoked responses to the CS+ and CS− is not significantly different (Fig. 2, Table 1). During A_1 the response to the CS+ is significantly enhanced, while the response to the CS− remains unchanged. The change in response to the CS+ during blank trials is similar to the enhanced response during the reinforced trials when both the CS+ flashes and the clicks are presented. During the extinction blocks, the difference between the CS+ and the CS− becomes progressively smaller and is enhanced again during the second acquisition period.

An analysis of variance and a trend test (*5*) for amplitude C show that the conditioning (CS+) and control (CS−) curves are significantly different at $P = 0.01$ ($F = 10.34$; 1, 15 d.f.). The cubic component of the interaction between curves and blocks of trials is also significant as predicted ($F = 4.27$, $P < .05$; 1, 15 d.f.). The significant cubic component reflects the double inflection in the conditioning curve at A_1 and E_2. The only noticeable change in the control curve is a drop from A_1 to E_1. The generally lower amplitudes of the control curve during the later blocks, as well as the smaller increase in the conditioning curve during reconditioning, possibly reflect some habituation of the cortical response.

The CS− trials also provide a control for possible sensitization effects caused by the UCS during conditioning. Since the response to the CS− remains unchanged from base line to A_1, the significant enhancement in the response to the CS+ suggests that the change in wave form is not due to pseudoconditioning. The change in wave form appears to be a differentiated response conditioned only to the specific stimulus

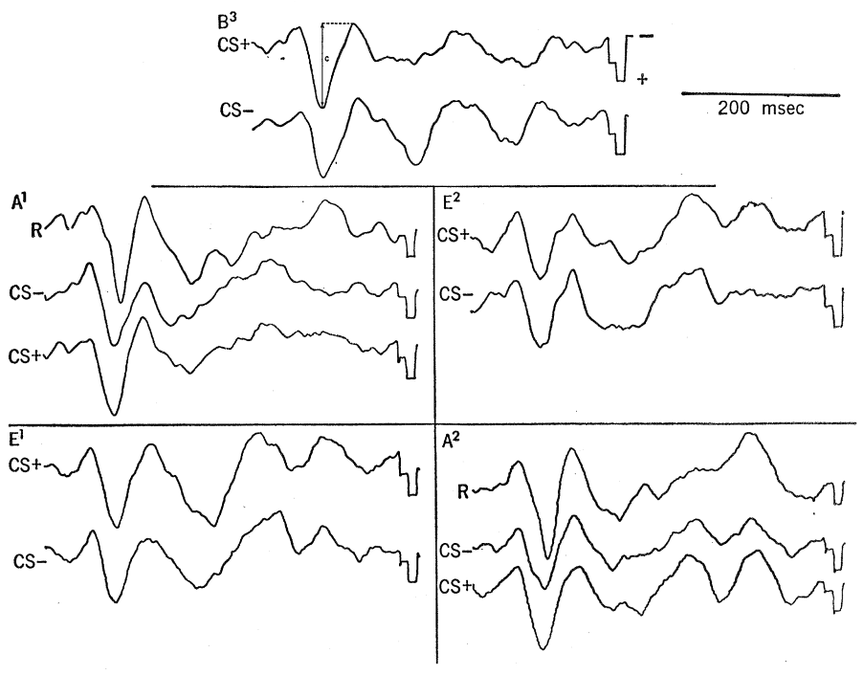

Fig. 1. Visual evoked potentials to positive conditioned stimulus (*CS+*) and negative conditioned stimulus (*CS−*) for one subject during base line (B_3), acquisition (A_1), extinction (E_1 and E_2), and reacquisition (A_2). Reinforced trials (*R*) were given only during A_1 and A_2. Negative deflections are up; time base is 500 msec. The calibration pulse at the end of each wave form is equal to 5 μv.

(CS+) previously paired with the UCS.

The comparable points for the conditioning and control curves within each block were subsequently evaluated by *t*-tests. The two curves were not significantly different at base line or at E_2. At A_1 the curves differed significantly at $P < .001$ ($t = 4.15$; 60 d.f.), and at E_1 and A_2 they differed at $P < .01$ ($t = 2.78$ and 3.04 respectively).

Analyses of variance for amplitudes A (negative peak at 65 to 70 msec) and B (positive trough at 105 to 110 msec) showed no differences related to either blocks or trials. Amplitude D (positive trough at 210 to 220 msec) was significantly different at $P = .05$ ($F = 5.20$; 1, 15 d.f.) for the combined CS+ and CS− curves, but the cubic component of the curves was not significantly different. The latencies were not significantly different between curves or across training blocks.

Four additional subjects were tested according to the same procedure so that eye movements and changes in myogenic potential during acquisition and extinction could be recorded. The failure to find changes in these measures paralleling the evoked potential suggests that the observed changes in the cortical response are not mediated by peripheral response mechanisms but reflect changes within the central nervous system accompanying conditioning and extinction.

The late components of the VEP have been related to the conscious perception of the external stimulus (*6*), the cognitive meaning of the stimulus (*7*), the conditioned affective meaning of figures (*4, 8*), and the affective meaning of words (*9*). Thus, the later activity of the wave form appears to reflect the psychological significance or meaning of the stimulus to the organism. John (*10*), in a series of studies on cats implanted with electrodes, showed that changes in the late components of the evoked potential are also particularly important during conditioning. His findings suggest that the wave form of the cortical response to the CS has two major determinants, one reflecting exogenous activity evoked by the physical stimulus and a second reflecting endogenous activity which is released by the stimulus. The latter is dependent upon the past association of the stimulus and may represent the storage of information in the form of a representational system formed during conditioning. When compared to the cortical response to the CS before conditioning, the occurrence of additional released activity to the same stimulus after conditioning is most easily seen in the modifications of the late component of the wave form (*10, 11*).

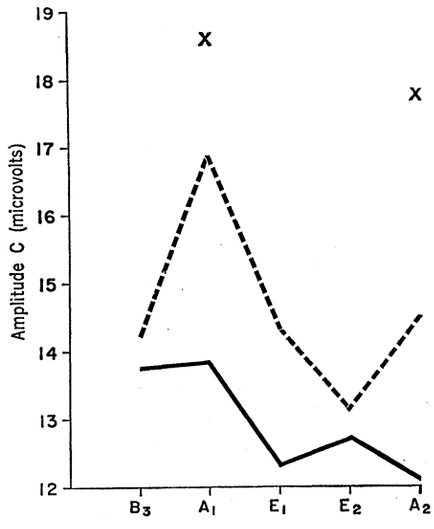

Fig. 2. Changes in amplitude C of the visual evoked potentials during conditioning and extinction. Mean values are based on 16 subjects. Cross, CS+, reinforced trials; broken line, CS—, blank trials; solid line, CS—, never reinforced.

Our study demonstrates a similar effect in human conditioning. Before conditioning, the CS+ and CS− elicit similar wave forms. After the flash and clicks have been paired, the CS+ when presented alone shows an enhancement of the late component similar to the response to the combined CS+ and UCS. Since this increase occurs only after conditioning and is reduced during extinction, it may be argued that two separate processes are determining the wave form: (i) the neuronal activity normally evoked by the flash; and (ii) the neuronal activity that represents the storage of information produced by the previous pairing of the CS+ and the UCS, now released by the former.

Perhaps the specific mechanism involved in the conditioning observed in our study is suggested by Yoshii and Ogura (*12*) who report that approximately a third of the neurons in the reticular formation of the cat are polysensory in function and that repeated pairing of stimuli from different modalities produces a change in responsiveness in the majority of the cells to either stimulus when presented alone. Similarly, Morell *et al.* (*13*) recorded single unit responses in the visual cortex to flash and shock presented alone and in combination. After pairing, the pattern of firing to the flash alone appeared to be a simple summation of the responses elicited by the flash and shock alone prior to pairing. In both studies the response to the CS after pairing appears primarily additive, with the CS now eliciting both its former response (exogenous or evoked) plus the response elicited by the second stimulus during pairing (endogenous or released).

Although the surface cortical potential is recorded over a relatively large anatomical area, there is some reason to believe that the wave form reflects the overall activity of its constituent units (*14*). As a result, the increased responsiveness of single cells after stimulus pairing would be manifested in the heightened amplitude of the surface response. Analogous to the findings of Morell *et al.* (*13*), the evoked potential to the flash alone in our study is significantly larger after the flash has been paired with the clicks (A_1) than before (B_3). This result appears generally consistent with a hypothesis of increased rate of neuronal firing to the CS+ after conditioning. Thus, the late components of the VEP reflect the release of patterns of neuronal activity which relate to the perception of the stimulus and to the previous relevant experience of the organism.

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References and Notes

1. F. Morrell, *Physiol. Rev.* **41**, 220 (1961); E. R. John, *Annu. Rev. Physiol.* **23**, 451 (1961).
2. ———, D. S. Ruchkin, J. Villegas, *Ann. N.Y. Acad. Sci.* **112**, 362 (1964).
3. The visual evoked response is multiphasic and yields four successive peak-to-trough amplitudes, measured in terms of the perpendicular distance between the successive peaks (designated A to D) and four successive latencies measured from time zero (designated as 1 to 4). K. A. Kooi and B. K. Bagchi, *Electroencephalogr. Clin. Neurophysiol.* **17**, 638 (1964).
4. H. Begleiter, M. M. Gross, B. Kissin, *Psychophysiology* **3**, 336 (1967).
5. D. A. Grant, *Psychol. Bull.* **53**, 141 (1956).
6. D. B. Lindsley and W. H. Emmons, *Electroencephalogr. Clin. Neurophysiol.* **10**, 359 (1958).
7. E. R. John, R. N. Herrington, S. Sutton, *Science* **155**, 1439 (1967).
8. H. Begleiter, M. M. Gross, B. Porjesz, B. Kissin, *Psychophysiology* **5**, 517 (1969).
9. H. Begleiter and A. Platz, *ibid.*, in press.
10. E. R. John, *Mechanisms of Memory* (Academic Press, New York, 1967).
11. ———, M. Shimokochi, F. Bartlett, *Science* **164**, 1534 (1969).
12. N. Yoshii and H. Ogura, *Med. J. Osaka Univ.* **11**, 1 (1960).
13. F. Morrell, J. Engel, W. Bouris, observations presented at Neurosciences Research Program's Intensive Study Program Conference, Boulder, Colorado (August 1966).
14. S. S. Fox and J. H. O'Brien, *Science* **147**, 888 (1965).
15. We thank Miss C. C. Yerre for assistance and Dr. E. R. John for comments. Supported by NIMH grants MH-13145 and MH-16477.

24 April 1969; revised 8 July 1969