3. The sample may not be independent for biological reasons (for example, habituation) or as an experimental artifact (for example, regular consecutive sampling). See: J. S. Bendat, *IEEE Inst. Electron. Elect. Eng. Trans. Bio-Med. Electron.* **11**, 72 (1964); D. F. Ruchkin, *ibid.* **12**, 87 (1965).
4. Particularly pertinent are the results obtained in averaging the random sinusoidal process. See: W. R. Bennett, *Quart. Appl. Math.* **5**. (1948).
5. I. Rapin, A. Schimmel, L. M. Tourk, N. A. Krasnegor, C. Pollak, *Electroencephalog. Clin. Neurophysiol.* **21**, 335 (1966).
6. I thank Drs. Weitzman, Rapin, and Vaughan for discussion. Research supported by NIH grants NB-03356 and 5T1 MH-6418.

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Electrocortical Correlates of Stimulus Response and Reinforcement

Abstract. *Three patterns of electrical response were identified in the occipital cortex of rhesus monkeys making a differential discrimination: an input pattern that identifies which stimulus has been displayed; a reinforcement pattern that indicates whether the outcome of the differential response was rewarded or in error; and an intention pattern that occurs prior to the response and predicts which response the monkey is about to make. Neither the reinforcement nor the intention pattern is present while the monkeys perform at chance; at this time, only the differences due to input can be distinguished. These results suggest that more than simple input transmission is occurring in the primary visual mechanism. The influence of the experience of the organism is apparently encoded in the averaged electrical potentials recorded from the striate cortex.*

To combine the techniques of electrophysiology with those of behavioral analysis of organisms subjected to cerebral ablations (*1*), we recorded potential changes that occur in the striate cortex of rhesus monkeys at various instants in a trial during which a visual discrimination is made. We placed a monkey in a restraining chair in front of, and within easy reach of, a 20- by 20-cm translucent panel split vertically down the center. Each half of the panel could be independently depressed; pressure closed a microswitch which sent a pulse to be recorded on magnetic tape (1.3 cm). The pulse also activated a circuit designed to deliver a food pellet into a cup placed under the panel whenever a correct response was made.

In front of the monkey, there was, attached to the chair, a small lever which, when pulled, activated a stimulus display. Thus there was reasonable assurance that the monkey would attend (make an observing response) to the display. Initially, during "shaping," the display covered the entire translucent panel until the animal pressed it; but the duration of exposure was gradually shortened until it lasted for only 0.01 msec. This short duration—in essence a flash—ensured that a transient response occurred in the visual pathways. A transient response was chosen because the techniques of analysis of neuroelectric phenomena are considerably more advanced at present for transients than for changes in steady state. Two stimulus patterns (vertical stripes and a circle) equated for area were generated in a relatively random sequence by slides in a modified Kodak Carousel projector facing the back of the panel. The order of the display of the two patterns was determined in advance, so that the report of the response would be collated by the reinforcing circuit with the pattern displayed. This collation determined whether the response made was correct or incorrect. The occurrence of reinforcement was also recorded on the magnetic tape.

Once "shaped," the monkeys were trained to press the right half of the panel whenever the circle was displayed and to press the left half of the panel whenever the vertical stripes were displayed. One monkey failed to learn the task (a difficult one because of the short duration of the display), and the other two monkeys reached a criterion of 85 percent correct in 200 consecutive trials after 1800 and 2800 trials. Two hundred trials were given daily 6 days a week.

The sequence of events that constitutes a trial is therefore as follows: (i) The monkey pulls a lever which initiates a pulse recorded on magnetic tape and (ii) turns on a stimulus display which lasts 0.01 msec. One of two patterns (vertical stripes or circle) is displayed; a pulse to indicate which display is flashed is reported to a reinforcing circuit and recorded on magnetic tape. (iii) After a variable period, the monkey depresses either the right or left half of the display panel. This pressure also initiates a pulse which is recorded on magnetic tape and reported to the reinforcing circuit. This circuit then delivers a food pellet whenever the vertical-stripe display is followed by a press of the left panel and whenever the circle display is followed by a press of the right side of the panel. Reinforcement is also recorded on the tape.

Recording of electrical activity from the brain was continuous over sample sessions of 200 trials and, of course, coincided with the recordings of the behavioral events. The sessions chosen were (i) at the beginning of training, after the monkey had been conditioned to press but while he was performing at chance, and (ii) after criterion performance was established. Recordings were made from 12 placements in the striate cortex. All were bipolar (depth of cortex to surface) from an insulated nichrome wire (300 μ in diameter). The electrical brain signals were adequately amplified before they were recorded on magnetic tape.

The tape-recorded results were processed on a small general-purpose digital computer (PDP-8). Brain activity was digitized by an A-to-D converter, and the results of conversion were stored on digital magnetic tape. We devised programs to average the digitized electrical activity forward in time from the onset of the stimulus display (the pulling of the lever) and from the response (the depression of either half of the display panel). Averages were also obtained by running the tape backward from the two time markers; these records indicated what was going on in the monkey's brain just prior to his turning on the display and making the differential response. Programs were also developed to equate records obtained from unequal numbers of trials, so that correct and incorrect performances could be compared at criterion. Finally, routines to smooth the curves were adapted for photographing the results.

For each of the samples recorded, compilations were made of the brain activity (i) after stimulus display, (ii) preceding differential response, and (iii) after differential response. These compilations were then broken down into three categories: circle as opposed to vertical stripes, right as opposed to left panel, and correct as opposed to incorrect outcomes (Fig. 1). Reliable differences (*2*) can be ascertained in the configuration of the brain record evoked by a stimulus display of 0.01 msec (*3*). In this instance, the circle generated a downward deflection; the two peaks of this deflection are more nearly equal than those generated by the vertical stripes. In the response to

stripes, the amplitude of the second peak always exceeded the first. This difference did not change appreciably between the sample taken before learning occurred and the one taken at criterion performance.

The records obtained before and after differential response are essentially flat before learning of the problem takes place. No characteristic deflections occur constantly. At criterion, however, a marked difference routinely characterizes correct and incorrect outcomes: nonreinforcement is accompanied by a marked burst of activity in the record (approximately 40 cycles per second). At this time, a difference can also be seen in the brain recording made just prior to the differential response. From this difference, one can predict whether the monkey is going to press the right or the left side of the panel (regardless of whether this will prove to be correct or incorrect). Because this difference in the record prior to response was never observed when the monkey was performing at chance, differences in movement per se probably cannot account for differences in the neuroelectric response.

Three types of brain activity were discerned: an input pattern related to the stimulus display and present before as well as after learning, a reinforcement pattern indicating correct or incorrect outcome of the trial, and an intention pattern which occurs prior to the differential response once it has become meaningful.

All the brain patterns were not recorded from all 12 electrode placements in the striate cortex. From some, input patterns were obtained best; intention patterns were derived from others, and reinforcement patterns were best obtained from still others. Yet all these brain patterns did occur in the striate cortex—the end station of the anatomically homotopic tracts originating in the retina. These findings suggest that much more than simple input transmission occurs in the primary visual mechanism. At the striate cortex, the neuroelectric signals encode the influence of experience not only with respect to input differences, but also with respect to the organism's intentions to respond and the outcome of behavior.

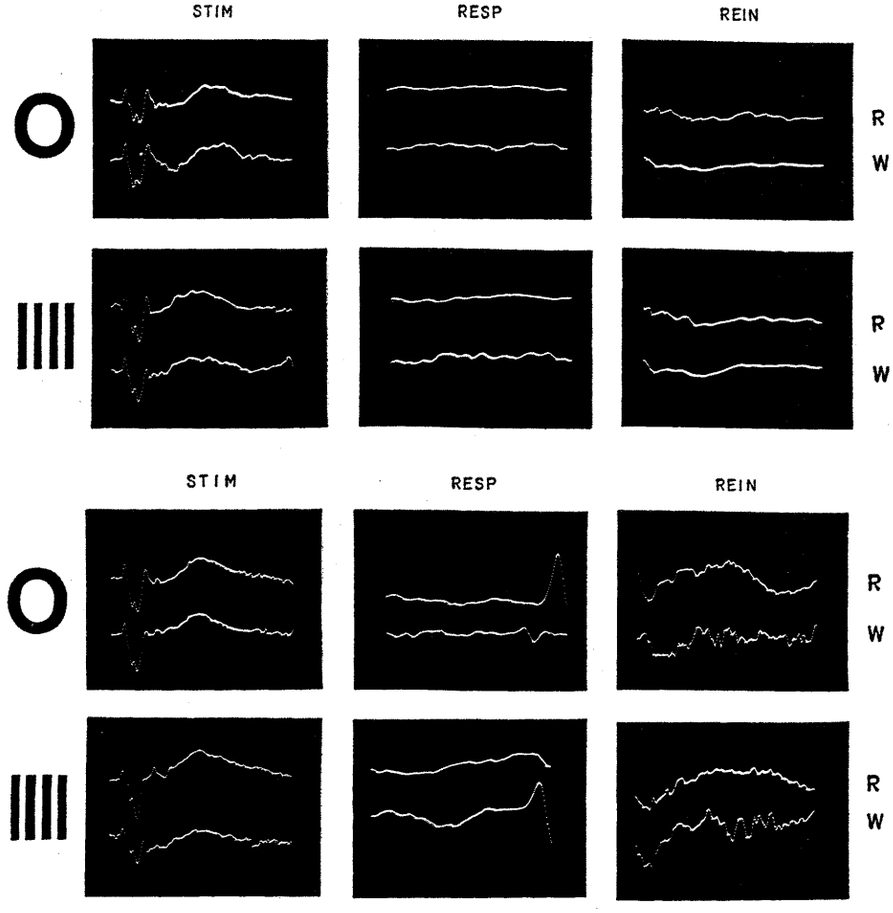

Fig. 1. Averaged recordings of electrical activity obtained from the occipital cortex of monkeys performing a differential discrimination: circle as opposed to vertical stripes. A standard 500 msec of activity is represented in each trace; the amplitude represented is variable, however, and depends on how many signals were averaged in order to make the record; for example, many more signals were obtained when the monkey made a correct response than when he made an error during criterion performance. The records under *STIM* are the waveforms evoked by a display lasting 1 msec; the records under *RESP* were generated just prior to the response; the records under REIN were generated after the response and during the period when reinforcing events occurred. The upper six panels were made from records obtained while the monkey was performing at chance; the lower six panels were made from records obtained after the monkey attained an 85 percent criterion (200 consecutive trials). The records in line with *R* were made when the monkey performed correctly; those in line with *W* were made when the monkey was wrong. The waves generated just prior to response (the intention waves) are similar whenever the monkey is about to press the right half of the panel, regardless of whether this is for the circle or vertical stripes, and regardless of whether this response proves to be correct or wrong.

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References and Notes

1. Neurobehavioral studies of the functions of the posterior "association" and frontolimbic formations of the forebrain have produced a wealth of evidence [K. H. Pribram, *Can. Psychol.* **7**, 326 (1966); ——, in *Advances in the Study of Behavior* (Academic Press, New York, in press); ——, A. Ahumada, J. Hartog, L. Roos, in *The Frontal Granular Cortex and Behavior* (McGraw-Hill, New York, 1964), p. 28]. Despite this abundance, or perhaps in part as a result of it, a series of dilemmas in regard to interpretation has arisen. Part of the problem has been the limited repertoire of techniques, consisting primarily of ablation of neural tissue and experimental analysis of behavior before and after surgery. Interpretation (as opposed to description) of results is always ambiguous when there is only one mode of presentation of data. Recently, additional methods of neurobehavioral study have become available, largely as the result of application of computer technology to analysis of electrophysiological recordings of the brain. The pioneering observations of D. B. Lindsley [in *Electrical Stimulation of the Brain* (Univ. of Texas Press, Austin, 1961), p. 331] and of E. R. John and K. F. Killam [*J. Nerv. Ment. Dis.* **131**, 183 (1960)] have paved the way for experiments in which behavior of the brain, as well as of the organism, during problem solving can be studied. Perhaps, by monitoring some internal as well as external responses of unoperated and lesioned primate solvers of problems, the choice among interpretations may be narrowed.
2. R. Spehlmann, *Electroencephalogr. Clin. Neurophysiol.* **19**, 560 (1965); E. R. John, R. N. Herrington, S. Sutton, *Science* **155**, 1439 (1967); S. Sutton, P. Tueting, J. Zubin, E. R. John, *ibid.*, p. 1436.
3. To determine quantitatively whether reliable differences existed between wave forms in the records, we used the following system of analysis of data. The record was averaged and displayed on the oscilloscope of a PDP-8 com-

puter system. A vertical line was then positioned by a computer program at each inflection point of the displayed wave pattern; these lines then served as break points for analysis of data. The amplitude values of the raw data of the segment of the wave between two break points was then averaged and stored. By this device, the relative amplitude of comparable segments of different waves could be statistically compared by using Student's t test. This method allows some estimate of the reliability (in the face of variability) of the difference between segments of the waveform, though, of course, it does not determine whether a total waveform is significantly different from another. When reported, a waveform differs from its control at least by $P < .05$.

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Pemoline and Magnesium Hydroxide: Lack of Effect on RNA and Protein Synthesis

Abstract. *Brain RNA polymerase isolated from rats treated with pemoline and magnesium hydroxide (Cylert) was not more active than enzyme from control animals. The drug did not increase enzymic activity in vitro. Pemoline did not significantly affect either RNA or protein synthesis in suspensions of Ehrlich ascites carcinoma cells.*

In a recent publication, Glasky and Simon (*1*) described the stimulating effect of magnesium pemoline (*2*) upon brain RNA polymerase. Data were presented which purported to show that the enzyme isolated from rats treated with the drug was more active than that from untreated animals, and that the chemical enhanced RNA synthesis when added to the in vitro assay system.

Repeated attempts by us to reproduce the original experimental observations have been unsuccessful. Typical experiments are described below (*3*).

The nuclear aggregate brain RNA polymerase as described by Barondes (*4*) was prepared from four groups of Sprague-Dawley white rats (100 to 150 g) which had received intraperitoneal injections of 20 mg of pemoline and magnesium hydroxide per kilogram of body weight in 0.25 percent Methocel, and which were then killed at varying times after the treatment. The brains from each of the groups were pooled for enzyme assay. The data obtained (Fig. 1) showed that (i) pemoline and magnesium hydroxide in vivo did not significantly affect brain RNA polymerase activity in any of the groups, (ii) there was significant enzyme activity at zero time, that is, in the group which was killed at the time of injection, and (iii) at no time was the enzyme more efficient in polymerizing a single triphosphate (1-NT reaction) than a mixture of all four triphosphates (4-NT reaction). Two other experiments with CTP-^{3}H confirmed these observations.

These findings are in contrast to the results obtained by Glasky and Simon in a similar experiment (*1*, Fig. 1). In addition to a positive drug effect, they reported almost no enzymatic activity without drug administration, and a 1-NT activity larger than 4-NT in their 30-minute groups. The latter two points also do not agree with the original observations on the rat-brain aggregate enzyme described by Barondes (*4*).

While this paper was in preparation, Morris *et al.* (*5*) reported that intraperitoneal administration of pemoline and magnesium hydroxide did not significantly affect either the concentration of rat-brain RNA or the uptake of tritiated uridine into brain RNA. We draw the same conclusions from similar experiments carried out in this laboratory.

Results obtained in 16 experiments in which drug was added directly to the in vitro assay system also failed to substantiate the original report. Table 1 shows that neither pemoline nor pemoline and magnesium hydroxide exerted a significant effect upon the polymerase activity.

Although it was not expected that pemoline and magnesium hydroxide would yield different results from that of pemoline alone in an in vitro system optimized with respect to divalent cation requirement, the former was included for comparison with the previous report (*1*). Most of our experiments were carried out with aqueous solutions of the drugs. Since the solubility of pemoline in water is approximately 0.2 mg/ml ($> 10^{-3}$ mole/liter) we felt that the use of dimethylsulfoxide, as suggested in the original report, was unnecessary (*1*). Nevertheless, some of our experiments did include pemoline and magnesium hydroxide "solubilized" in dimethylsulfoxide, but again no drug effect was observed.

Pemoline and magnesium hydroxide (1 and 5×10^{-5} mole/liter) did not increase the incorporation of CTP-α-^{32}P into RNA of isolated rat-brain nuclei in vitro.

While brain has a high rate of nucleic acid and protein synthesis, we

Table 1. Lack of effect of pemoline or pemoline plus magnesium hydroxide on brain RNA polymerase. The assay system in vitro was the same as in Fig. 1, except that the following ribonucleoside triphosphates were used per 2.0 ml: ATP, GTP, UTP, 0.9 μmole each; CTP-^{3}H, 100 μc/μmole, 0.02 μmole. Enzyme activity is expressed as picomoles CMP incorporated per milligram of protein in 15 minutes; mean values (triplicate) ± standard deviation are cited.

Addition (mole/liter)		Enzyme activity
Pemoline	Pemoline + $Mg(OH)_2$	
Reaction stopped at zero time		4 ± 0.4
0	0	43 ± 6
1×10^{-5}	0	41 ± 3
5×10^{-5}	0	37 ± 5
1×10^{-4}	0	39 ± 7
0	1×10^{-5}	46 ± 8
0	5×10^{-5}	44 ± 3
0	1×10^{-4}	38 ± 2

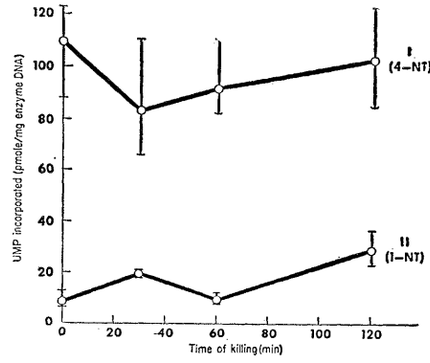

Fig. 1. RNA polymerase activity from rat brain of four groups, each containing ten rats. All the animals received 20 mg of pemoline and magnesium hydroxide per kilogram of body weight and were killed at the times shown after drug administration. The enzyme activity assay system for curve I (4-NT) contained per 2.0 ml: Tris buffer, pH 8.0, 200 μmole; $MnCl_2$, 4 μmole; KCl, 1000 μmole; ATP, CTP, GTP, 0.5 μmole each; UTP-α-^{32}P, 150 μc/μmole, 0.02 μmole. For curve II (1-NT), ATP, CTP, and GTP were omitted. The DNA content per 2.0 ml was 0.175, 0.176, 0.192, and 0.176 mg for the 0-, 30-, 60-, and 120-minute enzyme preparations, respectively. The mixtures were incubated for 15 minutes at 37°C; at this time, 0.1 ml of a 4-percent $Na_4P_2O_7$ and 0.5-percent RNA solution was added, and the reaction was terminated by the addition of 5 ml of 10 percent trichloroacetic acid. The precipitates were washed three times with cold 5-percent trichloroacetic acid on Millipore filters, and the radioactivity was determined in a liquid-scintillation counter. The activity is expressed as the number of picomoles of UMP incorporated per milligram of DNA in 15 minutes (1 picomole is equivalent to 200 count/min; the zero time control, 327 count/min). The vertical bars represent the range of activities, determined in triplicate. If $MnCl_2$ was eliminated from the incubation medium, the activity was reduced to 5, slightly above background.