Reports

Endogenous Conditioning to Abnormal Cerebral Electrical Transients in Man

Abstract. Ten subjects with epilepsy were trained to press a lever to avoid a mildly painful shock each time paroxysmal epileptiform activity occurred in their own electroencephalogram. All subjects learned to avoid the shock as long as they could hear the electroencephalogram and respond to the characteristic change in sound accompanying each spike-wave burst. When the audio component of the electroencephalogram was eliminated, only one subject was able to respond to the purely subjective effect of his own paroxysmal activity.

One of the most puzzling aspects of electroencephalography, particularly of epileptic subjects, is the common absence of the slightest subjective symptom or objective sign during high-voltage paroxysmal discharges recorded at the scalp. Focal or diffuse bursts of waves of 10 to 15 times the background voltage, associated with abrupt change in frequency and wave form, emerge and disappear in unpredictable sequence, enduring for fractions of a second up to several minutes without the least detectable objective or subjective sign in their human source. Furthermore, with the exception of the rhythmic diffuse spike and wave bursts of "centrencephalic" epilepsy, which regularly interrupt volitional behavior and speech if prolonged beyond 3 to 5 seconds, examinations of learning, behavior, or autonomic function often fail to reveal aberrations during these dramatic changes in cerebral electrical activity. Indeed, early expectations that important relationships would be demonstrable between the electroencephalogram (EEG) and concurrent psychic activity have on the whole been unrewarded. In recent years, conditioning techniques have been of increasing importance to electroencephalographers working to establish such correlations and have perhaps proved the most fruitful approach to this problem (*1*).

In the present series of experiments (*2*) we have reversed the conventional Pavlovian conditioning format in which paired external stimuli evoke specific motor, autonomic, or electroencephalographic responses. Instead, we designate as conditioning stimulus a specific EEG transient, spontaneously produced by the subject, namely, the random spike or rhythmic spike-wave pattern, and regularly follow these endogenous spontaneous paroxysms of the subject by a mildly painful electric shock to the wrist, the unconditioned stimulus. The unconditioned response is the subject's withdrawal or avoidance maneuver evoked by the shock, measured by electromyogram from ipsilateral arm flexors or by depression of a key which turns off the shock.

Several patients with epilepsy participated in the early stage of this study. These individuals were chosen because of suitable EEG tracings, characterized by relatively normal patterns of background activity which were interrupted from 30 to 200 times each hour by high-voltage focal or diffuse paroxysmal epileptiform complexes. Hundreds of these spontaneous spike or spike-wave paroxysms were followed after (2 sec) latency by the wrist shock, without any evidence of development of a conditioned withdrawal or avoidance response. We then borrowed a leaf from the conditioning program of certain investigators of smooth muscle conditioning in the U.S.S.R., who have shown that the specific sensory systems must often be enlisted to accomplish "interoceptive" conditioning (*3*). Thus we added an audio signal to our experimental design to aid the subject in recognition of his change in EEG pattern. The output from one channel of the Grass electroencephalograph was converted to an audio signal, the sound of which was characteristically altered by each paroxysmal discharge, and which both subject and experimenter heard throughout the pairing of EEG paroxysms and wrist shock. The same scalp derivations were simultaneously monitored with the conventional EEG write-out. The subjects were instructed to press a key to avoid the shock whenever this characteristic sound occurred, and were rewarded for each correct response both by avoiding the shock and by the experimenter's "Good!" Failure to press the key during the initial few seconds of the paroxysm was of course followed by the wrist shock.

All subjects learned to press the key to avoid the shock in response to the change in sound in their own EEG occurring with each paroxysmal discharge. After a successful avoidance response was formed to the epileptiform paroxysms of the "audio EEG," from 20 to 400 "overlearning" trials were given. These were interrupted at irregular intervals by trials in which the audio amplifier gain was either gradually diminished or entirely turned off, requiring the subject to respond only to some subjective effect of the paroxysmal changes in his EEG.

Ten subjects have been studied intensively with this procedure. Five individuals have clinical symptoms of petit mal or generalized attacks and intermittent 3-cy/sec rhythmic spike-wave paroxysms in their EEG's. Five subjects show focal spike or spike-wave discharge in their EEG's, all right temporal in location, and clinically present with psychomotor epilepsy. As noted above, all subjects learned to press the avoidance key in response to the characteristic sound of their spike paroxysms in the audio EEG. Early in the training period four of the five subjects with bilateral 3-cy/sec spike-wave discharges frequently failed to press the avoidance lever during the paroxysm, instead depressing the key at the termination of the abnormal paroxysm, after receiving several shocks. With repeated pairing of spike-wave with shock however, all of the subjects in this group finally were able to avoid the shock by depressing the key in response to the characteristic "clickety-click" sound of their spike-wave discharge in the audio EEG. A variable latency of 5 to 8 seconds was permitted for the avoidance response,

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Type manuscripts double-spaced and submit one ribbon copy and two carbon copies.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each. Submit three copies of illustrative material.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

as long as it was made during the epileptiform paroxysm, for this entire group of patients had longer response latency than the focal group. The five subjects with focal spike-wave bursts regularly responded within a 1- to 2-second latency period, even to very high voltage polyspike discharges. Occasional clinical ictus was observed in only one individual, a member of the 3-cy/sec diffuse group, who during seizure discharges lasting 5 seconds or more opened his eyes and blinked rhythmically—that is, he had a typical petit mal seizure. Remarkably enough, this is the only subject who was conditioned to press the avoidance key in response to his paroxysmal bursts after elimination of the audio EEG (Fig. 1*A*). Once conditioned, the key press responses were regularly performed during 85 percent of his diffuse spike-wave discharges whether of 1-second duration and asymptomatic, or of 20-second duration and accompanied by clinical petit mal. Furthermore, the key press was performed automatically, the subject never recalling whether or not he made the response during the prolonged bursts, or whether he had received the shock. He did report that for the first time in his life he occasionally experienced a warning sensation prior to his petit mal attacks. Flickered light readily induced seizure bursts in this subject, and such bursts also regularly precipitated the conditioned key press response, although flicker of intensity insufficient to provoke seizure bursts failed to evoke the conditioned response. No sustained alteration in the incidence of paroxysmal bursts occurred in any of the subjects during the experimental periods. Also, with the exception of the single individual just described, no subject became aware of any nuance or least sensation accompanying his bursts of epileptiform activity despite up to more than 500 paired spike-wave and shock sequences, with and without the audio signal. None of the other nine subjects have been able to establish a conditioned avoidance response to their own seizure activity in the absence of the audio EEG. Their conditioned responses continue to be made with remarkable reliability (85 to 90 percent) as the audio gain is diminished down to the threshold of auditory perception, at which time the key press response invariably disappears (Fig. 1, *B* and *C*). It has not yet been established whether an autonomic response may be formed to the endogenous stimulus.

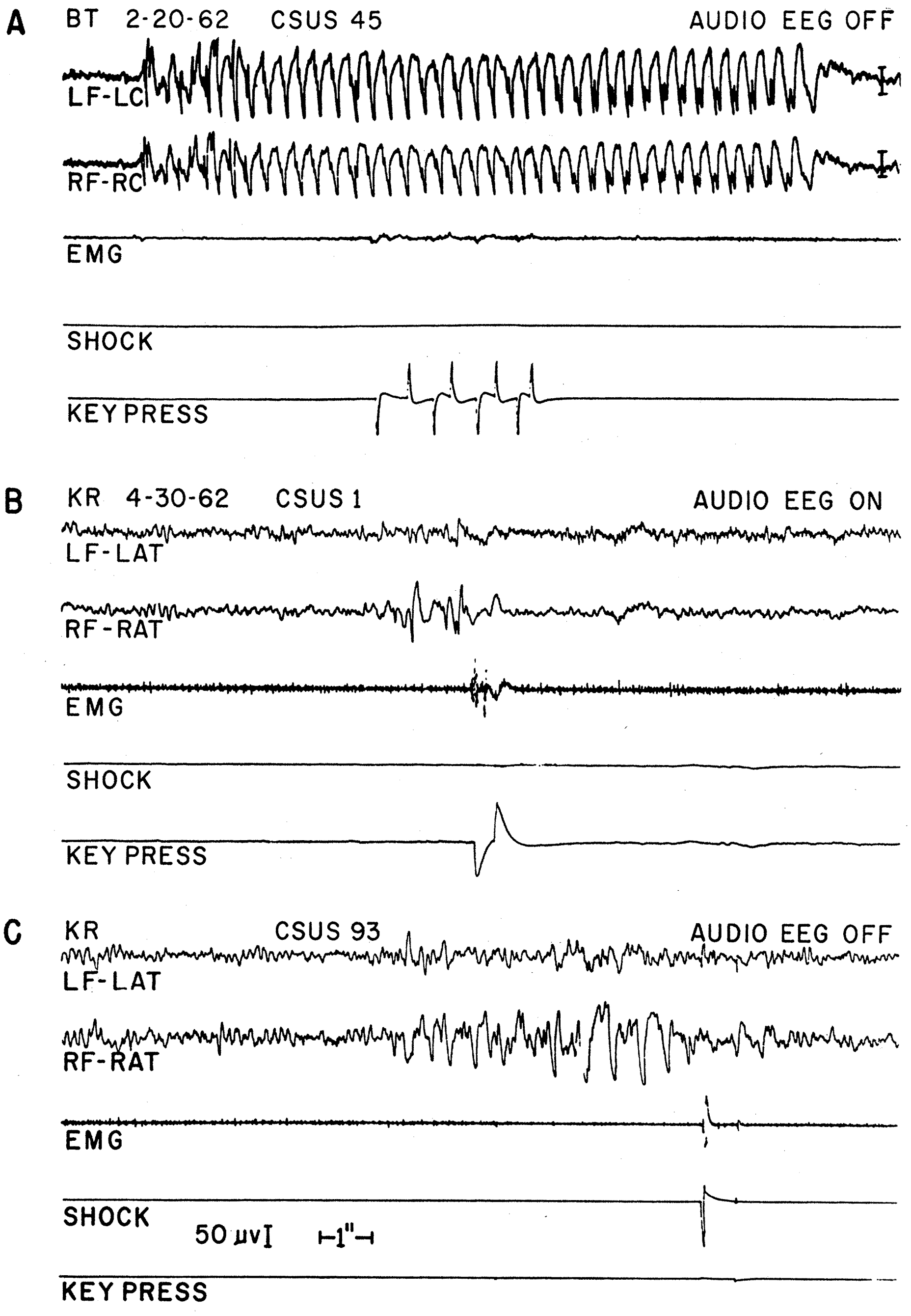

Fig. 1. Conditioned responses to endogenous epileptiform paroxysms. *CS*, conditioned stimulus (spike-wave paroxysm); *US*, unconditioned stimulus (shock); *LF*, left frontal; *LC*, left central; *RF*, right frontal; *RC*, right central; *LAT*, left anterior temporal; *RAT*, right anterior temporal; *EMG*, electromyogram. (*A*) Repeated conditioned avoidance responses during a paroxysmal diffuse spike-wave discharge—no audio signal. (*B*) Conditioned avoidance response to focal right temporal epileptiform paroxysm, audio EEG on. Such responses were regularly performed to more than 90 percent of paroxysms. (*C*) Same patient as in *B*, same experimental session, fails to respond to high voltage right temporal spike-wave paroxysm in absence of audio signal.

The evidence to date indicates that the striking alterations of cerebral electrical activity associated with a discharging epileptic focus do not reach a threshold of consciousness sufficient to permit their employment as signals. Furthermore, even the discharges of diffuse type can but rarely be so utilized.

This type of experiment offers a potential route to the exploration of a number of interesting relationships between psychic processes and cerebral electrical activity. Although epileptiform patterns have been chosen for these experiments because of their easy recognition, obviously the field of inquiry may be widened by the use of cortical or depth electrodes, the employment of other EEG patterns or specific frequency bands as conditioning stimuli, and by using the spontaneous variations in electrical activity of diverse cerebral loci as signals. Using this technique, we not only detect relatively slight changes in consciousness associated with certain electrical patterns but may also be able to influence the frequency of occurrence of certain normal and abnormal patterns. Furthermore, these methods may permit conscious realization of shifts in cerebral electrical activity which herald not only epileptic seizures but perhaps other more subtle and commonplace alterations in cerebral activity.

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References and Notes

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2. This work was supported by grant B-1140 from the U.S. Public Health Service. I gratefully acknowledge the assistance of Christa Oltman, J. Jansen, L. Mills, and Dr. Walter Stahl.
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26 June 1962

Ribonucleic Acid in "Transformation" of Lymphoid Cells

Abstract. When incubated with "reactive" ribonucleic acid extracted from lymph nodes of rabbits immunized by skin homografts, "neutral" lymph node cells from nongrafted rabbits were apparently altered to a state of transplantation immunity, as manifested by a positive skin reaction upon injection of these cells into the donor of the skin homografts.

Alterations in cell metabolism after the incorporation of foreign nucleic acids have been convincingly demonstrated. However, most previous work has been concerned with bacterial transformation (*1*) and with investigation of infectious nucleic acids extracted from plant and animal viruses (*2–4*). Recent results obtained in our laboratory suggest that adult mammalian lymph node cells may be altered with respect to transplantation immunity by incubation with homologous lymphoid RNA obtained from immune animals. While the evidence presented below is insufficient to support the conclusion that the observed alterations in cellular reactivity have resulted from a process analogous to bacterial transformation, nevertheless the possibility that a form of transformation has taken place cannot be ruled out.

White New Zealand rabbits were used as experimental animals. As an indication of transplantation immunity, a skin test was utilized similar to the "transfer reaction" successfully employed by Brent, Brown, and Medawar (*5*) in guinea pigs. In brief, a suspension of 4 to 10 million fresh immune lymphoid cells, obtained 8 days after grafting from a recipient rabbit's popliteal lymph nodes draining a skin homograft, were injected intradermally into the back of the rabbit which had donated the homograft. The appearance after 48 to 72 hours of a tuberculin-like skin reaction at the injection site was considered a positive test. On each occasion the donor rabbit received an intradermal injection of an equal number of lymph node cells from a nongrafted "neutral" rabbit into the opposite side of the back as well. Thus, the reaction produced by the "neutral" cells and that produced by the immune cells, stimulated by the skin homograft, could be compared in each case. An arbitrary scale of 0 to 5+ was used in grading the reactions. The results obtained with this test in 13 donor rabbits are summarized in Table 1. It is clear that the immune cells in every case but one produced a significant (2+ or greater) skin reaction in the donor animals, and that "neutral" cells failed to produce any reaction greater than 1+ (smallest reaction definitely perceptible). As a check on the specificity of the skin test, an equal number of immune cells (4 to 10 million) were injected intradermally into a "neutral" rabbit in each experiment. In six of 13 experiments the immune cells produced a significant (2+ or greater) skin reaction in the neutral rabbit as well as in the donor of the skin homograft. We have interpreted this result as indicating the presence of common tissue antigens in both the "neutral" and donor rabbits in some instances.

Utilizing the same skin test as evidence for transplantation immunity, we have subsequently explored the possibility of transforming "neutral" lymphoid cells to a state of transplantation immunity. The RNA used in these experiments was extracted with phenol from a homogenate of fresh lymph node tissue suspended in citrate buffered saline. The extraction procedure was carried out in the cold following the modification by Alexander *et al.* (*4*) of the method of Gierer and Schramm (*2*). The product has an ultraviolet absorption spectrum characteristic of RNA. The hyperchromatic effect on incubation with NaOH is 31 percent. The diphenylamine test for DNA is negative. In the ultracentrifuge the material forms three peaks sedimenting at approximately S-21, S-8, and S-4, respectively.

Table 1. Maximal skin reactions produced by immune or "neutral" lymph node cells 48 to 72 hours after intradermal injection into the rabbits indicated. Intensity of reactions graded on scale of 0 to 5+ (see text). The symbol ± denotes the doubtful presence of a perceptible reaction.

Rabbit No.	Donor rabbit		Neutral rabbit (right side: immune cells)
	Right side: immune cells	Left side: "neutral" cells	
8	4+	0	0
17	4+	1+	1+
16	1+	0	0
35	2+	0	0
40	4+	1+	2+
129	2+	±	1+
42	3+	0	0
48	4+	0	2+
149	5+	0	3+
164	4+	1+	3+
170	5+	0	2+
154	3+	1+	1+
151	3+	0	2+

Table 2. Maximal skin reactions produced 48 to 72 hours after intradermal injection into donor rabbit by "neutral" lymph node cells incubated with "reactive" RNA or with media alone. Intensity of reactions graded on same scale as Table 1 (see text).

Rabbit No.	Right side: cells + RNA	Left side: cells + media
63	2+	1+
65	4+	0
64	4+	0
79	2+	0
100	1+	1+
277	2+	0
116	2+	0
124	2+	0
119	2+	1+
126	3+	0
133	4+	0
105	2+	2+
137	1+	0
139	4+	1+
135	2+	1+
148	3+	±
145	2+	0
146	3+	2+
150	3+	±
95	1+	0