of protein synthesis, puromycin, prevents the induction by cortisone of such enzymes (*5*), as well as that of others concerned with gluconeogenesis (*6*). Puromycin also lowers the level of liver glycogen in normal mice (*7*).

Actinomycin, an inhibitor of RNA synthesis (*8*), also interferes with the rise of liver enzymes induced by cortisone. This suggests that the stimulation of the synthesis of certain RNA species may be one of the primary actions of the hormone (*5*). The effect of actinomycin is more specific than that of puromycin; it interferes with the increase in liver tyrosine transaminase and tryptophan pyrrolase which is brought about by cortisone, but it does not interfere with the rise in the amount of trytophan pyrrolase induced by the substrate.

We have now asked the question whether the prevention of the cortisone-induced rise in liver enzyme levels will inhibit the stimulation of glycogen deposition by the hormone. Therefore, cortisone was administered to starved rats, alone or in conjunction with the aforementioned inhibitors, and the amount of liver glycogen was estimated 6 hours later. Puromycin prevents the cortisone-induced rise in glycogen levels and actinomycin inhibits it by approximately 70 percent (Table 1). The differences observed are highly significant statistically. None of the treatments influenced the total liver or body weight significantly. To illustrate the effect of the same treatments on an individual enzyme, the tyrosine transaminase activity of some of the livers is also included in Table 1. Puromycin and actinomycin largely prevented the cortisone-induced elevation of this enzyme (*5*).

It is not known which of the enzymes controlled by cortisone regulate the rate of glycogen deposition in the liver. Some of them have a direct effect on gluconeogenesis (*9*) or on the regulation of glucose concentration (*10*). The elevation of the level of transaminases may be responsible for enhanced gluconeogenesis (*11*, *12*). The rate of increase of the activity of some of these enzymes is significantly lower than the rate of glycogen deposition, but it is possible that the small initial rise which does coincide with rapid glycogen deposition is important. It is possible that the amounts of other enzymes whose response to cortisone treatment has not yet been studied will prove to be more important in the regulation of glycogen deposition. We did not attempt to evaluate the role of individual enzymes but demonstrated that substances which generally prevent the cortisone-induced rise in liver enzyme levels also inhibit the cortisone-induced glycogen deposition. Such inhibition would not be anticipated if cortisone controlled glycogen deposition by influencing the speed of catalytic processes without alteration of enzyme amounts. Thus, these results are compatible with the suggestion (*1*) that hormonal regulation of metabolism in vivo may be brought about by changes in enzyme concentrations. Such a dependence on protein synthesis would explain the inability (commonly experienced) to demonstrate physiologically meaningful hormone action in cell-free systems in which the biosynthesis of macromolecules is largely interrupted (*15*).

Table 1. Effect of puromycin and actinomycin on the rise of liver glycogen and tyrosine transaminase levels induced by cortisone. Adult male Sprague Dawley rats starved for 24 hours were injected intraperitoneally with saline suspensions of actinomycin (0.14 mg/100 g) 7 hours before the rats were killed, puromycin (multiple injections, 6 mg/100 g each) at 1, 2, 3, 4, 5, and 6 hours before the rats were killed, and cortisone (25 mg/100 g) 6 hours before the rats were killed. The glycogen content of each liver and the tyrosine-α-ketoglutarate transaminase activity [micromoles reaction product, *p*-hydroxyphenylpyruvate (HPP), formed per gram of liver per minute] of three livers in each group were measured (*13*, *14*). Each glycogen value is a mean (± standard error) of results obtained with 14 to 16 separate rats; each of the three tyrosine transaminase values was obtained from a separate liver.

Substances administered	Glycogen (g/100 g liver)	Tyrosine transaminase (μmole HPP g^{-1} min^{-1})		
None	0.32 ± 0.06	1.1	1.3	0.9
Cortisone	2.56 ± 0.39	3.7	3.5	4.2
Cortisone + puromycin	0.43 ± 0.07	1.4	1.5	1.8
Cortisone + actinomycin	0.96 ± 0.14	1.7	1.6	1.4

OLGA GREENGARD
G. WEBER
R. L. SINGHAL

Institute for Muscle Disease, New York 21, New York, and *Pharmacology Department, Indiana University School of Medicine, Indianapolis*

References and Notes

1. W. E. Knox, V. H. Auerbach, E. C. C. Lin, *Physiol. Rev.* **36**, 164 (1956).
2. F. T. Kenney, *J. Biol. Chem.* **237**, 1610 (1962).
3. H. L. Segal, R. G. Rosso, S. Hopper, M. M. Weber, *ibid.*, prelim. commun. 3303 (1962).
4. P. Feigelson and O. Greengard, *ibid.*, 3714 (1962).
5. O. Greengard, M. A. Smith, G. Acs, *ibid.* **238**, 1548 (1963).
6. G. Weber and R. L. Singhal, *Federation Proc.* **22**, 636 (1963).
7. J. Hofert, J. Gorski, G. C. Mueller, R. K. Boutwell, *Arch. Biochem. Biophys.* **97**, 134 (1962).
8. E. Reich, R. M. Franklin, A. J. Shatkin, E. L. Tatum, *Science* **134**, 556 (1961).
9. G. Weber, G. Banerjee, S. B. Bronstein, *J. Biol. Chem.* **236**, 3106 (1961).
10. G. Weber, C. Allard, G. Lamirande, A. Cantero, *Endocrinology* **58**, 40 (1956).
11. F. Gavosto, A. Pileri, A. Brusca, *Biochim. Biophys. Acta* **24**, 250 (1957).
12. F. Rosen, N. R. Roberts, L. E. Rudnik, C. A. Nichol, *Science* **127**, 287 (1958).
13. J. H. Roe, *J. Biol. Chem.* **212**, 335 (1955).
14. Z. N. Canellakis and P. P. Cohen, *ibid.* **222**, 53 (1956).

15. Supported by grants Ca-05034 and Ca-07-37-01 from the U.S. Public Health Service and by the American Cancer Society (E-254).

27 May 1963

Cerebral Heterostimulation in a Monkey Colony

Abstract. *In an established colony a subordinate monkey repeatedly pressed a lever which stimulated the caudate nucleus of the boss monkey by radio and inhibited his aggressive behavior. In other experiments, timed stimulations of the posteroventral nucleus of the thalamus of the boss monkey, paired with a tone, increased his aggressiveness and established conditioned escape responses of the whole group. Both types of experiments may be useful in neurophysiological and pharmacological investigations.*

Electrical stimulation of some areas of the brain induces positive reinforcement, and rats, cats, and monkeys learn to stimulate themselves by pressing a lever repeatedly for hours or even days (*1*). This method is very valuable for physiological and pharmacological analysis of the central nervous system. Since animals are capable of self-stimulation, they might stimulate the brain of a cagemate if suitable means were provided.

In a colony of four monkeys (*Macaca mulatta*) the boss, Ali (5.2 kg), was an ill-tempered, powerful male who often expressed his aggressiveness by grimacing and biting his own right hand (Fig. 1). Ali had friendly relations with the female, Sarah (4.0 kg), was hostile toward the other female, Elsa (4.6 kg), who ranked No. 3 in the group, and paid less attention to the male, Lou (3.8 kg), who was lowest in social rank, as determined by the peanut test and by offensive-defensive reactions. The colony was housed for several weeks in a cage 7 by 3 by 3

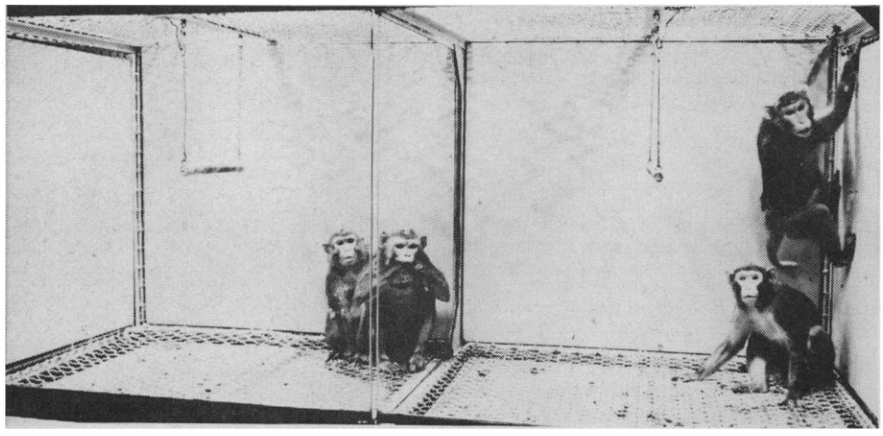

Fig. 1. Control. Left to right: Sarah, Ali (the aggressive boss, biting his own hand), Lou, and Elsa (hanging from ceiling).

ft in a soundproof air-conditioned room with constant day and night cycles of 12 hours each. The monkeys were observed through a one-way window from an adjoining room, and their behavior recorded by time-lapse photography (one picture every 2 seconds for 8 hours daily); the data were analyzed with the aid of automation (*2*). Elsa and Lou were the controls. Multilead electrodes were implanted in the head of the caudate nucleus, thalamus, central gray, reticular substance, and other cerebral areas of Ali and Sarah. Two additional subcutaneous leads made connections between any cerebral contact and a small stimulating device strapped to the back of the animal. This device is a modified radio receiver (*3*) with a sensitivity of 2 to 4 μv, and it is reliable within a range of several hundred feet. Its only function is to close a switch to activate a transistorized stimulator whose output intensity is adjusted before the experiments. In this way cerebral stimulations are reliable and independent of possible variations, such as antenna orientation and changes in radio signals. Further technical details have been published elsewhere (*4*).

Controls were established first with the monkey lightly restrained in a chair. Each cerebral point was electrically stimulated; voltage and milliamperage were monitored in an oscilloscope, and bipolar recordings of the electrical activity before and immediately after the stimulations were obtained with an eight-channel Grass electroencephalograph. None of the effects described was accompanied by electrical afterdischarges. In all cases stimulations were monopolar, unidirectional, with exponential fall, 0.5 msec of pulse duration, and 100 cy/sec. The electrode resistance was about 50,000 ohms, and intensities up to 4 ma were used.

After stimulation under restraint, Ali and Sarah were both released in the colony, one wearing a radiostimulator and the other a dummy. In both, telestimulation of the central gray and of the posteroventral nucleus of the thalamus evoked antisocial behavior with increased chasing, jumping, biting, and fighting which was quantified and analyzed (*2*). Stimulation of the caudate nucleus induced behavioral inhibition (*5*) and was selected to test possible heterostimulation. In Ali and Sarah caudate stimulation produced similar effects including slight ipsilateral head turning, loss of interest in food, and inhibition of activities, such as drinking, taking of pellets, walking, picking, self-grooming, and diminution of spontaneous aggressiveness. The latter was more impressive in Ali because of his greater size and ferocity. Caudate stimulation did not modify the behavioral categories of nestling (two monkeys embracing each other), balling (one monkey with head down on knees), lying down, or being groomed by another. During caudate stimulation it was possible to touch the animal with one's bare hands, and in this manner the monkeys were caught repeatedly. The animals were not completely inhibited and on occasion withdrew a few steps without attempting to retaliate or bite.

After controls were established a lever was attached to the cage. At any time any monkey could press this bar, which automatically started a tone which was followed 2 seconds later by a 5-second radiostimulation. Tone and stimulation ended simultaneously. In the first series of experiments, the tone was set at 600 cy/sec, and Ali carried the stimulator connected to the caudate nucleus for 4 consecutive days. The lever was located close to a feeding tray, and the first day monkeys exploring the area occasionally touched the lever, starting the tone-simulation cycle. The second day, Elsa pressed the bar 12 times, while each of the other monkeys, including Ali, touched the lever only 0 to 5 times. On the third and fourth days, Elsa pressed the lever 17 and 25 times, and the other monkeys 0 to 7 times. Figure 2 shows one example of Elsa stimulating Ali (*6*). After the fourth

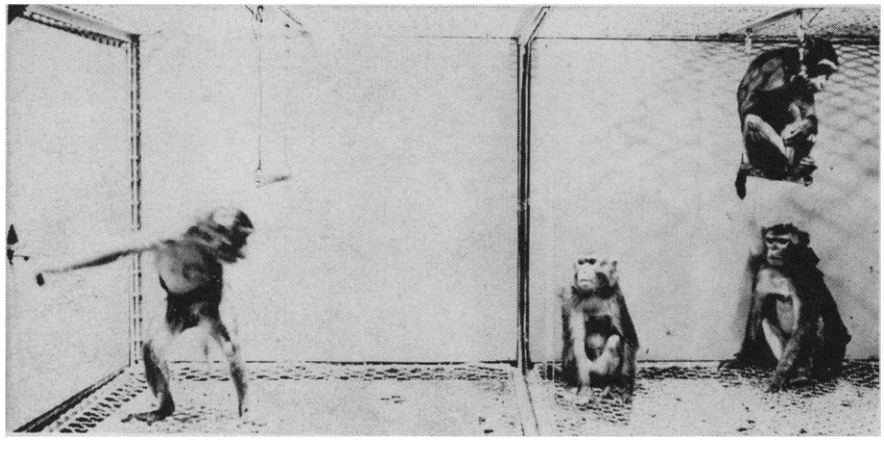

Fig. 2. Elsa, pressing the lever, stimulates by radio the caudate nucleus of Ali (on right side of cage), producing behavioral inhibition. Elsa's attitude is significant because her attention is directed not to the lever but to Ali. It is unusual for lower-ranking monkeys to look straight at the boss of the colony because this evokes retaliation.

day, radiostimulation was discontinued, but the tone remained connected for 1 week. The next two recorded lever pressings started the tone and produced some head-turning and behavioral inhibition in Ali, but extinction appeared quickly, and successive tones were not effective. The number of lever pressings by Elsa diminished to 9 the first day and to 1 to 8 per day during the following 6 days. No evidence of increased self-stimulation appeared in these studies. Immediately after these experiments the radiostimulator was attached to Sarah for three consecutive days, and the tone was changed to 500 cy/sec. For each monkey, including Sarah, 0 to 6 daily lever pressings were recorded with no significant increase in the number for any animal during the 3 days.

Curiosity probably was not the cause of the increase in lever pressing because fewer were recorded on the first than on the fourth experimental day. Correlation with radiostimulation seems more probable because lever pressings during the third and fourth days were more than twice as many as during any of the seven extinction days when only the tone could be activated. The lever was permanently attached to the cage and competed for the monkeys' attention with padlocks and food on the floor, swings, and other parts of the living quarters. This competition may explain the low number of lever pressings, and it makes more significant the increased pressing resulting when Ali was radiostimulated. Observation of the colony and analysis of films showed that several times Ali's threatening attitude was followed by Elsa's lever pressing (*6*).

The studies continued with the radiostimulator again strapped on Ali, this time connected to a contact in the posteroventral nucleus of the thalamus, and with the tone set at 900 cy/sec. Previous radiostimulations of this area had increased Ali's aggressiveness. When the lever was attached to the cage, it was triggered only seven times during three consecutive days. Then the lever was removed and was actuated by a timer once every minute for half an hour. After the fourth trial, signs of conditioning were evident. At the onset of the tone, Ali showed increased aggressiveness, and the other three monkeys grimaced and climbed to the cage ceiling. On several occasions this escape reaction to the tone started before Ali initiated any threat. Later the stimulation was discontinued, and during 30 trials the tone continued to sound once every minute and induced a reaction 25 times in Elsa, 11 times in Ali, and 7 times in both Sarah and Lou. This experiment was duplicated on three different days with results showing similar characteristics and indicates that conditioning may be established through association of the tone with aggressive behavior evoked in Ali. In another series of investigations, there was no individual or social conditioning when motor areas were stimulated in Ali and in Sarah by radio-timed control.

Performance of instrumental responses may be induced by cerebral stimulation and may be conditioned to auditory or visual cues (*7*). The fact that "spontaneous-like" behavior evoked by brain excitation may also be conditioned to an indifferent stimulus is a relatively new finding. These results have been confirmed in further experiments (*8*). Behavioral conditioning has also been established on a time basis by programmed stimulations of the superior vestibular nucleus of the thalamus without giving the monkey any cue other than fixed interval of 1 minute between stimulations (*9*).

Social conditioning may help in the analysis of cerebral stimulation because each member of the colony is an interpreter of the reactions of the stimulated animal. Heterostimulation presents obvious questions about hierarchical control, reciprocal punishment, instrumental self-defense, and other problems related to human behavior (*10*).

JOSÉ M. R. DELGADO

Department of Physiology,
Yale University School of Medicine,
New Haven, Connecticut

References and Notes

1. J. V. Brady, in *Reticular Formation of the Brain*, H. H. Jasper *et al.*, Eds. (Little, Brown, Boston, 1958), p. 689; B. Bursten and J. M. R. Delgado, *J. Comp. Physiol. Psychol.* **51**, 6 (1958); J. Olds and P. Milner, *ibid.* **47**, 419 (1954); J. Olds, *ibid.* **51**, 675 (1958); M. Sidman, J. V. Brady, J. J. Boren, D. G. Conrad, A. Schulman, *Science* **122**, 830 (1955).
2. J. M. R. Delgado, in *Pharmacological Analysis of Central Nervous Action*, W. D. M. Paton, Ed. (Pergamon, Oxford, 1962), vol. 8, p. 265; J. M. R. Delgado and R. Rodriguez Delgado, *Phil. Sci.* **29**, 253 (1962).
3. "Venus" frequency-modulated radio receiver modified with miniature coils (Lafayette Radio MS-828) and miniature relays (Elgin National Watch Co. type NMIC-50).
4. J. M. R. Delgado, in *Bio-Telemetry*, L. Slater, Ed. (Pergamon, New York, 1963), p. 231.
5. N. A. Buchwald, E. J. Wyers, C. W. Lauprecht, G. Heuser, *Electroencephalog. Clin. Neurophysiol.* **13**, 531 (1961); J. M. R. Delgado, *Federation Proc.* **16**, 29, (1957); R. G. Heath and R. Hodes, *Trans. Am. Neurol. Assoc.* **77**, 204 (1952); B. W. Robinson and M. Mishkin, *Proc. Intern. Union Physiol. Sci.* **2**, 362 (1962); E. H. Rubinstein and J. M. R. Delgado, *ibid.* **2**, 366 (1962); J. R. Stevens, C. Kim, P. D. MacLean, *A.M.A. Arch. Neurol.* **4**, 47 (1961).
6. Two months later Elsa and Ali were part of a new colony with three other monkeys. The radiostimulator was then strapped to Ali and connected to his caudate nucleus. Heterostimulation of Ali by Elsa was recorded 22 times in 1 day, and during the bar pressing Elsa's attention was usually directed toward Ali, in a way similar to that shown in Fig. 2. Reproducibility of the phenomenon of heterostimulation was thus demonstrated.
7. J. M. R. Delgado, W. W. Roberts, N. E. Miller, *Am. J. Physiol.* **179**, 587 (1954); J. M. R. Delgado, H. E. Rosvold, E. Looney, *J. Comp. Physiol. Psychol.* **49**, 373 (1956); D. E. Sheer, Ed., *Electrical Stimulation of the Brain* (Univ. of Texas Press, Austin, 1961).
8. J. M. R. Delgado, in Symposium on *Pharmacology of Conditioning, Learning and Retention*, 2nd Intern. Congr. Pharmacol., Prague, August 1963.
9. J. M. R. Delgado, in *Electroencephalog. Clin. Neurophysiol.* Suppl. 24, in press.
10. Supported by grants from the U.S. Public Health Service (M2004-06) and the Office of Naval Research (ONR 609-08).

16 May 1963

Relationship between Nuclear Volumes, Chromosome Numbers, and Relative Radiosensitivities

Abstract. *An inverse relationship between a volume estimated to be associated with interphase chromosomes and acute lethal exposure to x- or gamma radiation has been found in 16 plant species. The apparent differences in radiosensitivities found would seem spurious, since the estimated average energy absorbed in the nucleus per chromosome (3.6×10^6 ev) approaches a constant (variation less than fourfold) in spite of wide ranges of lethal exposures (0.6 to 75 kr), of nuclear volumes (43 to 1758 μ^3), and of somatic chromosome numbers (6 to 136). The regression line obtained can be used to predict the radiosensitivities of other plant species if their nuclear volumes and chromosome numbers are known.*

The radiosensitivity of a species, as indicated by degree of growth inhibition, is correlated with the average volume of interphase nuclei and with chromosome number (*1*, *2*). If these variables are controlled one at a time, an increase in nuclear size with chromosome number constant increases sensitivity and an increase in chromosome number with nuclear volume constant decreases sensitivity. Apparently the number and size of targets in the nucleus (excluding the nucleoli) are the major factors determining radiosensitiv-