6. M. A. Greer and E. B. Astwood, *Endocrinology* **43**, 105 (1948).
7. E. B. Astwood, M. A. Greer, M. G. Ettlinger, *J. Biol. Chem.* **181**, 121 (1949).
8. Hazleton Laboratories.
9. W. E. Griesbach, *Brit. J. Exptl. Pathol.* **22**, 245 (1941).
10. W. E. Griesbach, T. H. Kennedy, H. D. Purves, *ibid.* **26**, 18 (1945).
11. A. Axelrad and C. P. Leblond, *Proc. Am. Assoc. Cancer Research* **1**, 2 (1953); **1**, 2 (1954).
12. H. P. Morris and C. D. Green, *Science* **14**, 44 (1951).
13. H. D. Purves and W. E. Griesbach, *Brit. J. Exptl. Pathol.* **28**, 46 (1947).
14. W. L. Money and R. W. Rawson, Cancer **3**, 321 (1950).
15. E. B. Astwood, *J. Am. Med. Assoc.* **172**, 1319 (1960).
16. M. Hoshino, *Nature* **186**, 174 (1960).
17. K. E. Paschkis, A. Cantarow, D. Stasney, *Cancer Research* **8**, 257 (1948).

18 April 1960

Discrimination of Tones during Reinforcing Brain Stimulation

Abstract. Hungry animals were trained to press a lever for brain stimulation. Different tones were presented concurrently with the stimulation. A second lever delivered food only during critical tone periods. Animals were able to discriminate tones presented concurrently with rewarding intracranial stimulation, and they also interrupted self-stimulation behavior to respond appropriately under other reinforcements.

Since the report by Olds and Milner (*1*) that rats will perform tasks if rewarded by electrical stimulation of specific subcortical brain areas, a number of studies have demonstrated the phenomenon with guinea pigs (*2*), cats (*3, 4*), and monkeys (*5, 6*). The phenomenon may be referred to as self-stimulation when the animal is permitted to determine the rate of intracranial stimulation by its rate of responding. Previous studies have reported the distribution of rewarding sites within the central nervous system (*7*), the effect of various schedules of intermittent reinforcement on self-stimulation behavior (*8, 9*), the rate of extinction following the withholding of intracranial stimulation (*8, 10*), and the effect of various states of deprivation such as food and sex deprivation (3, *11*).

Little information is available, however, concerning the ability of an animal to respond to stimuli during intracranial stimulation. There is evidence that animals are unresponsive to environmental cues while receiving rewarding brain stimulation. In conditioned emotional response experiments, for example, animals stop working for food if a clicking sound which had previously been associated with a painful shock is presented (*12*), but Brady (*5*) has recently demonstrated that when rewarding brain stimulation is substituted for food, animals continue responding during the period of clicker presentation. Are the animals capable of sensing and interpreting stimuli presented concurrently with intracranial stimulation? The experiment reported here was designed to answer this question. The results also shed some light on the ability of animals to interrupt self-stimulation to respond under other kinds of reinforcement.

Seven male albino rats, weighing approximately 300 gm each at the beginning of the experiment, served as the subjects. Bipolar electrodes were implanted stereotaxically in the middle and posterior hypothalamus. Postoperatively, the rats were deprived of food until their weight was reduced to 80 percent of their weight on an ad libitum feeding regimen, and then were maintained at this weight. They were then trained to press a lever to receive a 0.2 ml cup of diluted condensed milk.

Tone discrimination training consisted of presenting 1.5-minute periods of tone A (1000 cy/sec) and tone B (200 cy/sec) in a random sequence. Responses were rewarded with milk only during tone A periods. When more than 80 percent of the responses occurred during the tone A periods, the subjects were trained to press a second lever for brain stimulation. The electrical stimulus consisted of a 0.5-second train of paired biphasic square waves presented at a frequency of 100 presentations per second. The pulse pairs were 0.2 msec in duration, and they were separated by an interval of the same duration. Responsiveness to the intracranial stimulation varied, and an intensity was selected which yielded stable lever-pressing rates.

After stable response rates for brain stimulation were established, 1.5-minute periods of either tone A or tone B were randomly presented with an average interval of 3 minutes between periods. During each tone period, every response on the brain-stimulation lever produced the appropriate tone. The onset of the tone followed the onset of the brain stimulus by 15 msec and ended 30 msec before the termination of the stimulus. If the animal switched to the food lever during a tone period, the tone started beeping independently of lever presses for the balance of the 1.5 minutes. The ending of the beeping tone served as a signal to the animal that food was no longer available. Thus, until the animal had switched to the food lever, tones were presented only concurrently with brain stimulation. Pressing the food lever was rewarded only during the tone A periods, while brain stimulation followed all responses on the brain-stimulation lever. Each test contained nine tone A and nine tone B periods.

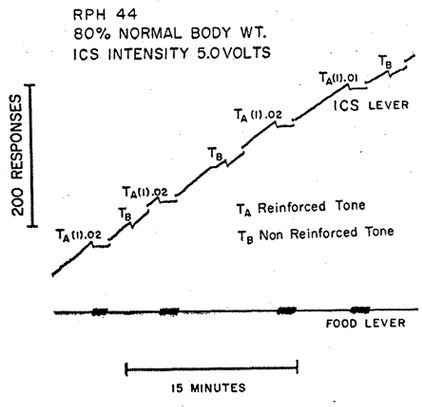

Fig. 1. Typical record illustrating responses on the intracranial stimulation (ICS) and food levers.

There is clear evidence that the rats were able to discriminate between tones presented concurrently with brain stimulation. Figure 1 shows the activity of a typical rat on the brain-stimulation and food levers after eight testing sessions. Pressing of the food lever is indicated on the horizontal, while activity on the brain-stimulation lever is cumulated in the upper curve. The pen recording brain-stimulation-lever activity was deflected downward during the tone periods; these can be identified from the labels. It can be seen that the animals switched to the food lever only during tone A periods and returned

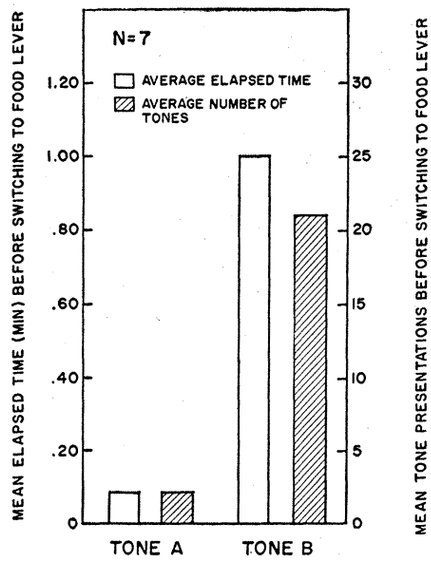

Fig. 2. Bar graph illustrating mean elapsed time and mean number of tone presentations before the switch to the food lever.

immediately to the brain-stimulation lever at the termination of this period. The figure in parenthesis gives the number of tone presentations before the animal switched to the food lever; this figure is followed by the time (in minutes) which elapsed between the first tone presentation and the response on the food lever.

Figure 2 summarizes the data for all animals during their last four tests. Bar graphs were plotted for the mean elapsed time and for the mean tone presentations before the switch to the food lever. The animals required an average of 2.1 tone A presentations, with an average elapsed time of 0.07 minutes, before switching to the food lever, while they rarely switched to the food lever during the tone B period. For purposes of calculation, 1.5 minutes was scored when the animals did not respond on the food lever during a tone period.

The results demonstrate not only that the animals were capable of distinguishing between the two tones during intracranial stimulation, but that, at least under certain conditions, they were capable of terminating self-stimulation to respond to other reinforcements. In subsequent experiments animals were found to differentiate between stimuli appearing simultaneously with brain stimulation, but whether or not they responded depended upon the reinforcing effect of the brain stimulation and the consequence of responding to the second lever.

BERNARD BEER
ELLIOT S. VALENSTEIN
Department of Psychology, Walter Reed Army Institute of Research, Washington, D.C.

References

1. J. Olds and P. Milner, *J. Comp. and Physiol. Psychol.* **47**, 419 (1954).
2. E. S. Valenstein, "Proceedings of the Eastern Psychological Association, 1958," *Am. Psychologist* **13**, 521 (1958).
3. J. V. Brady, J. Boren, D. Conrad, M. Sidman, *J. Comp. and Physiol. Psychol.* **50**, 134 (1957).
4. H. C. Nielson, R. Doty, L. Rutledge, *Am. J. Physiol.* **194**, 427 (1958).
5. J. V. Brady, "Temporal and emotional factors related to electrical self-stimulation of the limbic system," in *Reticular Formation of the Brain*, H. Jasper *et al.*, Eds. (Little, Brown, Boston, 1958).
6. J. C. Lilly, "Learning motivated by subcortical stimulation: The start and stop pattern," in *Reticular Formation of the Brain*, H. Jasper *et al.*, Eds. (Little, Brown, Boston, 1958).
7. J. Olds, *J. Comp. and Physiol. Psychol.* **49**, 281 (1956).
8. D. A. Brodie, O. M. Moreno, J. L. Malis, J. J. Boren, *Science* **131**, 929 (1960).
9. M. Sidman, J. V. Brady, J. J. Boren, D. G. Conrad, *ibid.* **122**, 830 (1955).
10. J. P. Seward, A. Uyeda, J. Olds, *J. Comp. and Physiol. Psychol.* **52**, 294 (1959).
11. W. Hodos and E. S. Valenstein, *ibid.*, in press; J. Olds, *ibid.* **51**, 320 (1958).
12. W. K. Estes and B. F. Skinner, *J. Exptl. Psychol.* **29**, 390 (1941).

6 May 1960

Allelic Genes in the Housefly Producing Modified Enzymes That Cause Organophosphate Resistance

Abstract. In all of six phosphate-resistant strains of *Musca domestica* L. a mutant gene is present which produces an altered ali-esterase. The modified enzymes are no longer irreversibly inhibited by the oxygen analogs of the insecticides to which the strains are resistant but can slowly convert them. In five of the strains the resistance is caused by this gene only.

It is generally conceded that the fundamental effect of gene mutation is the production of altered proteins. However, in only a few cases has this actually been demonstrated (*1*). From the study presented here, it appears that organophosphate resistance in the housefly, *Musca domestica* L., is mainly due to the production of such an altered protein. The adaptive value of this change is of great importance to the species under the altered conditions of life.

In the housefly the use of the organophosphate insecticides Parathion, Diazinon, and Malathion (*2*) has led to the development of resistant strains (*3*). Since these phosphorothionates, after conversion to the corresponding oxygen analogs paraoxon, diazoxon, and malaoxon, act as strong inhibitors of different esterases in the insect, a comparison was made between the esterase activities shown by homogenates of resistant and susceptible strains. A curious difference was found: homogenates of all the phosphate-resistant strains studied showed a very low ali-esterase activity toward substrates such as methyl- and phenylbutyrate, whereas high activity was found in all the susceptible strains investigated (*4*). Low activity has now been observed in five strains resistant to Parathion and Diazinon from Europe and North America and in two strains from North America resistant to Malathion. High activity is present in six organophosphate-susceptible strains, some of which are resistant to the chlorinated hydrocarbons DDT and γ-BHC (benzene hexachloride).

The low esterase activity was found to be caused by an autosomal gene (*5*) (indicated as *a*; the corresponding gene for high esterase activity is indicated as a^+). The mean esterase activities toward methylbutyrate of a homogenate of single male flies of the genetic constitutions *aa*, aa^+, and a^+a^+, respectively, are about 50, 175, and 300 (expressed as microliters of CO_2 produced in the Warburg manometer in 30 minutes under certain conditions). Furthermore, it was found that in four out of five strains studied the phosphate resistance depends mainly on one gene (*5*, *6*), which is identical with the *a* gene. In the fifth strain the *a* factor is responsible for only a part of the resistance; in addition, one or more other resistance genes are present which do not affect esterase activity.

Although the *a* genes seem to be equal in their effect on the esterase activity, they differ widely in the specificity and degree of resistance which they confer on the strains. At least three different *a* genes have been discerned so far (Table 1, strains D, C, and G). The fact that they have the same influence on the esterase activity and all cause resistance suggests that they may be alleles. Nguy and Busvine (*6*) studied this possibility for the Parathion-Diazinon resistant strain C and the Malathion resistant strain H. Hybrids of these strains were backcrossed with the susceptible strain. Since the offspring consisted of about 50 percent Malathion resistant and 50 percent Parathion-Diazinon resistant flies (resistance is semidominant), these workers concluded that the two genes are allelic or otherwise closely linked.

We studied the possible allelism in the Parathion-Diazinon resistant strains F and C and the Malathion resistant strain G, estimating the esterase activity instead of resistance. The crosses F × G and C × G were made, and f_1 and f_2 generations were obtained. The f_1 flies had low esterase activity. Low

Table 1. Susceptibility and in vitro breakdown capacity in some strains of houseflies. The in vitro breakdown was measured in the following way. To a series of samples of homogenates, increasing amounts of inhibitor were added. After 2 hours of incubation the presence or absence of inhibitor was tested by adding fresh cholinesterase. The breakdown capacity was calculated by taking the mean of the amount of the inhibitor that had been converted completely and the next higher amount which had not yet been completely degraded. In strains without a breakdown enzyme a certain amount of inhibitor was bound by the homogenate. However, this amount (indicated by an asterisk) does not increase with time.

LD_{50} Diazinon (μg/jar)	Breakdown diazoxon (mμg/2 hr)	LD_{50} Malathion (μg/fly)	Breakdown malaoxon (mμg/2 hr)
	Strain S		
2	5*	0.6	4*
	Strain D		
12	15		
	Strain C		
62	72	2.5	13
	Strain G		
4	7*	25	130
	Strain F		
100	24		
	Strain F_a		
11	17		
	Strain F_b		
11	5*		