bulbospinal as well as ascending projections (*10*), and the increasing responses may relate more to spinal reflex functions or to generalized arousal states. However, my results indicate that the activity of many of these bulboreticular neurons is not associated with specific movements or with inputs from several sensory modalities; rather, the magnitude of their synchronized discharge to somatic stimuli appears to be systematically related to a behavior operationally indicative of pain sensation. Moreover, it has been shown in cat that lesions in the NGC or its thalamic projection (*11*) significantly increase escape latency without affecting motor performance. Finally, stimulation of NGC in rats elicits escape (*12*), and similar effects have been observed in the cats used for unit recording in this study. Indeed, the effect of NGC stimulations is immediately generalized, for cathodal stimulation of 25 to 100 μa through the recording microelectrode (100 pulses per second, 0.2-msec pulse duration; skull screw anodal) elicits escape on the first stimulus trial, the points with lowest threshold being located in the region of responsive bulboreticular units described in this report (*13*). Taken together, the results lend further support to the hypothesis that the bulboreticular formation in the NGC region plays an important role in pain sensory mechanisms. In view of the relative lack of somatotopic organization in this area, it seems possible that the NGC and its rostral projections are more concerned with the motivational, rather than the spatial discriminative aspects of pain (*14*).

KENNETH L. CASEY

Department of Physiology, University of Michigan, Ann Arbor 48104

References and Notes

1. W. F. Collins, F. E. Nulsen, C. T. Randt, *Arch. Neurol.* **3**, 381 (1960); A. G. Swanson, G. C. Buchan, E. C. Alvord, *ibid.* **12**, 12 (1965).
2. P. R. Burgess and E. R. Perl, *J. Physiol. (London)* **190**, 541 (1967); E. R. Perl, *ibid.* **197**, 593 (1968); P. Bessou and E. R. Perl, *J. Neurophysiol.* **32**, 1025 (1969).
3. M. Manfredi and V. Castellucci, *Science* **165**, 1020 (1969).
4. B. Pomeranz, P. D. Wall, W. V. Weber, *J. Physiol. (London)* **199**, 511 (1968); B. N. Christensen and E. R. Perl, *J. Neurophysiol.* **33**, 293 (1970).
5. K. L. Casey, *Exp. Neurol.* **25**, 35 (1969); J. J. Keene and K. L. Casey, *ibid.* **28**, 155 (1970).
6. A. Lundberg, in *Physiology of Spinal Neurons*, J. C. Eccles and J. P. Schade, Eds. (Elsevier, Amsterdam, 1964).
7. J. D. Green, *Nature* **182**, 962 (1958).
8. R. Spears, G. Smith, K. L. Casey, *Physiol. Behav.* **5**, 1327 (1970).
9. R. R. Sokal and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969), pp. 571–575. The KS statistic is the more powerful of the two tests and is most appropriate for small sample sizes that may be encountered in the case of infrequently discharging cells. The χ^2 has been more commonly used in studies of this kind, so it is included for comparison and as an additional response criterion.
10. A. Brodal and G. F. Rossi, *AMA Arch. Neurol. Psychiat.* **74**, 68 (1955); R. Nyberg-Hansen, *J. Comp. Neurol.* **124**, 71 (1965).
11. C. L. Mitchell and W. W. Kaebler, *Amer. J. Physiol.* **210**, 263 (1966); B. P. Halpern and J. D. Halverson, *Physiologist.* **10**, 193 (1967); D. Bowsher, A. Mallart, D. Petit, D. Albe-Fessard, *J. Neurophysiol.* **31**, 288 (1968).
12. J. J. Keene and K. L. Casey, *Exp. Neurol.* **28**, 155 (1970).
13. K. L. Casey, *Int. J. Neurosci.*, in press.
14. ———, *J. Neurophysiol.* **29**, 727 (1966).
15. These normalizing procedures were necessary because of variations among cats in the current required to elicit escape and because of differences in the level of unit activity and response.
16. Supported by NIH grant NS06588. I thank J. D. Matthews and R. Macklin for technical assistance.

1 February 1971; revised 1 April 1971

Caudate Nucleus Stimulation Retroactively Impairs Complex Maze Learning in the Rat

Abstract. *Rats, with permanent electrodes implanted bilaterally in the caudate-putamen complex, were stimulated with single pulses after reinforcement of each maze learning trial or were stimulated with multiple pulses after each choice point or after reinforcement. Single pulses retarded the development of learning only when stringent learning criteria were required, whereas multiple pulses interfered with acquisition when the criteria for learning were less difficult.*

Data indicate that a variety of experimental treatments administered shortly after a learning trial can retroactively impair the memory for that event (*1*). Much of this research is based on the effects of electroconvulsive shock (ECS) on shock motivated passive avoidance learning. Whereas many results indicate that such treatments are most effective when administered immediately after the learning trial and that the amnesic effect diminishes as the time between learning and ECS increases, little is known about the primary anatomical site of action of ECS, or of any other amnesic agent. Because the treatments that produce these apparent memory deficits are frequently gross systemic ones, it has been difficult to ascertain the specific structures involved in various stages of memory formation. A number of results suggest that stimulation of the basal ganglia (*2*), thalamic reticular formation (*3*), and various limbic system structures (*4*) may affect learning and memory. However, it is extremely difficult to assess such experiments in terms of direct effects on specific brain structures or on memory mechanisms because of the use of relatively high current densities or long stimulation durations, because of the failure to control for spread of current to other structures, and because of the possibility that stimulation of brain structures during learning produces direct effects on performance.

Several experiments have indicated that it is possible to impair one-trial inhibitory avoidance learning retroactively by brief electrical stimulation of the caudate-putamen complex (CPU) (*5*, *6*) or by placement of small electrolytic lesions in the cingulate field (*7*). These studies (*5*–*7*) were directed specifically at the retroactive and time-dependent effects of such manipulations on memory; they included more adequate controls than the earlier investigations. In addition to demonstrating neural specificity resulting from electrical stimulation of specific structures, this technique has the advantage of avoiding aversive effects that result from multiple administrations, such as observed with repeated ECS treatments (*8*); thus, it is of use in multiple trial learning paradigms.

We investigated the effects of electrical stimulation of the CPU on multiple alley maze learning in food-motivated rats. Because a previous investigation demonstrated that the impairment resulting from CPU stimulation is not the result of current spread to surrounding structures (*5*), this experiment focused on stimulation characteristics, such as timing in the response-reinforcement sequence and frequency of presentation, rather than on control electrode placements. Finally, the subtlety of this technique permitted a more careful analysis of the development of the learning process than has traditionally been possible with other retrograde amnesic agents, namely, assessing the disruptive effect of different stimulation characteristics as the learning process proceeded through a graded series of learning criteria.

Male albino rats (Sprague-Dawley) between 150 to 225 days old were individually housed and maintained.

Bipolar, concentric electrodes (with the electrode barrel constructed of 26-gauge tubing and with a 0.25-mm exposure on both the tips and the barrels) were placed bilaterally in the head of the CPU of the animals (DeGroot stereotaxic coordinates (*9*) were AP, 8.4; H, 1.0; L, 2.5). Rats were assigned to one of six groups—three control groups and three brain stimulation groups. The control groups were normal controls with no implanted electrodes, sham-operated controls with head connectors but without implanted electrodes, or controls with electrodes positioned in the CPU but never stimulated. The brain stimulated groups received single pulse stimulation at the end of each maze trial, after the rat had eaten for 30 seconds (PTS); stimulation after passing through each choice point between successive units (CPS); or multiple stimulations at the end of each maze trial, after the rat had eaten (MPTS).

The apparatus was a Lashley III maze modified to be used with animals with cables attached to their heads. These modifications included increasing the height of the walls to 10 inches and shortening the alleys to 40 inches. The alleys were 5 inches wide; the plywood maze was painted flat gray and was placed on the floor of the laboratory. Various relay racks and other laboratory equipment were visible to the rats and provided rich extramaze cues. Low, indirect room illumination was provided by a 100-watt light directed at the ceiling. Food deprivation began at least 1 week after surgery and weights were maintained throughout training at 85 percent of the levels prior to the operations. First, each rat was trained for 2 days to eat wet mash in the goal box. Then, on the following 4 days, training consisted of traversing one-half and then all of a 40-inch straight alley from starting box to goal box. No brain stimulation was administered during the preliminary training.

During maze learning, one trial per day was given to each rat for the first 3 days. For the next 3 days, two trials were administered each day, and on each of the remaining days, three trials were administered. The intertrial interval was 5 to 10 minutes for multiple-trial days. Head cables were attached to the animals with implanted electrodes and the animals with head connectors only, just prior to placement into the starting box. Trials were timed from leaving the starting box to entering the goal box, and errors were recorded each time the head and forepaws entered a blind cul. Rats in the brain stimulated groups were given bilateral pulses of 500 μa for 2 msec (*10*). This current level was chosen because it was similar to the current levels used in the prior study that demonstrated a memory deficit from such stimulation (*5*). For the MPTS group, the number of stimulations given on each trial was the median number of stimulations that the CPS group had received on the same trial. The number of pulses per trial presented to the MPTS group was 9, 7, 7, 5, 7, 5 (trials one through six, respectively) and 4 for the seventh and all the remaining trials. Multiple stimulations were separated by 1 second.

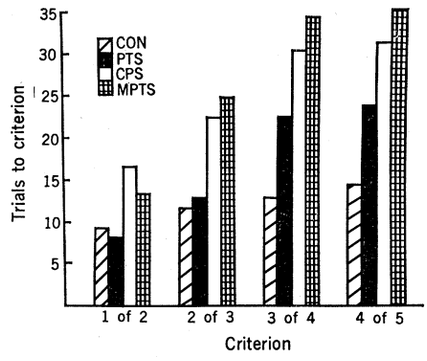

Fig. 1. Median number of trials to different criteria of learning (one out of two, two out of three, three out of four, and four out of five errorless trials) for groups of rats administered no, single, or multiple stimulations of the caudate-putamen at various times in the response-reinforcement sequence. *CON*, control; *PTS*, single stimulation after each trial; *CPS*, single stimulation after each choice point; *MPTS*, massed simulation after each trial.

Rats with electrodes were killed and perfused. The brains were frozen, and 80-μm sections were cut until the electrode tracks were visible. Photomicrographs were then made to permit verification of tip placement. Verified CPU electrode placements for each of the groups were: PTS, $n = 15$; CPS, $n = 13$; and MPTS, $n = 11$. Of the control groups there were four with CPU placed electrodes, six sham-operated with head connectors but no electrodes, and ten normal controls. Because there were no trends toward differences on any measure among these groups they were combined into one control group (Con) for the purpose of statistical comparisons. The number of errors and the number of trials to criteria yielded similar results, so only the number of trials to criteria are discussed.

All rats were run to a criterion of four errorless trials out of any five. (Running was discontinued if this criterion was not met by the 50th trial.) However, in reaching this criterion each animal also fulfilled criteria of three perfect trials out of any four, two perfect trials out of three, and one out of any two (the first errorless trial). The data for each criterion level is presented in Fig. 1. For the easiest criterion, that is, the number of trials to the first errorless trial, an overall nonparametric analysis of variance (Kruskal-Wallis) demonstrated a significant difference among the four groups ($P < .01$). Two-tailed Mann-Whitney U tests were performed between pairs of groups. No difference was found between groups PTS and Con, but both CPS and MPTS groups took reliably more trials to reach the first errorless trial than did the controls ($P < .002$ and $P < .05$, respectively). Groups CPS and MPTS also took more trials to reach this criterion than did group PTS (P between .05 and .10 and $P < .02$, respectively). There was no difference between groups CPS and MPTS. These results demonstrate that multiple stimulations of the CPU during or after a training trial interferes with learning, whereas a single stimulation after a trial does not interfere when the criterion of learning is an easy one. For the results of the next most difficult criterion, two errorless trials out of three, the pattern of results was identical to that for the criterion of one trial out of two.

For the third criterion, three errorless trials out of four, the differences shown by individual group comparisons after the significant overall test ($P < .001$) begin to show a different pattern. Groups CPS and MPTS are still both reliably slower to reach this criterion than the control group ($P < .001$ in both cases), but they did not differ from each other, and they both are different from group PTS ($P < .05$ and $P < .002$, respectively). However, group PTS is, at this criterion level, reliably slower to reach criterion than the control group ($P < .001$). For the criterion of four errorless trials out of five, the pattern of results was identical to that for three trials out of four. At these more difficult criterion levels, stimulation of the CPU whether by single or multiple stimulations retards the rate of learning, and multiple stimulations are more detrimental than single pulses.

These results demonstrate that stimu-

lation of the CPU can retroactively impair the acquisition of a maze learning habit. Multiple stimulations administered either during the maze trial, contingent upon passing through choice points, or after the completion of the trial and reinforcement, were effective in producing this impairment regardless of the criterion of learning employed. Single pulse stimulation after a trial produced a deficit only in the later stages of learning, after the initial few errorless trials had been run. These results suggest that the different stimulation conditions represent a dose effect, similar to that observed with other amnesic agents (*11*). In this experiment, single pulse stimulation disrupted learning only late in training, when errorless performance was being perfected, whereas multiple stimulations were effective in interfering with acquisition from the earliest stages of learning.

These observations emphasize a major contribution that such studies can make which is impossible with the gross treatments used previously in studies of memory impairment, that is, administration of the amnesic agent after or during each trial in a multiple trial experiment. Such treatments as ECS proved to be, in themselves, aversive after several administrations (*8*). This does not appear to be the case with stimulation of the CPU insofar as such stimulation appears to be neither aversive nor positively reinforcing (*12*).

These results confirm and extend the basic findings that stimulation of the CPU after a trial in rats produces retroactive memory impairment. This has been demonstrated in several single trial, passive avoidance paradigms (*5, 6*) and now in a food-motivated complex maze situation. It would appear that the CPU has a major involvement in the formation of memory. Whether this complex contains some discrete (or stochastic) neural activity representing the stimuli associated during learning, or whether its involvement is the result of its influence [probably inhibitory (*13*)] on other sites or systems is still very much open to question.

HARMAN V. S. PEEKE
MICHAEL J. HERZ
Department of Psychiatry, University of California and *Laboratory of Psychobiology, Langley Porter Neuropsychiatric Institute, San Francisco 94122*

References and Notes

1. J. L. McGaugh, *Science* **153**, 1351 (1966); M. R. Rosensweig and A. L. Leiman, *Annu. Rev. Psychol.* **19**, 55 (1968).
2. R. Thompson, *J. Comp. Physiol. Psychol.* **51**, 421 (1958).
3. H. Mahut, *ibid.* **55**, 472 (1962).
4. R. P. Kesner and R. W. Doty, *Exp. Neurol.* **21**, 58 (1968).
5. E. J. Wyers, H. V. S. Peeke, J. S. Williston, M. J. Herz, *ibid.* **22**, 350 (1968).
6. E. J. Wyers and S. A. Deadwyler, *Physiol. Behav.* **6**, 97 (1971).
7. W. J. Hudspeth and W. E. Wilsoncroft, *J. Neurobiol.* **1**, 221 (1969).
8. W. J. Hudspeth, J. L. McGaugh, C. W. Thomson, *J. Comp. Physiol. Psychol.* **57**, 61 (1964).
9. J. DeGroot, *The Rat Forebrain in Stereotaxic Coordinates* (N. V. Noord-Hollandsche Uitgevers Maatschappij, Amsterdam, 1959).
10. Stimulations were biphasic, that is, a positive 1-msec square wave 250 μa peak to baseline followed immediately by an identical wave inverted.
11. A. Cherkin, *Proc. Nat. Acad. Sci. U.S.* **63**, 1094 (1969).
12. M. P. Olds and J. Olds, *J. Comp. Neurol.* **120**, 259 (1963); A. Bruner, *ibid.* **131**, 615 (1967).
13. N. A. Buchwald and C. D. Hull, *Brain Res.* **6**, 1 (1967).
14. We thank R. Crawford and E. Moffet for assistance in the early stages of this research, and Dr. D. L. Jewett of University of California, San Francisco, for making histology facilities available. Supported in part by NIMH grants MH-17784, MH-18636, and 5-TI-MH-7082.

4 December 1970

Allergic and Classically Conditioned Asthma in Guinea Pigs

Justesen, Braun, Garrison, and Pendleton (*1*) state that the "pressure differential between inspiratory peak and expiratory peak of each respiratory cycle is . . . greater during an attack of asthma than when the airway is patent." This is so provided rates of air flow are similar in both instances. Peak pressures are increased not only during asthma, but also during hyperventilation without airway obstruction. The authors present no data that permit one to distinguish between these two processes. This paper does not contain convincing evidence that asthmatic attacks can be induced in guinea pigs by conditioning techniques. The study of such responses requires simultaneous recordings of rate of air flow, driving pressure, and lung volume. Several well-established techniques are available for this purpose (*2*).

AREND BOUHUYS
John B. Pierce Foundation, Yale University, New Haven, Connecticut 06519

References

1. D. R. Justesen, E. W. Braun, R. G. Garrison, R. B. Pendleton, *Science* **170**, 864 (1970).
2. M. O. Amdur and J. Mead, *Amer. J. Physiol.* **192**, 364 (1958); S. D. Murphy, J. K. Leng, C. E. Ulrich, H. V. Davis, *Arch. Environ. Health* **7**, 60 (1963); M. W. Dennis, J. S. Douglas, J. U. Casby, J. A. J. Stolwijk, A. Bouhuys, *J. Appl. Physiol.* **26**, 248 (1969).

18 January 1971

If a suspected attack of conditional asthma were found upon direct examination to be caused by a form of hyperpnea (for example, the hyperventilation syndrome), no damage would be done the thesis that classical conditioning had been demonstrated because conditional responses often differ greatly, both quantitatively and qualitatively, from the unconditional reflexes from which they are elaborated (*1*). Although this argument is partly responsive to Bouhuys' central challenge, it proves to be academic. Inhaled isoproterenol, a bronchodilator, invariably controlled the higher peak pressures associated with allergic and conditional attacks of asthma. The drug's established specificity as a dilator of smooth muscle of the airway (*2*) thus permitted a strong operational distinction to be drawn between an obstructed airway and mere hyperventilation. In demonstration of conditioning, (i) only animals with a history of immunologically competent, hypersensitizing treatments developed plethysmographic signs of allergic, then conditional asthma; (ii) the conditional response was resistant to experimental extinction, but could be extinguished; and (iii) once extinguished, it could be reconditioned. The convergence of the original data with other data drawn from immunology and pulmonary physiology (*3*), pharmacology (*2*), and Pavlovian psychology (*4*) leaves little doubt that classical conditioning of an obstructed airway was indeed accomplished in the guinea pigs.

DON R. JUSTESEN
Neuropsychology Laboratories, Veterans Administration Hospital, Kansas City, Missouri 64128

References

1. G. A. Kimble, *Hilgard and Marquis' Conditioning and Learning* (Appleton-Century-Crofts, New York, 1961), chap. 3, p. 52.
2. I. R. Innes and M. Nickerson, in *The Pharmacological Basis of Therapeutics*, L. S. Goodman and A. Gilman, Eds. (Macmillan, New York, ed. 3, 1965), chap. 24, p. 498.
3. P. Kallos and W. Pagel, *Acta Med. Scand.* **91**, 292 (1937).
4. E. R. Hilgard and G. H. Bower, *Theories of Learning* (Appleton-Century-Crofts, New York, 1966), chap. 3, p. 48.

5 April 1971