Brain stimulation and behavior: Controls and consequences*

MICHAEL J. HERZ, KEATS E. MARSHALL†, and HARMAN V. S. PEEKE

*Department of Psychiatry and Laboratory of Psychobiology
Langley Porter Neuropsychiatric Institute, University of California, San Francisco, California 94143*

Groups of rats ranging from unimplanted controls to caudate-putamen (CPU) stimulated experimental animals were compared in terms of the number of trials required to acquire a brightness discrimination habit. The results indicated that the degree of deficit produced was a function of the magnitude of experimental intervention imposed. Animals with headplugs and cables, but without electrodes required significantly more trials to learn than unimplanted controls. The addition of electrodes without a headcable produced no greater deficit, while electrodes with attached cable resulted in significantly greater disruption than plug and cable alone. Stimulation of the PCU with one or three bilateral pulses following each discrimination produced significantly greater interference than did the electrode lesion and headcable. The results are discussed in terms of the control conditions required for studies of the effects of brain stimulation on behavior.

A basic assumption implicit in investigations of the effects of various physiological manipulations on behavior is that the results are directly attributable to the experimental treatment. However, numerous experiments have indicated that such conclusions often are not warranted and that a variety of experimental observations can more appropriately be attributed to unforeseen artifactual interactions. For example, Schwartzbaum and Donovan (1965) and Schwartzbaum and Gustafson (1970) have pointed out that unless intracranial electrodes are adequately isolated from ground while implanted animals receive peripheral shocks, it is quite possible that brain stimulation will be delivered each time the peripheral shocking circuit is activated. Similarly, the results of an investigation by Denti et al (1970) indicate that the lesions produced by the implantation of chronic intracranial electrodes can themselves produce modifications of learning and memory processes. Consequently, it is always necessary to compare the behavior of brain-stimulated animals with that of implanted nonstimulated as well as nonimplanted controls to assess fully the effects of stimulation. It should also be pointed out that since the effect of lesions resulting from implantation alone may be minimal, sensitive behavioral indices may often be required to detect their behavioral consequences.

This report presents additional observations on the consequences of various procedures typically employed in investigations of the behavioral effects of electrical stimulation of the brain.

Over the past several years, research in our laboratory has concentrated on the role of the rat caudate-putamen (CPU) in learning and memory. In a variety of experimental situations, it has been demonstrated that single-pulse stimulation of this structure can interfere

with learning (Wyers et al, 1968; Herz & Peeke, 1971; Peeke & Herz, 1971). In all of these investigations, the behavior of implanted, stimulated Ss has been compared with that of implanted, nonstimulated as well as with nonimplanted control animals, and in no case have the latter two control groups differed significantly from each other. However, in an investigation that utilized a relatively complex spatial learning situation, the Lashley III maze, requiring multiple learning trials and multiple brain stimulation (Peeke & Herz, 1971), it was observed that implanted, nonstimulated control Ss tended to make slightly, though not significantly, more errors in reaching learning criteria than did controls. One of the purposes of the present investigation, which utilized a visual discrimination paradigm, was to determine whether the lesion resulting from implantation resulted in behavioral deficits.

METHODS

Sixty-five Long-Evans male rats (Simonson Laboratories, Gilroy, California), ranging in age from 100 to 180 days, were utilized in the investigation. The Ss were individually housed and maintained on ad lib food and water for at least 1 week prior to surgical and/or experimental treatment. Animals were run under one of five conditions: nonimplanted control (C, N = 18); headplug plus headcable (PO, N = 7); implanted, no headcable (SNH, N = 8); implanted, with headcable (SHC, N = 17); or implanted, stimulated (\$, N = 15).

Ss in implanted groups (SNH, SHC, and \$) were anesthetized with sodium pentobarbital, positioned in the stereotaxic instrument, and implanted bilaterally with bipolar, twisted wire (0.127 mm diam) electrodes (de Groot coordinates: AP, 8.4; H, 1.0; L, 2.5), which were terminated in Amphenol female pin connectors and a receptacle that was fastened to the skull with acrylic cement. Electrodes were not implanted in Group PO Ss, but animals were anesthetized, placed in the stereotaxic instrument, had the scalp incised, and headplugs cemented to the skull. A recovery period of at least 1 week was permitted prior to the beginning of the food-deprivation regimen, which was designed to maintain Ss at 85% of their preoperative weights.

The experimental apparatus was an elevated linear discrimination runway modified from Brown and Ghiselli (1934). It consisted of a series of five elevated octagonally shaped platforms, 50.6 cm long x 40.4 cm wide, tapering to

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†Now at the Department of Pharmacology, University of Minnesota, Minneapolis, Minnesota 55455.

Fig. 1. Median number of trials (discriminations) to criterion. C, control; PO, plug only; SNH, electrodes, no headcable; SHC, electrodes plus headcable; and S, stimulated.

7.6 cm wide where each joined the adjacent platform. At the center of each platform was a 20.2-cm-high barrier, which contained two 10.1 x 20.2 cm doorways. The doors were split vertically and hinged on the outside lateral edges, permitting unimpeded passage for animals carrying headcables. At one end of the apparatus was a 13.9 x 22.8 x 19.6 cm high startbox, and at the far end was a 12.6 x 26.6 cm goalbox with 19.6-cm-high sides which contained a Petri dish full of wet mash. The headcable was supported by a slider attached to a tight wire running the length of the runway, 114 cm above the animal, and was pulled along the wire by the S.

The animals were first permitted to eat wet mash in the goalbox on two 30-sec trials on the 1st day. The headcable was attached to the animals in the appropriate groups from the 1st day of pretraining. On the next day (two trials), they were placed in front of the open gray doorways of the partition closest to the goalbox and permitted to run to the goalbox and to eat for 30 sec. This procedure was repeated on the 3rd day (three trials) with gray doors closed. On successive days, pretraining trials were begun with the animals placed one partition further from the goalbox until they were running through all of the closed doorways (two trials per day on the 4th through the 7th days). On the 8th day, black-white discrimination training was begun; the animals were permitted two runs down the runway (10 trials or discriminations) with the white doors as the positive discriminanda and the black doors blocked. The position of the white doors was systematically varied each day according to a predetermined random sequence. The stimulated group was administered one set of bilateral CPU pulses (either one pulse, 0.2 msec duration, or three pulses, 1.0 msec duration, 0.5 mA) 1 sec after passing through each doorway. (Monophasic square waves were administered simultaneously but independently to each electrode with two Grass SD-5 stimulators and constant current units.) Training continued until a criterion of 9 out of 10 errorless choices had been made within one set of daily discriminations. At the

completion of training, animals were sacrificed with pentobarbital, perfused with Formalin, and the brains removed and stored in Formalin until histology was performed. All implanted Ss had verified bilateral CPU electrode placements.

RESULTS

An overall analysis of variance (Kruskal-Wallis) of the total number of trials (discriminations) required to reach criterion revealed a significant effect ($p < .001$). Comparisons between various conditions indicated that each of the treatments resulted in significantly more trials being required to reach the learning criterion (see Fig. 1) than was necessary for the control group. Even the group with only a headplug and cable (PO) differed significantly from the controls ($p < .02$),¹ although this group did not differ significantly from the implanted animals which were run without a headcable (SNH), suggesting that the requirement that Ss pull the long headcable through the apparatus significantly interfered with the rate of learning to as great a degree as did the implantation of electrodes, as long as implanted animals were not required to carry the cable (SNH). However, addition of electrodes (SHC) to the plug and headcable (PO) resulted in significantly more trials required to reach criterion ($p < .02$). Since there was not even a trend toward a difference between the two different stimulation conditions, the animals from the two subgroups were combined and, compared with all of the other groups, found to be significantly different in all cases (C, $p < .002$; PO, $p < .05$; SNH, $p < .05$, one-tailed; SHC, $p < .05$).

The results of the present investigation are important for a number of reasons. First, from the point of view of the control conditions which are required for investigations of the effects of brain stimulation on behavior, the observation that the addition of a headplug and cable (Group PO), without invasion of the cranial cavity, is sufficient to produce deficits in learning indicates that complex learning paradigms are extremely sensitive to interference. In the absence of such a control, it could be concluded that the deficit resulting from brain stimulation, or even from the implantation of electrodes without stimulation, was entirely due to the effects of neural damage. The present results indicate that the addition of a headplug and cable to control animals produces almost as much interference with acquisition as does the presence of electrodes if implanted animals are run without a headcable. While it seems quite likely that at least those deficits resulting from the headplug and cable are attributable to effects upon performance, it is impossible to distinguish clearly between learning and performance without further experimentation.

The observation that implanted, nonstimulated Ss require significantly more trials to acquire the learning criterion is consistent with the trend observed in at least one previous investigation from this laboratory (Peeke & Herz, 1971). In fact, the present brightness

discrimination paradigm was developed to be a more sensitive learning index than the spatial discrimination utilized in the earlier study, with the anticipation that it would permit more detailed analysis of the deficits resulting from small lesions and brief electrical stimulation. These results are also consistent with previous research indicating that lesions involving the caudate nucleus are capable of disrupting learning (Green, Beatty & Schwartzbaum, 1967). The results of the present investigation also indicate that stimulation of CPU with brief, bilateral pulses following brightness discrimination trials is sufficient to produce deficits in acquisition. While these observations are consistent with previous results in which such stimulation retroactively impaired passive avoidance learning (Wyers et al, 1968; Wyers & Deadwyler, 1971) and extinction (Herz & Peeke, 1971), the relationship between frequency of stimulation and degree of disruption obtained in Lashley III learning (Peeke & Herz, 1971) was not observed. However, the confounding of pulse duration and frequency in the present investigation makes it difficult to resolve this discrepancy.

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NOTE

1. All comparisons between pairs of groups were performed using the Mann-Whitney U test, and two-tailed probability levels are reported unless otherwise noted.

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