12

Stimulation Studies

DIRECT stimulation of the hippocampus, by chemical or electrical means, is being used in an increasing number of behavioural studies. As a technique stimulation lacks several of the drawbacks associated with surgical lesions. Its effects are at least theoretically transient, so that the function of a neural region can be disrupted at any stage of a learning experiment at the whim of the investigator. Further, the problem of recovery of behavioural function after surgical lesions, whatever its basis (see pp. 235-6), need not arise with stimulation. Brain stimulation has also been used as a means of assessing the effects of initiating or disrupting normal physiological function upon various aspects of general behaviour, the latter with more success than the former.

These uses of stimulation all suffer from the possibility that, though applied locally, stimulation can elicit important effects in distant structures. This occurs, for instance, through propagated seizure discharges or the diffusion of injected drugs. The effects of electrical stimulation often last considerably longer than the stimulation itself (e.g. Gergen and MacLean 1961), particularly when seizures are evoked. In practice, this means that the electrical activity of other brain areas should be recorded during the course of stimulation in order to detect the propagation of seizure after discharges. As it is now well established that seizure thresholds decrease with repeated stimulation (cf. Goddard, MacIntyre, and Leech 1969), these control records should be taken throughout the course of any stimulation study. Few of the pioneering studies, and too few of the more recent studies, provide this important control.

Chemical stimulation suffers from fewer difficulties once the possibility of diffusion has been controlled. Local application, through implanted cannulae, of minute quantities of drugs can have marked behavioural effects, and this technique conveys the added advantage that one might be able to draw quite specific conclusions regarding the pharmacological basis of these changes. These advantages, however, only hold when the injection site is limited and the quantity of drug is small. Few of the studies presently available fulfill these criteria.

Both electrical and chemical stimulation techniques are continually being improved, and more recent studies employ controls against many

of the objections raised above. For this reason it is worth devoting a chapter to the discussion of studies using these techniques. However, our discussion cannot be exhaustive. The interested reader is referred to Table A29 for a list of studies using stimulation techniques and to recent review articles which present these studies in a more complete fashion (e.g. Izquierdo 1975).

In view of the paucity of 'clean' data we have not relied upon stimulation experiments in the development of the present theory. Here, we shall attempt to interpret stimulation studies in terms of the theory and shall discuss three different types of study: (1) studies investigating the general behavioural effects of stimulation, seen either as an initiator or disrupter of normal activity; (2) studies investigating the influence of stimulation during the performance of a previously trained behaviour; (3) studies investigating the effects of stimulation, given either during or after learning, upon learning and/or retention.

12.1. General effects

In describing any effect of stimulation it is essential to separate those effects seen during stimulation from those seen after stimulation has ceased. This is most easily done with electrical stimulation where the dividing line between the two is under the direct control of the investigator. This differentiation between *on* and *off* reactions seems particularly important in regard to hippocampal stimulation where quite different effects can be seen in the two phases. Thus, the most commonly observed effect of mild stimulation of the hippocampus is an alerting, or *arrest*, reaction (e.g. Kaada, Jansen and Andersen 1953, MacLean 1957b, Bland and Vanderwolf 1972b*) during stimulation, while active exploration can be seen as an off reaction (Milgram 1969a). Associated with the alerting response one can observe cortical desynchronization, respiratory acceleration, and heart rate increases (Kaada, Feldman, and Langfeldt 1971). Direct application of cholinergic drugs (typically carbachol) can also elicit this alerted state (Grant and Jarrard 1968), but is often followed by seizures and a catatonic state (MacLean 1957a,b) as we noted before. Local application of anticholinergic drugs (e.g. neostigmine or methylscopolamine) leads to a decrease in exploratory behaviour (Van Abeelen *et al.* 1972), as does strong electrical stimulation involving after discharges in the hippocampus (Leaton 1968). All of these effects are consistent with the notion that hippocampal integrity is crucial to exploratory behaviour. Van Abeelen *et al.* concluded that

'the mouse hippocampus contains a cholinergic mechanism which regulates exploratory tendencies' (p. 474).

* According to Bland and Vanderwolf this arrest pattern is only seen with stimulation of 8 Hz or more.

Less easy to understand are the observations of eating and drinking elicited by hippocampal stimulation (electrical: Milgram 1969a,b, Oliver, Firestone, and Goodman 1973, Huston *et al.* 1974, Milgram, Grant and Stockman 1975; chemical: Fisher and Coury 1962, Coury 1967, Grant and Jarrard 1968, Mountford 1969, Huston *et al.* 1974, Siegfried *et al.* 1975). The present model holds that the hippocampus is not directly involved in the control of these behaviours, and we have already seen that hippocampal lesions do not generally affect eating or drinking. Hippocampal stimulation-elicited eating and drinking differ from that seen with hypothalamic stimulation in two ways. First, they typically occur some time after stimulation, either as a rebound effect upon cessation of electrically induced after discharges (Milgram 1969a; Oliver *et al.* 1973, Milgram *et al.* 1975), or spreading depression elicited by KCl (Huston *et al.* 1974, Siegfried *et al.* 1975), or after a seizure discharge has subsided (Mountford 1969). Second, the eating elicited by stimulation can be conditioned either to a signal (CS) or to the experimental situation itself (Siegfried *et al.* 1975, Milgram *et al.* 1975).

There is strong evidence that the stimulation-elicited drinking is an indirect effect. Routtenberg (1967) suggested that carbachol-elicited drinking was generally a function of diffusion of the drug into the third ventricle; activation of cells bordering on the ventricle would then be responsible for the consummatory pattern. While Mountford (1969) disputed this claim, Simpson and Routtenberg (1972) provided convincing evidence that the crucial site involved in elicited drinking is the subfornical organ; this has been confirmed by other investigators. Elicited eating, on the other hand, does not submit to this simple analysis, though it must be indirect in the sense of being a rebound effect. Our theory does not preclude such effects, but nor does it provide a clear basis for predicting them.

There are at least two ways to account for the rebound elicitation of eating (or drinking) after hippocampal stimulation. First, it could be assumed that the hippocampus has outputs to (presumably) hypothalamic sites driving these consummatory patterns and thus can modulate such behaviour. The fact that these specific behaviours are generally elicited after stimulation in CA1 (Grant and Jarrard 1968, Milgram 1969b, Jarrard 1973) suggests the possibility that this region of hippocampus normally blocks specific consummatory patterns, perhaps as a corollary to its primary function of driving exploration. The decreased latency to eat observed after hippocampal lesions (see p. 256) is consistent with this notion. Second, it could be assumed that the effects of hippocampal stimulation are non-specific in that they appear only when propagated seizures have been elicited. The rebound nature of the behavioural effects is consistent with this idea, as are data from studies of the effects of hippocampal stimulation upon hypothalamically elicited attack behaviour (Siegel and

Flynn 1968, Vergnes and Karli 1969, Nagy and Decsi 1974). Vergnes and Karli showed that

'the inhibitory effect of a hippocampal seizure discharge seems to be due to its propagation to other nervous structures, in particular to amygdala and hypothalamus' (p. 889).

Another effect which might be explained in this way concerns the self-stimulation sometimes (Ursin *et al.* 1966, Milgram 1969a, Brown and Winocur 1973, Oliver *et al.* 1973) but not always (Stein 1965, Margules and Stein 1968, Milgram 1969a, Livesey and Wearne 1973) seen with hippocampal placements. A study by Jackson and Gardner (1974) demonstrated that hippocampal stimulation could effect hypothalamic self-stimulation, suggesting that self-stimulation in the hippocampus works through the hypothalamus.

It is not possible to choose between these two alternatives, partly because they are not genuinely opposed. The very presence of such elicited effects indicates that activity started in the hippocampus can influence consummatory patterns. What must remain at issue is the specificity of this influence and the role of seizure after discharges in the triggering of behaviour. Should propagated seizures be essential to the effects then we can conclude that the hippocampus is not central to the control of consummatory patterns. Certainly, the lesion data provide no reason to assume otherwise.

12.2. The effects of stimulation upon performance

Hippocampal stimulation has been used as a functional lesion to assess the effects of disruption upon the performance of previously learned behaviours. The aim of much of this research was the determination of the role of the hippocampus in the long-term memory for such behaviours. The major problem with this use of stimulation concerns the propagation of seizure discharges to other brain structures. Thus, strong electrical stimulation can disrupt simple classically conditioned responses (e.g. Flynn and Wasman 1960, Vanegas and Flynn 1968), the learning of which is unaffected by hippocampal lesions. In most of the studies reporting this effect seizures were routinely elicited. The possibility that such effects upon performance owe to interference with structures other than the hippocampus is supported by the fact that decrements are usually elicited only by intense stimulation. With parameters specifically chosen to avoid at least overt behavioural seizures, or with spreading depression confined to the hippocampus, a different picture emerges. No disruption was seen in the performance of go–no-go discrimination, learning set and delayed alternation (Weiskrantz, Mihailovic, and Gross 1962), nor in a position habit (Olds and Olds 1961), nor in a T-maze brightness discrimination (Grossman and Mountford 1964), nor in lever-press avoidance (Margules and Stein 1968).

In the last study more intense stimulation, which likely evoked after discharges, did interfere with performance. Similarly, Nakao (1966) has shown that performance of an escape response motivated by hypothalamic stimulation was disturbed by hippocampal stimulation when after discharges invaded the amygdala. Thus, interference with performance in these studies could be dependent upon disruption of activity in other brain areas. Andy *et al.* (1968) provided strong support for this interpretation when they showed that the debilitating effect of hippocampal stimulation upon performance of a passive avoidance task was ameliorated by fornix lesions.

On the other hand, carbachol injected into the hippocampus interferes with the performance of an operant go–no-go alternation task (Overstreet, Vasquez and Russell 1974), while hippocampal spreading depression has been shown to interfere with one-way active avoidance (Bures *et al.* 1960), spatial alternation (Henderson, Henderson, and Greene 1973), and lever-press avoidance (Erickson and Chalmers 1966). In the active avoidance study deficits appeared to be due to the loss of place information; the authors suggested that the rats

'had lost the ability to differentiate the safe side of the apparatus and remember its position' (p. 223).

The deficit in the lever-press avoidance task is in disagreement with both lesion results and the data from a study using electrical stimulation (see above).* In agreement with lesion results, however, is the finding that electrical stimulation facilitates two-way active avoidance (Stein 1965) and disrupts performance on a VI-45 operant schedule (Oliver *et al.* 1973).

Most of these studies of the effects of stimulation upon performance have used tasks which, we can assume from the results of lesion studies, do not require hippocampal participation. The inappropriate conclusion has been drawn that the hippocampus is not involved in permanent memory storage, though it might be involved at some early stage of learning since stimulation does not disrupt performance in many tasks. We can only suggest that tasks should be chosen which demand the involvement of the hippocampus if one is to assess the effects of stimulation properly.

12.3. Effects of stimulation upon learning

Stimulation has been used to study the role of the hippocampus in learning in basically two ways. First, it has been used more or less as a

* A possible explanation for this discrepancy lies in the technique used to elicit spreading depression. Bures *et al.* (1960) note that when crystalline KCl is used depression is typically confined to the hippocampus, but that with the use of liquid KCl depression can easily spread. Erickson and Chalmers used liquid KCl and did not check for the spread of depression to neighbouring areas.

functional lesion, present during the entire course of learning but usually not during retention. Second, it has been used in a much more precise fashion, applied only at some critical time during/after learning or before/during retention. Here, the interest resides in the role of the hippocampus in some circumscribed stage of the learning/memorization process.

12.3.1. STIMULATION THROUGHOUT LEARNING

This application of stimulation was used in most of the early studies, unfortunately in conjunction with the learning of tasks not normally affected by surgical lesions. Flynn and Wasman (1960), for instance, showed that stimulation involving continual after discharges interfered with the performance of a classically conditioned response during the learning session but that retention was nearly perfect when stimulation ceased. This study shows the danger of ignoring the effects of seizure propagation; it is clear that the hippocampus is not involved in either the learning or the retention of such tasks, but that seizures elicited in this structure can interfere with performance. Stimulation below seizure threshold (Correll 1957), or below the threshold for eliciting overt behavioural responses (Weiskrantz *et al.* 1962), did not interfere with the learning of appetitive approach responses or simultaneous discrimination, respectively.

On the other hand, disruption of the hippocampus interfered with passive avoidance learning (Henderson *et al.* 1973, but see Bresnahan and Routtenberg 1972)*, reversal of a position habit (Olds and Olds 1961), extinction of an approach response (Correll 1957), the learning of a delayed response task (Hirano 1966), spatial alternation (Greene and Lomax 1970, Henderson *et al.* 1973) or jump avoidance (Whishaw and Deatherage 1971). Most of these results are in accordance with the lesion data, as is the finding that procaine injections into the hippocampus facilitate two-way active avoidance learning (Weiss and Hertzler 1973).

12.3.2. STIMULATION AT CRITICAL STAGES OF LEARNING

The most promising application of stimulation involves its use only at some critical stage of the learning, memorization, or retention process. This application typically rests on the assumption that the storage of memory depends upon processes extended in time after the learning experience and that these processes might be separable into several distinct components. The simplest use of this methodology involves stimulation just after learning and the measurement of retention as an indicator of

* These results accord well with the lesion data. Bresnahan and Routtenberg used a step-down passive avoidance task, which rarely elicits deficits in lesioned rats. Henderson *et al.* used the runway task known to produce mixed effects. They report only latency data which often indicate a deficit after lesions, but do not report upon the number of contacts with the water spout, which is rarely increased in lesioned rats.

any hippocampal role in the storage process. More sophisticated, and more recent, applications involve quite discrete stimulation given for brief periods. In either case retention can be tested at various times after learning and/or stimulation, thus distinguishing between the effects of the treatment upon short-term and long-term memory processes.

Many of the early studies using stimulation in this way are subject to the criticisms lodged earlier: the use of inappropriate tasks, the propagation of after discharges, and so on. This makes some sense of the early work, in view of the likelihood of seizure propagation to the amygdala and the now widely accepted role of this structure in most of the tasks used in these studies (cf. Goddard 1964, Bresnahan and Routtenberg 1972). Kesner and Doty (1968), for instance, found deficits in passive avoidance learning only when their post-trial stimulation in the dorsal hippocampus caused after discharges in the amygdala. Stimulation in the ventral hippocampus, even when it produced after discharges in the dorsal hippocampus, did not influence learning. The authors concluded that

'while the amygdala thus seems to have a critical role in the mnemonic processes pertinent to the present study, the hippocampus probably does not' (p. 65).

Nyakas and Endröczi (1970) reached the same conclusion, as did Vardaris and Schwartz (1971).

In several studies spreading depression was elicited in the hippocampus just after training a conditioned emotional response (Avis and Carlton 1968, Hughes 1969, Kapp and Schneider 1971). In all of these studies liquid KCl was used, and propagation of depression beyond the borders of the hippocampus likely. Considerable seizure activity was noted in the first and third studies, which provided EEG records, while Hughes reported that 17 per cent of his subjects died from the injection. Though purporting to demonstrate that the hippocampus is involved in the processes underlying long-term memory storage, these studies actually show little more than the dangers inherent in the poorly controlled use of stimulation techniques.*

* Numerous difficulties arise in the interpretation of studies involving the injection of puromycin, an inhibitor of protein synthesis (e.g. Cohen and Barondes 1967, Cohen, Ervin, and Barondes 1966). Interference with memory storage seems related to the epileptogenic action of the drug, rather than to its suppression of macromolecular synthesis. In a carefully controlled series of studies Nakajima (1969, 1972) showed that deficits induced by actinomycin-D injected into the hippocampus, in a T-maze position habit, were related to the seizures induced by the drug, rather than to its effects upon RNA synthesis. Thus, deficits were dependent upon the injection-test interval rather than on the training-injection interval. Maximal disruption appeared only after four days, when seizure activity begins to appear in the hippocampus. RNA synthesis, on the other hand, was suppressed within a few hours of injection, but this did not seem to influence learning or retention. Nakajima (1969) suggested that propagation of seizures into the amygdala might be an important factor in the decrements observed. Such an explanation is even more compelling with regard to those early studies in the mouse which limited injections merely to the temporal area *in toto* (e.g. Flexner, Flexner, and Stellar 1965). We do not deny the likelihood that RNA and/or protein synthesis are

While these early studies, and others we have not discussed, were subject to basic methodological flaws, more recent work incorporates some of the necessary controls. A consensus seems to be arising from these studies that stimulation of the hippocampus has disturbing effects only at certain stages of the memorization process. Most of this work has concentrated upon one-trial passive avoidance tasks; an unfortunate choice, for the involvement of the hippocampus in such tasks, as we have seen, is rather variable.

Several studies employed step-down passive avoidance, a task usually unaffected by hippocampal lesions (see Table A23). Wilson and Vardaris (1972), using currents above threshold for eliciting seizures, found only a small deficit in this task. Zornetzer and Chronister (1973) and Zornetzer, Chronister, and Ross (1973), however, found that bilateral stimulation in the fascia dentata at subthreshold intensities could interfere with learning. Lastly, Kapp, Kaufman, and Repole (1974) report that step-down passive avoidance is *not* affected by post-trial stimulation, even when the current level is quite high and when there are bilateral fascia dentata placements. Clearly, no firm conclusions can be drawn from these data.

Sideroff *et al.* (1974) used a different form of passive avoidance, akin to a discriminated step-through task, and found that stimulation given either 10 s or 3 h after a single electric shock caused retention deficits, but only in terms of latency to respond. In two studies directly aimed at differentiating between short-term memory and long-term memory Kesner and Conner (1972, 1974) trained rats to lever press for continuous reward and then shocked the final lever press of the session, after which the lever was withdrawn and hippocampal stimulation applied. Retention after 1 min was good, but after either 4 min or 24 h there were deficits. This was expressed in terms of the number of lever presses and did not show up in the measure of latency to the first lever press.

In a recent extension of this work Kesner *et al.* (1975) showed that while deficits are present with stimulation-test intervals of up to 60 min, performance is normal at 180 min. If stimulation was applied a week after learning, then deficits were not seen at any stimulation-test interval. These data were all obtained in the lever press passive avoidance situation. In an active avoidance task only limited effects of stimulation were reported, and then only up to one day after stimulation. In a purely

involved in long-term memory storage, nor that the hippocampus undergoes such changes. In fact a wide variety of experiments had demonstrated biochemical changes in the hippocampus correlated with learning; many of these are discussed by Nakajima (1975). Precise study of the hippocampal role in long-term memory *demands* the use of behavioural tasks requiring locale participation and control over all the effects of drugs used, and very few of the studies presently available fit this description.

appetitive task, the acquisition of lever pressing, deficits were elicited by stimulation given one, but not seven, days after completion of training. It must be stressed that in all these studies the stimulation employed inevitably elicited hippocampal after discharges.

These latest results invert the conclusions reached from the earlier work and suggest that hippocampal seizures can interrupt some intermediate memory stage. However, without controls providing information about the spread of these seizures it is impossible to state that the effects are due to an interruption of hippocampal function, as Kesner et al. (1975) do.*

Another study investigating the effects of post-trial stimulation upon avoidance learning is worth mentioning. Landfield, Tusa, and McGaugh (1973) trained rats on one-way active avoidance and stimulated them 5 s after a response; in a second study a discriminated avoidance task was used. In both studies the stimulated rats were significantly better than non-stimulated controls with electrodes implanted and the authors use this difference to justify the conclusion that hippocampal stimulation facilitates the consolidation of memory. However, there was no difference between stimulated rats and unoperated controls in either task. There seems to be no justification for their conclusion.**

Finally, Livesey and Wearne (1973) reported large deficits in rats trained on a simultaneous brightness discrimination and stimulated either just after a response or throughout the training trials. This deficit was related to the use of a maladaptive position habit, and is thus consistent with results seen in several lesion studies. Livesey and Meyer (1975) have replicated this effect using more restricted stimulation conditions; the maximally effective treatment involved stimulation during the choice period of each trial. All rats were then trained to criterion without stimulation and retested with stimulation. Decrements were seen in some, but not all, the subjects. The authors noted, but failed to comment further upon, this dichotomous result. Once again, deficits were related to the adoption of perseverative position habits. Livesey and Bayliss (1975), however, have shown that stimulation in the fascia dentata does not interfere with the learning of this task, though it can disrupt reversal. Here, the deficit was similar to that produced by lesions; no difficulty in giving up the old response, but the adoption of a maladaptive position habit.

Though much of this stimulation work is intriguing, it is plain that

* Zornetzer and Chronister (1973a) have reported the use of a food-finding task which seemed to involve place learning. Here, stimulation after a trial caused a clear deficit. It would be useful to have some data on the short-term and long-term memory characteristics of this effect.

** Zornetzer, Boast, and Hamrick (1974) have also shown that the mere implantation of an electrode in the hippocampus can lead to deficits in one-trial passive avoidance. It is possible that stimulation merely alleviates the irritating effects of electrode implantation.

clear-cut results are not yet available.* ** While we would agree that stimulation techniques can be useful and that real effects may have already been demonstrated, we would argue that at present there are too many unknowns in these studies to allow for any straightforward conclusions. In constructing a theory of hippocampal function based on the effects of its disruption it would seem better to rely upon lesion data at present.

* Deutsch and his collaborators (e.g. Deutsch, Hamburg, and Dahl 1966, Deutsch and Leibowitz 1966, Wiener and Deutsch 1968) have reported that various changes in retention are a function of changes in cholinergic systems, which can be manipulated by anti-cholinergic and anti-cholinesterase drugs injected into the hippocampus. In a recent study George and Mellanby (1974) have shown that the carrier used in such studies (peanut oil) can, by itself, affect memory. This throws the earlier work into some doubt, and shows once again the problems associated with stimulation and injection techniques.

** Many of the studies discussed in this chapter are explored at greater length in the review article by Nakajima (1975).