Memory Disruption by Electrical Stimulation of Substantia Nigra, Pars Compacta

Abstract. *Electrical stimulation of the substantia nigra, pars compacta, of albino rats while they were learning a simple foot shock task of withdrawal and response suppression disrupted retention of that task 24 hours after original learning. Stimulation in the reticular zone of the substantia nigra was without effect on retention performance. Stimulation through electrodes in the medial lemniscus, red nucleus, or brainstem regions surrounding the substantia nigra, pars compacta, was also ineffective. Original learning performance, measured as time to criterion, was unimpaired by the stimulation. Posttrial stimulation in the substantia nigra, pars compacta, but not in adjacent structures, also disrupted retention performance.*

Memory may take form as a result of activation of specific subcortical and cortical pathways after the occurrence of an event (*1*). If this physiological activity in specific pathways could be disrupted, memory formation should be blocked. Evidence compatible with this view comes from experiments on the effect of electroconvulsive shock on memory (*2*), in which such neural pathways were probably disrupted. Regional brain stimulation (*3, 4*) has been used with success to disrupt memory in an effort to achieve a more precise anatomical statement of the pathways involved in memory consolidation.

Although stimulation of certain brain regions such as the amygdala (*5*), hippocampus (*6*), and caudate nucleus (*7*) leads to memory disruption, the particular nuclear groups within a structure which are responsible for disruption have not been identified. Recently, however, we found that low-level unilateral, subseizure stimulation of the medial nucleus of the amygdala had a disruptive effect on retention of a learned withdrawal task (*8*). Since memory disruption was not complete, it seems likely that other brain regions are involved in learning and retention of the withdrawal task. In this report we suggest that one other such structure is the substantia nigra, pars compacta.

Subjects were 79 male adult albino rats (Holtzman Research, Madison, Wisconsin), weighing 250 to 350 g at the start of the experiment. Of these, 15 served as unoperated controls. The remaining 64 animals were deprived of food for 24 hours before surgery; pentobarbital sodium (50 mg per kilogram of body weight) was the anesthetic. Bipolar electrodes made of 254-μm enamel-insulated Nichrome wire were aimed at the ventral midbrain tegmentum in the region of the substantia nigra. Following a 1-week week recovery period, animals were tested according to the schedule described by Bresnahan and Routtenberg (*8*), as follows. Immediately before the training session, a spring-clip connector was attached to the bipolar electrode of the animal, and sine-wave 60-hertz peak current of 10, 15, 20, or 25 μa was applied (*9*). The rat was then placed on the platform of the training apparatus, which had an electrified grid floor that delivered 0.5 ma to the feet of the animal when it stepped down from the platform. In order to preclude electrical interaction between foot shock and brain stimulation, a microswitch under the platform was used so that brain stimulation and foot shock were never given at the same time; in addition, both sources were isolated with a separate transformer and were thus floating with respect to ground. The apparatus was 27.9 cm long, 27.3 cm wide, and 31.8 cm high; a platform 7.6 cm wide and 9.5 cm above the grid floor extended 27.6 cm across one end of the apparatus.

The initial learning performance of both operated and unoperated control subjects and experimental animals was similar. During original learning, animals typically descended in the first 10 seconds (75 of 79 animals descended in less than 10 seconds), received the 0.5-ma foot shock, and climbed back on the platform within 5 to 10 seconds. Animals invariably received a second shock before remaining on the platform for the 2-minute learning criterion period. Some animals descended three or four times before reaching criterion. This criterion was used in an attempt to equalize the degree of learning in all animals.

Because we wished to apply brain stimulation just after the initiation but before termination of the trial, animals received the brain stimulus before placement on the platform and during the period while they were on the platform. Brain stimulation was delivered each time the animal ascended the platform after receiving foot shock and during the 2-minute learning criterion period. Since animals received only a few seconds of foot shock before climbing back on the platform, the total time during which brain stimulation was given was about 20 seconds less than the total time to reach the learning criterion (Table 1).

Retention was evaluated 24 hours later by placing the animal on the platform with electrode clip attached and determining if it would descend within a 3-minute retention test period. Since 39 of 40 unoperated animals in our previous study (*8*) remained on the platform for the entire test period, and 14 of 15 did so in the present experiment, we consider retention unimpaired when the animal remains on the platform for 180 seconds and consider it impaired when the animal descends in less than 180 seconds.

Animals were tested for retention for four additional days. With the same levels of brain stimulation used during the memory experiments, selected subjects were tested for rewarding or aversive motivational effects in a tilt cage and for intracranial self-stimulation (ICSS) in a Skinner box as described (*10*). Both approach to and escape from brain stimulation was measured in the tilt cage, while active seeking of the stimulus was determined in the Skinner box. The criterion for approach or escape was receiving or escaping more than two-thirds of the delivered stimuli. Intracranial self-stimulation rate was defined as before (*10*), in responses per 15 minutes: neutral, 0 to 49; low, 50 to 199; and medium, 200 to 499. In a separate test, the behavior of some subjects was subsequently observed and quantified by time-sampling procedures before, during, and after brain stimulation to determine whether stimulation had any noticeable motor effects (*11*). Following completion of testing, each animal was killed with an overdose of barbiturate, the brain was fixed in formalin, and frozen sections were taken and stained for Nissl substance or myelin to identify the location of the electrode tip; this was done without knowledge of the behavioral results.

We found that electrical stimulation of the substantia nigra, pars compacta (SNC), led to an impairment in performance during the retention test, whereas stimulation of an adjacent nuclear group, substantia nigra, pars reticulata (SNR), or of the adjacent fiber tract, the medial lemniscus, had

no disruptive effect on performance. Some placements outside of the SNC did give rise to disruption, but the number of cases was insufficient to evaluate the significance of this disruption in relation to that at the SNC.

Electrode locus (Fig. 1) was classified according to the deepest penetration of the electrode tip. If the tip was not located in the SNC, SNR, or medial lemniscus, placements were classified in the brainstem category, which included the field of Forel, medial forebrain bundle, red nucleus, ventral tegmentum, midbrain reticular formation, brachium of the inferior colliculus, and brachium conjunctivum. Electrodes of operated controls were in the SNC, SNR, medial lemniscus, red nucleus, or midbrain reticular formation. The total time to reach the criterion of learning did not differ among groups (Table 1); a one-way analysis of variance was not statistically significant ($F = .91$; d.f. $= 5$, 73; $P > .20$). There was no difference in total time to learn between animals stimulated in the SNR and those stimulated in the SNC ($F = .02$; d.f. $= 1$, 24; $P > .20$). Finally, during training there was no difference among groups with respect to latency of the first descent ($F = .92$; d.f. $= 5$, 73; $P > .20$) or total number of descents before criterion was reached ($F = .29$; d.f. $= 5$, 73; $P > .20$). Thus, whatever the effects of brain stimulation on subsequent retention, they could not be related to an alteration in the degree of original learning as measured by the total time to learn, initial descent latency, or number of descents.

After 24 hours, an obvious decrement in first-descent latency was seen in animals with electrodes associated with the SNC; this decrement in latency is taken as an impairment in memory. Without exception, electrodes in the SNC gave rise to disruption. Stimulation in adjacent brain structures during learning had no effect on retention 24 hours after learning. An overall analysis of variance of first-descent latency on day 1 of retention testing was significant ($F = 16.4$; d.f. $= 5$, 73; $P < .001$), as were the individual comparisons between stimulation to SNC and brainstem ($F = 22.4$; d.f. $= 1$, 26; $P < .001$) and between stimulation to SNC and SNR ($F = 34.6$; d.f. $= 1$, 24; $P < .001$). No difference existed between animals receiving brainstem stimulation and operated controls ($F = 1.57$; d.f. $= 1$, 24; $P > .20$). On days 2 to 5, first-descent performance of animals given SNC stimulation was similar to the performance of animals with medial amygdala electrodes [figure 10 in (*8*)]. These results indicate that electrical stimulation of the SNC during learning led to disruption of retention 24 hours after the task was learned. Stimulation in another nuclear group of the substantia

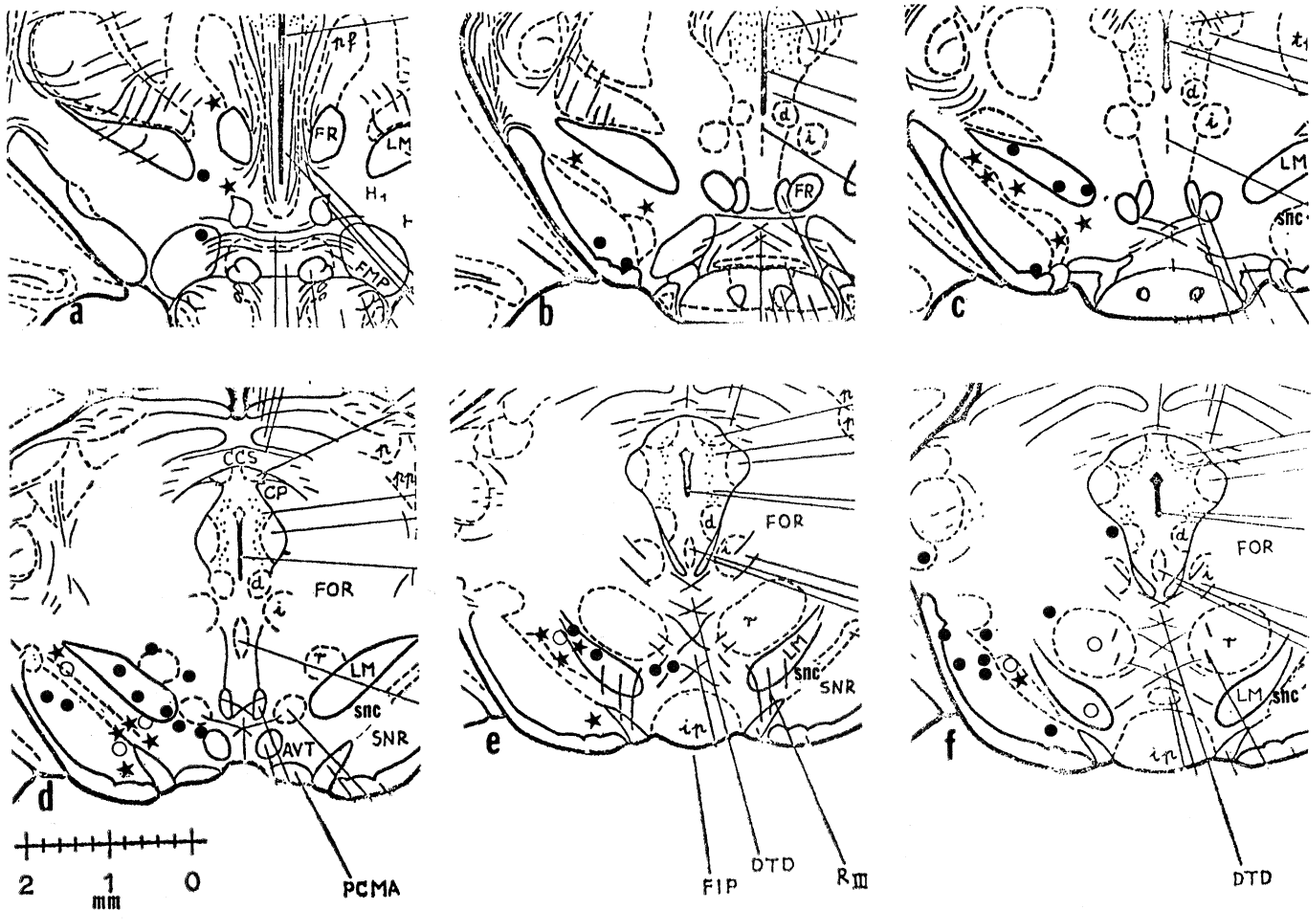

Fig. 1. Electrode placements in subjects with disruption of retention (stars) or no disruption (solid circles), and in operated control subjects (open circles). Electrodes in three operated controls and five subjects with brainstem placements were posterior to drawing (f) and are not shown. Drawings are taken from König and Klippel (*19*): (a) figure 42B; (b) figure 44b; (c) figure 45b; (d) figure 46b; (e) figure 48b; and (f) figure 49b. Selected abbreviations: *SNC*, substantia nigra, pars compacta; *SNR*, substantia nigra, pars reticulata; *LM*, medial lemniscus; *FOR*, midbrain reticular formation; *r*, red nucleus; *FMP*, medial forebrain bundle; *FR*, fasciculus retroflexus; *pf*, parafascicularis nucleus; and *ip*, interpeduncular nucleus.

nigra, the reticular zone, had no disruptive effect on retention performance, even though the groups given SNC or SNR stimulation did not differ in the amount of time to reach criterion during original learning.

The memory disruption associated with SNC stimulation was not complete amnesia. The difference between the 5.4-second mean latency during original learning and the 33.2-second latency on day 1 of retention testing was statistically significant ($t = 3.35$; d.f. $= 12$; $P < .01$). Thus, while the memory deficit following SNC stimulation was clear, the performance during retention testing suggested that some aspects of the original learning were not entirely forgotten.

Because the stimulation was applied throughout the learning trial in somewhat different patterns for different animals, in another experiment we delivered brain stimulation for 2 minutes immediately after the animal reached the 2-minute learning criterion. The animal remained on the platform during this additional 2-minute period. This procedure also permitted an assessment of the effects of posttrial stimulation on retention (*2*). Eleven animals were implanted with electrodes aimed at the ventral tegmentum, and ten animals were unoperated controls. Five of the implanted animals had electrodes in the SNC and six had electrodes in surrounding regions. There were no significant differences in time to learn among the three groups ($F = .47$; d.f. $= 2, 18$; $P > .20$). During retention testing 24 hours later, the mean descent latency for the SNC group was 79.8 seconds, whereas that for each of the other two groups was 180.0 seconds. This difference was significant ($F = 7.1255$; d.f. $= 2, 18$; $P < .01$). Thus, immediate posttrial stimulation of the SNC disrupted retention performance 24 hours after learning.

The ventral tegmentum, particularly the region of the substantia nigra, in addition to its important role in motor coordination, may be involved in learning and memory. Several studies on the effect of brain lesions on learning have implicated the substantia nigra (*12*). Studies on the effect of brain stimulation on memory have shown disruptive effects from one projection field of the SNC, namely, the caudate and putamen complex (*7*). Recording from neurons in awake, freely moving animals, Olds *et al.* (*13*) implicated this region in the early stages of learning. Finally, in a pharmacological study, it was shown that manipulation of the concentration of the dopamine transmitter in the SNC can alter the learning and performance of cats in a delayed response task (*14*). These results and the present study suggest a relation between monoamine-containing neurons and memory consolidation processes (*15*). Taken together, they provide a basis for the view that the SNC may be active physiologically during learning and that this activity, along with that in other brain structures (*8*), leads to memory consolidation.

Table 1. Effects of stimulation at different brain loci during original learning on subsequent retention. Means and standard deviations are given; *N*, number of rats.

Locus	*N*	Original learning: time to criterion (seconds)	24-hour retention descent latency (seconds)
Substantia nigra, pars compacta	13	170.5 ± 57.9	33.2 ± 36.7
Substantia nigra, pars reticulata	13	173.4 ± 27.9	175.6 ± 11.7
Medial lemniscus	12	174.8 ± 47.6	165.4 ± 50.2
Brainstem	15	206.2 ± 70.1	153.6 ± 58.9
Operated controls	11	195.2 ± 65.3	171.8 ± 27.1
Unoperated controls	15	181.7 ± 49.2	169.7 ± 40.0

The present results do not allow any detailed discussion of the mechanism of the observed disruption. It is not likely to be related to epileptiform activity, because 60-hertz sine-wave stimulation of the substantia nigra at 140 μa, nearly six times the current we used, did not produce afterdischarges (*16*). Nor do the motivational consequences of the stimulation appear to play a role in the present study; in animals in which disruption occurred, either neutral, low, or moderate rates of ICSS were found. In animals without disruption, ICSS at the same current used in the memory experiment was present in some animals and absent in others. No escape behavior was demonstrated in the tilt cage. This lack of relation of memory disruption to ICSS behavior is consistent with our previous study (*8*). Also, the disruption cannot be related to the motor effects of stimulation, since none were observed in our time-sampling tests at the currents used. In addition, when retention was tested no brain stimulation was used.

While we do not claim to have ruled out all competing hypotheses related to mechanisms other than memory, we think it reasonable to propose that stimulation of the SNC disrupts the normal physiological activity that occurs during the original learning situation (*17*). Whatever the mechanism for the disruption, the present results emphasize that memory disruption can be obtained by stimulation of restricted loci in the mammalian central nervous system with low current. It is a prerequisite, perhaps, that the latter be used to demonstrate the former. We do not believe that the SNC is the site of memory storage, but it may be viewed as part of a system important for the memory storage of processed inputs and executed responses (*18*).

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17. The physiological activity may be related, in part, to the response characteristics required of the animal; in this case, it is withdrawal and suppression of the natural tendency to step down from a platform. This disruption may take the form of simultaneous activation of SNC and other fiber pathways, such as those which we have described from the lateral hypothalamus [Y. Huang and A. Routtenberg, *Physiol. Behav.* **7**, 419 (1971)], which perforate through the SNC. The disruption could occur because simultaneous activation of SNC and descending lateral hypothalamic fibers normally does not occur. Another possibility is that the efferent nigral outflow to the ipsilateral midbrain tegmentum and tectum [R. Y. Moore, R. K. Bhatnager, A. Heller, *Brain Res.* **30**, 119 (1971)] is responsible for the disruption, since Glickman (*3*) showed that direct stimulation of midbrain tegmentum could cause impairments in performance in a passive avoidance task.
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Hibernation: Effects on Memory or Performance?

McNamara and Riedesel (*1*) report that ground squirrels which hibernate during an 11-day period of exposure to cold perform better on a test designed to measure retention of a previously learned visual discrimination than do animals which do not hibernate during the cold retention interval. They interpret this result in terms of an alteration in memory. This interpretation is interesting because it extends previous research indicating that in some cases exposure to cold facilitates retention of learning in poikilotherms (*2*). However, there are deficiencies in McNamara and Riedesel's experimental design which make alternative interpretations possible.

After an initial 2-week period of adaptation to the experimental situation and a 1-week rest, all animals were trained on a visual discrimination for 7 weeks, after which they received 8 weeks of reversal training before being subjected to the first of two 11-day periods of exposure to cold. Thus, all the animals received exactly the same amount of training. However, equivalent amounts of training do not guarantee equivalent amounts of learning. If the animals that subsequently hibernated learned the reversed discrimination more thoroughly than those that did not hibernate, the observed difference in retention performance could be interpreted more parsimoniously in terms of this initial difference than in terms of any supposed effect upon memory. McNamara and Riedesel state that "before the cold-exposure periods there were no differences between those animals that later hibernated and those that did not hibernate ($F = 4.49$, d.f. = 1,16, $P > .05$)." This conclusion is apparently erroneous, since the stated value of the F statistic is equal to the critical value for rejection of the null hypothesis at the .05 level of significance (*3, 4*). Thus, it is quite likely that the difference in retention-test performance can be accounted for by a difference in degree of learning.

However, even if there were no difference in performance at the end of training, it would still be unsafe to assume that the hibernators and nonhibernators had learned the task equally. For example, if the hibernators had learned more rapidly than the nonhibernators, they would have been overtrained to a greater degree than the nonhibernators; and this differential overtraining could account for the observed difference in retention-test performance (*5*). To ensure equivalent degrees of initial learning and to avoid differential overtraining, McNamara and Riedesel should have trained each of their animals to the same criterion of initial correct performance. For example, training for each animal might have been discontinued when it first reached the criterion of eight correct responses in ten successive trials.

Another uncontrolled variable is the differential effect of exposure to cold upon the general physical condition of hibernators and nonhibernators and possibly upon their level of motivation (*6*). "All animals spent the same amount of time in the cold. For the first few days the animals had free access to food. Subsequently, food was withdrawn in varying amounts to encourage hibernation. Some animals hibernated while others did not." Since hibernation reduces metabolic rates and conserves bodily stores of nutrients and since the nonhibernators were not fed for some unspecified but apparently significant portion of the cold 11-day retention interval, it seems certain that during the retention test the nonhibernators must have been thinner and generally in poorer physical condition than the hibernators. Indeed, McNamara and Riedesel themselves recognize that "the cold environment acted as a stressful situation to awake animals." The differential stress to which hibernators and nonhibernators were subjected may have left them unequally able to perform the discrimination. Alternatively, the two groups of animals may have been unequally motivated to escape from the mixture of water and detergent which was their "reward" for correct performance. Clearly, a control experiment is needed to determine whether the general aftereffects of their stressful experience could have produced the relatively poor retention-test performance of the nonhibernators before differences in memory are posited as an explanation. The necessary control experiment would be a comparison of acquisition of the visual discrimination by previously untrained hibernators and nonhibernators that had just been exposed to cold for 11 days.

Finally, even if the other necessary control procedures had been followed and it was clear that some kind of memory effect had been found, the fact that McNamara and Riedesel's animals were tested for retention of a reversed discrimination would make the effect difficult to interpret. When animals make errors on a reversed discrimination, they do so by responding in the fashion that was correct in original learning. Thus, unless a control group of animals is tested for retention of the unreversed discrimination and comparisons are made between the performance of these controls and that of the reversed animals, it is impossible to tell whether animals that make errors in reversed discrimination have forgotten both of their training experiences or only the second one.

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4. All the F values reported by McNamara and Riedesel are exactly equal to the critical values for the .05 level of significance with the corresponding degrees of freedom. Moreover, the inconsistency with which they interpret these F values is also puzzling. Some are interpreted as giving $P > .05$, as in the instance cited; others are interpreted as giving $P < .05$. Since McNamara and Riedesel list critical values labeled