SHORT TITLE:

SUBCORTICAL CHEMICAL INJECTION AND CONSOLIDATION OF LEARNING

THE EFFECT OF SUBCORTICAL CHEMICAL INJECTION ON THE CONSOLIDATION OF LEARNING

by

Elizabeth Barkentin Gardner

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Department of Psychology
McGill University
Montreal.

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INTRODUCTION

There is evidence that the long-term memory of an event is established by processes that continue in the nervous system for a period of time after the event has occurred. Müller and Pilzecker (1900) postulated the existence of such a consolidation process as an explanation for retroactive inhibition, which is caused by the learning of interpolated material.

McDougall (1901) suggested that retrograde amnesia, loss of memory for events just preceding a blow on the head or other insult to the nervous system, might be explained in terms of interference with consolidation. Among the causes of retrograde amnesia in human patients listed by Russell and Nathan (1946) are concussion, epilepsy, meningitis, acute cerebral anoxia, carbon monoxide poisoning, severe loss of blood, and electro-convulsive shock (ECS).

Duncan (1949), using ECS, showed that retrograde amnesia could be produced in rats and attempted to measure the duration of the consolidation process by giving ECS at various times after learning. He found maximum interference with learning when ECS was given 20 seconds after each trial, the interference becoming progressively less

until, when ECS was delayed for an hour, there was no significant effect.

The theoretical framework for understanding consolidation suggested by Müller and Pilzecker is still commonly accepted, in a slightly modified form. They suggested that following an event or a stimulus, a pattern of neural activity which was established during the stimulus continues for a time, and this activity serves to "fix" the event in memory. Hebb (1949) and others have modified this hypothesis by suggesting that information is held in reverberating patterns of neural activity until more permanent changes can occur (at the synapses, or, as recently suggested, in the neurons).

The disruptive effects on memory of many agents have primarily been interpreted, following Müller and Pilzecker, in terms of a disturbance of patterned neural activity. More recently it has been recognized that although many treatments that disrupt consolidation affect patterns of neural firing, they also affect other aspects of neural functioning, and in some cases disruption of consolidation may be attributable to one of these other effects. Any treatment that greatly alters the rate of neural firing could be expected to disrupt firing patterns, but treatments

that increase firing would also affect, for instance, metabolic functions, blood flow, local oxygen availability, pH, local temperature, electrical resistance, and steady electrical (D.C.) potential gradients. It seems likely that the rate of synthesis of proteins and nucleic acids by individual cells might be affected as well. Because of this multiplicity of effects of most experimental manipulations, it is difficult to isolate causes for the disruption of consolidation. Nevertheless, it is possible that various methods of interfering with consolidation may have common modes of action. An examination of the physiological and biochemical effects of treatments or agents which disrupt consolidation might clarify the nature of the consolidation process.

Treatments that Disrupt Consolidation

Electroconvulsive shock is a rather extreme treatment which produces massive firing and a multitude of interconnected physiological effects, among which are hypoxia (Himwich & Fazekas, 1942), hemorrhage (Madow, 1956), and a shift in D.C. potential (Goldring, Ulett, O'Leary, & Greditzer, 1950). ECS has usually been found to be very effective in disrupting recent learning, but it is not unanimously agreed that this is due to interference

with consolidation. Alternatively, it has been suggested that ECS acts as negative reinforcement (Coons & Miller, 1960; Miller & Coons, 1955), or that inactivity becomes conditioned to the ECS (Adams & Lewis, 1962a; Adams & Lewis, 1962b; Lewis & Maher, 1965).

These suggestions may explain part of the effect of ECS in certain experiments, but the universality of these hypotheses as explanations for the effect of ECS is limited, as shown by the evidence for disruption of consolidation obtained with ECS in one-trial passive avoidance learning experiments, in which the consolidation and alternative hypotheses point to different predictions (Bureš & Burešová, 1963; Chorover & Schiller, 1965; Chorover & Schiller, 1966; Heriot & Coleman, 1962; King, 1965; Madsen & McGaugh, 1961; McGaugh & Alpern, 1966; Pearlman, 1966; Quartermain, Paolino, & Miller, 1965; Weissman, 1963; Weissman, 1964). In these experiments, if ECS were acting as negative reinforcement, it would presumably summate with peripheral shock, making it less, not more, likely that the animals would respond in the retention test. The conditioning explanation also predicts that the animals receiving ECS would be less likely to respond. These one-trial passive avoidance learning experiments, then, indicate that ECS does indeed

disrupt consolidation.

Chorover and Schiller (1965), using a one-trial, step-down, passive avoidance situation, found that at intervals of less than 10 seconds between foot shock and ECS, the degree of impairment varied inversely with the foot shock - ECS interval (and that ECS alone at comparable intervals had no such effect). However, they found that ECS was ineffective when administered 30 seconds after the shock. In contrast, other investigators have found ECS to disrupt consolidation when administered as late as one hour or more after learning trials (Bureš & Burešová, 1963; Gerard, 1955; Hayes, 1953; Heriot & Coleman, 1962; Leukel, 1957; Tenen, 1965a; Thompson & Dean, 1955). Chorover and Schiller (1966) suggest that the effectiveness of ECS at longer intervals in other passive avoidance experiments may be accounted for by a decrease in locomotor inhibition produced by ECS, rather than a disruption of consolidation per se. Although it is not clear how ECS might act on the nervous system to produce a decrease in locomotor inhibition, Vanderwolf (1963) reports a related finding, that ECS reduced the tendency of animals to "freeze" in an avoidance situation. Similarly, Routtenberg and Kay (1965) found some indication that one noncontingent ECS decreased the latency with which

rats stepped down from a platform. However, as this result was significant only once in three replications, and as one ECS also produced, conversely, decreased open-field responding, verification of the former result would be desirable.

Although the studies of Chorover and Schiller raise the question of whether ECS, even in one-trial passive avoidance situations, may in some cases be acting not on the memory trace, but on some other component of the response, neither the hypothesis that ECS decreases locomotor inhibition and thereby increases responding, nor the negative reinforcement, nor conditioned immobility hypotheses can explain deficits produced by ECS in experiments using discrimination learning (e.g. Bureš & Burešová, 1963; Corson, 1963; Hayes, 1953; Thompson & Dean, 1955). Another experiment in which the results are not easily explained by any of these hypotheses is that of Tenen (1965a; 1965b). Tenen reports that water-deprived rats given ECS one hour after receiving water for a brief period at a hole in the side of a box explored the hole less than did rats given foot shock but no ECS. The hypothesis that ECS decreases locomotor inhibition would require a prediction of more responding, not less. It is

also doubtful that negative reinforcement or conditioned immobility would be associated with the response in view of the length of the response-ECS interval. Tenen's experiments and experiments using discrimination learning provide further evidence that ECS can disrupt consolidation, and not necessarily only within 10 seconds of the response.

There is some indication that the disruptive effect of ECS on consolidation may be due primarily to the passage of electrical current, rather than to the occurrence of a convulsion (Gottlieb & Wilson, 1965; McGaugh & Alpern, 1966). The physiological effects of ECS are so multitudinous, however, that it would be folly to single out any one as the active factor - at least on the basis of ECS experiments alone.

In addition to electric shock, auditory stimulation (in some animals) or injection of drugs can also cause convulsions. Audiogenic seizure has been shown to disrupt consolidation (Essman & Hamburgh, 1962). Metrazol-induced convulsions were shown by Pearlman, Sharpless, and Jarvik (1961) to completely suppress avoidance responding when administered at 2, 4, or 8 hours after the shock, and to produce a significant deficit when administered at four days. It is not clear, however, whether the effect of

Metrazol at four days reflects a disruption of consolidation or of performance; the authors point out that the rats at the time of retention testing (24 hours after the convulsion) may have been in a confused state, similar to that which follows convulsive "therapy." A further experiment in which retention is tested 48 hours after the convulsion might clarify this point.

Most of the physiological and biochemical effects of convulsions elicited by ECS also accompany convulsions elicited by the injection of drugs or by auditory stimulation. They include massive neural firing, and changes in cortical steady D.C. potentials (Goldring, Ulett, O'Leary, & Greditzer, 1950; Vanasupa, Goldring, & O'Leary, 1959).

D.C. potential changes are also a conspicuous feature of spreading depression (SD). Grafstein (1956) finds that cortical SD starts with a short burst of neural firing. This is followed by depression of both spontaneous and evoked neural activity - a prolonged refractory period. The depression spreads across the cortex at 3-6 mm./min., accompanied by a slight positive and then a 5-20 mV. surface negative shift of the steady D.C. potential, which is replaced, after about a minute, by a positive potential shift of longer duration and smaller amplitude.

However, the initial burst of firing is not always seen (Burešová, Shima, Bureš, & Fifková, 1963), the period of marked depression of cortical activity is sometimes replaced by paroxysmal discharge (Winokur, Trufant, King, & O'Leary, 1950), and the depression does not necessarily spread (Marshall, 1959). Marshall (1959) cautions that the only sure diagnosis of SD is the correlation between depression of evoked potentials and the characteristic steady potential change.

SD has been elicited in several structures in addition to the cortex, but the only structures in which it has been used to disrupt consolidation are the hippocampus (Bureš, 1959) and the cortex (e.g. Albert, 1966a; Bureš & Burešová, 1963). Albert (1966a) used SD to disrupt the consolidation of the interhemispheric transfer of an active avoidance response. He found that there was a relationship between the learning - treatment interval and the length of the period of SD required to disrupt consolidation. Albert (1966a) also found that a short period of SD, although without effect in itself, lengthened the time during which a longer period of SD was effective in disrupting condolidation.

The physiological concommitants of SD are almost

as numerous as those of ECS. SD is accompanied by metabolic changes, such as a decrease in cortical glycogen, free glucose, and phosphocreatine, and an increase in lactic acid (Křivánek, 1962). SD remains primarily confined to the structure or hemicortex in which it is elicited (Bureš, 1959; Bureš & Burešová, 1960; Fifková, 1966; Weiss & Fifková, 1960), although neocortical SD spreads to the amygdala and sometimes to the caudate nucleus (Fifková & Syka, 1964). However, electrical activity is altered in regions of the brain to which SD does not actually spread. Spontaneous and evoked EEG activity is decreased in the thalamus and hypothalamus (Weiss & Fifková, 1961). Reticular formation EEG is unchanged during cortical SD in unanesthetized rats (Weiss, 1961), but the activity of a large percentage of single reticular neurons is altered (mostly increased) during cortical SD (Burešová, Bureš, & Fifková, 1962).

Another treatment which interferes with consolidation is cathodal (surface negative) electrical polarization of the cortex. Albert (1966a; 1966b) found that the disruptive effects of spreading cortical depression on the consolidation of interhemispheric transfer of an active

avoidance response were closely paralleled by those of cathodal polarization of the medial part of the cortex. Electrical potential gradients are thought to affect the excitability of neurons (Goldring & O'Leary, 1951; Marshall, 1959), but aside from this, little is known of their physiological or biochemical effects.

Hypoxia has also proved to be effective in disrupting consolidation. Hayes (1953) found that both ECS and hypoxia (produced by manual compression of the rat's chest) were effective when given 1 hour after each single daily trial on a 3-choice T maze. Ransmeier and Gerard (1954) reported that both ECS and hypoxia produced retention deficits when administered 12 hours after learning. In contrast, Thompson and Pryer (1956) found that hypoxia equivalent to an altitude of 20,000 feet, maintained for 10 minutes, disrupted consolidation only when administered within 4 minutes after the rats had reached criterion, even though Thompson and Dean (1955) had found ECS to be effective at 1 hour. Similarly, Leukel and Quinton (1964) found that a two-minute period of CO_2-produced hypoxia was effective only when started within 10 sec. of each trial, and that the CO_2 treatment acted as negative reinforcement. Thompson (1957), however, varied the degree of hypoxia by

simulating 20,000 and 30,000 foot altitudes. His findings suggest that the discrepancy in effective intervals may be attributable to differences in degree of hypoxia.

It is difficult to assess the role of hypoxia in the disruption of consolidation. Hypoxia is one of the concommitants of ECS. It has also been shown that severe cortical hypoxia produces a negative steady potential shift with a latency of about two minutes (Meyer & Denny-Brown, 1955). To further complicate the interpretation of the mechanism by which hypoxia disrupts consolidation, Marshall (1959) states that hypoxia "probably conditions for SD and under appropriate conditions triggers the reaction."

Anesthetics sometimes have a weak effect on consolidation if they are administered promptly after learning takes place. Abt, Essman, and Jarvik (1961) report a deficit in the retention of a one-trial passive avoidance response with ether anesthesia administered as late as 20 minutes after the shock. Pearlman (1966) reports that ether anesthetization 10 minutes after the shock in a one-trial passive avoidance task is as effective as a short period of SD in disrupting consolidation. Pearlman et al. (1961), however, using a different one-trial passive avoidance task, found ether

effective at 5 minutes but not at 10 minutes. In the same study, Pearlman et al. found that sodium pentobarbital anesthesia begun within 10 minutes produced a deficit which was greater than that produced by ether at 5 minutes. On the other hand, Ransmeier and Gerard (1954) report that neither ether nor Nembutal anesthesia produced any effect one to two minutes after learning (time of onset of unconsciousness is unspecified), although ECS and hypoxia produced deficits when administered 12 hours after learning. Similarly, Leukel (1957) found only a weak effect with sodium pentothal anesthesia administered 6 minutes after each trial, whereas ECS at 2 hours produced a significant deficit. Bureš and Burešová (1963) found no effect of ether anesthesia immediately after learning, although ECS produced a deficit at 6 hours. Differences in task, and length, depth, and time of onset of anesthesia may account for much of the variation in results, but in any case, the disruptive effects of anesthesia are evidently not strong.

Heat narcosis in goldfish (Cerf & Otis, 1957), tranquilizers (Doty & Doty, 1964), and an anticholinesterase drug, diisopropyl fluorophosphate (Deutsch, Hamburg, & Dahl, 1966), have also been reported to disrupt consolidation.

It is a well-established finding in learning experiments, particularly in those using human subjects, that the learning of new material can interfere with the retention of previously-learned material (Müller & Pilzecker, 1900). Little is known about the physiological nature of this interference, however, or its relationship to the process of consolidation.

Treatments that Facilitate Consolidation

Several stimulants have been reported to facilitate consolidation. Paré (1961) found improved retention of the learning of a visual discrimination after administration of caffein. McGaugh and co-workers have demonstrated improved learning with post-trial administration of strychnine sulphate (McGaugh, Thomson, Westbrook, & Hudspeth, 1962), picrotoxin (Breen & McGaugh, 1961), physostigmine (Stratton & Petrinovich, 1963), and 1-5-diphenyl-3-7-diazadamantan-9-ol (McGaugh, Westbrook, & Burt, 1961; McGaugh, Westbrook, & Thomson, 1962; Westbrook & McGaugh, 1964). Similar results have been reported for low doses of Metrazol (Irwin & Benuazizi, 1966).

The primary evidence that injection of stimulants can affect consolidation is the finding of McGaugh, Thomson,

Westbrook, and Hudspeth (1962) that strychnine decreased the number of errors made in a maze when injected within 15 minutes after each trial, but not at 30 or 90 minutes. Cooper and Krass (1963), however, found that rats injected with strychnine sulphate 3 days before maze training were superior to untreated rats. On the basis of these results, they suggested that strychnine administered after each trial acts, not upon the consolidating trace of the preceding trial, but rather upon the learning which takes place during the next trial. As these two sets of results seemed inconsistent, Greenough and McGaugh (1965) devised an experiment to settle the issue. Rats were given 2 trials in a maze, followed 1 week later by 5 retention trials. Strychnine was injected 2 days before the training trials, immediately after the training trials, or 2 days before the retention trials. The prediction of the residual effect hypothesis, that the performance of the first and third groups would be superior, was not borne out. Neither was the immediate post-trial injection group significantly superior, although the differences were in this direction. Female rats were more affected by strychnine than were males. Greenough and McGaugh attribute the negative results to an incorrect dose level

for the strain (Sprague Dawley) of rats used. This study illustrates that improved consolidation due to administration of stimulants is an elusive phenomenon, vastly sensitive to dosage (Breen & McGaugh, 1961), strain differences (Breen & McGaugh, 1961; Greenough & McGaugh, 1965; McGaugh, Westbrook, & Burt, 1961; Stratton & Petrinovich, 1963), and sex (Greenough & McGaugh, 1965; McGaugh, Thomson, Westbrook, & Hudspeth, 1962). Improved consolidation, or an increase in rate of consolidation due to stimulants, might be observed as a decrease in the effectiveness in disrupting consolidation of another treatment such as ECS at a given time after learning. Gerard (1961) mentions an informal communication from Krech concerning a finding of this nature, but no such study has been published.

McGaugh and associates have attributed improved consolidation caused by administration of stimulants to enhancement of perseveration in the patterns of neural firing which are hypothesized to continue for a time after the stimulus or event. This explanation cannot, at present, be ruled out, but further confirmation, perhaps electrophysiological, would strengthen the

hypothesis. The fact that strychnine was effective in facilitating learning when injected as late as 15 minutes after each trial (McGaugh, Thomson, Westbrook, & Hudspeth, 1962) makes an explanation of its effect in terms of enhanced perseveration in patterns of neural firing perhaps unlikely. It seems doubtful that a pattern of firing would continue undisturbed for that long.

Anodal (surface positive) polarization of the cortex has also been found to facilitate consolidation in that it can restart or re-establish consolidation which has been stopped with cathodal (surface negative) polarization or with SD (Albert, 1966b). Unfortunately, however, little is known at present about the effects of polarizing currents on neural functioning.

Common Aspects of Treatments that Affect Consolidation

Many of the treatments or agents which have been shown to interfere with consolidation can be expected to alter pre-existing patterns of neural firing. Morrell (1961b; 1963), however, has pointed out that many of these treatments also share the characteristic of being accompanied by a shift in D.C. potential, thus raising the interesting possibility that many of the agents that affect the consolidation process do so, not by interfering

with patterns of neural firing, but by reversing or eliminating the potential gradients normally found in the brain. There is evidence that imposed anodal (surface positive) potential gradients can facilitate the formation of temporary functional stimulus-response connections (Morrell, 1961a; Rusinov, 1953). There are also reports that polarization of the cortex can retard or facilitate learning, depending upon the orientation or sign of the polarization (Kupfermann, 1965; Morrell, 1963; Morrell & Naitoh, 1962; Stamm, 1964). More direct evidence bearing on the hypothesis that steady potential changes specifically affect the consolidation phase of learning is the finding (Albert, 1966a; Albert, 1966b) that the disruptive effects of SD on consolidation were closely paralleled by those of cathodal (surface negative) polarization of the cortex. An even more dramatic indication of the importance of steady potential gradients in consolidation is, of course, Albert's (1966b) finding that anodal polarization can restart or re-establish consolidation which has been disrupted by cathodal polarization or by SD.

The way in which potential gradients might affect the consolidation process is not yet known. Morrell

has suggested (1961b) that normally-occurring potential gradients may serve to place populations of cells in a similar state of responsiveness, and that this might facilitate the development of functional connections between the cells. Marshall (1959) states that "it is also generally accepted that variations in the gradients can modulate the all-or-none discharges of axons from the cell body, and also modulate activity of other neuron elements." It would seem that this hypothesis, that potential gradients place cells in similar states of responsiveness, would be inadequate to account for the restarting of consolidation, however.

The effect of potential gradients on the functioning of subcortical structures is also uncertain. Some treatments which seem to affect primarily subcortical structures, such as electrical stimulation and chemical injection, have been shown to disrupt consolidation. It is not entirely clear how graded electrical fields could have a meaningful effect on subcortical structures in which there is no orderly arrangement of neurons such as that found in the cortex.

In short, the effects of potential gradients are still almost completely unknown. That steady potential

shifts are the mechanism of the disruption of consolidation by various agents is certainly a possibility, however, for which Albert's findings provide strong support, but further experimental confirmation is clearly called for before it can be considered the final or only explanation. Furthermore, although there is good evidence from many experiments that soon after the event - in a matter of seconds, probably - other processes take over, a pattern of neural firing may be the sustaining mechanism of the engram for at least a short period.

The studies of Mahut (1957; 1962; 1964) bear on this point. Mahut (1957; 1962) found that 15 sec. of low-level electrical stimulation of intralaminar and midline thalamic nuclei in rats, immediately after each trial on Hebb-Williams maze problems, resulted in increased errors, whereas 15 sec. of stimulation begun at 2 min. 45 sec. after each trial had no effect. In her 1964 study, Mahut found that 10 sec. of stimulation of intralaminar and midline thalamic nuclei in cats, immediately after each response on object discrimination problems, produced deficits, but that 10 sec. of stimulation begun 20 sec. after each response did not. Similar results were reported by Thompson (1958) who found that position habit reversals in cats were more impaired

by caudate stimulation delivered immediately than by stimulation which was delayed 17 sec. The time during which consolidation can be disrupted by electrical stimulation is much shorter than times during which other treatments, such as ECS, or even anesthetization, have been shown to be effective. It is quite possible, of course, that electrical stimulation is just a very much less effective way of disrupting the same processes which are affected by ECS, or it may be that low-level electrical stimulation disturbs a different and shorter-lived process, such as patterned neural firing.

Consolidation: A Chemical Process?

The results of some experiments with hypothermia support another line of speculation about the physiological-biochemical mechanisms involved in consolidation: that consolidation may be, at least in part, a chemical process.

Ransmeier and Gerard (1954) found that cooling hamsters to 5°C. (reached in 40 minutes) after they had learned a maze had no effect on consolidation, although it eliminated electrical activity in the hypothalamus, thalamus, and cortex, and was maintained for up to 12 hours, but that cooling the animals after learning prolonged the period during which ECS was effective in disrupting consolidation

(Gerard, 1955; Gerard, 1961). A similar finding has been reported with goldfish (Davis, Bright, & Agranoff, 1965). That the period of effectiveness of ECS could be prolonged appeared to implicate some biochemical or metabolic process which could be slowed by cooling. Further evidence in support of this hypothesis is Albert's (1966a) finding that a short period of SD, although without effect in itself, lengthened the time during which a longer period of SD was effective in disrupting consolidation.

If consolidation is a chemical process which can be slowed down, and possibly speeded up, perhaps it involves primarily intracellular elements. If so, it might be interfered with or facilitated by agents which directly affect synthesis of intracellular components, such as proteins and nucleic acids.

In support of this line of reasoning, a number of chemicals, such as puromycin, acetoxycycloheximide, actinomycin-D, 8-azaguanine, magnesium pemoline, and 1,1,3-tricyano-2-amino-1-propene (U-9189), which act directly on intracellular components, have recently been shown to affect learning and memory (Agranoff, Davis, & Brink, 1965; Agranoff & Klinger, 1964; Barondes & Cohen, 1966; Barondes & Jarvik, 1964; Biersner, 1966;

Chamberlain, Rothschild, & Gerard, 1963; Cohen & Barondes, 1966; Davis, Bright, & Agranoff, 1965; Dingman & Sporn, 1963; Flexner & Flexner, 1966; Flexner, Flexner, & Stellar, 1963; Plotnikoff, 1966). Most studies using these chemicals have used pre-trial administration, and therefore it is not possible to make definite statements as to their effects on consolidation. However, puromycin and acetoxycycloheximide, protein synthesis inhibitors, and actinomycin-D, which inhibits RNA synthesis, have been used in studies on consolidation.

Puromycin has been found to decrease retention when injected intracranially into goldfish within 5 minutes after 20 shuttlebox trials (Agranoff & Klinger, 1964). This finding was extended by Agranoff et al. (1965) and Davis et al. (1965) who found that the amount of deficit varied with amount of puromycin, and that puromycin was effective in disrupting consolidation when injected up to 30 minutes after training trials (ECS was effective at longer intervals, however).

Dramatic results with puromycin were obtained by Flexner et al. (1963) and Flexner and Flexner (1966) with mice. They trained mice to criterion, giving massed trials on a position habit in a Y maze, then injected puromycin intracerebrally. Bilateral temporal

injections of puromycin decreased retention of a position habit learned two days earlier, and combined temporal, ventricular, and frontal injections up to 43 days after learning significantly decreased retention, which was tested 3 days after injection. It is by no means clear, however, that the retention deficit caused by puromycin injected 43 days after learning is attributable to disruption of consolidation, or that consolidation continues for that long a time. Puromycin may well be affecting processes other than consolidation, or perhaps somehow "undoing" the consolidation of recently-learned material, or even causing permanent damage. For example, Flexner et al. state that "most animals treated with effective doses of puromycin were demonstrated to be capable of relearning after loss of memory, though the process of relearning, particularly with high doses of puromycin, often required considerably more trials than in the initial training experience."

Although intracerebral injections of acetoxycycloheximide caused as much inhibition of protein synthesis as did puromycin, acetoxycycloheximide failed to affect retention. It also seemed to protect against the effects of puromycin, for combined acetoxycycloheximide and puromycin injections

were without effect (Flexner & Flexner, 1966).

Actinomycin-D was found to have no effect on learning in mice (Barondes & Jarvik, 1964; Cohen & Barondes, 1966), despite 83% and 94-96% inhibition of brain RNA synthesis, respectively. Cohen and Barondes also failed to find evidence of impairment in mice injected intracerebrally with actinomycin-D 24 hours after maze learning, and tested for retention 8 or 24 hours after injection. On the other hand, Biersner (1966) found deficits when actinomycin-D was injected into rat brains one or five days after the learning of a pattern discrimination, and retention was tested five days after injection. At 20 days after injection, however, retention was normal. As the deficit in retention proved to be temporary, it is evident that consolidation was able to proceed unhindered by injection of actinomycin-D. There is, as yet, no evidence that actinomycin-D disrupts consolidation.

Speculation that memory may be coded in macromolecules, in a manner perhaps analogous to the coding of genetic information, has recently gained support, tempered by reports of negative findings (e.g. Luttges, Johnson, Buck, Holland, & McGaugh, 1966), from experiments with

mammals showing transfer of response tendencies by injection of extracts of "trained" brain tissue (e.g. Albert, 1966c; Babich, Jacobson, Bubash, & Jacobson, 1965; Røigaard-Petersen, Fjerdingstad, & Nissen, 1965; Rosenblatt, Farrow, & Herblin, 1966; Ungar & Oceguera-Navarro, 1965). These studies extend the earlier findings of McConnell (McConnell, Jacobson, & Humphries, 1961; McConnell, 1962) and Corning and John (1961) with planaria. However, it is not at present clear just what is transferred, whether the RNA or peptide fraction (or neither) is critical, or how the chemical agent acts on the recipient nervous system. Nevertheless, if the engram is coded in macromolecules, it seems likely that consolidation is, at least in part, a chemical process.

An interesting bit of evidence linking the transfer experiments with what is known about consolidation is a report by McConnell (1966) that Albert "finds that if he extracts the cortical tissue from his donor animals an hour or less after they have run their final training trial, he gets no transfer effect; if he waits 7 hours or more after the final trial before making the extraction, he gets positive results."

The suggestion that memory is stored as a biochemical

change rather than a structural one, such as growth of synaptic endings as proposed by Hebb (1949) and others, is certainly provocative. Barondes (1965), however, has suggested that although the memory-fixing process may involve chemical changes, memory may reflect structural changes. Barondes (1965) proposes the hypothesis that memory is based on the development of new synaptic connections, and that protein molecules are involved in this process, but that "the molecule involved in memory is not believed, in itself, to contain the specific information which has been stored. Rather, the new synaptic connexion which is established by this biological alteration is felt to be the repository of the psychic event." This would seem to be an hypothesis worthy of investigation.

Albert has suggested a link between evidence that the "fixing" of the engram may involve the proteins or nucleic acids manufactured by the cells and evidence indicating the importance of potential gradients in the consolidation process. He suggests (Albert, 1964) that consolidation is an intracellular physico-chemical activity, and that potential gradients might maintain or alter "the charge or orientation of molecules, or their

migration within cells." Certainly this possibility should not be disregarded, but this hypothesis also is, as yet, without direct experimental confirmation.

The Structures Involved in Consolidation

Most consolidation studies have employed treatments which directly affect neurons in most or all of the brain. It is also possible to employ agents which selectively affect different parts of the brain, and to investigate the locus of the consolidation of different types of learning.

Because SD remains primarily confined to the structure in which it is elicited, it has been used, for example, to show that responses learned during unilateral spreading cortical depression are elaborated in the intact hemicortex, since depression of the "trained" cortex, after the previously depressed hemicortex has recovered, abolishes the response (Russell & Ochs, 1963). Albert (1966a), using cathodal polarization, was able to locate the consolidation of the interhemispherically-transferred avoidance response as taking place in the medial part of the cortex. Possible subcortical participation in consolidation cannot be ruled out in these experiments with either SD or cortical polarization, however. Using

puromycin, Flexner et al. (1963) found that bilateral temporal injections were more effective in disrupting consolidation than frontal or ventricular injections. Other techniques which are useful for indicating the structures involved in the consolidation of the learning of a particular response are electrical stimulation and cannulation of chemicals into subcortical structures.

Most of the studies which have used subcortical electrical stimulation do appear to implicate subcortical structures in the consolidation of learning, as do reports of deficits of recent memory in human patients with bilateral temporal lobe damage (Milner, 1959; Milner, 1965; Scoville & Milner, 1957). The studies of Mahut and Thompson, which are relevant to this point, have already been mentioned; Glickman (1958) found that stimulation of the reticular formation produced deficits in avoidance learning in rats; Williston, Herz, Peeke, and Wyers (1964) report deficits produced by brief stimulation of the caudate nucleus immediately after the shock in a one-trial passive avoidance task, and Goddard (1964) found that stimulation of the amygdala impaired the consolidation of a conditioned emotional response. It is not clear, however, whether electrical stimulation is activating a

system or disrupting it, and whether its effect on consolidation is due to interference with the function of the structure stimulated, or whether it is due to disruption of the function of remote structures to which the stimulated structure sends afferents. For example, Mahut (1958) suggests that in her studies (1957; 1962; 1964), in which she stimulated the nonspecific ascending systems, the disruption of consolidation was achieved by interference with cortical functioning. Because Glickman (1958) also stimulated the reticular formation, this explanation would apply to his results also. The use of chemicals makes it possible to selectively depress or excite an area. There have been only two studies which have used subcortical injection of chemical agents to study consolidation: those of Albert (1966a) and Hirano (1965).

Albert (1966a), using the interhemispheric transfer paradigm, failed to find any effect on consolidation of procaine hydrochloride injected into the midline thalamus or amygdala, or of hippocampal spreading depression elicited by the injection of 12% potassium chloride. Perhaps the interhemispheric transfer paradigm is characterized by more specifically cortical involvement

than are other learning situations.

The only successful disruption of consolidation using subcortical chemical injection was that of Hirano (1965) who found that such injections into the hippocampus of rats within 1 min. of the shock disrupted the consolidation of a one-trial, step-down, passive avoidance response. However, since he obtained the same results with 1.11% potassium chloride, which he assumed would increase neural firing, and 1.26% calcium chloride, which he assumed would decrease firing, the mechanism by which these chemicals disrupted consolidation is no more clear than the mechanism by which subcortical electrical stimulation disrupts consolidation.

THE PRESENT INVESTIGATION

Studies using electrical stimulation appear to implicate subcortical structures in the consolidation of learning. It is not clear, however, whether low-level electrical stimulation facilitates or blocks the functioning of the area stimulated, or whether the effect on consolidation is attributable to interference with the functioning of the area stimulated or of remote structures. It was therefore decided to employ the technique of chemical injection to determine the effect on consolidation of depressing neural firing in several subcortical structures: the amygdala, septal area, midline thalamus, and hippocampus. The chemical chosen for this purpose was 5% procaine hydrochloride; procaine, applied iontophoretically from a saturated solution to cat motoneurons has been found to prevent the initiation of neuronal spikes by raising the threshold of the neuronal membrane to direct, orthodromic, and antidromic stimulation, without changing the membrane potential (Curtis & Phillis, 1960).

It was also decided to investigate the effect on consolidation of injection into the same structures of 3% potassium chloride (KCl) which, it was thought, would produce a slight sustained increase in firing.

PROCEDURE

General Procedure

The subjects were naive male Long-Evans type hooded rats, 200-300 grams, obtained from Quebec Breeding Farm. Of the original 292 subjects, 95 were rejected in accordance with 3 criteria stated below, leaving 197 Ss.

After the Ss had been handled for at least a week, 23-gauge stainless-steel cannula guide tubes, similar to those used by Nakajima (1964), were stereotaxically implanted with their tips 1 mm. above the desired injection sites. One cannula guide tube was implanted at the midline, for midline-thalamic injections; for injections into other structures, bilateral cannulae were implanted. Close-fitting plugs of 30-gauge stainless-steel tubing, 1 mm. shorter than the cannulae, were inserted at the time of cannula implantation and removed before the first trial. Ss were given one or two days to recover before testing began.

Injections were made through 30-gauge needles inserted into each of the cannula guide tubes and connected by polyethylene tubing to microsyringes mounted in a holder. The needles extended 1 mm. beyond the lower tips of the cannulae. Needles, tubing, and syringes were filled with solution and the needles were inserted into the cannulae before each trial

to minimize the interval between trial and injection.

The apparatus consisted of a two-compartment avoidance box, 36" x 10" x 18" deep, with a grid floor. One compartment was black and the other white, separated by a sliding partition. Ss were placed in the black compartment for 45 seconds before the first trial. Before each trial the injection needles were inserted, the partition was removed, and S was placed in the end of the white compartment, facing the white end wall. Five seconds later S was given brief 0.33 ma. shocks until it entered the black compartment. The partition was replaced, and the chemical was injected over a 20-30 second period. S was then removed from the apparatus, the injection needles were removed, and S was placed on a stand for observation. Trials, usually four per day, were spaced at a minimum interval of three hours. When a rat made two consecutive avoidance responses (crossing to the black compartment before shock was presented), it was considered to have learned the task. Ss were always shocked after being placed in the white compartment on the first trial, and this was counted as an error. Therefore the smallest possible number of trials to criterion, including criterion trials, was 3; errors, 1.

Retention was tested the day after S reached the

learning criterion. The same procedure was used except that no needles were inserted and the intertrial interval was reduced to approximately five minutes. About 6% of the Ss made more errors during retention testing than during learning and were discarded. This eliminated animals which might have accidentally met the relatively low learning criterion through exploration or hyperactivity.

Following the retention test the animals were sacrificed and perfused with formalin. The brains were removed, sectioned, mounted on slides, and stained with thionin. Each slide was projected at a standard setting and the largest area of tissue damage was measured with a planimeter. A certain amount of damage due to the implantation of the guide cannulae was inevitable. In addition, there was sometimes considerable damage caused by infection or possibly by the injection of solution. As a large amount of damage in a given area would decrease the amount of tissue in that area on which the chemicals could act, and as such lesions, present at all times, could not be said to be affecting only the consolidation period, it was necessary to reject a number of Ss because of excessive tissue damage. If the area of tissue damage in any one section exceeded 10 mm^2 in either half of the hippocampus

or amygdala (approximately 20% and 50% of these structures, respectively), or in the entire septal area or thalamus (40% and 20%), S was eliminated from the analysis. About 14% of the Ss were rejected by this criterion.

Ss with misdirected guide cannulae were also discarded; the injection sites were required to have been within the intended area in both hemispheres. About 16% of the Ss were rejected because of misdirected cannulae.

Experiment I: Effect of Immediate Post-Trial Injections

Three main groups were tested in the first experiment, each having four subgroups, representing the amygdala, septal area, midline thalamus, and hippocampus. A group of 20 Unoperated Normal rats was also tested, half at the beginning, and half at the end of Experiment I.

The Immediate KCl group received injections of 3 µl of sterile 3% KCl solution into each hemisphere (except the midline thalamic subgroup, which received one 3 µl injection, and the septal area subgroup, which received injections of 2 µl of solution on each side). The injections were given immediately after each trial. The Procaine group similarly received 5 µl (septal subgroup: 3 µl) of sterile 5% procaine hydrochloride solution immediately after each trial. The No Injection group

was treated in exactly the same manner except that they received no injections. This was to control for the effects of implantation of the guide cannulae as well as any effect of inserting the injection needles which protruded 1 mm. beyond the tips of the guide cannulae. Three No Injection subgroups were trained with no solution in the injection system. The Hippocampus No Injection subgroup was trained with the injection system filled with KCl solution to control for the possibility that solution might be leaking into the brains of the Ss during trials, since the injection needles were inserted before each trial.

Ss were operated upon and tested in batches of 10 or 12, representing one or more subgroups, in no systematic order.

At the dose levels used, there were usually observable behavioral effects of the chemicals. Behavioral effects were usually not seen until 30 seconds or more after S had been removed from the apparatus. Occasionally KCl produced running fits; when this occured, the dosage for that S was decreased. The behavioral effects of the chemicals varied somewhat from rat to rat, and from time to time in the same rat. Both chemicals typically

produced hypoactivity, rapid clicking of the teeth, or occasionally gnawing on food pellets. Procaine sometimes elicited crawling or circling behavior.

An attempt was also made to find out how far the chemicals were spreading or being carried in the brain. Several Ss representing each of the four subcortical areas were injected with 3 µl of 5% ferric chloride solution, then sacrificed and perfused with 1% potassium ferrocyanide in 10% formalin within five minutes or less of injection. The two chemicals react to produce a dark blue (Prussian blue) stain, easily visible when the brain is sectioned. At least one S representing each subcortical area was injected with 3 µl of 10% neutral red stain, then sacrificed but not perfused. There was no apparent difference between the two methods in amount or distribution of dye found in the brain. The extent of the Prussian blue or neutral red stain can only be regarded as a rough approximation of the spread of 3 µl of KCl solution, however.

Experiment II: Control for an Effect of the Chemicals on Learning

Experiment II investigated the possibility that any effect of the chemicals on learning might be due, not to

interference with processes taking place after the trial, but to interference with processes occurring before and during the next trial - i.e. that the effects of the chemicals had not "worn off" in three hours. In this experiment, four subgroups were trained as in Experiment I except that instead of receiving injections immediately after each trial, they were injected with KCl three hours before each trial. Injections were given not less than one hour after the previous trial. If the chemicals were affecting the trial following the injection rather than the trial preceding the injection in Experiment I, one would expect the animals injected with KCl one hour after each trial in Experiment II to have learning scores indistinguishable from those of animals injected with KCl immediately after each trial in Experiment I, as the injection - trial interval was three hours in both cases.

Experiment III: Electrophysiological Effects of Injection of the Chemicals

It was originally predicted that injection of 3% KCl might cause a slight sustained increase in neural firing. As the electrophysiological effect of KCl injection was not certain, however, Experiment III examined the effects of injection of both chemicals

into unrestrained, unanesthetized rats by recording the electrical activity of tissue near the lower tips of implanted cannulae.

Recordings were taken from the amygdala and midline thalamus. Data were obtained from four Ss with electrodes made of 64μ diameter nichrome wire, insulated except for the tips, and from three Ss with electrodes made of 250μ diameter insulated stainless-steel wire. Electrodes were fastened to a guide cannula with Insl-X so that the electrode tips extended 1 mm. beyond the tip of the cannula. Amphenol plugs connected the electrodes to a cable which permitted movement restricted only by the confines of the plastic bucket in which S was placed for recording. Recordings were taken two or more days after implantation of the cannula and electrodes. Only one chemical was injected in any recording session, and at least one day elapsed between sessions.

The signal was led off through low-noise Micro-Dot cable and fed through a Tektronix 122 low-level pre-amplifier into a standard cathode-ray oscilloscope. Signals below 500 cps were filtered out by a high pass filter between the preamplifier and the oscilloscope. The output of the oscilloscope amplifier was then fed

into a Ballantine Root-Mean-Square meter. The mean-square voltage (MS) output of the meter is proportional to the average power between the electrodes, and to the number of impulses (Arduini & Pinneo, 1962). The MS output was recorded on a D.C. channel of a Grass Model 7 polygraph. Increases or decreases in activity, which primarily reflected changes in amount and amplitude of all-or-none neural firing, were observed as deflections in baseline.

In some cases the low-frequency EEG signal was led off in parallel and fed directly into an EEG channel of the Grass polygraph with filters set at 1 - 75 cps.

RESULTS

Potassium chloride (3%) disrupted consolidation of the learning of a one-way active avoidance response when injected subcortically immediately (within 30 seconds) after each learning trial. Immediate procaine injections had no effect on learning, nor did KCl injections one hour after each trial.

All statistical computations were performed on number of trials to criterion and on errors to criterion. Since in all cases the two measures yielded the same levels of significance, only comparisons of trials to criterion will be reported.

As shown in Table 1, animals in Experiment I that received KCl injections immediately after each trial required more trials to reach criterion than did animals that received procaine injections, or animals that received needle insertion only. An analysis of variance showed that differences between the three treatments were significant ($p < .001$; all probability values are based on two-tailed tests). Comparison of treatment means by the Newman-Keuls method (Winer, 1962) showed that the Immediate KCl group was significantly different from both Procaine ($p < .01$) and No Injection

($p < .01$) groups, but that the latter two groups did not differ from each other (Table 2).

There was no significant difference between the scores of subgroups with cannulae in different areas (Table 1).

Ss rejected from the analysis of Experiment I because of excessive tissue damage or guide cannula misdirection were placed in three Damage and three KCl Misses subgroups, which were then compared with the appropriate subgroups in Experiment I with separate analyses of variance. (Four was arbitrarily chosen as the minimum number of Ss required to constitute a subgroup.) As shown in Table 3, Ss rejected from the analysis because of excessive tissue damage required significantly more trials to criterion ($p < .025$) than did Ss that received KCl injections into the same areas immediately after each trial. Table 4 shows that, at least in the cases of the septal area, midline thalamus, and hippocampus, KCl injections were as effective when delivered into the immediate vicinity of an area as when delivered into the area itself.

The dye injection indicated that there was sometimes considerable spread of the injected fluid, which may

account for the fact that there was no significant difference between the Immediate KCl and KCl Misses groups. If the dye entered a ventricle, it was carried some distance from the structure into which it had been injected. There was no consistent spread to any specific structure, however.

Figures 1-4 represent the distribution of dye in four brains, each with cannulae in a different area. In several brains dye was found outlining the cannula tracts; it may be that this was merely dye left behind when the dye-filled cannulae were withdrawn from the brain. In several cases dye injected into the hippocampus and midline thalamus entered a ventricle and was carried as far as the anterior portion of the septal area, and posterior to the lateral surface of the hippocampus. Dye injected into the septal area remained there in four of six brains; in two brains dye entered a ventricle, but was not carried as far posterior as was dye injected into the hippocampus and midline thalamus. Dye injected into the amygdala tended to be fairly well localized in the amygdala and around the cannula tracts; however in one case (out of eight) it did enter a ventricle. In summary, then, it seems probable that the chemicals sometimes directly

affected structures other than those into which they were injected. Injection of KCl solution one hour after each trial did not have the same effect on learning as did KCl injection immediately after each trial, even though both groups were tested three hours after injection. An analysis of variance (Table 5) showed that animals which received KCl injections immediately after each trial (Immediate KCl group, Experiment I) required significantly more trials to reach criterion ($p < .001$) than did animals injected with KCl solution one hour after each trial (Delayed KCl group, Experiment II). Thus the deficit of the Immediate KCl group cannot be attributed to interference with the trial following injection.

There was no significant difference between the Unoperated Normal group in Experiment I and any of the Procaine, No Injection, or Delayed KCl subgroups in Experiments I and II (Table 6) as shown by Dunnett's t statistic (Winer, 1962). Furthermore, since the Hippocampus No Injection subgroup, trained with the injection system filled with KCl solution, did not differ significantly from the Unoperated Normals, it is clear that the injection system did not leak solution

into the brains of Ss during trials.

There were no significant differences (Dunnett's t) in retention scores between the Unoperated Normal group and any of the subgroups in Experiments I and II or any of the KCl Misses or Damage subgroups (Table 7).

Figures 5 - 7 show the electrophysiological effects of injection of the chemicals in the four Ss with 64 μ diameter electrodes.

Procaine typically produced a decrease in activity, accompanied by a decrease in EEG amplitude (Figure 5). KCl also produced decreased activity, although to varying extents (Figure 6). At any rate, KCl was never observed to cause the predicted sustained increase in firing. Control saline injections had no effect (Figure 7). The results obtained from the three Ss with the larger (250 μ) electrodes were similar.

The decreased firing caused by KCl probably reflects a collapse in membrane potential or depolarization block due to the increased concentration of extracellular K^+ ions. Since both chemicals were observed to produce a decrease in electrical activity, it would be questionable to attribute the difference in the effect of the two chemicals on consolidation to a difference in effect

on neural firing.

It seems possible that there might have been some initial firing after KCl injection due to influx of K^+ ions. No marked increase of firing during or immediately following KCl injection was observed, however, nor was there any effect on EEG recordings. Therefore any initial firing, if such existed, would probably have been too weak to disrupt patterned firing in remote structures.

DISCUSSION

If both procaine and potassium chloride cause a decrease in neural firing, why, then, did only the immediate KCl injections disrupt the consolidation of the learning of an active avoidance response? The most likely possibility is that KCl altered subcortical steady potential gradients and thereby disrupted consolidation, whereas procaine did not.

Steady potential changes are known to occur subcortically. Furthermore, steady potential changes are known to occur in the particular structures investigated in the present experiment. Spreading depression, accompanied by its characteristic steady potential change, has been observed in the thalamus (Aquino-Cias, Belceva, Bureš, & Fifková, 1966; Bureš, Burešová, Fifková, & Rabending, 1965; Fifková, 1966), hippocampus (Bureš, 1959; Fifková, 1964; Monakhov, Fifková, & Bureš, 1962; Weiss & Fifková, 1960), and amygdala (Fifková & Syka, 1964). There are no published reports of septal area SD; on the other hand, neither are there reports of failure to elicit septal area SD. No data on the threshold concentration of KCl for the elicitation of subcortical SD have been reported, but, as Bureš

(1959) is able to obtain cortical SD with 0.6% KCl, it seems not unreasonable to suppose that 3% KCl might trigger subcortical SD. Whether there is actually a spread of the depression is not important, however. Potassium chloride was observed in Experiment III to cause a decrease in firing, as would be expected with SD, no doubt for the same reason: a depolarization block due to increased extracellular concentration of K^+ ions. It is probable that 3% KCl in the present experiment produced changes in subcortical steady potentials similar to those accompanying subcortical SD. Procaine, on the other hand, would be expected to have little effect on steady potential gradients because its effect is to decrease neural firing, and ionic concentrations are unchanged.

The data of the present experiment, then, offer support for the hypothesis (Albert, 1964; Morrell, 1961b) that steady potential gradients are somehow important in consolidation. However, as the way in which potential gradients affect the consolidation process is still unknown, and it is not clear how potential gradients might have meaningful effects on non-layered subcortical structures, further research in this area would seem desirable.

An alternate interpretation of these data is that KCl did not disrupt consolidation; rather, injection of KCl served as negative reinforcement for performance of the response, whereas injection of procaine was not negatively reinforcing. This argument is weakened by Hirano's (1965) finding that injection of 1.11% KCl into the hippocampus within one minute of the shock in a one-trial, step-down, passive avoidance task increased the tendency of Ss to step down onto the grid in a retention test. Had KCl been negatively reinforcing, the rats would have been less, not more, likely to respond in the retention test. This result lends support to the contention that KCl in the present experiment disrupted consolidation and did not merely act as a negative reinforcer.

It is unlikely that the interference with consolidation was due to disruption of the functioning of remote structures, since subcortical firing was not increased by KCl injection. Therefore, subcortical structures themselves must have been directly involved in consolidation of the one-way active avoidance response. It is extremely doubtful, however, whether consolidation took place in subcortical structures exclusively. Cortical

involvement in the consolidation and storage of this response is suggested, although not conclusively shown, by Albert's findings (1966a; 1966b) that SD and cathodal polarization of the cortex disrupted the consolidation of the interhemispheric transfer of an active avoidance response, and by the fact that removing part of the cortex in the hemisphere in which an active avoidance response was elaborated abolished the response (Albert, 1966c). It is probable that both cortex and subcortical structures are involved in the consolidation of the learning of this task. However, the data of the present experiment do not indicate whether subcortical structures were involved in the storage of the learning as well as in its consolidation.

It is of interest that "running fits" occasionally occurred, mostly after injection of KCl rather than procaine, and that the majority of the running fits which occurred involved midline thalamic Ss. It might appear that the occurrence of such fits would imply an increase rather than a decrease in neural firing in the midline thalamus, but there are indications that the latter is more likely the case. Vanderwolf (1961) reports, and Goddard (personal communication,

1966) confirms, that rats given midline thalamic lesions often show running behavior upon recovering from the anesthetic. Therefore, in causing running fits, KCl could well have mimicked a lesion, or caused a decrease, rather than an increase, in neural firing in the thalamus. Also, there is known to be a region in the subthalamus, which, if electrically stimulated, produces walking or running movements in a suspended, lightly-anesthetized cat (Grossman, 1958; Waller, 1940). Grossman demonstrated that stimulation of the nonspecific nuclei of the thalamus decreased or temporarily arrested repetitive locomotor movements elicited by such subthalamic stimulation. He suggests (Grossman, 1958) that "the non-specific thalamic nuclei have descending connections capable of rapidly and briefly inhibiting those neural elements of the subcortical locomotor mechanism essential for the rhythmic repetition of locomotor movements." Perhaps the midline thalamus in the rat normally exerts a damping influence on this "subcortical locomotor mechanism;" if this were the case, functionally eliminating the midline thalamus with a lesion or chemical injection might release the locomotor mechanism from this inhibitory influence and give

rise to a running fit. However, it is not clear why, in the present experiment, running fits occurred occasionally but by no means invariably; why procaine injections seldom caused running fits; or why a few running fits also occurred after injection of KCl into the septal area and hippocampus.

That there was no significant difference between the learning scores of Immediate KCl subgroups with cannulae in different areas is, perhaps, a bit surprising, but undue importance should not be attached to the failure to demonstrate a difference. Diffusion of the chemicals to other areas would tend to increase the variance and obscure any differences between subgroups. It does not appear to be the case, however, that chemicals injected into one area affected all four areas equally: running fits occurred primarily after KCl injections into the midline thalamus. On the other hand, the fact that these four areas are closely connected anatomically suggests that they may participate jointly in consolidation. If this were the case, one might expect injection of KCl to have a similar effect on consolidation in each structure.

Finally, the low scores of the Amygdala Procaine

subgroup (Table 1) deserve brief mention. Since neither the difference between areas nor the interaction in the analysis of variance proved to be significant, it is not strictly proper to perform further statistical tests of this apparent difference. However, if one were to do *t* tests, one would find that the Amygdala Procaine subgroup differed significantly from the Amygdala No Injection subgroup ($t=3.67$, $df=18$, $p < .01$) and the Unoperated Normal group ($t=2.50$, $df=28$, $p < .02$). The Amygdala Procaine subgroup did not, however, differ from the Unoperated Normal group when tested with Dunnett's *t* statistic (Table 6). Therefore the low score of the Amygdala Procaine subgroup may well have been a chance occurrence. This result is intriguing, however, in view of the fact that permanent lesions in the amygdala impaired avoidance learning (Damage subgroup, Table 3). In spite of the questionable validity of the differences between the Amygdala Procaine subgroup and other groups, the possibility that quieting the amygdala after a trial prevents the trace from being interfered with by subsequent events may be worth investigating.

SUMMARY

Five percent procaine hydrochloride or 3% potassium chloride (KCl) were injected through implanted cannulae into the amygdala, septal area, midline thalamus, or hippocampus of rats after each spaced trial on a one-way active avoidance task.

Ss that received KCl injections immediately after each trial required more trials to reach criterion than did Ss that received immediate procaine injections, Ss that received KCl injections one hour after each trial, or Ss that received no injections. There was no significant difference between the scores of subgroups with cannulae in different areas.

Electrical recordings indicated that both chemicals produced decreases in neural firing. It is suggested that KCl produced a depolarization block, accompanied by changes in subcortical steady potential gradients which disrupted consolidation.

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Table 1

Experiment I: Two-way analysis of variance of trials to criterion (including 2 criterion trials).

	Amygdala	Septal Area	Midl. Thal.	Hippocampus
Immediate KCl	$\bar{X}$ = 10.10 s = 3.75 n = 10	$\bar{X}$ = 9.63 s = 3.81 n = 8	$\bar{X}$ = 10.00 s = 3.80 n = 17	$\bar{X}$ = 9.90 s = 1.37 n = 10
Procaine	$\bar{X}$ = 4.10 s = 1.58 n = 10	$\bar{X}$ = 8.00 s = 2.00 n = 5	$\bar{X}$ = 7.55 s = 3.11 n = 11	$\bar{X}$ = 8.10 s = 1.45 n = 10
No Injection	$\bar{X}$ = 7.50 s = 2.29 n = 10	$\bar{X}$ = 7.71 s = 1.58 n = 7	$\bar{X}$ = 6.22 s = 2.39 n = 9	$\bar{X}$ = 7.50 s = 1.58 n = 8

Source of variation	Degrees of freedom	Mean square	F ratio	p
Areas	3	9.2232	1.1368	ns*
Treatments	2	94.8459	11.6899	$p < .001$
Areas by treatments	6	13.7420	1.6937	ns
Within subgroups	103	8.1135		
Total	114			

*$p > .05$

Table 2

Comparison of treatment means in analysis of variance of trials to criterion in Experiment I by the Newman-Keuls method.

Group	Treatment mean (Mean of all 4 subgroups)	Difference and p value
Immediate KCl	9.93	
		3.12 $p < .01$
Procaine	6.81	
		.40 ns
No Injection	7.21	
		2.72 $p < .01$

Table 3

Immediate KCl subgroups in Experiment I *vs.* Ss rejected from all three groups in Experiment I because of excessive tissue damage: Two-way analysis of variance of trials to criterion (including 2 criterion trials).

	Amygdala	Septal Area	Hippocampus
Immediate KCl	$\bar{X}$ = 10.10 s = 3.75 n = 10	$\bar{X}$ = 9.63 s = 3.81 n = 8	$\bar{X}$ = 9.90 s = 1.37 n = 10
Damage	$\bar{X}$ = 13.57 s = 6.78 n = 14	$\bar{X}$ = 12.43 s = 4.37 n = 7	$\bar{X}$ = 13.89 s = 5.80 n = 9

Source of variation	Degrees of freedom	Mean square	F ratio	p
Areas	2	4.3378	0.1664	ns
Treatments	1	161.9740	6.2127	$p < .025$
Areas by treatments	2	1.6288	0.0625	ns
Within subgroups	52	26.0713		
Total	57			

Table 4

Immediate KCl subgroups in Experiment I vs. Ss rejected from KCl subgroups because of misdirected cannulae: Two-way analysis of variance of trials to criterion (incl. 2 criterion trials).

	Septal Area	Midline Thal.	Hippocampus
Immediate KCl	$\bar{X}$ = 9.63 s = 3.81 n = 8	$\bar{X}$ = 10.00 s = 3.80 n = 17	$\bar{X}$ = 9.90 s = 1.37 n = 10
KCl Misses	$\bar{X}$ = 7.83 s = 1.57 n = 6	$\bar{X}$ = 10.38 s = 4.92 n = 13	$\bar{X}$ = 9.50 s = 1.12 n = 4

Source of variation	Degrees of freedom	Mean square	F ratio	p
Areas	2	8.5557	0.6216	ns
Treatments	1	4.2004	0.3052	ns
Areas by treatments	2	4.6877	0.3406	ns
Within subgroups	52	13.7632		
Total	57			

Table 5

Experiment II: Immediate KCl group in Experiment I vs. Delayed KCl group: Two-way analysis of variance of trials to criterion (including 2 criterion trials).

	Amygdala	Septal Area	Midl. Thal.	Hippocampus
Immediate KCl	$\bar{X}$ = 10.10 s = 3.75 n = 10	$\bar{X}$ = 9.63 s = 3.81 n = 8	$\bar{X}$ = 10.00 s = 3.80 n = 17	$\bar{X}$ = 9.90 s = 1.37 n = 10
Delayed KCl	$\bar{X}$ = 8.00 s = 2.88 n = 7	$\bar{X}$ = 7.20 s = 2.40 n = 10	$\bar{X}$ = 6.78 s = 2.10 n = 9	$\bar{X}$ = 6.86 s = 2.29 n = 7

Source of variation	Degrees of freedom	Mean square	F ratio	p
Areas	3	1.9624	0.1925	ns
Treatments	1	132.2046	12.9669	$p < .001$
Areas by treatments	3	1.2504	0.1226	ns
Within subgroups	70	10.1955		
Total	77			

Table 6

Comparison of the learning scores of the Procaine, No Injection, and Delayed KCl subgroups in Experiments I and II with the learning scores of the Unoperated Normal group, using Dunnett's t statistic: Trials to criterion (including two criterion trials).

Subgroup	n	$\bar{X}$	t	p
Unoperated Normals	20	6.15	----	--
Amygdala Procaine	10	4.10	1.80	ns
Septal Area Procaine	5	8.00	1.62	ns
Midline Thalamus Procaine	11	7.55	1.23	ns
Hippocampus Procaine	10	8.10	1.71	ns
Amygdala No Injection	10	7.50	1.18	ns
Septal Area No Inj.	7	7.71	1.37	ns
Midline Thal. No Inj.	9	6.22	0.06	ns
Hippocampus No Injection	8	7.50	1.18	ns
Amygdala Delayed KCl	7	8.00	1.62	ns
Septal Area Delayed KCl	10	7.20	0.92	ns
Midline Thal. Delayed KCl	9	6.78	0.55	ns
Hippocampus Delayed KCl	7	6.86	0.62	ns

Table 7

Comparison of the retention scores of all subgroups in Experiments I and II, including Damage subgroups and KCl Misses subgroups, with the retention scores of the Unoperated Normal group, using Dunnett's t statistic: Trials to criterion (including two criterion trials).

Subgroup	n	$\bar{X}$	t	p
Unoperated Normals	20	2.10	----	--
Amygdala Imm. KCl	10	3.10	1.85	ns
Septal Area Imm. KCl	8	2.88	1.44	ns
Midline Thalamus Imm. KCl	17	3.35	2.31	ns
Hippocampus Imm. KCl	10	2.70	1.11	ns
Amygdala Procaine	10	2.50	0.74	ns
Septal Area Procaine	5	2.00	0.19	ns
Midline Thalamus Procaine	11	2.18	0.15	ns
Hippocampus Procaine	10	2.00	0.19	ns
Amygdala No Injection	10	3.50	2.59	ns
Septal Area No Injection	7	3.14	1.93	ns
Midline Thalamus No Inj.	9	2.89	1.46	ns
Hippocampus No Injection	8	2.25	0.28	ns
Amygdala Delayed KCl	7	2.57	0.87	ns
Septal Area Delayed KCl	10	2.40	0.56	ns
Midline Thal. Delayed KCl	9	2.44	0.63	ns
Hippocampus Delayed KCl	7	2.00	0.19	ns
Amygdala Damage	14	2.71	1.13	ns
Septal Area Damage	7	2.71	1.13	ns
Hippocampus Damage	9	3.33	2.28	ns
Septal Area KCl Misses	6	3.00	1.67	ns
Midline Thal. KCl Misses	13	2.46	0.67	ns
Hippocampus KCl Misses	4	2.25	0.28	ns

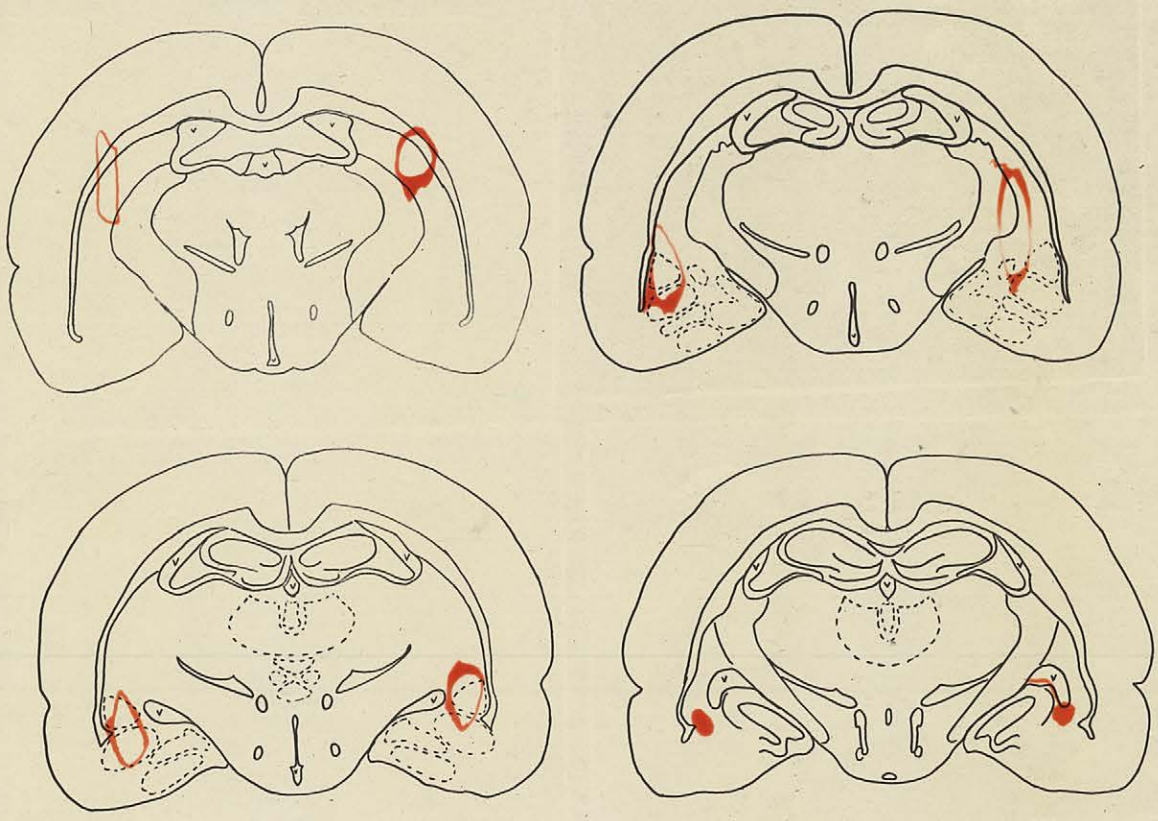


Figure 1. Distribution of dye in the brain of one rat with cannulae in the amygdala. (Drawings adapted from de Groot, 1959)

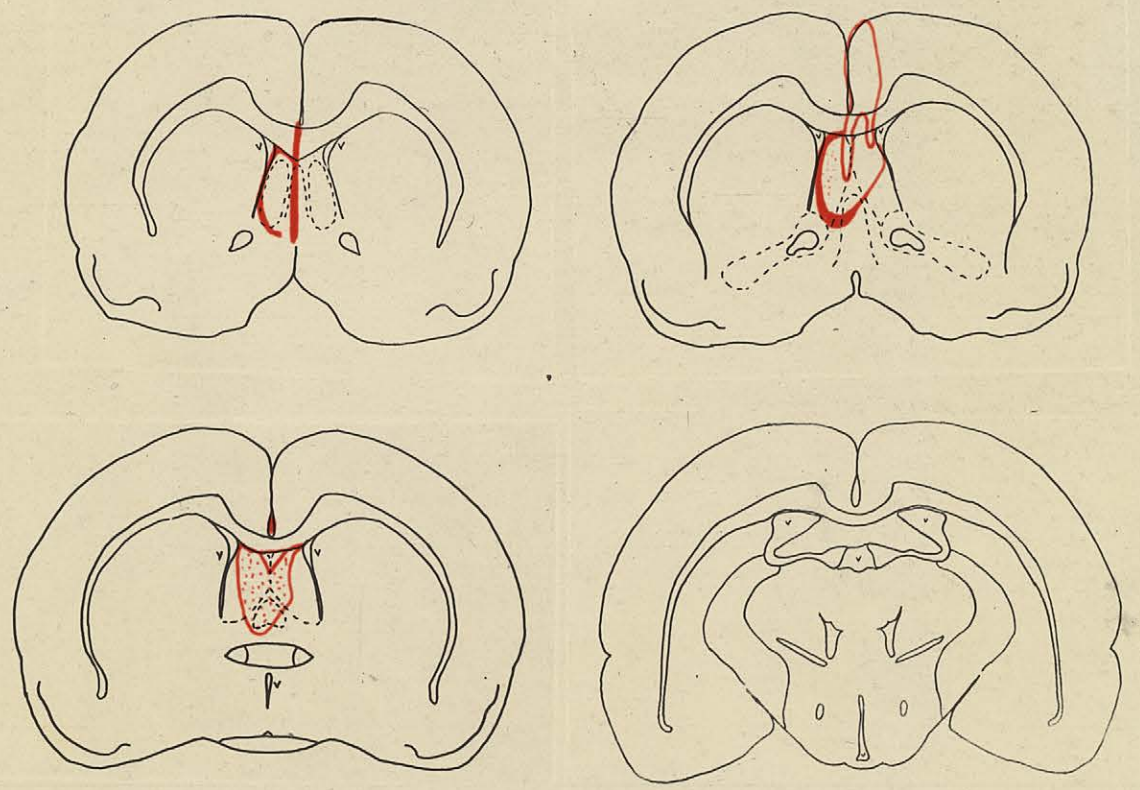


Figure 2. Distribution of dye in the brain of one rat with cannulae in the septal area. (Drawings adapted from de Groot, 1959.)

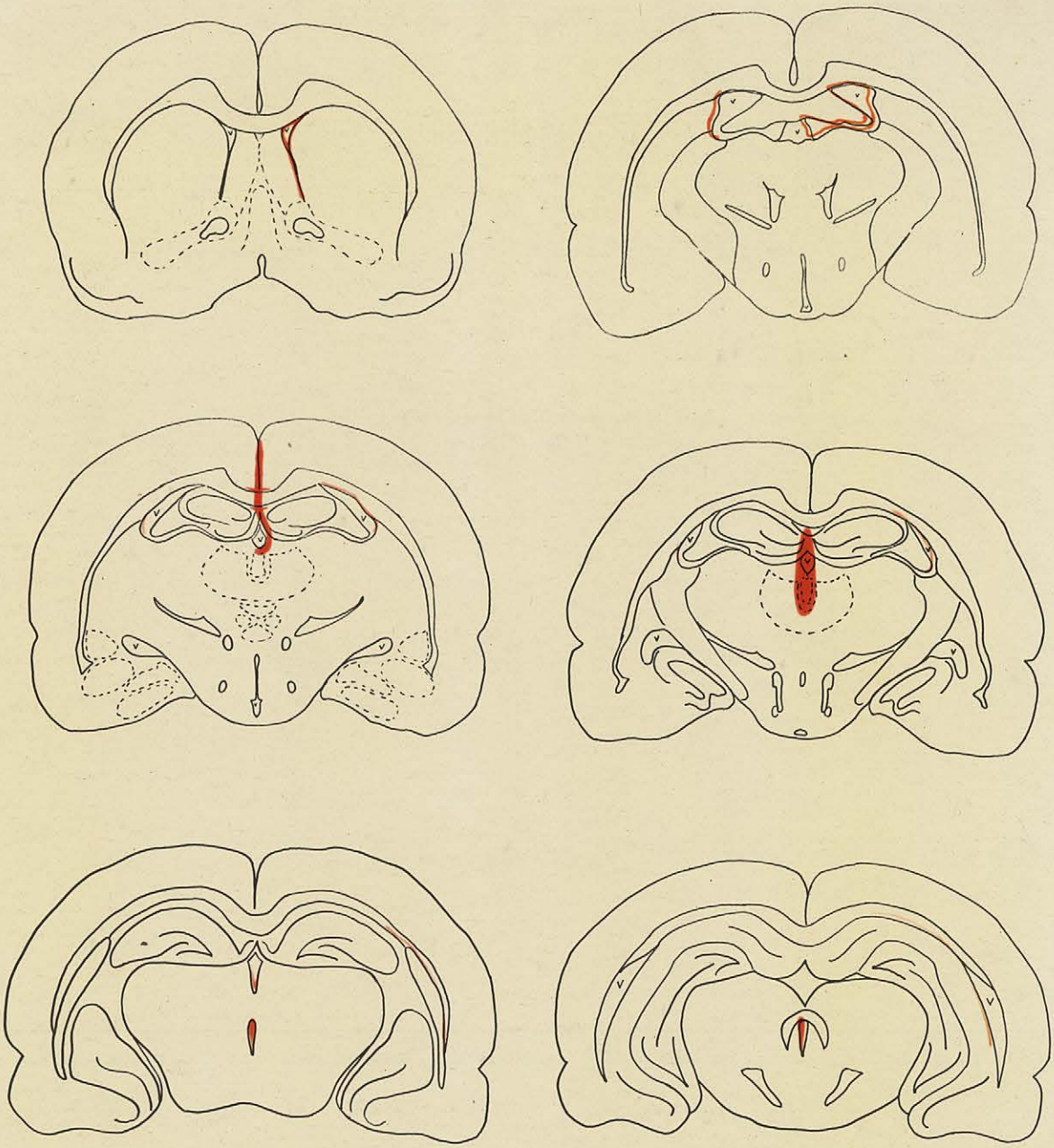


Figure 3. Distribution of dye in the brain of one rat with cannulae in the midline thalamus. (Drawings adapted from de Groot, 1959.)

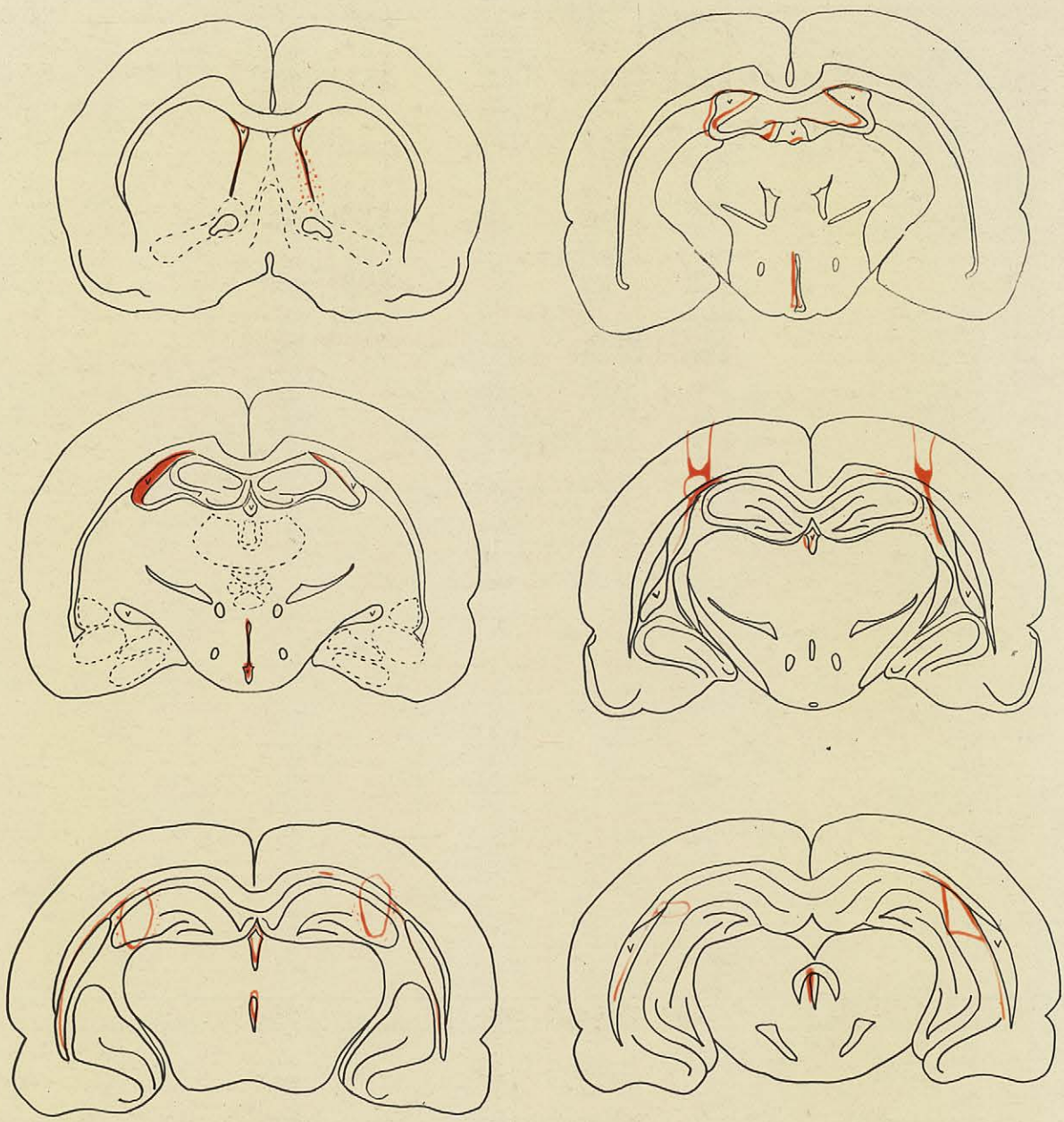


Figure 4. Distribution of dye in the brain of one rat with cannulae in the hippocampus. (Drawings adapted from de Groot, 1959.)

(facing page 87)

Figure 5. Effect on mean square voltage (MS) and EEG of injection of 5 µl of 5% procaine hydrochloride solution.

Rat A: bipolar electrode in midline thalamus

1: before injection

2: during injection

3: 1 min. after injection

4: 5 min. after injection

5: 10 min. after injection

6: 30 min. after injection

Rat B: bipolar electrode in midline thalamus

1: during injection

2: 1 min. after injection

3: 5 min. after injection

4: 10 min. after injection

5: 30 min. after injection

6: 60 min. after injection

Rat C: bipolar electrode in amygdala

1: before injection

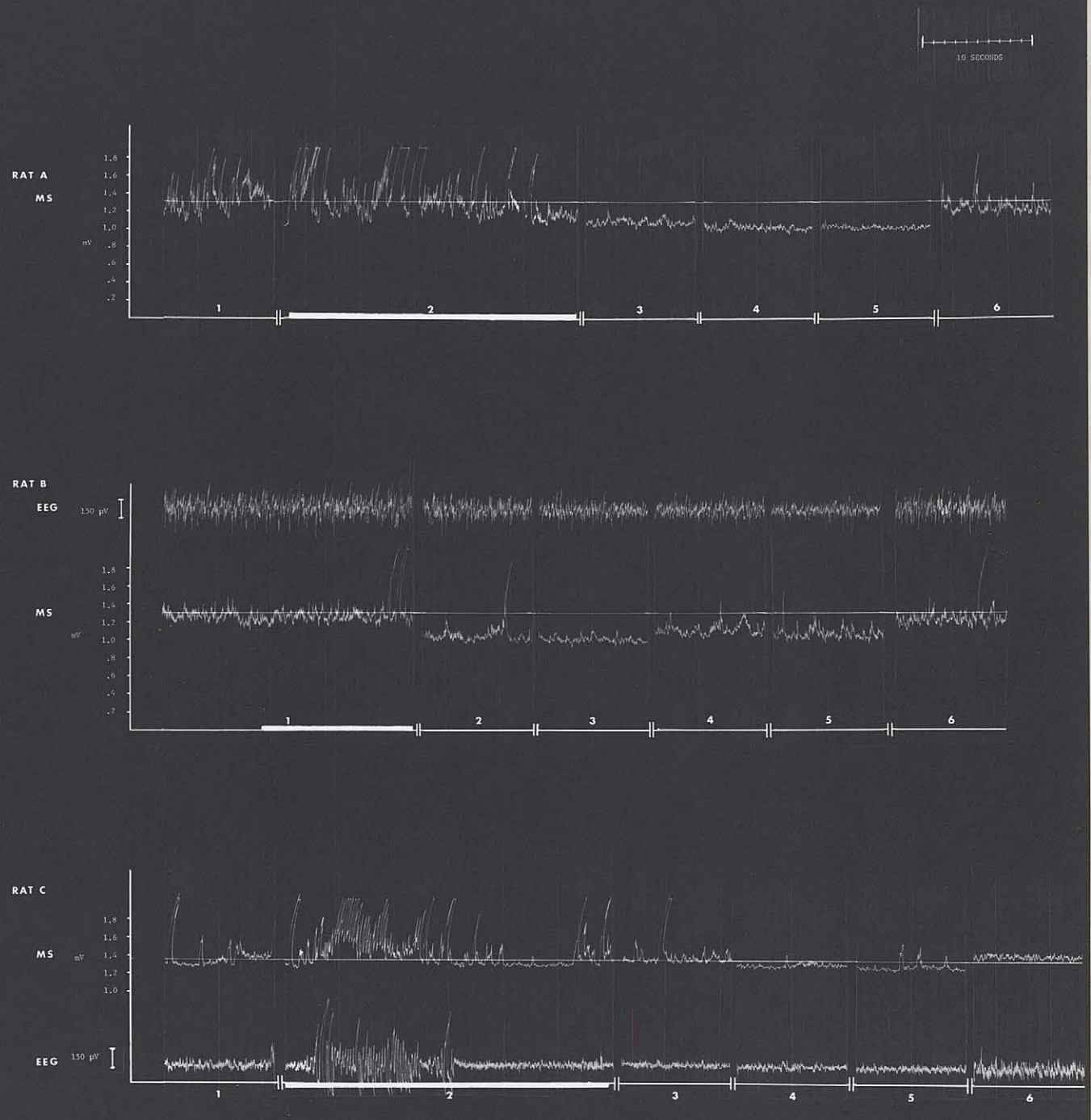
2: during injection

3: 1 min. after injection

4: 5 min. after injection

5: 15 min. after injection

6: 30 min. after injection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RAT B
EEG
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RAT C
MS
mV
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EEG
150 μV
1
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6

(facing page 89)

Figure 6. Effect on mean square voltage (MS) and EEG of injection of 3 µl of 3% KCl solution.

Rat A: bipolar electrode in midline thalamus

1: before injection

2: during injection

3: 1 min. after injection

4: 5 min. after injection

5: 10 min. after injection

6: 30 min. after injection

Rat B: bipolar electrode in midline thalamus

1: during injection

2: 1 min. after injection

3: 5 min. after injection

4: 30 min. after injection

5: 75 min. after injection

6: noise level with 50 K resistance (approximate resistance of electrodes as tested in saline)

Rat C: bipolar electrode in amygdala

1: during injection

2: 1 min. after injection

3: 5 min. after injection

4: 10 min. after injection

5: approximately 45 min. after injection

Rat D: monopolar electrodes in amygdala (top record) and midline thalamus (bottom record)

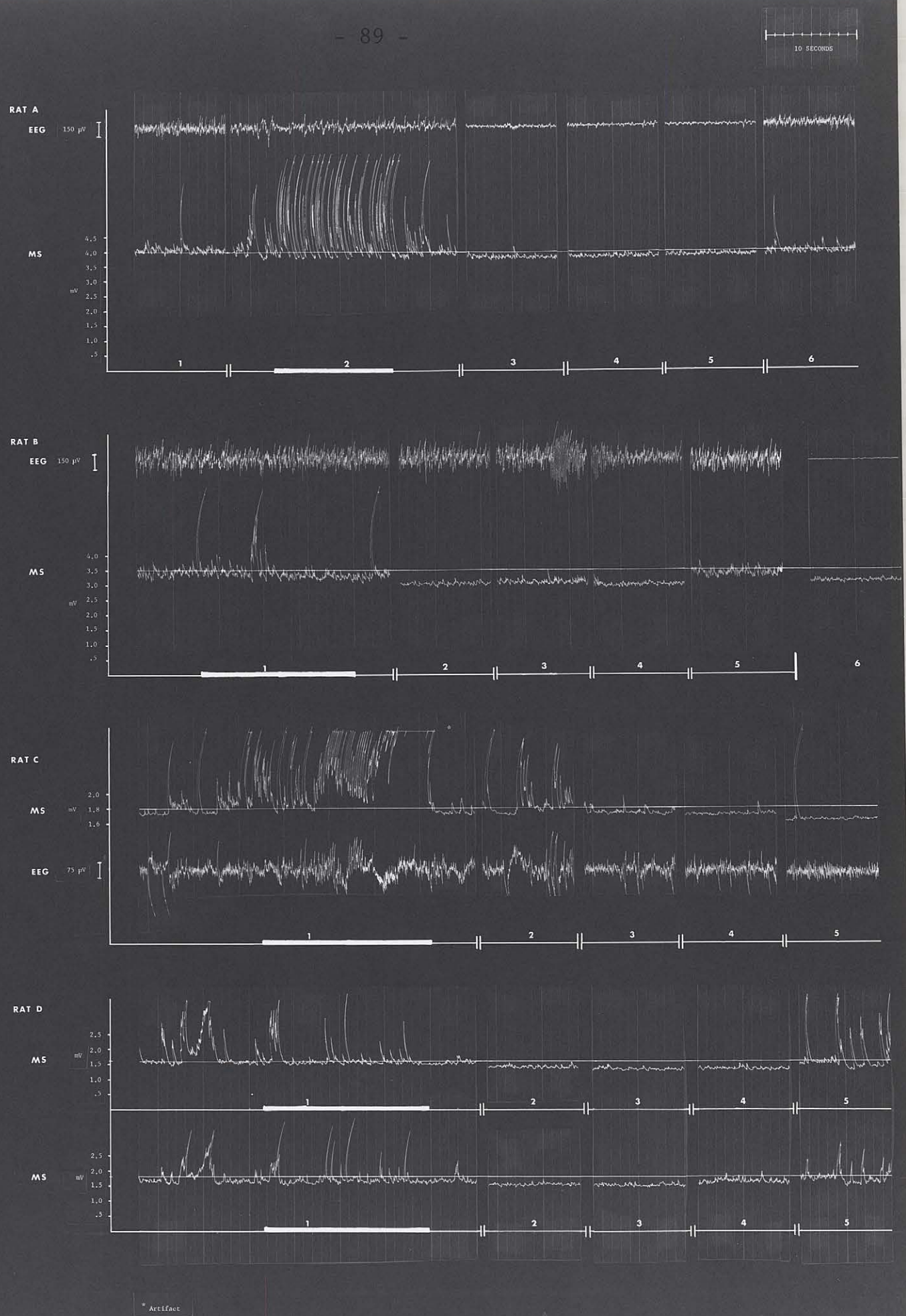
1: during injection into thalamus

2: 1 min. after injection

3: 8.5 min. after injection

4: 17 min. after injection

5: 35 min. after injection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2.0
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4
5
RAT D
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2.5
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4
5
MS
2.5
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mV
1.5
1.0
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* Artifact

(facing page 91)

Figure 7. Effect on mean square voltage (MS) and EEG of injection of 5 μl of 0.9% saline solution.

Rat A: bipolar electrode in midline thalamus

1: during injection

2: 1 min. after injection

3: 5 min. after injection

4: 10 min. after injection

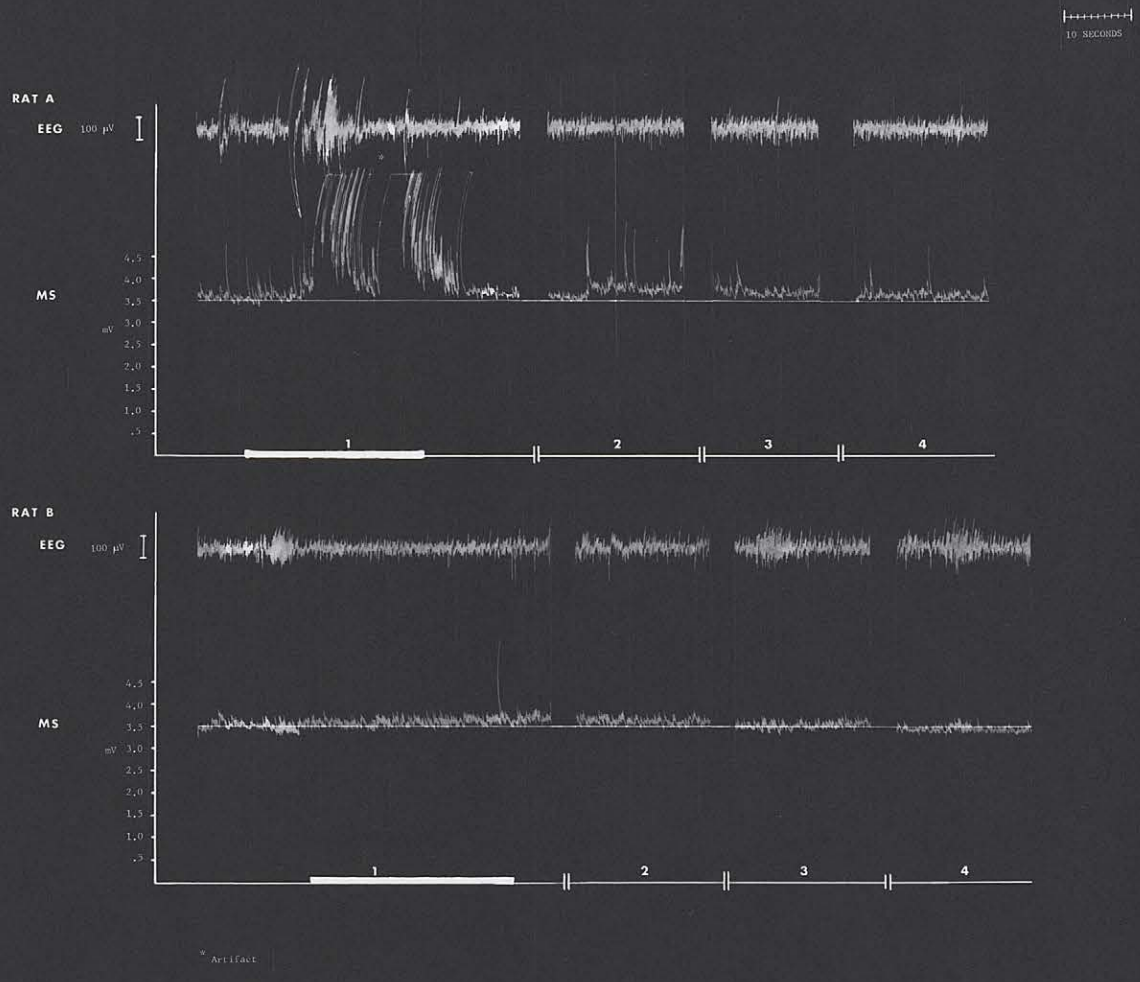
Rat B: bipolar electrode in midline thalamus

1: during injection

2: 1 min. after injection

3: 5 min. after injection

4: 6 min. after injection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1
2
3
4
* Artifact