INVESTIGATIONS OF OPERANT CONDITIONING OF SINGLE UNIT ACTIVITY IN THE RAT BRAIN

Thesis by

David Ellis Hiatt

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DEDICATION: TO CALTECH

Eros and Logos were friends at one time.
They had risen together from the primordial slime.
As Yin is to Yang were they entertwined.
Eros and Logos were one at one time.

Then in Logos an idea came to,
A bit of the trick Eros could not do,
So Logos split,
convoluted,
grew and grew.
This is what happened in me and you.

When in the real world all progress was complete,
Logos turned his thoughts to the very last feat.
Refusing to lower himself from the seat,
He pitted his ideas against Eros --- to beat.

By the sweat of the brow, Logos did try,
The existence of Eros entirely to deny.
But the closer he came to this ultimate high,
Into the Void his thoughts returned to fly.

ACKNOWLEDGMENTS

Both this work* and I have profited and grown in manifold ways from the suggestions, interest, covert hostility, humor, censure, support, judgment, apathy, zeal, criticism, passion, confidence... and love of many individuals. These ingredients were found in varying proportion at various times. Confining space prohibits me from attempting to convey the true richness of the blend I experienced, but then, it would only be painted fire anyway. Let me show that primacy-recency is psychological truth by first thanking my parents for starting my life off exceedingly well, and then jumping to the army of advance readers of the thesis who suffered through eyestrain and ennui to give me their wise suggestions. These are Margaret Scott, Prof. Anthonie Van Harreveld, Duncan Stuart, Prof. Ray Owen, Carol Kornblith, Prof. Felix Strumwasser, Menahem Segal, Prof. Richard Russell, Mary Ann Linseman, and Prof. James Olds. The last person deserves special mention for he has been my advisor for the last five years, and given me a personal example of profound enthusiasm for brain research. His sharp mind has been a great asset in pushing through problems in my research. The techniques and facilities he provided were indispensible. I appreciate the guidance he has given, both direct and indirect, in helping me become a more intelligent observer of nature's mysteries. Dr. Marianne Olds has also educated me with her practical suggestions

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Abstract

The aim of these studies was to show that the capacity for operant responses is distributed differentially in the brain and that such capacity is maintained in the absence of feedback from movement in specific parts of the brain. The experimental subjects were rats chronically implanted with microelectrodes for single unit recording from several different brain structures. There were three experimental paradigms. In Experiments I and II positive reinforcement was applied following bursts of activity of an arbitrarily selected unit during periods indicated by a discriminative stimulus. All such units in cerebellum and brain stem displayed significant conditioned rate increases while only about half those in hippocampus, midbrain and superior colliculus did so indicating that operant conditioning is more a property of "motor" units. Experiment II was a direct continuation of Experiment I with some of the rats which had conditioned units. The contribution of the bodily movement which seemed inevitably correlated with the conditioned unit response was determined by inducing skeletal muscle paralysis with Flaxedil. Conditioned responses were maintained under paralysis in all 5 rats with an experimental unit in the brain stem, but in only one of the 6 rats with an experimental unit in the cerebellum, and none in the other 7 rats with experimental units divided among hippocampus, midbrain and superior

colliculus. This indicated that the conditioned responses of most of the units were fed-back from movement which the conditioned activity of the brain stem units probably preceded. A control experiment with non-contingent reinforcement showed that these conditioned responses were probably not entirely due to operant conditioning. This ambiguity was absent in Experiment III which showed clearly operant activity. The rate of units, predominantly in the cerebellum, was increased or decreased depending upon the contingency of reinforcement. However, Experiment III used active animals and tested no units in the brain stem. A final experiment demonstrated clearly operant activity of a brain stem unit under paralysis. Reinforcement was made contingent upon rapid alternation between activity and inactivity of the unit. After acquisition, this behavior was brought under the control of a discriminative stimulus, and then maintained under paralysis, which eliminated the alternation of stereotyped movements that had been correlated with the unit activity.

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Table of Contents

Page

Page

INTRODUCTION

Operant conditioning* has been one of the most fruitful experimental approaches to the understanding of behavior. In this conceptualization, developed by B. F. Skinner and his associates (Ferster and Skinner, 1957), complete control of behavior by manipulation of environmental stimuli, rewards, and punishments, is viewed as tantamount to the understanding of behavior. Utilizing empirically derived laws, operant conditioning allows prediction of behavior with high probability. Understanding the cellular basis of conditioning as well as how brain activity initiates and controls operant responses would be of great interest.

Although the present research mainly addresses the problem of initiation and control, some interesting approaches which seem to point the way to eventual understanding of the cellular basis of conditioning are mentioned here. Because of the tremendous complexity of the mamallian nervous system, researchers have operated with the assumption that the same underlying mechanisms may be found in simplified (analog) preparations. Horridge (1962) found that the leg of a headless cockroach possessing only a ventral ganglion, "learned" to avoid shock by maintained flexion, whereas the leg of a yoked

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*Appendix A describes the basic ideas in operant conditioning.

control receiving the same number of shocks on a non-contingent basis did not. This intriguing study suffered from the finding that the cockroach leg exhibited the response with the prothoracic ganglion removed (Eisenstein and Cohen, 1965). This means that the effect must be due to the muscles being directly shocked, rather than to actual neural changes, which are undoubtedly the basis of conditioning in the central nervous system. It remains possible, however, that the intact prothoracic ganglion might participate in an effect analogous to conditioning. Hoyle (1965) made intracellular recordings of muscle junction potentials from an identified leg muscle of the headless locust and shocked the afferent nerves whenever the spontaneous rate of discharge fell below a certain "demand" level. This resulted in an augemented discharge frequency from the leg muscle, but not in a yoked control which received the same number of shocks non-contingently. A trouble with this was that attempts to subsequently increase the discharge frequency of the yoked control by contingent shocks were not successful.

A seemingly more promising analog for the study of the cellular basis of conditioning comes from the work of Kandel and his associates (1967). These experiments were done with intracellular recording in the abdominal ganglion of Aplysia of an identifiable cell which has a fairly regular pattern of spontaneous bursting and quiet periods. No matter when it was applied, strong stimulation to the connectives produced changes in the bursting pattern lasting

up to 20 minutes. However, weaker stimulation of the connectives or of a single interneuron producing an elementary monosynaptic IPSP, produced different changes in the bursting pattern, depending upon the contingency of application of the stimuli. Stimulation at burst onset decreased inter-burst intervals, while stimulation during silent periods prolonged inter-burst intervals. These effects outlasted stimulation for one to fifteen minutes depending on how long contingent stimulation was applied. Although the cellular mechanisms of these effects remain unknown, Kandel (1967) suggests that pacemaker cells may possess intraneuronal mechanisms for temporarily maintaining modifications in their endogeneous rhythms, which these experiments have shown can be initiated by impinging synaptic activity. This would mean that temporary information storage would be in the post-synaptic cell not in the synaptic changes.

Separate from the question of how conditioning causes physical change in nervous tissue is that of how brain activity initiates and controls the pattern of operant responses. Much of the answer to this question may be inferred from what is known about the brain organization of skilled and "voluntary" movement. ("Voluntary" is the word usually applied by physiologists to non-reflexive -- and therefore operant -- behaviors.) It is known that the impulses that initiate voluntary movement in mammals are carried by the pyramidal and extrapyramidal tracts. Severing the pyramidal tract produces loss of fine volitional movements and flacidity of the muscles;

severing the extrapyramidal tract produces varying degrees of paralysis of volitional movements, but differs from pyramidal tract loss in that resistance to passive movements is increased (Fulton, 1949). Operant control of neuro-muscular activity in humans can be exceedingly fine. Trained subjects can control the activity of a single motor unit of the finger muscle (Basmajian, 1963), and fire the unit in predetermined sequences, sometimes even rotating firing between two or more units (Basmajian, 1967).

Certain parts of the brain such as the motor cortex, the cerebellum, and the basal ganglia seem more involved than others in the production of impulses which pass over the pyramidal and extra-pyramidal tracts. However, no one of these structures is essential (Ruch, Patton, Woodbury and Towe, 1965). In control of these essentially motor structures seem to be higher control features, which take into account the affective state of the organism and relationships between present and past circumstances before "deciding" the direction of behavior. Penfield (1954) coined the term "centrencephalic" to describe the subcortical systems which seemed to regulate and coordinate cortical-cortical information flow, and presumably originate the impulses that result in voluntary movement. No specific anatomy of the centrencephalic system was given, but the reticular formation of the midbrain and the thalamus are strongly implicated.

Behavior seems to be organized in a hierarchical fashion, wherein reflexes are integrated into more and more complicated behaviors with "decision" making apparatus at the top, functioning to "release" or "trigger" the integrated behavior pattern at the appropriate time (Bullock and Horridge, 1965; Glickman and Schiff, 1967). Willows and Hoyle (1969) provide a good demonstration of this triggering principle. Activity of a group of electrically coupled cells in the pleural ganglion of the nudibranch Tritonia was found to initiate an elaborate fixed action pattern -- the "swimming escape response". In more complicated organisms, "triggered" behavior patterns are also more complicated and take on a "purposive" character, wherein they are adapted to the particular features of the environment. Thus, Delgado (1969) found that certain electrical stimulation of the brain of a monkey would cause aggressive behavior only if there were other monkeys present.

In humans, the functional hierarchy of organization of behavior seems to start with a "psychic plan" for movement that mobilizes "kinetic formulas", which in turn produce specific movement control signals (Paillard, 1960). Cortical lesions can produce selective interference with these things, a condition called apraxia (a = neg. + Gr. prassein = to do). Paillard describes three kinds of apraxia: 1) "a perturbation of psychic planning of action" associated with damage to the anterior frontal areas (ideational apraxia); 2) "the incapacity to mobilize correctly the kinetic

formulas in accordance with the established plan" associated with damage in the region of the supromarginalis gyrus (ideokinetic apraxia); and 3) "the dissolution of the kinetic formulas themselves" associated with damage to frontal areas (motor apraxia). Rhinencephalic structures which communicate with the cortex and which are integrated with the brain stem arousal mechanisms, are thought to supply an essential affective component in the organization of skilled behavior (Paillard, 1960).

Despite a traditional conviction to the contrary, operant conditioning seems to extend to some degree beyond the skeletal muscle to the autonomic nervous system. For example, the electrodermal activity in man has been operantly conditioned, and an immobilizing dose of d-tubocurarine did not interfere with the control of the activity (Birk, Crider, Shapiro, and Tursky, 1966). Operant conditioning of heart rate changes has been shown in rats fully curarized to eliminate the possible influence of skeletal muscle activity (Trowill, 1967; Miller and DiCara, 1967). The fact that these changes are not particularly facile suggests kinetics other than those involved in normal operant activity. Human subjects successful in augmenting heart rate often report that they did it by "thinking of exciting things", although one very successful subject felt he was exerting the control directly much as he would in moving his arm (Shapiro, personal communication). When

first learned, complex behaviors are executed in a primative, fumbling way and only gradually, with practice, become more refined and skilled. Normal voluntary behavior probably "feels" direct and immediate because the complex motor patterns called upon are relatively automatic, perhaps combinations of reflexes, so well learned that they are very easy to use. This subjective aspect of ease belies the underlying complexity and possible contribution or "indirect" factors such as "thinking of exciting things".

One experimental approach to the question of brain pattern during operant behavior is simply to look for the brain correlates of operant behavior. Thus, Jasper and Penfield (1949) discovered that the rolandic beta rhythm is blocked specifically in the precentral areas during the kinetic component of intentional movement. (Later Penfield and Jasper (1954) pointed out the probable subcortical (centrencephalic) regulation of such cortical rhythms.) Utilizing, restrained monkeys, trained to make an operant response to avoid shock, Jasper, Ricci, and Doane (1960) found acceleration of certain single units in the motor cortex prior to the response. Later acceleration of units was found in sensory cortex, a finding which would be expected on the basis of proprioceptive feedback from the operant response. Evarts (1966, 1968) operantly conditioned specific movements of the hand, and wrist of monkeys, requiring some of the movements to be made against greater resistances than others.

Recording from pyramidal tract neurons identified by antidromic stimulation of the pyramidal tract, Evarts found consistent acceleration of neural activity before the overt response. He also found that pyramidal tract neuron activity was more related to the force of the movement than the displacement, and that some cells were specially related to flexor, others to extensor movements. Using the same preparation Thach (1970a,b) studied activity of the cerebellar Purkinje cells and neurons in the dentate nucleus. The activity of cells in the lateral portions of cerebellar cortex and in the dentate nucleus was found to anticipate movement while activity of more medial portions of cerebellar cortex tended to coincide with movement. Jasper and Bertrand (1966) discovered single units in the ventral lateral nucleus of the thalamus of conscious human patients which were active during self-initiated arm movements, but not when the arm was moved passively or palpated.

There is another approach to the exploration of neural patterning during operant behavior which is potentially more powerful than the correlational method. This consists of applying operant conditioning directly to the neural activity, which in effect "short-circuits" the response which has to be made. The power of this method lies in its ability to clarify the operant character of the particular activity which is being recorded. Operant conditioning of the brain gives the organism free range to do anything it requires to modify

the activity, so that after a reasonable opportunity for modification has been allowed, some degree of confidence can be held in the judgment that the activity is or is not operant. Since being operant implies an adaptive function of the response, this method also allows stronger inferences than the correlation method about the functional significance of brain activity patterns. Finally, if it is used with paralyzed animals, it allows judgments to be made about whether the activity is excited by sensory feedback from behavior. The correlational method has the definite advantage of allowing behavior to be well specified. However, Evarts (1968) who has used this method with great sophistication to study motor neurons, points out that ideally the monkeys should be trained to produce a very wide repertoire of movements so that the particular muscle which the neuron activates can be discerned, but that this is not possible in practice. Operant conditioning of the brain activity perhaps could be used here as a preliminary tactic to give a better indication of the particular muscle commanded by the neuron.

Operant conditoning of brain activity has been studied in several different ways. Olds and Olds (1961) were the first to use this approach. In several subcortical and paleocortical structures, single unit rates have been found to augment when positive reinforcing electrical brain stimulation was given following spontaneous rate increases of the unit. Such augmentation was observed both in sedated rats (Olds and Olds, 1961), and in awake

active rats with either food or electrical brain stimulation as rewards (Olds, 1965, 1967). Fetz (1969) found augmented unit rates in motor cortex of monkeys when food reinforcement was given following rate increases of the units, but not when it was given at random. These rate elevations were found to extinguish, returning to normal levels when reinforcement was withdrawn. Fetz and Finocchio (1971) used the same preparation and found relationships between motor cortex units and specific arm movements, and then showed that a relationship between a precentral cell and a muscle movement could be dissociated by reinforcing cell activity and muscle suppression. This elegant demonstration of dissociation shows the unique power of operant conditioning.

Operant conditioning of the amplitude of cortical potentials evoked by light flash in cats given food reinforcement has been reported by Fox and Rudell (1968, 1970) and Rudell and Fox (1970). In an attempt to find a dynamic relationship between the evoked potential and overt behavior, Rosenfeld and Fox (1971) first found a portion of an evoked potential in somato-sensory cortex which was related to a particular reaching movement by the cat. Alterations of the reaching movement then resulted from operant conditioning

of changes in the evoked potential. Working with human subjects, Rosenfeld, Rudell, and Fox (1969) demonstrated operant control of a late component of the cortical potential evoked by an auditory stimulus.

The EEG has also been operantly conditioned. Wyrwicka and Sterman (1968) found operant conditioning of slow wave spindles (12-20 cps) in sensorimotor cortex of cats using milk reinforcement, and Sterman, Howe and MacDonald (1970) found that this procedure produced longer than normal epochs of undisturbed sleep. In an unpublished doctoral thesis, Carmona (1967) found that active cats could be operantly conditioned to increase or decrease the voltage of their EEG when reinforced with brain shock. Carmona ruled out the possible feedback from activity of skeletal muscles by repeating the experiment with rats paralyzed with d-tubocurarine. Also paralyzed to avoid the contaminating effects of skeletal muscle movement feedback, dogs have been operantly conditioned to increase and decrease the percentage of hippocampal theta rhythm (Black, Young, and Batenchuk, 1970; and Dalton, 1969). "Voluntary" blocking of the alpha rhythm of the EEG in humans has been shown (Jasper and Shagass, 1941b), as well as "voluntary" enhancement of it (Kamiya, 1962, 1968). (See Appendix B for a more detailed account of this work.)

Although some general things are already known, full elucidation

of the brain's activities during operant conditioning and behavior is a rather remote goal. The application of operant conditioning to the activity of the brain appears to be a direct method of investigating the functional limits of the brain. However, the general impression from the research so far accomplished with this method is that all brain activity is to some degree operant. It seems that given contingent reinforcement for doing so, the brain can modify any of its own activity. The present research sought to show that this is not necessarily true. First, it attempted to show that the capacity for operant change is distributed differentially in the brain of the active animal. Second, by the use of paralysis, it attempted to show that the class of activity that is operantly changed is not all initiated directly from within the brain -- so that a "respondent" portion can be distinguished which relies on excitation produced outside of the brain.

The experimental subjects were rats, chronically implanted with microelectrodes for single unit recording. Microelectrodes were used for discrete localization not as a means of analyzing the physiology of individual neurons. They were variously placed in order to sample from "sensory", "motor", "arousal" and "integrative" systems. Three different experimental paradigms were used which were referred to as "Experiments I, II, and III". Experiment I was an application of the procedure of operant conditioning with a

discriminative stimulus ("discriminative operant conditioning") to single unit activity in 5 different brain areas of active rats, with reinforcement contingent upon increases in rate. Experiment II was a continuation of Experiment I using some of the successful subjects under the condition of skeletal muscle paralysis. Experiment III was an application of the operant conditioning procedure to single unit activity in 4 of the 5 brain areas used in Experiment I, with reinforcement contingent upon rate increases and decreases of the same unit.

METHODS (GENERAL)

Subjects and Surgical Procedure

The subjects were adult, male, albino rats from the Holtzman Co. weighting from 250 to 350 grams. At the time of surgery, the rats were from three to four months old. The rats were anesthetized with pentobarbital (38 mg/kg i.p.) and placed in a stereotaxic instrument. Electrodes were implanted through small holes made in the skull with a hand drill. The implantation of the rather large microelectrodes for long-term chronic extracellular recording of single unit activity has been described by Olds (1967, 1969) and Olds and Hirano (1969) and was adapted from the method of Strumwasser (1958). Enamel-insulated nichrome wire, 62.5 μ diameter, was cut with scissors to form a blunt recording tip. The electrode was lowered into the brain with a microdrive, and recording was started when the tip was approximately 0.5 mm above the intended position. The recording apparatus consisted of a cathode-follower, preamplifier, an amplifier passing frequencies from 500 to 10,000 Hz., and an oscilloscope. The sound made by the signal over a loud speaker was also used in the detection of units. The probe was advanced slowly until unit spikes of at least 3:1 signal-to-noise ratio could be consistently recorded. The wire was then fixed to the skull by spreading acrylic dental cement around a prepared deformity in the

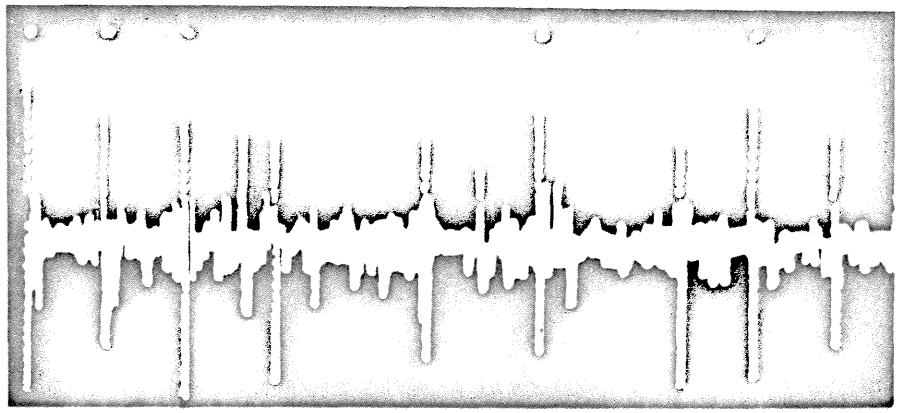
wire and nearby stainless steel jeweler's screws imbedded in the skull. When the cement hardened the microdrive connection to the wire was loosened and the process was begun again with the next probe. Recording electrodes were aimed at the brain stem (medulla oblongata and pons), cerebellum, hippocampus, midbrain reticular formation, and superior colliculus. Four to seven recording electrodes were implanted in each subject. An uninsulated 250 μ nichrome wire was implanted to a depth of about 6 mm in the anterior region of the brain to be used as a reference electrode for recording and a ground for stimulation. Two stimulating electrodes made of 250 μ nichrome wire, factory-insulated with epoxy, and bare only at the cross section were aimed at the medial forebrain bundle. After electrodes were cemented to the skull, they were attached to a delrin plaque cemented to the skull. This was used for later connections to recording and stimulating apparatus.

Experimental Apparatus

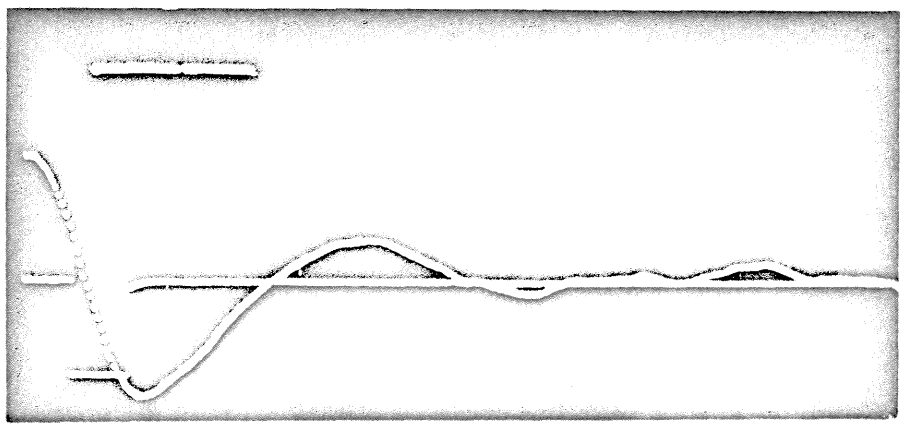
During experiments the rats were kept in an open-topped plexiglass cylinder 35 cm in diameter and 25 cm high inside of an electrically shielded, sound-attenuated box, with water continuously available. Food was delivered to a small tray from a mechanical pellet dispenser outside of the box through a chute made of tygon tubing. A 12 inch cable made of 10 pieces of low-noise Microdot antistatic wire led from the rat to an overhanging counter-balanced arm with a multiple slip ring assembly, allowing the rat relatively free movement about the cage. Two cathode-follower preamplifiers were plugged into the arm for recordings while other plugs in the arm were used to introduce electrical stimuli into the brain. Simultaneous recordings were made from two electrodes. Binary logic systems were used to automate the experiments. After amplification, the signals from each channel were fed into a "waveform discriminator" described by Olds (1967) which produced a binary pulse whenever the voltage level passed through an "amplitude window" and fell back to ground level through a "time window" which measured the duration of the wave (see Figure 1). With a two-beam oscilloscope, the device could be set to discriminate a unit of a given amplitude and fall time from other units and from background noise with good reliability. For each recording channel used in an experiment, Polaroid photographs were made of traces saved temporarily on the screen of a special oscilloscope (Tektronix, type 564, storage oscilloscope). The oscilloscope traces were

Figure 1.

Photographs of typical oscilloscope traces recorded from the hippocampus. The upper trace duration is 50.0 msec, and the lower trace duration is 1.0 msec. The dots in the upper trace mark units which passed the criteria set on the waveform discriminator device. As seems to be the case here, often more than one unit is discriminated. These units had to fall to ground potential through a falltime window which can be seen in the lower trace as the offset portion of the baseline. An amplitude window was also used, the lower threshold of which can be seen as the point at which the trace was triggered. The long line above the lower trace represents the output of the discriminator device which in the upper trace was compressed to a dot. This digital signal was counted electronically and used as the basis upon which reinforcement was automatically delivered to the animal.



50 MSEC TRACE



1.0 MSEC TRACE

initiated by the "lower" amplitude detector of the waveform discriminator. Two representative traces of each channel were photographed, one trace at a sweep speed of 5 msec/cm and the other at 0.1 msec/cm. Figure 1 shows typical results for a recording from hippocampus. The slower sweep rate showed the relation between signal to noise amplitude, and the faster sweep showed the shape of the unit. The units were continuously monitored on the oscilloscope throughout the experiment to insure uniformity and guard against artifacts. Episodes of special interest were sometimes recorded on magnetic tape for later oscilloscope display and photography. Separate binary counters received outputs of the waveform discriminator used for each recording channel, and a mark was made by a pen on a moving paper chart whenever the count reached a pre-set level (usually = 100). Other events to be described later were recorded simultaneously on the paper chart.

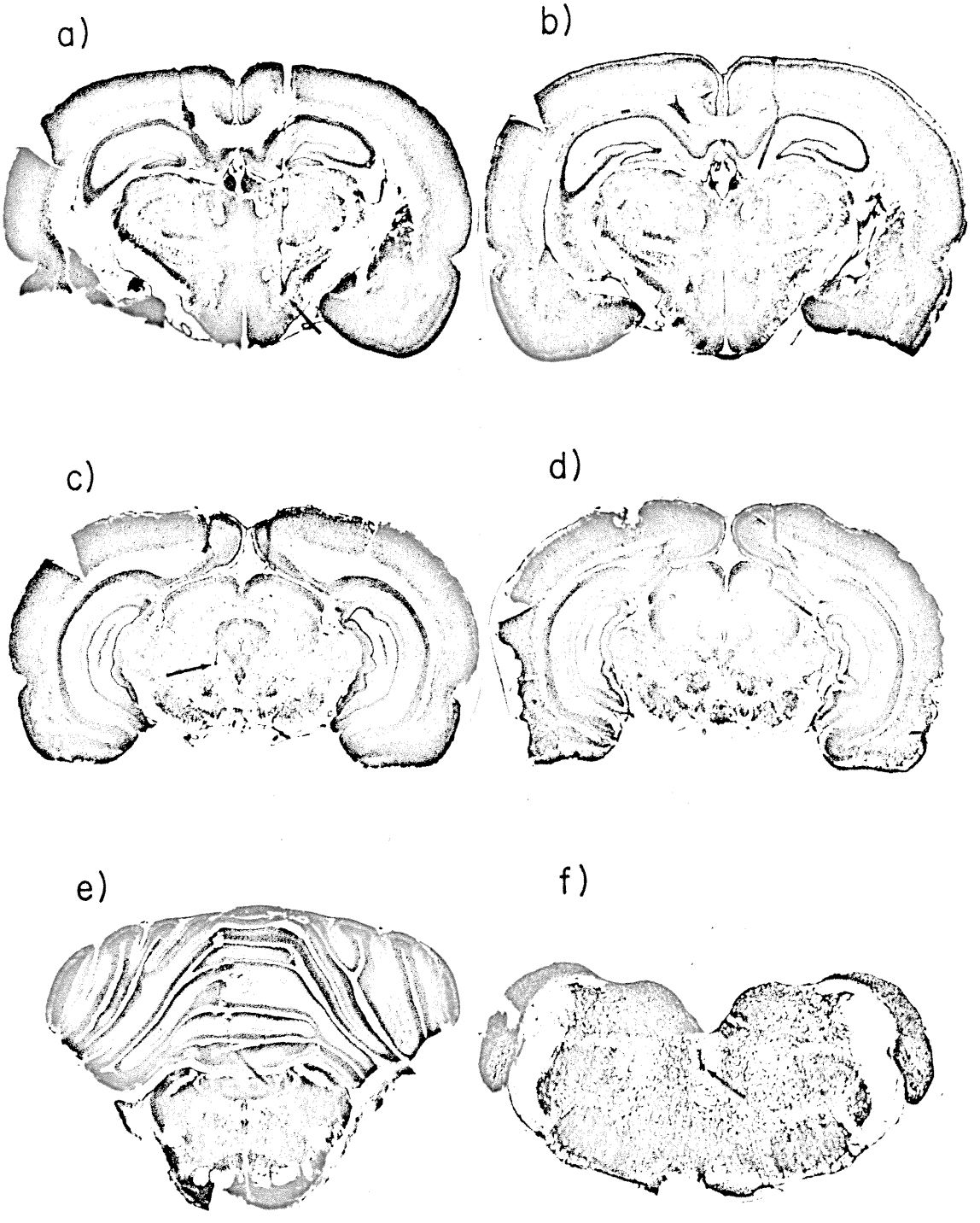
Histology

After the experiments the rats were sacrificed with an overdose of pentobarbital. In most instances the recording sites were marked by small lesions made by passing a 10 μamp DC current through the electrode for 15 sec grounding the cathode to the body of the rat. Perfusion with physiological saline was followed by a 10% formalin solution. The brains were fixed in formalin for at least 3 days

and then transferred to ethanol, frozen and cut in 60 μ coronal serial sections. Alternate sections were stained with Weil and cresyl violet which stained for fibers and cell bodies respectively. The point of recording or stimulation could be determined from the terminus of the electrode track. Typical histological material is shown in Figure 2.

Figure 2.

Examples of histological material from each of the brain areas used: a) stimulation probe in the medial forebrain bundle at the level of the lateral hypothalamus, b) recording probe in hippocampal gyrus, c) recording probe in midbrain, d) recording probe in superior colliculus, e) recording probe in cerebellum, and f) recording probe in brain stem.


a)
b)
c)
d)
e)
f)

EXPERIMENT I

Somewhat arbitrary decisions had to be made in choosing what asepct of unit activity to reinforce. Since changes in the rate of units seem to be the vehicle of information transmission, it seemed likely that peak rates had high information value and would be amenable to conditioning. Thus it was decided to define a half second period of relatively fast activity (a "burst") as the response to condition.

Identification of operantly conditioned overt behavior is ordinarily not difficult because of the usual low frequency of the behavior before conditioning. Unfortunately, rate increases of units are not improbable, so it is sometimes difficult to distinguish an operantly conditioned increase from one occurring spontaneously if a simple operant conditioning procedure is used. To clarify the effect of conditioning, it was decided to employ a discriminative stimulus (Appendix A) which signaled periods when reinforcement became available. This imposed a clear on-off demarcation and made conditioned responses easier to distinguish against background variability.

Procedure

Pre-training: In order to produce subjects with a high likihood of success in unit conditioning, each rat was given experience with operant conditioning of overt behaviors and the discriminative stimulus which was used in the experiment. To train some generalization and avoid fixation on one response pattern, three different operant behaviors were conditioned in pre-training: (1) pedal pressing with a forepaw; (2) lever pressing with the nose; and (3) gross body movement.

Food reinforcement was used initially because of its clear reinforcing properties. Each rat was maintained on a limited diet so that the weight was kept at 70-80% of the preoperative level. After a post-surgery period of at least 4 days, training on the first operant reponse began: pedal press responses were reinforced with a 45 mg food pellet. This training continued for 1 hour a day until a criterion rate of 300 food pellets earned in an hour was reached. Training of this operant response to the discriminative stimulus ("discriminative operant conditioning") then began. The animal was reinforced for a pedal press only during the 2 min on period of a light which remained off for the next 5.5 minutes. The animal was tested 4 hours a day until it earned 300 pellets within a given session. Following this, to teach response substitution, the rat

was trained to earn food pellets by pressing its nose through a hole (against a lever); and after acquisition it was again trained in the light-dark discrimination for the new task. Finally the rat was trained to earn food by making gross body movements. This was instrumented by amplifying the signal generated by a special "noisy" wire attached to the rat's cable. This wire was open-circuited at the bottom and generated spikes of electrostatic noise when it was jiggled by slight movements of the animal. High amplitude spikes were automatically discriminated by a Schmitt trigger, the output of which fed into a special circuit used to discriminate bursts of activity. This circuit consisted of a spike counter which produced an output signal if the pre-set count was reached, but which was reset to zero 0.5 sec after the beginning of each count if the pre-set number was not reached. The rat was reinforced with a food pellet whenever the "burst discriminator" produced an output. The number of spikes in a burst was set at the beginning of training so that the rat received a pellet about once a minute due to spontaneous activity. Training continued until the rate of reinforcement was increased markedly. The discriminative stimulus was then reinstated for the final phase of pre-training.

Unit Conditioning: Two good units were used for each experiment, selected from among the probes of the rat on the basis of where the best units could be recorded at the time of the experiment. One was arbitrarily designated the "experimental" unit, and reinforcements of the animal were made contingent upon its activity. The other unit was designated the "control" unit, and no reinforcements were intentionally made contingent upon its activity. The units which were used had at least a 2:1 signal to noise ratio and a regular waveform, free from artifacts.

Bursts of the experimental unit were detected by the burst discriminator circuit which has already been described. The burst discriminator received outputs from the waveform discriminator of the experimental unit and it in turn triggered the reinforcement. Bursts and reinforcements were marked separately on the moving paper chart. The burst criterion was set so that the initial spontaneous unit rate triggered a reinforcement about once a minute. The rat was given a 30 min acquisition period followed by at least 5 hours of discriminative operant conditioning, using the discriminative stimulus for 2 min out of every 7.5 min. Often there was drift in the background rate. If the rate changed so that the burst occurred too seldom (fewer than about 4 times in a light-dark cycle) or too frequently (more than about 25 times in a light-dark cycle), the burst criterion was changed until bursts again occurred about

once a minute. This procedure seemed sufficient to detect the presence or absence of conditioning.

The experiment was repeated on many of the acceptable recording channels from each rat by either reversing the experimental and control electrodes or by using other electrodes from which good quality units could be recorded. Sometimes when brain stimulation reinforcement was used and the rat appeared fresh and continued to be motivated by the reinforcement, it was used immediately for a second experiment with different units. No more than two experiments were run consecutively with the same animal. Otherwise there was at least a 12 hour period between experiments. At any given time about 10 rats were available for experimentation; each was used in rotation until it was no longer useful.

It was recognized that behavioral pre-training and use of the same rat in a second experiment might pre-condition units. In fact, a considerable tendency for correlation between experimental and control units was observed during the experiment. However this was not considered detrimental to the present experiment. It would be objectionable only if the experiment were attempting to examine the process of operant conditioning of units in isolation from overt behavior. The aim of the experiment was simply to distinguish units displaying operantly conditioned responses from those which did not. Within this experiment there was no way of distinguishing what was primary and what was secondarily involved

in the acquisition of the operant response, so no attempt was made to exclude pre-conditioned units. There was no attempt to select for pre-conditioned units either.

Positive Reinforcement by Brain Stimulation: Electrical stimulation of the medial forebrain bundle has strong positive reinforcing properties (Olds, Travis, and Schwing, 1960). It was substituted for food reward in some of the cases where units were apparently under operant control. This was in preparation for Experiment II in which successful animals in Experiment I were paralyzed to test for maintenance of the conditioned responses. This necessitated the use of a reinforcement requiring no overt behavior. Validation of the reinforcing properties of the stimulation was done by testing its effectiveness in conditioning an overt operant behavior. Each entry of the animal into a predetermined area of the cage was reinforced with 1 sec of 50 μamp (r.m.s.) 60 Hz sine wave stimulation. If the animal quickly learned to remain in this area, and if by using the same method it could then be quickly conditioned to a second area, the brain stimulation was adopted for experimental use.

Special Tests: 1. To test for the possibility that the reinforcement itself caused the unit activity to increase and thereby created the appearance of operant responding during discriminative stimulus, a "discrimination probe" (see Appendix A) was used as a

control procedure. If after training, a differential response did not occur during the discrimination probe, the unit could not be considered to be conditioned.

2. To test for specific acceleration of the "experimental" unit, as opposed to general activation of an area or general activation of the brain, a "control" unit was recorded simultaneously with no reinforcement contingent upon its activity. Whenever possible, the control recording was made from the same structure as the experimental one. Occasionally they were both recorded from the same probe in which case the activity was always correlated.

Data Analysis: A count was made of the number of times the unit fired during the 2 min period just prior to the period of the discriminative stimulus ("discrimination period"). This was subtracted from the number of times it fired during the discrimination period to yield a measure which will be called an "inflection differential." The algebraic sign was positive for rate increases and negative for decreases. This measure was made on the first discrimination period and every fourth period thereafter so that activity was sampled every 30 min of the experiment. A Wilcoxen test for matched pairs (Siegel, 1956) was made on the first ten of these inflection differentials; selection of significant cases was made at the 5% probability level (one-tailed, because only rate increases supported the hypothesis of conditioning). This statistical

test was employed simply as a method of objective decisions as to differences between units.

Results

The data reported here are from 79 repetitions of the experiment. A total of 34 rats were used; 10 were used in more that three experiments. An example of the paper chart record of a conditioned unit is shown in Figure 3.

Experimental Units: There were cases of signficant rate increases of experimental units in each area tested. All 8 cases in the brain stem and all 22 in the cerebellum were significantly increased, compared to 8 out of 21 in hippocampus, 8 out of 14 in midbrain, and 7 out of 14 in superior colliculus (see Table 1 and Figure 4 for the localization of recording points). These proportions differ significantly (Chi-square = 25.2, df = 4, $p < 0.001$), indicating that location is a significant variable.

Of the total number of inflection differentials of experimental units sampled in each brain area, there were significantly more positive than negative (see Table 2). However the proportion of positive inflection differentials differed significantly between areas (Chi-square = 91.89, df = 4, $p < 0.001$), ranging from 98% in brain stem and 96% in cerebellum to 74% in midbrain, 63% in hippocampus and 60% in superior colliculus.

Figure 3.

Samples of the paper chart record made during Experiments I and II. A spike was marked in the upper trace each time a pre-set number of unit spikes recorded from the experimental probe was counted, and in the second trace each time a pre-set number of unit spikes recorded from the control probe was counted. Upward deflections were made in the third trace for bursts (a pre-set number of unit spikes within 0.5 sec) of the experimental unit. The pen remained deflected for the 5.0 second delay period imposed after each burst during which time no other bursts were detected. Downward deflections in the fourth trace marked the application of reinforcement. The 2 min discrimination periods were recorded as simultaneous upward displacements of all four traces for the duration of the period.

These samples were taken during an experiment where the conditioned acceleration of unit activity was maintained under paralysis. The times of Flaxedil injection and beginning of artificial respiration of the animal are marked by dashed lines. The first three discrimination periods were with reinforcement available, and the last two were with reinforcement withdrawn. The first discrimination period following reinforcement withdrawal was designated the "discriminative probe."

SAMPLE OF RECORD

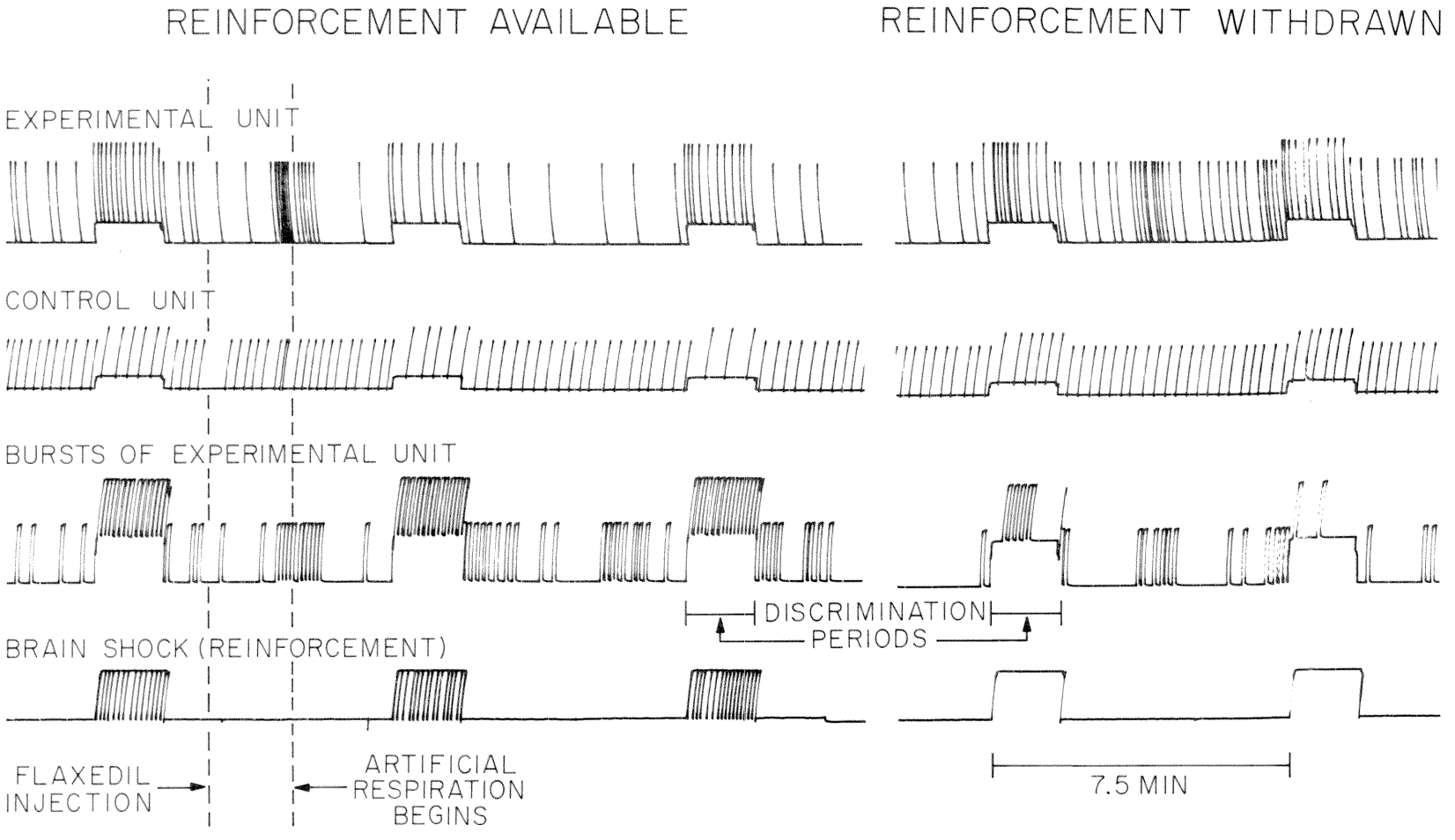


Table 1.

Summary of outcomes of tests for rate increases of each unit. The test for significance was a Wilcoxen test for matched pairs (Siegel, 1956; selecting at $p < 0.05$). Each unit was tested separately by using 10 "inflection differentials" (the total number of times it fired during the discrimination period minus the total for the equivalent time immediately preceding) sampled at 30 min intervals.

The results for the experimental and control units are depicted separately, as are the results from each brain area. The results for the cases when the rats were reinforced with food are shown apart from those when brain stimulation was used for reinforcement. Totals for each brain area and method of reinforcement are also shown.

Table 1

		Experimental Units			Control Units		
		Food	Brain Stim.	Total	Food	Brain Stim.	Total
Brain stem	Sig.	3	5	8	2	1	3
	Not Sig.	0	0	0	0	1	1
Cerebellum	Sig.	9	13	22	1	6	7
	Not Sig.	0	0	0	0	4	4
Hippocampus	Sig.	3	5	8	1	3	4
	Not Sig.	5	8	13	6	5	11
Midbrain	Sig.	3	5	8	5	6	11
	Not Sig.	3	3	6	2	1	3
Sup. Colliculus	Sig.	2	5	7	1	3	4
	Not Sig.	1	6	7	0	11	11
Total	Sig.	20	33	53	10	19	29
	Not Sig.	9	17	26	8	22	30

Figure 4.

Histological localization of recording points used in Experiment I. The upper 8 coronal sections are drawings covering the points used in the hippocampus, midbrain and suprior colliculus. They are designated by the distance in microns anterior to the coronal section of the interaural line (König and Klippel, 1963). The lower 4 drawings are also in the coronal plane and cover the points used in the brain stem and cerebellum. They are designated by the figure numbers from Craigie (1963).

CONDITIONED UNIT DISCRIMINATION

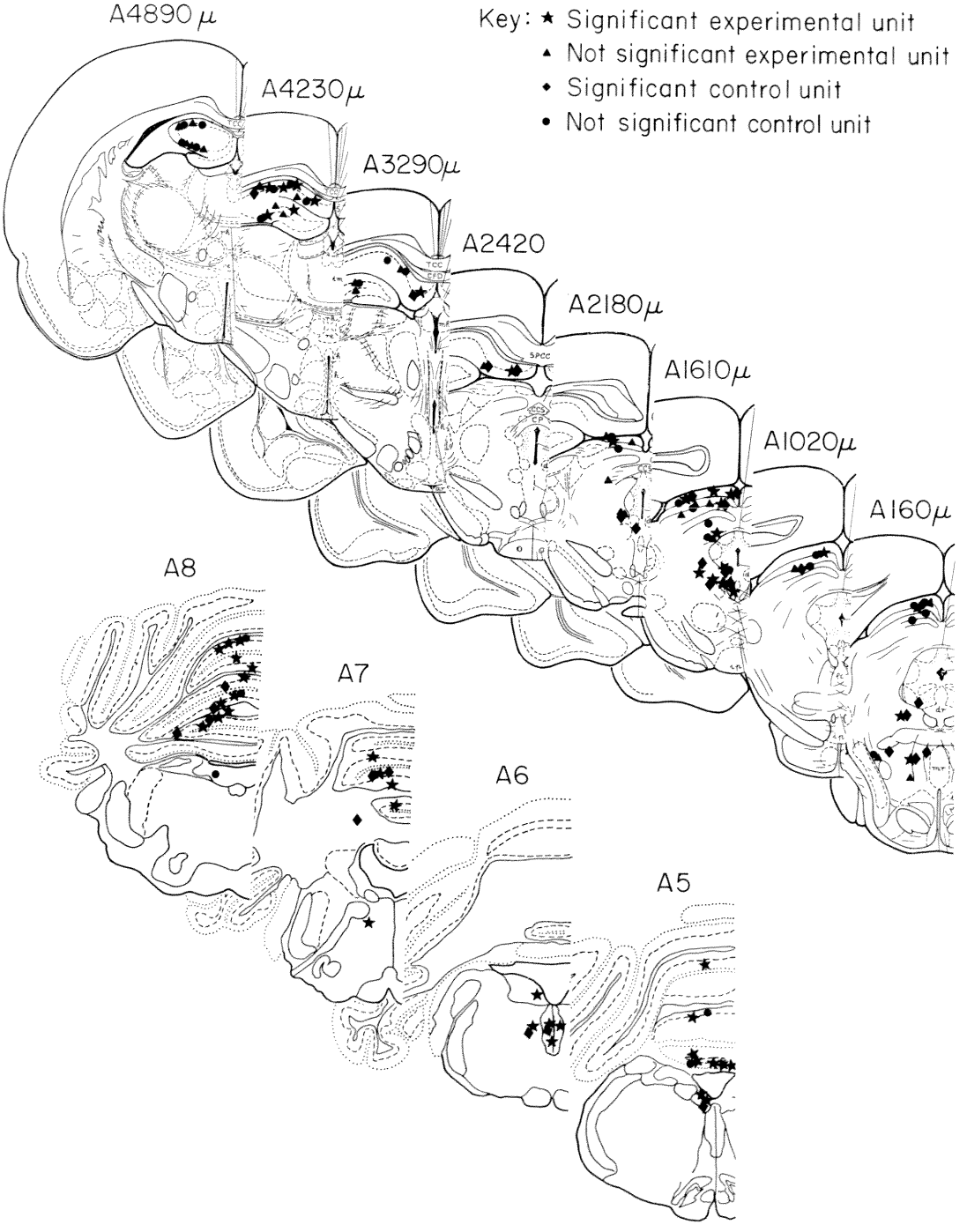


Table 2.

Totals of the number of positive and negative inflection differentials sampled in each brain area with respective percentages, z-scores, and probability values (binomial test, two-tailed).

Table 2

	Total Number of Inflection Differentials		Positive Total	Z-score	Probability (two-tailed)
	Positive	Negative			
			Experimental Units		
Brain stem	49	1	98%	6.65	<0.00006
Cerebellum	199	9	96%	13.10	<0.00006
Hippocampus	113	65	63%	2.85	=0.0044
Midbrain	86	31	74%	4.99	<0.00006
Sup. Colliculus	71	47	60%	2.12	=0.034
			Control Units		
Brain stem	21	2	91%	3.75	<0.00022
Cerebellum	76	14	84%	6.42	<0.00006
Hippocampus	69	37	65%	3.01	=0.0026
Midbrain	98	22	82%	6.85	<0.00006
Sup. Colliculus	33	69	32%	-3.55	<0.00046

Control Units: There are fewer control units than experimental units reported here because in some experiments, histology showed some were not in one of the experimental brain areas. There were cases of significant rate increases of the control units in each of the brain areas (see Table 1 and Figure 4 for localization of recording points), but the proportions differed significantly between areas (Chi-square = 12.85, df = 4, $p < 0.02$), ranging from 3 out of 4 in brain stem, 7 out of 11 in cerebellum, and 11 out of 14 in midbrain to 4 out of 15 in hippocampus and 4 out of 15 in superior colliculus. These proportions are lower than for the experimental units in all areas but the midbrain. The total number of positive inflection differentials sampled in each brain area was significantly greater than the total of the negative in all brain areas except the superior colliculus where there were 32%, a significantly smaller proportion ($p < .00046$, binomial test) (see Table 2).

Comparison of Experimental and Control Units: The proportion of significant control units was 49% (29 out of 59) compared to 67% (53 out of 79) of the experimental units. The difference is small but statistically significant (Chi-square = 4.5, df = 1, $p < 0.05$). Occasionally the same experimental unit was used in a second experiment with a different control unit, and vice versa. Including these experiments, there were 65 where the test of significance could be made on both the experimental and control units. Of the

65 experiments, both experimental and control units were significant in 24, and neither was significant in 10; the experimental unit was significant in 19 when the control was not, and the control was significant in 12 cases when the experimental was not (Table 3a). The proportion of control units tracking or following their experimental counterparts was about the same whether or not the control unit was in the same brain areas as the experimental unit (see Tables 3b and 3c).

Food vs. Brain Stimulation Reinforcement: The total proportion of significant experimental units was about the same for food reinforcement (20 out of 29 = 69%) as for brain stimulation (33 out of 50 = 66%) (see Table 1). In each brain area the proportions were also quite similar. Similarly, for the control units, brain stimulation was about as effective as food: 10 out of 18 = 56% were significant using food reinforcement and 29 out of 59 = 49% were significant with brain stimulation.

Practice: Since many rats were used in more than one experiment, it was of interest to determine whether or not the chances of success were affected by the past history of success. Table 4 shows a break-down of the significant and not significant cases according to whether or not they were preceded by at least one success. The proportions were very similar in every case; the history of success

Table 3a.

Contingency table summarizing the outcomes of all experiments where the Wilcoxen test of significance could be made on both experimental and control units. A given unit may have been used more than once if it was used with a different unit or if it was switched from experimental to control or vice versa.

Table 3b.

Same as Table 3b except that it includes only those experiments where experimental and control units were in the same brain area.

Table 3c.

Same as Table 3a except that it includes only those experiments where experimental and control units were in different areas.

Table 3a.

		Experimental Units		
		Significant	Not	Total
Control Units	Significant	24	12	36
	Not	19	10	29
	Total	43	22	65

Table 3b.

		Experimental Units		
		Significant	Not	Total
Control Units	Significant	6	4	10
	Not	7	9	16
	Total	13	13	26

Table 3c.

		Experimental Units		
		Significant	Not	Total
Control Units	Significant	18	8	26
	Not	12	1	13
	Total	30	9	39

Table 3d.

Same as 3a except that each brain area is listed separately.

Table 3d.

			Brain Stem		Cerebellum		Hippo-campus		Mid-Brain		Superior Colliculus		Total	
			Sig.	Not	Sig.	Not	Sig.	Not	Sig.	Not	Sig.	Not	Sig.	Not
Control Units	Brain Stem	Sig.	0	0	0	0	0	0	0	1	1	0	1	1
		Not	1	0	0	0	0	0	0	0	0	0	1	0
	Cerebellum	Sig.	0	0	2	0	0	2	2	2	1	1	5	5
		Not	0	0	1	0	0	0	2	0	0	0	3	0
	Hippocampus	Sig.	0	0	0	0	1	2	0	1	2	0	3	3
		Not	2	0	0	0	4	5	0	0	0	0	6	5
	Midbrain	Sig.	3	0	4	1	2	0	2	1	0	0	11	2
		Not	2	0	2	0	0	0	0	1	0	0	4	1
	Superior Colliculus	Sig.	0	0	2	0	1	0	0	0	1	1	4	1
		Not	1	0	2	0	1	1	0	0	1	3	5	4
	Total	Sig.	3	0	8	1	4	4	4	5	5	2	24	12
		Not	6	0	5	0	5	6	2	1	1	3	19	10

Table 4.

Summary of the outcomes of the Wilcoxen test on experimental units according to whether or not the experiment was preceded by at least one other experiment on the same rat when the experimental unit outcome had been significant.

Table 4

	Brain stem		Cerebellum		Hippocampus		Midbrain		Superior Colliculus		Total	
	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.
Preceded by Another Sig. Experiment Unit	4	0	10	0	4	7	2	4	4	4	24	15
Not Preceded by Another Sig. Experimental Unit	4	0	12	0	4	6	6	2	3	3	29	11

did not seem to help in predicting success. However, all rats had considerable pre-training for discrimination before the unit conditioning experiments began which may have rendered this subsequent training relatively inconsequential.

Sensory Effects and Discriminative Probes: The effect of light alone was analyzed by looking at successive inflection differentials without reinforcement, usually in rats where conditioning was extinguished. Figure 3 shows an example of record with reinforcement withdrawn. Wilcoxen tests for matched pairs were done on 20 successive inflection differentials for each of 34 units. In only one case, a unit in the cerebellum, did a significant increase occur during the discrimination period (see Table 5). However, the hippocampus, midbrain and superior colliculus each had 2 units that were significantly decreased. Since a significant rate increase happened only once, the light alone could not account for the experimental results.

In cases when conditioning appeared to be successful, often the reinforcement was withdrawn for one of the discrimination periods to test whether or not the rate increase was then associated with the light alone. In all 23 cases when the experimental unit was tested this way, the rate increase continued to occur during the light alone (see Table 6), indicating that the rate increases were a learned consequence of the reinforcement. In all 12 cases

Table 5.

Summary of outcomes of tests for rate changes when the discriminative stimulus (light) was presented in the absence of reinforcement. * = significant rate decrease (Wilcoxen tests on 20 successive inflection differentials; 2-tailed, $p < 0.05$).

Table 5

	Brain Stem	Cerebellum	Hippocampus	Midbrain	Superior Colliculus
Significant	0	1	2*	2*	2*
Not Significant	5	5	7	7	3

*Significant rate decreases

Table 6.

The number of positive and number of negative discriminative probes according to whether or not the unit had also displayed significant rate increases.

Table 6

	Brain Stem		Cerebellum		Hippocampus		Midbrain		Superior Colliculus		Totals	
	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.	Sig.	Not Sig.
					Experimental Units							
Positive Discriminative Probe	2	0	8	0	3	3	5	0	5	3	23	6
Negative Discriminative Probe	0	0	0	0	0	1	0	1	0	0	0	2
					Control Units							
Positive Discriminative Probe	0	0	4	1	2	1	4	0	2	3	12	5
Negative Discriminative Probe	0	0	0	1	0	2	0	0	0	2	0	5

when discriminative probes were made on control units which had shown significant increases, the results were also positive. In cases categorized as not significant negative, discriminative probes were expected and occurred in 7 out of the 18 units tested.

Discussion

Contrary to the impression found in the literature on operant conditioning of brain activity, the results of the experiment made clear that the operant conditioning procedure does not always produce changes in the brain activity to which it is applied. Under the conditions of this experiment, it was clear that units from certain parts of the brain were more likely to be conditioned. In fact, there were no failures ever found with experimental units in the cerebellum or brain stem. The distinguishing feature of these parts of the brain is their relation to motor activity.

It is dangerous to infer from this that those units which were not "significantly" augmented were absolutely refractory. Perhaps different procedures would have brought these units into play. However, when the experiments with refractory units were greatly prolonged or repeated on another day, improvement was seen very seldom.

Surprisingly little specificity of effect was produced. It was anticipated that the control units would be generally unaffected, because in many cases they were quite distant from the companion experimental unit, but this idea was contradicted by the results.

Regardless of the proximity of the control unit to the experimental unit, the control unit tended to be conditioned. Sometimes the control unit was conditioned even when the experimental unit was not! A significantly lower percentage (49%) of control units were conditioned that experimental units (67%), but the difference was not great. The explanation for the high percentage of conditioned control units seems to be that a whole behavioral pattern was being conditioned, not just that fraction of activity on which reinforcements were contingent -- the experimental unit. Overt behavior always was conditioned to some degree when the experimental unit was conditioned. The experimental unit was not necessarily even an important part of the totality that was conditioned, which obviously included many control units.

Control experiments where the discriminative cue - the light - was presented alone to extinguished animals showed that the results were not due to simple sensory stimulation. Discriminate probes (presenting the discriminative cue alone after acquisition) showed that the responses indeed were conditioned and were not the result of simple activation by the reinforcement. However, there was an anomaly in the results which prompted another control test. A few experimental units in hippocampus and superior colliculus were depressed during the discriminative period even though the animal was obviously motivated by the reinforcement. It could be shown by a discriminative probe that the reinforcement itself was not

directly causing the rate depression, and it could be shown by extinguishing the animals, that the light alone was not producing the results. This meant that it was a conditioned effect, and since it was not in the direction which would be expected for operant conditioning, it was by exclusion, respondent conditioning. That is, it was caused by the association of the discriminative stimulus with some kind of "reflex" which depressed the rate. Since the reflex could just as easily have acted to increase unit activity, operant conditioning was called into question as the exclusive interpretation of the results. Although the presence of reinforcement was certainly important, its contingency upon unit rate may not have been. To further explore this possibility, a group of 8 rats were run under the conditions of Experiment I except that reinforcement was programmed at random during the discrimination periods instead of contingent upon an experimental unit. In 6 of these cases a significant rate increase occurred (see Table 7), showing that respondent conditioning was a powerful effect in this situation.

Although this made it plain that it was not possible to distinguish operantly conditioned unit responses in Experiment I, there was good circumstantial evidence that operant conditioning had taken place. First, many rats repeated stereotyped overt behaviors just before reinforcement which indicated that the unit activity thus generated was operant, not a reflex evoked by the discriminative stimulus. Second, non-contingent reinforcement

Table 7.

Summary of the outcomes of tests for rate increases of each unit when the reinforcement was programmed at random during the discrimination period.

Table 7

	Brain stem	Cerebellum	Hippocampus	Midbrain	Superior Colliculus	Total
Sig.	0	2	1	1	2	6
Not Sig.	0	1	0	0	1	8

failed to condition all the cerebellar units, while all 22 had been conditioned with contingent reinforcement. It appears likely that both operant and respondent conditioning of units occurs simultaneously when the discriminative operant method is used. Although negative results using this method may be taken as evidence that operant conditioning does not work, positive results may not all be due to operant conditioning.

EXPERIMENT II

Testing of some of the successful subjects of Experiment I was continued under paralysis induced by Flaxedil (gallamine triethiodide, a synthetic curare-like compound which blocks cholinergic transmission at the neuro-muscular junctions (Goodman and Gilman, 1965)) to find whether the conditioned unit accelerations were reflexly caused by feedback from conditioned overt responses.

Procedure

Removing the animal from the experimental chamber only long enough for an injection, it was paralyzed with Flaxedil (50 mg/kg i.p.) and artificially respirated through the nasal openings with a pump at 60 strokes per min and 30 cc stroke volume with 5% CO_2 added to maintain stimulation of the respiratory centers in the brain. The animal was connected to the respirator with a nosepiece made of a flared section of tygon tubing which fit snugly, but comfortably around its snout. The rat was held onto the respirator with a rubber band around its skull plaque and the nosepiece. Paralysis was checked by constant surveillance and periodic tail pinches, touching the cornea of the eye, and most effectively by tickling the ear opening with a cotton swab. If any movement was noticed, a supplementary injection of 25 mg/kg i.p. Flaxedil was given.

Often a great change occurred in unit rate when the animal was paralyzed, so it was necessary to readjust the criteria for

reinforcement until it occurred about once a minute. If no light-dark discrimination was then noticeable, an extended period of 20 minutes of remedial training was initiated where the light remained on. This was followed by another test for light-dark discrimination lasting at least an hour. The animal was then allowed to recover from paralysis. If the rat had other good units which had not been tested, it was returned to its home cage after recovery of breathing. If not, it was sacrificed.

Data Analysis: The units of the rats tested under paralysis for discrimination were analyzed for statistical significance as in Experiment I, except that the Wilcoxen test was made on successive inflection differentials instead of every fourth one.

Results

Occasionally units would stop when the animal was paralyzed so they could not be tested. Of the units it was possible to test, two-thirds failed to maintain the discrimination which had been clear just before paralysis (see Table 8 and Figure 5 for localization). Specifically, only 1 of the 6 experimental units in cerebellum maintained discrimination, an area where all 22 units tested in Experiment I had been successful. Neither of the 2 experimental units in the hippocampus or the 2 units in superior colliculus continued to maintain discrimination. In the midbrain, none of the

Table 8.

Summary of outcomes of tests for maintained rate increases of each unit of the paralyzed rats. Each unit was tested separately with the Wilcoxen test on successive inflection differentials.

Table 8

Experimental Units

	Brain Stem	Cerebellum	Hippocampus	Midbrain	Superior Colliculus	Total
Significant	5	1	0	0	0	6
Not Significant	0	5	2	3	2	12

Control Units

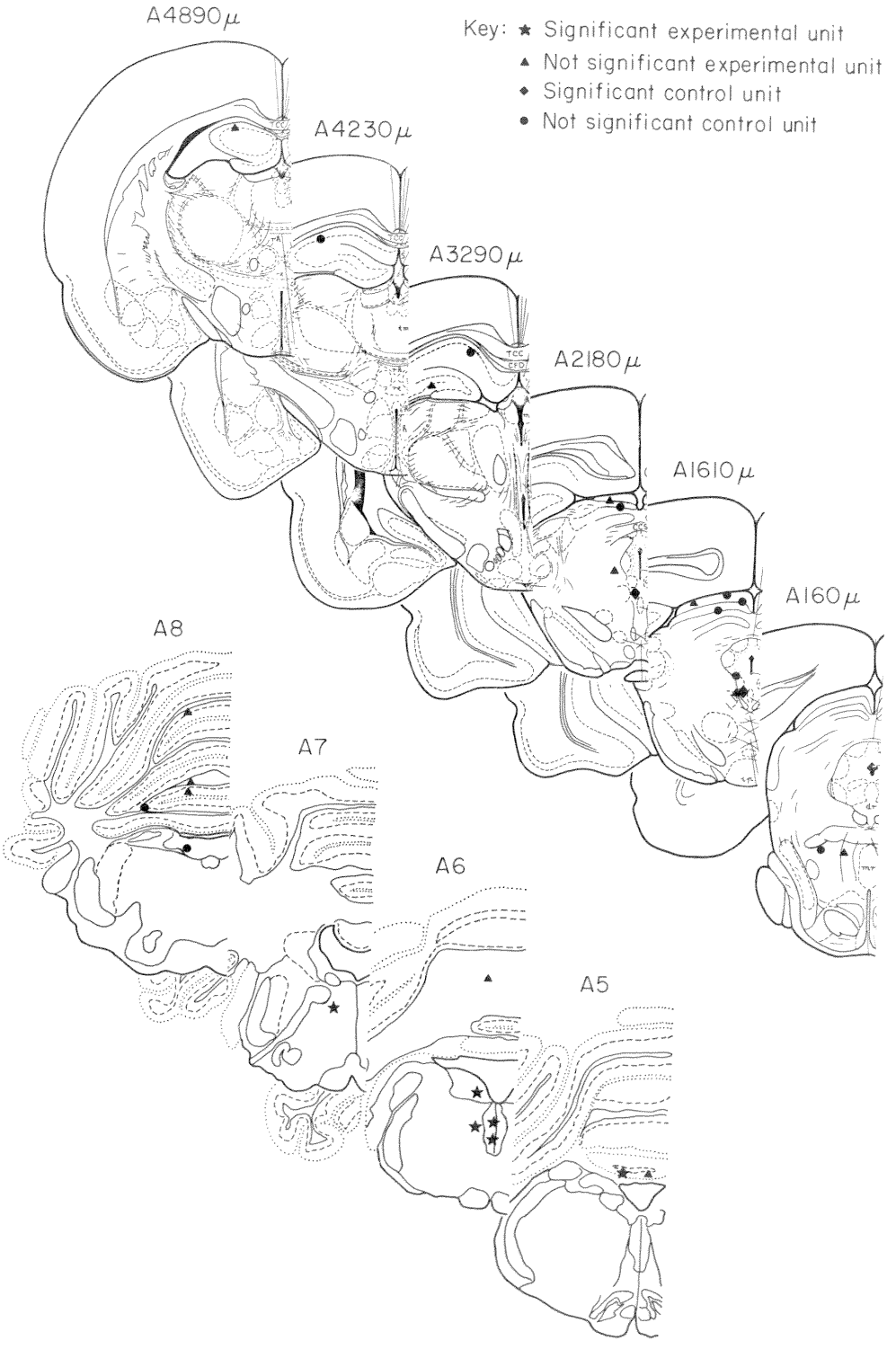
Significant	0	0	0	2*	0	2
Not Significant	1	1	2	4	4	12

*both cases accompanied significant experimental units in the brain stem.

Figure 5.

Histological localization of recording points used in Experiment II. The upper 6 drawings are from König and Klippel (1963) and the lower 4 drawings are after Craigie (1963).

DISCRIMINATION MAINTAINED DURING PARALYSIS



3 cases were positive, but many units in this area stopped firing when the Flaxedil was given. All 5 experimental units in the brain stem successfully maintained discrimination, which clearly distinguished this area from the others. Each of these units was recorded from a different rat. Continuous photographs of an experimental unit in the brain stem of a rat under paralysis before and during and after a reinforced discrimination period appear in Figure 6, and continuous photographs of the same unit before, during and after a discriminative probe appear in Figure 7. Of the 14 control units tested under paralysis, only 2, both in the midbrain, displayed significant rate increases during the discrimination periods (see Table 8). Each of these accompanied one of the experimental units in the brain stem.

General Rate Changes: Including pilot studies, 64 units in the 5 brain areas were recorded before and during paralysis. The drug-induced paralysis noticeably accelerated spike rates in 5 cases (8%), noticeably slowed rates in 27 cases (42%) and stopped activity altogether in 9 cases (13%) although the activity returned with movement ability (see Table 9, and Figure 8 for histological localization). Four of the 5 accelerated cases were in cerebellum making up 28% of the total cerebellar group). All 9 of the stopped units were in the midbrain. (This was 53% of the midbrain group.) A case of a midbrain unit whose activity was abolished by paralysis is depicted in Figure 9.

Figure 6.

Illustration of an experimental unit in the brain stem of a paralyzed animal (rat #9584) which displayed a conditioned rate acceleration. A continuous photographic record of the oscilloscope trace of the unit is shown before, during and after a discrimination period with reinforcement available. "Light on" and "light off" mark the beginning and end of the discrimination period respectively. A total of 17 brain stimulation reinforcements were applied during the discrimination period. These stimulation artifacts appear as a block with slightly greater height than the units themselves. The approximate times when the burst criterion was met are indicated by triangles above the units.

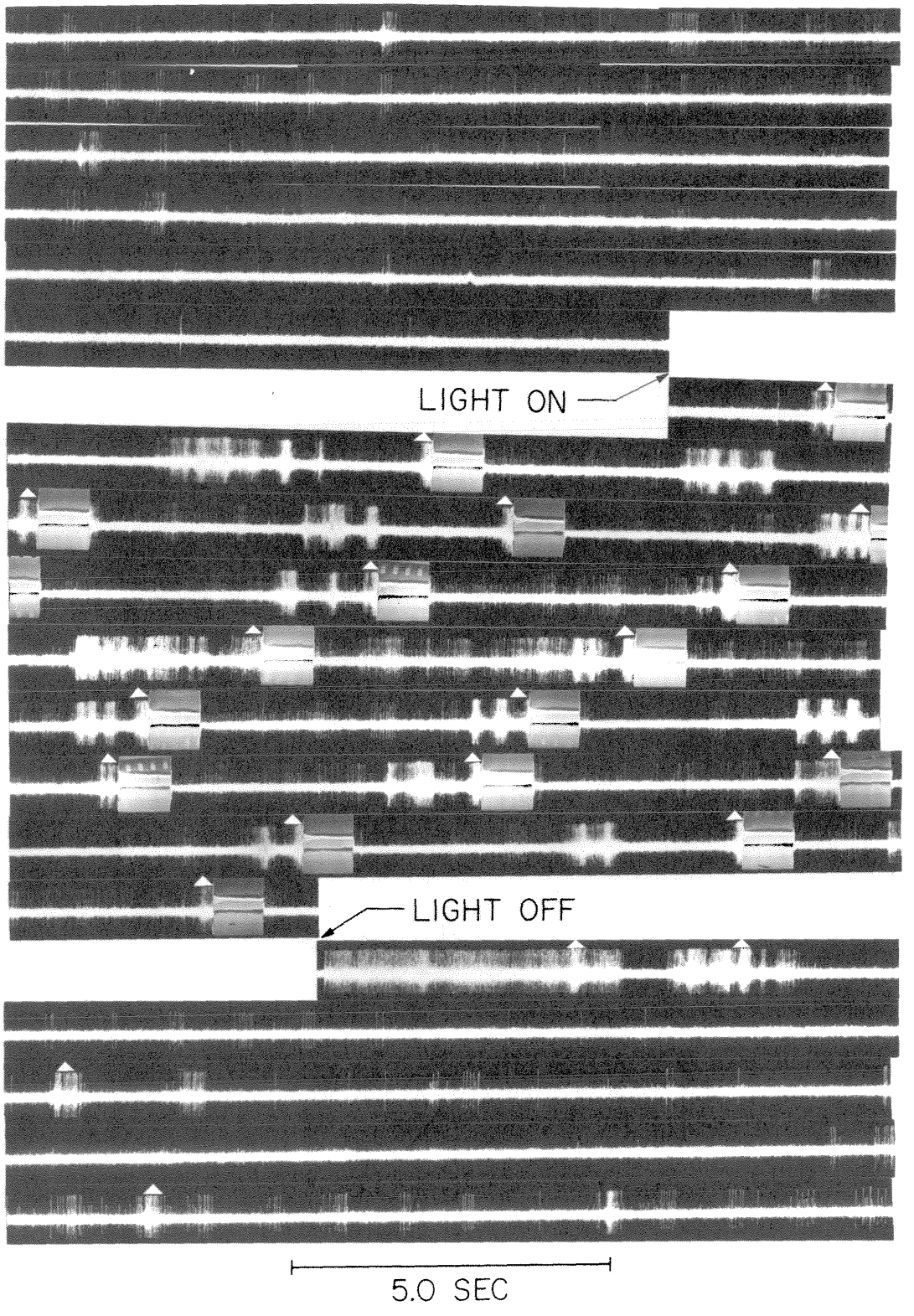

LIGHT ON
LIGHT OFF
5.0 SEC

Figure 7.

An illustration of the same unit as in Figure 6, before, during, and after a discriminative probe (just after reinforcement was withdrawn), showing the maintenance of a conditioned response elicited by the discriminative stimulus. After repetitions of the discriminative stimulus with reinforcement withdrawn, the differential unit response ceased.

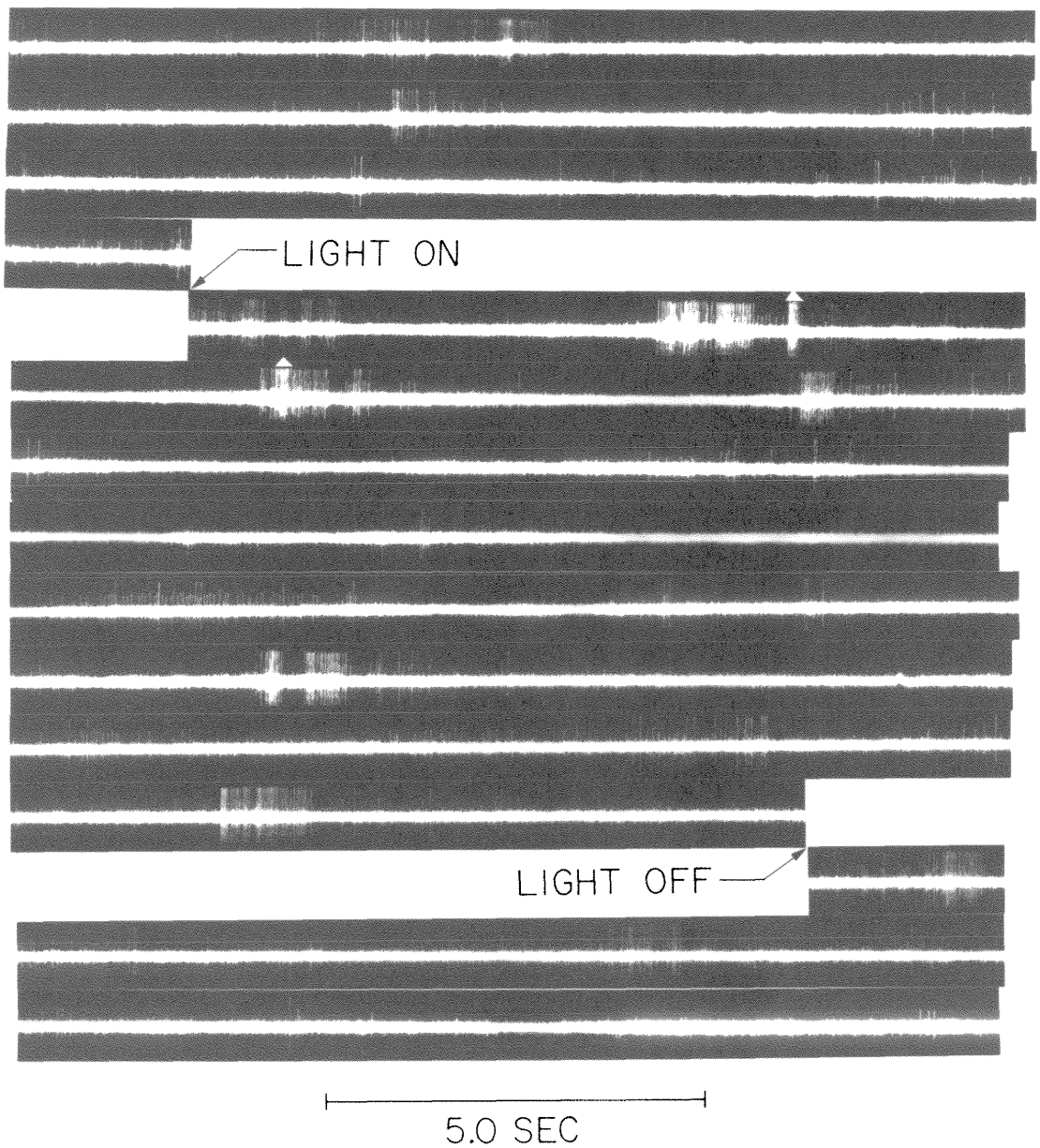

LIGHT ON
LIGHT OFF
5.0 SEC

Table 9.

Summary of how many units were affected in rate in which way immediately after the Flaxedil-induced paralysis. The table includes many units used only in pilot studies and not for the test of maintained discrimination under paralysis.

Only clearly noticeable changes are categorized as rate increased and rate decreased. "Temporarily abolished" units were completely inactive for at least 30 min.

Table 9

	Brain Stem	Cerebellum	Hippocampus	Midbrain	Superior Colliculus	Total	Percentage Total
Rate Increases	0	4	1	0	0	5	8%
Same Rate	4	6	5	3	5	23	36%
Rate Decreased	4	5	6	5	7	27	42%
Temporarily Abolished	0	0	0	9	0	9	14%
Totals	8	15	12	17	12	64	100%

Figure 8.

Histological localization of the units which were observed for rate changes at paralysis onset. The upper 5 drawings are from König and Klippel (1963), and the lower 4 drawings are after Craigie (1963).

UNIT RATE CHANGES AT PARALYSIS ONSET

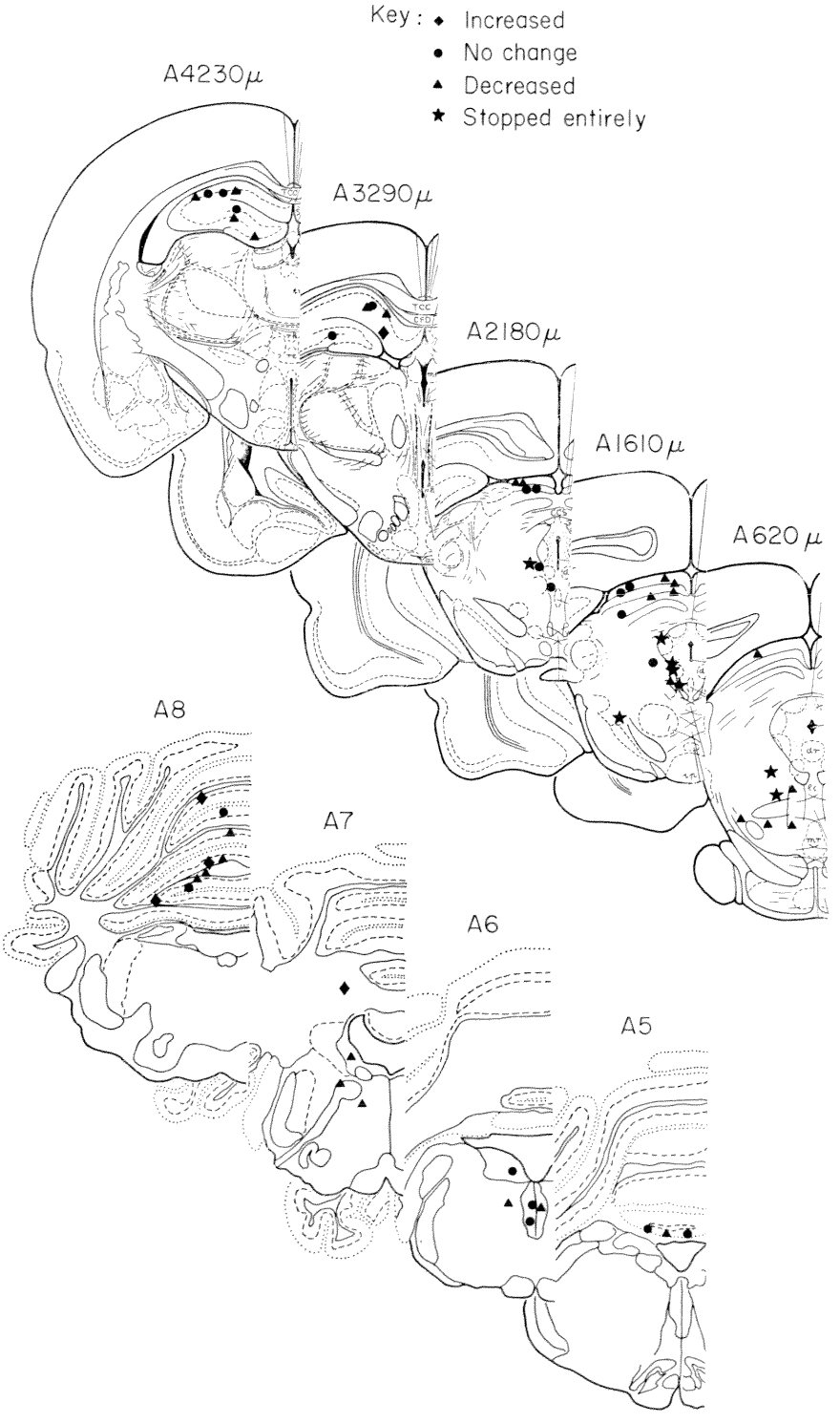
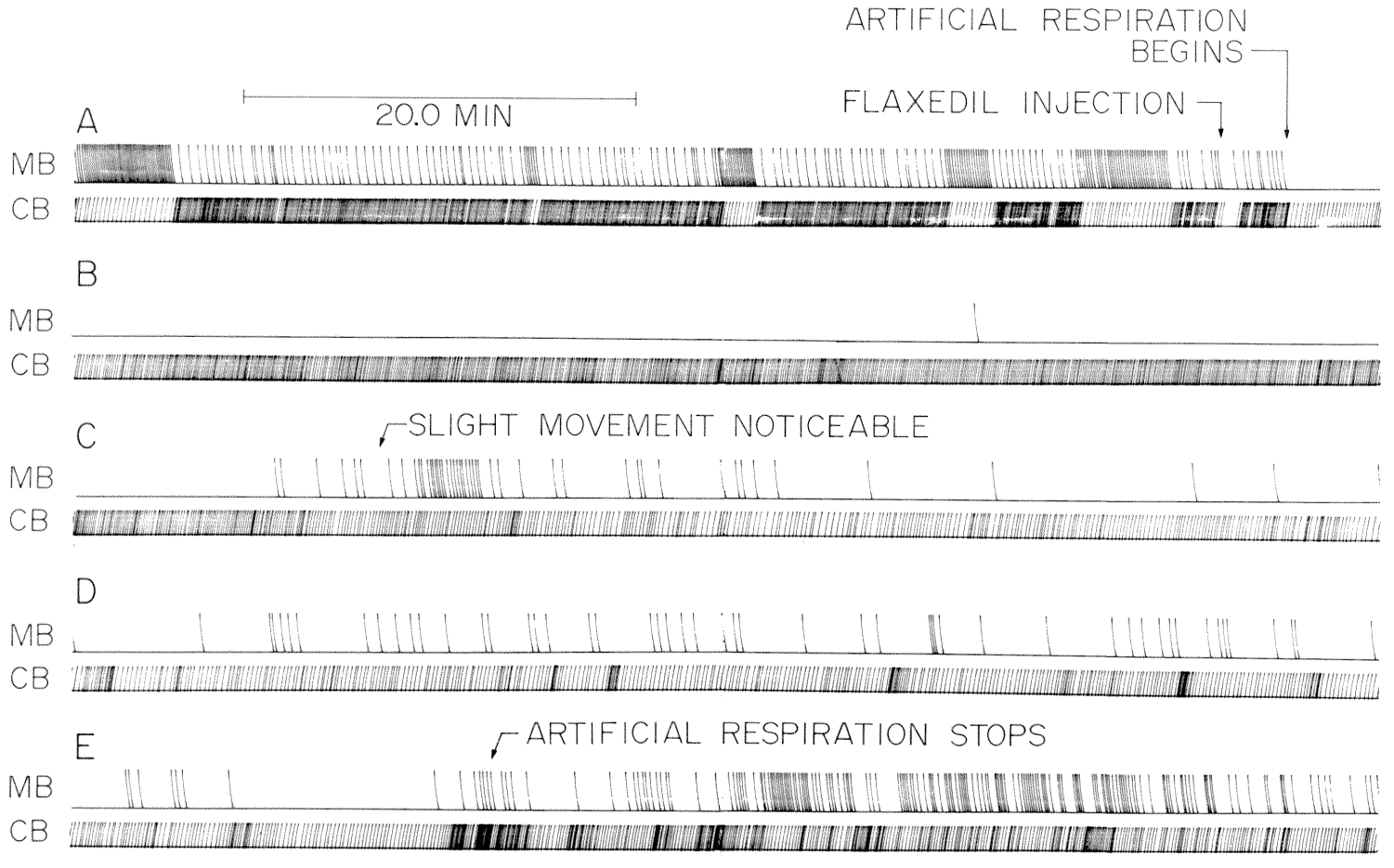


Figure 9.

Illustration of a case of a midbrain unit whose rate was temporarily abolished by paralysis. Each count of 50 units recorded from the midbrain probe resulted in a spike on the traces marked "MB"; and each count of 50 units simultaneously recorded from a probe in the cerebellum resulted in a spike on the traces marked "CB". A) Before paralysis these units had an interesting reciprocal relationship with one another. When the midbrain unit was accelerated and the cerebellum unit was slowed, the animal appeared to be sleeping. The midbrain unit stopped shortly after the Flaxedil injection when the artificial respiration was started. B) Only one spike appeared in the midbrain trace for more than an hour, although the cerebellar unit rate remained rather high. C) Activity begins again in the midbrain trace as the animal begins to regain the ability to move, although the activity remained low in D). E) The animal was able to move well enough to pull loose from the respirator and breath on its own at which time both units picked up in rate somewhat although they no longer seemed to be reciprocally related.

TEMPORARY ABOLITION OF A MIDBRAIN UNIT BY PARALYSIS



Discussion

The main interest of these results is that feedback excitation from conditioned movements was shown to be the source of most of the conditioned unit responses. Secondly, it is interesting that all but one of the experimental units which maintained the conditioned activity under paralysis were found in one region -- the brain stem. None of the five animals tested under paralysis with experimental units in the brain stem failed to maintain the conditioned response. This is in marked contrast with the 5 out of 6 animals tested under paralysis with experimental units in the cerebellum which did fail. Before paralysis, conditioning had been successful with every experimental unit in both the brain stem and cerebellum. The difference, it seems, is that most of the cerebellar units were activated by conditioned overt movements while the brain stem units were not.

Outside of the brain stem, besides the one unit in cerebellum, conditioned responses were also maintained under paralysis in 2 control units in the midbrain. Thus, the brain stem is not unique in this property, but it does stand out as an area where such units were found in high concentration. The question of the location of the origin of these impulses is difficult. It is possible that the autonomic nervous system is a link in the chain of command, because Flaxedil has little or no effect on it (Goodman and Gilman, 1965).

The present method cannot distinguish primary from secondary units in the chain of command. However, it does allow the units activated by movement to be eliminated, and this seems to amount to a large percentage.

An incidental finding of interest was that more than half (9 of 17) of the midbrain units were temporarily abolished in animals subjected to paralysis. When the animal could not move, the units were inactive; when it began recovering the ability to move, the units began to return. None of the 47 units from other parts of the brain were abolished, although many were slowed and several were accelerated. Apparently, the midbrain units were especially dependent upon movement feedback.

EXPERIMENT III

Introduction

Experiment I had made it clear that operant conditioning did not always produce effects on single unit activity, and that units in certain parts of the brain were more likely to be conditioned. However, it became evident that the procedure used tended to produce respondent as well as operant conditioning of units. The "reflexive" changes in unit rates which the reinforcement sometimes produced seemed to be respondently conditioned to the discriminative stimulus. Thus, a new experimental procedure was designed to eliminate the possibility for respondent conditioning. This was done to show conditioning that was unequivocally operant and to further explore the hypothesis of the first experiment, which was that the capacity for operant conditioning is distributed differentially in the brain.

Since it had been observed that unit rate often drifts spontaneously, and that reinforcement of the animal often directly excites or inhibits units, a rigorous definition of an operant unit was adopted, based on the argument of Black (1971) that operant control of behavior implies the ability to turn it off as well as on. Under this definition a unit was considered operant only if its rate could be systematically increased and decreased by simply changing the contingency of reinforcement and not the rate of reinforcement. It will be noticed that this definition agrees with the observable properties of overt operant behavior.

Procedure

The experiment was performed at least 4 days after surgery, and each rat was used only once. There was no behavioral pre-training. Reinforcement was only by electrical stimulation of the medial forebrain bundle which was prevalidated and used as described in Experiment I. Units were recorded from 4 brain areas: cerebellum, hippocampus, midbrain, and superior colliculus. Two units were recorded simultaneously, both from the same brain structure in all but one rat. One unit was arbitrarily designated the "experimental" unit, and reinforcements were made contingent upon its activity; the other unit was designated the "control" unit, and no reinforcements were intentionally made contingent upon its activity.

The experiment contained three 30 minute "treatment" periods which were always in the same order: "pseudo-conditioning", "incremental conditioning", and "decremental conditioning". Following each of these treatments was an "extinction" period; the first two were 7 minutes long, and the last was at least 10 minutes long. Prior to the first treatment was a 30 minute "baseline" period. Reinforcement was applied only during the treatment periods, and no other stimulus was ever used. The only difference between the treatments was the contingency of reinforcement. During pseudo-conditioning it was applied at random intervals. During incremental conditioning it was contingent upon "bursts" of the experimental unit, and during decremental conditioning it was contingent upon "pauses"

of the experimental unit. Bursts were defined as in Experiment I. Pauses were defined as periods of up to 5 seconds during which the experimental unit did not fire. Pauses were detected by a clock which was reset to zero whenever the unit fired and which produced an output pulse when it reached the pre-set time of up to 5 seconds. When either the burst or pause criterion was met, there followed a 5 sec "time-out" period before it could be detected again. Both the burst and pause criteria were adjusted during pseudo-conditioning until each criterion was reached about once every 30 seconds. Pens marking on a moving paper chart recorded experimental and control unit activity as in Experiment I; each burst, pause, and reinforcement was also marked.

Data Analysis: A count was made of the number of times the unit fired in each successive 200 second time bin throughout each treatment period. "Operant" units were found by comparing the total rate of a given unit during the last half of incremental conditioning to its respective total during pseudo-conditioning, and decremental conditioning. Operant units were those which had incremental rates higher than pseudo-conditioning rates which in turn were higher than decremental conditioning rates. The last half of the period was used, because this is when conditioned effects should be largest. A Wilcoxen test for matched pairs (Siegel, 1966) was also applied to the individual unit scores for a test of statistical significance. For each unit,

the matched pair was formed with respect to time, using one score from each of the treatments undergoing comparison.

So that the unit scores could be grouped and compared, the scores for each unit were then converted to percentile scores wherein the maximum rate observed equaled 100% and the minimum 0%. The units in each brain area were analyzed as a group, except that experimental and control units were analysed separately. To judge the effect of non-contingent reinforcement, scores from the last half of the pseudo-conditioning period were compared to the respective scores of the baseline period. To judge the effects of incremental and decremental conditioning, each treatment was compared to pseudo-conditioning. The pseudo-conditioning rate was used as the basis of comparison to control for the effects of non-contingent reinforcement. Extinction was judged by comparing the extinction scores to those of the previous treatment period. Each of these comparisons was tested for statistical significance at the 5% probability level with a T-test for matched pairs (Hays, 1966). Matched pairs were formed as described above.

Results

The following data are based on 8 rats with experimental units in the cerebellum, 9 with experimental units in hippocampus, 11 with experimental units in midbrain and 8 with experimental units in superior colliculus. Each of these experimental units was paired

with a control unit from the same area except in one case where the control unit was found in the superior colliculus and the experimental unit had been in the midbrain.

Individual Operant Units: More "operant" units were found in the cerebellum than in any other area. Out of the 8 experimental units in the cerebellum, 5 had totals during the last half of incremental conditioning which exceeded the respective pseudo-conditioning total it had, which in turn exceeded the respective decremental conditioning score it had (see Table 10). This was true of only 2 out of 9 units in hippocampus, 1 out of 11 units in midbrain and none out of 8 in superior colliculus. Fewer control cerebellar units satisfied this criteria (1 out of 8), but the number of control units which satisfied the criteria in the other areas was the same as the number of experimental units. These proportions differ significantly between brain areas (Chi-square = 10.86, df = 3, $p < 0.02$).

A good example of an operant unit in the cerebellum is illustrated in Figure 10. Here the experimental and control unit rates were very similar in the baseline, pseudo-conditioning and final extinction periods, but during the incremental and decremental conditioning periods, only the experimental unit showed clear effects. The pattern of bursts and pauses of the experimental unit, also illustrated in the lower half of Figure 10, showed the operant

Table 10.

a.) Number of individual units in each brain area which had incremental conditioning scores (total of last half of the period) which were greater than the respective score for pseudo-conditioning. b.) same with decremental conditioning less than pseudo-conditioning. c.) same with incremental conditioning greater than decremental conditioning. d.) number of individual units which met both a.) and b.) criteria.

Table 10

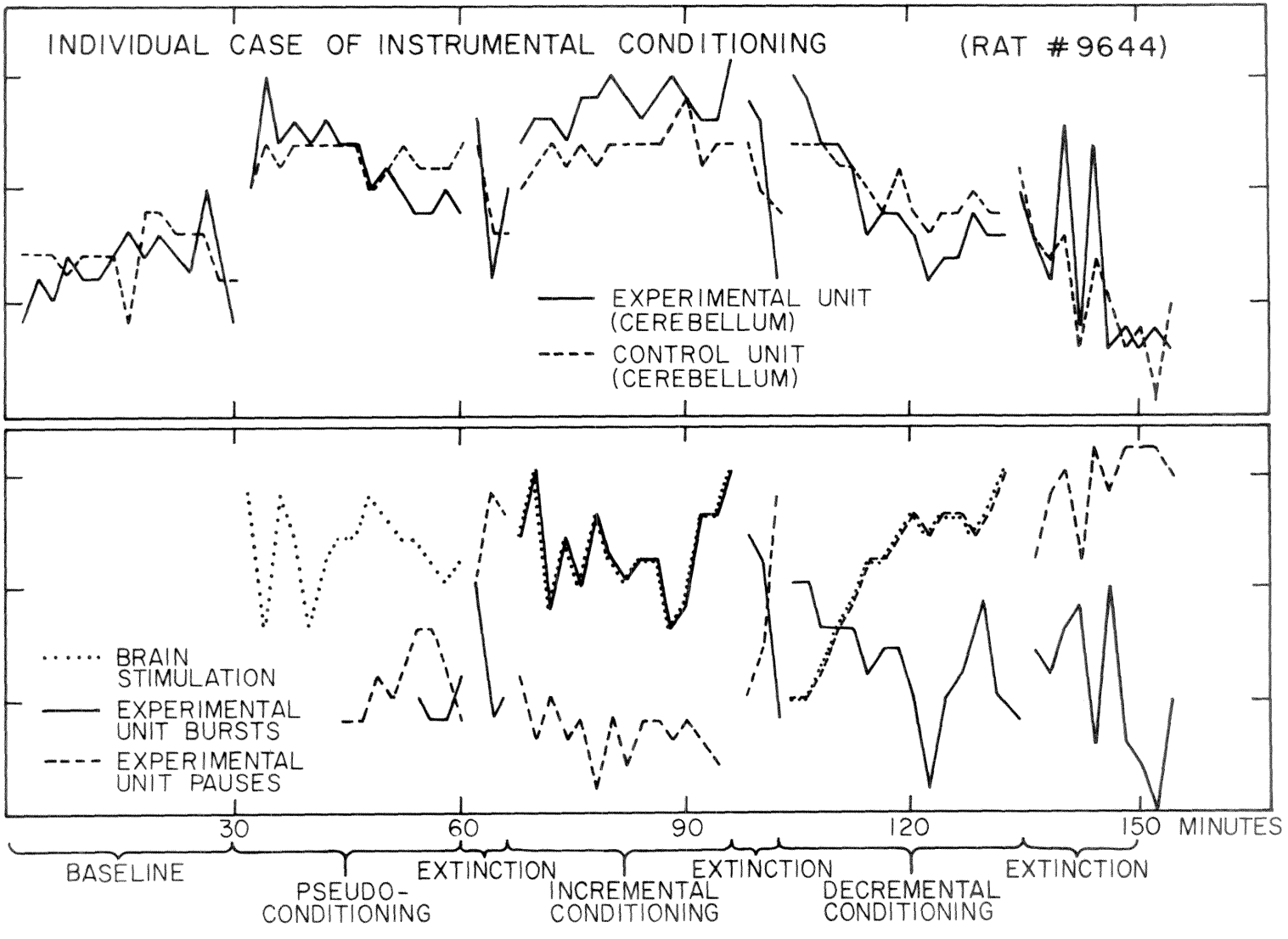
	N = 8 Cerebellum	N = 9 Hippocampus	N = 11 Midbrain	N = 8 Superior Colliculus
	Experimental Units			
a) Cases of Incremental Conditioning> Pseudo-conditioning	7	5	4	2
b) Cases of Decremental Conditioning< Pseudo-conditioning	6	5	4	6
c) Cases of Incremental Conditioning> Decremental Conditioning	8	5	8	5
d) Cases of Incremental Conditioning> Pseudo-conditioning > Decremental Conditioning	5	2	1	0

Table 10 (continued)

	N = 8 Cerebellum	N = 9 Hippocampus	N = 10 Midbrain	N = 9 Superior Colliculus
	Control Units			
a) Cases of Incremental Conditioning> Pseudo-conditioning	4	5	5	3
b) Cases of Decremental Conditioning< Pseudo-conditioning	5	5	6	3
c) Cases of Incremental Conditioning> Decremental Conditioning	5	3	6	4
d) Cases of Incremental Conditioning> Pseudo-conditioning > Decremental Conditioning	1	2	1	0

Figure 10.

Illustration of an "operant" experimental unit displaying both rate increases and rate decreases (Wilcoxen test for matched pairs, $p < 0.05$, for both rate increase and decrease). Both experimental and control units were recorded from the cerebellum. The curves showing the experimental and control unit rates and the brain stimulation rate are for the duration of Experiment III. The curves showing the rates at which the experimental unit met the burst and pause criteria are shown beginning in the pseudo-conditioning period after the settings had been fixed for the remainder of the experiment.


INDIVIDUAL CASE OF INSTRUMENTAL CONDITIONING
(RAT #9644)
EXPERIMENTAL UNIT (CEREBELLUM)
CONTROL UNIT (CEREBELLUM)
BRAIN STIMULATION
EXPERIMENTAL UNIT BURSTS
EXPERIMENTAL UNIT PAUSES
30
60
90
120
150 MINUTES
BASELINE
PSEUDO-CONDITIONING
EXTINCTION
INCREMENTAL CONDITIONING
EXTINCTION
DECREMENTAL CONDITIONING
EXTINCTION

conditioning better. The rate of bursts rose markedly during incremental conditioning and fell during decremental conditioning while the rate of pauses remained low during pseudo-conditioning and incremental conditioning, but climbed steadily during decremental conditioning. The rate of brain stimulation was of course determined by the bursts during incremental conditioning and the pauses during decremental conditioning, but was independent of the animal during pseudo-conditioning. The amount of brain stimulation in each of the conditioning periods was quite similar to the amount during pseudo-conditioning. However, the bursts and pauses were both noticeably lower than the brain stimulation rate during pseudo-conditioning. This shows they were not directly produced by the stimulation. Rather, it seems that they were produced operantly when required.

When more stringent criteria were applied (incremental conditioning exceeded pseudo-conditioning, Wilcoxen test, $p < 0.05$; and pseudo-conditioning exceeded decremental conditioning, Wilcoxen test, $p < 0.05$), there were 2 "operant" units in the cerebellum, 2 in the hippocampus, 1 in the midbrain and none in the superior colliculus (see Table 11 and Figure 11 for histological localization of these points). No control units passed these criteria.

Table 11.

Same as table 10 except that Wilcoxen tests for matched pairs were used ($p < 0.05$) on scores from last half of each period.

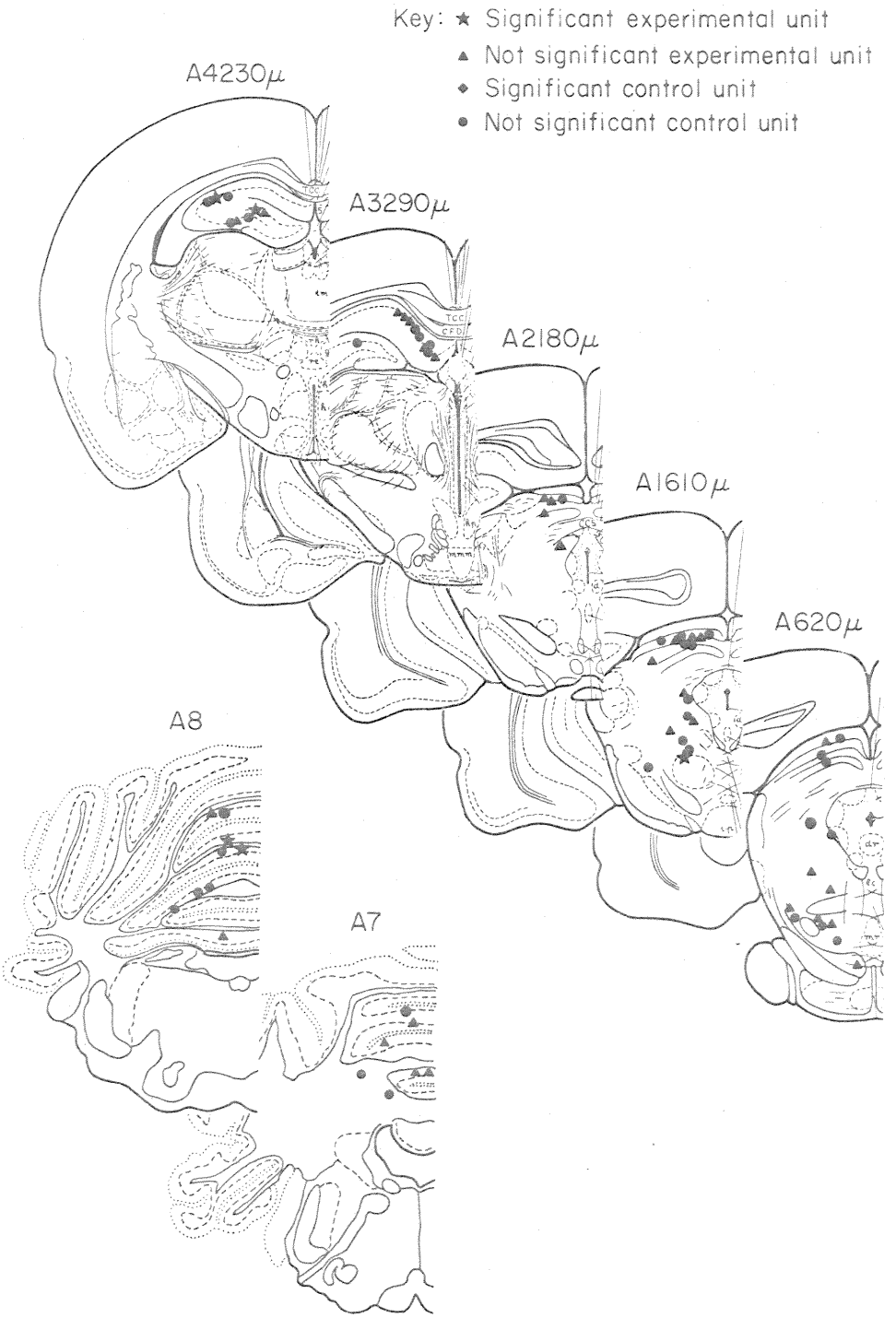
Table 11

	N = 8 Cerebellum	N = 9 Hippocampus	N = 11 Midbrain	N = 8 Superior Colliculus
Experimental Units				
Cases of incremental rate > pseudo-conditioning	5	5	5	2
Cases of decremental rate < pseudo-conditioning	4	4	6	5
Cases of incremental rate > pseudo rate> decremental rate	2	2	1	0
Control Units				
Cases of incremental rate > pseudo-conditioning	2	2	3	3
Cases of decremental rate < pseudo-conditioning	5	3	4	1
Cases of incremental rate > pseudo-rate > decremental rate	0	0	0	0

Figure 11.

Histological localization of the recording points used in Experiment III. The points are classified according to whether or not the units recorded there showed both rate increases during incremental conditioning and rate decreases during decremental conditioning which were statistically different from the pseudo-conditioning rate of the same unit as determined by the Wilcoxen test.

INSTRUMENTALLY CONDITIONED RATE INCREASES AND DECREASES OF THE SAME UNIT



Operant Groups of Units: As might have been expected from the proportions of individual units which were operant, the group of 8 experimental units in the cerebellum was the only one of all the experimental and control groups which satisfied the operant criteria. That is, the mean incremental conditioning score significantly exceeded the mean pseudo-conditioning score which, in turn, significantly exceeded the decremental conditioning score in only the experimental cerebellar group (T-tests, $p < 0.05$; see Table 12 and Figures 12a,b,c,d).

Separate Effects of Treatments: "Pseudo-conditioning" is an effect which looks like conditioning, but is actually due to a general "sensitizing" effect of the reinforcement in this case. Some was found in the cerebellum and midbrain but not in the hippocampus and superior colliculus when random reinforcement was applied. This was a statistically significant effect in the cerebellum and midbrain groups (T-test, $p < 0.05$), except for the control midbrain group, which nevertheless had an average which was very close to the experimental group (see Table 12 and Figures 12a,b,c,d).

During the incremental conditioning period, significant rate increases of units above the pseudo-conditioning level occurred only in the cerebellar and hippocampal experimental groups (see Table 12). During the decremental conditioning period, a significant rate decrease of units below the pseudo-conditioning level occurred only in the cerebellar and superior collicular experimental groups

Table 12.

Results of T-tests ($p < 0.05$, one-tailed) of the differences between the main experimental conditions in each brain area. Individual unit rates were first transformed to percentile scores. The average percentile score of each unit for the last half of each experimental condition was used for the T-test. Arrows up indicate significant outcome of test; dashes indicate not significant outcome of test.

Table 12

Experimental Units

	Cerebellum	Hippocampus	Midbrain	Superior Colliculus
Pseudo-conditioning Random Stimulation >Baseline	↑	–	↑	–
Incremental Conditioning >Pseudo-conditioning	↑	↑	–	–
Decremental Conditioning <Pseudo-conditioning	↑	–	–	↑

Control Units

Pseudo-conditioning Random Stimulation >Baseline	↑	–	–	–
Incremental Conditioning >Pseudo-conditioning	–	–	–	–
Decremental Conditioning <Pseudo-conditioning	–	–	–	–

Figure 12a.

Curves for the duration of Experiment III for the 8 rats with both experimental and control units recorded from the cerebellum. The minimum and maximum rates for each unit were transformed to scores of 0.0 and 100.0 percentile respectively, and the intermediate rates were transformed to proportional percentile scores. The curves show the mean percentile rates of the experimental and control units as well as the mean rate of brain stimulation for these animals.

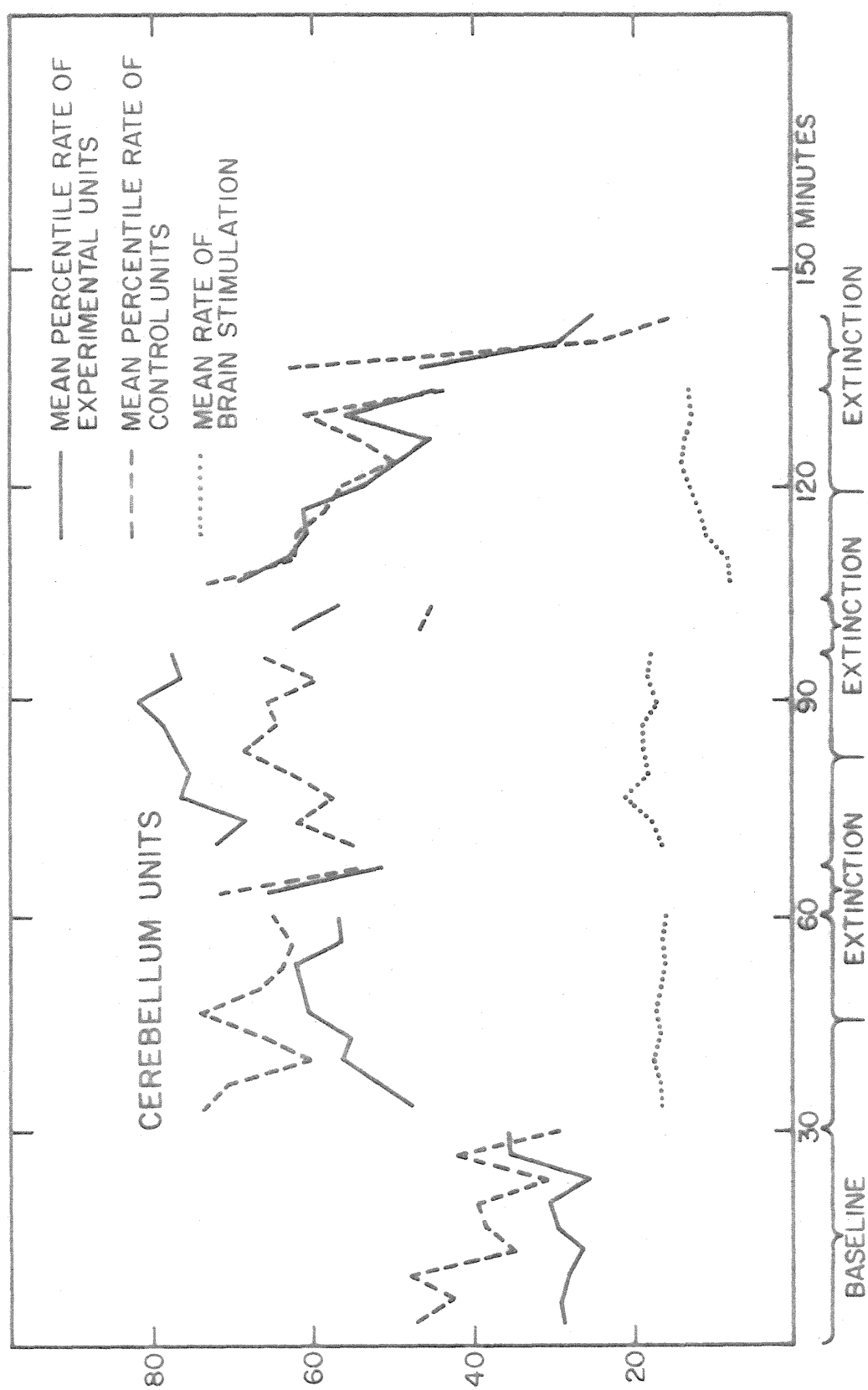

MEAN PERCENTILE RATE OF EXPERIMENTAL UNITS
MEAN PERCENTILE RATE OF CONTROL UNITS
MEAN RATE OF BRAIN STIMULATION
CEREBELLUM UNITS
80
60
40
20
30
60
90
120
150 MINUTES
BASELINE
PSEUDO-CONDITIONING
EXTINCTION
INCREMENTAL CONDITIONING
EXTINCTION
DECREMENTAL CONDITIONING
EXTINCTION

Figure 12b.

Same as for Figure 12a for the 9 rats with both experimental and control units in hippocampus.

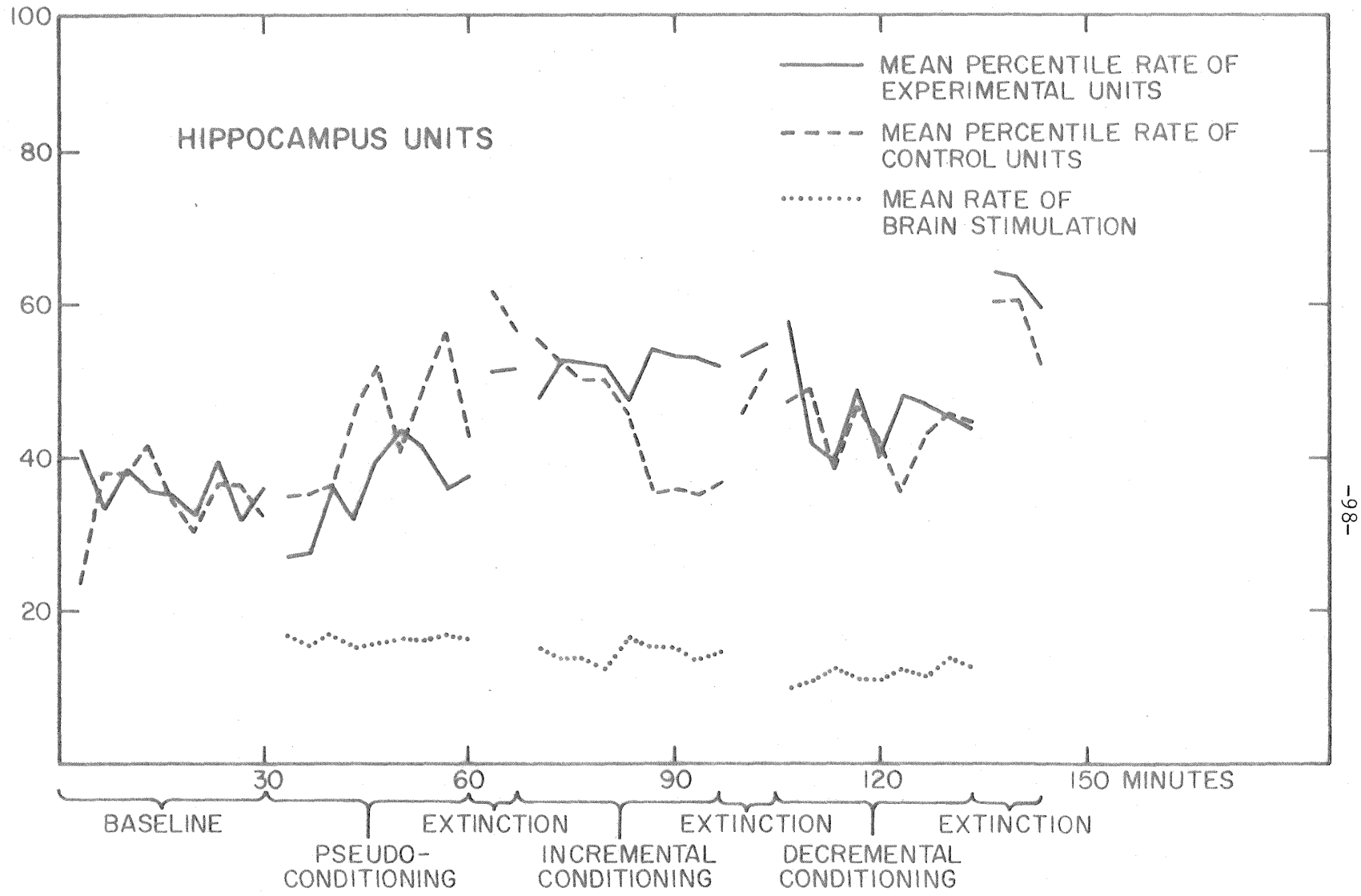

100
80
60
40
20
HIPPOCAMPUS UNITS
MEAN PERCENTILE RATE OF EXPERIMENTAL UNITS
MEAN PERCENTILE RATE OF CONTROL UNITS
MEAN RATE OF BRAIN STIMULATION
30
60
90
120
150 MINUTES
BASELINE
PSEUDO-CONDITIONING
EXTINCTION
INCREMENTAL CONDITIONING
EXTINCTION
DECREMENTAL CONDITIONING
EXTINCTION

Figure 12c.

Same as for Figure 12a for the 11 rats with experimental units in the midbrain and for the 10 of these rats which also had control units in the midbrain. The control unit of the eleventh rat was found by histology to be in the superior colliculus and was included in those scores.

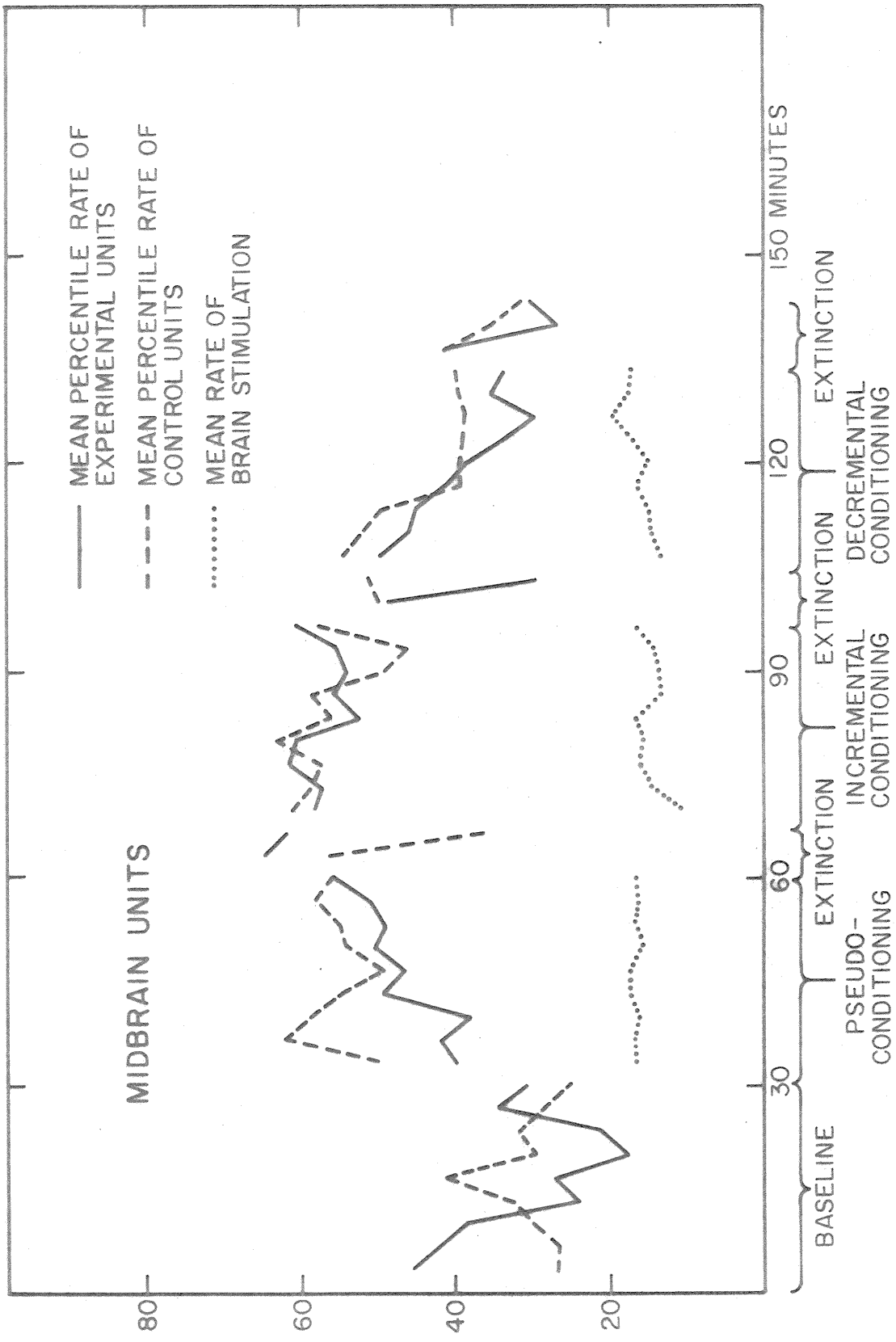
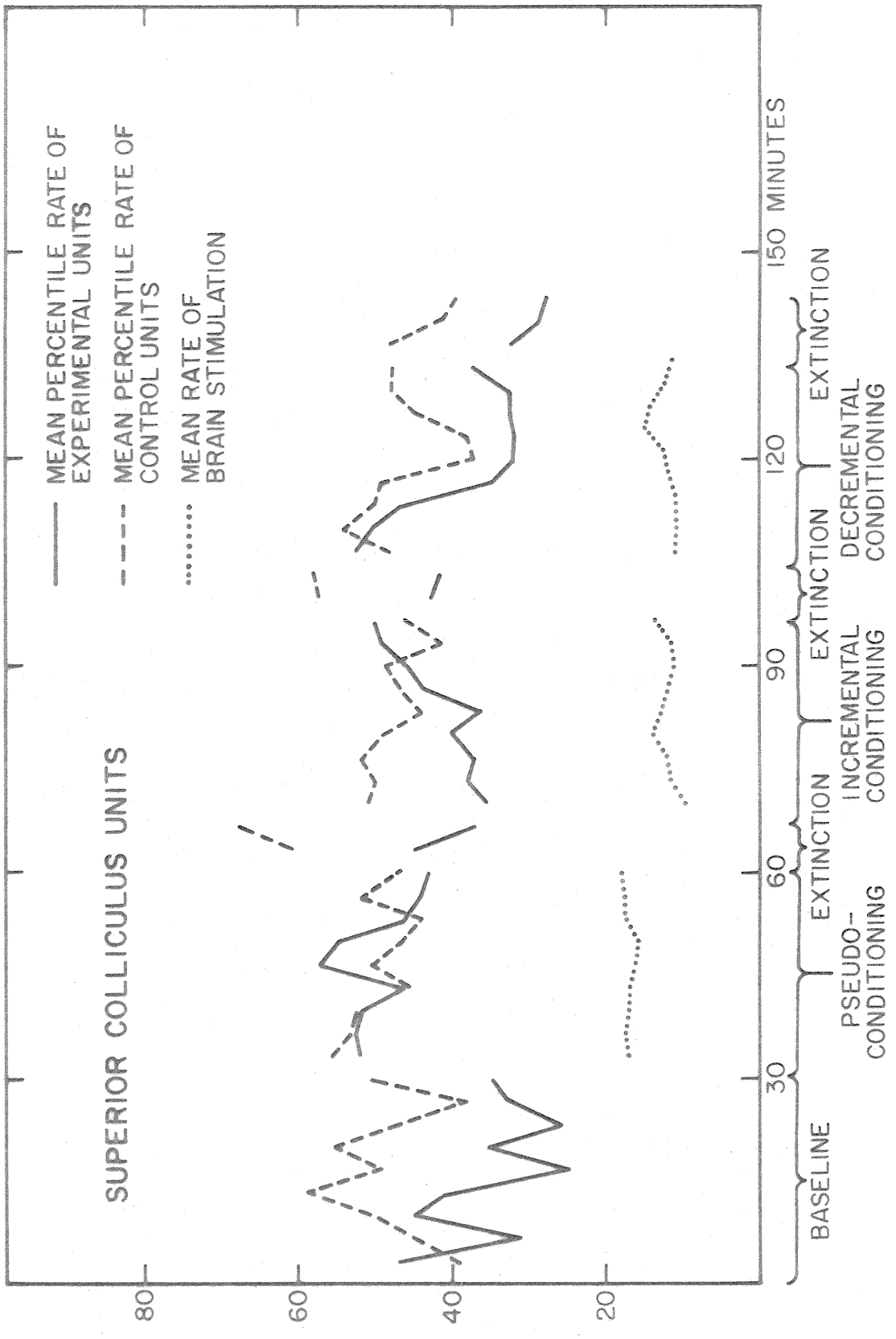

MEAN PERCENTILE RATE OF EXPERIMENTAL UNITS
MEAN PERCENTILE RATE OF CONTROL UNITS
MEAN RATE OF BRAIN STIMULATION
MIDBRAIN UNITS
80
60
40
20
30
60
90
120
150 MINUTES
BASELINE
PSEUDO-CONDITIONING
EXTINCTION
INCREMENTAL CONDITIONING
EXTINCTION
DECREMENTAL CONDITIONING
EXTINCTION

Figure 12d.

Same as for Figure 12a for the 8 rats with experimental and control units in the superior colliculus. A ninth control unit recorded from the superior colliculus was included in these scores even though the experimental unit which it accompanied was recorded from the midbrain.


SUPERIOR COLLICULUS UNITS
MEAN PERCENTILE RATE OF EXPERIMENTAL UNITS
MEAN PERCENTILE RATE OF CONTROL UNITS
MEAN RATE OF BRAIN STIMULATION
80
60
40
20
30
60
90
120
150 MINUTES
BASELINE
PSEUDO-CONDITIONING
EXTINCTION
INCREMENTAL CONDITIONING
EXTINCTION
DECREMENTAL CONDITIONING
EXTINCTION

(see Table 12). No group of control units exhibited significant deviations from the pseudo-conditioning levels during either incremental or decremental conditioning.

In most brain areas there was an abrupt rise in the experimental unit rate at the outset of incremental conditioning. In all areas except the suprior colliculus, there was little improvement for the remainder of the period. In contrast, during decremental conditioning there were gradual downward slopes in all groups but hippocampus. In other words, the rate increase behavior seemed to be conditioned fast (if at all) and was "skilled" at the end of three minutes while the rate decrease behavior was conditioned slowly (if at all) and it improved gradually throughout the decremental conditioning period.

Extinction: The rate of units in the cerebellum and midbrain tended to fall off during extinction. In the cerebellum, there were significant rate decreases which occurred in the experimental unit group following the incremental and decremental conditioning periods, (T-test, $p < 0.05$, two-tailed) (see Table 13 and Figure 12a). Significant rate decreases were found in the cerebellar control group only after the final conditioning period (see Figure 12a). A significant unit rate change occurred in midbrain only once. The experimental unit rate decreased following incremental conditioning (see Figure 12c).

Table 13.

Results of T-tests ($p < 0.05$, two-tailed) of the differences between the rates during each extinction period and the period immediately preceding. The average percentile score for the extinction period for each unit was used for the T-test against the average percentile score for the last half of the preceding period. Arrows up indicate significant increase; arrows down indicate not significant decreases; dashes indicate not significant outcome of the test.

Table 13

Experimental Units

	Cerebellum	Hippocampus	Midbrain	Superior Colliculus
Extinction Rate Following Pseudo-conditioning	–	↑	–	–
Extinction Rate Following Incremental Conditioning	↓	–	↓	–
Extinction Rate Following Decremental Conditioning	↓	↑	–	–

Control Units

Extinction Rate Following Pseudo-conditioning	–	–	–	↑
Extinction Rate Following Incremental Conditioning	–	–	–	–
Extinction Rate Following Decremental Conditioning	↓	↑	–	–

In contrast, instead of the rate falling off during extinction among the hippocampal and superior colliculus units, the rate often increased (see Table 13). Specifically, in hippocampus significant rate increases occurred in both the experimental and control groups following decremental conditioning and in the experimental group following the pseudo-conditioning period (see Figure 12b). In superior colliculus, the only significant increase occurred in the control group following the pseudo-conditioning period (see Figure 12d).

Behaviors Observed: During conditioning, behaviors were often noted which seemed to relate to the reinforcement (see Table 14). Most often the general activity level was augmented during incremental conditioning and depressed during decremental conditioning. In a few cases, especially during incremental conditioning, there were obviously stereotyped behaviors preceding reinforcement, such as rearing up on the hind legs, or leaning on the side of the cage and then turning clockwise.

Table 14

Some Behavioral Observations During Experiment III

Rat #	During Incremental Conditioning Period	During Decremental Conditioning Period
CB 9620	non-specific, active	non-specific, very active
9649	non-specific, active, walking, sniffing	holds still
9644	non-specific, very active	active, walks close to the floor
9624	holds still before reinforcement	holds very still until reinforcement, very stereotyped
9608	rears up for reinforcement, stereotyped	holds still for reinforcement
9596	often turns rapidly to the left	leans out front window with forepaws on window ledge and holds still for reinforcement
9581	non-specific, active	holds still
9538	often reinforcement follows rearing up, but sometimes it occurs with all feet on the floor	sniffs, does not rear, not-specific
HP 9639	non-specific, active	non-specific, active
9630	non-specific, active	leans out front window
9628	non-specific, active	

Table 14 (continued)

Rat #	During Incremental Conditioning Period	During Decremental Conditioning Period
HP 9600	reinforcement seems to follow small head movements, but not specific; sometimes just when holding still	appears to be sleeping
9594	non-specific, active	non-specific, active
9563	non-specific, sniffing, moving head	non-specific, sniffing, moving head
9562	non-specific, often swings head to the right	sniffing at right side of cage
MB 9591	stereotyped, gets reinforcement when grooming, touching mouth to forepaws	non-specific, active, much rearing
9641		holds still at cage front
9652		sniffing at cage left
9582	stereotyped, climbs window ledge with back feet and jumps off	strains forward with front paws on window ledge
9653	walks close to floor	
9549	non-specific, active, much rearing	non-specific active
9451	rears up on front window ledge	rears up on front window ledge, occasionally jumps off

Table 14 (continued)

Rat #	During Incremental Conditioning Period	During Decremental Conditioning Period
MB 9609	stereotyped, leans on window ledge with forepaws, then turns clockwise for reinforcement	
9539	stereotyped, rears up for reinforcement	active, walking, rears occasionally
SC 9645	faces left rear and moves head slowly to right then quickly to left again; occasionally reinforced	same behavior facing front
9638	non-specific, active	non-specific, very active, walking
9615	remains in cage rear; rearing up occasionally	
9595	non-specific, active	
9583		sniffing and lying down at rear of cage
9498	very active, moving around cage, rearing up usually precedes reinforcement	same behavior

Discussion

The discriminative operant method used previously in Experiment I and in earlier studies of Olds (1965, 1967) to identify operant activity of single units had been shown to be confounded by a tendency for respondent conditioning. Unit rate augmentation had been shown to result when positive reinforcement was made contingent upon increases in the rate (Olds and Olds, 1961; Olds, 1965, 1967), but these studies did not show clearly that the contingency of reinforcement was a crucial variable, and thus represent an equivocal demonstration of operant conditioning. Fetz (1969) demonstrated augmentation in unit rates in motor cortex in awake monkey when reinforcement was contingent upon increases of rate which returned to normal levels when the reinforcement was withdrawn. Fetz showed that the contingency of reinforcement was a crucial variable in producing the rate augmentation, because the same quantity of reinforcement given at random produced no augmentation of rate, and thus made a good case for operant conditioning. However, if random stimulation alone had produced rate augmentation, there would have been a problem in interpretation. Thus, the main importance of the present results is that they showed single unit activity in rats which was clearly operant. The results also showed that one part of the brain -- the cerebellum -- was more apt to display operantly conditioned effects that certain other parts of the brain.

The control group of cerebellar units taken from these same animals, showed the same rate augmentation effect of non-contingent reinforcement as the experimental group, but no significant changes during the conditioning periods. In other words, the operant conditioning tended to selectively change the rate of experimental units. However, it should be noticed that the experimental and control group of units show a marked rate correlation during decremental conditioning, even though during incremental conditioning, they appear relatively independent. This suggests that the decreased rates were achieved by behaviors which decreased the cerebellar activity as a whole, whereas the increased rates might have been achieved by more specific behaviors.

Since non-contingent brain stimulation produced significant unit rate increases in the cerebellum, it might seem that the significant rate changes during the conditioning periods were due only to changes in the rate of brain stimulation and not to operant conditioning. Indeed, the mean brain stimulation rate in the cerebellum was somewhat higher during the incremental conditioning period and somewhat lower during the decremental conditioning period than it was in the pseudo-conditioning period. It was not possible to insure that the brain stimulation rate was equal in all treatments because in fact, it was dependent on the rat. However, these differences in rate of brain stimulation were not statistically significant (T-test $p > 0.05$, one-tailed). In addition, during

the decremental conditioning period, the brain stimulation rate rose while the unit rate fell, the reverse of what would be expected if the unit rates were directly related to the brain stimulation rate. Finally, while random stimulation produced significant rate increases in both experimental and control cerebellar unit groups, significant conditioned effects occurred only in the experimental unit group. If the rate of cerebellar units had been related only to the brain stimulation rate, both groups should have been the same.

It should not be inferred from these results that operant conditioning is the exclusive property of the cerebellum. Although the experimental units in cerebellum were the unique "operant group," not all the units tested in the cerebellum passed the somewhat arbitrary criteria set for an "operant unit". There were 2 units in hippocampus, and 1 in midbrain which also passed the criteria of displaying both rate increases and decreases, depending on the contingency of reinforcement. Furthermore, there are various indications of selective conditioning of experimental groups in other brain areas. For instance, the experimental hippocampal group was significant during incremental conditioning, as was the experimental superior collicular group during decremental conditioning, whereas no control groups were. It seems more accurate to say that the probability of finding operant units is higher in the cerebellum.

The experiment was improperly designed in that the incremental conditioning always preceded decremental conditioning; it would have been better to have the reverse order in half the experiments so that order effects, if any, could be discerned. Conceivably, it might have been the case that random reinforcement could have created the appearance of conditioning if its excitatory effects continually increased to the end of the incremental conditioning period, at which time the effect began to gradually attenuate. In fact, something like this coincidence may have been the case with some of the midbrain units, except that the peak effect seemed to occur early in the incremental conditioning period. It is quite unlikely that this was a very pervasive effect, because it was often shown that following the decremental conditioning period, a second incremental conditioning period again would produce rate increases.

Overt Behavior: The behavior of the animals during the experiment was instructive in understanding what was going on. Typically an animal was somewhat activated when first put into the experimental chamber and attached to the amplifier leads, but by the end of the baseline period, the behavioral activity was not great. Then with the onset of random stimulation, it increased greatly. There was considerable walking forward and sniffing. This heightened activity lasted throughout the pseudo-conditioning

period and abated during the short extinction period which followed. During the incremental conditioning,stereotyped patterns often began to develop in successful subjects, such as rearing up on the hind legs or turning the head and torso rapidly in one direction. As soon as the behavior was executed, the animal would regularly receive a reinforcement. These stereotyped patterns often developed early in the period and became more "skilled" by the end. Occasionally, 2 or more specific behavior patterns would be seen in the course of the period, each seemingly an operant behavior associated with the reinforcement. During the following extinction period, the behavioral activity levels would again decrease typically. The decremental conditioning period often began with behaviors similar to those just seen, and these might continue to be repeated from time to time throughout the period. However, successful subjects tended to become quite still, perhaps appearing tense, but not making large movements. Again extinction produced decreased activity levels.

The general picture from the behavioral observations is that while random reinforcement produces a general behavioral activation, reinforcement contingent upon unit activity produces seemingly operant behaviors which apparently have the effect of controlling the rate of the unit. To engage in a bit of anthropromorphism, it may seem to the rat like the reinforcement is triggered by overt behavior, rather than something in its brain. Indeed, as the

paralysis data in Experiment II show, overt behavior often may be a necessary link in the chain.

DISCUSSION (GENERAL)

The overall aim of these experiments was to make the method of operant conditioning of brain activity more interesting by showing that distinctions could be made by varying brain location and utilizing paralysis. The first experiment employed the discriminative operant method and showed brain location was important for finding conditioned units. Experimental units in the brain stem and cerebellum all were conditoned, while only about half were in hippocampus, midbrain and superior colliculus. However it was discovered that this method did not distinguish between operant and respondent conditioning. The second experiment was a continuation of the first with paralyzed animals. It showed that brain location and paralysis were both important variables. Feedback from conditioned overt movements was necessary for conditioned units in cerebellum, hippocampus, midbrain, and superior colliculus, but not necessary for conditioned units in brain stem. Here too the conditioned response was not clearly operant. Hence the third experiment eliminated the possibility of respondent conditioning. Units were found which increased and decreased in rate according to the reinforcement contingency, and thus satisfied the rigorous definition of "operant" which was used. Of all the groups, only the experimental cerebellar group satisfied the criteria. This time brain location was shown to be important specifically for finding operantly conditioned

responses. Finally, the aim of showing clear operant responses of a unit under paralysis was reached in the experiment described in Appendix C. Here a brain stem unit was conditioned to rapidly alternate between rate increases and decreases to show convincing operant conditioning. The alternation behavior was then conditioned to a discriminative stimulus. The animal was paralyzed and the unit behavior was maintained.

Behavioral Activation and Conditioning

A fact which became obvious with the pseudo-conditioning results of Experiment III was that brain stimulation reinforcement has general behavioral activating properties. In the cerebellum for instance, the pseudo-conditioning effect was larger than either the incremental or decremental conditioning effects. Obviously, behavioral activation is an adaptive response to reinforcement, because it tends to increase the organism's chances of finding the source of reinforcement. Behavioral activation requires excitation of the brain and in particular the motor systems, so it comes as no surprise that the cerebellar units were so much affected by pseudo-conditioning. Brain stem units would probably also fit in this category, although there are no data from these experiments bearing on the question. The midbrain units were also accelerated by non-contingent brain stimulation. In this case, it might be related to the suggested "arousal" function of the midbrain reticular

formation (Moruzzi and Magoun, 1949), rather than to a motor relationship. The hippocampal and superior collicular units were not much affected by non-contingent stimulation, but had a definite tendency to accelerate following the periods of brain stimulation, which was difficult to understand.

In the experiments which looked specifically for operant conditioning, the cerebellum units excelled, and the brain stem units also might have been expected to do so, judging from the case of the operant brain stem unit described in Appendix C. The midbrain units did poorly in these experiments, but rather well when respondent conditioning had been possible. A general explanation of the differences in conditioning between brain areas might be that those parts of the brain more related to overt behavior are best conditioned operantly. Those parts of the brain related more to "arousal" level such as perhaps the midbrain reticular formation might be more amenable to respondent conditioning. Respondent conditioning would also tend to occur with motor units such as in cerebellum and brain stem, which are excited during the behavioral activation produced by non-contingent stimulation. The superior colliculus and hippocampal units may be related strongly to neither arousal nor overt movement. After observing the behavioral correlates in many experiments, it was difficult to imagine that anything other than overt behavior or the brain's representation of it would ever be possible to condition operantly, at least in the rat.

Conditioning in Cerebellum and Brain Stem

The brain stem and cerebellum units stood apart from the units in the other areas in the degree of success in these experiments, and are considered together here for that reason and the fact that both areas are related to motor function.

All experimental units in the cerebellum and brain stem were conditioned by the discriminative operant method in Experiment I. Most (5 or 8) of the experimental units in cerebellum displayed both rate increases and decreases and met the rigorous definition for "operant", as did the experimental cerebellum units as a group. Unfortunately no brain stem units were in Experiment III, but the experiment described in Appendix C clearly shows an operant brain stem unit. The universal success of conditioning of units in these areas in the active animal is an interesting backdrop to the contrasting findings in the paralysis experiment. All the cerebellum units, save one, stopped showing the conditioned activity, while the brain stem units continued. The cerebellum units thus appeared to rely on feedback stimulation from movement while the brain stem units did not.

The simplest explanation for the conditioning of these units is that they were motor-related and were called into play simply as a result of the animal learning to make movements in which they participated. Since all but one of the cerebellum units failed to maintain the conditioning under paralysis, their normal role

in motor activity might be supposed to require feedback from movement, such as would have to occur for regulation of ongoing movements. These findings fit with those of Thach (1970a,b) who studied the activity of Purkinje cells in lateral and intermediate areas of the cerebellum in monkeys trained to make specific arm movements. He found much activity in lateral areas which preceded movement but little intermediate activity which did so. Instead, intermediate area cells were most active during movement. Thus, lateral cerebellar units might be expected to maintain conditioning under paralysis like the brain stem units while intermediate area cells would lose it. The present study found the expected failures in intermediate cerebellum, but did not test any units in lateral cerebellum. The lateral cerebellar areas (neocerebellum) are massively innervated from association cortex, and project to the motor cortex by way of the dentate nucleus and the ventral lateral nucleus of thalamus (Evarts and Thach, 1969). The findings of Thach (1970a,b) coupled with the clinical evidence in man that cerebellar lesions sometimes impair movement initiation (Holmes, 1939), and the anatomical data combine to suggest that the neocerebellum plays a role in movement initiation (Evarts and Thach, 1969). There is anatomical evidence that the intermediate parts of the cerebellum receives input from sensory-motor cortex and indirectly from somatosensory receptors in the spinal cord, and outputs largely to the magnocellular portion of red nucleus,

which projects to the spinal cord. Somato-motor cortex also projects to the red nucleus, so intermediate cerebellum might act in a feedback fashion to stabilize movement (Evarts and Thach, 1969). The neurophysiological data of Thach (1970a,b) and the present findings support this notion.

The brain stem units are located in a position which indicates that they are part of the extrapyramidal system, which might be relaying movement initiating signals from higher brain centers. Most of the units were in the medial longitudinal fasciculus which serves as a communication link between brain stem motor nuclei as well as a major component of the extrapyramidal system. Two additional units were in the reticular formation of the brain stem, and one was in the vestibular nucleus. Electrophysiological studies have shown a definite role is played by this part of the brain in the production of movement. Thulin (1953) found that electrical stimulation of the vestibular and brain stem reticular formation had effects on spinal motoneurones by recording elicited impulses in the ventral roots. These impulses were shown to be conducted in both the vestibulo-spinal and reticulo-spinal tracts. It is also known that electrical stimulation of the medial portions of the brain stem in this region can produce inhibition in the spinal reflex pathways (Lundberg and Vyličký, 1966) presumably by depolarization of primary afferents. These studies indicate that

the units in this area have direct motor effects.

Two of the units maintaining discrimination during paralysis were located in structures related to vestibular function. One was in the nodulus of the cerebellum, and the other was in one of the brain stem vestibular nuclei. The vestibular system helps to maintain posture and is sometimes characterized as reflexive, which suggests that it lacks functional plasticity. Conditioned responses of vestibular units in Experiment I might have been produced by conditioned postural changes which would be detected by the vestibular apparatus and instigate changes throughout the vestibular system. However, since paralysis eliminated this kind of activation, these units must have been receiving excitation from other sources. One possibility is that the vestibular system might be given direct information about the movement before it occurs, which would allow the system to adapt more smoothly to a new posture.

Abolition of Midbrain Units by Paralysis

Experiment II was aimed at finding what paralysis did to conditioned units, but many units were found which changed rate substantially as soon as the animal was paralyzed. Halpern and Black (1967) have shown that Flaxedil has a direct action on the central nervous system, so it is possible that these observed changes were caused directly. Alternatively, they could have been produced indirectly by the removal of feedback from skeletal muscle

movement that was either tonically inhibitory or excitatory to those units. The most general effect was slowing, which would be expected from the removal of a tonically excitatory input. This was seen in all the brain areas to some extent. The most dramatic result, however, was the temporary abolition of activity which occurred with half of the midbrain units, but with units in no other area. The question of why Flaxedil-induced paralysis would result in abolition rather than slowing the unit to an "idling" rate as it frequently seemed to in other brain areas is intriguing, because it seems to reflect something distinctive about the physiology of this area. As a result of this abolition, many conditioned midbrain units were eliminated by default from Experiment II. This was disappointing because 2 midbrain control units had displayed conditioning in paralyzed animals, indicating that others might also be found.

Conclusions

This research has shown that the rat brain is differentiated with regard to conditioning, and specifically with regard to operant conditioning procedures applied directly to single unit activity. Conditioned responses were most frequent in the motor areas studied, presumably because whenever reinforcement is applied to the animal, it seems to reinforce an ongoing behavior pattern, and among the units studied these motor units were related most strongly to the behavior pattern. While it would certainly be hasty to assume that all units in such places as hippocampus and superior colliculus are not amenable to maintaining conditioned responses under paralysis, they seem less promising places to look than the parts of the movement initiating system described by Evarts and Thach (1969).

However, the activity controlling "motor" units - the "decision-making" or "triggering" elements, and in humans perhaps the "psychic plan" - should also respond to operant conditioning. Research on the isolation of these elements is perhaps the most interesting potential use of the operant conditioning method. The present research helps set the stage by showing that the method works to differentiate different brain areas in the active rat and that paralysis can further reduce this population by eliminating activity which requires feedback from overt movement. Obviously, much activity remains besides that of the primary initiating elements. One source is feedback from the autonomic nervous system. Drugs with

selective effects might be used on the brain and the autonomic nervous system to quell unwanted activity and generally explore the limits of the "preparation" in maintaining conditioned responses. Eventually, providing that the concept of discrete decision making elements holds up under investigation, the problem might be investigated using selective lesions of the brain and, with "luck", complete isolation of a block of neural tissue which continues to demonstrate the properties of operant conditioning. Perhaps something fundamental might then present itself or have become clear by then, about the "chain of command" in the brain. However, despite evidence from invertebrate investigations (Hoyle, 1966 and Kandel, 1967) that neural analogs of operant conditioning are possible with very limited amounts of tissue, the following statement rings true: "you can only slice cheese so thin, and then it isn't cheese anymore" (Kunz, personal communication).

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APPENDIX A

Operant Conditioning*

In the parlance of operant conditioning, all behavior is divided into two kinds of responses: those which are elicited by environmental stimuli (reflexes) are called "respondent" behaviors; those which are freely emitted are called "operant" (operating; producing effects) or "instrumental" (serving as an instrument or means) behaviors.

Respondent behaviors are said to be "conditioned" when they occur with regularity following a previously neutral stimulus. To establish the conditioning, the neutral stimulus is made to occur slightly in advance of the stimulus which elicits the respondent reflexly, until the neutral stimulus alone is sufficient to produce the response. This conditioning is also called "classical" or "Pavlovian" because of Pavlov's early and important laboratory investigation of it.

In contrast to respondent behaviors, operant behaviors are said to be conditioned by their consequences if and when the consequences have the property of being reinforcing. "Positive reinforcements" (roughly equivalent to rewards) tend to increase

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*This material was taken from Ferster and Skinner (1957), Reynolds (1968) and Boring (1957).

the probability of the behaviors they follow, while "negative reinforcements" (roughly equivalent to punishments) tend to decrease the probability of the behaviors they follow. It will be noticed that the definition of reinforcement suffers from being somewhat circular.

Operant behaviors can be controlled by "discriminative stimuli." These are environmental cues which the organism learns are associated with the reinforcement of the operant response. For example, the word "sit" is a discriminative stimulus to the dog that only then performs the operant of sitting after which he often receives the reinforcement of food. Discriminative stimuli are thus useful for turning operant behavior on and off and can be used to distinguish conditioned responses from those which occur spontaneously. When a discriminative stimulus has been learned, its presentation alone is sufficient to cause the operant response to occur. The initial presentation of the discriminative stimulus without the reinforcement is called a "discriminative probe" and is useful in checking the progress of conditioning.

The fact that the probability of behaviors are changed by the rewards and punishments which follow them is a common sense observation used by animal trainers, parents and dictators among others. Before Skinner, operant conditioning was called "trial and error" learning by Thorndike when it was used in "puzzle-boxes"

and mazes, and it was called "Type II" learning by Pavlov who called respondent conditioning "Type I" learning (Boring, 1957).

APPENDIX B

Operant Conditioning of the Alpha Rhythm

In man, the occipital alpha rhythm of the EEG has been the subject of much research on operant control. Durup and Fessard (1935) had accidentally discovered that the EEG desynchronization response to light flashes in the cat could be classically conditioned to the sound of a camera which preceded the flash. After more rigorous replications (Loomis et al., 1936; Jasper and Cruikshank, 1937), Jasper and Shagass (1941a) showed it could be obtained with EEG alpha rhythm in human subjects. Jasper and Shagass (1941b) then demonstrated "voluntary" blocking of the alpha rhythm. The subject pressed and released a button and subvocally said the work 'block' when he pressed the button and 'stop' when he released it. Initially this produced no response, but during a period of training the button controlled a light flash. After this the alpha was blocked when the button was pressed even though no light flash occurred.

The recent reports of voluntary alpha control seem to have ignored this initial finding. The topic received no further attention until Kamiya (1962, 1968) reported that the percentage of alpha rhythm could be voluntarily increased when subjects were informed of its presence by a tone (a procedure which has been dubbed

'bio-feedback'). Ostensibly, because alpha rhythm was found to predominate during the meditation states of expert Yogis (Anand, et. al., 1961), the prospect of being able to learn to directly control it has led to many more papers on the subject, and a public enthusiasm for simplified electronic devices which have been made for the purpose of alpha feedback "in the home". In support of the notion that the meditation state is approximated by increasing alpha, Kamiya (1969), Nowlis and Kamiya (1970), and Brown (1970) have reported changes in "states of consciousness" associated with enhanced alpha activity.

Some constraints have been investigated to determine how the operant control is exerted. The investigations have shown alpha enhancement possible with eyes open or closed (Nowlis and Kamiya, 1970), and with light instead of tone feedback (Brown, 1970). Eye position was found to be not necessarily related (Fenwick, 1966), nor were eye movements (Brown, 1970). However, Dewan (1967) and Mulholland and Peper (1971) have shown that alpha blocking is related to lens accommodation, processes of fixation and pursuit tracking, which could mean that alpha enhancement is accomplished by the inverse state: allowing vision to blur and not tracking.

APPENDIX C

Operant Conditioned Unit Maintained Under Paralysis

The initial discovery of maintained conditioned responses in brain stem under paralysis was somewhat of an accident. The probes were intended to lodge in the cerebellum, but extended beyond into the brain stem due to overestimation of the depth of the cerebellum. Experiment III did not include brain stem locations, because their importance was not then appreciated, so that without further experimentation, no firm statement could be made regarding the importance of the operant training given the rats with these units. The following experiment was designed in order to show a clear operant effect which would be obvious under paralysis.

Pilot studies had shown that the method used in Experiment III was difficult to employ with paralysis. It seemed better to have the behavior pre-conditioned before paralyzing the rat. The clear demarcation of effect with the discriminative operant conditioning method was also very desirable. Using an animal with an experimental unit in the brain stem (later localized in the medial longitudinal fasciculus) and rewarding electrode in the medial forebrain bundle, operant training was first applied to increase the unit rate as in Experiment III. Then following each reinforced rate increase, a decrement in rate was required, which was itself reinforced, and

which in turn required a rate increment for the next reinforcement. Constant surveillance and adjustment of the reinforcement criteria was necessary to obtain levels which made the decremental rate sufficiently distinguishable from the incremental rate while maintaining a high enough rate of reinforcement to motivate the animal. With prolonged training (two 12-hour sessions) this goal was stably achieved. The rate of alternation was quite rapid, achieving up to 8 reinforcements a minute (with a 5 second delay period following each reinforcement). Rate increases were accompanied by behavioral activation while rate decreases were accompanied by behavioral inactivity. The activity of the unit was unmistakably operant. This behavior was then placed under discriminative operant control so that a distinctive onset and offset of activity could be seen. A light was turned off for a 2.0 minute discrimination period out of every 7.5 minutes during which time reinforcement became available. When this discrimination was clear, the rat was paralyzed. The result was that the operant alternation of rate was maintained in the absence of skeletal muscle movement. A sample of activity before, during and after a discrimination period is shown in Figure 14. An equivalent sample taken during a Pavlovian Probe is shown in Figure 15. And a final test showing that non-contingent reinforcement does not result in burst-pause alternation is illustrated in Figure 16. This test was made after the alternation response was extinguished by repeated presentations of the discriminative stimulus period with

the reinforcement withdrawn.

This was perhaps a more elaborate demonstration than was necessary, because it had already been established by Experiment II that these units were not particularly affected by lack of skeletal muscle feedback. The equivocal point had been whether or not the conditioning was operant. This was settled before paralysis when it was clear that the unit was turned on and off much more rapidly than would occur spontaneously, and that the activity of the unit was directly associated with stereotyped overt behavior of the animal which preceded the reinforcement. The unit rate increased when the animal reared up on its hind legs, and the unit stopped when the animal held still. These behaviors were done in rapid succession and were maintained for long periods when reinforcement was available, but stopped when reinforcement was not available. The appearance of operant behavior was unmistakable. The fact that the unit activity was maintained in the absence of the overt behavior, however, shows that the units in the brain stem were both operant and independent of feedback stimulation from movement.

Figure 13.

Illustration of an experimental unit in the brain stem of a paralyzed animal (#0050) which shows operantly conditioned alternations of burst and pauses. Continuous photographic records of an oscilloscope trace of the unit are shown before,during, and after a discrimination period with reinforcement available. Triangles pointing upward indicate the approximate times the burst criterion was met; triangles pointing downward indicate the approximate times the pause criterion was met. "Light off" and "light on" mark the onset and offset of the discrimination period; in all the previous experiments, onset of the light marked the beginning of the discrimination period.

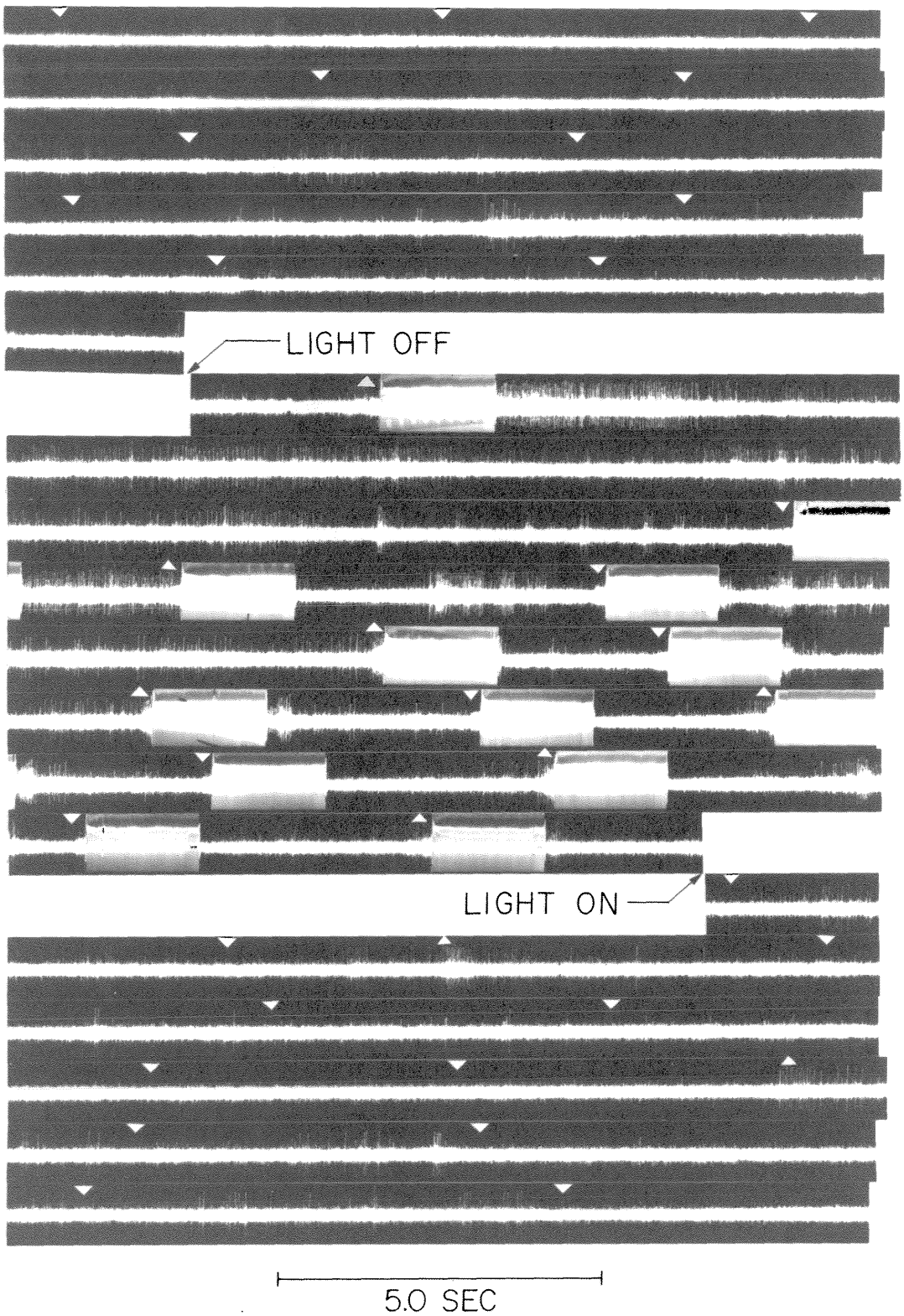
LIGHT OFF
LIGHT ON
5.0 SEC

Figure 14.

Same conditions as in Figure 13 before, during, and after a discriminative probe (reinforcement withdrawn).

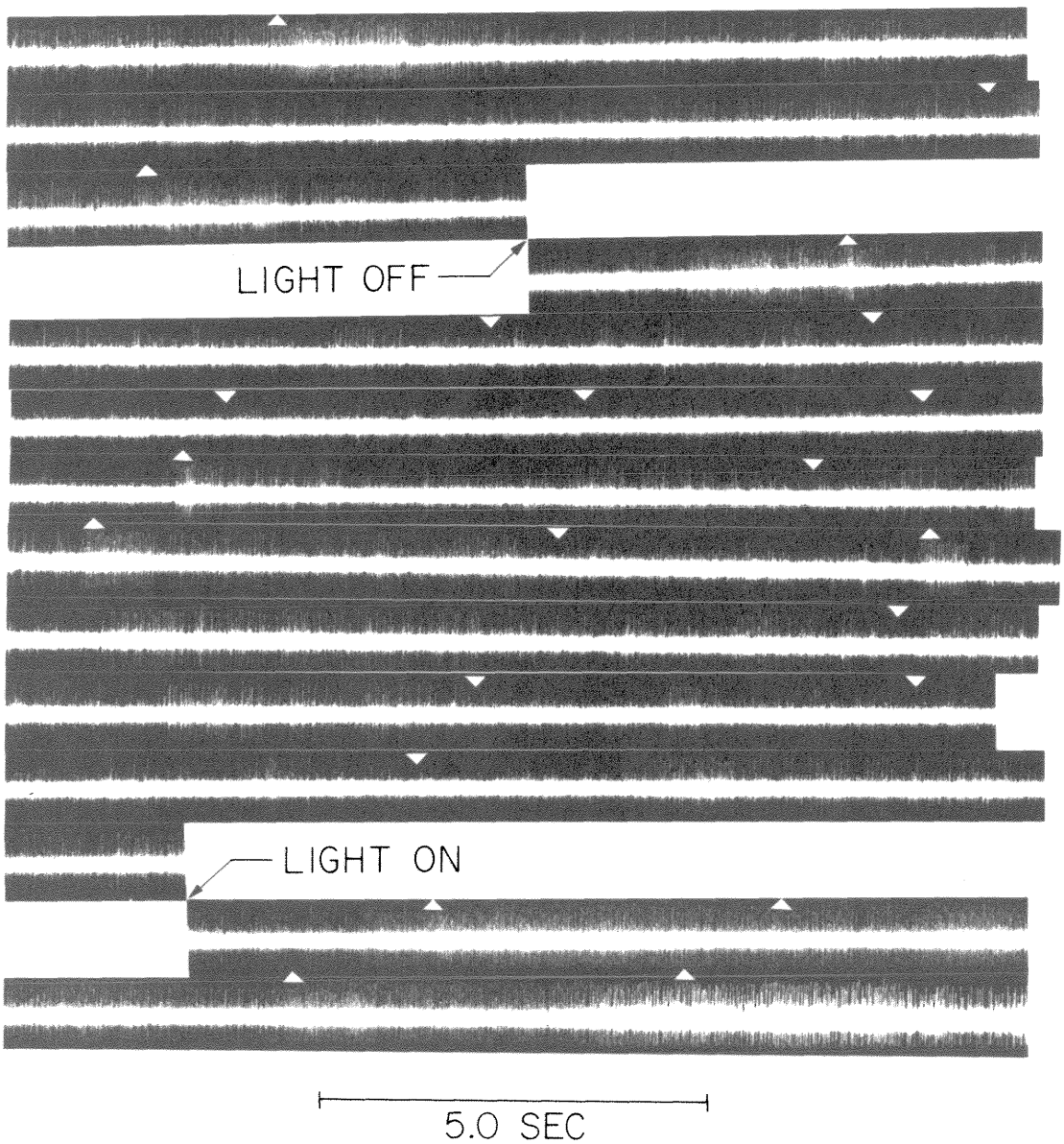
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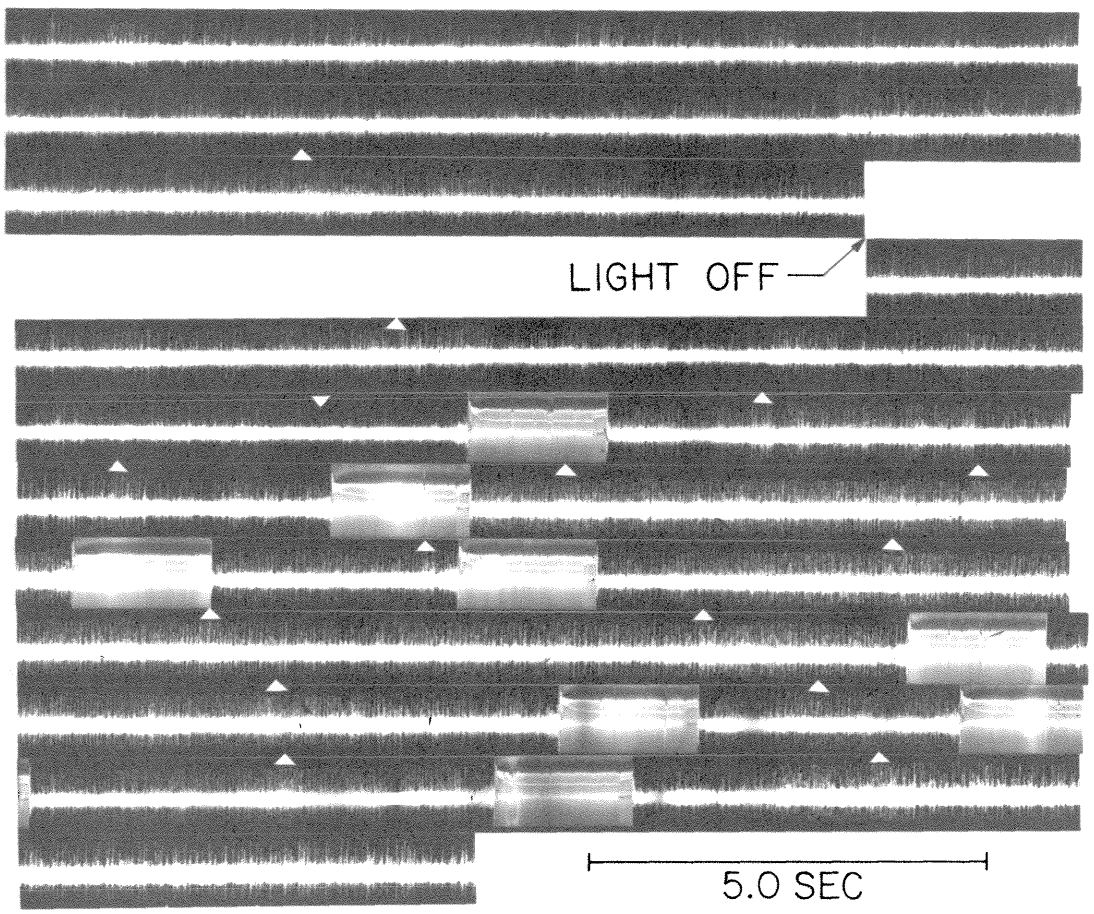
Figure 15.

Same as in Figure 13 before and during a discrimination period following extinction of the burst-pause alternation behavior in which the same brain stimulation was applied at random.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