AD-A151 077 NEURONAL MECHANISMS OF INTELLIGENCE(U) CALIFORNIA UNIV 1/1
IRVINE DEPT OF PHARMACOLOGY L STEIN ET AL. NOV 84
AFOSR-TR-85-0073 F49620-81-K-0015

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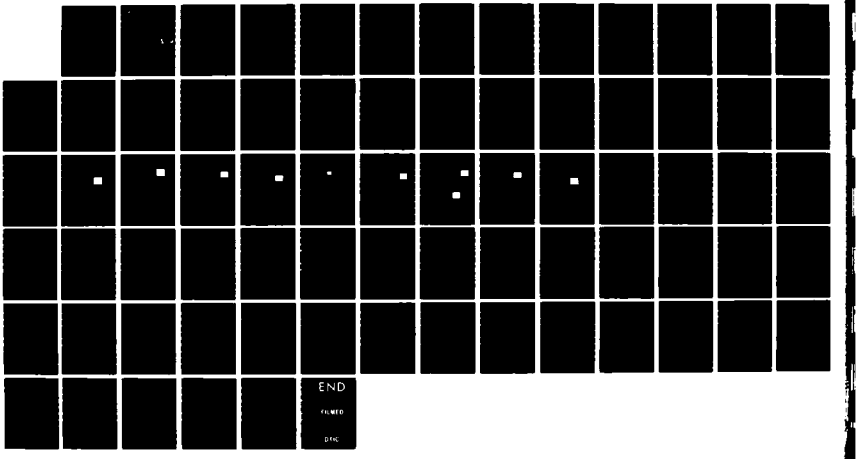

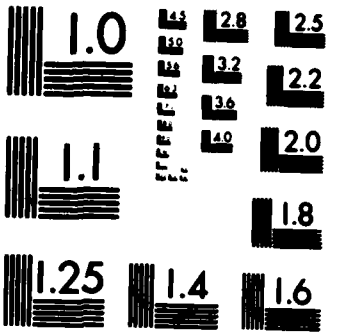

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER AFOSR-TR- 85-0078	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) NEURONAL MECHANISMS OF INTELLIGENCE	5. TYPE OF REPORT & PERIOD COVERED Final Technical Report 30 JUNE 81 - 29 JUNE 84
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Larry Stein & James D. Belluzzi	8. CONTRACT OR GRANT NUMBER(s) F49620-81-K-0015
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Pharmacology University of California, Irvine Irvine, CA 92717	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 2312/A1 61102F
11. CONTROLLING OFFICE NAME AND ADDRESS UASF Office of Scientific Research/NL Bolling Air Forces Base, DC 20332	12. REPORT DATE November 1984
	13. NUMBER OF PAGES 69
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report) UNCLASSIFIED
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DTIC ELECTE MAR 1 1985 S D A

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Operant Conditioning, Neuronal Conditioning, Reward, Adaptive Networks, Positive Reinforcement, Learning.

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The aim of this research program was to identify the functional unit in the brain for reward or positive reinforcement. On the assumption that the simplest possible unit is the single brain cell, we have attempted to reinforce individual neuronal firing patterns by direct applications of neurotransmitters or drugs to the cell soma. Our most satisfactory experiments have been performed on large pyramidal cells in hippocampal brain slices. The probability of neuronal firing increased sharply when rein-

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Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

AFOSR Grant Number F49620-81-K-0015
Final Technical Report
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NEURONAL MECHANISMS OF INTELLIGENCE

DEPARTMENT OF PHARMACOLOGY
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IRVINE, CA 92717

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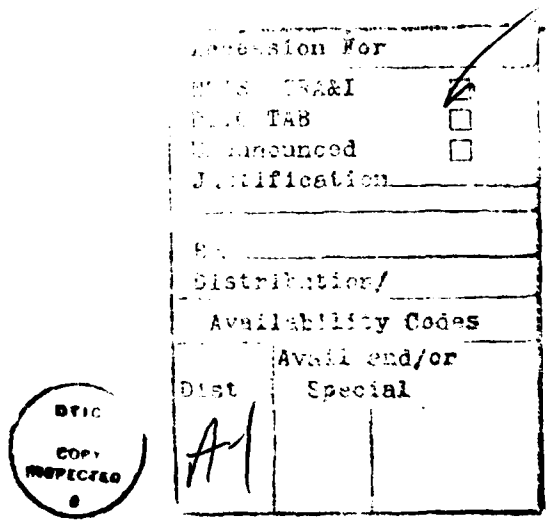
ACKNOWLEDGEMENTS

We thank Dr. A. Harry Klopf, whose theoretical work provided a basis for the present research, for insightful advice and criticism at every stage. Our program managers, Col. George W. Irving, III and Dr. William O. Berry, provided valuable encouragement and support. We also thank Charlotte Hoffmann for expert technical assistance and Walt Otto for development of computer programs.



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NEURONAL MECHANISMS OF INTELLIGENCE

I. SUMMARY

The aim of this research program was to identify the functional unit in the brain for reward or positive reinforcement. On the assumption that the simplest possible unit is the single brain cell, we have attempted to reinforce individual neuronal firing patterns by direct applications of neurotransmitters or drugs to the cell soma. Our most satisfactory experiments have been performed on large pyramidal cells in hippocampal brain slices. The probability of neuronal firing increased sharply when reinforced by contingent applications of dopamine or cocaine; the same injections applied independently of neuronal firing had no such effect and in fact tended to suppress activity. There is an indication of pharmacological specificity: included among substances that are ineffective are GABA, serotonin, acetylcholine, imipramine, ethanol, and saline. Some features of behavioral operant conditioning are not observed in the neuronal experiments; reinforcement schedules are ineffective and relearning is not enhanced. Such features thus may reflect properties of neuronal systems rather than of individual cells. Finally, we have begun to consider the biochemical events that may mediate the cellular reinforcement process. Proteins that control cellular firing rates may be modified (phosphorylated) via a biochemical cascade involving the conjunction of Ca^{++} influx and dopamine receptor stimulation. Originator Supplied Keywords include:

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II. INTRODUCTION

Our overall aim is to understand how the brain produces intelligent behavior. What are the essential properties and functions of brain cells, cellular and organizational, on which intelligence depends? The present research is based on the premise that human intelligence has evolved from the goal-seeking brain functions of lower forms, and that these functions in turn depend on a capacity for behavior to be strengthened or positively reinforced by certain stimulus consequences or rewards. Our immediate aim is to identify and characterize the smallest functional unit in the brain for reward or positive reinforcement. Knowledge of this presumed unit of organization of biological intelligence could then be used to define a suitable unit of organization of machine intelligence.

The phenomenon of positive reinforcement is well established at the level of the whole animal. If a response is closely and regularly followed by a reinforcing stimulus, the behavior is strengthened or its probability is increased. A behavioral response obviously reflects the activity of many neurons. Is it the integrated activity of these neurons that is reinforced; that is, is reinforcement exerted at the level of neuronal systems? Or is it the individual activities of the relevant neurons that is reinforced; that is, is reinforcement exerted at the cellular level (Klopf, 1982)?

It is commonly believed that reinforcement is exerted at the systems level. According to this view, the reinforcement of behavioral responses is paralleled at

the neuronal level by the strengthening or reorganization of complex neuronal circuits or assemblies (Hebb, 1949). Unfortunately, no such circuit or cell assembly for any reinforced behavior has yet been identified. Where in the rat's brain, for example, would one find the circuit for a lever-press response? And how would one measure the changes in circuitry that are induced when lever-pressing is reinforced? These anatomical and physiological considerations, as well as difficulties at the behavioral level associated with response definition, have led some psychologists to ask whether it really is correct to say that the whole response is the functional unit for reinforcement. According to Skinner (1953), a more useful conceptual scheme assumes that all responses are made up of elements, and that it is these elements or "behavioral atoms" (p. 94), and not whole responses, that are the units strengthened by reinforcement. If so, and if atoms of behavior can be represented by the activity of individual neurons, then positive reinforcement may actually occur at the level of individual cells, as suggested by Klopf (1982).

The cellular hypothesis suggests an analogy between reinforced behavior and an ant colony. If you watch an ant colony from a distance as it descends on a pile of sugar, it looks much like one large creature moving towards the sugar. However, close up, the colony is seen to be no single creature, but rather an aggregated mass of individual ants. In the terms of the analogy, the important question for a theory of reinforcement is, who tastes the sugar? Obviously it is not the ant colony as a whole, but the individual ants themselves that actually taste the sugar. Similarly, it could be argued that in the brain it is the behavior of

individual neurons, and not the integrated activity of neuronal circuits, that is directly modified by reinforcing signals.

III. REFLEXES AND OPERANTS

According to Skinner (1938), environmental stimuli exert control over behavior by two fundamentally different processes--respondent and operant. The distinction is based entirely on the temporal relationship between the controlling stimulus and the response (Table 1). In the case of respondent (reflex) processes, the controlling stimulus precedes the response and elicits it, as, for example, food in the mouth elicits salivation. In the case of operant processes, the controlling stimulus follows the response and reinforces it, as, for example, food following a lever-press response makes lever pressing more probable.

Operant or reinforced behavior thus is controlled by its consequences, which change the likelihood of future occurrences of the behavior. Consequences that increase response probabilities are termed positive reinforcers. Only the temporal relationship of stimulus and response, and not the nature of the response itself, distinguishes respondent from operant behavior. For example, blinking elicited by a cinder in the eye is respondent, whereas the same eyelid closure, winking to attract a member of the opposite sex, is operant.

From an evolutionary point of view, the emergence of operant behavior had profound implications. With only a reflex brain, an organism is essentially reactive and controlled by impinging stimulation. Through Pavlovian conditioning, responses could come under the control of new stimuli as conditioned reflexes, but the

Table 1. Temporal relationships in classical and operant conditioning.

Type of Process	Controlling Stimulus	Effect on Behavior	Hypothesized Linkage of Stimulus (S) and Response (R)	Hypothesized Locus of Conditioning
Classical	Antecedent	Elicitation	S-R	Synapse
Operant	Consequent	Reinforcement	R-S	Intracellular pacemaker and/or Synapse

organism remains stimulus bound. As Skinner (1981) points out, operant conditioning must have evolved in parallel with two other products of natural selection -- "a susceptibility to reinforcement by certain kinds of consequences and a supply of behavior ...which has little or no relation to (eliciting or releasing) stimuli." (p. 501). As a consequence, the organism with operant capability is not stimulus bound but intrinsically active, and can learn to find more favorable stimulation than that provided by its current environment.

It is interesting to consider whether or not fundamental changes in brain physiology were required for the evolution of operant behavior. Respondent mechanisms derive directly from classical Sherringtonian physiology--a specific stimulus elicits a particular response by activation of a reflex arc. Operant processes, on the other hand, are not obviously compatible with classical physiology. Impulses in a reflex arc are conducted in an afferent to efferent

direction; hence, the neuronal circuitry requires that the stimulus precede the response (S-R). But operants are controlled by stimuli that follow the response. Since the reinforcing stimulus occurs only after the response has been completed, operant behavior would seem to require an unconventional circuitry in which efferents are activated before afferents (R-S).

The classical solution to this problem has been to assume that all responses (operants as well as respondents) are elicited by antecedent stimuli, and that the reinforcing stimulus acts merely to strengthen the connectivity or linkage between these eliciting stimuli and the reinforced response (Thorndike, 1911; Hull, 1943). According to this view, operant responses are little more or less than Pavlovian conditioned reflexes; as such, operants can obviously be mediated by reflex arcs, and both operant and classical conditioning can be explained at the physiological level by the same fundamental mechanisms of synaptic change.

Skinner (1953) has criticized this view on the grounds that it is probably impossible to show that any single stimulus invariably precedes an operant response. (What stimulus, for example, will reliably elicit a lever-press response from an untrained rat?) In the absence of eliciting stimuli, operants must be generated intrinsically--in Skinner's terminology, operants are said to be "emitted" rather than elicited. Furthermore, any incidental stimulation that may have occurred prior to the operant response has no necessary relationship to future reinforcement. In the case of operants, only responses have fixed relationships to

reinforcement. Therefore, the neural connections important for operant behavior must be those between responses and reinforcing stimuli (or, more precisely, between the brain elements that represent responses and those that represent reinforcers). Thus, if the classical view conceives all behavior to be mediated by S-R connections, Skinner's view conceives only respondents to be mediated by S-R connections and requires, for the mediation of operants, a new functional unit that can act in an R-S mode. This new unit will be designated the "operant element".

IV. NEURONAL MODELS OF OPERANT REINFORCEMENT

The operant element proposed here resembles in many ways the "elementary heterostat" posited in Klopf's (1982) theory of intelligent systems, although, as we shall see, the heterostat functions in the classical, Thorndikean S-R mode rather than in a Skinnerian R-S mode. Both units represent neuronal models of operant conditioning, and both are functional elements intended to serve as fundamental building blocks for complex behavior. In both cases, operant conditioning is conceived as an adaptive change in neuronal activity induced by reinforcement signals received after the neuron has discharged.

Klopf (1982) has put his idea vividly and succinctly: "After a neuron fires, it waits for a few hundred milliseconds or more to see how it will be affected by the action it has taken. If it experiences further depolarization within a second or so, it increases the effectiveness of the excitatory synapses that led to its firing in the first place, thereby increasing the probability that it will fire the next time that some fraction of these synapses is active" (p. 5). Klopf's heterostat is diagrammed as a 3-neuron assembly in Fig. 1, which shows a "reinforced" neuron and its requisite synaptic connections with distinct, but chemically unspecified, excitatory "input" and "reinforcement" neurons.

The heterostat may be compared with the operant element of the present proposal, diagrammed as a 2-neuron assembly in Fig. 1. Again, we have a neuron

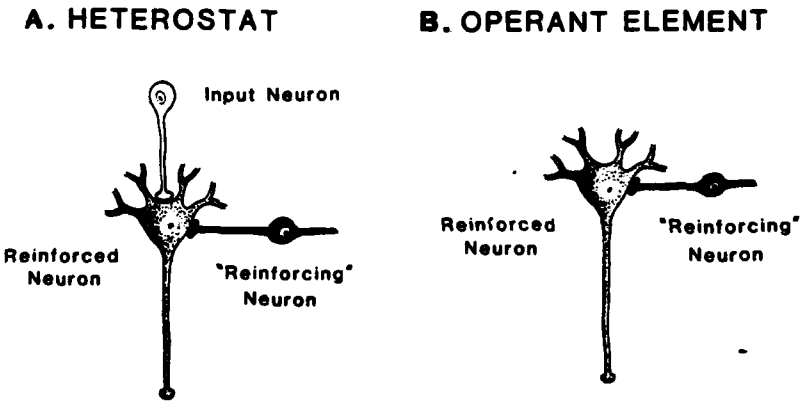

Figure 1

Neuronal models of operant conditioning. (A) Diagram of Klopf's (1982) elementary heterostat as a 3-neuron assembly, with "input", "reinforced", and "reinforcing" neurons which subserve stimulus, response, and reinforcement functions, respectively. Sequential firing of "input", "reinforced" and "reinforcing" neurons in appropriate temporal relation increases the effectiveness of the excitatory synapse between the "input" neuron and the "reinforced" neuron. (B) Diagram of operant element. Only two neurons are required: a "reinforced" or command neuron capable of spontaneous activity and a catecholamine- or endorphin-releasing neuron which is chemically specialized for "reinforcing" functions. When the spontaneous activity of the command neuron is regularly followed by activation of its catecholamine or endorphin receptors (due to contingent firing of the "reinforcing" neuron), the intracellular pacemaker of the command neuron is activated. See text for further explanation.

that receives reinforcement signals from a "reinforcement" neuron, but the reinforcement neuron is chemically specialized and the "reinforced" neuron (which we will now call a command neuron) must exhibit intrinsically generated spike activity, even in the absence of excitatory synaptic inputs. The operation of the

operant element is relatively simple: if the spontaneous firing of a command neuron is shortly followed by activation of its chemically specialized reinforcement receptors (due to release of endorphins or catecholamines from a reinforcement neuron), the intracellular pacemaker of the command neuron will be energized and its spontaneous activity will therefore be increased. On the other hand, if reinforcement receptors are activated during periods of silence, the intracellular pacemaker will not be energized and spontaneous spike activity will not be affected or may even be reduced. Finally, if the augmented spike activity of a previously reinforced command neuron is no longer followed by reinforcement, the intracellular pacemaker will be gradually deactivated or extinguished and spontaneous firing will return to a baseline level.

Keeping in mind the many points of similarity, it is instructive to contrast the heterostat and the operant element. First, the two models differ with respect to the nature and locus of the reinforcing effect. In the case of the heterostat, operant reinforcement is assumed to act at the synapse and to increase excitatory conductances; in the case of the operant element, reinforcement is assumed to act intracellularly and to energize an action potential generator or pacemaker. The two models also differ with respect to the sign and chemical specificity of the reinforcement message. The heterostat requires depolarizing reinforcement signals, but their chemical nature is not important. The operant element requires chemically specified reward messages, but the sign of the postsynaptic potential is not important. Indeed, it is interesting that the enkephalin and catecholamine

reinforcement transmitters proposed in the operant-element model generally exert acute hyperpolarizing effects in neurophysiological experiments. Whether such inhibitory actions are a necessary characteristic of reinforcement transmitters remains to be determined. In any case, it seems clear that the immediate synaptic effects of reinforcement transmitters, which often inhibit cellular activity, must be distinguished from their longer-term reinforcing actions, which facilitate cellular activity.

V. NEUROCHEMICAL SUBSTRATES OF REINFORCEMENT

An important role for catecholamines and endorphins in the reinforcement process is suggested by evidence from self-stimulation and self-administration experiments (for reviews see Crow, 1973; Fibiger, 1978; Stein, 1978; Stein & Belluzzi, 1979; Wise & Bozarth, 1982). In the self-stimulation experiments, electrodes were implanted in brain pathways which release catecholamines or endorphins upon stimulation. Although the sole reward for operant behavior was an electrical brain stimulus, very high response rates were observed (Olds & Milner, 1954; Stein, 1964; Crow, 1972; Belluzzi & Stein, 1977). Anatomical mapping of positive and negative sites was generally consistent with the suggestion that catecholamines and endorphins were involved with reinforcement. This idea was further supported by pharmacological experiments. Antagonists of catecholamines and endorphins, such as chlorpromazine and naloxone, would be expected to block chemical transmission of reinforcement messages. In support of the model, these drugs blocked self-stimulation behavior (Olds, 1962; Holtzman, 1976; Belluzzi & Stein, 1977).

In the self-administration experiments, the sole reinforcement for operant behavior was central or systemic injections of endorphins (Belluzzi & Stein, 1977; Mello & Mendelson, 1979; Olds & Williams, 1980) or dopamine-like compounds such as apomorphine and piribedil (Baxter et al., 1974; Yokel & Wise, 1978). Animals work avidly for the chemical injections, just as they do for electrical stimulation. The patterns of self-administration often resemble the patterns observed when

cocaine, amphetamine or morphine are self-administered (Baxter et al., 1974). In addition, drugs such as chlorpromazine, which block the reinforcing effects of cocaine and amphetamine, also block apomorphine self-administration (Baxter et al., 1976; Wise, 1978).

Although these anatomical and pharmacological findings seem consistent with the idea that reinforcing functions are performed by specialized systems of catecholamine and endorphin neurons, major problems remain. The anatomical data are primarily correlational and insufficiently detailed; it has not yet been shown that any map of positive self-stimulation sites overlaps precisely the map of catecholamine or endorphin pathways in that region. Furthermore, effective fields of stimulation are so large relative to the size of the units stimulated that self-stimulation electrodes must cause the simultaneous release of many different transmitters, including some that are still unknown. At the same time, almost all of the pharmacological reports can be criticized because it is difficult to distinguish specific drug effects on reinforcement from nonspecific effects on performance (Phillips & Fibiger, 1978; Wise, 1978). In addition, there are findings which seem to directly contradict the norepinephrine and dopamine theories of reinforcement. Lesions of the dorsal noradrenergic bundle failed to eliminate locus coeruleus self-stimulation (Clavier & Routtenberg, 1976) and lesions of the nigro-striatal bundle failed to eliminate substantia nigra self-stimulation (Clavier & Fibiger, 1977). Self-administration studies generally provide a stronger line of evidence for catecholamine involvement in reinforcement processes, but difficulties have been raised by conflicting findings with the norepinephrine agonist

clonidine (Davis & Smith, 1977; Yokel & Wise, 1978) and the paradoxical observation that the dopamine antagonist haloperidol supports self-administration (Glick & Cox, 1975).

Such difficulties have led to the suggestion that exclusive reliance on conventional self-stimulation and self-administration methods is inappropriate for the study of reinforcement mechanisms, and that new and more powerful methods must be developed (Hall, Bloom & Olds, 1977). It is possible that a simpler preparation than the whole animal may be required to provide definitive tests of the catecholamine-endorphin reinforcement theory. Since the simplest biological system whose behavior may be capable of reinforcement is the single cell, a second objective of the present research was to provide more definitive tests of the catecholamine-endorphin reinforcement theory.

VI. OPERANT CONDITIONING OF SINGLE NEURONS

Olds (1965) was the first to report apparent evidence for the operant conditioning of single neurons. In these experiments, rats with implanted microelectrodes received food or rewarding brain stimulation contingent on appropriate bursts of single unit activity. Firing rates were increased in a number of cases, suggesting reinforcement of the single unit response. Unfortunately, it is not clear whether it was the behavior of the individual neuron that was being reinforced or whether some more complex response or movement, of which the neuron's activity was a part, actually was being reinforced. In some of Olds' tests a restriction system was used to limit movement: electronic detectors were discharged by most movements and these precluded reinforcement. Although operant conditioning was still obtained under these conditions, one cannot rule out the possible reinforcement of behaviors involving undetected movements, such as postural adjustments or attentional responses. Like other investigators who have attempted to demonstrate operant conditioning of single unit activity (Fetz, 1969; Wyler & Robbins, 1980), Olds recognized that, if a reinforcing stimulus is delivered to a behaving animal, it is impossible to separate the reinforcement of single units from the reinforcement of more complex responses.

One solution to this problem is to deliver a reinforcing stimulus only to the neuron being conditioned. But what is effective reinforcement for a single neuron? According to the catecholamine-endorphin theory (see Section IV above), reinforcement signals normally are delivered to their neuronal targets by the

release of catecholamines or endorphins. If so, it might be possible to duplicate these natural signals of reinforcement by the direct application of the transmitters to appropriate neurons. In such experiments catecholamines or endorphins would be applied via micropipettes to individual units in various regions of the brain following particular rates or patterns of activity. These rates or patterns should be strengthened only by contingent application of the transmitters, and not by noncontingent application.

As indicated above, the neuronal operant conditioning procedure could provide more definitive answers to a number of basic questions concerning the neurochemical nature of the reinforcement substrate. For example, the relative efficacy of dopamine and norepinephrine as reinforcers could be assessed by means of the neuronal operant conditioning method. And if both catecholamines proved effective, their sites of reinforcing action could be determined by testing units in various locations. In the same way, the question of which opiate peptide may be specialized for reinforcing functions could be answered by determining the relative reinforcing potency of the different endorphins. Similarly, identification of the precise opiate receptor that might be specialized for reinforcement functions could be assessed by use of highly specific opiate receptor agonists. It would also be possible to provide information about the possible interactions of catecholamine and endorphin transmitters in the mediation of reinforcement functions. For example, if synergism were the case, subthreshold doses of a catecholamine and an endorphin might be combined in the same micropipette to produce effective reinforcement.

VII. OBJECTIVES

The main objectives of this research program include the following: 1) demonstration that the activity of single neurons can be reinforced by cellular applications of transmitters or drugs, 2) determination of the properties and limits of such neuronal operant conditioning, and 3) investigation of the physiological and biochemical events that may mediate the cellular reinforcement process. These objectives correspond to and fall naturally into three phases of investigation. As will be described in detail below, good progress has been made in accomplishing the objectives of phase 1, a start has been made with regard to the objectives of phase 2, and conceptual work has begun in preparation for phase 3, in that a plausible and testable working hypothesis of the biochemistry underlying the cellular reinforcement process has been formulated.

VIII. METHODS

1. Surgery and Brain Slice Preparation

In whole brain experiments, rats were anesthetized with chloral hydrate, artificially respirated, and placed in a stereotaxic instrument. Using a Leitz micropositioner, a micropipette was lowered into different brain regions through a small hole in the skull, and slowly advanced until suitable action potentials were displayed on the oscilloscope. In brain slice experiments, rats were decapitated, and their brains were rapidly removed (30-45 sec) and chilled to 6° C in oxygenated artificial cerebrospinal fluid (ACSF; Dingledine, et al., 1980). Using plastic tools, the hippocampal region was dissected out and rinsed repeatedly with cold ACSF to minimize cell damage. A 4-mm block was isolated by parasagittal cuts using a tissue chopper. One cut end of the block was glued with cyanoacrylate to the bottom of the cutting chamber and supported by surrounding it with agarose (1.8%, 43° C). Once secured, the tissue was again immersed in ACSF at 6° C and further sectioned with a vibratome to yield about six 400-μ slices. The slices were individually transferred to a static chamber and supported on nylon mesh at the surface of ACSF solution in an oxygenated atmosphere (95/5 O_2/CO_2, 500 ml/min) at 35° C. At least 1 hr of incubation was allowed for recovery of physiological activity prior to the start of experiments (Schwartzkroin, 1981; Teyler, 1980). Fresh ACSF was infused into the static chamber every 30-45 min.

2. Extracellular Recording and Pressure Microinjection

Single-barrel micropipette blanks (Omega Dot) were pulled on a Kopf electrode puller and then back-filled with test solution or vehicle (165 mM saline). The micropipette was connected to a pressure injector, and the tip broken back under microscopic control to produce a droplet 18μ in diameter at an injector setting of 15 p.s.i and 35 ms. Using a Leitz micropositioner, the micropipette was visually guided to targeted cells and slowly lowered until a suitable action potential was obtained. Unit activity was displayed on a Tektronix 5223 digital storage oscilloscope, monitored on a loud speaker, and recorded on a Gould 220 strip chart recorder. Important criteria for the selection of suitable cells included a signal/noise ratio of at least 4:1 and relatively stable levels of baseline activity. Action potentials were led into a Haer 74-45-1 amplitude analyzer, whose output provided digitized input to the computer and to a Haer 74-40-3 rate/interval analyzer. Firing rates were displayed on a Houston B-5000 strip chart recorder. A Data General Eclipse S/120 minicomputer with 128kB of memory and 12.5MB disk was programmed to count unit activity and activate the injection pump. Both the S/120 and a Data General Eclipse S/140 computer with 512kB main memory and 25MB disk was used for on-line data storage and analysis. An Ampex PR260 7-channel FM recorder provided a permanent record of all essential experimental events in sequence for later analysis.

A high-pressure microinjection system (Picospritzer II, General Valve Corp.) was used for rapid extracellular delivery of picoliter volumes of neurotransmitters

and drugs. Pressure injection is required for immediate delivery of reinforcing solutions with injection durations as short as 5ms. High pressure nylaflow tubing was used to connect the injection pump to the micropipette.

3. Single-Unit Operant Conditioning Procedures

A somewhat arbitrary decision was made in choosing which aspect of unit activity to reinforce. Since firing rates are likely to be an important vehicle for information transmission, peak rates should have high information value and might be amenable to conditioning. Thus, in these experiments, we defined a half-second period of relatively fast activity as the neuronal response to reinforce. These neuronal responses or "bursts" were individually determined for each unit studied. Prior to the start of conditioning, 500 successive half-second samples of neuronal activity were recorded and a frequency distribution of the number of spikes per sample was compiled. A "burst" was defined as that spike number that was equalled or exceeded in only 2 percent of the samples.

In a few experiments, we have made use of a new computer program to detect bursts of firing in a relatively precise way (see Appendix II). In this program, a burst is defined as $\underline{n}$ or more spikes in a train with a maximum interspike interval of $\underline{t}$ ms. (In the case of the CA1 pyramidal neuron, preliminary data indicate that satisfactory results may be obtained with $\underline{n} = 3$ and $\underline{t} = 15$ms, although these values will vary somewhat depending on the neuron under investigation). Since the duration of each burst will be determined precisely, it

will be possible to reinforce the neuronal response immediately or at a fixed delay after its completion. (For a related analysis of delay of reinforcement at the behavioral level, also supported by this project, see Black, Belluzzi, & Stein, submitted for publication, and Appendix I).

The basic operant conditioning method involved six stages: 1) Baseline. The number of "bursts" in the absence of reinforcement (operant level) was determined during a baseline period of approximately 10 minutes. 2) Operant Conditioning. Each "burst" was now followed by an injection of the reinforcing solution. If conditioning failed to occur after 5 minutes, the duration of the injection (and hence the dose) was increased until evidence of conditioning was obtained, or until direct pharmacological or mechanical effects interfered with recording. 3) Extinction. Reinforcement was terminated, and recording continued until the baseline was recovered. 4) Matched "Free" injections. Noncontingent injections of the reinforcing solution were made at regular intervals to determine direct pharmacological effects on rates of firing and probability of "bursts." The pattern and number of "free" injections were matched to the pattern and number of reinforcing injections in the preceding phase of operant conditioning. The presentation of programmed free injections was delayed for three sec after the occurence of "bursts" to minimize their adventitious reinforcement. 5) Washout. A second baseline period without injections was given to allow residual effects of the noncontingent drug administrations to be dissipated. 6) Reacquisition. Whenever possible, a second period of reinforcement was scheduled in order to compare rates of original acquisition and reacquisition.

IX. RESULTS

1. Whole Brain

Initial work was performed in the whole animal to maximize the probability of successful experiments. In the absence of experience with locally applied reinforcement, it was felt that surgical reduction of the preparation, or the use of cells in culture, should be deferred until positive results could be demonstrated in the intact brain. An early positive experiment in an intact, anesthetized preparation with cocaine (a dopamine enhancer) as the reinforcing solution is displayed in Figure 2. This unit, probably located in dorsal hypothalamus, exhibited a seven-fold increase in the number of "bursts" and a two-fold increase in overall firing rate following approximately 20 cocaine reinforcements. The same number of free cocaine injections had no effect on the number of "bursts" or overall spike frequency. Another apparently positive experiment with amphetamine (a dopamine releaser) as the reinforcing agent is shown for a unit presumably located in hippocampus (Fig. 3). In this case, a dramatic increase in the rate of "bursts" was observed after approximately 6 reinforcements. Twenty similar amphetamine injections, administered noncontingently in the free injection phase, produced no significant increase in the number of "bursts" or overall spike rate. Introduction of an FR-2 schedule caused a decline in "bursts" in this and other experiments. Reintroduction of the regular reinforcement schedule increased the number of "bursts" which again decreased when the FR-2 schedule was reinstated. A similar experiment with the dopamine receptor agonist apomorphine serving as the

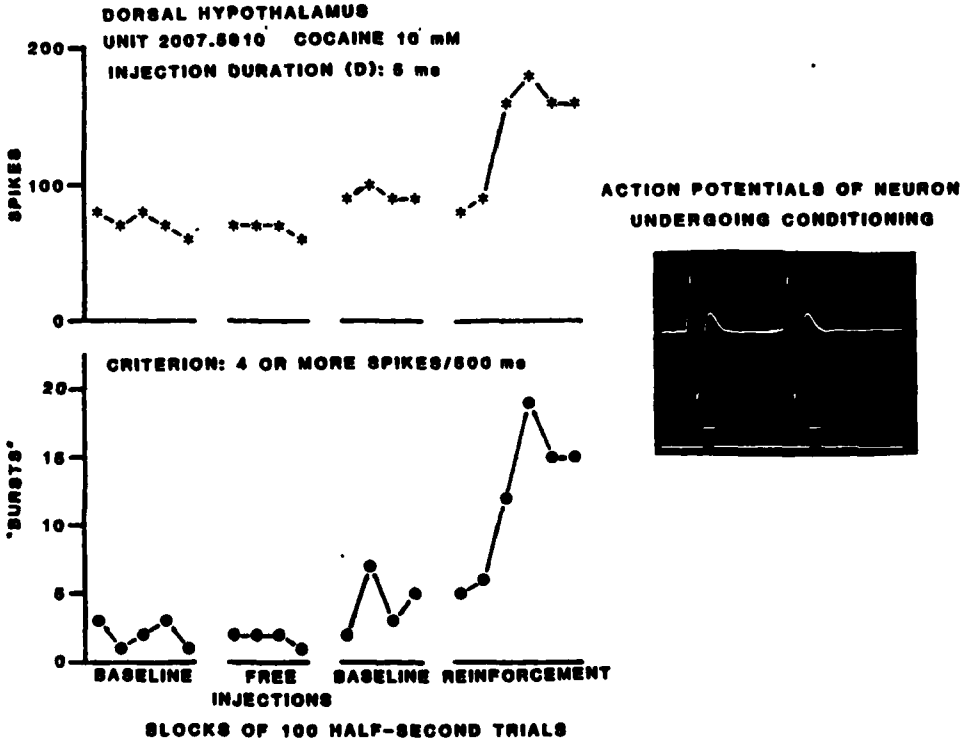

Figure 2

Operant conditioning of a neuron in rat brain presumed to be located in dorsal hypothalamus using local injections of cocaine as reinforcement. Unit activity throughout four phases of a complete experiment is shown. Prior to the first baseline phase, the "burst" criterion of 4 or more spikes per half-second sample was established. Each point shows the number of "bursts" (lower graph) and number of spikes (upper graph) in blocks of 100 half-second samples or trials. In the second phase, free injections of cocaine HCl (10 mM in 165 mM saline) were made after every 20th half-second trial for a total of 20 free injections. The injection duration was 5 ms. Following a second baseline period, the 5-ms duration cocaine injections were delivered after each "burst" (reinforcement phase). "Bursts" and overall spike rate were increased by the contingent cocaine injections during the reinforcement phase, but were not increased when the same injections were administered noncontingently in the free injection phase. Inset: (upper trace) photograph of digital oscilloscope display of two action potentials from the unit undergoing conditioning, and (lower trace) 1-ms time markers.

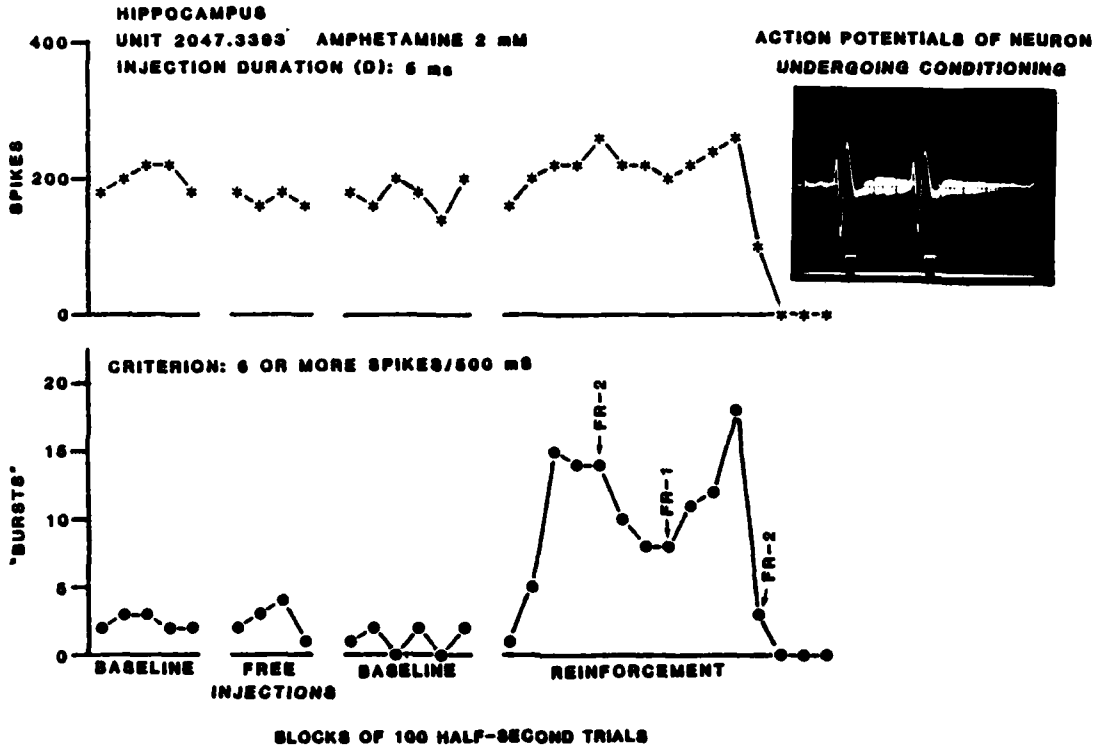

Figure 3

Operant conditioning of a presumed hippocampal neuron in rat brain using local injections of amphetamine as reinforcement. For details, see text and Fig. 2. FR-1 = regular reinforcement schedule, FR-2 = fixed-ratio 2 reinforcement schedule.

reinforcing drug is shown for a unit presumably located in the reticular nucleus of the thalamus (Fig. 4). In this case, an increase in injection duration from 70 to 100 ms was required for evidence of operant conditioning. Free injections of apomorphine, both preceding and following the reinforcement phase, failed to increase the number of "bursts" or the overall spike rate above baseline, suggesting that direct stimulation cannot explain apomorphine's reinforcing action. Finally, a negative control experiment, in which saline was substituted for the reinforcing drugs, is shown in Figure 5. No evidence of operant conditioning was observed

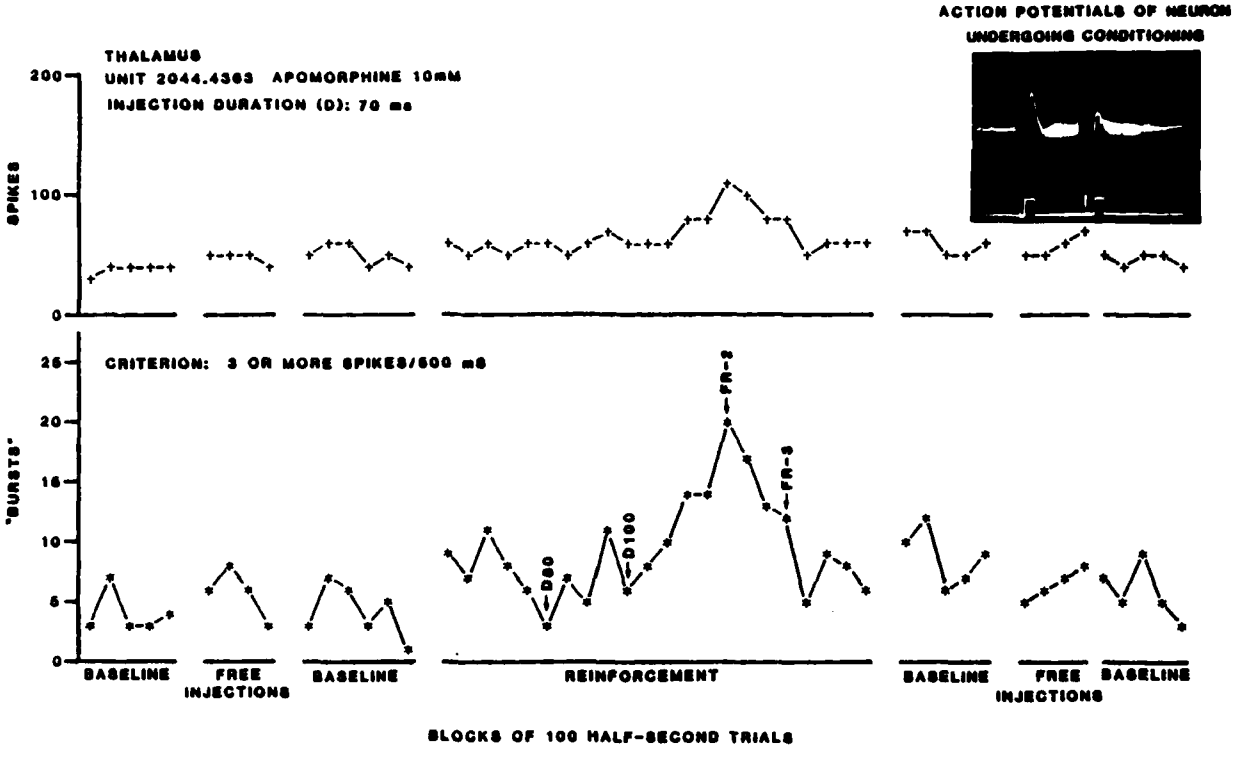

Figure 4

Operant conditioning of a presumed thalamic neuron in rat brain using local injections of apomorphine as reinforcement. For details, see text and Fig. 2. At D80 injection duration increased from 70 to 80 ms, at D100 injection duration increased from 80 to 100 ms. FR-2 = fixed ratio 2 reinforcement schedule, FR-3 fixed ratio 3 reinforcement schedule.

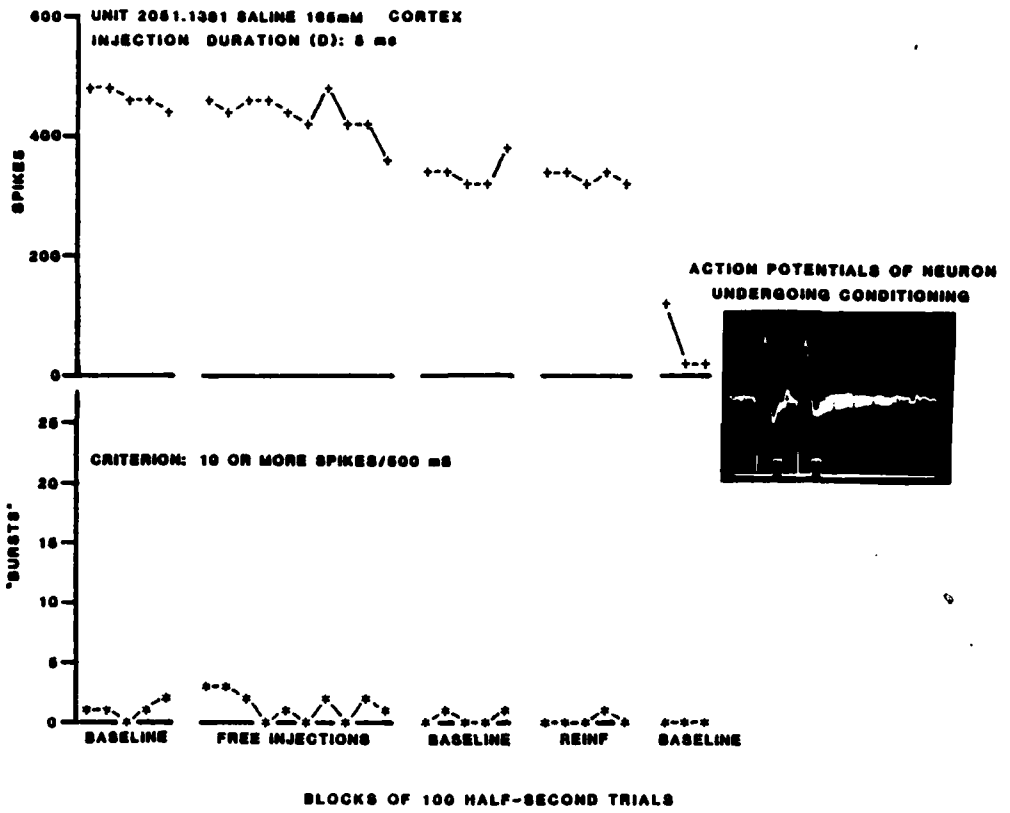

Figure 5

Saline control experiment. Failure to obtain evidence of operant conditioning of a presumed cortical neuron in rat brain using local injections of saline as reinforcement. For details, see text and Fig. 2.

suggesting that a chemically specific message, and not the mere contingency of pressure microinjection following bursts, is required for reinforcement.

2. Brain Slices

Contrary to initial expectation, our most satisfactory experiments have been performed in brain slices. This reduced preparation isolates the cell undergoing

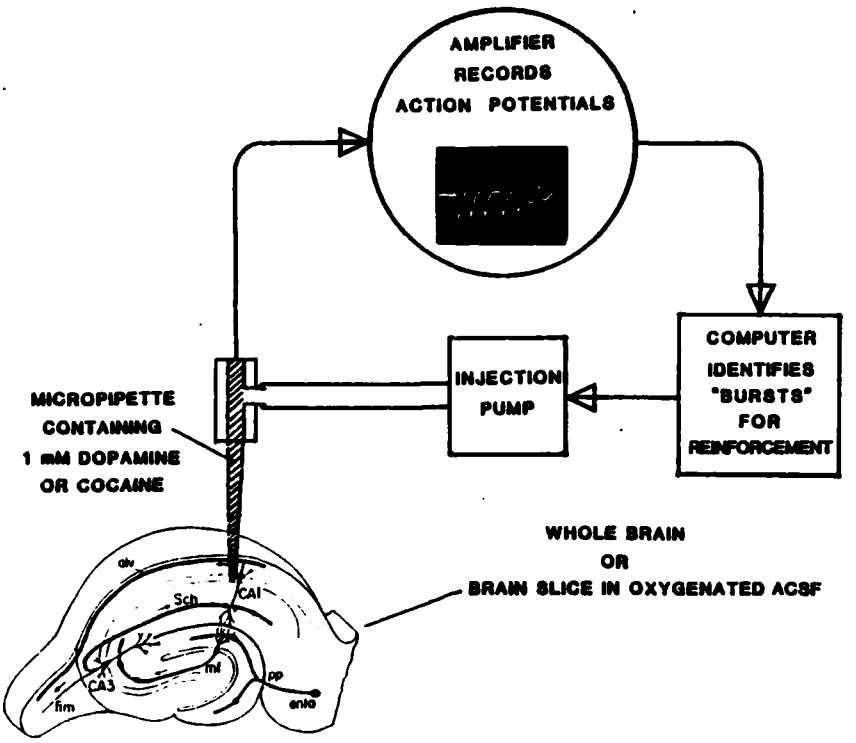

Figure 6

Protocol for operant conditioning of individual neurons.

conditioning from many of its normal connections. Use of brain slices furthermore eliminates the artifacts associated with anesthesia and the possibility that, even under anesthesia, animals may be influenced by environmental stimulation. Still another major advantage of the brain slice technique is the ability to return to the same cell type from experiment to experiment since microelectrode placement is under direct visual control. At present we have focused our efforts on hippocampal slices, aiming our micropipettes at the large pyramidal cells in the CA1 field of dorsal hippocampus (see Fig. 6 for a diagram of the hippocampal slice and experimental protocol).

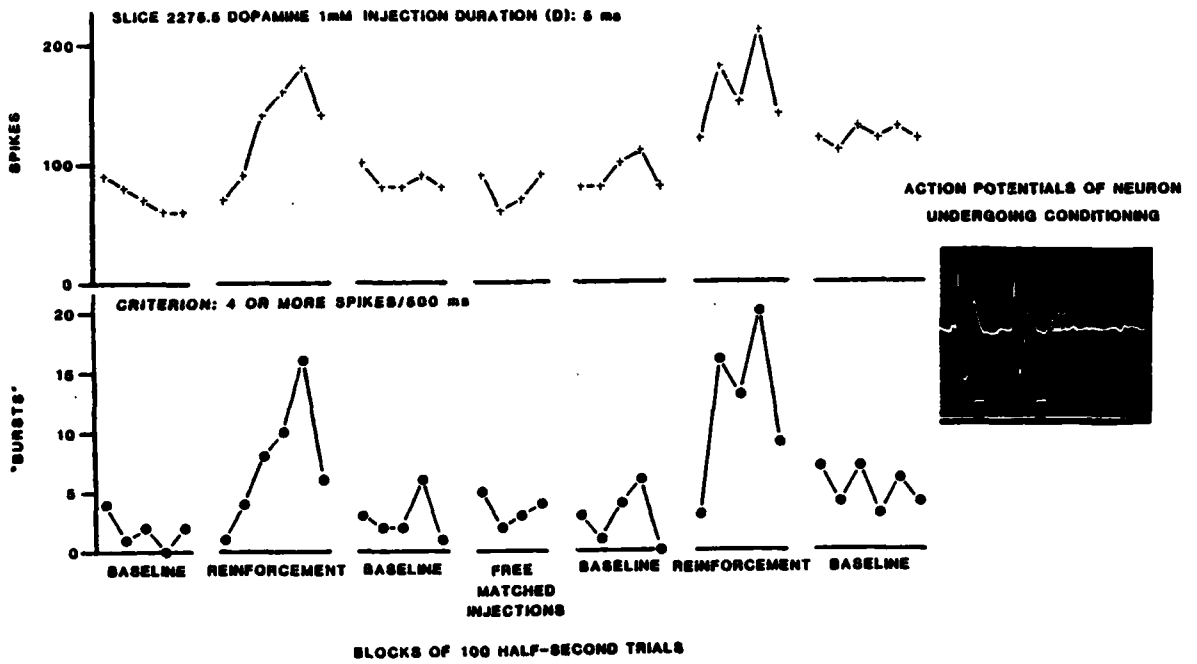

Figure 7

Operant conditioning of a pyramidal neuron in a dorsal hippocampal slice using local injections of dopamine as reinforcement. For details, see text and Fig. 2. Free = free injections phase, reinf. = reinforcement phase.

A positive experiment using dopamine as the reinforcing solution is shown for a hippocampal unit in Figure 7. The frequency of "bursts" and overall firing rates were rapidly increased after approximately 10 reinforcements in two separate phases of operant conditioning. The same dopamine injections administered noncontingently failed to increase either "burst" frequency or overall firing rate. It may also be noted that extinction occurred rapidly following both instances of operant conditioning. A second positive experiment using dopamine is shown in Figure 8. An initial period of free injections delivered at a rate of approximately 5 per minute had no effect on the frequency of "bursts" or on overall firing rate. During a first phase of operant conditioning, "bursts" and firing rates were sharply

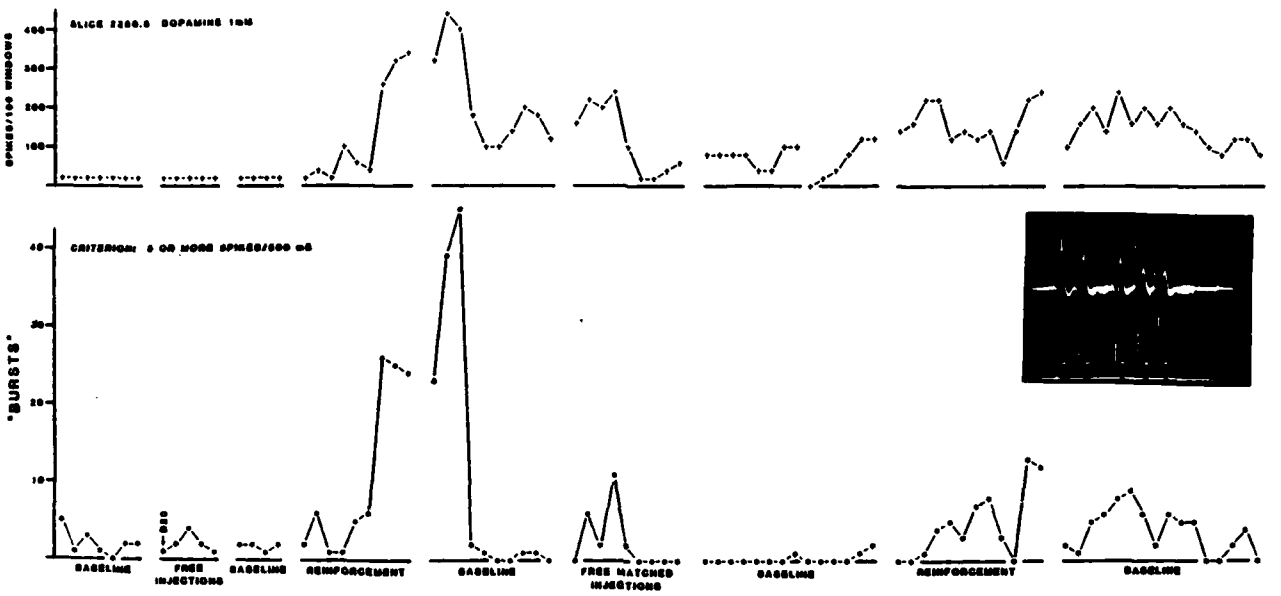

Figure 8

Operant conditioning of a pyramidal neuron in a dorsal hippocampal slice using local injections of dopamine as reinforcement. For details, see text and Fig. 2. D20 = injection duration of 20 ms.

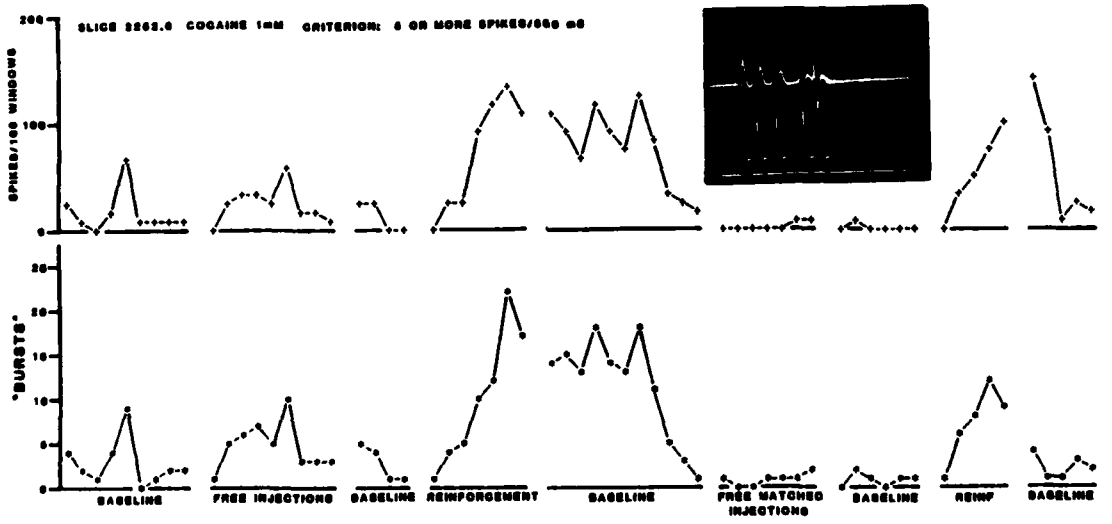

Figure 9

Operant conditioning of a pyramidal neuron in a dorsal hippocampal slice using local injections of cocaine as reinforcement. For details, see text and Fig. 2. Free = free injections phase, reinf. = reinforcement phase.

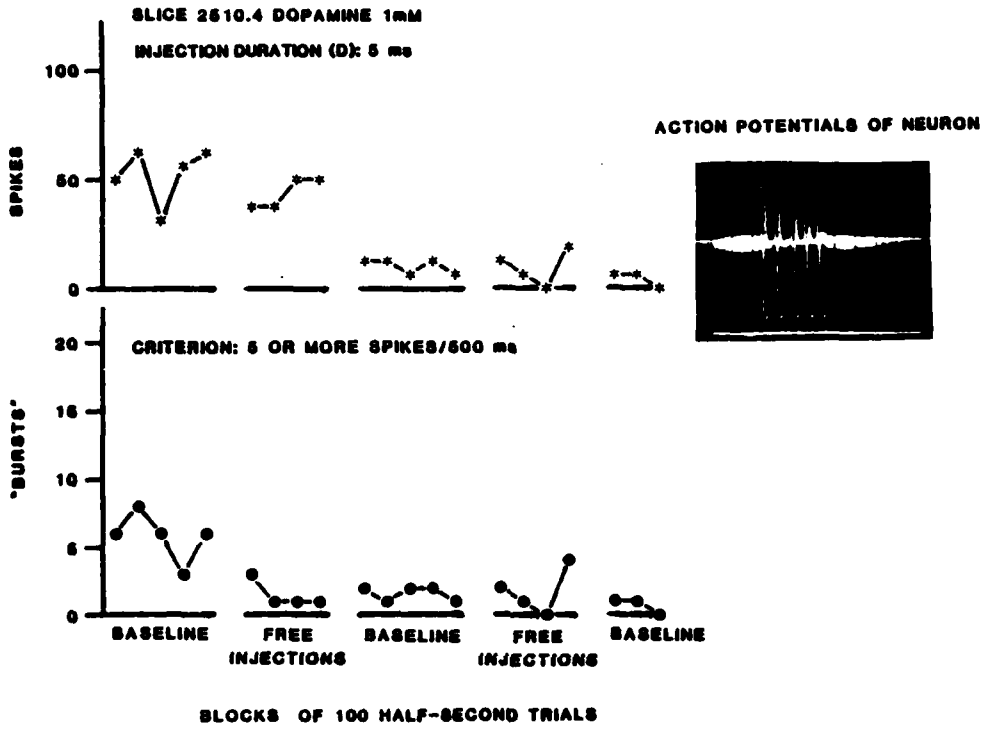

Figure 10

Control experiment with dopamine administered noncontingently to a pyramidal neuron in hippocampal slice. For details, see text and Fig. 2.

increased after approximately 20 applications of dopamine. Extinction following the first phase of reinforcement initially produced a further increase in "bursts" and spike frequency, but then a sharp decline. Free dopamine injections again were given in a second phase, at the rate of approximately one every 5 seconds to match the peak rate obtained in the preceding reinforcement phase. Initially, these densely packed free injections slightly increased the number of "bursts" and the overall firing rate, but then depressed them. In a second phase of operant

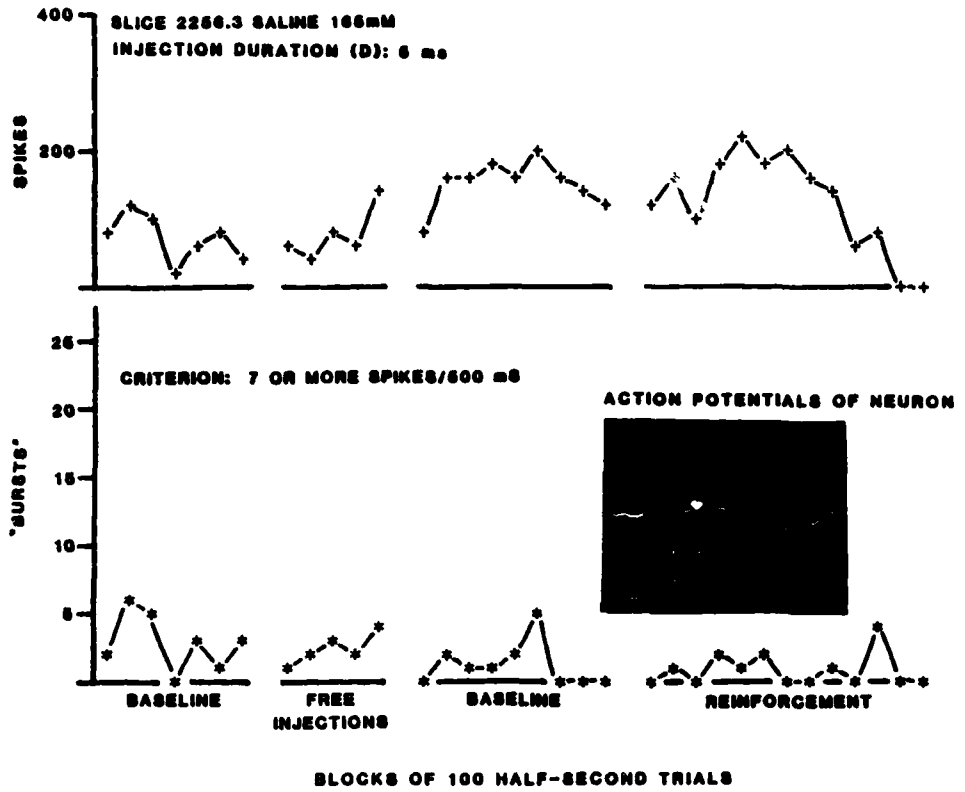

Figure 11

Saline control experiment. Failure to obtain evidence of operant conditioning of a pyramidal neuron in dorsal hippocampal slice using local injections of saline as reinforcement. For details, see text and Fig. 2.

conditioning, contingent injections of dopamine again increased the frequency of "bursts" and overall firing rate, but not to the level observed in the first phase of reinforcement. A similar pattern of results was obtained in an experiment with cocaine (Fig. 9). Injections of cocaine during 2 phases of operant conditioning sharply increased the frequency of criterion responses and the overall firing rate, whereas free injections had no facilitatory effect on either response measure and even depressed response rates below the baseline level. In control experiments, dopamine was administered noncontingently throughout the experiment (Fig. 10) or

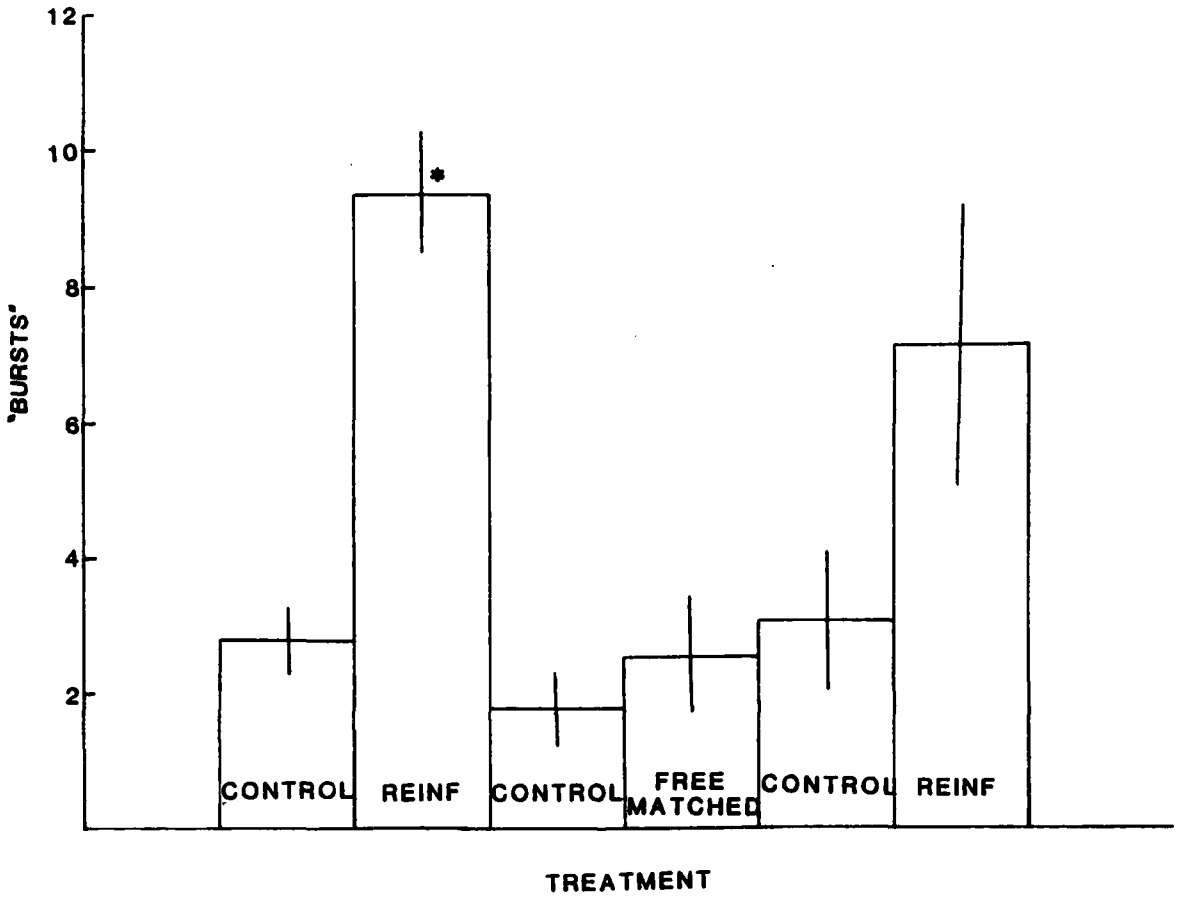

Figure 12

Summary of positive dopamine experiments.

saline was substituted for dopamine (Fig. 11). In neither case was "burst" frequency or overall firing rate increased. We have also observed that effective doses of reinforcing solutions in hippocampal slice experiments seem to be lower by an order of magnitude than those in whole brain experiments.

A summary of 8 positive dopamine experiments is shown in Figure 12. Plotted here are the means of the peak rates obtained in each phase of the

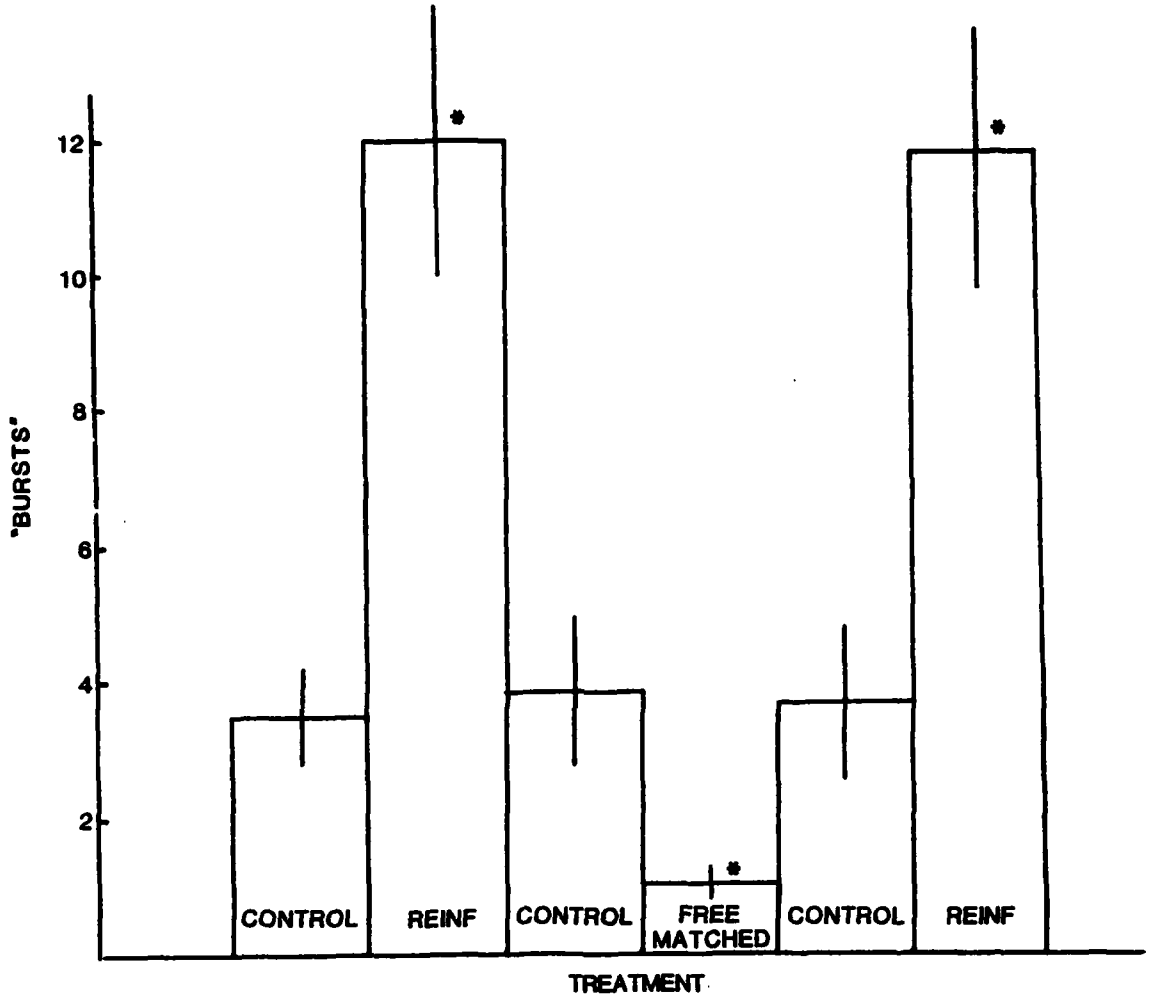

Figure 13

Summary of positive cocaine experiments.

experiment for each neuron. Highly significant increases were obtained in each of the reinforcement periods when compared either to control periods or periods when the dopamine was presented independently of neuronal bursting. A similar summary of the positive cocaine experiments is shown in Figure 13.

Table 2. Summary of hippocampal slice experiments.

Drug	Dose (mM)	No. of Exps.	RESULTS* +	?	-
Cocaine	1	48	11	12	25
Cocaine (Free)	1	13	0	0	13
Dopamine	1	17	9	2	6
Dopamine (Free)	1	12	0	1	11
Norepinephrine	1	4	1	1	2
Acetylcholine	1	6	1	1	4
Serotonin	1	3	0	0	3
GABA	1	4	0	0	4
Amphetamine	1	3	0	2	1
Imipramine	1	2	0	0	2
Ethanol	1	3	0	0	3
Saline	165	5	0	0	5

*Columns are defined as follows:

+ = conditioning-like changes (increased probability of bursts following reinforcement) plus noncontingent controls,

? = conditioning-like changes but no controls,

- = no evidence of conditioning.

Finally, all hippocampal slice experiments containing useful data are summarized in Table 2. Excluded from this Table are experiments in which suitable action potentials could not be obtained or held, or in which artifacts, such as drug overdoses or clogging of the micropipette, caused experiments to fail. In the columns labelled "RESULTS", the designations are as follows: + = conditioning-like changes (increased probability of bursts following reinforcement) plus noncontingent controls, ? = conditioning-like changes but no controls, and - = no evidence of conditioning. The table thus indicates that 9 of the 17 dopamine experiments were positive and contained noncontingent controls. According to Benardo & Prince (1982), only 75 percent of CA1 hippocampal cells are responsive to dopamine in the first place; hence, our success rate actually represents 9/(17 x .75) or approximately 70 percent of all completed experiments. We consider this rate of success about as high as can be expected at this stage, given the difficulties involved. In the cocaine experiments, the success rate was substantially lower than in the dopamine experiments. We do not know the explanation, but speculate that cocaine, which acts via dopamine, may require a physiologically active dopamine system to exert its full reinforcing effect. Since the dopamine axons are severed in the hippocampal slice preparation, it is conceivable that their responsivity to cocaine is reduced.

X. CONCLUSIONS

After a long phase of exploratory experimentation, we have found that individual neurons may be operantly conditioned by direct cellular applications of reinforcing transmitters or drugs. It seems unlikely that a brain cell would display a gratuitous capacity for operant conditioning; hence, the individual neuron may represent the functional unit for positive reinforcement in the brain.

Applications of the reinforcing substances had opposite effects on subsequent firing rates, depending on the activity pattern of the neuron at the time of administration. If the neuron had been firing rapidly just before the injection, the firing rate was increased; if the neuron had been firing slowly or was silent just before the injection, the firing rate was unaffected or decreased. In other words, the action of locally applied reinforcing transmitters or drugs on brain cells was activity-related in a way that formally resembles the action of conventional reinforcers on behavior. A food pellet delivered after a lever-press response increases lever pressing, whereas the same pellet delivered independently of the behavior has no effect or even may suppress lever pressing.

We have begun to work out the conditions that will demonstrate neuronal operant conditioning on a reliable basis. Thus, we find at present the most satisfactory preparation to be the brain slice, the best neurons for operant conditioning to be the large pyramidal cells in the CA1 field of dorsal hippocampus, and the most reliable reinforcing agents to be dopamine and cocaine. The

probability of CA1 firing increased sharply when reinforced by contingent applications of dopamine applied directly to the cell soma by pressure injection; the same dopamine injections applied independently of neuronal firing had no such effect and, in fact, tended to suppress activity, as previously reported by Benardo & Prince (1982). The reinforcing action of dopamine, which is a long-term facilitatory effect, must thus be demonstrated in the face of its short-term inhibitory action. This observation raises the interesting possibility that the direct synaptic action of reinforcement transmitters may generally be inhibitory rather than excitatory.

There is already an indication of dopaminergic specificity: included among substances that were ineffective are GABA, serotonin, acetylcholine, imipramine, ethanol, and saline, while cocaine, the only effective reinforcer other than dopamine, is thought to act by enhancing the actions of endogenous dopamine. On a preliminary basis, dopamine seemed to be a more effective agent than norepinephrine. Some evidence suggests that reinforcement schedules may have a different effect in single neurons than they do in the whole animal. Even a simple fixed-ratio 2 schedule caused decrements, rather than increments, in firing rates. Our neuronal preparation also seemed to differ from conventional operant conditioning with regard to reacquisition after a period of extinction. More rapid reacquisition or "savings", typically observed in behavioral experiments, is not observed in the neuronal studies. The effects of reinforcement schedules and reacquisition thus may reflect properties of neuronal systems rather than of individual cells.

A troublesome feature of these experiments was the fact that relatively high concentrations (1mM) of dopamine and cocaine were required for reinforcement. However, it should be clear that total drug dose is determined not only by the concentration of the solution injected but also by other injection parameters, such as duration and volume. Because drug injections in our experiment had to be delivered to individual cells in close contingency to bursts of activity, it was necessary to use exceedingly short injection durations (5-20ms) and small volumes (0.5-3 picoliters). After diffusion to action sites, these minute droplets of drug presumably are diluted to the same concentrations effective in other studies, where lower initial concentrations of drug are applied in greater volumes and for much longer durations.

In future experiments, we plan systematically to map a large number of cell types in different brain areas for their ability to undergo operant conditioning. We also plan to determine the chemical specificity of single unit reinforcement by investigating a wide variety of transmitters and drugs. In a later stage of investigation, suitable neurons in cell culture, which are not organized into physiologically meaningful networks, will be tested for their ability to undergo operant conditioning in an attempt to provide final proof that reinforcement takes place at the level of the single cell.

We have begun to consider the biochemical events that may be involved in cellular reinforcement. What is required is a mechanism that will satisfy the following conditions: 1) if a cell fires in some characteristic pattern of activity,

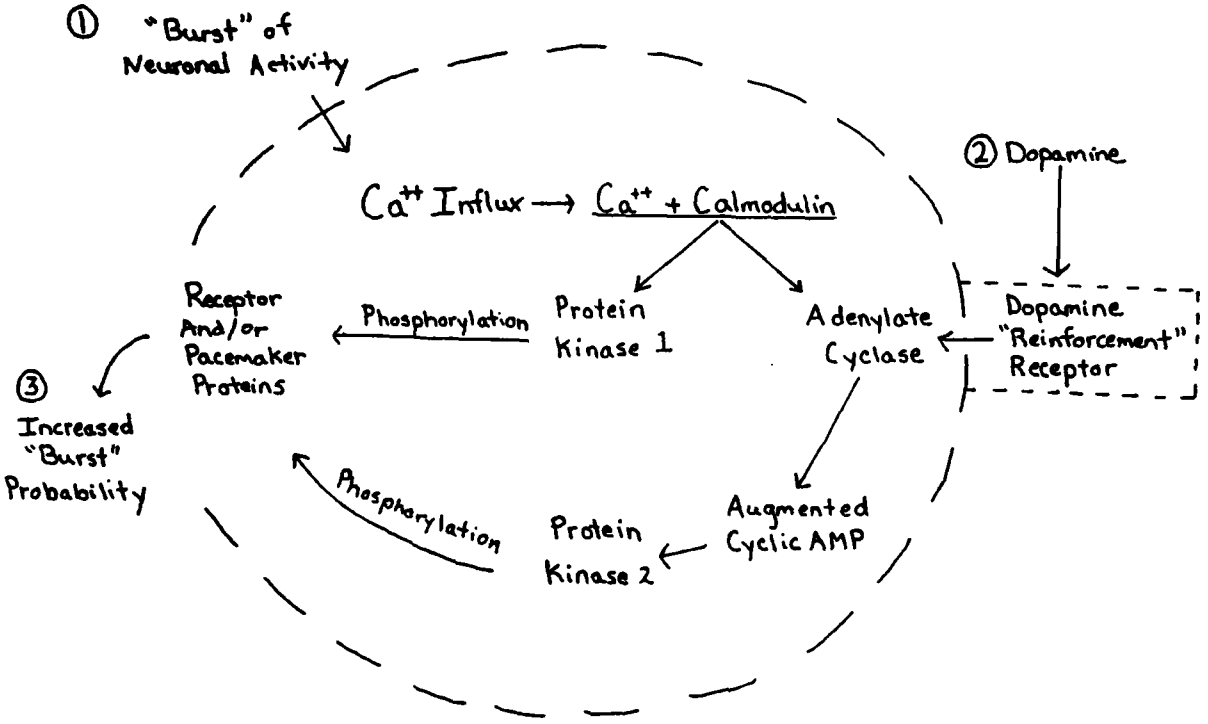

Figure 14

Proposed biochemical cascade in neuronal reinforcement.

such as a "burst", and 2) if the cell's (dopaminergic) reinforcement receptors are then activated, then and only then, 3) will the receptor or pacemaker proteins that control firing be modified (in all probability, by phosphorylation) so that the probability of a "burst" will be increased. Clearly, only recently active cells can be eligible for reinforcement. We do not know which aspect of the activity pattern renders a cell eligible for reinforcement. Four possible markers of recent activity are: depolarization-induced changes in membrane associated proteins, K^+ efflux, Na^+ influx, and Ca^{++} influx. As a working hypothesis, we propose that the influx of

Ca^{++} with each "burst" may signal eligibility for reinforcement (Fig. 14). Evidence (Krnjevic, 1983) consistent with this hypothesis includes the following: 1) hippocampal pyramidal cells exhibit a remarkable Ca^{++} influx, 10-fold greater than any other cell studied, 2) this high-rate Ca^{++} influx may be specifically associated with pyramidal cell bursting activity -- the "burst" must contain 3 or more spikes to be effective. It has been shown in CA3 cells that the first two spikes are tetrodotoxin sensitive, and presumably mainly mediated by sodium; the third and subsequent spikes are presumably mainly mediated by Ca^{++} (Wong & Prince), and 3) activation of the large Ca^{++} current by stimulation of CA1 inputs has an abrupt threshold, both for stimulation frequency and intensity; such abrupt thresholds are consistent with discrete states of reinforcement eligibility and noneligibility. We further assume that the Ca^{++} binds to calmodulin and that the Ca^{++}-calmodulin complex interacts with dopamine-sensitive adenylate cyclase in the cell body so that the cyclase subsequently produces more cAMP in response to dopamine (Greengard, 1978). The final step involving phosphorylation of receptor or pacemaker proteins may be jointly catalyzed by both cAMP-dependent and calcium-calmodulin-dependent protein kinases. Precisely such joint activation of a neuron-specific protein, Protein I, has been reported by Huttner, DeGennaro and Greengard (1981). A similar requirement for activation of receptor or pacemaker proteins would provide the necessary specificity for reinforcement-induced protein phosphorylation.

In summary, by positing a contingency requirement at two points in the biochemical cascade, the proposed mechanism provides two-fold insurance that

only recently active cells can be reinforced. First, because Ca^{++} is required for activation of adenylate cyclase, it insures that only recently active cells will generate high intracellular levels of cAMP in response to dopamine. And secondly, by requiring the joint action of calcium-calmodulin-dependent and cAMP-dependent protein kinases for reinforcement-induced modification of the receptor and pacemaker proteins that control firing, it again insures that Ca^{++} influx and dopamine receptor stimulation must be contingent.

We have formulated a working hypothesis of the reinforcement mechanism sufficiently detailed to permit experimental testing. Needless to say, this hypothesis will be modified by experimental results. Major experiments to be performed include: 1) substitution of cAMP for dopamine in neuronal operant conditioning studies to determine if, in fact, dopamine's action is mediated via cAMP, 2) prevention of neuronal operant conditioning by use of calcium-free media, or by pretreatment with calcium chelators or calcium channel antagonists such as nitrendipine to determine if, in fact, Ca^{++} influx is required for reinforcement, 3) blockade of neuronal operant conditioning by the highly specific calmodulin inhibitor R-24571 (Van Belle, 1981) to determine if, in fact, reinforcement is a calmodulin-dependent process, 4) substitution of a calcium channel activator (BAY K8644) for spontaneous firing in neuronal operant conditioning experiments. If silent cells could be treated with BAY K8644 to mimic a burst-induced influx of Ca^{++}, and if these cells could then be reinforced with dopamine (that is, if they later exhibited enhanced bursting), this would be a powerful proof that the influx of Ca^{++} is the biochemical marker for reinforcement

eligibility. Finally, in vitro phosphorylation studies of hippocampal tissue may permit us to identify those proteins which are specifically phosphorylated by combined administration of calcium-calmodulin and cAMP. Such experiments could help us to identify the reinforcement-sensitive receptor and pacemaker proteins that control cellular firing rates. Furthermore, if these proteins could be purified, antibodies could be raised to reveal their anatomical localization in the cell.

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XIII. APPENDIX I: Ancillary Experiments

Delay of Reinforcement

The effect of delayed reinforcement on the acquisition of lateral hypothalamic self-stimulation was investigated. Brain stimulation reinforcement minimizes cues associated with reinforcement delivery (secondary reinforcement) and, by eliminating consummatory responses, permits precise temporal control of the interval between the operant response and reinforcement. Different groups were trained in daily 1-hr sessions for brain stimulation reinforcement at one of four delay intervals (1, 2, 3, or 6 sec). Responses made during the delay interval were not reinforced and reset the delay timer. Control groups were reinforced immediately, but were required to space responses—according to a DRL schedule—for an interval corresponding to one of the delay of reinforcement intervals. The DRL schedule equalized opportunities for reinforcement and non-reinforcement. At all intervals, rats trained with delayed reinforcement had significantly lower bar-press rates than controls trained under DRL. When reinforcement schedules were switched (DELAY groups now get DRL and vice versa), response rates rapidly shifted to levels appropriate to the new schedule. The pre-switch results indicate that delays even as short as 1 second markedly impede the acquisition of self-stimulation behavior. The post-switch results suggest that delay of reinforcement, like stimulation intensity, may determine the strength of hypthalamic reinforcement and hence final levels of performance.

Cell Culture Experiments

A number of experiments were carried out to develop a neuronal cell culture model in which operant conditioning could be tested. The requirements to be met by this system were: (i) cells should exhibit spontaneous electrical activity, (ii) they should possess opiate and catecholamine receptors, (iii) cells should be large enough to permit lengthy recording times with an intracellular electrode.

Our first approach was to use the neuroblastoma x glioma hybrid cell line NG108-15, a tumor derived cell line, the properties of which satisfy conditions (ii) and (iii). Our goal was to manipulate the chemical environment of the NG108-15 cells in order to induce spontaneous electrical activity, since cyclic AMP (cAMP) is thought to be involved in the process of neuronal cell maturation and the expression of electrical activity. Firstly, cells were grown in a medium containing dibutyryl cAMP - an analogue of cAMP. Secondly, cells were cultured in the presence of prostaglandin E and Theophylline, which increase endogenous cAMP levels. NG108-15 cells were then tested electrophysiologically for spontaneous activity; none was detected, however.

Another, alternative approach was attempted. Since single cell operant conditioning was detected in the cerebellum of adult rats, we embarked on a series of experiments to develop a cell culture preparation of hippocampal and cerebellar cells. Hippocampus and cerebellum of neonatal animals were dissected out into a cell suspension and plated in standard media for primary neuronal cultures. The

use of neonatal material was essential since neurons from adult animals do not survive the dissociation procedure. Our aim was to find conditions which would enable neurons to survive in cell culture for prolonged periods of time in order to differentiate and acquire electrical activity characteristic of neurons in adult animals. Difficulties were encountered, such as glial cell proliferation, which tended to decrease neuronal survival. This was overcome by use of mitotic inhibitors. Neuronal cultures from hippocampi and cerebella grown in culture for up to 4 weeks were finally obtained and these were tested electrophysiologically. These cells, however, proved to be too small and fragile to survive microelectrode impalement and attempts to obtain recordings from them proved unsuccessful.

We intend to continue our search for a viable cell culture model by using approaches such as cell hybridization. This will involve fusing two different cell types each of which has some, but not all, of the desirable properties outlined above. A hybrid will be created which will hopefully retain all the properties of its parent cell lines.

Studies on Calcium Channels.

A crucial role for calcium as the signal for reinforcement eligibility has already been discussed. Calcium channels in the periphery can be inactivated by the calcium channel blockers D600 (methoxyverapamil) and nitrendipine (DHP). However, D600 is not suitable for neuronal operant conditioning studies because

the drug also affects radioligand binding of brain muscarinic, α_1-adrenergic and opiate receptors. It was thus of interest to determine whether DHP drugs have more selective calcium-channel blocking actions without actions on brain receptors.

The DHP drugs used in this study were nicardipine, nitrendipine, nimodipine, felodipine, nifedipine and nisoldipine. They were examined for activity in inhibiting the specific binding of ^{3}H-QNB and ^{3}H-WB401 to the muscarinic and α_1-adrenergic receptors of rat-brain. It was found that nicardipine was the most active of these DHP drugs in inhibiting receptor binding function and that this inhibition was competitive and stereoselective, with the (+) isomer of nicardipine being 28 times as active as the (-) isomer on the brain muscarinic receptor and 3 times as active on the α_1-receptor. This stereoselectivity suggests direct interaction with the receptors. An allosteric effect occurring indirectly at the receptors via a primary reaction with the DHP high-affinity binding site presumably associated with a brain Ca^{++} channel was ruled out experimentally. These results suggest (a) that nicardipine should not be used in brain reinforcement studies where effects due solely on Ca^{++} channels are being sought, and (b) other DHP drugs such as nifedifine or nisoldipine would be better choices in such experiments, since they have appreciably less effect on brain receptors (Biochemical Pharmacology, submitted).

XIV. APPENDIX II: NEURONAL CONDITIONING CONTROL PROGRAM (NCCP)

TABLE OF CONTENTS

1.0 INTRODUCTION

1.1 SYSTEM OVERVIEW

The NCCP System is designed to provide the capability to perform real-time data acquisition, analysis, control, and reporting of Neuronal Response Tests and non real-time data reduction, program development, and computation. The system is based on Data General's S/120 Eclipse Computer, Data General's RDOS operating system, and XYCOM's Neuronal Conditioning Control software. Digital inputs and outputs are made through a Data General Digital I/O Card (4040). Digital inputs can be pulses or switches, and the outputs can be used for controlling or monitoring the experiment.

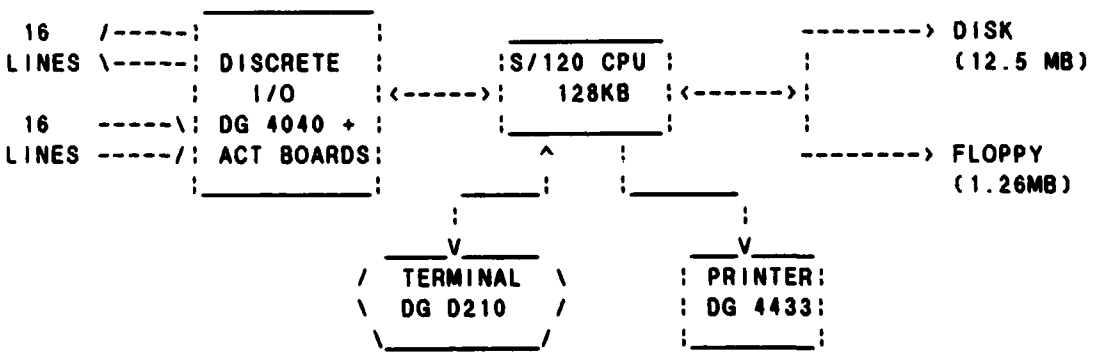
1.2 SCOPE OF DOCUMENT

This manual is limited in scope to describing and explaining the set-up and operation of the NCCP software. Detailed descriptions of the computer hardware and peripherals as well as operating RDOS are contained in the manuals provided by Data General.

2.0 HARDWARE DESCRIPTION

2.1 HARDWARE CONFIGURATION

The NCCP system is based on Data General Hardware, Data General operating system software, and XYCOM application software. Figure 1 is a block diagram of the hardware and is comprised of the following components:



The experiment interfaces to the NCCP through the digital I/O lines which require standard TTL logic levels, and connect to the ACT boards to provide -24VDC inputs and outputs to the environment. Input/Output lines can be assigned anywhere provided the software definitions are changed accordingly. Current assignments are shown below.

2.2 LINE ASSIGNMENTS:

ACT NO.	OCTAL VALUE	BIT	INPUT LINE FUNCTION	OUTPUT LINE FUNCTION
200	100000	00	--	Spike Out (1msec echo)
100	040000	01	--	Reinforcement Duration Indicator
40	020000	02	--	Reinforcement Pump On
20	010000	03	--	Print Marker
10	004000	04	--	Spike in Window
4	002000	05	Manual Switch	Window Over
2	001000	06	"B" spikes	Burst Found
1	000400	07	"A" spikes	-Spare-

3.0 SYSTEM OPERATION

3.1 Startup

1. Turn Cabinet Power ON
2. Turn Computer Power ON
3. Turn Disk Power ON
4. Turn Printer Power ON
   Adjust Printer Top of Form
   Press Printer "On-Line"
5. Turn Terminal Power ON
   It should respond:
   "D210 Self Test OK"
   Press "Alpha Lock" key
6. After the disk unit ready light comes "ON"
   Press Computer "Reset"
   Press Computer "PRLOAD"
7. The Terminal should now be displaying
   FILENAME
   Type in a "NEW LINE"
   (From now on, "NEW LINE" will be shown as (NL))
8. The system will request date & time; respond accordingly.
   Terminate each complete entry with a (NL)
9. The system will now display an "R" for "READY"
   Type: UP(NL)
   The "UP" command clears discrete outputs, clears files, initializes the system and the UCI directories, and puts the system in the UCI directory. At this point, any RDOS command or program can be executed or the Neuronal Conditioning Control Program can be executed as described in Secton 3.2.

3.2 Test Operation

The program is initiated by typing
NCCP(NL)
This causes the main program to be read into memory, common area to be cleared, and default values to be initialized. The setup overlay (PRTST1) is then loaded and executed. Setup displays the menu shown in Figure 2. A description of each selection is on the next page.

FIGURE 2: MAIN MENU

```
                    NCCP PRERUN MENU

                EDIT SETUP       - 1
                PRINT SETUP      - 2
                RUN TEST         - 3
                HELP (DRUGS)     - 4
                HELP (COMMANDS)  - 5
                EXIT             - 6

                SELECTION - _____

            (ERROR IN SELECTION - SELECT 1 - 6)
```

3.3 MAIN MENU DESCRIPTION

1 - EDIT SETUP displays the current value of all parameters that can be modified by the operator. Figure 3 shows the screen after this selection has been made. The cursor is then positiond at the first entry and the operator can effect changes to the setup by keying in the new value followed by a New Line. The cursor will then advance to the next parameter.

If no change is required, just key in a (NL) without a preceding value. Keying in an (ESC) will cause the EDIT program to return to the menu. All changes entered prior to the (ESC) will be in effect.

2 - PRINT SETUP prints the setup information on the printer in the format shown in Figure 3.

3 - RUN TEST causes the SETUP program to be exited and actual testing to begin using the parameters defined by SETUP. Each time you begin testing, the computer will request the name of the file you wish to use to store your test data. Up to 20 characters are allowed. Once a file has been used, it cannot be reused until you delete it wih the RDOS "DELETE" command. STOPPING the test is accomplished through an on-line CLI command.

4 - HELP (DRUGS) displays all of the drugs defined to the system and their corresponding symbols to be used during setup. To continue after viewing the display, strike any key (see Figure 4).

5 - HELP (COMMANDS) displays all of the on-line commands available to the operator and a brief description of each command. To continue after viewing the display, strike any key (see Figure 5).

6 - EXIT causes the NCCP program to stop and control is returned to RDOS.

Each command, except 3 & 6, returns to the menu.

FIGURE 3: EDIT SETUP

```
                    PRERUN SETUP
                    ------------
RAT #                                   UNIT #
DRUG ID
DOSE                          PSI
WINDOW DESCRIPTION
 MODE (M/F): M    SIZE: 500  WINDOWS/PRINT: 100  INTERSPIKE INTERVL: 10
HISTOGRAM DESCRIPTION
 BIN SIZE: 300      # OF BINS: 10      DURATION (D3) 3000
REINFORCEMENT DESCRIPTION
 MODE (R,B,F,) B      WINDOW/FREE REINF. 40    CRITERIA: 3
 RATIO: 1              INJ DURATION: 50
                              DELAYS
 POST WINDOW (D1) 0          POST REINFORCEMENT (D2) 0
            (CR) -> NO CHANGE, (ESC) -> RETURN TO MENU
```

FIGURE 4: DRUG CODES

CODE	DRUG	CODE	DRUG	CODE	DRUG
01	Acetylchol	08	Enkephalin	15	Norepineph
02	Amphetamine	09	Endorphin	16	Saline
03	Apomorphine	10	GABA	17	Serotonin
04	Carbachol	11	Glutamate	18	Tofranil
05	Chlorpromaz	12	Imipramine	19	Other
06	Cocaine	13	Methamphet	20	EOD
07	Dopamine	14	Morphine		

FIGURE 5: COMMAND TABLE

CODE	FUNCTION	RANGE	DEFAULT
BM	Burst Submode	(F or M)	F
CR	Set Criterion (# spikes for rein)	(1 - 100)	3
D1	Set Post Window Delay	(0 - 500)	0
D2	Set Post Reinforcement Delay	(0 - 500)	0
D3	Histogram Duration	(1 - 10000)	3000
FW	Set Fixed Window Mode (msec)	(10 - 2000)	500
HB	Set Number of Histogram Bins	(1 - 50)	10
HE	Display Command HELPs	--	--
HS	Set Histogram Bin Size (msec)	(1 - 200)	100
II	Interspike Interval (msec)	(5 - 100)	10
MD	Set MODE (Treatment)	(B, R, F)	B
MW	Start Moving Window "BURST" Mode	(10 - 2000)	500
RC	Graphics Dump to Printer	--	--
RD	Set Reinforcement Duration (msec)	(5 - 1000)	50
RP	Replot Graph on the Terminal	--	--
RT	Set Ratio (Bursts/reinf)	(1 - 99)	1
ST	Stop Test	--	--
WC	Set Windows/Free Reinforcement	(1 - 100)	40
WP	Set Windows/Print	(10 - 200)	100
WS	Set Window Size (msec)	--	--
XX	Exit CLI Active Mode	(0 - 500)	

3.4 COMMAND LINE INTERPRETER (CLI)

Following initiation of a test, the screen is used for two functions: 1) to display real time events as described later, and 2) to display operator commands. In order to prevent the two functions from interfering with each other, two separate tasks have been created and a control algorithm has been implemented that allows only one or the other to be in control of the screen.

COMMAND ENTRY:
The CLI task is initiated by the main program which in turn issues a request for an input from the keyboard. To make a request for a command entry the operator must key
<CNTL W> or the up arrow.

Any other entry is ignored (except CLI commands, e.g., CNTL A, etc.)

After finding a CNTL W, CLI will check the "RT Display Request" flag which will be false from the time the real time display completes its update until 10 seconds before the next update. If it is false, CLI will display "=>" on line 22, sound the terminal bell (beep) and set the "terminal in use" flag. At this time, the operator can enter commands. Every 15 seconds CLI will check to see if a command has been input and if not, it will release the display and the operator must key in another <CNTL W>. If the CLI input task was terminated because of the timeout feature, then the CNTL W must be preceded by a character (any alpha/numeric) before the CNTL W will be recognized by CLI.

3.4.1 IMMEDIATE COMMANDS:

Normally, changes in SETUP parameters do not take effect until the next print marker occurs; however, provision has been made to allow instantaneous action. For numeric values, simply key in a negative value and for non-numeric values, follow the letter with an I.

Figure 5 lists all of the on-line commands and a one sentence description and the following paragraphs expand the description when applicable.

BM - The BURST MODE of detecting cell activity has two submodes, ALL (A) and MINIMUM (M). Every test starts in the "ALL" mode and the mode can be changed by the BM command. MINIMUM means that a Burst will be detected when the number of consecutive pulses that are separated by less than the "interspike interval" has reached the criteria level. ALL means that a Burst will be detected when the number of consecutive pulses that are separated by less than the "interspike interval" has reached the criteria level and that "interspike interval" milliseconds has elapsed since the last pulse has occurred.

CR - SET CRITERION
CR specifies the number of pulses (spikes) required within a window before the system will count a burst and apply reinforcement. Other parameters, such as mode or ratio, could prevent reinforcment from occurring.

D1 - Set POST-WINDOW DELAY
This Delay is applied after a window has been detected and before reinforcement is started.

D2 - Set POST-REINFORCEMENT DELAY
This Delay is applied after reinforcement is turned off and before starting Histogram data collection.

D3 - HISTOGRAM DURATION DELAY
This Delay is applied after beginning Histogram collection and is used by the system to determine when to start searching for another window. D3 must be greater than or equal to Histogram bin size (HS) times the number of Histogram bins (HB).

FW - FIXED WINDOW
Starts "FIXED" window and allows window duration (in msec) to be changed. A "Burst" equals criterion number (CR) of spikes falling in the window, irrespective of interspike interval (II).

HB - Set the NUMBER OF HISTOGRAM BINS
A "Bin" is a period of time equal to the value defined below under HS. Bins occur during D3.

HE - Display the "HELP Commands" display.

HS - Set the duration (in msec) of each Histogram bin.

II - Set the Interspike Interval (in msec).

MD - Change MODE to BASELINE (B), REINFORCE ON (R), OR FREE INJECTIONS ON (F).

MW - MOVING WINDOW
Sets the window MODE to "BURST" and allows the window duration (in msec) to be changed for print determination. There will be a printout every N windows, where N is set using the WP command.

RC - PRINT GRAPHIC DISPLAY on the printer. If this is done while windows are still being located and printed, this printout may by interspersed with the window data.

RD - Set the REINFORCEMENT DURATION (in msec).

RP - REDISPLAY GRAPH PLOT on the CRT terminal from the beginning.

RT - Set the REINFORCEMENT RATIO, i.e., # Bursts/reinf.

ST - STOP TEST; requests post-test comments, and then returns to the menu.

WC - Set WINDOWS/FREE REINFORCEMENT, i.e., the number of windows between consecutive free injections.

WP - Set WINDOWS/PRINT,i.e., the time between printouts.

WS - Set WINDOW DURATION (in msec).

XX - EXIT COMMAND entry mode and allow the graphics display to return.

4.0 OPERATIONAL SOFTWARE

This section is designed to present the users with an overview of the NCCP software in order to assist the user in making simple changes. A complete list of all the NCCP programs is shown in Appendix I, and each module's source file (.FR) contains header information that lists or describes the functions of the module. In addition, most modules contain comments to describe the FORTRAN commands to facilitate understanding of the program.

The NCCP software runs under RDOS and is, therefore, subject to all of the restrictions and limitations of RDOS. Figure 6.1 is a block diagram representative of the software structure. NCCP operates in two basic modes, SETUP and TBST (see Flowchart, Figure 6.2). In the SETUP mode, pulse detection is not functional and the operator can define the test parameters. After SETUP, NCCP goes into the TBST mode, and three major functions are then activated:

RTCON - Test Control
RTCLI - Real Time Command Line Interpreter
REAL - Real Time Processing

RTCON performs initialization of parameters and starts the real time process. It then waits for a response from the real time process and performs the post-event processing. Events are windows, bursts, and histogram completions (see Flowchart, Figure 6.2).

REAL is an assembly language program that is connected to the RDOS clock interrupt routine; therefore, it runs at the interrupt level and at the 1 KHZ rate. REAL handles all input and output discrete processing, detection of BURSTS (if in BURST mode), timing of durations, counting pulses, and communicating to RTCON the status of real-time activity.

RTCLI allows the operator to interact with the test as the test progresses. Most parameters and modes can be changed through individual commands as described previously.

As the test runs, data is saved in a data file whose name is:
RAT NO. $ UNIT .DT (e.g., 123481.DT)

The structure of the data file and its records is shown in Appendix C.

4.1 SOFTWARE BLOCK DIAGRAM

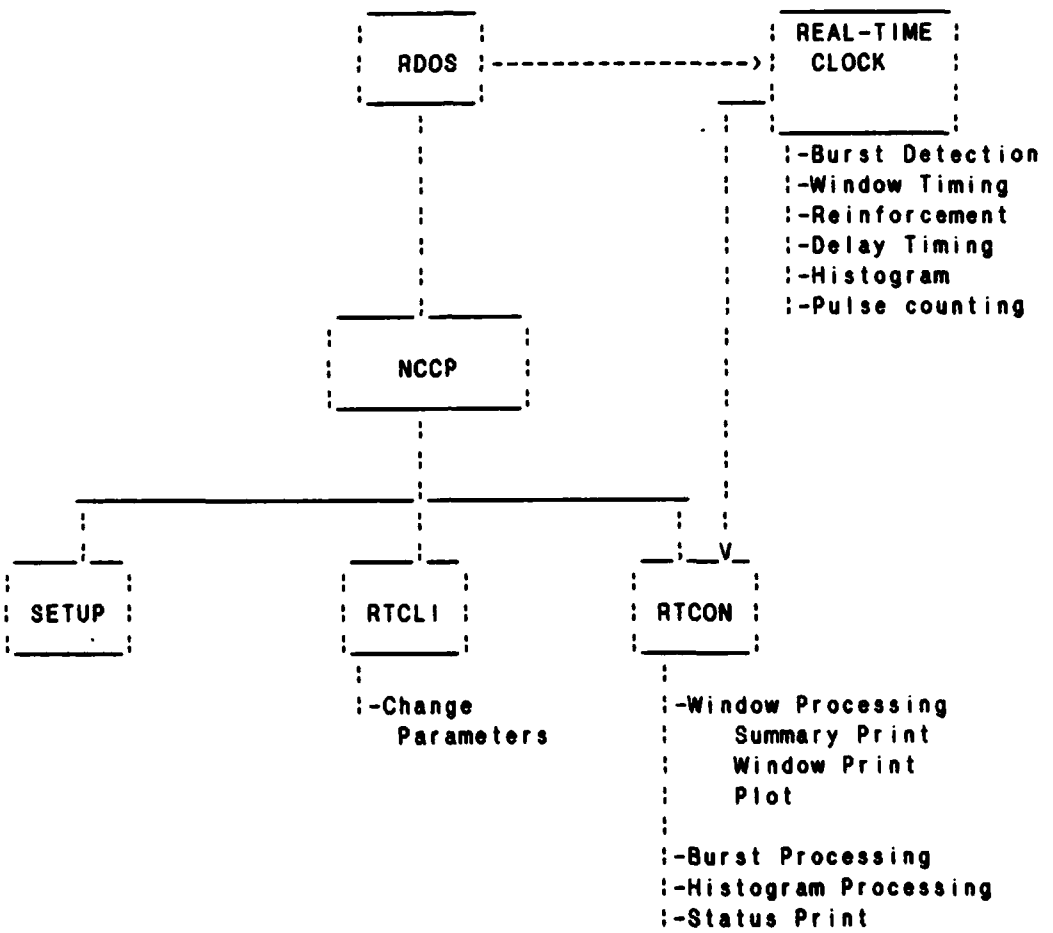


FIGURE 6.1
SOFTWARE BLOCK DIAGRAM

4.2 MAIN PROGRAM (NCCP) FLOWCHART

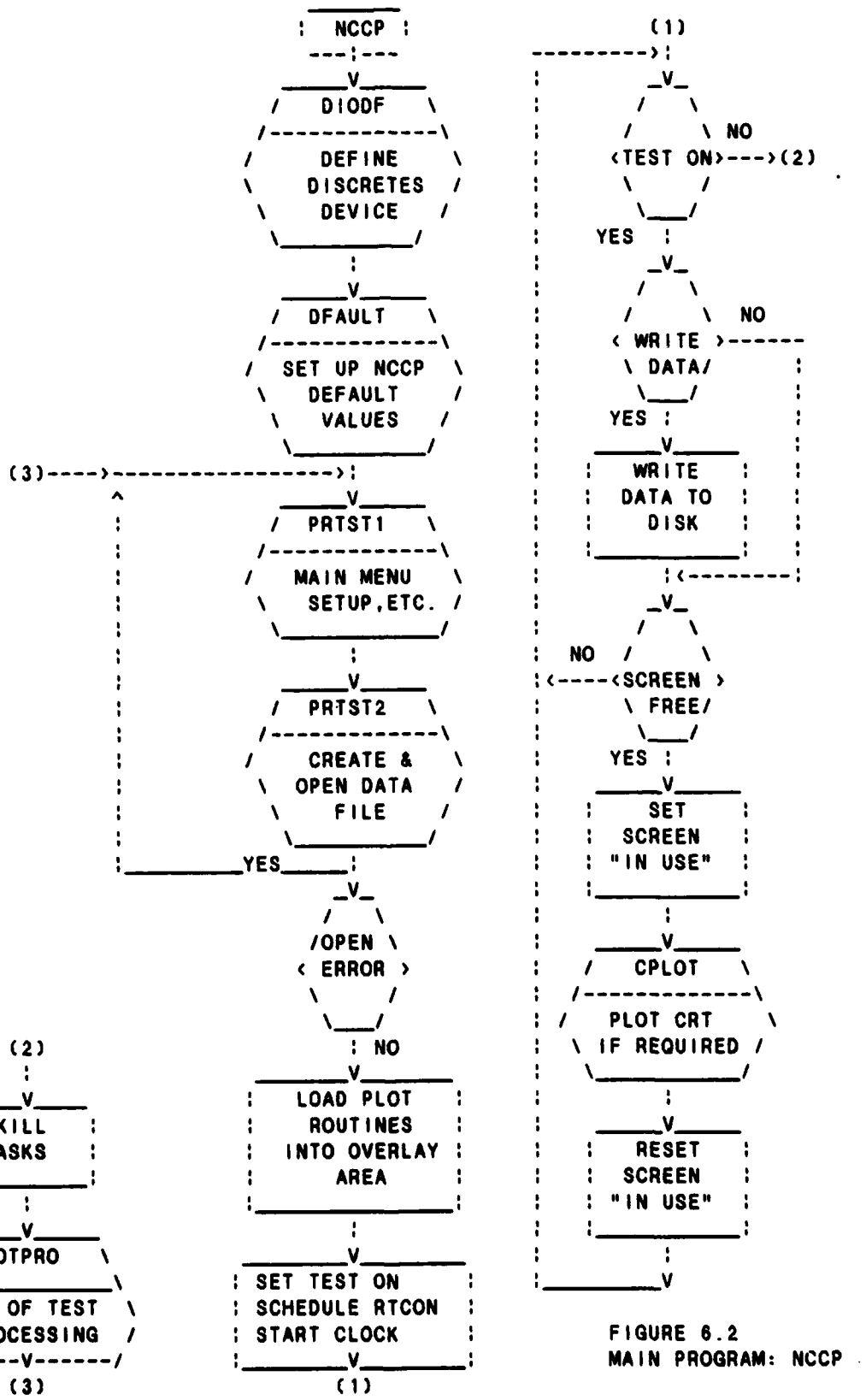


FIGURE 6.2
MAIN PROGRAM: NCCP

4.3 CONTROL PROGRAM (RTCON) FLOWCHART

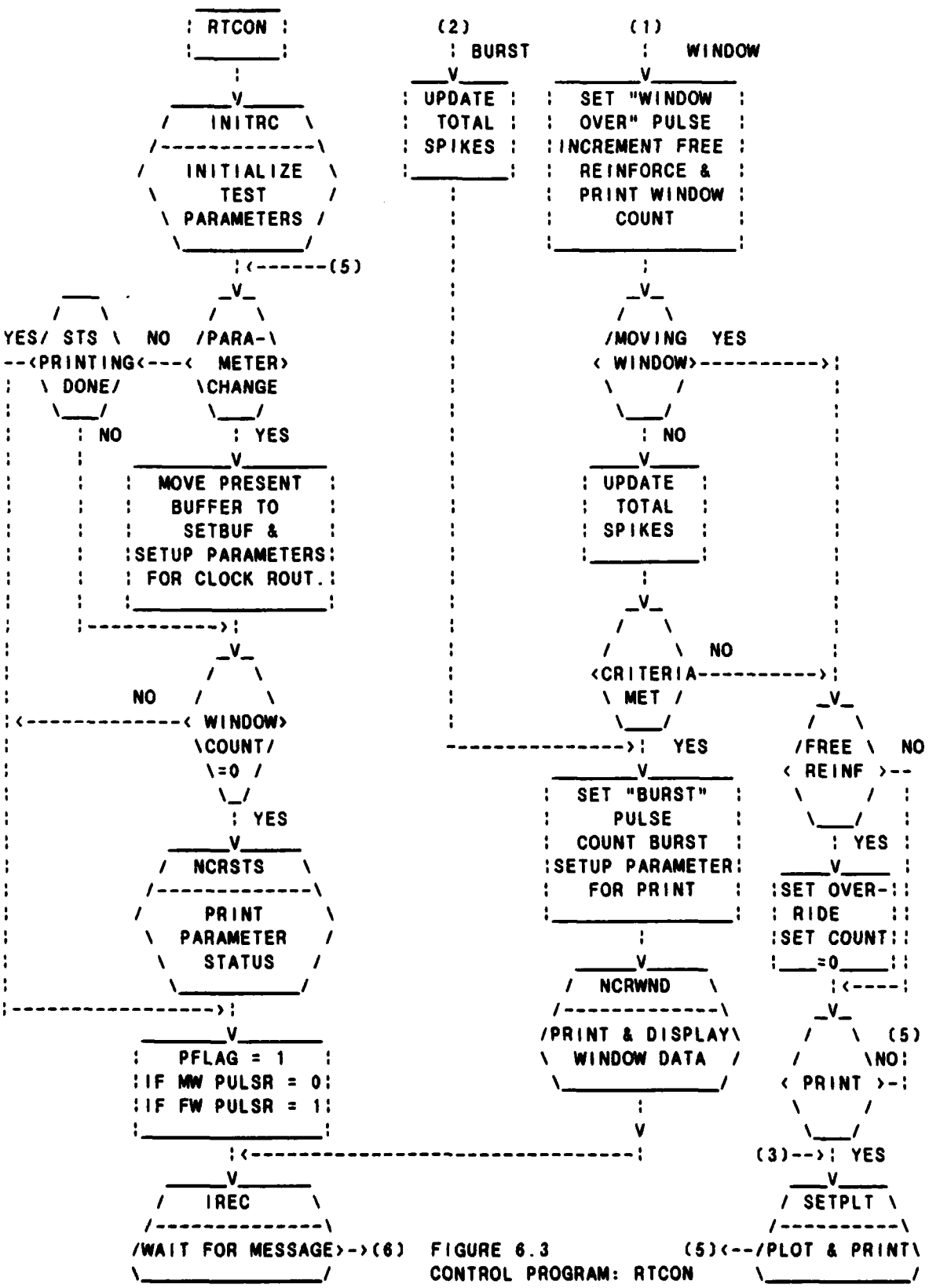


FIGURE 6.3
CONTROL PROGRAM: RTCON

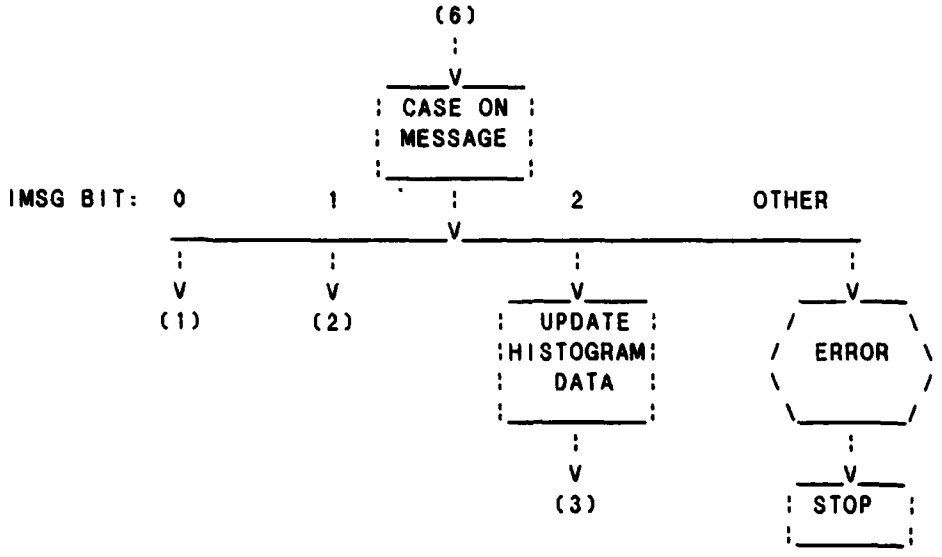


FIGURE 6.3 (CONTINUED)

APPENDIX A

NCCP MODULES

CHEADR.FR	-	CRT Header
CLCOM.FR	-	Common used by Assembly Language Routines
CLIMOD.FR	-	CLI Value Modification Subroutine
CLRSCR.FR	-	Clear Screen
CONCAT.FR	-	Concatenate Strings
CPLOT.FR	-	CRT Plot - Main Routine & Control
CPLOTCOM.FR	-	Common Used by Plot Routine
CPLOTP.FR	-	CRT Plotting Routine
CURSOR.FR	-	Position Cursor
D7PLOT.FR	-	Display Window Data Subroutine
D7PRNT.FR	-	Print Window Data Subroutine
DFAULT.FR	-	SETUP Default Values
DIODF.SR	-	Define Discrete I/O Device
DIOINT.SR	-	Discrete Interrupt Routine
EOTPRO.FR	-	End of Test Processing - Write Out Data, Get Comments
FSETUP.FR	-	Generalized Setup Control Program
GVAL.FR	-	Input a Value from Keyboard, Check Limits
GVAL1.FR	-	Input a Value from Keyboard, Check Limits, Check Sign
HELPCO.FR	-	Command Helps
HELPDR.FR	-	Drug Helps
IDSPLY.FR	-	Display Certain Setup Parameters on Screen
INITRC.FR	-	Initialize Test Variables
KEYBRD.FR	-	Get Two Characters from the Keyboard
MOVMSG.FR	-	Move a Message from One Array to Another Array
MPLOT.FR	-	Mode Change Plot Handler
NCCP.FR	-	MAIN PROGRAM - Controls Program Flow, Calls Plot, Writes Data to Disk
NCCPCOM.FR	-	Common Main
NCCPPAR.FR	-	System Parameters
NCRHED.FR	-	Printer Header
NCRPG.FR	-	Printer Page Control
NCRSTS.FR	-	Printer Parameter Status Report
NCRSUM.FR	-	Printer Summary Report (Every Print Cycle)
NCRWND.FR	-	Printer Window Report
OUTVAL.FR	-	Output a Value @ Specified Position on CRT
POSTP.FR	-	Setup Post Processor
PPLOT.FR	-	Print Plot Routine
PRTST1.FR	-	Pre-test Overlay #1 - Main Menu
PRTST2.FR	-	Pre-test Overlay #2 - Open Disk File
RDFLD.FR	-	Set-up Read Field Subroutine
REAL.SR	-	Real Time Clock Control Program
RTCLI.FR	-	Real Time CLI Processor
RTCON.FR	-	Real Time Control Program (Windows, Histogram)
SELECT.FR	-	Get a Value for Menu Response
SETP.SR	-	Set-up Table
SETPLT.FR	-	Set-up Plot Table
STUPRT.FR	-	Prints Set-up Values
WRFLD.FR	-	Set-up Write Field Subroutine
ZTRANS.FR	-	Converts Numbers to ASCII with Leading Zero's

APPENDIX B

NCCP BUILD PROCEDURE

1. Bring up RDOS as described in Section 3.1

2. Edit .SR Files and .FR Files as required

3. a) Assemble .SR Files (MAC/L)
   b) Compile .FR Files (FORTRAN)
   c) PRINT Listing Files (.LS) or Source Files (.FR)
   d) Delete Listing Files

4. Type:

   ```
   NCCPLD (NL)
   ```

   If SETP.SR was the module changed then, after assembling SETP, type:

   ```
   SETPLD (NL)
   ```

5. When the above step is done (about 5 minutes), type:

   ```
   PRINT NCCP.MP (NL)
   ```

   Save the printout, this is your Load Map.

   A printout of the macro command NCCPLD can be obtained by typing:

   ```
   PRINT NCCPLD.MC (NL)
   ```

   It should be noted that if any of the system common or parameter definitions are modified, it might be necessary to recompile all modules that use common. This can be done using one of the following MACRO's:

   ```
   TOTALFOR - Compiles all programs
   NCCPFOR  - Compiles all programs that use NCCPCOM Common
   CLCOMFOR - Compiles all programs that use CLCOM Common
   ```

   There are other macro commands and the user can look at macro's ending with -LD.MC to determine their use.

APPENDIX C

DATA FILE STRUCTURE

III-1 DATA FILE STRUCTURE:

The data file contains several different types of data records:

* Initial Setup Data
* A Spike History
* B Spike History (when defined)
* PLOT Table
* Post Test Comments

INITIAL SETUP DATA:
The first record in the file is the initial setup record. This is a fixed length record of 50 words (200 bytes) and a description of the record is contained in NCCPPAR.FR.

SPIKE HISTORY DATA:
The spike history data is formated in 1001 word blocks with the first word of each block indicating the type of record:

1 = A Spikes/Buffer 0
2 = A Spikes/Buffer 1
3 = B Spikes/Buffer 0
4 = B Spikes/Buffer 1

Each entry in the table is a pair of words representing the time of the spike in milliseconds relative to the start of the test. The first word (bits 1-15) contain the least significant bits and the second word (bits 1-15) contain the most significant bits of the time. If the first word of a pair is equal to -1, then that is the last valid entry in that block.

PLOT TABLE DATA:
PLOTTBL, the buffer which contains the information from which the plots are generated, is written to the disk periodically throughout the running of the test and at the end of the test. A plot table record is identified by the first word of the record being greater than or equal to six, but not 999. This number times 2 plus 1 equals the number of words in the record. The format of the data is described under III-2 "Plot Table Structure" below.

POST TEST COMMENTS:
The post test comments are written in ASCII to the file as the last record in the file and their record type is 999. Eighty characters are written even though the user is restricted to 72 characters of input.

III-2 PLOT TABLE STRUCTURE:

All data that is plotted, including "ON", "OFF", etc., is stored in the Plot Table, "PLOTTBL". The 1st word of the table contains the number of entries in the table and this is immediately followed by the 1st entry. Each entry consists of two words defined as follows:

		-1	-2	-3
First Word:	# of Criteria	"ON" Plot	"OFF" Plot	Variable Change
Second Word:	# of Spikes	0;"ON" Only not 0; "ON"+ Windows/Free Reinforcement	No Meaning	Bits 0-5 = Criteria Bits 6-15 = Reinforcement Duration

The table is sized for 160 entries.

SPOKEN PAPERS BY L. STEIN

DATE	TITLE AND INSTITUTION	LOCATION
10/28/81	"ENDORPHINS AND MEMORY FORMATION" Opioids in Mental Illness Conference	New York,NY
12/10/81	"REINFORCEMENT TRANSMITTERS" UCI Biological Chemistry Seminar	Irvine,CA
2/1/82	"OPERANT CONDITIONING AND HIGHER MENTAL FUNCTIONS", UCI Philosophy of Brain Meeting	Irvine,CA
3/23/82	"CATECHOLAMINE REINFORCEMENT TRANSMITTERS AND DEPRESSION", Denghausen Depression Meeting	Martinique
5/24/82	"MECHANISMS OF POSITIVE REINFORCEMENT" UCI Psychobiology Seminar	Irvine,CA
6/29/82	"MECHANISMS OF DRUG ABUSE" ADAMA Science Press Seminar Series	Washington, D.C.
10/7/82	"BEHAVIORAL ACTIONS OF ENDORPHINS" DuPont Central Research Seminar	Wilmington, DE
11/9/82	"PHARMACOLOGY OF BENZODIAZEPINES" Anaheim Memorial Hospital Seminar	Anaheim,CA
3/25/83	"OPERANT CONDITIONING OF INDIVIDUAL NEURONS",Society of Experimental Psychologists	La Jolla,CA
4/6/83	"POSITIVE REINFORCEMENT OF NEURONS" Denghausen Depression Group, New York University	New York,NY
5/10/83	"REINFORCEMENT MECHANISMS" Center for Neurobiology of Learning & Memory Seminar	Irvine,CA
7/28/83	"POSITIVE REINFORCEMENT: CELLULAR OR SYSTEMS PRIORITY", Air Force Office of Scientific Research Review	Irvine,CA
8/30/83	"REWARD SYSTEMS" Psychiatry Department Seminar, UCI Medical Center	Orange,CA

9/14/83	"MECHANISMS OF POSITIVE REINFORCEMENT" Neurology Department Seminar, UCI Medical Center	Orange,CA
12/1/83	"NEUROCHEMISTRY OF REWARD" UCI Department of Microbiology Seminar	Irvine,CA
3/5/84	"POSITIVE REINFORCEMENT OF SINGLE UNITS", Center for Neurobiology of Learning & Memory Seminar	Irvine,CA
3/15/84	CELLULAR BASIS OF REWARD" Anhedonia & Affect Deficit States	Chicago,IL
3/23/84	"CELLULAR BASIS OF REWARD" Denghausen Conference	
5/17/84	"CELLULAR BASIS OF POSITIVE REINFORCEMENT", UCLA Psychology Depart. Seminar	Los Angeles,CA
6/9/84	"OPERANT CONDITIONING OF SINGLE NEURONS" 7th Harvard Symposium on Quantative Analysis & Behavior	Boston,MA

SPOKEN PAPERS BY J.D. BELLUZZI

11/8/83	"OPERANT CONDITIONING: CELLULAR OR SYSTEMS PROPERTY", Society for Neuroscience Paper	Boston,MA
10/12/83	"PHARMACOLOGY OF LEARNING" UCI Department of Neurology Seminar	Orange,CA

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