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Neurobiology of Drug Abuse: Learning and Memory

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Neurobiology of Drug Abuse: Learning and Memory

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Preface

The study of the neurobiology of learning and memory has not been fully exploited in terms of drug abuse research. Studies of learning and memory can add to our understanding of drug abuse by demonstrating: the consequences of drug abuse on performance and cognitive function; the role(s) of endogenous opioids on memory processes; how drug effects on perception, arousal, and motivation modify learning and memory; and the mechanisms involved in the development of tolerance and sensitization. There are a number of animal models available to researchers; the choice of a model depends on the aim of the experiment. Thus, simple, one-trial learning is appropriate for studying effects of drugs and endogenous opioids on the stages of memory formation, while complex tasks can be used to test cognitive function analogous to that seen in humans. By using appropriate animal models, studies can be directed at pinpointing the anatomical, physiological, and pharmacological substrates mediating effects on learning and memory.

This monograph is the result of a technical review that was held September 28-29, 1988 to assess the available model systems and the current research on the neurobiology of learning and memory as it relates to the study of drug abuse. This technical review and monograph complement Monograph 84, "Learning Factors in Substance Abuse," which presents the clinical perspective on this area. The reports in this monograph present studies by researchers in the field of drug abuse and neuroscientists in the area of learning and memory whose model systems are relevant to drug abuse research.

A convincing. case was presented by Dr. Rosenzweig for the use of a simple peck-aversion task in the chick in studying the different stages of memory formation. The use of the chick model is well suited for determining dose-effects functions of pharmacological agents and mapping the regions in the chick brain that participate in memory formation. Studies are under way using the chick model to characterize the effects of endogenous opioids and agonists and antagonists selective for opiate receptor subtypes on learning and memory processes.

While one-trial learning tasks can be used to advantage to elucidate the dynamic aspects of the memory process, the use of complex tasks is

necessary to model human memory performance. Dr. Kesner described the use of spatial location tasks in the rat to model human list learning. Rats display serial position curves, serial anticipation learning, coding of temporal information, repetition lag functions, and the use of retrospective and prospective codes that are very similar to those seen in human subjects. In addition, there are comparable memory-deficit patterns observed in brain-damaged humans and in rats with lesions in homologous brain regions.

Dr. Olton presented a strategy that might be useful for probing for deficits produced by chronic exposure to drugs of abuse. He described the use of a drug challenge with scopolamine to disclose a latent impairment produced by previous chronic treatment with a cholinesterase inhibitor. While chronic cholinesterase treatment alone produced no decrements in successive reversals of a two-choice simultaneous discrimination or in a delayed match-to-sample task, previous cholinesterase treatment did potentiate the effects of scopolamine on these tasks. Dr. Olton also delineated some of the factors that must be considered in developing animal models for evaluating the effects of drugs of abuse on learning and memory.

Enkephalins are endogenous opioid peptides that have a modulatory influence on the strength of the memory trace and may affect the specific sites of memory storage by affecting hippocampal plasticity. Dr. Martinez's laboratory is investigating both of these processes. Leu-enkephalin (LE) may be a stress as well as a learning hormone. It has a modulatory effect on the acquisition and retention of conditioned responses that is mediated, at least in part, by a site outside the blood-brain barrier. In addition to this learning modulatory role, opioid peptides are involved in the induction of mossy-fiber long-term potentiation in the CA3 region of the hippocampus. Long-term potentiation is a change in synaptic strength that is thought to underlie memory storage.

Dr. Deadwyler stressed the importance of studying the underlying neurobiology of the effects of both short- and long-term drug exposure on learning and performance. Drugs of abuse may affect cognitive and behavioral processes while the subject is intoxicated, during withdrawal, and long after drug exposure has ceased. Since much of the study of the neurobiology of learning and memory focuses on the hippocampus, Dr. Deadwyler's laboratory has focused on understanding how disruptions in hippocampal function mediate the effects of delta-9-tetrahydrocannabinol (delta-9-PTHC), the psychoactive constituent of marijuana, on learning and memory. Dose-dependent delta-9-THC-induced behavioral deficits were demonstrated in the detection and processing of conditioned sensory stimuli as well as in the retrieval of trial-specific information. Hippocampal electrophysiological correlates were altered over the same dose range as was behavior,

The hippocampus is also involved in associative learning, and Dr. Disterhoft describes experiments to elucidate the hippocampal cellular bases of rabbit

eyeblink conditioning. To this end, behavioral testing is combined with *in vivo* hippocampal electrophysiological recording and *in vitro* recording from a hippocampal slice preparation. Recording from slices enables the study of the ionic mechanisms underlying associative learning. Dr. Disterhoft reported conditioning-specific reductions in the CA1 pyramidal cell after hyperpolarization, a response that is due primarily to calcium-mediated potassium currents. Data were also presented that nimodipine, a calcium channel blocker, facilitates acquisition of the conditioned eyeblink response in aged and young rabbits, suggesting that it may be possible to affect learning and memory through pharmacological manipulation of calcium flux.

Dr. Levy presented an information computation perspective for relating hippocampal cellular physiology and anatomy to system physiology. The brain's function is to ensure survival of the organism by controlling the environment through the ability to make predictions. Prediction is possible because of the spatiotemporal regularities and physical constraints, i.e., the redundancies in the environment. This approach analyzes the hippocampus in terms of the storage of redundancy at synapses to yield the predictive representations and the reduction of signal redundancy.

The enhancement of the short-latency acoustic startle reflex by prior fear conditioning is an excellent paradigm for investigating the effects of anxiolytic drugs as well as for studying where in the nervous system plastic changes occur that enable a conditioned stimulus to affect behavior. In this paradigm, a cue previously paired with a shock produces an augmented startle response elicited by a loud tone. Dr. Davis presented data on the temporal specificity of fear-potentiated startle that demonstrated that the conditioned stimulus in fear conditioning is not the light alone but rather the temporal pattern of events that are paired with the unconditioned stimulus (shock) in training. Lesion and electrical stimulation studies have mapped the primary startle circuit as well as the point at which the visual conditioned stimulus modifies transmission in the startle pathway. Other studies using lesions and stimulation of the central nucleus of the amygdala suggest that a neutral stimulus will elicit a state of fear when that stimulus activates the amygdala after being paired with an aversive stimulus.

Dr. Koob suggested that, in addition to its role in ACTH release from the anterior pituitary, CRF is a neurotransmitter in a brain arousal system that, under conditions of extreme activity or chronic activation, produces the behavioral manifestations of stress. In a number of behavioral tests (open field, operant conflict, conditioned suppression, and acoustic startle) CRF appears to be anxiogenic. CRF seems to play a role in learning where arousal and/or an aversive stale are required for animals to learn an association such as in two-way active avoidance or the conditioned emotional response paradigm. Thus, the brain CRF system may be required for forming associations between aversive events and previously neutral stimuli.

Drawing on several studies in human subjects in which the levels of arousal of the subjects modified drug effects on cognitive performance, Dr. Kornetsky stressed that, when studying the effects of drugs on cognitive performance, investigators should always consider whether the observed effect is due to a direct action on the neurobiological substrate of learning and memory or is due to alterations of motor or perceptual-attentional systems. Dr. Kornetsky then described a series of studies in animals in which it was possible to dissociate drug effects on the threshold for rewarding brain stimulation from effects on the threshold for detection of intracranial stimulation. Thus cocaine lowers (and pimozide raises) the threshold for rewarding stimulation at doses that have no effect on stimulus detection. This suggests that attentional-perceptual functioning is not impaired at doses of cocaine that are euphorigenic.

Dr. Lê presented evidence that learning factors play a role in the development of functional tolerance, a neuroadaptive process in which there is a reduction in CNS sensitivity to the drug. Evidence that learning plays a role in tolerance comes from the demonstration that behavioral manipulations such as practice while intoxicated and classical conditioning and neurobio logical manipulations (brain lesions or changing neurotransmitter levels) that affect learning can influence tolerance development. Dr. Lê suggested that tolerance that develops through learning involves different mechanisms than tolerance that results pharmacologically. To clarify whether there are two separate mechanisms, studies using neurobiological manipulation should be conducted under conditions in which either pharmacological or learning factors predominate.

In his treatment of the role of conditioning in sensitization produced by psychomotor stimulants, Dr. Pert presented a historical perspective on the development of current thinking in this area. Stimulant-induced behavioral sensitization is due to neuropharmacological changes resulting from repeated drug administration, such as changes in neurotransmitter release or reuptake and conditioned drug effects elicited by situational cues including both environmental and procedure-induced interoceptive stimuli. Pharmacological and lesion studies from Dr. Pert's laboratory suggest that dopamine in the nucleus accumbens and amygdala are essential for the acquisition of conditioned locomotor excitation. Dopamine in the amygdala appears to modulate the attachment of emotional significance to a given stimulus and may determine which stimuli gain access to structures afferent to the amygdala, while dopamine in the accumbens determines which limbic inputs gain access to motoric output.

I would like to thank all of the authors of chapters in this monograph for their thoughtful presentations. This monograph highlights many areas of research that the National Institute on Drug Abuse wishes to encourage, and I hope it will stimulate interest in these areas.

Contents

The Chick as a Model System for Studying Neural Processes in Learning and Memory

Mark R. Rosenzweig

INTRODUCTION

Reports of learning by young chicks and other precocial birds were made in the last century, but it was only in the 1970s and 1980s that extensive experimental work was done on brain mechanisms of learning and memory in the chick, and this research is gaining in volume and importance. In 1873, Spalding published the first report of what is now called imprinting in domestic fowl. In 1896, Lloyd Morgan commented on rapid learning and lasting memory in young chicks. He reported that if young chicks pecked once at conspicuously colored but bad tasting cinnabar caterpillars, they refrained from pecking if presented with the caterpillars on subsequent days. In the late 1890s, Edward L. Thorndike (1898) studied learning in chicks as well as in dogs and cats. For the chicks, he used simple mazes constructed of books standing on end.

All of these kinds of learning, as well as several other paradigms, are being studied by contemporary investigators. During September 19 through 22, 1988, the author participated in a conference at the University of Sussex that brought together most of the investigators from around the world studying learning, memory, and neural plasticity in chicks. There were 35 papers and about 20 posters. Several of the contributions will appear in a book to be edited by Professor Richard J. Andrew of the University of Sussex. Some of the participants' conclusions about the advantages and disadvantages of the chick subject will be summarized in the last section of this chapter.

The two learning situations most frequently used with chicks are imprinting and peck-aversion learning. Research on imprinting and its brain mechanisms has been reviewed recently in a book by Horn (1985). Peck-aversion learning is related to Lloyd Morgan's observation; it was made into an experimental method by the pharmacologist Arthur Cherkin (1969). Cherkin's method was to allow a chick to peck at an attractive lure-in the first experiments, a tiny light, and, in later experiments, a small metallic bead. If

the lure was coated with a bitter substance, the chick showed a disgust response, and it refused to peck at a similar lure a second time, whether the next presentation occurred seconds, minutes, hours, or days after the original trial. The chick pecked at other targets, even a bead of a different color, so it did acquire a discrimination in a single trial. Cherkin and his co-workers used this method to study the effects of anesthetics and other pharmacological agents on the formation of memory (Cherkin 1969; Cherkin and Lee-Teng 1965; Davis et al. 1982). Since this learning can be timed accurately, it offers a useful technique for the study of the temporal course of memory formation, including the stages of formation of memory. Use of this task is affording a cohesive body of data on the processes of memory formation. (It may be noted that this tactic is the opposite of that discussed by Kesner (this volume) in that it eschews complexity for brevity and simplicity; both tactics are appropriate for studying different aspects of learning and memory.)

This chapter will consist of four main parts: (1) evidence for multiple stages in memory formation, including some new findings; (2) participation of different regions of the chick brain in stages of memory formation; (3) effects of opioid agonists and antagonists on learning and memory in the chick; and (4) advantages and disadvantages of the chick for studies of brain mechanisms of behavior and for studies of the effects of pharmacological agents.

EVIDENCE FOR MULTIPLE STAGES IN MEMORY FORMATION

A Review of Research and Hypotheses on Multiple Stages in Memory Formation

Evidence of many sorts has been offered since the work of Ebbinghaus for the existence of at least two stages of memory, now called short-term memory (STM) and long-term memory (LTM), and research with chicks is now contributing to this topic. Parametric differences between STM and LTM in human verbal learning convinced many that these are two quite different stages. A basis for this work had been provided by Mueller and Pilzecker (1900) in their perseveration-consolidation hypothesis of the formation of memory. References to the term "short-term memory" began to appear in the *Psychological Abstracts* by 1964, and they continued at a high rate through the 1970s reaching a peak of over 10 per 1,000 references in 1980, then falling in the present decade to a level of about 2 per 1,000 references. Some continue to view the distinction between STM and LTM as fundamental or at least highly useful (e.g., Horton and Mills 1984; Simon 1986), but others believe that the concept of a separate STM has outlived its usefulness (e.g., Crowder 1982).

Neuropsychological studies also support a dissociation between STM and LTM. Deficits of memory often affect formation of LTM but leave STM

intact, as in the well-known case of H.M. (Corkin 1984). On the other hand, there are reports of impairment of STM with LTM intact (Baddeley and Warrington 1970; Warrington 1982). Thus, the effects of certain brain lesions may dissociate the two stages.

Studies with animal subjects on consolidation of memory began with the work of Duncan in 1949. In the same year, Hebb (1949) and Gerard (1949) independently set forth dual-trace hypotheses of memory. Although neither Hebb nor Gerard mentioned Mueller and Pilzecker, their 1949 hypotheses appear to be based on that of the earlier workers; perhaps Mueller and Pilzecker's formulation had become so much a part of the background knowledge of investigators in learning and memory that it had become generic. In 1968, McGaugh published a schematic graph (figure 1) suggesting that performance after a learning trial is held very briefly by a sensory buffer and then by three overlapping stages: STM, intermediate-term memory (ITM), and LTM; this figure has been adapted and reprinted by many authors, often without acknowledgment of the original.

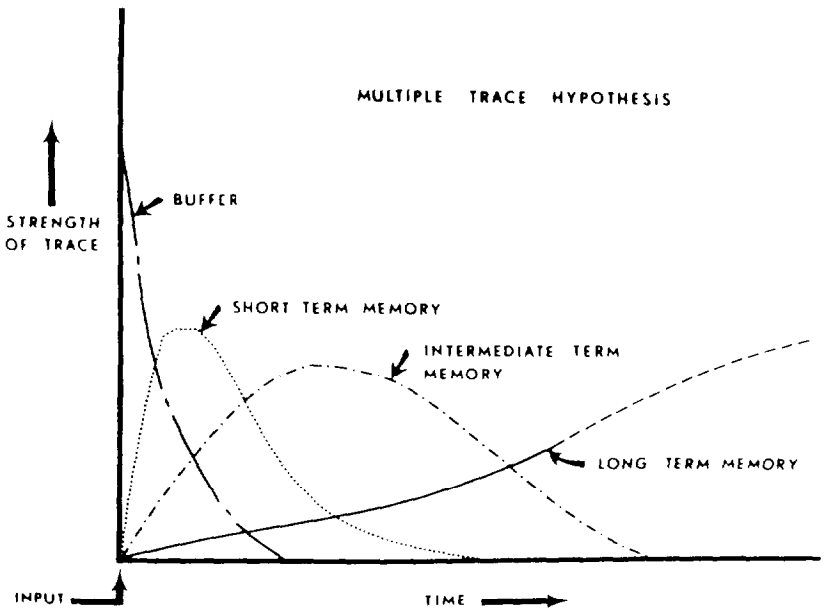

FIGURE 1. *Diagram of a multiple-trace hypothesis of memory storage*

SOURCE: McGaugh 1968, copyright 1968, Academia Nazionale dei Lincei.

In 1972, McGaugh and Herz edited an important volume that reviewed animal research on consolidation of memory. By that time, the concept of consolidation had almost disappeared from research on human memory. Since then, there has been some revival of research on consolidation in normal human subjects, and there has also been interesting research on consolidation in patients with impaired memory, as is reviewed in a book edited by Weingartner and Parker (1984). In animal research, Bennett et al. (1972) introduced the use of the protein synthesis inhibitor (PSI) anisomycin for work on formation of LTM. (It is true that, in the previous year, Schwartz et al. (1971) had shown that anisomycin does not prevent short-term sensitization in Aplysia, but they did not show that anisomycin can prevent formation of LTM, nor did they deal with associative memory.) The findings of Bennett el al. (1972) appeared to demonstrate that anisomycin can prevent formation of LTM for associative learning in mammals. Anisomycin, a relatively nontoxic agent, overcame several of the difficulties that had complicated interpretation of research using other inhibitors such as puromycin and cycloheximide. Flood, Rosenzweig, and collaborators added evidence that protein synthesis must occur in a narrow time window following training if LTM is to be formed (Flood et al. 1972; Flood et al. 1973; Flood et al. 1975; Rosenzweig and Bennett 1984a; Rosenzweig and Bennett 1984b). Rosenzweig and Bennett (1984a) suggested that the protein might be required, in part at least, for the changes seen in synaptic number and structure as a result of differential experience (Diamond et al. 1975) or of formal training (Chang and Greenough 1982). Control experiments were performed to test whether the PSIs affected formation of LTM as such, or whether the effects could be attributed to influences on factors such as perception, motivation, learning, or motor performance. For example, administration of PSIs an hour or more before training was found not to affect learning, even though it affected other aspects of behavior. A large dose of anisomycin 5 hours pretraining altered the motivational state of mice but did not interfere with retention, whereas a small dose that blocked protein synthesis in the immediate posttraining period prevented formation of LTM (Davis et al. 1980).

Meanwhile in the 1970s, the neurochemical bases of formation of the earlier stages as well as of LTM were investigated by Mark and Watts (1971) and Watts and Mark (1971) and then by Gibbs (formerly Watts) and collaborators (Gibbs and Ng 1978; Gibbs and Ng 1979; Gibbs and Ng 1984). This research employed the one-trial taste avoidance training (or peck-aversion training) of chicks introduced by Cherkin (1969). In 1977, Gibbs and Ng set forth a comprehensive model of memory formation, including three sequentially dependent stages, each depending upon different neurochemical mechanisms. Formation of LTM in chicks required synthesis of proteins in the brain shortly after training, as Flood, Bennett, Rosenzweig, and others had shown with rodents (Flood et al. 1973; Flood et al. 1975; Rosenzweig and Bennett 1984a; Rosenzweig and Bennett 1984b). An early stage (STM), lasting about 10 minutes, appeared to depend upon hyperpolarization

due to potassium conductance; a later stage (called the labile stage but in later articles ITM), lasting about 30 minutes, was hypothesized to depend upon hyperpolarization associated with sodium pump activity. Research in the author's laboratory with chicks has confirmed and extended many of the observations of Gibbs and Ng (Patterson et al. 1986) but has also indicated the importance of calcium influx and calmodulin for the early stages of memory formation (Patterson 1987).

Preliminary work of Patterson et al. (personal communication) has also yielded indications that some agents lead to a decline of memory by 30 seconds after training. If confirmed, this may reveal a stage of memory formation in the chick that resembles STM of human learners in its temporal parameters. For the present, however, the author will continue to use the term STM, in research with chicks, to designate a stage that lasts about 10 minutes.

The existence of three main stages in memory formation is indicated by the fact that use of a variety of amnestic agents yields only three curves of appearance of amnesia and not a different curve for each agent. Table 1 shows some of the agents that produce each of the three time curves. These data suggest that there are three main stages of memory formation, each of which can be affected by a number of different agents.

TABLE 1. *Groups of amnestic agents that cause identical curves of decay of memory*

STM	I T M	LTM
Glutamate	Ouabain	Anisomycin
$LaCl_3$	Scopolamine	Cycloheximide
KCl	Trifluoroperezine	Emetine
	Ethaainic Acid	Aminoisobutyrate

Evidence from Animal Behavior for the Existence of Stages of Memory

Could direct behavioral evidence for the existence of stages of memory be found? Behavioral studies by Kamin (1957a; Kamin 1957b; Kamin 1963), and others indicated that the course of memory strength following training may not be monotonic but may include one or more dips (Gisquet-Verrier 1983). In general, however, the complex timecourse was interpreted as indicating motivational factors rather than stages of memory formation. Furthermore, studies of the Kamin effect did not investigate in detail the changes in the strength of memory within the first hour after training. Gibbs and Ng (1977) and Gibbs and Ng (1984), however, reported that

memory of chicks for peck-aversion training shows dips or "fissures" at about 12 and 55 minutes posttraining, and they interpreted the earlier dip as marking the transition from STM to ITM and the second dip as marking the transition from ITM to LTM. They also repotted that the timing of these dips could be shifted by various pharmacological agents. The existence of two sharp dips has not yet been confirmed by publications from other laboratories, and doubts have been raised about them (Roberts 1987; Ng and Gibbs 1987).

Evidence has recently been obtained that bears upon this question. In order to conduct research on enhancing memory formation by pharmacological agents including opioid agonists and antagonists, Diane W. Lee, in our laboratory, has been giving chicks weaker training by using dilute solutions of methyl anthranilate (MeAn) as the aversive substance. The results at 24 hours revealed, as Gherkin (1971) had reported, that animals trained with diluted MeAn showed less retention than animals trained with 100 percent MeAn. We then wondered whether testing their memory at different times following training might reveal successive drops in performance like the hypothetical cures of McGaugh (1968) and, thus, indicate stages in memory formation. This research is in progress; the results to date (based on 20 to 25 animals per point) are shown in figure 2. They do suggest successive stages in memory formation, but the presumed stages do not drop off monotonically as do McGaugh's curves. Now that we have indications of cusps around 15 minutes and 60 minutes, further work is being done to define these time regions more clearly. It seems quite possible that weaker training allows the transitions between stages to appear rather clearly, whereas stronger training pushes behavior toward the ceiling at all time points. The effects of various amnestic agents on performance after weaker training will also be tested in an attempt to define more completely the shapes of each of the component curves. The first component is seen more clearly in a graph that expands the time scale (figure 3); this is a limb that descends steeply to 60 seconds. It appears to be like the component labeled "sensory buffer" in McGaugh's presentation, but note that it has approximately the duration of STM in studies of human verbal learning. Perhaps this should be labeled STM, and perhaps two intermediate-term stages follow before protein-synthesis-dependent LTM appears.

Clearly, there is much to do along these lines. The appearance of distinct successive stages in the behavioral curves opens up new possibilities for research and for understanding of memory, and these possibilities have begun to be pursued in research with chicks.

ROLES OF DIFFERENT BRAIN REGIONS IN MEMORY FORMATION IN THE CHICK

It has been found that not all sites in the chick brain have the same significance for formation of memory. This research was made possible by the

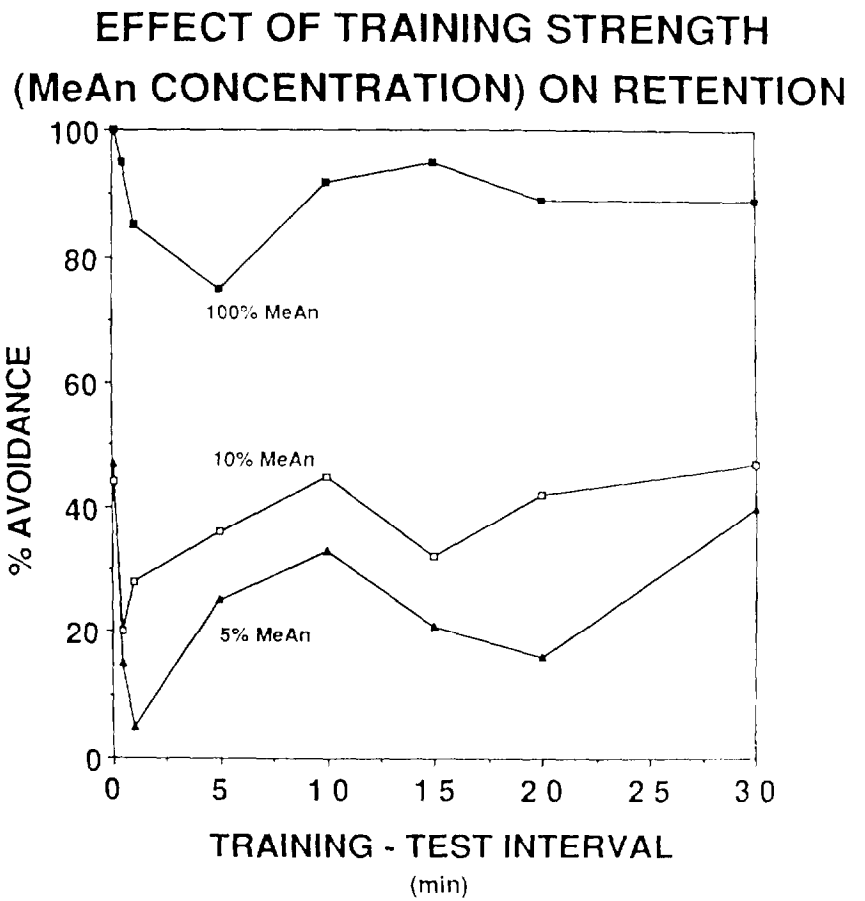

FIGURE 2. *Effect of training strength on retention of the peck-aversion response*

NOTE: Training strength was varied by diluting the concentration of the bitter substance on the training bead (MeAn 100 percent, 10 percent. and 5 percent). Training-to-test intervals were the following: 10 and 30 seconds; 1, 5, 10, 15, 20, 30, 45, 60, 90, and 120 minutes; and 24 hours. Percent avoidance is the percentage of chicks that refrained from pecking in the 10-second test period. The results are cumulative data from three successive experiment in which 8 to 10 chicks were tested at each time point. Separate groups of chicks were tested for each time pint.

finding that certain amnestic agents, administered in rather low doses, affect only a small volume of tissue around the site of injection, as has already been reported (Patterson et al. 1986). Among such agents are glutamate, which impairs formation of STM; ouabain, which impairs formation of ITM; and emetine, which impairs formation of LTM. (These designations of memory stages are used here as the author and others have used them in

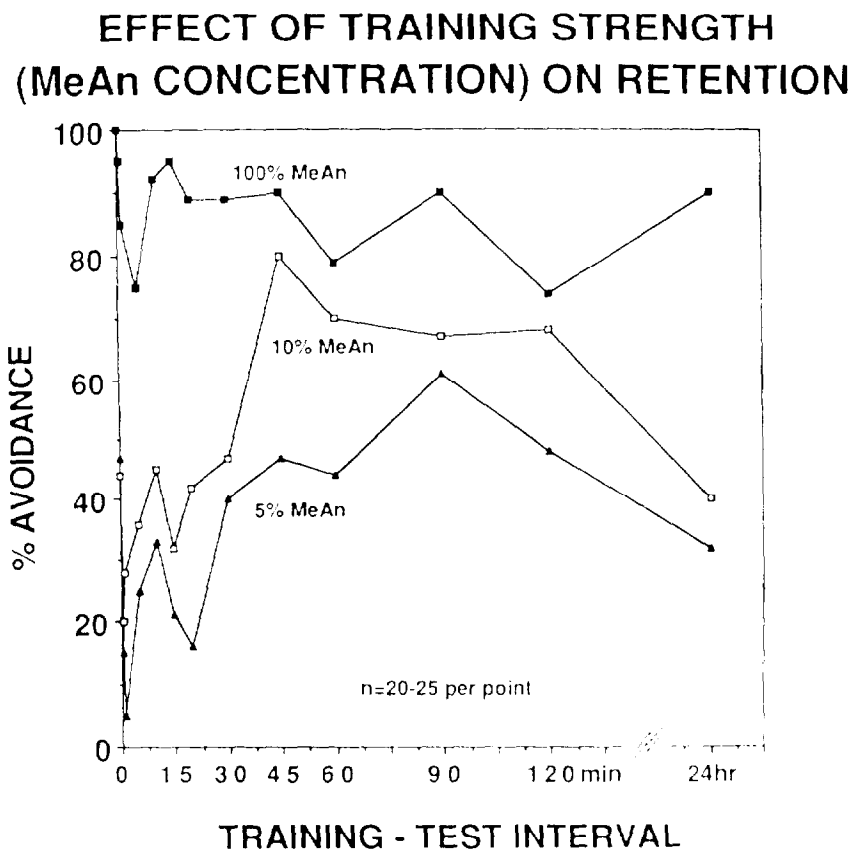

FIGURE 3. *Effect of training strength on retention of the peck-aversion response for the first eight time points*

NOTE: The data of figure 2 are replotted here for the first eight time points

previous publications, although the author is not convinced that they are correct.) The first studies on this topic in our laboratory, made by Teresa Patterson, used injections into two regions of the chick forebrain that are analogous to cerebral cortex in mammals: the intermediate medial hyperstriatum ventrale (IMHV) and the lateral neostriatum (LNS). With bilateral injections, IMHV and LNS showed rather similar dose-response functions for amnesic effects (table 2). Unless otherwise noted, all injections were made 5 minutes before training, and testing was done 24 hours later. The dose-response functions in the table have been abbreviated, to show some of the main data without making the tables too full.

TABLE 2. *Abridged dose-response curves for three amnestic agents in two regions of chick brain (percent chicks showing avoidance at 24-hour test)*

	IMHV				LNS		
GLUTAMATE (mM)	Sal	25	37	50	Sal	50	
Experimenter							
TP	8.5	70	55*	38*	76	49*	
PS	86			42*			
OUABAIN (mM)	Sal	007	014	027	Sal	027	
TP	85	87	49*	25*	76	28*	
PS	82			46*			
EMETINE (mM)	Sal	0.08	1.5	2.25	30	Sal	15	225
TP	82	70	24*	40*	40*	76	45*	40*
PS	85		55*	54*	4.0 50*			

*Statistically significant amnesia.

NOTE: TP=T.A. Patterson; PS=P. Serrano.

The fact that IMHV and LNS yield similar results with bilateral injections does not allow one to conclude that these two sites play identical roles in memory formation. Unilateral injections produce mirror-image effects at these two loci (figure 4); injections in the left IMHV cause amnesia, whereas injections into the right IMHV produce no significant effect. At the LNS, the situation is reversed; injections into the left LNS are without effect, but injections into the right LNS cause amnesia. At each site, each amnestic agent causes amnesia to appear according to the temporal characteristic of that agent—STM, ITM, or LTM. Note also that effects at each site showed sequential dependence; that is, preventing formation of one stage of memory also prevented the appearance of the later stage or stages.

Peter Serrano, in the author's laboratory, has been exploring further brain sites. One of these is the cerebellum, because recent research (Thompson 1986) has shown that mechanical or chemical lesions of the deep cerebellar nuclei prevent formation of conditioning of somatic responses. Serrano,

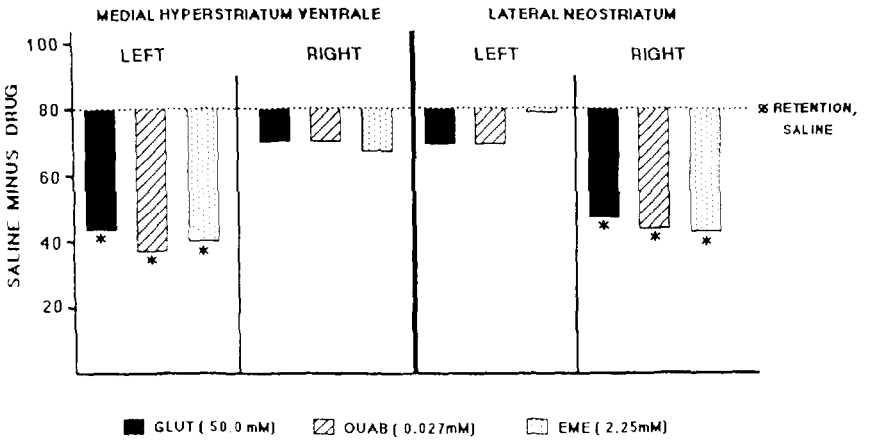

FIGURE 4. *Comparison of effects of unilateral injection of amnestic agents into the left or right IMHV or the leji or right LNS*

NOTE: Injections were made 5 minutes pretraining, and memory was tested at 24 hours. Saline control group were run with each of the 12 drug groups. Since the retention of the saline control groups varied from 74 to 86 percent, each of these values has been normalized to 80 for clarity of presentation. but the magnitude of the difference between each drug group and its control group has not been altered. Numbers of chicks ranged from 39 to 58 per group. Each of the three agents caused significant amnesia when injected into the left but not the right IMHV, each caused significant amnesia when injected into the right but not the left LNS.

SOURCE: Patterson, unpublished doctoral dissertation.

therefore, tested the same three amnestic agents with deep cerebellar injections (table 3). The results showed a lack of amnesic effect for doses that are amnestic in IMHV or LNS; even tripling effective doses for the other sites did not cause amnesia when the agents were injected into the cerebellum.

Thus far, it appeared that a brain site was either involved in memory formation for the peck-aversion response in the chick (as is the case for IMHV and LNS) or that it was not involved (as is the case for the cerebellum). But exploration at a further site revealed another possibility (table 4): bilateral injections of glutamate into the lobus paraolfactorium (LPO) did not cause amnesia within or even beyond the dose range that was amnestic in IMHV or LNS. Injections of emetine into LPO were largely without

TABLE 3. *Abridged dose-response curves for three amnestic agents in cerebellum (percent of chicks showing avoidance at 24-hour test)*

GLUTAMATE (mM)	Sal	45	60	90
	81	68	65	68
OUABAIN (mM)	Sal	.025	035	05
	94	77	89	92
EMETINE (mM)	Sal	1 . 0	2.0	8 . 0
	81	71	94	88

effect, although at a high dose a small amnesic effect was observed. However, ouabain produced amnesia when injected into LPO at the same dose used for most of the experiments with IMHV and LNS. The lime curve of amnesia produced by ouabain in LPO was similar to the curves found with injections into IMHV and LNS. Moreover, scopolamine, another agent found to prevent formation of ITM in IMHV, was also found to be effective in LPO. Thus, there is presumptive evidence that LPO plays a role in formation of ITM but not in the formation of STM or LTM. It should be noted that, although LPO does not appear to be involved directly in formation of LTM, the same sequential dependence is seen here as at IMHV and LNS; that is, prevention of formation of ITM by intervention at LPO causes failure of LTM, since the test was made at 24 hours.

TABLE 4. *Abridged dose-response data for three amnestic agents in lobus paraolfactorium (percent of chicks showing avoidance at 24-hour test)*

GLUTAMATE (mM)		Sal	35	50	70
		89	78	77	83
OUABAIN (mM)	Sal	.02	.03	.04	.05
	87	65	50*	38*	40*
EMETINE (mM)		Sal	2.25	3.0	6.0
		82	79	72	61*

*Statistically significant amnesia.

Exploration is continuing at other brain sites. Possibly some will be found that appear to have special significance for formation of STM or LTM, as LPO appears to have for ITM. Even among the sites already identified, it will be necessary to trace their connections and intereactions and to try to find how each fits into an overall picture of the formation of the stages of memory.

A note of caution must be added here. The dosages stated in tables 2 through 4 are based upon amounts injected into the chick brain, but we found recently that half or more of the injected material may leak rapidly out of the brain through the track of the hypodermic needle unless special precautions are taken. This was discovered when a measurement of the rate of metabolism of radioactively labeled [leu]enkephalin injected into one hemisphere of the chick brain was sought. Chicks were sacrificed from 1 to 60 minutes after injection, the brains were dissected into several samples, and the radioactivity was determined by scintillation counting. It soon became apparent that much of the activity could not be recovered from the brain, even at the earliest analyses. Activity was found on the surface of the head and on the headholder. This problem had not been encountered in work with rodents, where injections were made through indwelling cannulas. The loss of material from the chick brain was not only relatively large but was also was variable from animal to animal. Because of these losses, the doses stated in this article and in published reports from the author's laboratory and other laboratories are probably too high by at least a factor of two.

In spite of the low and variable recovery, it has been possible to determine the transfer of radioactivity from the site of injection to neighboring regions, to the other hemisphere, and into the hindbrain. Over 90 percent of the recovered activity is found in the injected hemisphere, and nearly 90 percent of this amount is found close to the site of the injection. The distribution does not appear to be time dependent. It should be noted that preliminary studies have shown that [leu]enkephalin is metabolized to smaller peptides within 1 to 2 minutes after injection into the chick brain. When radioactively labeled glutamate or ouabain were injected into one hemisphere, data for localization of the recovered activity were similar to those found for [leu]enkephalin. Thus, in spite of the loss of material, the data support the localization of the regions of the brain involved in memory formation.

EFFECTS OF OPIOID AGONISTS ON LEARNING AND MEMORY IN THE CHICK

Because many similarities were found between chicks and laboratory rodents in their responses to amnestic agents, the author collaborated with Dr. Joe L. Martinez, Jr., and his research group to determine whether chick learning and memory formation might provide a useful model system for the study of the effects of opioid agonists and antagonists. Preliminary findings

appear in Patterson et al. (1989), and other findings have been reported at the Society for Neuroscience (Bennett 1988); some of the results will be reported briefly here.

ß-endorphin (ß-END) injected bilaterally 5 minutes before training (10 µl/ hemisphere into the region of the IMHV) made the chicks amnesic at tests conducted 24 hours later. Three doses of ß-END produced significant amnesia, compared to the retention of control animals that received injections of physiological saline solution: 0.01 nmole/hemisphere, O-END vs. saline, $\chi^2(4, n=63)=4.8$, $p<.05$.; 0.10 nmole/hemisphere, ß-END vs. saline, $\chi^2(4, n=64)=5.8$, $p<.05$; 1.0 nmole/hemisphere, ß-END vs. saline, $\chi^2(4, n=63)=9.5$, $p<.01$.

To test whether the effect of P-END was an opioid effect and not due to some other type of action of the drug, we tested to see whether it could be reversed by naloxone in experiments conducted like the previous one. P-END given alone or in combination with a low dose of naloxone (16.0 nmole/hemisphere) produced significant amnesia compared to saline controls. But a higher dose of naloxone (50.0 nmole/hemisphere) reversed the amnesia caused by P-END. Flood et al. (1987) have reported that naloxone administered immediately posttraining enhanced memory formation in both chicks and mice. In their experiments, naloxone was approximately 1,000 fold more potent when administered intracerebroventricularly than subcutaneously, so it appears to exert its effect within the central nervous system.

[Leu^5]enkephalin (LEU), employed in similar experiments, was found to be amnestic at a dose of 1.0 nmole/hemisphere. Since LEU works mainly on delta receptors, the effects of an agonist that is highly selective for delta receptors, [D-Pen^2, L-Pen^5]enkephalin (DPLPE), were tested. Injections of 10 @/hemisphere of DPLPE produced significant amnesia.

The reversal of amnesia produced by either LEU or DPLPE by the use of an agonist that is selective for the delta receptor, ICI 174,864, was then attempted. Figure 5 shows that LEU and DPLPE again produced significant amnesia compared with saline-injected controls. ICI 174,864 reversed these amnesias in a dose-dependent manner.

These indications of clear effects of opioid agonists and antagonists on learning/memory formation in chicks have encouraged the author to further develop a chick model to investigate effects and mechanisms of drugs of abuse. Several lines of research are being followed. Whether the effects are on learning or on memory formation is being investigated, and, in the latter case, the effects on different stages of memory formation will be tested. These experiments are studying enhancement as well as impairment of learning and memory. Different kinds of learning and different tests of memory are being investigated for their effectiveness with chicks. The

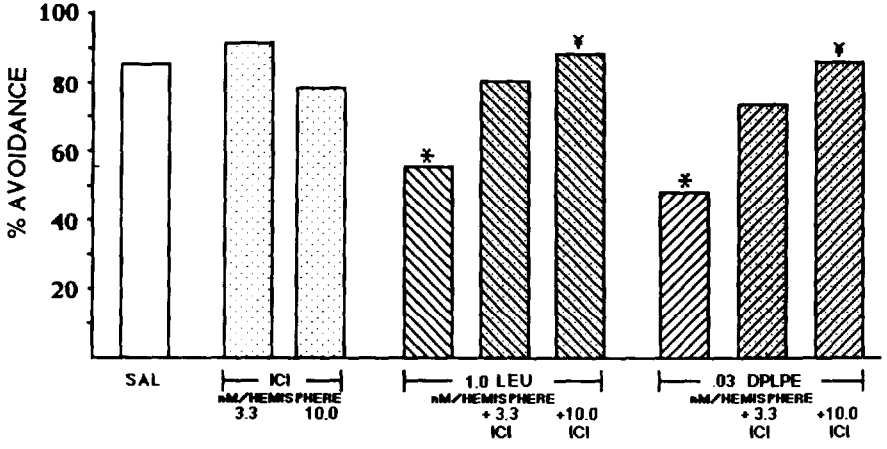

FIGURE 5. *Reversal of amnesia produced by DPLPE or LEU by the delta selective antagonist ICI 174,864*

NOTE: ICI administered alone did not produce either enhancement or amnesia. LEU or DPLPE given alone produced significant amnesia compared to saline controls (LEU vs. saline χ^2 [4,N=41]=6.7; DPLPE vs. saline χ^2 [4,N=43]=8.9, $p<.01$). ICI 174.864 reversed amnesia produced by either LEU or DPLPE in a dose-dependent manner (χ^2 [4, N=45]=6.2, mixture of LEU and ICI compared to LEU alone; χ^2[4,N=41)=6.4. mixture of DPLPE and ICI compared to DPLPE alone; ¥, $p<.05$). All injections (10 µl/hemisphere) were made bilaterally into the region of the IMHV 5 minutes before training, and chicks were tested 24 hours after training.

SOURCE: Patterson et al. (1989), copyright 1989, American Psychological Association.

concentrations of endogenous opioids and of different opioid receptors in the chick brain are being mapped. Among other things, this mapping will suggest further sites to investigate for their roles in learning and memory formation. The effects of peripheral administration of opioid agonists and antagonists in the chick are also being investigated, following findings of effects of peripheral injections in rodents. Clearly there is much to explore and investigate with the chick preparation, and the author will be happy to communicate with others who wish to work in this field.

ADVANTAGES AND DISADVANTAGES OF THE CHICK LEARNING MODEL

Following are some of the advantages and disadvantages of the chick learning and memory system for investigation of effects of pharmacological agents and for the study of brain processes in learning and memory formation, drawn from the author's observations and from the comments of his colleagues at the Sussex conference.

Advantages

- Chicks are readily available and inexpensive. Many hatcheries keep only female chicks and dispose of cockerels, so they are willing to sell cockerels cheaply. Some investigators incubate their chicks in laboratory brooders in order to know the exact times of hatching.

- Commercially available chicks are F1 hybrids between inbred lines and they are phenotypically and genetically standard. For this reason, experimental results tend to be robust. Information about the exact crosses is proprietary and, therefore, not available.

- The young chick is highly competent; it sees, hears, and tastes well, and it makes well-coordinated pecking and locomotor responses. It also learns and remembers well. Its precocial state at hatching means that it can be tested readily shortly after hatching. This in turn means low costs of housing and maintenance.

- Previous experience of the chick is limited and can readily be controlled, especially if the chick is hatched in the laboratory. The small amount of prior experience may make early training particularly significant and its cerebral effects relatively easy to measure.

- The brain is relatively large for a small animal. About the size of the brain of an adult mouse, the chick brain is suitable for localized lesions or recording, for localized intracerebral injections, and for biochemical studies. There is little blood-brain barrier in the young chick.

- The unossified skull roof permits rapid intracerebral injections without the necessity of anesthesia. This allows training and/or testing shortly after injections, affording precise and close timing of events following administration of drugs.

- Because young chicks can be injected rapidly and can learn discriminations in a single trial, as many as 200 can be injected and trained in a single session, so 8 to 10 experimental conditions can be compared directly within a single batch of chicks,

- Chicks recover rapidly from surgery, and they are resistant to operative infection.

- Although detailed mapping of concentrations of endogenous opioids and of opioid receptors in the brain of the chick remains to be done, preliminary reports indicate that opioid receptors are present in relatively high concentrations in the brains of chicks and other birds (Bardo et al. 1982; Felix et al. 1979; Pert et al. 1974).

- Chicks have complete optic decussation, so that visual input can be restricted to one cerebral hemisphere by covering one eye. There is evidence that the two hemispheres process visual information differently.

- Although the brain of the young chick is immature and is developing rapidly, the young chick can be substituted for juvenile and adult mammals in many behavioral and physiological studies. Thus, Thompson et al. (1983), in a review of "Cellular Processes of Learning and Memory in the Mammalian CNS," cited work with chicks in several places, and, in a footnote to their first page (p. 447), they conferred honorary membership in the class Mammalia upon the class Aves.

- A final advantage of the young chick, pointed out by Kastin (Kastin et al. 1981), an investigator who had used rats in his previous research, was that chicks do not bite.

Disadvantages

- Because the young chick is clearly immature in its behavior, neuroanatomy, and neurochemistry, it is possible that, although it is capable of learning and of other behaviors that resemble those of juvenile and adult mammals, the underlying processes may differ from those that are obtained in juvenile and adult mammals.

- The neuroanatomy and neurochemistry of the chick have been less completely investigated than have those of mammals.

- Some differences are found from batch to batch of chicks from the same hatchery, and there are seasonal differences in activity and excitability of chicks.

- Chicks grow rapidly into large animals that are expensive to feed and house, so studies of posthatch development are costly and are rarely undertaken.

- When injections are made into the brain of the unanesthetized chick with a hypodermic needle, much of the injected material promptly leaks out unless special precautions are taken. For this reason, published dose-response data must be interpreted with caution.

- The chick is not yet well known as a research subject, so investigators and granting agencies may be wary of undertaking or sponsoring projects with them. The publication of the Sussex conference and the increasing numbers of research reports with chicks should help to overcome this reluctance to use chicks.

CONCLUSION

On balance, the young chick has much to recommend it for studies of neural bases of learning and memory; in particular, it may serve as an excellent model system to study drugs of abuse. The author invites other investigators to join him in developing and exploiting the advantages of the chick system.

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New Approaches to the Study of Comparative Cognition

Raymond P. Kesner

INTRODUCTION

There has been and there continues to be a genuine interest in the development of animal models of human memory. This interest is based on the assumption of evolutionary continuity between animals and humans in terms of brain-memory functional relationships. To the extent that the above assumption has validity, animal models can provide the impetus (a) to understand the neurobiological basis of memory in animals and humans; (b) to develop new neurobiological theories of memory representation; and (c) to develop potential therapeutic approaches, including pharmacological ones, aimed at alleviating memory problems in humans.

Of the many animals that have been studied, rodents have been used most extensively. Can the memory performance of rats provide the basis of an animal model of human memory? There have been a number of approaches. One approach emphasizes the importance of learning and memory storage or retrieval within tasks that can be learned in a single trial, e.g., active avoidance, inhibitory avoidance, and taste aversion learning. The advantage of this approach is that, because acquisition is rapid, one can evaluate the efficacy of a pharmacological treatment with a single drug dose. By injecting animals before or after training or before retention testing, one can assess whether the treatment produced state-dependent effects or affected storage, short-term memory, long-term memory, or retrieval. One can thus evaluate the dynamic aspects of the memory process. The disadvantages are that one often needs large numbers of animals, variability in memory performance is often quite high, and one often needs to use negative reinforcement. Finally, these tasks are not used to assess memory function in humans, in part because acquisition is too rapid.

A different approach emphasizes the use of complex tasks, e.g., list-learning tasks. The advantages of this approach are that fewer subjects are required, performance is quite stable, and comparisons can be made more readily with human memory performance. The disadvantages are that training takes a long time, requiring multiple injections of specific pharmaceutical agents or the use of long-acting agents. Even though the latter approach has not

been used extensively, the ease of between-species comparisons makes this approach attractive.

In this chapter, a number of examples of animal memory performance in a variety of list-learning tasks will be presented. These examples include the display of serial position curves, serial anticipation learning, coding of temporal information, repetition lag function, and utilization of retrospective and prospective codes.

SERIAL POSITION CURVES

One commonly used method to assess memory function in humans is to present a list of items (e.g., pictures, words) of information to a subject and ask for recall or recognition of the items. In normal subjects, one observes a serial position curve with better performance for the first items (primacy effect) and the last items (recency effect) compared to items located in the middle of the list. It has been proposed by some theorists that the primacy effect reflects information storage in long-term memory, while the recency effect reflects information processing in short-term memory (Atkinson and Shiffrin 1968). However, it should be noted that there are other interpretations for the serial position effect. For example, it has been suggested that the recency effect reflects automatic availability of information requiring little attention, whereas the primacy effect reflects the utilization of controlled or effortful attention-directing activity (Hasher and Zacks 1979; Shiffrin and Schneider 1977). Others have suggested that the ends (primacy and recency) of a list are more distinctive and, therefore, are remembered better than the interior positions (Ebenholtz 1972).

How do rats perform on an item- or order-recognition task for a list of spatial locations? Rats were placed on an eight-arm radial maze and were given experience exploring all eight arms. Reinforcements could be obtained at the end of each arm and consisted of pieces of sweetened cereal. After receiving extensive exposure to the radial arm maze, the item-recognition memory task was begun. Each animal was allowed to visit a sequence of five arms on each trial (one per day), which was selected on a pseudorandom basis. The sequencing of the five arms was accomplished by sequential opening of Plexiglas doors (one at a time) located at the entrance of each arm. This constituted the study phase. Immediately after the animal had received reinforcement from the last of the five arms, the test phase began. Only one test was given for each trial; it consisted of opening two doors simultaneously, with one door representing an arm previously visited for that trial and the other door representing a novel arm for that trial. For half of the animals, the rule to be learned, leading to an additional reinforcement, was to choose the arm that had been previously visited during the study phase of the trial (win-stay). For the other half of the animals, the rule leading to a reinforcement was to choose the arm that had not previously been visited during the study phase of the trial (win-shift).

Each animal received 2 sets of 40 trials, with 8 tests for each of the serial positions.

For the order-recognition memory task, each animal was allowed on each trial (one per day) to visit all eight arms in an order that was randomly selected for that trial. The sequencing of the eight arms was accomplished by the sequential (one at a time) opening of Plexiglas doors located at the entrance of each arm. This constituted the study phase. Immediately after the animal had received reinforcement from the last of the eight arms (i.e., completed the study phase) the test phase began. Only one test was given for each trial; it consisted of opening two doors simultaneously. On a random basis, either the first and second, fourth and fifth, or seventh and eighth doors that occurred in the sequence were selected for the test. The rule to be learned, leading to an additional reinforcement, was to choose the arm that occurred earlier in the sequence.

The animals were initially given 8 trials per serial position for a total of 24 trials. If, after these 24 trials, the animal had not performed at 75 percent or better on tests of both the first and second (primacy) and seventh and eighth (recency) serial positions (criterion), then additional blocks of 6 trials (2 trials per serial position) were given until the criterion was met for the most recent block of 24 trials or until 100 trials total had been given.

The results of the item-recognition memory task are shown in figure 1 and indicate that by the second block of 40 trials rats display a serial position curve for memory of spatial locations given that a win-stay rule is required. However, when a win-shift rule is required, animals display only a recency effect, which can already be observed within the first block of 40 trials. With continued training, rats display excellent retention for all serial positions for either the win-stay or win-shift rules. DiMattia and Kesner (1984) have interpreted these results as reflecting differential processing requirements for the win-stay and win-shift rules. They suggest that the win-stay rule necessitates relatively more effortful, elaborative processing than does the win-shift rule, which is used automatically. A more detailed description of the experimental methods can be found in DiMattia and Kesner (1984).

The results of the order-recognition memory task are shown in figure 2 and indicate that rats again display a serial position curve for the sequential order of spatial locations. This serial position function is maintained even with extensive training (Kesner et al. 1984). Recently, Dale (1987) tested humans in a task analogous to the order-recognition memory study for rats. In this case, eight lights were turned on (one at a time) in a specific sequence. Subjects were then tested for memory for the order of the first and second, fourth and fifth, or seventh and eighth serial positions. The results are shown in figure 2 and indicate a serial position function parallel to that obtained with rats. Thus, it is clear that rats, like humans, display serial position curves for a list of items, suggesting that list-learning experiments

can provide an excellent paradigm in which to develop animal models of human memory.

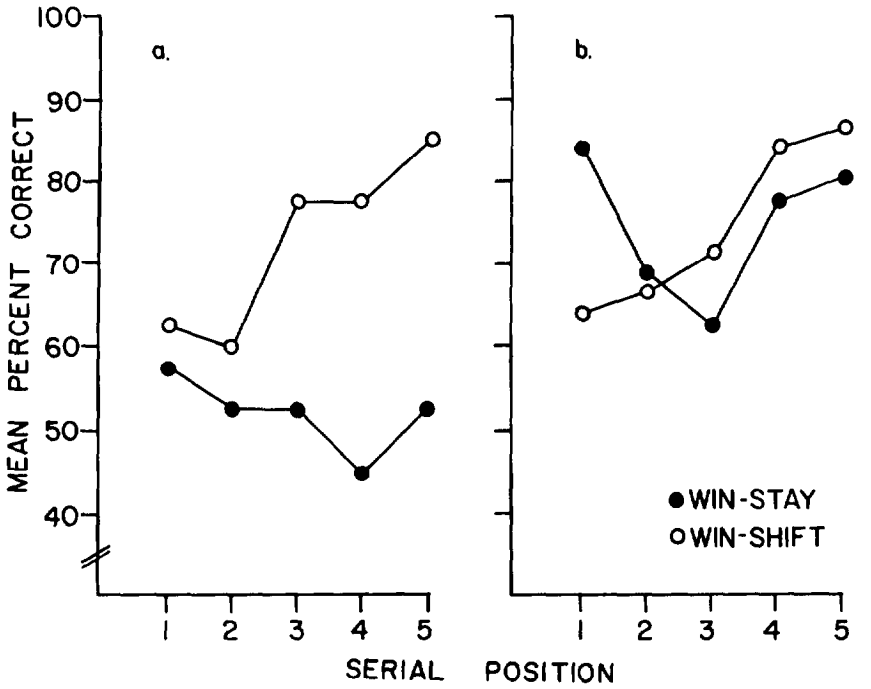

FIGURE 1. *Mean percent correct as a function of serial position for the win-stay (closed circles) and win-shift (open circles) for the first 40 trials (a) and for the second 40 trials (b)*

SOURCE: DiMattia and Kesner 1984, Copyright 1984, American Psychological Association.

Since similar serial position curves for item memory for lists of information have been reported for pigeons and monkeys (Sands and Wright 1980; Wright et al. 1985), these animals could also serve as useful animal models.

SERIAL ANTICIPATION LEARNING

Another commonly used procedure to assess human memory is to present a single nonvarying list of items that must be recalled in the order presented. One method used to study serial recall requires that a subject remember the second item in the list when the first is presented alone, the third item in the list when the second is presented, and so on. This method is called the

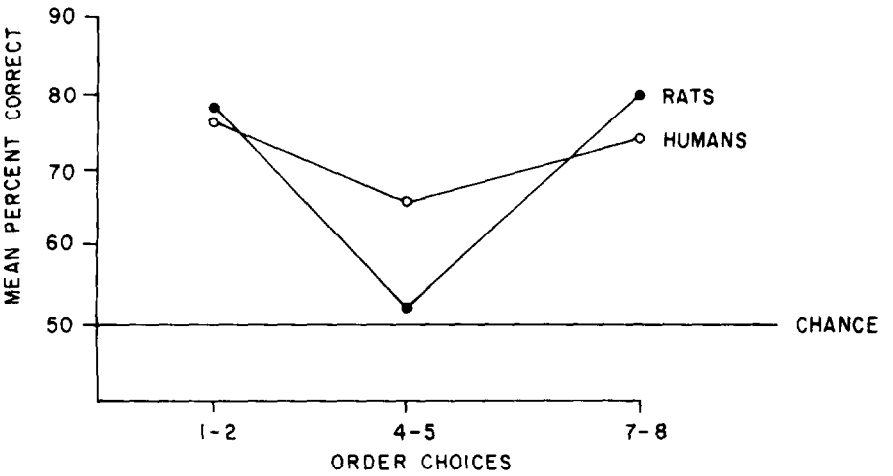

FIGURE 2. *Mean percent correct as a function of serial order position in rats and humans*

anticipation method. Thus, a subject is required to anticipate the next item of a nonvarying list. During the learning of such a list, subjects often make errors by reporting an incorrect serial position item within the list. These errors are called intralist intrusion errors. The results of an experiment with 12 words using the serial anticipation method are shown in figure 3. The results indicate that there are more errors in the middle compared to the end items of the list (Deese and Kresse 1952).

In order to test how rats perform in a serial list task using the serial anticipation method, rats were tested on an eight-arm radial maze. After extensive experience with the maze, animals were given 40 trials in the S-arm maze. On each trial, the animals were presented with a constant sequence of five arms. Serial anticipation memory was measured as a pattern of correct or incorrect orienting responses in anticipation of the ensuing doors in the constant sequence. Since the sequence of spatial locations did not vary from trial to trial, it was assumed that the emitted orienting responses reflected the operation of memory for sequential spatial information. An intralist intrusion error reflected an orienting response in front of an incorrect door that was part of the set of spatial locations within the constant sequence. The results based on 40 trials are shown in figure 4 and indicate that most of the errors occurred for the middle serial position compared to the end positions (Kesner and Beers 1988). A more detailed description of the experimental methods can be found in Kesner and Beers (1988). Thus, performance of the rats parallels the results achieved with humans, suggesting that measurement of intralist intrusion error in a serial anticipation

learning task might represent another example of a potential animal model of human memory.

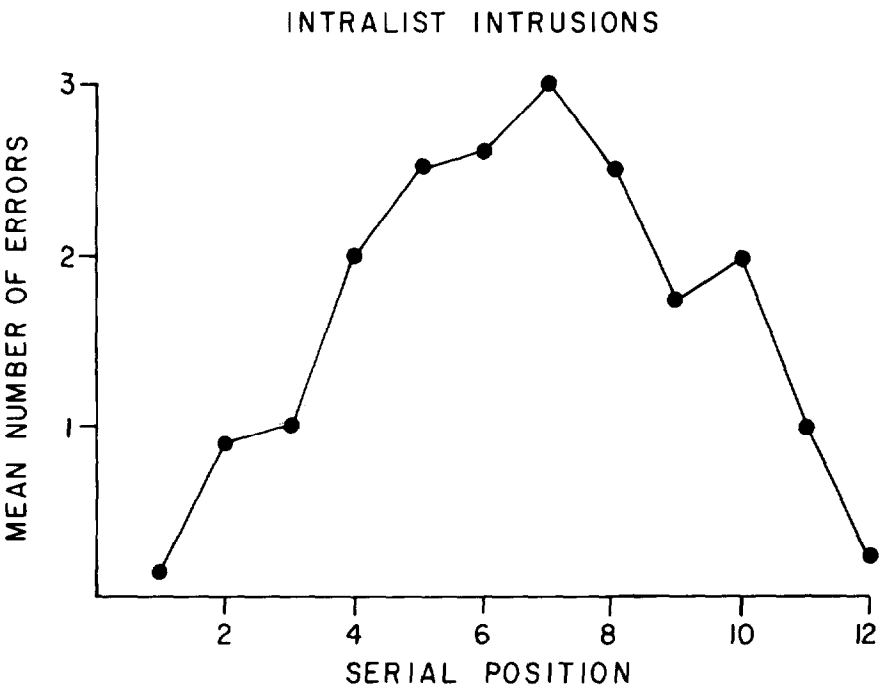

FIGURE 3. *Mean number of intralist intrusion errors as a function of serial position in humans*

SOURCE: Deese and Kresse 1952, copyright 1952, American Psychological Association.

CODING OF TEMPORAL INFORMATION

In a somewhat different experiment, Estes (1985) has summarized data in humans demonstrating that order or sequential information is remembered better with more items (lag) between any two items to be tested for order memory. In order to test this temporal lag function for spatial location information, rats were trained on an eight-arm maze as described for the order-recognition memory task. After extensive training, the rats were allowed on each trial (one per day) to visit all eight arms in an order that was randomly selected for that trial. This constituted the study phase. Immediately after the animal had received reinforcement from the last of the eight arms (i.e., completed the study phase), the test phase began. Only one test was given for each trial; it consisted of opening two doors

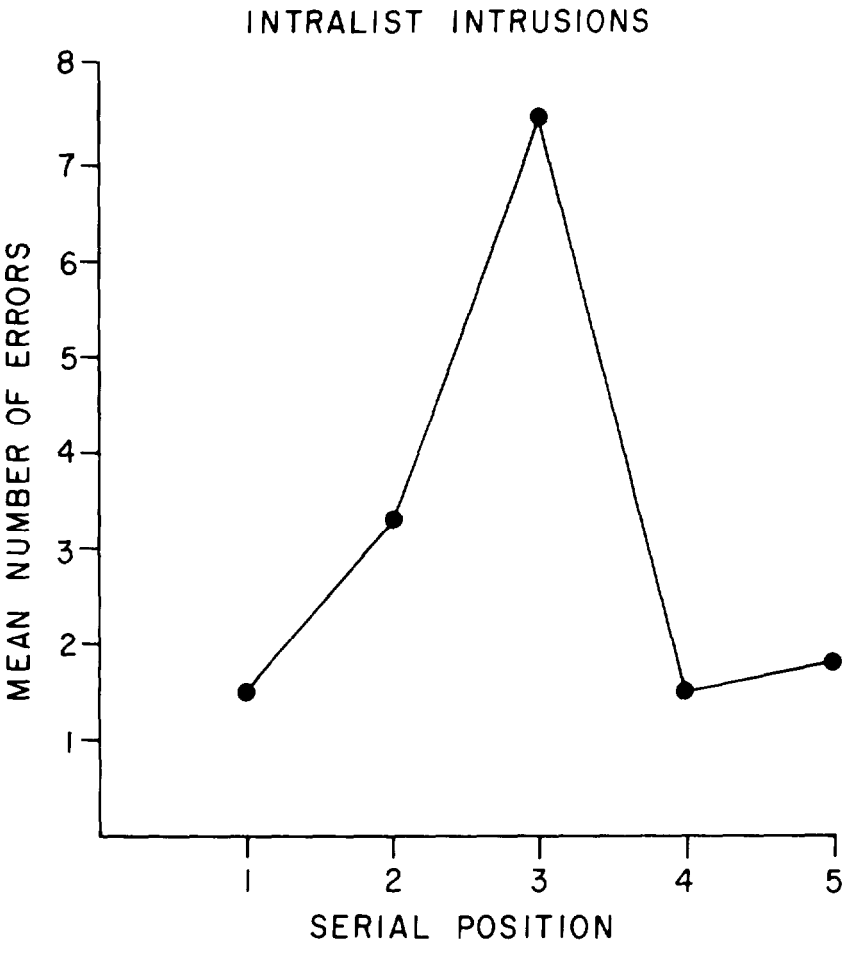

FIGURE 4. *Mean number of intralist intrusion errors as a function of serial position in rats*

SOURCE: Kesner and Beers 1988, copyright 1988, Academic Press.

simultaneously. On a random basis, lags of zero, one, two, three, four, five, or six arms were selected for the test. In the case of a lag of zero arms, the arms followed each other in the study phase; in the case of a lag of six arms, six items occurred between the two selected arms in the study phase. The rule to be learned, leading to an additional reinforcement, was to choose the arm that occurred earlier in the sequence. The animals received a total of 56 trials, with 8 tests randomly selected for each lag

condition. An identical procedure was used for college students, but, in this case, spatial locations were presented one at a time as X's on a grid of 16 squares. The study phase consisted of the presentation of X's in eight different locations. During the test phase, subjects had to select from two X's that occurred during the study phase and that varied in terms of lag. There were 56 trials, with 8 tests randomly selected for each lag condition. The results are shown in figure 5 and indicate that both rats and college students perform poorly for lag of zero, but perform well for lags of one to six. College students, however, continue to improve with longer lags, whereas rats do not show such an improvement. Again, there appear to be comparable memory functions for rats and humans.

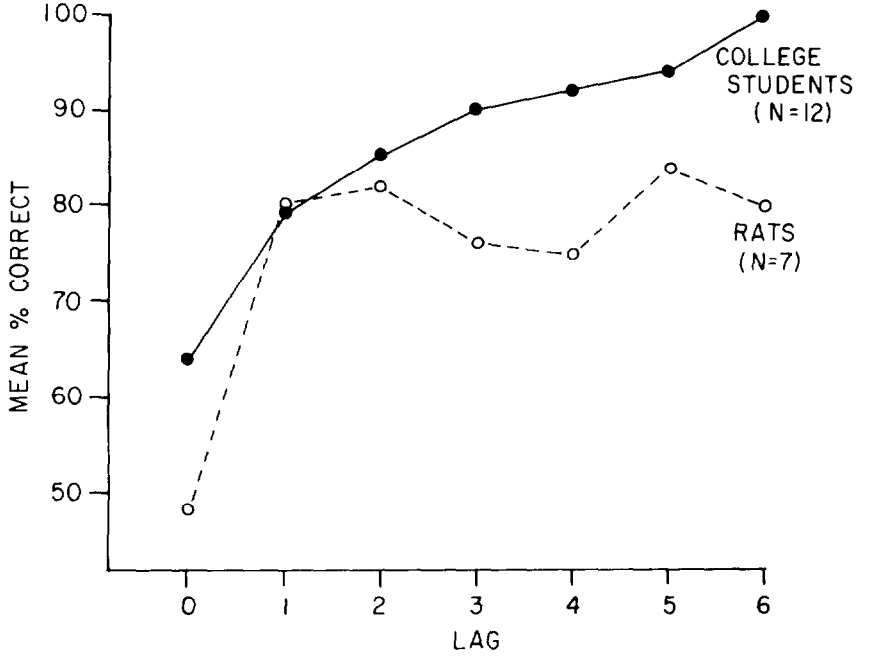

FIGURE 5. *Mean percent correct as a function of lag between occurrences of items selected for the test phase in college students and rats*

REPETITION LAG FUNCTION

In a final experiment emphasizing the importance of temporal coding of information, it has been shown that memory for a repetition or frequency of occurrence of specific items is a function of lag (number of items) between

repetitions. The more items between a repetition or the greater the lag, the better the memory for the repetition. Results of such an experiment conducted with humans are shown in figure 6 (Madigan 1969). It is clear that there is a monotonic increasing-repetition-lag function.

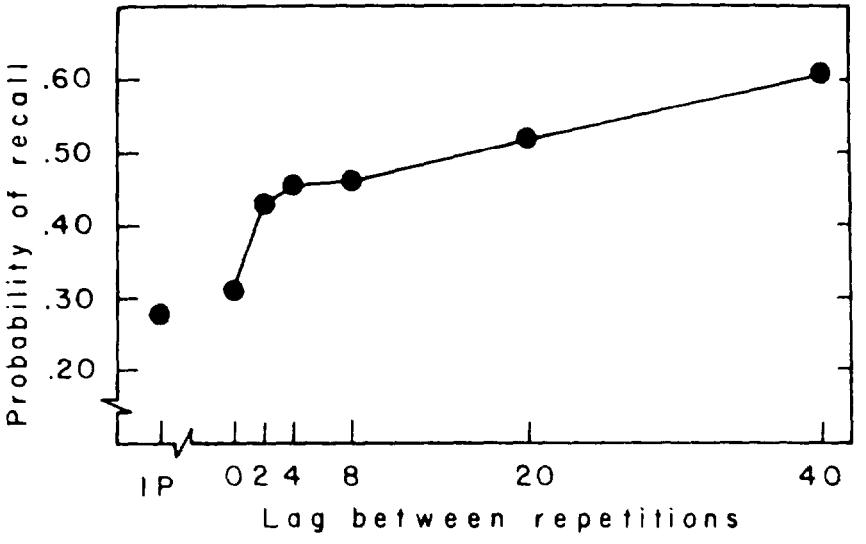

FIGURE 6. *Recall probability as a function of repetition and the lag between occurrences of repeated items in humans*

NOTE: 1P is one presentation.

SOURCE: Madigan 1969. Copyright 1969, Academic Press

In order to test whether rats could show a repetition lag function, rats were tested for memory for the frequency of occurrence of specific spatial locations. In this task, the animals were tested on an eight-arm radial maze. On each trial (one per day), each animal was allowed to visit four arms to receive reinforcement in an order that was randomly selected for that trial. One of the arms was repeated with a lag of one, two, or three arms in between a repetition. This constituted the study phase. Upon completion of the study phase, the door in front of the repeated arm and a door in front of a nonrepeated arm were opened simultaneously. This constituted the test phase. The rule to be learned, leading to an additional reinforcement, was to choose the arm that had been *repeated* in the study phase sequence.

The results after about 60 trials of training are shown in figure 7 and indicate that animals show excellent memory for the repetition with a lag of 3

arms between a repetition, but poor performance for a lag of 1 or 2 arms, even when the data were analyzed for those trials on which the repetition occurred in the last serial position. This appears to be yet another example of a comparable memory function in animals and humans.

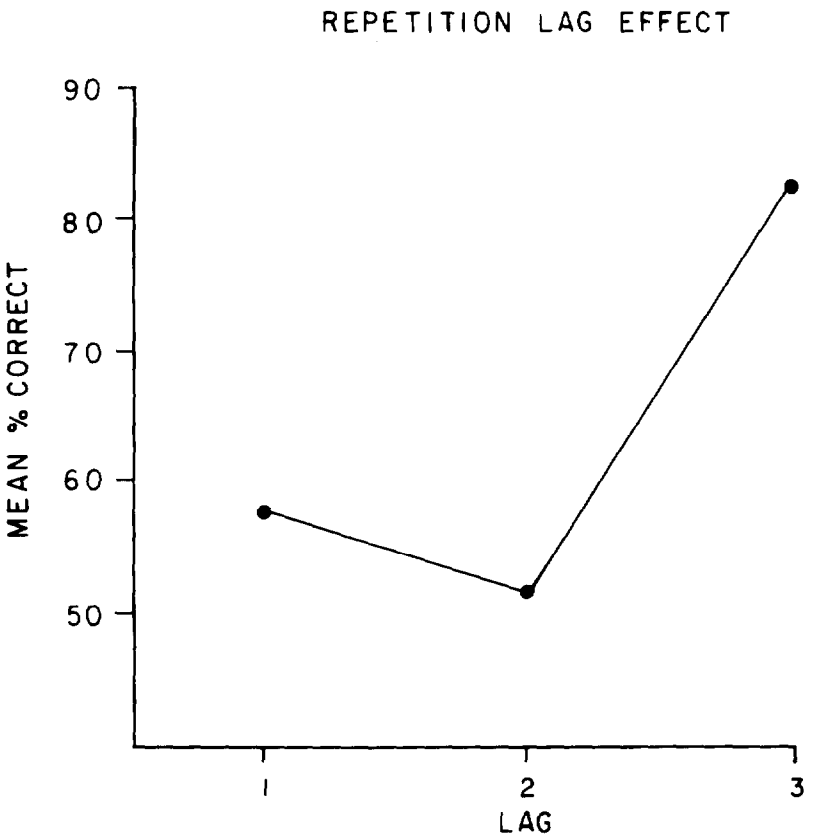

FIGURE 7. *Mean percent correct as a function of repetition and the lag between occurrences of repeated items in rats*

RETROSPECTIVE AND PROSPECTIVE CODING OF INFORMATION

As a final example of the possibility of comparability of memory functions between animals and humans, one can point to the recent observation that rats can use retrospective and prospective memory codes when asked to re-

member items within long lists (Cook et al. 1985). More specifically, on any one trial, a rat is presented with 2, 4, 6, 8, or 10 items (places) on a 12-arm radial arm maze followed 15 minutes later by 2 win-shift tests comprising choices between a place previously visited and a novel place. The animal is reinforced for entering the novel spatial location. During learning, animals showed an increase in errors as the number of places to be remembered increased from two to six, reflecting the use of a retrospective memory code. These animals also showed a decrease in errors as the number of places to be remembered increased from 6 to 10, reflecting the use of a prospective memory code.

In an analogous mnemonic task, college students were presented with either 2, 4, 6, 8, 10, 12, or 14 consecutive pages of X's marked on a grid of 16 squares. On subsequent tests, college students were asked to choose between a novel X and one that had appeared previously. Subjects were asked to circle the novel X. Based on verbal reports, subjects were divided into two groups reflecting different memory coding strategies. The results arc shown in figure 8 and indicate that the subjects reporting a retrospective coding strategy showed an increase in errors as the list length increased.

The subjects reporting a retrospective and prospective strategy showed an initial increase in errors for a list length of 2 to 8 items, followed by a decrease in errors for a list length of 8 to 14 items. This latter error pattern is seen in rats, with the largest number of errors occurring in the middle of the list. Even though there are a few differences in procedure between the rat and human study, a short vs. long delay between study phase and test phase and the use of a different type of reinforcement, there are a sufficient number of similarities in the procedure, including the use of spatial location information and two tests for each study phase, that a comparison between rats and humans can be made. Thus, the similarity in the pattern of results suggests the possibility that rats indeed utilize both retrospective and prospective codes in remembering long lists of information. This explanation could account for the excellent ability to remember all spatial locations in a 17-arm maze (Olton et al. 1977). In conclusion, the present study provides for yet another demonstration that comparable mnemonic functions can be found in animals and humans.

There arc other memory and cognitive phenomena that have been studied in animals and humans that provide for nearly equivalent functional patterns. For example, rats, monkeys, and humans display similar functions in memory scanning tasks based on the Sternberg paradigm (Ellis et al. 1984; Sands and Wright 1981). Pigeons display the use of mental imagery similar to representational imagery in humans (Neiworth and Rilling 1987; Shepard and Cooper 1982; Jagacinski et al. 1983). Pigeons and monkeys can, like humans, learn to categorize items utilizing conceptual schemas (Hermstein 1984; Medin and Dewey 1984).

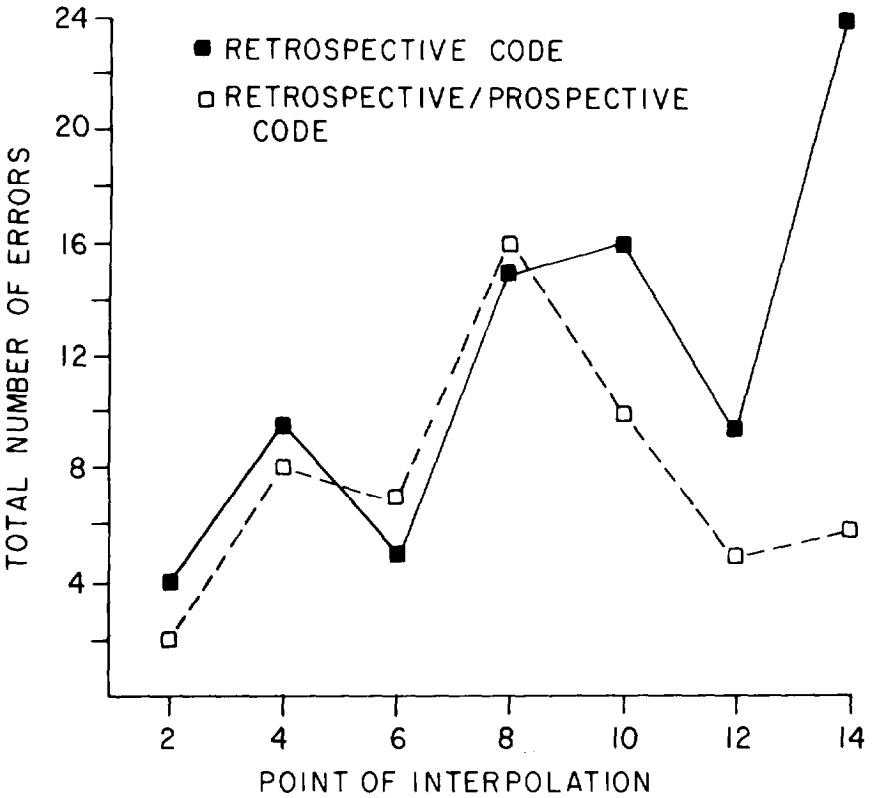

FIGURE 8. *Total number of errors as a function of point of interpolation (list length) for humans using retrospective and retrospective/prospective codes*

SOURCE: Kesner and DeSpain 1988, Copyright 1988, Psychonomic Society, Inc.

NEURAL MEDIATION OF MEMORY FUNCTION

Since the rat can serve as an animal model for a variety of memory functions based on lists containing temporal-spatial information, it should be possible to compare the performance of animals with specific lesions or drug treatment with humans that have sustained brain damage or are under the influence of specific drugs. A couple of examples will suffice. Damage to the medial prefrontal cortex in the rat impairs memory for order information, as evidenced by a total deficit in the order-recognition memory, temporal coding, and repetition lag tasks (Kesner and Holbrook 1987;

Kesner, in press). Similar order-memory deficits have been found in humans with frontal lobe damage (Milner 1971; Milner et al. 1985).

In the item-recognition memory task, small lesions of the medial septum or dorsal hippocampus in rats produce a deficit in the primacy but not recency component of the serial position curve (Kesner et al. 1988). A similar deficit pattern is seen in humans with damage to the hippocampus and patients diagnosed as having mild dementia of the Alzheimer's type (Milner 1978; Kesner et al. 1989). Large lesions of the medial septum, dorsal hippocampus, or parietal cortex in rats produce a deficit in both the primacy and recency component of the serial position curve (Kesner et al. 1986; DiMattia and Kesner 1988). A similar deficit pattern has been observed in patients diagnosed as having moderate dementia of the Alzheimer's type (Kesner et al. 1987). These data demonstrate not only specific neural mediation of memory function but also comparable memory-deficit patterns between brain-damaged humans and lesioned rats.

SUMMARY

The rat's memory capacity and performance render it an excellent model of human memory. Rats display serial position, serial anticipation learning, temporal coding, and repetition lag functions as well as utilization of retrospective and prospective codes that are nearly equivalent to that observed for humans. Furthermore, based on the above-mentioned memory performance functions, there are comparable memory-deficit patterns between brain-damaged rats and humans. Thus, the rat can serve as an excellent animal model to evaluate the efficacy of pharmacological treatments or brain damage upon memory.

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Long-Term Effects of Cholinergic Agonists on Memory

David S. Olton and Kathleen C. Raffaele

INTRODUCTION

Long-term exposure to drugs and other chemical compounds can produce significant and enduring changes in neurotransmitter systems. Of particular interest to the current project is the decrease in the number of receptors following prolonged exposure to a neurotransmitter agonist. The agonist produces excessive stimulation of postsynaptic receptors, which decrease in number, a phenomenon called "downregulation of receptors." This downregulation decreases the sensitivity of the postsynaptic neuron to endogenously released neurotransmitters, which should in turn produce a decrease in the output of the postsynaptic neuron for any given input that stimulates those receptors. This decrease in the "gain" of the synaptic receptor mechanism might be expected to produce substantial functional impairment.

However, the brain is a very plastic organ and maybe able to compensate for at least some alterations in a neurotransmitter system. Long-term exposure to a drug provides ample opportunity for both neural and behavioral compensation. Indeed, some recovery of function is the expected outcome of brain damage (Finger and Stein 1982). If excessive stimulation by a neurotransmitter agonist is viewed as abnormal brain activity, then some kind of compensation is expected.

In a relatively benign environment, one that does not challenge the organism excessively, the impairments expected from decreased synaptic gain may be fully compensated by recovery of function so that no behavioral impairment follows. However, the neural system may still have a significant latent impairment, one that can be made manifest by challenging the system in some way. One obvious challenge is to give an antagonist for the neurotransmitter system in question. The downregulation of receptors should produce increased sensitivity to the antagonist, resulting in a leftward shift of the dose-response curve, as compared to the normal condition (one in which no exposure to the drug has taken place prior to testing with the antagonist).

In the context of learning and memory, the cholinergic neurotransmitter system is an obvious one in which to carry out this manipulation (Bartus et al. 1985; Davies 1985; Pontecorvo et al. 1985). Long-term exposure to a cholinergic agonist should result in excessive stimulation of the postsynaptic neurons, downregulation of the postsynaptic receptors, increased sensitivity to an anticholinergic drug such as scopolamine, and a leftward shift of the dose-response curve, so that a given dose of scopolamine causes a greater impairment in tasks requiring memory.

The experiments summarized here tested this hypothesis (Raffaele et al., in press). The chronic treatment used diisopropylfluorophosphate (DFP), a compound that irreversibly inhibits acetylcholinesterase (AChE), the enzyme that breaks down acetylcholine. Chronic treatment with DFP produces downregulation of muscarinic receptors (Lim et al. 1986; Sivam et al. 1983; Yamada et al. 1983). Scopolamine, a cholinergic antagonist, produces significant impairments in many different tests of memory (Bartus et al. 1985; Pontecorvo et al. 1985). Consequently, following chronic exposure to DFP, rats given scopolamine should show a leftward shift of the dose-response curve.

SUCCESSIVE REVERSALS

Successive reversals of a two-choice simultaneous discrimination (TCSD) were conducted in an apparatus that had a runway connected to a goal platform with two interchangeable goal boxes. One goal box had black and white stripes, the other had black spots on a white background.

Each rat was first shaped in the usual testing procedures to obtain reinforcement, which was chocolate milk placed in a cup at the far end of each goal box. Next, each rat was given a TCSD. Each day's testing had two parts. The first part was *retention.* During retention, the goal box that was correct at the end of the previous day remained correct. If the rat met any one of several different criteria, indicating accurate memory of the previous day's discrimination, the second part, *reversal,* was begun. For the discrimination reversal. the previously correct goal box was incorrect, and the previously incorrect goal box was correct. Testing continued until the rat reached criterion in the reversed discrimination or a total of 30 trials had been given. This procedure was continued for each rat until stable performance was reached, using an intertrial interval of 1 minute.

The cholinergic antagonist, scopolamine, was used to produce an impairment of choice accuracy in this task, Scopolamine, at doses of .15, .35, or .75 mg/kg, was given at least twice prior to treatment with DFP to establish a pretreatment baseline; at least four times during treatment with DFP to determine the effects of this treatment on the sensitivity to scopolamine; and at least twice following treatment with DFP to determine the recovery from the DFP treatment. Scopolamine was given once every 6 days.

Methylscopolamine or saline was given on the third day following the scopolamine treatment. No acute treatment injections were given during the intervening days.

DFP was given in two doses. For the first day of DFP treatment, the dose was 1.0 mg/kg. For all subsequent days of DFP treatment, the dose was 0.5 mg/kg. DFP was given after behavioral testing every third day. Consequently, in a block of 6 days, the drug treatment procedure looked like that outlined in table 1.

TABLE 1. *Summary of experimental procedures*

Days	Chronic Treatment	Acute Treatment
1	DFP or Saline	None
2	None	None
3	None	Methylscopolamine or Saline
4	DFP or Saline	None
5	None	None
6	None	Scopolamine or Saline

NOTE: The chronic treatment was given after the day's behavioral testing. The acute challenge was given 20 minutes prior to the day's behavioral testing.

The use of a between-subjects design produced eight groups, as outlined in table 2. These groups are identified by the abbreviations SAL-O, SAL-15, SAL-35, SAL-75, DFP-0, DFP-15, DFP-35, and DFP-75. The first half of each abbreviation indicates the chronic treatment (SAL or DFP), the second half indicates dose of scopolamine (0, .15, .35, and .75 mg/kg (table 2)).

TABLE 2. *Summary of the experimental groups*

Chronic Treatment	Acute Challenge: mg/kg Scopolamine			
	0	.15	.35	.75
Saline	SAL-0	SAL-15	SAL-35	SAL-75
DFP	DFP-0	DFP-15	DFP-35	DFP-75

At the end of behavioral testing, each rat was sacrificed. Total muscarinic receptor binding was measured by modifications of [^{3}H]-QNB binding (Yamamura and Snyder 1974). M_1 muscarinic receptor binding was

measured by modifications of [^{3}H]-pirenzepine binding (Watson et al. 1984). AChE activity was determined spectrophotometrically (Ellman et al. 1961).

Each rat learned the discrimination rapidly, and the mean number of errors decreased with continued behavioral testing. Prior to the first injection of scopolamine, the mean number of errors to criterion was 2.4 for retention and 5.2 for reversal.

Scopolamine produced a slight increase in the number of errors, with .75 mg/kg producing the greatest increase. However, this difference was not statistically significant. Methylscopolamine and saline had no effect on choice accuracy. A similar pattern of performance occurred during reversal. DFP itself produced no significant change in behavior. The DFP-0 group performed similarly to the SAL-0 group. Thus, the chronic treatment itself did not produce a significant change in behavior.

However, the chronic treatment did produce a marked increase in sensitivity to scopolamine, especially at .75 mg/kg (figure 1). During retention, the control group (SAL-75) had a mean of five errors or less. In contrast, the DFP-75 group had a mean of seven errors or more. As can be seen in figure 1, this increase in the number of errors was consistent throughout testing during DFP treatment. Furthermore, it continued to the posttreatment days, after DFP treatment was stopped. The magnitude of the effect showed some signs of decreasing during this posttreatment phase and might have eventually disappeared if testing was continued. However, it clearly persisted for at least 2 weeks after DFP was stopped.

A similar pattern of results occurred during the reversal of the discrimination; the DFP-75 group was more sensitive to scopolamine than was the saline group. The effects at other doses (.15 and .35 mg/kg scopolamine) were not as large. The lowest dose (.15 mg/kg) produced no differential effect. The intermediate dose (.35 mg/kg) produced a slight increase in errors in rats receiving chronic DFP, but the magnitude was significant for only one block of tests.

The neurochemical assessments indicated a decrease in cholinergic receptors in the cortex of rats given DFP. On the second day following the last DFP treatment, muscarinic receptor concentration was reduced by 22 percent, M_1 receptor concentration was reduced by 18 percent (not a significant difference), and AChE activity was reduced by 60 percent.

DELAYED MATCH-TO-SAMPLE

An additional experiment tested performance in delayed match-to-sample. The apparatus was a rectangular tank filled with water. The start area was located at one end. Two choice areas were located at the other end. In each choice area, there was a goal box. Each goal box had a distinctive

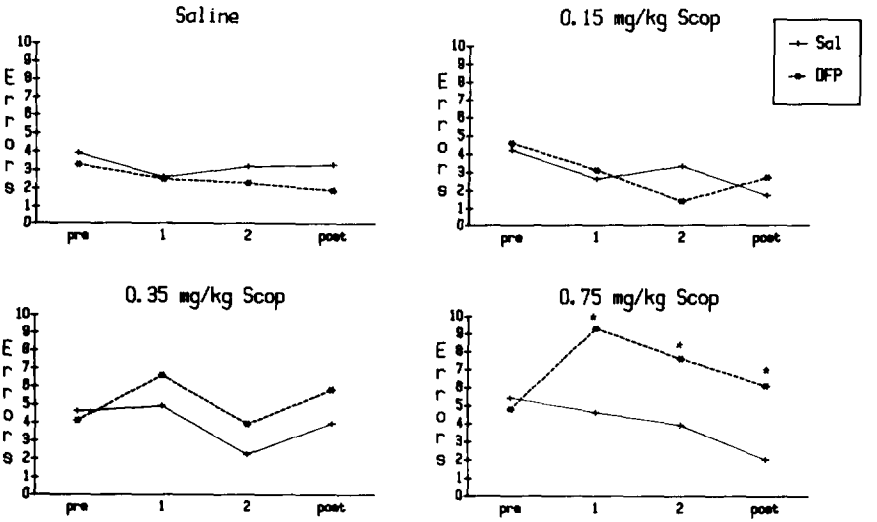

FIGURE 1. *Response of rats to a scopolamine challenge*

NOTE: Rats given a chronic treatment of DFP show an enhanced response to an acute challenge with .75 mg/kg scopolamine. The data are the number of errors made during retention testing when the acute challenge was given before the treatment with DFP ("pre"), during the treatment with DFP (1 and 2, each two injections of scopolamine), and after the treatment with DFP had ended ("post"). As indicated in the bottom right graph, .75 mg/kg scopolamine produced a significant increase in the number of errors for all injections during treatment with DFP and for the two injections after DFP was stopped.

pattern, similar to the ones used in the preceding experiment. One goal had vertical black and white stripes, the other had large black spots against a white background. In the correct goal box, a platform was hidden under the water, allowing the rat to escape. In the incorrect goal box, no platform was available, so the rat could not escape.

Each trial began with a *forced swim,* during which the rat was forced to go to the goal box containing the platform. After reaching the goal box, the rat climbed onto the platform and escaped from the water. For the subsequent *choice swim,* the rat was allowed to go to either goal box. However, only the goal box entered during the immediately preceding forced swim had the platform. Consequently, the correct response for the rat was to return to the goal entered during the immediately preceding forced swim.

Because the preceding experiment had established a dose-response curve and the major goal of the present experiment was to test the generality of the interaction between chronic exposure to DFP and acute exposure to scopolamine, only a single dose of scopolamine (3.0 mg/kg) was used in the

present experiment. One group of rats received chronic treatment with DFP, the other did not. All other aspects of the procedure were similar to those described for the previous experiment.

DFP produced a similar increase in sensitivity to scopolamine. Rats receiving saline had a mean of 68 percent correct during scopolamine test days, rats receiving DFP had a mean of only 51 percent correct.

These results consistently demonstrate a significant increase in sensitivity to a cholinergic antagonist as a result of chronic treatment with DFP, a compound that inhibits AChE, which normally breaks down acetylcholine. DFP produced a downregulation of cholinergic receptors, which in turn produced an increased sensitivity to scopolamine in two different tasks. Consequently the observed phenomenon is probably a general one and may occur in many different situations.

ANIMAL MODELS

Benefits and Risk

Animal models, like all models, have both benefits and risks. The benefits include the opportunity to manipulate and measure parameters that cannot be examined with people. Indeed, the very term "abuse" implies an inappropriate use of drugs, one that should not be undertaken voluntarily by a person, or imposed by one person upon another person. In addition, animals provide the opportunity for important and necessary controls, because all aspects of the experiment can be controlled, including the genetic characteristics, developmental experience, drug exposure, etc.

Animal models also provide important opportunities for carrying out measurements that cannot be conducted with people. Ultimately, drugs interact with elements in the brain, and these neural events are the critical links between the drug and behavior. Although considerable information can be obtained from purely psychological and behavioral analyses, these analyses arc ultimately limited by their inability to examine directly each event that intervenes between the stimulus and the response (the behavioral language) or each of the cognitive modules lying between the input and the output of the system (the cognitive/computational language). Although a variety of strategies have been developed to try to assess the intervening steps in the chain, such steps are fundamentally limited in their ability to access the details of the mechanisms interrelating stimuli and responses.

In contrast, neurobehavioral analyses in animal models can examine directly each element in the chain via manipulation or measurements. An electrode or a cannula can be placed at every point along the pathway between the receptors and effectors, allowing each step to be described completely.

Animal models, like all models, have the risk that the phenomena observed in the model do not generalize to the situation of primary interest, namely human drug abuse. Awareness of these risks has led to considerable effort to create procedures to minimize them. Especially in the analysis of animal models in memory, substantial progress has been made in two directions. First, criteria to evaluate the validity of any given model have been identified. Second, individual models have used these criteria to design procedures that are highly valid (Ingram 1985; Kesner 1985; Olton 1985; Squire and Zola-Morgan 1985). These points are illustrated by some animal models that examine recent memory. Further examples are provided by Kesner (this volume).

Recent memory, as described earlier, is the memory for information presented several minutes to hours previously. In everyday life, it is involved in answering questions such as: What did you have for breakfast? When did you last see your brother? Where did you park your car? In the laboratory, it is usually assessed by giving a person some information to be remembered, such as a list of words, set of pictures, or information in a short story. After a delay interval, during which the person is engaged in other kinds of tasks, retrieval of the information presented earlier is assessed.

Some of the dimensions used for assessing validity and a specific example of a manipulation using that dimension are given in table 3. The discussion below comments briefly on each of these.

TABLE 3. *Types of validity and examples from tests of recent memory*

Type of Validity	Example
Operational	Number of items to be remembered Length of time each item is to be remembered
Psychological	Interference: proactive, retroactive Serial order effects
Ethological	Optimal foraging in natural environment
Neuroanatomical	Hippocampus
Neurotransmitter	Cholinergic system

Validity Assessment

Operational validity describes the specific parameters manipulated in an experiment. These parameters include the amount of information to be

remembered and the delay interval during which the information is to be remembered. As might be expected, choice accuracy decreases as the amount of information and the delay interval increase.

Psychological validity describes the psychological/cognitive/computational processes that mediate performance in the memory tasks. Proactive interference refers to processes that result in negative transfer, so that previously leamed information interferes with currently learned information. Retroactive interference refers to processes that underlie another form of negative transfer, the fact that currently learned information can interfere with previously learned information.

Ethological validity reflects considerations of optimal foraging and ethological analyses of animal behavior. The issue is whether the kinds of problems given to animals in the laboratory are so odd that they distort the normal behavior of the animal. Examination of the kinds of problems faced by animals in their natural habitat has consistently shown that many animals are faced with situations in which recent memory is required. The ability to solve these problems influences the ability of the animal to survive, and different species of animals have developed highly sophisticated foraging strategies to solve these problems (Sherry and Schacter 1987).

Neurological validity refers to the brain mechanisms involved in recent memory. Considerable evidence implicates the hippocampus and associated temporal lobe structures in the circuit for recent memory.

Neurotransmitter validity refers to the transmitter substances involved in recent memory. As mentioned earlier, the cholinergic system is implicated as one of the important neurotransmitters required for normal recent memory (Bartus et al. 1985). Kesner (this volume) provides numerous examples of the similarity in operational manipulation and measurement and psychological/cognitive/computational description. The other references in this chapter provide documentation for ethological, neuroanatomical, and neurotransmitter validity.

A large number of experimental procedures and conceptual frameworks are available to bring to the study of drug abuse. Obviously, some alterations will have to be made to address the issues important in drug abuse. However, the fact that these analyses of animal models have been undertaken so extensively in other situations means that much of the preliminary work has already been completed, and the additions to address issues in drug abuse can proceed quickly and effectively. Finally, of course, the behaviors in these models have the advantage of direct similarity to many of the behaviors of interest in humans. Thus, the results obtained from these models should be more readily applicable to the human situation than the results from models that use behaviors for which the human analog is less

clear, and for which the issues of validity have not yet been as carefully considered.

SUMMARY

Animal models provide important information about strategies that can be used to assess the effects of chronic exposure to drugs or other compounds. Futhermore, when used in conjunction with analyses designed to utilize the many advantages of animal models to examine learning and memory, these kinds of experiments can have significant ramifications for assessing the mnemonic effects of chronic drug use in people. Finally, given that aging is accompanied by significant deterioration of neurochemical systems, which may compromise the functions of those systems, the results from the experiments with DFP predict that changes in learning and memory abilities that are not apparent in young individuals may begin to surface as those individuals age or may appear following any other kind of insult to their nervous system.

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Enkephalin Influences on Behavioral and Neural Plasticity: Mechanisms of Action

Joe L. Martinez, Jr., Patricia H. Janak, Susan B. Weinberger, Gery Schulteis, and Brian E. Derrick

INTRODUCTION

The anatomical basis of memory storage must involve the functional connections of neurons. The concept of circuits of neurons forming the neural substrate of memory was clearly elaborated by Hebb (1949) in his postulation of a dual trace mechanism. In this conception, memory was first encoded as a reverberating cellular assembly of neurons. The activity of this assembly would decrease over time unless exercised by repetition; this assembly formed the basis of short-term memory. Through exercise, this assembly would become a functionally intact system through the growth of synaptic knobs and would become long-term memory (Hebb 1949). Although it is easy to imagine a cellular assembly forming the neural substrate of a memory, this cellular assembly is not of much use on its own. It must be functionally connected to other nervous system components; for instance, an effector system is necessary. In addition, there also must be an input from a sensory or other modality that regulates in some way the activity of the circuit and, hence, determines its strength. Conceptualized in this way, this modulatory input can be recognized as an integral part of the memory storage process.

In this view of learning, nervous system changes most certainly involve specific neural sites of information storage as well as those modulatory inputs that serve to mark the importance of the event to the organism. This chapter will examine the role of enkephalins in both of these processes, beginning with a consideration of [leu]enkephalin (LE) as a component of a modulatory input system and concluding with a discussion of enkephalin influences on neural plasticity in the hippocampus.

ENKEPHALINS AND BEHAVIORAL PLASTICITY

Kety suggested in 1970 that there might be modulatory inputs to the memory trace; that is, an input might act to make memory traces stronger. Of

course, if this modulatory input can make memory traces stronger, then it is possible that this input can make memory traces weaker as well (Martinez 1986). Although the memory trace itself is probably located within the central nervous system (CNS), the inputs that modulate the activity of this trace may originate from locations more widely distributed throughout the body. Extensive evidence supports the role of some hormonal systems as modulators of learning and memory, such that administration of low doses of a hormone may increase or decrease the strength of training. These hormones may therefore play a role in normal learning by providing the modulatory input that influences the strength of a memory trace.

An examination of the opioid peptide, LE, in the periphery suggests that it may be a stress hormone as well as a learning modulatory hormone (McGaugh and Martinez 1981; Martinez et al. 1988b). For example, the enkephalins are coreleased with the adrenal medullary hormones epinephrine and norepinephrine (Chaminade et al. 1984; Corder et al. 1982; Hanbauer et al. 1982; Viveros et al. 1980); in addition, enkephalins are localized with norepinephrine in sympathetic nerve terminals (Klein et al. 1982). Conditioning studies in animals suggest that LE has a modulatory influence on the memory circuit, and that this modulatory influence originates, at least in part, outside the blood-brain barrier (BBB).

Peripherally Administered LE Alters Conditioning

LE influences the acquisition or retention of a variety of conditioned responses in a number of species (deWied et al. 1978; Yamamoto et al. 1982; Rigter 1978; Izquierdo and Dias 1981; Martinez and Rigter 1982; Martinez et al. 1981; Rigter et al. 1980a; Rigter et al. 1980b; Rigter et al. 1981; Martinez et al. 1984; Martinez et al. 1988a). For example, figure 1, panel A, shows the impairing effect of peripheral administration of LE administered prior to the acquisition of a one-way active avoidance response in mice (Schulteis et al. 1988). In these studies, mice are given 10 seconds to avoid delivery of a footshock by crossing to a safe chamber. The number of avoidances an animal makes during 14 training trials is taken as a measure of acquisition performance.

An examination of these same animals 1 day after training suggests that LE affects learning rather than performance variables. As seen in figure 1, panel B, the animals showing impaired acquisition on the first day of training still exhibited impaired performance in the same paradigm 24 hours later. Thus, the interaction of the peptide treatment and the training experience produced an as-yet-unidentified enduring effect that outlasts the residence time in the body of the exogenously administered LE.

LE produced a U-shaped dose-response function (figure 1) in this study, as it does in most studies; that is, doses lower and higher than the behaviorally active dose had no effect. This type of function is commonly seen in

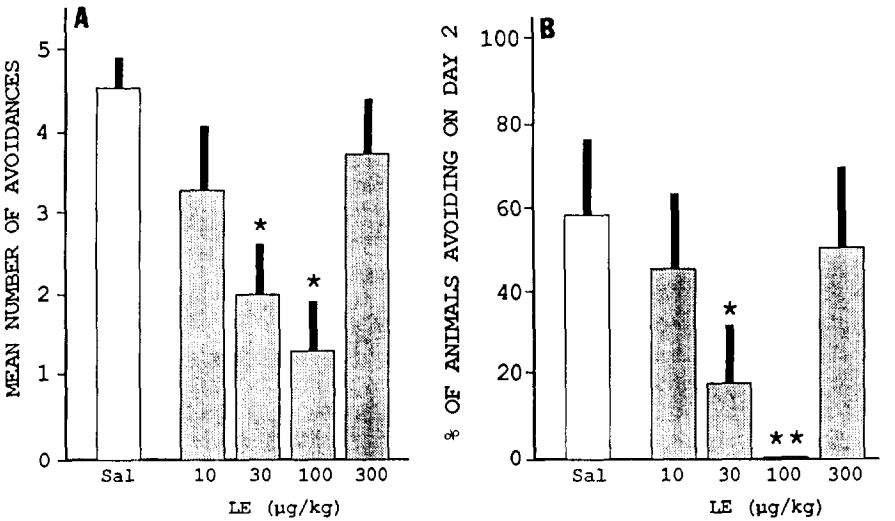

FIGURE 1. *LE dose-response functions*

*p<.01

**p<.0001

NOTE: A LE impairs acquisition of a one-way active avoidance task in mice at doses of 30 and 100 µg/kg. Saline control group. n=26; all LE groups, n=10 to 12. B. These same doses (30 and 100 µg/kg.) result in poor retention when animals are given a one-trial retention test 24 hours after injection and training.

SOURCE: Adapted from Schulteis et al. 1988, copyright 1988, American Psychological Association.

studies examining the effects of hormone administration on conditioning and is consistent with the proposed modulatory influence of LE on the actual memory trace (see Martinez 1986 and Koob 1987 for further discussion of U-shaped curves and learning and memory).

In addition to mice, enkephalin effects on conditioning are seen in three strains of rats (Rigter et al. 1980a; Rigter et al. 1981; Martinez et al. 1985a), in chicks (Patterson et al. 1989), and in primates (Olson et al. 1979; Olson et al. 1981). Therefore, LE conditioning effects exhibit considerable generality across species.

LE's conditioning effects also exhibit generality across tasks. For example, LE impairs Y-maze conditioning in mice. In a discriminated Y-maze shock-escape task, mice receiving a pretraining injection of 100 µg/kg. LE made significantly more errors than saline-treated control animals (Martinez

et al. 1984). Likewise, in an appetitively motivated Y-maze discrimination task, posttraining injections of LE (300 μg/kg) impaired retention of the maze response. The impairing effect of LE has also been observed by others in a nonshock-motivated, nonassociative-learning situation in which LE (10 μg/kg IP) injected into rats following habituation training impairs later performance (Izquierdo et al. 1980). However, it is important to note that LE has been reported to produce enhancement of learning and memory as well (Rigter et al. 1980b).

These data suggest that LE's conditioning effects may be important to learning in general, since, in addition to affecting conditioning in many species, LE alters conditioning in both aversive and nonaversive tasks.

Control Studies That Strengthen the Interpretation That LE Affects Learning

All of the conditioning studies described thus far involve multiple-trial tasks during which the experimenter must handle the animal multiple times. Plasma levels of stress hormones such as corticosterone (Ader et al. 1967; Dobrakovova and Jurcovicova 1984) and catecholamines (McCarty and Gold 1981) are elevated in rodents when they are handled. We therefore studied the effects of LE on acquisition of an automated shelf-jump active avoidance response in rats. Handling of the animals in this paradigm occurs only during drug administration and initial placement of the animal into the testing chamber. As in the standard active avoidance paradigm, LE (1 and 3 μg/kg) impairs acquisition of the automated shelf-jump avoidance response (Weinberger et al., in press; Weinberger et al. 1988). Experimenter handling, therefore, probably does not contribute significantly to the effect of LE on behavior in our experiments.

A number of additional control studies strengthen the suggestion that LE affects learning. Since enkephalins are opioid agonists, impaired performance could be due to analgesia seen when they are administered before training in a shock-motivated situation. However, since LE also impairs conditioning in the appetitively motivated Y-maze task, this is unlikely. More direct evidence is supplied by experiments in which the effect of LE on shock sensitivity was examined. LE, at doses up to 10 times the avoidance-impairing doses, injected IP immediately before testing of flinch-jump thresholds, did not produce evidence of analgesia in rats (Rigter et al. 1980b) or mice (Schulteis et al. 1988). Thus, changes in shock sensitivity cannot explain the avoidance-impairing effects of LE.

Since we commonly administer the peptide before training, it is also important to examine possible effects of LE on locomotor activity. In mice, 100 μg/kg LE usually does not alter shock-induced locomotor activity (Martinez et al. 1984; Schulteis et al. 1988; Weinberger and Martinez 1988) although, in one study, 100 μg/kg LE was found to enhance locomotion

(Weinberger and Martinez 1988). In addition, 10 μg/kg LE can enhance shock-induced locomotor activity in rats (Martinez et al. 1985). These findings raise the possibility that motor activity changes could contribute to the avoidance conditioning impairment seen after LE administration. However, an increase in locomotor activity is difficult to reconcile with an overall decrease in the number of avoidances seen in the LE-treated animals.

In summary, the above data suggest that LE can alter the strength of a memory trace. This effect is seen in many species and tasks, suggesting that LE may play an important role in learning in general. How might LE act to modulate learning? The following discussion examines the possible mechanisms involved in the effects of LE on conditioning.

Delta Receptors Mediate the Effects of LE on Conditioning

LE exhibits greater affinity for delta than for mu opioid receptors; LE's affinity for kappa receptors is negligible (Kosterlitz and Hughes 1978; Kosterlitz and Paterson 1985; Paterson et al. 1983). Thus, it is reasonable to hypothesize that the effects of LE on avoidance conditioning are mediated by delta receptors. Indirect support for this hypothesis comes from the finding that a number of opioid peptides and opioid analogs with affinity for mu receptors do not impair acquisition of the one-way active avoidance response when injected peripherally over a wide dose range. These peptides include [d-ala^2-met]enkephalin and [d-met^2,pro^5]enkephalinamide; beta-LPH_{62-65} and beta-LPH_{62-60}; alpha, gamma, and beta endorphin (Rigter et al. 1981); and Tyr-D-Ala-Gly-MePhe-Gly-ol (DAGO) (Schulteis and Martinez 1988).

More direct evidence for the involvement of delta receptors comes from recent studies employing delta-selective agonists and antagonists. As can be seen in figure 2, peripheral injections of [D-Pen^2,D-Pen^5]enkephalin (DPDPE), an enkephalin analog with very high selectivity for delta receptors (Hruby 1986; Mosberg et al. 1983; Corbett et al. 1984; Cotton et al. 1985), impair acquisition of one-way active avoidance conditioning in mice at one-tenth the maximally impairing dose of LE (Schulteis et al. 1988). The greater potency of DPDPE in this study is most likely due to its resistance to the peptidases that rapidly degrade LE and [met]enkephalin (ME) (Corbett et al. 1984; Weinberger and Martinez 1988).

DPDPE also impairs acquisition of an automated shelf-jump avoidance task in male Sprague-Dawley rats (Weinberger et al., in press; Weinberger et al. 1988). Furthermore, chicks receiving DPDPE prior to training on a taste-avoidance task show amnesia when tested 24 hours later (Patterson et al. 1989), although these effects are seen after intracerebral, rather than IP, injection. Like LE, DPDPE produces a U-shaped dose-response curve in all three tasks and species. Evidence to date, therefore, indicates that delta receptor activation can impair conditioning in several tasks and species.

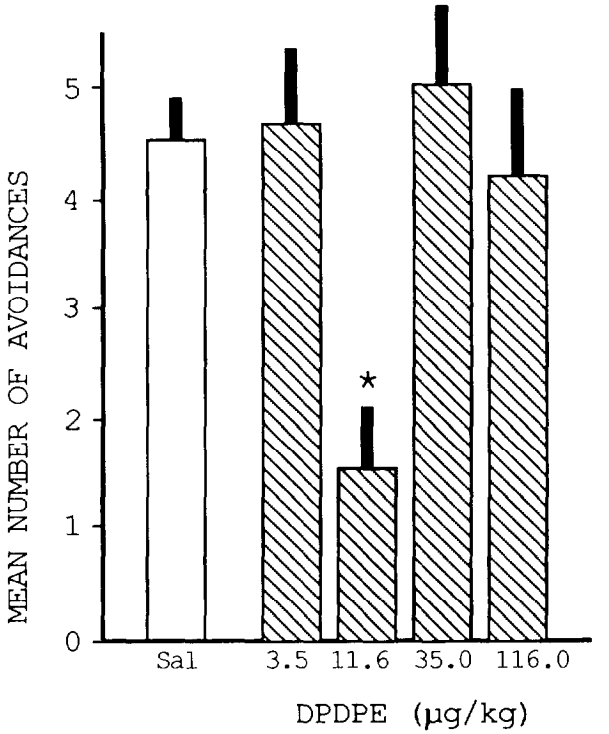

FIGURE 2. *DPDPE dose-response function*

*p<.001

NOTE: DPDPF, a selective delta receptor agonist. impairs acquisition of one-way active avoidance tasks in mice at 11.6 µg/kg. a dose equimolar to 10 µg/kg. of LE; thus DPDPE is more potent than LE in impairing learning (figure 1). Saline control group, n=24; all DPDPE groups, n=10 to 12.

SOURCE: Adapted fmm Schulteis et al. 1988, copyright 1988, American Psychological Association.

Consistent with the above data, ICI 154,129 (Schulteis et al. 1988) and ICI 174,864 (Schulteis and Martinez 1988) two delta-selective antagonists (Cotton et al. 1985; Gormley et al. 1982; Shaw et al. 1982), enhance one-way active avoidance conditioning in mice when injected systemically shortly before training (see figure 3). ICI 174,864 enhances learning at a lower dose than ICI 154,129. a finding that is consistent with the *in vitro* bioassay potencies of these two compounds (Cotton et al. 1985). Thus, activation of delta opioid receptors by selective agonists impairs learning in a manner similar to that of LE, whereas selective blockade of delta receptors has the opposite effect.

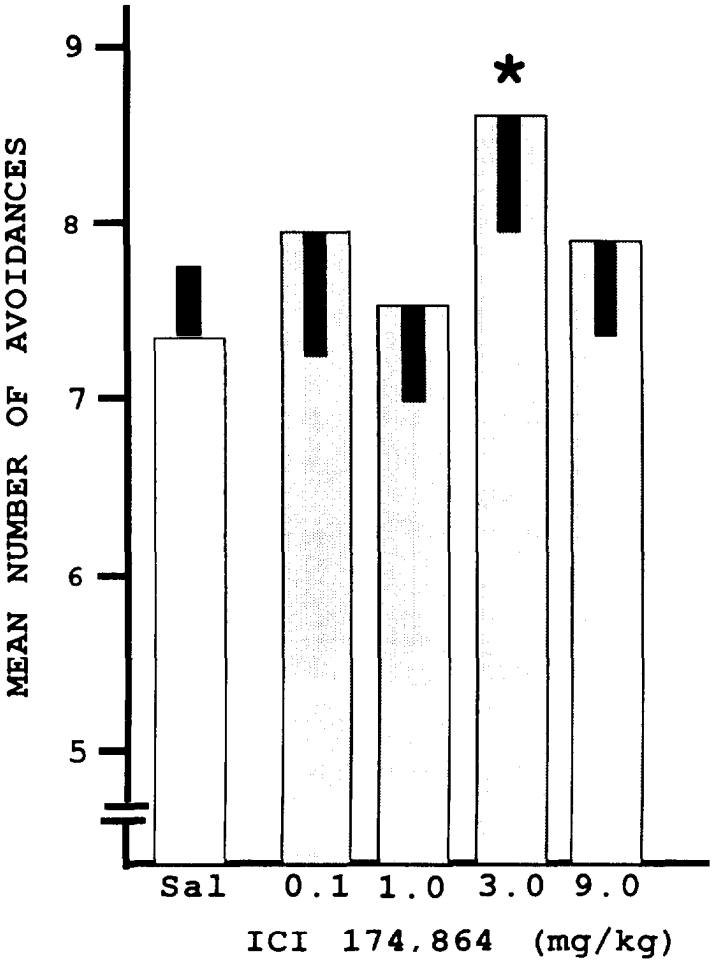

FIGURE 3. *ICI 174,864 dose-response functions*

*p<.02

NOTE: ICI 174,864. a selective delta receptor antagonist, enhances acquisition of one-way active avoidance tasks in mice at 1.0 mg/kg. Thus, blocking delta receptors produces an effect on learning opposite to that seen with stimulation of delta receptors using agonists (figures 1 and 2). Saline control group, n=17; all ICI 174,864 groups. n=15 to 19.

SOURCE: Developed from data presented in Schulteis and Martinez 1988

Perhaps the most compelling evidence that delta receptors mediate LE's conditioning effects comes from recent studies in which the effects of LE

on avoidance responding in mice were reversed in a dose-dependent manner by the concurrent administration of ICI 174,864 (Schulteis and Martinez 1988). A comparison of figures 3 and 4 reveals that a 1 mg/kg dose of ICI 174,864 that completely reverses the impairment produced by LE is without effect when administered by itself. This suggests that the reversal of LE's effect is due to a competitive receptor antagonism. The findings in mice are supported by studies in chicks, in which the amnesia for taste-avoidance training produced by LE is blocked by concurrent administration of ICI 174,864 (Patterson et al. 1989). Taken together, these studies provide strong evidence that the effects of LE on avoidance conditioning involve delta receptor activation.

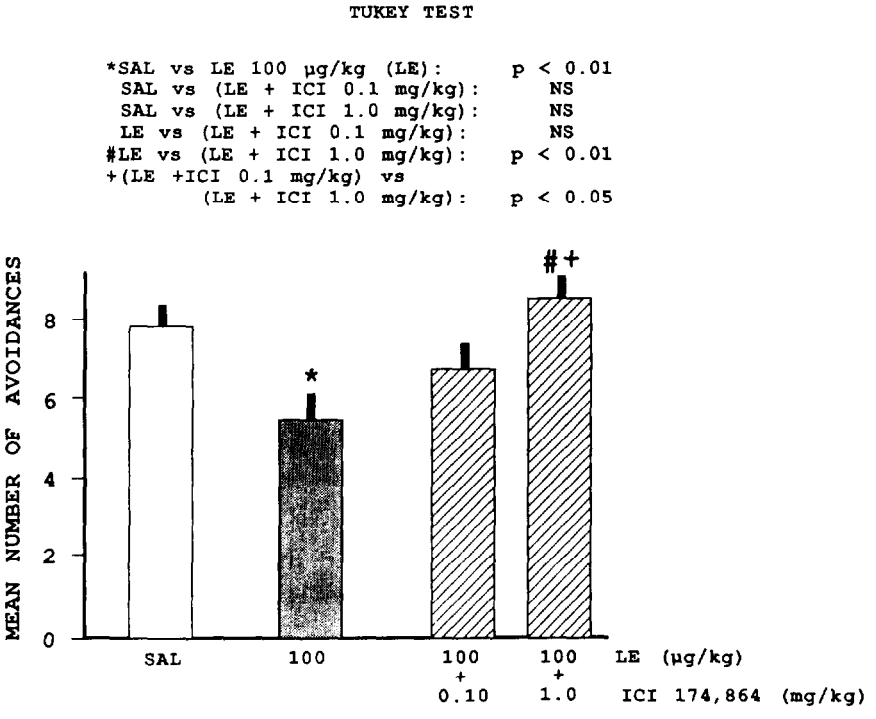

FIGURE 4. *Reversal of LE effects by ICI 174,864*

NOTE: The effects of LE (100 µg/kg) on one-way active avoidance acquisition in mice are attenuated by concurrent administration of a 0.10 mg/kg dose of ICI 174,864 and are completely reversed by a 1.0 mg/kg dose of ICI 174,864. All possible pairwise comparisons were made using a Tukey test, and the results are summarized in the legend above the histogram. Saline control group, n=16; all other groups, n=14 to 16.

SOURCE: Developed from data presented in Schulteis and Martinez 1988

LE Affects Conditioning Through a Site Outside the BBB

As the above evidence indicates, either peripheral or central administration of LE affects conditioning. This suggests that both central and peripheral opioid systems influence learning. The discussion below will focus on peripheral mechanisms of action following IP administration of LE.

Enkephalins affect avoidance conditioning when administered peripherally in microgram doses. When given in these amounts, it is unlikely that LE crosses the BBB in sufficient quantities to be of physiological significance. Studies measuring penetration of LE into the brain using the brain uptake index (BUI) failed lo reveal significant entry of LE into the CNS (Pardridge and Mietus 1981; Cornford et al. 1978; Zlokovic et al. 1985). Furthermore, LE is rapidly hydrolyzed by aminopeptidases present on the walls of brain microvessels (Pardridge and Mietus 1981; Zlokovic et al. 1985), and any radioactivity found in the brain following peripheral administration of labeled LE is almost entirely in the form of free tyrosine. The preponderance of evidence to date suggests that LE crosses the BBB poorly, if at all, and, therefore, effects on conditioning seen following peripheral administration of LE likely occur at a site outside the BBB (for other interpretations see Kastin et al. 1976; Zlokovic et al. 1987).

A number of behavioral pharmacology studies support the notion that LE acts to impair conditioning at a site or sites outside the BBB. Intracerebroventricular ((ICV) administration of LE fails to alter passive-avoidance retention (Belluzzi and Stein 1981), active avoidance learning (Martinez et al. 1985a), and extinction of a pole-jump response (Yamamoto et al. 1982). Similarly, D-ala^2-met-enkephalinamide, an analog of ME, impairs acquisition of a conditioned heart-rate response in rabbits when injected directly into the amygdala, but equivalent doses of the LE analog D-ala^2-[D-leu] enkephalin (DADLE) are without effect (Gallagher et al. 1982).

The conditioning effects of LE are attenuated by opioid antagonists that do not cross the BBB. Mcthylnaloxonium, a quatemary form of naloxone with limited ability to cross the BBB (Brown et al. 1983), attenuates the effects of LE on active avoidance conditioning (Martinez et al. 1985b) and place preference conditioning (Heinrichs and Martinez 1986) in mice. Similar results were reported by Introini et al. (1985), who found that a quatemary form of naltrexone, naltrexone methyl bromide, reversed the impairment of passive avoidance retention produced by posttraining injections of ME or LE in mice. Thus, the lack of effect of centrally administered LE, combined with the reversal of the effects of peripherally injected LE by quaternary antagonists, strongly suggests that the receptors that mediate the effects of LE on conditioning lie outside the BBB. The precise locations and nature of these opioid receptors are currently under investigation.

The Role of Endogenous Peripheral Enkephalin Systems in the Conditioning Effects of LE

Administration of quaternary antagonists prevents endogenous opioids access to their receptors in the periphery and, therefore, allows for an assessment of the role of these endogenous peptides in learning and memory. In this regard, it is interesting to note that methylnaloxonium enhances acquisition in Y-maze and one-way active avoidance conditioning tasks in mice (Martinez and De Graaf 1985; Mendieta and Martinez 1988), as well as enhancing retention of this same active avoidance task in mice (Mendieta and Martinez 1988). Mendieta and Martinez (1988) also report that naltrexone methylbromide enhances both acquisition and retention of one-way active avoidance conditioning, whereas passive avoidance in mice (Introini et al. 1985) and conditional opioid analgesia in rats (Calcagnetti et al. 1987) are unaffected by peripheral injedions of naltrexone methyl bromide. These different results may reflect differences in the conditioning tasks employed. Although these results require further investigation, the data suggest that, in some cases, preventing access of opioids to their receptors in the periphery produces an action opposite to that seen with exogenously administered LE. This provides evidence that endogenous opioid systems normally participate in the acquisition and retention of conditioned responses. Since neither mcthylnaloxonium nor naltrexone methyl bromide is notably selective for specific opioid receptor types, these results do not indicate which endogenous opioid peptide ligand or ligands are important.

However, studies using an antiserum to LE indicate that LE is an endogenous peptide important in avoidance conditioning. Passive immunization of endogenous peripheral LE systems enhances acquisition of active avoidance conditioning in mice (Martinez et al. 1985), an effect qualitatively the same as that observed with methylnaloxonium and opposite that produced by LE. This suggests that an important source of endogenous LE exists in the periphery and that this LE normally acts to impair conditioning.

The source of this LE might well be the adrenal medulla (Yang et al. 1980; Pelto-Huikko et al. 1985; Schultzberg et al. 1978; Viveros et al. 1980). Martinez and Rigter (1982) found that the effects of both ME and LE were abolished by adrenal medullectomy (ADMX). Tenfold to a hundredfold increases in the dose of LE restored its impairing action in demedullated rats, suggesting that ADMX had removed an endogenous source of LE that normally acts in concert with the injected peptide. In contrast, the effect of ME could not be restored. In summary, the available evidence suggests that peripheral enkephalin systems normally act to modulate conditioning and that an important source of these enkephalins might be the adrenal medulla. Since hormones are distributed to their targets by the circulatory system, the findings discussed thus far suggested that we examine LE in the blood circulatory system.

Changes in Plasma LE Concentrations With Conditioning May Be Associated With a Regulatory Enzyme System

The changes in conditioning seen with systemically injected enkephalins suggest that peripherally located endogenous enkephalin systems may participate in regulating an animal's response to a conditioning situation. In accordance with this suggestion, evidence has been found in rats for a plasma enzyme system whose activity, as measured by LE hydrolysis, is highly correlated with performance in an active avoidance conditioning task. As seen in figure 5, a correlation of 0.82 was measured between the latency of rats to escape on the first active avoidance trial and the rate at which LE is hydrolyzed in plasma (Martinez and Weinberger 1988). Of particular interest are the changes we observed in the activity of this enzyme system following the conditioning experience. Following the first avoidance trial and shock exposure, hydrolysis activity in plasma is significantly altered (figure 6) (Martinez and Weinberger 1988). Importantly, both increases and decreases in hydrolytic activity were seen. These data point to the existence in plasma of a regulatory enzyme system that modulates behavior, and, accordingly, we have begun to characterize the enzymes that hydrolyze LE in rat plasma and that may be involved in this modulatory relationship with conditioning behavior.

Rat plasma was found to have its own unique pattern of enkephalin hydrolysis. These findings are based on differences in total metabolite accumulation measured in the presence or absence of selective peptidase inhibitors, as assayed by thin layer chromatography. Blood was collected through an indwelling femoral artery cannula. Eighty-five to 90 percent of the hydrolysis of LE is attributed to the combined action of aminopeptidase M (E.C. 3.4.11.2) and angiotensin-converting enzyme. The remaining hydrolysis of LE in plasma is at present unaccounted for; it does not involve "enkephalinase" (E.C. 3.4.24.1) or aminopeptidase MII activity, but it could include dipeptidyl aminopeptidases (Weinberger and Martinez 1988). These data suggest that either aminopeptidase M or angiotensin-converting enzyme potentially could be involved in the relationship observed in rats between the rate of LE hydrolysis in plasma and performance in an avoidance-conditioning situation.

LE is Rapidly Metabolized After Peripheral Injection

In order to understand the mechanisms responsible for the effects of LE on conditioning, it is important to characterize the fate of LE after peripheral injection. Recent studies have examined the kinetics of uptake and metabolism of radiolabeled LE and DADLE following IP administration in rats (Martinez et al. 1988a; Schulteis et al., in press). Interestingly, 1 minute following IP injection, 95 percent of the LE in plasma is in the form of metabolites. By 2 minutes a plateau is reached, at which approximately 97.5 percent of the LE is metabolized.

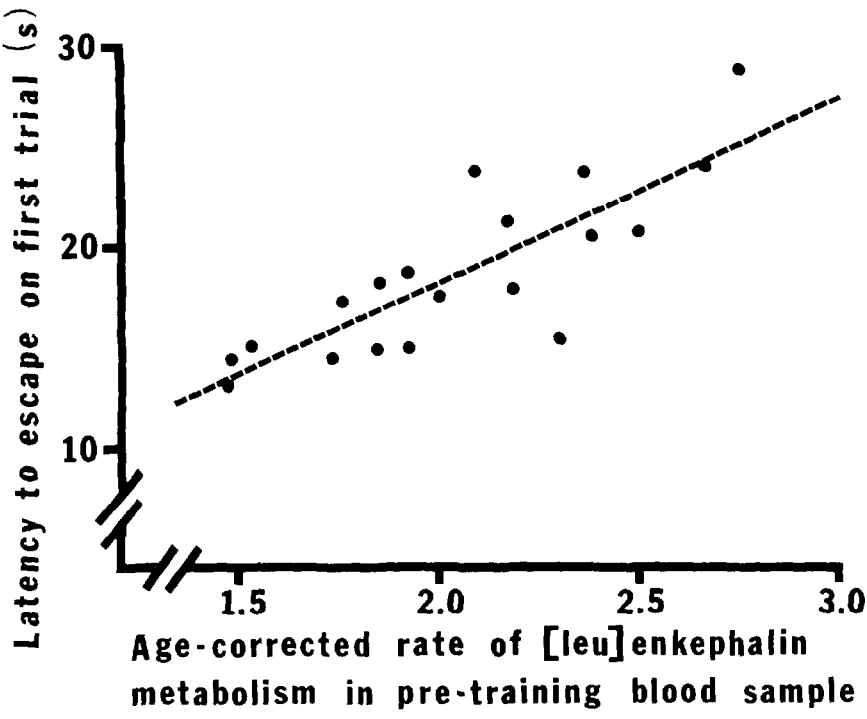

FIGURE 5. *Correlation between escape latency and LE metabolism*

NOTE: Corellation between the latency of rats to escape on the first trial of a one-way active avoidance task and the amount of LE metabolized in 1 minute, as corrected for age. in plasma collected through an indwelling femoral artery cannula immediately before the conditioning trial; r(19)=.82, p<.001. Correlation between age and LE metabolism rate: r(19)=.58, p<.005.

SOURCE: Martinez and Weinberger (1988), copyright 1988, American Psychological Association.

In contrast, LE is 50 percent metabolized by 2.5 minutes in plasma *in vitro* (Martinez et al. 1988a; Martinez and Weinberger 1988; Schulteis et al., in press). DADLE is metabolized more slowly than LE following IP administration. The percent of DADLE that is metabolized at 1 minute is 12.7 percent, and, at 15 minutes, it is 63.7 percent. Like LE, DADLE is much more rapidly hydrolyzed *in vivo* (63.7 percent at 15 minutes) than in plasma *in vitro* (16 percent at 150 minutes) (Martinez et al. 1988; Schulteis et al., in press).

Because the *in vivo* metabolism of both enkephalins following IP injection is much more rapid than in plasma *in vitro,* it is highly unlikely that plasma enzymes alone account for the rapid hydrolysis of these peptides after

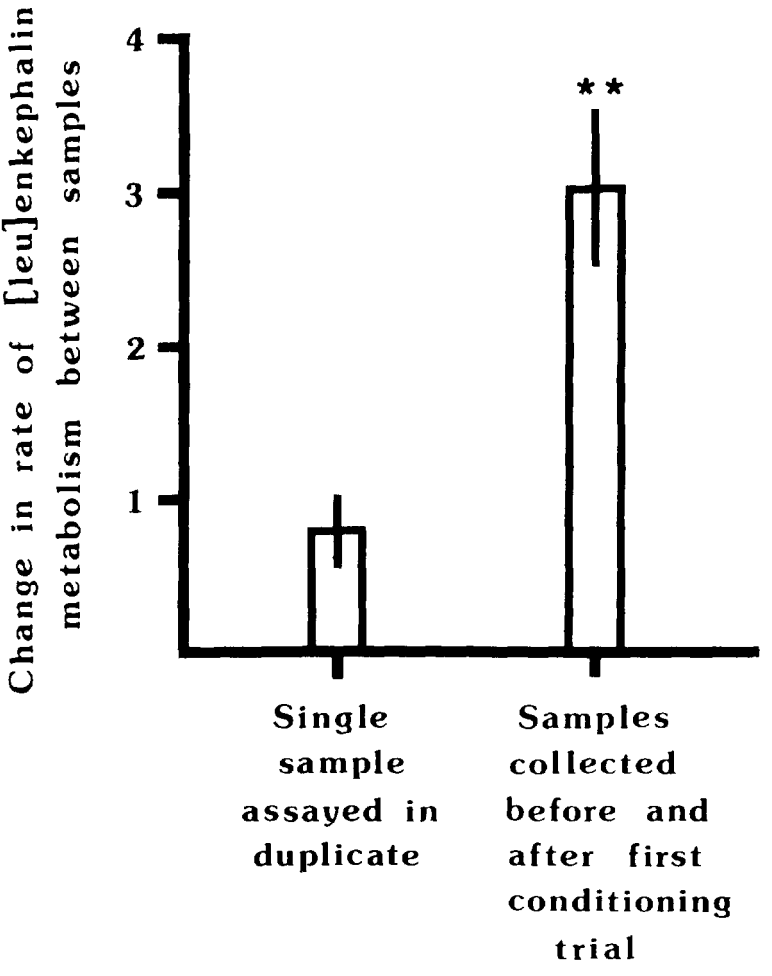

FIGURE 6. *Changes in LE metabolism associated with conditioning*

NOTE: Changes in percentage of LE metabolized between blood samples collected from rats through an indwelling femoral artery cannula (mean absolute value of the difference between samples, as corrected for age by analysis of covariance, ± SEM). Control group: difference in enzyme activity between sample pairs when a single sample was divided in half for duplicate assay. n=12. Experimental group: difference in enzyme activity in blood samples collected immediately before and after the first conditioning trial, n=18. Analysis of covariance with age as covariate: F(l.27)=10.61, p=.003.

SOURCE: Martinez and Weinberger (1988), copyright 1988, American Psychological Association.

peripheral administration. In accordance with this suggestion, we found that bestatin (10 mg/kg), an inhibitor of aminopeptidase M, a major participant

in the hydrolysis of LE, inhibits LE hydrolysis more effectively when administered IP along with LE than when it is injected intra-arterially about 50 seconds before an IP injection of LE. This indicates that a major portion of LE hydrolysis occurs as LE crosses membranes on its way from the site of injection into the bloodstream.

These results have important implications for studies of the behavioral effects of LB following its IP administration. First, although the injected peptide, when administered at a behaviorally active dose of 3 μg/kg, is 95 to 98 percent metabolized at all time points after injection, the amount of LE remaining intact in plasma is within the range of endogenous plasma concentrations of LE (30 to 120 pg/ml plasma) (figure 7) (Martinez et al. 1988a; Martinez et al. 1988b). This suggests that a behaviorally active dose of LE achieves a plasma LE concentration that is within a normal physiological range. Likewise, a dose of DADLE (0.6 μg/kg) that is approximately one-fifth the dose of LE on a molar basis yields plasma levels of intact peptide that range from 2.4 to 5.1 times the levels achieved by LE across the 15-minute sampling interval, consistent with the greater potency of DADLE in conditioning studies (figure 7) (Rigter et al. 1980a; Rigter et al. 1980b). This indicates that the effects of LE on learning are not simply due to the pharmacological effects of large doses of injected peptide, but rather may mimic the action of endogenous peripheral enkephalin systems. A second important consideration of the metabolism data described above is the possible contribution of enkephalin metabolites to the observed behavioral effects of LE. This possibility is discussed in detail in the following section.

Some LE Metabolites Impair Active Avoidance Conditioning

De Wied and coworkers were the first to suggest that metabolic fragments of peptides may have biological activity (Burbach et al. 1983). Since LE is rapidly degraded after release, the question of whether any LE degradation products can also influence an animal's behavior becomes important. We examined the effects of a number of LE metabolites on conditioning in mice. In an appetitively motivated Y-maze task, LE (300 μg/kg) but not its metabolite des-tyr-LE (Gly-Gly-Phe-Leu (GGPL)) impairs retention (Linden and Martinez 1986). Similarly, in the active avoidance task, LE (100 μg/kg) impairs acquisition, while GGPL is without effect (Weinberger and Martinez 1988). Since GGPL has less than one-thousandth the opioid potency of LE, as measured in bioassays and binding assays (Hambrook et al. 1976). these findings are to be expected if LE's effects on conditioning are mediated through opioid receptors.

In contrast, three other potential LE metabolites impair acquisition in the one-way active avoidance paradigm (table 1). The enkephalin metabolites Tyr-Gly (TG), Tyr-Gly-Gly (TGG), and Tyr-Gly-Gly-Phe (TGGP) all impair acquisition of the active avoidance response in mice (Janak et al. 1987;

Weinberger and Martinez 1988). Both TG and TGGP impair avoidance conditioning at a dose equimolar to the behaviorally active 100 µg/kg dose of LE; TGG is effective at a dose equimolar to the 30 µg/kg dose of LE.

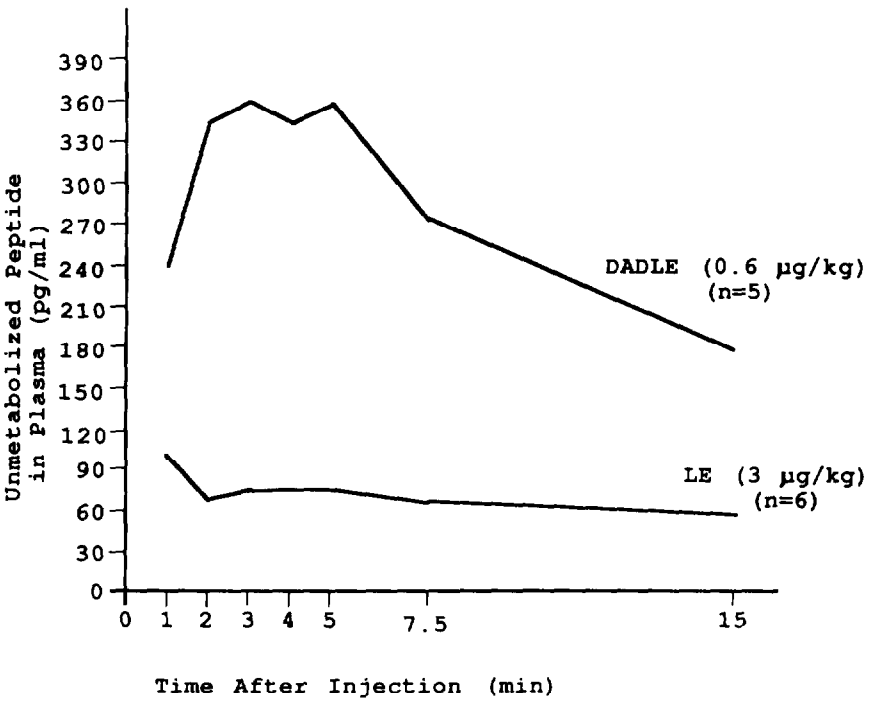

FIGURE 7. *Concentrations of unmetabolized LE and DADLE in plasma as a function of time after IP injection*

SOURCE: Developed from data presented in Martinez et al. 1988.

In addition, as the table indicates, the dose-response function for the impairing effect is consistently U-shaped. Thus, three potential metabolites of LE influence conditioning in a manner similar to that of the parent pep tide. Neither GGPL nor tyrosine itself (Janak and Martinez in preparation) has an effect on avoidance conditioning when tested over a wide dose range. These results together indicate that the TG portion of the LE molecule is the minimum sequence necessary for producing acquisition impairment.

As table 1 illustrates, the avoidance impairments produced by TG and TGG are not attributable to drug-induced changes in locomotor activity. Animals that received the impairing dose of TG or TGG prior to testing for shock-induced locomotor activity in an open field did not differ from saline-control animals (Janak et al. 1987; Weinberger and Martinez 1988). However, animals that received the avoidance-impairing dose of TGGP showed a decrease in shock-induced locomotor activity (Janak et al. 1987). Therefore, the avoidance-impairing effects of TGGP may be secondary to the activity decreases produced by the metabolite. Interestingly, both GGPL and free tyrosine also produce a decrease in shock-induced locomotor activity in mice but do not affect conditioning (Weinberger and Martinez 1988; Janak and Martinez, in preparation).

TABLE 1. *Effects of enkephalin metabolites on one-way active avoidance conditioning in mice*

Peptide	Dose Range (μg/kg)	Maximally Effective Dose (μg/kg)	Avoidance Impairment	Decreased Locomotion
Tyr-Gly-Gly-Phe-Leu ([leu]enkephalin)	30-300	100	Yes	No
Tyr-Gly-Gly-Phe	24-80	80	Yes	Yes
Tyr-Gly-Gly	16-160	16	Yes	No
Tyr-Gly	4.3-43	43	Yes	No
Tyr	3.9-390	none	No	Yes
Gly-Gly-Phe-Leu	7.1-710	none	No	Yes

The avoidance impairing effects of some of these LE metabolites are puzzling. As discussed previously, LE has strong affinity for and appears to produce its avoidance impairment through the delta opioid receptor. In contrast, TG, TGG, and TGGP have almost no opioid activity as measured in a guinea pig ileum or mouse vas deferens bioassay (Dewey 1982). The behaviorally active LE metabolites may, therefore, be exerting their effects through some mechanism other than activation of opioid receptors. Such effects are common in the literature, in that other short peptides are observed to produce opioid effects without seeming to activate directly opioid receptors. For example, kyotorphin (Tyr-Arg) and kentsin (Thr-Pro-Arg-Lys) both produce naloxone-reversible analgesia (Takagi et al. 1979a; Takagi et al. 1979b; Satoh et al. 1985; Fox et al. 1987), yet neither peptide appears to occupy opioid receptors. These compounds are hypothesized to exert their opioid effects by inducing release of endogenous enkephalins (Takagi et al. 1979a; Satoh et al. 1985; Fox et al. 1987). An alternative explanation is that the LE metabolites compete with their parent compound for binding to degradative enzymes, as in the model proposed by LaBella et al. (1985), thereby effectively increasing the levels of endogenous peptide. TG, TGG, and TGGP also may increase circulating levels of endogenous LE by binding to the regulatory proteins in plasma that appear to offer LE

some protection from enzymatic degradation (Venturelli et al. 1986). Further investigation is necessary to differentiate among these possible mechanisms.

Summary of LE Effects on Behavioral Plasticity

The above evidence suggests that LE may directly affect memory traces by an action on the modulatory inputs to the neural substrate of memory storage, even though LE itself does not cross the BBB in sufficient quantities to be of physiological significance. One major way to modulate the strength of the memory trace is for the concentration of enkephalin to decrease or increase at its delta receptors that lie outside the BBB. A potential mechanism involved in regulating LE concentrations in the blood and, hence, at its receptors is the enkephalin-hydrolyzing enzyme system in plasma. This enzyme system appears to be able to alter its activity as a result of learning.

ENKEPHALINS AND NEURAL PLASTICITY

Activity within the memory-modulating peripheral systems discussed above must ultimately affect the memory trace. One site in the mammalian CNS that is strongly implicated in memory function is the hippocampus. Synapses in the hippocampus are highly plastic, and the experimental manipulations that change synaptic strength may be quite long lasting. We are investigating the involvement of opioid peptides in long-term potentiation (LTP) (Bliss and Lomo 1973; Bliss and Lynch 1988), a synaptic phenomenon thought to underlie memory storage at the neural level. LTP can be induced by repetitive, high-intensity stimulation of afferents to the hippocampus and dentate gyrus. An important aspect of LTP is that its induction conforms to the idea of a Hebbian synapse. Generally, induction of LTP requires a combination of pre- and postsynaptic activity. Hippocampal synapses will potentiate if, and only if, they are active at a time when the regions of the dendrite where the synapses are located are strongly depolarized (Bliss and Lynch 1988).

Current evidence suggests that induction of LTP in both the CA1 region of the hippocampus and the dentate gyrus involves activation of the N-methyl-D-aspartate (NMDA) receptor and the subsequent entry of calcium postsynaptically (Lynch et al. 1983). This receptor is a voltage-dependent subtype of the glutamate receptor, and it requires both presynaptic transmitter release and postsynaptic depolarization for its activation (Collingridge and Bliss 1985). It thus resembles a conjunctive mechanism of associative memory postulated by Hebb 40 years ago (Hebb 1949), in that the efficacy of the synapse is increased once the NMDA receptor is activated by repetitive stimulation, presumably through the release of a magnesium block (Collingridge and Bliss 1985).

LTP is also observed in the mossy fibers, a major projection to the CA3 region of the hippocampus. The mossy fibers contain a relatively high content of proenkephalin and prodynorphin derivatives (Gall et al. 1981). Release of these derivatives is Ca^{++}dependent (Chavkin and Bloom 1985). LTP of mossy-fiber responses, however, differs from LTP in other hippocampal regions, in that antagonists of NMDA receptors fail to block LTP in the mossy-fiber pathway (Harris and Cotman 1986). This agrees with the finding that the area where mossy fibers terminate has a low density of NMDA receptors (Monaghan and Cotman 1985).

LTP of Mossy-Fiber-CA3 Responses May Be Dependent on Release of Endogenous Opioid Peptides

The action of naloxone on LTP of both the mossy-fiber and commissural-CA3 response in the rat hippocampus *in vivo* was investigated. Under pentobarbital anesthesia, CA3 pyramidal cell responses were evoked both by stimulation of the mossy fibers and by stimulation of the contralateral CA3 region to activate commissural fibers. A 1 µl volume of naloxone (1, 5, or 10 mM) was applied via pressure ejection to the pyramidal layer. Naloxone, at all concentrations, did not attenuate field responses of either the mossy-fiber or commissural pathway. However, LTP of the monosynaptic mossy-fiber response (induced by two l-second, 100-Hz stimulation trains) was blocked by naloxone at concentrations of 5 and 10 mM. LTP of the commissural response was unaffected by naloxone at these concentrations (figure 8) (Derrick and Martinez 1988). Importantly, (+)-naloxone (10 mM) did not block mossy-fiber LTP, indicating that the effect of naloxone is stereospecific and, therefore, mediated by opioid receptors.

Exogenous application of enkephalin and its analogs produces excitation of pyramidal cells, presumably due to an attenuation of recurrent inhibition (Nicoll 1980; Ziegleganslberger et al. 1979; Haas and Ryall 1980; Derrick et al. 1987). The induction of LTP is facilitated when GABAergic inhibition is blocked (Wigstrom and Gustafsson 1985) and application of GABA can prevent the induction of LTP in several hippocampal regions (Scharfman and Sarvey 1985). Naloxone could effectively block the induction of LTP by antagonizing the disinhibitory effects of endogenously released opioid peptides (Martin 1983; Segal 1988). In this view, opioid peptides are necessary for mossy-fiber LTP, but they act indirectly, allowing a sufficient level of postsynaptic depolarization for the induction of LTP via actions on inhibitory mechanisms. GABAergic inhibitory mechanisms are well-established postsynaptic processes (Alger and Nicoll 1982; Bliss and Lynch 1988). If an influence of opioid peptides on GABAergic inhibitory mechanisms is necessary for the induction of mossy-fiber LTP, it would suggest that LTP induction is dependent on postsynaptic mechanisms, similar to the mechanism of induction observed in the dentate gyrus and CA1 region.

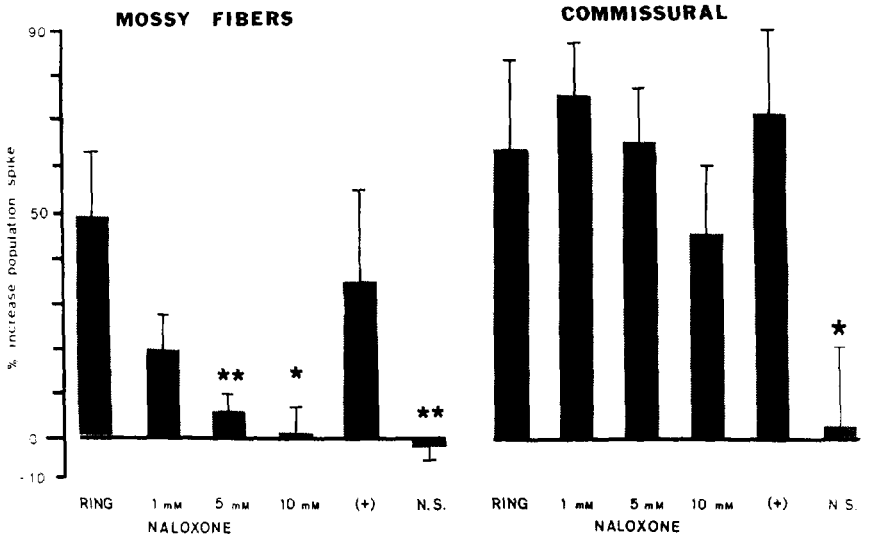

FIGURE 8. *LTP of mossy-fiber and commissural responses following application of 1.0 ul lactated Ringer's or naloxone*

*p<.01.

**p<.001.

NOTE: Each bar represents the average percent increase in population spike 20 minutes posttetanus. (+) Naloxone was at a concentration of 10 mM. n=6 to 12 for each concentration.

SOURCE: Derrick and Martinez 1988.

However, a naloxone blockade of an opioid peptide-mediated disinhibition is unlikely for several reasons. First, our studies indicate that LE and its metabolically stable analog, DADLE, produce excitation of the mossy fiber response without attenuating either feedback (figure 9) (Derrick et al. 1987) or feedforward inhibition (figure 10) (Derrick et al. 1988). Second, if opioid peptides play a role in the initiation of LTP solely via a disinhibitory mechanism, then one would expect opioid antagonists to attenuate LTP in other hippocampal regions where excitatory and, presumably, disinhibitory effects of opioid peptides are observed. However, opioid antagonists do not attenuate LTP in the CA1 field *in vivo* (Stringer et al. 1983; Linesman et al. 1981).

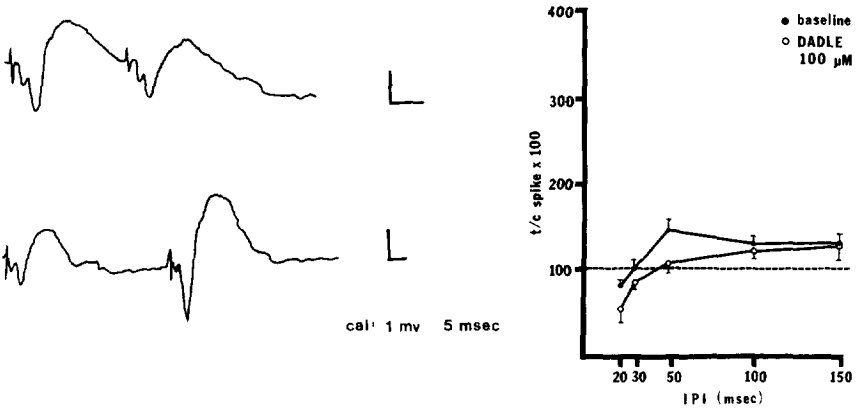

FIGURE 9. *The effect of DADLE (1.0 µl of 100 µM) on attenuation of the mossy fiber-CA3 population spike amplitude in the second (tart) response following paired-pulse stimulation*

NOTE: Ordinate=percent of first population spike amplitude; n=5 for each group.

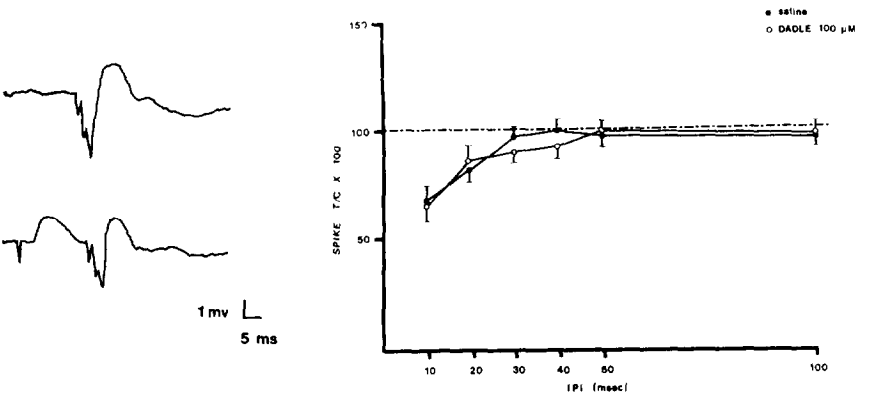

FIGURE 10. *Change in paired-pulse measures of feedforward inhibition following 1.0 µl of 100 µM DADLE*

NOTE: First (conditioning) pulse was at an intensity that did not elicit a population spike, thereby preventing activation of recurrent inhibitory mechanisms.

Opioid Peptides May Play a Direct Role in the Initiation of Mossy Fiber-CA3 LTP

If a disinhibitory influence is not the mechanism of naloxone blockade of mossy fiber-CA3 LTP, then how could opioid peptides serve in the expression of LTP in the mossy-fiber pathway? One possibility is that the excitatory effect produced in the CA3 region by exogenous opioid peptides and, presumably, by those that are released endogenously as well, may be necessary for the induction of LTP in the mossy-fiber pathway. In this view, the excitation produced by endogenously released opioid peptides could allow a level of depolarization sufficient to induce LTP. Recently, enkephalins were shown to block the M-current in CA3 pyramidal cells (Siggins 1988). Through actions on such a mechanism, opioid peptides could effectively amplify the postsynaptic response. Thus, a presynaptic release of opioid peptides could be necessary to produce a level of postsynaptic depolarization sufficient to contribute to the induction of LTP in the mossy fibers.

However, this mechanism needs the specificity of a Hebbian synapse. Opioid peptides do not contribute to mossy-fiber responses during low-frequency stimulation, as evidenced by the lack of an effect of opioid antagonists on single-pulse-evoked mossy fiber responses (Chavkin and Bloom 1985; Dalkara and Krnjevick 1984; Derrick and Martinez 1988). This is similar to the effects observed with NMDA antagonists, which have little effect on hippocampal-evoked responses during low-frequency stimulation (Collingridge and Bliss 1985). The release of some peptide cotransmitters, including opioid peptides, may be dependent on intense or repetitive presynaptic activity (Ishida 1970; Mitchell et al. 1987; Bramham et al. 1988). The release of opioid peptides contingent on intense or repetitive presynaptic activity, such as is needed to induce LTP, coupled with an increase in postsynaptic responsiveness resulting from opioid-peptide actions postsynaptically, may serve as a necessary condition for the induction of LTP at the mossy fiber-CA3 synapse. In this way, mossy-fiber LTP may occur through a unique, opioid-peptide-dependent mechanism of LTP induction within the hippocampus. If the activity of the postsynaptic cell was changed by the conjunction of mossy-fiber activation and the postsynaptic action of opioid peptides, then this could be considered a Hebbian synapse.

Activation of the NMDA receptor is believed to induce LTP by allowing a postsynaptic increase in calcium (Lynch et al. 1983). Similarly, an increase in postsynaptic calcium would presumably be required for induction of LTP in the mossy fibers. Opioid peptides could promote entry of calcium through two possible mechanisms. First, activation of opioid receptors can directly influence calcium conductance (North 1986) which can increase the intracellular concentration of calcium (Henderson 1983). Second, opioid receptor activation could lead to an increase in calcium via the second messenger systems that underlie M-current blockade. A variety of peptides,

including opioid peptides, produce M-current blockade. These peptides usually act via the receptor-stimulated metabolism of phosphoinositides (PI turnover) (Nicoll 1982; Brown 1988). Activation of some opioid receptors has recently been shown to stimulate PI turnover (Satoh et al. 1988). The products of PI turnover can also rapidly release intracellular calcium from nonmitochondrial stores (Nishizuka 1984). Thus, actions of opioid peptides on this second messenger system could block the M-current and allow an increase in intracellular calcium postsynaptically.

Mossy Fiber LTP May Represent a Fundamentally Distinct Form of LTP in the Hippocampus

This distinct form of LTP induction observed in the mossy fibers suggests that LTP in this system may represent a distinct form of synaptic plasticity in the hippocampus. In line with this suggestion are the unique characteristics of mossy-fiber LTP seen in studies using pentobarbital-anesthetized rats. Following tetanic stimulation of commissural afferents, the commissural-CA3 response shows a rapid increase of both the population spike and the field EPSP. LTP of commissural responses is decremental, with a half-life of about 70 minutes *in vivo*. In contrast, mossy-fiber LTP attains maximal amplitude more slowly, reaches a smaller maximal amplitude, and does not decrease over 3 hours posttetanus (Washington et al. 1988). These differences in the decay rate of LTP, in separate afferents to the same population of pyramidal cells, induced with identical stimulation parameters, suggest that distinct mechanisms may underlie both the induction and maintenance of mossy-fiber LTP. These mechanisms are currently being investigated.

Mossy-fiber LTP is also differentially affected by other pharmacological agents. Urethane, at anesthetic doses, was found to prevent the induction of mossy-fiber but not commissural LTP (Washington et al. 1988). Others report that pertussis toxin prevents LTP in the mossy fibers, while potentiation of Schaffer collateral-CAl synapses remains unimpaired (Ito et al. 1988). This toxin selectively inactivates certain G-proteins, and the results suggest that G-proteins may be involved in the induction of mossy-fiber LTP. Interestingly, anesthetics can also interfere with G-protein-receptor coupling (Anthony et al. 1988). Thus, the mechanism of blockade of both urethane and pertussis toxin may be the result of similar actions on specific G-proteins.

In summary, induction of mossy-fiber LTP may be dependent on the release of opioid peptides. The exact role of opioid peptides in the expression of LTP is unknown, but they may contribute to mossy fiber LTP as a result of actions on GABAergic inhibitory mechanisms or postsynaptic mechanisms that augment depolarization. It seems unlikely that opioids affect LTP through a release from inhibition in the mossy-fiber pathway, since opioids do not affect either feedforward or feedback inhibition. The frequency-dependent release and the subsequent postsynaptic effects of these peptides

may function as a Hebbian conjunctive mechanism. as do NMDA receptors in other hippocampal regions.

CONCLUSION

The evidence presented here suggests that enkephalins are involved in two different processes important to learning and memory. Enkephalins may influence the memory trace that represents the actual memory in a manner similar to their effects on plasticity in the hippocampus. Enkephalins may also play an integral role in a hormonal modulatory system; this system influences the modulatory input that regulates the strength of the memory trace. The study of the mechanisms underlying both the memory trace itself and its modulatory input may reveal some basic characteristics of how mammalian nervous systems store information.

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The Effects of Delta-9-THC on Mechanisms of Learning and Memory

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INTRODUCTION

One of the most important concerns regarding drug abuse by the individual in society is the potential impairment in learning and performance while under the influence of these agents. The added threat of long-term exposure to these substances suggests that drug-induced altered neurobiological mechanisms may not only change cognitive and behavioral processes when the drug is present, but also may have persistent effects on these mechanisms; effects that outlast the duration of drug exposure. It is, therefore, important to ascertain the action of abused substances on known neurobiological mechanisms of learning and memory in order to evaluate both cognitive and performance deficits that accrue following short- and long-term exposure to these substances.

Modem neurobiological conceptualizations of learning processes have used information-processing models to approximate the manner in which the brain encodes and retrieves experiences (Hopfield 1984). This approach has been applied to animal models of learning and memory. To the extent that such models achieve a high degree of representativeness of human information processing, they can serve as a neurobiological base for manipulations of neural processes and as a determination of neurophysiological correlates (Donchin et al. 1986). Such processes, however, are not easily identified. In the past, one limitation regarding the appropriateness of animal models for human information processing has been the inability to construct the task for the animal to test the same cognitive function(s) that can be assessed in humans. Recently, however, there have been important breakthroughs in this capability (Kesner and Beers 1988; Olton 1984) that suggest that certain cognitive functions associated with specific brain regions in animal models have similar functions in humans.

The hippocampus has been one of the brain structures most consistently implicated in the neurobiological basis of learning and memory (Thompson

et al. 1983). There is abundant evidence suggesting that this structure is critically involved in many aspects of the spatial and temporal coding of sensory information (Eichenbaum and Cohen 1988). Both human and animal models have recently yielded convergent information showing the requirement of an intact hippocampus to encode and retrieve task relevant sensory information (Winocur 1984). With the current emphasis on behavioral tasks that measure specific types of memory, the precise nature of the memory deficits caused by disruption of the hippocampus and related structures is becoming better characterized (Olton, in press; Squire and Cohen 1984; Mishkin and Petri 1983). An important goal for eventually understanding the manner in which drugs of abuse influence memory processes is knowledge of how the hippocampus processes sensory information and which aspects of hippocampal function are altered by exposure to these substances.

Understanding how drugs of abuse alter performance and cognition in humans may rely greatly on knowledge of how these drugs influence hippocampal mechanisms of learning and memory. If it can be shown that disruptions in performance brought about by drug exposure are related in a dose-dependent manner to alterations in hippocampal function, it is likely that the actions of the abused substance can then be directly related to other forms of hippocampal disruption.

EFFECTS OF DELTA-9-THC ON DETECTION OF SENSORY EVENTS

There are several aspects of learning and memory processes that could be altered by drugs abused in society. Delta-9-THC, the psychoactive ingredient in marijuana, has a long history of being associated with memory impairments in humans (Miller and Branconnier 1983). Delineation of the exact manner in which a substance like delta-9-THC affects learning and performance is, therefore, an important consideration and a first step in determining the neurobiological basis of the memory-disruptive effects of this agent. Such endeavors require determination of the possible influence of delta-9-THC pharmacology on critical stages of information processing and retrieval (Dewey 1986). As a first step, it is important to determine whether delta-9-THC disrupts memory and performance by distracting the animal from attending to the critical features of the environment.

Animals were trained to detect a very short duration (250 milliseconds) tone stimulus that varied in intensity. Tone intensity ranged across seven different levels, with the midpoint anchored each day at the behavioral detection threshold (defined as 50 percent responding). This paradigm generated a series of input-output curves ranging from zero correct at the lowest intensity (no tone) to 96 percent correct responses to the highest tone intensity (figure 1). In addition, it was possible to determine the relationship of hippocampal activity to behavioral detection of tone stimuli. In control

sessions, sensory-evoked potentials were present in the hippocampus only on those trials in which the tone stimulus was detected (responded to) behaviorally but not when the stimulus was undetected. When the tone was responded to, a long (onset) latency (70-millisecond) component (N2) of the well-characterized averaged stimulus-evoked potential was registered in the dentate gyrus of the hippocampus (Deadwyler et al. 1981; Deadwyler et al. 1985). The acute effects of delta-9-THC and recovery from that exposure were examined on the behavioral aspects of tone detection in this task.

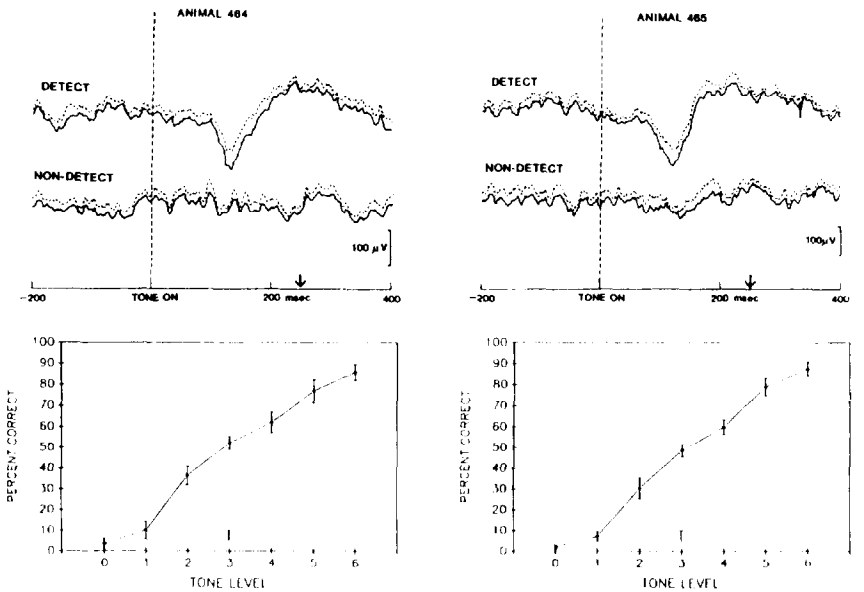

FIGURE 1. *Behavioral performance curves and hippocampal correlates of auditory signal detection*

NOTE: Upper: Hippocampal sensory-evoked potentials OM AEPs from two different animals averaged over tone level 3 (below) and sorted on the basis of behaviorally defected (responded to) and nondetected stimuli. The upper trace shows averages from detect trials only in which a typical sensory-evoked potential recorded from the outer molecular layer of the dentate gyrus is present. The bottom trace indicates the absence of sensory-evoked activity on nondetect trials at the same tone level (3) on trials in which the tone was not detected behaviorally. Each trace is the result of over 50 averaged single-trial potentials. The dotted trace represents the standard error over all averaged trials. Bottom: Behavioral performance curves for each of the seven tone-intensity levels for each animal. Level 3 is designated as threshold (T) on the basis of a 50-percent response criterion. Note, however, that tone level 2 was detected 30 percent of the time, and levels 4 to 6 were detected more than 50 percent of the time. Bars represent ± SEMs over all sessions.

Delta-9-THC had a marked effect on behavioral performance in this task. Figure 2 shows the effects of delta-9-THC for each of four different dose levels. The 0.5 mg/kg dose was not effective in altering sensory detection thresholds, while doses of 1.0, 1.5, and 2.0 mg/kg systematically increased detection threshold and decreased performance accuracy. Importantly, these changes in detection were not accompanied by alterations in the latency to respond to the tone stimulus on detection trials at any dose level. The effects of delta-9-TIlC were, therefore, not the result of a direct motor impairment that incapacitated responding. Since the performance curves were shifted to the right as a function of increasing dose of administration, the decrease in behavioral sensitivity appeared to be linearly related to increasing systemic levels of delta-9-THC. At the higher doses of delta-9-THC (1.5 mg/kg), there was no recovery of the tone detection behavior within 2 to 4 hours after injection (figure 2). Preliminary evidence showed that the change in detection threshold was accompanied by a decrease in amplitude and appearance of hippocampal sensory-evoked potentials under the influence of delta-9-TllC.

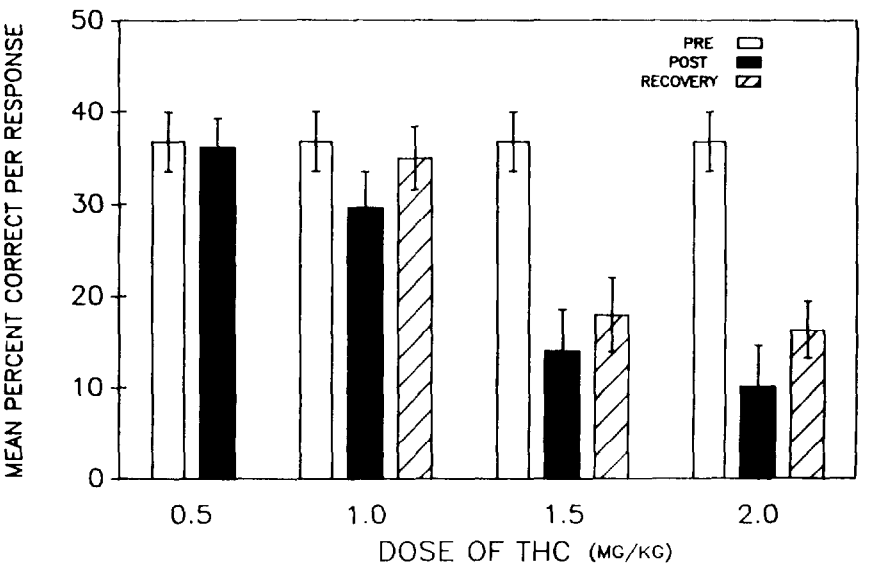

FIGURE 2. *Dose-response effects of delta-9-THC on signal detection performance during predrug, postdrug, and recovery sessions*

NOTE: Mean percent correct responses for each dose level are shown averaged over all tone-intensity levels. Note that significant recovery did not occur following 2 to 4 hours, the 1.5 and 2.0 mg/kg doses. Bars indicate SEMs.

These findings show that delta-9-THC raised the threshold for detecting auditory stimuli in a dose-dependent manner. The deficit in tone detection produced by delta-9-THC can be described as due to a decrease in recognition, since there were no changes in latency to respond. This leads to the question of whether or not delta-9-THC selectively influenced the ability of animals to discriminate as a function of dose levels that produce the recognition deficit.

EFFECTS OF DELTA-9-THC ON TONE DISCRIMINATION PERFORMANCE IN RATS

In previous studies, delta-PTHC produced a dose-dependent disruption of auditory discrimination performance in rats. The deficit was manifested as a decreased responding to the positive (i.e., reinforced) tone in a successive (two tone) discrimination paradigm. This deficit was not accompanied by an increase in responding to the negative (unreinforced) tone (Campbell et al. 1986a). The decrease in discrimination performance in this paradigm was accompanied by a significant dose-dependent increase in behavioral latency (figure 3). Even though the task can be performed at about 90 percent efficiency in animals with hippocampal lesions, this behavioral paradigm is sufficient to evoke considerable task-related changes in extracellularly recorded hippocampal synaptic and cellular activity (Deadwyler et al. 1981; Deadwyler 1985). Hippocampal electrical correlates of performance accuracy were disrupted during behavioral impairment by acute injections of delta-9-THC. An intraperitoneal dose of 0.5 mg/kg of delta-9-THC produced minimal alterations in behavior and in the components of sensory-evoked synaptic processes, as well as in granule cell discharges to the conditioned tone stimulus. As in the tone detection task, a dose of 2.0 mg/kg produced a marked (60 percent) disruption in behavioral performance and in hippocampal tone-evoked electrical activity (figures 3, 4, and 5), while 0.5 mg/kg had little effect. Baseline (predrug) levels of discrimination performance and hippocampal electrical activity recovered within 2 to 4 hours after a single acute injection (Campbell et al. 1986a; Campbell et al. 1986b) for all dose levels.

The changes manifested in hippocampal electrical correlates during the two-tone discrimination task were similar over the same dose range as the behavioral deficits. However, a marked specificity of the delta-9-THC influence on hippocampal processes was shown by the differential nature of the effect on the amplitudes of two separate components of the well-characterized tone-evoked potential (figure 4). Recently, it has been determined how such changes in sensory-evoked potential amplitudes were produced by delta-9-THC. The effects were the result of changes in the sequential dependency of trial-to-trial amplitude fluctuations in the hippocampal sensory-evoked potentials (Deadwylcr et al. 1985). Fluctualions in amplitude of these components are predictable in the sense that they are determined by the preceding sequence of trials and not by the

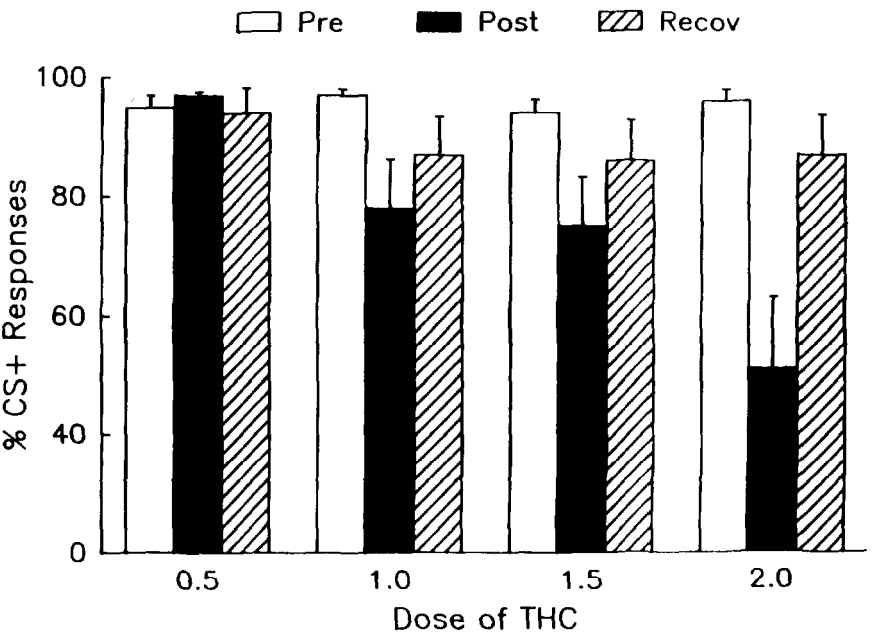

FIGURE 3. *Dose-response effects of delta-9-THC on dual tone discrimination performance for predrug, postdrug, and recovery sessions as shown in figure 2*

NOTE: Mean percent responses to the positive conditioned stimulus (CS+) tone are shown. Bars indicate SEMs.

actual tone stimulus (positive or negative) presented on any given trial (West et al. 1982). Thus, runs of positive or negative tones produce respective decreases or increases in amplitude of the early (N1) component of the sensory-evoked potential relative to its (intermediate) amplitude on single-alternating trials (figure 6). Double-alternating trials produce maximal and minimal fluctuations in N1, presumably because the sequence appears to oscillate between "runs" of positive or negative trials (i.e., two like trials in a row). This sequential dependency suggests that information is continually processed on a trial-by-trial basis and that the temporal context in which a particular trial occurs determines the magnitude of its effect on hippocampal cellular and synaptic activity (Foster et al. 1988).

Since delta-9-THC had a pronounced dose-dependent influence on hippocampal electrical correlates, it is likely that the disruption resulted from a change in the sequential dependency of the electrical events. This was examined using previous methods of sorting and combining single trial data.

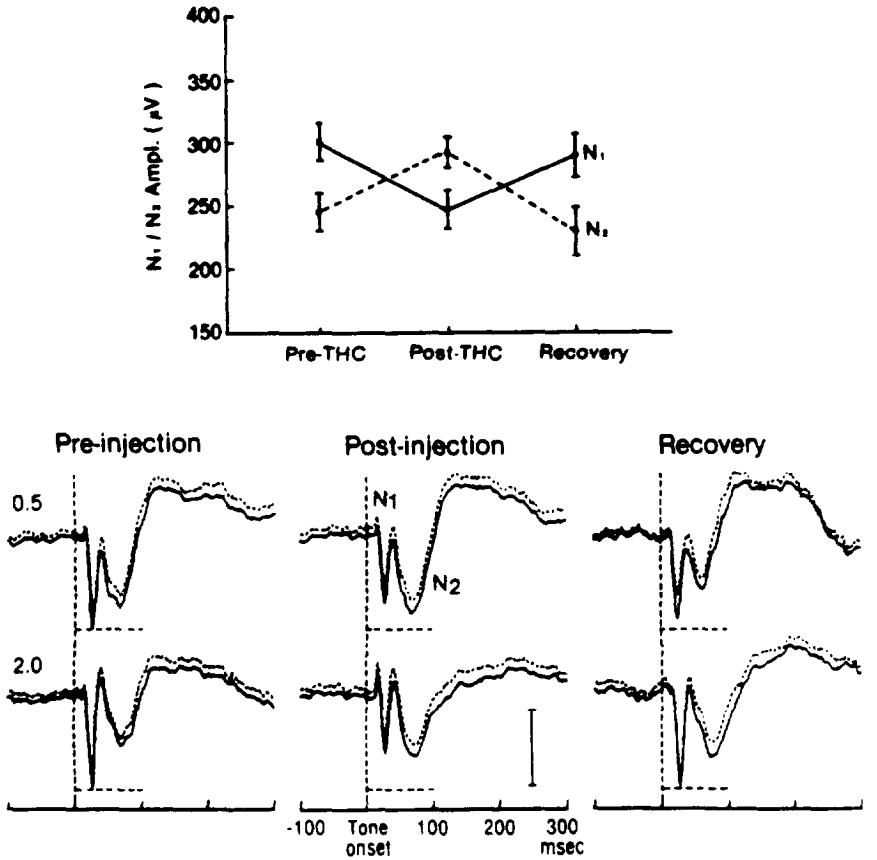

FIGURE 4. *Effects of delta-9-THC on hippocampal sensory-evoked potentials recorded daring performance of dual tone discrimination task as shown in figure 3*

NOTE: Upper panel shows marginal and maximal effects related to the lowest (0.5 mg/kg) and highest (2.0 mg/kg) doses of delta-9-THC. Graph at the top shows mean amplitude changes measured over all animals and doses for the three types of sessions. Bars are ± SEMs.

Delta-9-THC (1.0 mg/kg dose) altered the sequential fluctuations in the early component (N1) of the sensory-evoked potential. This component, which reflects transmission of sensory information from the entorhinal cortex to the dentate gyrus via the perforant path (Deadwyler et al. 1981; Foster et al. 1988), exhibited two major changes from the normal pattern of trial-to-trial fluctuation: (1) the degree of amplitude change during single-alternating trial sequences was drastically exaggerated, and (2) there was a

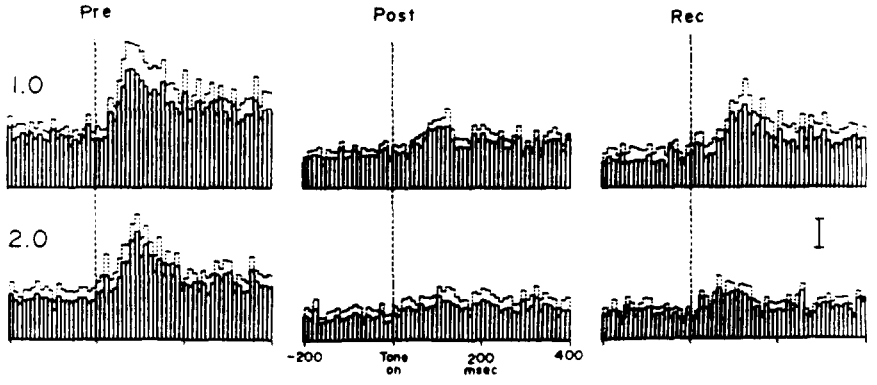

FIGURE 5. *Effects of delta-9-THC on tone-tvoked dentate granule cell (g-cell) activity recorded in the dentate gyrus of the hippocampal formation*

NOTE: Poststimulus histograms are shown for predrug, postdrug, and recovery sessions at two dose levels indicating decreased g-cell discharge that recovered differentially as a function of dose level. In each case, an isolated g-cell was recorded during a 100-trial session. Poststimulus histograms are normalized across trials, sessions, and animals. Dotted tracings above histogram indicate SEM-of-unit discharges. Calibration: 0.2 spikes/bin.

shift in the pattern of N1 amplitude fluctuation such that N1 amplitude switches appropriate to changes in particular sequences did not occur on the same trial as in control sessions (figure 6). The shift in amplitude during single-altemation trials was due to a change from intermediate-amplitude N1 potentials on unrewarded stimulus (CS-) trials to virtually no response on those same types of trials under the influence of delta-9-THC (1.0 mg/kg). This occurred without any significant change in N1 amplitude on CS+ trials during either single- or double-alternation trial sequences (figure 6). Although the general pattern of amplitude fluctuation for other types of sequences remained similar to that in the control condition, there was a one-trial "lag" in the large fluctuations across different sequences with respect to predrug sessions. This is illustrated in figure 6, where the pattern of change for each sequence type is shifted slightly to the right by one trial. Since the pattern and not the amplitude of these potentials was altered after delta-9-THC, it is likely that the drug action was specific to information-processing mechanisms in the hippocampus.

The change in pattern of evoked potential amplitude fluctuation may have reflected a decreased mnemonic capacity to track changes in trial sequences produced by delta-9-THC. This was assessed by examining the serial dependence of N1 amplitude as a function of the number of trials preceding the current trial (Deadwyler et al. 1985). This analysis showed that under control conditions the most recent trial (position 1) exerted the largest

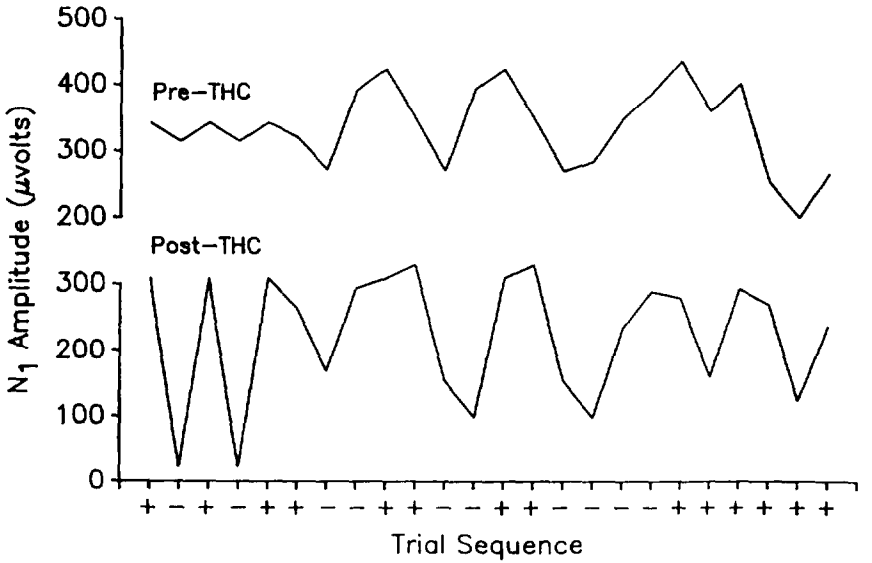

FIGURE 6. *Effects of delta-9-THC (1.0 mg/kg) on trial-to-trial fluctuations in N1 amplitude provoked by specific types of trial sequences*

NOTE: Mean amplitude of the N1 component (see figure 4) is plotted for potentials in which the trial evoking the potential was preceded by five trials of the type indicated on the axis marked trial sequence. Note that single-alternating trial sequences were most affected by delta-9-THC (post-THC), causing maximal-to-minimal amplitude changes that do not normally occur for type-of-sequence in predrug control sessions (pre-THC). Means are averaged over animals, trials. and sessions.

influence on the amplitude of N1, while the next four serial positions in the sequence were much less influential, showing only about a 10- to 12-percent contribution for each successive position. Figure 7 illustrates the serial position curve for N1 amplitude in the predrug and 1.0 mg/kg-delta-9-THC condition. The contribution of the most recent and the most remote (i.e., the one furthest removed in time) trials were significantly altered by injections of delta-9-THC. The manifestation of these changes in serial position effect is verified by the altered pattern of sequential influences discussed above (figure 6). These findings suggest that the mechanics of serial processing of sensory events in the hippocampus were disrupted by delta-9-THC. Given this observation, it was important to determine whether or not this disruption would be manifested in tasks in which the retrieval of trial-specific information is required.

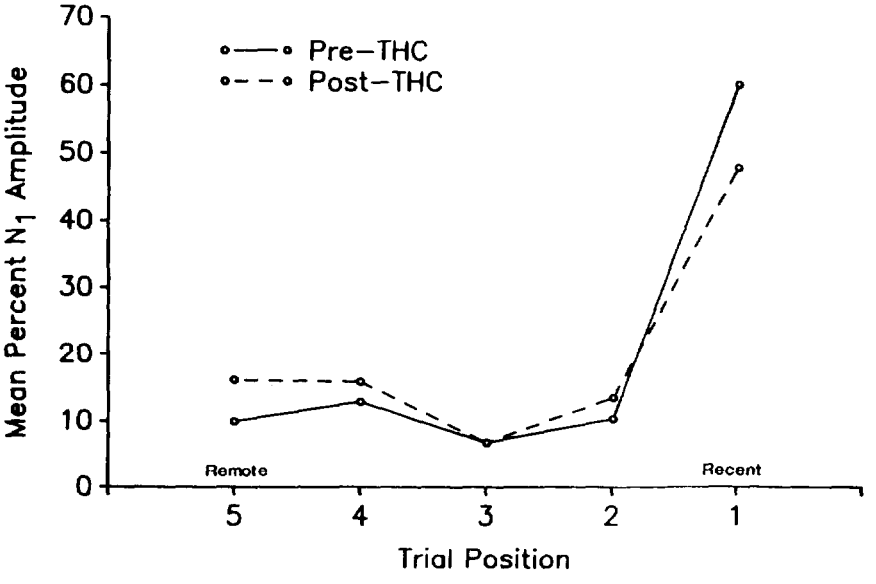

FIGURE 7. *Influence of delta-9-THC trial-position effect in the prior sequence on N1 amplitude*

NOTE: The most influential trial averaged over all sequences was most recent (i.e., the trial immediately preceding the trial on which amplitude was assessed). The remaining positions contributed relatively equal amounts but were much less influential. Delta-9-THC (1.0 mg/kg) decreased the percentage contribution of the most recent trial (position 1), but increased the contribution of the more remote trials (positions 4 and 5) in the sequence.

EFFECTS OF DELTA-9-THC ON RETENTION IN A DELAYED-MATCH-TO-SAMPLE (DMTS) TASK

The demonstration that the same dose range of delta-9-THC systematically increased the detection threshold for tones presented at different intensities and decreased responding in the presence of the positive tone in a successive discrimination paradigm supports previous suggestions of delta-9-THC's capacity to alter the perception of stimulus features as demonstrated in human studies (Hollister 1974; Pertwee 1983). Previous investigations in humans have shown that the most consistent effect of exposure to delta-9-THC on performance is the disruption of short-term memory tasks requiring retention of item-specific information (Miller and Branconnier 1983). The subtle but significant change in sequential dependency of tone-evoked electrical activity produced by delta-9-THC, however, implicates the disruption of retention as well as perceptual processes.

This possibility was investigated by testing animals in a DMTS task that required retention of spatial information over a l- to 31-second delay interval. Performance in this task is critically dependent on the length of the delay interval and decreases from 92 percent at l- to 5-second delays to 70 percent at the 25- to 31-second delay period. If delta-9-THC produced an impairment in retention of the DMTS task under the influence of delta-9-THC should be selectively reduced at long, but not at short, delay intervals.

Figure 8 shows the effects of delta-9-THC on DMTS performance. There was a marked dose-by-delay effect, which was manifested across the 0.5- to 2.0-mg/kg dose range. The 2.0-mg/kg dose of delta-9-THC disrupted performance at the longer delay intervals; however, performance during l- to 5-second delay intervals was not significantly altered. Thus, the deficit at longer delay intervals was not due to a generalized effect of the delta-9-THC on the spatial discrimination performance. Numerous studies have established that a deficit in DMTS performance of the type demonstrated here is the hallmark of hippocampal disruption (Rawlins 1985; Eichenbaum and Cohen 1988). The results of the DMTS task further implicate the hippocampus as a target of delta-9-THC action.

DELTA-9-THC AND HIPPOCAMPAL CORRELATES OF LEARNING AND MEMORY

In the three types of studies, the dose-dependent behavioral effects of delta-9-THC were associated with behavioral deficits characteristic of hippocampal damage. In addition, hippocampal electrophysiological correlates were altered over the same dose range of delta-9-THC as the behavior. The signal detection results suggest that, at higher doses (above 0.5 mg/kg in the rat) of delta-9-THC, behavioral impairments may arise from the lack of registration of the sensory information in the hippocampus (Wilkison and Pontzer 1987). In this connection, it is curious that recovery from acute exposure to THC was slower in the signal detection task than in the two-tone discrimination task at the same dose levels. Thus, delta-9-THC may have progressively more severe influence on tasks requiring a high degree of vigilance than on tasks where the stimulus termination is response contingent (discrimination and DMTS tasks). The disruptive effects of delta-9-THC in the tone discrimination task were manifested as a decrease in responsiveness to the positive tone stimulus (Campbell et al. 1986a) but not as an increase in responding to the negative stimulus. Given the tone detection results, an increase in the detection threshold of the positive stimulus may have been partially responsible for these results. However, since recovery on the tone discrimination task was more rapid than in the signal detection task over the same dose range, the effects of delta-9-THC differed with respect to the behavioral requirements of the task, even though the presentation of sensory stimuli in the two tasks was quite similar. Performance in all three tasks was not affected by cannabidiol, the

nonpsychoactive derivative of marijuana, even at twice the highest dose of delta-9-THC (Martin 1986)

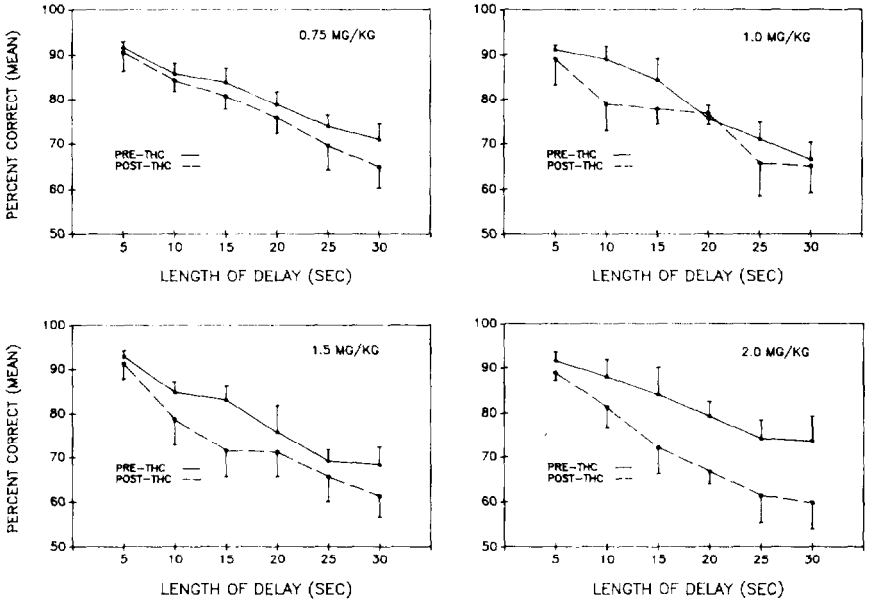

FIGURE 8. *Effects of delta-PTHC on DMTS performance at four different dose levels*

NOTE: There is a progressive increase in effects of delta-9-THC at the longer delay intervals. Performance was not affected at the 1- to 5-second delay intervals, indicating no gross disruption in the two-lever spatial discrimination capacity.

It is also important to note that the dose range shown to be effective in all three of the behavioral paradigms described was sufficient to produce a full range of disruption in behavior and hippocampal neural correlates. The degree to which the various behaviors were sensitive to delta-9-THC is reflected in the rank ordering of the severity of deficits at each dose level: the signal detection task was the most sensitive, the tone discrimination task was next, and the DMTS task was the least affected at each of the four dose levels. This ranking agrees, in general, with the recent reports of delta-9-THC action in the monkey on similar behavioral tasks (Schulze et al. 1988). In terms of the tasks that have been shown to be the most dependent on the integrity of the hippocampus, however, the DMTS deficit was the most similar to that following a hippocampal lesion (Gaffan et al. 1984; Etherington et al. 1987; Rawlins 1985).

CONCLUSION

In conclusion, it can be stated that delta-9-THC can differentially alter behavioral processes requiring information processing by the hippocampus, including the detection and processing of conditioned sensory stimuli, as well as the retrieval of trial-specific information. Recent reports have shown the hippocampus to be a target for neuropathological changes following long-term exposure to delta-9-THC (Landfield et al. 1988; Scallet et al. 1987). Tasks that require a higher degree of vigilance, such as the tone detection task, exhibited a more marked influence at intermediate- and high-dose levels (1.0 to 2.0 mg/kg). A similar relationship was also reported in the recent studies on monkeys (Schulze et al. 1988). This same dose, however, did not affect recall of recently acquired information over brief time intervals in the DMTS task. In the latter case, delta-9-THC differentially altered responding a! longer delay intervals. Thus, delta-9-THC appears to be a potent centrally acting agent that has critical consequences for the maintenance of optimal performance in tasks requiring either the detection and/or the recall of sensory information. One of the actions of this compound appears to be directed toward disruption of hippocampal neural processes involved in the performance of such tasks.

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Calcium-Mediated Events in Associative Learning

John F. Disterhoft

INTRODUCTION

This chapter will summarize a series of experiments analyzing the cellular bases of rabbit eyeblink conditioning with a combination of *in vivo, in vitro,* and neuropharmacological approaches. The hippocampus was chosen as a starting point. Previous experiments analyzing the involvement of the hippocampus in associative learning have generally analyzed the effects of dorsal hippocampal lesions on the human's or animal's ability to acquire or to retain a learned behavioral task (O'Keefe and Nadel 1978; Squire 1987). A second major approach has utilized extracellular recording of single or multiple neurons in the behaving animal during or after learning (Segal 1973; Berger et al. 1980). These approaches have demonstrated a profound involvement of the hippocampus in many associative learning tasks.

The experiments that will be described have added a new approach to those two basic techniques. Our purpose is to analyze the cellular events that underlie the increased excitability reported to occur in hippocampal neurons during and after associative learning. In addition, we wish to evaluate cellular alterations that *we* know *a priori* are local to the hippocampus and are not secondary reflections in this region of alterations located elsewhere on the conditioned reflex arc. This localization issue is a severe problem in attempting to localize neural substrates of learning with lesions or recording in the intact animal.

Therefore, an *in vitro* approach has been used in combination with *in vivo* experiments. Hippocampal neurons in brain slices from trained animals were evaluated biophysically (Disterhoft et al. 1984). We knew beforehand that any conditioning-specific alterations observed in the hippocampal slice had to be localized there. They cannot be interpreted as secondary reflections of changes projected to the hippocampus from some other brain region-neurons in the slice are separated from their normal interconnections with other brain regions.

Drain slice experiments have delineated the importance of conditioning-specific reductions in slow calcium-mediated potassium currents during

learning (Disterhoft et al. 1988a). These results encouraged us to test the effect of nimodipine, a calcium channel blocker, on acquisition of eyeblink conditioning. This chapter will conclude by describing the facilitation in learning eyeblink conditioned responses observed in rabbits treated with this dihydropyridine (Deyo et al. 1989). The facilitation is particularly interesting, because it is most clearcut in aging rabbits.

EYEBLINK CONDITIONING IN RABBITS

Behavioral training was done with standard procedures for rabbit eyeblink or nictitating membrane conditioning (Disterhoft et al. 1977). Experiments were controlled, behavioral data stored online, and data processed and displayed on- or offline by computer. Subjects were young adult male albino rabbits, *Oryctolagus cuniculus,* weighing 1.5 kg at the start of training (except, of course, in the aging studies). The pure tone conditioned stimulus (CS) was a 2,000 Hz pure tone presented at 85 db in a sound-attenuated chamber. In delay conditioning, the 400-millisecond tone CS was paired and coterminated with a 150-millisecond air puff or periorbital shock unconditioned stimulus (UCS) just sufficient to cause a reliable blink unconditioned response (UCR). Pseudoconditioned control rabbits received explicitly unpaired presentations of the CS and UCS in a random fashion.

Trace conditioning combined a 100-millisecond, 6,000 Hz tone pip CS followed after 300 milliseconds with a 150-millisecond corneal air puff. Pseudoconditioning control training again consisted of explicitly unpaired presentations of the CS and UCS. In the nimodipine experiments, the trace interval was 500 milliseconds. An example of the type of behavioral responses observed on individual trials early and late in trace conditioning as well as the acquisition curve for one group of eight rabbits is shown in figure 1. Trace conditioning (even with only a 300-millisecond trace interval) has been found to be much more difficult for the rabbits to acquire than delay conditioning. For example, almost all rabbits trained for 3 days in the delay task reached 80 percent of the total CRs on the third training day. However, it takes, on average, 10 training sessions for rabbits to reach an 50-percent behavioral criterion in the trace paradigm with a 500-millisecond trace interval (Moyer et al., in press).

SINGLE NEURON RECORDING

Berger and Thompson and their colleagues have described a striking neural modeling of the eyeblink conditioned response (CR) in CA1 and CA3 pyramidal neurons in both multiple and single unit recordings (Berger et al. 1980; Berger et al. 1983). Recording has begun of single neurons in CA1 hippocampus of trace-conditioned rabbits (Akase et al. 1988). A variety of responses have been seen in the complex spike cells of CA1 hippocampus (presumed pyramidal cells). Almost all neurons are functionally

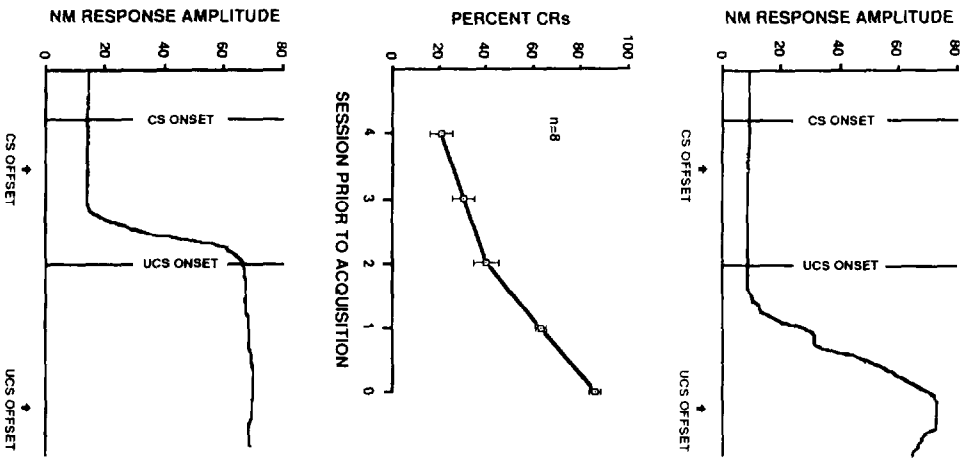

FIGURE 1. *Trace eyeblink conditioning using a 300-millisecond trace interval*

NOTE: Examples of eyeblink responses on individual trials early in training (left) and during asymptotic acquisition. The center panel shows an acquisition curve for the last 5 days of training for a group of eight rabbits. Note that trace eyeblink conditioning, even with only a 300-millisecond trace interval. is a considerably more difficult task for the albino rabbit. This group of animals took an average of 7.5 days to teach an 80 percent CR performance criterion; a comparable group trained in the delay task required 2 to 3 days.

modulated during the trials. Some cells show clear modulation similar to the type described by Berger and colleagues, except with an additional sensory response to the tone CS (figure 2). Others show clear inhibitory components in their response profiles, which is especially marked during the tone CS air puff UCS interstimulus interval and may be followed by enhanced responses to the air puff UCS (figure 2). This type of inhibition during the trace interval may be important in the control of CR temporal characteristics disrupted in hippocampal-lesioned rabbits trained in the trace paradigm (Graves and Solomon 1985; Moyer et al., in press). The distinctive sensory response to the tone in the trace paradigm is unique to this situation; it was not reported previously in studies of delay conditioning. Perhaps this response reflects a registration of the onset of the CS that will be useful in the temporal patterning of the CR after the appropriate trace interval.

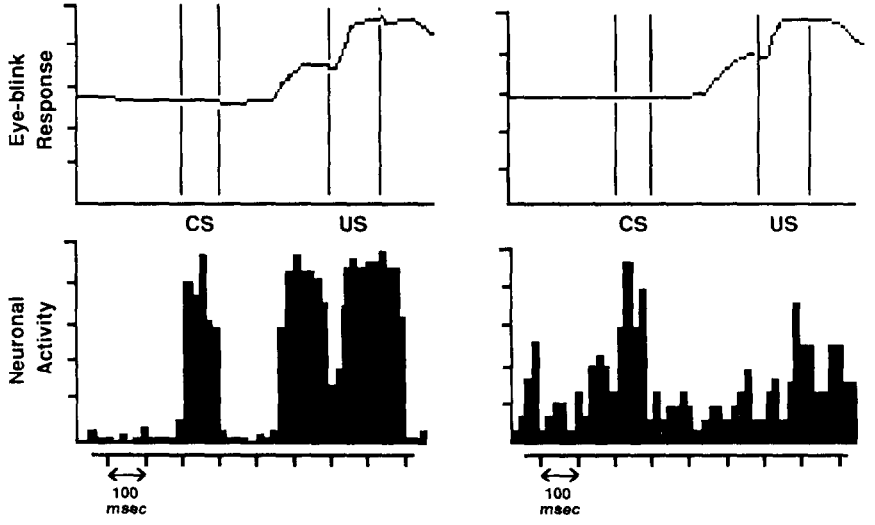

FIGURE 2. *CA1 pyramidal neuron activity during asymptotic trace eyeblink conditioning*

NOTE: Averaged behavioral response is shown above. poststimulus time histogram during the same time period is shown below for each panel. Both neurons showed components of "neural modeling" in their responses. The neuron on the left showed a noticeable sensory response. to the tone CS. That on the right showed an inhibitory response, especially during the trace interval, followed by enhanced firing during the air puff UCS presentation.

SOURCE: Figure developed from data presented in Akase et al. 1988.

BRAIN SLICE EXPERIMENTS

Initial Observations

A similar procedure has been used in the several replications of the brain slice experiment that have been done to date (Disterhoft et al. 1986; Coulter et al. 1989). Animals first were trained to behavioral criterion or received pseudoconditioning. Naive controls were also used. Twenty-four hours later. slices were made and maintained with standard procedures. Next, cells were impaled and their membrane properties were studied. The studies that will be summarized concern CA1 pyramidal neurons and dentate gyrus granule cells, two of the major cell types in the classic trisynaptic hippocampal circuit (figure 3) (Andersen et al. 1971).

The major conditioning-specific effect observed in CA1 neurons recorded in slices from trained rabbits was a reduction in the afterhyperpolarization (AHP) response observed after action potentials were elicited with intracellular current pulses. This response was smallest in trained animals and approximately the same in the pseudoconditioned and naive groups (Disterhoft et al. 1986; Coulter et al. 1989). The AHP duration, as well as the amplitude, was decreased after conditioning. Another important feature of the AHP decrease was that it was present in the conditioned group of cells in the absence of any alteration in spike height, resting potential, or input resistance from the other two groups. Since the AHP increases as the number of spikes increases (it is additive with increasing numbers of spikes) (Hotson and Prince 1980), the conditioning effect was more marked as the number of spikes in the burst became larger. Finally, the AHP reduction was present in cells from trained animals, even when sodium-dependent synaptic transmission was blocked by bathing the slices in tetrodotoxin (TTX) and tetracthylammonium chloride (TEA) (Coulter et. al. 1989).

Functionally, the AHP is presumed to shut the hippocampal pyramidal cells off during and after bursts of action potentials (Hotson and Prince 1980). A reduction in AHP after conditioning would make the cells more excitable. As mentioned above, *in vivo* recording shows that hippocampal pyramidal cells are more responsive to the tone CS after conditioning (figure 2). A reduction in the AHP could certainly cause, or contribute to, this increased excitability.

Acquisition

Next, a series of experiments was performed to determine when, during the course of CR acquisition, the decrease in AHP size occurs (Disterhoft et al. 1988b). In all of the work discussed thus far, rabbits were trained for three 80-trial sessions in the short-delay paradigm to insure that the animals were very well conditioned. In the acquisition studies, rabbits were trained for

two 80-trial sessions. Some rabbits were very well trained at this point, while others showed almost no CRs.

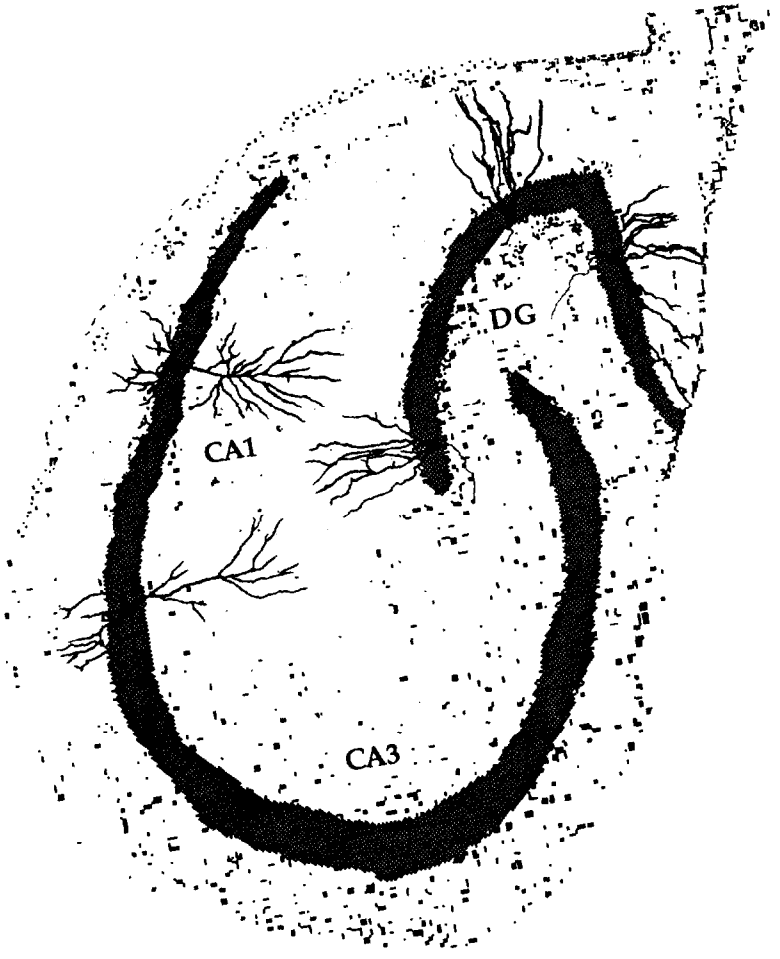

FIGURE 3. *A schematic drawing of a hippocampal slice*

NOTE: Camera lucida drawings of Gogi-stained individual cells have been placed as they are actually situated in the brain slice. Examples of the cell types studied in the brain slice experiments discussed here, CA1 pyramidal neurons and dentate gyros granule cells, are presented.

SOURCE: Thompson and Disterhoft, unpublished observations.

Behavioral acquisition curves for the rabbits were used to separate them into two groups—low and high acquisition (figure 4). Note that the low group showed about 10 percent CRs on the second day of training. The high group showed 80 to 90 percent CRs after the same number of training trials. The AHP was markedly reduced in both amplitude and duration in the high CR group as compared to comparable measurements taken in the low group (figure 5). Again, these shifts in AHP amplitudes and durations occurred in the absence of alterations in other biophysical indices such as spike height, membrane resting potential, or input resistance.

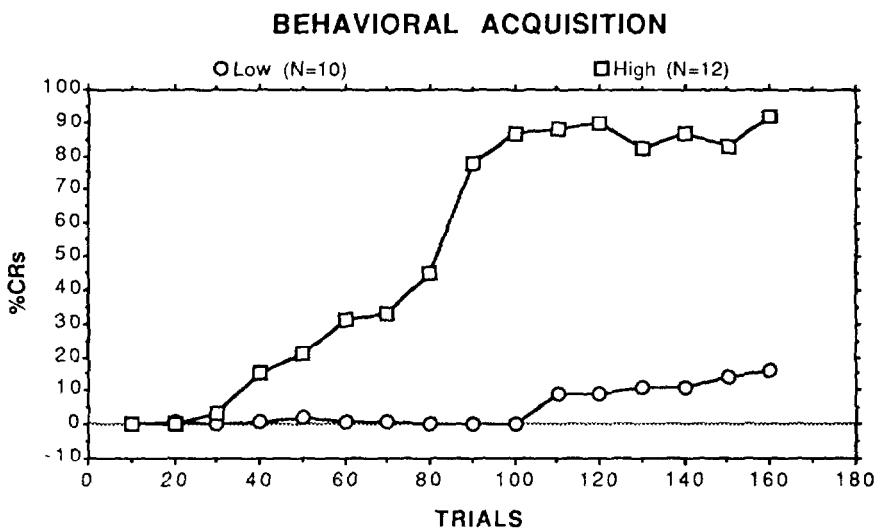

FIGURE 4. *Learning curves for the high- and low-acquisition groups*

NOTE: Note that each session consisted of 80 trials, therefore, trials 81 to 160 were in the second training session. On each trial, a CR was an eyeblink that occurred during the CS-US interval.

SOURCE: Disterhoft et al. 1988b, copyright 1988, Elsevier.

When histograms were made of the distribution of AHP amplitudes in the two acquisition groups, a clear shift in the distribution of maximum AHPs was seen between the low and high groups. This observation was consistent with what was observed in earlier comparisons made between cells from conditioned, pseudoconditioned, and naive rabbits (Disterhoft et al. 1986). It is clear that large numbers of cells show increased excitability after conditioning. But not all cells are changed; there is an overlap in the distributions. This is consistent with the single neuron data that have been

reported *in vivo.* Berger and his colleagues reported that about 60 percent of neurons showed enhanced firing in the CS-UCS interval in trained rabbits (Berger et al. 1983).

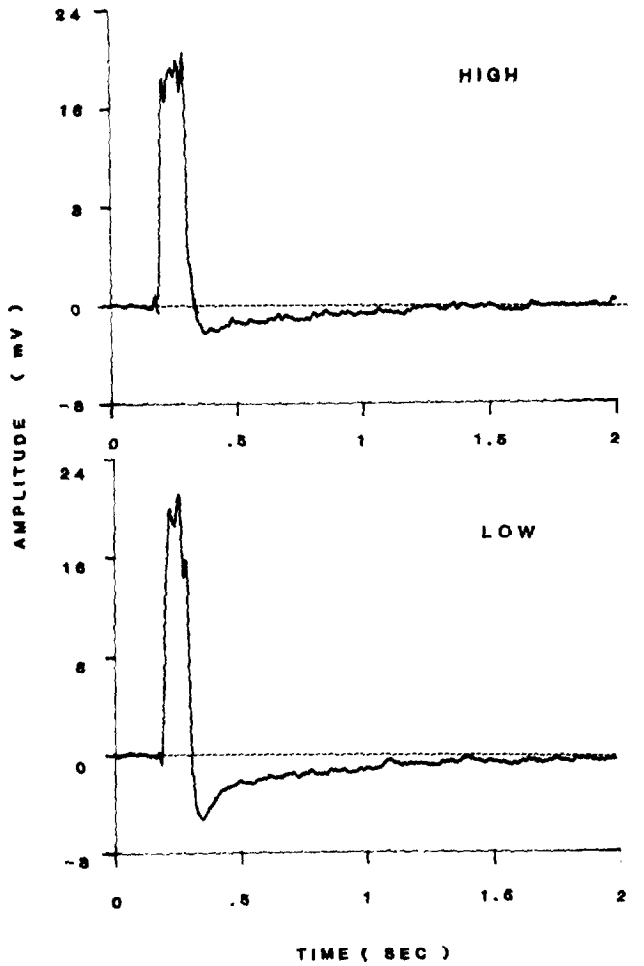

FIGURE 5. *Computer-averaged traces from neurons in the high- and low-acquisition groups*

NOTE: Four action potentials were elicited by intracellular current injection, and the AHP response averaged five times. The frequency response of the analysis system (500 Hz) was adequate for accurate analysis of the afterhyperpolarization response during the 1.7 seconds after the depolarizing pulse but did not follow the action potentials elicited during the pulse.

SOURCE: Disterhoft et al. 1988b. copyright 1988, Elsevier.

The important things about this study are (1) that it links the decreased AHP after conditioning more closely to behavioral performance, and (2) it is further evidence that the decreased AHP is not some aberration of the conditioning paradigm, All animals in the acquisition study received 160 CS-UCS pairings. Only those that learned, as evidenced by the number of CRs they showed, had CA1 pyramidal cells with reduced AHPs. This links the NIP reduction closely to CR acquisition and actual performance of the response. The acquisition group serves as another type of "control" in addition to the pseudoconditioned and naive groups.

Trace Conditioning

It is well known that hippocampal lesions do not affect the acquisition of short-delay conditioned eyeblink responses (Schmaltz and Theios 1972; Solomon and Moore 1975; Akase et al. 1989). There is no doubt that the AHP alterations that have been observed reflect conditioning-specific alterations that are local to the hippocampus. They are present in hippocampal slices separated from their normal afferent and efferent connections and even in slices where all sodium spike-dependent synaptic activity is absent (Coulter et al. 1989). It has been shown that hippocampectomy does retard retention even in the short-delay paradigm, if rabbits arc not overtrained (Akase et al. 1989). However, we wished to insure that the biophysical alterations that have been seen are intimately related to the behavior that the rabbit was learning. We also wanted to determine if the changes observed in rabbits learning a task that may more clearly involve the hippocampus (as indicated by the effects of hippocampectomy) were the same as those that occurred after the short-delay paradigm. Therefore, examination has begun of the conditioning-specific changes in ionic currents that might be seen during trace conditioning, a paradigm whose acquisition Solomon, Thompson, and their colleagues have shown is blocked by hippocampal lesions (Solomon et al. 1986). Lesion data with trace conditioning indicate that hippocampectomy markedly retards extinction of trace conditioning with a relatively short, 300-millisecond trace interval (Moyer et al., in press). When the longer 500-millisecond trace interval studied by Solomon et al. (1986) was used, hippocampectomized rabbits were unable to acquire the trace-conditioned response, even after 25 training sessions (figure 6).

An initial survey has now been completed of CA1 pyramidal neurons studied in hippocampal slices after trace conditioning (de Jonge et al., in press). It has been found that both the AHP amplitude and area are reduced in a conditioning-specific manner (figure 7). The effects of conditioning on both measurements were larger as the number of action potentials in the intracellularly elicited burst increased from one to four. Again, as in the studies of the biophysical alterations seen after short-delay conditioning, no differences were seen among the behavioral groups in terms of other indices that might be expected to affect the AHP measurements directly,

i.e., resting potential, input resistance, action potential height, or amount of current required to elicit the action potentials.

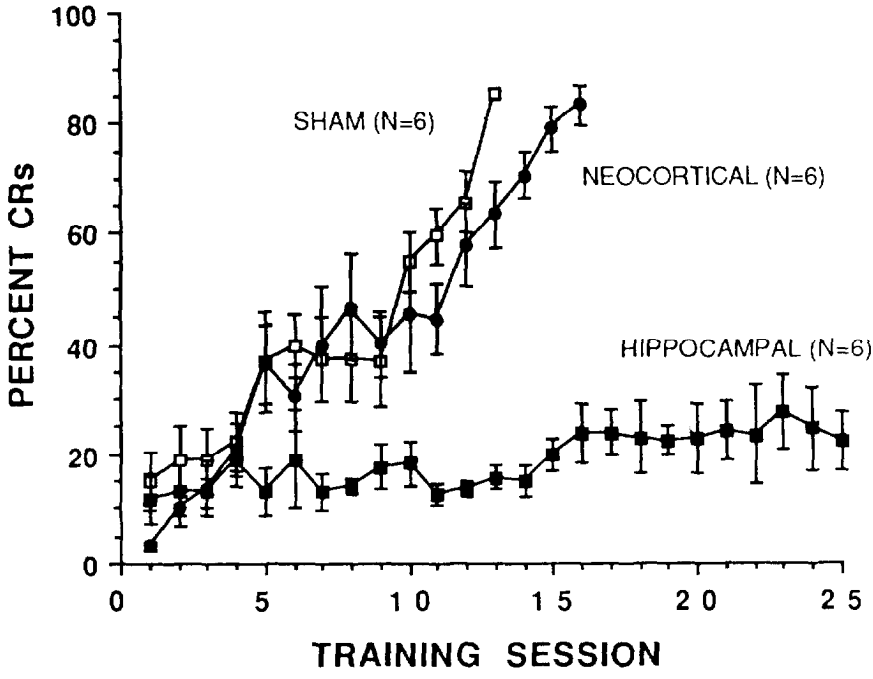

FIGURE 6. *Learning curves for rabbits in three surgical conditions (hippocampectomized, neocortical, sham) when trained in the trace-conditioning paradigm with a 500-millisecond trace interval*

NOTE: The curves are "Vincentized" according to the slowest learner in each group. The major point is that no hippocampectomized rabbit acquired the response even after 25 training sessions. A mean number of 10 acquisition sessions was required by the other two groups to reach 80 percent CRs in a training session.

SOURCE: Figure developed from data presented in Moyer et al. 1989.

The role of CA1 pyramidal neurons in mediating eyeblink conditioned responses has been stressed. It is the CA1 and CA3 pyramidal neurons that exhibit the striking "neural modeling" of the CR as learning occurs (Berger et al. 1983). Multiunit recordings that have been made from the dentate gyrus granule neurons during eyeblink conditioning suggest that this cell type is involved more in changing responsiveness to the sensory significance of the CS during learning (Berger and Weisz 1987). Dentate neurons show

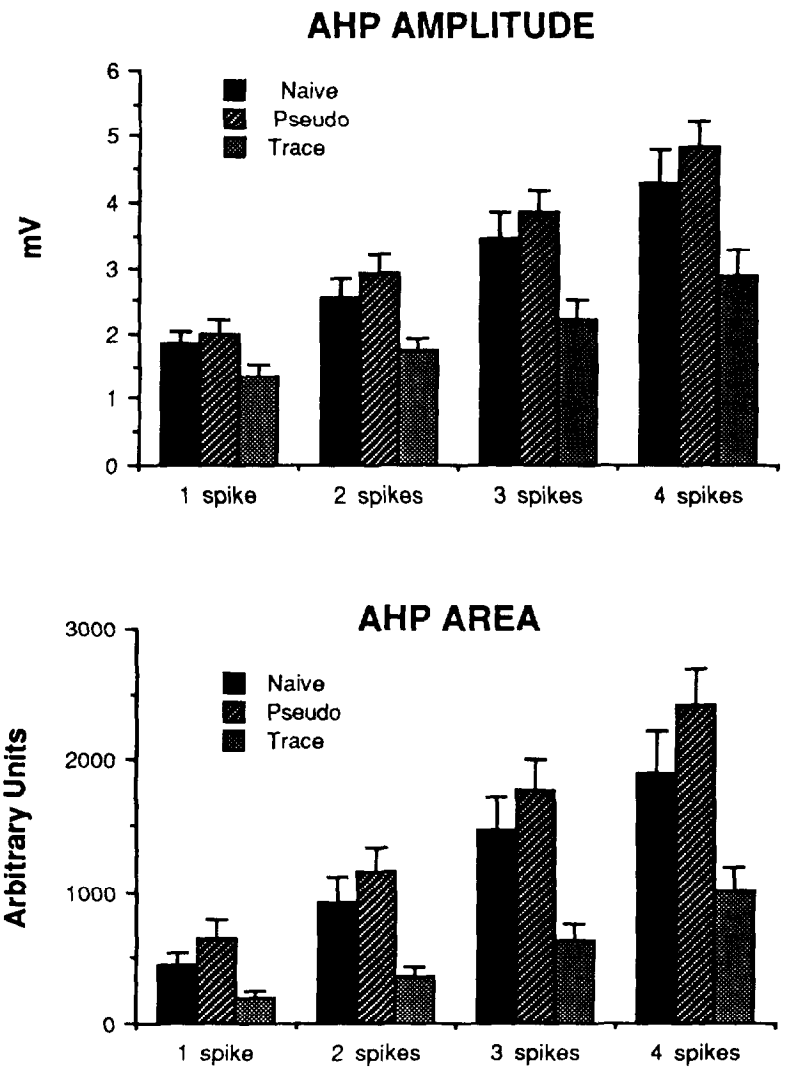

FIGURE 7. *AHP amplitude and area for CA1 pyramidal neurons in the three behavioral groups*

NOTE: The average responses were significantly smaller for both amplitude and area at all levels. Note that the response reduction became larger as the number of action potentials in the burst became larger.

SOURCE: Figure developed from data presented in de Jonge et al. 1988

an enhanced response to the presentation of the CS very early in training. The response does not mimic the shape of the CR, as the pyramidal cell response does, nor does the amplitude of the response increase markedly as behavioral learning proceeds, as is the case for pyramidal neurons (Weisz et al. 1982).

Given this interesting functional differentiation, which has been demonstrated for two adjacent cell types with *in vivo* recording, the next test was whether dentate granule neurons did or did not demonstrate a similar AHP reduction, as was the case for CA1 pyramidal neurons (Black et al. 1988). First, it was confirmed that dentate granule neurons have a substantial AHP response (figure 8) as was reported previously (Durand and Carlen 1984; Fricke and Prince 1984). More important, after thoroughly examining about 50 dentate gyrus granule cells, no evidence was found for conditioning-specific alterations in the AHP response (figure 9). Again, there was no difference among groups in any of the other biophysical indices examined, including membrane resting potential, spike height, input resistance, or current required to elicit action potentials.

These data clearly demonstrate an anatomical differentiation in the AHP reductions occurring within the hippocampus. They also show an interesting convergence with the functional data gathered with unit recording techniques in the conscious animal. It would appear that the AHP reductions could be an ionic change underlying the increased excitability reflected in the neural modeling of the behavioral response. Since the evidence is quite good that the CA1 pyramidal neuron AHP reduction is local and postsynaptic, the lack of such a conditioning-specific change in dentate gyrus granule neurons may indicate that the increased firing these cells show *in vivo* is not local. The enhanced responsiveness to the tone CS may reflect altered responsivity in entorhinal cortex neurons, which project to dentate gyrus granule neurons via the perforant path and have been shown to increase firing during conditioning (Berger et al. 1980). Finally, the results in the dentate gyrus region lead to the hypothesis that CA3 pyramidal neurons will demonstrate a conditioning-specific reduction in the slow AHP, since these cells demonstrate the neural modeling of the CR, as do CA1 pyramidal neurons. This hypothesis is presently being tested.

Temporal Analysis of AHP Changes

Alterations in the slow AHP response have been emphasized to this point. But it has become obvious that there are several potassium currents that contribute to the action potential repolarization and whose reduction could cause enhanced excitability in hippocampal neurons (Adams and Galvan 1986; Schwindt et al. 1988). There are two calcium-mediated potassium currents, I_C and I_{AHP}. I_C is a relatively fast current lasting milliseconds that is active on the lower half of the action potential downward trajectory (Storm 1987). I_{AHP} is a relatively slowly activating current that is

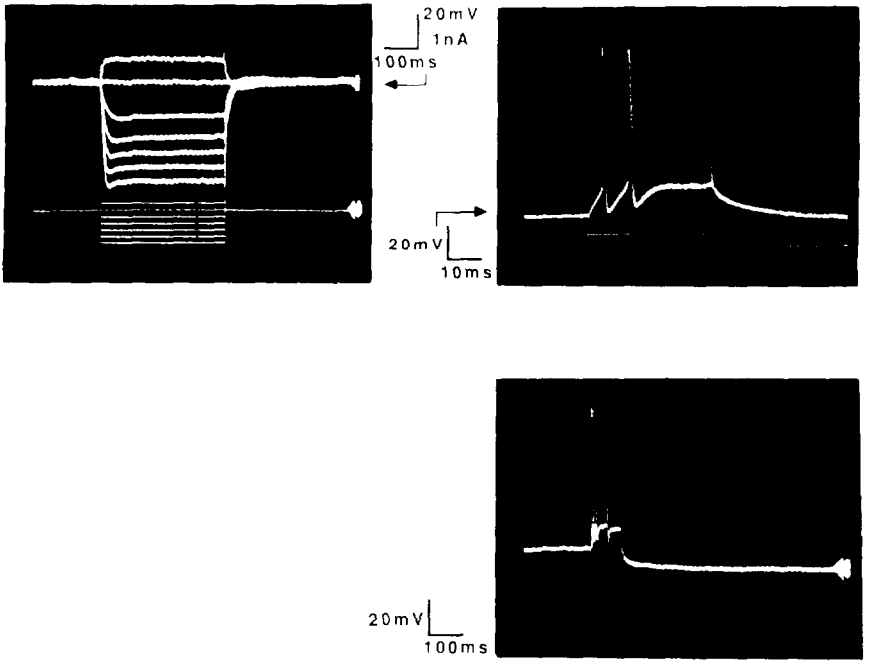

FIGURE 8. *The functional response of a dentate granule neuron*

NOTE: Note the typically high input resistance (100 MΩ to a .2 na hyperpolarizing pulse) and the relatively large and long-lasting AHP after four action potentials (8 mv) in this neuron.

SOURCE: Figure developed from data presented in Black et al. 1988.

evident for hundreds of milliseconds to seconds after a train of action potentials (Lancaster and Adams 1986; Storm 1987). There is also a medium AHP, evident in the 50 to 100 milliseconds after the action potential train, which is calcium independent (Gustaffson et al. 1982; Storm 1987). Finally, there are the fast, voltage-dependent potassium current I_A that is active in the upper half of the downward trajectory of the action potential and the slower, delayed rectifier, a potassium current that is also active in the lower portion of the downward portion of the action potential (Storm 1987).

We were interested in determining whether the alteration in the calcium-mediated potassium currents was specific to a particular portion of the AHP response or was a more generalized phenomenon. We also wanted to explore the comparability between the cellular mechanisms underlying the excitability changes that have been observed in the hippocampus after classical conditioning and those that have been reported in invertebrates. Alkon and his colleagues have observed reductions in both the fast,

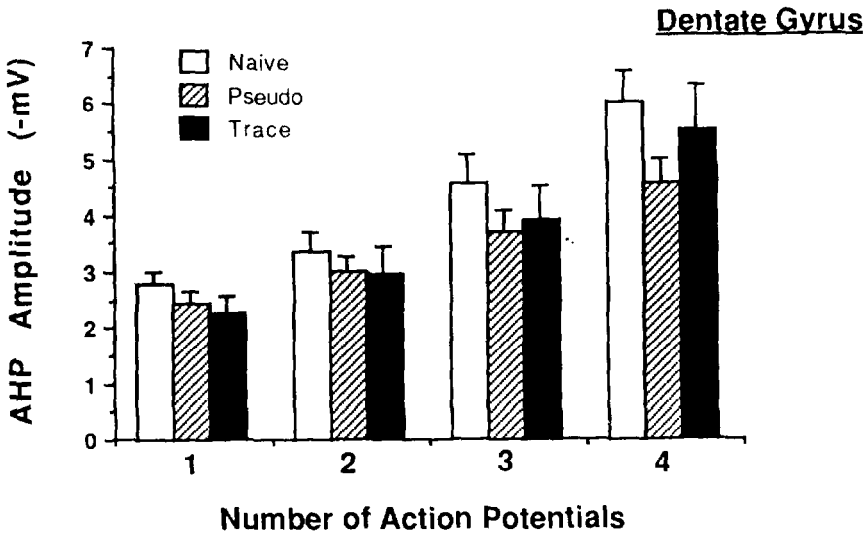

FIGURE 9. *AHP amplitude after four action potentials in dentate gyrus granule neurons recorded from the three behavioral groups*

NOTE: No statistically detectable difference in AHP amplitude was observed.

SOURCE: Figure developed from data presented in Black et al. 1988.

voltage-activated potassium current IA and in the somewhat slower, calcium-mediated potassium current I_C after associative conditioning in *Hermissenda* (Alkon et al. 1982; Alkon 1984). Kandel and his colleagues have reported reductions in a voltage-activated potassium current, apparently the serotonin-sensitive I_{KS}, in *Aplysia* after sensitization (Klein and Kandel 1980). It is of interest to determine if the same pattern of changes occurs in the mammalian brain during associative learning, particularly as attempts are made to unravel the subcellular and molecular chain of events mediating the alterations.

Therefore, the timecourse of the AHP reductions observed in CA1 pyramidal neurons was looked at in some detail. First, the timecourse of the AHP reduction was examined after a train of four action potentials (figure 10). The AHP reduction was present in the conditioned group as early as 50 milliseconds after the action potential train and remained throughout the entire 750 milliseconds that were analyzed in detail. This was an indication that both the medium and slow AHP responses were reduced in a conditioning-specific fashion. This detailed analysis clearly confirmed the reduction in the calcium-mediated slow AHP. Second, the upward and

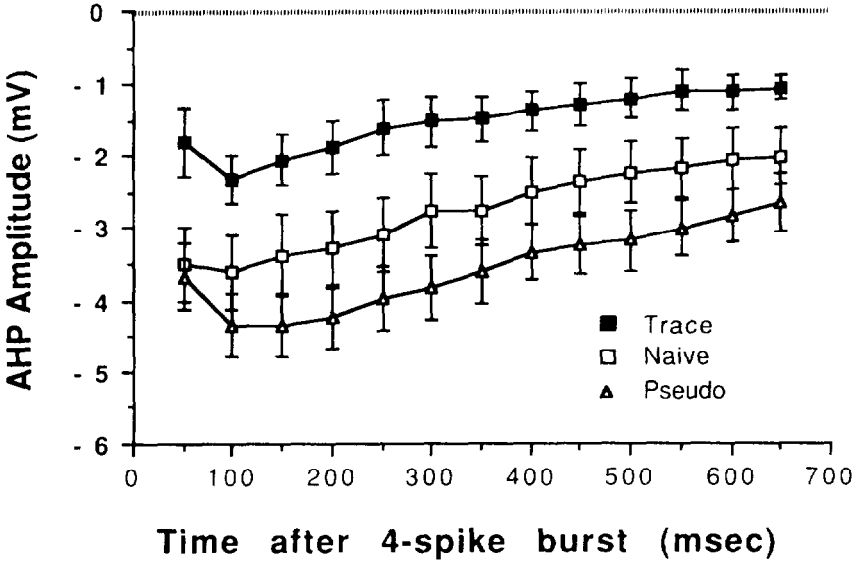

FIGURE 10. *An analysis of the average AHP amplitude in the 750 milliseconds after the four action potential train*

NOTE: The response for each neuron was averaged over five trials, then the average for each behavioral condition was derived. The AHP amplitude was smallest in the trace-conditioned group at each 50-millisecond time point examined. This indicated that both the medium and slow AHP responses were reduced in the trace eyeblink conditioning group.

SOURCE: de Jonge et al.. in press

downward trajectory of the action potential was examined, as well as its width, for evidence of any alteration due to conditioning. Because of the contribution of I_A, I_C, and the delayed rectifier to the action potential repolarization, an alteration in any of these currents should have been evident in the action potential waveform (Storm 1967). Unlike the case for the medium and slow AHP responses, there was no alteration evident in the action potential in any of the behavioral groups (figure 11). This lack of effect was found in both the CA1 pyramidal neuron and the dentate gyrus granule cell populations. It should be noted that this experiment was not a definitive evaluation of the possibility of alterations in the early currents. A more detailed examination using pharmacological techniques to isolate the various currents and/or single electrode voltage clamp studies will be required to make a definitive statement. At this point, it can be said that the learning-induced AHP reduction appears to be confined to the medium and slow AHP responses.

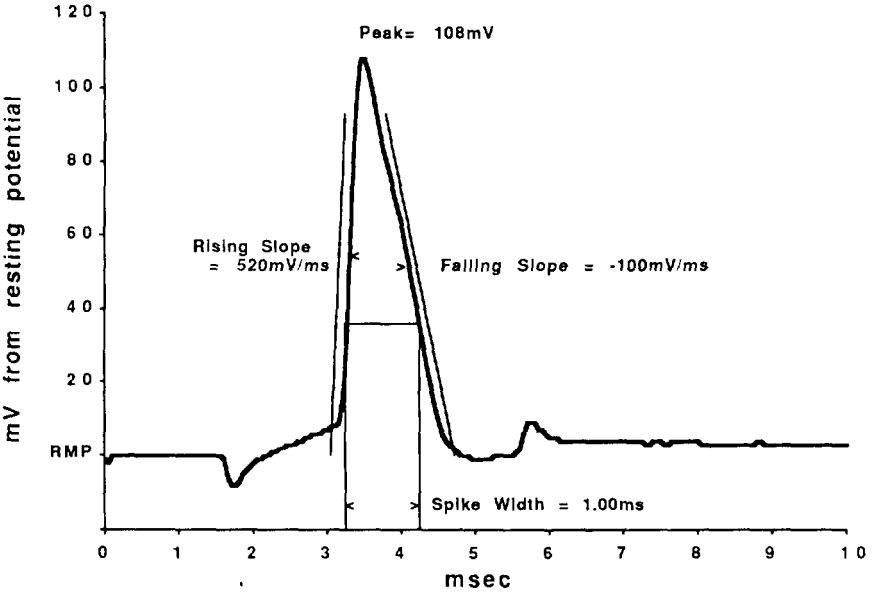

FIGURE 11. *A computer-generated display of a single action potential in a dentate granule neuron to demonstrate how the detailed analysis of the action potential was done*

NOTE: The rising and falling slopes, the action potential width, and the action potential height were determined for each neuron five times and averaged. A d-millisecond pulse was used to elicit the action potential. The data were gathered at 10-μsecond intervals, yielding sufficient accuracy for this analysis. The action potential width and falling slope measurements were particularly sensitive to possible alterations in the fast currents, I_A, I_C, and the delayed rectifier. No differences were seen between the behavioral groups in either CA1 pyramidal or dentate gyms granule neurons with this detailed analysis.

SOURCE: Figure developed fmm data presented in Black et al. 1988.

NIMODIPINE FACILITATES EYEBLINK CONDITIONING IN AGING RABBITS

The final set of experiments that will be described involves an evaluation of the effect of intravenous administration of nimodipine on the acquisition of the trace eyeblink conditioned response in aging rabbits. Nimodipine is a dihydropyridine known to be a calcium channel blocker (Scriabine et al. 1985). It is being tested in humans for use in controlling the consequences of stroke in the period immediately after the cerebrovascular event. What interested us was the preliminary report that learning ability was enhanced in aging humans with chronic cerebrovascular disease to whom nimodipine was administered (Bono et al. 1985). We were intrigued by the possibility that this effect could be a result of a reduction of calcium-mediated

potassium currents as a result of direct blockade of the calcium current itself. Some evidence exists for nimodipine blockade of calcium currents in hippocampal neurons *in vitro* (Docherty and Brown 1986; Gahwiler and Brown 1987). Eyeblink conditioning in the rabbit is a good preparation in which to evaluate a pharmacological agent that may counteract learning deficits during aging. Eyeblink conditioning was initially developed as an experimentally controlled behavioral test in the human; its parameters are rather similar in humans and rabbits (Gormezano 1966). Aging also slows behavioral acquisition similarly in both species (Woodruff-Pak and Thompson 1985).

It was found that intravenous administration of nimodipine (1 μg/kg/minute) markedly facilitated the acquisition of eyeblink conditioning in aging rabbits (retired breeders averaging 36.3 months old) when compared to vehicle controls (mean age 37.7 months) (figure 12) (Deyo et al. 1989). As expected from previous studies (Graves and Solomon 1985; Woodruff-Pak and Thompson 1985), the aging vehicle-control rabbits learned significantly slower than did the young adult (3 months) controls. It was also found that young adult rabbits receiving nimodipine learned faster than young vehicle controls, at about the same performance levels as the aging nimodipine rabbits. Nimodipine had no effect on the size of CRs that did occur nor on the size of unconditioned eyeblink responses to the air puff. There was no effect of nimodipine in pseudoconditioned rabbits; i.e., there was no increased sensory responsivity as a result of drug application. These data suggested that the nimodipine facilitated whatever central pathway was involved in mediating the learned association itself, rather than facilitating responsivity in a generalized fashion.

We do not yet have a definitive understanding of the mechanism for the facilitation of learning that has been observed. There seem to be two obvious possibilities. First, as mentioned above, nimodipine may be reducing AHP responses directly in brain regions critical for mediating the tone-air puff association via a reduction of calcium currents. There is some evidence for nimodipine effects on calcium currents in the hippocampus (Docherty and Brown 1986; Gahwiler and Brown 1987). Possible reductions of the AHP response, which have not yet been examined, are currently being evaluated. Consistent with the brain slice work summarized above (Disterhoft et al. 1986; de Jonge et al., in press), it is suggested that nimodipine could then increase cellular excitability directly in a fashion appropriate to facilitate acquisition. The second major possibility could be that nimodipine is enhancing cerebral blood flow and acting as a vasodilator. Many of the "nootropic" substances that have been described to facilitate learning apparently act via this mechanism (Hock 1987). Direct blockade of central nervous system calcium channels is the hypothetical mechanism that seems most theoretically appealing at this point.

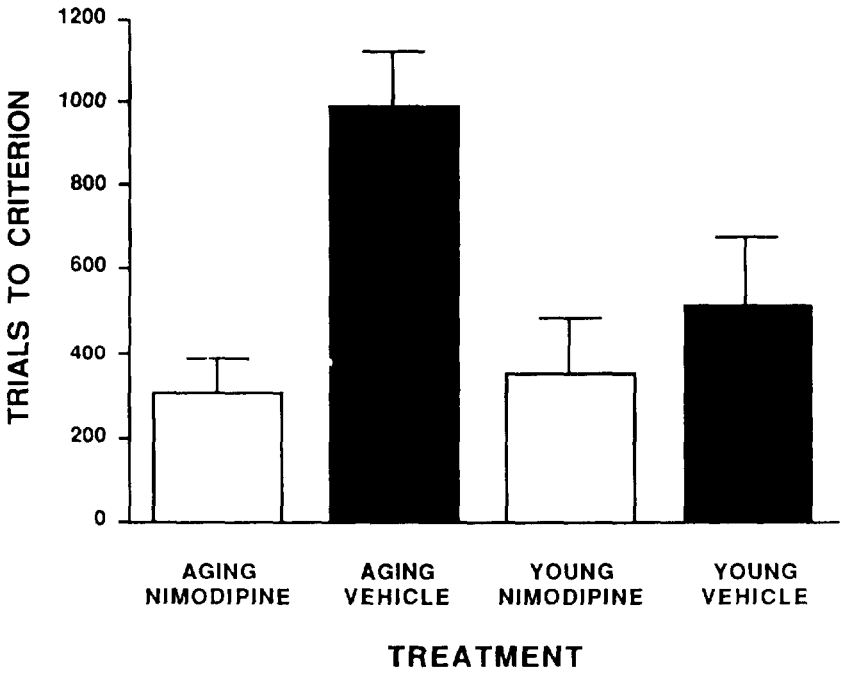

FIGURE 12. *The mean number of trials (plus the standard error) to reach behavioral criterion (8 CRs in a block of 10 trials) in each treatment group in the nimodipine study*

SOURCE: Deyo et al. 1989, copyright 1989, AAAS.

CONCLUSION

An ionic mechanism that would appear to underly associative learning in the mammalian brain has been demonstrated *in vitro.* The AHP response in hippocampal cells is primarily caused by a calcium-mediated potassium current. Functionally, this AHP is presumed to shut the hippocampal pyramidal cells off during bursts. A reduction in the AHP could certainly cause, or contribute to, the increased excitability (responsivity to the CS) seen in hippocampal pyramidal neurons after conditioning. It was observed that the slow, but not the fast, AHP response was reduced after conditioning. This demonstrates an important specificity in the ionic current that is modulated during learning: I_{AHP}, but apparently not I_C or the delayed rectifier, was reduced. It has also been seen that there is anatomical localizability, even within the hippocampus, in the AHP reduction. CA1 pyramidal neurons

demonstrate this postsynaptic reduction, while dentate granule neurons do not. The AHP reduction is similar in trace and eyeblink conditioning.

Finally, evidence exists that a calcium channel blocker, nimodipine, facilitates the acquisition of the eyeblink conditioned response. This facilitation is especially marked in the aging rabbit. It is appealing to speculate that this learning enhancement may be mediated by directly causing an AHP reduction with drug administration. If so, studies of the cellular mechanisms of learning may have practical application considerably sooner than anticipated. All the studies we have done thus far emphasize an important role for calcium flux in mediating associative learning in the mammalian brain.

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Hippocampal Theories and the Information/Computation Perspective

William B. Levy

INTRODUCTION

Through the years, there seems to be no end of theories about the function of the hippocampus. Such functions include olfactory processing, recent memory, memory consolidation, response inhibition, selective attention (see Douglas 1967 for review), spatial mapping (O'Keefe and Nadel 1978), stimulus mismatch operations, temporal and sequence learning (Schmajuk 1987), storage of declarative knowledge, and working memory. Squire (1982) reviewed many of the psychological theories of hippocampal function. Worse still, the functioning rat's hippocampus may not do what H.M.'s hippocampus does (compare Scoville and Milner 1957 to above citations).

The point is that, after 30 plus years of serious experimental and intellectual assaults, we have a theory of hippocampal function that is only a little better than when we started. Certainly none of these theories is very sharp nor is there any particular trend for such theories becoming precise statements of hippocampal function. Even more bothersome is the fact that none of these theories relates function to the rather special anatomy of the hippocampus or to what we have learned or even to what we will learn about the cellular physiology of the hippocampus. Clearly this old approach to understanding hippocampal function alone will not do.

As a reductionistic neuroscientist, I would like to understand how groups of cells function to form an integrated system. This approach generally tries to relate cellular physiology and anatomy to system physiology. Within the context of hippocampal function, such an approach seeks to understand the input-output characteristics of the hippocampus as a system from its parts and to relate this system's behavior to the behavior of other brain regions and to brain functions. If such an abstract approach is properly framed, certain questions will be natural, while other questions will be easily avoided. Consider the four examples that follow.

First, we should not have to know the function of the entorhinal cortex (EC), the major extrinsic input to the hippocampus, or the Hubel and Wiesel-like feature-detecting properties of entorhinal neurons before we can study the hippocampus. Otherwise, by a natural regression, we would have to know the feature codings of all the brain regions that precede the hippocampus in sensory processing before we formulate a theory of hippocampal function. This approach certainly would postpone research on the hippocampus for a long time.

Second, we should be able to compare input-output functions between areas and between species. For example, how do primary visual cortex (area 17) and the hippocampus differ in the way they process signals? How do the rat and human hippocampi differ in their signal-processing abilities?

Third, a proper framework should stimulate quantitative as well as qualitative statements of function. Thus, when there are thousands of stimulating and recording electrodes in the future, there will be predictions worth testing.

Finally, independently obtained results from various brain regions should easily fit together. That is, if a proper language of input-output functions is used consistently for different regions, then we should be able to plug various parts of the brain together after studying individual systems and obtain a prediction of total brain function.

Therefore, the idea underlying the proposed approach is to study the abstract input-output transformations of systems like the hippocampus at the level of cells and groups of cells over physiologically relevant ranges of parameters. Even this is not enough of a framework, however, to gain an understanding of the function of the hippocampus or of any other brain region. We have to include, in our framework, questions of some central but general issue of brain function itself. The issue should guarantee relevance to the entire organism and must be identifiable in a consistent way in isolated brain regions.

But what is it that is special about the brain's function?

The function of the brain is to control the environment and the organism so that the organism survives and produces fertile offspring, thereby ensuring the survival of the species. This control is accomplished in large part by the brain's abilities to predict (1) future states of the environment, (2) how current and future states of the environment will affect the organism, and (3) how the organism's interactions with the environment will alter the environment.

Central to all of this predicting is our ability to find, store, and use the predictiveness—the regularities-that exist in the environment. Successful

prediction is possible, because there are spatiotemporal regularities and physical constraints in the environment. We will refer to these regularities and constraints as "redundancies" for four reasons: (1) the formal mathematical measures suggested by this term; (2) the ease of identifying this formalized redundancy with what we call correlations, concepts, associations, regularities, and constraints; (3) the formalization applies both to the environment and to individual brain regions; and (4) the meaning of the word redundancy, at least at its extremes in these mathematical formulations, agrees with the meaning of the word in conversation. Thus, successful prediction of the environment depends upon environmental redundancy.

Stored redundancies can be used to form predictions. When a new signal arrives, it is transformed to a less redundant signal based on past associations. These transformed signals are called predictive representations and are synonymous with recodings and with coded representations and are, in a mathematical sense, predictions.

It is, then, the storage of redundancy at synapses and predictive representation by reduction of signal redundancy that finally frames the abstract approach to understanding brain function. This issue gives our abstract approach to the brain and its regions that extra something that takes it beyond simple input-output questions to a general problem central to brain function that is applicable to almost any brain region as well as to the environment itself.

APPLICATION OF THE APPROACH TO THE HIPPOCAMPUS

As a general issue, the reduction of redundancy is particularly easy to apply when there is only one class of high-information-carrying inputs. As an example, let's consider the dentate gyrus (DG)-CA3 system of the hippocampus.

Given this approach, the theory of DG-CA3 function is, loosely stated, as follows. The layer-II stellatc cells of the EC provide information to the neurons of the DG-CA3 system. These neurons then reduce the signal redundancy while maintaining a distinctiveness of coding that allows for minimal loss of information. That is, the DG-CA3 system recodes the EC afferent information before sending it to the hippocampal CA1 region. The recoding is based on synaptically encoded associations, i.e., environmental redundancies, that have been learned via associative synaptic modification. This recoding is helpful to CA1, or to any other brain area, because of the severely restricted size and procesing speed of CA1 (and of every other brain region for that matter; see below). Figure 1 schematically illustrates these simplifications.

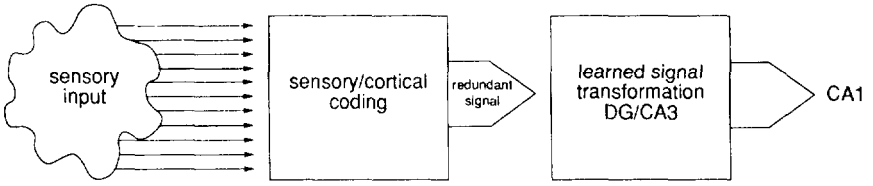

FIGURE 1. *Recoding a signal to remove redundancy*

Note that there is only one class of inputs in figure 1. This simplification comes from a basic idea in information theory (Shannon and Weaver 1949) that information capacity increases with both the number of channels and the maximum frequency of each channel. In this view, the EC afferents to the DG-CA3 system carry essentially all of the information to this part of the hippocampus because of their large numbers and their high-frequency firing rates. This is not to say that the monoaminergic and cholinergic afferents are unimportant-indeed, I am as certain as anyone that the hippocampus would not function properly without them-but that their function is not to carry high-density information to the hippocampus. This job is left for entorhinal afferents. Note that this view does not take away from the importance of monoaminergic or cholinergic inputs. To gain a better understanding, consider a metaphor of a TV set with two wires going in: the power cord, identified with the nonspecific inputs, and the antenna wire, identified with the entorhinal afferents. The antenna wire carries the information, but cut the power cord and nothing works.

Since there is no other high-density, extrahippocampal information source interacting with the entorhinal input, the EC signal is interacting only with itself: but to what end? Certainly the EC is the beginning of the funnel that brings together signals from all the diverse sensory systems. The DG-CA3 helps in this funneling, so obviously a recoding is going on, but why? Action potentials are sure, so there is no reason to insert redundancy. Textbooks to the contrary, there are no relay areas that are not processors. To merely pass on a signal not only wastes time and processing units, but it risks the almost certain injection of noise into the signals. On the other hand, reducing signal redundancy will help CA1, whatever its task. By the way, CA1 desperately needs help, because the number of cells in CA1 is woefully inadequate to create, much less compare, all possible hypotheses.

Now, note that we concern ourselves not with the environment that the entire organism experiences but with the environment that the hippocampus itself experiences. This is a major simplification of the framework compared to the old approach. That is, aside from hormonal influences, the environment of the hippocampus is no more and no less than the information provided by its afferents.

Thus, we no longer must identify cells by their feature-detection properties relative to the environment external to the organism. Instead, we determine the redundancy reduction at each stage of processing, though we always have the option of speculating on the redundancy of the environment and the feature-detection codings that are, in fact, predictive representations.

It is not that environmental redundancy is an uninteresting question; it is just that it requires solving a more difficult problem than understanding the brain itself, i.e., understanding the redundancy or organization of the entire world. Also, it implicitly evokes an almost dogmatic belief that we know what we see and that what we consciously see are the proper dimensions of the world. I am suspicious that we no more know the component features of what we see than we know the component features of what we smell.

A LITTLE MORE ABOUT REDUNDANCY

The regularities of the world are, through learning and heredity, stored in the connections—indeed as the quantitative connections—between neurons. Thus, we should understand that the associations that are learned and stored at synapses are synonymous with the regularities, correlations, and constraints, i.e., the redundancies, of the environment. Information and coding theories allow us to abstract and quantify the predictability of the environment using the idea of redundancy.

More precisely, redundancy is the amount of statistical dependence in a group of signals. When a linear measure of redundancy is desired, the mathematical formulations are quite explicit (Watanabe 1969).

The importance of predictive reduction of redundancy deserves comment. The reasons are both practical and philosophical. Practically, the brain is too small and too slow. This may seem strange, since we have always tried to impress the lay public and our students with the notion of how big the brain is—more than 10^{10} cells, more than 10^{14} synapses—but consider how many possible games of chess there are, and the brain is small in comparison. Consider how many possible configurations of the environment there are, each one you might encounter and need to represent, and the brain is minuscule by comparison.

Because of the overwhelming numbers of possible patterns in the world, it is critical to remove as much redundancy as possible from the representations of the environment. In addition, reducing redundancies will speed up computations and decrease storage requirements. Furthermore, when redundancies are removed from representations, there are some safe, fast, and easy ways to process representations and make predictions. Even these improvements will not be enough, however, and neural computations will usually have to "cheat" to compute fast enough for real time interactions. By cheating I mean make predictions, or equivalently do constraint

satisfaction, without examining each individually possible configuration of the environment. Such incomplete examination risks error. By removing redundancies, the average size of this error can be minimized.

The final argument may appeal only to the more philosophically minded but has application far beyond the hippocampus. First, note that the configurations of the environment that reach our sensory receptors are in fact signals. Then, the redundancy in these special signals is all there is to learn about the environment. In signals with no redundancy or in an environment with no redundancy, there is nothing to learn. Obviously, then, if we can recode the environment by removing and storing the redundancies (or use the predictive redundancies we have inherited) while maintaining pattern distinctiveness, we understand all there is to know about these signals with regard to themselves. Thus, identifying and storing redundancy is what learning and, therefore, much of synaptic modification is all about.

AN EXAMPLE OF REDUNDANCY IN OUR DAY-TO-DAY LIFE

Imagine this scene-you are at home, reading a book, waiting to be picked up and driven to the airport. A horn sounds from outside. Honk! You get up and go to the door to leave. Note how the honk predicts the car outside. Now suppose we recreate this scene again except you are not reading but sitting at the window. Just at the same time the horn sounds, you see the arrival of the expected car. Note that the sound alone was enough to predict the car so that, in this second case, hearing the horn and seeing the car are to a large extent redundant in the information that your ride has arrived.

AN EXAMPLE OF NEURAL REDUNDANCY

In figure 2, we consider a very simple nervous system with two afferent lines of signals.

An event is an active cell; PROB ( ) stands for probability of.

(A) There is zero redundancy if there is total independence; e.g.,

PROB (EVENT 1 AND EVENT 2) = PROB (EVENT 1) . PROB (EVENT 2)

(B) There is redundancy if there is not statistical independence, e.g.,

PROB (EVENT 1 AND EVENT 2) ≠ PROB (EVENT 1) . PROB (EVENT 2)

FIGURE 2. *Examples of neural redundancy*

If the probability of joint activity, i.e., the probability of both cells being active, is equal to the product of the individual probabilities of cell activity, as in (A) in figure 2, then there is statistical independence and no redundancy in the signals. If the equality does not hold, as in (B) in figure 2, then there is redundancy in the system. When there is no redundancy, conditional prediction is a waste of time, because there are no useful correlations or associations. When there is redundancy, we have something to gain from predictions about activity at one cell conditional on the activity of the other cell.

This idea of redundancy vs. no redundancy can be refined to formal measurements quantifying gradations that vary between no redundancy and total redundancy. One such measurement is an entropy called mutual information.

Using mutual information, we now state the theory more precisely:

> The object of the DG-CA3 transformation is to minimize the mutual information of the CA3 output signals with respect to themselves, while maximizing the mutual information between EC input signals and CA3 output signals.

What we are proposing is that subsystems of the brain like the hippocampus face a dual optimization problem that involves (1) minimizing the mutual information of CA3 cell activities with regard to themselves, while, at the same time, (2) maximizing the mutual information of the layer-II EC input relative to the CA3 output. Part (1) is the optimization that reduces signal redundancy. The transformation performed by the dentate gyrus and CA3 recodes the EC representation of whatever into a CA3 representation of this same whatever. Notably the CA3 representation is less internally redundant than the EC representation. Part (2) is the optimization that maintains pattern distinctiveness. That is, those patterns that are easily separable or distinguishable in the EC representation are still easily distinguishable by their CA3 representations.

Just to show that this dual optimization problem is well formulated, we point out that there actually is a unique solution to this problem if the world is Gaussian (of course it is not!). For zero mean Gaussian environments, the Karhunen-Loeve transformation is the optimal mapping that uniquely solves this dual optimization (Watanabe 1969). This transformation codes the environment so that all output lines considered pairwise are independent and no higher order interactions exist. Because the transformation is invertible, the input-output mutual information is maximal. A more general solution suitable for optimally transforming mote complex environments is desirable.

CONCLUSION

Finally, we should realize that this new framework can happily exist with the old game. The following are examples of this coexistence.

1. Spatial sequences, such as encountered by small animals running through the maze of the underbrush and through drain pipes, require bringing together codings of rather diverse spatial relations and sequences. These spatial and spatiotemporal patterns are likely represented differently by the different senses (e.g., visual and olfactory codings must be very different initially). Recoding for an efficient compatible melding of these diverse dimensions seems essential to using all the cues quickly and accurately. Recoding to achieve redundancy reduction in DG-CA3 and other limbic structures may be critical to the degree of success or failure in such an environment.

2. Stimulus mismatch is a trigger for selective attention and an attempt to recode our stored, predictive redundancies. Mismatch activates certain nonspecific inputs to the hippocampus that in turn facilitate associative synaptic modification in the DG-CA3. This facilitation of modification in a mismatch situation is an opportunity to store redundancies that more accurately coincide with those of the environment.

3. Short-term memory incorporates a redundancy reduction process. This process saves space later when memories are transferred from short-term to long-term storage.

HISTORICAL NOTES

The ideas expressed here have appeared at various times in various places. Included below are the authors who have been most influential on me or who, discovered in retrospect, presented relatively clear expositions of their ideas.

The ideas here are strongly related to those of S. Watanabe (1969) relating pattern recognition and inference to reduction of entropy.

The theme of redundancy reduction was quite explicitly introduced to neuroscience by Barlow (1959) and is still occasionally found as an issue in the visual system.

W.R. Ashby (1956) and Richard Bellman (1967) clearly expressed the idea that the nervous system or any other device was too small and too slow compared to the possibilities of the environment.

Marr (1970) mentions the issue of redundancy reduction in terms of the overwhelming encoding problems faced by the brain.

The unsupervised concept formation systems of various authors, including Anderson (1968), Amari (1977), Kohonen (1984), and Marr (1970) strongly resemble the problem and the system considered here. However, predictive reduction of redundancy is a more general issue than concept formation as it is usually presented. That is, the redundancy issue includes concept formation (as Marr points out). However, predictive redundancy reduction is less restrictive, and the mathematics are much better developed.

The equivalency between total understanding and recoding for minimum redundancy is almost a direct quote from the work by Rissanen (1986), while the germ of the idea appears in the writings of several philosophers.

J.A. Gray (1982) has emphasized the importance of prediction and related it to the limbic system.

The ideas of this chapter appeared previously in abstract form (Levy 1985).

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Pharmacological and Anatomical Analysis of Fear Conditioning

Michael Davis

INTRODUCTION

A major challenge in neuroscience is understanding the biological substrates of learning and memory. This will eventually involve a detailed cellular and biochemical description of the events in the nervous system that result in a relatively permanent change in neural transmission—a change that allows a formerly neutral stimulus to produce or affect some behavioral response. The most definitive work on the cellular and biochemical analysis of learning and memory has been carried out in invertebrate nervous systems (Alkon 1979; Carew 1984; Castellucci et al. 1970; Crow and Alkon 1980; Hawkins et al. 1983; Walters and Byrne 1985). A major advance in the analysis of these questions was choosing a simple reflex behavior that could be modified by experience and then determining the neural circuit that mediated the behavior being measured. Once this was done, it was possible to isolate where different types of plasticity occurred and then to determine how these changes were brought about at the cellular level.

Comparably detailed biochemical analyses of learning and memory that relate directly to behavioral output have not been possible in vertebrate nervous systems. In large part, this is because it has been very difficult to isolate where plastic changes take place in complex vertebrate nervous systems. Thus, at this stage, it is important to develop, in complex vertebrates, simple models of learning and memory. Such models can be used to isolate loci within the nervous system where plastic changes take place that allow a conditioned stimulus to induce or affect behavior. The short-latency acoustic startle reflex enhanced by prior classical fear conditioning may be an especially promising model system with which to carry out this type of analysis.

The purpose of this chapter is to describe the fear-potentiated startle paradigm and the advantages it provides for a neuroanatomical analysis of fear conditioning. The neural pathways involved in the startle reflex will be described. The role of the amygdala in fear-potentiated startle and its possible connections to the startle pathway and critical visual structures that carry information about the conditioned stimulus will be reviewed. Finally,

the importance of the central nucleus of the amygdala and its efferent projections to several brainstem target areas for the expression of fear will be outlined.

THE FEAR-POTENTIATED STARTLE PARADIGM

Brown et al. (1951) demonstrated that the amplitude of the acoustic startle reflex in the rat can be augmented by presenting the eliciting auditory startle stimulus in the presence of a cue (e.g., a light) that has previously been paired with a shock. This phenomenon has been termed the "fear-potentiated startle effect" and has been replicated using either an auditory or visual conditioned stimulus, when startle has been elicited by either a loud sound or an airpuff (Davis 1986).

In this test, a central state of fear is considered to be the conditioned response (McAllister and McAllister 1971). Conditioned fear is operationally defined by elevated startle amplitude in the presence of a cue previously paired with a shock (figure 1). Thus, the conditioned stimulus does not elicit startle. Furthermore, the startle-eliciting stimulus is never paired with a shock. Instead, the conditioned stimulus is paired with a shock, and startle is elicited by another stimulus either in the presence or absence of the conditioned stimulus. Fear-potentiated startle is said to occur if startle is greater when elicited in the presence of the conditioned stimulus. Potentiated startle only occurs following paired, rather than unpaired or "random," presentations of the conditioned stimulus and the shock, which indicates that it is a valid measure of classical conditioning (Davis and Astrachan 1978). Discriminations between visual and auditory conditioned stimuli (Hitchcook and Davis 1986) or between auditory cues that differ in duration (Siegel 1967) have also been demonstrated with potentiated startle. Generalization decrements resulting from a change in the frequency of a pure tone conditioned stimulus between training and testing have also been reported (Siegel 1967). Increased startle, in the presence of the conditioned stimulus, still occurs very reliably at least 1 month after original training, making it appropriate for the study of long-term memory as well (Cassella and Davis 1985).

TEMPORAL SPECIFICITY OF FEAR-POTENTIATED STARTLE

Recent data indicate that fear-potentiated startle shows a good deal of temporal specificity (Davis et al. 1989). To test this, separate groups of rats were given 10 pairings of a light and a shock on each of 3 successive days. In one group, the light-shock interval (conditioned stimulus-unconditioned stimulus (CS-US) interval) was 200 milliseconds (i.e., a 700-millisecond light terminating with a 500-millisecond shock); in the other group, the light-shock interval was 51,200 milliseconds. A third group, the random group, had lights and shocks presented in a nonsystematic fashion. Two days later, all groups were tested identically by eliciting startle in the

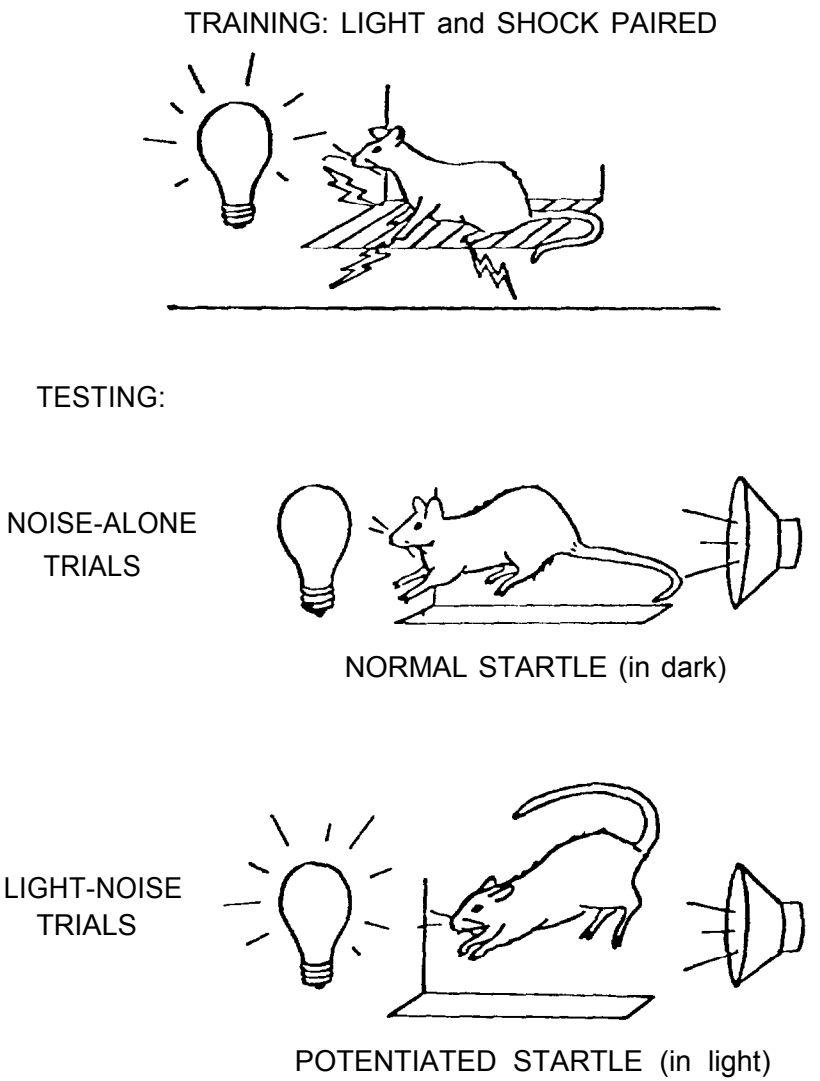

FIGURE 1. *Cartoon depicting the fear-potentiated startle paradigm*

NOTE? Bring training, a neutral stimulus (conditioned stimulus) such as a light is consistently paired with a footshock. During testing, startle is elicited by an auditory stimulus (e.g., a 100-dB burst of white noise) in the presence (light-noise trial type) or absence (noise-alone trial type) of the conditioned stimulus. The positions and postures pictured may not represent the actual behavior of the animals.

absence of the light (noise-alone trials) or 25, 50, 100, 200, 400, 800, 3,200, 12,800, or 51,200 milliseconds after onset of the light. The 10 to 20 occurrences of each of the 10 different test trial types were presented in a balanced, irregular order across a 1.5-hour test session.

Unconditioned Effect of Light on Startle

The left panel of figure 2 shows the mean amplitude startle response at each of the various test intervals for each of the three groups. The solid line was drawn between the average values of the noise-alone trials for the three groups to facilitate visualizing the effects of the light in the different groups. In the random group, startle was markedly depressed from about 25 to 200 milliseconds after light onset (prepulse inhibition) and then slightly elevated at the long test intervals. These data were consistent with earlier reports showing that the presentation of a visual stimulus in untrained animals leads to prepulse inhibition at short test intervals and facilitation at long test intervals (Ison and Hammond 1971).

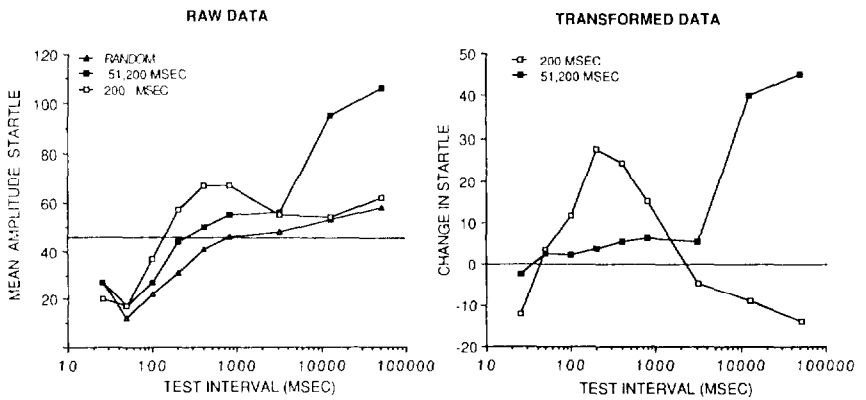

FIGURE 2. *Temporal specificity of fear-potentiated startle*

NOTE The left panel shows the mean amplitude startle response at each of the various light-noise test intervals (25, 50, 100, 200, 400, 800, 3,200, 12,800, or 51,200 milliseconds plotted on a log scale) for animals trained with either a 200-millisecond (open squares) light-shock interval, a 51,200-millisecond (closed squares) light-shock interval, or a random relationship between lights and shocks (triangles). The solid line was drawn between the average values of the noise-alone trials for the three groups, which did not differ significantly from each other. The right panel shows these same data transformed in the following way. First, the startle level on the noisealone trials was subtracted from the startle level on each of the light-noise trials for every animal, allowing startle at each light-noise test interval to be expressed relative to the individual noise-alone baseline in that group. Next, using these difference scores, the data from the random group at each test interval were subtracted from the data of each group at every test interval, thus factoring out unconditioned effects of the light (i.e., prepulse inhibition at short intervals, facilitation at long intervals) so as to visualize the effects of prior conditioning.

Conditioned Effects of Light on Startle After Training With Short vs. Long CS-US Intervals

In the 200- and 51,200-millisecond groups, there was also a decrease in startle at the 25-, 50-, and 100-millisecond test intervals. This indicates that the light still produced prepulse inhibition, even in groups that had the light previously paired with a shock. In the 200-millisecond group, there was a sharp increase in startle from 50 to 400 milliseconds, which declined back to the level of the random group at the later test intervals. In contrast, in the 51,200-millisecond group, the largest increase in startle occurred much later, at the 12,800- and 51,200-millisecond test intervals.

To more fully visualize the conditioned changes in startle at various light-noise test intervals after previous training at different light-shock intervals, it is necessary to factor out the complex unconditioned effects of the light on startle seen in naive animals or the random group. To accomplish this, the following data transformations were made. First, the startle level on the noise-alone trials was subtracted from the startle level on each of the light-noise trials for every animal. In this way, changes in startle at each of the light-noise test intervals, relative to the individual noise-alone baseline for that animal, could be compared after various light-shock training intervals. Using these difference scores, the data from the random group at each test interval were then subtracted from the data of each group at each test interval. This was done to factor out the unconditioned effects of light on startle at the various test intervals (i.e., prepulse inhibition, facilitation at long test intervals) to visualize differences based on prior conditioning.

The right panel of figure 2 shows the transformed data for the 200- and 51,200-millisecond groups derived from the subtractions listed above. Any points above the zero line represent potentiated startle relative to an animal's individual baseline and the random group. This panel shows quite clearly that potentiated startle was maximal about 200 milliseconds after light onset in rats trained with a 200-millisecond light-shock interval, with no potentiation at long test intervals. In contrast, potentiated startle was maximal about 51,200 milliseconds after training with a 51,200-millisecond light-shock interval, with little or no potentiation at short test intervals.

To analyze the degree of temporal specificity of potentiated startle, other groups of 10 rats were each given light-shock intervals of either 0, 25, 50, 100, 800, 3,200, or 12,800 milliseconds. They were tested 2 days later at each of the several light-noise intervals, as described above. Figure 3 shows the transformed data for each of nine different training groups. Again, data points above the zero line indicate increased startle at that test interval relative to the random group. Figure 3 shows three important findings. First, potentiated startle was obtained over a very wide range of light-shock training intervals, from 25 to 51,200 milliseconds. Secondly, the test interval of maximum potentiation within a group tended (although certainly

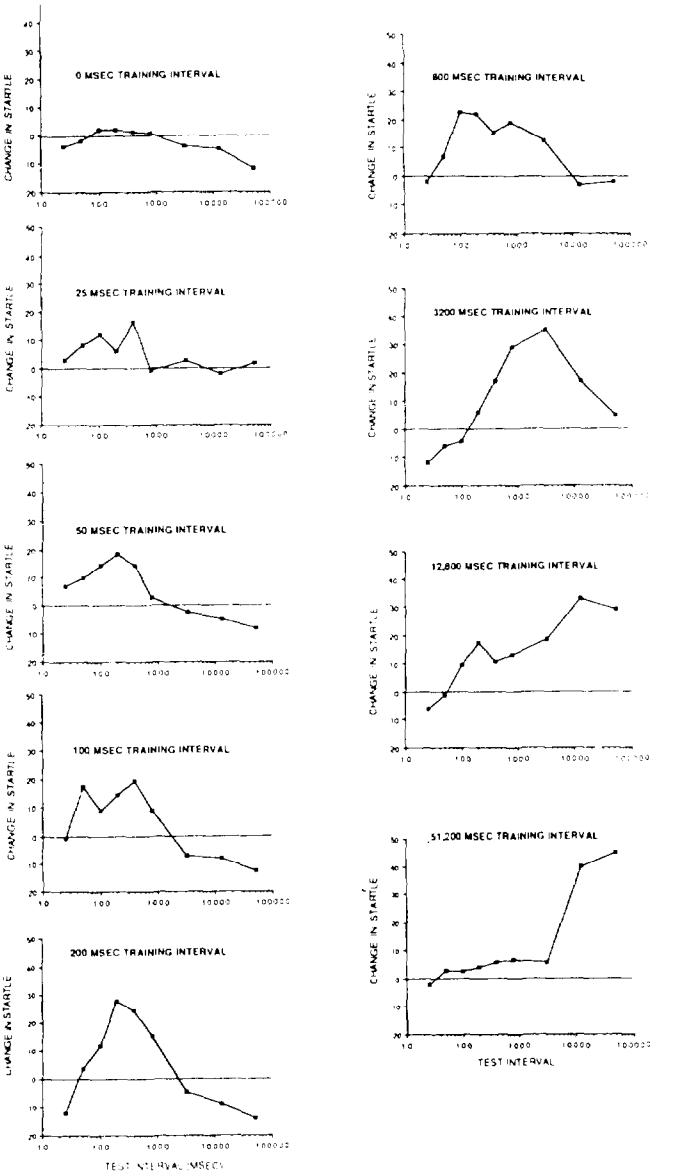

FIGURE 3. *Mean change in startle amplitude at each of the various light-noise test intervals after the nine different light-shock training intervals*

NOTE: The data have been transformed for each group as described in figure 2.

not in every group) to match the CS-US interval used in training. For example, with a 200-millisecond training interval, potentiation was maximal about 200 to 400 milliseconds after light onset but was completely gone at the long test intervals. In contrast, with a 51,200-millisecond training interval, potentiation was greatest at the 12,800- and 51,200-millisecond test intervals but was very weak at the 200- to 400-millisecond test intervals. Finally, the magnitude of potentiation tended to grow with longer training intervals. Thus, at the 0-millisecond training interval (i.e., simultaneous light and shock onset), no data points were above the line, indicating no potentiation at any interval. With a 200-millisemnd training interval, the maximal change was about 30 units, whereas, with a 51,200-millisecond training interval, the maximal change was about 40 to 45 units.

These data indicate, therefore, that fear-potentiated startle shows considerable temporal specificity, since potentiated startle tends to be maximal at the test interval that matches the training interval. Other studies showed that temporal specificity tended to "sharpen" with an increasing number of training trials. Remarkably, a temporal pattern of potentiated startle similar to that shown in figure 2 for the 51,200-millisecond group could be obtained after a single training trial when the long, 51,200-millisecond light-shock interval was used. Taken together, these data indicate that the conditioned stimulus in fear conditioning is not simply the presence or absence of the light, but instead is the temporal pattern of events that are paired with the US in training.

THE PHARMACOLOGY OF FEAR-POTENTIATED STARTLE

Advantages of Fear-Potentiated Startle for Studying the Pharmacology of Fear or Anxiety

Fear-potentiated startle offers a number of advantages for analyzing how drugs affect fear or anxiety. First, potentiated startle is defined as a within-subjects difference in startle amplitude in the presence (light-noise trials) vs. the absence (noise-alone trials) of the visual conditioned stimulus. This makes it a sensitive measure, because it reduces problems caused by between-subjects variability in startle. Second, it allows an evaluation of specific effects (reduction of startle on light-noise trials) vs. nonspecific effects (reduction of startle on noise-alone trials), so that qualitative as well as quantitative drug profiles can be compared. Third, different intensities of the auditory stimulus can be used within the same test session to elicit startle. This allows potentiated startle to be assessed at several points on the measurement scale, circumventing problems of interpretation that can arise when markedly different parts of the measurement scale are involved (e.g., rate-dependent drug effect in operant paradigms; percent figures used with very different baselines). Fourth, no shocks are given in testing. Thus, drug effects observed in testing cannot be explained in terms of changes in sensitivity to shock. Fifth, the separation between training and

testing sessions allows one to evaluate whether a drug alters original learning or performance, and tests for state-dependent learning can be easily evaluated (Davis 1979a). Sixth, potentiated startle does not involve any obvious operant. Thus, the animal is not required to make or withhold a voluntary response to indicate fear or lack of fear, and, consequently, drug-induced effects that might be expected to alter operant performance (e.g., rate-dependent, motivational, disinhibitory motor effects) are circumvented. In addition, most animal tests of fear or anxiety involve a suppression of ongoing behavior in the presence of a fear stimulus (e.g., suppression of bar pressing in the conditioned emotional response test or the operant conflict test; suppression of licking in the lick-suppression test; suppression of movement measured by freezing; suppression of normal activity in the social interaction test). Hence, certain treatments (e.g., decreases in serotonin transmission) might appear anxiolytic in these tests if they interacted with neural systems common to each of these tests (e.g., response inhibition), even though they might not reduce anxiety clinically. Because fear in the potentiated startle paradigm is reflected by enhanced response output, it may provide an important alternative test with which to analyze potential anxiolytic compounds.

Effects of Different Drugs on Fear-Potentiated Startle

Table 1 shows that a variety of drugs that reduce fear or anxiety in humans decrease potentiated startle in rats. Clonidine, morphine, diazepam, and buspirone, which differ considerably in their mechanism of action, all block potentiated startle. In most cases, these treatments do not depress startle levels on the noise-alone trials (figures 4 and 5), although clonidine (figure 6) does have marked depressant effects on both types of trials. In addition, yohimbine and piperoxane (figure 6), which induce anxiety in normal people and exaggerate it in anxious people (Chamey et al. 1984; Goldenberg et al. 1947; Holmberg and Gershon 1961; Soffer 1954), actually increase potentiated startle in rats. Thus, at very low doses, these drugs increase startle amplitude on the light-noise trials without having any effect on startle on the noise-alone trials; this only occurs in rats conditioned to fear the light (Davis et al. 1979).

On the other hand, table 1 shows that a variety of treatments that alter serotonin (5-HT) transmission do not affect potentiated startle. This is important because treatments that decrease 5-HT transmission have an anxiolytic profile in several animal tests of fear or anxiety (e.g., operant-conflict test, lick-suppression test, and social interaction test), perhaps by interfering with response inhibition (Soubrie 1986). These effects may represent false positives in these tests, because treatments like p-chlorophenylalanine do not appear to be anxiolytic when tested clinically (Shopsin et al. 1976). In addition, although the anxiolytic effects of buspirone have been suggested to be mediated through the serotonin system, the ability of buspirone to selectively decrease fear-potentiated startle does not seem to be

TABLE 1. *Effects of different treatments that alter different neurotransmitters on fear-potentiated startle*

Treatment Effects on Potentiated Startle	Dose Range (mg/kg)	Reference
Block		
Sodium amytal	10-40	Chi 1965
Diazepam	0.3-2.5	Davis 1979a Berg and Davis 1984
Flurazepam	2.5-20	Davis 1979a
Morphine	2.5-10	Davis 1979b
Alcohol	7.5-22.5 cc/kg	Miller and Barry 1960
Nicotine*	0.4 mg/kg	Sorenson and Wilkinson 1983
Buspirone	0.6-10	Kehne et al. 1988
Gepirone	0.6-10	Kehne et al. 1988
Clonidine	0.01-0.04	Davis et al. 1979
Do Not Block		
Cinanserin	10	Davis et al. 1988
Cyproheptadine	5	Davis et al. 1988
Ipsapirone	5-20	Davis et al. 1988
8-0H-DPAT	2.5-10	Davis et al. 1988
p-chloroamphetamine	5	Davis et al. 1988
p-chlorophenylalanine	400x2	Davis et al. 1988
Naloxone	2.0	Davis et al. 1988
Ro-15-1788	1.0	Davis et al. 1988
Raphe lesions	---	Davis et al. 1988
1-PP (buspirone metabolite)	0.5-40	Kehne et al. 1988
Imipramine (chronic or acute)	5-10	Cassella and Davis 1985
WB-4101	1.0	Davis et al. 1979
Propranolol*	20	Davis et al. 1979
Increase		
Piperoxane	0.25-1.0	Davis et al. 1979
Yohimbine	0.125-.25	Davis et al. 1979

*Partial blockade.

attributable to its actions at either pre- or postsynaptic 5-HT receptors (Davis et al. 1988). Thus, other drugs such as ipsapirone or 8-OH-DPAT,

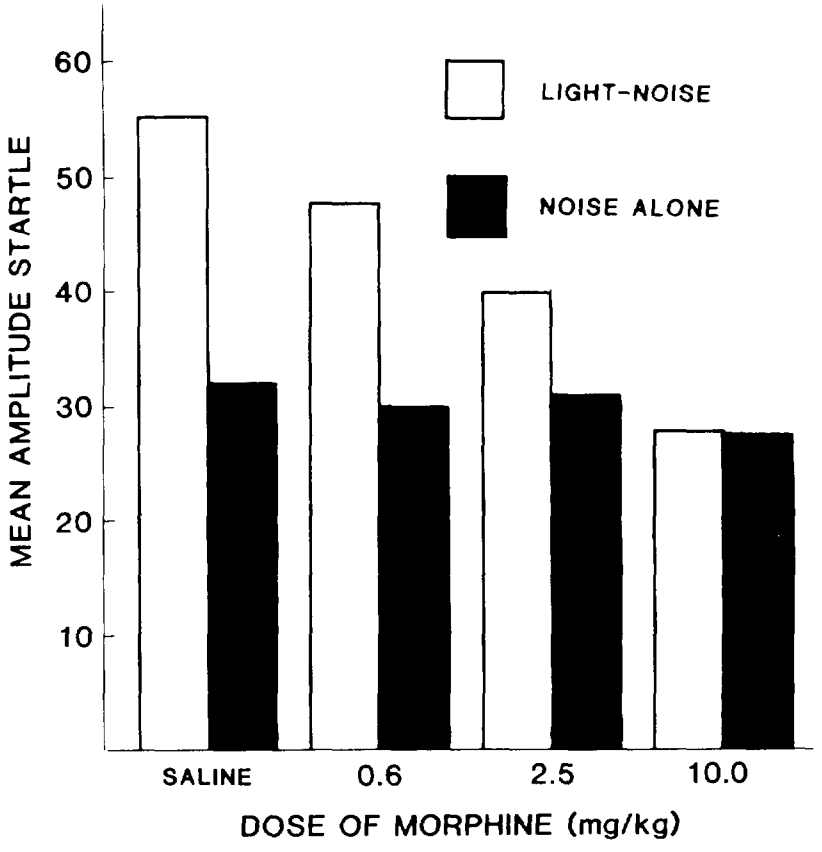

FIGURE 4. *Mean amplitude startle on noise-alone trials (dark bars) or light-noise trials (open bars) following IP injection 15 minutes earlier of saline or morphine (0.6, 2.5, 10 mg/kg)*

NOTE: Rata were given 45 light-shock pairings (1,000-millisecond light, 500-millisecond, 0.25-mA shock, 500-millisecond light-shock interval) on each of 2 days. One day later, a total of 40 noise-alone and 40 light-noise trials (20 at 105 dB and 20 at 110 dB) were given in an irregular order at a 30-second interval. Data were collapsed across the two stimulus intensities used to elicit startle.

SOURCE Adapted from Davis 1979b.

which bind to 5-HT_{1A}-binding sites as buspirone and gepirone do, do not block fear-potentiated startle. Moreover, raphe lesions by themselves do not block fear-potentiated startle, nor do they prevent buspirone from having its usual anxiolytic effect in this test. Currently, the effects of drugs that

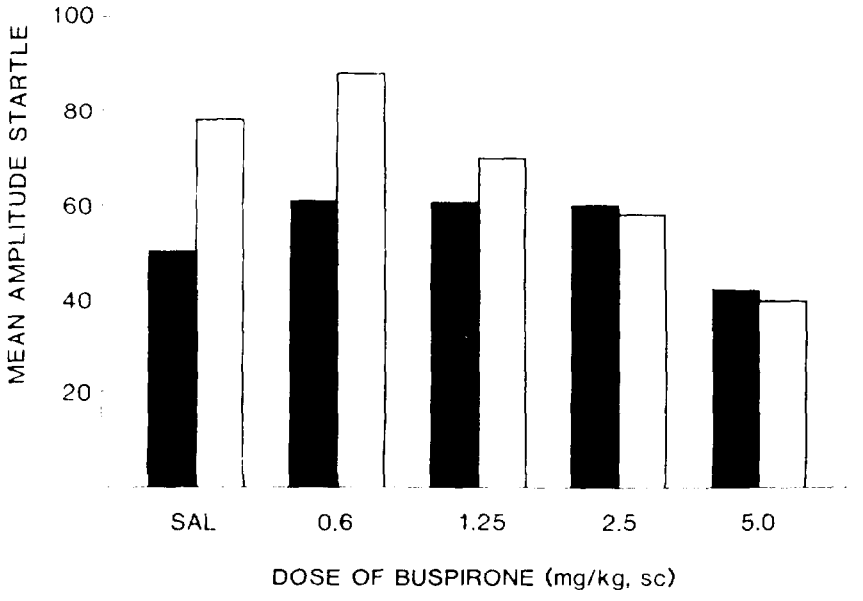

FIGURE 5. *Mean amplitude startle on noise-alone trials (dark bars) or light-noise trials (open bars) 5 minutes after SC injection of saline or buspirone (0.6, 1.25, 2.5, 5.0 mg/kg)*

NOTE: Rats were given 10 light-shock pairings (3,700-millisecond light, 500-millisecond later, 0.6-mA shock, 3,200-millisecond light-shock interval) on each of 2 days. Two days later a total of 30 Norse-alone and 30 light-noise trials (10 at 90, 95, or 105 dH) were given in an irregular order at a 30-second interval. Data were collapsed across the three stimulus intensities used to elicit startle.

SOURCE: Kehne et al. 1988, Copyright 1988, Springer-Verlag.

act on dopamine, vs. dopamine, receptors on fear-potcntiated startle that may be relevant to the actions of drugs like buspirone (Taylor et al. 1982) are being tested.

THE ACOUSTIC STARTLE PATHWAY

A major advantage of the potentiated startle paradigm is that it measures fear by a change in a simple reflex. Hence, with potentiated startle, fear is expressed through some neural circuit that is activated by the conditioned stimulus and ultimately impinges on the startle circuit. These two neural

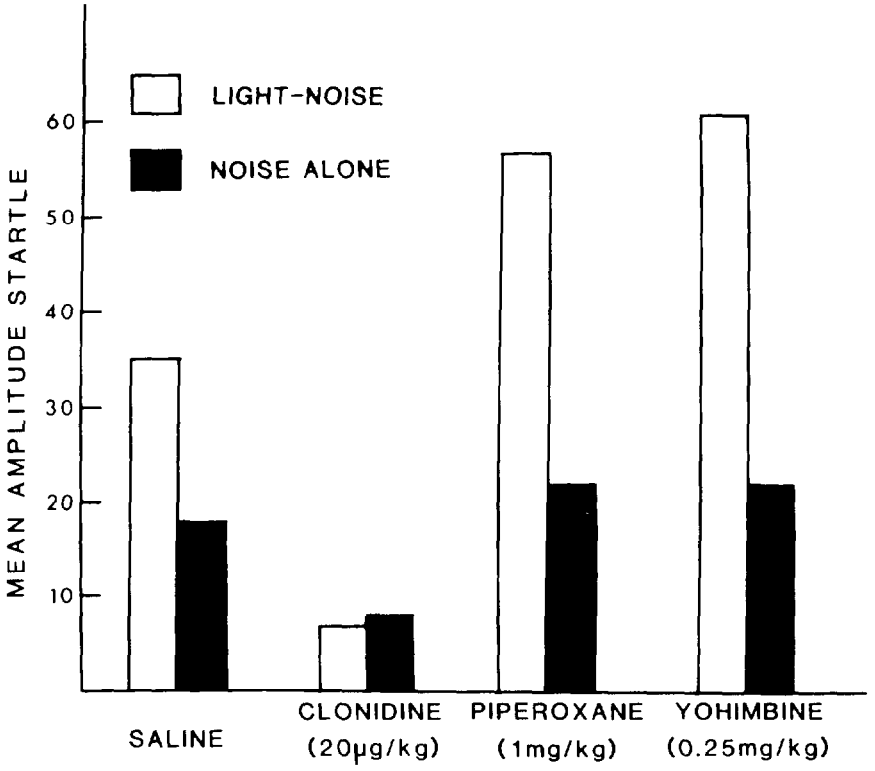

FIGURE 6. *Mean amplitude startle on noise-alone trials (dark bars) or light-noise trials (open bars) 5 minutes after IP injection of saline, clonidne (20 pg/kg), piperoxane (1.0 mg/kg), or yohimbine (0.25 mg/kg)*

NOTE: Rats were given 45 light-shock pairings (1,000-millisecond light, 500-millisecond later, 0.25-mA shock, 500-millisecond light-shock interval) on each of 2 days. One day later, a total of 40 noise-alone and 40 light-noise trials (20 at 105 dB and 20 at 110 dB) were given in an irregular order at a 30-second interval. Data were collapsed across the two stimulus intensities used to elicit startle.

SOURCE: Adapted from Davis et al. 1977.

pathways are being delineated to see how they interconnect to mediate potentiated startle.

In the rat, the latency of acoustic startle is 6 milliseconds recorded electromyographically in the foreleg and 8 milliseconds in the hindleg (Ison et al. 1973). This is an extraordinarily short latency and indicates that only a few synapses can be involved. Figure 7 illustrates the nuclei and fiber tracts

believed to mediate the acoustic startle response in the rat (Davis et al. 1982).

The posteroventral cochlear nucleus (VCN) appears to be the first synapse in the primary acoustic startle circuit. Bilateral lesions of the VCN, but not the neighboring dorsal cochlear nuclei, abolish acoustic startle. In awake rats, bilateral, single-pulse stimulation of the VCN (1-millisecond pulse width, 10 to 25 μA) elicits startlelike responses with a latency of 7.0 to 7.5 milliseconds (figure 8).

The next synapse appears to occur in the ventral nucleus of the lateral lemniscus (VLL), which is known to receive direct projections from the VCN. Bilateral lesions of the VLL eliminate acoustic startle, and electrical stimulation of this nucleus elicits startlelike responses with an average latency of about 6.0 milliseconds (figure 8).

The next synapse may occur in a ventromedial region of the nucleus reticularis pontis caudalis (RPC). Bilateral lesions of this area abolish acoustic startle. Electrical stimulation of points within the RPC elicits startlelike responses with an average latency of about 5 milliseconds. Cell bodies in the RPC send their axons to all levels of the spinal cord (SC) by way of reticulospinal tract. This tract courses near or through the medial longitudinal fasciculus (MLF) on the midline and then bifurcates to form the ventral funiculi in the SC. Complete lesions of the MLF eliminate acoustic startle, and electrical stimulation on the midline through the MLF elicits leg movements with a latency of about 4.0 to 4.5 milliseconds (figure 8).

Fibers from the reticulospinal tract synapse in the spinal cord, forming the final synapse before the neuromuscular junction. Direct monosynaptic connections onto motorneurons, as well as indirect ones through an interneuron in the cord, are possible. To date, it has not been determined whether spinal interneurons are involved.

More recently, it has been found that infusion of ibotenic acid, which destroys cell bodies without damaging fibers of passage (Schwarcz et al. 1979; Zaczek and Coyle 1982), into either the VCN, VII, or RPC also eliminates acoustic startle (Cassella and Davis 1986).

DETERMINING THE POINT WITHIN THE STARTLE PATHWAY WHERE FEAR ALTERS NEURAL TRANSMISSION

Having delineated the startle reflex circuit involved in fear-potentiated startle, the next task was to determine the point within the startle circuit where the visual conditioned stimulus modulates transmission following conditioning. To do this, startlelike responses were elicited electrically from various points along the startle pathway before and after presentation of a light that was either paired or not paired with a shock in different groups of

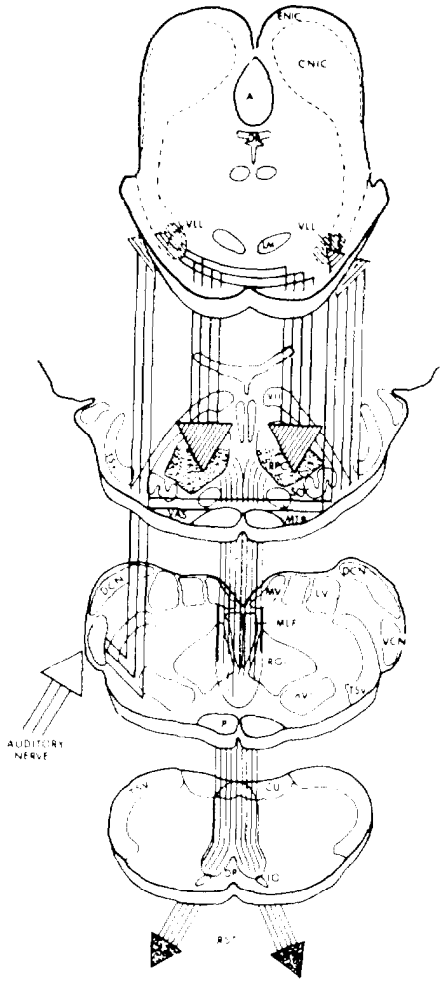

FIGURE 7. *Schematic diagram of a primary acoustic startle circuit consist& of the VCN, VLL, and RPC*

NOTE: Other abbreviations used are: A, aqueduct; CNIC, central nucleus of the inferior colliculus; CU, cuneate nucleus; DCN, dorsal cochlear nucleus; DP, decussation of pyramids; DR, dorsal raphe nucleus; ENIC, external nucleus of the inferior colliculus; HRP, horseradish peroxidase; IO, inferior olive; LL, lateral lemniscus; LM, medial lemniscus; LV, lateral vestibular nucleus; MLF, medial longitudinal fasciculus; MTB, medial nucleus of the trapezoid body; MV, medial vestibular nucleus; nVII, nucleus of the seventh nerve; P, pyramids; RGI, nucleus reticularis gigantocellularis; RPO, nucleus reticularis pontis oralis; RST, reticulospinal tract; RSTm medial reticulospinal tract; SO, superior olive; TSV, spinal tract of the fifth nerve; VAS, ventral acoustic stria; VII, seventh nerve.

SOURCE: Davis et al. 1982, Copyright 1982, Society for Neuroscience

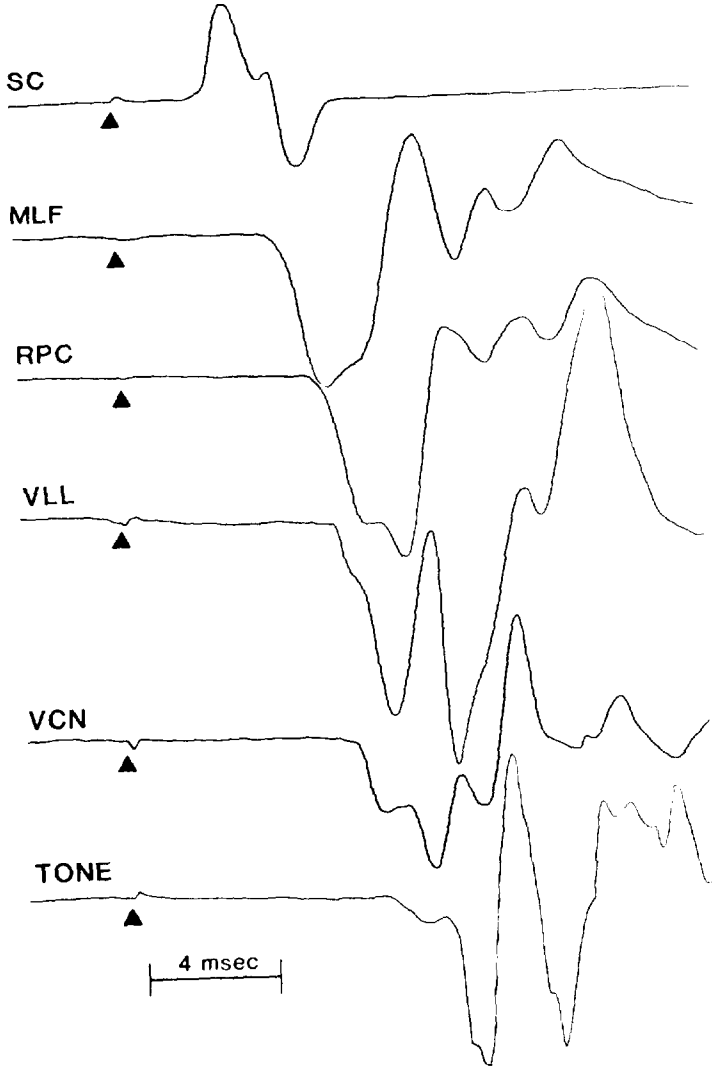

FIGURE 8. *Electromyographic recording from the quadriceps femoris muscle complex of the "startle" response elicited by electrical stimulation in the SC, MLF, RPC, LL, VCN, or by a tone*

SOURCE: Davis et al. 1982, Copyright 1982, Society for Neuroscience

rats (Berg and Davis 1985). Startle elicited by electrical stimulation at or before the point in the startle circuit where the light modulates transmission should show potentiation, whereas startle elicited beyond this point should not (see footnote to figure 9 for the rationale of this experiment). In this experiment, separate groups of rats were implanted, at least 24 hours prior to training, with bilateral monopolar electrodes (0.25 mm diameter, 0.5 mm uninsulated tip) in either the VCN, VLL, or RPC. In addition, other groups were implanted with electrodes aimed at lateral as well as medial aspects of the ventral acoustic stria (VAS), the fibers that connect the VCN to the VLL. Following recovery, all groups were trained to be fearful of a light by pairing it with a footshock.

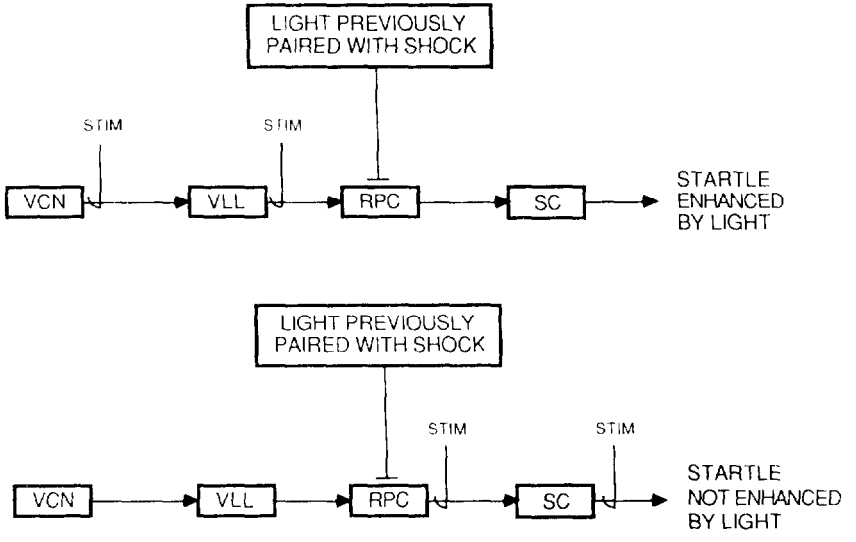

FIGURE 9. *Schematic diagram of modulation of electrically elicited startle by presentation of a light previously paired with a shock*

NOTE: The point of electrical elicitation of startle is indicated by the "stim" symbol. The model predicts that if the light ultimately modulates neural transmission at the RPC, then startle elicited electrically in the VCN or VLL will be enhanced by the light, since the signal produced by electrical stimulation must travel through the area where the light modulates neural transmission. In contrast, startle elicited electrically in the RPC beyond the area of modulation or startle elicited electrically in spinal motor neurons will not be enhanced by the light, since the signal produced by electrical stimulation will bypass the area where the light modulates neural transmission.

Figure 10 shows that potentiation of electrically elicited startle was equivalent to potentiation of acoustic startle at all locations up through the VLL.

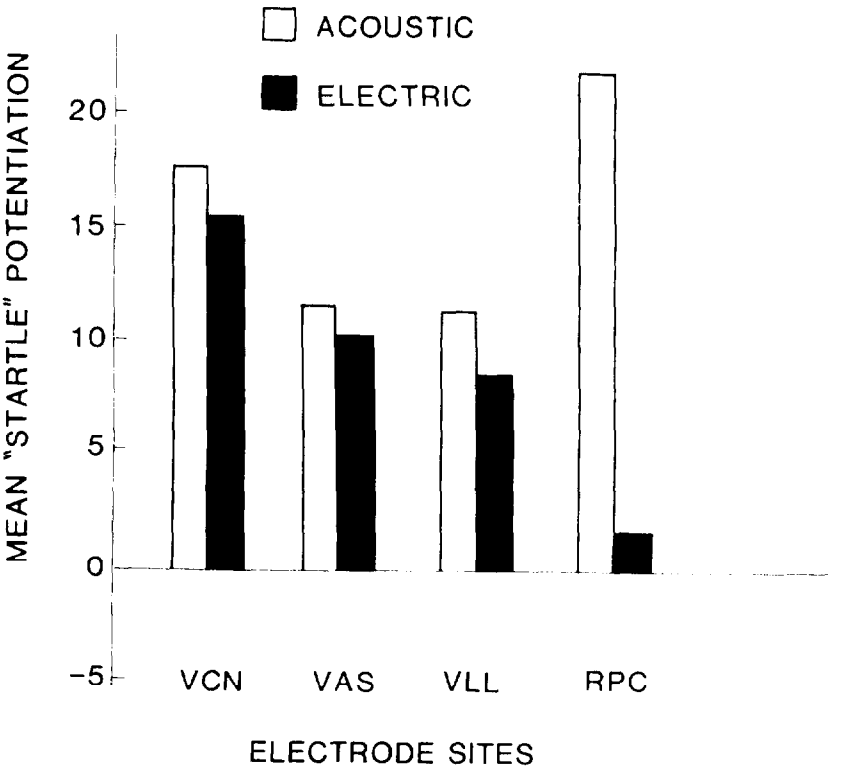

FIGURE 10. *Magnitude of fear-potentiated startle effect when startle was elicited either acoustically (white bars) or electrically (black bars) in different groups of rats that had electrodes implanted in the VCN, VAS, VLL, or RPC*

NOTE: The white bars represent the degree of potentiation of startle elicited acoustically in tats with electrodes implanted into different parts of the startle pathway. The black bars represent the degree of potentiation of startle elicited electrically through electrodes implanted in different parts of the startle pathway. Degree of potentiation is expressed as the mean difference in startle amplitude in the presence vs. absence of the visual conditioned stimulus.

SOURCE: Berg and Davis 1985, Copyright 1985, American Psychological Association.

In contrast, startle elicited from the RPC was not potentiated. The difference in potentiation of electrically and acoustically elicited startle at sites beyond the VLL cannot be attributed to the extent of effective conditioning, because potentiation of startle elicited acoustically was just as great in the RPC group as it was in the VCN, VAS, and VLL groups. Furthermore, the potentiation of electrically elicited startle was specific to an explicit pairing

of light and shock during training. Animals with electrodes implanted in the VCN and trained with a random temporal relation between light and shock showed no sign of potentiation of either electrically or acoustically elicited startle. Finally, other studies showed that drugs like diazepam, which are known to block fear-potentiated startle elicited acoustically, also block fear-potentiated startle elicited electrically through the VCN (figure 11) (Berg and Davis 1984). Taken together, these data indicate that the VLL or the RPC is the point in the startle pathway where a visual conditioned stimulus ultimately modulates transmission following conditioning so as to affect the startle reflex.

THE ROLE OF THE AMYGDALA IN FEAR-POTENTIATED STARTLE

Lesions of the Central Nucleus of the Amygdala Block Fear-Potentiated Startle

It was hypothesized that lesions of the amygdala, a structure long implicated in fear, should block potentiated startle, because potentiated startle is a measure of fear. In fact, lesions of the central nucleus of the amygdala following fear conditioning were shown to completely eliminate potentiated startle (figure 12) (Hitchcock and Davis 1986; Hitchcock and Davis 1987). In contrast, transection of the cerebellar peduncles or lesions of the red nucleus had no effect on potentiated startle. Another experiment using a visual prepulse test indicated that the blockade of potentiated startle observed in animals with lesions of the amygdala could not be attributed to visual impairment. A third experiment indicated that the absence of potentiation in the amygdala-lesioned animals did not simply result from a lowered startle-level ceiling, because the amygdala-lesioned animals could show increased startle with increased stimulus intensity and with administration of strychnine, a drug that reliably increases startle (Kehne et al. 1981). Taken together, the results of these experiments support the hypothesis that the amygdala is involved in potentiated startle—a measure of conditioned fear.

Enhancement of Acoustic Startle By Electrical Stimulation of the Amygdala

At the present time, it is not clear how the amygdala participates in fear-potentiated startle. It is possible that the light, after being paired with shock, activates the amygdala, which would then increase startle. Short-latency visual-evoked potentials have been recorded in the amygdala (Pollock et al. 1976; Sanghera et al. 1979), and electrical stimulation of the amygdala has been reported to produce fearlike behaviors in animals (Applegate et al. 1983; Gloor 1960), to mimic conditioned and unconditioned cardiac effects in rabbit heart-rate conditioning (Kapp et al. 1982), and to elicit feelings of anxiety in humans (Chapman et al. 1954). Consistent with this, it was recently found that low-level electrical stimulation of

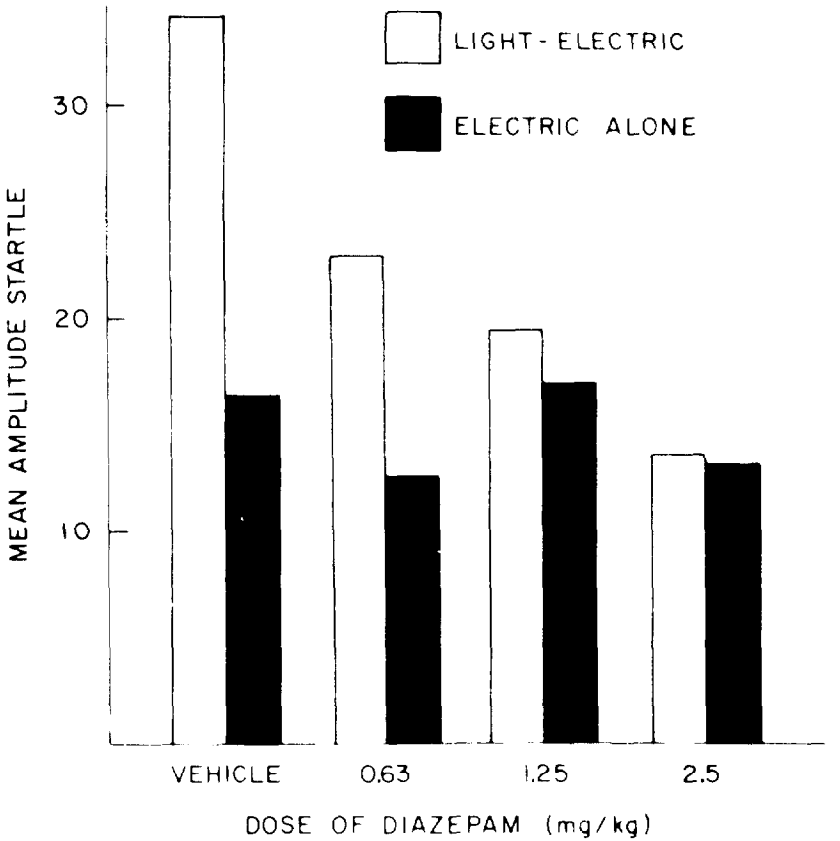

FIGURE 11. *Mean amplitude of startle elicited by electrical stimulation of the VCN in the presence (white bars) or absence (black bars) of the light after administration of various doses of diazepam or its vehicle*

SOURCE: Berg and Davis 1984, Copyright 1984, Pergamon Press.

the amygdala (e.g., 40 to 400 μA, 25-millisecond trains of 0.1-millisecond square-wave cathodal pulses) markedly increases acoustic startle amplitude (Rosen and Davis 1988). This excitatory effect has occurred in every rat tested so far in which electrodes had been placed in the central, intercalated, or medial nucleus of the amygdala or in the area just medial to the amygdaloid complex. Stimulation of the area just medial to the amygdala had the lowest threshold for increasing acoustic startle. This area coincides with the initial part of the ventral amygdalofugal pathway as it begins its projection to the lower brainstem (Krettek and Price 1978; Schwaber et al.

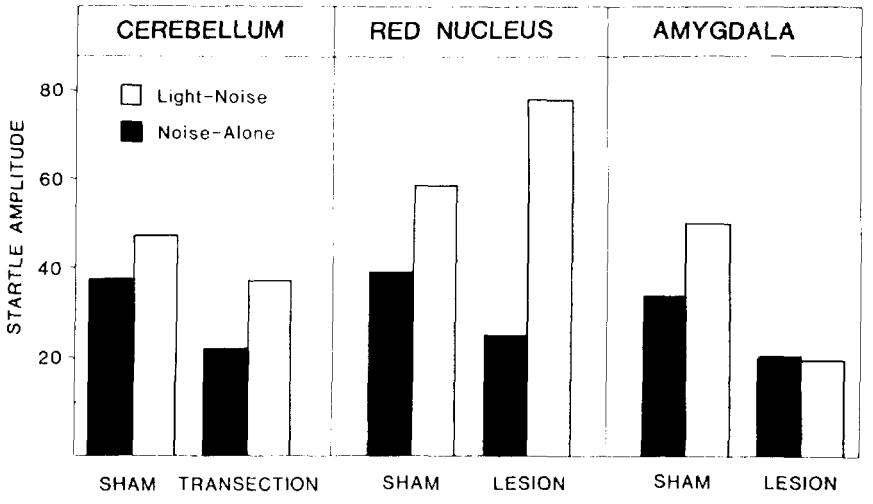

FIGURE 12. *Mean amplitude startle on the light-noise or noise-alone trials in rats in which the cerebellum was surgically transected from the brainstem or in rats with electrolytic lesions of the red nucleus or the central nucleus of the amygdala*

NOTE: Sham animals for the cerebellar experiment had the transection knife inserted under the cerebellum hut the fiber pathways were not cut. Sham animals for the other experiments had the electrodes lowered into the brain. but no current was passed.

SOURCE: Hitchcock and Davis 1986, Copyright 1986, American Psychological Association

1982; Post and Mai 1980). Low-level electrical stimulation at this site would be expected to activate a large number of fibers projecting to the brainstem, since they are highly concentrated at this part of the pathway. In contrast, stimulation of the amygdala nuclei themselves, where the neurons of these fibers originate, would require higher currents (i.e., more current spread) to activate the same number of brainstem projections, because the neurons are dispersed throughout the nuclei. Further studies involving stimulation of the area just medial to the amygdala where the ventral amygdalofugal pathway begins, in combination with lesions of cell bodies (e.g., ibotenic acid lesions) in the central, medial, intercalated, or basolateral nucleus, would help clarify whether enhancement of startle by stimulation in this pathway actually results from activation of the axons originating from these amygdaloid nuclei.

Electrical stimulation of the amygdala did not produce any signs of behavioral activation except for an enhancement of startle at these stimulation

currents and durations, indicating that startle is an extremely sensitive index of amygdala stimulation. Moreover, the duration of stimulation is well below that used to produce kindling in rats (Handforth 1984) so that the effects on startle are not associated with convulsions.

The excitatory effect on startle occurs within a few milliseconds from the onset of amygdala stimulation, This rapidity of action means that the increase in startle is not secondary to autonomic or hormonal changes that might be produced by amygdala stimulation, because these would have a much longer latency. In addition, electrical stimulation of the amygdala alone does not elicit startle, even at high currents. Finally, electrical stimulation of several other nearby brain areas such as the endopiriform nucleus, fundus striati, internal capsule, or some sites in the basolateral nucleus of the amygdala do not increase startle.

The Role of Different Amygdala Efferent Projections in Fear-Potentiated Startle

As mentioned earlier, lesions of the central nucleus of the amygdala block fear-potentiated startle (figure 12). Recently, the sensitive *Phaseolus vulgaris*-leucoagglutinin (PHA-L) anterograde tracing technique (Gerfen and Sawehenko 1984) and lesion studies have been used to delineate possible connections between the amygdala and the startle pathway in relation to fear-potentiated startle (Hitchcock and Davis, in press). The central nucleus of the amygdala projects to a variety of brain regions via two major efferent pathways, the stria terminalis and the ventral amygdalofugal pathway. Lesions of the stria terminalis itself, or of the bed nucleus of the stria terminalis, a major projection area of this pathway, do not block potentiated startle. Knife cuts of the rostral part of the ventral amygdalofugal pathway, which would interrupt its projections to the rostral lateral hypothalamus and substantia innominata, also fail to block potentiated startle. On the other hand, lesions of the caudal part of the ventral amygdalofugal pathway, at the point where it passes through the subthalamic area and cerebral peduncles, completely block potentiated startle. Interestingly, Jarrell et al. (1986) found that lesions of this area also block heart-rate conditioning, another measure of fear conditioning. Finally, lesions of the substantia nigra, which receives central nucleus projections as well as fibers of passage from the central nucleus of the amygdala to more caudal brainstem regions, also block potentiated startle. This blockade does not seem to involve dopamine cells in the zona compacta, since infusion of the dopamine neurotoxin 6-0HDA into the substantia nigra did not block potentiated startle despite a more than 90 percent depletion of dopamine in the caudate nucleus.

These lesion experiments indicate that the pathway from the central nucleus of the amygdala to the startle circuit travels through the caudal part of the ventral amygdalofugal pathway, which is known to project directly to many parts of the pons, medulla, and probably the SC (Krettek and Price 1978;

Mizuno et al. 1985; Post and Mai 1980; Price and Amaral 1981; Sandrew et al. 1986; Schwaber et al. 1982). In fact, Inagaki et al. (1983) have reported direct connections between the central nucleus of the amygdala and the exact part of the RPC (an area just dorsal to the superior olive) that is critical for startle. This projection has been verified through the use of both anterograde tracing techniques.

Relationship Between Fear-Potentiated Startle and Startle Increased By Electrical Stimulation of the Amygdala

It is not clear whether startle enhanced by electrical stimulation of the central nucleus of the amygdala is related to fear-potentiated startle. Several experiments could be performed to evaluate this hypothesis. For example, lesions of points along the ventral amygdalofugal pathway that block fear-potentiated startle should also block startle enhanced by stimulation of the amygdala. In fact, in a preliminary experiment, lesions of the caudal ventral amygdalofugal pathway at the level of the subthalamic nucleus and substantia nigra blocked the enhancement of startle normally produced by electrical stimulation of the central nucleus of the amygdala.

Relationship of the Amygdala to the Visual Structures Involved in Fear-Potentiated Startle

Thus far, there is no direct information linking the amygdala to any visual structures that appear critical for fear-potentiated startle. The central nucleus of the amygdala is known to receive input from the lateral nucleus of the amygdala, which receives afferents from the insular cortex (Turner and Zimmer 1984). The insular cortex receives visual information (Turner and Zimmer 1984) through a pathway probably involving the lateral geniculate nucleus, visual cortex, and visual association cortex. Thus, it is possible that the visual conditioned stimulus would activate the central nucleus of the amygdala after being relayed through these structures. Hitchcock et al. (unpublished observations) have found that lesions of the lateral geniculate nucleus and insular cortex block fear-potentiated startle. In contrast, lesions of superficial layers of the superior colliculus, the pretectal area, the parietal cortex, or the dorsal lateral lemniscus did not block potentiated startle (Tischler and Davis 1983). It was also reported in that study that lesions of deep and intermediate layers of the superior colliculus blocked fear-potentiated startle. Recently, this result was replicated with testing 6 to 7 days after lesions of deep and intermediate layers of the superior colliculus (Hitchcock and Davis, unpublished observations). At this time, however, baseline levels of startle (i.e., on the noise-alone trials) were markedly elevated after lesions of the superior colliculus. In fact, when these animals were retested using a very weak, 75-dB startle stimulus, they each showed increased startle in the presence of the light. Moreover, lesions of deep and intermediate layers of the superior colliculus did not prevent electrical stimulation of the amygdala from enhancing startle

(Hitchcock et al., unpublished observations). Hence, it is now believed that deep and intermediate layers of the superior colliculus, which project directly to the VLL (Henkel and Edwards 1978; Henkel 1981), tonically inhibit acoustic startle. This effect may interfere with the measurement of fear-potentiated startle unless special test conditions are arranged. However, the superior colliculus does not appear to be either an obligatory visual relay in startle potentiated by a visual-conditioned fear stimulus or part of the pathway connecting the central nucleus of the amygdala to the startle circuit. Moreover, Hitchcock and Davis (unpublished observations) have recently found that complex removal of the visual cortex does not block fear-potentiated startle. Further studies will try to determine the role of connections between the lateral geniculate nuclei and the perirhinal cortex in fear-potentiated startle.

ENHANCEMENT OF STARTLE BY FOOTSHOCKS

Effects of Footshocks on Acoustic Startle

Fear-potentiated startle is defined by an increase in startle in the presence of a cue previously paired with shock. One might expect, therefore, that shock itself should increase startle amplitude, so that fear-potentiated startle would reflect the familiar finding that the conditioned response mimics the unconditioned response. Curiously, however, Brown et al. (1956) reported that acoustic startle was actually depressed when elicited from 15 to 60 seconds after a footshock, although longer intervals were not tested. Recently, Fanselow and colleagues (Fanselow 1981; Fanselow 1982; Fanselow 1984; Fanselow and Bolles 1979) have shown that shock leads to an increase in freezing, a traditional measure of fear in rats. Interestingly, however, freezing does not occur immediately after the shock, but develops gradually over several minutes. Since the magnitude of fear-potentiated startle correlates highly with the amount of freezing measured in the same experimental situation (Leaton and Borszcz 1985), it was reasoned that startle should increase for several minutes following footshock with a timecourse similar to that of freezing reported by Fanselow. Consistent with this expectation, figure 13 shows that a series of 10 shocks (0.6 mA, 0.5 seconds in duration presented at a rate of 1 shock per second) markedly increased startle measured over a 20-minute period after the shocks. Interestingly, this effect did not occur immediately, but developed over several minutes, similar to the timecourse of freezing seen by Fanselow. However, unlike Fanselow's results where freezing appears to be context specific, several laboratory experiments have led to the conclusion that this increase in startle does not result from a rapid conditioning to the context. Therefore, it is thought that the increase in startle seen after footshocks may represent the unconditioned effects of the shock on startle, and that this becomes conditioned to a light that is consistently paired with that shock.

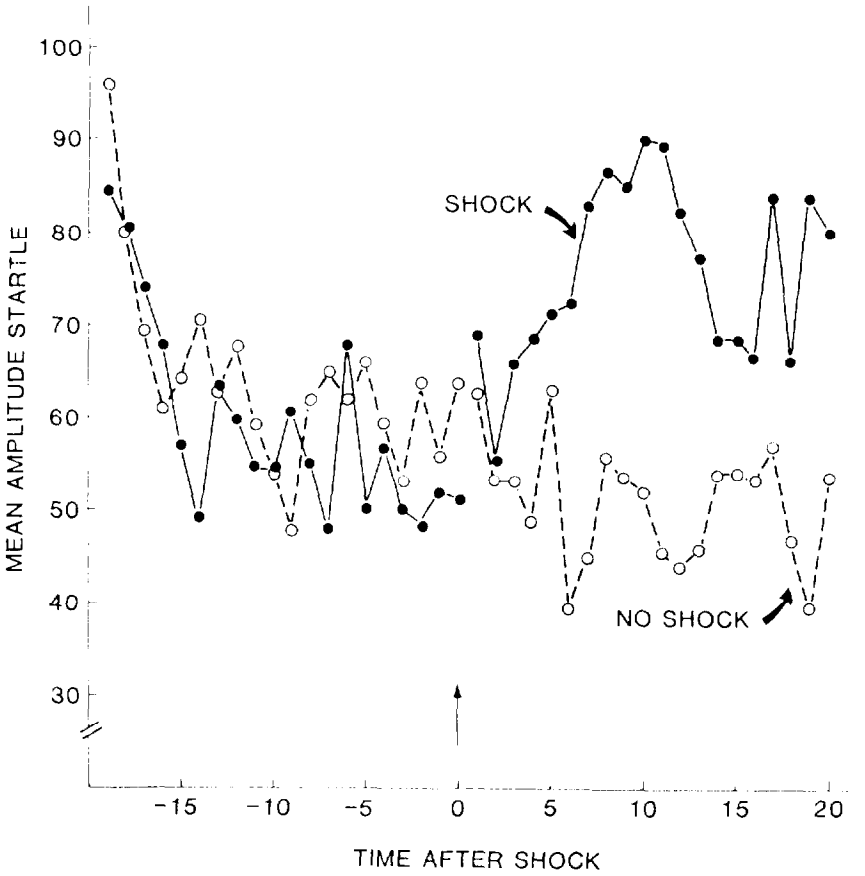

FIGURE 13. *Enhancement of startle by footshock*

NOTE Mean amplitude startle response prior to and following a series of five 0.6-mA, 500-millisecond footshocks (indicated by the arrow) presented once per second (solid) or no intervening shock (open). Forty 105-dB noise bursts were presented at a 30-second interval both before and after the shocks.

The Role of the Amygdala in Footshock-Induced Enhancement of Startle

Because footshock is both aversive and fear producing, it is possible that footshock activates the amygdala, which then leads to an elevation of startle via connections between the amygdala and the startle pathway. In fact, figure 14 shows that lesions of the central nucleus of the amygdala prevent footshocks from enhancing startle (Hitchcock et al. 1989). Other studies showed that this effect could not be attributed to a lesion-induced change in

shock sensitivity. Moreover, lesions of the immediately adjacent lateral amygdala nucleus did not block shock sensitization. Finally, lesions of the caudal ventral amygdalofugal pathway, which connects the central nucleus of the amygdala to the startle circuit, also completely blocked shock sensitization.

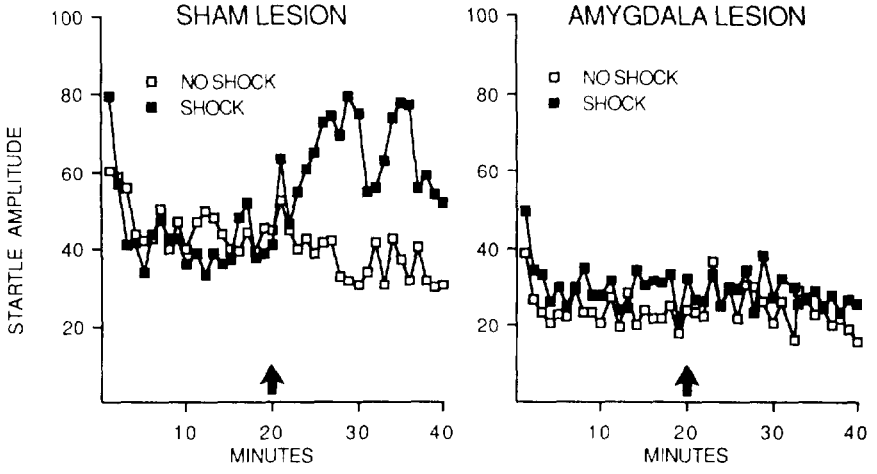

FIGURE 14. *Enhancement of startle by footshock in lesioned or unoperated animals*

NOTE: Mean amplitude startle response over successive 2-minute periods preceding and following presentation (arrow) of 10 0.6-mA footshocks (solid) or no footshocks (open) in unoperated (left panel), sham-lesioned (center panel), and amygdala-central-nucleus-lesioned animals (right panel).

SOURCE: Hitchcock et al. 1989, Copyright 1989, American Psychological Association.

THE ROLE OF THE AMYGDALA IN INNATE AND CONDITIONED FEAR

A variety of animal models have been used to infer a central state of fear or anxiety. In some models, fear is inferred when an animal freezes, thus interrupting some ongoing behavior such as pressing a bar or interacting socially with other animals. In other models, fear is measured by changes in autonomic activity such as heart rate, blood pressure, or respiration. Fear can also be measured by a change in simple reflexes or a change in facial expressions and mouth movements. Thus, fear appears to produce a complex pattern of behaviors that are highly correlated with each other.

Anatomical Connections Between the Amygdala and Brain Areas Involved in Fear

Reflecting suggestions of several previous reviews (Gloor 1960; Kapp and Pascoe 1984; Kapp et al. 1984; Sarter and Markowitsch 1985), figure 15 summarizes work done in many different laboratories indicating that the central nucleus of the amygdala has direct projections to a variety of brainstem areas that might be expected to be involved in many of the signs of fear. Thus the central nucleus of the amygdala projects to a region of the central grey (Beitz 1982; Post and Mai 1980) that has been implicated in fear in a number of behavioral tests (Iwata et al. 1986; Liebman et al. 1970). Direct projections to the dorsal motor nucleus of the vagus (Hopkins and Holstege 1978; Schwaber et al. 1982; Takeuchi et al. 1983; Veening et al. 1984) may be involved in several autonomic measures of fear, since the vagus nerve controls many different autonomic functions.

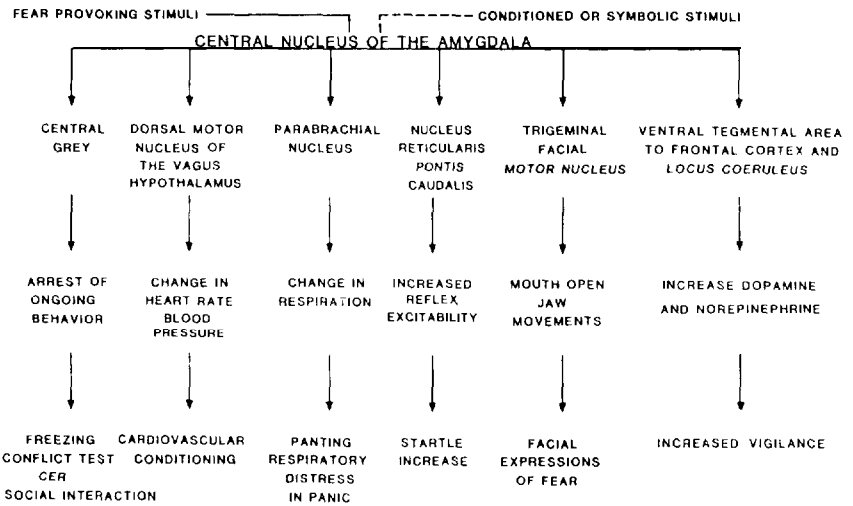

FIGURE 15. *Schematic diagram of connections of the central nucleus of the amygdala to target areas probably involved in the pattern of behaviors typically associated with fear*

Projections of the central nucleus of the amygdala to the parabrachial nucleus (Krettek and Price 1978; Price and Amaral 1981; Takeuchi et al. 1982) may be involved in respiratory changes during fear, since electrical stimulation of the parabrachial nucleus is known to alter respiratory rate (Cohen 1971; Bertrand and Hugelin 1971). Direct projections to the trigeminal (Post and Mai 1980) and perhaps the facial motor nuclei may mediate

some of the facial expressions of fear. As outlined earlier, projections of the amygdala to the RPC (Inagaki et al. 1983) probably are involved in fear potentiation of the startle reflex. Finally, projections from the central nucleus of the amygdala to the ventral tegmental area (Phillipson 1979) may mediate stress-induced changes in dopamine turnover in the frontal cortex (Thierry et al. 1976). Moreover, this projection might also link the central nucleus of the amygdala to the locus coeruleus (Deutch et al. 1986), which itself has been implicated in fear and anxiety (Redmond 1977) or increased vigilance and attention (Aston-Jones and Bloom 1981).

Fear Produced by Electrical Stimulation of the Amygdala

Significantly, it has also been shown that electrical stimulation of the central nucleus of the amygdala can produce a complex pattern of behavioral and autonomic changes that, taken together, constitute a state that closely resembles a state of fear. Thus, electrical stimulation of the central nucleus of the amygdala produces a cessation of ongoing behavior (Applegate et al. 1983; Gloor 1960). In fact, cessation of ongoing behavior is the critical measure of fear or anxiety in several animal models such as the operant conflict test (Geller and Seifter 1960). the conditioned emotional response (Estes and Skinner 1941), the social interaction test (File 1980), and freezing itself (Fanselow and Bolles 1979). Stimulation of the amygdala can also alter heart rate (Applegate et al. 1983; Kapp et al. 1982) and blood pressure (Morgenson and Calaresu 1973), both of which are measures used to study cardiovascular conditioning. Electrical stimulation of the central nucleus of the amygdala also alters respiration (Applegate et al. 1983; Harper et al. 1984) a prominent symptom of fear, especially in panic disorders. Electrical stimulation of the amygdala also elicits jaw movements (Applegate et al. 1983; Gloor 1960; Ohta 1984), which often accompany the fear response. Amygdala stimulation can also produce gastric ulceration (Henke 1980a; Henke 1980b; Innes and Tansy 1980; Sen and Anand 1957), which may result from chronic fear or stress. As outlined earlier, electrical stimulation of specific parts of the amygdala increases the acoustic startle reflex, which is elevated during fear. Finally, it has been reported in humans that electrical stimulation of the amygdala elicits feellngs of fear or anxiety as well as autonomic reactions indicative of fear (Chapman et al. 1954; Gloor et al. 1981).

Taken together, the highly correlated set of behaviors seen during fear may result from activation of a single area of the brain (the central nucleus of the amygdala), which then projects to a variety of target areas that are critical for each of the specific signs of fear. Moreover, it must be assumed that all of these connections arc already formed in an adult organism, since electrical stimulation produces these effects in the absence of prior explicit fear conditioning. Given this innate wiring diagram, it would seem most parsimonious to assume that a neutral stimulus will elicit a state of fear when that stimulus comes to activate the amygdala after being paired with

an aversive stimulus. Thus, plasticity following fear conditioning probably results from a change prior to or in the amygdala rather than a change in its efferent target areas.

The Role of the Amygdala in Fear Elicited by a Conditioned Stimulus

Consistent with this interpretation, several studies have shown that a neutral stimulus paired with aversive stimulation will now alter neural firing in the amygdala, especially in the central nucleus of the amygdala (Henke 1983; Pascoe and Kapp 1985). Moreover, lesions of the central nucleus are known to eliminate or attenuate conditioned changes measured by a cessation of ongoing behavior such as freezing (Iwata et al. 1986); reduced bar pressing in the operant conflict test (Shibata et al. 1986); or the conditioned emotional response paradigm (Kellicut and Schwartzbaum 1963; Spevack et al. 1975). Lesions of the central nucleus also block conditioned changes in heart rate (Cohen 1975; Gentile et al. 1986; Kapp et al. 1979), blood pressure (Iwata et al. 1986) or ulceration induced by immobilization stress (Henke 1980a; Henke 1980b). Data outlined earlier indicate that lesions of the central nucleus of the amygdala block fear-potentiated startle (Hitchcock and Davis 1986). Lesions of the amygdala are known to block several measures of innate fear in different species (Blanchard and Blanchard 1972; Ursin et al. 1981). This, along with a large literature implicating the amygdala in many other measures of fear such as active and passive avoidance (Kaada 1972; Sarter and Markowitsch 1985; Ursin et al. 1981) and evaluation and memory of emotionally significant sensory stimuli (Bennett et al. 1985; Bresnahan and Routtenberg 1972; Ellis and Kesner 1983; Gallagher and Kapp 1978; Gallagher and Kapp 1981; Gold et al. 1975; Handwerker et al. 1974; Kesner 1982; Liang et al. 1985; Liang et al. 1986; Mishkin and Aggleton 1981), compellingly indicates a crucial role of the amygdala in fear.

SUMMARY AND CONCLUSIONS

The potentiated startle paradigm measures conditioned fear by an increase in the amplitude of a simple reflex (the acoustic startle reflex) in the presence of a cue previously paired with shock. This paradigm offers a number of advantages as an alternative to most animal tests of fear or anxiety, since it involves no operant and is reflected by an enhancement rather than a suppression of ongoing behavior. Lesion and electrical stimulation studies on fear-potentiated startle and startle increased by electrical stimulation of the amygdala are being used to define the neural pathways necessary for a visual conditioned stimulus to alter the acoustic startle reflex. The current working hypothesis is that the conditioned stimulus activates the central nucleus of the amygdala through a pathway involving the lateral geniculate nucleus and insular cortex. The central nucleus of the amygdala may then project directly to the acoustic startle pathway, modulating the startle response. More work has to be done to define conclusively the relevant

neural pathways involved in fear-potentiated startle. Nonetheless, by combining behavioral, anatomical, physiological, and pharmacological approaches, it will be possible to determine each step along the pathway that mediates the ability of a stimulus signaling fear to alter behavior. Once the exact structures are delineated, it should be possible to determine the neurotransmitters that are released during a state of fear and how this chemical information is relayed along these pathways to affect behavior. Eventually, this approach should help to determine where plastic changes take place along these pathways to mediate the conditioned effects that are being measured and the biochemical processes that are involved.

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Stress, Performance, and Arousal: Focus on CRF

George F. Koob, Belinda J. Cole, Neal R. Swerdlow, Michel Le Moal, and Karen T. Britton

INTRODUCTION

Corticotropin releasing factor (CRF) is a 41-amino-acid peptide (Vale et al. 1981) that has potent activating effects on the pituitary adrenal axis, as shown by its ability to release adrenocorticotropin hormone (ACTH) and ß-endorphin from the anterior pituitary (Rivier et al. 1984). CRF is now thought to be the primary hypothalamic releasing factor with the specific function of activating the release of ACTH from the anterior pituitary.

The significance of the chemical identification of CRF can perhaps best be elaborated in terms of classical "stress" theory. Two generally accepted definitions of stress are that a "stress is the nonspecific (common) result of any demand upon the body (usually, but not always noxious)" (Selye 1980, p. vii) or "anything which causes an alteration of psychological homeostatic processes" (Burchfield 1979). Using this definition, one of the most reliable and sensitive indications of a state of stress is an increase in the production of ACTH (Selye 1936).

Internal or external demands are conveyed in the form of stimuli to the anterior pituitary via neurohumoral means (presumably CRF), and the pituitary responds with a secretion of ACTH. ACTH, in turn, stimulates the adrenal cortex to secrete glucocorticoids that have widespread effects on metabolism, such as gluconeogenesis, hyperinsulinemia, lysis of lymphoid tissue, increased gastric secretion, and reduced inflammatory and antibody responses. These physiological changes, in response to increased hypothalamic-pituitary action, are paralleled by alterations in behavior that have been associated with increases in alertness and attention (De Wied 1977).

However, an alternative means by which behavioral or physiological responses to arousing stimuli and/or stressors might involve CRF is via a direct neurotropic action of CRF in the brain itself. Thus, just as pathways project to the hypothalamus from limbic areas to activate the pituitary adrenal axis via CRF release in the hypothalamus, so may CRF release in

the central nervous system (CNS) mediate behavioral arousal and behavioral responses to stress.

AROUSAL-STRESS CONSTRUCT CONTINUUM

The concept of stress and arousal as intervening variables or hypothetical constructs that have important similarities has been elegantly elaborated in terms of psychoendocrine measures (Hennessy and Levine 1979). These authors argued that "the pituitary adrenal system is part of the arousal system of the animal and [activity in this system] may prove to be an extremely reliable and sensitive measure of arousal as well as stress" (Hennessy and Levine, p. 137). These authors further argued that, while the concept of arousal may involve a wide range of independent and dependent variables, stress primarily refers to the excess consequences of arousal that produce tissue damage and toxic effects. The concept of stress, then, may be considered a parallel continuum subsumed by a basic arousal theory.

The thesis of this chapter is that the pituitary adrenal axis has a homologous representation in the brain in a basic brain arousal system that utilizes the neurotransmitter CRF. Thus, just as CRF controls the release of ACTH from the pituitary, and activity in the pituitary adrenal axis has proven to be a reliable and sensitive measure of arousal as well as of stress, it is hypothesized that CRF in the CNS is part of a basic arousal system that, under conditions of extreme activity or chronic activation, can produce the behavioral manifestations of stress as well as, ultimately, pathophysiology.

CRF AND BEHAVIORAL AROUSAL

CRF administered directly into the CNS produces behavioral and physiological activation. Intracerebroventricular (ICV) injections of CRF produce elevations of plasma epinephrine. norepinephrine, and glucose (Brown et al. 1982a; Brown et al. 1982b). These effects are reproducible in hypophysectomized animals but are abolished by ganglionic blocking agents (Brown and Fisher 1983). suggesting an involvement of the sympathetic autonomic nervous system. CRF injected ICV also produces a profound dose-dependent activation of the electroencephalogram (EEG) (Ehlers et al. 1983). Doses of 0.015 to 0.15 nmol produced a long-lasting activation of EEG. At the cellular level, CRF produces increases in the firing rate of norcpinephrine containing neurons within the locus coeruleus (Valentino et al. 1983), a system thought to be of importance in the mechanisms by which the brain is able to attend selectively to certain novel external events (Foote et al. 1983).

The autonomic and electrophysiological activation produced by central administration of CRF is paralleled by a dose-dependent increase in locomotor activity (Sutton et al. 1982; Koob et al. 1984; Sherman and Kalin 1987). This effect also appears to be independent of the pituitary-adrenal system,

since locomotor activation is still observed in hypophysectomized and dexamethasone-treated rats (figure 1) (Eaves et al. 1985; Britton et al. 1986). Given that this activation is not seen with systemic administration of CRF, these observations suggested that this peptide exerts some of its effects by a direct action on the CNS.

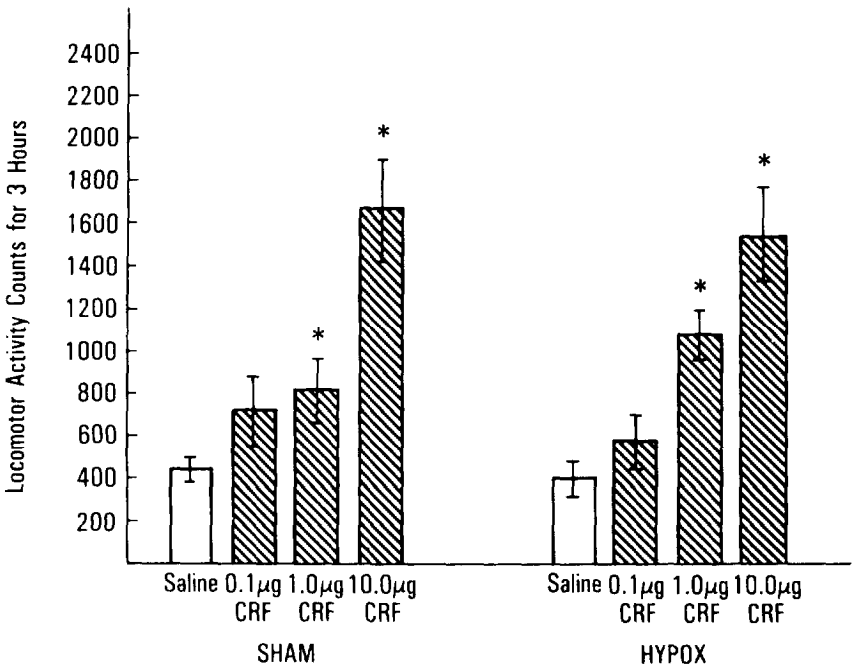

FIGURE 1. *Effects of CRF injected intracerebroventricularly on locomotor activity in photocell cages in hypophysectomized rats treated chronically with rat growth hormone and in sham-operated animals*

*Significantly different from saline injection. $p<.05$, main effects ANOVA.

NOTE: Results represent the total activity counts over 3 hours (mean ± SEM), n=9 in each group.

SOURCE: Eaves et al. 1985, copyright 1985, Pergamon Press.

Although other peptides such as the endorphins and ACTH have been shown to produce increases in spontaneous behavioral activity, the locomotor activation caused by CRF is not antagonized by the opiate antagonist naloxone or by low doses of the dopaminc-receptor antagonist alpha

flupenthixol (Koob et al. 1984). Nor is this activation reversed by 6-hydroxydopamine lesions of the region of the nucleus accumbens, even though such lesions reverse the locomotor-stimulating effects of indirect sympathomimetics (Swerdlow and Koob 1985). ACTH injected centrally failed to increase locomotor activity but instead produced an increase in grooming behavior, as has been observed by others (Gispen et al. 1975).

To delineate further the site of action for this behaviorally activating effect of CRF, experiments were conducted that examined lateral ventricular and cistema magna injections of CRF combined with obstruction of the cerebral aqueduct. Rats injected intracerebroventricularly with CRF at the level of the lateral ventricle or cistema magna showed a dose-dependent increase in locomotor activity. The increase in locomotor activity from injections of CRF into the cistema magna was blocked by a cold cream plug in the cerebral aqueduct (Tazi et al. 1987a). An identical plug failed to block the increase in locomotor activity produced by CRF injected into the lateral ventricle. These results suggest that the activating effects of CRF depend on forebrain CRF receptors and may not depend on hindbrain sites.

In a subsequent study, rats were surgically implanted with cannulae aimed at either the lateral ventricle, frontal cortex (FC), nucleus accumbens, amygdala centralis, substantia innominata/lateral preoptic area (SI/PO), or pedunculopontine nucleus (PPN). Injection of 0.5 μg rat CRF into discrete brain regions produced increases in locomotor activity that were inversely related to the distance of the injection site from the SI/PO: activation was least intense when produced by CRF injection into the FC and PPN and most intense when produced by CRF injection into the SI/PO. Only SI/PO injection sites yielded activation levels that exceeded those produced by ICV CRF injection (Tazi et al. 1987a). These results suggest some degree of neuroanatomical specificity for the neural substrales of CRF-induced behavioral activation.

CRF AND BEHAVIORAL RESPONSES TO STRESS

Early work with CRF showed that while CRF produced a behavioral activation in some situations, in other more stressful situations, the same doses of CRF produced behavioral inhibition. For example, when mildly food-deprived rats are exposed to an open-field situation where food is available, they will eventually approach the food and eat. In contrast to untreated animals that made increasingly frequent forays into the center of the field to consume food (Britton and Britton 1981), rats previously injected intracerebroventricularly with CRF showed a marked decrease in exploratory and ingestive behavior and remained close to the comers of the field (Britton et al. 1982). CRF also decreases food intake and muscimol-, norepinephrine-, dynotphin-, and insulin-induced feeding, effects attributed to a stress-related suppression of food intake (Levine et al. 1983; Morley and Levine 1983).

Similar anxiogeniclike results were observed with nondeprived rats in an open field without food (Sutton et al. 1982). Rats tested in a novel open field, following ICV injection of doses of CRF (0.0015 to 0.15 nmole), showed responses that were consistent with increased "emotionality" or sensitivity to the stressful aspects of the situation. Here, rats showed *decreases* in locomotion and rearing (Sutton et al. 1982). In this open-field test, a typical saline-injected rat rapidly circled the outer squares of the open field during the first 3 to 4 minutes of the 5-minute test. During the last 1 to 2 minutes of the test, these saline-injected animals then made some forays into the center of the open field, usually also rearing on their hind legs. Typically, a rat injected with 0.15 nmole of CRF and placed 60 minutes later in the open field moved hesitantly to the outer squares and then either circled the open field, remaining close to the floor, or remained in one of the comers, grooming or hesitantly moving forwards and backwards (Sutton et al. 1982). These behavioral changes are consistent with heightened "emotionality" or increased sensitivity to the presumably stressful aspects of the test.

CRF has also been tested in other behavioral paradigms that are sensitive to antianxiety (anxiolytic) compounds such as the benzodiazepines. In an operant conflict test, CRF produced a significant decrease in punished and unpunished responding, an effect opposite to that observed with benzodiazepines; this "anxiogenic" effect was reversed by concomitant treatment with a benzodiazepine (figure 2) (Thatcher-Britton et al. 1985). However, this increased sensitivity to aversive events was not paralleled by an increased sensitivity to pain. These effects appear to be independent of the pituitary adrenal axis, in that dexamethasone treatment also failed to alter the suppression in operant behavior produced by CRF (Britton et al. 1986a).

In a recent study, CRF injected intracerebroventricularly in rats produced a further suppression during the conditioned stimulus (CS) presentation in a conditioned suppression task. The observed decrease in the suppression rate is of significance, because it shows that the decrease in performance was more dramatic during the CS period that signaled impending shock (Cole et al., unpublished manuscript).

Another paradigm differentially sensitive to anxiogenic and anxiolytic compounds is the acoustic startle test. The acoustic startle reflex is an easily quantified muscular contraction in response to an intense acoustic stimulus. CRF (1 μg ICV) significantly potentiated acoustic startle amplitude (Swerdlow et al. 1986). Pretreatment with the benzodiazepine chlordiazepoxide (CDP) in doses that did not by themselves lower startle baseline attenuated this effect (figure 3). These results also supported the hypothesis that CRF might act to potentiate behavioral responses normally expressed during states of enhanced fear or anxiety.

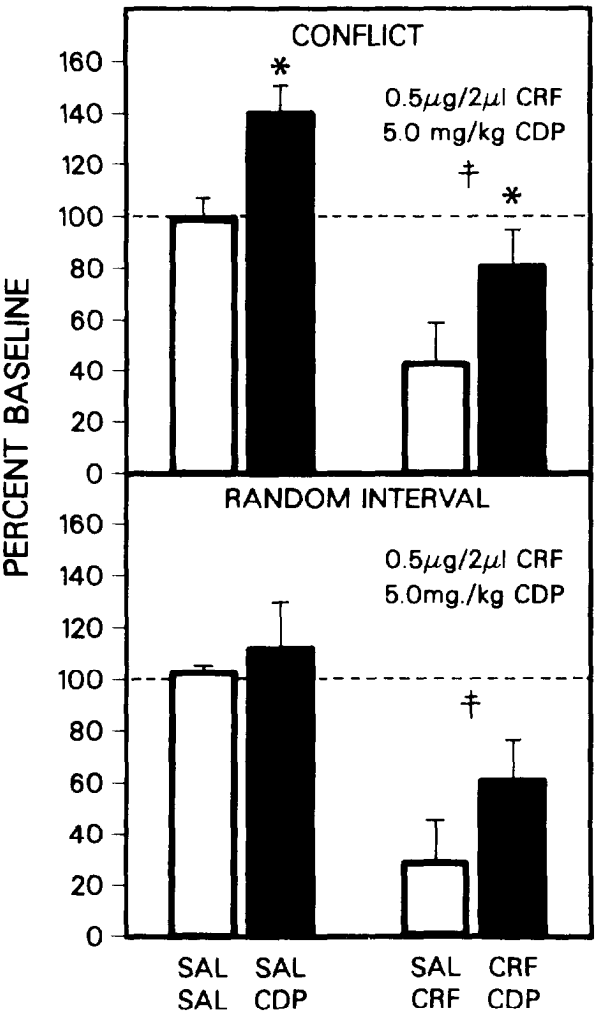

FIGURE 2. *The interaction of 0.5 μg corticotropin-releasing hormone injected intracerebroventricularly and of 5.0 mg/kg CDP injected intraperitoneally on responding during the random interval and conflict components of an operant conflict test*

*Significant difference from saline, main effect CDP.

‡Significant difference from saline, main effect CRF. $p<.05$, two-way ANOVA.

NOTE: Results are expressed as percent of baseline from the previous day.

SOURCE: Thatcher-Britton et al. 1985, copyright 1985, Springer Verlag.

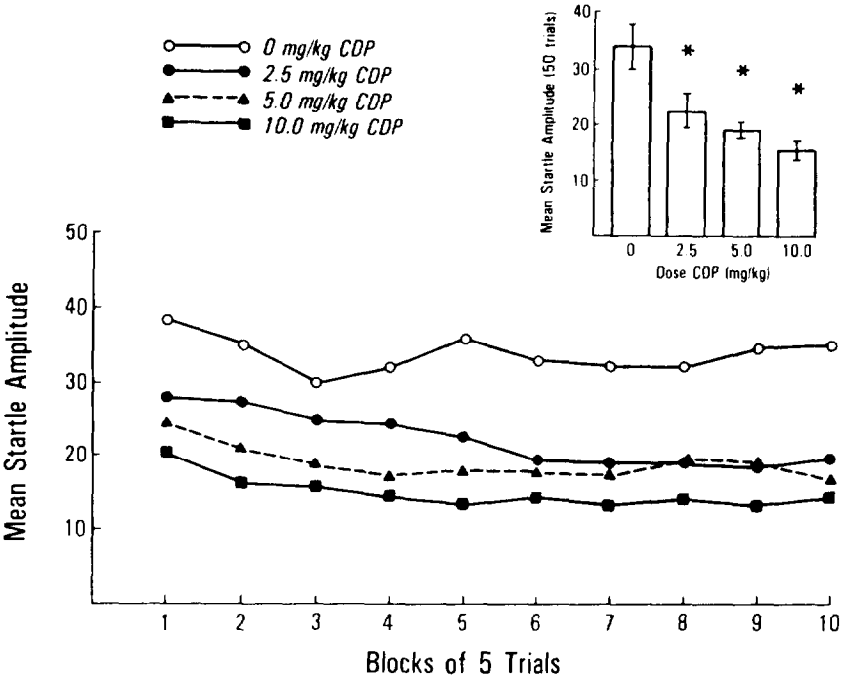

FIGURE 3. *Acoustic startle amplitude in rats following ICV infusion of 1 μg CRF and IP injection of chlordiazepoxide (0, 2.5, 5.0, or 10.0 mg/kg*

*p<.05, Newman-Keuls individual means comparison.

NOTE: Insert histogram indicates mean startle amplitude over 50 trials.

SOURCE: Swerdlow et al., 1986, copyright 1986, Springer-Verlag.

Other more recent work has extended and elaborated this stresslike effect of CRF. CRF injected intracerebroventricularly into mice allowed to explore a novel environment (multicompartment chamber) produced a decreased mean time per contact with novel stimuli (Bet-ridge and Dunn 1986). Interestingly, this effect was reversed by naloxone, but it should be noted that previous studies have shown that naloxone can produce the opposite result on its own, i.e., increased exploratory behavior (Arnsten and Segal 1979).

Although the effects of exogenous CRF in potentiating behavioral responses to stress are interesting, the hypothesis that behavioral responses to stress depend on activation of endogenous CRF in the brain would be bolstered by

evidence of an antistress effect of an antagonist to CRF. A peptide antagonist to CRF, α helical CRF 9-41 (Rivier et al. 19843, has recently been shown to have behavioral actions in some tests. This antagonist is 10 times less potent than CRF in binding to CNS CRF receptors (De Souza 1987). Alpha helical CRF has been shown to partially reverse stress-induced decreases in feeding (Krahn et al. 1986), and it attenuated stress-induced fighting (Tazi et al. 1987b). This antagonist also appears to have activity in various open-field tests. Alpha helical CRF injected into mice reversed stress-induced changes in a novel environment (multicompartment chamber) (Berridge and Dunn 1987). Alpha helical CRF also decreased the latency for rats to emerge into a novel open field from a dark familiar enclosure (Takahashi et al. 1988).

Howevcr, these results with the CRF antagonist in aversive situations are reasonably subtle particularly in light of a significant amount of negative results in some classic animal models sensitive to antianxiety drugs. Alpha helical CRF ICV by itself does not produce a reliable release of punished responding (Britton et al. 1986b). Nor does a helical CRF ICV block the suppression of responding observed in a conditioned emotional response (CER) (Cole et al., unpublished manuscript).

CRF AND LEARNING

There is a significant amount of empirical and theoretical evidence that suggests that performance of learned behavior can vary with the level of activation or "arousal" of the animal. Correlations have been obtained between independent measures of arousal such as exploratory behavior, EEG, galvanic skin response, and the speed of acquisition of a response (Berlyne 1960). Indeed, it has been hypothesized that both animals and humans strive to maintain intermediate amounts of arousal potential, and it follows that optimal learning will correspond to these optimal levels of arousal. Other treatments of this theoretical issue have centered on arousal as the intervening variable in the incentive-motivation required for all learning (Killeen et al. 1978).

Recent results in our laboratory with both CRF and the CRF antagonist have revealed more robust effects in learning situations where arousal and/or an aversive state are required for animals to learn an association at an optimal rate. First, rats pretreated with CRF and trained on a two-way active avoidance task showed an improvement in performance at low doses of CRF (10 and 100 ng ICV) but less of an improvement at a higher dose of 1,000 ng (figure 4). At this higher dose, the rats appeared incapable of responding in a coherent way and, in fact, reacted to the CS as if it were the shock itself by jumping back and forth across the two compartments.

In the CER paradigm, a baseline of food-reinforced operant responding is established and then a CS is presented prior to the delivery of a brief,

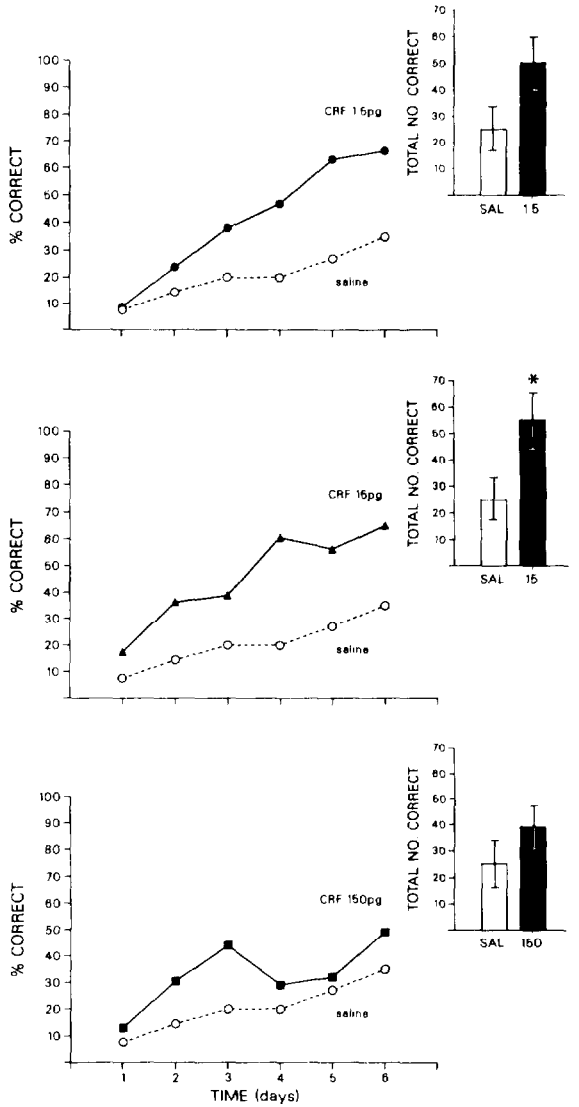

FIGURE 4. *Effects CRF injected intracerebroventricularly on acquisition of two-way active avoidance in rats*

*Significantly different from saline, $p<.05$ ANOVA.

NOTE: CRF was injected 1 hour prior to testing. Rats received 20 trials per day. Each trial consisted of 5 seconds of a compound CS (tone and light) followed by 0.3 mA of AC shock if the rat did not jump to the other side within 5 seconds. The intertrial interval was 20 seconds. Saline group is the same in each panel, n=8 per group.

inescapable foot shock (unconditioned stimulus (UCS)) while the animal is responding on the baseline schedule. This results in a reduction of food-reinforced responding during the presence of the CS. During acquisition, this reduction in performance is acquired rapidly. However, rats treated intracerebroventricularly with the CRF antagonist a helical CRF, at doses of 1 to 25 μg, had significantly impaired acquisition of the CER. High doses of the antagonist injected intravenously sufficient to attenuate plasma corticosterone levels did not impair acquisition, although a low-intravenous dose did impair acquisition. Also, dexamethasone treatment, in doses sufficient to block the pituitary adrenal axis, failed to alter the acquisition of the CER. These results, taken together, suggest that the ability of *a* helical CRF to attenuate the development of conditioned fear is independent of its action on the hypothalamic pituitary adrenal axis. The results also suggest a possible role for peripheral CRF in the development of conditioned fear.

CRF AROUSAL AND PERFORMANCE

To summarize, CNS manipulation of the putative neurotransmitter CRF produces changes in activation, behavioral responses to stress, and learning. CRF administered directly into the CNS also produces physiological activation and can potentiate behavioral responses to stress. This activation can result in a facilitation of performance in learning situations, especially those involving aversive motivation. However, CRF administered prior to the daily acquisition of an appetitively motivated visual-discrimination task also significantly improved acquisition (Koob et al. 1987).

The effects of the CRF antagonist a helical CRF have been more subtle. This peptide antagonist appears to be particularly active in situations where plasticity of associations is involved. For example, a helical CRF blocks the development of stress-induced fighting and the development of the CER but has little effect when the behavioral response is well established, e.g., lack of effect of a helical CRF on established conflict or CER performance.

These results suggest that CRF itself may be part of a fundamental activation system that is engaged in situations of high arousal and stress. This brain system may be required for animals to form associations between aversive events and previously neutral stimuli. The fact that the CRF antagonist does not have basic sedative properties and does not produce a benzodiazepinelike profile of disinhibition suggests that this system may be active only under situations requiring behavioral adaptation. Clearly, CRF effects on activation, anxiety, and learning could all be interpreted as independent effects (mediated by separate neural substrates), or they all could be considered different behavioral manifestations of a common process-arousal. Whether activation of the CRF system proves crucial to learning in general or simply to the learning associated with stress reduction remains to be determined.

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Attentional and Motivational Effects of Psychoactive Drugs

Conan Kornetsky, Joseph E.G. Williams, and Michael Bird

INTRODUCTION

An important question in the study of abuse substances is how and to what extent these drugs affect learning and memory. Is an enhancement or impairment of performance due to drug effects on motivation or attention? This chapter will deal with some of the problems associated with the multiple effects of abuse substances.

Learning and memory usually do not take place in the absence of reward or reinforcement, and, for the regulation of a stimulus in the central nervous system (CNS), the attention of the subject must be engaged. A possible exception is classical or type I conditioning. This type of learning is obligatory, in that reinforcement does not seem to play a direct role. Even in classical conditioning, however,' the conditioned stimulus, as well as the unconditioned stimulus, must be of sufficient intensity to register and elicit a response. If the subject is not attending to the stimulus, it fails as a conditioned stimulus. Thus, in considering the effects of drugs on learning and memory, factors other than the direct effect of the drug on those neurons in the brain that are specifically related to learning and memory must be considered.

AROUSAL AND ATTENTION

Although there seems to be a paucity of specific information on the effects of arousal level on learning and memory, there arc a number of studies that have manipulated the arousal level of the subject by looking at the effects of drugs on various cognitive, motor, and attention tasks. Arousal level may be changed by manipulating the motivational level. A classic study of this type, in which motivation was manipulated, thus changing inferred arousal levels, was carried out by Hill et al. (1957). In this experiment, they determined the effects of 250 mg of pentobarbital in human volunteers on reaction time (RT) under three motivational conditions. The subjects who volunteered to participate in the experiment were prisoner-patients at the U.S. Public Health Service hospital. These subjects were recidivists,

heroin addicts who had multiple admissions at the hospital. At the time of the experiment, they were not dependent on any drug, although it was most likely that once released from the hospital they would once again become dependent. Subjects were often rewarded for participation in the experiments with small amounts of morphine, which would be given a number of days after the completion of the experiment. In this experiment. one group of subjects (standard incentive) received the usual fixed amount of morphine at the end of the experiment. Another group (low incentive) received the same amount of reward, only it was given a number of weeks prior to participation in the experiment. A third group of subjects (high incentive) received payment for participation at the end of the experiment, but, in this case, the amount of payment was contingent upon their latency of response in a simple RT test. Figure 1 summarizes the results of this experiment. As shown, changing the motivational conditions altered the speed at which the subjects responded.

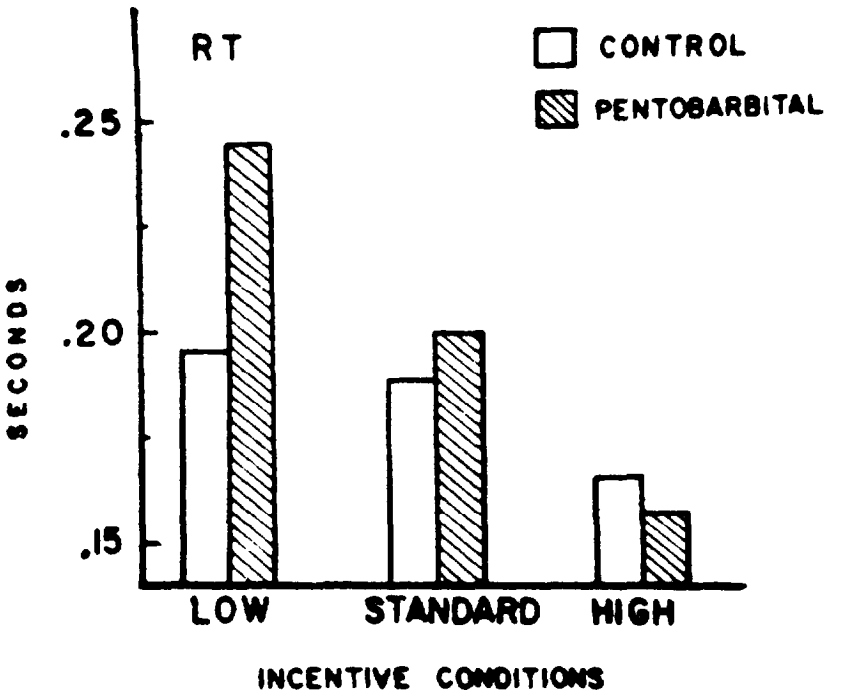

FIGURE 1. *Effects of pentobarbital on RT under three levels of motivation*

SOURCE: Adapted from Hill et al. 1957 in Kornetsky 1976. copyright 1976, John Wiley & Son, Inc.

Although this effect was not surprising, the effects of pentobarbital under these three conditions were. Under the highest motivating conditions, the RT after pentobarbital was not only faster than that under control conditions for low and medium levels of motivation, but was actually faster than that seen in the unmedicated group in the high-motivation condition. The operant conditioning "maven" would point out that the three levels of motivation resulted in changes in the schedule, and that schedule differences have been clearly shown to product differential drug effects.

A series of experiments demonstrating that changing the arousal level of the subject can produce markedly different drug effects were carried out; in these experiments, arousal level was changed by direct manipulation of the mesencephalic reticular formation. In the first of these experiments (Kometsky and Eliasson 1969), low-level electrical stimulation delivered by means of bipolar electrodes impaired the performance of rats on a simple go-no-go discrimination test that was an animal version of the continuous performance test (CPT) (Rosvold et al. 1956). However, when a dose of chlorpromazine that impaired performance on this test was combined with the low-level electrical stimulation to the reticular formation, the result was an animal whose performance was not different from that seen after saline alone. The results of this experiment are shown in figure 2.

The role of the arousal level of the subject is further demonstrated in an experiment on the effects of d-amphetamine on attention, simple and complcx RT, and learning in sleep-deprived subjects (Kometsky et al. 1959). In an earlier experiment in nonsleep-deprived subjects, it was found that d-amphetamine caused no significant improvement in the performance of the subjects (Kometsky 1958) a finding that generally corroborated previous observations (Hauty and Payne 1957). In the sleep deprivation experiment, normal volunteers were tested on the CPT, RT, Digit Symbol Substitution Test (DSST), and a learning task after 44 and 68 hours of sleep deprivation. The learning task consisted of having the subjects learn the proper association between 10 lights and 10 buttons. These were arranged on a console in front of them, with the lights at eye level and the buttons arranged in a row at desk level. For each learning test, a different association of lights and buttons was presented to the subjects. Each subject was sleep deprived twice, 1 week apart. In one case, the subjects were administered a placebo 60 minutes prior to both the 44-hour and 68-hour tests. In the second experiment, the subjects were administered 10 mg of d-amphetamine 60 minutes before the 44-hour test and 15 mg 60 minutes before the 68-hour test. The experiment was a simple double-blind cross-over design. After 44 hours of sleep deprivation, there was significant impairment in performance on the CPT and RT but not on the learning task. Only the RT performance was significantly improved by *d*-amphetamine as compared to placebo at 44 hours of sleep deprivation. At 68 hours of sleep deprivation, performance after placebo on the two procedures and on learning was significantly impaired. This impairment on all procedures was significantly

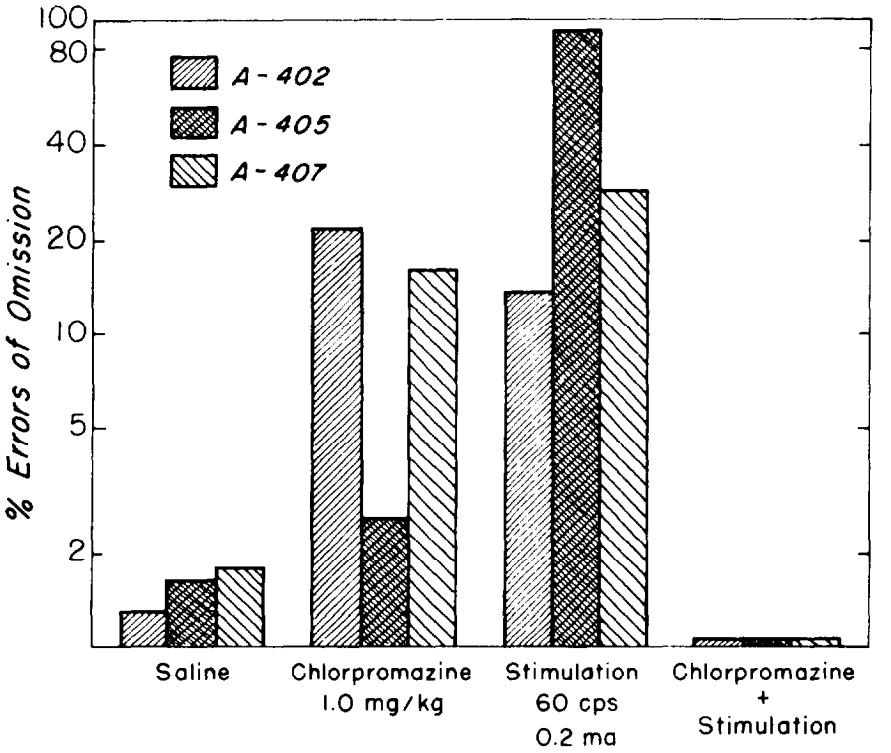

FIGURE 2. *The percentage of errors of omission for the three animals after administration of chlorpromazine (1.0 mg/kg) or during 60 Hz 0.2 ma of stimulation and the effect of this dose and this amount of stimulation combined*

NOTE Although errors are indicated for the combined treatment, none of the animals made errors of omission when chlorpromazine and stimulation were given together.

SOURCE: Kometsky and Eliasson 1969, copyright 1969, AAAS.

improved by d-amphetamine. Figure 3 shows the percent of control (nonsleep-deprived) errors in learning for the four conditions in this sleep deprivation experiment.

The significance of these studies is that the effects of a drug on a variety of performance tasks is very dependent on the arousal level of the subject. A question that must be asked by every investigator who attempts to study the effects of a drug on cognition is whether the observed effect is a direct result of the action of the drug on those areas of the brain that have

specific functions in learning and memory or is due to alterations of motor or perceptual-attentional systems. The sleep deprivation study described above would suggest that the drug effects were not due to a direct effect on cognitive functioning.

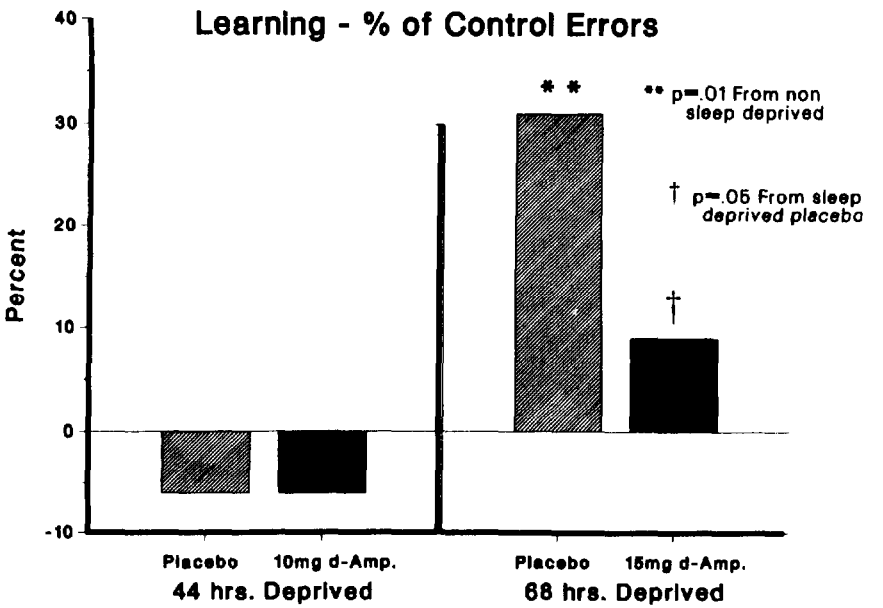

FIGURE 3. *Effects of* d-*anlphetanzine on learning (percent errors in acquisition) after 44 hours and 68 hours of sleep deprivation*

SOURCE: Adapted from Kornetsky et al. 1959.

THE BRAIN REWARD SYSTEM AND PERCEPTION OF BRAIN STIMULATION

All drugs that act in the CNS at some dose will decrease the registration of stimulation. Whether a drug can enhance the perception of a stimulus in a normal, well-motivated subject is not clear. The sleep deprivation study described in the previous section is an example of the enhancement of the registration of a stimulus by *d*-amphetamine in an impaired subject. Some drugs are believed, especially by the users, to enhance the awareness of the physical environment and, specifically, to lower the threshold for the perception of colors, sounds, and smells. Lysergic acid diethylamide (LSD) was believed by many users in the 1950s and 1960s to enhance awareness

of all sorts of stimuli. Despite these claims, there is no experimental evidence of such enhancement of perception (Abramson et al. 1955). Experimental studies of the effects of LSD on perception demonstrated that it either had no effect or impaired perception in a number of modalities. What subjects may have been reporting is an apparent enhancement of perception resulting from an impairment in perceptual constancies. Thus, instead of perceiving a room that they are in as a large cube, they see it as trapezoidal in shape, the image that is actually impinging on the retina. The walls of a room that are painted one color are seen as being of different colors. Subjects become very much aware of differences caused by different amounts of light on the walls. Normally, these differences are not noticed unless there is specific attention directed toward these differences.

The laboratory work of Kometsky has focused on the study of the effects of drugs on the reward system, more specifically, the effects of drugs on the threshold for the perception of rewarding brain stimulation. This system was first described in an experiment by Olds and Milner in 1954, in which they demonstrated that animals will work to obtain electrical stimulation of lateral hypothalamic areas of the brain. Almost all, if not all, drugs that lower the threshold for such rewarding brain stimulation are abused or have the potential for abuse; animals will self-administer them and, when such drugs are given to human subjects, the subjects report feelings of euphoria (Kometsky 1985). This has been interpreted as evidence that the drug has hedonic effects. Drugs that raise the threshold are believed to be anhedonic (Wise 1982). The question that is raised by studies of the effects of drugs on brain-stimulation reward is whether the effects observed are due to (1) an effect of the drug on motor systems; (2) in the case of threshold studies, the ability of the animal to make the perceptual discrimination; or (3) the result of a combination of these effects with an effect on the motivational system.

When rate of response is the dependent variable in intracranial self-stimulation studies, it is difficult to determine whether facilitation of responding is the result of psychomotor stimulation and whether decreased responding is due to psychomotor slowing. Many investigators using rate of response as the dependent variable have found that, after the first exposure to morphine, facilitation of rate of response only occurs 3 hours after the morphine is administered (Lorens and Mitchell 1973; Hand and Franklin 1386). In our earliest studies using a rate-independent threshold procedure, morphine caused an increased sensitivity to the stimulation that was observed within 30 minutes after the drug was administered for the first time (Marcus and Kometsky 1974).

Although the threshold method used gives results that are independent of rate of response, the method does not completely answer the question of whether observed changes are due to an effect of the drug on the attentional-discriminative aspect of cognitive functioning. In order to

answer that question, a technique, brain-stimulation detection, was employed that is similar to the procedure used for determining the threshold for rewarding brain stimulation, but asks the animal a different question. In the brain-stimulation reward paradigm, the threshold for a rewarding brain stimulation is determined. In the brain stimulation detection paradigm, the threshold for detecting an intracranial stimulation that has no reinforcing effects of its own is determined.

In the brain-stimulation reward procedure, Marcus and Kometsky (1974) used bipolar electrodes stereotaxically implanted into the medial forebrain bundle at the level of the lateral hypothalamus (MFB) or the ventral tegmental area (VTA) of F-344 rats. Animals were trained and tested in an experimental chamber in which there was a cylindrical manipulandum in one wall. A maximum of a quarter turn of the manipulandum operated a microswitch that resulted in the animal receiving a reinforcing electrical stimulation via the bipolar electrode.

Determination of the reinforcement threshold made use of a rate-free, discrete-trial procedure. A schematic of the procedure is indicated in figure 4. A trial began with the delivery of a noncontingent 0.5-second pulse train. A response within 7.5 seconds of the onset of the noncontingent stimulus resulted in the immediate delivery of a contingent stimulus that was identical to the noncontingent stimulus in all parameters. This response terminated the trial. If the subject failed to respond, there was no scheduled consequence; the trial was terminated after 7.5 seconds. Intervals between trials averaged 15 seconds (range: 7.5 to 22.5 seconds), and responses during the inlet-trial interval resulted in a 15-second delay before the start of the next trial. Stimulus intensities were varied, and thresholds were determined according to a modification of the psychophysical method of limits. For further details of the procedure, see Esposito and Kometsky (1977).

In the brain-stimulation detection procedure, the paradigm used was a modification of the technique first used by Doty et al. (1956). The experimental chamber, including the manipulanda, was identical for brain-stimulation reward and detection. Also, the surgical preparation of the animals was identical, with some variation if the area of the brain in which the cueing electrode was implanted was different from the electrode used for reinforcing the behavior.

When only a single stimulating electrode was used (Kometsky and Esposito 1981), the procedure for determining the brain-stimulation detection threshold was only slightly different from that used and indicated in figure 4 for brain-stimulation reward. In the detection procedure, the noncontingent stimulus was varied in intensity but at current levels that were not reinforcing, while the second or response-contingent stimulus was held constant at a highly reinforcing level. Thus, the noncontingent stimulus functioned purely

as a cue or discriminative stimulus, indicating the presence of a rewarding level of stimulation, The intensity of the initial stimulus was varied according to a modification of the psychophysical method of constant stimuli.

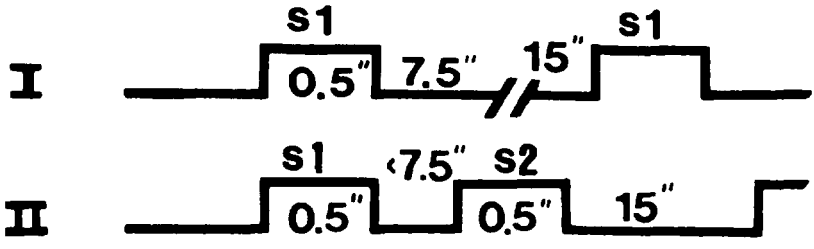

FIGURE 4. *A schematic representation of the two possible outcomes during a single trial of the brain-stimulation reward procedure*

NOTE: In the first example (I), the subject is presented with a nonreinforcing stimulus (S1), after which it does not respond during the 7.5-second available response time. The animal's failure to respond has no scheduled consequences, and a new trial begins after a 15-second intertrial interval. In the second example (II), a reinforcing stimulus (S1) is presented, and the subject responds by turning the wheel manipulandum within the 7.5-second available response interval and receives a second stimulus (S2) of the same intensity as the S1.

By employing two electrodes (Wheeling and Kometsky 1983), detection thresholds could be determined from one intracranial locus, while behavior was maintained by rewarding brain stimulation to a second site. The advantage of the two-electrode model is that it allows for the determination of the threshold for detection from sites where stimulation is never reinforcing at any intensity. Other than the fact that the cueing electrode is not the same electrode that delivers the reinforcing stimulation that drives the behavior, the two-electrode procedure is identical to that employed when only one electrode is used. Using this technique, marked differences in drug effects on absolute threshold for detection and reward were found, but detection was from different brain sites (Wheeling and Kometsky 1984).

Comparison of the Effects of Cocaine and Pimozide on the Thresholds for Brain-Stimulation Reward and Brain-Stimulation Detection

In these experiments, the single electrode procedure was used. In the first of these experiments, Kometsky and Esposito (1981) compared the effects of cocaine, a drug that increases an animal's sensitivity to rewarding brain stimulation, on the threshold for rewarding and detecting electrical stimulation to the MFB. An important question posed by this experiment was whether the threshold-lowering effects of cocaine for rewarding brain

stimulation was specific for the brain site being stimulated or specific for the type of stimulation, which, in this case, was rewarding. The former would be borne out, if the threshold for detection also was lowered with the potency approximately the same.

The threshold for rewarding brain stimulation after various doses of cocaine was determined in four animals. The results replicated a previous experiment (Esposito et al. 1978) that demonstrated that cocaine lowered the threshold. After a cocaine dose-response curve was generated for each animal, the animals were retrained on the brain-stimulation detection procedure using the same electrode as was used for determining the threshold for rewarding stimulation. The range of thresholds for the four animals after saline was 65 to 125 μA for reward and 7 to 22 μA for detection.

Threshold change scores (postdrug threshold minus predrug threshold) for each experimental day were converted to a standard score (z-score) based on the mean and standard deviation of all the change scores after saline treatment for each animal respectively. This was done for both reward and detection. The mean effects of cocaine on both the reward and detection thresholds expressed as z-scores are shown in figure 5, which indicates two interesting phenomena. The first is the complete dissociation between the effects of cocaine. While cocaine increases an animal's sensitivity to rewarding brain stimulation, as indicated by the lowering of the threshold, it decreases the sensitivity of the animal to the detection of stimulation to the same intracranial site by means of the same electrode. Further, cocaine will lower the threshold for rewarding brain stimulation at doses that have little or no effect on the detection threshold. These results suggest that attentional-perceptual functioning is not impaired by cocaine at doses that are probably euphorigenic; however, as doses increase, there is impairment of such functioning.

If drugs that increase dopamine availability at the synapse have euphorigenie properties, are self-administered by humans and animals, and lower the threshold for rewarding brain stimulation, do dopamine blockers decrease euphoria or, as suggested by Wise (1982), are they anhedonic? In every case where the effects of a neuroleptic have been tested on brain-stimulation reward, the neuroleptic has either decreased response rate for the stimulation (Wise 1982) or it has raised the threshold (Esposito et al. 1981), suggesting that it decreases the animal's sensitivity to the stimulation. The problem of the specificity of the effect is even greater when looking at a behavior that is impaired by the drug, rather than a behavior that is facilitated. Some investigators have attempted to deal with this problem of performance capability in the study of neuroleptic effects on brain-stimulation reward. For example, in attempts to dissociate the motivational from the performance effects of pimozide, some investigators (Franklin 1978; Stellar et al. 1983; Gallistel and Karras 1984) have determined that the pulse frequency (the higher the frequency, the greater the amount of stimulation)

required to initiate running was raised at doses that did not decrease the speed of the running.

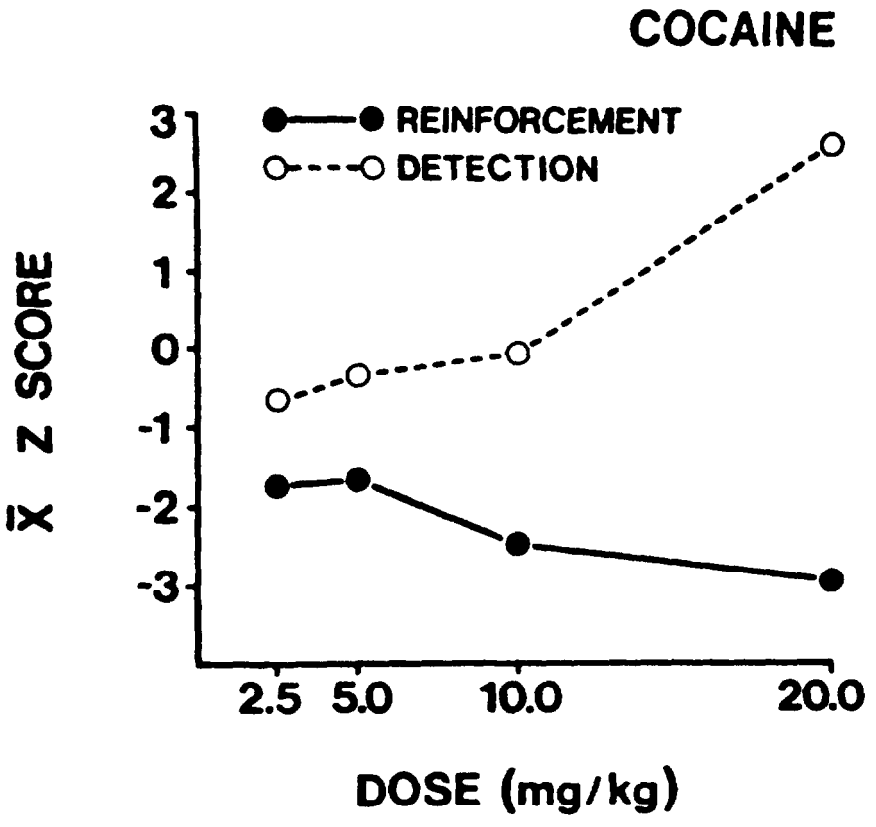

FIGURE 5. *Mean effect of various doses of cocaine on the threshold for brain-stimulation reward (reinforcement) and on the threshold for brain-stimulation detection*

NOTE: Data are expressed as z-scores based on the respective mean and standard deviation of the effects of saline (n=4).

SOURCE: Kornetsky and Bain 1982, copyright 1982, Elsevier.

Although these findings suggest a specific decrease in the reinforcing value of the electrical stimulation, they did not control for the possibility that pimozide was impairing attentional capacity, and this could account for the increase in pulse frequency necessary to initiate speed of running. In order

to more directly address the problem of performance deficits accounting for the decreased sensitivity of animals to rewarding brain stimulation, the effects of pimozide on both the reward and detection threshold were determined in the same animals (Bird and Kometsky 1988). The experiment was similar to the above described experiment with cocaine. Also, because of the delay in the distribution of pimozide in the CNS, testing was not begun until 4 hours after injection. In this experiment, psychophysical power functions were determined using the least-squares regression analyses of the probit of percent response versus the log of the total electrical charge of the stimulus (pico Coulombs). The threshold in pico Coulombs was interpolated from the line of best fit using the 50-percent response criteria (probit, 5). These thresholds were transformed to z-scores based on the mean and standard deviation of all vehicle treatment days for each animal, respectively.

The results of one of the four animals used in the experiment are shown in figure 6. The figure shows the power function lines of best fit for both detection and reward. At doses of pimozide that significantly raise the threshold for brain-stimulation reward, there are no significant effects on the detection of stimulation. The mean dose-response curve for the four animals studied is shown in figure 7. Using this technique, it can be concluded that there is clearly no effect on the ability of the animal to detect stimulation at doses that cause a loss of sensitivity to rewarding stimulation. To the extent that the brain-stimulation detection procedure reflects an important part of cognitive functioning, these data indicate a relatively specific effect of pimozide on the sensitivity of the animal to rewarding stimulation.

Effects of Chlorpromazine on the Threshold for Brain-Stimulation Reward and Brain-Stimulation Detection

Chlorpromazine, also a neuroleptic but with less specificity than pimozide as a dopamine antagonist, was tested on brain stimulation reward as well as brain stimulation detection. As expected, chlorpromazine raised the threshold for both types of stimulation (Esposito et al. 1981; Williams and Kometsky 1985). Figure 8 shows the mean dose-response curve for the effects of doses of chlorpromazine from .03 to 2.0 mg/kg intraperitoneal (IP), on the threshold for brain-stimulation reward with stimulating electrodes in the MFB-lateral hypothalamic area, detection from the MFB, and also from the mesencephalic reticular formation (MRF). Although not indicated, higher doses of chlorpromazine raised the reward threshold even higher; however, higher doses of chlorpromazine resulted in the animal's inability to do the detection task, as indicated by the vertical arrows. Of interest is that, in contrast to the results obtained with pimozide, chlorpromazine caused marked impairment in detection from at least two sites in the brain, at doses far below those that decreased sensitivity to rewarding

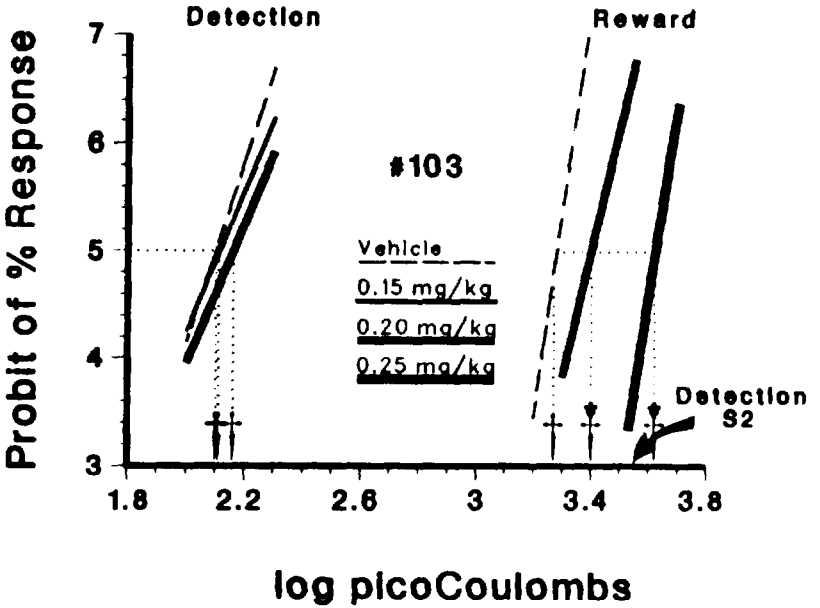

FIGURE 6. *The effects of pimozide on the detection and reward power functions and threshold for a single animal is shown*

NOTE: The intensity of stimulation needed to reinforce responding to the S1 in the detection procedure is indicated by the large arrows. Probit of percent response is plotted against the stimulation intensity expressed in log 10 pico Coulombs units. Threshold for each treatment is indicated by daggers.

brain stimulation. From these two experiments, it can be concluded that there seems to be a relatively specific effect of the pimozide on brain stimulation, while the much less specific dopamine antagonist chlorpromazine affects reward only at doses that are impairing the perceptual attentional ability of the animals.

CONCLUSIONS

The above described experiments, although not directly measuring the effects of drugs on learning and memory, clearly demonstrate that motivation and attention influence the effects of abuse substances on a subject's cognitive performance. A significant aspect of cognitive function is the ability of the animal to make fine discriminations and attend to the possible presentation of a stimulus. This detection of stimuli is easily impaired by both abuse and nonabuse substances. This decrease in sensitivity to stimuli,

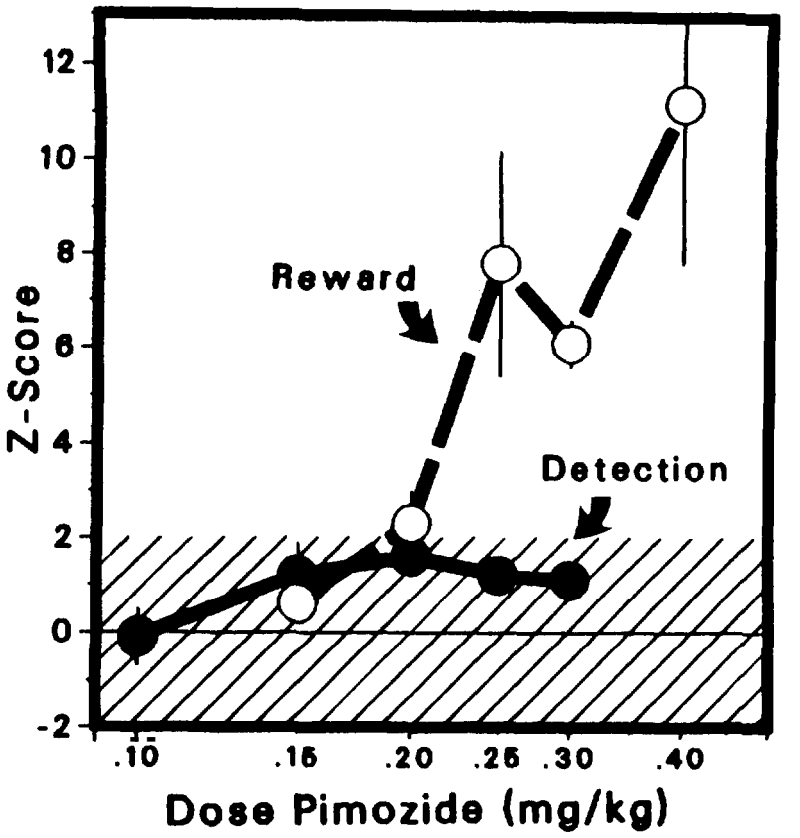

FIGURE 7. *Mean z-scores ±SEM in the detection and reward thresholds as a function of punozide dose for four animals*

NOTE: Error bars not shown indicate SEM less than the diameter of the symbols in this illustration. Each z-score is based on the difference between the mean postvehicle and postpimozide threshold. A z-score of 2 denotes the standardized 95-percent confidence interval of postvehicle thresholds and indicates a significant deviation from vehicle-treated sessions.

however, may be selective, in that doses of cocaine that impaired detection were able to increase the animal's sensitivity to rewarding brain stimulation, and doses of pimozide that decreased sensitivity to rewarding brain stimulation failed to impair the detection of nonrewarding brain stimulation to the same site. Thus, it is important to realize that, in all experiments designed to elucidate the effects of drugs on performance, at least at lower doses, behaviors can be dissociated, and, although sensitivity of an animal can be increased in one modality, it may be impaired in others.

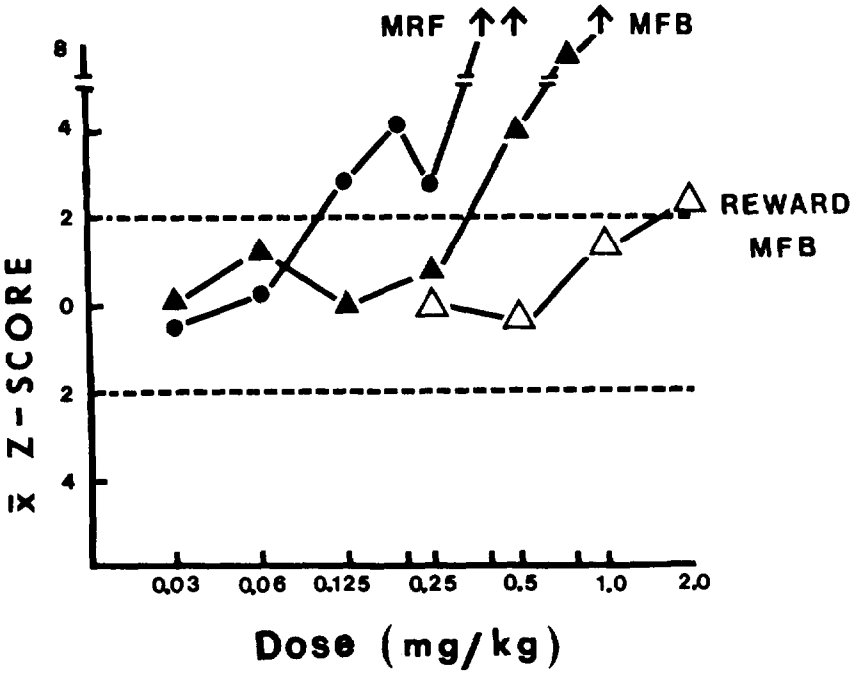

FIGURE 8. *Effects of chlorpromazine on threshold for rewarding stimulation to the MFB and detection threshold to the MFB and to the MRF*

NOTE: Data are expressed as the mean z-scores of two animals for each of the detection placements. For the reward threshold level, the data are the mean of four animals. Z-scores are based on the mean and standard deviation for all saline treatments for the respective animal and task.

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Learning as a Factor in Ethanol Tolerance

A.D. Lê and H. Kalant

INTRODUCTION

Tolerance is usually defined as a reduction in the behavioral and/or physiological effects produced by a given dose of a drug as a consequence of chronic exposure to the drug. Tolerance can result from either a diminution in the sensitivity of the central nervous system (CNS) to the drug (i.e., functional tolerance), an increase in the rate of elimination of the drug (dispositional or metabolic tolerance), or both (Kalant et al. 1971).

Functional or CNS tolerance has been a subject of interest for multiple reasons, ranging from clinical pharmacological to forensic issues. From the research viewpoint, however, tolerance has been studied for two main reasons. First, tolerance is a cardinal sign of drug addiction and is believed to contribute to the strength of addiction. Therefore, a knowledge of its mechanism(s) may facilitate the development of rational measures for treating addiction (Cappell and LeBlanc 1981). Secondly, tolerance is generally viewed as a form of neuroadaptation (Kalant et al. 1971; LeBlanc and Cappell 1977) and knowledge of its mechanism(s) may facilitate or contribute to our understanding of CNS adaptive mechanisms or neuronal plasticity in general.

CNS tolerance has been a subject of investigation for more than 60 years. In earlier times, drug tolerance was an exclusive domain of pharmacologists or neurobiologists. It was viewed as a general physiological adaptation to the presence of the drug in the CNS (Himmelsbach 1943). Such adaptation was thought to occur at the molecular level and to be specific for the type of drug used or to the mechanism of action of the drug (Collier 1965; Goldstein and Goldstein 1968; Cochin 1971). It was assumed that tolerance developed equally to various drug effects following chronic drug treatment.

Beginning in the late sixties, the traditional view of tolerance was challenged by the work of Mitchell and colleagues on morphine tolerance (Kayan et al. 1969; Kayan and Mitchell 1972) and that of Chen on ethanol tolerance (Chen 1968). Essentially, these investigators demonstrated that pharmacological treatment might not necessarily produce tolerance (Chen

1968), or that similar pharmacological history does not necessarily lead to the same level of tolerance development (Kayan et al. 1969). Factors such as repeated testing while under intoxication or resemblance between the procedure and circumstances associated with drug treatment and with testing conditions (Gebhart and Mitchell 1972) might dictate the manifestation of tolerance or the level of tolerance attained. These findings led to the view that tolerance might be a learning process rather than a physiological adaptation. This, of course, makes the questionable assumption that learning is not based on physiological adaptation.

Research in the last several years has indicated that tolerance is a multifaceted phenomenon. At present, it is quite clear that tolerance can develop as a consequence of drug exposure per se (Lieber et al. 1965; Goldstein and Pal 1971). For example, tolerance to ethanol (Goldstein and Pal 1971) can be induced by continuous vapor inhalation, and tolerance to morphine can follow morphine pellet implantation (Bhargava 1978). In such cases, behavioral factors are absent or minimally involved. However, various learning processes such as associative learning or Pavlovian conditioning and opportunity to practice while under intoxication can be critical factors in tolerance development.

There are two different bodies of experimental data that support the role of learning in drug tolerance. The direct involvement of learning in tolerance was demonstrated by the fact that behavioral manipulations such as practice while under intoxication (Chen 1968; LeBlanc et al. 1973; LeBlanc et al. 1976a) and Pavlovian conditioning (Siegel 1975) can affect tolerance development. The second body of data was derived from experimental procedures that demonstrated that neurobiological manipulations that affect learning also affect tolerance development in a similar manner. Although the behavioral and neurobiological factors in tolerance have been studied on a variety of drugs or drug classes, this chapter will concentrate mainly on how these factors affect ethanol tolerance. Where relevant, tolerance to other drugs will be discussed.

BEHAVIORAL MANIPULATIONS

Intoxicated Practice

The Before-and-After design has been used extensively to demonstrate the role of intoxicated practice or response contingency in ethanol tolerance. Essentially, this design involves two groups of subjects receiving the same total drug exposure, but differing with respect to the temporal relation between the drug and the test behavior. On the treatment or training days, subjects in the Before (or Behavioral) group are injected with ethanol and then tested on the task concerned. The subjects in the After (or Physiological) group perform the test in the sober condition and are then injected with

ethanol afterward. On the test days, both groups receive the alcohol before performing the test.

Employing this design, Chen (1968) demonstrated that tolerance to the disruptive effect of ethanol on maze performance did not develop unless ethanol was given prior to the test on training days. In other words, tolerance did not develop unless the animal had the opportunity to practice the task while under ethanol intoxication. For this reason, he suggested that tolerance was a consequence of learning to compensate for drug-induced impairment, rather than of simple drug exposure per se.

Subsequent work by LeBlanc and his coworkers using food-motivated or shock-avoidance tests (LeBlanc et al. 1973; LeBlanc et al. 1976a) essentially confirmed Chen's finding. In these studies, however, LeBlanc and his coworkers found that the After group also developed tolerance to the same extent as that of the Before group, if the ethanol treatment was extended over a long enough duration. LeBlanc et al. 1973, therefore, suggested that practice while intoxicated only enhanced the rate of tolerance development, but may not be essential for tolerance development. The enhancement of tolerance development by intoxicated practice was referred to as behavioral augmentation of tolerance.

Intoxicated Practice and the Development of Ethanol Tolerance

The role of intoxicated practice or response contingency on ethanol tolerance has also been shown in a variety of ethanol effects such as analgesia (Jorgensen and Hole 1984; Jorgensen et al. 1986), impairment of thermoregulation (Alkana et al. 1983; Lê et al. 1986b), sexual behavior (Pinel et al. 1988), and anticonvulsant action (Pinel et al. 1983; Pinel et al. 198.5). For example, Pinel and coworkers have demonstrated that tolerance to the anticonvulsant effect of ethanol in a kindled seizure model develops only when amygdaloid stimulation has been administered during ethanol intoxication (Pinel et al. 1983; Pinel et al. 1985).

There is, however, considerable debate on whether intoxicated practice is an absolute prerequisite for, or simply accelerates, tolerance development. Some of the difference in the interpretation might be accounted for, in part, by the ethanol dose level employed. For example, in a recent study (Lê et al. 1987a). it was found that the influence of intoxicated practice on tolerance to the motor impairment effect of ethanol was dependent on treatment dosage employed. When animals were treated daily with a 3.5 g/kg dose of ethanol for 30 days, the extent of tolerance was essentially similar between the intoxicated practice and nonpractice groups. However, when a low treatment dose was used (1.5 to 2.5 g/kg intrapetitoneal (IP) daily for 30 days), or when tolerance was not maximal, intoxicated practice resulted in a significant enhancement of tolerance development. In a collaboration with Pinel's laboratory, it was also found that tolerance to the anticonvulsant

effect of ethanol in a kindled seizure model did not require amygdaloid stimulation during ethanol intoxication if the treatment dosage employed was high (Mana et al. 1988). Similarly, work by Jorgensen et al. (1986) showed that tolerance to the analgesic effect of ethanol was contingent upon the administration of the painful stimulus during ethanol intoxication when the daily ethanol treatment dose was 2.5 g/kg, but not when the treatment dose was 5 g/kg.

These studies thus demonstrated that intoxicated practice can influence tolerance development, but that the extent or nature of the influence of such practice on ethanol tolerance is dependent on the ethanol treatment regimen employed.

PAVLOVIAN CONDITIONING AND ETHANOL TOLERANCE

The role of Pavlovian conditioning in morphine tolerance was demonstrated by Siegel during the mid-seventies in a series of elegant experiments (Siegel 1975; Siegel 1976). The central hypothesis of the Pavlovian model of tolerance is that an unconditioned compensatory response to the drug effect becomes conditionally linked to the environmental cues accompanying drug administration, and that tolerance reflects an antagonism of the acute drug effect by this conditioned response (Siegel 1975; Siegel 1976).

Work from our laboratory (Lê et al. 1979b; Lê et al. 1987a) and from others (Mansfield and Cunningham 1980; Crowell et al. 1981; Melchior and Tabakoff 1981; Melchior and Tabakoff 1985) has shown that tolerance to ethanol-induced hypothermia is also subject to Pavlovian control. Essentially, in these studies, tolerance to the hypothermic effect of ethanol was observed only, or to a greater extent, when animals were tested in the presence of environmental cues previously associated with ethanol treatment, not in an environment previously associated with saline administration. In the presence of the environmental cues associated with ethanol administration, a saline injection elicited an anticipatory hyperthermia in the ethanol-treated animals (Lê et al. 1979b; Mansfield and Cunningham 1980; Crowell et al. 1981).

Conditioning has been shown to influence tolerance to other effects of ethanol such as narcosis (Melchior and Tabakoff 1981) and analgesia (Tiffany et al. 1987). In addition, tolerance to other sedative-hypnotic drugs, such as barbiturates and benzodiazepines (Cappell et al. 1981; Greeley and Cappell 1985; King et al. 1987) as well as cross-tolerance between ethanol and barbiturate (Cappell et al. 1981; El-Ghundi et al. 1989), have been shown to be subject to Pavlovian control.

Similar to the effect of intoxicated practice on tolerance, the influence of Pavlovian conditioning on ethanol tolerance varies with the size of the alcohol treatment dose used to produce tolerance. At low treatment dose (12 to

14 trials of 2 g/kg of ethanol), tolerance was demonstrable only in the presence of environmental cues previously associated with ethanol administration. At high treatment dose (12 to 14 trials of 4 g/kg of ethanol), however, tolerance was manifested when tested in the presence or absence of environmental cues previously associated with ethanol administration (Lê et al. 1987b).

NEUROBIOLOGICAL MANIPULATIONS

The role of learning in drug tolerance has also been supported by a different body of data derived from a different experimental strategy. Basically, this strategy consists of examining similarities or parallels in the characteristics of drug tolerance and learning and in their susceptibility to neurobiological modification. For example, a task once learned, then forgotten, can be reacquired more rapidly than on the original learning. In a similar way, tolerance to ethanol has been shown to be reacquired more rapidly on subsequent cycles of exposure (Kalant et al. 1978; de Souza Moreira et al. 1981). Similarly, a number of neurobiological manipulations, ranging from cortical ablation to neurotransmitter modification, which have been shown to affect learning, modify tolerance in the same pattern. A summary of these studies is shown in table 1. It is clear from this table that the manipulations employed did not influence tolerance to a specific drug but affected the development of tolerance to a variety of drug classes.

It is beyond the scope of this chapter to examine in detail the findings from all these studies. The role of serotonin and the pituitary peptide vasopressin, and their interaction in the regulation of ethanol tolerance, will be examined as an example of the approach that attempts to draw parallels between learning and tolerance.

INTERACTION BETWEEN SEROTONIN AND VASOPRESSIN IN THE REGULATION OF ETHANOL TOLERANCE

Various studies have suggested that the brain serotonergic system may be involved in learning and adaptive processes (Khanna et al. 1980). Depletion of brain serotonin by administration of patachlorophenylalanine (pCPA), which inhibits the synthesis of serotonin, or by intracranial administration of 5,7-dihydroxytryptamine retarded the development of tolerance to the motor impairment and hypothermic effects of ethanol (Frankel et al. 1975; Frankel et al. 1978a; Lê et al. 1980a). Conversely, administration of L-tryptophan, in daily doses known to increase brain 5-hydroxytryptamine (5-HT) content, also increased the rate of tolerance development (Lê et al. 1979a). The retardation of ethanol tolerance by depletion of serotonin appears to be due to an acceleration of the loss of tolerance: once tolerance was fully acquired, administration of pCPA accelerated its loss (Frankel et al. 1978b).

TABLE 1. *Effects on drug tolerance of neurobiological manipulations that have been shown to disrupt learning*

Inhibition of Protein Synthesis:	Inhibits tolerance development
Morphine tolerance:	Ginsberg and Cox 1972; Feinberg and Cochin 1977
Ethanol tolerance:	LeBlanc et al. 1976b; Speisky and Kalant 1987
Barbiturate tolerance:	Hitzemann and Loh 1973
Electroconvulsive Shock:	Impaired tolerance development
Morphine tolerance:	Kesner et al. 1976; Stolerman et al. 1976
Frontal Cortical Ablation:	Impaired tolerance development
Ethanol tolerance:	LeBlanc et al. 1976b
Amphetamine tolerance:	Glick 1973
Depletion of Brain Acetylcholine:	Impaired tolerance development
Barbiturate tolerance:	Brezenoff and Mycek 1976
Depletion of Brain Norepinephrine:	Impaired tolerance
Morphine tolerance:	Friedler et al. 1972
Ethanol tolerance:	Tabakoff and Ritzmann 1977; Melchoir and Tabakoff 1981
Barbiturate tolerance:	Tabakoff et al. 1978
Depletion of Brain Serotonin:	Retarded tolerance development
Morphine tolerance:	Cheney and Goldstein 1971; Ho et al. 1972
Ethanol tolerance:	Frankel et al. 1975; Frankel et al. 1978a; Lê et al. 1980a; Lê et al. 1981
Barbiturate tolerance:	Frankel et al. 1975; Lyness and Mycek 1978; Lê et al. 1980b

TABLE 1. (Continued)

Vasopressin and Its Analogs:	Prolong the retention of tolerance
Morphine tolerance:	Krivoy et al. 1974; de Wied and Gispen 1976
Ethanol tolerance:	Hoffman et al. 1978; Hoffman and Tabakoff 1984; Rigter et al. 1980; Lê et al. 1982; Pittman et al. 1982; Hung et al. 1985; Speisky and Kalant 1985

The cell bodies of central serotonin neurons are clustered in three raphe nuclei, which project their fibers to different parts of the CNS. By lesioning the raphe nuclei selectively, it is possible to determine whether or not the development of ethanol tolerance requires the participation of all central serotonergic neurons. Destruction of the mesolimbic serotonergic pathway by means of electrolytic lesions of the median raphe nucleus, which mainly innervates the septum and hippocampal areas, was found to be necessary and sufficient to retard tolerance development. Lesioning of the dorsal raphe nucleus, which projects to the anterior cortex, the hypothalamus, and the striatum, or of the raphe magnus, which projects fibers to the spinal cord, did not affect tolerance development (Lê et al. 1981).

The pituitary peptide hormone vasopressin, and its analogs such as desglycinamide lysine vasopressin (DGLVP) or desglycinamide arginine vasopressin (DGAVP), have been shown to be able to prolong the retention of learned avoidance behavior in the rat (de Wied et al. 1972). Brattleboro rats, which in their homozygous state are deficient in vasopressin, also show impairment in their learning ability (Bohus et al. 1975).

Similarly, administration of vasopressin (Hoffman et al. 1978; Hoffman and Tabakoff 1984) or its analog DGAVP (Lê et al. 1982) can retain tolerance to the hypothermic, hypnotic, or motor impairment effects of ethanol despite the termination of ethanol treatment. Brattleboro rats also fail to develop tolerance to ethanol (Pittman et al. 1982). The effect of vasopressin on the retention of ethanol tolerance is mediated centrally by its interaction with V_1 receptors, and intracerebral administration of vasopressin or the specific V_1 agonist can maintain ethanol tolerance (Hung et al. 1985; Tabakoff and Hoffman 1988).

The effects of vasopressin or its analogs on learning might be mediated, in part, by its interaction with brain serotonin (Ramaekers et al. 1977). It was

also found that the retention of ethanol tolerance by DGAVP required an intact mesolimbic serotonergic pathway; administration of DGAVP failed to retain ethanol tolerance in median raphe-lesioned rats. Lesions of the dorsal raphe nucleus did not affect the maintenance of ethanol tolerance by DGAVP (Lê et al. 1982).

As mentioned earlier, the mesolimbic 5-HT pathway innervates other structures such as the parietal cortex, as well as the hippocampus. The mesolimbic 5-HT pathway splits into two branches before reaching the hippocampus. The infracallosal branch, traveling via the fornix and fimbria, innervates the larger ventral hippocampus, while the supracallosal branch traveling via the cingulum bundles innervates the dorsal hippocampus (Azmitia and Segal 1978). Selective depletion of hippocampal serotonin by means of intracranial injection of 5,7-dihydroxytryptamine into these two pathways blocked the retention of ethanol tolerance by DGAVP (Speisky and Kalant 1985). Thus, the actions of DGAVP in the retention of tolerance to ethanol might be mediated through its interaction with hippocampal serotonin.

The exact nature of the cellular processes for which 5-HT and DGAVP are required to regulate tolerance are still unknown. However, the fact that their interaction in the regulation of tolerance occurs mainly in the hippocampus, an important structure for learning and memory, further heightens the analogy between learning and drug tolerance.

CONCLUSION

From all the foregoing data on the effects of behavioral and neurobiological manipulations on tolerance, one must conclude that, depending on certain experimental conditions, learning can play a critical role in tolerance development. However, it is also clear that tolerance can develop under treatment conditions in which learning has little opportunity to take place. One of the important questions that needs to be answered is whether these factors interact to regulate the development of a single process of tolerance or whether learning constitutes a different type or mechanism of tolerance. In a situation in which both learning and pharmacological factors are optimal, the same maximum degree of tolerance can be achieved (LeBlanc et al. 1976a; Lê et al. 1987a; Lê et al. 1987b). For this reason, it has been proposed that there is no need to invoke more than one mechanism of tolerance. A large body of data has suggested that the stimulus for tolerance development is the functional disturbance induced by the drug rather than the drug itself (Kalant et al. 1971; Kalant 1987). If this is the case, then neurons or synapses in an altered state associated with functional activity, due to either behavioral or physiological manipulations, are more sensitive to the effects of ethanol and other drugs and, therefore, experience a greater stimulus to adapt to these effects (Kalant 1985; Kalant 1987).

Recent studies, however, have suggested that learning might reflect a separate mechanism of tolerance. Tolerance acquired under learning conditions can be retained for a much longer time than that acquired by purely pharmacological means. For example, tolerance to the hypothermic effect of ethanol induced by purely pharmacological means dissipated by 3 to 6 days after the termination of ethanol treatment (Hoffman et al. 1978; Lê et al. 1982). Conditioned tolerance to the hypothermic effect of ethanol, however, persisted for months after the termination of ethanol treatment (Crowell et al. 1981). Furthermore, learned tolerance also resulted in a cross-tolerance to the effects induced by other drugs that pharmacological tolerance failed to confer. When tolerance to various effects of ethanol was produced by treatment conditions that involved minimal learning opportunity, little or no cross-tolerance to pentobarbital or hydralazine was observed (Gougos et al. 1986; Lê et al. 1987b). However, in a conditioning paradigm of tolerance, or when opportunity to practice while under ethanol intoxication was provided, cross-tolerance to the hypothermic effects of pentobarbital and hydralazine (Lê et al. 1987b; El-Ghundi et al. 1989) or to the motor impairment effect of pentobarbital (Lê et al. 1986a) could be readily demonstrated. Similarly, work by Jorgensen et al. (1986) showed that cross-tolerance to morphine- or clonidine-induced analgesia following chronic ethanol treatment was demonstrable in animals that had been exposed repeatedly to thermal stimulation under ethanol intoxication, but was not demonstrable in those that had received the same ethanol treatment without the repeated thermal stimulation.

The data derived from studies with neurobiological manipulation provide evidence for the involvement of learning in tolerance. However, the experimental paradigms employed to produce tolerance in these studies confounded learning and pharmacological factors. In these studies, tolerance to ethanol was induced by high treatment doses and involved repeated testing of the animals under the intoxicating effects of ethanol (Frankel et al. 1975; Lê et al. 1980a; Lê et al. 1981). It is, therefore, difficult to determine which factor these manipulations affected. It is clear that further work is required to clarify the manner in which these neurobiological manipulations affect tolerance development. The effects of these manipulations on tolerance should be studied under experimental conditions in which either learning or pharmacological factors are operating exclusively. The results from these studies will be of help in clarifying the question of unitary or multiple mechanisms of tolerance.

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Conditioning as a Critical Determinant of Sensitization Induced by Psychomotor Stimulants

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INTRODUCTION

The fact that pharmacological agents can act as unconditioned stimuli has been known for some time. Collins and Tatum (1925) were the first to report that stimuli associated with drug administration in dogs could develop the ability to elicit conditioned reactions. These investigators found that situational cues associated with multiple injections of morphine eventually acquired the power to induce salivation, an effect produced by the drug itself. Pavlov (1927) and his colleagues observed similar effects in the dog following morphine as well as apomorphine injections:

> A dog was given a small dose of apomorphine subcutaneously and after one or two minutes a note of a definite pitch was sounded during a considerable time. While the note was still sounding the drug began to take effect upon the dog: the animal grew restless, began to moisten its lips with its tongue, secreted saliva and showed some disposition to vomit. After the experimenter had reinforced the tone with apomorphine several times it was found that the sound of the tone alone sufficed to produce all the active symptoms of the drug, only in a less degree. (Pavlov 1927, p. 35)

These observations were followed shortly by a more detailed analysis of the conditioned salivary reflex following morphine injections in the dog (Kleitman and Crisler 1927). These investigators extended the previous Endings by showing that conditioning would also occur to stimuli (situational) that were presented as much as 2 hours prior to morphine injections and that extinction would take place if the unconditioned stimulus (UCS) (morphine) was no longer presented. In addition, it was also found that the conditioned reflex was not as robust if sessions in which the conditioned stimulus (CS) was not followed by morphine were interspersed

with conditioning sessions. These phenomena were entirely analogous to those seen in classical alimentary reflex conditioning (Pavlov 1927).

In addition to the salivary reflex, it soon became apparent that other drug reactions were also amenable to conditioning, especially those associated with psychomotor stimulants. Tatum and Seevers (1929) reported that after a month of cocaine injections in dogs, the animals became very excitable whenever the attendant came near, especially if they saw the hypodermic syringe. Downs and Eddy (1932) also found that dogs injected with cocaine showed increased activity, excitement, and eagerness as the experimenter entered the room. Both of these early studies appear to suggest that situational stimuli associated with cocaine had acquired incentive motivational properties. Surprisingly, little attention was paid to these intriguing findings over the next three decades, even though they suggested the development of rather striking environmental control over addictive behaviors.

CONDITIONING OF PSYCHOMOTOR-STIMULANT-INDUCED MOTORIC EFFORTS

Contemporary studies with psychomotor stimulants have focused on conditioning the motoric effects of these compounds. A variety of studies with amphetamine and cocaine have assessed the ability of stimuli temporally paired with these drugs to elicit locomotor effects or to potentiate the motoric actions of the psychomotor stimulants. Irwin and Armstrong (1961) reported the development of conditioned locomotor excitation in response to an environment where rats had been injected with methamphetamine. Surprisingly, however, little extinction was observed following testing with saline. Furthermore, Pickens and Dougherty (1971) have offered alternative explanations to account for the increase in activity during tests with saline in this study. Ross and Schnitzer (1963) also claimed to have demonstrated conditioning of locomotor activity following exposure to amphetamine. Three groups of rats were employed in their study. Two of the groups were injected with amphetamine. Rats from one group were placed in a locomotor activity monitor, while those from the other group were returned to their home cage. The third group had a needle introduced intraperitoneally (IP) and were placed in the test apparatus. One week later, when all animals were placed in the apparatus, the group that had experienced the apparatus under amphetamine had higher activity scores than the needle-insertion controls. Unfortunately, however, no differences were found between the conditioned and unconditioned amphetamine groups. In this study, it appeared that exposure to the drug in either environment was sufficient to enhance activity 1 week later under no-drug conditions.

Rushton et al. (1963) also reported enhanced exploratory activity in a Y-maze following prior amphetamine plus barbiturate exposure. However, they interpreted their results in terms of alterations in habituation processes.

They asserted that the drug mixture may have interfered with the habituation of the animals to the apparatus. Thus, when these animals were tested subsequently with saline, their locomotor responses would be expected to be higher than those trained (habituated) under saline. Pickens and Crowder (1967) attempted to control for lack of habituation as a variable in conditioning paradigms. In their study, rats were first habituated to the activity chamber for 6 hours. One group was then injected with amphetamine and the other with saline. Both groups were placed in the activity monitor for 30 minutes immediately following injections. Four to five hours following placement in their home cages, the amphetamine rats were injected with saline, while the saline rats were injected with amphetamine. After six daily conditioning sessions, all rats were tested in the activity monitors following saline injections. Rats that had been trained under amphetamine had higher activity scores than those trained under saline despite extensive habituation.

While conditioning does appear to occur in situations where drugs are used as unconditioned stimuli, it is often uncertain which environmental cues serve as the conditioned stimuli. Pickens and Dougherty (1971) have very elegantly demonstrated the development of conditioned locomotor behavior to a more defined CS. In this study, the CS was a 2-minute tone that was followed by a 25-second infusion of 0.5 mg/kg methamphetamine that overlapped the CS. A progressive increase in locomotor activity in response to the tone was seen following repeated CS-UCS pairings. Pseudoconditioning controls did not show such conditioning. Schiff (1982), Bridger et al. (1982), and Barr et al. (1983) have also described conditioning of locomotor activity to auditory stimuli associated with injetions of amphetamine or apomorphine. In addition, Hayashi et al. (1980) have reported that a flickering light that had been associated with amphetamine injections enhanced the locomotor stimulatory actions of amphetamine.

Conditioned locomotor behaviors in studies in which psychomotor stimulants were used as the unconditioned stimuli follow many of the principles of classical conditioning. Tilson and Rech (1973), for example, reported that the magnitude of the conditioned locomotor effects were related directly to the dose of amphetamine used during conditioning. The CS-UCS interval also appears to be a critical variable that determines the strength of conditioning to the motoric effects elicited by amphetamine (Pickens and Crowder 1967). In addition, the presentation of the CS in the absence of the UCS has been found to result in the extinction or weakening of conditioning. Barr et al. (1983) as well as Hinson and Poulos (1981) and Hayashi et al. (1980) reported that situational cues associated with cocaine and amphetamine injections lost their effectiveness to elicit conditioned locomotor behavior when the drugs were no longer presented. Finally, the strength of the conditioned response (CR) appears to decay with time (Barr et al. 1983).

Gross locomotor activity is not the only motor behavior that is amenable to conditioning. Carey (1986a; Carey 1986b), for example, has recently demonstrated conditioned rotational behavior following the administration of amphetamine or apomorphine to rats with unilateral 6-hydroxydopamine lesions of the substantia nigra. Interestingly, rats were found to rotate ipsilaterally to the lesion in the environment in which they experienced amphetamine and contralaterally in the environment in which they had experienced apomorphine. Thus, the appropriate conditioned stimuli are able to mimic either directly or indirectly acting dopamine (DA) agonists. Conditioned rotational behavior has also been reported by Casas et al. (1989) following a single injection of apomorphine in lesioned rats. This type of conditioning appears to be relatively resistant to decay, since significant CRs have still been detected 24 (Carey 1986b) or 180 days (Casas et al. 1988) following conditioning. Drew and Glick (1987) as well as Elias et al. (1983) have also found conditioned rotational behavior in unlesioned rats following amphetamine administration. Robinson (1984), on the other hand, found little evidence for significant conditioning of rotational behavior following amphetamine, although a clear trend in that direction is evident in the data. A variety of cocaine-elicited motor behaviors, including stereotypy, have also been conditioned (Schiff 1982; Barr et al. 1983; Wagner et al. 1982).

Psychomotor stimulants such as amphetamine and cocaine are not the only drugs that can be used to produce conditioned increases in locomotor activity. Kamat et al. (1979) were the first to report that apparatus and situational cues paired with low doses of morphine were able to elicit increases in locomotor activity when the drug injections were discontinued. Conditioned increases in locomotor activity in rats produced by morphine have also been reported by Mucha et al. (1981) and Hinson and Siegel (1983). Conditioned locomotor activity has been observed also following direct injections of morphine into the ventral tegmental area of the rat mesencephalon (Stewart 1983). Interestingly, in this study, pretreatment with pimozide prevented the development of morphine-induced conditioning, suggesting that dopaminergic mechanisms are critically involved. This is not surprising considering the considerable amount of evidence suggesting that the locomotor stimulatory actions of opiate agonists involve mesolimbic DA (Broekkamp et al. 1979; Joyce and Iversen 1979; Kalivas et al. 1983; Kelley et al. 1980).

CONDITIONED DECREASES IN LOCOMOTOR BEHAVIORS

In contrast to psychomotor stimulants, neuroleptics and other behavioral depressants have been found in a few studies to be effective unconditioned stimuli for producing conditioned suppression of activity or motor performance. Irwin and Armstrong (1961) reported that situational cues associated with acute and chronic injections of perphenazine or chlorpromazine developed the ability to suppress behavior. However, appropriate

control groups were not employed, and the conditioned response was unpredictable in a series of replications. Conditioned cataleptic effects have been reported for bulbocapnine (Perez-Cruet and Gantt 1959), while apparatus cues associated with haloperidol injections acquired the ability (at least at low doses) to suppress motor behaviors (Carey and Kemey 1987). The authors of the latter study, however, interpreted this effect in terms of operant conditioning processes. Finally, stimuli associated with scopolamine injections, which normally suppress behavior, have been found to disrupt operant lever pressing in rats (Hermstein 1962). Although the findings from the studies above are suggestive, it is not readily apparent whether the results are due to conditioned decreases in locomotor activity or conditioned suppression of ongoing behavior by stimuli that have been associated with aversive events, i.e., pharmacological agents that produce aversive states.

NEUROPHARMACOLOGICAL SUBSTRATES OF CONDITIONED LOCOMOTOR BEHAVIORS

Psychomotor stimulants such as cocaine and amphetamine are thought to enhance locomotor output by increasing the availability of extracellular mesolimbic dopamine (Kelly and Iversen 1975) either through uptake blockade and/or enhanced release (Hadfield and Nuggent 1983; Moore 1977; Reith et al. 1986). Opiate agonists are also thought to increase locomotor output by enhancing the activity of the mesolimbic DA system (Broekkamp et al. 1979; Di Chiara and Imperato 1988; Joyce and Iversen 1979; Kalivas et al. 1983; Kelley et al. 1980). These are the mechanisms underlying the unconditioned responses of these drugs. Relatively little is known, however, regarding the neurochemical mechanisms that determine the conditioned response. Do conditioned stimuli activate the same neurochemical systems that are activated by the unconditioned stimuli (drugs), or are other systems recruited as well? Only a few studies have attempted to assess the neurochemical concomitants of conditioned locomotor behavior. Perez-Cruet (1976), for example, found that DA metabolism could be increased by an auditory stimulus previously associated with methadone, morphine, and bulbocapnine, which by themselves had similar effects on this parameter of DA function. Schiff (1982) has also found increases in mesolimbic and striatal DA following presentation of stimuli associated with apomorphine and amphetamine injections. The changes found in this study could reflect either increases in DA turnover or increases in release without concomitant alterations in synthesis induced by the conditioned stimuli. Alterations in DA metabolism, however, were not found following presentation of stimuli that had been paired with cocaine injections (Barr et al. 1983). It is still uncertain whether the CR in locomotor activity studies is determined solely through DA pathways or whether there is an involvement of additional mechanisms that are recruited during the conditioning process. In light of the following recent studies, as well as others, the latter seems to be the more likely alternative.

PSYCHOMOTOR-STIMULANT-INDUCED SENSITIZATION

Conditioning also appears to play a rather important role in the expression of certain behavioral effects associated with the repetitive administration of psychomotor stimulants. It has been known for some time that certain behavioral effects of drugs such as amphetamine and cocaine are augmented with repetitive injections (behavioral sensitization). Tatum and Seevers (1929) as well as Downs and Eddy (1932) found that, in dogs, there was a progressive increase in the severity of reactions to cocaine with repeated administration. Subsequent to these early reports, numerous other studies also have reported sensitization to the behavioral effects of repetitive cocaine and amphetamine injections. Repetitive administration of cocaine has been found to produce progressive increases in the locomotor output of rats (Post and Rose 1976; Post 1981; Kalivas et al. 1988) as well as mice (Shuster et al. 1977; Riffee et al. 1988). Similar effects in rats have been found following chronic administration of either amphetamine (Browne and Segal 1977; Segal and Mandell 1974; Rebec and Segal 1979; Gold et al. 1988) or monoamine oxidase inhibitors (Irwin 1961). Stereotypic effects have also been reported to increase in intensity with repetitive administration of either cocaine (Stripling and Ellinwood 1977; Post et al. 1981) or amphetamine (Nelson and Ellison 1978; Browne and Segal 1977; Segal and Mandell 1974; Magos 1969). Rotational behavior in either lesioned (Robinson 1984; Robinson et al. 1982) or unlesioned rats (Glick et al. 1986; Drew and Glick 1989) also increases in intensity with repeated injections of amphetamine. With high doses of cocaine, sensitization is also seen for the convulsant effects of this agent (Stripling and Ellinwood 1977; Post et al. 1988; Tatum and Seevers 1929). Such behavioral alterations are relatively long lasting and have been shown to be related to dose (Shuster et al. 1977), gender (Post and Contel 1983), and, in part, to the intermittency of administration (Post 1980; Post 1981; Post and Ballenger 1981; Post and Kopanda 1976).

CONDITIONING AS A DETERMINANT OF SENSITIZATION

At least two factors appear to contribute to the development of behavioral sensitization. One set of factors is related to possible neuropharmacological alterations induced by the repetitive administration of pharmacological agents. These would include alterations in receptor sensitivity, enhanced release, enhanced reuptake blockade, or decreases in autoreceptor sensitivity (Robinson and Becker 1986). The other set of factors contributing to the development of sensitization are those related to the conditioned drug effects elicited by situational and environmental cues. The relative contribution of each set of factors is determined by the experimental conditions and setting.

One of the best illustrations of the relative importance of conditioning as a determinant of sensitization is found in a study by Post and his colleagues (Post et al. 1981). In this report, two groups of rats were injected with

cocaine daily for 10 days. One group received cocaine in a locomotor test apparatus and saline in the home cage, while the other group received saline in the test apparatus and cocaine in the home cage. Both groups, therefore, received equal exposure to the drug as well as the experimental chamber and differed only in terms of where they were injected with cocaine. In subsequent sessions, the animals that had experienced cocaine in the test chamber had adivity scores that were considerably higher than those that had experienced cocaine in their home cages, when tested under either saline or cocaine. A number of other studies have also revealed the importance of conditioning in the development and expression of sensitization induced by psychomotor stimulants. Tilson and Rech (1973), for example, reported that rats that had experienced amphetamine in the test chamber had activity levels that were considerably higher than those of animals that had experienced amphetamine in the home cage when both were tested subsequently with amphetamine in the test chamber. In fact, the latter group was not different from rats treated with saline during the initial phase of the study. Hinson and Poulos (1981) also found that sensitization to the behavioral effects of cocaine was more pronounced following drug administration in the presence of cues previously associated with the drug. Hayashi et al. (1980) have reported that a flickering light that had been presented concurrently with amphetamine injections enhanced sensitization during the test day. Context-dependent sensitization to amphetamine has also been reported by Drew and Glick (1989) and Elias et al. (1983). This is not surprising, considering the fact that rotational behaviors induced by psychomotor stimulants can be conditioned readily.

Although there is considerable evidence to indicate that conditioning plays an important role in the sensitization process, a number of investigators have failed to find support for the operation of this factor. Segal and Mandell (1974), for example, found that rats that were continuously exposed to the experimental chambers showed a progressive augmentation of locomotor output as well as stereotypy following chronic administration of amphetamine. A saline challenge (presumably the CS) did not induce locomotor behavior that was quantitatively different from that seen prior to the initiation of drug injections. They concluded that conditioning is not a critical factor in locomotor sensitization and also found it difficult to understand how conditioning could contribute to the replacement of ambulatory responses by stereotypy with repeated administration of amphetamine. Browne and Segal (1977) also found that animals treated with high doses of amphetamine in three different environments still exhibited enhanced stereotypy when tested in a standard experimental chamber. Past and Rose (1976) also failed to find significant conditioning in chronically cocaine-treated rats when saline injections and apparatus exposure were used as the CS. Finally, Robinson (1984) has else failed to find that conditioning plays a critical role in the sensitization of amphetamine-induced increases in rotational behavior. Rats that had been injected with high doses of amphetamine in the test apparatus were not different from animals

that had received the amphetamine in the home cage when tested subsequently with the drug in the test chamber.

Several factors may account for the failure of some studies to have found an important contribution of conditioning in sensitization. First, conditions should be optimal for the operation of the wnditioning process. Segal and Mandell (1974), for example, injected their rats in the cages in which the animals lived. The strongest conditioning, however, would be expected to develop to environmental cues that are discriminable. Conditioning would not be expected to occur in a paradigm in which the animals were injected in their home environment. This possibility has also been acknowledged by Browne and Segal (1977) in a subsequent publication. Second, while conditioning undoubtedly does play a critical role in the sensitization process under a variety of conditions, it is also apparent that high doses of psychomotor stimulants given over a longer period of time will contribute to sensitization through mechanisms independent of conditioning, perhaps involving alterations in neurochemical or neurophysiological processes (Robinson and Becker 1986). The emergence of steteotypy following repeated administration of high doses of amphetamine, for example, may be determined solely by such mechanisms (Segal and Mandell 1974). Third, the use of a habituation period prior to conditioning (Post and Rose 1976) may also work against the acquisition of a conditioned locomotor response. The presentation of a CS prior to conditioning has been shown to significantly retard the acquisition of a CR in a variety of paradigms (Anderson et al. 1969; Carlton and Vogel 1967; Kremer 1971; Lubow and Moore 1959; Siegel 1969). Finally, it has commonly been assumed that the injection of saline in the context of the test chamber, needle insertion, or exposure to the apparatus are adequate conditioned stimuli to test for the presence of conditioning (Browne and Segal 1977; Carey 1986a; Carey 1986b; Casas et al. 1988; Drew and Glick 1987; Hayashi et al. 1980; Post and Rose 1976; Robinson 1984; Ross and Schnitzer 1963; Schiff 1982). Generally speaking, however, the conditioned responses (locomotor activity) under such conditions are rather modest in most cases or not seen at all. This may be due to the fact that a saline injection is not always the appropriate CS.

What is conditioned precisely when a drug is used as an UCS (figure l)? The drug itself, of course, produces a variety of physiological and behavioral actions, all of which can serve as the UCR. Locomotor activity is one of a number of reactions (Stewart and Eikelboom 1987) that are conditioned to situational stimuli. It is the only one, however, that is measured in the majority of studies. It may be important to consider how these conditioned reactions and behaviors interface and interact during the process of conditioning. There are also problems in precisely defining the stimulus complex that comes to serve as the CS. In the majority of studies, the apparatus cues are generally considered to be the primary stimuli in

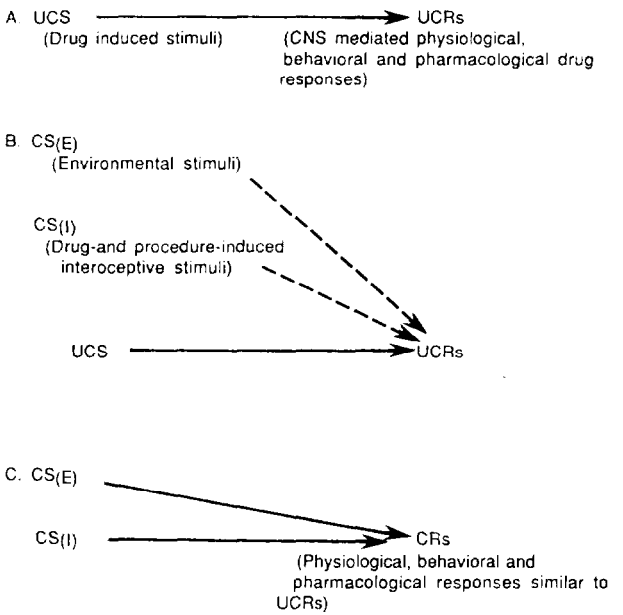

FIGURE 1. *Classical conditioning paradigm in which drugs acting at specific receptor sites in the brain produce UCS that result in the production of various unconditioned physiological, behavioral, and pharmacological states (UCR)*

NOTE: External environmental ($CS_{(E)}$) as well as interoceptive ($CS_{(I)}$) stimuli produced by the drug through peripheral sites take on the ability to elicit responses [CR) that are similar to those produced by the drug (UCS) itself when the CS (E + I) and UCS occur in temporal contiguity.

the total complex. Stimuli produced by the injection procedure, however, as well as interoceptive cues accompanying the needle insertion, must also play a role in the entire complex. More important, however, drugs such as cocaine and amphetamine produce interoceptive cues through actions on peripheral targets (i.e., cardiovascular system) that also enter into determining the entire complex of cues to which conditioning occurs. All of the above stimulus components should be capable of eliciting the conditioned response to some extent depending on their relative saliency. It is also apparent that the most robust conditioned reactions should be seen when the entire stimulus complex is reinstated during the test for conditioning. Injections with saline only reinstate a part of the total complex and would not necessarily reveal the presence of conditioning in every situation. The inability of a number of investigators to find conditioning following a saline challenge may be due simply to the fact that the CS is not entirely appropriate.

The authors recently introduced a rather simple and powerful one-session conditioning paradigm with cocaine that illustrates the importance of conditioning in sensitization more clearly by reinstating the interoceptive drug-produced stimulus component of the CS during the test phase (Weiss et al., in press). Three groups of rats were employed in this design. One group was injected with a high dose of cocaine (40 mg/kg, IP) prior to placement in the activity test chamber and with saline upon return to the home cage (conditioning group). The second group was injected with saline prior to placement in the test chamber and cocaine upon return to the home cage (pseudoconditioning group). The third group received saline in both environments (conditioning control group). It is important to note that the first two groups received equal exposure to cocaine as well as to the two different environments and differed only in terms of where they experienced cocaine. The next day animals in all three groups were injected with a low dose of cocaine (10 mg/kg, IP) and were placed in the test chamber. Rats that were treated with cocaine in the test chamber on the first day showed a marked increase in locomotor activity, compared to controls, following an injection of 10 mg/kg of cocaine on the second day (figure 2). Animals that received injections of cocaine in the home cage on day 1, however, were no different than the saline controls during the second day. The difference between the two groups that had been exposed to cocaine can be attributed entirely to conditioning.

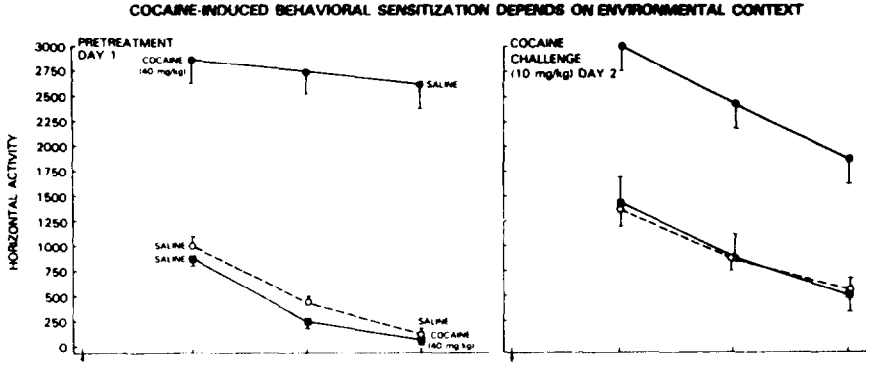

FIGURE 2. *The role of conditioning in cocaine-inched sensitization*

NOTE: Left panel illustrates the mean horizontal activity of rats (n=10/group) that received the following treatment on day 1: cocaine (40 mg/kg, IP) in the activity chamber and saline in the home cage (filled circles). Saline in the test chamber and cocaine in the home cage [filled squares). and saline in both environments (open circles). Right panel illustrates horizontal activity for these three groups following a cocaine challenge (10 mg/kg, IP) on day 2. Only the rats that received cocaine in the test chamber on day 1 showed sensitization to cocaine on day 2.

As noted above, the strength of the CR is dependent on the similarity of the stimulus complex during conditioning to that presented during the test for conditioning. Figure 3 illustrates the locomotor response of four groups of rats to an injection of 10 mg/kg of cocaine after experiencing 40 mg/kg of cocaine in different environments during the previous day. Rats that were tested on day 2 in an environment identical to that present during training had activity levels that were significantly higher than groups trained and tested in different environments. Besides illustrating the presence of discrimination in this paradigm, the findings also rule out the possibility that failure to habituate to apparatus cues on day 1 under the influence of cocaine could be a possible explanation for the differences found between the two cocaine exposure groups in the previous study. The group that was exposed to the apparatus under cocaine on day 1 was in fact more active than any of the other groups on day 2.

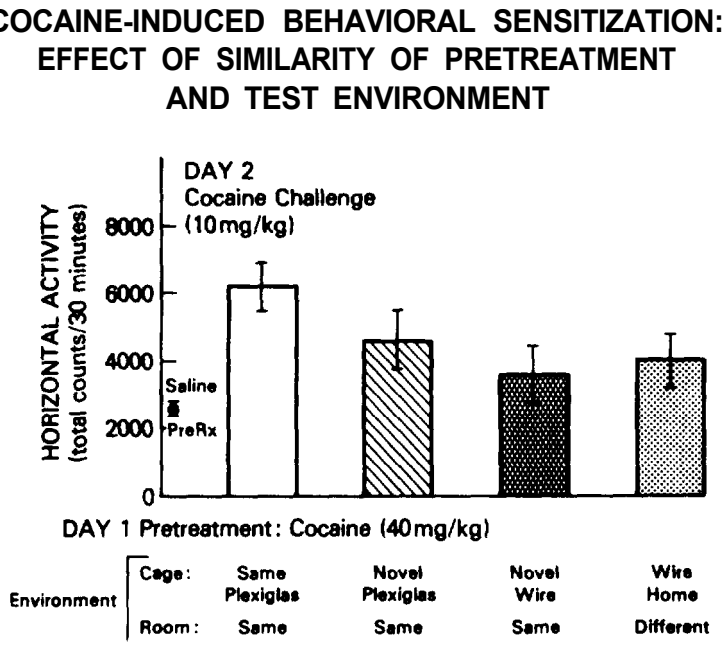

FIGURE 3. *The effect of similarity of pretreatment and test environment on cocaine-induced behavioral sensitization*

NOTE: Group means and standard error values for horizontal activity over a 30-minute period are illustrated for four different pretreatments. All groups were injected with 40 mg/kg of cocaine on day 1, but in different environments as described on the abscissa. For comparison, the mean value and standard error for a group of animals that received Saline on day 1 is also illustrated by the filled circle on the left of the figure.

While conditioning plays an important role in behavioral sensitization, it is unlikely to be the only factor. Under certain circumstances, noncontextual variables also appear to determine the increase in responsiveness to repeated injections of cocaine. For example, when rats are pretreated for 3 days with 40 mg/kg of cocaine instead of just 1 day and challenged on day 4 with 10 mg/kg of cocaine, then, even animals that experienced the high dose of cocaine in their home cage have activity levels as well as stereotypy scores significantly higher than the saline controls (figure 4). Noncontextual factors may become determinants of sensitization when relatively high doses of psychomotor stimulants are administered over a longer period of time.

NEUROPHARMACOLOGICAL SUBSTRATES OF' CONDITIONED SENSITIZATION

Since mesolimbic DA appears to be involved in mediating the locomotor stimulant actions of cocaine (Kelly and Iversen 1975), it follows that this system may also be involved in mediating cocaine-induced sensitization as well as its conditioned components. If this assumption is correct, then it should be possible to attenuate the development of cocaine-induced sensitization and the expression of its conditioned components with DA blockers such as haloperidol. In a recent study (Weiss et al., in press), the one-session conditioning paradigm was utilized to test these assumptions. It was found that haloperidol administered concurrently with cocaine on day 1 blocked the cocaine-induced hyperactivity and also prevented the development of cocaine-induced sensitization on day 2 (figure 5). Rats treated with cocaine (40 mg/kg) plus saline on day 1 had a significantly greater response to a 10 mg/kg cocaine challenge on day 2 than rats that had been pretreated with cocaine plus halopetidol (0.5 mg/kg). Diazepam administered concurrently with cocaine on day 1 also blocked cocaine-induced hyperactivity and prevented the development of cocaine-induced conditioned sensitization on day 2. Surprisingly, however, haloperidol administered concurrently with 10 mg/kg of cocaine on day 2 did not block the expression of conditioned cocaine-induced sensitization (right side of figure 5). Thus, blockade of DA receptors with haloperidol prevents the development of conditioned sensitization to cocaine, whereas it was incapable of preventing the expression of the CR once established. Blockade of DA receptors with pimozide has also been found to block the establishment of conditioned locomotor excitation but not the expression of conditioned activity elicited by saline injections following conditioning with either amphetamine (Beninger and Hahn 1983) or cocaine (Beninger and Herz 1986).

Not all studies, however, support the position that the conditioned locomotor reactions elicited by stimuli paired with psychomotor stimulants ate resistant to DA blockade. Schiff (1982), for example, found that small doses of haloperidol were able to block conditioned motor responses such as head-bobbing, sniffing, and hyperactivity elicited by stimuli that had been paired

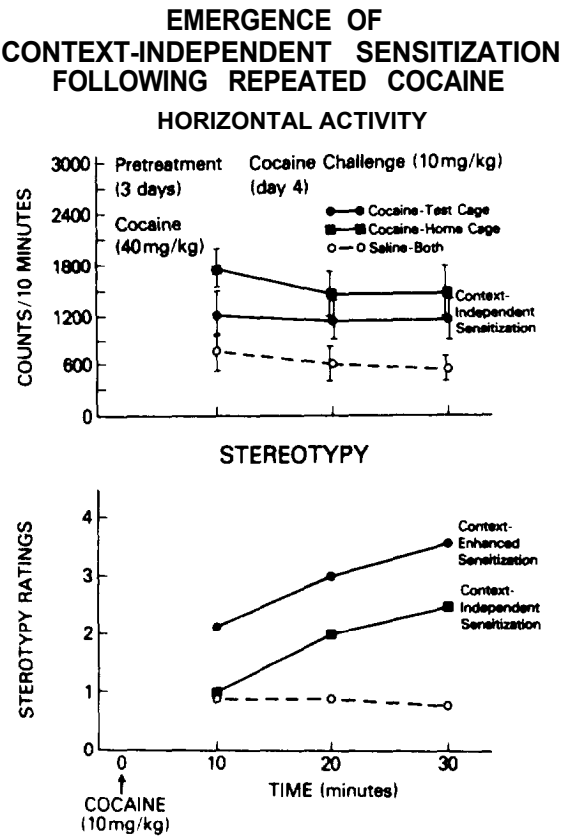

FIGURE 4. *Development of context-independent sensitization*

NOTE: Three groups of rats received the following treatments on days 1 to 3: Cocaine (40 mg/kg, IP) in the activity chamber and saline in home cage (filled circles), saline in test chamber and cocaine in home cage (filled squares). and saline in both environments (open circles). On day 4, all animals were injected with 10 mg/kg cocaine in the test apparatus Following 3 days of treatment with high doses of cocaine, even animals that had been injected with cocaine in their home cages showed sensitization to both stereotypy (bottom) as well as horizontal locomotor activity (top). The difference in locomotor activity between the two cocaine-pretreated groups can be attributed to the development of more robust stereotypy in the group that had received injections of cocaine in the test apparatus. Stereotypy interfered with the expression of horizontal locomotor activity.

with amphetamine injections. However, the authors concluded that only the blockade of head-bobbing and sniffing were pharmacologically specific, since haloperidol also produced a significant attenuation of motor activity in the pseudoconditioned controls. Drew and Glick (1987) have also reported that 0.1 mg/kg of haloperidol was effective in blocking conditioned rotational behavior in unlesioned rats. Finally, Gold et al. (1988) have found

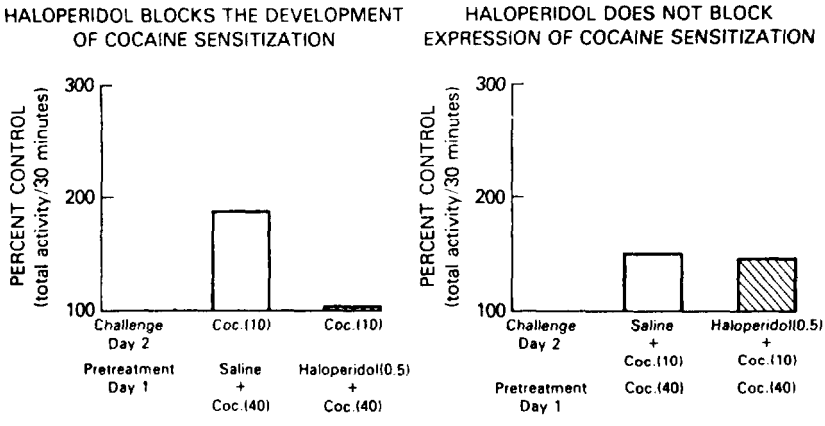

FIGURE 5. *The effect of haloperidol on the development and expression of conditioned cocaine-induced sensitization*

NOTE: The figure illustrates horizontal activity on day 2 in response to a 10 mg/kg cocaine challenge of activity in the control group. *Left side:* The control group for day 1 cocaine (40) group (open column) was pretreated with Saline on day 1. The control group for the haloperidol plus cocaine group (shaded column) was the group pretreated with haloperidol plus saline on day 1. Haloperidol pretreatment on day I blocked sensitization to cocaine on day 2 when all animals were challenged with 10 mg/kg of cocaine. *Right side:* Haloperidol (0.5 mg/kg) was administered to animals only prior to cocaine rechallenge on day 2 (shaded column). The control group for rats that received haloperidol prior to cocaine on day 2 following treatment with cocaine (40 mg/kg) on day 1 (shaded column) received similar treatment on day 2 but were pretreated with saline on day 1. As illustrated, these haloperidol-pretreated (day 2) rats showed as robust sensitization, based on prior cocaine exposure, as did animals tested without haloperidol (open column). Thus, haloperidol did not block the expression of cocaine sensitization (right panel) but did block its development (left panel).

that 6-OHDA lesions of the nucleus accumbens, which decreased DA content by 90 percent, prevented the expression of a conditioned locomotor response that had been conditioned with amphetamine as the UCS. The authors concluded that an intact DA system within the region of the nucleus accumbens is necessary for the expression of conditioned locomotor behavior.

MECHANISMS UNDERLYING NEUROLEPTIC BLOCKADE OF CONDITIONING

The mechanisms whereby DA blockers inhibit the development of psychomotor-stimulant-induced conditioning are not fully understood. Several possibilities exist. Neuroleptics, for example, could disrupt the acquisition of a conditioned response by interfering with associative processes, by altering the nature of the conditioned stimuli, or by disrupting the unconditioned response (locomotor activity). The simplest explanation for the ability of haloperidol or pimozide to prevent the development of conditioned

locomotor activity is that they block the expression of the unconditioned response. Hirabayashi and Alam (1981) have in fact found that mice whose activity is physically restricted do not develop sensitization. These findings, however, could be confounded by the development of aversive conditioning to the apparatus cues induced by restricted movement. The aversive motivational state elicited by the situational cues could interfere with the expression of locomotor behavior. In addition, Swerdlow and Koob (1984) have failed to replicate these findings. They reported that restrained rats demonstrated significant conditioned locomotor activation in an amphetamine-associated environment. A simple inhibition of the unconditioned reaction to psychomotor stimulants may not be sufficient to account for the acquisition deficits seen. Blockade of the unconditioned response in other conditioning paradigms has not been sufficient to prevent the appearance of a conditioned reaction during subsequent testing. For example, pretreatment of dogs with atropine, which blocks salivation, prior to presentation of morphine or acid as the UCS does not prevent the development of conditioned salivation to stimuli associated with these two manipulations (Crisler 1930; Finch 1938). In addition, flexion reflexes are still elicited by conditioned stimuli in animals that had ventral roots innervating the limb crushed during training (Light and Gantt 1936; Beck and Doty 1957). Finally, curare, a drug that blocks skeletal reactions, does not prevent the conditioned expression of such reactions when the drug has worn off (Solomon and Turner 1962). If conditioning of locomotor behaviors follows the principles of classical conditioning, it is unlikely that merely preventing the occurrence of the locomotor behavior during conditioning is sufficient to disrupt the formation of the association. It is possible, however, that centrally mediated effects of drugs such as neuroleptics and diazepam could inhibit or block the initiation of motor programs that could result in a deficit in conditioning.

Neuroleptics also have been found to retard the acquisition of other conditioned reactions. Harvey and Gormezano (1981) and Schindler et al. (1985) have found that haloperidol as well as pimozide inhibit the rate of acquisition of the nictitating membrane response in rabbits. Such disruptive effects were not determined by interference with associative processes but by the ability of the drugs to attenuate the conditioned and unconditioned excitatory properties of the stimuli used as the CS. Neuroleptics such as chlorpromazine have also been reported to block the unconditioned as well as conditioned excitatory effects of auditory stimuli as measured by changes in the threshold for behavioral and electroencephalogram arousal (Key and Bradley 1960; Killam and Killam 1958). A decrease in the excitatory properties of stimuli would decrease their ability to enter into conditioning. Strength of conditioning has been shown to be directly related to the strength of the CS (Barnes 1956; Kamin and Schaub 1963; Kamin and Brimer 1963). The main effect of CS intensity may be to increase its discriminability from the background.

Even more likely is the possibility that haloperidol and pimozide by blocking DA receptors alter the strength of the UCS. It has been well established that strength of conditioning is directly related to the intensity of the UCS (Wagner et al. 1961; Ost and Lauer 1965; Annau and Kamin 1961; Kamin and Brimer 1963). Increases in UCS probably strengthen the conditioning process by increasing overall levels of arousal or drive (Spence 1956).

NATURE OF THE CONDITIONED RESPONSE

What is the nature of the conditioned response in situations in which psychomotor stimulants are used as unconditioned stimuli? In the simplest formulation, the conditioned response mimics, at least in part, the unconditioned response. Since psychomotor stimulants induce increases in locomotor activity and stereotypy as part of their unconditioned effects, the conditioned responses could simply mimic these reactions. It is possible, however, that the motor behaviors elicited by conditioned stimuli associated with psychomotor stimulants reflect more than just simple conditioned motor reactions. One possibility is that such conditioned behavior actually represents the operation of underlying incentive motivation mechanisms. It has been known for some time that stimuli associated with primary reinforcers, such as food, for example, can increase locomotor output in rats (Sheffield and Campbell 1954; Baumeister et al. 1964; Bolles 1963; Finger et al. 1960). It has been suggested that such stimuli acquire the ability to arouse incentive-motivational mechanisms that play a role in energizing instrumental responding (Bindra 1968; Rescorla and Solomon 1967). Loviband (1983), for example, has demonstrated that stimuli associated with sucrose reinforcement in rabbits enhanced subsequent instrumental responding. Drugs such as cocaine and amphetamine can also serve as primary reinforcers by acting directly on the reward systems in the brain. It is apparent that stimuli associated with administration of psychomotor stimulants also acquire motivational properties. For example, animals injected with amphetamine (Reicher and Holman 1977; Sherman et al. 1980), cocaine (Mucha et al. 1982). or opiate agonists (Kumar 1972; Katz and Gormezano 1979; Mucha et al. 1982; Rossi and Reid 1976) in a specific environment will spend more time in that environment when given a chance in subsequent test sessions without the drug (conditioned place preference). Furthermore, specific stimuli that are paired with injections of opiates and psychomotor stimulants in self-administration paradigms are capable of maintaining instrumental responding when they are made contingent on such behavior (Davis and Smith 1976; O'Brien 1976; Schuster and Woods 1968). It is possible that stimuli associated with enhanced DA activity acquire the ability to generate positive affective motivational states that are reflected by increases in general locomotor activity.

Neuroleptics not only block the conditioning of locomotor reactions to stimuli associated with psychomotor stimulants (above), but also inhibit the

ability of stimuli associated with primary rewards such as food and drugs to acquire incentive-motivational properties (incentive learning). For example, administration of pimozide during the pairing of an auditory stimulus with food prevented the stimulus from acquiring the ability to maintain instrumental responding following removal of the drug (Beninger and Phillips 1980a). Pimozide has also been shown to prevent a conditioned stimulus from acquiring incentive-motivational properties in an operant discrimination paradigm (Beninger and Phillips 1980b). Incentive learning has also been shown to be inhibited by haloperidol when amphetamine has been used as an UCS (Davis and Smith 1975; Davis and Smith 1977). Thus, the ability of haloperidol to prevent conditioned locomotor behavior in the authors' studies above as well as in the studies reported by Beninger and his colleagues (Beninger and Hahn 1983; Beninger and Herz 1986) could reflect its ability to interfere with incentive learning. Haloperidol may have blocked the acquisition of incentive-motivational qualities by stimuli associated with either cocaine or amphetamine.

NEUROCHEMICAL SUBSTRATES OF THE CONDITIONED RESPONSE

The inability of haloperidol to block the expression of conditioned locomotor activity in the authors' studies as well as in those reported by Beninger and Hahn (1983; Beninger and Herz 1986) would suggest that the neural events underlying the conditioned response are somewhat different than those produced by the drug itself. Since cocaine produces its effects on locomotor activity by enhancing the availability of DA in the nucleus accumbens (Kelly and Iversen 1975). it appears that conditioned stimuli activate the motor system through mechanisms that are independent of mesolimbic DA. Alternatively, it is possible that the acquisition process is more sensitive to DA blockade than is the expression of established behavior. In fact, it has been shown that well-established instrumental behaviors are relatively resistant to disruption by neuroleptics (Beninger 1983).

As noted above, there are very few studies that have evaluated alterations of DA function by stimuli associated with psychomotor stimulants. Unfortunately, the results from those that have are somewhat equivocal (Schiff 1982; Barr et al. 1983). Studies with opiates, on the other hand, do seem to suggest that stimuli associated with morphine acquire pharmacological properties that are highly similar to those produced by the drug itself. Roffman et al. (1973), for example, reported that an auditory stimulus paired with morphine injections acquired the ability to prevent withdrawal symptoms in the rat. Blockade of morphine withdrawal by stimuli that had been paired with morphine has also been reported by Tye and Iversen (1975). In addition, hyperthermia induced by stimuli associated with morphine has been found to be antagonized by naloxone (Lal et al. 1976). All of these observations indicate that conditioned stimuli are capable of

eliciting neuropharmacological states similar to those produced by the drug itself, Paradoxically, it would seem that these stimuli had acquired the ability to release endogenous opiates in the CNS. On the other hand, it is possible that conditioned stimuli, under certain circumstances, may acquire the ability to produce effects that are similar to the UCS (drug) by acting on pathways beyond the initial site of action of the drug.

NEUROANATOMICAL SUBSTRATES UNDERLYING CONDITIONED SENSITIZATION

While dopamine appears to be critically involved in the acquisition of conditioned locomotor behaviors in which the UCS is a psychomotor stimulant, little is known regarding the neuroanatomical substrates. One likely candidate is nucleus accumbens, which has been shown to mediate the excitatory (Kelly and Iversen 1976) as well as reinforcing effects (Roberts et al. 1977) of cocaine as well as other psychomotor stimulants. Additional structures that may play a role in such conditioning include the amygdala, hippocampus, and cerebellum. The amygdala had been implicated in mediating the acquisition of a variety of conditioned behaviors. Amygdala lesions, for example, have been shown to produce deficits in the learning of active avoidance (Brady et al. 1954; Coover et al. 1973; Goldstein 1974; Ursin 1965) as well as passive avoidance (Coover et al. 1973; Grossman et al. 1975; Slotnick 1973; Ursin 1965) behaviors. Deficits in these tasks probably reflect disruption of conditioned fear mediated by the amygdala. Lesions of the amygdala have also been found to attenuate the acquisition of conditioned emotional responding (Kellicutt and Schwartzbaum 1967; Spevack et al. 1975) as well as heart rate conditioning in rabbits (Kapp et al. 1979). More recently, amygdalectomy has been reported to block the potentiation of a startle response by stimuli that had been associated with shock (Hitchcock and Davis 1986; Hitchcock and Davis 1987).

There is considerable evidence to indicate that the cerebellum may also be involved in conditioned motor reactions. Lesions of the dentate and interpositus nuclei of the cerebellum or superior cerebellar peduncle have been found to disrupt the acquisition and expression of the conditioned nictitating membrane response in rabbits (Lincoln et al. 1982; McCormick et al. 1981; McCormick et al. 1982) and lesions of the deep cerebellar nuclei have been shown to abolish conditioned leg-flexion responses in the rabbit (Donegan et al. 1983). Lesions of the hippocampus have also been found to disrupt the acquisition of a variety of learned behaviors (Isaacson 1974).

In order to evaluate the participation of these neural structures in the conditioned aspects of sensitization, the authors lesioned each region and observed the effect of this manipulation on the acquisition of conditioned increases in locomotor output elicited by cocaine. In the first study, three groups of rats were used. One group was lesioned bilaterally in the amyg-

dala with radiofrequency procedures, while the other group was injected bilaterally in the nucleus accumbens with 6-hydroxydopamine. The third group received sham lesions. The radiofrequency lesions destroyed a substantial portion of the amygdala, including most of the basolateral and corticomedial nuclei. Thc 6-OHDA lesion of the nucleus accumbens decreased DA content in this structure by approximately 65 percent. The 1-day conditioning paradigm described above was used to assess the effect of the lesions on context-dependent, cocaine-induced sensitization. On day 1, half of the animals in each group were injected with 40 mg/kg of cocaine, while the other half received saline. On day 2, both subgroups received 10 mg/kg of cocaine. Neither lesion on day 1 appeared to modify the response to the high dose of cocaine (figure 6). This was somewhat surprising, since 6-OHDA lesions have previously been found to attenuate the motoric effects of cocaine (Kelly and Iversen 1975). The DA depletion in our study, however, was considerably smaller than that reported by these investigators. Apparently a considerable degree of dopaminergic destruction is needed to attenuate cocaine-induced increase in locomotor output. Although neither lesion had an effect on the initial response to cocaine, both effectively prevented the appearance of context-dependent sensitization. There was a clear difference in locomotor activity between the saline- and cocaine-pretreated rats only for animals that had received sham lesions.

Some of these findings were confirmed and extended in a subsequent study. Rats were lesioned in the amygdala with either radiofrequency procedures or 6-OIIDA injections that reduced the DA content by approximately 60 percent. These lesions also had relatively little effect on the initial response to 40 mg/kg of cocaine on day 1. When tested on day 2 with 10 mg/kg of cocaine, both types of lesions were found to have prevented the development of sensitization (figure 7). Thus, it appears that DA in the amygdala is a critical neurotransmitter involved in the process by which environmental, interoceptive and situational stimuli acquire the ability to elicit conditioned responses similar to those produced by cocaine. Neither dorsal and ventral hippocampal lesions (figure 8) nor lesions of the deep cerebellar nuclei (data not shown) had a significant effect on cocaine-induced sensitization in this paradigm, although the dorsal hippocampal lesion did appear to attenuate it to some degree.

The two primary structures involved in mediating context-dependent sensitization appear to be the amygdala and nucleus accumbens. It is likely that these two structures mediate different aspects underlying the development of this phenomenon (figure 9). It is probably unlikely that lesions of the nucleus accumbens attenuate the conditioning process by interfering with associative mechanisms. Wilson (1983) has, in fact, shown that lesions of the nucleus accumbens actually enhance conditioned leg flexion and vocalization in the cat elicited by electrical shock delivered to the leg. Lesions of the nucleus accumbens have also been shown to enhance the acquisition

of avoidance responses (Lorens et al. 1970) that are dependent on aversive conditioning.

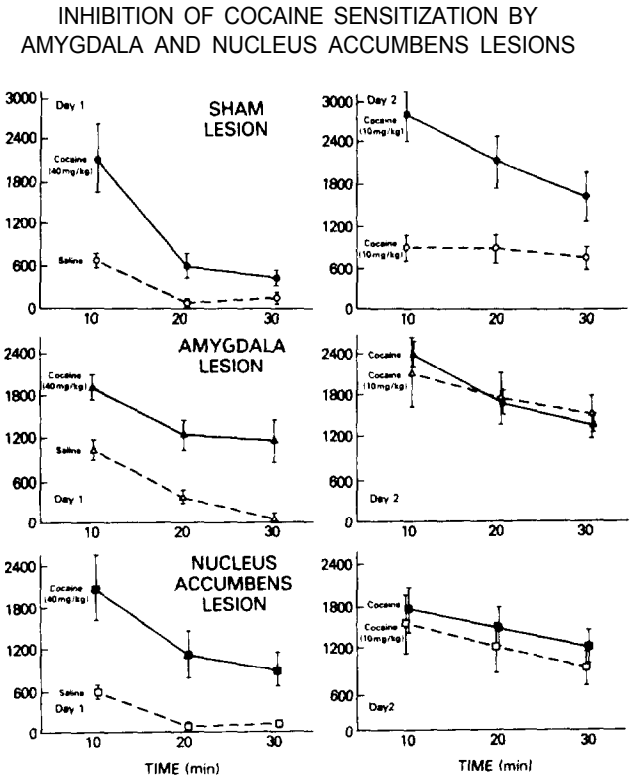

FIGURE 6. *The effect of amygdala (radiofrequency) and nucleus accumbens (6-OHDA) lesions on the development of conditioned cocaine-induced sensitization*

NOTE: The left side of the figure illustrates the effects of saline or cocaine (40 mg/kg, IP) injections on horizontal activity in the three different lesion groups. Neither lesion appeared to have a marked effect on cocaine-induced increases in locomotor output. When all animals were tested on day 2 with 10 mg/kg of cocaine, it was apparent that the lesions were effective in preventing the development of cocaine-induced sensitization.

Mogenson (1987), as well as Stevens (1973), has proposed that the nucleus accumbens may serve to gate motivational input from limbic structures such as the amygdala and hippocampus to the motor system. Presumably the function of such a gating mechanism is to regulate how biologically significant stimuli gain access to the motor system. There is evidence that

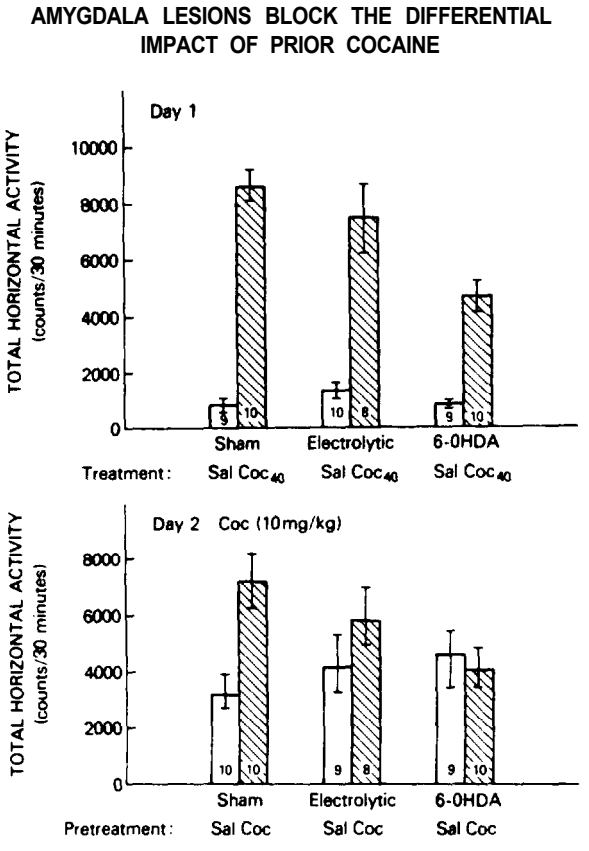

FIGURE 7. *The effect of radiofrequency and 6-OHDA lesions of the amygdala on the development of cocaine-induced sensitization*

NOTE: The top part of the figure illustrates total horizontal activity in 30 minutes following injection of saline or cocaine (40 mg/kg) to the various lesion groups. Neither radiofrequency- or 6-OHDA-induced destruction of the amygdala had a marked effect on the initial response to cociane. However, when all animals were tested with 10 mg/kg of cocaine on day 2 (bottom), it was apparent that both lesions had prevented the development of sensitization. Thus, dopamine in the amygdala appears to be critical in mediating this behavior.

activation of mesolimbic DA neurons has a modulatory influence on the transmission of information from the amygdala and hippocampus to the ventral pallidum via the nucleus accumbens. The ventral pallidum, in turn, is thought to be involved in the control of motor behaviors through its connections with the mesencephalic locomotor region (Mogenson 1987). Thus, DA input to the nucleus accumbens may serve to translate the motivational

determinants of behavior that are mediated by the limbic system into biologically relevant actions. In this respect, mesolimbic DA may serve not to mediate the hedonic quality of rewards (Wise 1982) but their ability to arouse and activate behaviors (incentive motivation).

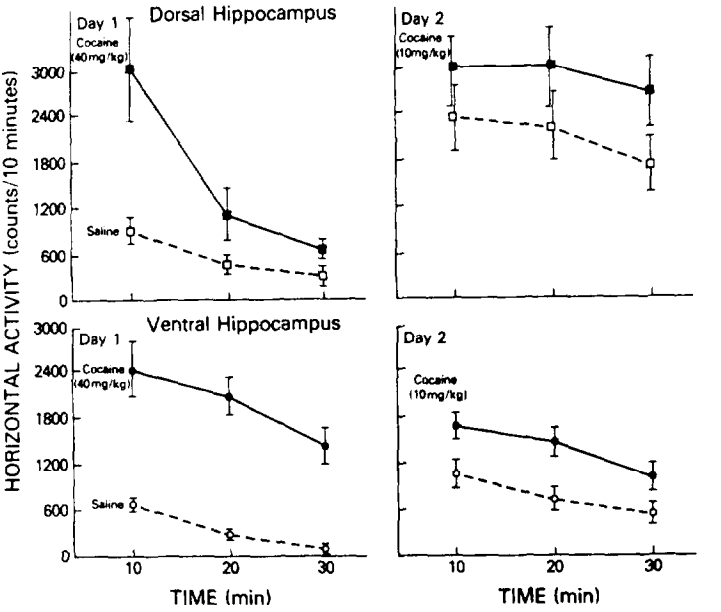

FIGURE 8. *Effects of ventral and dorsal hippocampal lesions on cocaine-induced sensitization*

NOTE: Left side of the figure illustrates the response of lesioned animals to the initial injection of cocaine (40 mg/kg). Neither lesion appeared to have an effect on cocaine-induced hyperactivity on day 1. On day 2 sensitization to 10 mg/kg of cocaine was still evident in both lesion groups.

The amygdala is a structure that receives polysensory input from the cortex and may be a focal point in the brain in which stimuli from all sensory modalities are assigned an emotional valance (Mishkin et al. 1984). Monkeys with amygdala lesions, for example, have been shown to be slow to learn the association between a visual stimulus and reward (Aggleton and Mishkin 1985). It may be that this is the brain region in which stimuli gain either positive or negative association values (expectations).

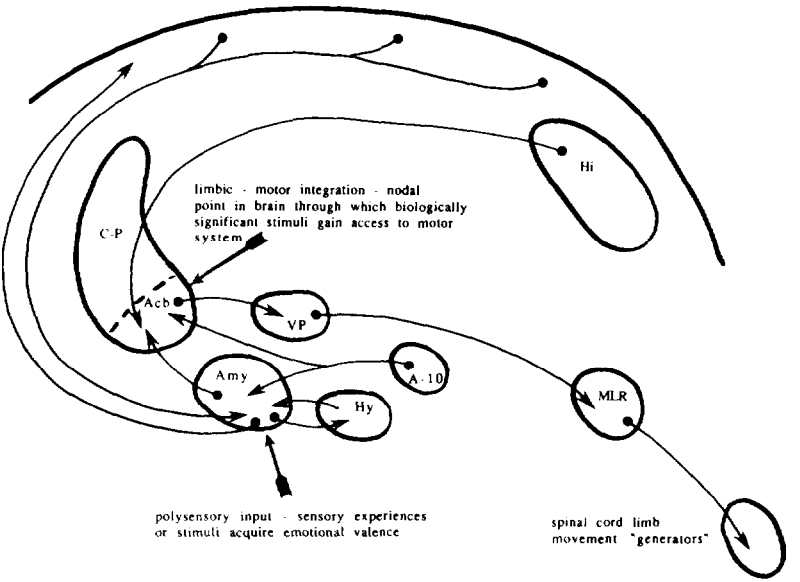

FIGURE 9. *Schematic illustrating some of the interrelationships between structures that play a role in mediating the conditioned components of cocaine sensitization*

NOTE: The nucleus accumbens may serve to gate motivational input from limbic structures to motor system. Dopamine input to the nucleus accumbens from the ventral tegmentum may be involved in translating the motivational determinants of behavioral mediated by the limbic system into biologically relevant actions. In the amygdala, mesolimbic DA might modulate the attachment of emotional significance to a given stimulus.

Interestingly, the amygdala also receives DA input from the same mesencephalic perikaryal region (A-10) that provides DA innervation to the nucleus accumbens. Dopaminergic pathways arising from the ventral tegmentum might have somewhat different functions in these two terminal regions, although the behavioral endpoints are ultimately related. In the amygdala, DA might modulate the attachment of emotional significance to a given stimulus. In this sense, amygdala DA input might serve as a gating mechanism to determine which stimuli gain access to structures efferent to the amygdala including the nucleus accumbens. Dopamine in the nucleus accumbens, on the other hand, serves to determine which limbic inputs gain access to the motor pathways.

SUMMARY AND CONCLUSIONS

It is apparent that stimuli associated with psychomotor stimulants as well as opiates acquire the ability to elicit motor behaviors that probably reflect the

acquisition and operation of incentive motivational processes. Such conditioning also appears to be a critical determinant of behavioral sensitization seen with repetitive administration of these agents. The conditioning of motor excitation to stimuli associated with psychomotor stimulants follows the principles of classical conditioning and is relatively long lasting. Dopaminergic mechanisms appear to be involved in the acquisition of such conditioned behaviors, since neuroleptics are effective blockers of the process. Dopaminergic blockade probably disrupts conditioning through several different mechanisms including attenuation of the conditioned and unconditioned excitatory properties of the CS and blockade of the US. DA blockade prevents stimuli associated with psychomotor stimulants from acquiring and subsequently generating positive affective motivational states that are reflected by increases in motoric output. While dopamine appears to be necessary for the formation of conditioned motor excitation, it is not critically involved in the expression of the conditioned effects. This seems to suggest that DA may serve only to modulate the formation of motivationally significant associations but is not involved in the expression of conditioned drug effects that may be mediated through DA-independent pathways.

The amygdala and nucleus accumbens are two structures in the CNS involved in the acquisition of conditioned motor excitation. Interestingly, both of these brain regions are the recipients of mesolimbic DA input. Dopamine probably plays different roles in each region during the conditioning process. In the amygdala, mesolimbic DA may serve to modulate processes that attach emotional significance to environmental stimuli; futher, DA may play a role in determining which stimuli gain access to structures afferent to the amygdala, including the nucleus accumbens. Dopamine in the nucleus accumbens, on the other hand, serves to determine which limbic inputs gain access to the motor pathways. In this way, DA in the nucleus accumbens may translate the motivational determinants of behavior that are mediated by limbic structures into biologically relevant actions.

Understanding the mechanisms that determine the conditioning of drug effects to associated stimuli also has possible relevance for elucidating processes underlying addictive behaviors. For example, it has been proposed (Stewart et al. 1984) that the acquisition of incentive motivational properties by stimuli associated with drugs determines the craving in addicts. Craving may simply be the induction of incentive motivational states by environmental stimuli that have been classically conditioned to elicit physiological states resembling those produced by the drug itself. In this context, it has been shown that conditioned stimuli have the power to initiate drug-seeking and drug-taking behaviors or to reinitiate them following abstinence (de Wit and Stewart 1981). Further analyses of conditioned behaviors associated with psychomotor stimulant administration will hopefully reveal new pharmacological and behavioral strategies for extinguishing addictive behaviors.

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