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GRADUATE COLLEGE

RELEASE OF NOREPINEPHRINE AND SEROTONIN FROM THE AMYGDALA DURING REWARDING MEDIAN FOREBRAIN BUNDLE STIMULATION

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

BY

JOAN ARLENE BUCHANAN HOLLOWAY

Oklahoma City, Oklahoma

1972

RELEASE OF NOREPINEPHRINE AND SEROTONIN FROM THE AMYGDALA DURING REWARDING MEDIAN FOREBRAIN BUNDLE STIMULATION

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RELEASE OF NOREPINEPHRINE AND SEROTONIN FROM THE AMYGDALA DURING REWARDING MEDIAN FOREBRAIN BUNDLE STIMULATION

CHAPTER I

INTRODUCTION

Psychopharmacological studies traditionally have been somewhat atheoretical in character, being motivated by applied, usually clinical interests. These studies, often involving an arbitrary drug, examined a subject's performance on some task in order to determine whether the drug had any effect on behavior associated with the task. Such studies have yielded a great deal of information regarding specific effects of drugs on specific behaviors, and occasionally, they have led to the discovery of neural systems which seem to be mediated by one class of compounds; i.e. a cholinergic drinking system and a noradrenergic eating system within the limbic-hypothalamic areas of the rat brain (Grossman, 1960).

Currently there is a trend to use techniques and strategies suited to obtaining information about basic biochemical mechanisms in an organism which is relatively unperturbed by the administration of a large dose of a drug.

In line with this approach, the present investigation attempted to obtain information on the relative importance of adrenergic and serotonergic systems for self-stimulation (SS) behavior in rats by examining release of the monoamines during either rewarding or non-rewarding electrical stimulation of the brain (ESB) or during auditory and visual stimulation (AVS).

In 1954 Delgado, Roberts, and Miller (1954) reported that electrical stimulation of some thalamic and hippocampal sites provided aversive motivation for several forms of learning. In the same year, Olds and Milner (1954) found that electrical stimulation of parts of the hypothalamus and septal regions had rewarding or reinforcing effects on behavior, since rats would press a lever at very high rates to obtain such stimulation. Subsequent work has demonstrated similar effects in a number of different species of animals including subjective reports of pleasure and pain in humans after electrical stimulation of subcortical regions (Heath & Mickle, 1960). These first reports fostered a vast number of research projects concerned with the phenomena of SS. Factors investigated have included brain loci from which either positive or negative effects can be elicited (Olds, Travis, & Schwing, 1960; Olds, 1962; Roberts, 1958; Bower & Miller, 1958; and Poschel, 1966); electrical parameters (Stein, 1962; Keesey, 1962; Keesey, 1964; Plutchik, McFarland, & Robinson, 1966); manipulation of certain basic drives such

as hunger, thirst, and sex (Olds, 1962; Valenstein, 1966); lesion effects (Lorens, 1966); and the effects of various drugs and chemicals.

The results of these investigations now constitute an important body of evidence related to the theoretical issues of reinforcement and motivation, with at least two review papers making major contributions to the field, one by Olds in 1962 and another by Glickman and Schiff in 1967.

Biological Theories of Reinforcement

While many traditional theories postulate drive reduction (Hull, 1943) or reduction of drive stimuli (Miller & Dollard, 1941) as the essential requirement for reinforcement, a somewhat different view has been taken by Glickman and Schiff (1967). In the tradition of response theories such as Sheffield's (1948) they place the emphasis on species-specific responses. Relying heavily on contributions made by Valenstein (1966), they conclude that reinforcement consists of activation of neuronal systems within the brainstem mediating the expression of the species-specific responses. They view reinforcers as those stimuli which are capable of facilitating the activity of these neuronal systems. For these authors, approach behavior is positively reinforcing and withdrawal behavior is negatively reinforcing. Various other theories of reinforcement have emphasized such factors as incentive and activation.

At the neurochemical level, what might be the mediator of such neural systems? A number of studies to be enumerated further in this dissertation strongly suggest that noradrenergic systems are specifically involved in SS behavior. It seems likely that norepinephrine (NE) is the responsible agent for initiating and sustaining the approach and consummatory behavior which results in reinforcement. Stein (1970) has evidence that NE acts on the amygdala in rats as an inhibitory transmitter. This, he believes, results in disinhibition of approach-consummatory behavior mediated by the forebrain.

Drug and Biochemical Effects on Self-Stimulation

The mass of experiments concerned with drug effects on SS has produced a tentative hypothesis that adrenergic and/or anticholinergic compounds tend to potentiate SS while cholinergic and/or antiadrenergic compounds tend to decrease SS. Moreover, it appears that serotonin is also involved in the neural systems responsible for SS. Findings for this monoamine have been somewhat less consistent, however, possibly because mechanisms of drug action and antagonism are less well understood for serotonergic systems.

Neurochemistry of Acetylcholine, Norepinephrine, and Serotonin

In order to sort out the effects on neural mechanisms produced by various pharmacological agents it is necessary

to examine some of the neurochemistry of the biogenic compounds before continuing with a review of their effects on SS.

The three neurohumors to be considered, acetylcholine, norepinephrine, and serotonin, all occur naturally within the central nervous system, specifically in the region of the lateral hypothalamus, amygdala, and the median forebrain bundle (MFB) (Eiduson, Geller, Yuwiler, & Eiduson, 1964; Hillarp, Fuxe, & Dahlstrom, 1966). Furthermore, the enzyme systems responsible for the biosynthesis and metabolism of these substances are found in these same regions. Briefly, for each neurohumor, the normal pathway of biosynthesis and degradation will be considered as will the compounds which either simulate or potentiate its primary effects and the compounds which antagonize or deplete the substance.

Acetylcholine. The biosynthesis of acetylcholine requires acetyl CoA and choline which combine to form acetylcholine in the presence of the enzyme choline acetylase. (see figure 1) It is degraded in the presence of cholinesterase into choline and acetate.

Acetylcholine is the neurotransmitter for the parasympathetic division of the autonomic nervous system. Released into the synaptic space upon the arrival of a neural impulse, it acts on the postsynaptic membrane resulting in the initiation of another impulse in the postsynaptic

Figure 1 - The biosynthesis and degradation of Acetylcholine.

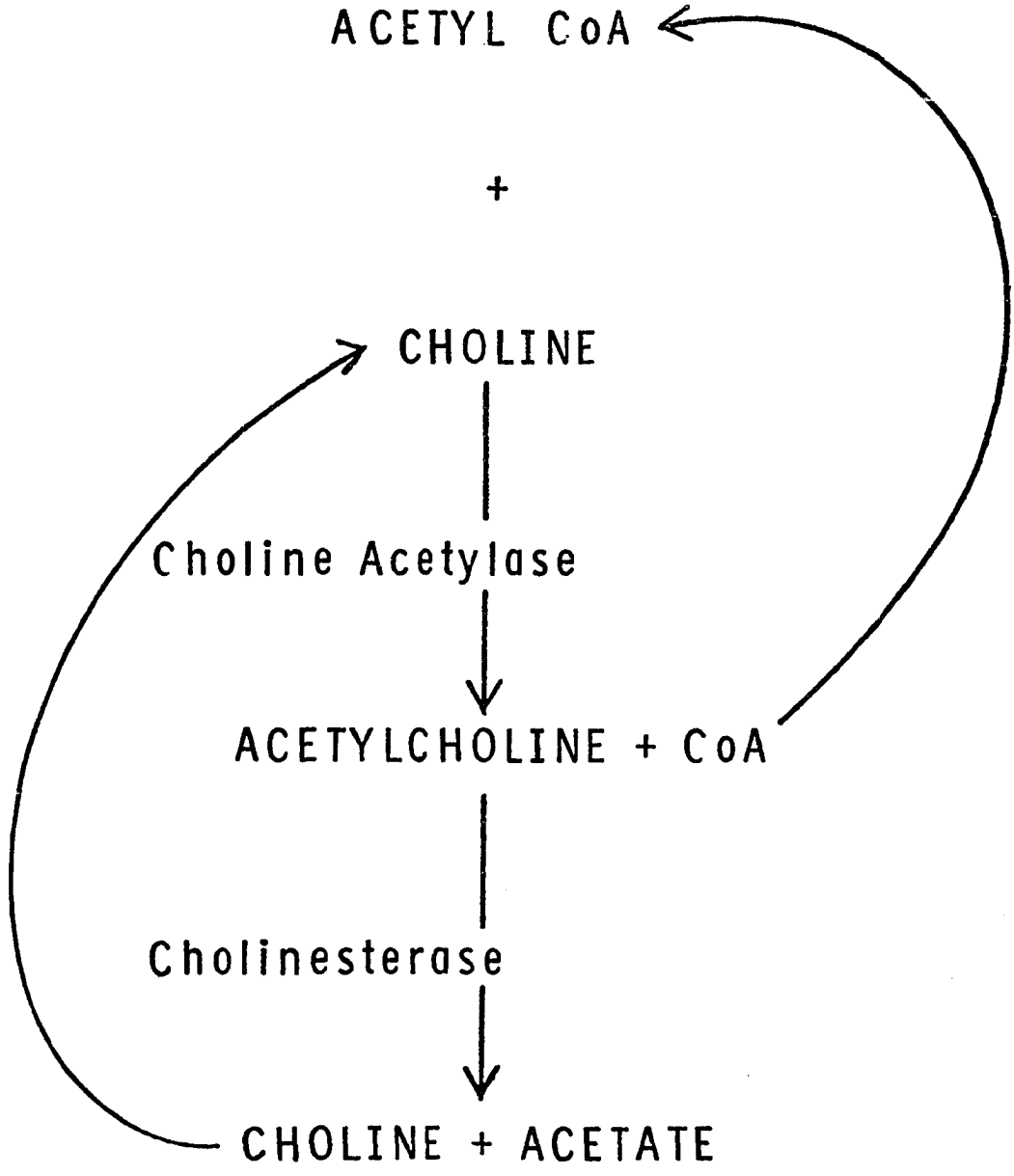

ACETYL CoA
+
CHOLINE
Choline Acetylase
ACETYLCHOLINE + CoA
Cholinesterase
CHOLINE + ACETATE

Figure 1

neuron. Its removal from the synaptic space is accomplished by degradation by the enzyme cholinesterase.

Cholinergic postsynaptic receptors appear to be of two types; muscarinic and nicotinic. The cholinomimetic effects of drugs on autonomic effector cells are called muscarinic while a stimulation effect followed by a blockade of autonomic ganglia and skeletal muscle are called nicotinic.

Carbachol is a synthetic compound which mimics the effects of acetylcholine, whereas atropine is a muscarinic cholinergic antagonist which is in competition with acetylcholine at the postsynaptic site. Physostigmine (eserine) an anticholinesterase, blocks the degradation of acetylcholine.

Norepinephrine. The catecholamine, norepinephrine (NE), is synthesized from the amino acid phenylalanine. Phenylalanine is hydroxylated into tyrosine which is further hydroxylated into dihydroxyphenylalanine (DOPA). DOPA is then decarboxylated into dopamine which is further hydroxylated into NE. One degradation pathway for NE involves o-methylation in the presence of catechol o-methyl transferase resulting in normetanephrine. (see figure 2) This compound is further metabolized and excreted as a glycol. This degradation pathway is believed to be the primary one for extracellular NE. Another degradation pathway for NE occurs inside the cell in the presence of monoamine oxidase

Figure 2 - The biosynthesis and degradation of norepinephrine.

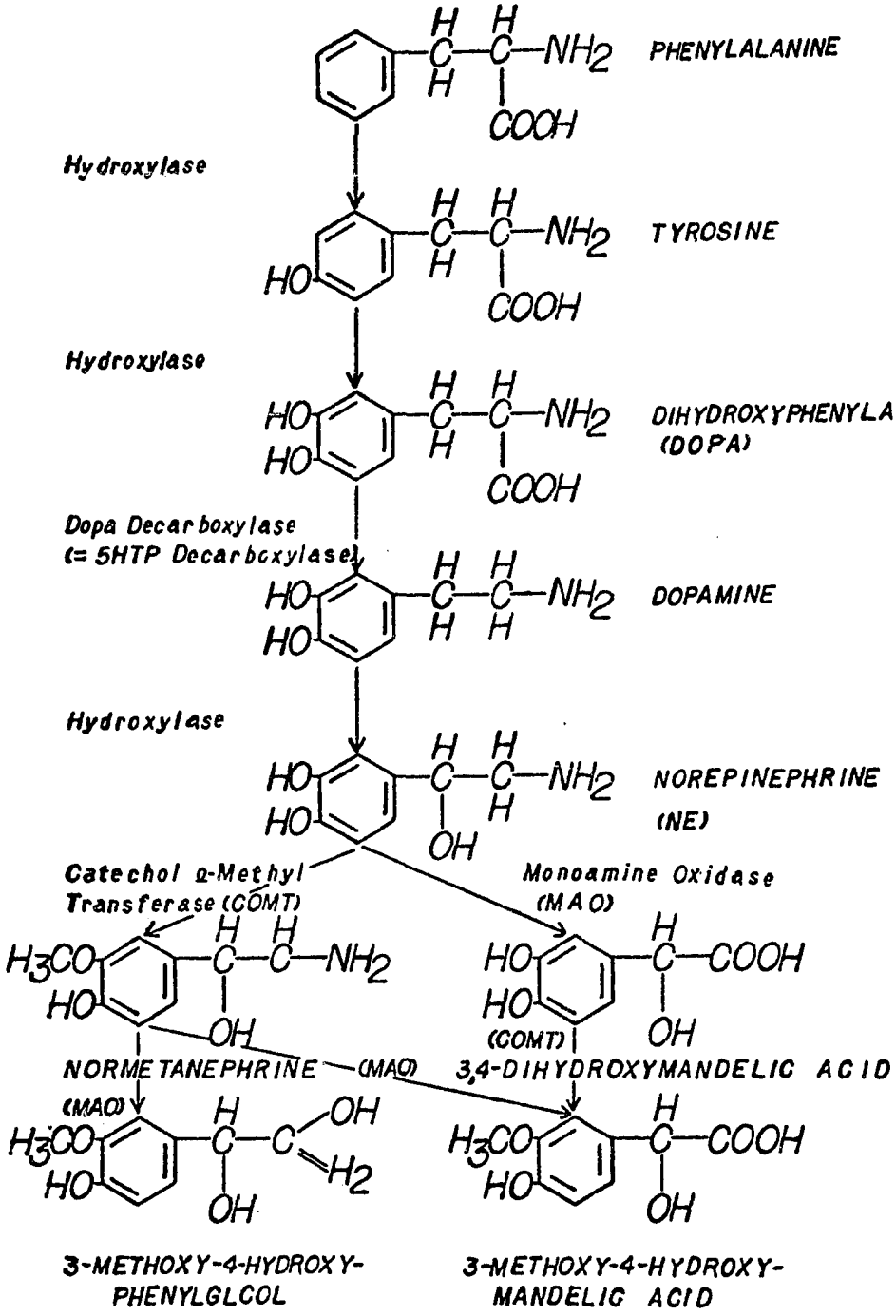

PHENYLALANINE
Hydroxylase
TYROSINE
Hydroxylase
DIHYDROXYPHENYLALANINE
(DOPA)
Dopa Decarboxylase
(= 5HTP Decarboxylase)
DOPAMINE
Hydroxylase
NOREPINEPHRINE
(NE)
Catechol o-Methyl
Transferase (COMT)
Monoamine Oxidase
(MAO)
NORMETANEPHRINE
(MAO)
(COMT)
3,4-DIHYDROXYMANDELIC ACID
(MAO)
3-METHOXY-4-HYDROXY-
PHENYLGLCOL
3-METHOXY-4-HYDROXY-
MANDELIC ACID

Figure 2

(MAO), and results in deaminated biproducts.

With the arrival of a neural action potential, NE is released into the synapse to act upon postsynaptic sites. Then it is removed either by reabsorption into the presynaptic cell, the primary method (and thus subject to metabolism by intracellular MAO), or by extracellular metabolism with catechol o-methyl transferase.

Alpha-methyl-meta-tyrosine is an inhibitor of decarboxylase and thus lowers the concentration of NE, dopamine, and serotonin.

Reserpine depletes NE, dopamine, and serotonin (Holzbauer & Voft, 1956; Carlson, Lindquist, Magnusson, & Waldeck, 1958) by impairing the vesicular binding sites for the monoamines, releasing them into the cell and preventing their reabsorption.

Amphetamine has adrenergic potentiating properties acting to release NE and possibly also as a MAO inhibitor (Goodman & Gilman, 1965). Dibenzaline is an adrenergic blocking compound acting at the postsynaptic receptor sites. Six-hydroxydopamine results in a long lasting depletion of NE due to neuron end-terminal damage.

Serotonin. The indoleamine serotonin or 5-hydroxy-tryptamine (5-HT) is synthesized from the amino acid trypto-phan (see figure 3), first by hydroxylation to 5-hydroxy-tryptophan (5-HTP) then by decarboxylation to 5-HT. Its degradation is accomplished either by monoamine oxidase

Figure 3 - The biosynthesis and degradation of serotonin.

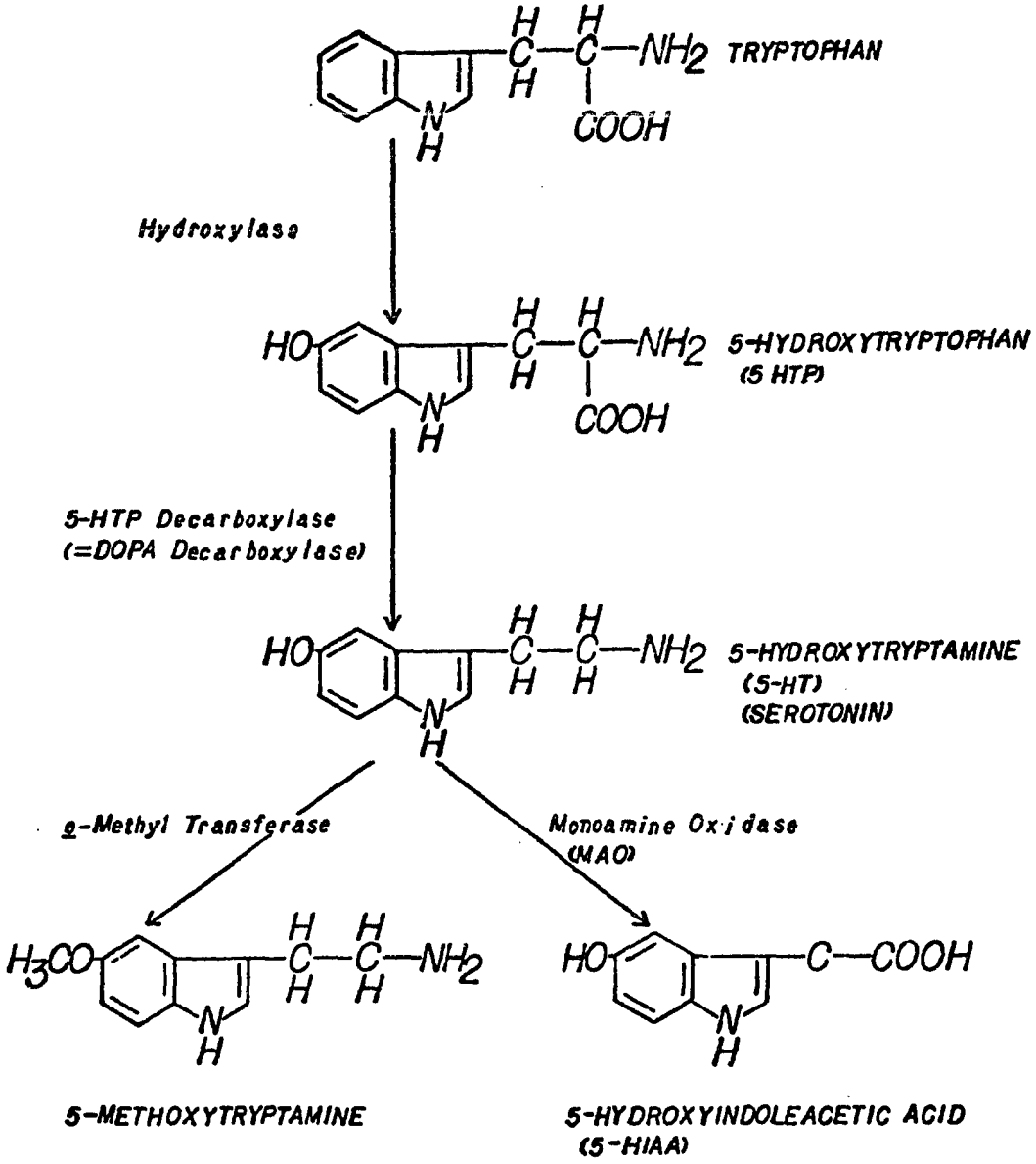

TRYPTOPHAN
Hydroxylase
5-HYDROXYTRYPTOPHAN
(5 HTP)
5-HTP Decarboxylase
(=DOPA Decarboxylase)
5-HYDROXYTRYPTAMINE
(5-HT)
(SEROTONIN)
o-Methyl Transferase
Monoamine Oxidase
(MAO)
5-METHOXYTRYPTAMINE
5-HYDROXYINDOLEACETIC ACID
(5-HIAA)

Figure 3

(the major pathway) producing 5-hydroxy-indoleacetic acid (5-HIAA) or by catechol o-methyl transferase. Both the synthesizing and degradation enzymes in the NE and 5-HT pathways are quite similar (i.e. DOPA and 5-HTP decarboxylase) and in many cases are found to be identical.

Para-chlorophenylalanine (PCPA) is a drug which blocks the synthesis of 5-HT by inhibiting the rate limiting step of the 5-hydroxylation of tryptophan (Koe & Weissman, 1966), thus lowering the concentration of 5-HT in body and brain. LSD is structurally similar to 5-HT and in many cases blocks its effects, and bromolysergic acid diethylamide (Brom-LSD) is in some instances also a 5-HT antagonist. Para-chloroamphetamine is a compound which releases serotonin (Miller, Cox, Snodgrass, & Maicke, 1970).

Drugs acting on both NE and 5-HT. In general MAO inhibitors potentiate the effects of both NE and 5-HT by inhibiting the metabolism of both substances. Iproniazid and pargyline hydrochloride are exemplary MAO inhibitors. However, it has been reported that phenylisopropylhydrazine (JB-516), an MAO inhibitor in dogs, can cause an elevation of 5-HT levels with no increase in catecholamine levels (Mailing, Highman, & Spector, 1962; Spector, Shore, & Brodie, 1960).

Reserpine, as previously mentioned, releases and impairs the bonding sites for both the catecholamines and serotonin.

Imipramine and amitryptyline alter the permeability of the cell membrane to catechol and indole amines (Goodman & Gilman, 1965) potentiating the effects of "free" intercellular NE and 5-HT.

Upon intravenous administration of alpha-methyl-meta-tyrosine or alpha-methyl dopa to guinea pigs, rats, pigeons, and mice the decarboxylase enzyme is inhibited and levels of NE, 5-HT, and dopamine are reduced (Sourkes, Murphy, Chavez, & Zielinska, 1961; Hess, Connamacher, Ozaki, & Udenfriend, 1961; Porter, Totaro, & Leiby, 1961; Sourkes, 1954; Hinggen & Aprison, 1963). After administration of these decarboxylase inhibitors the three humors return to their preadministration level at different times; dopamine at six to eight hours, and 5-HT at three and one half to twenty hours, whereas NE remains low at twenty hours and does not return to normal levels before seventy-two to ninety-six hours.

Studies Relating to Self-Stimulation

Four general techniques have been employed to investigate drug or biochemical effects on SS. In the first, the drug is introduced systemically after which alterations in response rates or electrical current thresholds for SS are measured. The second technique, a procedure which has met with many difficulties, is self-injection of chemicals directly into the brain. The main difficulty with this method is that the amount injected at one time is usually

so large or has such profound behavioral effects as to make it difficult to use SS response rate as a measure of its action. Often, some of the chemical remains in the cannula for a long period of time, making it unnecessary for the animal to respond during this time. The third method is to inject chemicals into the site of the stimulating electrode and again measure alterations in response rate or differences in current thresholds. The fourth method is to allow the animal to SS and measure various substances in his brain. A modification of this later method was the approach used in this research.

Studies reviewed in the following sections involve drugs which are known to affect both SS and either the cholinergic, adrenergic, or serotonergic systems. All studies used rats as subjects except as indicated otherwise.

SS cholinergic mechanisms: pharmacological studies. Some of the most striking effects on SS behavior have been found with chlorpromazine (Olds, Killam, & Bach-Y-Rita, 1956; Miller, 1957; Olds, Killam, & Eiduson, 1957; Olds, 1957; Olds, 1958; Olds & Olds, 1958; Olds, 1959b; Olds & Travis, 1960; Stein & Ray, 1960; Stein, 1961, Stein & Seifter, 1961; Stein, 1962; Olds, 1962; Olds & Olds, 1964). This drug, a major tranquilizer in man, reliably increased the threshold for SS and lowered the animal's rate of responding. In general, other phenothiazines have the same effect on responding. It is interesting to note that five phenothiazines which seem

to have varying degrees of effectiveness on psychotic symptoms bear the same relationship in the same order to SS (Stein, 1961; Olds, 1962). Promethazine increased SS, promazine had no effect, chlorpromazine reduced, and prochlorperazine and trifluoroperazine were extremely powerful in eliminating responding. The last two drugs were also found to be especially effective in treating psychotics. A similar ranking was found for these drugs in the modulation of cholinergic effects. Although Bradley (1965) does not explain the exact nature of the antagonism, he reports that promethazine has a strong antagonism to the effects of acetylcholine. Promazine has a somewhat less antagonistic effect along with chlorpromazine. And, trifluoroperazine has no antagonism to acetylcholine. Further, these drugs are ordered in the same way for their relative pharmacological antagonism to serotonin. One notes that those phenothiazines which have largest antagonistic effects on acetylcholine and serotonin have the least effects and may even have a potentiating effect on SS.

Consistent with the hypothesis that cholinergic stimulating drugs decrease SS, research by Olds and Domino (1969) demonstrated that muscarinic cholinergic drugs depressed SS whereas nicotinic cholinergic drugs had a more complex depressant and stimulant effect. Brophy and Todd (1970) found that when drugs were deposited at the site of SS electrodes, carbachol markedly and acetylcholine moderately

reduced SS rates. Moreover, this effect could be attenuated by atropine.

SS adrenergic mechanisms: pharmacological studies. Amphetamine has also been widely investigated with the finding that it markedly decreased the threshold for SS and increased response rates (Miller, 1957; Olds, 1959b; Stein & Ray, 1960; Stein, 1961; Stein & Seifter, 1961; Stein, 1962; Olds & Olds, 1964; Stein, 1964). Miller (1957) reported an interesting relationship in which methamphetamine acts to increase the time to turn the stimulation off while chlorpromazine acts to increase the time to turn it on. When methamphetamine was administered subsequent to imipramine hydrochloride (a potentiator of "free" intracellular and extracellular NE and 5-HT), the potentiating effects of methamphetamine on SS parameters were augmented (Stein, 1961; Stein, 1962). Stein (1962) administered chlorpromazine, which decreases SS, and found that its effect was antagonized by methamphetamine. This was true regardless of which drug was administered first.

Phenethylamine, the basic adrenergic structural compound, increased SS (Stein, 1964). Also, if phenylalanine was administered after iproniazid (an MAO inhibitor) the potentiating effect on response rate was especially strong.

Reserpine, releasing both the catecholamines and 5-HT, produced a biphasic effect in that it reduced response rates within forty-five minutes after administration but subse-

quently caused a persistent elevation in responding lasting for as long as twelve days (Stein, 1962). When reserpine was followed by amphetamine, its initial inhibitory effect on SS was reversed (Stein, 1962). The administration of atropine followed by amphetamine also increased responding, but not as much as the administration of imipramine followed by amphetamine. Stein reported that amitriptyline (also a potentiator of intracellular and extracellular NE and 5-HT) enhanced the effect of amphetamine on SS.

Six-hydroxydopamine, which functionally destroys adrenergic end terminals, produced a progressive decrease in SS behavior over time (Stein, 1971).

These studies just cited indicate that those compounds which in some manner potentiate the effects of NE, augment SS behavior while those compounds which suppress the effects of NE, decrease SS behavior.

Although for reasons mentioned earlier, the self-injection studies must be evaluated critically, one substance which caused substantial self-injection responding was iproniazid. This MAO inhibitor would be expected to increase the concentration of NE. In addition, NE seemed to cause some self-injection behavior, although this finding is somewhat uncertain (Olds, 1958; Olds, 1959a).

Paradoxically Brophy and Todd (1970), depositing drugs at the site of SS found a decrease in responding after both NE and dibenzyline (an adrenergic blocking agent).

However, histological examination indicated the drugs had caused lesions at the sites of the electrodes, making any conclusions questionable.

SS adrenergic mechanisms: biochemical analysis. A new approach to the investigation of adrenergic mechanisms in SS was reported by Bliss, Wilson, and Zwanziger (1966). Stimulating electrodes were implanted in the posterior hypothalamus and the medial lemniscus of rats. Stimulation sites were evaluated for their tendency to induce SS or escape behavior. Animals that stimulated themselves spontaneously more than 2000 times per hour were labeled self-stimulators, whereas those who responded less than five times per hour and showed startle or withdrawal reactions were called aversive animals. Biochemical analysis revealed drops of twenty per cent in NE levels in the hypothalamus as well as in the rest of the brain in both SS and aversive animals. Animals in the Bliss, Wilson, and Zwanziger study were yoked so that one rat was allowed to self-stimulate, receiving "active" stimulation, while his partner received "passive" stimulation. Passively stimulated animals showed a small reduction in NE just bordering on statistical significance ($p<0.10$). It was also determined that brain levels of NE did not differ between unimplanted animals and animals who were implanted but received no stimulation.

Rats, which had not proven to be self-stimulators, but which had electrodes implanted in the hypothalamus, were

then stimulated at a rate of 3000 per hour. These animals also showed a decrease in NE as well as emotional responses to the stimulation comparable to the SS group.

Finally, animals were implanted with electrodes located slightly to one side of the hypothalamic SS sites. Automatic stimulation of these animals produced no or few emotional responses, and no decrease in NE levels in the brain. The authors suggested that the decrease in NE levels found in both SS and aversive animals reflects the depletion of NE occurring with the normal release of the neurohumor during synaptic transmission. The failure to differentiate self-stimulators from aversive animals on the basis of NE levels leads one to question whether NE itself is specific to the affective component of intracranial reinforcement. A major difficulty with this study, however, was that placement of the electrodes was not confirmed. Thus, the meaning of "in the hypothalamus" or "just outside" is not clear.

An ingenious study by Stein and Wise (1969) helps clarify the results of the Bliss, Wilson, and Zwanziger (1966) investigation. Since the research reported in this dissertation employed methods similar to those used by Stein and Wise, their study will be examined in detail. These investigators implanted stimulating electrodes in the MFB of rats. In addition, a needle for injection of radioisotopes was inserted into the lateral ventricle, and a push-pull cannula implanted into either the lateral

hypothalamus or the amygdala. Rats were taught to SS and evaluated for their SS performance during a one hour test. Animals displaying rates of 1000 responses per hour or more were classified as being in a rewarding group. Others were classified in the non-rewarding group.

H^3norepinephrine and C^{14}norepinephrine were injected into the lateral ventricle of each animal and forty-five minutes later the animal was given electrical stimulation in the MFB delivered at a fixed rate of one train of 100 cps pulses per second. Locke-Ringer's solution was perfused into the push-pull cannula and samples were taken every five minutes. The samples were assayed for NE, its deaminated metabolites, and normetanephrine. Stimulation in the rewarding group caused a marked increase in the radioactivity of the perfusates in both the hypothalamus and amygdala, the primary component being o-methylated derivatives of NE. Non-rewarding stimulation did not cause an increase in radioactivity and in some cases, caused an inhibition of release. They further found that amphetamine injected i.p. caused release of NE from amygdaloid sites but not from hypothalamic sites.

On the basis of the release of NE from the hypothalamus and the amygdala during rewarding ESB the authors concluded that NE is released in central synapses at the terminal sites of the MFB in the hypothalamus and is responsible in part for the positive reinforcement of behavior.

The Stein and Wise study suggests that the rewarding effect of SS in rats is mediated by release and turnover of NE. However, conclusions about the specificity of this relationship depend on answers to the following questions: (1) Is the release of NE specific to rewarding ESB? Stein and Wise utilized only ESB, but it is possible that the release is not specific to rewarding ESB but might occur with the presentation of any stimulus. (2) Is the release of biogenic substances limited to NE or are other biogenic amines which are present in that area of the brain (i.e., 5-HT) also released? If so, they may be equally important for reinforcement processes. (3) Does ESB, and/or other peripheral stimuli, promote the "release" of other compounds (e.g., urea) which are relatively inert with respect to normal nervous functioning? If the latter were found to be true, Stein's concept of the specificity of NE in the mechanisms of reinforcement would be gravely challenged.

SS serotonergic mechanisms: pharmacological studies. The combined effects of LSD and 5-HT have been examined by Olds, Killam & Eiduson (1957); Olds (1959b) and Olds & Olds (1964). There were electrode sites in which LSD reduced responding but this effect could be antagonized by pre-administration of 5-HT one hour earlier. At these same sites Brom-LSD, a 5-HT antagonist, had no effect. These sites included the septal region, the preoptic region, and the extreme posterior-ventral hypothalamus. With other electrode placements,

however, both LSD and Brom-LSD reduced responding and this effect was not blocked by prior administration of 5-HT.

Stark, Boyd, and Fuller (1964), using dogs, studied the effects of three drugs: (1) alpha-methyl dopa, three and one half, eight, and twenty-four hours after administration; (2) JB-516 (a MAO inhibitor relatively specific to serotonergic mechanisms), twenty-four hours to five days after administration; and (3) Brom-LSD, ten minutes after administration.

Alpha-methyl dopa, which depletes 5-HT and NE, caused complete inhibition of SS at three and one half hours. Performance returned almost to control levels at eight hours and showed complete recovery at twenty hours. Brain levels of 5-HT were fifty-three per cent of control levels at three and one half hours, seventy per cent at eight hours, and eighty-three per cent at twenty hours. However, at twenty hours the level of NE in the brain was still only sixty-three per cent of control levels. Thus, inhibition of responding was associated with depletion of both NE and 5-HT, but response recovery followed the recovery of 5-HT to control levels more closely than that of NE.

Forty-eight hours after JB-516 was administered, the electrical threshold for SS was lowered, and response rates (with fifty to one hundred microamps current intensity) were increased in two of three animals. Rates with 125 microamps were about at pre-drug level and rates with 150 microamps

were lower than control levels. In non-electrode dogs, brain serotonin levels forty-eight hours after administration of the drug were 154 per cent of control.

With Brom-LSD, high doses tended to inhibit SS, while low doses were found to lower the threshold of SS in the same two of three dogs. (The third animal's electrode was in a slightly different anatomical location.) Stark, Boyd, and Fuller suggested that 5-HT may be responsible for the reinforcing effect of SS in that inhibition of responding with alpha-methyl dopa followed the curve of 5-HT depletion, while responding recovered before NE levels began to return to normal levels. The investigators failed to stress, however, that during the period of response inhibition, both NE and 5-HT concentrations were lowered. Therefore, NE cannot be ruled out as a mediator of SS. Their study does suggest that both NE and 5-HT may mediate the neural mechanisms of reinforcement.

Poschel and Ninteman (1968) found that SS thresholds were lowered after administering the MAO inhibitor, pargyline hydrochloride followed by 5-HTP. The difference in results on SS threshold levels of administering a MAO inhibitor and raising brain levels of 5-HT between the Poschel and Ninteman (1968) and Stark, Boyd, and Fuller (1964) studies may be explained by the use of different current levels. The Poschel and Ninteman study used seventeen and one half and twenty-five microamps and the Stark, Boyd, and Fuller study used

fifty to one hundred-fifty microamps.

Paradoxically, further research by Poschel and Ninteman (1971) employed PCPA, which inhibits 5-HT synthesis, and found an excitatory effect on SS behavior from three to seven days after administration. In addition, the serotonin releasing compound para-chloroamphetamine produced an inhibitory effect on SS. As a result of their last studies, these researchers postulated a theory that placed emphasis on both NE and 5-HT mechanisms for regulating reward thresholds. NE lowers thresholds for reinforcement and 5-HT raises thresholds.

One may note from the studies on 5-HT mechanisms and SS that the results are anything but consistent and much further research will have to be done in order to clarify the previously cited literature.

General Considerations for the Proposed Research

The psychopharmacological research reviewed above led to the hypothesis that an adrenergic mechanism is importantly involved in the maintenance and enhancement of SS behaviors. Moreover, it appears that there is a strong likelihood that serotonergic mechanisms also may play a part in SS behavior, although its precise role is more obscure. Thus, it is proposed that both NE and 5-HT are necessary for the phenomenon of SS, perhaps each in a slightly different manner from the other.

Although the Stein and Wise (1969) study found a release of NE during rewarding ESB and no release or a decrease

in release of NE during non-rewarding ESB, the Bliss, Wilson, and Zwanziger (1966) study found a decrease in brain levels of NE in both stimulators and non-stimulators. The decrease in NE found in the stimulators could be due to depletion produced by the release and rapid metabolism of the substance, whereas the decrease in the non-stimulators could be the result of an inhibition of the synthesis of NE. Studies cited by Page (1968) showed that the manufacture of neurohumors is dependent on neural impulses in an intact nervous system. Had Bliss and his co-workers measured NE metabolites in the brain as Stein and Wise did, this issue might have been settled.

The Stark, Boyd, and Fuller (1964) depletion data suggested that 5-HT was more closely associated with SS behavior than NE. Nevertheless, at the time inhibition of SS behavior occurred both NE and 5-HT were depleted. Furthermore, the increase in 5-HT induced by an MAO inhibitor made the animals much more sensitive to the effects of electrical stimulation. Their threshold for responding was decreased and their response rate increased at low current levels. At higher levels of current, however, which presumably produced more excitement, the increase in 5-HT did not change or, in fact, decreased rates of responding. Thus, the relationship between rates of responding and current levels after administration of a 5-HT specific, MAO inhibitor was not a simple monotonic function.

This dissertation research focused on the specificity

with which monoamines are released from the amygdala during rewarding ESB. Electrode placements in rats were behaviorally evaluated to determine the rewarding value of ESB. Isotopically labeled 5-HT, isotopically labeled NE, and a control drug, isotopically labeled urea, were injected into the lateral ventricle, and the amygdala was perfused during presentation of the ESB or other sensory stimuli, namely a paired tone and light.

The following questions concerning the specificity of release of the biogenic amines with respect to drug conditions were investigated:

(1) Is NE released during rewarding electrical stimulation of the brain? A finding that NE is released in these circumstances would constitute a replication of part of the Stein and Wise (1969) study.

(2) Is 5-HT released during rewarding electrical stimulation of the brain? Since other investigations have found 5-HT to be systematically related to SS, a positive finding can be anticipated here.

(3) Does ESB also induce the release of a relatively neutral substance, urea? If so, concepts which advocate the monoamines as specific mediators of reinforcement on the basis of the Stein and Wise (1969) findings will be considerably challenged, and the method of examining released substances will require close scrutiny in order to determine how much the release might be due to an actual physiological release,

and how much to other factors such as stress to the animals or physical insult to their brains.

(4) Will simultaneous injection of both labeled NE and labeled 5-HT alter the results? It is desirable to inject both monoamines simultaneously in order to examine possible interaction effects or possible differential involvement of adrenergic and serotonergic systems while holding conditions of electrode placement and SS rates constant. Further, it may be that injecting both NE and 5-HT produce changes in the amines or in the way in which they are absorbed and utilized.

Finally, the following questions concerning the specificity of release of biogenic amines with central and peripheral stimuli will be investigated:

(1) Is the release of biogenic amines specific to rewarding ESB or do other sensory stimuli (AVS), presumably neutral in their rewarding value, such as a paired tone and light, cause an analogous release? In other words, is it merely a non-specific property of stimuli which causes the release of biogenic amines, or is the release contingent upon rewarding ESB?

(2) Does the current level of the ESB affect the release of the monamines? One might expect that the higher the current (within certain limits), the greater the amount of monoamines released.

CHAPTER II

METHODS

General Design of the Research

Table 1 presents the design of the research. There are four categories of isotopically labeled substances which were injected into the lateral ventricle of rats prior to amygdaloid perfusion. One group (NES) had only NE injected, a second group (5-HTS) had only 5-HT injected, a third (NE+5-HT) had both NE and 5-HT injected simultaneously, and the last group (US) had only urea (U) injected. The first two groups (NES and 5-HTS) were used to examine the release of these amines during ESB and also during auditory and visual stimulation (AVS). The third group (NE+5-HT) was used to examine possible interaction effects of the two amines, while holding the electrode placement and rewarding value of the ESB constant. The last group (US) was used as a control to examine the chemical specificity of the release phenomenon and to investigate the possibility that any release of the biogenic amines was an artifact due to some aspect of the electrical stimulation other than induction of neuronal firing.

TABLE 1

GENERAL DESIGN OF THE RESEARCH

	Rewarding (R)	Non-Rewarding (NR)	Control or background release (C)
H^3norepinephrine (NES)	N=4	N=2	N=0
H^3serotonin (5-HTS)	N=2	N=2	N=0
H^3norepinephrine and C^{14}serotonin (NE+5-HT)	N=4	N=2	N=3
C^{14}urea (US)	N=4	N=0	N=1

The two categories of rewarding (R) or non-rewarding (NR) ESB were determined during behavioral testing one to five days prior to perfusion. A third group, background (B) release, was given no ESB and no AVS during the perfusion in order to examine the nature and shape of the release curve under conditions of minimal stimuli. Table 1 also lists the number of rats in each condition.

Some of the data from the NES,R group and the NES, NR group represent a replication of the research reported by Stein and Wise (1969). The other groups represent original research.

Subjects and Surgery

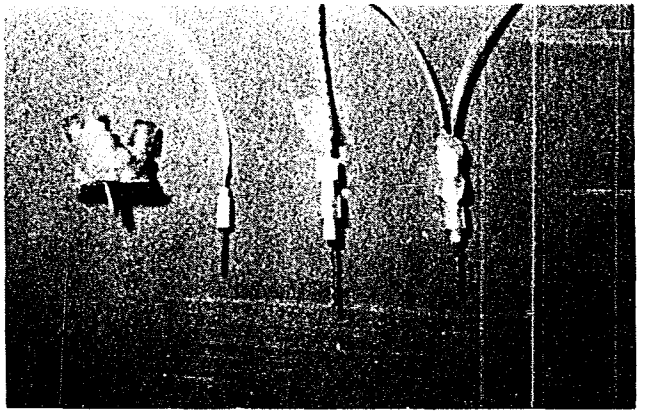
Subjects were male rats of the Sprague-Dawley (Schmitt) strain implanted with bipolar stainless steel electrodes (0.008 inches in diameter) in the MFB under pentobarbitol anesthesia (50 or 60 mg/kg) approximately one to four weeks prior to behavioral testing. In each animal, a stainless steel push-pull guide cannula (18 gauge tubing) also was implanted in the amygdala and a stainless steel injecter guide cannula (22 gauge tubing) was implanted in the lateral ventricle. All implants (obtained from Plastic Products, Roanoke, Va.) were on the left side. The stereotaxic coordinates for the electrode varied somewhat but two placements were routinely used: 1) with incisor bar elevation at the level of the intra-aural line, the placements were 3.5 mm posterior to bregma, 1.3 to 1.7 mm lateral

from the midline, and 8.5 mm down from the top of the skull, and 2) using deGroot (1959) reference points the placement was AP +3.8, V -3.5, and L 1.3. The push-pull placement with deGroot (1959) reference points was AP +5.0, V -2.5, and L 4.0. The injecter cannula placement was AP 6.0, V +3.1, and L 1.4. Figure 4 (a) illustrates the implanted electrode and cannulae with accompanying apparatus necessary to use them.

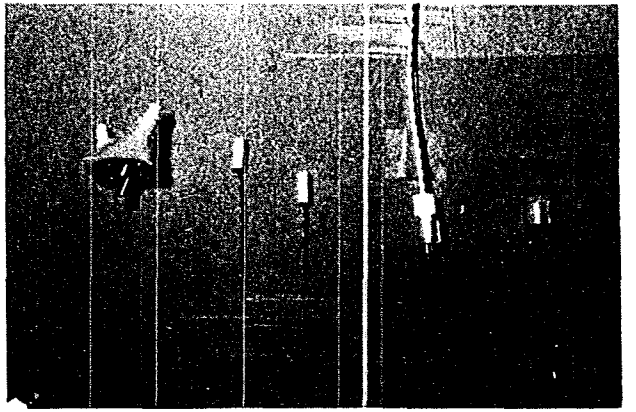
Behavioral Evaluation

After the surgical recovery period of one to four weeks, the electrode sites were evaluated for R or NR using operant techniques. Ten animals received training in a Skinner box where delivery of an ESB was contingent on a lever press. Learning the lever-press response and asymptotic rate of responding was the index of R or NR for these animals. Fourteen animals were tested in a runway equipped with photocells. The animal received ESB when the designated photo cell at one end of the runway was broken. The ESB continued at the rate of one per second until the animal broke another photocell. The end of the runway where ESB was received was systematically varied to ensure the animal was not merely exibiting an end-position preference. Testing was done primarily in five-minute sessions. Only those sessions in which the animal sampled both the ESB end and the other non-stimulation end of the runway were included in the behavioral analysis. The per cent of time

Figure 4 - Chronically implanted apparatus. (a) Shows from left to right a skull cap, the injecter cannula, the electrode, and the push-pull cannula. Each implant is shown assembled as it is used. (b) Shows the component parts of the electrode assembly. From left to right, a skull cap, the electrode as it is supplied from Plastic Products, Inc., the electrode cut off ready for implantation, the electrode connector, and the electrode cap. (c) Shows the components of the injecter cannula. From left to right a skull cap, the guide cannula which is implanted into the brain, the internal cannula, the internal cannula incerted into the guide cannula ready for isotope injections, and the dummy cannula. (d) Shows the component parts of the push-pull cannula assembly. From left to right the push cannula with connector, the pull cannula, the push and pull cannula screwed together, the push-pull cannula assembly after it has been sealed to prevent leaking and screwed into the guide cannula, and the push-pull cannula inserted into the skull cap.



(a)



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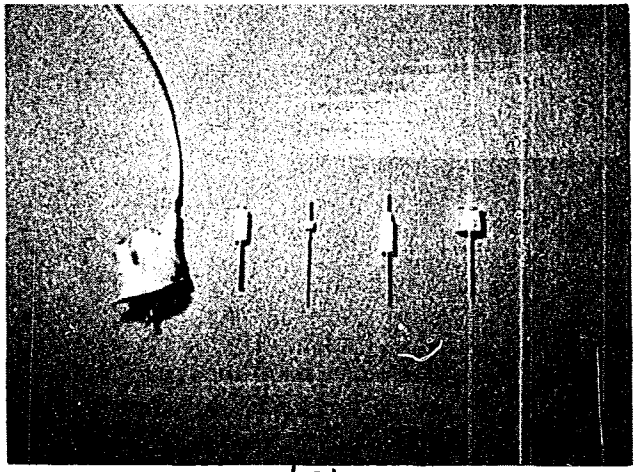
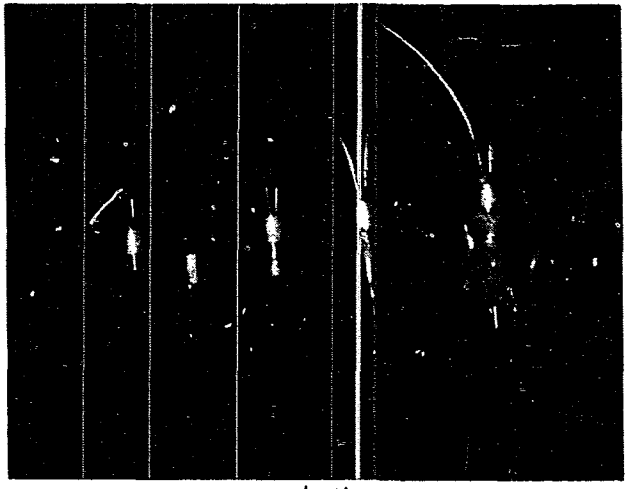


(c)



(d)

Figure 4

the animal spent in the ESB end of the runway was taken as an index of R or NR. The ESB parameters were: train duration = 100 msec., pulse frequency = 100 cps, and monophasic pulse duration = 0.2 msec. The current was varied for each animal based on his behavior and ranged from 50 microamps to 450 microamps. Figure 5 (a) shows an animal in the runway testing apparatus.

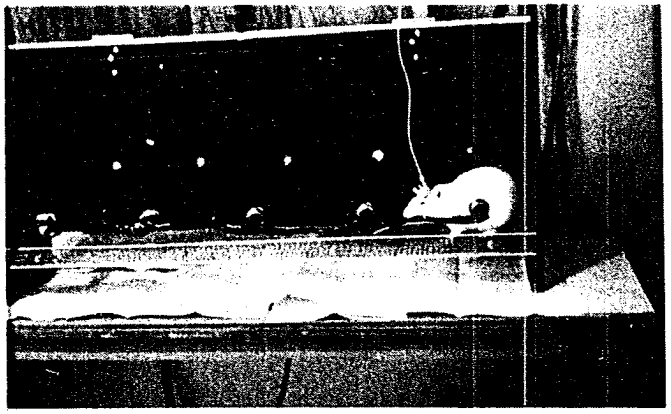
Injection of the Radio-Isotopes and Hypothalamic Perfusion

On the perfusion day the animal was placed in the restraining device shown in Figure 5 (b) and received lateral ventricle injections of trace amounts of either H^3NE, $H^3$5-HT, both H^3NE and C^{14}5-HT, or C^{14}U (Amersham-Searle). Figure 5 (c) illustrates the injection proceedure. Amounts of the compounds injected varied from 1.55 micrograms to 3.87 micrograms for NE, from 0.03 milligrams to 0.06 milligrams for 5-HT, and from 10 micrograms to 15 micrograms for U. Volumes injected were either 20, 30, or 50 microliters of solution.

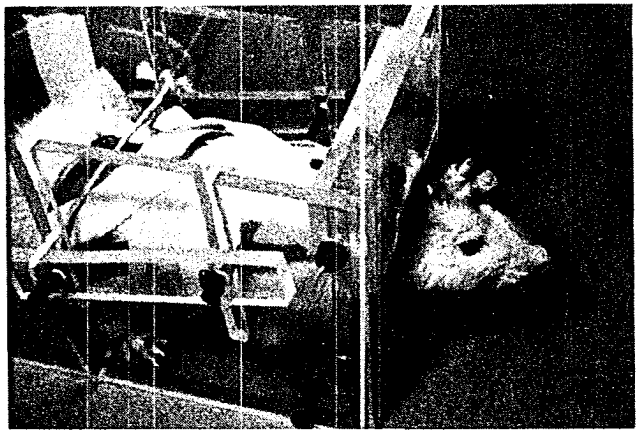
Recent studies (Glowinski & Iversen, 1966; Iversen & Glowinski, 1966; Palaic, Page, and Khairallah, 1967; Axelrod & Inscoe, 1963; and Chase, Katz, & Kopin, 1969) have demonstrated that labeled NE and labeled 5-HT injected in this manner mix with the endogenous stores and are probably released in a manner analogous to the naturally occurring monoamines.

Forty-five minutes after the ventricle injection, the

Figure 5 - An animal during behavioral testing and push-pull perfusion. (a) Shows the rat in the evaluation runway. It is remaining in the end of the runway where it is receiving ESB at the rate of one train every second. (b) Shows the animal which has been placed in the restraining device prior to isotope injection. The device allows some movement while the animal's head is relatively immobile and is easily accessible. (c) Shows the animal being injected with trace amounts of H^3NE. (d) Shows the rat during push-pull perfusion. The electrode connector and the push-pull cannula have been screwed in.



(a)



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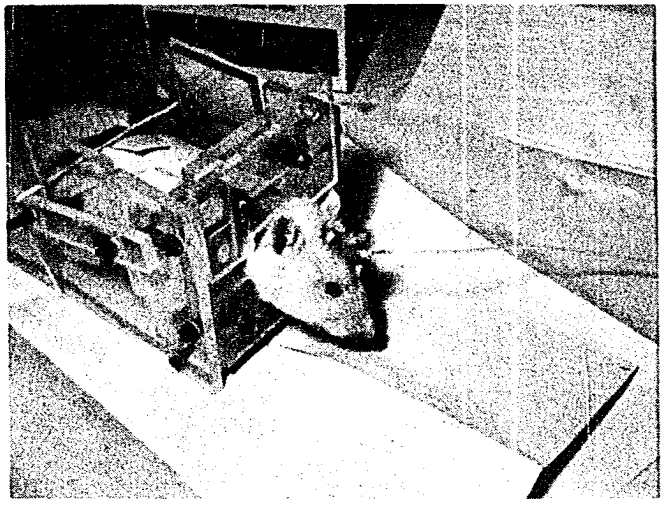
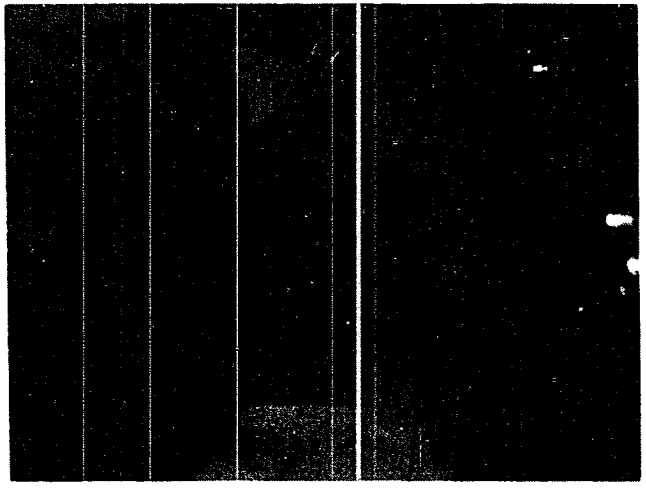


(c)



(d)

Figure 5

animal was placed in a dimly illuminated and sound attenuated chamber. The tubing of the push and pull cannulae were attached to syringes on a Harvard infusion-withdrawal pump and the flow of the solution through the push-pull cannula was initiated and checked for uniformity of flow. The push-pull cannula was inserted into the guide cannula and perfusion of the amygdala was begun. Figure 5 (d) shows an animal being perfused.

The push-pull cannula (seen in figure 6) consisted of concentrically arranged 22 and 28 gauge stainless steel tubing. Locke-Ringer's solution flowed down the inner tube under positive pressure, was exposed to the amygdaloid tissue, and flowed up the outer tube under negative pressure. A portion of the released neurohumor is picked up in the solution and carried out the push-pull cannula to be collected in a syringe on the pump. The rate of perfusion was 0.15 ml per minute or 9 ml per hour.

The amygdala was perfused in this manner for a period of five to seven hours. For all animals, except those in the group receiving no stimuli during perfusion, there were three stimulation treatment periods, each lasting approximately 45 minutes. During the first of these (ESB-1), animals were given ESB at a train rate of one per second using the same parameters as during the behavioral evaluation proceedure. The current was set slightly higher than threshold level. During the second stimulation period (ESB-2) all

Figure 6 - Construction of the push-pull cannula. During the perfusion the push-pull cannula shown on the left is inserted and screwed into the chronically implanted guide cannula shown on the right. Locke-Ringer's solution is infused through the push or inner cannula under positive pressure. The fluid is exposed to brain tissue at the tip of the cannula and picks up released neurohumors. The solution is then withdrawn through the pull or outer cannula under negative pressure.

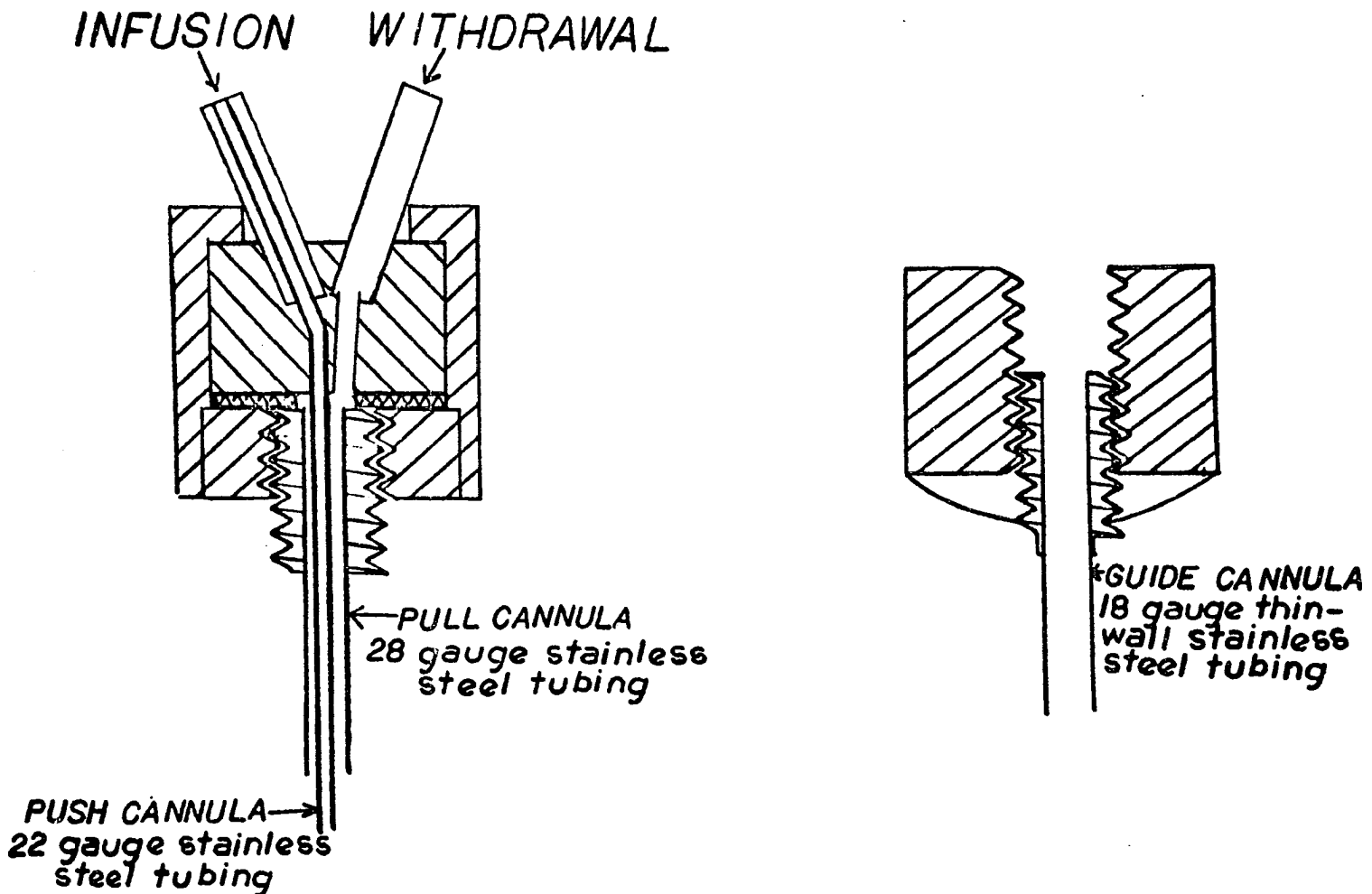

INFUSION
WITHDRAWAL
PULL CANNULA
28 gauge stainless steel tubing
PUSH CANNULA
22 gauge stainless steel tubing
*GUIDE CANNULA
18 gauge thin-wall stainless steel tubing

Figure 6

animals were given the same ESB trains but at a higher current intensity. During the last stimulation period (AVS) animals were subjected to intermittant lights and tones at the same rate they received the ESB. For all animals except three which were in the NES,R group the stimulation periods alternated with background control periods (B) of no stimulation, also lasting approximately 45 minutes. The other three animals received one hour B control periods before and after the three sucessive stimulation treatment periods.

During the perfusion, samples of the perfusate were collected every fifteen minutes. Five hundred microliters of each sample was added to 15 ml of Triton X-100 counting solution and was counted in a liquid scintillation counter to determine the amount of radioactivity present in each sample.

Occasionally during the perfusion of some animals the cannula would cease to function properly because of clogging, producing a reduction in the rate of flow of the solution. When this occurred the push-pull cannula was removed, cleaned and reinserted. After perfusion was restarted the treatment condition underway at the onset of flow retardation was usually reinitiated.

At the end of the perfusion period all animals were perfused with formalin. Later, the brains were removed and histological examination of the location of the implants was performed on all animals.

CHAPTER III

RESULTS

The data will be examined first for individual animals, second for general characteristics of the background release function, third for differences in release induced by stimulation in the single and double labeled groups, and finally for stimulation effects in the labeled groups combined.

Correction of Data

Each sample obtained from the push-pull cannula perfusion was counted in a liquid scintillation counter. The results were corrected for quenching. In addition where the sample time deviated slightly from the nominal fifteen minutes the data were corrected to a standard sampling period of fifteen minutes. Data were also corrected for the exact amount of perfusate obtained in each sample if the amount differed from the standard 2.2 ml.

Individual Results

Figures 7-9 present raw data from representative individual animals in counts per minute (CPM) to illustrate the overall shape of the release curves of the injected

Figure 7 - Individual results of an animal in the rewarding group: NE & 5-HT. This is a plot of the raw data in CPM of animal # BA-37. This animal spent 82% of its time in the ESB side of the evaluation runway when the current was set at 150 microamps. It was injected with 20 microcuries (1.55 micrograms) of H^3NE and 4.6 microcuries (0.03 milligrams) of C^{14}5-HT. During the ESB-1 stimulation period when the current was set at 150 microamps a slight potentiation of release of NE and inhibition of release of 5-HT can be seen. During the ESB-2 stimulation when the current was set at 200 microamps there was a potentiation of release of both NE and 5-HT. During the AVS period when lights and tones were presented there was very little release or inhibition of release of NE or 5-HT.

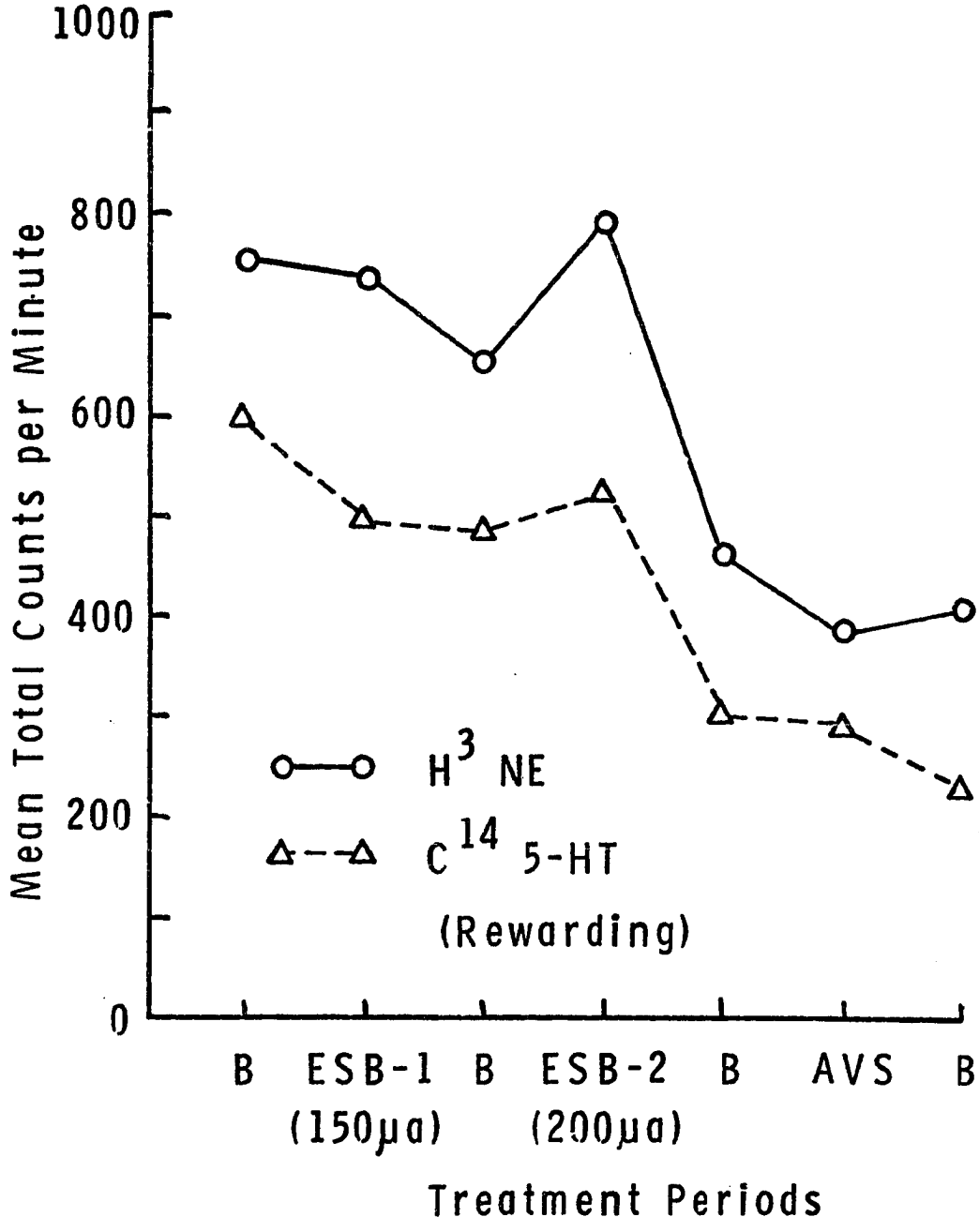

1000
800
600
400
200
0
Mean Total Counts per Minute
H3 NE
C14 5-HT
(Rewarding)
B
ESB-1
(150μa)
B
ESB-2
(200μa)
B
AVS
B
Treatment Periods

Figure 7

Figure 8 - Individual results of an animal in the non-rewarding group: 5-HT. This is a plot of the raw data in CPM of animal # B-51. This animal spent 22% of its time in the ESB side of the evaluation runway when the current was set at 150 to 400 microamps. It was injected with 75 microcuries (0.06 milligrams) of $H^3$5-HT. During both the ESB-1 period with the current set at 250 microamps and ESB-2 with the current set at 300 microamps there was an inhibition of release of 5-HT. During the AVS period when lights and tones were presented there was no release or inhibition of release.

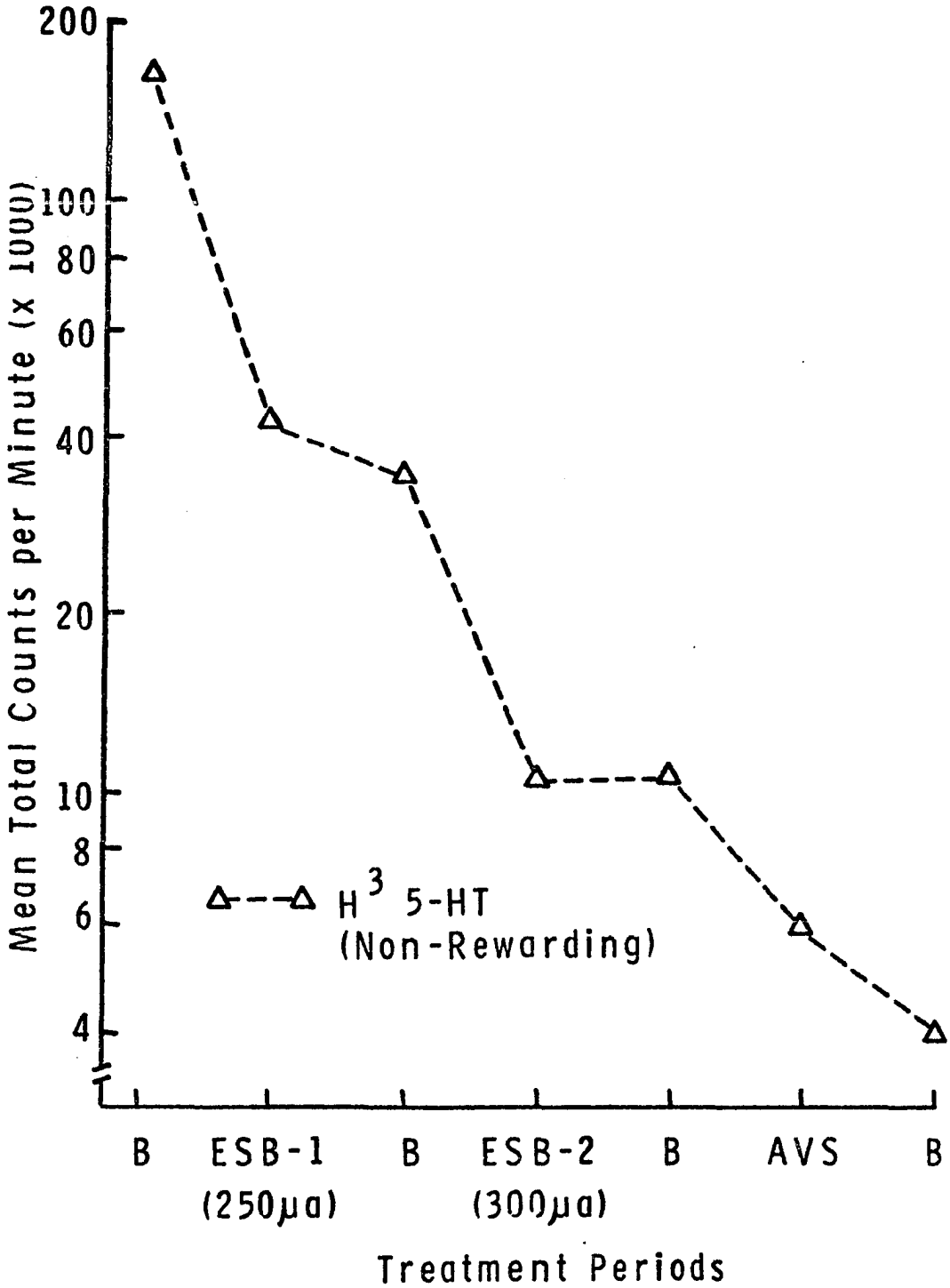

200
100
80
60
40
20
10
8
6
4
Mean Total Counts per Minute (x 1000)
H 3 5-HT
(Non-Rewarding)
B
ESB-1
(250μa)
B
ESB-2
(300μa)
B
AVS
B
Treatment Periods

Figure 8

Figure 9 - Individual results of an animal in the rewarding group: urea. This is a plot of the raw data in CPM of animal # C-44. This animal spent 86% of its time in the ESB side of the evaluation runway when the current was set at 250 microamps. In addition its asymptotic self-stimulation rate was 3220 responses per hour when the current was set at 300 microamps. It was injected with 10 microcuries (10micrograms) of urea. There was no release or inhibition of release of urea during either the ESB-1 or ESB-2 stimulation periods with the current set at 250 and 300 microamps respectively. A slight inhibition of release of urea is apparent during the AVS stimulation period when lights and tones were presented.

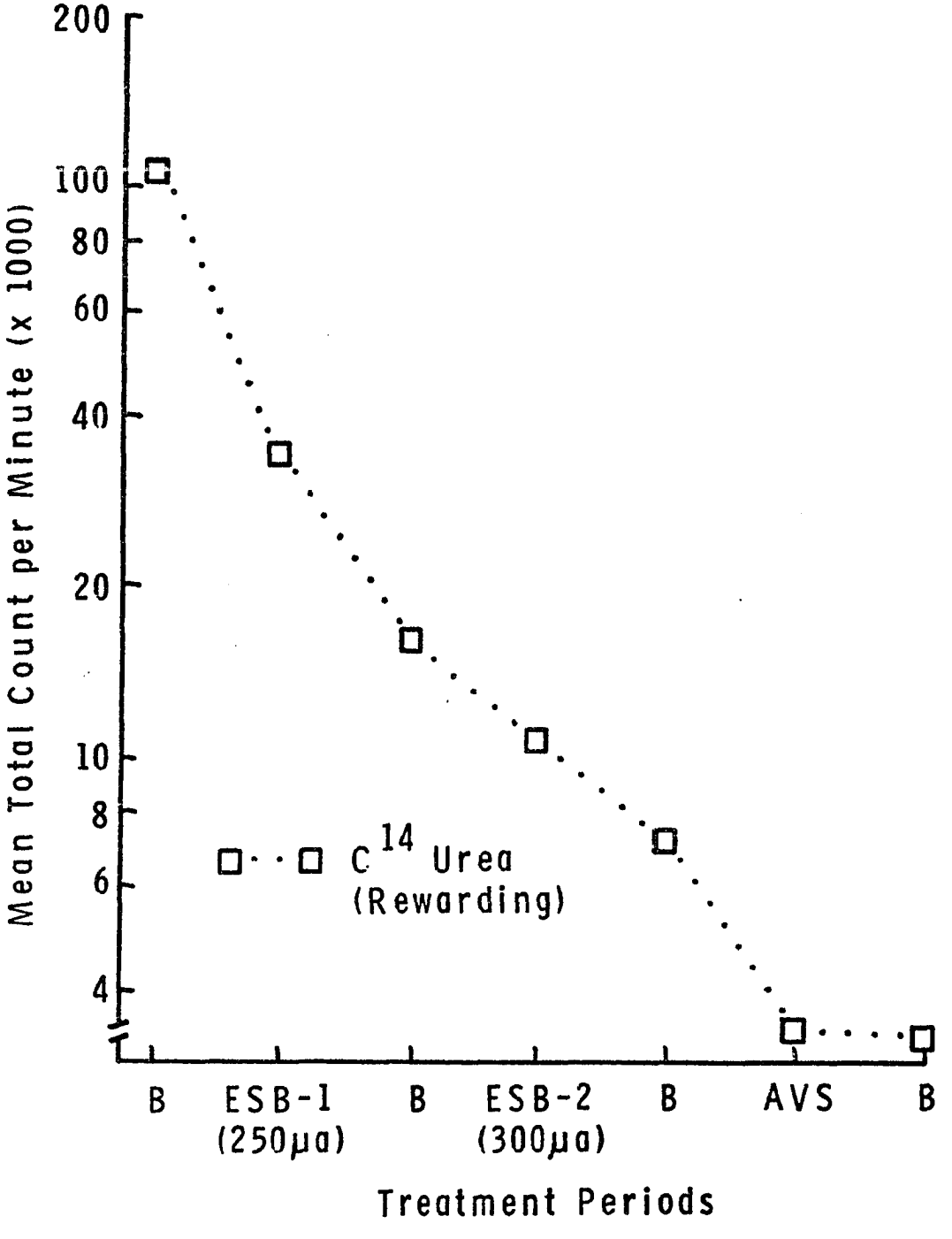

200
100
80
60
40
20
10
8
6
4
Mean Total Count per Minute (x 1000)
C 14 Urea
(Rewarding)
B
ESB-1
(250μa)
B
ESB-2
(300μa)
B
AVS
B
Treatment Periods

Figure 9

compounds. Figure 7 presents the data obtained on an animal injected with both H^3NE and C^{14}5-HT in the R group. This animal had spent 82 per cent of the time in the evaluation runway on the side which delivered ESB (current intensity set at 150 microamps). The total amount of H^3NE injected was 1.55 micrograms while the total amount of C^{14} 5-HT was 0.03 milligrams. This figure illustrates that there was a rapid fall in the background rate of release of both NE and 5-HT, presumably due to the radioisotope's disappearance from the CNS. The background release for this subject is nearly linear and the mean CPM is low (604 with a standard deviation of 176 for NE, and 425 with a standard deviation of 139 for 5-HT). It can be seen that the curves for NE and 5-HT are very similar. It should be noted that during the ESB-1 period when the current intensity is close to threshold for rewarding effects there appears to be a slight potentiation of NE release and a slight inhibition of 5-HT release. During the ESB-2 period, however, with the current 50 microamps higher, there is substantial release of both 5-HT and NE. During the AVS period there appears to be a slight inhibition of NE and a slight release of 5-HT over background levels.

Figure 8 presents the data obtained on an animal injected with $H^3$5-HT in the NR group. This animal's electrode was classified as NR because he had spent only 22 per cent of his time on the ESB side of the evaluation

runway where current intensities ranged from 150 to 400 microamps. He was injected with 0.06 milligrams of $H^3$5-HT. Again there is a rapid fall in the background rate of release. The mean level of CPM is much higher than in the preceeding animal (39,789 and a standard deviation of 61,941). It should be noted that data for this animal are plotted on a logarithmic scale because the rate of background release changes considerably over time. When plotted in this manner, the log-rate of background release is nearly linear over time. There appears to be an inhibition of release of 5-HT during both ESB-1 and ESB-2 but not during AVS.

Figure 9 presents the data obtained on a control animal injected with $C^{14}U$ in the R group. This animal was tested in both the Skinner box bar-press task and in the evaluation runway. He had spent 86 per cent of the time on the ESB side of the runway (at a current intensity of 250 microamps). In addition his rate of SS was 3220 responses per hour (at 300 microamps current intensity). Again the rate of background release changes over time but is nearly linear when plotted on a logarithmic scale. The mean level of CPM was also high in this animal (25,899 with a standard deviation of 49,459). Neither the ESB-1 nor ESB-2 caused detectable potentiation or inhibition of release but during AVS, a slight inhibition of release may have occurred.

Grouped Data

In general, when an animal was injected with both NE and 5-HT the background release declined faster for 5-HT than for NE, and in most cases there was an exponential decline in the rate of release for both amines.

Nature of Background Release

Figure 10 is a graph of all background points averaged over all animals. The data are portrayed in three different ways. First, there is a plot of raw CPM scores converted to standard scores. Second, there is a semi-logarithmic plot of the same data using the same standard scores on the y-axis and the log of the sample number (which is an index of time) on the x-axis. Third, there is a log-log plot using the logs of standard scores of CPM on the y-axis and the log of the sample number on the x-axis. The correlation coefficient for each line is also given. It is evident that the plot of standard scores vs. the sample number produces a curvilinear relationship. The semi-logarithmic plot produces a relationship nearly linear until the last point. The log-log plot is relatively linear when compared with the semi-log plot but is perhaps not as good a fit as the semi-log relationship. The correlation coefficient for the semi-log plot also indicates that it is probably the best fit in terms of a straight line, although the log-log relationship is virtually as good. Therefore, the rate of background release of these compounds is most probably a

Figure 10 - Three plots of background control data. For each of these plots every background data point for every animal was included. The plot keyed as "raw" refers to a plot of the raw standard scores on the y-axis vs. the sample number (an index of time) on the x-axis. The plot keyed as "Semi-log" refers to a plot of the raw standard scores on the y-axis vs. the log of the sample number on the x-axis. The plot keyed as "Log-log" refers to a plot of the log of the raw standard scores on the y-axis vs. the log of the sample number on the x-axis. The R's indicate that the Semi-log plot is probably the best fit of the data for a straight line. It is apparent that it is the best fit especially for the first three points.

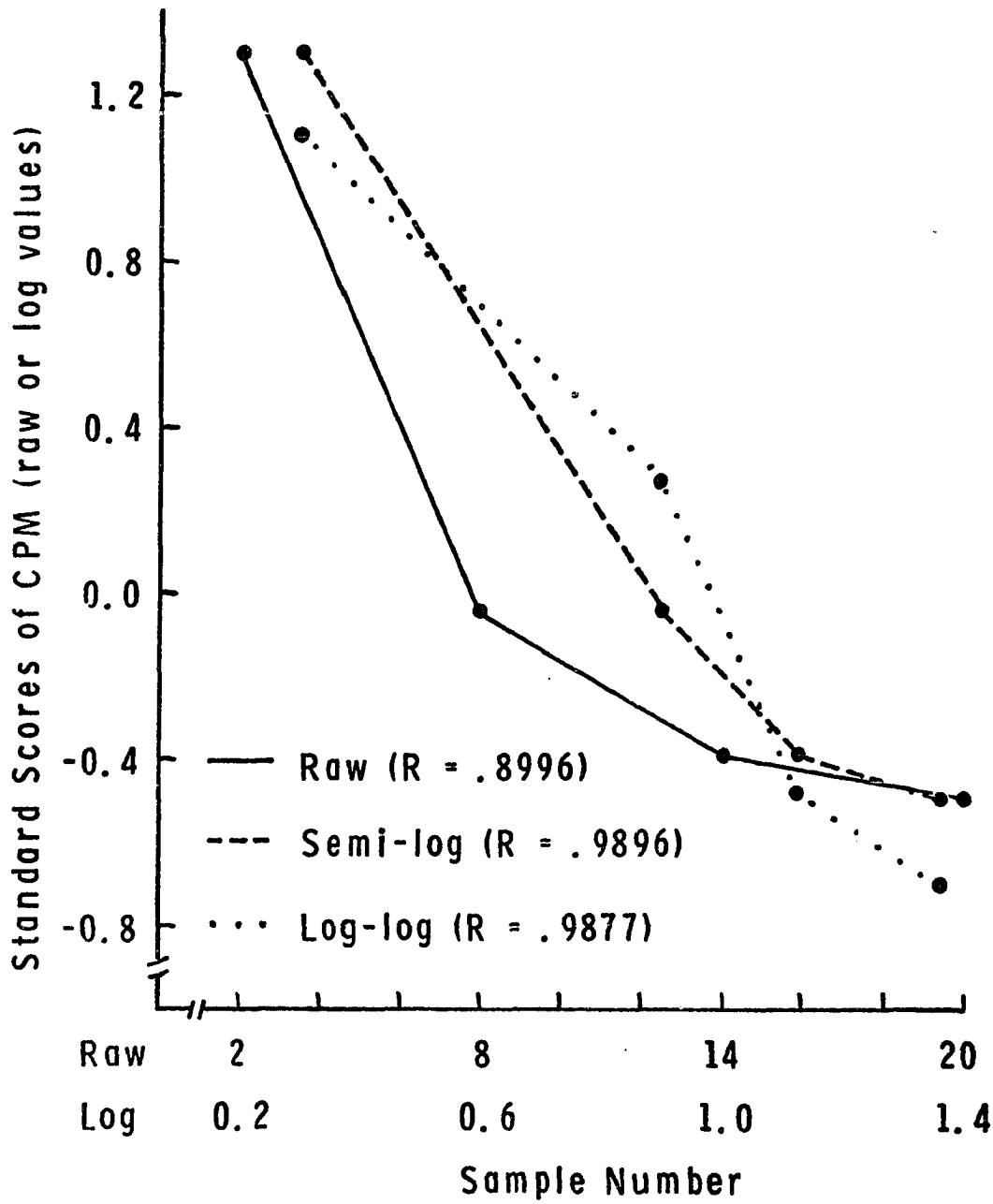

Standard Scores of CPM (raw or log values)
1.2
0.8
0.4
0.0
-0.4
-0.8
Raw (R = .8996)
Semi-log (R = .9896)
Log-log (R = .9877)
Raw 2 8 14 20
Log 0.2 0.6 1.0 1.4
Sample Number

Figure 10

semi-logarithmic one.

Plots of Means

Figures 11-13 are graphs of the means of standard scores of CPM per sample of perfusate. First the CPM scores for each animal were converted to standard scores. Then the mean of the standard scores for each treatment in the R,NR, or control groups was determined. It is evident that during ESB-2 for the R group, release of both NE and 5-HT was potentiated. In addition, it appears that there was an inhibition of release for ESB-2 in the NR groups. To evaluate the statistical significance of these treatment effects, residual change scores were computed for each animal, for each treatment condition.

Expected Minus Observed Difference Scores (Residual Change Scores)

Due to the large variation in the level of CPM between animals and the exponential disappearance of the compounds from the CNS, standard scores for each animal were derived from the distribution represented by the three samples of the control period prior to the stimulation period (C_1), the three samples during the stimulation period (S), and the three samples of the control period after the stimulation period (C_2). The mean of the before and after control periods was taken as an expected score, $\bar{E} = \frac{C_1 + C_2}{2}$. Difference scores were then computed between

Figure 11 - Plots of the means of standard scores of CPM for each group and treatment for NE. Mean = 0, S.D. = 1.0

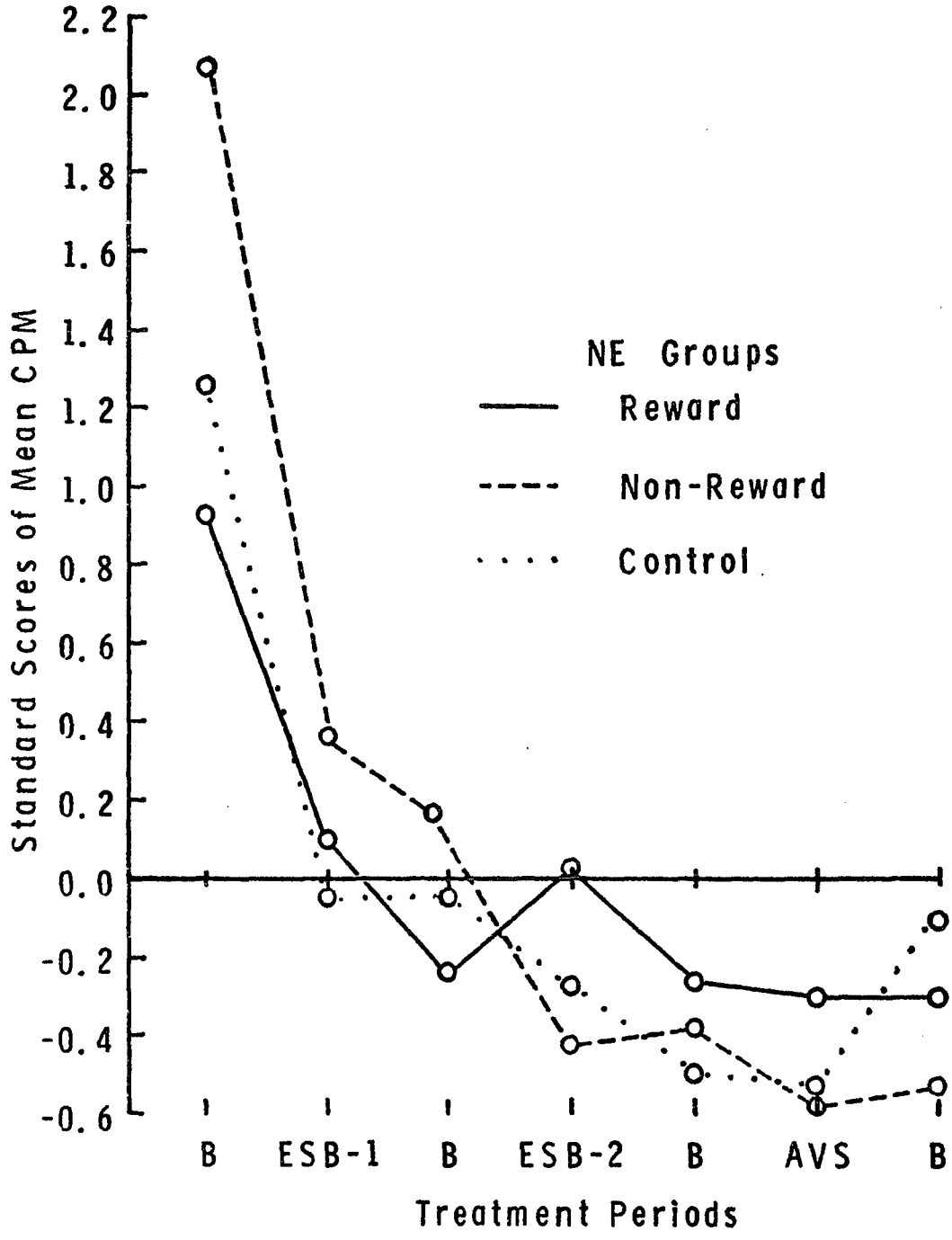

2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Standard Scores of Mean CPM
NE Groups
Reward
Non-Reward
Control
B
ESB-1
B
ESB-2
B
AVS
B
Treatment Periods

Figure 11

Figure 12 - Plots of the means of standard scores of CPM for each group and treatment for 5-HT. Mean = 0, S.D. = 1.0

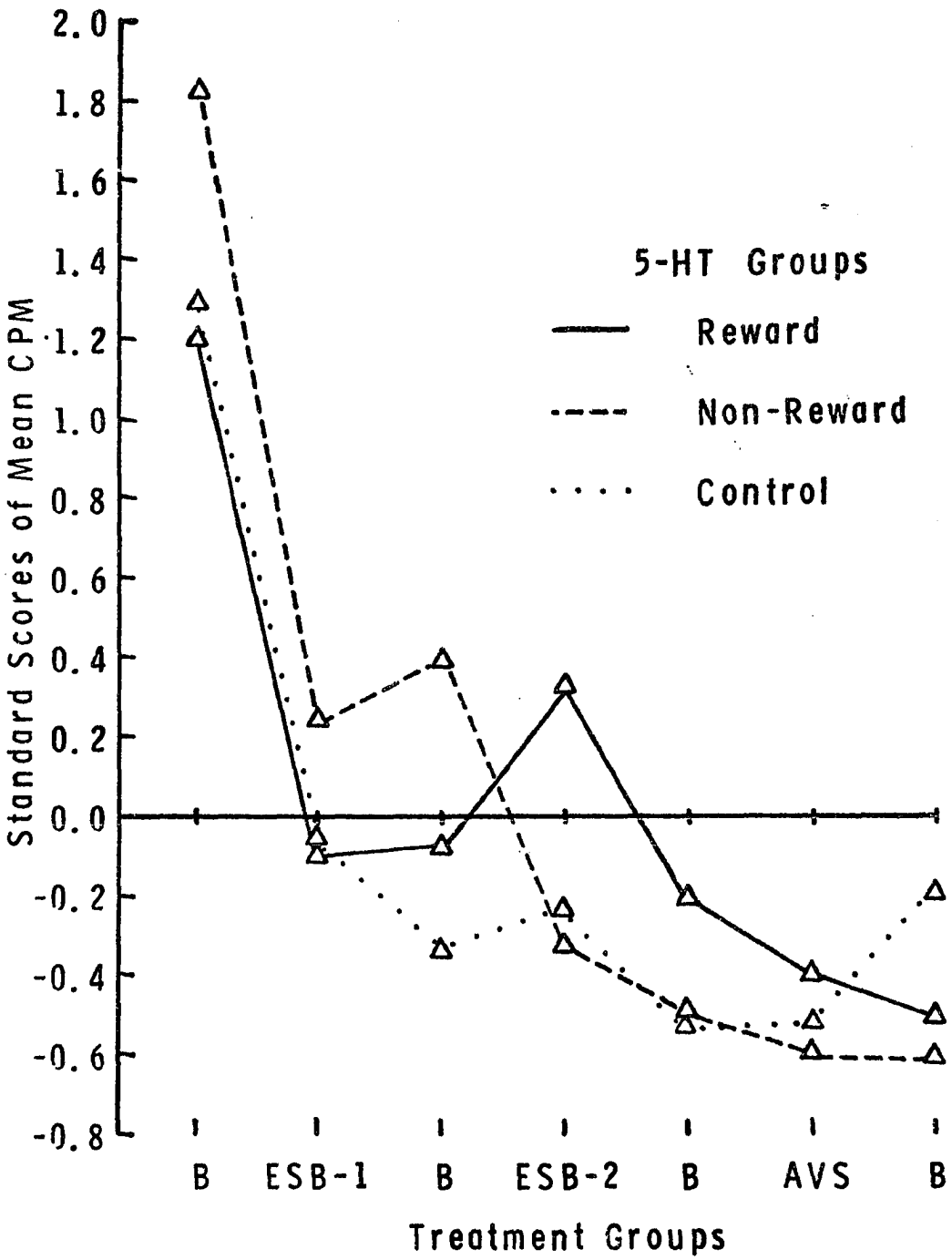

2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Standard Scores of Mean CPM
5-HT Groups
Reward
Non-Reward
Control
B
ESB-1
B
ESB-2
B
AVS
B
Treatment Groups

Figure 12

Figure 13 - Plots of the means of standard scores of CPM for each group and treatment for urea. Mean = 0, S.D. = 1.0

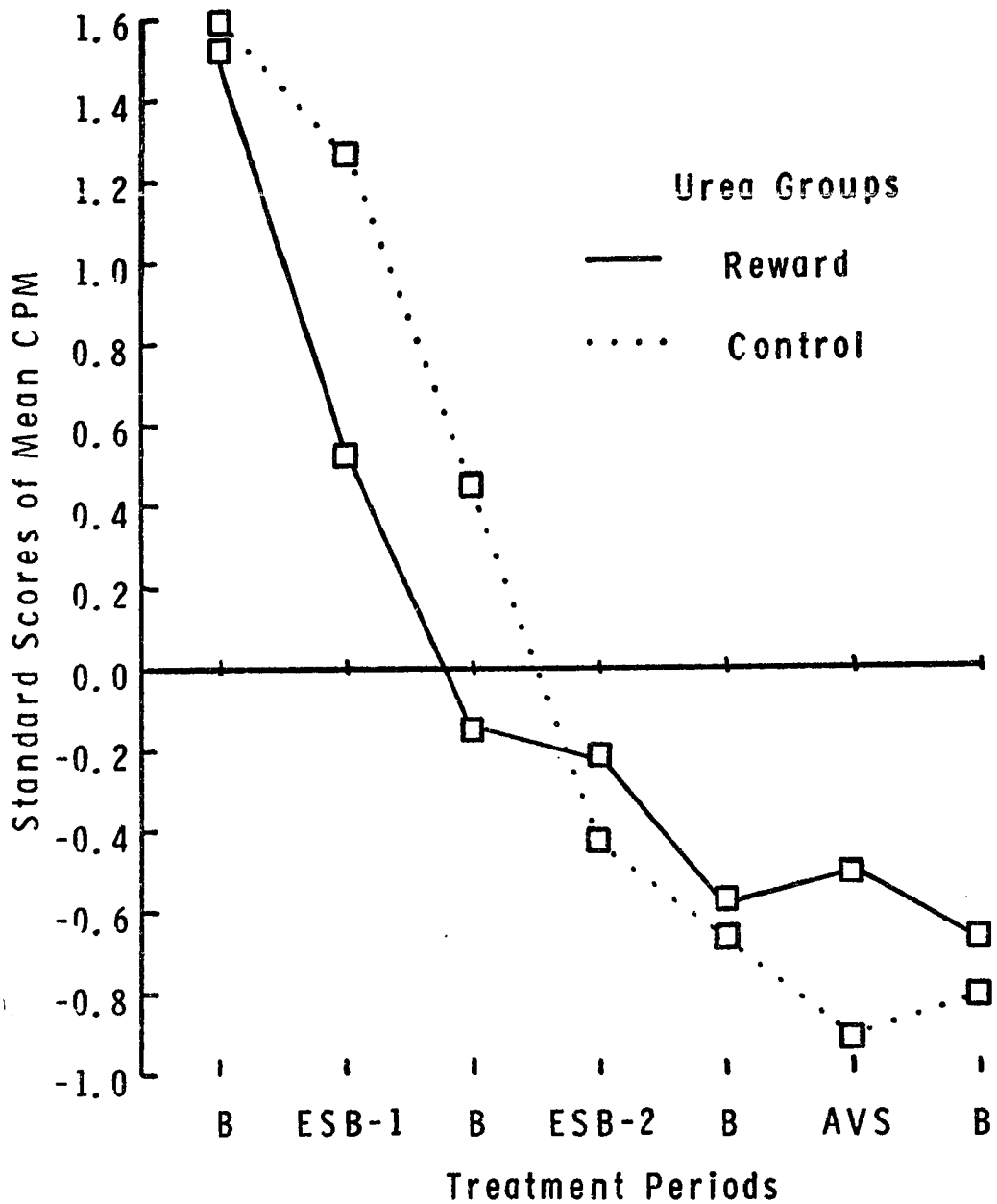

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
Standard Scores of Mean CPM
Urea Groups
Reward
Control
B
ESB-1
B
ESB-2
B
AVS
B
Treatment Periods

Figure 13

the expected and observed means $\bar{D}_r = \bar{S} - \bar{E}$. A positive residual change score indicates potentiation and a negative score indicates inhibition of release of a compound. The t test was used to assess the significance of the observed minus expected or residual change scores ($\bar{D}_r$).

Single vs. dual labeled data. Figures 14 and 15 present the $\bar{D}_r$ scores and standard errors for the three treatment conditions ESB-1, ESB-2, and AVS for single vs. dual labeled animals (NES vs. NE+5-HT for NE and 5-HTS vs. NE + 5-HT for 5-HT). Figure 14 shows the data for R placements and figure 15 shows data for NR placements. Zero mu t tests for these data indicate that the only significant effects were an inhibition of release of 5-HT during ESB-1 for the R, dual labeled group; release of NE during ESB-2 for the R, single labeled group; and an inhibition of release of NE for the NR, dual labeled group.

T tests for independent means indicate that there was no significant difference for any dual vs. single labeled group during any ESB period (t for ESB-1, NE, R = 1.06, df = 5; t for ESB-2, NE, R = 0.25, df = 6; t for ESB-1, 5-HT, R = 0.84, df = 3; t for ESB-2, 5-HT, R = -0.09, df = 4; t for ESB-1, NE, NR = 2.77, df = 2; not computable for ESB-2, NE, NR; t for ESB-1, 5-HT, NR, = 3.09, df = 2; t for ESB-2, 5-HT, NR = -0.10, df = 2). Therefore in further data analysis the single and dual labeled data for any one amine were combined and treated as

Figure 14 - Expected minus observed difference scores (residual change scores) for single vs. dual labeled animals for rewarding groups. *$p<0.10$, **$p<0.05$ Numbers in parentheses indicate the number of animals in each group.

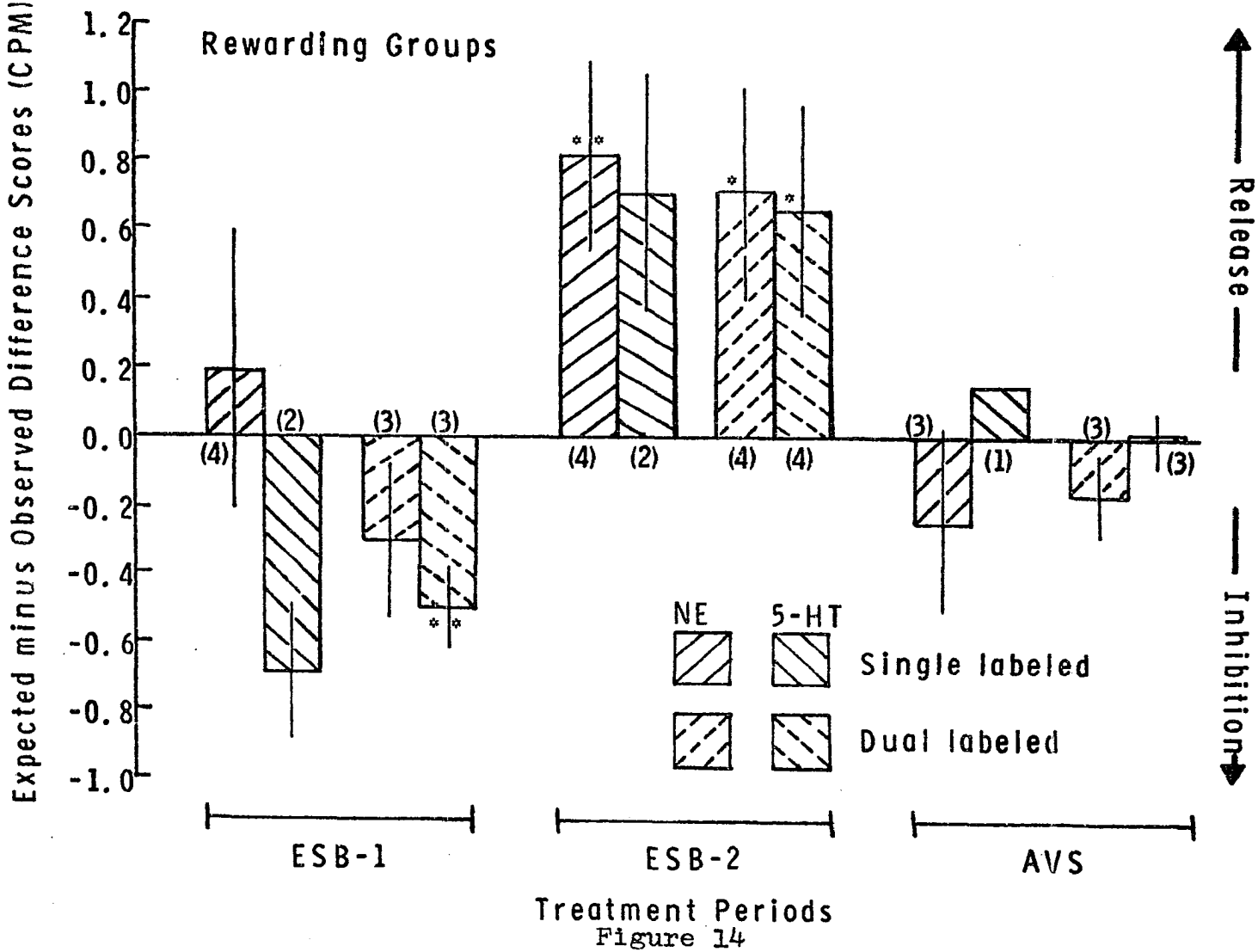

Rewarding Groups
Expected minus Observed Difference Scores (CPM)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
(4)
(2)
(3)
(3)
(4)
(2)
(4)
(4)
(3)
(1)
(3)
(3)
NE
5-HT
Single labeled
Dual labeled
Release
Inhibition
ESB-1
ESB-2
AVS
Treatment Periods

Figure 14

Figure 15 - Expected minus observed difference scores (residual change scores) for single vs. dual labeled animals for non-rewarding groups. *p<0.10, ****p<0.01 Numbers in parentheses indicate the number of animals in each group.

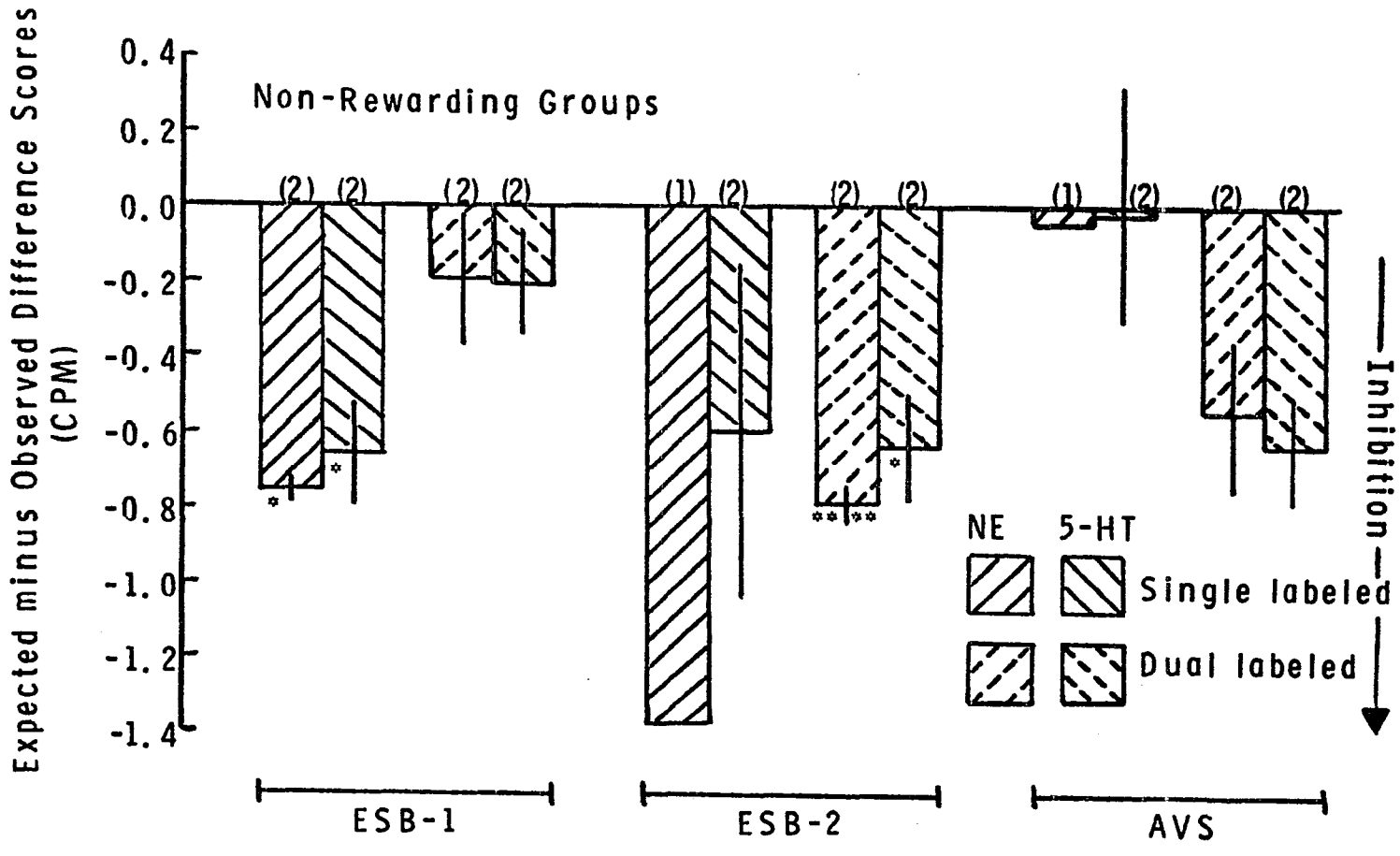

Non-Rewarding Groups
Expected minus Observed Difference Scores (CPM)
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
(2) (2) (2) (2) (1) (2) (2) (2) (1) (2) (2) (2)
NE 5-HT
Single labeled
Dual labeled
Inhibition
ESB-1
ESB-2
AVS
Treatment Periods

Figure 15

one group.

Combined data. Table 2 presents the combined data ($\bar{D}_r$) for each group, the number of subjects in that group, the standard error of the mean difference, and the zero mu t with its level of significance. The same data are represented in figures 16-18.

Combined data: zero mu t tests. In the R group there was a significant release of NE during ESB-2 at the higher current intensity, but not during ESB-1 when the current intensity was only slightly above threshold levels for rewarding effects.

There was a significant release of 5-HT during ESB-2 and contrary to the expected result, a significant inhibition of release of 5-HT during ESB-1 at the lower current level.

In the NR group there was a significant inhibition of release of both NE and 5-HT during both ESB-1 and ESB-2. No significant changes in the perfusate radioactivity of any compound were obtained during the AVS. Further, any increases or decreases in the perfusate radioactivity of U during ESB-2 and ESB-1 respectively were not significantly different from zero.

For the control groups there was a significant inhibition of release for ESB-1 for both NE and 5-HT. As will be discussed later, this inhibition may be more apparent than real. No other stimulation period produced any significant differences.

Combined data: R vs. NR. Table 3 presents the data

TABLE 2

RESIDUAL CHANGE SCORES ($\bar{D}_r$) FOR TREATMENT CONDITIONS FOR EACH COMPOUND

	Number of Animals	Mean	Standard Error of the Mean Difference	Zero Mu T
NE,R,ESB-1	7	-0.03	0.26	-0.12
NE,R,ESB-2	8	0.78	0.23	3.41[d]
NE,R,AVS	6	-0.22	0.14	-1.56
5-HT,R,ESB-1	5	-0.58	0.10	-5.72[h]
5-HT,R,ESB-2	6	0.66	0.23	2.84[c]
5-HT,R,AVS	4	0.04	0.06	0.63
NE,NR,ESB-1	4	-0.47	0.17	-2.41[b]
NE,NR,ESB-2	3	-0.97	0.21	-4.59[c]
NE,NR,AVS	3	-0.39	0.21	-1.80
5-HT,NR,ESB-1	3	-0.53	0.21	-2.58[b]
5-HT,NR,ESB-2	4	-0.97	0.21	3.14[b]
5-HT,NR,AVS	4	-0.33	0.25	-1.35
U,R,ESB-1	4	-0.16	0.31	-0.54
U,R,ESB-2	4	0.52	0.28	1.83
U,R,AVS	4	0.31	0.55	0.57
NE,C,ESB-1	3	-0.53	0.05	-9.82[c]
NE,C,ESB-2	2	-0.02	0.44	-0.06
NE,C,AVS	1	0.19		
5-HT,C,ESB-1	3	-0.42	0.07	-6.21[f]
5-HT,C,ESB-2	2	0.04	0.43	0.07
5-HT,C,AVS	1	-0.71		
U,C,ESB-1	1	0.40		
U,C,ESB-2	1	-0.47		
U,C,AVS	1	-1.29		

[a] $p<0.10$, [b] $p<0.05$, [c] $p<0.025$, [d] $p<0.01$ for one-tailed test
[e] $p<0.10$, [f] $p<0.05$, [g] $p<0.02$, [h] $p<0.01$ for two-tailed test

Figure 16 - Expected minus observed difference scores (residual change scores) for all groups for the first stimulation period (ESB-1). **p<0.05, ***p<0.02, ****p<0.01 Numbers in parentheses indicate the number of animals in each group.

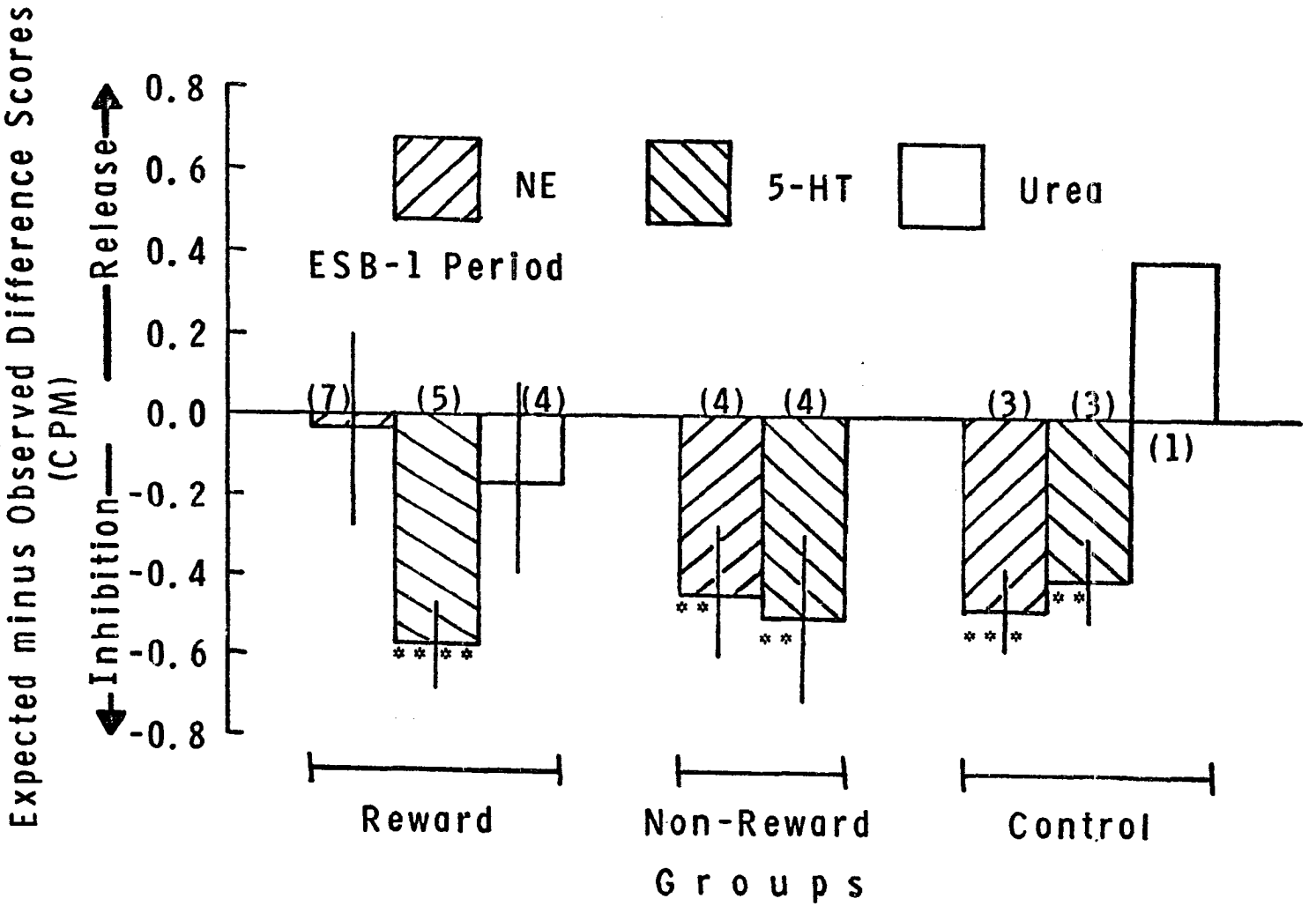

Expected minus Observed Difference Scores
(CPM)
Release
Inhibition
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
NE
5-HT
Urea
ESB-1 Period
(7)
(5)
(4)
(4)
(4)
(3)
(3)
(1)
Reward
Non-Reward
Control
Groups

Figure 16

Figure 17 - Expected minus observed difference scores (residual change scores) for all groups for the second stimulation period (ESB-2) **p<0.05, ***p<0.25, ****p<0.01 Numbers in parentheses indicate the number of animals in each group.

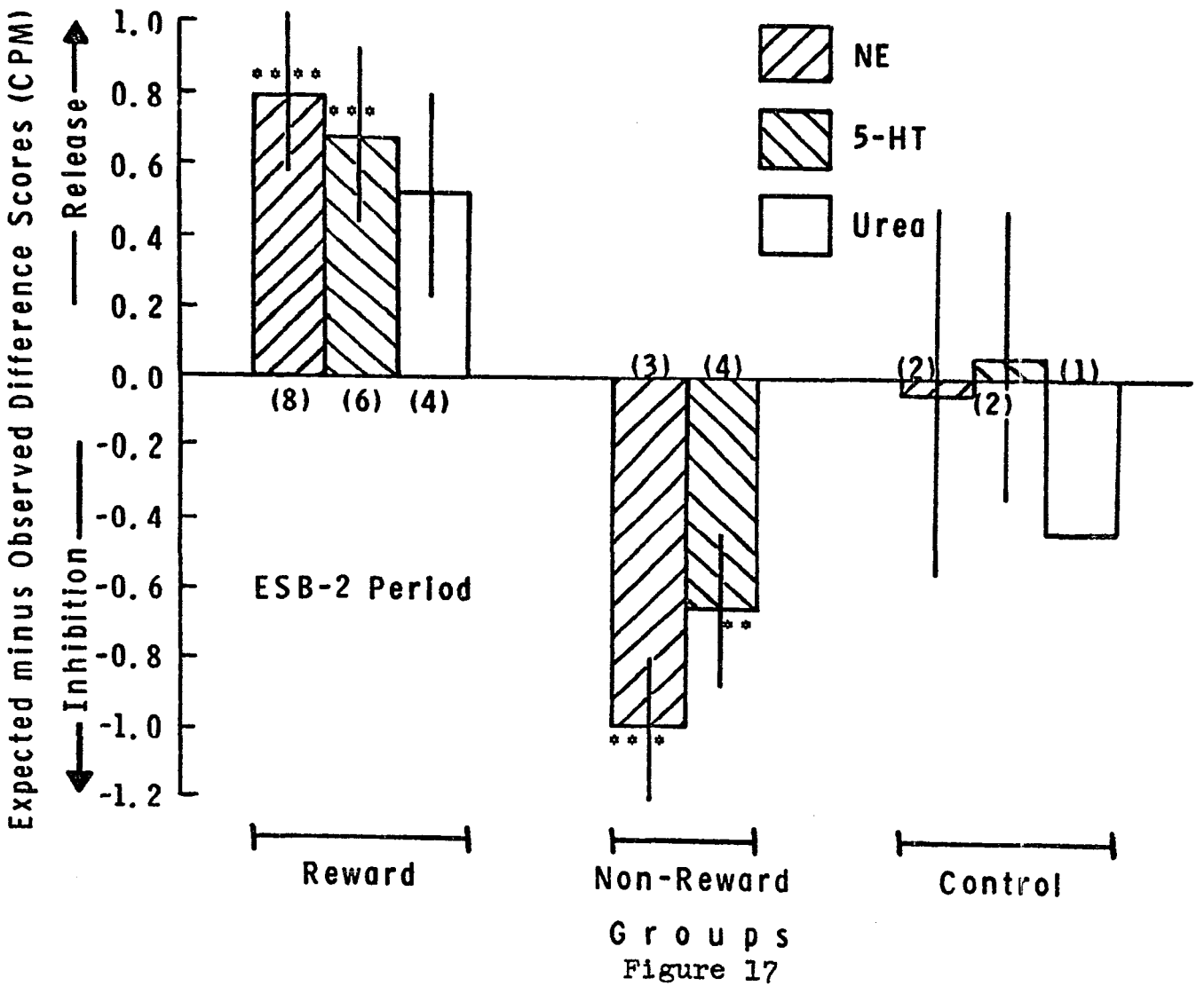

Expected minus Observed Difference Scores (CPM)
Release
Inhibition
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
NE
5-HT
Urea
(8) (6) (4)
(3) (4)
(2) (2) (1)
ESB-2 Period
Reward
Non-Reward
Control
Groups

Figure 17

Figure 18 - Expected minus observed difference scores (residual change scores) for all groups for the third stimulation period (AVS). None of the differences are significant. Numbers in parentheses indicate the number of animals in each group.

Expected minus Observed Difference Scores
(CPM)
—Release—
—Inhibition—
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
NE
5-HT
Urea
(6)
(4)
(4)
(3)
(4)
(1)
(1)
AVS Period
Reward
Non-Reward
Control
Groups

Figure 18

TABLE 3

T'S FOR BETWEEN GROUP COMPARISONS

Comparison	df	t	p
R vs. NR			
NE, ESB-1	9	1.50	<0.10
NE, ESB-2	9	5.62	<0.0005
5-HT, ESB-1	7	-0.59	N.S.
5-HT, ESB-2	7	5.19	<0.005
NE,R & 5-HT,R vs. U,R			
NE vs. U, ESB-1	9	0.34	N.S.
NE vs. U, ESB-2	10	0.71	N.S.
5-HT vs. U, ESB-1	7	-1.30	N.S.
5-HT vs. U, ESB-2	8	0.39	N.S.
ESB vs. AVS			
NE, R, ESB-1	4	0.28	N.S.
NE, R, ESB-2	5	5.04	<0.005
5-HT, R, ESB-1	2	-5.03	<0.025
5-HT, R, ESB-2	3	1.91	<0.10
NE, NR, ESB-1	4	-0.03	N.S.
NE, NR, ESB-2	3	-1.46	N.S.
5-HT, NR, ESB-1	3	-0.45	N.S.
5-HT, NR, ESB-2	3	-0.77	N.S.
ESB-1 vs. ESB-2			
NE, R	7	-3.42	<0.02
5-HT, R	4	-4.03	<0.02
NE, NR	4	4.79	<0.01
5-HT, NR	4	-0.36	N.S.

TABLE 3-- Continued

Comparison	df	t	p
NE & 5-HT vs. Control (C)			
NE, R vs. C, ESB-1	8	1.91	<0.10
NE, R vs. C, ESB-2	8	1.39	<0.20
5-HT, R vs. C, ESB-1	6	-1.37	N.S.
5-HT, R vs. C, ESB-2	6	1.29	N.S.
NE, NR vs. C, ESB-1	5	-0.38	N.S.
NE, NR vs. C, ESB-2	3	-1.94	<0.20
5-HT, NR vs. C, ESB-1	5	-0.54	N.S.
5-HT, NR vs. C, ESB-2	4	-1.39	N.S.

for between group comparisons. T tests for independent means indicate that there was a significant difference between the R group displaying NE release and the NR groups displaying inhibition of NE release for ESB-2. The level of significance for ESB-1 for the same comparison approached but did not reach statistical significance.

Likewise there was a significant difference between the R group displaying 5-HT release and the NR group displaying inhibition of 5-HT release for ESB-2 but not for ESB-1.

Combined data: NE & 5-HT vs. Urea. Contrary to expectations, there were no significant differences between NE, R and U, R groups or between 5-HT, R and U, R groups for any treatment.

Combined data: ESB vs. AVS. For the NE and 5-HT, R groups there was a significant difference between the ESB-2 periods, displaying release, and the AVS periods. For 5-HT, R groups the difference between ESB-1 periods displaying inhibition of release, and AVS periods was also significant. In the NR group none of the ESB vs. AVS comparisons were significant.

Combined data: ESB-1 vs. ESB-2. There was a significant difference between ESB-1 and ESB-2 periods for the NE, R and 5-HT, R groups with ESB-2 showing more release.

For the NE, NR group there was a significant differ-

ence between ESB-1 and ESB-2 with a greater inhibition of release during ESB-2. For the 5-HT, NR group there was no significant difference between ESB-1 and ESB-2. Both showed inhibition of release.

Combined data:NE & 5-HT vs. Control. For the NE, R group the release of NE over control group levels only approached significance, no other comparisons with control groups indicated any significant differences, perhaps due to the small number of animals in each group.

NE vs. 5-HT. Because of the mixture of correlated and uncorrelated scores for the NE and 5-HT treatments, the data cannot be combined to perform single t tests to determine the statistical significance of any differences between these groups. Therefore seperate t tests were carried out on the correlated and uncorrelated data. None of the t tests indicated any significant differences between NE and 5-HT groups for any treatment. One comparison between NE,R and 5-HT,R for ESB-1 approached significance ($t = 1.94$, $df = 4$, $p<0.10$). It is likely that with a larger N the difference might have been significant.

Combined data: non-parametric tests. In order to further access the magnitude of the treatment effects the Walsh test was used. This non-parametric test utilizes both the magnitude and sign of the difference scores. Table 4 presents the D_r scores for each animal in each group and the

TABLE 4

INDIVIDUAL D_r SCORES IN EACH GROUP AND RESULTS OF THE WALSH AND SIGN TESTS

Scores in Group	Walsh Test p	Sign Test p	Scores in Group	Walsh Test p	Sign Test p
NE,R,ESB-1	N.S.	0.500	5-HT,R,ESB-1	<0.062	0.031
-0.58			-0.91		
-0.54			-0.68		
-0.51			-0.54		
-0.31			-0.50		
+0.17			-0.29		
+0.25					
+1.31					
NE,R, ESB-2	<0.012	0.035	5-HT,R,ESB-2	<0.016	0.016
-0.12			+0.03		
+0.02			+0.31		
+0.56			+0.44		
+0.65			+0.52		
+0.89			+1.07		
+1.14			+1.59		
+1.44					
+1.67					
NE,R,AVS	N.S.	0.344	5-HT,R, AVS	N.S.	0.25
-0.54			-0.13		
-0.53			+0.03		
-0.44			+0.11		
-0.09			+0.14		
+0.02					
+0.28					
NE,NR,ESB-1	<0.062	0.0625	5-HT,NR,ESB-1	<0.62	0.0625
-0.75			-1.00		
-0.72			-0.72		
-0.39			-0.36		
-0.003			-0.05		

TABLE 4--Continued

Scores in Group	Walsh Test p	Sign Test p	Scores in Group	Walsh Test p	Sign Test p
NE,NR,ESB-2 -1.39 -0.77 -0.74		0.125	5-HT,NR,ESB-2 -1.08 -0.77 -0.54 -0.13	<0.62	0.0625
NE,NR,AVS -0.79 -0.32 -0.05		0.125	5-HT,NR,AVS -0.78 -0.53 -0.38 +0.37	N.S.	0.25
U,R,ESB-1 -0.75 -0.55 +0.03 +0.61	N.S.	0.50	U,C, ESB-1 +0.40		
U,R,ESB-2 -0.14 +0.27 +0.83 +0.83	N.S.	0.25	U,C,ESB-2 -0.47		
U,R,AVS -0.76 -0.38 +0.75 +1.63	N.S.	0.50	U,C,AVS +1.29		
NE,C,ESB-1 -0.62 -0.54 -0.43		0.125	5-HT,C,ESB-1 -0.54 -0.40 -0.31		0.125
NE,C,ESB-2 -0.46 +0.41		0.50	5-HT,C,ESB-2 -0.40 +0.46		0.50
NE,C,AVS -0.19			5-HT,C,AVS -0.71		

level of significance as determined by the Walsh test. In addition results of the non-parametric sign tests are given.

For the NE, R group only three of the seven scores indicate potentiation of release of NE for ESB-1 while seven of the eight scores indicate release for ESB-2. However, for the 5-HT, R group all five scores for the ESB-1 treatment indicate inhibition of release, and all six scores for the ESB-2 treatment indicate release of 5-HT. Therefore it appears that the effects for 5-HT are more consistent than they are for NE for the R groups although the difference is not great for ESB-2.

For the NR groups the signs of every score in every ESB treatment condition for both NE and 5-HT is negative indicating a very consistent inhibition of release of both NE and 5-HT.

None of the treatments in the U,R group had a significant effect. The signs for these groups are all mixed indicating no consistent effects.

Again none of the AVS treatment periods indicate any significant effects and the signs are mixed except for the NE, NR, AVS group in which all signs are negative with only three scores in that group.

For the control groups for the ESB-1 treatment period all scores are negative with only three scores in each group but for the ESB-2 treatment period the signs are mixed.

Histology

Figure 19 shows the placements of electrodes for all animals except one non-stimulation control animal whose electrode was apparently bent laterally. The brain tissue of this animal was damaged by removing the skull cap and exact histological location of the electrode tip was impossible. One NR electrode located in AP 4.0 was almost through the brain tissue.

Photographs of representative sections for some of the animals are shown in figure 20. Figure 20 (a), (b), and (c) show sections of animal BA-37 whose data were represented in figure 7. Part (a) shows the injecter cannula which does not quite reach the lateral ventricle and probably explains the low level of overall activity for this animal. This section also shows the placement of the push-pull cannula. Section (b) further shows the placement of the push-pull cannula, and section (c) shows the electrode placement. A typical lateral ventricle placement is seen in section (d). It is from animal C-44 whose data are represented in figure 9. Sections (e) and (f) show other typical placements of the push-pull cannula. Section (e) is from an animal injected with 5-HT in the R group and section (f) is from an animal injected with both NE and 5-HT in the NR group.

Figure 19 - Placements of the electrodes. All electrode placements (except one which is explained in the text) are indicated in this figure. Adapted from Pellegrino and Cushman (1967)

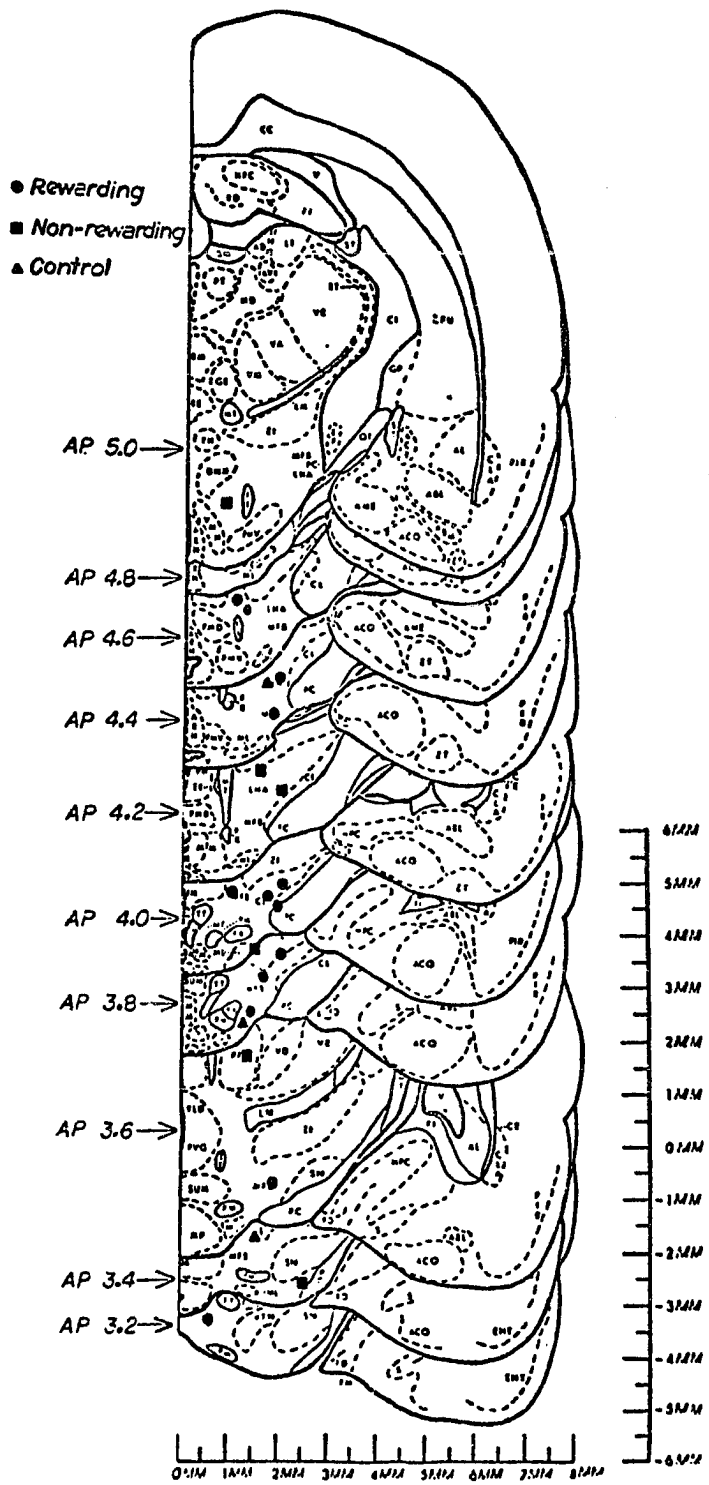
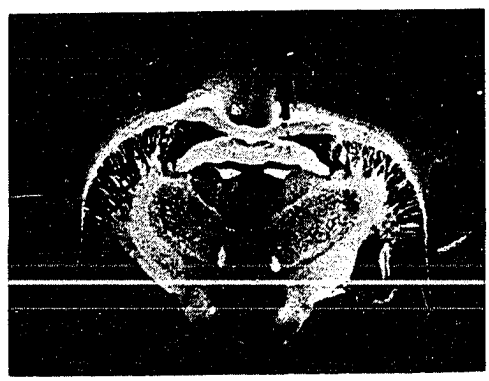
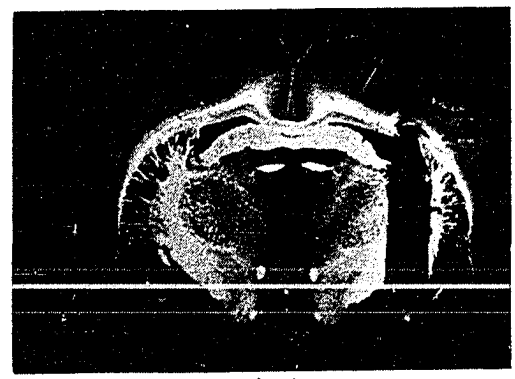

● Rewarding
■ Non-rewarding
▲ Control
AP 5.0
AP 4.8
AP 4.6
AP 4.4
AP 4.2
AP 4.0
AP 3.8
AP 3.6
AP 3.4
AP 3.2

Figure 19

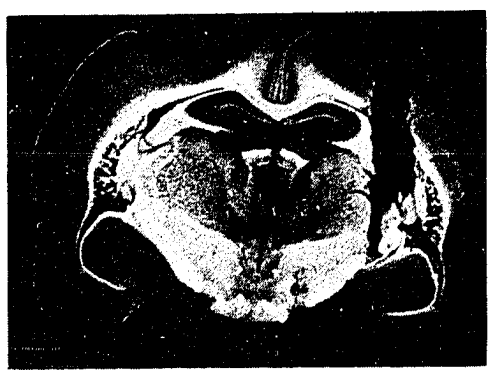
Figure 20 - Photographs of representative rat brain sections. (a) (b) and (c) illustrate the injecter cannula placement, the push-pull cannula placement, and the electrode placement respectively for animal # BA-37 whose raw data are presented in Figure 7. (d) is the placement of the injecter cannula for animal C-44 whose raw data are presented in Figure 9. (e) and (f) show the placements of push-pull cannulas in two other animals. Magnification = X3 3/4



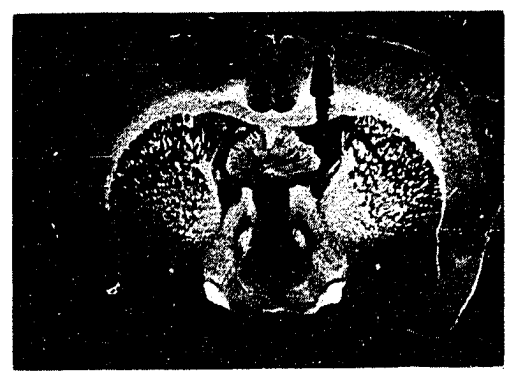
(a)



(b)



(c)



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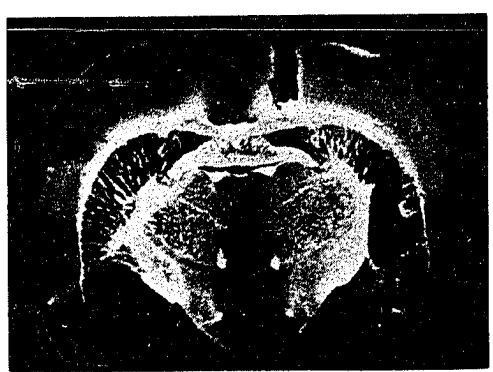
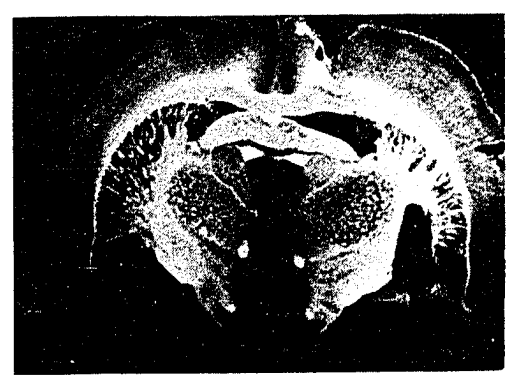


(e)



(f)

Figure 20

CHAPTER IV

DISCUSSION

General Discussion of the Results

The most consistent and significant findings of this research are that during periods of stimulation with higher current intensities, there was a significant potentiation in the release of both NE and 5-HT in animals receiving rewarding (or reinforcing) ESB as determined by prior behavioral testing. Furthermore, during these stimulation periods there was also a significant inhibition of release of both NE and 5-HT for animals receiving non-rewarding (or non-reinforcing) ESB.

During stimulation periods with lower current intensities there was no consistent potentiation in the release of NE with rewarding ESB. However, there was a consistent, statistically significant inhibition of release of 5-HT. In addition, there was an inhibition of release of both NE and 5-HT during stimulation periods with lower current intensities for animals receiving non-rewarding ESB. Furthermore, there was no statistically significant or consistent potentiation or inhibition of release of either NE or 5-HT during auditory and visual stimulation. Finally,

during rewarding ESB at either high or low current intensity there was no consistent or statistically significant change in the perfusate radioactivity of a control compound, urea.

Significant differences in NE and 5-HT activity were found between rewarding and non-rewarding stimulation groups during stimulation with higher current intensities. Additionally, for both biogenic amines there were significant differences between higher current intensity ESB periods and auditory and visual stimulation periods, and between the lower and higher current intensity ESB periods.

The failure to find significant release of NE during the lower current intensity stimulation period or differences between R and NR groups for NE during the lower current intensity stimulation period and between ESB during the lower current intensity stimulation period and auditory and visual stimulation may be due to either one or both of the following factors: (1) the lower current intensity does not reliably produce a potentiation of release of NE or (2) the rapidly declining, semi-logarithmic nature of the background rate of release of compounds during the initial stimulation period obscured any potentiation of release of NE. The latter possibility appears plausible. The only significant potentiation of release of NE or 5-HT in the non-stimulation control animals occurred during the lower current intensity stimulation period and was an inhibition of release. Perhaps, then, all scores during the first stimulation period appear

lower (due to the measuring technique) than they actually are. If this is the case, then the inhibition of release of 5-HT with R ESB during the lower current intensity stimulation period and even the inhibition of release of NE and 5-HT with NR ESB during this same period must take on less theoretical significance, and major theoretical considerations should be based on results obtained during the higher current intensity stimulation period.

The failure to find statistically significant differences either between the NE, R and U, R groups or 5-HT, R and the U, R groups is probably due to the small number of animals in these groups. The trends are in the direction of more potentiation of release of NE and 5-HT than U and the effects are more consistent for NE and 5-HT than they are for urea. Likewise, the failure to find expected statistically significant differences in potentiation or inhibition of release of compounds between any NE or 5-HT group and the non-stimulation control groups probably is due to the small number of subjects in the control groups, especially for the higher current intensity stimulation period where the stimulated groups show the expected potentiation or inhibition of release but the control groups show inconsistent effects.

Specificity of Results

The results of this research are consistent with the proposition that the rewarding effects of ESB are

mediated by the biogenic amines, NE and 5-HT. The results indicate that the potentiation of release of NE and 5-HT are specific to rewarding ESB. The finding that NE is released more during ESB which has been shown to be rewarding to the animals than during background release periods replicated the Stein and Wise (1969) study. Furthermore, the potentiation of release of 5-HT during R ESB implies that 5-HT too is involved in the reinforcing effects of ESB. The failure to find a consistent or statistically significant potentiation or inhibition of release of the control compound, U argues for the specificity of release of the biogenic amines examined in this research. In addition the non-release of U confirms that the push-pull cannula method can be a very useful tool in investigating brain-behavior relationships as it demonstrates that the technique itself does not cause a substantial release of a functionally non-significant compound.

The findings that there were no differences between the data obtained from the singly or doubly labeled animals implies that the proceedure of injecting both amines simultaneously does not alter the results. This finding also demonstrates the usefulness of the push-pull technique when investigating behaviors which have been reported to be effected by both NE and 5-HT such as sleep patterns. The simultaneous injection of both amines allows investigations of the differential involvement of the noradrenergic and

serotonergic systems while holding treatment conditions constant within one animal.

The problem of incorporation of the biogenic amines into the endogenous stores bears on the interpretation that the release of both NE and 5-HT accompanies rewarding ESB. As already cited a number of studies indicate that both NE and 5-HT injected in this manner do mix with the endogenous stores, but precise determination of the extent to which each store is labeled remains difficult. Some evidence indicates that noradrenergic sympathetic neurons are able to take up 5-HT as well as NE (Cooper, Bloom, & Roth, 1970). If the central noradrenergic neurons also have this ability, it is possible that 5-HT injected into the ventricle could have been taken up by the noradrenergic as well as serotonergic neurons. This makes the interpretation of the release of 5-HT somewhat unclear. It is theoretically possible that the release obtained during the ESB-2 periods could have been due to NE containing neurons only. This possibility seems less likely, however, when the inhibition or release of 5-HT during the ESB-1 period is considered. The inhibition of release of the two amines was not parallel. There was a significant inhibition of release of 5-HT and no significant inhibition of release of NE. In addition, the comparison for the ESB-1 period for R stimulation in the single labeled group indicated a trend for a difference between NE and 5-HT activity ($p<$

0.10). (Due to the experimental design the single and dual labeled scores could not be combined for these statistical comparisons.) Since the inhibition or potentiation of release of these two amines was not completely parallel, it implies that more than one mechanism of release or inhibition of release was in operation. Thus, it seems probable that the release of the two amines during rewarding ESB-2 does, at least in part, reflect potentiation of release from both NE and 5-HT containing neurons, and that this effect is functionally significant.

Another aspect of the uptake of compounds injected into the lateral ventricle concerns the uptake of a neurally nonfunctional compound such as U. While this compound is commonly used as a control substance in such studies, no proof of its uptake into any brain tissue exists. Thus although no significant release of U was obtained in this study, the absence of specific knowledge of its location within the cell makes it less than a perfect control compound. Perhaps the d isomer of NE which is apparently functionally inactive in the brain, would serve as a better control in further research of this typ

The finding of potentiation or inhibition of release of NE and 5-HT during R or NR ESB respectively, and no potentiation or inhibition of release of these amines during auditory and visual stimulation is further substan-

tiation that the potentiation and inhibition of release is contingent on R or NR stimulation and not due to a non-specific effect of stimuli.

The current intensity level profoundly affected the potentiation or inhibition of release of both NE and 5-HT. The low current intensity produced no or relatively less potentiation or inhibition of release than did the higher current intensity for each group tested, and in one group (5-HT, R) the effect of increasing current intensity apparently changed the inhibition of release of 5-HT to a relatively strong potentiation of release of this amine during the higher current intensity ESB. While for reasons discussed earlier, the inhibition of release of 5-HT during the lower intensity may be more apparent than real; nevertheless, there was a considerable change in the results depending on the current level for this amine. As will be discussed more fully after results of other studies are examined, this considerable change in the behavior of 5-HT with different current intensities could theoretically reflect a somewhat different role for 5-HT than for NE in reward mechanisms.

To summarize the results of this research with respect to specificity, the potentiation of release of both biogenic amines, NE and 5-HT, appears to be specific to rewarding ESB while the inhibition of release of NE and probably 5-HT is specific to non-rewarding ESB. The data support the

proposition that there is both chemical specificity, as U did not show any potentiation of release during rewarding ESB and stimuli specificity, as the potentiation or inhibition of release depended on the rewarding properties of the ESB and auditory and visual stimulation produced no inhibition or potentiation of release of compounds. Furthermore the potentiation or inhibition of release depended on the current intensity of the ESB.

The specificity of these findings appears to eliminate the possibility that the release of amines is due to some nonspecific stress factor of the ESB. The opposite results for rewarding and non-rewarding ESB argue against this hypothesis as well as the fact that auditory and visual stimulation produced no consistent results. In addition, if the potentiation of release were due to a stress factor, one would expect that the non-rewarding stimulation (which in some animals appeared to be very aversive) would produce a greater stress on the animal than would the rewarding ESB and thus should have resulted in a greater potentiation of release of the amines than the positive ESB did.

In the remaining discussion the release of NE and 5-HT will be assumed to be specific to the rewarding aspects of the ESB.

Biogenic Amines in Reinforcement

Studies reported in the literature indicate that

two different models with respect to the relationship of NE and 5-HT mechanisms to reinforcement are possible. (1) Some evidence indicates that NE systems and 5-HT systems play opposite roles with respect to reinforcement mechanisms, i.e., agonist and antagonist. (2) Other evidence indicates, however, that the two systems have similar or complementary effects on reinforcement mechanisms. In the following section of the discussion the evidence which bears on these two alternative hypotheses will be discussed with emphasis on three of the factors which appear to be important when considering 5-HT mechanisms in reinforcement: the current intensity, electrode location, and level of amines present in the brain and methods of inducing these levels.

NE Mechanisms

Psychopharmacological and biochemical studies cited in the introduction and the findings of this research are consistent with the proposition that NE is involved specifically in reinforcement mechanisms and that a noradrenergic neural system controls rewarding or reinforcing behavior. Manipulations which potentiate noradrenergic effects consistently increase SS behavior while those manipulations which have antagonist effects to NE decrease SS behavior. Moreover, as long as the stimulating electrode is in a rewarding area the specific electrode location or the current intensity usually does not interact with manipulations of CNS noradrenergic levels to produce differential effects

on SS behavior.

The potentiation of release of NE found in this study may reflect the fact that reinforcement mechanisms are under the control of neural pathways which employ NE as the primary neural transmitter. Thus, the potentiation of release of NE with rewarding stimulation is due to the increase in firing of neurons within this neural system.

5-HT Mechanisms

The findings of this research and other studies cited in the introduction and below indicate that the relationship of 5-HT mechanisms to reinforcement processes may not be as simple and direct or potent as it is for NE. Although none of the differences between NE and 5-HT in this research are statistically significant, the trend appears to be for greater release of NE during rewarding ESB and greater inhibition of release of NE during non-rewarding ESB than for 5-HT. In addition there are conflicting reports in the literature of the role of 5-HT systems in reinforcement mechanisms.

Stein (1971) found that 5-HT injected into the lateral ventricle reduced SS rates to forty or fifty per cent of the pretreatment level. This result as well as that of Wise, Berger, and Stein (1970) which reports that increasing brain levels of 5-HT potentiated conditioned suppresion of drinking while reduced levels of brain 5-HT reduced conditioned suppression of drinking, led Stein to postulate that 5-HT

mediates suppression of goal directed behavior while NE mechanisms mediate its release. Thus, Stein views NE and 5-HT systems as having opposite effects on reinforcement processes.

A recent study by Poschel and Ninteman (1971) in which brain levels of 5-HT were reduced with p-chlorophenylalanine reported excitatory effects on SS behavior, a result opposite to their earlier findings. This excitatory effect was especially pronounced with electrode placements in the ventral tegmental area of Tsai or the MFB. No such effect was found in the dorsomedial hypothalamus or the midbrain reticular formation. These investigators postulate reciprocal effects of NE and 5-HT on reinforcement behavior, such that NE acts to increase and 5-HT to decrease the excitability of the reward system. They do emphasize, however, that at any given time level of excitation depends on the balance of the two amines and not just the presence of sufficient brain levels of NE.

An earlier study by Poschel and Ninteman (1968) found that after MAO inhibition by pargyline, 5-hydroxytryptophan (the precursor of 5-HT) produced excitatory effects on SS behavior, a result which argues against the position that 5-HT systems are antagonistic to SS behavior. They further reported that other investigators have found inhibitory effects on behavioral responding after administering pargyline followed by higher doses of 5-hydroxytryptophan

than were used in the Poschel and Ninteman (1968) study. Thus, it could very well be that effects of 5-HT mechanisms on SS and other behavioral responding are dose specific, i.e., very sensitive to the specific levels of 5-HT in the CNS.

As discussed in the introduction, the Stark, Boyd, and Fuller (1964) research clearly implicates 5-HT systems as being necessary for SS behavior, as responding for ESB declined with declining CNS levels of NE and 5-HT but responding returned with returning levels of 5-HT rather than NE. These investigators also found that increasing brain levels of 5-HT had differing effects depending on current levels. With relatively high levels of 5-HT in the CNS, rates of responding were lowered at high current intensity levels and increased at lower current intensity levels. The research of these investigators argues for a 5-HT system which has similar or complementary effects on reinforcement processes rather than strictly opposite effects.

The present study found that 5-HT release was inhibited during the ESB-1 period at the lower current intensity but was very substantially released during ESB-2 in the R group. This result is difficult to explain by any simple relationship between 5-HT systems and reinforcement processes. It is not consistent with the position that 5-HT mechanisms act in opposition to NE mechanisms, but is consistent with the position that the two systems work in concert when

exerting their effects on reinforcement processes. One theoretical possibility is that 5-HT mechanisms are not operational until current intensities are considerably above threshold levels for rewarding effects, and may act more in an auxiliary capacity to NE systems rather than as direct mediators of reinforcement processes.

A study just reported by Antelman, Lippa, and Fisher (1971) utilizing 6-hydroxydopamine which irreversibly destroys noradrenergic end terminals, found first a suppression of SS responding corresponding to a depletion of brain NE, but with additional testing he found a return of SS even though levels of NE never returned. Obviously some other compound or system must be substituting for NE. It could very well be a 5-HT system.

The differential effects on responding produced by current intensity changes in the Stark, Boyd, and Fuller (1964) study as well as the reversal of direction from inhibition to potentiation of release of 5-HT with increasing current levels found in this study are consistent with the hypothesis that as current intensity is raised a serotonergic inhibitory system is invoked; i.e., a homeostatic steady-state mechanism for response control. The release of 5-HT produced by the higher current intensities in this study might reflect the activation of that system. Then, the decreased responding found in the Stark, Boyd, and Fuller (1964) study at higher current intensities with

higher brain levels of 5-HT would be the behavioral result of that system's activation.

The effects produced by higher current intensities could of course also be due to the spread of current into adjacent neural areas not as directly related to reinforcing effects.

Theoretical Speculations on Neural Mechanisms in Reinforcement

At the molecular and functional neurophysiological levels, how do biogenic amine brain systems coincide with behavioral constructs? Presumably the neurohumors released activate certain neural circuits within the limbic area of the brain. The question then becomes one of what circuits are activated. It is proposed that both NE and 5-HT are necessary for the phenomenon of SS, perhaps in a slightly different but complementary manner.

Assuming that the NE and 5-HT systems mediate reinforcement processes it would be helpful to be able to delineate the precise manner in which each system functions with respect to reinforcement mechanisms. The neural systems may be either completely separate systems or they may be overlapping systems both anatomically and functionally.

Evidence cited by Routtenberg (1968) implies that there may be two functionally distinct systems which mediate reinforcement. There are sites in the septal area which when stimulated will support SS. Stimulation to

these sites also lowers heart rate and inhibits behavioral responding for an ongoing task. On the other hand, stimulation sites in the hypothalamus which produce SS, raise heart rate and potentiate behavioral responding for ongoing behaviors. It is possible that each separate system is mediated by a different neural transmitter; i.e., NE and 5-HT.

Research by Deutsch (1964) directly implies that there are two anatomically distinct neural systems responsible for SS, one system with shorter refractory periods of 0.5 to 0.6 msec. which is responsible for reinforcement effects and another system with longer refractory periods of 0.8 to 1.1 msec. which is responsible for drive aspects of SS. Again it is theoretically possible that a different transmitter is involved in each of these separate but complementary neural systems. Again the transmitter substrate could be NE and 5-HT respectively.

Theoretical Noradrenergic Mechanisms

NE has historically been associated with activation and indeed the SS phenomena reviewed above seem congruent with Hess' concept of an ergotropic substance. Thus, perhaps in limbic regions of the brain the organism is incited to action via the effects of an adrenergic system. The readiness to respond and the maintenence of excitement could be under the control of NE circuits. The species specific behaviors described by Glickman and Schiff (1967) i.e., the activation of the consummatory and approach

behaviors, which are reinforcing, might be initiated by NE mechanisms.

Theoretical Serotonergic Mechanisms

If one considers behaviors in which 5-HT has been found to have an effect (Page,1968), many of them involve an affective or sensitizing component. Often 5-HT mechanism effects depend on the conditions of the biological substrate prior to 5-HT's application or release. Perhaps serotonin mechanisms function in different ways, depending on the current state of the neural system. This might account for the sometimes contradictory results for various behaviors obtained in different investigations. If 5-HT acts as a sensitizing agent it might be expected to heighten pleasure in some states of the neural system, and pain in others. Thus, one might speculate that 5-HT may be the neurohumor which is responsible to a large extent for the affective components of reinforcement, the pleasure or pain associated with a reinforcer.

Theoretical Interaction of 5-HT and NE Mechanisms

The notion that response readiness aspects of SS are mediated by noradrenergic mechanisms and that affective, pleasure, or satisfying aspects of SS are in part mediated by 5-HT mechanisms does not imply mutually exclusive functions for the two monoamines. Indeed based on their

similar molecular structures, similarity of manufacture and degradation, and some similarities at the neurophysiological level, they are probably both inextricably involved in any behavior in which either is implicated. Thus, at some functional level the indoleamine, 5-HT may be able to substitute for the catecholamine, NE (e.g., when it is depleted as in the Stark, Boyd, and Fuller (1964) and Antelman, Lippa, and Fisher (1971) studies) and vice versa.

Perhaps some of Gerbrandt's (1965) concepts are relevant to effects NE and 5-HT may have. He proposes that there are responses which are highly stabilized and represent the normal mode of action of the animal. (Perhaps these may be compared to those species-specific behavior patterns postulated by Glickman and Schiff.) During the learning process the animal has many competing responses which must be controlled. Thus, the Papez circut functions to control competing behavior during stabilization. He further proposes that the nonspecific projection system functions in the release of stabilized behaviors. For Gerbrant, a reinforcing stimulus serves to activate limbic and midbrain circuts which control or inhibit prepotent competing responses.

Within his system the prepotent normal responses might be released by NE systems. During reinforcement 5-HT systems come more into use and the 5-HT systems act to control or inhibit competing responses. Serotonergic

mechanism effects might be likened to the effects of surround inhibition. Those neural systems which have some bias to be inhibited would be even more inhibited while those systems which tend to be activated would be even more activated to a certain level beyond which 5-HT mechanisms may act to restore equilibrium.

If the above proposed functions of 5-HT and NE mechanisms are correct, one is then faced with finding a molecular and neurophysiological model whereby 5-HT and NE systems could function in this manner.

The molecular structures of NE and 5-HT are very similar. If there are sites on the postsynaptic membrane which have an affinity for 5-HT on the basis of certain structural characteristics, they are likely to have a similar though somewhat modified affinity for NE. It might even be possible that both neurohumors act on the same postsynaptic or presynaptic receptor sites, but in a slightly different manner. Indeed there is some evidence to suggest that some of the effects of the application of 5-HT on SS behavior is due to serotonin's action on the receptor sites of NE (Poschel & Ninteman, 1968).

One manner in which these two biogenic amines could function and be compatible with the above speculations is that NE may have a greater affinity for the postsynaptic sites. Thus, when 5-HT and NE are released in essentially equal quantities, the primary effects produced on the postsynaptic

sites are those produced by NE by virtue of it being preferentially bound to these sites. If however, 5-HT has a more potent action on the postsynaptic site than does NE, and 5-HT is released in greater quantity than NE, some of the 5-HT will become bound with the postsynpatic sites and produce its stronger effect (a heightened excitation or inhibition, as the case may be) on the postsynaptic membrane. Thus, two aspects of these neurohumors might account for their varied actions; the molecular affinity of the postsynaptic sites for each of the substances, and the potency of action for inhibition or excitation of each substance.

This is only a very general mechanism of action, and many of its aspects could vary such as, in neural inhibitory mechanisms NE might be the more potent humor whereas in neural excitatory mechanisms 5-HT might be the more potent humor. It does offer a new manner of examining the effects of drugs and neurohumors which have been considered merely occupying two opposite sides of the coin. The implication of such a functioning mechanism would be that behavioral and neural effects of NE and 5-HT systems must be examined more in concert and under conditions which do not substantially alter CNS amine levels than has previously been the case.

Further Research Suggestions

Further research involving the push-pull technique should probably center on examining other brain areas than the

amygdaloid region. In addition a wider variety of current levels, and treatment orders might provide useful information on the relative importance of NE and 5-HT mechanisms. It would also be helpful to examine the release of the biogenic amines during ongoing behavioral responding for ESB.

More research with other control compounds such as d-norepinephrine would also further delineate the specificity of release of the biogenic amines.

The use of precursor compounds for both NE and 5-HT in the ventricular injections would be helpful in assuring that the labeled stores are indeed those of the newly manufactured NE and 5-HT which are the functionally significant fraction. Additionally, analysis of the perfusate for the amount and character of the metabolites would further clarify the functional relationship of NE and 5-HT systems to reinforcement.

The push-pull cannula method lends itself to the investigation of the release of other compounds believed to affect the 5-HT and NE systems, and might more precisely delineate the effects of compounds altering brain levels of these amines.

Other research which would be especially helpful in delineating the relationship of the two amines would employ double-pulse stimulation designed to differentially affect the two systems Deutsch (1964) describes and determine the relative amounts of NE and 5-HT released under each

condition.

Additional research might also employ drugs which are known to affect SS behavior and also affect biogenic amine levels. The drug's effects on SS would be examined, its effect on the release of the biogenic amines would be examined using the push-pull cannula technique, and finally its effects on the release of the biogenic amines during ongoing bar pressing for ESB would be examined using the push-pull cannula technique. This paradigm would allow a more complete evaluation of NE and 5-HT mechanisms involved in reinforcement under conditions of altered brain amine levels which have been employed in psychopharmacological studies.

CHAPTER V

SUMMARY

Previous psychopharmacological and biochemical research has strongly implicated norepinephrine mechanisms in reinforcement behavior. There is also some evidence to indicate that serotonergic mechanisms are importantly involved. Using a push-pull cannula technique Stein and Wise (1969) found that during rewarding electrical stimulation of the brain the biogenic amine NE was released from both the lateral hypothalamus and the amygdala.

The purpose of this present research was to determine the degree of specificity of release obtained using the push-pull technique. Specifically the questions addressed were: 1) Is the biogenic amine, serotonin, also released during rewarding electrical stimulation of the brain? 2) Is a control compound, urea, released during rewarding ESB? 3) Will auditory and visual stimulation which are presumably neutral in value also elicit release of these biogenic amines? 4) Will these biogenic amines be released during ESB which is not rewarding?

First, rats were stereotaxically implanted with electrodes in the posterior median forebrain bundle, injecter

cannulae into the lateral ventricle, and push-pull cannulae in the amygdala. The electrode sites were then evaluated as to whether they were rewarding or non-rewarding using operant techniques.

Trace amounts of either H^3NE, C^{14}5-HT, $H^3$5-HT, or C^{14}urea were injected into the lateral ventricle. After a period of about forty-five minutes the amygdala was perfused for a period of five to seven hours. During the perfusion there were three treatment periods during which the animals received stimulation alternating with control periods of no stimulation. During the first two stimulation treatment periods the animals were presented with electrical stimulation of the brain at the rate of one train of 100cps stimulation per second. The current was raised during the second stimulation period. During the third stimulation treatment period the animals were presented with auditory and visual stimulation at the same rate they received the electrical stimulation of the brain.

During the perfusion, samples of the perfusate were collected every fifteen minutes and counted in a liquid scintillation counter to determine the amount of radioactivity present in each sample. The amount of radioactivity present during stimulation periods relative to non-stimulation control periods was taken as an index of release or non-release of the compound.

There was a potentiation of release of NE and 5-HT

but not urea, during rewarding electrical stimulation of the brain, and an inhibition of release of NE and 5-HT, but not urea, during non-rewarding electrical stimulation of the brain. There was no potentiation or inhibition of release of these amines during auditory and visual stimulation. During rewarding electrical stimulation of the brain at current intensities near threshold levels for rewarding effects there was an inhibition of release of 5-HT but no potentiation or inhibition of release of NE or urea.

Both NE and 5-HT mechanisms appear to be involved in reinforcement processes since the potentiation of release displayed both stimulation specificity for higher current intensity electrical stimulation of the brain and chemical specificity for the biogenic amines NE and 5-HT.

It is theoretically possible and even probable that there are separate neural systems mediating different aspects of the reinforcement processes. Each system could be mediated by a different neural transmitter (i.e., NE and 5-HT). These systems may act in opposition. However, evidence from this study in conjunction with other evidence cited in the literature is congruent with the proposition that the two systems may act is a similar or complementary manner on reinforcement processes rather than in an antagonistic manner.

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APPENDIX

ABBREVIATIONS

AVS	auditory and visual stimulation
B	background control period
BromLSD	bromolysergic acid diethylamide
C	non-stimulation control
C_1	mean of standard scores from the control period prior to a stimulation period
C_2	mean of standard scores from the control period after a stimulation period
CNS	central nervous system
CPM	counts per minute
D_r	residual change or difference score ($\bar{S} - \bar{E}$)
DOPA	dihydroxyphenylalanine
E	expected score
ESB	electrical stimulation of the brain
ESB-1	treatment period during which lower current intensity ESB was given
ESB-2	treatment period during which higher current intensity ESB was given
5-HIAA	5-hydroxy-indole acetic acid
5-HT	serotonin (5-hydroxytryptamine)
5-HTP	5-hydroxytryptophan
5-HTS	group injected only with $H^3$5-HT (single labeled)

JB-516	phenylisopropylhydrazine
LSD	lysergic acid diethylamide
MAO	monoamine oxidase
MFB	median forebrain bundle
NE	norepinephrine
NES	group injected only with H^3NE (single labeled)
NR	non-rewarding
NE + 5-HT	group injected with H^3NE and C^{14}5-HT (dual labeled)
PCPA	para-chlorophenylalanine
R	rewarding
S	mean of standard scores during a stimulation period
SS	self-stimulation
U	urea
US	group injected with C^{14}urea