Neurochemical changes associated with schedule-controlled behavior[1,2]

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ABSTRACT

Studies of monoamine metabolites within the cerebrospinal fluid compartment have indicated that this approach may be useful in examining central metabolic changes in vivo. By combining the technologies of radioisotope chemistry, operant behavior control and modification, and brain perfusion with push-pull cannulas, we have been able to examine minute to minute changes in the disposition of radiolabeled monoamine transmitter candidates and their metabolites. These substances appear to co-vary with changes in complex behavior maintained by operant schedules of reinforcement and affected by changes in schedules or administration of psychotropic drugs. In agreement with other perfusion studies, we have observed changes in fractional distribution of radiolabeled urea, a so-called extracellular marker, along with shifts in monoamines; but the former appear more transient. These observations nevertheless support the concept of dynamic changes within the extracellular environment of the CNS that may be part of a hormone-like communicating system with functional significance. Furthermore, the presence of peaks and/or troughs, in perfusates, of [^{14}C]urea or similar substances should not be taken as a priori evidence for nonspecificity of the technic, since selective release or inhibition of release of monoamines can be shown with appropriate drugs that are thought to act through these aminergic systems. Destruction of catecholamine nerve terminals with 6-hydroxydopamine likewise attenuates the signal-locked release of radiolabeled norepinephrine by a conditioned stimulus after conditioning occurs. No such release is seen on presentation of the to-be-conditioned neutral stimulus in control or 6-hydroxydopamine treated rats. These initial studies indicate the availability of a powerful tool for the study of drug-neurochemical-behavioral interactions using subjects as their own controls for extended periods of time so that phenomena of plasticity, tolerance and dependence may likewise be examined.—SPARBER, S. B. Neurochemical changes associated with schedule-controlled behavior. *Federation Proc.* 34: 1802–1812, 1975.

The concept of individual differences underlies, to a great extent, the philosophy of an operant approach to the analysis of behavior. Accordingly, when studying the behavioral effects of manipulating environmental variables or administering drugs which may or may not alter behavior by interacting with those variables identified as eliciting or maintaining behavior (i.e., deprivation, stimulus control, reinforcing character and density, and so on) it would likewise appear advantageous to adhere to the aforementioned philosophy (30).

Until recently, the closest approximation to an examination of the biochemical changes that occur within the central nervous system (CNS) consequent to manipulating external and/or internal environmental variables has centered around a technology that depends on the instantaneous or at least the rapid sacrifice of experimental subjects during or after behavioral sessions. Although much information on the mechanism of action of drugs, at the biochemical level, has been gleaned by this approach, much information about the close temporal relationship between biochemistry and behavior is lost. In addition, the destruction of an animal that required much time and effort to train is a costly way of obtaining the desired information. I should like here to describe some work in which we have been engaged that attempts to circumvent many of the obvious disadvantages just stated. We have attempted to superimpose on the technology that has evolved around the study of operant behavior, the additional technologies associated with radioactive isotope labeling of neurotransmitter candidates and their precursors, as well as that of perfusion of individual organs or parts thereof so that in vivo conditions might be approached. I am convinced that, together with other, more traditional approaches to psychopharmacological problems, the utilization of the technics described herein will add much to our understanding of the biochemical substrate of CNS function and ultimately behavior and drug action on such function and behavior.

BIOGENIC AMINES AND CONDITIONED BEHAVIOR

The evidence for involvement of the biogenic amines (dopamine, norepinephrine, and serotonin (5-HT)) in the maintenance of conditioned behavior stems from variations of original experiments first performed by Carlsson and co-workers (11, 50, 51) and Brodie and co-workers (10) who found that behavioral depression coincided with the administration of drugs that produced depletion of these amines. Specific inhibitors of the

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synthesis of the catecholamines norepinephrine and dopamine, which likewise depress operant behavior, as well as studies in which depressed behavior has been at least partially reversed by the administration of the precursor of dopamine and norepinephrine, *l*-dopa, have led many to conclude that it is mainly the catecholamines that are involved in the behavioral depression seen after the administration of drugs that lower steady-state levels of both phenethylamines and indolealkylamines (12, 21, 47). Findings contrary to the prediction that destruction of catecholamine containing neurons with 6-hydroxydopamine would lead to a deficit in performance of operant behavior (42, 48) have increased the level of ambiguity regarding the specific involvement of the amine transmitter candidates in the mediation of operant behavior.

Because of an important pharmacological maxim—that drugs can do nothing that the organism (or parts thereof) is incapable of in the absence of drugs—we set about to determine if catecholamines could be shown systematically to co-vary with behavior controlled by a specific reinforcement schedule. This allowed us to manipulate behavior by changing the consequences of that behavior (18) without having to introduce the *confounding* drug variable. To determine if the radioactive norepinephrine within the push-pull perfusate we were collecting during performance of an operant was released selectively, control experiments were performed with the so-called extracellular marker, [^{14}C]urea; in addition we compared the effects of drugs with apparently different mechanisms of action, in dosages that disrupted the operant behavior to an equal extent (58). One of these drugs, *d*-amphetamine, appears to act via facilitation of release of catecholamines and, additionally, through prevention of reuptake by nerve terminals, while no good evidence exists to suggest that mescaline, the second drug, acts in a similar fashion. We subsequently have examined the relationship between equieffective doses of *d*-amphetamine, mescaline and *d*-lysergic acid diethylamide (*d*-LSD-25) as they relate to changes in the diffusible extracellular content of norepinephrine, 5-HT and some of their metabolites during operant sessions. The relationship between tolerance to the operant behavioral action of *d*-amphetamine and its ability to alter the disposition of radiolabeled norepinephrine and 5-HT introduced into the brains of rats prior to the operant sessions is likewise discussed.

I will also touch briefly on some newer perfusion experiments that allow a finer dissection of the relationship between isomers of drugs like the amphetamines and their effects on conditioned behavior.

CHOICE OF METHODS

Having witnessed the use of Gaddum's push-pull cannula (23) in the laboratory of Dr. F. Sulser (56), it became immediately apparent that a potentially powerful tool was available for psychopharmacological research. The main obstacles to be overcome included the design of a system that would allow perfusion, with chronic indwelling cannulas, of brains of animals who were engaged in complex behavior tasks that would be sensitive to specific and nonspecific stimuli. The sensitivity to specific stimuli would enable the experimenter to manipulate, at will, those variables thought to maintain the behavior. Sensitivity to nonspecific stimuli would allow for a built-in control against changes in intracranial pressure, tissue integrity, and the like, which have been suggested as possible contaminants of experiments in which brain tissue is perfused (13, 29, 43).

Most of our work to date has been centered around perfusion of the lateral ventricular space in the rat. The decision to stay within the ventricular space was prompted in part by the aforementioned objections to the nonphysiological conditions produced by perfusion of solid tissues, as well as the theoretical questions raised about the research and clinical use of cerebrospinal fluid constituents as a measure of periventricular brain metabolism (37).

In addition, the relatively small size of the rat ventricular system allows for more rapid diffusion and mixing of substances released from periventricular structures with the perfusion medium delivered by the tip of a relatively large device (cannula) inserted into the space. Finally, this species has been used in our laboratory, and many others, for studies of effects of psychotropic drugs on operant behavior and it would be foolhardy to attempt to manipulate too many variables without a strong base of information on which one can build. As you will see, this systematic approach has allowed us to compare and contrast data generated in our own laboratories, as well as others, on the behavioral effects of drugs of separate pharmacologic classes and drugs within a class.

I will not spend any time on the specific perfusion methodology, other than to refer the interested individual to the excellent chapter of Myers' (39) and the original publications from our own laboratory cited at appropriate places within the text of this communication. We have subsequently modified our cannulas, collection procedures, and separation technics as described in a forthcoming communication.

Manipulating schedule parameters and perfusion of lateral ventricle

[^{3}H]norepinephrine injected into the lateral ventricle of rats is taken up into catecholamine-containing neurons and is distributed in a parallel fashion to endogenous norepinephrine (1, 26, 27). Several laboratories have taken advantage of these observations to combine push-pull perfusion technics with isotope pulse labeling procedures in order to follow the release of transmitter candidates after electrical stimulation of brain (28, 55), handling and injection (thought to act as stressor stimuli) (60), drug administration (56) or stimulation of olfactory neurons with odorous substances (13). We reasoned that if substances injected into the cerebrospinal fluid (CSF) gained access to brain structures in a selective way, and substances within CSF reflected metabolism of brain structures in a fairly selective manner (38), sampling of the ventricular space would allow us to infer metabolic or other dynamic changes occurring concurrently with operant behavior output or drug action. Our initial studies were designed to determine whether changes in operant behavior that would be compared to an *emotional* reaction would show changes in the disposition of [^{3}H]norepineph-

rine that had been previously injected and allowed to equilibrate with endogenous stores. Withholding of positive reinforcement after a history of low fixed ratio schedule control of behavior was chosen as the manipulation because of its behavioral consequences, which have been interpreted within an emotional and autonomic framework. Possible CNS catecholamine involvement in emotional stress in many species has been reported (7, 8, 46).

After slowly injecting [^{3}H]norepinephrine or [^{14}C]urea into the lateral cerebral ventricles of rats trained to bar-press for 45 mg Noyes food pellets on a relatively low fixed ratio schedule, the site of injection within the lateral ventricle was perfused at a rate of approximately 20 μl/min (57). Total radioactivity in aliquots of 2 min collection samples was then determined. Unlike some reports in the literature that claim a lack of *release* of extracellular marker in push-pull or ventriculocisternal perfusion (12, 28, 56) we observed slight increases in [^{14}C]urea in the perfusate as a consequence of handling for injection (Fig. 1) or changing bar-pressing rates by imposing an extinction period during fixed ratio performance (Fig. 2) (54). Our initial observations indicated a definite peak in [^{3}H]norepinephrine (and metabolites) only in cases in which extinction resulted in large increases in responding (post-extinction bursts). When the behavioral consequences (bursts) of extinction were minimal, only slight changes in the profile of sample to sample contents of ^{3}H could be seen (Fig. 3). However, these data also confirmed the suggestion by Chase and Kopin (13) that perfusion experiments may be confounded to an extreme extent by nonspecificity of *release* and/or that "local changes within the extracellular compartment may attend neural stimulation." At about the same time we were doing these experiments Winson and Gerlach (60) were observing similar results with extracellular marker substances but their data, like those of Stein and Wise (55) and Chase and Kopin (13), were derived from experiments in which cannula size and/or flow rates through solid tissue were so large as to question the specificity of release based on these criteria alone (39). However, Winson and Gerlach did make an interesting observation which reinforced our own thoughts regarding the necessity and sufficiency of showing changes in putative transmitter profiles in the absence of extracellular marker profiles. Although they could not cause *release* into push-pull perfusates of the hypothalamus by *stressing* their subjects, which did cause changes in radioactivity from [^{3}H]norepinephrine, ^3H_2O or [^{14}C]urea origin in the amygdala perfusates, they were able to partially replicate the work of Sulser et al. (56) by injecting desmethylimipramine and reserpine, which did increase [^{3}H]norepinephrine content in hypothalamic push-pull perfusate. Unlike Sulser et al. (56) however, Winson and Gerlach did report increases of [^{14}C]urea. It occurred to us that in the few cases in which extracellular marker substances were not *released*, the perfused subjects were either unconscious or paralyzed. In those cases in which immobilized subjects did show so-called nonspecific release, the severe perfusion parameters could just as likely have caused movement of substances through extracellular channels by bulk flow and damage alone (9). What had to be attempted was an experimental protocol that utilized drugs as tools to determine if specificity of release could be demon-

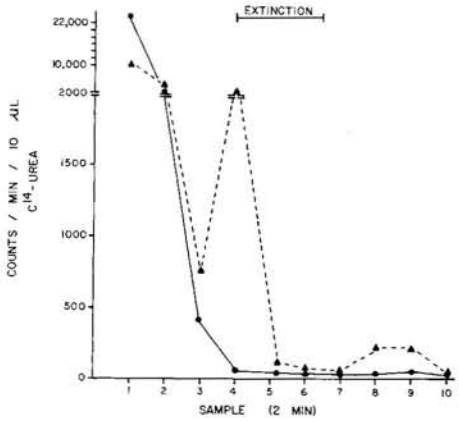
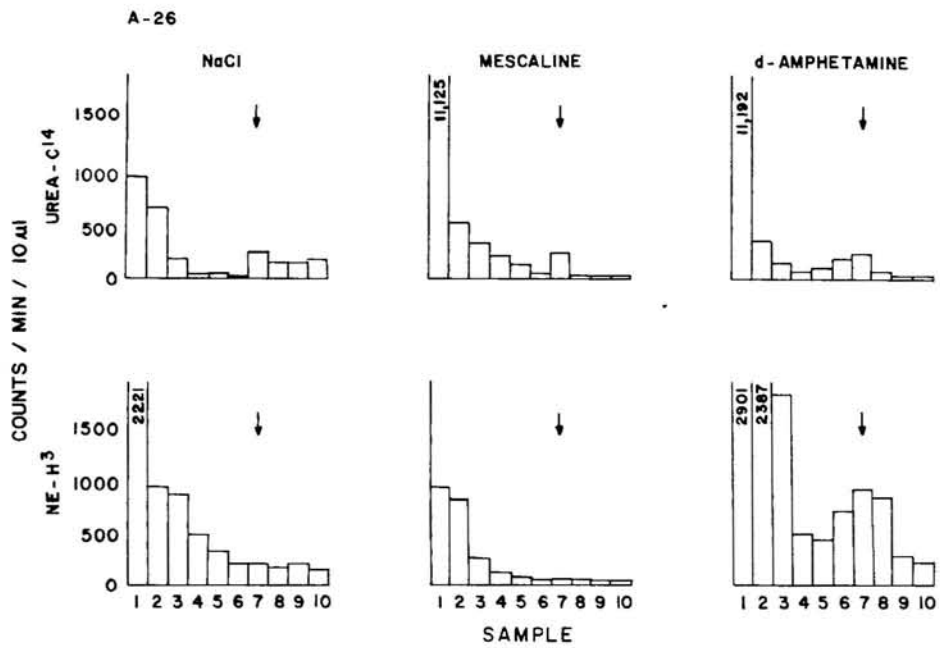


Figure 2. Increases in radioactivity from [^{14}C]urea resulting from extinction. Each point represents the average of the three rats during control sessions (circles) and 5 min of extinction (triangles) while bar-pressing for food on a fixed ratio (FR) 5 schedule of reinforcement. In all three cases there were postextinction increases in bar-pressing (see Fig. 3 for identical records) followed by pauses in this behavior prior to resumption of bar-pressing on reinstallation of reinforcement. The appearance of the second peak is suggested as resulting from increased motor activity and lowering of the rats' heads to the food delivery trough. (Reprinted with permission (54).)

Figure 1. Radioactivity histograms for one rat showing slight increases in [^{14}C]urea efflux in push-pull perfusate resulting from injection of saline (NaCl), mescaline HCl (15 mg/kg) or (+) amphetamine SO_4 (2.5 mg/kg). Only (+) amphetamine produced an increase in [^{3}H](from [^{3}H]norepinephrine (^{3}H-NE)) in the ventricular perfusate. Arrows indicate approximate time of injection of drug. Samples were collected every 2 min during the operant session which started 1 hr after injecting [^{3}H]norepinephrine into the lateral ventricle. (Reprinted with permission (54).)



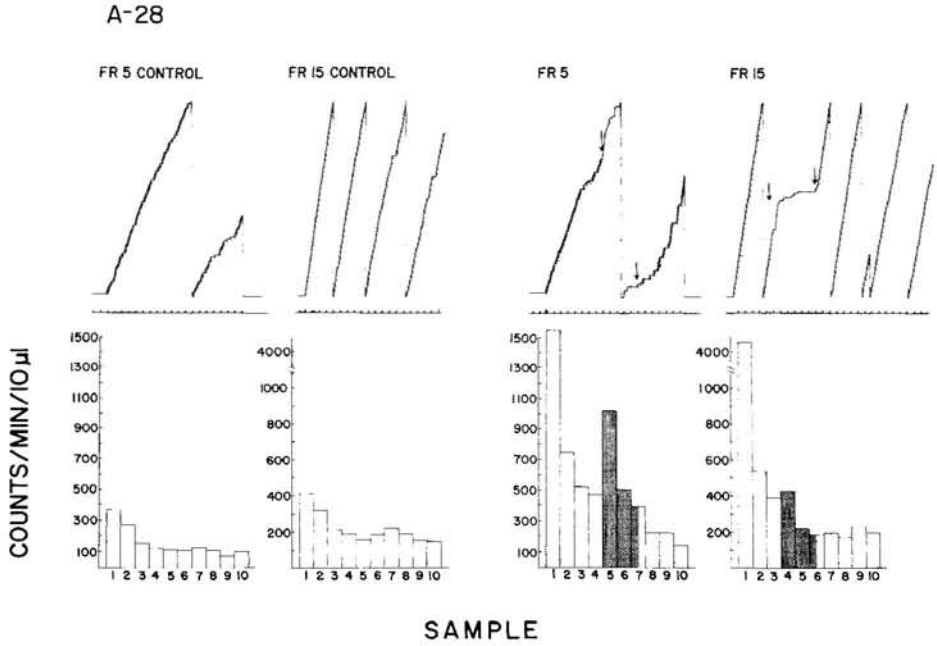


Figure 3. Cumulative records and radioactivity histograms for control and extinction (arrows) sessions for rat A28. Tracings of event pens beneath the cumulative records indicate a 1 min time base and each bar of the histograms represents the radioactivity, in 10 µl of perfusate, from [7-^{3}H]norepinephrine injected 1 hr prior to the sessions. Perfusion rates were 20 µl/min, using artificial CSF as the medium. Shaded areas indicate the extinction-perfusion samples. (Reprinted with permission (54).)

strated. From our own studies (52), and those of others (6, 20), with fixed ratio schedule control and effects of psychotomimetic and psychomotor stimulants, it was becoming increasingly clear that similarities in the various drugs' disrupting effects on operant behavior, maintained under some schedules of reinforcement, could not be explained through similarities in biochemical mechanisms (22, 24, 32, 35, 41). Therefore, a series of experiments was designed in which the fixed ratio was kept constant and the effect of *d*-amphetamine, mescaline and *d*-LSD-25 upon the disposition of [^{3}H]norepinephrine and 5-[^{14}C]HT in push-pull perfusate could be examined over short periods of time, along with such variables as latency to the onset and duration of the behavioral effects of these drugs (58). Additionally, we attempted an analysis of aliquots of perfusate by thin layer chromatography to determine if appropriate metabolites could be observed (25).

Figure 4 shows the type of behavioral response to drugs we were dealing with. By defining disruption as the point in time, after drug administration (52), at which 2 min have elapsed without the organism receiving a reinforcer, we were able to choose equieffective dosages for further study. Table 1 shows the relationships between the various doses of *d*-amphetamine and mescaline, as well as a dose of *d*-LSD of approximate equieffectiveness on fixed ratio behavior, and latency to disruption. Figures 5 and 6 show the effects of the two phenethylamines on ^{3}H from [^{3}H]norepinephrine and ^{14}C from 5-[^{14}C]HT injected into the lateral ventricles prior to perfusion and drug administration. Interestingly, this dose (0.4 mg/kg) of *d*-LSD caused an increase in neither ^{3}H nor ^{14}C in the perfusates during its course of action, as was seen with *d*-amphetamine or mescaline, respectively. On the other hand, it produced a significant decrease in the ^{14}C (from 5-[^{14}C]HT) in perfusates subsequent to injection and during its behavioral action (Table 2). The data in Tables 3 and 4 indicate that changes in the disposition of total radioactivity in push-pull perfusates could also be attended by changes in apparent metabolism of these putative transmitters. When *d*-amphetamine was injected, there followed an almost immediate significant increase in the percentage of unchanged [^{3}H]norepinephrine in perfusate, as well as a significant increase in the percentage of radioactivity in the spot where authentic normetanephrine co-chromatographed. This was accompanied by a corresponding decrease in other spots on the thin layer chromatograms that are essentially *O*-methylated-de-

Figure 4. Sample cumulative records from one rat showing dose-related disruption of fixed ratio (FR) 30 responding. Mescaline, (+) amphetamine or NaCl were injected i.p. 10–15 min into the session (↓). Each excursion of the event pen at the bottom of the figure indicates a 1 min time base and the recorder pen automatically reset after 550 responses. The rate of responding before drug injection is similar to that obtained before and after NaCl, indicating that the perfusion procedure and injection per se did not affect food-reinforced responding. (Reprinted with permission (58).)

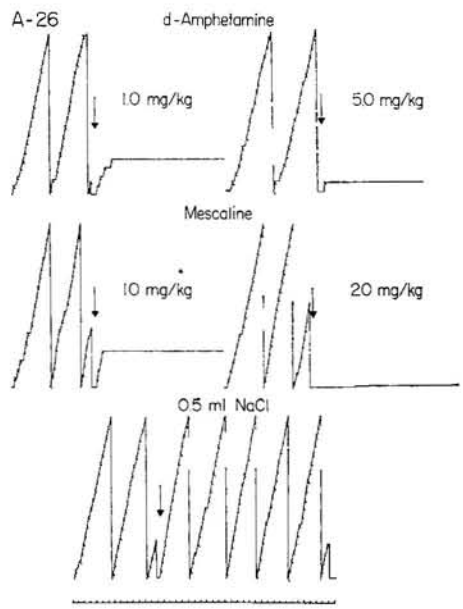


TABLE 1. Latency to onset of behavioral disruption after drug administration

Treatment	Latency, min ± SEM
Mescaline	
10 mg/kg (7)	4.5 ± 0.3
15 mg/kg (7)	3.6 ± 0.2
20 mg/kg (13)	2.9 ± 0.2
d-Amphetamine	
1.0 mg/kg (7)	6.5 ± 0.8
2.5 mg/kg (7)	3.9 ± 0.2
5.0 mg/kg (13)	2.7 ± 0.2
d-LSD	
0.4 mg/kg (6)	2.8 ± 0.2

Rats were injected intraperitoneally (i.p.) with drugs 10 to 15 min into the perfusion session. Latency to drug action is defined as the period between injection of the drug and the point in time when no reinforcers were delivered for 2 min. Each point is the mean ± SEM. Numbers in parentheses represent the number of experiments performed at that dose.

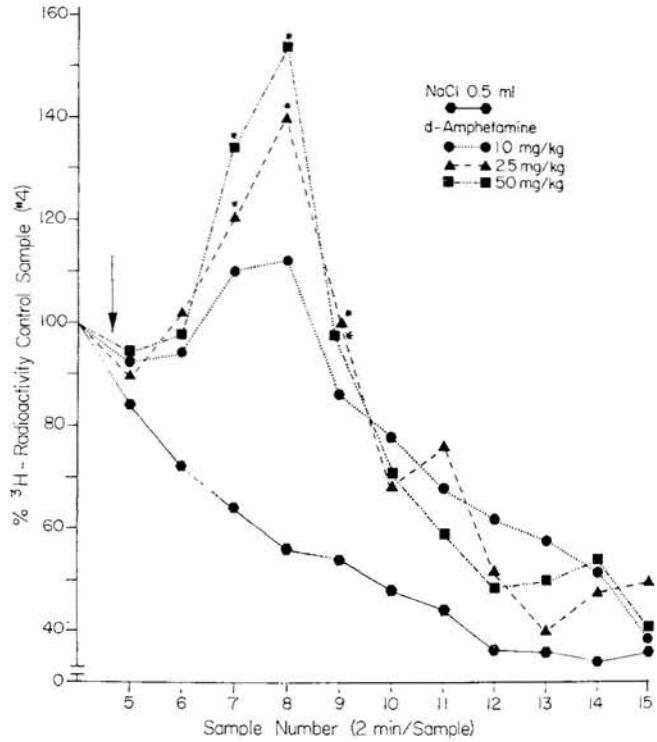


Figure 5. Release of radioactivity from [³H]norepinephrine into the perfusate from the ventricles after the i.p. injection of various doses of (+) amphetamine or NaCl. Results are expressed as a percentage of radioactivity compared to sample no. 4 (100%). Each point is the mean of three experiments for the two lower doses and six for the 5.0 mg/kg dose and the NaCl experiments. The * indicates a significant difference from NaCl levels using a matched pair *t*-test, $P < 0.05$. The ↓ indicates the approximate point of injection. (Reprinted with permission (58).)

aminated metabolites and assorted unidentified compounds. At no time did *d*-amphetamine, in the doses used, cause significant changes in 5-[¹⁴C]HT or metabolites in acute experiments of this type. However 5-[¹⁴C]HT and its metabolite(s) were significantly shifted by the two higher doses of mescaline (15 and 20 mg/kg). Unlike the effect on [³H]norepinephrine produced by *d*-amphetamine, mescaline produced a significant decrease in the percentage of unchanged 5-[¹⁴C]HT and a corresponding increase in its acid (5-[¹⁴C]HIAA) and other metabolites. Even at the time when the peak in radioactivity was no longer evident (sample 15), there were continued significant differences in the ratio of the distribution of radioactivity on the chromatograms, indicating that changes in transmitter chemistry continued to accompany the protracted behavioral disruption. Interestingly, while *d*-LSD decreased the overall output of ¹⁴C from 5-[¹⁴C]HT, the distribution of 5-HT metabolites on the chromatogram remained unchanged, indicating that, at least within the period sampled, 0.4 mg *d*-LSD/kg seemed to decrease the rate of release (turnover?) of 5-HT without altering significantly its compartmentalization or metabolism. Our interpretation of these last data suggests a presynaptic releasing effect of mescaline, similar perhaps to *d*-amphetamine and tyramine effects on catecholaminergic neurons, while *d*-LSD may be affecting mainly serotonergic receptors (postsynaptic?) directly and thereby decreasing serotonergic nerve function through some feedback inhibitory or direct presynaptic inhibitory action. Biochemical and electrophysiological evidence for this interpretation has likewise been offered (2–4, 19, 33, 45, 59). In a follow-up experiment we examined the effects of continued administration of *d*-amphetamine on [³H]norepinephrine, 5-[¹⁴C]HT and their metabolites (53). After about a week and a half of daily injections of 2.5 mg *d*-amphetamine sulfate/kg, the drug no longer produced its disruptive effect on the operant. On the other hand, mescaline (15 mg/kg) did produce the characteristic abrupt cessation of fixed ratio responding, indicating no or incomplete cross-tolerance between the two phenethylamines (Fig. 7). Table 5 shows the continued release of [³H]norepinephrine (and metabolites) into the perfusate to about the same extent both during disruption of the operant and after tolerance to the disruptive action of *d*-amphetamine. However, unlike the absence of an effect by *d*-amphetamine on 5-[¹⁴C]HT prior to tolerance, there was a significant

Figure 6. Release of radioactivity from 5-[¹⁴C]HT into the perfusate from the ventricles after the i.p. administration of various doses of mescaline or NaCl. Results are mean percentage of radioactivity compared with sample no. 4 taken as 100%. Four animals were used for the experiments involving lower doses of mescaline and seven for the 20 mg of mescaline per kg and NaCl experiments. The * indicates a significant difference from NaCl levels using a matched pair *t*-test, $P < 0.05$. The ↓ indicates the approximate point of injection. (Reprinted with permission (58).)

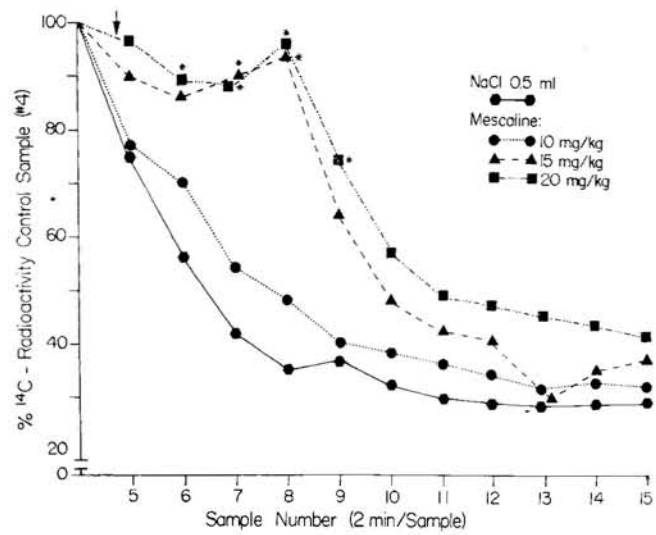


TABLE 2. Effect of intraperitoneal drug administration on appearance of radioactivity in the ventricular perfusate

Treatment	Mean % radioactivity of sample no. 4	
	^{14}C	^{3}H
NaCl	38.0 ± 2.8 (7)	51.1 ± 5.3 (6)
d-Amphetamine		
1.0 mg/kg	42.6 ± 1.5 (4)	72.6 ± 0.7[a] (3)
2.5 mg/kg	47.1 ± 5.4 (4)	78.5 ± 3.2[a] (3)
5.0 mg/kg	41.9 ± 1.8 (7)	89.3 ± 8.6[a] (6)
Mescaline		
10 mg/kg	46.2 ± 3.4 (4)	56.0 ± 4.5 (3)
15 mg/kg	65.7 ± 6.5[a] (4)	51.4 ± 3.9 (3)
20 mg/kg	68.9 ± 5.7[a] (7)	56.8 ± 4.7 (6)
d-LSD		
0.4 mg/kg	28.7 ± 3.6[a] (3)	42.6 ± 6.3 (3)

[^{3}H]-norepinephrine or 5-[^{14}C]HT were infused intraventricularly 1 hr or 30 min before perfusion sessions, respectively, and drugs, or NaCl were injected i.p. 12 to 15 min into the session. Results are expressed as mean ± SEM percentage of radioactivity in samples subsequent to the injection relative to sample no. 4. Number of subjects is in parentheses. [a] Significantly different from NaCl value, matched pair *t* test, $P < 0.05$.

increase in 5-[^{14}C]HT (and metabolites) in the perfusates subsequent to *d*-amphetamine administration (Fig. 8). Additionally, although 15 mg of mescaline HCl/kg was able to increase 5-[^{14}C]HT in perfusates to 65% from an average of 45% in samples subsequent to NaCl injection prior to tolerance to *d*-amphetamine, the same dose of mescaline (15 mg/kg) increased it further, to an average of 85% of the sample prior to injection, indicating perhaps greater reactivity of serotonergic neurons to pharmacological manipulation. The interpretation of these data can include the possibility that refractoriness of catecholaminergic receptors has developed and/or indicate a functional antagonism between catecholaminergic and serotonergic central pathways. Alternately, repeated doses of *d*-amphetamine could mimic large acute doses in their ability to alter brain serotonin and could be linked to some aspects of toxic psychosis produced by high doses of amphetamine (5, 16, 17). If mobilization of serotonin is part of a homeostatic mechanism, blockade of synthesis of 5-HT or its receptors should inhibit tolerance to at least some of amphetamine's actions.

TABLE 3. Effect of intraperitoneal administration of drugs on percentage of [^{3}H]norepinephrine and [^{3}H]metabolites of total radioactivity in the perfusate

Treatment	Sample no.	Mean percent-total radioactivity			R = DOM/(NE + NM)
		% NE	% NM	% DOM	
NaCl, 0.5 ml	4	28.2 ± 2.4	9.7 ± 2.8	63.9 ± 1.6	1.78 ± 0.18
	8	32.8 ± 5.2	8.8 ± 0.6	58.4 ± 5.8	1.52 ± 0.40
	15	29.4 ± 2.2	8.8 ± 1.0	62.0 ± 2.0	1.65 ± 0.15
d-Amphetamine, 5.0 mg/kg	4	26.9 ± 0.7	8.2 ± 2.0	67.4 ± 2.9	2.07 ± 0.08
	8	55.4 ± 3.9[a]	21.9 ± 2.4[a]	22.8 ± 2.7[a]	0.30 ± 0.05[a]
	15	33.5 ± 5.1	38.3 ± 10.9[a]	28.2 ± 9.4[a]	0.45 ± 0.21[a]
Mescaline, 20 mg/kg	4	28.8 ± 0.9	5.5 ± 1.5	65.8 ± 1.0	1.93 ± 0.09
	8	29.6 ± 2.3	5.8 ± 1.2	64.6 ± 2.6	1.86 ± 0.22
	15	30.1 ± 0.8	5.6 ± 0.7	64.3 ± 1.8	1.81 ± 0.10
d-LSD, 0.4 mg/kg	4	33.6 ± 1.8	9.0 ± 0.6	60.1 ± 2.6	1.53 ± 0.17
	8	32.5 ± 1.1	4.0 ± 1.2	63.6 ± 2.3	1.76 ± 0.16
	15	30.1 ± 0.5	5.7 ± 1.5	63.4 ± 1.3	1.74 ± 0.09

Samples of perfusate before and after injection of psychoactive substances were analyzed by TLC for percentage of [^{3}H] norepinephrine (NE) and [^{3}H]normetanephrine (NM) of total counts per minute on the TLC plate. All other counts on the plate are [^{3}H]DOM. DOM = deaminated-*O*-methylated metabolites. Results are mean ± SEM of percentage of total radioactivity and each value is the mean of the same three subjects. [a] Significantly different from predrug value of same treatment, matched pair *t* test, $P < 0.05$.

With the availability of chemicals that can specifically destroy presynaptic catecholamine nerve terminals, experiments could be attempted to support or refute the concept of specificity of release from appropriate nerve terminals when [^{3}H]norepinephrine (and metabolites) appears after manipulation of behavior by antecedent environmental variables or drug administration. To this end, animals were trained to avoid a shock by jumping onto a platform during presentation of a previously neutral visual stimulus which was subsequently paired with a 2 mA shock delivered to their feet through a stainless steel grid floor. Prior to training and implantation of push-pull perfusion cannulas, half the animals were injected intraventricularly with about 250 μg of 6-hydroxydopamine in an ascorbic acid solution while the other half were injected with only the ascorbic acid solution (0.1%). Two weeks later they were implanted with push-pull cannulas according to methods previously described (54). When perfused, samples were collected every 4 min instead of every 2 min. Aliquots of samples 4 (before experimental manipulation), 7 and 10 (12 and 20 min after stimuli presentation, respectively) were spotted on cellulose thin layer chromatography (TLC) plates and co-chromatographed with authentic norepinephrine and normetanephrine.

In the first experiment (*I*), the animals' brains were pulse labeled with *d,l*-[^{14}C]norepinephrine 1 hr prior to perfusion and their lateral ventricles were perfused prior to conditioning of the visual stimulus-unconditioned shock (CS-US) pairings. The purpose of this portion of the study was to determine if *a*) injection with 6-hydroxydopamine altered the disposition of total ^{14}C (from [^{14}C]norepinephrine) or the ratio of unchanged norepinephrine to normetanephrine in perfusates generally, and *b*) presentation of a neutral visual stimulus would cause alterations in the parameters described in *a*). The second experiment (*II*) examined the effects of the US (shock) on those parameters examined in the first experiment. Be-

TABLE 4. Effect of drugs administered intraperitoneally on percent 5-[^{14}C]HT and 5-[^{14}C]HIAA of total radioactivity in the perfusate

Treatment	Sample no.	Mean percent-total radioactivity		
		% 5-HT	% 5-HIAA	% other
NaCl, 0.5 ml	4	62.3 ± 3.3	3.9 ± 0.4	33.8 ± 3.6
	8	59.9 ± 6.7	1.6 ± 0.1	48.7 ± 6.5
	15	61.5 ± 1.3	1.2 ± 0.1	37.3 ± 1.2
d-Amphetamine, 5.0 mg/kg	4	57.2 ± 3.2	3.9 ± 0.1	38.9 ± 3.2
	8	63.0 ± 3.6	2.3 ± 0.5	34.7 ± 6.3
	15	60.9 ± 5.3	2.8 ± 0.7	36.3 ± 4.6
Mescaline, 20 mg/kg	4	62.6 ± 1.5	5.4 ± 1.8	31.9 ± 0.3
	8	35.5 ± 0.4[a]	8.5 ± 0.9[a]	55.4 ± 3.5[a]
	15	30.5 ± 3.7[a]	16.4 ± 2.6[a]	53.0 ± 5.7[a]
d-LSD, 0.4 mg/kg	4	54.8 ± 7.2	3.1 ± 0.5	43.9 ± 6.9
	8	55.9 ± 3.0	2.8 ± 0.3	41.4 ± 2.7
	15	55.0 ± 8.9	2.9 ± 0.8	42.3 ± 7.8

Samples of perfusate before and after i.p. injection of psychoactive substances were analyzed by TLC for percentage of 5-[^{14}C]HT or 5-[^{14}C]HIAA. Results are mean ± SEM of percentage of total radioactivity and each value is the mean of the same three subjects. [a] Significantly different from preinjection level of same treatment, matched pair *t* test, $P < 0.05$.

tween the second and third experiments the rats were given 10 escape-avoidance trials a day for 5 days with the platform available. By the third day all rats in both groups were making an avoidance response during the 40 seconds of CS presentation.

The third and fourth experiments (*III, IV*) were essentially identical to the first experiment except that only the CS was presented (without shock or the opportunity to make the avoidance response by jumping onto the platform). In addition, the fourth experiment was carried out after the avoidance response to the CS was extinguished by forcing the rats to experience the presentation of the CS without its being paired with the US. Ten daily sessions were utilized for the extinction procedure. Perfusions were not carried out during training or extinction sessions.

This dose of 6-hydroxydopamine did not affect the rate at which conditioned avoidance behavior was acquired. By the end of the second daily session of 10 trials, both groups of rats were avoiding on the average of 90%, remaining above 90% avoidance responding on the subsequent three sessions. Likewise, the profiles of total ^{14}C-radioactivity in 4 min perfusion samples were very

TABLE 5. Effect of tolerance to the behavioral suppressant action of *d*-amphetamine and attempted cross-tolerance with mescaline on the appearance of radioactivity in the perfusate

Treatment	% Radioactivity relative to sample #4[a]	
	[^{3}H] norepinephrine (and metabolites)	5-[^{14}C]HT (and metabolites)
NaCl	42.2 ± 2.6	45.6 ± 9.7
d-Amphetamine (2.5 mg/kg)		
Pre-tolerance	69.5 ± 2.1[b]	49.6 ± 8.4
Tolerance	64.2 ± 4.3[b]	70.1 ± 10.4[b]
Mescaline (15 mg/kg)		
Prior to *d*-amphetamine tolerance	51.4 ± 3.9	65.7 ± 6.5[b]
After tolerance to *d*-amphetamine	54.0 ± 3.1	83.1 ± 3.8[c]

[a] Results are expressed as mean ± SEM of three subjects in subsequent samples related to sample #4, taken as 100%. [b] $P < 0.05$, matched pair *t*-test. [c] $P < 0.01$, matched pair *t*-test.

Figure 7. Sample cumulative records showing tolerance to the behavioral effects of 2.5 mg (+) amphetamine SO_4/kg (i.p.) and lack of cross tolerance to mescaline HCl (15 mg/kg). Intraperitoneal isotonic NaCl did not affect fixed ratio (FR) 30 responding (*A*), while (+) amphetamine initially produced abrupt cessation of responding (*B*), followed 8–12 days later by tolerance to the daily injections of (+) amphetamine (*C*). Mescaline, the following day, produced behavioral disruption (*D*). Arrows (and recorder reset) indicate the point of injection and the event marks are a 1 min time base. (Reprinted with permission (53.)

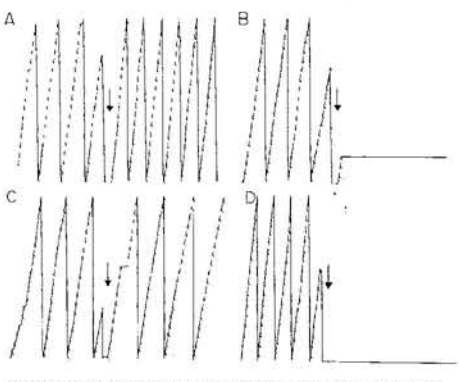
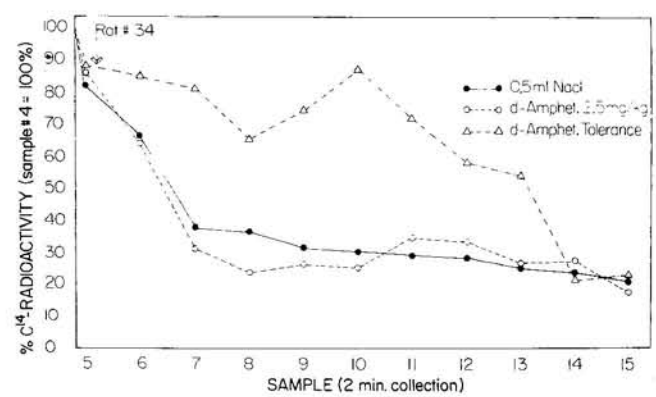


Figure 8. Releasability of 5-[^{14}C]HT (and metabolites) by (+) amphetamine after tolerance. Before tolerance to (+) amphetamine the profile of 5-[^{14}C]HT is identical to that after an injeciton of NaCl and decreases in a multiexponential fashion. The arrow denotes the point of injections and radioactivity, expressed as percent of radioactivity in sample #4, derived by counting 10 μl aliquots of 2 min samples (20 μl/min perfusion rate). (Reprinted with permission (53).)



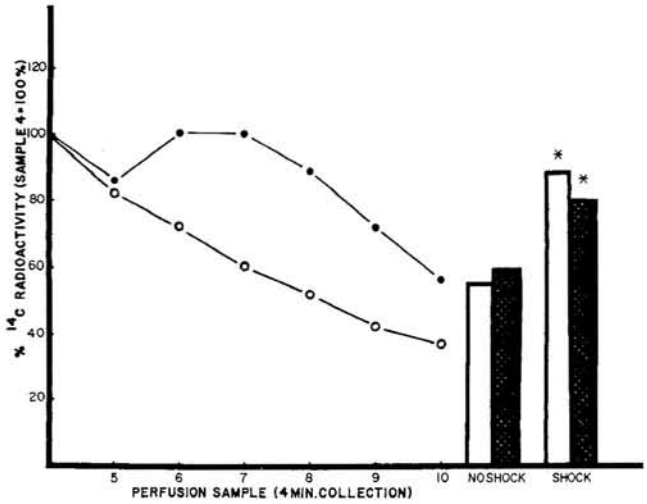
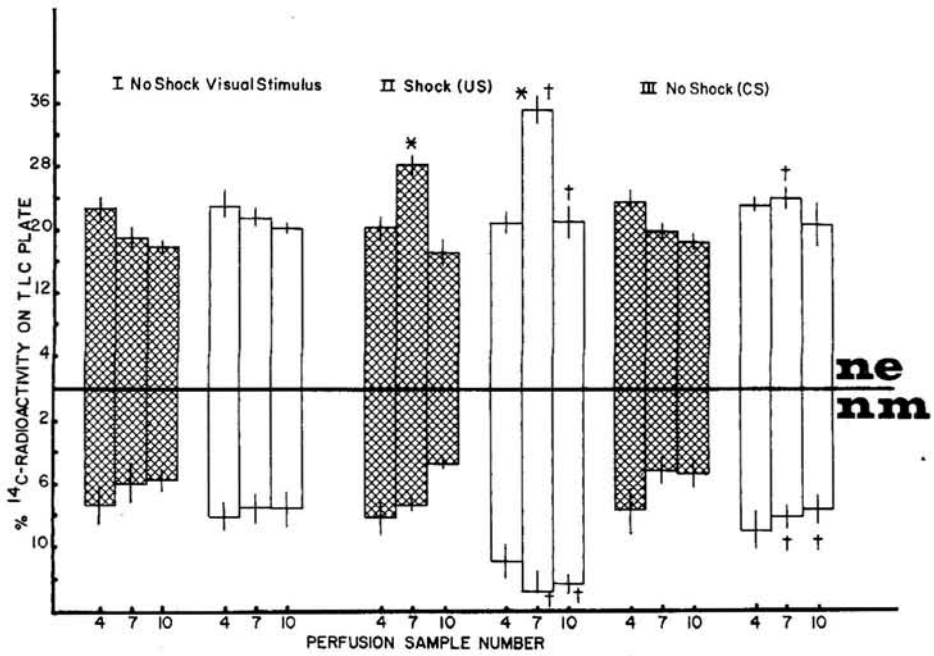


Figure 9. Distribution of [^{14}C]norepinephrine (and metabolites) in 10 μl aliquots of perfusion samples after presentation of a neutral visual stimulus for 60 sec (o ——— o) in experiment I or a neutral visual stimulus for 60 sec, the last 20 sec of which were paired with a 2 mA shock to rats' feet (• ——— •) in experiment II. The stimuli presentation was between samples 4 and 5. Both ascorbic acid vehicle and 6-hydroxydopamine groups are combined (N = N = 4). The open histograms represent the control group's average and the cross hatched histograms represent the 6-hydroxydopamine group's average radioactivity in samples after presentation of stimuli, relative to sample 4. Shock presentation resulted in a statistically reliable increase in the average amount of [^{14}C] in samples 5–10 relative to sample 4 ($P < 0.05$, correlated 2-tailed t-test). Analyzed separately, both groups likewise showed significantly elevated [^{14}C] activity in perfusates after shock presentation, *$P < 0.05$, correlated 2-tailed t-test. (Tilson, Rech and Sparber, unpublished observations.)

Figure 10. Radioactivity histograms for norepinephrine (ne) and normetanephrine (nm) in samples 4 (prior to presentation of neutral visual stimulus in experiment I, both neutral stimulus and shock, US, in experiment II, and the conditioned visual stimulus alone, CS, in experiment III), 7 (12 min after stimuli presentation) and 10 (20 min after stimuli presentation). Data are expressed as a % of [^{14}C] at spots where authentic ne and nm cochromatographed on the cellulose plates relative to the total amount of [^{14}C] on the plate. The * indicates a significant difference between that sample and its corresponding control (sample 4), $P < 0.05$ correlated 2-tailed t-test. The † indicates a significant difference between that sample and identical sample in the experimental group, $P < 0.05$, 2-tailed t-test. Vertical lines represent ±1 SEM for groups of 4 rats each. (Tilson, Rech and Sparber, unpublished observations.)



similar for both groups during experiments *I* and *II*. For both groups, radioactivity at the sites on the TLC plates corresponding to authentic norepinephrine and normetanephrine showed a distribution of 24% and 8% of the total counts on the entire plate for sample number 4. The 6-hydroxydopamine-treated group tended to decrease in percent of norepinephrine and normetanephrine a little more rapidly by sample 10 than the vehicle controls (Fig. 10). Presentation of the unconditioned shock stimulus (US) produced a significant increase in ^{14}C radioactivity (Fig. 9), which was also seen on the TLC plates (Fig. 10). However, although both groups showed a significant increase in ^{14}C at the norepinephrine spot, the increase in the percentage of radioactivity at this spot was significantly greater for controls than the 6-hydroxydopamine-treated group. To further support this suggestion are the data from experiment *III*, in which just the CS (visual stimulus) was presented under conditions in which avoidance was not possible. Figure 11 shows the significant increase in ^{14}G in samples 5–10, relative to sample 4, only in the control group, the experimental group's profile being very similar to that of experiment *I*. In addition, analysis of samples 4, 7 and 10 by TLC separation indicate significantly more norepinephrine and normetanephrine in the control group perfusate as a consequence of exposure to the CS compared with the 6-hydroxydopamine group (Fig. 10). Extinction sessions produced a washout curve of total radioactivity that paralleled that of experiment *I* for both groups.

These data indicate that periventricular limbic structures may be responsible for release of catecholaminergic transmitters in the absence of overt motor movement. The inability to show differences in avoidance behavior (after the 6-hydroxydopamine treatment regimen used) with this paradigm is not surprising, since many reports have appeared that indicate the relative resistance of conditioned and unconditioned behavior to severe alterations in catecholamine levels with the use of this compound (31, 49). It appears, however, that in these cases a more

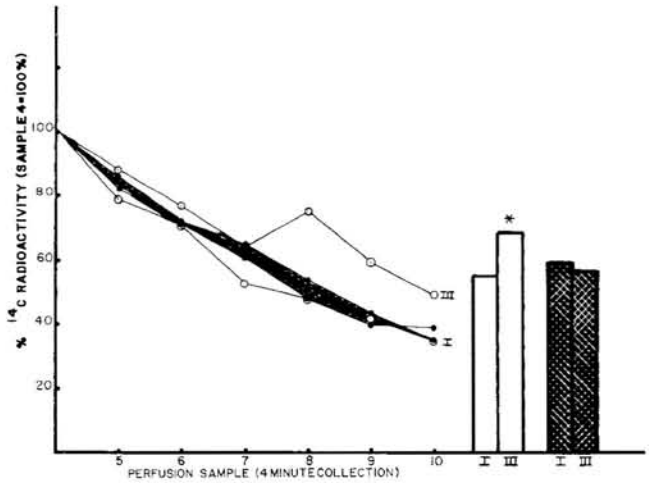


Figure 11. Effect of presentation of the CS (visual stimulus only), after avoidance learning had occurred, on the total radioactivity in each sample relative to sample 4 for the control (open area) and 6-hydroxydopamine group (cross-hatched area). In each case, the control group showed greater amounts of [^{14}C]norepinephrine (and metabolites) subsequent to presentation of the CS compared to experiment I, when the same visual stimulus was neutral and had no consequences. The average amount of radioactivity in samples collected after presentation of the CS is significantly greater only for the control group when compared to radioactivity collected in identical samples during experiment I. * $P < 0.05$ correlated t-test, N = N = 4. (Tilson, Rech and Sparber, unpublished observations.)

plausible explanation is that the behavioral measures were too insensitive to pick up the chronic effects of 6-hydroxydopamine (14, 15) as is also likely with the experimental paradigm employed in our study. Interestingly, Peterson and Sparber (unpublished observations) have found a significant increase in fixed ratio responding subsequent to 6-hydroxydopamine in which brain norepinephrine was significantly depleted while dopamine levels were hardly affected. Additionally, daily extinction sessions, in which positive reinforcement was completely withheld, resulted in a significant reduction in nonreinforced responding on the part of 6-hydroxydopamine treated (norepinephrine depleted) rats (Table 6). If one interprets these data as indicating enhanced learning of the consequences of barpressing in the absence of reinforcement, they are in agreement with those of Cooper et al. (14) who also showed enhancement of shuttle-box avoidance acquisition after preferential norepinephrine depletion with 6-hydroxydopamine. Regardless of the interpretation of the altered extinction performance by the 6-hydroxydopamine animals performing the operant, the data nevertheless support our contention that stimuli that have come to maintain conditioned behavior are in one way or another interacting with central catecholaminergic function (and/or other transmitters) that, in turn, may influence the degree to which those stimuli initiate or maintain that same behavior.

TABLE 6. Effects of intraventricular 6-hydroxydopamine (6-OHDA) on responding during extinction

Time after treatment, (days)		Extinction behavior[a]		
		Complete suppression		Nonreinforced responding (5 min/day)
		(minutes)	(responses)	
12	Vehicle	19 ± 2[b]	511 ± 125	
	6-OHDA	20 ± 2	561 ± 82	
13–17	Vehicle			607 ± 130
	6-OHDA			415 ± 57
107	Vehicle	28 ± 5	671 ± 141	
	6-OHDA	30 ± 7	637 ± 126	
108–112	Vehicle			620 ± 58
	6-OHDA			427 ± 58[c]

[a] On days 12 and 107 animals were put in extinction after receiving 10 reinforcers (300 responses on fixed ratio-30) and the time and response output were measured to the point where responding was completely suppressed for 4 min. On 5 subsequent days (13–17 and 108–112) animals were placed into the operant chamber and the number of nonreinforced responses were measured during a 5 min period. The value presented represents the sum of each block of 5 sessions. [b] Mean ± SE for 9 animals in each group. [c] Two tailed t-test, $P < 0.05$.

In ending, I might say that the question of whether or not useful information regarding the release or metabolism of transmitter substances with the use of technologies similar to that described here was really answered about 10 years ago when McLennan (36) showed release of endogenous acetylcholine and dopamine with perfusion technics and, more recently, by the demonstration by McKenzie and Szerb (34) that endogenous dopamine could be found in push-pull perfusate from striatal tissue.

Furthermore, variations of these technics in which homeostatic CNS mechanisms are used as a bioassay for active substances in perfusate (40) can be extended to the degree of elegance recently demonstrated in a report by Yaksh and Myers (61). Perfusates of monkeys' hypothalamuses taken during periods of food deprivation (hunger) were able to initiate fixed ratio responding after satiation, in the same monkeys during reperfusion, indicating a biologically active substance that is catecholamine-like, in that similar behavior could be reproduced by injection of norepinephrine into those same sites. It remains to be determined if the biologically active substance is indeed norepinephrine, as suggested by their bioassay, using blood pressure responses in a pithed rat. In any event, their data are extremely attractive regarding the probability that norepinephrine is the active substance.

Some of the many problems we have encountered while doing the perfusion work include the inability, so far, of maintaining a sustained level of newly synthesized catecholamines in perfusate after pulse labeling with tyrosine or l-dopa. This has

been due in part to the physical-chemical and ^3H-exchange problems we have encountered when using high specific activity compounds. We have been able however, to separate appropriate metabolites when dopamine has been injected intraventricularly. There are virtually no norepinephrine metabolites to speak of in the perfusates. With time, unchanged dopamine diminishes and homovanillic and dihydroxyphenylacetic acid as well as 3-methoxytyramine tend to increase (Peterson and Sparber, unpublished observations). With better separation technics we have been able to pick up relatively increasing amounts of dihydroxyphenylglycol after pulse labeling with norepinephrine. It appears, therefore, that much more information might be forthcoming from ventricular perfusions in the rat, regarding drug-behavioral-neurochemical interactions, with this approach. Like Myers and co-workers, we have had success in perfusing solid tissue during performance of an operant and are currently examining the relationship between perfusate constituents from caudate and bilateral motor activity after unilateral nigroneostriatal lesioning. The utilization of a right-left lever operant discrimination schedule should enable us to more precisely study presumptive excitatory and inhibitory transmitter disposition and their relationship to normal and abnormal behavior. FP

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