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Release of ^{14}C-Norepinephrine into the Lateral Cerebroventricle of Rats by Exposure to a Conditioned Aversive Stimulus[1]

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TILSON, H. A., R. H. RECH AND S. B. SPARBER. *Release of ^{14}C-norepinephrine into the lateral cerebroventricle of rats by exposure to a conditioned aversive stimulus.* PHARMAC. BIOCHEM. BEHAV. 3(3) 385–392, 1975. – Rats chronically implanted with push-pull cannulas were injected with a pulse of ^{14}C-norepinephrine (NE) into the lateral cerebroventricle under a variety of pretreatment and behavioral conditions. Animals pretreated intraventricularly with 6-hydroxydopamine (Group A) or ascorbic acid vehicle (Group B) were subsequently perfused under four conditions: (1) presentation of a novel, visual stimulus in a one-way avoidance chamber; (2) presentation of the light (CS) followed by shock; (3) training to a high level of avoidance behavior, after which the CS was presented in the absence of opportunity for an avoidance response and in the absence of shock; and (4) after forced extinction, followed by CS without opportunity to avoid and without presentation of shock. Samples of perfusate from rats subjected to the four test conditions were analyzed by thin-layer chromatography for total ^{14}C in a scintillation counter and for proportion of NE and normetanephrine (NM). During Tests 1 and 4 the ^{14}C perfusion wash-out did not differ from control values for either Group A or B. During Test 2, total radioactivity as well as the proportions of NE and NM increased in the perfusate for both Groups A and B. Presenting the CS without shock (3) resulted in an increase in ^{14}C and NE and NM for Group B (vehicle), but not for Group A (6-OHDA). To test for non-specific release unrelated to a brain catecholaminergic function, another group of rats was subjected to identical treatments with the exception that ^{14}C-urea replaced ^{14}C-NE as a pulse-label. In these animals Test 2 (shock) induced an increase in ^{14}C in the perfusate, while Tests 1, 3 and 4 yielded wash-out curves essentially identical to controls.

CNS Catecholamine efflux Ventricular perfusion Push-pull cannula Conditioned aversion

THERE have been numerous reports indicating that aversive environmental conditions enhance the utilization of brain biogenic amines [4, 6, 7, 10, 18]. However, the study of the neurochemical effects of stress in the conscious and freely-moving animal has been impeded by technical difficulties involved in the measurement of neurotransmitter release in the central nervous system [16]. One means of investigating neurochemical and behavioral events concurrently has been to perfuse brain with indwelling push-pull cannulas and measure the disposition of putative brain neurotransmitters marked or pulse-labelled previously with a radioisotope. This combination of procedures has been used to study various interactions between behavior and brain neurochemistry [16, 17, 23] as well as the neuropharmacological effects of psychoactive drugs [16, 19]. However, few investigators have used the perfusion technique to study the effects of stress or aversive conditions upon brain neurochemistry. Winson and Gerlach [22] reported that the apparent stress of injecting isotonic saline intraperitoneally produced changes in radioactivity from ^{3}H-norepinephrine (NE) in push-pull cannula perfusates from the amygdala, but not the hypothalamus. In a study

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using rats responding for food on a fixed-ratio schedule of reinforcement, it was observed that withholding reinforcement from these animals resulted in an increased efflux of ^{3}H-NE (and metabolites) probably from structures surrounding the cerebral lateral ventricles [16]. However, it is not clear in the latter study to what extent the increased motor output or food deprivation contributed to the efflux of ^{3}H-NE (and metabolites) during extinction.

In the present communication, we have studied the effects of electric footshock on the appearance of ^{14}C-NE and metabolites from pulse-labelled stores in cerebral lateral ventricular perfusate. In addition, we have investigated the disposition of this amine following the onset of a visual stimulus having acquired aversive properties.

METHOD

Animals

Sixteen male Sprague-Dawley rats (Spartan Farms, Haslett, Mich.) weighing approximately 300–350 g were used. The rats were housed in groups of 2 or 3 in air-conditioned quarters having a 12 hour light–dark cycle (light on 8:00 a.m. to 8:00 p.m.). Food and water were freely available in the home cages. The animals were divided into 4 groups of 4 rats each and designated Groups A, B, C and D.

Pretreatments and Intraventricular Cannular Implantation

Group A was pretreated intraventricularly with 252 μg of 6-hydroxydopamine hydrobromide (6-OHDA; dose expressed as the base). The 6-OHDA was dissolved in a 0.1% solution of ascorbic acid and infused into the right lateral ventricle through a stainless steel needle tube (30 g) attached to an infusion pump by means of polyethylene tubing. Infusion of the solution (10 μg of 6-OHDA/cc) was conducted under anesthesia (35 mg/kg sodium pentobarbital and methoxyflurane) at a rate of 8.4 μl/min for 3 min. Coordinates for the procedure were determined by measuring 1 mm laterally and 1 mm posteriorly from the bregma and lowering the tip of the needle 3.55 mm into the brain through a small hole drilled through the skull. Group B was infused intraventricularly in an identical manner, except that the infusion contained only 0.1% ascorbic acid. Groups C and D received no pretreatment.

At least two weeks after pretreatment procedures all 16 rats were anesthetized and implanted with chronic, indwelling push-pull cannulas using the coordinates for the lateral ventricles described above. Description of the cannulas and other details of the implantation procedure have been reported elsewhere [16].

Apparatus

Experiments were conducted in a one-way shuttle box (LaFayette Instrument Co., Model 85200). Activation of a motor by a hand switch moved the back wall of the box away from the chamber, permitting access to an escape platform. Electric footshock (0.2 – 2.0 mA) was applied to the grids by means of a shock generator and scrambler (BRS-Foringer, Inc., Models SG-903 and SC-902, respectively). The onset of a stimulus light in the shuttle box and the application of electric shock to the grids were controlled by standard electromechanical equipment.

Procedure

In order to minimize the possibility that release of NE during critical stages of the experiment was the result of novel environmental conditions, the animals were given several sessions to acclimate or adapt to the shuttle box and the perfusion procedure. Two weeks after the implantation of the push-pull cannulas, the rats were given 2 successive daily acclimation sessions in which they were connected to the perfusion device (but not perfused) and placed in the darkened shuttle box for 45 min. On the following day, the lateral ventricles of the rats were perfused with artificial cerebrospinal fluid (artificial csf) [14] at a rate of 20 μl/min for 45 min. Details of the perfusion apparatus and method of sample collection have been reported elsewhere [16,19]. In the present study, sequential samples of perfusate were collected every 4 min in cups containing 100 μl of 5N acetic acid.

Shock Level

In preliminary studies, we were concerned with the level of shock intensity required to produce a measurable neurochemical effect. Thus, the four rats in Group C were subjected on a random basis to four intensities of electric footshock via the grids of the shuttle box. At least 4 days separated the test sessions. On the day of the perfusion, the animals were pulse-labelled with 10 μl (1 μC) of ^{14}C-d, l-norepinephrine (specific activity 44 mC/mmol; 3.8 μg; New England Nuclear) 1 hr prior to the session. They were placed in the darkened chamber and perfused with artifical CSF as described above for the acclimated perfusion session. Following the collection of the fourth sample of perfusate, 0.2, 0.5, 1.0 or 2.0 mA of shock were applied to the grids for a period of 20 sec. The perfusion was terminated following collection of the tenth sample of perfusate. Since 2.0 mA shock was effective in producing an efflux of ^{14}C-NE in all animals, it was used in the subsequent series of four experiments.

Experiment 1 (Novel light + no shock). On the day after the last acclimation session in the shuttle box, rats in Groups A and B were infused intraventricularly with 10 μl (1 μC) of ^{14}C-NE via the inner cannula tube one hour prior to testing. Sixteen min after the collection of the first sample of perfusate, a novel visual stimulus was activated for 60 sec and then turned off for the remainder of the session.

Experiment 2 (Novel light + shock). Two days later the procedure used in Experiment 1 was repeated. However, in this session electric footshock (unconditioned stimulus; US) was applied to the grids during the last 20 sec of the 60 sec as a test for the effects of the shock on the release of ^{14}C-NE.

Experiment 3 (CS + no shock after conditioning). For the next 5 daily sessions, during which no perfusions were conducted, the rats were given 10 conditioning trials per day. Each trial consisted of 60 sec of darkness followed by 60 sec of light (conditioned stimulus; CS), the last 20 sec of which was paired with electric footshock. Following the onset of the CS, the sliding wall at the rear of the chamber was moved, providing access to the platform. An avoidance response was defined as mounting the platform at anytime during the first 40 sec of the CS and remaining there until the wall was moved back to its original position following termination of the shock and the CS. By the third day of

conditioning, the rats were successfully avoiding the shock during 90 percent of the trials. The day after the last conditioning session, the rats were infused with 1 μC of ^{14}C-NE 1 hr before perfusion. The procedure of this session was identical to the test for the effects of shock on the release of NE (Experiment 2) except that electric footshock did not follow the onset of the CS and avoidance responding was not permitted. This experiment was designed to test for the effects of a stimulus with acquired aversive properties (CS) on the release of ^{14}C-NE.

Experiment 4 (Extinction). Following completion of Experiment 3 rats in Groups A and B were subjected to 10 additional daily sessions in the shuttle box during which perfusions were not performed. These sessions were identical to the conditioning procedure except that the floor grids were not electrified during the latter portion of the light period, nor was the platform available for avoidance responding (forced extinction). The day following the last extinction session, the rats were again pulse-labelled with ^{14}C-NE 1 hr before perfusing in the shuttle box. During this session the onset of the CS (light) was not followed by shock, nor was the avoidance platform available.

^{14}C-Urea control. The last group of rats (Group D) was used to test the possibility that the appearance of the ^{14}C-radioactivity in the perfusate following the CS in Experiment 3 may have been nonspecific. These animals were treated as described above for Groups A and B except that 0.1 μC of ^{14}C-urea (Sp. Act. 16.7 μC/mM; New England Nuclear) was infused intraventricularly 30 min before the sessions.

Analysis of the Perfusate

Immediately after each perfusion, 10 μl aliquots from each of the 10 samples were placed in counting vials containing 15 ml scintillation liquid (6 g of 2,5-diphenyloxazole per liter of toluene) and 0.180 ml of sample solubilizer (BBS-3; Beckman Instruments, Inc.). ^{14}C-Radioactivity in each vial was counted in a Beckman LS-100 scintillation spectrometer. The remaining perfusate from Groups A and B was frozen until thin layer chromatographic (TLC) separation of perfusate was performed. Twenty μl aliquots from samples of perfusate taken before the onset of the light (Sample no. 4) and two after the termination of the light (Samples no. 7 and 10) were analyzed for their proportion of ^{14}C-NE and ^{14}C-normetanephrine as described previously [20]. The solvent system used in the present experiment consisted of n-butanol:methanol:1N formic acid (60:20:20) [8]. The average recovery of ^{14}C-NE for 12 observations was 41.6% ± 6.2 (mean ± S. E.), and counting efficiency ranged from 57.5 – 66.4%.

RESULTS

The ^{14}C-radioactivity in the perfusate was characterized by a washout profile, with large amounts of ^{14}C-radioactivity in the first 3 samples and smaller amounts in Samples 4–10. As reported previously, the profile appeared to have at least 2 dimensions [16,19]. There were large decreases in radioactivity between Samples 1 and 3 followed by a more uniform and moderate rate of decline in radioactivity in Samples 4–10. Thus, the behavioral manipulations were conducted after the collection of the fourth sample and the radioactivity in Samples 5 through 10 are expressed relative to Sample 4. The absolute amount of ^{14}C-radioactivity recovered in the perfusate was variable, but an analysis of the average ^{14}C-radioactivity in Sample 4 indicated no statistical differences between any of the groups.

In the pilot study of the effect of shock level on release of ^{14}C-NE into the perfusate (Group C), 0.2 mA delivered via the grids of the shuttle box caused no apparent alteration in the pattern as compared to that observed from perfused, non-shocked rats (Fig. 1). Greater shock levels of 0.5, 1.0 and 2.0 mA produced intensity-related increases of ^{14}C-radioactivity in the perfusate. Since the 2.0 mA level of shock produced easily quantifiable increases, it was chosen for subsequent experiments utilizing the avoidance paradigm.

The purpose of the series of four experiments was to determine if the release of NE could be elicited by a stimulus with acquired aversive properties (CS) and if the release could be blocked by destroying catecholamine nerve terminals at the site of perfusion with 6-OHDA.

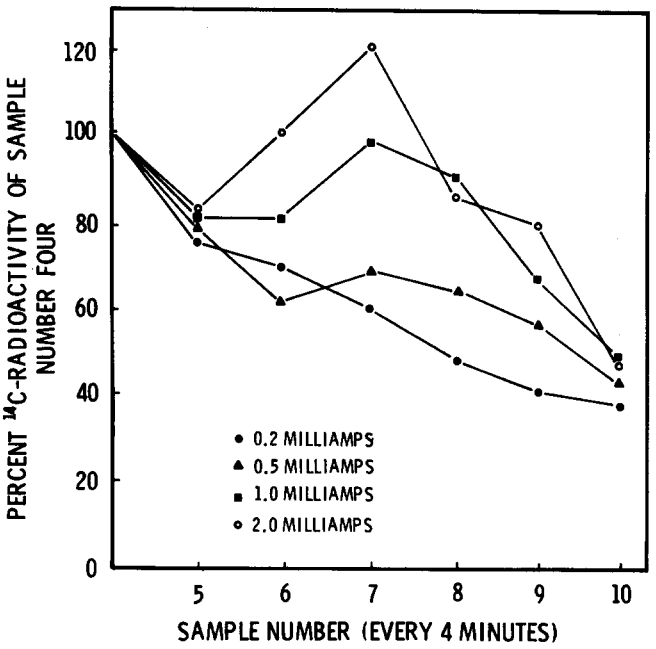

FIG. 1. The effects of various levels of shock intensity on the appearance of ^{14}C-radioactivity from ^{14}C-NE in cerebral ventricular perfusate. Rats were pulse-labelled with 1 μC of ^{14}C-NE 1 hr before perfusion and, following the fourth 4 min sample, 0.2–2.0 mA of shock was applied to the grids of the chamber for a 20 sec period. Each point is the mean of four rats for percentage of ^{14}C-radioactivity in Samples 5–10 relative to Sample 4. The absolute radioactivity (mean dpm ± S.E.) in the fourth sample for the 0.2, 0.5, 1.0 and 2.0 mA experiments were 1392 ± 181, 1451 ± 217, 1223 ± 352 and 1859 ± 380 respectively.

Experiment 1 (Novel Light + No Shock)

The presentation of the novel, visual stimulus did not influence the pattern of ^{14}C recovered in the perfusate, and there were no statistical differences between Group A (6-OHDA) and Group B (ascorbic acid) (Figs. 2 and 3).

Experiment 2 (Novel Light + Shock)

When 2mA of footshock was presented during the last 20 sec of the 60 sec light both the 6-OHDA pretreated (A)

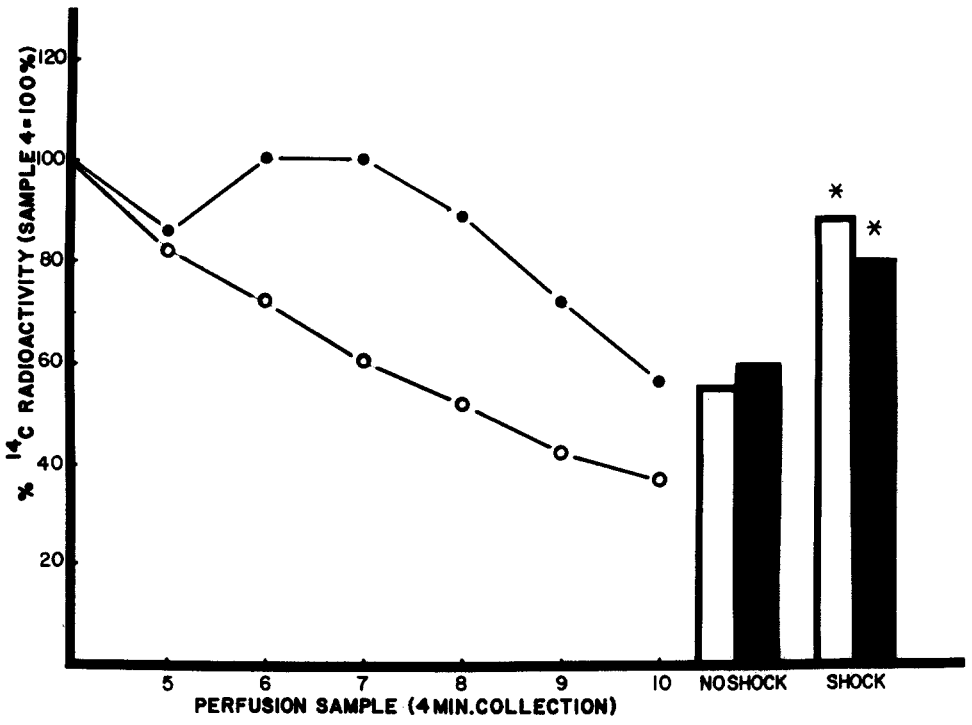

FIG. 2. Profile of ^{14}C-radioactivity from ^{14}C-NE (and metabolites) in 10 μl aliquots of perfusion samples after presentation of a neutral visual stimulus for 60 sec o———o (Experiment 1) or a neutral visual stimulus for 60 sec, the last 20 sec of which were paired with a 2 mA shock to rats' feet •———• (Experiment 2). The stimulus presentation was between Samples 4 and 5. Data from Group A (6-OHDA) and Group B (ascorbic acid) were combined (N = N = 4) to construct these curves. The open histograms represent Group B's average radioactivity and the cross-hatched histograms represent Group A'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). The asterisks (*) indicate that both groups showed significantly elevated ^{14}C-radioactivity in perfusates after shock presentation ($p<0.05$, correlated 2-tailed t test). The average (mean dpm ± S. E.) ^{14}C-radioactivity in Sample 4 for Group A was 1157 ± 412 and 972 ± 310 for Experiments 1 and 2, respectively. The average dpm is Sample 4 for Group B was 1659 ± 427 and 1064 ± 208, respectively.

and vehicle pretreated (B) groups exhibited an increase in the rate of efflux of ^{14}C into the perfusate (Fig. 2). Again, there were no statistical differences between the two groups. When the average amount of ^{14}C, relative to Sample 4, was determined for Samples 5 through 10, the light + shock condition provoked a significantly greater release of radioactivity than was observed in perfusates collected from these rats under the light + no shock condition (Experiment 1). For both Group A and Group B the radioactivity for Sample 4 on the TLC plates at an R_f value for authentic NE and NM showed a distribution of 24 percent and 8 percent of the total counts on the plate. The 6-OHDA treated group (A) tended to decrease in percent of NE and NM slightly more rapidly by Sample 10 than the ascorbic acid group (B; Fig. 3). The presentation of shock resulted in a significant increase in ^{14}C on the TLC plate that was attributable to NE; though both groups showed this increase, that derived from Group B was significantly greater than that from Group A.

Experiment 3 (CS + No Shock)

During the conditioning portion of the experiment, there were no differences in the rate at which the two groups (A and B) acquired the avoidance response. During the last 3 training sessions the animals maintained an avoidance performance averaging around 90 percent of the trials. During the test session the shock was not presented, nor were the rats given the opportunity to make an avoidance response (platform not available). Under these conditions there was an increase of ^{14}C in the perfusate of Group B but not of Group A (Fig. 4). The average ^{14}C in Samples 5–10, relative to Sample 4, was statistically greater for Group B than the comparable value in Experiment 1 (novel light + no shock). On the other hand, the average ^{14}C in perfusates of Group A (6-OHDA) did not differ significantly when values from this experiment and Experiment 1 were compared. In addition, the analysis of Samples 4, 7 and 10 by TLC separation indicated significantly more NE and NM in the perfusate from Group B exposed to Experiment 3 as compared to these values in Group A (Fig. 3).

Experiment 4 (Extinction)

After the forced extinction trials for Groups A and B, the test session involving ventricular perfusion yielded a

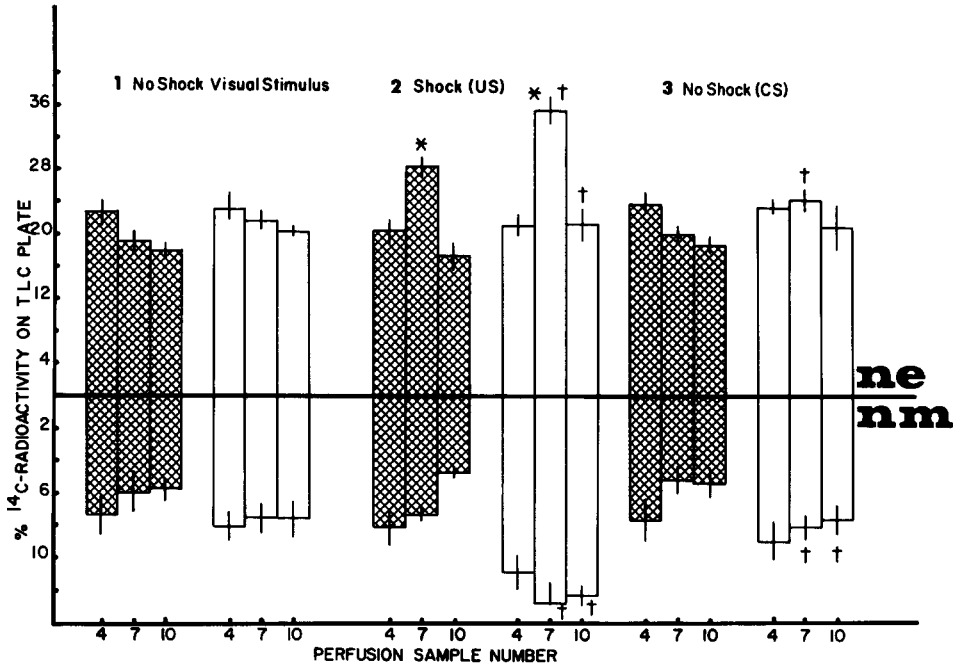

FIG. 3. Radioactivity histograms for NE and normetanephrine (NM) in Samples 4 (prior to presentation of neutral visual stimulus in Experiment 1, both neutral stimulus and shock, US, in Experiment 2, and the conditioned visual stimulus alone, CS, in Experiment 3), 7(12 min after stimuli presentation) and 10(20 min after stimuli presentation). The cross-hatched histograms represent values from Group A (6-OHDA), while the open histograms denote values from Group B (Ascorbic acid). Data are expressed as a percent of ^{14}C at spots where authentic NE and NM were located when cochromatographed on the cellulose plates, relative to the total amount of ^{14}C on the plate. The asterisk (*) indicates a significant difference between that sample and its corresponding control (Sample 4) ($p<0.05$, correlated 2-tailed t test). The cross (+) indicates a significant difference between that sample and the identical sample in the experimental group ($p<0.05$, 2-tailed t test). The vertical lines represent ± 1 S. E. M. for groups of 4 rats each.

wash-out curve of ^{14}C for both groups that resembled that of Experiment 1. For Group B the results of this last procedure suggested that the CS had lost its conditioned aversive properties as evidenced by a lack of effect on the rate of ^{14}C release into the perfusate.

^{14}C-Urea Control

The animals pulse-labelled with ^{14}C-urea instead of ^{14}C-NE (Group D) were similar to Groups A and B in acquiring the avoidance response within two or three daily sessions. The profile of ^{14}C from ^{14}C-urea was different than that of ^{14}C-NE, as described previously [16]. That is, in the first test (same behavioral paradigm as in Experiment 1), the first 3 samples of the perfusion contained more than 98 percent of the total ^{14}C recovered in the 10 samples, while the remaining samples contained very low counts from ^{14}C. The presentation of the novel visual stimulus did not appear to alter the efflux of ^{14}C in ventricular perfusate. In the second test (same behavioral paradigm as in Experiment 2), presentation of the visual stimulus plus footshock produced a large efflux of ^{14}C in Sample 7 followed by a rapid decline in Samples 8 through 10. A similar effect has been described following I P injection of isotonic saline and various psychoactive drugs to rats lever-pressing for food reinforcement in an operant procedure [16]. After learning to avoid the electric shock, rats in Group D did not show an efflux of ^{14}C from ^{14}C-urea when the CS was given alone without shock (same behavioral paradigm as in Experiment 3) (Fig. 5) or following forced extinction (same behavioral paradigm as in Experiment 4).

DISCUSSION

In a previous study, it was reported that withholding positive reinforcement from an animal lever-pressing for food produced an efflux of NE (and metabolites) from periventricular structures [16]. However, the efflux of radioactivity during extinction was associated with changes in motor output or extinction-induced bursts of responding. Since extrapyramidal structures surrounded the site of perfusion, it was not clear whether the efflux was related to purely emotional, psychological phenomena or whether it was more a consequence of the extinction-induced change in motor activity. The data of the present experiment suggest that the catecholamines are released from periventricular structures at least in part as a consequence of emotion-producing environmental contingencies and that the release is not necessarily associated with increases in motor activity. Following the repeated pairing of a novel visual stimulus (i.e., initially having no behavioral or neurochemical significance) with imminent electric footshock, the light (CS) rapidly began to elicit avoidance behavior. When the opportunity for avoidance responding was blocked, and

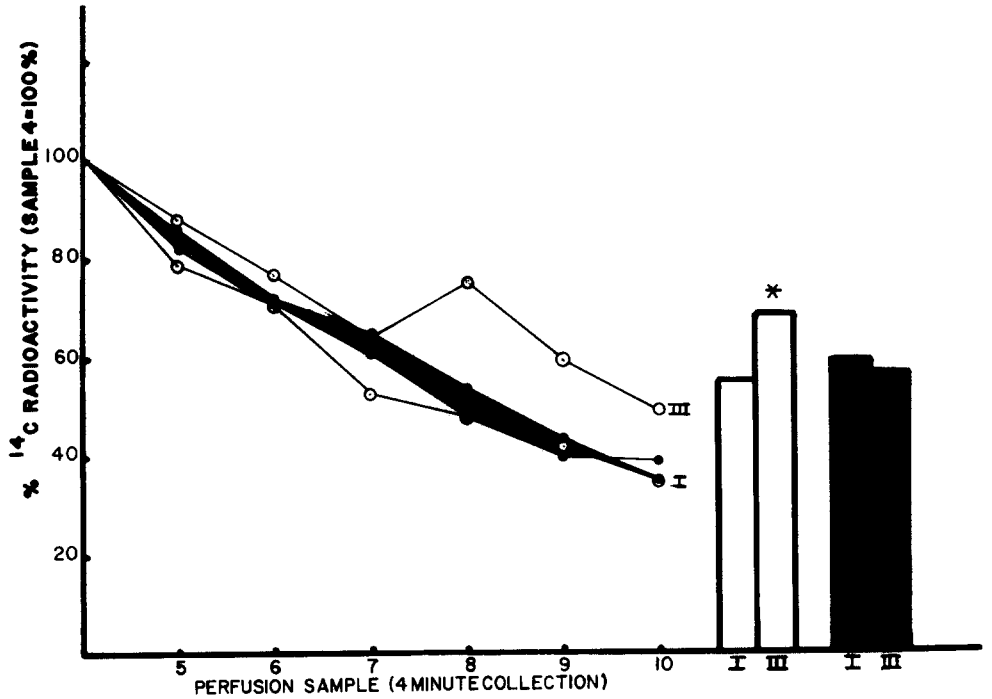

FIG. 4. Effect of presentation of the CS (visual stimulus only) after avoidance learning had occurred, on the total radioactivity in each sample relative to sample 4 for Group B (ascorbic acid; open symbols and open histograms) and Group A (6-OHDA; filled symbols and cross-hatched histograms). In each case, Group B showed greater amounts of ^{14}C-NE (and metabolites) subsequent to presentation of the CS as compared to Experiment 1, 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 Group B when compared to radioactivity collected in identical samples during Experiment 1. The asterisk indicates statistical significance ($p<0.05$, correlated t test). During Experiment 3, the average dpm ± S. E. in Sample 4 for Group A and B was 1630 ± 709 and 1171 ± 352, respectively. See Fig. 1 for the absolute ^{14}C in Sample 4 for Experiment 1.

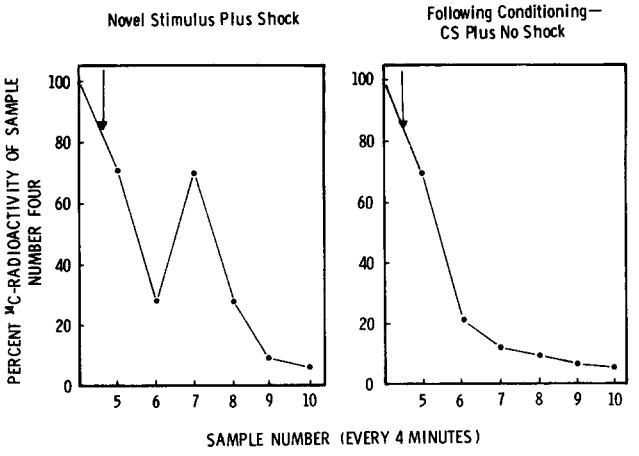

FIG. 5. The effects of electric footshock and a conditioned aversive stimulus (CS) on the appearance of ^{14}C-radioactivity from ^{14}C-urea in cerebral ventricular perfusate. Experiments were conducted as with animals infused intraventricularly with ^{14}C-NE, except 0.1 μC of ^{14}C-urea was infused via the inner cannula tube 30 min prior to perfusion. Data are mean percentage of ^{14}C-radioactivity in samples 5–10, relative to Sample 4, for 4 animals in Experiments 2 and 3. The profile of ^{14}C after presentation of a novel visual stimulus or after the stimulus (CS) subsequent to forced extinction resembled that of the CS + no shock condition (behavioral paradigm similar to Experiment 3). The mean dpm ± S. E. in Sample 4 during the first (2) and second (3) tests was 236 ± 36.2 and 310.3 ± 45.4, respectively.

in the absence of shock, there was an efflux of ^{14}C from NE and metabolites in the perfusate on presentation of the CS. Furthermore, the conditioned release of NE appeared to be from functional catecholaminergic nerve terminals. An efflux of ^{14}C was not observed following onset of the CS alone for animals pretreated with 6-OHDA (Group A) or for animals pulse-labelled with ^{14}C-urea (Group D), an extracellular marker. However, it is likely that the efflux of ^{14}C following electric shock (US) alone in Experiment 2 may have been partly non-specific. That is, the presentation of electric footshock increased ^{14}C in the perfusate of all animals, regardless of pretreatment or source of ^{14}C-radioactivity. This observation tends to support a previous finding that non-specific efflux of extracellular markers such as ^{14}C-urea or ^{14}C-inulin is often associated with movement of the experimental animal or with changes in fluid dynamics during the perfusion [16].

In general, the results of the present investigation are in accord with numerous studies showing changes in the disposition of catecholamines following various kinds of physical (4, 6, 7, 10, 18] and psychological [2,21] stressors. The data from the present investigation tend to implicate basal ganglia and limbic structures as being responsible for the efflux of ^{14}C-radioactivity from ^{14}C-NE. For example, one periventricular limbic structure lying near the site of our perfusion is the hippocampus which is clearly implicated in the control of some emotional responses [11]. Furthermore, electrical footshocks have been reported to increase

the turnover of NE in this area of the rat brain [12]. These latter investigators also reported that the stress-induced changes in the hippocampus were dependent upon the integrity of the locus coeruleus. This structure is made up of cell bodies for NE-containing neurons [9] and supplies noradrenergic nerve terminals to the hippocampus, as well as the cerebral cortex [1]. The caudate nucleus, which contains dopamine (DA) nerve terminals, also is in juxtaposition to the site of perfusion. Assuming that the ^{14}C-NE used for pulse-labelling was also taken up into these DA-containing neurons and preferentially released, then this structure could have contributed to the observed efflux. This would be in accord with another study indicating that aversive conditions increase the metabolism of DA, regardless of changes in motor activity [3].

The relatively large dosage of ^{14}C-NE used to pulse-label catecholamine stores could have been taken up into non-catecholamine nerve terminals or bound non-specifically to extraneuronal tissue. This perturbation of normal amine concentrations may have resulted in a displacement of other neuronal substances, producing a non-specific or non-functional release. There are, however, several observations that argue against these possibilities, particularly during the crucial conditioned release experiment (Experiment 3). For example, the rapidly declining or wash-out phase of radioactivity under control conditions appears to originate from non-specific sites and the efflux of radioactivity above this baseline of non-specific wash-out appears to be related to a functional release [20]. That the release of catecholamines was associated with a functional catecholaminergic system is indicated by the alterations in the proportion of ^{14}C-NE and ^{14}C-NM following the CS, as well as the US. In addition, destruction of nerve terminals at the site of perfusion with 6-OHDA blocked both increases in efflux and changes in proportions of NE and NM in the perfusate subsequent to the CS. Finally, the absence of a conditioned efflux from rats pulse-labelled with ^{14}C-urea also argues against the emotion-induced appearance of radioactivity as originating from non-neuronal sites.

The unilateral intraventricular administration of 6-OHDA to rats under similar conditions in a previous study in our laboratory has been found to decrease NE and DA whole brain levels to 15 and 44 percent of control, respectively [20]. Thus, it was somewhat surprising that the 6-OHDA pretreatment was effective in blocking the neurochemical response to the conditioned aversive stimulus, while there was no difference in the rate at which 6-OHDA (Group A) and ascorbic acid control rats (Group B) learned the avoidance response. Other investigators have also reported that some conditioned and unconditioned behaviors are relatively resistant to the long-term effect of intraventricularly administered 6-OHDA [13]. However, the most likely explanation for our inability to observe a behavioral deficit following 6-OHDA is that our behavioral test was not sensitive enough. Other studies have shown that intracisternal administration of 6-OHDA is effective in reducing responding during acquisition of a two-way shuttle box avoidance [5]. In other experiments, 6-OHDA treatment has been found to affect the maintenance and control of operant behavior. A depletion by 6-OHDA of brain NE, without affecting DA, resulted in a significant increase in food-reinforced responding and significant decrease in the number of responses emitted when positive reinforcement was withheld (extinction) [15]. Thus, these observations tend to support the contention that stimuli which acquired a control over conditioned behavior interact in some manner with a brain catecholaminergic function. This function may likewise influence the extent to which these stimuli may initiate or maintain that same behavior.

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