Pharmacology Biochemistry & Behavior, Vol. 16, pp. 131–137, 1982. Printed in the U.S.A.

Measurement of Metabolites of Dopamine and 5-Hydroxytryptamine in Cerebroventricular Perfusates of Unanesthetized, Freely-Moving Rats: Selective Effects of Drugs[1]

J. A. NIELSEN AND K. E. MOORE

Department of Pharmacology and Toxicology, Michigan State University East Lansing, MI 48824

Received 20 July 1981

NIELSEN, J. A. AND K. E. MOORE. *Measurement of metabolites of dopamine and 5-hydroxytryptamine in cerebroventricular perfusates of unanesthetized, freely-moving rats: Selective effects of drugs.* PHARMAC. BIOCHEM. BEHAV. 16(1) 131–137, 1982.—A method was developed for measuring endogenous acid metabolites of dopamine (dihydroxyphenylacetic acid, DOPAC; homovanillic acid, HVA) and of 5-hydroxytryptamine (5-hydroxyindoleacetic acid, 5HIAA) in serial samples of cerebroventricular perfusates from unanesthetized, freely-moving rats by the use of high performance liquid chromatography with electrochemical detection. Systemic administration of probenecid increased while pargyline reduced the efflux of all of the acid metabolites. Intraperitoneal injection of L-tryptophan increased the perfusate content of 5HIAA, decreased HVA and was without effect on DOPAC. Injections of haloperidol increased the efflux of DOPAC and HVA but did not alter the efflux of 5HIAA. Administration of apomorphine decreased the concentration of all three compounds. These drug-induced changes are generally consistent with those reported to occur in brain tissues. The results indicate that this method can be employed to monitor the activity of dopamine and 5-hydroxytryptamine neurons in unanesthetized, freely-moving rats.

Brain perfusion Dihydroxyphenylacetic acid Homovanillic acid 5-Hydroxyindoleacetic acid HPLC

THE activities of aminergic neurons in the central nervous system (CNS) have been estimated from measurements of amines and/or their metabolites in cerebrospinal fluid (CSF) or perfusates of the cerebroventricular system (e.g., [6, 13, 17, 20]). These studies have generally been performed in anesthetized animals. Anesthesia facilitates the withdrawal of CSF and the perfusion of the ventricles, but it also alters the activity of aminergic neurons [5,16] and the concentrations of neurotransmitter metabolites in the CSF [4,9]. Until recently the lack of sensitivity of analytical techniques dictated the use of radioactive tracer techniques in order to measure the small amounts of biogenic amines and their metabolites in reasonable volumes of cerebroventricular perfusates. The recently developed technique of combining high performance liquid chromatography with electrochemical detection (LCEC) makes possible the direct measurement of endogenous amines and their metabolites in CSF [21] or brain perfusates [10].

This report describes a procedure utilizing push-pull perfusion and LCEC for measuring metabolites of dopamine (DA) and 5-hydroxytryptamine (5HT) in serial samples of cerebroventricular perfusates from unanesthetized, freely-moving rats on a chronic basis.

METHOD

Cannula Construction

The construction of the perfusion cannula base, cap and stylus was slightly modified from that described previously [11]. Briefly, a stainless steel injection cannula (Model 220, D. A. Kopf, Tujunga, CA) was modified to serve as the perfusion cannula base (Fig. 1). The flat portion of the base was trimmed and the needle tubing shortened to extend 6 mm below the base. Two O-rings (Small Parts Inc., Miami, FL) were fitted into the base to assure a complete seal between the cap and the base during perfusion. Caps were

[1]This research was supported by USPHS grant NS 15911. Preliminary reports were presented at the 22nd Annual Meeting of the American Society for Pharmacology and Experimental Therapeutics, Rochester, MN, August 17–21, 1980 and the 10th Annual Meeting of the Society for Neuroscience, Cincinnati, OH, November 9–14, 1980.

Copyright © 1982 ANKHO International Inc.—0091-3057/82/010131-07$03.00/0

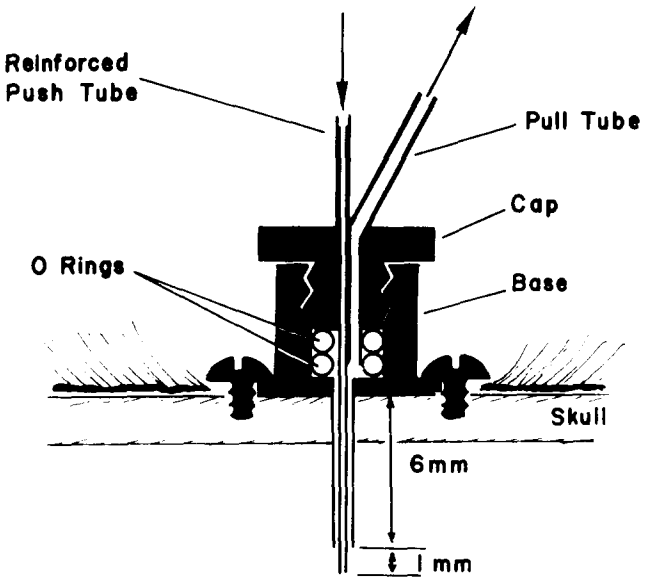

FIG. 1. Schematic diagram of a cross-section of the cannula base containing 2 O-rings and the perfusion cap screwed into the base (not drawn to scale).

made by drilling a hole through a stainless socket head screw (1/8 inch, 6-32 thread size) and securing two pieces of stainless steel tubing (a push and a pull tube) in this hole with retaining compound (RC680, Loctite, Newington, CT). The push tube consisted of 33 gauge tubing which extended 0.75–1.0 mm past the tip of the needle tubing in the base; above the cap the push tubing was reinforced for about 20 mm by cementing 23 gauge tubing around the 33 gauge tubing. The pull tube consisted of 23 gauge tubing which extended 0.5 mm below the bottom of the cap and about 20 mm above the cap. The stylus was prepared by cementing 33 gauge tubing into the socket head screw and trimming this tubing so that it extended approximately 0.5 mm beyond the needle tubing in the base when securely screwed down. The stylus was kept in place whenever the ventricular system was not being perfused.

Implantation and Perfusion

Male Sprague-Dawley rats (Spartan Research Animals, Haslett, MI) weighing 225 to 275 g were anesthetized with Equithesin (3 ml/kg, IP) and implanted with the cannula base such that the tip of the needle tubing was within a lateral cerebroventricle. The stereotaxic coordinates [12] were A 0.0, L 1.6. Other details of implanting the cannula base have been described [11] previously.

Unanesthetized rats were placed in large plastic bowls and the push and pull tubes in the cannula cap were attached with silicone tubing (Dow-Corning Silastic Medical Tubing, Dow-Corning Corp., Midland, MI) to a four channel peristaltic pump (Minipuls II, HP4, Gilson Electronics, Middleton, WI). The lateral ventricles were perfused at a rate of 20 μl/min with a perfusing solution (129 mM NaCl, 2.5 mM $NaHCO_3$, 0.5 mM Na_2HPO_4, 3 mM KCl, 0.8 mM $MgCl_2$, 1.3 mM $CaCl_2$ dissolved in redistilled water). This solution was bubbled with 95% O_2-5% CO_2 and filtered (0.22 μM filter membrane, Millipore Corp., Bedford, MA) prior to use.

Sequential 15 minute samples of perfusate were collected into polyethylene microsample tubes containing 400 μl of the LCEC mobile phase. Samples were frozen at −20°C until analyzed. At the conclusion of the perfusion the animal was disconnected from the perfusion apparatus, the stylus was screwed into the cannula base, and the animal was returned to its home cage.

LCEC Analysis of Perfusate

Perfusate samples were filtered (0.22 μM filter membrane, Millipore) and then 500 μl were injected directly onto a liquid chromatography column. A model LC 40 liquid chromatograph with a TL-3 carbon paste amperometric detector (Bioanalytical Systems, West Lafayette, IN) was employed with a 30 cm × 3.9 mm i.d. μBondapak C_{18} column (Waters Associates, Milford, MA). Detector potential was +0.75 V versus a $Ag^+/AgCl$ reference electrode. The detector was generally set at 1 nA full scale. Sample injection was accomplished using a 500 μl loop. All chromatographic experiments were performed at ambient temperature (20–22°C) in an electrically shielded room.

The mobile phase consisted of 0.1 M citric acid and 0.1 M phosphate (sodium phosphate dibasic anhydrous) plus 12.5% methanol and 0.004% sodium octyl sulfate (SOS) (Bioanalytical Systems), pH 3.5. The citrate-phosphate buffer was filtered through a 0.22 μM filter membrane (Millipore), while the methanol was filtered through a 0.5 μM teflon filter membrane (Schleicher and Schnell, Inc., Keene, NH).

Pharmacological Evidence for the Authenticity of DA and 5HT Metabolites in Ventricular Perfusate

Five rats were implanted with cannula bases and perfused at 5–7 day intervals for 60 to 90 minutes. Initially no systemic injection was administered during the perfusion. Subsequently, intraperitoneal (IP) injections were made 15 minutes into the perfusion. In separate experiments the rats were administered 0.9% saline (once or several times), 3×10^{-3} N hydrochloric acid (pH 3.5), 3.5×10^{-3} N sodium hydroxide (pH 10.0), probenecid (250 mg/kg), L-tryptophan (100 mg/kg) or pargyline HCl (75 mg/kg). All injections were IP in a volume of 1 ml/kg.

Six different rats were implanted with cannula bases and perfused several times at 5–7 day intervals for 90 minutes. Forty-five minutes into the sessions the rats received an injection (subcutaneously; 1 ml/kg) of 0.9% saline, apomorphine (0.5 mg/kg), or haloperidol (0.1 mg/kg).

Calculations

The amount of biogenic amines and their metabolites in each perfusate sample was determined by measuring peak heights and comparing them with standards which were analyzed the same day. Little deviation from linearity was detected over a range of 1 to 30 ng of the compounds. The amount of the metabolites in perfusate was expressed as ng/ml of perfusate. The sensitivity of the assay for the metabolites varied inversely with the age of the μBondapak column, but it was easy to detect 1.5 ng HVA, 0.6 ng DOPAC and 0.3 ng 5HIAA per ml perfusate.

Treatments were compared using the Friedman test and the Wilcoxon T statistic [8]. A critical value of 0.05 was set as that required to indicate a statistically reliable effect of experimental manipulation.

Chemicals

Analytical reagents were used in the perfusing solution (Mallinckrodt, Inc., Paris, KT). Dopamine HCl (DA), 5-hydroxytryptamine creatinine sulfate (5HT), 5-hydroxyindole-3-acetic acid (5HIAA), 5-hydroxytryptophan (5HTP), 3-methoxy-4-hydroxyphenyl acetic acid (homovanillic acid, HVA), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3-methoxytyramine HCl (3MT) (Sigma Chemical Co., St. Louis, MO) were dissolved in the perfusion solution or in the mobile phase. The standard solutions were stored at −20°C.

Pargyline HCl (Sigma; final pH 3.8) and apomorphine HCl (Eli Lilly Co., Indianapolis, IN; final pH 5.2) were dissolved in 0.9% saline. Probenecid (Merck Sharp and Dohme Research Lab, Rahway, NJ; final pH 7.0) and L-tryptophan (Sigma; final pH 10.0) were dissolved in 1.0 N sodium hydroxide. Haloperidol (McNeil Lab, Fort Washington, PA) was dissolved in 0.3% tartaric acid (final pH 3.5).

Anatomical Effects of Perfusion

At the completion of the perfusion studies eight rats were anesthetized with Equithesin (3 ml/kg, IP) and perfused via cardiac puncture with 10% neutral buffered Formalin. Coronal slabs containing the cannula track were post-fixed in Formalin, dehydrated and embedded in paraffin. Serial sections were cut at 10 μ and stained with hematoxylin and eosin.

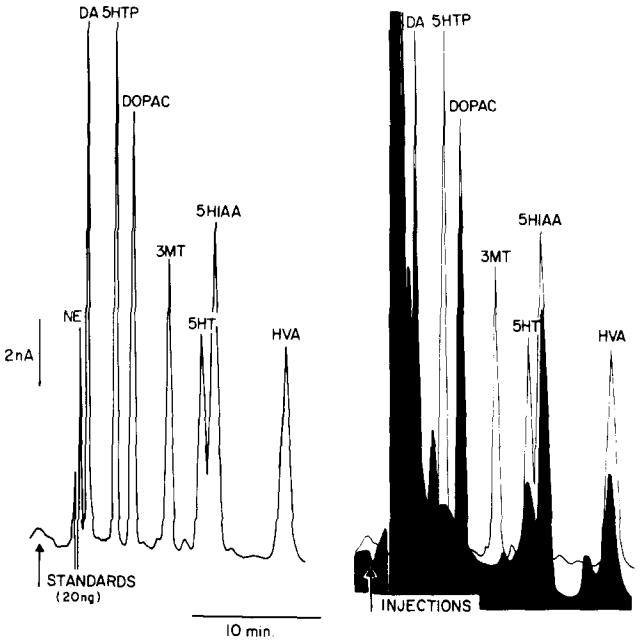

FIG. 2. Chromatograms of standards (on left) and a brain perfusate sample (solid chromatogram) superimposed on the standards (on right). The sensitivity for the brain perfusate chromatogram was 2 nA full scale.

RESULTS

Chemical Evidence for the Authenticity of DA and 5HT Metabolites in Ventricular Perfusate

Figure 2 shows a chromatogram of a standard containing the metabolites of interest and some of their precursors, plus a chromatogram from a typical perfusate sample superimposed on the standard chromatogram. The perfusate contained peaks which co-chromatograph with DA and its metabolites DOPAC and HVA, as well as 5HT and its metabolite 5HIAA. In some instances, DA eluted near the "solvent front" and could not be differentiated. For this reason values for DA are not presented. The peak in the perfusate sample which co-chromatographed with authentic 5HT is probably not exclusively endogenous 5HT because when the LCEC mobile phase was changed so that the compounds eluted with different retention times, there was no peak in the perfusate sample which co-chromatographed with authentic 5HT. Therefore, values for 5HT are not reported. Unidentified peaks were observed in perfusate samples while there were no peaks which co-chromatographed with authentic 5HTP or 3MT.

Pharmacological Evidence for the Authenticity of DA and 5HT Metabolites in Ventricular Perfusate

Several determinations were made before any drugs were administered. The injection of saline, an acid solution (pH 3.5) used as the vehicle for pargyline, or a basic solution (pH 10.0) used as the vehicle for L-tryptophan, did not alter the quantity of metabolites in the perfusate (Table 1). Therefore, data from the experiments where saline was injected was used as the control for the drug effects.

The concentrations of metabolites in the perfusates varied markedly between rats, as evidenced by the high variance about the means (Table 1), but there was little variability in the day-to-day values from individual animals. Therefore, each rat was used as its own control. Since each rat was perfused several times, it was important to determine if the perfusate content of the compounds varied as a function of time, number of perfusions, or injections. Data from rat No. 17 (Table 2) illustrates that there was little variability in any of the metabolites as a function of these variables.

Several drugs with established effects in the central nervous system were used to determine if constituents in the perfusate would reflect expected changes in the dynamics of DA or 5HT metabolites (Fig. 3). Probenecid, an acid transport blocker, significantly increased the quantity of the acid metabolites HVA, DOPAC and 5HIAA in perfusate. The probenecid effect occurred within the first 15 minutes and persisted for at least 75 minutes. Pargyline, a monoamine oxidase inhibitor, significantly decreased the quantity of HVA, DOPAC and 5HIAA. The effects generally did not occur until 15 to 30 minutes after injection, but lasted the entire perfusion. L-Tryptophan increased the efflux of 5HIAA between 30 and 75 minutes after injection. Unexpectedly, L-tryptophan decreased the efflux of HVA but did not alter the efflux of DOPAC.

The quantity of monoamine metabolites in some of the initial nondrug studies decreased from the first to second, and occasionally to the third perfusate sample. For this reason in studies with haloperidol and apomorphine the injections were delayed until after the third sample was collected. Haloperidol, a DA receptor antagonist, and apomorphine, a DA receptor agonist, cause an increase and decrease, respectively in the concentrations of HVA and DOPAC in brain tissue [3, 14, 16, 19]. As anticipated, haloperidol increased and apomorphine decreased the efflux of HVA and DOPAC (Fig. 4). Unexpectedly, apomorphine reduced the efflux of 5HIAA.

TABLE 1

LACK OF EFFECT OF VEHICLE INJECTIONS ON THE EFFLUX OF DOPAC, HVA AND 5HIAA

	DOPAC		HVA		5HIAA	
Perfusion time (min)	0–15	15–60	0–15	15–60	0–15	15–60
No injection	11.3 ± 4.1	9.1 ± 1.1	9.4 ± 2.9	8.4 ± 2.1	15.1 ± 5.7	14.9 ± 3.1
0.9% NaCl	13.8 ± 6.8	11.8 ± 3.6	11.9 ± 2.4	8.3 ± 3.1	19.0 ± 4.7	20.6 ± 1.8
3×10^{-3} N HCl (pH 3.5)	10.0 ± 3.0	9.2 ± 2.1	9.3 ± 3.1	9.1 ± 2.7	16.0 ± 1.7	15.5 ± 1.1
3.5×10^{-3} N NaOH (pH 10.0)	9.7 ± 2.2	8.1 ± 4.7	10.2 ± 1.1	9.9 ± 2.0	17.1 ± 3.6	16.9 ± 2.4

Values (mean ± 1 S.E.; N=4) represent metabolite concentrations (ng/ml). The same 4 rats received each treatment at 4–5 day intervals in the order listed. Injections were made IP after collecting the first 15 min perfusion sample. The values for the 15–60 min period represent the mean values of three 15 min samples from each animal. There was no significant difference in the efflux of metabolites among any of the treatment groups ($\chi^2>0.05$; Friedman test).

TABLE 2

CONSISTENCY OF PERFUSATE CONCENTRATIONS OF METABOLITES UPON REPEATED PERFUSIONS

	DOPAC		HVA		5HIAA	
Perfusion time (min)	0–15	15–60	0–15	15–60	0–15	15–60
Day 1	13.9	8.2	4.8	7.3	18.8	22.4
Day 2	6.3	7.5	6.1	5.4	16.2	20.1
Day 31	7.8	7.5	5.1	4.6	15.1	21.9

Values respresent the perfusate concentration of each metabolite (ng/ml) from rat No. 17. The perfusion was interrupted at 15 min by an IP injection of 0.9% NaCl. Between days 2 and 31 the rat was perfused 5 times at approximately 5 day intervals during which time it received IP injections of dilute hydrochloric acid, sodium hydroxide, pargyline, probenecid and L-tryptophan.

Anatomical Effects of Perfusion

Little damage occurred to the ventricular wall or nearby surrounding tissue after repeated perfusions. Figure 5 shows a brain section indicating the entry of the cannula into the ventricle (rat No. 9) and a higher magnification of the cannula entry into the ventricle (rat No. 18). In these and other rats there was little difference in the size of the perfused and nonperfused ventricles but occasional mild gliosis and infiltrations of macrophages were seen near the ventricular lining or choroid plexus.

DISCUSSION

A procedure utilizing push-pull perfusion and LCEC was employed to measure metabolites of DA and 5HT in cerebroventricular perfusates of unanesthetized, freely-moving rats. Peaks in the chromatograph of the cerebroventricular perfusate co-chromatographed with authentic DOPAC, HVA, and 5HIAA, while several peaks remained unidentified. Experiments have revealed that these latter peaks are not γ-aminobutyric acid, norepinephrine or its metabolites 3-methoxy-4-hydroxyphenylethylene glycol and normetanephrine (Nielsen, unpublished). The lack of 5HTP and 3MT in perfusate samples is not surprising as these compounds are rapidly metabolized to 5HT and HVA, respectively.

There are numerous reports of drug-induced alterations in CSF concentrations of DA and 5HT metabolites, but most of these studies have been performed in anesthetized animals. Probenecid has been reported to increase ventricular perfusate contents of HVA, DOPAC and 5HIAA in dogs, rabbits and rats [1, 2, 7, 21], and L-tryptophan is reported to increase the CSF concentration of 5HIAA [22]. The time course of the effects of probenecid and L-tryptophan noted in the present report was similar to that reported in the earlier studies [1, 2, 22].

Monoamine oxidase inhibitors reduce concentrations of deaminated metabolites of DA and 5HT in brain tissue [15, 18, 19] so it was not surprising that pargyline reduced the quantities of DOPAC, HVA and 5HIAA in the brain perfusates. Another monoamine oxidase inhibitor, clorgyline, is reported to reduce the concentration of 5HIAA in cisternal CSF [22].

The concentrations of DA metabolites in ventricular perfusates might reflect the activity of neurons which use these amines as neurotransmitters. Apomorphine, a DA agonist, and haloperidol, a DA antagonist, decrease and increase, respectively, impulse traffic in ascending DA neurons and cause concomitant changes in the brain concentrations of DOPAC and HVA [5,16]. Changes in ventricular perfusate concentrations of DOPAC and HVA produced by haloperidol reported in the present study are consistent with a previous report [4]. The apomorphine-induced decrease in HVA and DOPAC was also consistent with its effects on brain tissue. Apomorphine's effect on the efflux of 5HIAA was unexpected and in a subsequent study has not been replicated (Nielsen, unpublished).

Push-pull perfusion of the cerebroventricular system of unanesthetized, freely-moving rats and LCEC analysis of the perfusate allows for a chronic, non-invasive monitor of DA and 5HT neurons. Repeated perfusions appeared to have little effect on brain anatomy while the efflux of DOPAC, HVA and 5HIAA remained fairly stable over a period of one month of intermittent sampling. The procedure described in

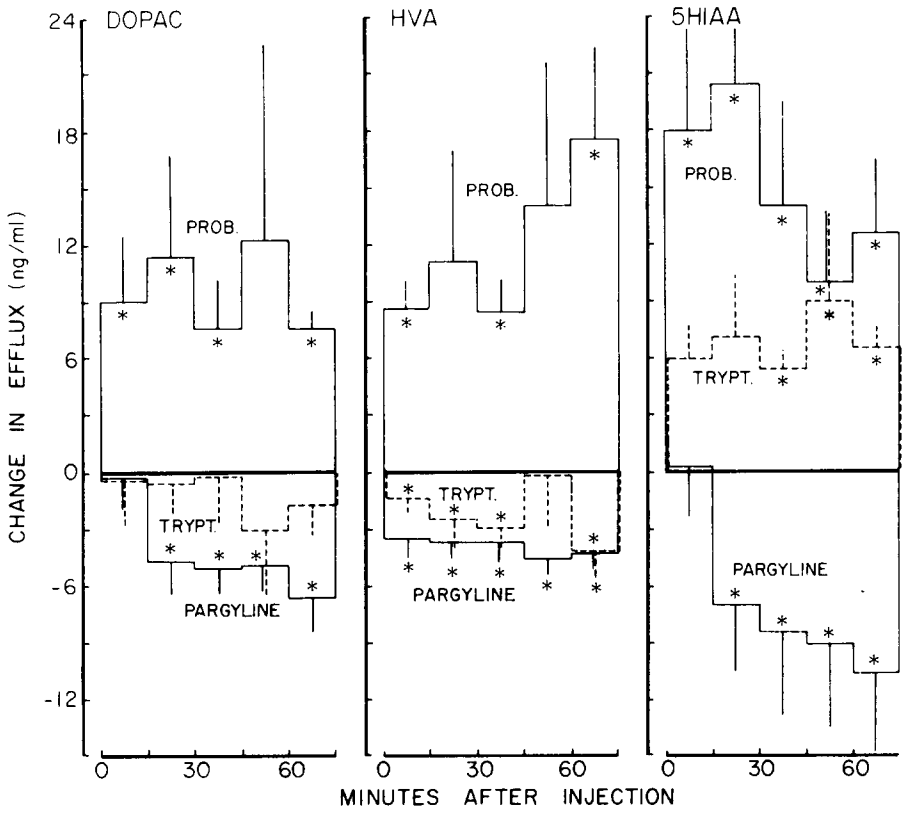

FIG. 3. Effects of systemically administered probenecid, L-tryptophan or pargyline on the efflux from the brain of DOPAC, HVA and 5HIAA. The lateral cerebral ventricles of 5 rats were perfused for 90 min with sequential samples collected at 15 min intervals. After collection of the first sample the rats were injected IP with either 0.9% saline, probenecid (250 mg/kg), L-tryptophan (100 mg/kg) or pargyline (75 mg/kg). At weekly intervals each rat was injected with each of the drugs in a random design. There were no statistical differences among the predrug perfusate concentrations of DOPAC, HVA and 5HIAA from any of the treatment groups; the values (ng/ml; mean±1 S.E., N=5) were:

	DOPAC	HVA	5HIAA
Saline	9.6 ± 3.0	10.0 ± 2.4	13.8 ± 2.5
Probenecid	8.9 ± 1.0	9.9 ± 2.6	12.8 ± 3.1
L-tryptophan	11.4 ± 2.4	10.2 ± 1.5	12.3 ± 1.4
Pargyline	11.3 ± 2.7	8.3 ± 2.5	18.0 ± 4.2

The drug-induced changes are depicted in the figure above; probenecid and pargyline by the continuous lines and tryptophan by the dash lines; (standard errors are indicated by vertical lines). Changes in the values from the saline-treated group were not significantly different from zero and are not depicted. *Significantly different from the saline-treated group ($p<0.05$; Wilcoxon T statistic).

this report should be useful for studying the effects of chronic drug treatments as well as the effects of physiological and behavioral variables on the activities of 5HT and DA neurons.

ACKNOWLEDGEMENT

The authors would like to thank Kathy Lovell, Department of Pathology, Michigan State University, for assistance in the histological studies.

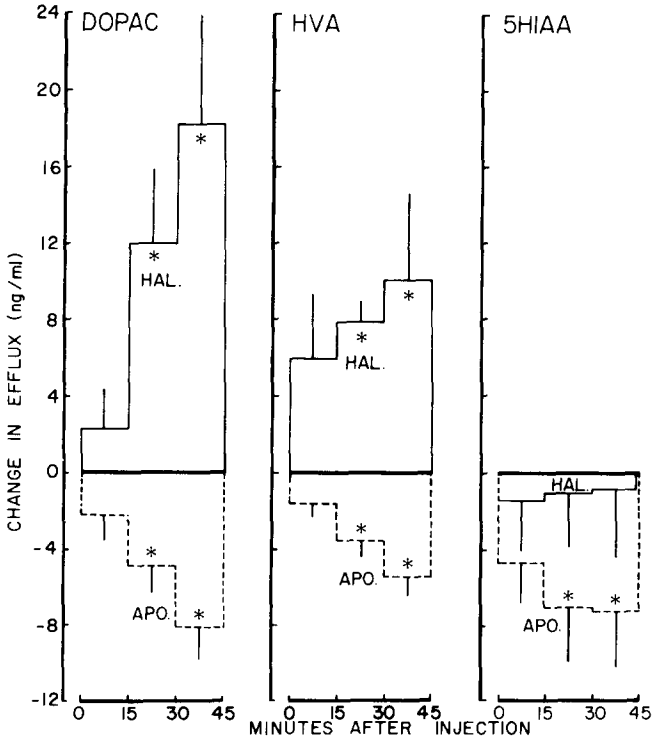

FIG. 4. Effects of systemically administered haloperidol or apomorphine on the efflux from the brain of DOPAC, HVA and 5HIAA. The lateral ventricles of 6 rats were perfused for 90 min with sequential samples collected at 15 min intervals. After collecting 3 samples the rats were injected SC with either 0.9% saline, haloperidol (0.1 mg/kg) or apomorphine (0.5 mg/kg). At weekly intervals each rat was injected with each of the drugs (or saline) in a random design. There were no statistical differences among the predrug concentrations of DOPAC, HVA or 5HIAA from any of the treatment groups; the values (ng/ml; mean±S.E.; N=6) were:

	DOPAC	HVA	5HIAA
Saline	11.8 ± 2.9	5.9 ± 1.3	13.6 ± 4.3
Apomorphine	10.1 ± 2.8	7.8 ± 1.0	13.4 ± 4.5
Haloperidol	15.2 ± 2.4	6.2 ± 0.9	16.7 ± 5.9

The drug-induced changes are depicted in the figure above; haloperidol by the continuous lines and apomorphine by the dash lines (standard errors are indicated by vertical lines). Changes in the values from the saline-treated group were not different from zero and are not depicted. *Significantly different from the saline-treated group ($p<0.05$; Wilcoxon T statistic).

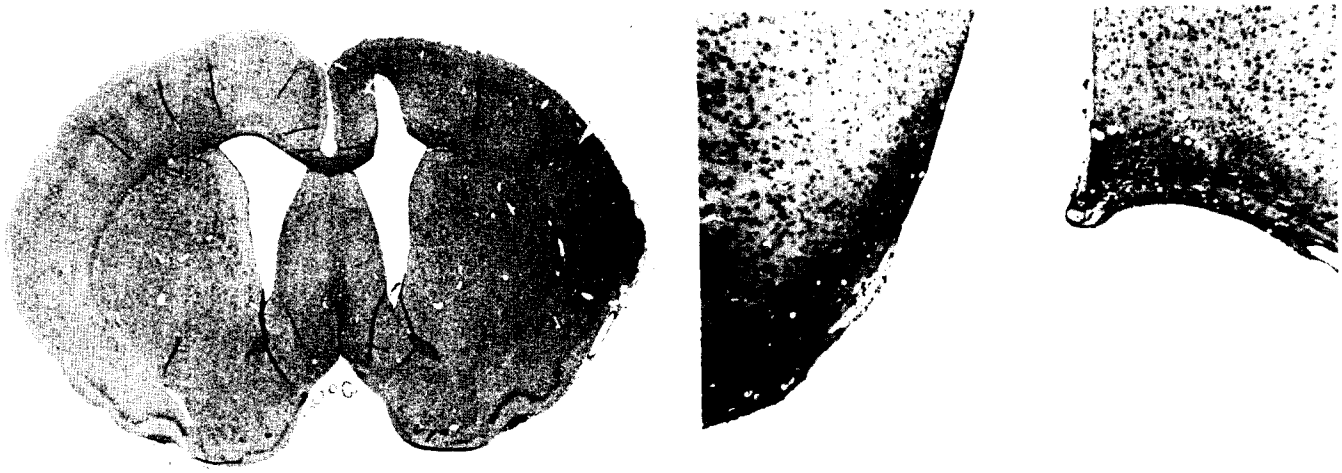

FIG. 5. Two frontal brain sections showing the cannula track entering the ventricle in rat No. 9 (on left) and a close-up of the anatomically normal ventricular lining by the cannula track in rat No. 18 (on right).

REFERENCES

1. Aizenstein, M. L. and J. Korf. Aspects of influx and efflux of homovanillic acid of rat cerebrospinal fluid. *Brain Res.* **149:** 129–140, 1978.
2. Aizenstein, M. L. and J. Korf. On the elimination of centrally formed 5-hydroxyindoleacetic acid by cerebrospinal fluid and urine. *J. Neurochem.* **32:** 1227–1233, 1979.
3. Andén, N.-E., B.-E. Roos and B. Werdinius. Effects of chlorpromazine, haloperidol and reserpine on the levels of phenol acids in rabbit corpus striatum. *Life Sci.* **3:** 149–158, 1964.
4. Barkai, A. I. Dopamine turnover in the intact rabbit brain: Effect of pentobarbital or haloperidol. *J. Pharmac. exp. Ther.* **205:** 133–140, 1978.
5. Bunney, B. S., J. R. Walters, R. H. Roth and G. K. Aghajanian. Dopaminergic neurons: Effect of antipsychotic drugs and amphetamine on single cell activity. *J. Pharmac. exp. Ther.* **185:** 560–571, 1973.
6. Carr, L. A. and K. E. Moore. Effects of amphetamine on the contents of norepinephrine and its metabolites in the effluent of perfused cerebral ventricles of the cat. *Biochem. Pharmac.* **19:** 2361–2374, 1970.
7. Guldberg, H. C., G. W. Ashcroft and T. B. B. Crawford. Concentrations of 5-hydroxyindoleacetic acid and homovanillic acid in the cerebrospinal fluid of the dog before and during treatment with probenecid. *Life Sci.* **5:** 1571–1575, 1966.
8. Harshbarger, T. R. *Introductory Statistics.* New York: MacMillan, 1971, pp. 334–342.
9. Isaac, L. Temperature alteration of monoamine metabolites in cerebrospinal fluid. *Nature (New Biol.)* **243:** 269–271, 1973.
10. Loullis, C. C., J. N. Hingtgen, P. A. Shea and M. H. Aprison. *In vivo* determination of endogenous biogenic amines in rat brain using HPLC and push-pull cannula. *Pharmac. Biochem. Behav.* **12:** 959–963, 1980.
11. Nielsen, J. A., L. H. Fossom and S. B. Sparber. Metabolism of ^{3}H-dopamine continuously perfused through push-pull cannulas in rats' brains: Modification by amphetamine or prostaglandin $F_{2\alpha}$. *Pharmac. Biochem. Behav.* **13:** 235–242, 1980.
12. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain.* New York: Appleton-Century-Crofts, 1967.
13. Portig, P. J. and M. Vogt. Release into the cerebral ventricles of substances with possible transmitter function in the caudate nucleus. *J. Physiol., Lond.* **204:** 687–715, 1969.
14. Roos, B.-E. Decrease in homovanillic acid as evidence for dopamine receptor stimulation by apomorphine in the neostriatum of the rat. *J. Pharm. Pharmac.* **21:** 263–264, 1969.
15. Roos, B.-E., N.-E. Andén and B. Werdinius. Effect of drugs on the levels of indole and phenolic acids in the central nervous system. *Int. J. Neuropharm.* **3:** 117–122, 1964.
16. Roth, R. H., J. R. Walters and G. K. Aghajanian. Effect of impulse flow on the release and synthesis of dopamine in the rat striatum. In: *Frontiers in Catecholamine Research,* edited by E. Usdin and S. H. Snyder. New York: Pergamon, 1973, pp. 567–574.
17. Sweet, R. D. and D. J. Reis. Collection of [^{3}H]norepinephrine in ventriculo-cisternal perfusate during hypothalamic stimulation in cat. *Brain Res.* **32:** 584–588, 1971.
18. Tozer, T. N., N. H. Neff and B. B. Brodie. Application of steady state kinetics to the synthesis rate and turnover time of serotonin in the brain of normal and reserpine-treated rats. *J. Pharmac. exp. Ther.* **153:** 177–182, 1966.
19. Umezu, K. and K. E. Moore. Effects of drugs on regional brain concentrations of dopamine and dihydroxyphenylacetic acid. *J. Pharmac. exp. Ther.* **208:** 49–56, 1979.
20. Von Voigtlander, P. F. and K. E. Moore. Involvement of nigro-striatal neurons in the *in vivo* release of dopamine by amphetamine, amantadine and tyramine. *J. Pharmac. exp. Ther.* **184:** 542–552, 1973.
21. Wightman, R. M., P. M. Plotsky, E. Strope, R. Delcore, Jr. and R. N. Adams. Liquid chromatographic monitoring of CSF metabolites. *Brain Res.* **131:** 345–349, 1977.
22. Young, S. N., G. M. Anderson and W. C. Purdy. Indoleamine metabolism in rat brain studied through measurements of tryptophan, 5-hydroxyindoleacetic acid, and indoleacetic acid in cerebrospinal fluid. *J. Neurochem.* **34:** 309–315, 1980.