Pharmacology Biochemistry & Behavior, Vol. 17, pp. 445–450, 1982. Printed in the U.S.A.

Behavioral Observation and Intracerebral Electrochemical Recording Following Administration of Amphetamine in Rats

J. D. SALAMONE,* W. S. LINDSAY,† D. B. NEILL*[1] AND J. B. JUSTICE†

*Departments of *Psychology and †Chemistry, Emory University, Atlanta, GA 30322*

Received 1 July 1981

SALAMONE, J. D., W. S. LINDSAY, D. B. NEILL AND J. B. JUSTICE. *Behavioral observation and intracerebral electrochemical recording following administration of amphetamine in rats.* PHARMAC. BIOCHEM. BEHAV. 17(3) 445–450, 1982.—Intracerebral electrochemistry (chronoamperometry) was performed on rats that were administered 1, 4, and 8 mg/kg doses of amphetamine. Graphpoxy working electrodes were implanted bilaterally in nucleus accumbens (ACC) and ventral anterior striatum (VAS). Following drug injection, locomotor and stereotyped behaviors were observed. Intracerebral electrochemical signals reliably increased following injection of amphetamine. The magnitude of these increases did not change significantly across the dose range tested for VAS electrodes. ACC electrodes had increases similar in magnitude to VAS electrodes at 1 and 4 mg/kg. At 8 mg/kg increases obtained from ACC electrodes were significantly lower than those recorded from VAS. Onset of the change in electrochemical signal paralleled the onset of activity or stereotypy but the subsequent declines in signal and behavior were only loosely correlated. At the 4 mg/kg dose, the magnitude of signal increase from striatum was negatively correlated with indices of stereotypy and positively correlated with locomotor counts.

Intracerebral electrochemistry Amphetamine Dopamine Stereotypy Nucleus accumbens Striatum Locomotion

AMPHETAMINE is a phenethylamine whose pharmacological and behavioral effects have been widely studied. The drug is known to stimulate release and block reuptake of central nervous system dopamine (DA) and norepinephrine [4,6]. DA in particular has been implicated as a central mediator of some of the behavioral effects of amphetamine, including increased locomotion and stereotyped behavior [10].

Evidence indicates that different DA terminal areas mediate amphetamine-induced locomotion and stereotypy. Reduction of neostriatal DA with intrastriatal injections of 6-hydroxydopamine (6-OHDA) attenuates amphetamine stereotypy while not affecting amphetamine locomotion [11]. In contrast, 6-OHDA injections into nucleus accumbens (ACC), another DA terminal region, decrease amphetamine-produced locomotion, but no stereotypy [11]. Injection of amphetamine or DA directly into ACC stimulates locomotor activity [21], while intrastriatal DA application induces the sniffing and biting response characteristic of stereotypy [5].

Despite extensive data on the behavioral and neurochemical results of amphetamine administration, little is known about the pattern of neurotransmitter activity during the course of drug-induced behavior. The majority of studies on amphetamine neuropharmacology have employed tissue punches, tissue slices, or synaptosomal preparations, and thus preclude direct comparison with behavioral events. Push-pull cannula methodology allows for assessment of neurotransmitter release in an intact organism [19] but few such studies have provided detailed comparisons with the behavior of the organism. For these reasons, intracerebral (also known as in vivo) electrochemistry has been receiving much attention recently. Intracerebral electrochemistry involves the monitoring of compounds via electrodes chronically implanted in the brain. This technique was originally developed by Adams [1], and is continuing as a focal research area in a number of laboratories [3, 7, 14, 15, 18].

Intracerebral electrochemical recording in DA terminal areas following amphetamine administration has been reported previously [3, 7, 9]. Huff *et al.* [9] recorded increases in neostriatal electrochemical signals following injection of a variety of doses of amphetamine. Unilateral destruction of the nigrostriatal DA systems with 6-OHDA attenuates the amphetamine-induced increase in the striatal signal on the lesioned side [7]. No behavioral observations were reported in these experiments.

In the present study, intracerebral electrochemistry was performed via electrodes implanted in ventral anterior striatum (VAS) and ACC while the behavior of the subjects was observed. The doses of amphetamine used were chosen

[1]Send reprint requests to Darryl B. Neill, Department of Psychology, Emory University, Atlanta, GA 30322.

on the basis of their ability to induce locomotion (1 mg/kg) and intense stereotypy (8 mg/kg). An intermediate dose (4 mg/kg) was also tested.

METHOD

Subjects

Ten male albino CFE rats (Charles River) weighing 300–350 g were used. The animals were housed individually and maintained on ad lib food (Rodent Lab Chow, Ralston Purina) and water. A 12 hour light/dark cycle (lights on 0800 hrs) was maintained in the animal colony, and temperature was kept at 72°F.

Electrochemical Techniques and Apparatus

The electrochemical method used in this study was chronoamperometry. A more complete account of the theory behind this technique can be found elsewhere [15]. Briefly, a voltage is applied which causes compounds to oxidize at an electrode surface. The current which results from this oxidation is proportional to the concentration of the electroactive species. Working electrodes were prepared by packing a graphite-epoxy mixture into the tip of a glass pipette in a manner previously described [16]. The tip diameters of the working electrodes were approximately 100 μm. A miniature glass-enclosed Ag/AgCl electrode served as reference electrode, and the control electrode was a stainless steel wire.

While pre-implantation electrochemical testing was performed with a Model 170 Electrochemistry System (Princeton Applied Research), chronoamperometric measurements during the experiment were conducted using a laboratory-built potentiostat/amplifier equipped with a 16 channel multiplexer. The potentiostat/amplifier was interfaced to an Intel microcomputer (SBC 80/20) which controlled the measurement process and data collection. A complete description of the instrumentation can be found in Lindsay *et al.* [15]. The applied voltage was a one second square wave pulse from 0–0.6 V. Oxidation current was measured during the last 16 msec of the pulse.

Pre-Implantation Testing

In order to minimize the possibility of implanting defective working electrodes and to insure that the electrodes for a given rat responded similarly to electroactive species in solution, a pre-implantation test was performed on each electrode. Electrodes were tested in a 10^{-3} M solution of 4-methylcatechol (4-MC) in pH 7.4 phosphate-citrate buffer, as well as a blank solution containing only the buffer. Electrodes were selected for implantation only if they gave a higher oxidation current to the 4-MC than to the blank, and showed consistent chronoamperometric output over three successive pulses. 4-MC was used instead of DA because 4-MC is very similar to DA in its electrochemical properties but is less prone to air oxidation. The applied potential parameters for this test were the same as those used for the intracerebral tests. Pre-implantation data were not used to convert the electrochemical signals to concentration values.

Surgery

For surgery, animals were anesthetized with sodium pentobarbital (50 mg/kg, Nembutal) given IP. Working electrodes were stereotaxically placed bilaterally in ACC (AP 9.2, H −0.3, L 2.0) and VAS (AP 8.6, H 1.0, L 2.7) according to the atlas of Pellegrino and Cushman [20]. The reference and control electrodes were placed in left and right parietal cortex, respectively. Wires from each electrode were connected to the metal contacts of a 6-pin socket (Plastic Products). The entire assembly was anchored to the skull using screws and dental cement.

Procedure

The animals were allowed two days of post-surgical recovery before testing began. Two hours after the lights went on, animals were placed in a 30.5 × 30.5 cm test chamber and connected to a commutator (Airflyte, Bayonne, NJ) to allow free movement during testing. Electrochemical measurements were obtained every 3 minutes. Three hours of baseline electrochemical data were recorded before injection.

The following intraperitoneal injections were given in random order: 1 mg/kg d-amphetamine sulfate (Sigma Chem.); 4 mg/kg amphetamine; 8 mg/kg amphetamine; 1 ml/kg physiological saline. Amphetamine was dissolved in a vehicle of deionized water. Animals received the 4 injections on alternate days over an eight day period. Locomotion was quantified by marking the cage floor into 4 quadrants and counting each time the animal crossed from one into another. Total crossings for each 3 min interval between electrochemical measurements were recorded. Stereotypy was rated every 3 min according to the following scale: 0—asleep, 1—awake but not moving, 2—occasional locomotion and sniffing, 3—continuous locomotion and sniffing, 4—continuous sniffing and head bobbing with little or no gross locomotion, 5—continuous sniffing with occasional licking or tongue protrusion, 6—continuous sniffing with occasional biting or gnawing. Behavioral observation ceased after the animal had received a few consecutive ratings of 2 or less, or stopped locomoting. Usually, electrochemical data were still collected after the termination of behavioral recording. After saline injection, electrochemical data were gathered for at least two hours.

Histology

After all four tests had been completed, the animals were anesthetized with sodium pentobarbital and perfused with physiological saline followed by 10% Formalin-saline. The brains were preserved in 10% Formalin for several days, then blocked in the angle of the atlas of Pellegrino and Cushman [20]. Frozen sections 50 μm thick were cut through the areas of the electrodes, mounted on microscope slides, and stained with thionin.

Data Analysis

Histological analysis was used to verify anatomical location of the working electrodes. In addition any electrodes which had oxidation currents of zero nanoamperes or whose signals went beyond the scale of the potentiostat/amplifier were not considered in the data analysis. For electrodes operating properly, the highest electrochemical signal occurring after injection was considered to be the peak of the signal increase. The increase of the peak over baseline was calculated as a percent increase over the last pre-injection point. The percent change datum for each brain locus was the mean of the responses of the two electrodes implanted in that structure for a given animal. When only one of the two electrodes was operating for a test session, then that electrode was used. The Wilcoxon test for matched-pair data

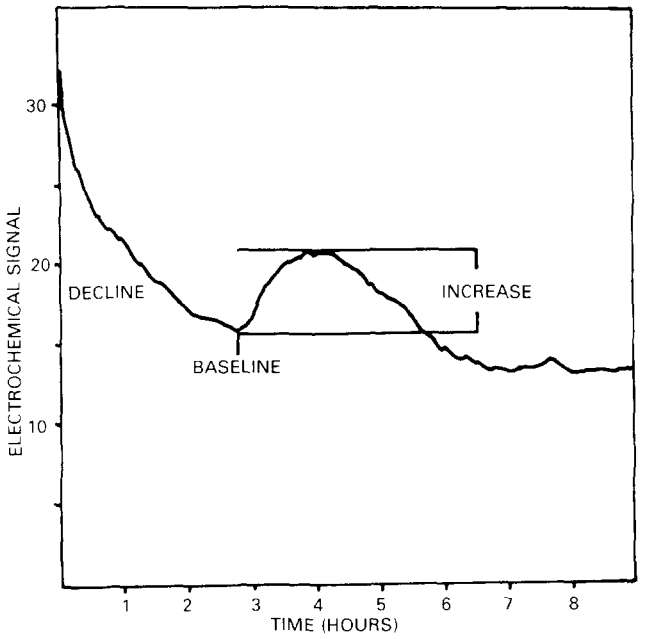

FIG. 1. Electrochemical response of a single ACC electrode to a 1 mg/kg injection of amphetamine. Electrochemical data are expressed in nanoamperes.

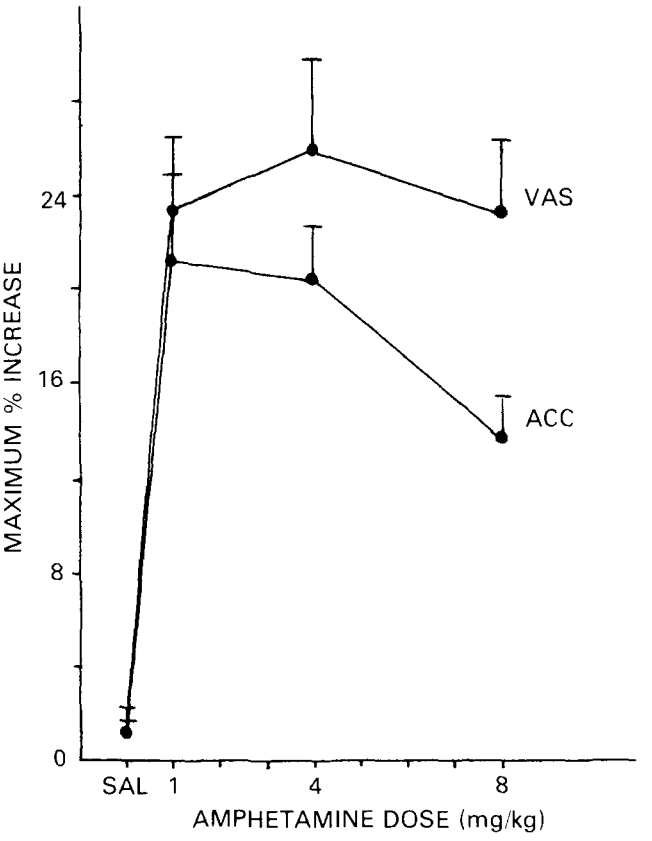

FIG. 2. Mean percent increase (±SEM) in electrochemical signal obtained from each brain region following injection of saline, 1, 4, or 8 mg/kg amphetamine.

was used to test for differences between the percent change data across regions and doses. Time required for the electrochemical signal to peak was also calculated.

Composite graphs showing both behavioral and electrochemical data were also constructed for each individual test run so that these data could be compared.

Electrochemical and behavioral data were correlated across animals using the Pearson product-moment correlation. The behavioral indices used were total locomotor counts/session and number of stereotypy ratings in the session greater than or equal to a certain score. These behavioral data were correlated across subjects with the percent increase in electrochemical signal obtained from each region.

RESULTS

Histological analysis revealed that the electrodes had been placed in the appropriate anatomical locations. The destruction of neural tissue and resulting gliosis in the vicinity of the working electrodes was no greater than that typically seen with chronic implantations of electrodes or cannulae. However, tissue around the tip of the reference electrode was damaged.

A typical electrochemical response for an experimental run is shown in Fig. 1. These data are from an electrode implanted in ACC. As shown in previous work [16,17], the current declined from the initial value as baseline data were gathered. After about 3 hours, a relatively constant signal was obtained. At this point a 1 mg/kg dose of amphetamine was administered, causing an increase in signal due to an increase in the concentration of electroactive compounds in the extracellular fluid at the working electrode tip. The signal increase lasted about 3 hours.

Figure 2 shows the mean percent increase (±SEM) in the electrochemical signal as a function of amphetamine dose for both ACC and VAS. Mean response to saline is also included. Percent increase for ACC electrodes at 8 mg/kg amphetamine was significantly lower than the VAS response of 8 mg/kg ($W=4$, $n=10$, $p<0.02$).

The time (in minutes) required for the increase in electrochemical signal to peak showed no significant differences as a function of amphetamine dose or brain region. Mean times to peak (±SEM) for each region and dose were as follows: ACC 1 (84.5±7.8), ACC 4 (91.8±22.3), ACC 8 (74.8±11.9), VAS 1 (82.6±9.1), VAS 4 (89.4±23.1), VAS 8 (65.8±6.9).

Composite graphs showing both behavioral and electrochemical data were constructed for each individual test run so that these data could be compared. Very few such comparisons yielded consistent point-by point correspondences between signal and behavior. Figures 3–5 show comparisons of electrochemical and behavioral data from individual test runs for each dose and brain region. In general, the initial increase in electrochemical signal paralleled the onset of increased locomotion or stereotypy. However, the declining phases of the electrochemical and behavioral responses to the drug did not consistenly correspond. Usually the electrochemical signal remained above baseline after the behavioral response had subsided.

At the 1 mg/kg dose of amphetamine the only behavioral

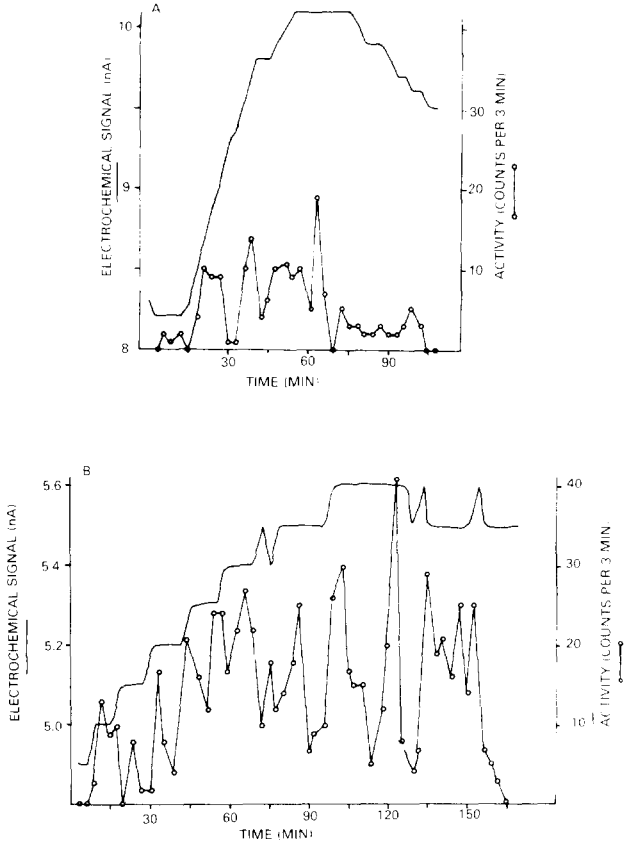

FIG. 3. All data in Figs. 3–5 are from individual test runs. A. Locomotor counts per 3 min interval and electrochemical signal (in nanoamperes) from ACC following injection of 1 mg/kg amphetamine. B. Locomotor counts and electrochemical signal from VAS following 1 mg/kg amphetamine.

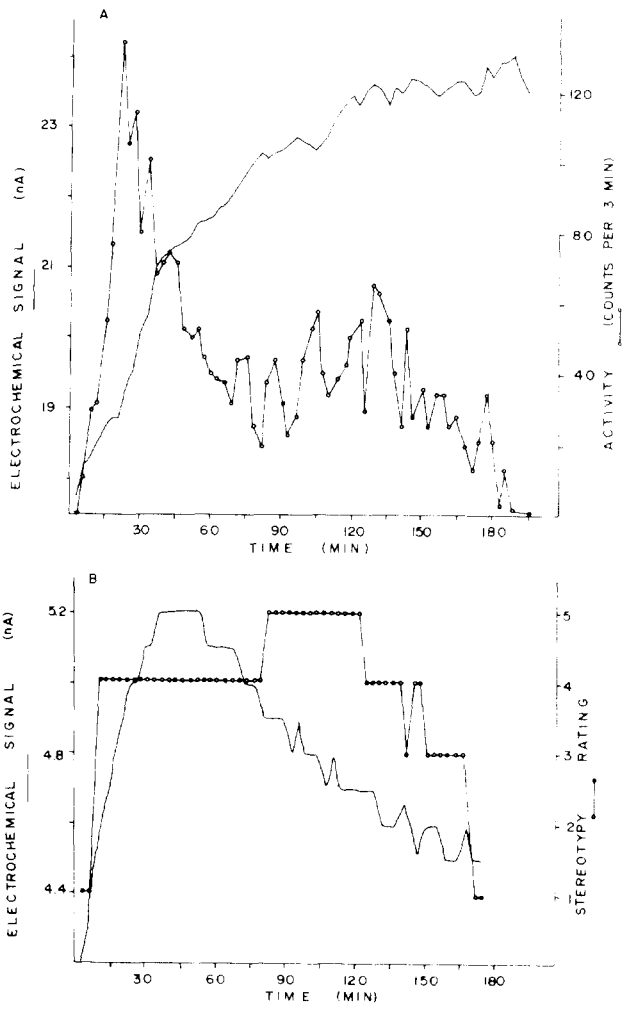

FIG. 4. A. Electrochemical signal from ACC and locomotor counts per interval from an animal who showed a locomotor response to 4 mg/kg amphetamine. B. Electrochemical signal from VAS and stereotypy ratings observed in an animal who showed focused stereotypy following 4 mg/kg amphetamine.

response shown was increased locomotion, and at 8 mg/kg every subject displayed consistent stereotyped behavior which was rated as a 4 or above by the observer. However, at 4 mg/kg some animals maintained continuous locomotion throughout the test, while others engaged in stereotyped movements in the absence of gross locomotion.

There were no significant correlations between indices of behavioral performance and electrochemical signal increase at the 1 mg/kg or 8 mg/kg doses. However, at the 4 mg/kg dose there was a direct relationship between the magnitude of the locomotor response and the electrochemical increase across subjects. Percent increase in electrochemical signal was positively correlated with total locomotor counts in the session for both VAS ($r=.78$, $p<0.05$) and ACC ($r=.68$, $0.05<p<0.10$). In contrast, percent increase in electrochemical signal was negatively correlated with the number of stereotypy ratings greater than or equal to "4" each animal received in the session for VAS ($r=-.89$, $p<0.05$) and ACC ($r=-.74$, $0.05<p<0.10$). Percent increases in signal for ACC and VAS were highly correlated with each other at 1 mg/kg ($r=.83$, $p<0.05$), and 4 mg/kg ($r=.78$, $p<0.05$), but not at 8 mg/kg ($r=.31$).

DISCUSSION

Systemic injections of *d*-amphetamine induced reliable increases in electrochemical (chronoamperometric) signals obtained from VAS and ACC. The percent increase over baseline for VAS electrodes did not change significantly across the dose range tested. ACC electrodes showed response increases similar in magnitude to those at VAS electrodes at 1 and 4 mg/kg but yielded significantly smaller increases at 8 mg/kg. Huff *et al.* [9] previously showed dose-related increases in chronoamperometric response to amphetamine from striatum across the range of 0.5–3 mg/kg. However, the mean change in electrochemical signal with 5 mg/kg amphetamine in their study was actually lower than that obtained at 3 mg/kg (see Huff *et al.*, Fig. 1). It appears that increasing the dose of amphetamine into the range typically used to induce stereotypy (i.e. 5 to 8 mg/kg) does not further increase the electrochemical response to the drug. In

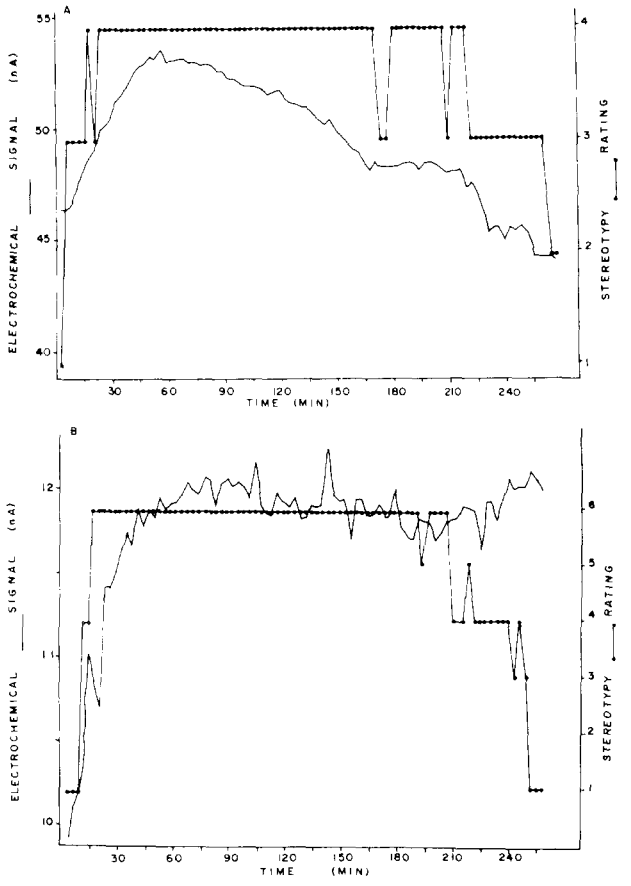

FIG. 5. A. Electrochemical signal from ACC and stereotypy ratings following injection of 8 mg/kg amphetamine. B. Electrochemical signal from VAS and stereotypy ratings following injection of 8 mg/kg amphetamine.

fact, the electrochemical response to amphetamine shows signs of attenuating at higher doses. Possibly, this reflects a "turning off" of DA neurons at high doses of amphetamine. Such a finding is consistent with electrophysiological evidence demonstrating that the rate of firing of midbrain DA neurons is decreased with increasing amphetamine dose [2], and evidence that high doses of amphetamine inhibit synthesis of DA [12].

The graphs comparing behavioral and electrochemical data for individual tests did not reveal a consistent relationship for either brain region. Generally, the onset of behavioral and electrochemical responses to amphetamine showed similar patterns. Yet as the effect of the drug was waning, the behavioral responses and electrochemical signals did not readily correspond.

At the 4 mg/kg dose amphetamine, percent increase in electrochemical signal was positively correlated with total locomotor counts in the session, and negatively correlated with the number of stereotypy ratings greater than or equal to 4, which is an index of focused, non locomotor stereotypy. In other words, animals who demonstrated a large locomotor response at this dose yielded greater increases in electrochemical signal than those animals who showed primarily focused, non-locomotor stereotypy. This suggests that less DA is released by animals in focused stereotypy than those engaged in locomotor activity. A similar scheme has been previously proposed based upon electro-physiological recording from striatal neurons [22].

One must be cautious in interpreting these results, since present chronoamperometric methods do not specify what compound is responsible for the signal increases observed. In the striatum, likely candidates are DA, homovanillic acid, (HVA), dihydroxyphenylacetic acid (DOPAC), and ascorbate [3]. It does seem less likely that HVA is involved in the signal increases seen in our study, since its peak oxidation potential is higher than the other compounds. It has been widely demonstrated that amphetamine enhances release and blocks reuptake of DA, so our results are consistent with the hypothesis that the electrochemical data obtained are reflective of the activity of DA projections. Further support for this hypothesis is that unilateral destruction of the nigrostriatal DA system with 6-OHDA attenuates the amphetamine-induced increase in electrochemical signal on the lesioned side [7].

Still, considerable controversy remains as to the relative contribution of ascorbate, DA, and DOPAC to the electrochemical signal. Lane *et al.* [13] developed an iodide-treated platinum electrode which could differentiate DA and ascorbate, and reported that intrastriatal injection of amphetamine near the working electrode tip caused an increase in the current obtained for DA but not ascorbate. Work from Adams's laboratory has shown that amphetamine does not induce release of ascorbate from striatal tissue slices [3]. More recently, however, Gonon *et al.* [8] suggest that the signal increases obtained with the methods used in our study actually reflect amphetamine-induced release of ascorbate, and note the possibility of a "functional relationship" between ascorbate levels and DA transmission. In our laboratory, we are currently utilizing push-pull perfusion methodology to determine what changes occur in the extracellular concentrations of various oxidizable compounds in VAS following amphetamine administration. Preliminary results indicate that extracellular levels of ascorbate do increase after injection of 4 mg/kg amphetamine. Additional studies are being undertaken to directly compare the neurochemical effects of amphetamine administration that are detected by push-pull cannula and intracerebral electrochemistry.

Based upon the evidence available at this time, our tentative conclusion is that the signal increases observed in this study reflect some aspect of the activity of DA terminals in striatum and ACC following amphetamine administration. Certainly further research must be performed to determine more precisely the source of the electrochemical signal increases. However, it is apparent that intracerebral electrochemical methods can be useful to scientists investigating the relationship between neurochemical and behavioral events.

ACKNOWLEDGEMENTS

This research was supported by the McCandless Fund of Emory University and the National Science Foundation (Grants BNS77-25495 to DBN and BNS79-06815 to JBJ/DBN).

REFERENCES

1. Adams, R. N. Probing brain tissue with electroanalytical techniques. *Analyt. Chem.* **48:** 1128A–1138A, 1976.
2. Bunney, B. S., J. R. Walters, R. H. Roth and G. K. Aghajanian. Dopaminerigic neurons: effect of antipsychotic drugs and amphetamine on single cell activity. *J. Pharmac. exp. Ther.* **185:** 560–577, 1973.
3. Conti, J. C., E. Strope, R. N. Adams and C. A. Marsden. Voltammetry in brain tissue: Chronic recording of stimulated dopamine and 5-hydroxytryptamine release. *Life Sci.* **23:** 2705–2715, 1978.
4. Coyle, J. T. and S. H. Snyder. Catecholamine uptake by synaptosomes in homogenates of rat brain: stereospecificity in different areas. *J. Pharmac. exp. Ther.* **170:** 221–231, 1969.
5. Fog, R., A. Randrup and H. Pakkenburg. Aminergic mechanisms in corpus striatum and amphetamine-induced stereotyped behavior. *Psychopharmacology* **11:** 179–183, 1967.
6. Glowinski, J. and J. Axelrod. Effects of drugs on the uptake, release, and metabolism of ^{3}H-norepinephrine in the rat brain. *J. Pharmac. exp. Ther.* **149:** 43–55, 1965.
7. Gonon, F., R. Cespuglio, J. L. Ponchon, M. Buda, M. Jouvet, R. N. Adams and J. F. Pujol. Mesure electrochemique continue de la liberation de dopamine realisee in vivo dans le neostriatum du rat. *C. r. hebd. Séanc. Acad. Sci., Paris.* **286:** 1203–1206, 1978.
8. Gonon, F., M. Buda, R. Cespuglio, M. Jovet and J. F. Pujol. Voltammetry in the striatum of chronic freely moving rats: detection of catechols and ascorbic acid. *Brain Res.* **223:** 69–80, 1981.
9. Huff, R., R. N. Adams and C. D. Rutledge. Amphetamine dose-dependent changes of in vivo electrochemical signals in rat caudate. *Brain Res.* **173:** 369–372, 1979.
10. Iversen, S. D. Brain dopamine systems and behavior. In: *Handbook of Psychopharmacology*, edited by L. Iversen, S. Iversen and S. Snyder. New York: Plenum Press, 1977, pp. 333–384.
11. Kelly, P. H., P. W. Seviour and S. D. Iversen. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. *Brain Res.* **94:** 507–522, 1975.
12. Kuczenski, R. Biphasic effects of amphetamine on striatal dopamine dynamics. *Eur. J. Pharmac.* **46:** 249–257, 1977.
13. Lane, R. F., A. T. Hubbard, K. Fukunaga and R. J. Blanchard. Brain catecholamines: detection by differential pulse voltammetry at surface modified platinum electrodes. *Brain Res.* **114:** 346–352, 1976.
14. Lane, R. F., A. T. Hubbard and L. D. Blaha. Application of semidifferential electroanalysis to studies of neurotransmitters in the central nervous system. *J. electro-Analyt. Chem.* **95:** 117–122, 1979.
15. Lindsay, W. S., B. L. Kizzort, J. B. Justice, J. D. Salamone and D. B. Neill. An automated electrochemical method for in vivo monitoring of catecholamine release. *J. Neurosci. Meth.* **2:** 373–388, 1980.
16. Lindsay, W. S., B. L. Kizzort, J. B. Justice, J. D. Salamone and D. B. Neill. Microcomputer controlled multielectrode system for *in vivo* electrochemistry. *Chem. Biomed. Environ. Instrum.* **10:** 311–330, 1980.
17. Lindsay, W. S., J. B. Justice and J. D. Salamone. Simulation studies of *in vivo* electrochemistry. *Comput. Chem.* **4:** 19–26, 1980.
18. Marsden, C. A. Functional aspects of 5-hydroxytryptamine neurons: application of electrochemical monitoring in vivo. *Trends Neurosci.* **2:** 230–234, 1979.
19. Myers, R. D. *Handbook of Drug and Chemical Stimulation of the Brain*. New York: Van Nostrand, 1974.
20. Pellegrino, L. J. and A. J. Cushman. *A Stereotaxic Atlas of the Rat Brain*. New York: Appleton-Century-Crofts, 1967.
21. Pijnenburg, A. J. and J. M. Van Rossum. Stimulation of locomotor activity following intracerebral injection of dopamine into the nucleus accumbens. *J. Pharm. Pharmac.* **25:** 1003–1004, 1973.
22. Rebec, G. V. and D. S. Segal. Dose-dependent biphasic alterations in the spontaneous activity neurons in the rat neostriatum produced by d-amphetamine and methylphenidate. *Brain Res.* **150:** 353–366, 1978.