Pharmacology Biochemistry & Behavior, Vol. 5, pp. 149–156.

Amphetamine, Chlorpromazine and Clonidine Effects on Self-Stimulation in Caudate or Hypothalamus of the Squirrel Monkey

JOHN SPENCER[1] AND ALVIN REVZIN

Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK

and

Aviation Toxicology Laboratory, Civil Aeromedical Institute, Federal Aviation Administration, Oklahoma City, OK 73125

(Received 15 October 1975)

SPENCER, J. AND A. REVZIN. *Amphetamine, chlorpromazine and clonidine effects on self-stimulation in caudate or hypothalamus of the squirrel monkey.* PHARMAC. BIOCHEM. BEHAV. 5(2) 149–156, 1976. – In 2 separate groups of squirrel monkeys and within 3 animals low rates of intracranial self-stimulation (ICSS) elicited from caudate or lateral hypothalamic brain sites were increased by as much as 200% above control levels by amphetamine (0.5 mg/kg). Thresholds for responding were decreased by 50%. Increasing the drug dose from 2 to 10 mg/kg produced response inhibition at both brain sites. The duration of inhibitory action of amphetamine (2.0 mg/kg) on ICSS from the medial forebrain bundle (MFB) area of the lateral hypothalamus was 6 hr. At caudate sites ICSS did not occur until 48 hr had elapsed. A 10 mg/kg dose of amphetamine produced a duration of action of 36 hr in the MFB and 84 hr in the caudate. Chlorpromazine (CPZ) doses of 0.5 and 1.0 mg/kg decreased caudate ICSS significantly more than lateral hypothalamic ICSS. At 1.0 mg/kg the duration of action of CPZ was 6 hr at lateral hypothalamic brain sites and 24 hr at caudate sites. At a 2.0 mg/kg CPZ dose the duration of action was 12 hr in the MFB and 36 hr in the caudate. A dose of 0.10 mg/kg of clonidine blocked high rates of MFB ICSS while within the same animal caudate ICSS was much less affected. Higher doses (0.25 mg/kg) sedated the animal and ICSS was equally inhibited at both sites. These findings, using ICSS as a behavioral measure, suggest that the effects of amphetamine and CPZ involve not only hypothalamic structures but more anterior telencephalic sites as well. The prolonged actions of amphetamine and CPZ on caudate ICSS suggest that drugs acting, in part, on dopamine containing neurons will interfere with certain caudate mediated behavior. Further, since hypothalamic but not caudate ICSS sites are more dose sensitive to drugs that selectively act on NE containing neurons, other amines in addition to NE may play a role in the support of ICSS.

Self-stimulation Caudate Hypothalamus Amphetamine Chlorpromazine Clonidine Squirrel monkey

INTRACRANIAL self-stimulation (ICSS) has been used as a behavioral technique to assess the effects of the psychomimetic drugs on operant response rate [13, 17, 18, 24, 26, 28, 29], neurophysiological correlates of behavior [19], and biochemical correlates of behavior [2, 20, 25, 27]. This literature has recently been reviewed by German and Bowden [8]. Generally, systemic administration of stimulant drugs such as amphetamine increases the rate of responding for hypothalamic ICSS in rats [17, 24, 26]. If the tranquilizing drug chlorpromazine (CPZ) is given, rates of responding are reduced [13, 18, 19]. A positive correlation appears to exist between the inhibitory effects of CPZ on hypothalamic ICSS and single unit activity within the hypothalamus [19], suggesting a hypothalamic locus of action for CPZ.

Because amphetamine and CPZ can influence the release and re-uptake of norepinephrine (NE), [3,9], it has been suggested [26] that the central site of action for these drugs is within areas such as the hypothalamus, that contain large amounts of this amine. Potentiation of hypothalamic ICSS by amphetamine can be significantly reduced if the animal has been pretreated with drugs (e.g., disulfiram) that inhibit the enzymatic synthesis of NE. When l-norepinephrine is then injected intraventricularly, ICSS is restored in 15 min and by 75 min the facilitating effect of amphetamine is again observed. Thus, amphetamine's

[1] This research was supported in part by Federal Aviation Administration task AM-A-TOX-22 and by USPHS Grant 5t01 MH 10322 (Biological Sciences Training Grant) from the Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK. The authors would like to thank Mr. Bill Henderson for his assistance with the histology. Reprint requests should be sent to John Spencer, Ph.D., Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, MD 20014.

stimulating action on behavior may involve, in part, alterations in NE release, although disulfiram may not be a pure inhibitor of NE synthesis [6].

It has also been shown that low doses of amphetamine induce greater facilitation in rate of ICSS from the locus coeruleus (LC) than from the hypothalamus [22]. The density of NE-containing neurons is quite high in the LC, and this region is one origin for a norepinephrinergic projection pathway with very widespread diencephalic and telencephalic connections [15]. This pathway forms a part of the medial forebrain bundle (MFB), a diffuse fiber tract which projects into and through the hypothalamus [10]. The finding that LC ICSS is very sensitive to amphetamine is not surprising assuming that the drug affects NE transmission in the brain and that NE is a necessary mediator of ICSS.

Other data have been presented though which show that the pharmacological and behavioral actions of amphetamine or CPZ may not be explicable purely in terms of NE. A release not only of NE but acetylcholine (ACH), dopamine (DA) and gammaaminobutyric acid (GABA) commonly occurs throughout much of the brain including the caudate nucleus, which has high proportions of DA, when the psychomimetic drugs are given [1, 11, 16]. Further, ICSS can occur at the caudate or substantia nigra [12,17] and evidence has been presented which shows amphetamine can influence rates of ICSS from either the hypothalamus or substantia nigra [17] thus suggesting some interaction between brain sites, amine content and ICSS. Based on these findings, it would appear that drug effects on several neurotransmitter systems can influence rates of responding for ICSS although the relative role of these systems in the mediation of ICSS remains to be clarified.

The present set of experiments analyze the dose effects and duration of action of amphetamine and CPZ on ICSS in the squirrel monkey, a species in which no information is available regarding ICSS and drug action. Two sites have been used to elicit ICSS, the head of the caudate and the hypothalamus. A third drug, clonidine, was also used in the present study to assess its effect on ICSS elicited from caudate and hypothalamic sites. This drug is thought to act principally on NE containing neurons [14]. If several brain pathways for ICSS do in fact exist, then those that contain large amounts of NE (e.g., hypothalamus) would be predicted to be more dose susceptible to clonidine's effects than those containing large amounts of DA (e.g., caudate).

METHOD

Animals and Housing Conditions

Twelve healthy, adult squirrel monkeys were used in the present experiments. Body weights were between 600 and 800 g. Each animal was housed in an individual cage (1½ × 1½ × 2 ft). All animals were fed standard squirrel monkey chow with supplements of fruit and vitamins. The cages were watered and cleaned daily. The room temperature was constant at 72°F. Light cycle was 12 hr on and 12 hr off.

Surgery

Each animal was initially anesthetized with pentobarbitol (25 mg/kg) and placed in a stereotaxic instrument. The cranium was exposed and stainless steel wire electrode pairs (0.25 mm dia.) insulated except for the tips (0.50 mm) were bilaterally implanted via small holes drilled in the skull. Coordinates used for implanting at the head of the caudate were (AP 15.0, L 2.5, V 17.0 mm) and for the lateral hypothalamus (AP 8.5 11.0, L 0.5 1.5, V 11.0 mm) [7]. The leads from the electrodes were connected to an ELCO plug and the entire assembly was embedded in dental repair acrylic. The incision was then closed and the animal was permitted to recover from the surgery. As a precautionary measure against infection, bicillin (0.25 cc) was given intramuscularly one day prior to surgery and several days thereafter. The recovery period was 2 weeks in duration. There was no evidence of postoperative infections.

Testing Apparatus

The experiment was conducted in a ventilated soundproof chamber (3½ × 3 × 4 ft, Lehigh Valley). The animals were held in a conventional two-plate restraint chair. A lever was mounted in a vertical position in front of and 5 cm above the animal. The required response was to reach out and up to grasp the lever and pull.

Each lever pull activated a microswitch that triggered a Tektronix waveform generator system. Rectangular trains of pulses (100 c/sec, pulse width 0.2 msec) were delivered via a multiconductor cable. The current intensity, pulse width, and waveform were monitored on a dual-beam oscilloscope. To provide a continuous record of the animal's response, a cumulative recorder (model CR2D, Scientific Prototype) was also activated by each lever pull. Minimum interstimulus interval was 630 msec. For the amphetamine administration studies, stimulus parameters were selected that produced low response rates (15–20/min), while for the CPZ and clonidine experiments maximal rates of responding (above 50/min) were used.

Dose Response Analysis

Absolute rate. Six animals were selected that had stable response rates to stimulation of the head of the caudate; 6 other animals were chosen with stable response rates from the hypothalamus. Three of these 12 subjects had positive sites in both the hypothalamus and the caudate.

Each animal was placed in the chamber and allowed to self-stimulate for 15 min on the CRF schedule. Saline was then injected into a small marshmallow and given to the animal. Recording was then continued for a further 60 min.

Following 3 days of saline control recording, either d-amphetamine sulphate (0.25, 0.50, 1.0, 2.0, or 10.0 mg/kg) dissolved in saline, or chlorpromazine hydrochloride (CPZ) was given in a randomized order in place of saline. Because of drug solubility problems, the 10.0 mg/kg dose of amphetamine was given interperitoneally (IP) instead of orally. Control studies showed that the stress of the IP injections did not alter rates of ICSS. All animals were food deprived for 12 hr prior to the test session so as to facilitate absorption of the drug. The minimum interval between successive drug administrations was 2 weeks. Later in the experiments, dosages of 0.05, 0.10, or 0.25 mg/kg of clonidine were given, as previously described.

Threshold changes. A separate analysis for changes in the threshold values of stimulus current was performed following amphetamine administration. A current intensity that produced a stable low rate, (15–20 responses per minute [resp/min]) was used as the starting point and 50 microampere stepwise decreases in the current were continued until the animal stopped responding. Amphetamine

dosages used were 0.25, 0.50, and 1.0 mg/kg, and the drug administration procedure was as described above.

Duration analysis. Analysis of duration of drug effects followed the above procedures initially. However, 3 hr after drug administration the animal was placed back in the experimental chamber and retested for 1 hr. This same procedure was repeated at 6, 12, and 24 hr postdrug and then every 24 hr until the animal's response rate had returned to its original baseline level. Each time the animal was placed in the chamber a marshmallow was given, following the procedure described above. For those animals with electrodes that yielded self-stimulation from both sites, each site was investigated separately for 40 min; the order of the site tested was counterbalanced, and if one site elicited ICSS but the other did not, the analysis for that particular dose of drug was replicated twice.

Histology

All animals were sacrificed with an overdose of Nembutal at the end of the experiment. Final electrode track verification was made by examining cross sections of the brain, cut frozen, at 100 micron section thickness. The sections were then mounted on slides and stained with cresyl violet acetate to facilitate localizing final electrode track penetration.

RESULTS

Amphetamine

Rate of response. Statistical analysis was performed on the rate of response per min (drug/saline ratio x 100). In all tests the maximum F value needed at the appropriate probability (p) level (conservative or negative biased test, Winer, 1973) was used. A 2 x 5 analysis of variance with repeated measures on the last factor [30] demonstrated a significant main effect for brain site ($F(1,10) = 8.90$, $p<0.05$) and for drug dose ($F(1,10) = 47.31$, $p<0.01$). The interaction between brain sites and drug dose was not significant ($F(1,10) = 4.81$, $p>0.05$). As dose of drug was increased from 0.25 to 0.50 mg/kg, the ratio of responding (low rate) was significantly increased at both caudate and hypothalamic sites and at 1.0 mg/kg, hypothalamic sites only. Doses higher than 1.0 mg/kg significantly decreased rate of ICSS from both sites, (Fig. 1A).

An individual cumulative response record for one animal is presented in Fig. 2. Amphetamine increased the response rate from both caudate and hypothalamic sites at dosages of 0.5 mg/kg and at hypothalamic sites at 1.0 mg/kg. Rate of ICSS from the caudate showed various periods of no responding followed by increases in responding at 1.0 mg/kg. Five animals showed various periods of rate increases or decreases while one animal showed a steady reduction in rate of response. The higher standard error of the mean for the caudate brain site at 1.0 mg/kg (Fig. 1) reflects the larger group variation in mean percent change in rate of ICSS.

Current threshold. Significant decreases in current threshold were obtained at 0.50 mg/kg of amphetamine at both brain sites and at 1.0 mg/kg in the hypothalamus (Fig. 1B) ($F(1,20) = 40.00$, $p<0.01$). Statistical analysis of changes in threshold for responding at caudate sites was not made at 1.0 mg/kg since there were periods of both rate increases and rate inhibition.

Duration of action. The duration of action of amphetamine on rate of ICSS is presented in Fig. 1C. Since the unit of measure (hours to return to baseline) was arbitrarily set in the experimental design, a planned comparison using nonparametric ranking procedures was used [23]. At doses of 2.0 and 10.0 mg/kg animals with electrodes implanted at the head of caudate did not self-stimulate for 48 hr at the 2.0 mg/kg dose and 84 hr at the 10 mg/kg dose. This

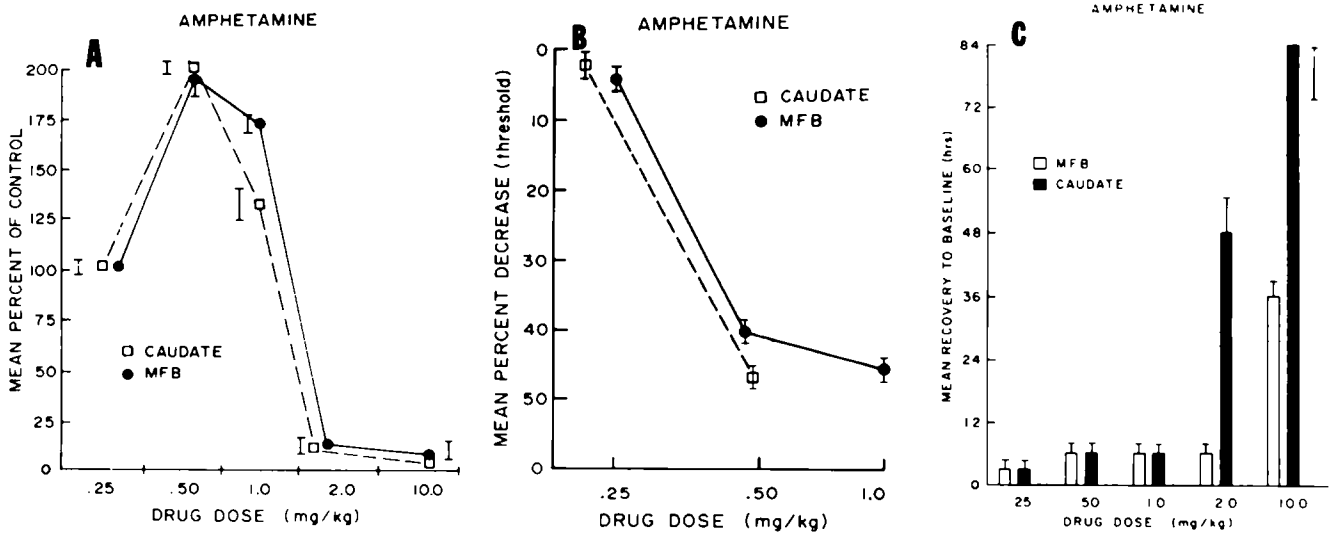

FIG. 1. (A) Dose-response curve for amphetamine effects on rate of self-stimulation from head of caudate (N = 6) or medial forebrain bundle (MFB) in the lateral hypothalamus (N = 6) of squirrel monkeys. Vertical bar represents standard error of the mean. (B) Dose-response curve for amphetamine effects on current threshold needed to elicit self-stimulation at caudate (N = 6) or MFB (N = 6). Vertical bar represents standard error of the mean. (C) Duration of action of amphetamine on rate of self-stimulation from head of caudate (N = 6) or the MFB (N = 6) brain sites. Vertical bar represents standard error of the mean.

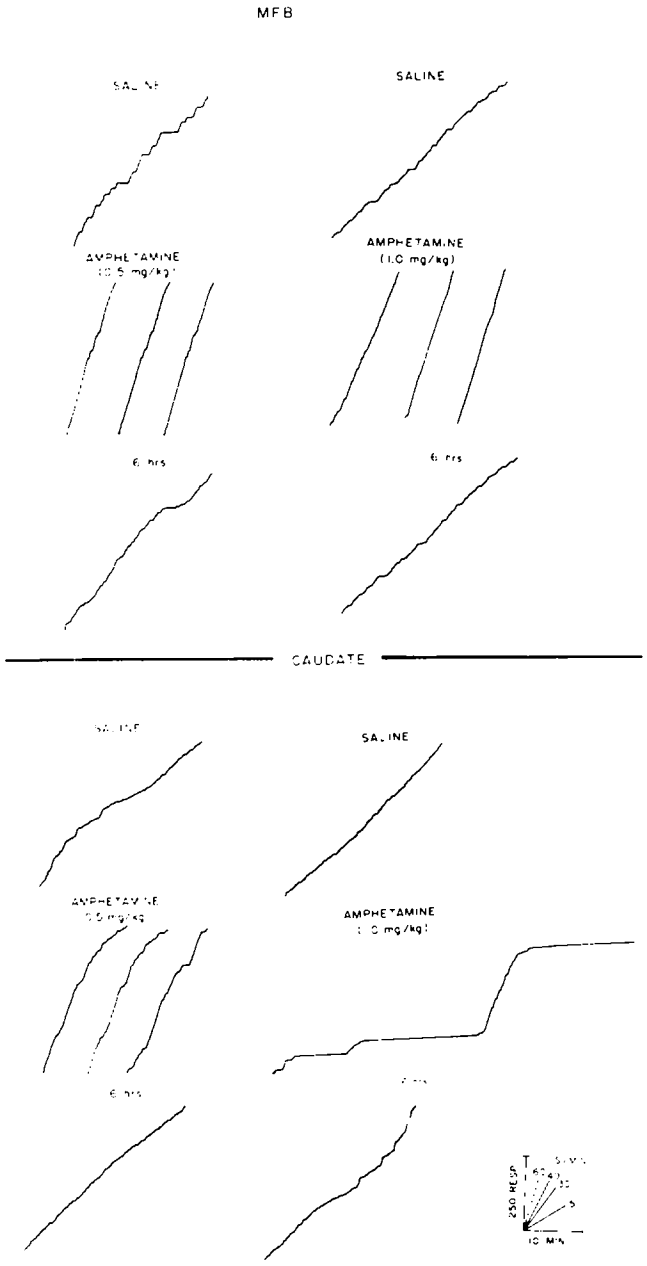

FIG. 2. Cumulative response record for one squirrel monkey that received dosages of 0.5 or 1.0 mg/kg of amphetamine. Brain site stimulated was the medial forebrain bundle (MFB) in the lateral hypothalamus or the head of the caudate.

behavioral duration of action was significantly longer than for those animals that had electrodes implanted within the lateral hypothalamus ($U = 0$, $p<0.001$). Although either low or high rates of ICSS could be elicited within the lateral hypothalamus by 36 hr, 3 of the animals that had electrodes implanted at both sites would not continue to respond if the active electrode was switched to the caudate. Between 84–96 hr all animals responded when either site was stimulated. The 10.0 mg/kg dose produced anorexia in all animals lasting for 24 hr. Another common behavioral sequela previously reported in cats [21] was that the monkeys appeared to sit quietly in their home cages and fixate into space.

Chlorpromazine (CPZ)

Rate of response. A 2 x 4 analysis of variance (brain site and drug dose), with repeated measures on the last factor [30] revealed a significant interaction between drug dose and brain site ($F(1,10) = 9.68$, $p<0.05$). After simple effects analysis was made, between-treatment totals analysis was done using the Scheffé test.

Doses of CPZ at 0.50 and 1.0 mg/kg produced a significant decrease in rate of responding when the head of the caudate was stimulated. These differences in caudate ICSS were also significantly different from ICSS elicited from the hypothalamus. Drug doses of 2.0 mg/kg produced a significant decrease in responding at both sites (Fig. 3A).

The duration of action of CPZ is presented in Fig. 3B. Again a nonparametric analysis based on signed ranks was performed [23]. At doses of 1.0 and 2.0 mg/kg self-stimulation was inhibited for longer periods of time from the caudate sites than from hypothalamic sites ($U = 0$, $p<0.001$; $U = 1$, $p<0.001$). These differences were observed both between groups and within 3 animals.

An individual cumulative record from an animal in which both the caudate and the hypothalamus supported self-stimulation is presented in Fig. 4. At a dose of 0.5 mg/kg CPZ blocked caudate self-stimulation for 6 hr while rate of ICSS from the hypothalamus was not changed. At 1.0 mg/kg the caudate site again failed to support ICSS and rate of ICSS from the hypothalamus was reduced. At 2.0 mg/kg both sites failed to support ICSS for 12 hr (MFB) and 36 hr (caudate).

Clonidine

Low doses of clonidine (0.05 mg/kg) did not change rate of responding for stimulation at either caudate or hypothalamic sites. A dose of 0.10 mg/kg of clonidine reduced hypothalamic ICSS for 30–45 min. Rate of ICSS elicited from the head of the caudate was not consistently reduced (Fig. 5). Higher doses (0.25 mg/kg) produced a sedative action usually lasting for 45 min. These results were observed between 2 groups of 3 animals each as well as within 2 animals in which both caudate and hypothalamic sites supported ICSS. The order of site stimulated was counterbalanced.

Histology

The final electrode locations for all 12 animals for caudate and hypothalamic sites are presented in Fig. 6. Maximum penetration for the caudate sites occurred at 14.5 through 15.0 A.P. For the hypothalamic sites 10.5 through 12.0 A.P.

DISCUSSION

The results of this study demonstrate that the squirrel monkey can be used to investigate drug effects on brain self-stimulation. Consistent and replicable results were obtained when either the head of the caudate nucleus or the MFB within the lateral hypothalamus was stimulated.

Since rates and thresholds of ICSS elicited from the caudate nucleus or the hypothalamus are affected by amphetamine or CPZ administration, these drugs probably act on DA as well as NE containing neurons. Low doses of amphetamine (0.5 mg/kg) facilitated low rates of ICSS

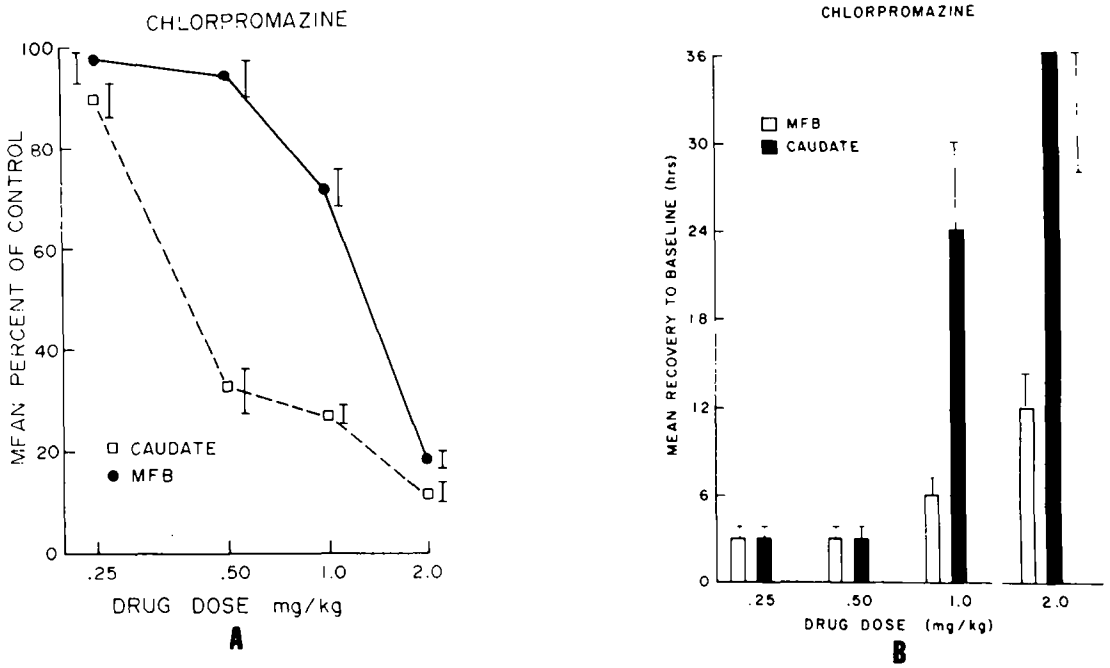

FIG. 3. (A) Dose-response curve for chlorpromazine effects on rate of self-stimulation from head of caudate (N = 6) or medial forebrain bundle (MFB) in the lateral hypothalamus (N = 6) of squirrel monkeys. Vertical bar represents standard error of the mean. (B) Duration of action of chlorpromazine on rate of self-stimulation from the head of the caudate (N = 6) or MFB (N = 6) brain sites. Vertical bar represents standard error of the mean.

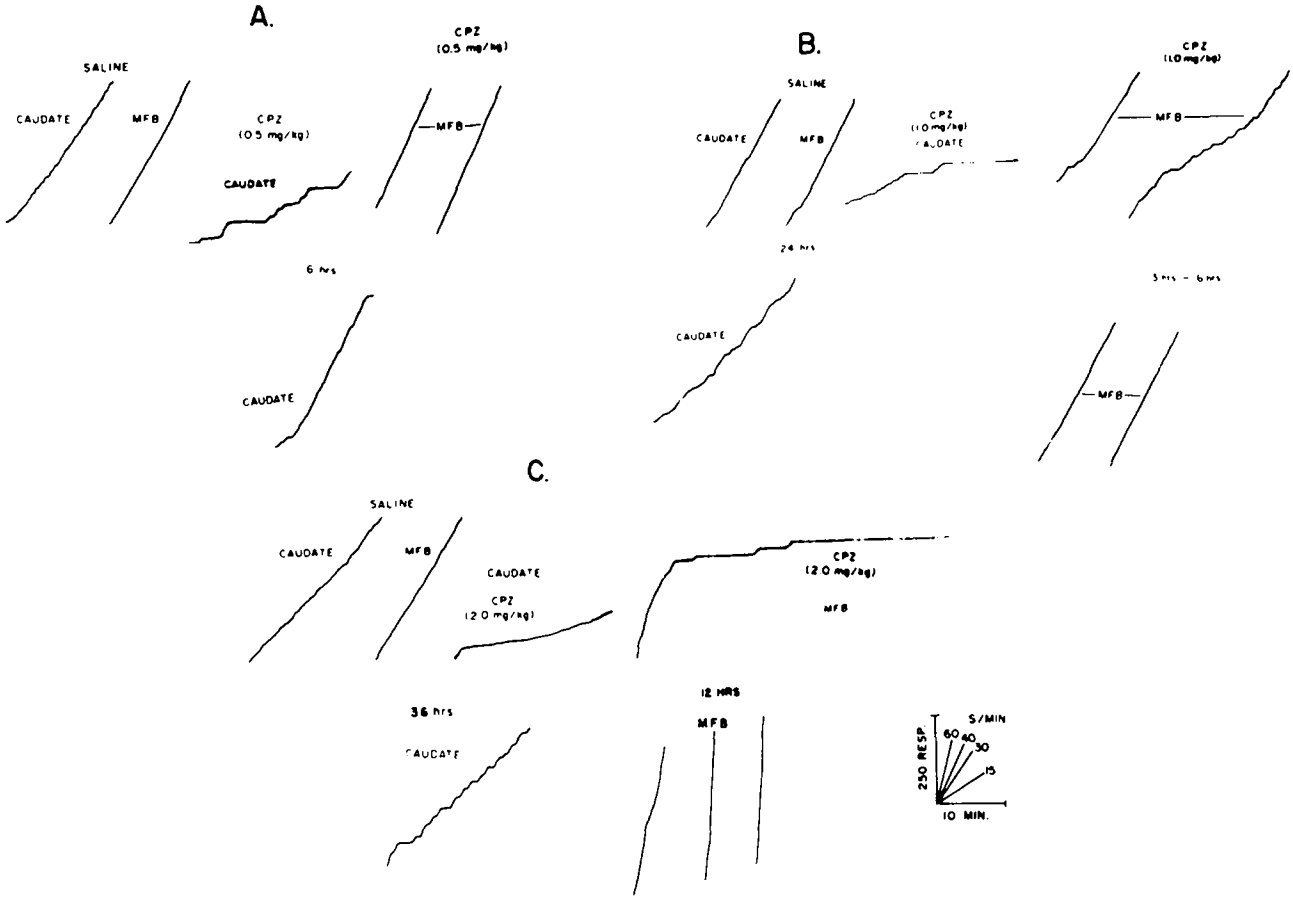

FIG. 4. Cumulative response record for one squirrel monkey that received dosages of 0.5 (A), 1.0 (B), and 2.0 (C) mg/kg of chlorpromazine. Brain sites stimulated were the head of the caudate and the medial forebrain bundle (MFB) in the lateral hypothalamus.

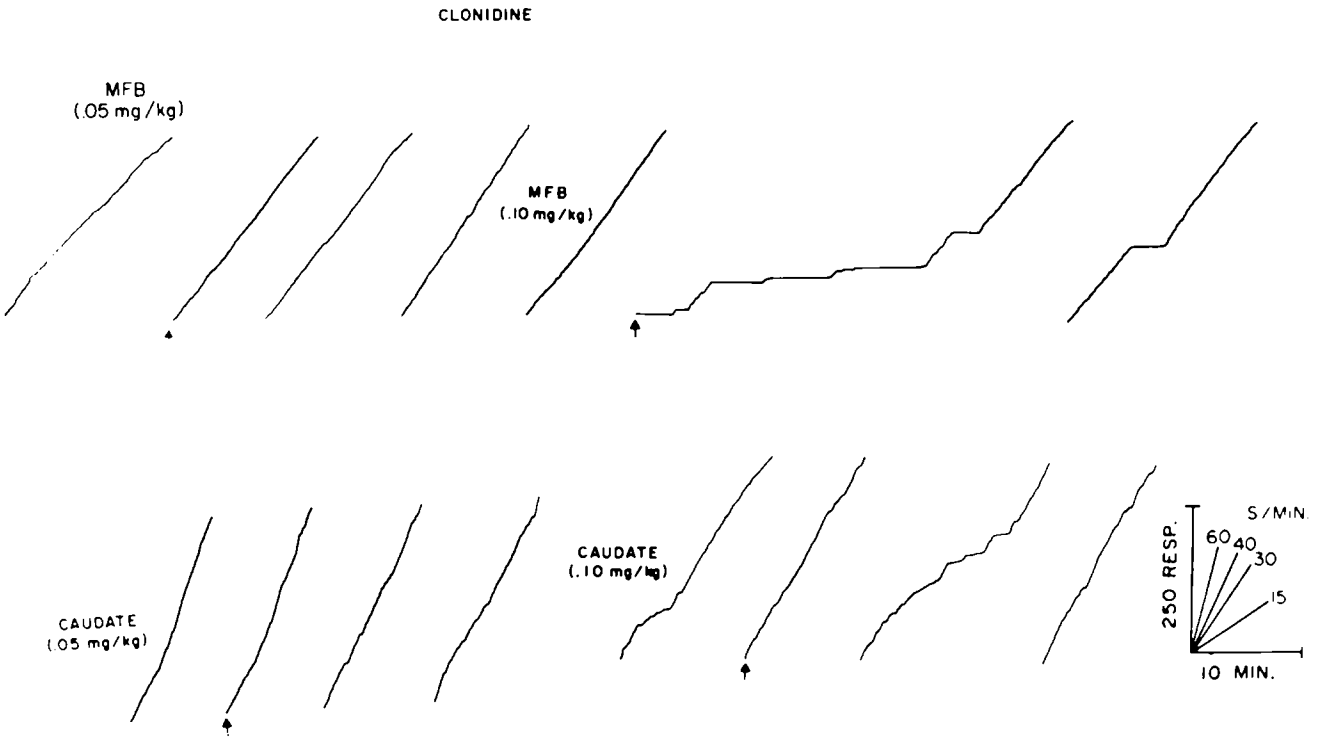

FIG. 5. Cumulative response record for one squirrel monkey that received dosages of 0.05 or 0.10 mg/kg of clonidine. Brain site stimulated was either head of the caudate or the medial forebrain bundle (MFB) in the lateral hypothalamus. Arrow indicates point when drug was given.

from both sites. One dose of amphetamine (1.0 mg/kg) facilitated ICSS from MFB while producing mixed effects of facilitation and inhibition at caudate sites. This result would suggest that the latency of the inhibitory effect of this particular dose of amphetamine on ICSS is shorter in the caudate than the MFB (possibly by acting on more or new aminergic pathways). A dose of 2.0 mg/kg blocked ICSS at both sites. Doses of 0.5 and 1.0 mg/kg of CPZ produced larger effects on caudate than MFB ICSS while at 2.0 mg/kg both sites failed to support ICSS. Although this finding might be partially explicable in terms of CPZ's known effects on dopamine-containing neurons [1,16] the issue is probably more complex. For example in rats, CPZ administered via implanted cannula directed into the caudate nucleus blocks ICSS elicited from the hypothalamus [13]. The action is fairly select since application of CPZ to the cortex did not affect rates of ICSS from the hypothalamus. The blockade of DA receptors in the caudate appears to inhibit ICSS in the hypothalamus. As would be expected, low doses of CPZ (0.5 mg/kg) also affect ICSS from caudate in the present experiment. At higher doses (i.e. 2.0 mg/kg) CPZ may have more general effects on ICSS at other brain sites via its more widespread influence on other amine systems.

High doses of amphetamine (2.0–10.0 mg/kg) or CPZ (2.0 mg/kg) had much longer durations of action on behavior elicited from the head of the caudate as compared with the hypothalamus. This result would suggest that high dosages of these drugs, using ICSS as a behavioral measure, tend to act on DA containing regions of the brain for longer periods than on noradrenergic sites. This interpretation is strengthened by the finding that caudate self-stimulation was inhibited for 4 days following a 10 mg/kg dose of amphetamine while within the same animal hypothalamic self-stimulation was observed after 36 hr. The hypothesis [26] that these drugs act primarily within the hypothalamus is at best incomplete. If these drugs do act on a common substrate for ICSS comparable thresholds and durations of drug action would be expected.

In contrast to the generalized effects of amphetamine or CPZ throughout the CNS, the hypotensive drug clonidine only appears to act on noradrenergic neurons [14]. Clonidine has known effects on both cardiovascular and behavioral processes mediated by NE neurons in the hypothalamus. For example, when clonidine is placed directly into the hypothalamus via cannula, a reduction in heart rate and a lowering of blood pressure occurs [4] and large amounts of eating behavior are observed [5]. The hypothesis that ICSS in the hypothalamus, which contains NE neurons, should be affected to a greater degree than caudate ICSS received support from the present findings. In animals with two functional electrodes, ICSS from hypothalamic sites was blocked while ICSS from the caudate was much less affected. The finding that caudate ICSS is still maintained while hypothalamic ICSS is blocked by clonidine would suggest that other transmitters besides NE and other pathways besides the MFB also play an important role in the support of ICSS.

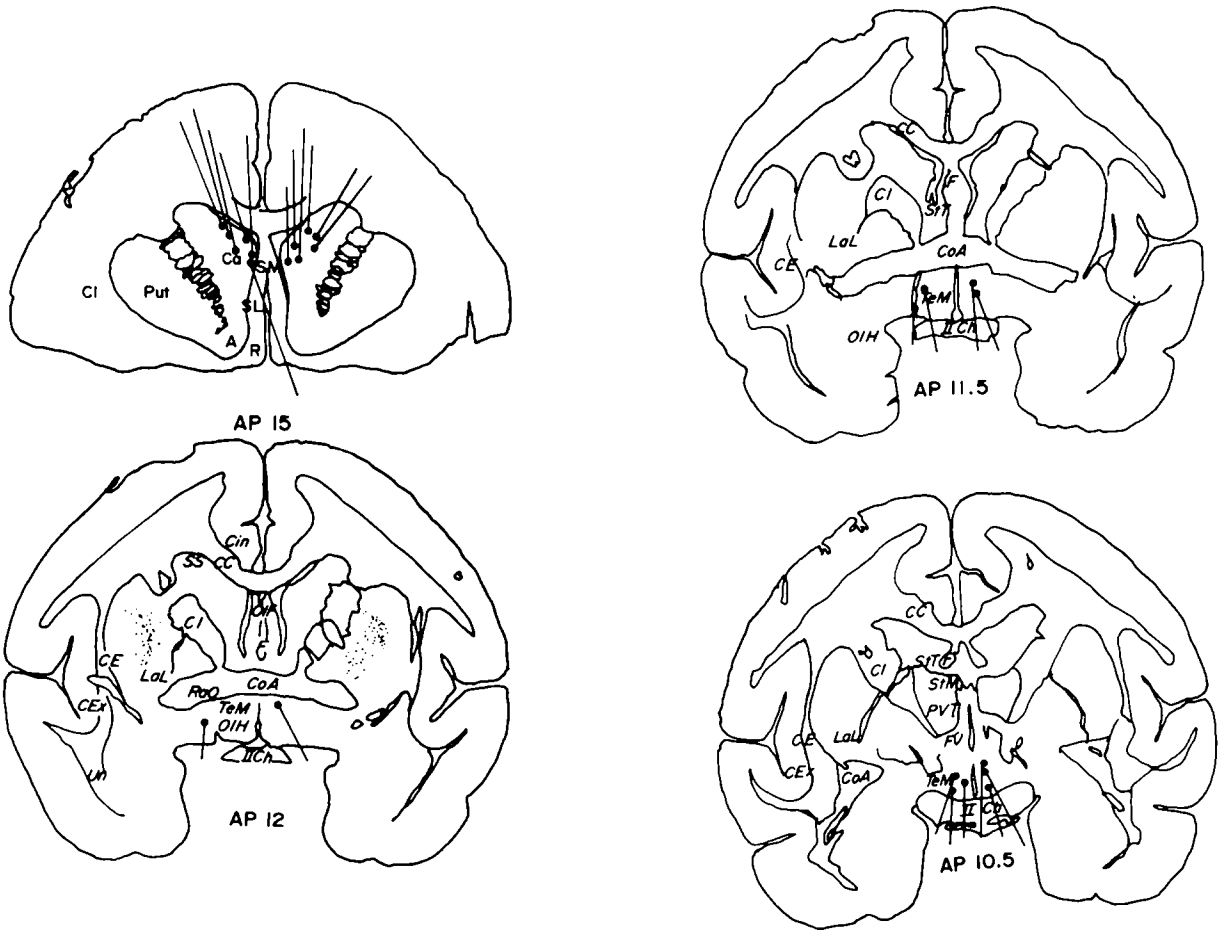

FIG. 6. Cross sections of squirrel monkey brain tissue taken through 4 anterior–posterior (AP) levels. Each black dot represents final electrode penetration. All electrode implants were bilaterally represented. Abbreviations: Ca: Nucleus caudatus; SL: Area septalis pars lateralis; SM: Area septalis pars medialis; A: Nucleus accumbens septi; R: Gyrus rectus; CL: Claustrum; Ci: Cingulum; SS: Stratum subcallosum; CC: Corpus callosum; CI: Capsula interna; Olf: Area olfactoria; F: Fornix; RaO: Radiatio olfactoria profunda; CoA: Commissura anterior; TeM: Fasciculus telencephalicus medialis (median forebrain bundle); OlH: Fasciculus olfactorius hippocampi (diagonal band of Broca); IICh: Chiasma nervorum opticorum (optic chiasma); CE: Capsula externa; CEx: Capsula extrema; Un: Fasciculus uncinatus; LaL: Lamina medullaris lateralis (pallidi); StT: Stria terminalis; StM: Stria medullaris thalami; PVT: Pedunculus ventralis thalami.

REFERENCES

1. Anlezark, G. M., G. W. Arbuthnott, J. E. Christie and T. J. Crow. Role of cerebral dopamine in the action of psychoactive drugs. *Br. J. Pharmac.* **41:** 406–407, 1971.
2. Arbuthnott, G. T., K. Crow, K. Fuxe, L. Olson and U. Ungerstedt. Depletion of catecholamine in vivo induced by electrical stimulation of central monoamine pathways. *Brain Res.* **24:** 471–483, 1970.
3. Axelrod, J. Noradrenaline: Fate and control of its biosynthesis. *Science* **173:** 598–606, 1971.
4. Boudier, H. A. J. and J. M. van Rossum. Clonidine-induced cardiovascular effects after stereotaxic application in the hypothalamus in rats. *J. Pharm. Pharmac.* **24:** 410–411, 1972.
5. Broekkamp, C. and J. M. van Rossum. Clonidine-induced intrahypothalamic stimulation of eating in rats. *Psychopharmacologia* **25:** 162–168, 1972.
6. Domino, E. F. Discussion of paper. In: *Psychotomimetic drugs,* edited by D. H. Efron. New York: Raven Press, 1970, pp. 146–148.
7. Gergen, J. A. and P. D. Maclean. A stereotaxic atlas of the squirrel monkey's brain (saimiri sciureus). U.S. Department of Health, Education, and Welfare, National Institutes of Health, Bethesda, Maryland. 1962.
8. German, D. and D. M. Bowden. Catecholamine systems as the neural substrate for intracranial self-stimulation: A hypothesis. *Brain Res.* **73:** 381–419, 1974.
9. Glowinski, J. and J. Axelrod. Effects of drugs on the uptake, release and metabolism of H3 norepinephrine in the rat brain. *J. Pharmac. exp. Ther.* **149:** 43, 1966.
10. Haymaker, W., E. Anderson and W. Nauta. *The Hypothalamus.* Springfield, Illinois: Charles C. Thomas, 1972.
11. Hemsworth, B. A. and M. J. Neal. The effect of stimulant drugs on the release of acetylcholine from the cerebral cortex. *Br. J. Pharmac.* **32:** 416, 1968.
12. Justesen, D. R., J. C. Sharp and P. B. Porter. Self-stimulation of the caudate nucleus by instrumentally naive cats. *J. comp. physiol. Psychol.* **56:** 371–374, 1963.

13. Keats, E. M. The effects of intracaudate injections of chlorpromazine on conditioned avoidance and self-stimulation behavior. Unpublished Ph.D. dissertation. University of California, San Francisco. Dissertation Abstract 2971-B, 34(6), 1973.
14. Laverty, R. and K. M. Taylor. Behavioral and biochemical effects of 2-(2,6-dichlorophenylamino)-2 imidazoline hydrochloride (ST 155) on the central nervous system. *Br. J. Pharmac.* **35:** 253–264, 1969.
15. Lindvall, O. and A. Bjorklund. The organization of the ascending catecholamine neuron systems in the rat brain. *Acta physiol. scand.* Suppl. **412:** 1–48, 1974.
16. Moore, K. Biochemical correlates of the behavioral effects of drugs. In: *An Introduction to Psychopharmacology*, edited by R. Rech and K. Moore. New York: Raven Press, 1971, pp. 79–133.
17. Phillips, A. G. and H. C. Fibiger. Dopaminergic and noradrenergic substrates of positive reinforcement: differential effects of d- and l-amphetamine. *Science* **179:** 575–576, 1973.
18. Olds, J., K. G. Killam and Bach-y-Rita. Self-stimulation of the brain used as a screening method for tranquilizing drugs. *Science* **124:** 265–266, 1956.
19. Olds, M. E. and M. Ito. Effects of chlorpromazine, chlordiazepoxide and pentobarbital on neuronal excitability in the medial forebrain bundle during self-stimulation behavior. *Neuropharmacology* **12:** 1117–1133, 1973.
20. Olds, M. E. and A. Yuwiler. Effect of brain stimulation in positive and negative reinforcing regions in the rat on content of catecholamines in hypothalamus and brain. *Brain Res.* **36:** 385–398, 1972.
21. Randrup, A. and I. Munkvad. Correlation between specific effects of amphetamines on the brain and on behavior. In: *Current Concepts on Amphetamine Abuse*, edited by E. Ellinwood and S. Cohen. Proceedings of a workshop, Duke University, Durham, N.C., Washington, D.C.: U.S. Government Printing Office, 1970, pp. 17–25.
22. Ritter, S. and L. Stein. Self-stimulation of the locus coeruleus. *Fedn Proc.* **31:** 820, 1972.
23. Siegal, S. *Nonparametric Statistics for the Behavior Sciences.* New York: McGraw-Hill, 1956.
24. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. *Fedn Proc.* **23:** 836, 1964.
25. Stein, L. Chemistry of reward and punishment. In: *Psychopharmacology: A Review of Progress 1957–1967*, edited by D. H. Efron. Washington, D.C.: U.S. Government Printing Office 1968, p. 105–123.
26. Stein, L. Facilitation of behavior by amphetamine. In: *Psychomimetic Drugs*, edited by D. H. Efron. New York: Raven Press, 1970, pp. 137–145.
27. Stein, L. and C. D. Wise. Release of norepinephrine from hypothalamus and amygdala by rewarding medial forebrain bundle stimulation and amphetamine. *J. comp. physiol. Psychol.* **67:** 189–199, 1969.
28. Unemoto, M. and R. Kido. Depressing effect of methamphetamine on self-stimulation in the cat. *Nature* **216:** 133–134, 1967.
29. Van Rossum, J. M. Mode of action of psychomotor stimulating drugs. *Int. Rev. Neurobiol.* **12:** 309–384, 1970.
30. Winer, B. J. *Statistical principles in Experimental Design.* New York: McGraw-Hill, 1973, p. 518.