Psychopharmacologia (Berl.) 23, 1—16 (1972)

Original Investigations

Effects of *d*-Amphetamine, Scopolamine, Chlordiazepoxide and Diphenylhydantoin on Self-Stimulation Behavior and Brain Acetylcholine*

EDWARD F. DOMINO and MARIANNE E. OLDS**

Michigan Neuropsychopharmacology Research Program, Department of Pharmacology, University of Michigan, Ann Arbor, Michigan 48104, U.S.A.

Received May 5, 1970; Final Version July 13, 1971

Abstract. The effects of *d-amphetamine* (0.25—8), scopolamine (0.25—8), chlordiazepoxide(2.5—40), and diphenylhydantoin (25—75), given i.p. or s.c. on a mg/kg basis, were studied on self-stimulation behavior in the male albino rat. The dose-effect relationships, the role of baseline rates of responding and their effects on brain acetylcholine (ACh) were determined in rats trained to self-stimulate for electrical reward in the lateral posterior hypothalamus. The effects of *d*-amphetamine were both dose and baseline-rate dependent. Low-moderate doses (0.5—2.0 mg/kg inclusive) facilitated self-stimulation and larger doses (2.0 to 8.0 mg/kg) depressed responding. Baseline rates before *d*-amphetamine administration were extremely important in the effect observed. Low rates of responding were facilitated and high rates were depressed by this agent. The effects of scopolamine in a wide range of dosage were less consistent. A small dose (0.5 mg/kg) facilitated only transiently self-stimulation and larger doses (1—8 mg/kg) tended to depress this behavior. Baseline rate effects were less important but high-rate responders were usually depressed by scopolamine.

The effects of chlordiazepoxide were dose-dependent. A dose of (5 mg/kg) caused facilitation but larger doses (10—40 mg/kg) produced depression of self-stimulation irrespective of baseline rates. However, high-rate stimulators showed the most dramatic increases with 5 mg/kg of chlordiazepoxide. In contrast, diphenylhydantoin (25—75 mg/kg) usually depressed self-stimulation. Low rate self-stimulators showed the most marked depressant effects.

Brain ACh was progressively reduced by handling of naive animals, injection of saline, and $^1/_2$ h of self-stimulation and escape behavior. Animals not allowed to self-stimulate but given *d*-amphetamine (2.0 mg/kg), scopolamine (2.0 mg/kg) showed a significant decrease in brain ACh. Self-stimulation, in addition to medication with the various drugs, showed a trend for further reduction in brain ACh but the differences were not statistically significant.

Key words: Self-Stimulation — Acetylcholine — Amphetamine — Scopolamine — Chlordiazepoxide.

* Supported in part by Grant MH-11846 (to EFD) and MH-11627 (to JO), U.S. Public Health Service.

** Present address: Division of Biology, California Institute of Technology, Pasadena, California 91109, U.S.A.

Introduction

Many different psychotropic drugs affect self-stimulation including various sedatives, antidepressants and psychomotor stimulants. These agents cause quantitative and qualitative changes in rates depending upon the specific brain sites of electrode placements (Stark *et al.*, 1969). One of the first agents described to dramatically increase self-stimulation was amphetamine (Stein, 1964, 1968). Antianxiety drugs of the benzodiazepine class in low doses also increase self-stimulation (Olds, 1966). Anticonvulsants like diphenylhydantoin have been reported to increase, under certain conditions, the number of responses emitted for brain reward (Reid *et al.*, 1964). The fact that similar effects are obtained with widely differing drug classes provides an opportunity to investigate whether changes in total brain acetylcholine (ACh) occur during very high rates of responding regardless of the pharmacological means employed to produce such high rates. It is well known that there is a relationship between brain ACh and self-stimulation for brain reward. Like other operant behaviors self-stimulation is depressed by cholinergic agonists (Vaillant, 1964, 1967; Stark and Boyd, 1963; Jung and Boyd, 1966; Domino and Olds, 1968; Olds and Domino, 1969a, b), and in the case of physostigmine-induced depression, has been correlated with high levels of brain ACh (Domino and Olds, 1968). Muscarinic cholinergic antagonists such as scopolamine are reported to facilitate responding for brain reward (Pradhan, personal communication, 1970). These reciprocal actions of ACh in the brain suggest the possibility of a modulating function in regions where self-stimulation behavior can be produced.

The present study was carried out in two parts. First, the dose-response relationships for *d*-amphetamine, scopolamine, chlordiazepoxide, and diphenylhydantoin were investigated to provide a comparative basis for their action on self-stimulation behavior and to select from the various doses tested the one most likely to produce high rates of responding. Next a dose for each drug was given to animals for measuring the total content of brain ACh with and without accompanying self-stimulation behavior. The results indicate that self-stimulation behavior reduces total brain ACh irrespective of the drug treatment used.

Methods

Ninety-six male adult Holtzman albino rats were used. They were individually housed and on an *ad lib.* feeding schedule. Body weight ranged from 250—300 g at the time of electrode implantation to a maximum of 500 g at the end of the drug tests. Surgery was performed using pentobarbital sodium anesthesia. The method of implantation and subsequent initial training for self-stimulation behavior have been de-

scribed previously in detail (Olds and Olds, 1963). Bipolar stimulating electrodes were placed in the lateral posterior hypothalamus.

Drug tests were conducted on rats which had been preselected on the basis of preliminary training as low, moderate, and high rate self-stimulators. Low rates were considered to be 100—350 responses/8 min, moderate rates 350—750/8 min and high rates 750—1100/8 min. In order to separate facilitation of gross behavior from facilitation of self-stimulation, drug sessions were also carried out in the same test chamber on animals without any electrodes, on animals with electrodes in other regions of the brain, and on animals which were classified as non-self-stimulators (below 100/8 min). The electric reward was a shock 0.25 sec in duration 60 Hz waves delivered to the hypothalamus via the indwelling electrodes. The current settings ranged from 40 to 60 μA selected to produce optimal rates of responding. Depression of the lever during the 0.25 sec duration of the sine waves did not produce another train. The schedule of reinforcement was FR_1, *i. e.*, each lever press after a reward produced another electric shock reward.

d-Amphetamine sulfate was given to groups of rats in doses of 0.25, 0.5, 1, 2, 4 and 8 mg/kg; scopolamine hydrobromide in doses of 0.25, 0.5, 1, 2, 4, 6 and 8 mg/kg; chlordiazepoxide hydrochloride in doses of 2.5, 5, 10, 20 and 40 mg/kg; and diphenylhydantoin sodium in doses of 25, 50 and 75 mg/kg. The drugs were dissolved in 0.9% saline and administered s.c. or i.p. Drug dosage is given as salt. The volume of the solution injected never exceeded 0.5 ml.

During testing each drug was administered after a 30—45 min period of self-stimulation and the effects of treatment were computed for an 80-min period. For the brain assay studies the drugs were similarly administered after an initial period of self-stimulation and the animals returned to the test chambers for a 30-min period before decapitation. The test animals were allowed to self-stimulate during that period. Control animals were placed in individual test chambers but no self-stimulation was made available to them.

All drug sessions were carried out on six to eight rats simultaneously. Drug sessions on the same animals were separated by at least six days. There was no indication that administration of one dose of the drug altered the response to a subsequent administration of the same compound of another. The ACh bioassay assay procedure was the same as that described by Domino and Olds (1968). Animals were sacrificed by decapitation and the brain quickly removed and homogenized in acid-alcohol using the method of Stone (1955) for total ACh.

Analysis of Data. Rates of responding for each rat were computed for blocks of 8 min before and after injection. Means scores for three blocks immediately preceding treatment constituted "baseline" rates

against which were compared rates per 8-min periods throughout the entire 80-min test session. The only animals included in these tests were self-stimulators with stable rates of responding during an extensive preliminary training period. Therefore, the three blocks of 8 min which constituted "baseline" rates were representative of the usual rate of self-stimulation of that animal. Drug effect was expressed as the percentage change in response output from the mean "baseline" rates. Both the student "*t*" for group comparison (Snedecor, 1956) and analysis of variance (Edwards, 1950) were used to evaluate the data.

Histology. Animals which were not sacrified for brain ACh assays were used to determine electrode placement at the conclusion of the experiments. Formalin fixed sections through the diencephalon were cut at 50 μ and stained with the cresyl violet method for determination of the regions stimulated. The electrodes were lodged in the median forebrain bundle in the vicinity of the posterior hypothalamus.

Results

Effects of d-Amphetamine. Groups of 6 to 8 rats were tested at levels chosen to produce optimal rates of self-stimulation of 100—1200/8 min. It was noted that 0.25 mg/kg *d*-amphetamine caused insignificant changes in the mean rate of self-stimulation. On the other hand, doses of 4 and 8 mg/kg caused a significant reduction in self-stimulation behavior at 16 and 24 min after injection which was the time of peak effect. In spite of marked variability, these effects were highly statistically significant ($P < 0.02$). It was obvious that the effects of *d*-amphetamine were not only dose-related but also dependent upon the baseline rate of self-stimulation. The effects of 2 mg/kg, i.p. of *d*-amphetamine in rats matched for rates of self-stimulation are illustrated in Fig. 1. *d*-Amphetamine in a dose of 2 mg/kg caused large increases in the rate of self-stimulation in those animals with low baseline rates. In contrast, *d*-amphetamine produced a slight increase and a subsequent decrease in rate in moderate self-stimulators. In the high self-stimulators *d*-amphetamine caused only a decrease in rate. A fourth group of animals with electrodes in the vicinity of the medial forebrain bundle, with rates of 0—100/8 min, which did not meet the criteria for self-stimulation, were also given *d*-amphetamine. These rats were previously tested at increasingly higher levels of electrical stimulation to assure that they did not normally self-stimulate. The administration of *d*-amphetamine in a dose of 2 mg/kg caused a striking increase in the number of responses observed 32 min after injection and subsequently. Facilitatory effects were still present at the end of 80 min when the test session was completed. These results were highly significant in a group

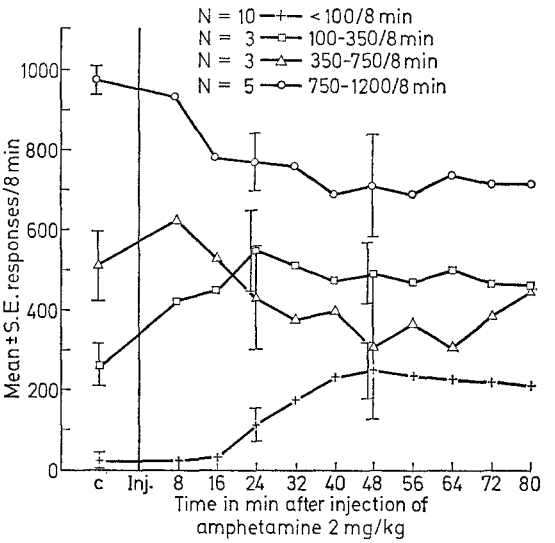

Fig. 1. Effects of *d*-amphetamine in rats matched for self-stimulation rates. Time in min after the injection of 2 mg/kg i.p. *d*-amphetamine is given on the x axis and the mean $\pm$ standard error of responding per 8 min is given on the y axis. Various rats are grouped on the basis of their rates of self-stimulation. Four groups are included: those animals that self-stimulated below rates of 100/8 min, those that self-stimulated between 100—350/8 min, those that self-stimulated 350—750/8 min, and those that self-stimulated 750—1200/8 min. Note that *d*-amphetamine caused a marked increase in the animals that self-stimulated at low rates but caused a depression of responding in those animals that stimulated at high rates

of 10 animals (DF 2, F 6.40; $P < 0.05$ at 32 min). Finally, the effects of 2 mg/kg of *d*-amphetamine were tested on a group of naive rats with no brain electrodes. Inasmuch as it is known that *d*-amphetamine causes gross behavioral activation, it seemed possible that simply by increased activity subjects might accidentally increase the rate of bar pressing. These animals showed a preinjection rate of approximately 5/8 min. After 2 mg/kg of *d*-amphetamine there was a gradual increase in the number of responses until it reached 50/8 min period 40 min after injection (see Fig. 2).

In summary, *d*-amphetamine clearly produced the most significant effects in rats with low rates of self-stimulation. Furthermore, *d*-amphetamine caused behavioral activation in naive animals although their rates of responding never approached those of animals given *d*-amphetamine but whose pre-injection rate did not meet criteria of self-stimulation.

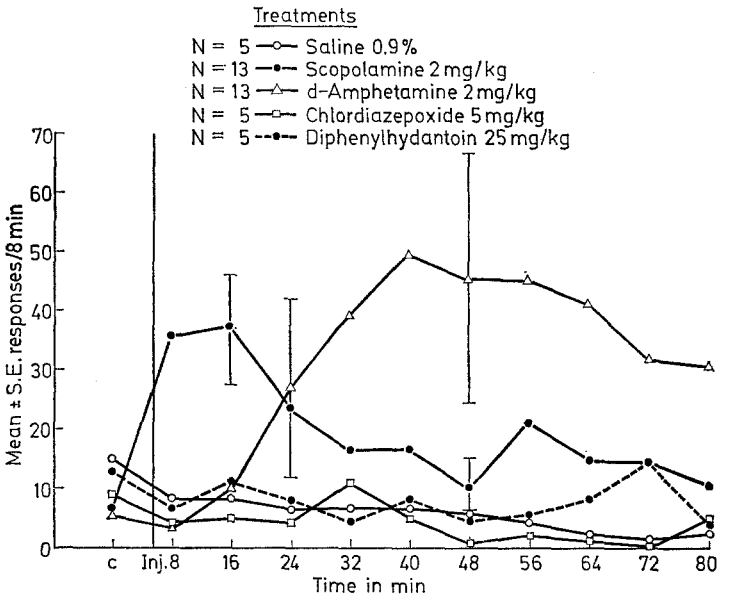

Fig. 2. Effects of various centrally active drugs on bar pressing behavior in naive rats. Data are plotted as in Fig. 1. Various groups of rats were given the drugs indicated i.p., except scopolamine was given s.c. Note that *d*-amphetamine and scopolamine produced an increase in bar pressing but never to the levels achieved in self-stimulation

Effects of Scopolamine. Scopolamine was given s.c. to groups of 6—8 animals with self-stimulation rates of 100—1200/8 min. Increases of 14 and 27% in the mean group rate occurred only with a dose of 0.25 mg/kg, 14 and 24 min after injection, but even at this dose not all animals showed increased responding. With higher doses, although there were individual cases of increased responding, the mean group effect was a decrease in self-stimulation behavior. These effects occurred promptly and were maximal 16 min after injection. With doses above 2 mg, there was less individual variability and a decrease in mean rate of responding. The mean decrease was generally less than 50% as was the case with *d*-amphetamine. Seldom was self-stimulation behavior abolished. A dose of 2 mg/kg was chosen for further tests because facilitation had been observed in some rats. The effects of 2 mg/kg of scopolamine were then tested on low, moderate and high-rate stimulators as previously defined. In contrast to the dramatic effects of *d*-amphetamine in causing increased self-stimulation of low rate stimulators, scopolamine in a dose of 2 mg/kg had no significant effect (see Fig. 3).

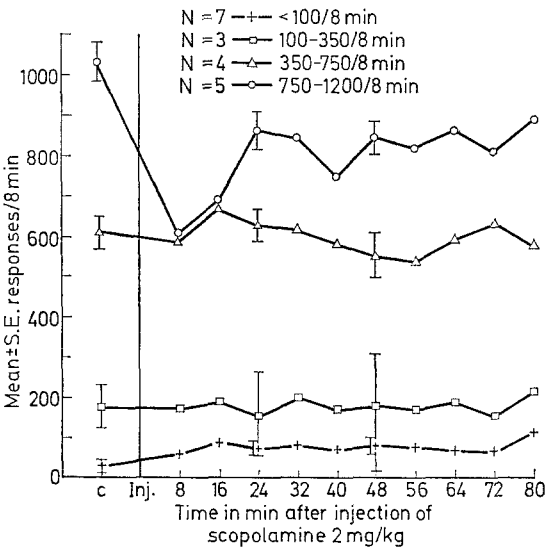

Fig. 3. Effects of scopolamine in rats matched for self-stimulation rates. Scopolamine was given in a dose of 2 mg/kg s.c. Note that scopolamine in this dose caused a slight increase in self-stimulation rates of those animals with baseline rates below 100/8 min. The drug had no significant effect on animals with low to moderate rates of responding but caused a definite decrease in high rate self-stimulators which lasted throughout the 80 min session

Scopolamine (2 mg/kg) also had no significant effect in moderate rate self-stimulators. In contrast, in high baseline self-stimulators, this dose of scopolamine caused a 50% decrease in the rate of self-stimulation within 8 min after injection. Subsequently, the rate of self-stimulation rose to about 80% of control levels but remained depressed from pre-injection rates throughout the entire test session. In a fourth group of animals with electrodes in the vicinity of the median forebrain bundle with rates of 0—100/8 min, but which did not meet the usual criteria for self-stimulation, 2 mg/kg of scopolamine caused a slight facilitation which reached a peak 16 min after injection, but this effect was not statistically significant. In a group of naive animals 2 mg/kg of scopolamine caused an increase in gross activity which again reached peak 16 min after injection. The rate of responding increased and was maintained above control levels over the 80-min test session (Fig. 2). However, these rates never reached the levels of responding of self-stimulators.

In summary, only very small doses of 0.25 mg/kg of scopolamine produced an increase in the rate of self-stimulation. This increase was

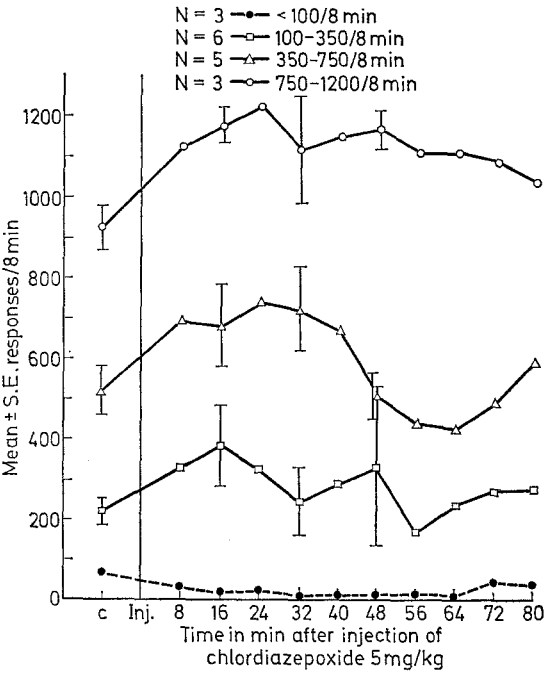

Fig. 4. Effects of chlordiazepoxide in rats matched for self-stimulation rates. Chlordiazepoxide was given in a dose of 5 mg/kg, i.p. Note that chlordiazepoxide caused the greatest increase in self-stimulation in animals with high baseline rates. In contrast, those animals with brain electrodes which self-stimulated below 100/8 min had a decrease in this behavior

very small in contrast to the effects of *d*-amphetamine. Doses of scopolamine in the order of 2 mg/kg had no consistent effects on self-stimulators of low and moderate rates but caused a definite decrease in the performance of high rate self-stimulators. These effects lasted throughout the 80-min test session. Although scopolamine caused an increase in gross behavioral movements and a random increase in bar pressing in naive animals, this rate never approached that of the self-stimulators. In contrast to the delayed action of amphetamine, the effect of scopolamine was rapid but transient.

Effects of Chlordiazepoxide. Chlordiazepoxide was given i.p. to groups of 6—8 rats self-stimulating between 100—1200/8 min. In general, the effects of chlordiazepoxide were facilitatory at low doses and progressively depressing with larger doses. With doses of 2.5 and 5 mg/kg of chlordiazepoxide the mean rate of self-stimulation increased to as much as 80% over preinjection rates 24 min after administration. Compared

with saline injected controls these effects were highly statistically significant using a group comparison "t" test ($P < 0.02$). After doses of 10 and 20 mg/kg of chlordiazepoxide some animals still showed facilitation. However, doses of 40 mg/kg of chlordiazepoxide depressed the rate of self-stimulation with a peak 24 min after injection ($P < 0.02$). Again, it was noted that there was marked individual variation. The effects of chlordiazepoxide in a dose of 5 mg/kg were studied in animals with low, moderate and high rates of self-stimulation as previously defined. As illustrated in Fig. 4, chlordiazepoxide increased the rate of self-stimulation behavior irrespective of baseline rates although there were quantitative differences. In animals with low rates of 100—350/8 min of self-stimulation chlordiazepoxide produced a slight increase followed by alternating facilitation and reduction of responding to approximately control rates. There were 2 periods when rates were approximately the same as before treatment, the first at 32 and the second at 56 min after injection. Chlordiazepoxide increased the self-stimulation rate of animals with moderate rates. Peak effects were observed 24 min after injection and lasted approximately 48 min with subsequent decrease to control levels. The most dramatic increases were seen in high rate responders. As illustrated in Fig. 4, this effect was greatest 24 min after injection but was still apparent at the end of the 80-min test session. Chlordiazepoxide injected in non-self-stimulators or in naive animals, depressed responding; the low initial rate was further decreased (see Fig. 2).

It would thus appear that the effects of chlordiazepoxide on self-stimulation were dose dependent with faciliatory effects at low doses and progressive depression at higher doses. The degree of facilitation of self-stimulation varied with the preinjection rates. Animals with low, moderate, and high rates showed progressive increase in self-stimulation following a dose of 5 mg/kg of chlordiazepoxide. The high rate animals showed especially dramatic facilitation.

Effect of Diphenylhydantoin. The effects of diphenylhydantoin given i.p. on self-stimulation behavior were determined in groups of 6—8 self-stimulators with rates of 100—1200/8 min. Each dose of diphenylhydantoin caused large decreases in the rate of self-stimulation for brain reward. The depressant effects were observed shortly after injection and for the highest dose lasted throughout the test session. There were only 2 animals injected with a dose of 75 mg/kg whose rates of self-stimulation were increased by more than 10%. With doses of 25—50 mg/kg, recovery was nearly complete at the end of the 80-min test session, whereas in the case of 75 mg/kg the peak depressant effect occurred toward the end of the 80-min session. The effects of diphenylhydantoin were not related to baseline rates. Nevertheless, a dose of 25 mg/kg was given to animals with low, moderate, and high rates of self-stimulation. As is

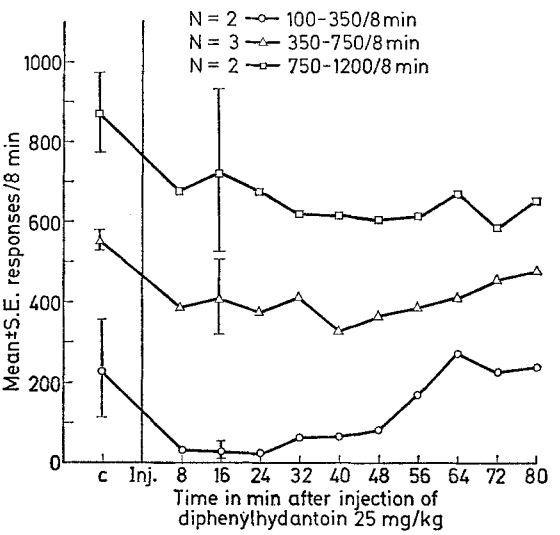

Fig. 5. Effects of diphenylhydantoin in rats matched for self-stimulation rates. Diphenylhydantoin was given in a dose of 25 mg/kg, i.p. Note that diphenylhydantoin decreased rates of self-stimulation, irrespective of baseline rates

evident in Fig. 5, animals in each of these categories showed depression in rates of responding. Diphenylhydantoin produced almost complete blockade of responding in the low rate animals, but 64 min after injection the rates returned toward control levels. When diphenylhydantoin was given to naive animals there was no significant effect on gross behavior and no change in the spontaneous rate of bar-pressing (see Fig. 2).

In summary, in doses of 25, 50 and 75 mg/kg of diphenylhydantoin, depression of self-stimulation behavior was usually observed. This occurred irrespective of the baseline rates of self-stimulation. Animals with low rates of self-stimulation showed an almost complete cessation of self-stimulation, whereas high-responders were bar-pressing at significantly decreased rates.

Effects of Drugs on Brain ACh in Self-Stimulating and Non-Self-Stimulating but Implanted Rats

Levels of ACh in Control Animals. The ACh values for endogenous content of the brain in 22 rats with chronic electrodes in posterior lateral hypothalamus for 1—6 months were 18.6 $\pm$ 0.9 nMol/g. These animals were not given saline injections but had been previously accustomed to the test chamber. The ACh content of animals treated exactly as the previous group but receiving a saline injection $^1/_2$ h before decapitation was slightly lower. Naive animals receiving a saline injection, but having

no previous experience in the test chamber and not being used to handling, had even lower mean levels of ACh than either of the 2 previous groups. Although the differences were not large, the trend suggests that handling and injection of saline were sufficiently stressful to produce a slight decrease in brain ACh. On the other hand, implantation of electrodes did not produce even slight depletion since naive animals given a saline injection had lower levels of ACh than animals with electrodes not given any injection.

The mean brain ACh content of 8 animals which self-stimulated for $^1/_2$ h, then received an injection of saline, and then again self-stimulated $^1/_2$ h before decapitation was markedly lower than that of animals in any of the previous experimental situations. The content of endogenous ACh was 14.5 ± 0.7 nMol/g, or $78\,\%$ of that in animals with electrodes in the same brain regions but not self-stimulating. This decrease was significant ($P < 0.05$, group comparison "*t*" test).

Eight rats which were in all cases identical with the previous group, but who responded to escape from shock punishment in the midbrain instead of self-stimulating for shock reward in the lateral hypothalamus had mean levels of ACh lower than those in self-stimulating rats and strikingly lower than those in rats implanted with electrodes and receiving saline injections. This decrease (13.7 ± 0.8) was also significant ($P < 0.05$, group comparison "*t*" test).

In summary, the four experimental procedures varying progressively in degree of psychological stress—handling of naive rats, injections of saline, self-stimulation and escape behavior—produced progressively lower levels of brain ACh.

Effects of d-Amphetamine. Assays for brain ACh in implanted but non-self-stimulating animals $^1/_2$ h after treatment with 2 mg/kg i.p. of *d*-amphetamine indicated a reduction of ACh levels compared to values obtained with non-self-stimulating implanted animals which received control injections of saline. In the drug treated animals ($N = 8$) the mean ACh content $\pm$ S.E. was 14.9 ± 0.6 nMol/g whereas in the saline-treated animals the mean content was 17.1 ± 0.6 nMol/g. The reduction in content was in the same range as that produced by self-stimulation behavior.

Eight animals which received *d*-amphetamine and self-stimulated had values of ACh which were only slightly lower (14.0 ± 0.3) than those which did not self-stimulate and were given *d*-amphetamine (14.9 ± 0.6), or animals which self-stimulated and received saline injections (14.5 ± 0.7).

These data indicate a trend for *d*-amphetamine to reduce the endogenous ACh content of the brain which is slightly greater when combined with episodes of self-stimulation.

Effects of Scopolamine. Treatment with this cholinergic antagonist in a dose of 2 mg/kg produced the lowest levels of brain ACh in animals which self-stimulated and those which did not but had implanted electrodes. It should be recalled this dose had an effect primarily on the high rate stimulators. Eight animals receiving an injection of scopolamine $^1/_2$ h before decapitation had a mean value of brain ACh of 10.1 ± 0.4 nMol/g, a level of ACh considerably lower than that of animals receiving saline injections (17.1 ± 0.6) and animals which had implanted electrodes but receiving no injections (18.6 ± 0.0). The brain ACh content of scopolamine treated rats was also lower than that of animals self-stimulating or escaping from brain shock. Scopolamine produced larger reductions in brain ACh content than any of the experimental procedures employed including escape from aversive brain shock, 13.7 ± 0.8.

Self-stimulating animals treated with scopolamine had a mean ACh content of 9.5 ± 0.4 nMol/g. This was considerably below that of animals in the various control and drug-treated groups and slightly below that of non-self-stimulating animals treated with scopolamine. Here also, as in the case of *d*-amphetamine treatment, the effects of the drug were enhanced by self-stimulation behavior.

Effects of Chlordiazepoxide. Eight non-self-stimulating animals with implanted electrodes which were given 5 mg/kg, i.p. chlordiazepoxide $^1/_2$ h before decapitation had mean levels of brain ACh comparable to animals with implanted electrodes receiving saline (chlordiazepoxide 17.7 ± 0.8 *vs.* saline 17.1 ± 0.6 A nMol/g).

The group of animals (N 8) which received the same dose of chlordiazepoxide and was allowed to self-stimulate had values of ACh almost identical to those of self-stimulating animals which received saline. These data provide evidence for the striking effects which can be produced on ACh content of the brain by self-stimulation behavior. While the ACh content in chlordiazepoxide-treated rats was not diminished as much as in animals treated with scopolamine, it was in the range of that in either animals treated with *d*-amphetamine or self-stimulating animals receiving saline injections. In other words, the ACh content was comparable to that of self-stimulating animals.

Effects of Diphenylhydantoin. Animals with electrodes but not self-stimulating prior to decapitation and treated with 25 mg/kg i.p. diphenylhydantoin had a mean value of brain ACh 15.9 ± 0.9 nMol/g. This was a level of ACh lower than with treatment with chlordiazepoxide or saline but slightly higher than that of animals self-stimulating or escaping from brain shock. In this case also, the brain content of ACh of animals treated with the drug and self-stimulating was lower than that of animals treated with the drug alone.

In all cases of drug treatment, the combination of self-stimulation with the drug caused an additional reduction in the brain levels of ACh. In most cases the differences were very slight, but a trend was observed.

Discussion

It is quite obvious that *d*-amphetamine, scopolamine, chlordiazepoxide and diphenylhydantoin affected self-stimulation behavior quite differently. Although *d*-amphetamine and scopolamine produced an increase in gross motor behavior, each had its own specific effects on self-stimulation. The dose-effect relationships of *d*-amphetamine indicate that low to moderate doses of the drug tend to facilitate self-stimulation behavior, whereas large doses tend to depress it. Our data, in part, support the observations of Stein (1964, 1968) that *d*-amphetamine dramatically increases self-stimulation behavior, particularly in animals self-stimulating at low rates near their electrical threshold.

The behavioral effects of a moderate dose of *d*-amphetamine are clearly related to previous baseline rates. This generalization concerning the behavioral effects of amphetamines has been proposed by Dews (1958) and Kelleher and Morse (1968). In animals where there was no self-stimulation or very low levels of responding *d*-amphetamine apparently lowered the threshold of the stimulus to such a degree that otherwise neutral stimuli became effectively rewarding. Rats with electrodes misplaced so that no responding occurred even when the intensity stimulation was raised to twice that originally applied, self-stimulated at rates far greater after *d*-amphetamine than naive rats given *d*-amphetamine. This would indicate that in addition to gross behavioral activation *d*-amphetamine has an important effect on the excitability threshold of hypothalamic neurons in the vicinity of the reward system in the brain. It would seem that *d*-amphetamine can transform a neutral or subthreshold electrical stimulus into one sufficiently "rewarding" to promote sustained self-stimulation behavior. Stein (1964) has suggested that *d*-amphetamine causes a shift to the left of the stimulus intensity-rate of responding curve. We have not had an opportunity to test this phenomenon for all doses of *d*-amphetamine, especially those that produce depression of high rates of responding.

Another explanation for the observation that *d*-amphetamine facilitates low rates of self-stimulation, but depresses high rates is related to the well known inverse "U" function of behavioral arousal and performance. When pre-drug arousal in an animal is low an elevation would be effective in increasing the number of responses, whereas one with high rates of self-stimulation might be adversely affected by the administration of *d*-amphetamine causing excessive arousal.

In contrast to the dramatic effects of *d*-amphetamine on self-stimulation behavior, the actions of scopolamine were quite small in spite of the fact that this drug produces stimulation of gross motor activity of naive animals. Only low doses of scopolamine produced an immediately excitatory effect on self-stimulation which decreased over time. Furthermore, scopolamine had no significant effect on the threshold of self-stimulation behavior. Animals with electrodes in which the stimulus had no significant motivational value were not facilitated by scopolamine as was the case with *d*-amphetamine. Scopolamine only slightly increased the number of responses. Large doses of scopolamine in general tended to reduce self-stimulation. The effects of scopolamine were not as dependent on preinjection baseline rates of behavior. However, animals that self-stimulated at very high rates tended to be depressed by the administration of scopolamine.

The effects of chlordiazepoxide on self-stimulation behavior were dose-related and biphasic. Low doses of chlordiazepoxide produced a very dramatic increase in self-stimulation. Paradoxically, the largest increases in self-stimulation behavior were obtained in animals with the highest rates of self-stimulation. Although chlordiazepoxide increased the rate of responding in low, moderate, and high rate responders, it was most dramatic in the latter group. These effects of chlordiazepoxide are particularly interesting in that the operant level of responding of naive animals was not enhanced. Furthermore, chlordiazepoxide had no significant effect on lowering the threshold for electrical reward.

In the doses used, diphenylhydantoin had no facilitatory effect on self-stimulation behavior. Only in an occasional animal was the drug slightly facilitating. In the vast majority of animals, diphenylhydantoin progressively decreased self-stimulation behavior. In this regard diphenylhydantoin had somewhat greater effects in those animals that self-stimulated at low and moderate rates as opposed to the high rate stimulators. It would appear that the anti-convulsant actions of diphenylhydantoin were not critical in facilitating self-stimulation behavior, but merely depressed it.

The effects of these drugs on brain acetylcholine in animals with and without self-stimulation indicate the extreme importance of ongoing behavior in any assessment of brain neurochemistry. In non-self-stimulating rats but with implanted electrodes, the data obtained for brain acetylcholine agrees with the literature for normal animals (see Votava, 1967).

Naive rats given saline, or animals which either self-stimulated or escaped midbrain tegmental shock showed a tendency towards decreased total brain ACh. *d*-Amphetamine, scopolamine, and diphenylhydantoin lowered brain ACh while chlordiazepoxide had no significant

effect. However, all self-stimulation animals, irrespective of the drug given, showed a trend for slightly lower levels of brain ACh.

This investigation supports the hypothesis of Stein (1968) of an adrenergic "go" mechanism in self-stimulation as evidenced by the actions of *d*-amphetamine. However, this applies only to low baseline rates. Further studies of very low doses of *d*-amphetamine on high rate responders are indicated. The muscarinic cholinergic system seems to be involved in self-stimulation behavior in two different ways as evidenced by the action of scopolamine. Low doses of this drug caused a behavioral disinhibition of a possible "no-go" cholinergic system (Domino, 1969) which had small facilitating effects on the rate of self-stimulation. However, large doses tended to reduce but not abolish this behavior. Thus, the cholinergic system appears to have a modulating role. An involvement of a cholinergic system with self-stimulation is also indicated from the brain ACh data. However, escape responding at similar rates produced a decrease in brain ACh to the same degree as self-stimulation suggesting stress or a brain "use" phenomenon.

References

Dews, P. B.: Studies on behavior. IV. Stimulant actions of methamphetamine. J. Pharmacol. exp. Ther. **122**, 137—147 (1958).

Domino, E. F.: Role of the central cholinergic system in wakefulness, fast wave sleep and "no-go" behavior in The present status of psychotropic drugs, A. Cerletti and F. Bovet (eds.), pp. 273—277. Amsterdam: Excerpta Med. Found. 1969.

— Olds, M. E.: Cholinergic inhibition of self-stimulation behavior. J. Pharmacol. exp. Ther. **164**, 202—211 (1968).

Edwards, A. L.: Experimental design in psychological research. New York: Holt, Rinehart and Winston 1950.

Jung, O. H., Boyd, E. S.: Effects of cholinergic drugs on self-stimulation response rates in rats. Amer. J. Physiol. **210**, 432—434 (1966).

Kelleher, R. T., Morse, W. H.: Determinants of the specificity of behavioral effects of drugs. Ergebn. Physiol. **60**, 1—56 (1968).

Olds, M. E.: Facilitatory action of diazepam and chloridazepoxide on hypothalamic reward behavior. J. comp. physiol. Psychol. **62**, 136—140 (1966).

— Domino, E. F.: Comparison of muscarinic and nicotinic cholinergic agonists on self-stimulation behavior. J. Pharmacol. exp. Ther. **166**, 189—204 (1969a).

— — Differential effects of cholinergic agonists on self-stimulation and escape behavior. J. Pharmacol. exp. Ther. **170**, 157—167 (1969b).

— Olds, J.: Approach-avoidance analysis of rat diencephalon. J. comp. Neurol. **120**, 259—295 (1963).

Reid, L. D., Gibson, W. E., Gledhill, S. M., Porter, P. B.: Anticonvulsant drugs and self-stimulating behavior. J. comp. physiol. Psychol. **57**, 353—356 (1964).

Snedecor, G. W.: Statistical methods applied to experiments in agriculture and biology, 5th ed., pp. 534. Ames, Iowa: Iowa State College Press 1956.

Stark, P., Boyd, E. S.: Effects of cholinergic drugs on hypothalamic self-stimulation response rates in dogs. Amer. J. Physiol. **205**, 745—748 (1963).

Stark, P., Turk, J. A., Redman, C. E., Henderson, J. K.: Sensitivity and specificity of positive reinforcing areas to neurosedatives, antidepressants and stimulants. J. Pharmacol. exp. Ther. **166**, 163—169 (1969).

Stein, L.: Amphetamine and neural reward mechanisms. In: Animal behavior and drug action. H. Steinberg, A. V. S. deReuck, J. Knight (eds.), Ciba Foundation Symposium, pp. 91—113. Boston: Little, Brown 1964.

— Chemistry of reward and punishment. In: Psychopharmacology, a review of progress 1957—1967. D. H. Efron, J. O. Cole, J. Levine, and J. R. Wittenborn, (eds.), pp. 105—135. PHS Pub. Nr. 1836, Supt. of Documents, U.S. Government Printing Office, Washington 1968.

Stone, W. E.: Acetylcholine in the brain. I. "Free", bound and total acetylcholine. Arch. Biochem. **58**, 181—192 (1955).

Vaillant, G. E.: Antagonism between physostigmine and atropine on the behavior of the pigeon. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak. **248**, 406—416 (1964).

— A comparison of antagonists of physostigmine-induced suppression of behavior in the pigeon. J. Pharmacol. exp. Ther. **157**, 636—648 (1967).

Votava, Z.: Pharmacology of the central cholinergic synapses. Ann. Rev. Pharmacol. **7**, 223—240 (1967).

Dr. E. F. Domino
Department of Pharmacology
University of Michigan
Ann Arbor, Michigan 48104, U.S.A.