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Dissociation of Dopaminergic and Non-Dopaminergic Substrates for Cues Produced by Electrical Stimulation of the Ventral Tegmental Area

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DRUHAN, J. P., M. T. MARTIN-IVERSON, D. M. WILKIE, H. C. FIBIGER AND A. G. PHILLIPS. *Dissociation of dopaminergic and non-dopaminergic substrates for cues produced by electrical stimulation of the ventral tegmental area.* PHARMACOL BIOCHEM BEHAV **28**(2) 251–259, 1987.—The present study provides evidence for the existence of multiple substrates for cues produced by electrical stimulation of the ventral tegmental area in rats. Two different procedures were employed to assess the effects of amphetamine and haloperidol on the discrimination of high and low intensity cues produced by electrical brain stimulation (EBS). When the procedure involved frequent presentation of brief trials, amphetamine and haloperidol had no effect on the discrimination of EBS. In contrast, when the trials were less frequent and extended in duration, amphetamine enhanced the perceived intensity of the cues whereas haloperidol had the opposite effect. These results indicate that the use of different discrimination procedures may result in the measurement of separate dopaminergic and non-dopaminergic substrates for cue properties of EBS in the ventral tegmental area.

Cue properties Electrical brain stimulation Ventral tegmental area Dopamine Amphetamine Haloperidol Discrimination ICSS Rats

IT is well established that electrical stimulation of certain brain regions may serve as a discriminative or conditional stimulus for instrumental or classically conditioned responses in animals [8, 15, 23, 29]. Recent studies of the stimulus properties of electrical brain stimulation (EBS) have sought to determine the relation between cueing and other functional properties of the stimulation such as reward and to identify the neurotransmitter substrates for the EBS cues [5, 6, 26, 29, 31, 32]. However, pharmacological studies attempting to modulate the cues produced by stimulation of the lateral hypothalamus (LH) have yielded inconsistent results [5, 6, 17, 26, 30]. Thus, reliable identification of neurotransmitter substrates has not been possible to date.

The present study investigated the possibility that the inconsistent effects of drugs on EBS cues may be due to the mediation of such cues by several neural pathways. Given the diversity of methods for measuring EBS cues in previous reports [5, 6, 17, 26, 30], it is conceivable that separate discrimination procedures may result in selective activation of individual substrates. Accordingly, the present study employed two different successive discrimination procedures to investigate the roles of dopaminergic (DA) and non-dopaminergic neurons in mediating cues produced by electrical stimulation of the ventral tegmental area (VTA) in rats. The possible contribution of DA neurons to the cue properties of VTA EBS was assessed by treatment with the indirect DA agonist, amphetamine [4,10], and the DA receptor antagonist, haloperidol [1]. If DA neurons were involved in mediating cue properties of VTA EBS, then amphetamine would be expected to enhance the perceived intensities of the cues, whereas haloperidol should attenuate them.

EXPERIMENT 1

This experiment employed a two-lever discrimination procedure in which the correct lever for food reward was

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signalled by four high or low intensity trains of EBS delivered during a brief (1.4 sec) interval. Amphetamine and haloperidol were injected prior to generalization tests with intermediate EBS intensities, to determine their effects on: (1) the psychophysical function describing the probability of choosing the 'high intensity' lever in response to each current intensity; and (2) the associated point of subjective equality (PSE), a measure of the stimulus value responded to by the rats as though it were midway between the two reference stimuli. A decrease in the perceived intensity of the currents should shift the psychophysical function and the PSE to the right, whereas an increase in the perceived intensity would be expected to produce a shift to the left. Generalization tests with different intensity ranges, in which the cues were altered physically rather than pharmacologically, confirmed the sensitivity of the present procedure for detecting changes in the perceived intensities of the EBS cues. Also, the efficacy of the drug doses employed was confirmed by observing the effects of amphetamine and haloperidol on self-stimulation thresholds.

METHOD

Subjects

The subjects were male hooded rats (Charles River, Long Evans strain) weighing 300 to 350 g at the time of surgery. Throughout the experiment, the rats were housed individually in stainless steel cages with tap water available ad lib. A 12 hr light/dark cycle was maintained in the animal colony, and all rats were tested in the light phase of this cycle. Rats were selected for each phase of the experiment from a general subject pool on the basis of their level of performance and the stability of their electrodes. Many of the rats were used in several phases of the experiment.

Surgery and Histology

Each rat was anesthetized with 65 mg/kg sodium pentobarbital, and a bipolar electrode (Plastic Products MS303/2) was implanted stereotaxically into the VTA. With the incisor bar set at −3.2 mm below the interaural line, the coordinates from stereotaxic zero were: A.P. = +2.8 mm; L. = −0.6 mm; D.V. = +2.1 mm. The electrode was anchored to the skull with jeweler's screws and dental cement. Upon completion of the experiment, all rats were killed and their brains were sectioned and stained with cresyl-violet for verification of electrode placements.

Apparatus

The rats were tested in six separate chambers (24×29×30 cm), each having Plexiglas walls and ceiling and a wire grid floor. Two levers (4.5×7 cm) were mounted 3.5 cm above the floor on opposite ends of the chamber. A 28 V houselight was positioned external to the chamber at the center of a side wall and a food-hopper positioned directly below it (3 cm above the floor). Each chamber was located within a sound attenuating enclosure (55×55×60 cm) and ventilation fans masked extraneous noise. The electrode leads (Plastic Products 303-302) were suspended from Mercotac commutators and passed through an opening in the ceiling. Constant current stimulation was delivered to the rats by a 10 channel, programmable sine wave stimulator. A Data General Nova 3 computer with MANX software was used to control the experimental events and record responses made by the rats. Stimulation currents were monitored continuously on a Telequipment D54R oscilloscope.

Pretraining

After a one week post-operative recovery period, the rats were trained to lever-press for 200 msec trains of 60 Hz sine wave EBS on a continuous reinforcement (CRF) schedule. Each rat received five daily 30 min sessions of CRF responding with the current intensity set at 20 μA. Following this phase of the experiment the rats were food deprived to 90% of their free-feeding weight and then given 7 to 11 intracranial self-stimulation (ICSS) sessions in which response rates were measured over a range of current intensities. The current intensity was set initially at 6 μA and subsequently increased by 2 μA every 5 min until 26 μA was reached. The same range of currents (26 to 6 μA) was again delivered in a descending order of presentation. Each change in the current level was signalled by the free delivery of 10 stimulation trains (2 trains/sec) at the new intensity.

The ascending and descending rate-intensity functions measured over the final 4 days of this phase were averaged to obtain a single function relating response rate to current intensity. From this function, one low current intensity (8 to 12 μA) and one high current intensity (10 μA higher than the low intensity) were chosen for use as discriminative stimuli. The low intensity supported threshold ICSS rates and the high intensity supported near asymptotic ICSS rates. Selection of the discriminative stimuli in this manner ensured that the intensities could be differentiated, at least with respect to their rewarding properties.

After the self-stimulation phase, all rats were trained to bar-press for 45 mg Noyes food pellets on a CRF schedule. This training was conducted in 30 min sessions on two consecutive days. During lever-press training for either food or brain stimulation, only one lever was inserted into the chamber. The location of the lever was alternated daily to prevent development of a side preference.

Discrimination Training

Initial daily discrimination training consisted of 90 trials given 15 to 25 sec apart (variable inter-trial interval with an average of 20 sec). Each trial was signalled by a brief (0.05 sec) flash of the houselight followed 1 sec later by delivery of four 200 msec trains of either high or low intensity EBS. The inter-train interval for the EBS was 200 msec and the total duration of cue presentation was 1400 msec. After a further 1 sec delay, the houselight was turned on. The next response made within a 10 sec period was recorded. A response on the lever appropriate for the cue on that trial led to the delivery of one 45 mg Noyes food pellet and termination of the trial (the houselight was turned off and the lever inactivated). The appropriate lever for each current intensity was counterbalanced between rats. If a rat did not respond within 10 sec on any trial, the houselight was turned off and the inter-trial interval (ITI) was started. During the first five training sessions, all correct responses were rewarded. Incorrect responses initiated a further 10 sec period in which the rats could respond appropriately and receive the food reward. In subsequent sessions, incorrect responses resulted in non-rewarded termination of the trial. When the rats were responding with a high rate of accuracy, only 75% of correct responses were reinforced. Responses during the ITI were recorded separately for each lever, but had no programmed consequences.

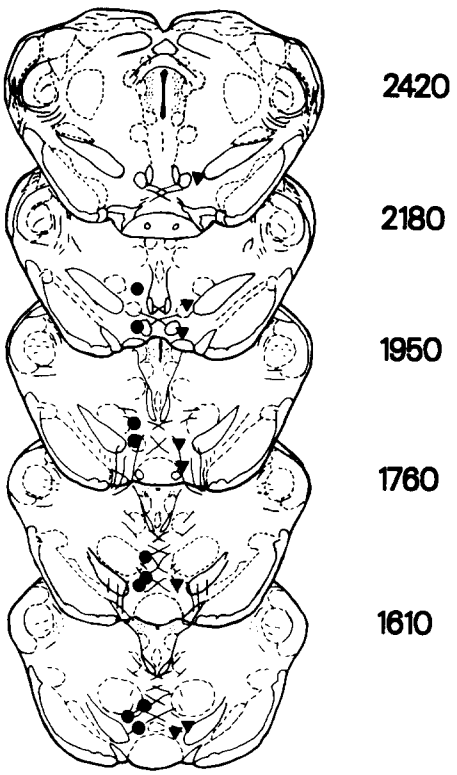

FIG. 1. Electrode placements. All electrodes were implanted in the left hemisphere. For clarity, the electrode placements from Experiment 1 are represented as circles on the left side of the brain, while placements from Experiment 2 are shown as triangles on the right side. The numbers to the right of the diagrams represent the anterior-posterior coordinates (μm) corresponding to the coronal sections from the brain atlas of König and Klippel [16].

Following training, rats that acquired the task (10 out of 16) were tested for stimulus generalization between the two training intensities. These tests consisted of 100 trials, with four equally spaced intermediate current levels (2 μA apart) delivered randomly on twenty of these trials (5 trials at each current). Responses made following intermediate intensities were not reinforced and resulted in termination of the trial. Accordingly, correct responses to the two training intensities were reinforced more frequently to ensure that the rats received an equivalent number of food pellets as on training days. Three baseline sessions were run in this manner, and these were alternated with regular training days.

EBS Generalization After Amphetamine and Haloperidol

Six rats were given generalization tests after receiving two separate doses of d-amphetamine sulphate (1.0 and 2.0 mg/kg) or haloperidol (0.075 and 0.1 mg/kg). These drug sessions were conducted on every third day, with the order of drug administration counterbalanced across animals. Regular training days were interposed between the drug tests. Amphetamine was dissolved in saline to a concentration of 1.0 or 2.0 mg/ml and injected intraperitoneally (IP) in a volume of 1 ml/kg 10 min before testing. Haloperidol (Haldol, McNeil) was diluted in sterile water to 0.075 mg/ml or 0.1 mg/ml and administered IP in a volume of 1 ml/kg, 45 min prior to testing. Three days after the fourth drug test, each rat was given a further generalization session following an IP injection of one of the drug vehicle solutions. Three rats received saline 10 min before the test, while the other four rats were given sterile water 45 min prior to testing.

EBS Generalization With Different Ranges of Current Intensity

Eight rats were given four generalization tests in which a different range of current intensities was delivered during each test. The new ranges differed from those received during baseline in that the absolute intensities presented were uniformly shifted up or down by either 2 or 4 μA. These transfer tests were given on every third day with the regular range of intensities presented on the intervening daily sessions. All rats received each of the four possible intensity ranges and the order in which the tests were given was counterbalanced across the subjects. Three days after the fourth transfer test, all rats were given a baseline generalization session with the regular range of intensities.

Effects of Amphetamine and Haloperidol on VTA ICSS Thresholds

Six of the rats used in the transfer tests were retained for this experiment. The apparatus was the same as in the previous experiments, but only one lever was provided in each chamber and illumination was provided by fluorescent lights attached to the ceilings of the outer enclosures. At the start of each self-stimulation session, the rats were placed in the chambers and baseline bar-pressing rates were measured for 5 min in the absence of brain stimulation. Following this period, the rats could respond on the lever to receive four 200 msec pulses of 60 Hz sine wave stimulation (200 msec inter-train interval) on a variable-interval 20 sec schedule of reinforcement (range=15 to 25 sec). The current intensity was initially set at 2 μA and thereafter increased by 2 μA every 5 min until an intensity of 24 μA was reached. Each increment in the intensity was signalled by the non-contingent delivery of 4 trains of EBS at the new current level.

After two weeks of baseline rate-intensity sessions, the rats were tested following IP injections of amphetamine (1.0 and 2.0 mg/kg) or haloperidol (0.075 and 0.1 mg/kg). The injection procedures were the same as described for the discrimination experiment. Test sessions were given on every third day, with the order of drug administration counterbalanced among rats. Three days following the final drug session, the rats were given injections of the drug vehicles (saline, N=3; sterile water, N=3) before self-stimulation testing. This test provided final baseline values against which to compare the drug effects.

Statistical Analyses

Comparisons between separate generalization tests were made using a two-way analysis of variance (ANOVA) with test session and current intensity as factors. Threshold, PSE and latency measures were analysed by a one-way ANOVA with test session as the factor. Differences revealed with these analyses were considered significant when the probability was less than 0.05. Newman-Keul's test was used for post-hoc comparisons among individual means when the ANOVA indicated significant differences. The less

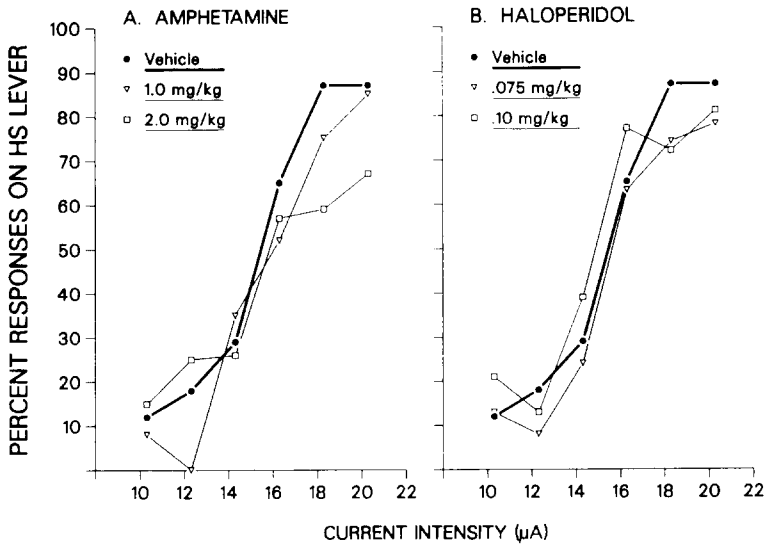

FIG. 2. Generalization functions obtained with the procedure involving frequent, brief trials after injections of: (A) vehicle, 1.0 and 2.0 mg/kg of amphetamine; or (B) vehicle, 0.075 and 0.1 mg/kg of haloperidol. The vehicle conditions in the two graphs represent the same data taken from a single session. The data are expressed in terms of the percentage of the responses that were emitted on the HS lever after the delivery of each current intensity. The data points along the abscissa correspond to the average intensity delivered at each current level.

conservative Duncan's multiple range test was used in this capacity in cases where the number of subjects was small.

RESULTS

Initial Training and Generalization

Ten of the original 16 rats learned the discrimination task to an accuracy of over 80% correct choices per session. These rats acquired the task within 2 to 4 weeks of training and performance remained stable thereafter throughout the study. The electrode placements of these rats are shown in Fig. 1. The other six rats failed to learn the discrimination and were dropped from the study.

For the purpose of analysis, the data from generalization tests are expressed as the percentage of responses at each current level emitted on the lever appropriate for the high intensity stimulation (HS). Individual PSE measures were obtained for each rat from the regression line plotted between the data points associated with the four intermediate intensities in each test. The PSE was defined as the interpolated current intensity which would be expected to elicit an HS response on 50% of trials.

During the initial three baseline generalization tests, the percentage of responses on the HS lever increased as a function of increasing current level, $F(5,45)=72.27$, $p<0.0001$. The overall tendency to respond on the HS lever did not differ across the 3 baseline sessions, nor did the responses to individual current intensities differ as a function of the separate tests. An analysis of the PSEs revealed no significant differences between the measures obtained in the separate tests (range of means=15.2 to 16.3 μA). The slopes of the regression lines also did not differ across tests (range of means=8.7 to 11.2). These results indicate that the generalization functions remained stable with repeated testing.

EBS Generalization After Amphetamine and Haloperidol

As is evident in Fig. 2, there were no differences in the tendencies to respond on the HS lever across different drug tests, or in responding at individual current intensities across days. There were also no significant differences between the PSEs (range of means=15.0 to 16.3 μA) or the slopes (range of means=6.7 to 12.2) obtained in the separate tests. However, there were significant effects of the treatments on the latencies for responding in the different sessions, $F(4,20)=4.09$, $p<0.02$. Post-hoc analysis indicated that response latencies were longer following administration of the higher doses of both amphetamine and haloperidol than after injection of vehicle or the low dose of amphetamine (Duncan's Multiple Range test, $p<0.05$).

EBS Generalization With Different Ranges of Current Intensity

The results from the transfer tests with different intensities are shown in Fig. 3. The percentages of HS responses differed significantly across the four transfer tests and the post-transfer baseline test, $F(4,28)=16.96$, $p<0.0001$. Post-hoc comparisons indicated that both a 2 (−2S) and a 4 μA (−4S) reduction in the range of current intensities resulted in significantly less overall responding on the HS lever relative to the baseline test. In contrast, increasing the range of current intensities by 4 μA (+4S) resulted in more responding on the HS lever. A 2 μA (+2S) increase produced only a

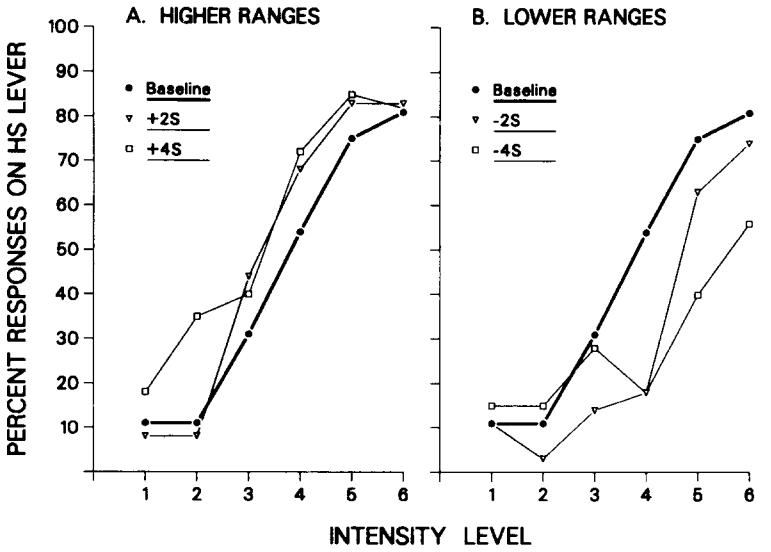

FIG. 3. Generalization functions obtained during tests with different intensity ranges with the procedure involving frequent, brief trials. (A) Tests with the intensity range shifted upwards by 2 μA (+2S), 4 μA (+4S) or 0 μA (baseline). (B) Tests with the intensity ranges shifted down by 2 μA (−2S), 4 μA (−4S) or 0 μA (baseline). The baseline curves in A and B represent the same data taken from a single generalization session.

nonsignificant trend toward more responding on the HS lever.

For this transfer experiment, the PSE was redefined as the interpolated current level (between 1 and 6) within each intensity range which would be expected to elicit an HS response on 50% of trials. Analysis of the PSE values obtained from the +2S, +4S, −2S and baseline conditions confirmed the pattern of results indicated by the analysis of HS responses, $F(3,19)=15.89$, $p<0.0001$. PSE values were not calculated from the generalization gradients obtained from the −4S condition, as the associated regression lines were flattened, $F(4,28)=6.91$, $p<0.001$, so that they did not rise to a level of 50% HS responding.

Effects of Amphetamine and Haloperidol on VTA ICSS Thresholds

Current threshold for ICSS was defined as the lowest intensity at which the mean response rate was 15 presses/5 min higher than the operant rate. A comparison of thresholds obtained from a pre-drug baseline session and the last vehicle test in 6 rats indicated that this measure yielded an index of threshold that did not change over the time during which the drug effects were assessed (both means=13.7 μA). The individual threshold currents for ICSS on the variable-interval 20 sec schedule ranged from the second to the fourth lowest stimuli used in the generalization procedure. All 6 rats self-stimulated at the highest three currents delivered during generalization tests.

Analysis of the effects of drug treatment on the ICSS thresholds (Fig. 4) revealed a significant overall difference between the amphetamine, haloperidol and vehicle tests, $F(4,20)=8.24$, $p<0.0005$. Post-hoc analysis (Duncan's Multiple Range test, $p<0.05$) revealed that the thresholds after

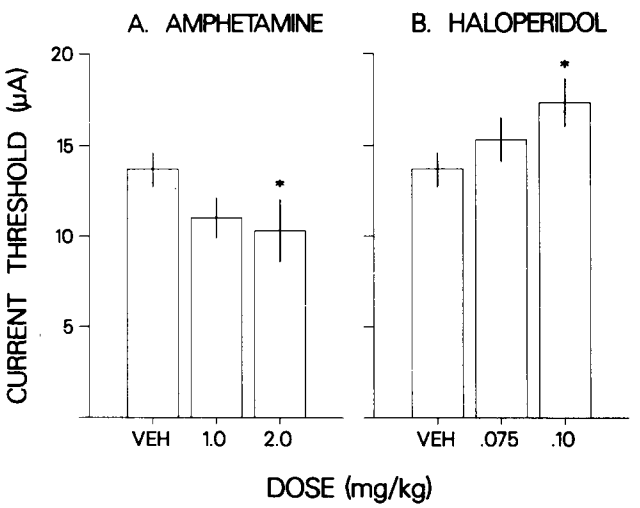

FIG. 4. Current thresholds (μA) for ICSS with a VI-20 schedule of reinforcement following injections of: (A) vehicle, 1.0 and 2.0 mg/kg amphetamine; or (B) vehicle, 0.075 and 0.1 mg/kg haloperidol.

2.0 mg/kg amphetamine (mean=10.3 μA) were significantly lower than those following vehicle injections (mean=13.7 μA). In contrast, 0.1 mg/kg haloperidol elevated ICSS thresholds relative to those observed after vehicle injections (mean=17.3 vs. mean=13.7 μA).

DISCUSSION

This experiment confirmed the utility of a discrimination

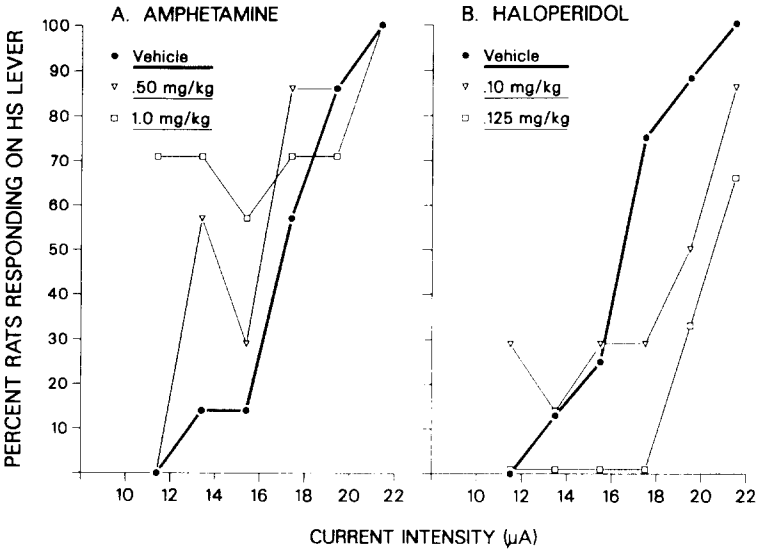

FIG. 5. Generalization functions obtained with the procedure involving trials that are less frequent and extended in duration after injections of: (A) vehicle, 0.5 and 1.0 mg/kg of amphetamine; or (B) vehicle, 0.1 and 0.125 mg/kg of haloperidol. The data are expressed in terms of the percentage of rats that responded on the HS lever at each intensity level. One rat did not respond after 0.1 mg/kg haloperidol, and three failed to respond after 0.125 mg/kg of this drug. These rats were omitted from the calculations of each respective generalization function.

procedure for providing a reliable measure of the stimulus properties of VTA EBS in rats. The sensitivity of the paradigm for detecting changes in the stimulus intensity was verified in transfer tests in which the range of currents was shifted relative to the range delivered in baseline tests. Despite the sensitivity of this procedure, no effects on the generalization gradients or their associated PSE values were observed following injections of amphetamine or haloperidol. This suggests that these drugs had no modulatory effect on the perceived intensities of EBS cues even at doses capable of altering both discriminated response latencies and ICSS thresholds. By inference, it may be concluded that DA neurons do not mediate VTA EBS cues measured with the procedure involving frequent, brief trials. Rather, such cues must be mediated by non-dopaminergic substrates within the VTA.

EXPERIMENT 2

Studies suggesting a non-DA substrate for EBS cues ([17,26], Experiment 1) have employed training procedures involving multiple brief trials within each daily session. In contrast, those studies supporting cue mediation by a DA substrate [5,6] trained animals during single, daily trials wherein the stimulation was delivered during an extended period of time (5 min) prior to access to the response levers. The rats then responded on the appropriate lever for a further 15 min in the continued presence of the EBS cue. The present experiment examined whether a variation of the latter procedure might permit the observation of dopaminergic substrates for VTA EBS cues. Accordingly a procedure was developed which incorporated certain characteristics of methods which have previously suggested a DA substrate for LH EBS cues [5,6].

METHOD

Pretraining

Nine experimentally-naive male hooded rats (Charles River, Long Evans strain) were used for this experiment. The housing conditions, surgery, histology, experimental apparatus and pre-discrimination training procedures were the same as those described in Experiment 1.

Discrimination Training

Initial training consisted of 12 discrimination trials given 120 to 180 sec apart (VI-150 schedule). The beginning of each trial was signalled by a brief (0.05 sec) flash of the houselight, followed 1 sec later by delivery of the first of 6 presentations of either high (20 or 22 μA) or low (10 or 12 μA) intensity EBS. Each presentation of the EBS consisted of four 200 msec trains of 60 Hz sine wave stimulation delivered 200 msec apart. The EBS was maintained at a constant high or low intensity throughout a given cueing period and was delivered at 20 sec intervals. A final 20 sec interval followed the sixth EBS presentation, after which the houselight was turned on to signal the availability of food. The houselight remained on for 30 sec during which time the rat could respond to obtain food on the lever appropriate for the EBS intensity presented during the cueing period. The appropriate lever for each current intensity was counterbalanced between rats. Responses on the incorrect lever had no programmed consequence. At the end of the 30 sec response period the houselight was turned off and the ITI was initiated. During ITIs and the cueing periods, responses on both levers were recorded but had no programmed consequence.

During initial training, each response on the correct lever

resulted in the delivery of one 45 mg Noyes food pellet. Following acquisition of this task, the reinforcement contingency was changed so that a food pellet was delivered after every third response on the correct lever, regardless of whether intervening responses were made on the other lever. When the rats had adapted to this contingency, a food pellet was delivered after every sixth response on the correct lever. The accuracy of the discrimination was assessed by recording the lever on which a rat first completed the FR requirement for the schedule in effect.

Following training, rats that acquired the task (8 out of 9) received three separate stimulus generalization tests. As was the case in Experiment 1, four equally spaced intermediate intensities (2 μA apart) were delivered randomly along with the usual training currents. Each intermediate intensity was delivered once and each training current was presented 4 times within a single generalization test. Because the rats could respond for food for 30 sec on each trial, the omission of reinforcement on some trials would likely have a substantial effect on discriminative performance. Thus, in contrast to Experiment 1, the rats were reinforced on all trials during generalization testing. When an intermediate intensity served as the cue, the rats were reinforced for continuing to complete the FR-6 requirement on the lever which was initially chosen on each trial. Thus, if the FR-6 requirement was initially completed on the left lever, then subsequent reinforcement would only occur for FR-6 responses on that lever within the 30 sec trial.

EBS Generalization After Amphetamine and Haloperidol

Seven rats were given generalization tests after receiving two doses of d-amphetamine sulphate (0.5 and 1.0 mg/kg) or saline solution. Following the tests with amphetamine, an eighth rat was added to the group and all rats were given generalization tests after injections of two doses of haloperidol (0.1 and 0.125 mg/kg) or sterile water. The order of dose administration was counterbalanced across animals, with at least two regular training sessions interposed between each test.

RESULTS

Initial Training and Generalization

Eight of the 9 rats learned the discrimination task to an accuracy of over 80% correct choices per session. The electrode placements of these rats are shown in Fig. 1. As in Experiment 1, the data from the 3 baseline generalization tests were analysed to determine the stability of the generalization functions. However, because the rats only received one trial with each intermediate intensity, the analysis could not be performed on the percent of HS responses emitted after each current level. Instead, threshold values were defined as the intensity at which each rat began to consistently select the HS lever at successive current levels. When HS responding was inconsistent across successive intensities, the threshold value reflected the average of the lowest intensity eliciting an HS response and the intensity at which the rat began consistently choosing the HS lever. Analysis of the thresholds obtained during baseline tests revealed no significant differences in threshold between the sessions (range of means=16.8 to 18.8 μA) indicating the reliability of the measure across separate tests.

EBS Generalization After Amphetamine and Haloperidol

The effects of amphetamine and haloperidol on the generalization functions obtained using extended cue presentations are shown in Fig. 5. The data are expressed as the percent of rats responding on the HS lever as a function of the current intensity. The two training intensities were each delivered 4 times in a session. An HS response was scored for these currents if 50% or more responses were made on the lever appropriate for high intensity stimulation. Rats that did not respond at all following a drug treatment were omitted from these calculations. Separate calculations of the results from the saline test, both with and without these rats, yielded similar generalization functions. Thus, shifts in the curves following drug treatments were not due to exclusion of the animals. As is evident in Fig. 5, amphetamine resulted in a greater percentage of the rats responding on the HS lever at lower intensities relative to tests following vehicle injections. This effect was most pronounced at the higher dose (1.0 mg/kg), with 60 to 70% of the rats responding on the HS lever at even the lowest intensities. In contrast, haloperidol reduced the number of animals responding on the HS lever at most intensities. The lower dose (0.1 mg/kg) produced reductions of 42%, 38% and 17% at the fourth, fifth and sixth intensity levels, respectively. The higher dose (0.125 mg/kg) produced reductions in the number of rats emitting HS responses at all but the lowest current level (range of differences=13 to 75%). Importantly, the response biases during the ITIs were not altered by either amphetamine or haloperidol. The shift in HS response tendencies only occurred following presentation of the EBS cues.

Statistical analyses revealed significant differences among threshold values obtained during both the amphetamine test phase, $F(2,12)=4.36$, $p<0.05$, and the tests with haloperidol, $F(2,10)=5.46$, $p<0.05$. Post-hoc analyses indicated that, relative to vehicle control tests, the thresholds for HS responding were significantly lower after the 1.0 mg/kg dose of amphetamine and higher after 0.125 mg/kg of haloperidol (Newman-Keul's, $p<0.05$).

DISCUSSION

In the present experiment, amphetamine and haloperidol both had significant effects on the rats' perception of EBS intensities. Following amphetamine injections the rats perceived the EBS intensities to be higher than during tests with saline, as indicated by the tendency for rats to respond on the HS lever at lower current levels. In contrast, haloperidol attenuated the perceived intensity of the EBS cues such that the rats began emitting HS responses at higher current values relative to saline tests. In view of the well known DA agonist properties of amphetamine [4,10], and the selective DA receptor antagonist effects of haloperidol at the doses used here [1], it would appear reasonable to attribute the actions of these drugs to modulation of the activity of a DA substrate for the cue properties of VTA EBS.

GENERAL DISCUSSION

The present study demonstrated that amphetamine and haloperidol may significantly alter the perceived intensity of cues produced by stimulation of sites in the VTA, but only under certain conditions. Significant drug effects were observed only when the discrimination procedure involved trials that were less frequent and extended in duration, and not when the trials were brief and presented frequently within a session. With the former procedure, the indirect DA

agonist, amphetamine, augmented the perceived intensity of the EBS cue whereas the DA receptor antagonist, haloperidol, had the opposite effect. These pharmacological effects are consistent with the hypothesis of a dopaminergic substrate for the EBS cue being measured with this procedure. It is unlikely that these drug effects represent a general disruption of the discrimination. Although previous work has suggested that neuroleptics and stimulants may disrupt the detection of very low intensity LH EBS cues [17,32], the lack of drug effects in the first experiment indicate that amphetamine and haloperidol do not alter the discrimination of VTA EBS at the intensities and doses employed in the present study. Furthermore, the lack of change in responses on the HS lever during the ITI refutes any suggestion that the drugs may be producing general changes in the rats' response biases.

The absence of pharmacological effects when the procedure involved brief and frequently presented trials may reflect the involvement of non-dopaminergic substrates for the EBS cues. This conclusion is supported by our preliminary observations that the perception of EBS intensities may be enhanced by the acetylcholinesterase inhibiter, physostigmine, when the discrimination trials are brief and frequent. In contrast, physostigmine has no effect on the perception of EBS when the trials are extended and less frequent [9]. This differential modulation of EBS cues by dopaminergic and cholinergic drugs provides support for the hypothesis that the cue properties of EBS may be mediated by several different neural substrates, with the relative contribution of each being determined by the training procedure employed. Unfortunately, because of the numerous differences existing between the two procedures, the critical variable determining which substrate predominates cannot be specified at present. However, it is possible to exclude variables that were held constant between experiments. For example, the range of intensities employed as cues and the placement of electrodes within the VTA were very similar. Furthermore, although different indices were used to assess the drug effects in the two experiments, the divergent results are unlikely to be a consequence of this factor. In fact, no significant differences were found between drug tests in Experiment 1 when the data were re-analysed in terms of the threshold intensities at which the HS lever was selected on more than 50% of the trials by each rat.

As noted above, the results of Experiment 2 suggest a role for DA in the cue properties of VTA EBS. Dopamine neurons may also serve as a substrate for the cue properties of psychomotor stimulant drugs [12–14, 27, 28] and the rewarding properties of both stimulants [18,25] and VTA EBS [22,24]. It is possible that all of these phenomena may involve the same DA neurons. In this regard, it is of interest that the rewarding effects of stimulants and VTA EBS and the cue properties of amphetamine all appear to involve DA projections to the nucleus accumbens [3, 18–21, 25]. On the other hand, a previous study has reported that VTA EBS does not substitute for the amphetamine cue [7], suggesting a dissociation of their neural substrates. However, these results should be interpreted with caution in view of the present evidence for multiple VTA EBS cues.

If artificial enhancement of DA neurotransmission with EBS or drugs can elicit cues for discriminative responding, then the question arises as to whether increases in DA activity induced by environmental events might also produce an internal stimulus with the capacity to influence behavior. For example, external stimuli signaling the imminent availability of food can increase DA turnover in the NAS [2]. In this context DA projections to the NAS may be involved in the modulation of emotional states associated with the anticipation of reward [11].

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