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# Differential Effects of Physostigmine on Cues Produced by Electrical Stimulation of the Ventral Tegmental Area Using Two Discrimination Procedures

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DRUHAN, J. P., M. T. MARTIN-IVERSON, D. M. WILKIE, H. C. FIBIGER AND A. G. PHILLIPS. *Differential effects of physostigmine on cues produced by electrical stimulation of the ventral tegmental area using two discrimination procedures*. PHARMACOL BIOCHEM BEHAV **28**(2) 261–265, 1987.—Two procedures were employed to assess the effects of physostigmine on the discrimination of cues produced by either high or low intensity electrical brain stimulation (EBS) of the ventral tegmental area in rats. When the procedure involved frequent presentation of brief trials, physostigmine enhanced the perceived intensities of the cues, causing the rats to respond to low intensities as though they had higher values. In contrast, physostigmine had no effects on the discrimination when the trials were less frequent and extended in duration. These results confirm the existence of multiple substrates for cues produced by stimulation of the ventral tegmental area in rats and implicate cholinergic neurons as substrates for the non-dopaminergic cues indentified in the companion paper.

Cue properties Electrical brain stimulation Ventral tegmental area Acetylcholine ICSS
Physostigmine Discrimination Rats

PREVIOUS pharmacological studies of the cue properties of electrical brain stimulation (EBS) have generated conflicting results with respect to drug effects on the discrimination of lateral hypothalamic (LH) EBS cues by rats [1, 2, 7, 14, 16]. This inconsistency may reflect the mediation of LH EBS cues by mutliple substrates, with the relative contribution of each being dependent on the training conditions employed. There is now strong evidence for the involvement of multiple substrates for cues produced by stimulation of the ventral tegmental area (VTA). Specifically, evidence for dopaminergic substrates of VTA EBS cues is found when the discrimination procedure involves trials that are presented infrequently and extended in duration [5]. In contrast, non-dopaminergic substrates are implicated when the trials are brief and presented frequently.

The present study was designed primarily to identify possible neurotransmitter substrates for VTA EBS cues when the discrimination procedure involves the frequent presentation of brief trials. Recent evidence suggests that activation of cholinergic receptors within the VTA may be rewarding [17]. Conceivably, cholinergic mechanisms might also contribute to the discriminative stimulus properties of VTA stimulation. Accordingly, the present study assessed the role of cholinergic neurons in mediating EBS cues by determining the effects of the acetylcholinesterase inhibitor physostigmine on the generalization of EBS intensities by rats trained with frequent, brief trials. The effects of physostigmine on VTA EBS cues when the trials were less frequent and extended in duration were also assessed. Evidence for selective cholinergic modulation of the cues associated with frequent presentation of brief trials would complement our previous results [5] by providing a double dissociation of substrates for the cue properities of VTA EBS.

## METHOD

### *Effects of Physostigmine on the Discrimination of EBS Cues Associated With Frequent Presentations of Brief Trials*

Two groups of male hooded rats were employed. The first group contained eight rats that had been used in previous

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experiments [5], whereas the second group consisted of 11 experimentally naive rats. Aside from different experimental histories, both groups were treated comparably throughout the training phase of the study. Details regarding the housing conditions, surgery, histology, apparatus and pre-discrimination training procedures are contained in the companion paper [5].

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. Both the high and the low current intensities supported intracranial self-stimulation (ICSS) on a continuous reinforcement schedule in previous experiments [5]. 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 again and the first lever-press made within a 10 sec period was recorded. During the first five training sessions, a single response on the lever appropriate for the cue on that trial always led to the delivery of one 45 mg Noyes food pellet and termination of the trial (the houselight was turned off and the pellet dispenser made inoperable). Incorrect responses initiated a further 10 sec period during which the rats could respond appropriately and receive the food reward. 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, initiating the inter-trial interval (ITI). During the ITI, responses were recorded but had no programmed consequences. After the fifth training session, incorrect responses always resulted in no reward and termination of the trial. When the rats were responding with a high rate of accuracy, only 75% of correct responses were reinforced. In addition, trials were conducted in the absence of the houselight to ensure that the rats were discriminating the lower intensities, rather than being cued by the signal light in the absence of an EBS stimulus.

Following training, rats that acquired the task were tested for stimulus generalization to current values between the two training intensities. These tests consisted of 100 trials, with four intermediate current levels (2 $\mu$A apart) delivered randomly on 20 of the trials (five trials at each current). Responses that followed intermediate intensities were not reinforced and resulted in termination of the trial. To maintain an overall rate of reinforcement similar to that obtained on training days, responses to the two training currents were rewarded on 85% of the trials during generalization tests. Three baseline sessions were run before the drug tests and alternated with regular training days.

Following the baseline phase, generalization tests were given after injections of either physostigmine or saline. The first group of rats received two doses of physostigmine (0.25 and 0.50 mg/kg). The second group received three doses (0.20, 0.35 and 0.50 mg/kg), thus providing a replication and extension of the results for Group 1. The order of dose administration was counterbalanced across animals. Scopolamine methylbromide (0.50 mg/kg) was injected in conjuction with physostigmine to reduce the peripheral effects of physostigmine. The rats were given a separate generalization test after administration of scopolamine methylbromide (0.50 mg/kg) alone, to control for the possible effects of this drug. Physostigmine was dissolved in saline to a concentration of 0.20, 0.25, 0.35 or 0.50 mg/ml and injected IP in a volume of 1 ml/kg, 20 min before testing. Scopolamine methylbromide was dissolved in saline to a concentration of 0.50 mg/ml and injected IP in a volume of 1 ml/kg 30 min before testing. All drug tests were given 3 days apart with regular training sessions occurring on the intervening days.

### *Effects of Physostigmine on EBS Cues Associated With Trials That are Less Frequent and Extended in Duration*

The six rats employed here were previously trained to discriminate between high and low intensity cues produced by intermittent (20 sec inter-train interval) EBS delivered over a prolonged (2 min) period [5]. In the present study, the EBS cue parameters were altered slightly and the inter-trial intervals shortened to accommodate more trials within each session. Accordingly, the training sessions consisted of 20 trials of 1.5 min duration given 60 to 120 sec apart (VI 90 sec). The beginning of each trial was signalled by a 0.05 sec flash of the houselight, followed 1 sec later by delivery of the first of 6 presentations of either high or low 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 10 sec intervals over a 1 min period. Ten seconds after the sixth presentation the houselight was turned on for 30 sec, during which time the rat could press the appropriate lever to obtain one food pellet after every sixth correct response (an FR-6 schedule).

The rats received five regular training sessions with the new stimulus parameters and were then given two generalization tests. The generalization tests were conducted two days apart with one regular training session interposed between them. The generalization tests involved the random delivery of four equally spaced intermediate intensities (2 $\mu$A apart) along with the usual training currents. Each intermediate intensity was delivered three times and each training current was presented four times within a single generalization session. 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 their discriminative performance. Therefore, 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 that particular trial. Thus, if the FR-6 requirement was initially completed on the left lever, then subsequent reinforcement would only occur after every sixth response on that lever within the 30 sec trial.

Following baseline testing all rats were given generalization tests after receiving two doses of physostigmine (0.25 and 0.50 mg/kg) or saline. The order of dose administration was counterbalanced across animals, with at least three regular training sessions interposed between each test.

### *Statistical Analyses*

Two methods of analysis were employed to compare the data obtained from the generalization tests. First, the percentage of responses emitted on the lever appropriate for high-intensity stimulation (HS) was determined for each current intensity delivered during a particular test session. The percentages from separate tests were then compared using a two-way analysis of variance (ANOVA) with test session and current intensity as factors. Secondly, the point of sub-

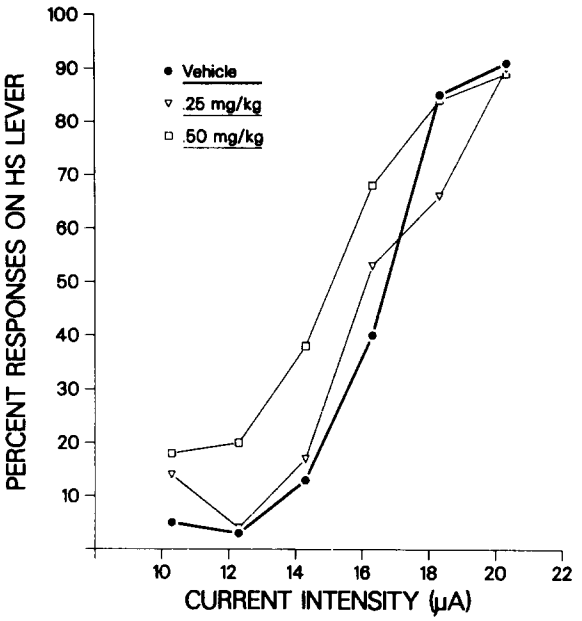


FIG. 1. Generalization functions obtained for Group 1 with the procedure involving frequent, brief trials after injections of vehicle, 0.25 and 0.50 mg/kg of physostigmine. The data are expressed in terms of the percentage of the responses emitted on the HS lever after the delivery of each current intensity. The data points are placed along the abscissa such that they correspond to the average intensity delivered at each current level.

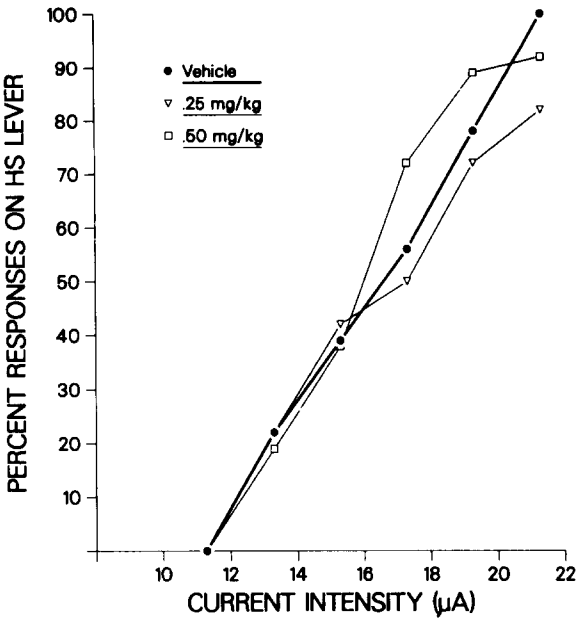


FIG. 2. Generalization functions obtained with the procedure involving less frequent trials that are extended in duration after injections of vehicle, 0.25 and 0.5 mg/kg of physostigmine.

jective equality (PSE) was determined for each rat under the separate test conditions and these PSEs were analysed either by a one-way ANOVA or a *t*-test where appropriate. The PSE was defined as the current intensity at which responding would occur on the HS lever on 50% of the trials. This intensity was interpolated from the regression line plotted between the data points associated with the four intermediate intensities in each test. Differences revealed by these analyses were considered significant where $p<0.05$. Newman-Keul's test was used to perform post-hoc comparisons among individual means when the ANOVA indicated significant main effects.

## RESULTS

### *Effects of Physostigmine on the Discrimination of EBS Cues Associated With Frequent Presentations of Brief Trials*

Ten of the eleven experimentally naive rats learned the discrimination task to an accuracy of over 80% correct choices per session. The acquisition rate (2 to 4 weeks) was comparable to the other eight rats employed in this phase of the study. The electrode placements for Group 1 are shown in the companion paper [5]. Group 2 had similar placements.

An ANOVA performed on the baseline generalization functions of all rats indicated that responding on the HS lever increased as a function of increasing current intensity, $F(5,85)=134.67$, $p<0.01$. There were no significant differences in responses on the HS lever, PSEs (range of means=16.1 to 16.9 $\mu$A), or slopes (range of means=10.7 to 11.7) across the three baseline sessions. Thus, the generalization functions remained stable with repeated testing.

The generalization functions obtained following injections of physostigmine or saline for Group 1 are shown in Fig. 1. The functions for Group 2 provide a close replication of the initial findings and are not presented. ANOVAs revealed significant effects of the treatments on HS responding between sessions for both Group 1, $F(2,14)=7.34$, $p<0.01$, and Group 2, $F(3,27)=4.00$, $p<0.02$. Post-hoc analyses indicated that rats in the first group made more responses on the HS lever after injections of 0.50 mg/kg physostigmine than after vehicle or 0.25 mg/kg physostigmine. In the second group, significantly more HS responses were made after injections of both 0.35 and 0.50 mg/kg physostigmine than after saline. Importantly, there were no increases in the percent of HS responses made during the ITI in either group. Thus, the shifts in the generalization functions cannot be attributed to a general change in the rats' response biases following drug treatment.

Analyses of the PSEs obtained after injections of physostigmine revealed significant drug effects for Group 2, $F(3,23)=4.76$, $p<0.01$, but not for Group 1 (range of means for Group 1=15.3 to 16.5 $\mu$A). Post-hoc analyses of the PSEs for Group 2 indicate that only the highest dose of physostigmine (0.50 mg/kg) produced a significant decrease in the PSE relative to saline injections (mean=14.1 vs. mean =16.7 $\mu$A). In neither group were there significant changes in the slopes of the regression lines.

Administration of scopolamine methylbromide did not significantly alter either HS responding or the PSEs relative to those observed during saline tests in either group of rats (Group 1 mean PSEs=16.5 $\mu$A vs. 16.7 $\mu$A; Group 2 mean PSEs=16.0 $\mu$A vs. 15.5 $\mu$A). This peripherally acting anticholinergic significantly flattened the slopes of the regression lines for Group 2, two-tailed, $t(9)=2.81$, $p<0.05$, but had no significant effects on the slopes for Group 1 (10.7 vs. 13.7).

*Effects of Physostigmine on EBS Cues Associated With Trials That are Less Frequent and Extended in Duration*

The electrode placements for the six rats used in this phase of the study are shown in the companion paper [5]. All rats acquired the discrimination of the altered cueing parameters within one to five days. This rapid adaptation suggests that the alteration of stimulation parameters did not produce a substantial change in the perceived intensities or nature of the EBS cues. Comparisons of the HS responses, the PSEs (mean=18.4 $\mu$A vs. mean=18.7 $\mu$A) or the slopes (9.1 vs. 8.3) measured during the separate tests did not reveal any significant differences between sessions. Thus, the generalization functions remained stable with repeated tests.

Analyses of the HS responses of the rats during the drug phase did not reveal any significant differences between tests conducted after physostigmine or saline injections (Fig. 2). Comparisons of the PSE measures also did not reveal any significant changes (range of means=16.6 to 18.2 $\mu$A), nor were there any differences in the slopes of the regression lines (range of means=7.9 to 9.8).

## DISCUSSION

The present study demonstrated that injections of physostigmine in combination with methylscopolamine altered discriminated responses to VTA EBS cues when the training procedure involved the frequent delivery of brief trials. Relative to saline tests, the EBS cues elicited significantly more HS responses after the high dose (0.50 mg/kg) of physostigmine in the first group of rats, and after the two higher doses (0.35 and 0.50 mg/kg) in the second group. Such effects were not observed during tests with methylscopolamine alone. This selective increase of HS responding following physostigmine injections may reflect an enhancement of the perceived intensities of the EBS cues measured with the procedure involving brief and frequent trials. Alternatively, the drug may have inhibited responding and accentuated an underlying response bias. Previous studies have shown that the doses of physostigmine that produced effects here can inhibit LH ICSS [3, 9–11]. In the present study these doses produced a decrease in the number of trials responded on, and also suppressed ITI responding. However, examination of responses emitted during the ITI did not indicate an underlying bias toward the HS lever. Furthermore, physostigmine did not alter the percent of HS responses emitted during the ITI and had no effects on HS responding in the procedure involving less frequent trials of extended duration. The absence of changes in HS responding under these latter conditions, refutes any interpretation of the present results as a general change in response biases or discrimination performance.

The ability of physostigmine to enhance the perceived intensities of cues when the discrimination trials are brief and frequent suggests that the substrates for these cues may involve cholinergic neurons. As physostigmine enhances cholinergic activity at both nicotinic and muscarinic receptor sites, the present results do not indicate which receptor type is responsible for mediating the enhanced perception of the EBS. However, it has recently been reported that nicotine does not alter the discrimination of LH EBS cues when the training procedure involves frequent presentation of brief trials [15]. If the VTA and LH EBS cues measured with this type of procedure involve a common substrate, then muscarinic mediation of the physostigmine effects may be inferred.

The lack of an effect of the same doses of physostigmine on cues produced by prolonged, intermittent stimulation indicates that this type of cue may not be mediated by a cholinergic mechanism. In the companion paper [5] it was shown that these latter cues may involve dopaminergic substrates which have no apparant role in mediating the cues associated with brief presentations of VTA EBS. Taken together, these results suggest that the cue properties of VTA EBS may be mediated by at least two neurochemically distinct substrates, with the relative contribution of each being dependent on the training conditions employed.

In all rats employed in this study, the EBS intensities used as discriminative stimuli were capable of supporting ICSS. Accordingly, it is possible that the cues produced by VTA EBS may be related to the rewarding effects of the brain stimulation. Previous studies have indicated that ICSS of the VTA is largely mediated by mesolimbic DA neurons projecting to the nucleus accumbens [8, 12, 13]. As DA neurons also mediate VTA EBS cues produced when trials are less frequent and of extended duration [4], it is possible that such cues may arise from the activation of DA reward pathways. In contrast, there is little evidence to suggest a relationship between VTA reward processes and the cues associated with frequent, brief trials. At present, there exists a paucity of data concerning the role of cholinergic neurons in VTA ICSS. Injections of muscarinic antagonists into the VTA can increase the thresholds for ICSS of the LH [17]. On the other hand, intraperitoneal injections of scopolamine decrease thresholds and increase the response rate for VTA ICSS [4]. Moreover, ICSS rates in rats with LH and VTA electrodes are reduced following intraperitoneal injections of physostigmine within the range of doses employed in the present study [3, 4, 9–11]. Until the role of cholinergic neurons in mediating the cue properties and rewarding effects of EBS is better understood, the relationship between these processes will remain unclear.

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## REFERENCES

1. Colpaert, F. C. Sensitization and desensitization to lateral hypothalamic stimulation. *Arch Int Pharmacodyn* **230:** 319–320, 1977.
2. Colpaert, F. C., C. J. E. Niemegeers and P. A. J. Janssen. Haloperidol blocks the discriminative stimulus properties of lateral hypothalamic stimulation. *Eur J Pharmacol* **42:** 93–97, 1977.
3. Domino, E. F. and M. E. Olds. Cholinergic inhibition of self-stimulation behavior. *J Pharmacol Exp Ther* **164:** 202–211, 1968.
4. Druhan, J. P., H. C. Fibiger and A. G. Phillips. Comparison of the effects of cholinergic agonists on the cue properties and rewarding effects of brain-stimulation in the ventral tegmental area. *Soc Neurosci Abstr* **12:** 931, 1986.
5. 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:** 251–259, 1987.

6. König, J. R. and R. A. Klippel. *The Rat Brain: A Stereotaxic Atlas*. Baltimore: Williams & Wilkins, 1963.
7. Kornetsky, C. and R. U. Esposito. Reward and detection thresholds for brain stimulation: dissociative effects of cocaine. *Brain Res* **209:** 496–500, 1981.
8. Mogenson, G. J., M. Takigawa, A. Robertson and M. Wu. Self-stimulation of the nucleus accumbens and ventral tegmental area of Tsai attenuated by microinjections of spiroperidol into the nucleus accumbens. *Brain Res* **171:** 247–259, 1979.
9. Newman, L. M. Effects of cholinergic agonists and antagonists on self-stimulation behavior in the rat. *J Comp Physiol Psychol* **79:** 394–413, 1972.
10. Olds, M. E. and E. F. Domino. Comparison of muscarinic and nicotinic cholinergic agonists on self-stimulation behavior. *J Pharmacol Exp Ther* **166:** 189–204, 1969.
11. Olds, M. E. and E. F. Domino. Differential effects of cholinergic agonists on self-stimulation and escape behavior. *J Pharmacol Exp Ther* **170:** 157–167, 1969.
12. Phillips, A. G. and H. C. Fibiger. The role of dopamine in maintaining intracranial self-stimulation in the ventral tegmentum, nucleus accumbens, and medial prefrontal cortex. *Can J Psychol* **32:** 58–66, 1978.
13. Phillips, A. G., A. Jakubovic and H. C. Fibiger. Increased *in vivo* tyrosine hydroxylase activity in rat telencephalon produced by self-stimulation of the ventral tegmental area. *Brain Res* **402:** 109–116, 1987.
14. Schaefer, G. J. and R. P. Michael. The discriminative stimulus properties and detection thresholds of intracranial self-stimulation: Effects of d-amphetamine, morphine, and haloperidol. *Psychopharmacology (Berlin)* **85:** 289–294, 1985.
15. Schaefer, G. J. and R. P. Michael. Task-specific effects of nicotine in rats: intracranial self-stimulation and locomotor activity. *Neuropharmacology* **25:** 125–131, 1986.
16. Stutz, R. M. and A. N. Maroli. Central mechanisms of the narcotic cue. In: *Stimulus Properties of Drugs: Ten Years of Progress*, edited by F. C. Colpaert and J. A. Rosencrans. Amsterdam: Elsevier/North-Holland Press, 1978, pp. 517–534.
17. Yeomans, J. S., O. Kofman and V. McFarlane. Cholinergic involvement in lateral hypothalamic rewarding brain stimulation. *Brain Res* **329:** 19–26, 1985.