Pharmacology Biochemistry & Behavior, Vol. 6, pp. 697–700.

Amphetamine Effects on Brain Slow Potentials Associated with Discrimination in the Rat[1]

JAMES H. PIRCH

Department of Pharmacology and Therapeutics, Texas Tech University School of Medicine, Lubbock, TX 79409

(Received 1 November 1976)

PIRCH, J. H. *Amphetamine effects on brain slow potentials associated with discrimination in the rat.* PHARMAC. BIOCHEM. BEHAV. 6(6) 697–700, 1977. – Slow potentials were recorded from the anterior cortex of rats during discrimination conditioning and the effects of various doses of d-amphetamine on these responses were examined. In the discrimination paradigm one tone (S^d) was followed at three sec after the onset by food reinforcement while another tone (S^Δ) indicated that no reinforcement would follow. Slow potential (SP) responses were measured during the three-sec period following onset of the stimulus. For the first several training sessions the SP responses demonstrated a phase of generalization during which responses were the same to both stimuli. Thereafter, the responses to S^d were significantly greater than responses to S^Δ. d-Amphetamine produced a dose-related depression of SP responses to the reinforced stimulus in doses of 0.25 to 2.0 mg/kg. The effect of amphetamine on SP responses to S^Δ was biphasic; the lower doses (0.25 and 0.5 mg/kg) enhanced responses, no change was seen after 1.0 mg/kg and the high dose (2.0 mg/kg) depressed responses. This study demonstrates that the rat develops differential slow potential responses to reinforced and nonreinforced stimuli in a discrimination paradigm and that d-amphetamine produces a differential and dose-related alteration of these SP responses. It is suggested that the actions of amphetamine may be produced through interference with mechanisms of discrimination, by an effect on subcortical activating systems involving norepinephrine, and/or by activation of inhibitory dopamine receptors on cortical neurons.

Brain slow potentials Discrimination conditioning Amphetamine Anterior cortex

THE EXISTENCE of brain slow potential (SP) changes associated with conditioning in animals has been amply demonstrated in behavioral paradigms ranging from classical conditioning to operant tasks [1, 4–7, 11, 13–17]. Very few studies have specifically examined SP responses accompanying procedures involving discrimination conditioning. Low and coworkers [11], in experiments with rhesus monkeys, found a clear differentiation of SP responses to two different auditory stimuli used in an operant discrimination task with either aversive (shock) or appetitive (dextrose pellets) reinforcement. One stimulus indicated that a lever press would provide reinforcement while the other stimulus was of no significance. Slow potential responses to the meaningful stimulus were significantly greater in magnitude than responses to the other stimulus. The nature of the brain mechanisms involved in generation of slow potential responses is uncertain, but processes such as arousal and attention are thought to play a role [19]. The fact that differential SP responses are observed in discrimination conditioning in the monkey suggests that some processing of the information provided by the stimulus also contributes to development of the SP response. It would be useful to know if similar differentiation of SP responses occurs in a lower mammal such as the rat.

Brain slow potential responses in the rat are altered by psychoactive drugs such as amphetamine and chlorpromazine [14]. The use of drugs in the study of behavior-related slow potentials may provide additional information regarding the brain processes involved in their generation as well as their possible role in behavior mechanisms. The pharmacology of amphetamine has been extensively studied and much is known concerning the actions of this agent, particularly with respect to its effects on neurotransmitter mechanisms. Amphetamine enhances the activity of noradrenergic and dopaminergic neuronal systems primarily indirectly by releasing norepinephrine and dopamine from nerve terminals. Norepinephrine and dopamine are thought to be involved in regulation of

[1] Supported in part by DHEW MH 23653, MH 29653 and by the Multidisciplinary Research Program in Mental Health at the University of Texas Medical Branch. The major portion of this work was performed in the Department of Pharmacology and Toxicology at The University of Texas Medical Branch, Galveston.

arousal level [9,10] and dopamine has been shown to inhibit neurons in the anterior cortex of the rat [8,20]. The present study was conducted to determine whether differential SP responses develop with discrimination conditioning in the rat and to investigate the effects of amphetamine on these responses.

METHOD

Female albino rats (Sprague-Dawley descent) were obtained from Holtzman Rat Co. and were housed in groups of three or four during the initial training period. After implantation of electrodes, the animals were maintained in individual cages. Silver-silver chloride electrodes were implanted under pentobarbital (35 mg/kg, IP) and ether anesthesia after atropine pretreatment according to procedures described previously [13]. Electrodes were in contact with cortex or bone via agar-saline pools. The active electrode was placed over the cortex 2–3 mm anterior to the bregma and to the right of midline. The bone reference was approximately 2 mm anterior to the parietal-interparietal suture on the left side. Animals were allowed one week for recovery before further experimentation was performed.

For conditioning, the animals were placed in a clear Plexiglas chamber 10 in. × 10 in. × 7.5 in. high with a grid floor. A Gerbrands pellet dispenser delivered 45 mg food pellets (P. J. Noyes Co.) to the food cup located within the chamber. The conditioned stimuli and delivery of reinforcement were programmed with relays and timers purchased from Grason-Stadler. Before electrode implantation, the animals were trained to associate delivery of reinforcement with the sound of the delivery device. Two different tones were employed in the discrimination paradigm; one tone (S^d) was followed after three sec by the delivery of a food pellet while the other tone (S^Δ) was not reinforced. The two stimuli were 400 Hz and 4500 Hz tones of 0.5-sec duration. For some animals the 400 Hz tone served as a S^d while the 4500 Hz tone was reinforced in others. S^d and S^Δ were given on alternate trials. Intertrial intervals varied between 45 and 90 sec. Thirty trials were given in a single session (15 trials with each stimulus) and only one session was conducted per day. The animals had free access to food during one hr at the end of each day.

The EEG was recorded with a Grass Model 7 polygraph using 7P1 d.c. preamplifiers. For measurement of SP responses, the fast EEG activity was filtered using the 1/2 amplitude high frequency setting on the driver amplifier. Voltage changes during the three-sec period following the onset of the conditioned stimulus were measured at each 0.25 sec interval using a Tektronix 31/53 Data Acquisition System. A print-out record of the measured voltage values and calculated response areas for each trial was obtained. At the end of each session an average SP response was calculated along with the mean area of response. Examination of the data indicated that the area measurement was the single most useful value for comparison of responses. Although analysis of the maximum amplitudes of the SP responses gave similar results, the areas were less variable and were selected as more representative of the overall SP response. The *t*-test for paired comparison was used to examine differences between SP responses, with each animal serving as its own control.

d-Amphetamine sulfate was donated by Smith, Kline and French Laboratories. The drug was dissolved in 0.9% saline in the appropriate concentration for an injection volume of 0.1 ml/100 g body weight. Doses were calculated on the basis of the salt. Animals were weighed immediately before each injection and all injections were administered intraperitoneally thirty min before trials began. Saline was administered before each control session during the drug testing phase.

RESULTS

Sample EEG recordings taken from one rat during trials initiated by the reinforced stimulus (S^d) are shown in Fig. 1. The negative slow potential response began immediately after the stimulus and returned to baseline following delivery of the food reinforcement. In some cases a positive shift developed after reinforcement, similar to the postreinforcement positive shift observed in cats by Marczynski *et al.* [12].

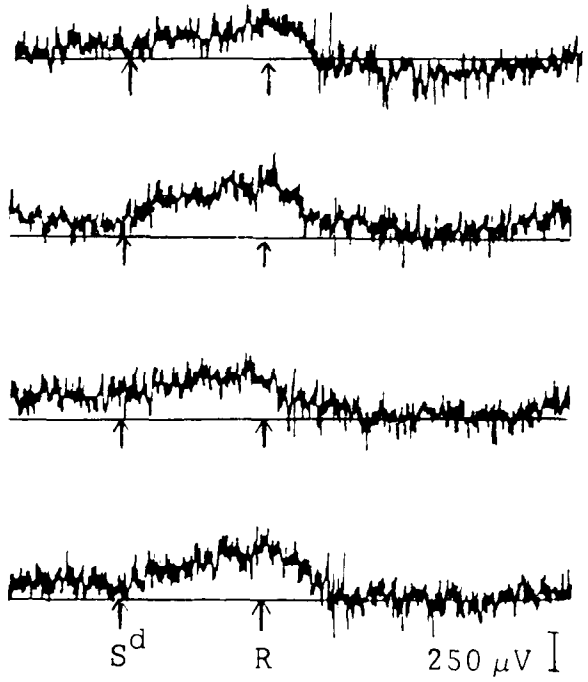

FIG. 1. EEG recordings during four trials initiated by the reinforced stimulus (S^d). First arrow in each recording indicates onset of 0.5-sec tone and second arrow indicates delivery of food reinforcement. Time between arrows is three sec. Negative is up.

The learning curve for five animals which were subjected to 25 training sessions is demonstrated in Fig. 2. Initially, four habituation sessions were conducted during which both tones were applied but no reinforcement was given. Slow potential responses during the last habituation session are plotted on the graph. The first ten training sessions show a phase of stimulus generalization during which SP responses were the same to both stimuli. By the twelfth session the responses to S^d were significantly greater than responses to S^Δ (paired comparison, $p<0.05$, $t>2.776$, 4 df). This difference increased and persisted throughout the remaining sessions.

Amphetamine was administered in doses of 0.25, 0.5, 1.0 and 2.0 mg/kg in a random order to each of eight animals subjected to 16 or more training sessions. At least

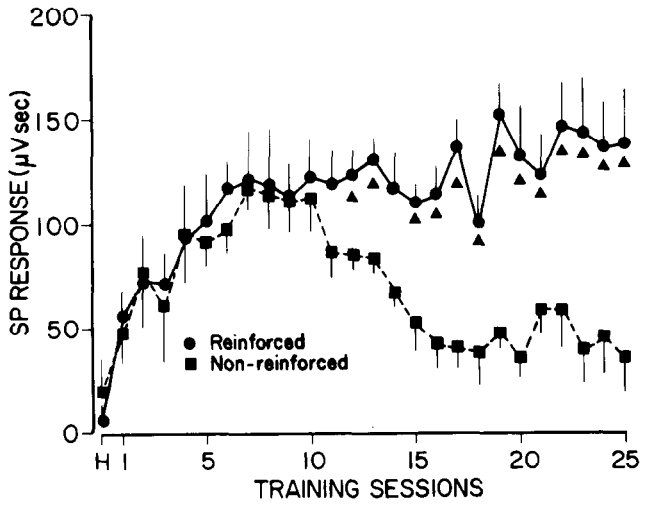

FIG. 2. Learning curve for animals trained on discrimination paradigm. Each point represents the mean of the SP response areas (μV sec) obtained in five animals. Vertical lines are standard errors. The first point of each curve shows the response during the fourth habituation session. Closed triangles indicate $p<0.05$ (paired comparison, $t>2.776$, 4 df).

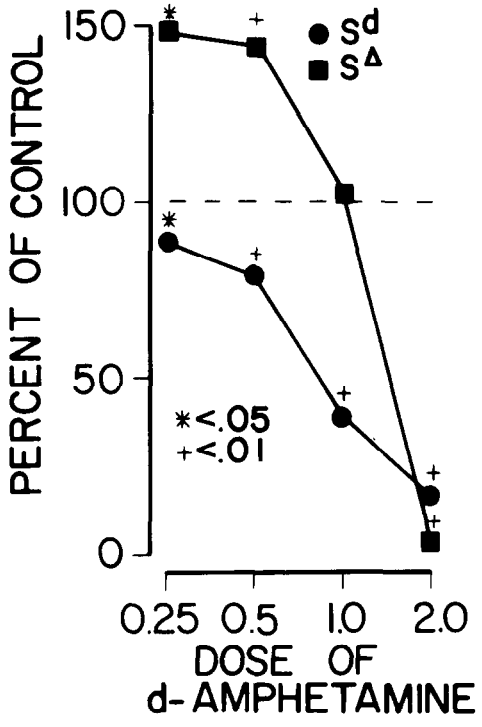

FIG. 3. The effect of amphetamine on SP responses associated with stimulus discrimination. Each point represents the mean of values obtained in eight animals. Probability is based on t-test for paired comparison of SP responses before and after drug treatment (see text for detailed description).

two saline control sessions were conducted between each drug session. The dose-response curves showing the effect of amphetamine on SP responses to S^d and S^Δ are illustrated in Fig. 3. For each animal the SP response (area) measured under drug was converted to a percent of the response observed during the saline control session immediately preceding the drug session. The values plotted represent the mean of calculated values obtained in eight animals. The p values were determined on the basis of t-tests performed directly on the SP response data (paired comparison; for $p<0.05$, $t>2.365$ with 7 df; for $p<0.01$, $t>3.499$ with 7 df). Each of the doses tested produced a depression of responses to the reinforced stimulus; the effect was dose-related. The action of amphetamine on responses to the nonreinforced stimulus was biphasic. The lower doses (0.25 and 0.5 mg/kg) enhanced the SP responses, no change was seen after the intermediate dose (1.0 mg/kg), and the high dose (2 mg/kg) depressed the responses. This differential action of amphetamine on the responses associated with S^d and S^Δ is more clearly seen in Fig. 4 where responses of individuual animals before and

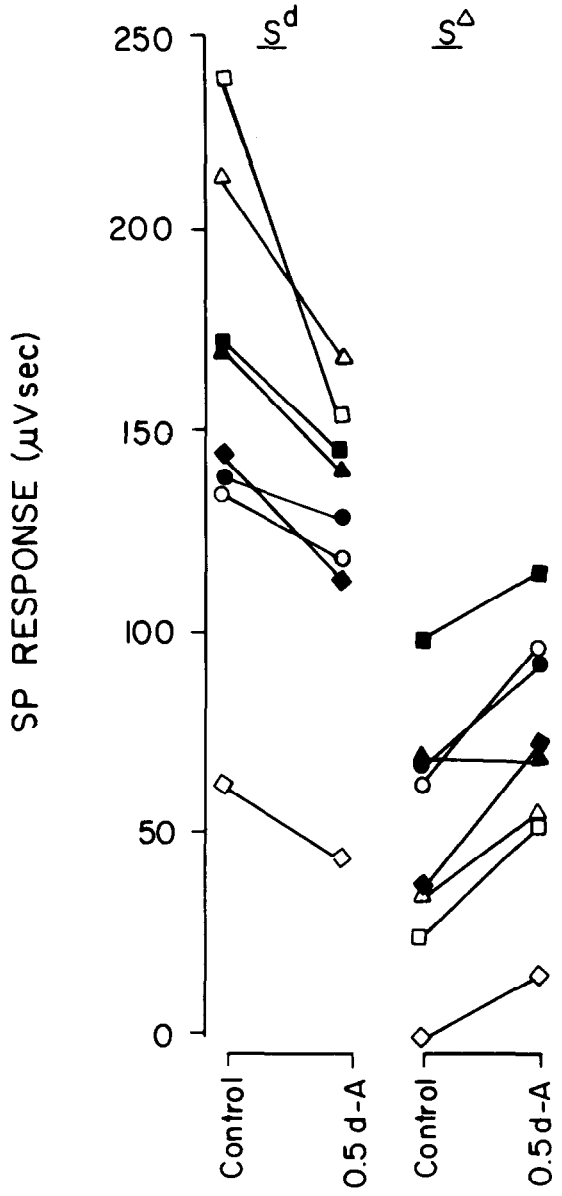

FIG. 4. Slow potential responses of individual animals to reinforced and nonreinforced stimuli before and after 0.5 mg/kg amphetamine.

after drug are plotted. It is apparent that 0.5 mg/kg amphetamine depressed the responses to S^d at the same time that responses to S^Δ were enhanced.

DISCUSSION

These experiments demonstrate that the rat develops differential slow potential responses to reinforced and nonreinforced stimuli in a discrimination paradigm. Thus, the capability of cortical SP responses to indicate that processing of stimulus information has occurred is not an exclusive property of the primate brain. Furthermore, these brain potentials can serve as a useful tool for studying the neurophysiological correlates of discrimination behavior and for investigating the effects of drugs on brain mechanisms involved in stimulus discrimination.

The actions of amphetamine on SP responses associated with this paradigm are complex. The effect on responses to the reinforced stimulus (S^d) was expected on the basis of a previous study which demonstrated that amphetamine produce a dose-related depression of SP responses to a stimulus which was always followed by reinforcement [14]. However, the effect of the lower doses (0.25 and 0.5 mg/kg) on responses to the non-reinforced stimulus (S^Δ) was not predicted. The enhancement of SP responses to S^Δ might be ascribed to an interference with mechanisms of discrimination by amphetamine or to an effect on subcortical activating systems. A failure to accurately discriminate between stimuli could lead to enhanced responses to S^Δ and decreased responses to S^d. On the other hand, if the SP response to S^Δ is normally small because of inhibitory mechanisms, facilitation of subcortical activating systems could counteract the inhibition, thereby producing increased responses to the nonreinforced stimulus.

Amphetamine is known to influence neuronal systems in which norepinephrine and dopamine are thought to function as transmitters. For example, amphetamine can mimic the effects of dopamine on neurons in the caudate nucleus or cortex [2,18], or the effects of norepinephrine on neurons in the locus coeruleus [3]. Dopaminergic terminals have been demonstrated in the anterior cortex of the rat [8,20] and iontophoretic application of dopamine to cortical neurons causes inhibition of firing [18]. The amphetamine-induced decrease in the magnitude of SP responses to S^d may result from activation of dopamine receptors on cortical neurons. Previous studies from this laboratory have shown that chlorpromazine and haloperidol (dopamine receptor antagonists) counteract amphetamine-induced depression of cortical SP responses [14, unpublished observations]. The effect of the high dose (2 mg/kg) on responses to S^Δ may also be related to activation of inhibitory dopamine receptors. It is more difficult to propose a mechanism at the neuronal level to explain the enhancement of responses to S^Δ by the lower doses of amphetamine. Norepinephrine is thought to function as a transmitter in the reticular activating system and infusion of norepinephrine into the midbrain reticular formation enhances the level of arousal of rats [10]. An effect of amphetamine on norepinephrine receptors in this area could lead to enhanced responsiveness to a previously irrelevant stimulus. These suggested mechanisms are clearly speculative and will require further investigation for confirmation.

REFERENCES

1. Borda, R. P. The effect of altered drive states on the contingent negative variation (CNV) in rhesus monkeys. *Electroenceph. clin. Neurophysiol.* **29:** 173–180, 1970.
2. Bunney, B. S. and G. K. Aghajanian. d-Amphetamine-induced inhibition of central dopaminergic neurons: Mediation by a stiato-nigral feedback pathway. *Science* **192:** 391–393, 1976.
3. Bunney, B. S., J. R. Walters, M. J. Kuhar, R. H. Roth and G. K. Aghajanian. d and l-Amphetamine stereoisomers: Comparative potencies in affecting the firing of central dopaminergic and noradrenergic neurons. *Psychopharmac. Comm.* **1:** 177–190, 1975.
4. Chiorini, J. R. Slow potential changes from cat cortex and classical aversive conditioning. *Electroenceph. clin. Neurophysiol.* **26:** 399–406, 1969.
5. Donchin, E., D. Otto, L. K. Gerbrandt and K. H. Pribram. While a monkey waits: Electrical events recorded during the foreperiod of a reaction time study. *Electroenceph clin. Neurophysiol.* **31:** 115–127, 1971.
6. Durkovic, R. G. and D. H. Cohen. Spontaneous, evoked and defensively conditioned steady potential changes in the pigeon telencephalon. *Electroenceph. clin. Neurophysiol.* **24:** 474–481, 1968.
7. Hablitz, J. J. Operant conditioning and slow potential changes from monkey cortex. *Electroenceph. clin. Neurophysiol.* **34:** 399–408, 1973.
8. Hokfelt, P., A. Ljungedahl, K. Fuxe and O. Johansson. Dopamine nerve terminals in the rat cortex: Aspects of the dopamine hypothesis of schizophrenia. *Science* **184:** 177–179, 1974.
9. Keane, P. E., J. M. Candy and P. B. Bradley. The role of endogenous catecholamines in the regulation of electrocortical activity in the encephale isole cat. *Electroenceph. clin. Neurophysiol.* **41:** 561–570, 1976.
10. Kornetsky, C. and R. Markowitz. Animal models and schizophrenia. In: *Model Systems in Biological Psychiatry*, edited by D. J. Ingle and H. M. Shein. Cambridge: MIT Press, 1975, pp. 26–50.
11. Low, M. D., R. P. Borda and P. Kellaway. "Contingent negative variation" in rhesus monkeys: An EEG sign of a specific mental process. *Percept. Mot. Skills* **22:** 443–446, 1966.
12. Marczynski, T. J., J. L. York and J. T. Hackett. Steady potential correlates of positive reinforcement: Reward contingent positive variation. *Science* **163:** 301–304, 1969.
13. Pirch, J. H. and P. R. Barnes. Steady potential responses from the rat cortex during conditioning. *Experientia* **28:** 164–165, 1972.
14. Pirch, J. H. and K. C. Osterholm. Drug-induced alterations of slow potential responses in the rat. *Pharmacologist* **17:** 189, 1975.
15. Rebert, C. S. and D. A. Irwin. Slow potential changes in cat brain during classical appetitive and aversive conditioning of jaw movement. *Electroenceph. clin. Neurophysiol.* **27:** 152–161, 1969.
16. Rowland, V. Cortical steady potential (direct current potential) in reinforcement and learning. *Prog. Physiol. Psychol.* **2:** 1–77, 1968.
17. Skinner, J. E. Abolition of a conditioned, surface-negative, cortical potential during cyrogenic blockade of the non-specific thalamocortical system. *Electroenceph. clin. Neurophysiol.* **31:** 197–209, 1971.
18. Stone, T. W. Responses of neurones in the cerebral cortex and caudate nucleus to amantadine, amphetamine and dopamine. *Br. J. Pharmac.* **56:** 101–110, 1976.
19. Tecce, J. J. Contingent negative variation (CNV) and psychological processes in man. *Psychol. Bull.* **77:** 73–108, 1972.
20. Thierry, A. M., G. Blanc, A. Sobel, L. Stinus and J. Glowinski. Dopamine terminals in the rat cortex. *Science* **182:** 499–501, 1973.