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Pipradrol Enhances Reinforcing Properties of Stimuli Paired With Brain Stimulation

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ROBBINS, T. W. AND G. F. KOOB. *Pipradrol enhances reinforcing properties of stimuli paired with brain stimulation.* PHARMAC. BIOCHEM. BEHAV. 8(3) 219–222, 1978. – The hypothesis that a psychomotor stimulant drug (pipradrol) enhances the reinforcing effects of stimuli paired with reinforcing brain stimulation was tested using a conditioned reinforcement paradigm. Rats were trained to discriminate between two stimuli (S+ and S–) to obtain ICS in the lateral hypothalamus by pushing a panel in the presence of S+. In a subsequent preference test, ICS was no longer available, but responding on one of two novel levers now produced S+, whereas responding on the other lever produced S–. Four groups of four rats received 0, 5, 10 or 15 mg/kg pipradrol. Doses of 5 and 10 mg/kg significantly enhanced the preference for S+ over S–. These doses increased responding for S+, but had no effect on responding for S–. These results support the hypothesis tested, and suggest that pipradrol potentiates the effects of conditioned reinforcement.

Pipradrol Self-stimulation Conditioned reinforcement Psychomotor stimulants

SEVERAL experiments have shown that the motivational state associated with intracranial stimulation (ICS) may become conditioned to external stimuli [2, 3, 7, 9, 14, 20]. The experiments in general have used one of two types of test paradigm. In the first type, an external cue such as a light or a noise is used to obtain stimulus control over operant responding reinforced by ICS. Stimulus control of behavior reinforced by ICS has been demonstrated in the rat [9], dog [14], and monkey [1]. In the second type of paradigm, a classical conditioning procedure has been used in which an external cue is paired with ICS, both being delivered independently of the responses of the animal. A neutral stimulus paired with reward in this way may acquire reinforcing properties of its own, and such stimuli are often termed conditioned reinforcers (CRs). The conditioned reinforcing properties of a stimulus can be assessed by determining whether the stimulus, by itself, can act as a reinforcer for the learning of a new response. Using this paradigm, Stein [20] was the first to demonstrate conditioned reinforcement for stimuli paired with ICS. Although one investigator [13] has failed to demonstrate conditioning of this type, the original finding of Stein has subsequently been confirmed and extended [7].

There has been little work into pharmacological factors which might affect conditioning to ICS. Stein [21] has proposed that amphetamine-like drugs enhance the behavioral effects of reward or reinforcement. This hypothesis suggests that psychomotor stimulant drugs would enhance the behavioral effects of stimuli associated with reward, a prediction supported by evidence that the psychomotor stimulant pipradrol can apparently potentiate conditioned reinforcement [6,16]. The purpose of the present experiment is to demonstrate that this potentiation can be extended to include stimuli which are paired with ICS.

A paradigm is employed in which differential stimulus control over behavior reinforced by ICS is first obtained. Rats are trained to respond for ICS in the presence of one stimulus (S+) but not in the presence of another (S–). The acquisition of possible CR effects for S+ or S– is measured in a test of preference between these two stimuli, which requires the learning of one novel response to obtain S+ and another to obtain S–. This paradigm thus combines elements of the two tests described above. The use of a method that initially establishes stimulus control over behavior ensures that the animals are attending to S+ and S– during training. The use of a preference test requiring that the animal should subsequently learn a new response to obtain S+ or S– is a stringent criterion of conditioned reinforcement [12]. Accordingly, the hypothesis that pipradrol potentiates conditioned reinforcement predicts that the drug will enhance the choice made for S+ over S–, when administered during the preference test.

METHOD

Animals

The animals were 19 male albino rats of the CFY strain, purchased from Carworth's Animal Suppliers when the rats weighed 250 ± 5 g. The rats were maintained on food and water ad lib in individual cages.

Surgery

The rats weighed 300–350 g at the time of surgery. Each rat was anaesthetised with Equithesin (3.75 ml/kg) and implanted with one bipolar stainless steel electrode (0.254 mm diameter, Plastic Products Co, Roanoke, VA) aimed at the lateral hypothalamus. Stereotaxic coordinates were –0.5 mm from bregma ± 1.7 mm lateral, and 8.3 mm below the dura of the brain, based on Pellegrino and Cushman [15].

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A week following surgery, the rats were screened for ICSS in an apparatus different from that used in the main experiment. The current producing asymptotic peak rates of responding was determined for each rat by a rate/intensity procedure [8]. These values varied from 20–40 μA for a 300 msec 50 Hz sine wave pulse, and were used in subsequent training.

The test apparatus was a standard operant chamber (Campden Instruments), 25 × 21 × 19 cm, housed in a sound-attenuating chest. On one wall was a Perspex panel, hinged at the top. To either side of the panel was a retractable lever. The discriminative stimuli were white noise (100 KHz, 66 ± 1 dB), and illumination of a lamp (2.8 W, 24 V) situated above and behind the panel. These stimuli were counterbalanced across rats as S+ or S−.

Procedure

Pretest training. Neither lever was present during this phase. On Session 1 each rat was shaped to panel-push according to a CRF schedule of brain stimulation. During Session 2, S+ and S− were introduced. S+ always preceded S− by 5 sec. S+ was initially 30 sec long, but was gradually reduced to 5 sec during Session 2. S− was always 5 sec long. This sequence of stimuli was presented at variable intertrial intervals averaging 4 sec in Session 2, gradually increasing to 12 sec in Session 3. Panel-pushing during S+ was reinforced on a CRF schedule. Responding during the intertrial interval lengthened it by 1 sec in Session 2, gradually increasing to 10 sec in Session 3. Responding during the interval between S+ and S−, or in S− itself also lengthened the intertrial interval by up to 10 sec. Session 3 ended when a stringent criterion had been met for performance during a single 5 min period: (1) the rat should average at least 4 responses per S+; (2) the proportion of responses during S+ to those during S− should average at least 95%; and (3) the proportion of responses during S+ to the total responses should average at least 85%. The final stage of training required each rat to attain the criterion for 3 consecutive 5 min periods on consecutive Sessions 4–6. Priming at the beginning of Sessions 4–6 was not generally necessary. Three rats of the 19 did not reach criterion performance and were used as untrained controls.

Test phase; preference test. The 16 remaining rats were divided into 4 groups of 4, balanced according to training performance and whether light or noise had been S+. The 4 groups received 0, 5, 10 or 15 mg/kg of pipradrol fifteen min prior to each of three 1 hr sessions, each session being separated by 48 hr. In the test phase no ICS was given and panel-pushing had no consequence. The two levers were now present, each requiring 12.5 g for switch closure. Each response on one lever produced S+ (CR+), and on the other lever, S− (CR−). The stimuli were 1 sec long, and if additional responses were made during this time, the duration was reset to 1 sec. Contingent presentation of CR+ or CR− was counterbalanced over the levers for each group, and rats received CR+ at a lever position random with respect to the side of their electrode placement. The untrained rats were used as controls for possible effects of the drug on responding for stimulus change. One of these rats was given control injections prior to each of three 1 hr sessions and the other two received doses of 10 mg/kg of pipradrol. Lever press responses and panel-pushes were recorded at 5 min intervals. Data were analysed with a 3-factor repeated-measures ANOVAR, and post hoc comparisons were made using the Newman-Keuls test [22].

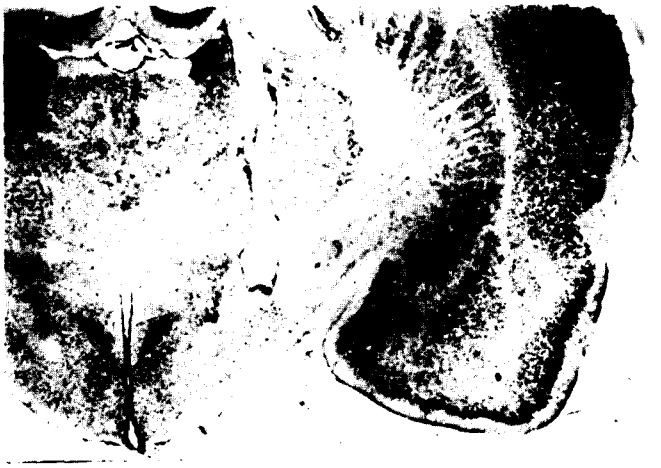

FIG. 1. Photomicrograph depicting a representative electrode placement in one of the rats.

Lever-press data were subjected to a square-root transformation to achieve homogeneity of variance [22], but panel-push data remained untransformed.

Histology

At the end of the experiment, all rats were sacrificed to verify electrode placement. The brains were perfused with Formalin, and every third 30 μ section was stained with cresylechtviolet. The histological examination verified that the tip of each of the electrodes implanted was in the vicinity of the lateral hypothalamus (see Fig. 1).

Drug. The drug employed was pipradrol hydrochloride (Meratran) dissolved in a 1 : 2 mixture of polyethylene glycol (BDH) and distilled water. The four doses of pipradrol employed (0, 5, 10 and 15 mg/kg) were injected intraperitoneally, fifteen min before each session, in a volume of 1 ml/1 kg body weight.

RESULTS

Pretest Training

A high level of differential responding between S+ and S− was obtained in all rats (mean 99.5 ± 0.3%), and each animal made the majority of its total panel-pushes during S+ (mean 89.5 ± 1.0%). The mean panel-pushing rate was 114.7 ± 8.2 responses per 5 min period.

Preference Test

Pipradrol produced a clear dose-dependent increase in responding on the lever providing CR+, but no marked effect on the lever providing CR− (see Fig. 2). This stimulatory action of the drug was highly significant, as assessed by the statistical interaction of drug dose with responding on the CR+ or CR− lever, $F(1,12) = 10.41$, $p<0.01$. Subsequent comparisons revealed that the interaction was attributable to the large increase in responding on the CR+ lever after 10 mg/kg relative to the other doses (Newman-Keuls, $p<0.001$). The facilitatory effect of 5 mg/kg just failed to reach significance ($p<0.10$). There were no significant effects of the drug on responding on the CR− lever, although it is interesting to note that responding after 10 mg/kg was less than that after 0 mg/kg in 3/4 cases. An additional analysis was performed to assess whether

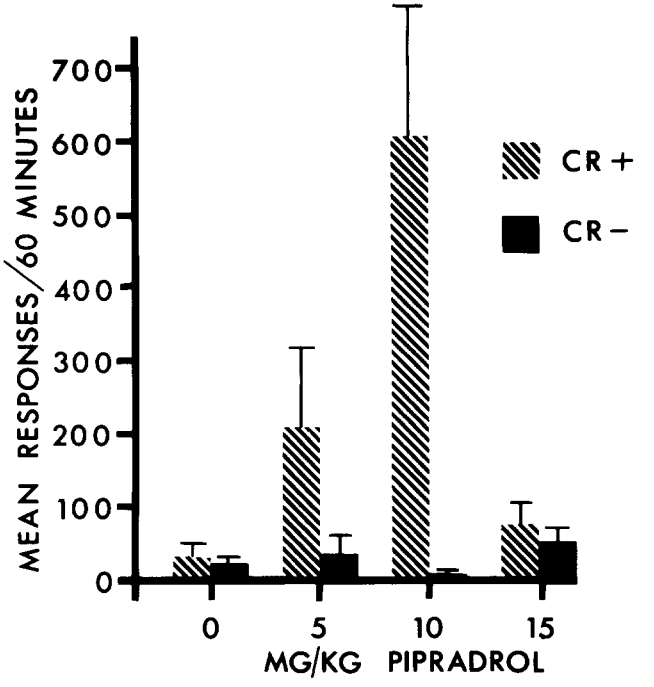

FIG. 2. Mean + SEM responses made by groups of rats receiving 0, 5, 10 or 15 mg/kg pipradrol over three 1 hour sessions. CR+ = responses made on CR+ lever. CR− = responses made on CR− lever.

there was a significant preference for CR+ over CR− at each of the drug doses. This revealed that although 3/4 rats at 0 mg/kg preferred CR+ over CR− there was no significant preference shown ($F<1.00$, ns). However, there was an enhanced preference for CR+ after both 5 mg/kg, $F(1,12) = 7.3$, $p<0.01$; and 10 mg/kg $F(1,12) = 51.6$, $p<0.001$) although not after 15 mg/kg ($F<1.0$, ns).

The results of both these analyses suggest that 5 and 10 mg/kg enhanced the choice made by rats for CR+ over CR−. The statistical analysis also revealed a significant general decline in responding over the three sessions, $F(2,24) = 4.67$, $p<0.025$, although this decrement was equivalent on the CR+ and the CR− levers, and was not affected by pipradrol.

Panel-Push Responding

A separate analysis of variance showed that pipradrol had no systematic effect on the previously trained panel-push when this response was extinguished, $F(3,12) = 1.23$. The mean ± SE scores were: 0 mg/kg, 84.5 ± 34.9; 5 mg/kg, 216.5 ± 116.9; 10 mg/kg, 82.5 ± 56.3; 15 mg/kg, 81.0 ± 33.3. It is noteworthy that 3 of 4 rats treated with 10 mg/kg showed reductions in panel-pushing compared with controls and yet were exhibiting high response rates on the CR+ lever. Panel-pushing extinguished rapidly over the course of the three sessions, $F(2,24) = 14.68$, $p<0.001$.

Untrained Controls

The rat treated with 0 mg/kg showed hardly any responding over three sessions (light lever, 20 responses, noise lever, 1 response). The rats treated with 10 mg/kg made 67 and 108 responses on the light lever, and 18 and 51 responses on the noise lever respectively both over three sessions.

Behavioral Observations

Observations made on closed circuit TV showed that each of the drugged animals developed stereotyped patterns of activity [10,11] including mainly repetitive sniffing and head movements. After 15 mg/kg, the stereotypy was intense and appeared to compete with lever-pressing. In one case, vigorous circling toward the left lever was induced, resulting in its activation with the animal's body, although it pressed the right (CR+) lever discretely, with its paws.

DISCUSSION

The major finding was that the psychomotor stimulant drug pipradrol, at doses of 5 and 10 mg/kg enhanced the reinforcing properties of external stimuli previously paired with ICS. These stimuli are termed conditioned reinforcers, and the results support the hypothesis [6] that psychomotor stimulants potentiate the effects of conditioned reinforcement. There was a highly selective facilitation of responding on the lever providing CR+ after 5 and 10 mg/kg, which could not be explained in terms of spatial preference for a particular lever being enhanced by the drug (e.g. [5]). In addition, responding for the light or noise, when both stimuli had minimal CR+ properties in the untrained group was increased only slightly in comparison after 10 mg/kg. The relative preference between responding for the light or noise was also greatly enhanced when one of the stimuli had been a CR+, than when neither stimulus had this property, as in the untrained group. Thus the enhancement appears to depend on the previously conditioned association of the stimuli with reinforcing brain stimulation.

Extinction of panel-pushing was not retarded significantly by pipradrol, although this was the response initially trained. Despite the higher rate of CR+ presentation after 10 mg/kg, this stimulus did not apparently act as a cue for more panel-pushing. This fact also suggests that pipradrol enhanced the reinforcing rather than the discriminative aspects of the CR+ stimulus. The experiment adds to a previous finding that pipradrol enhanced the acquisition of a new response reinforced by CR+, when the primary reinforcer during training had been water [17,18]. It extends the paradigm by providing a choice for the animal of responding for CR+ or CR−. The dose producing maximal responding in the earlier situation was 15 mg/kg [18]. The shifting of the dose-response curve in the present experiment could have been due to several differences, in training procedure, in primary reinforcer or in age of rats.

Stereotyped behavior was observed at all doses of pipradrol. At the 15 mg/kg dose, it was more constricted and intense than at lower doses and probably interfered with responding by response incompatibility [11]. After 5 and 10 mg/kg, the stereotypy was often blended with lever-pressing in a fashion similarly noted by others [4,10]. This observation raises the possibility that the lever-pressing itself is maintained in part by a perseverative tendency [17]. However, the selection of responding on a particular lever was clearly determined by CR+ after 5 or 10 mg/kg. This experiment illustrates how responding affected by a psychomotor stimulant can become focused onto particular responses [11], particularly those providing conditioned reinforcers.

The results also have implications for theories of motivation. No convincing evidence of conditioned reinforcement with ICS was found in undrugged animals, even

though stringent control over behavior reinforced with ICS had been previously obtained. Previous experiments have enhanced weak reinforcing effects of stimuli paired with ICS by training on a partial schedule of reinforcement or by increasing the level of food deprivation [7]. The present experiment shows that pipradrol is another manipulation which can exaggerate the conditioned reinforcing effects of stimuli previously paired with ICS. The precise mechanisms by which this psychomotor stimulant drug potentiates conditioned reinforcement however require further investigation. The behavioral excitatory effects of pipradrol have been shown to be dependent on an interaction with catecholamines stored in a reserpine-sensitive pool; and thus pipradrol is thought to belong to the methylphenidate class of stimulant drugs [19]. The paradigm employing ICS as the primary reinforcer provides a useful method for further analysis of this problem and a novel means for exploring the associative processes by which stimuli acquire rewarding properties.

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