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Effect of Minor Tranquilizers, Tryptamine Antagonists and Amphetamine on Behavior Punished by Brain Stimulation[1]

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MORATO DE CARVALHO, S., J. C. DE AGUIAR AND F. G. GRAEFF. *Effect of minor tranquilizers, tryptamine antagonists and amphetamine on behavior punished by brain stimulation.* PHARMAC. BIOCHEM. BEHAV. 15(3) 351–356, 1981.—Earlier observations have shown that septal lesions released operant responding punished by foot-shock, but did not change behavior punished by electrical stimulation of the dorsal periaqueductal gray (DPAG) substance of the rat brain. In contrast, chlordiazepoxide facilitated both kinds of punished responding. In order to further study the mechanism of brain stimulation punishment, dose-response curves of two minor tranquilizers, chlordiazepoxide and pentobarbital, of two tryptamine antagonists, methysergide and cyproheptadine as well as of amphetamine on lever-pressing behavior of rats maintained by water reinforcement and punished by DPAG stimulation were determined. A multiple schedule with a variable-interval 2 min (VI 2) non-punished component and a continuous reinforcement (CRF) component in which every response was both rewarded and punished was used. Chlordiazepoxide and pentobarbital caused dose-dependent increases in punished responding. Unpunished VI response rates were also moderately increased by the minor tranquilizers. In contrast, neither methysergide nor cyproheptadine increased punished or unpunished responding at doses that have been previously shown to markedly release behavior punished by foot-shock, in the rat. Conversely, amphetamine, a drug that usually does not release responding punished by peripheral noxious stimulation, caused dose-dependent increases in responding suppressed by DPAG punishment without affecting VI response rate. These and previous results with septal lesions suggest that neither the septo-hippocampal system nor its serotonergic input from the mesencephalon mediate response suppression by DPAG electrical stimulation, in contrast to their active role in peripheral punishment. This difference may also explain the marked facilitatory effect of amphetamine on responding punished by brain stimulation shown by the present results.

Punishment Dorsal periaqueductal gray stimulation Minor tranquilizers Tryptamine antagonists Amphetamine

IT has recently been shown [19] that the minor tranquilizer, chlordiazepoxide (CDP), released food-reinforced lever-pressing punished by either foot-shock or electrical stimulation of the dorsal periaqueductal gray (DPAG) substance, an aversive area of the rat brain [39, 42, 43, 49]. Septal lesions, however, increased responding punished by foot-shock, but did not change behavior punished by brain stimulation. The last result suggests that the septo-hippocampal system is not involved in brain stimulation punishment [19].

Both minor tranquilizers and tryptamine antagonists have been shown to markedly release responding punished by peripheral noxious stimulation [13, 14, 15, 17, 20, 36, 41, 45, 47, 48]. In contrast, amphetamine-like drugs do not usually increase low rates of punished responding [3, 13, 25, 30, 34], though similar response rates generated by other precedures are clearly enhanced by the psychostimulants [8]. Since the mechanisms of central and peripheral punishment may be different [19], it is interesting to know whether these drugs affect behavior punished by brain stimulation in the same way as responding suppressed by peripheral punishment.

Therefore, dose-response curves of two minor tranquilizers, CDP and pentobarbital, of two tryptamine antagonists, methysergide and cyproheptadine, as well as of amphetamine on rat lever-pressing behavior simultaneously rewarded by water presentation and punished by brief electrical stimulation of the DPAG were presently determined. A multiple schedule with punished and non-punished components, similar to that described by Geller and Seifter [13],

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was used. During the non-punished component, responses were rewarded at variable intervals averaging 2 min (VI 2), while in the punished component every response was immediately followed by both water presentation and DPAG electrical stimulation.

METHOD

Animals

Twelve male, albino Wistar rats, weighing 250–300 g at the beginning of the experiment, were housed in individual glass-walled cages and given water ad lib before lever-pressing training. During the experiments, the rats were deprived of water for 23 hr daily. Each animal was allowed to drink 2 to 15 ml of water fifteen minutes after each experimental session, in order to maintain body weight between 80 and 85% of its free-drinking weight. The experimental sessions were conducted daily from Monday through Friday. After the Friday experimental session, each rat was given 50 to 100 ml of water, to keep body weight within the above criterion on the next Monday.

Surgery

Rats were anesthetized with 40 mg/kg of sodium pentobarbital, IP, and operated in a stereotaxic instrument (David Kopf, U.S.A.). A bipolar electrode made of twisted stainless steel wires, 160 μm in diameter, Diamel-insulated except at the cross-section of the tip, was implanted in the dorsal midbrain. With the skull horizontal between bregma and lambda, the electrode was vertically introduced either at the lambda or 1 mm lateral to it. The lateral placement avoids piercing the venous sinus. The tip of the electrode was lowered 5.2 mm below the surface of the skull. The electrode was attached to the bone with stainless steel screws and methylmethacrylate polymer cement.

Apparatus

Brain stimuli were generated by a constant current, sine-wave stimulator [32]. The stimulation current was monitored by means of an oscilloscope (Heathkit, U.S.A.).

A standard, Grason-Stadler (U.S.A.) rat chamber (23×29×19 cm), placed inside an insulating chest provided with fan and an observing screen was used. The lever near the front door of the chamber was removed. The remaining lever was placed 9 cm above the grid floor and a minimum of 12 g vertical force was necessary for its operation. A circular opening in the divisional panel, near the floor, gave access to a liquid dipper. During the experimental session, the animal compartment was illuminated by either a 2 W white light, placed at the opposite side of the lever or a 2 W red panel light, placed above the lever. The rats inside the experimental chamber had their midbrain electrodes connected with the stimulator by means of a mercury swivel and a flexible, bite-proof cable. Temperature inside the chamber varied between 22 and 24°C. Standard electromechanical equipment (Grason-Stadler) and a cumulative recorder (Gerbrands, U.S.A.) were used for automatic programming and recording.

Procedure

Ten days after the surgery, the animals were placed inside the experimental chamber and stimulated with 60 Hz, AC electric current. The current intensity was gradually increased until either a behavioral change occurred or a ceiling of 70 μA (RMS) was reached. Only animals displaying aversive responses to the brain stimulation, such as running or jumping, were used in the experiment.

Following lever-pressing training, a multiple schedule similar to that originally described by Geller and Seifter [13] was used. During the non-punished component, the white panel light was on and lever-pressing was maintained by an arithmetical variable-interval schedule of reinforcement with average 2 min and range 5-240 sec (VI 2). Each reinforcement consisted in the presentation of 0.1 ml of tap water for 4 sec. During the punished component, the red light was on and each lever-press was immediately followed by water presentation (CRF) as well as by 1-sec electrical stimulation of the dorsal midbrain. The intensity of the brain stimulus was gradually increased over several sessions, until only 10 to 20 responses occurred in each experimental session. Eventually, intensities of 21–84 μA (r.m.s.) were used. In each session, six CRF (punishment) periods of 1 min duration were interspersed between 7 periods of non-punished VI, each of 7-min duration.

The experiments were conducted daily, from Monday through Friday. After performance on the punished and non-punished components was stable, drug injections were given on Tuesdays and Fridays, provided the punished responding in the day before the injection was within the criterion of 10 to 20 responses per session. If otherwise, the intensity of the brain stimulus was adjusted until the response criterion was resumed. Thursdays were used as control sessions.

Analysis of Results

Cumulative response records were inspected daily for shifts in response rate and patterns of responding.

Punished and non-punished responses were independently recorded in digital counters. For each rat, the number of responses per session was converted to a percentage of the control average. From these individual data group means and deviations were calculated.

Statistical analysis of dose-response functions was made using Friedman's rank sums test, followed by multiple comparisons between each dose and its control [26].

Histology

Rats were sacrificed under deep pentobarbital anesthesia and their heads removed after perfusion through the heart with saline, followed by 10% formalin solution saturated with potassium ferrocianide. After decapitation, a DC current was passed through the brain electrode for 15 sec. The brains were removed and fixated in 10% formalin for at least 3 days. Frozen sections of 50 μm were placed on a glass slide and enlarged photographies were taken with an amplifying projector. Electrode placements were localized in diagrams from König and Klippel's rat brain atlas [29].

Drugs

Chlordiazepoxide (Roche), sodium pentobarbital (Nembutal®, Abbott), methysergide (Sandoz), cyproheptadine hydrochloride (Merck) and *d-l*-amphetamine hydrochloride (Sigma) were used. Amphetamine was dissolved in 0.9% NaCl solution for injections. Pentobarbital, methysergide and cyproheptadine were dissolved in distilled water, while chlordiazepoxide was dissolved in 1% tween-80 solution. In

TABLE 1

CONTROL RESPONSE RATES IN THE MULTIPLE VI 2 CRF SCHEDULE OF WATER PRESENTATION WITH AND WITHOUT PUNISHMENT OF CRF RESPONSES BY BRAIN ELECTRICAL STIMULATION

Drug Treatment	VI 2 component		CRF component		N*
	No punishment	Punishment	No punishment	Punishment	
	Responses per minute (mean ± SEM)				
Chlordiazepoxide	14.04 ± 2.67	10.80 ± 1.36	10.87 ± 0.52	2.47 ± 0.09	27
Pentobarbital	14.04 ± 2.67	10.63 ± 1.66	10.87 ± 0.52	2.60 ± 0.09	27
Cyproheptadine	12.96 ± 2.28	9.80 ± 1.26	10.91 ± 0.42	2.47 ± 0.07	33
Methysergide	13.77 ± 3.01	10.42 ± 1.54	10.60 ± 0.50	2.54 ± 0.09	24
Amphetamine	14.65 ± 3.30	11.93 ± 1.16	10.02 ± 0.70	2.64 ± 0.11	21

*Number of control sessions (3) multiplied by the number of rats in each treatment group. With one exception, all rats used (12) were included in two or more groups.

each administration, a volume of 1 ml/kg of drug or control solution was injected, IP, 30 min before the experimental session. Doses of the drugs refer to salts. The different doses of each drug were given in non-systematic order.

RESULTS

Localization of the Brain Electrodes

The tips of the electrodes implanted at lambda were localized inside the DPAG substance or adjoining tectum of the mesencephalon, as previously reported [19,42]. In the animals with electrodes implanted 1.0 mm lateral to lambda, the electrode tips were localized inside or adjacent to the dorso-lateral periaqueductal gray matter.

Control Performance Under the Multiple VI2 CRF Schedule

The performance of the rats under the multiple VI CRF schedule, with and without punishment, was similar to that previously reported by Geller and Seifter [13], using electrical foot-shock as punisher. During the VI schedule component, the animals responded at a nearly constant rate with some oscillation in local rate. Responding was steadier during the CRF component without punishment. With the punishing brain stimulation, however, responses became rare and irregularly spaced during CRF. A typical performance of one rat is illustrated by the cumulative records in Fig. 3.

Control response rates for the different drug-treatment groups are shown in Table 1. It may be seen that punishment markedly suppressed CRF responding to approximately 25% of unpunished response rate. Rates in the non-punished VI component were also moderately reduced by CRF punishment. There was no difference between rats with brain electrodes implanted at the midline (n=6) and those with electrodes placed 1 mm lateral to lambda (n=6) as regards the punishing effect of brain stimulation.

Effect of Minor Tranquilizers and Anti-5-HT Drugs on Punished and Non-Punished Behavior

As shown in Fig. 1, CDP increased punished CRF responding in a dose-dependent way. Overall significance was observed during this component ($S'=26.60$, $p<0.001$). Comparison with control showed a significant increase in response rate at 10 mg/kg ($p<0.01$) and 17 mg/kg ($p<0.01$). Non-punished VI rate was moderately increased by CDP (Fig. 1) and this effect was overall significant ($S'=20.72$, $p<0.001$). Significant increases in respect to control were also observed at 10 mg/kg ($p<0.01$) and 17 mg/kg ($p<0.05$).

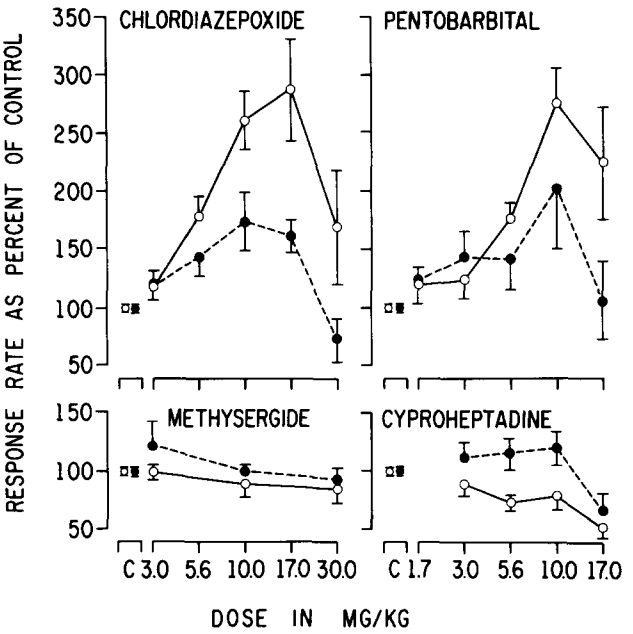

FIG. 1. Comparison between the effect of two minor tranquilizers, chlordiazepoxide and pentobarbital, and two 5-HT antagonists, methysergide and cyproheptadine, on punished (○) and unpunished (●) responding of rats under the multiple VI 2 CRF (punishment) schedule. During CRF, every response was followed by aversive electrical stimulation (1 sec, 60 Hz, 21–84 μA, r.m.s.) of the dorsal midbrain. Each point in the dose-response curves represents the mean of single determinations in 9 rats for chlordiazepoxide and pentobarbital, 8 for methysergide and 11 for cyproheptadine. The mean (made equal to 100%) and the variation of undrugged controls (C) were calculated from 3 observations for each rat in the different groups, made during the corresponding dose-response determination period. Vertical bars represent ±SEM. Drugs were injected IP, 30 min before the experimental session.

The dose-response curve of pentobarbital on punished CRF responding was similar to that of CDP, as also shown in Fig. 1. The effect of pentobarbital on punished responding

was highly significant overall ($S'=22.65, p<0.001$) and significant increases in response rate with respect to control were observed at 5.6 mg/kg ($p<0.05$), 10 mg/kg ($p<0.01$) and 17 mg/kg ($p<0.05$). The effect of pentobarbital on VI responding was also overall significant ($S'=15.91, p<0.01$), but only the dose of 10 mg/kg significantly increased response rate ($p<0.01$) in respect to control (Fig. 1).

In contrast to the effect of minor tranquilizers, punished responding was not significantly increased by either methysergide ($S'=0.54$, $p>0.05$) or cyproheptadine, though overall significance was observed for cyproheptadine ($S'=21.71$, $p<0.001$) because the dose of 17 mg/kg significantly decreased punished response rate ($p<0.01$) in respect to control (Fig. 1).

Non-punished VI responding was not significantly affected by methysergide ($S'=0.45$, $p>0.05$). Overall significance was observed with cyproheptadine ($S'=10.68$, $p<0.05$), though no individual dose significantly altered VI response rate in respect to control (Fig. 1).

Effect of Amphetamine on Multiple VI 2 CRF (Punishment) Responding

As shown in Fig. 2, amphetamine caused dose-dependent increases in punished responding. This effect was overall highly significant ($S'=23.26, p<0.001$). Significant increases in respect to control were observed after 1.7 mg/kg ($p<0.01$) and 3 mg/kg ($p<0.05$). Non-punished VI responding was not significantly affected by amphetamine ($S'=4.39$, $p>0.05$). Saline injection did not significantly affect either punished or non-punished response rates, as also shown in Fig. 2.

The releasing effect of amphetamine on CRF responding suppressed by DPAG punishment is illustrated by the lower cumulative record in Fig. 3.

DISCUSSION

Previously reported results have shown that a dose of 5 mg/kg of CDP significantly increased food-rewarded lever-pressing simultaneously punished by brief electrical stimulation of the DPAG, in the rat [19]. The present results extend this observation, since a complete dose-response function of the releasing effect of CDP on responding punished by DPAG electrical stimulation is described. These results also show that the barbiturate minor tranquilizer, pentobarbital, markedly released punished responding in the same way as CDP. Therefore, the ability to counteract the punishing effect of DPAG stimulation may be generalized to non-benzodiazepine minor tranquilizers. Since behavior punished by peripheral noxious stimulation is characteristically released by anti-anxiety drugs [13, 14, 36, 41, 45, 47, 48], the conclusion may be drawn that DPAG punishment is similar to peripheral punishment in regard to minor tranquilizer's action.

Nevertheless, there are clear differences between central and peripheral punishment in their sensitivity to 5-HT antagonists and septal lesions. It has been reported that septal lesions releasing comparable rates of foot-shock punished responding were ineffective on DPAG punishment [19]. Similarly, the present results show that two 5-HT antagonists, methysergide and cyproheptadine, did not increase responding punished by brain stimulation, at doses that have been previously shown to release lever-pressing behavior punished by foot-shock [17]. These results suggest that neither the septo-hippocampal system [23] nor its 5-HT input from the mesencephalon [1], seemingly mediating behavioral inhibition in the rat [21, 22, 23], play any important

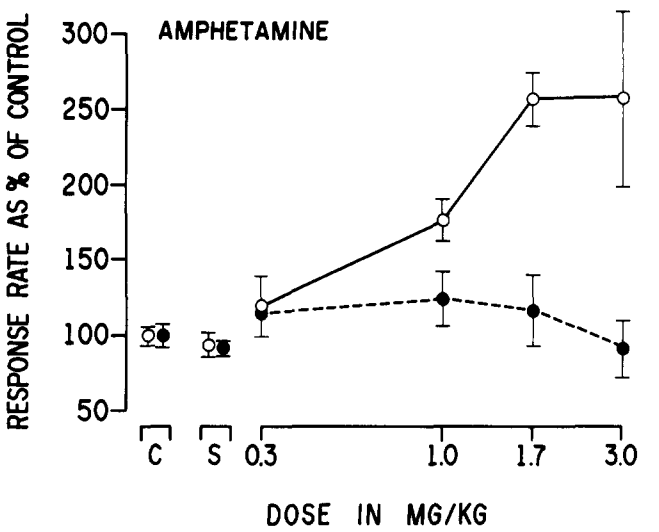

FIG. 2. Effect of amphetamine given IP, 30 min before the experimental session, on punished (○) and unpunished (●) responding of rats under the multiple VI 2 CRF (punishment) schedule. Each point in the dose-response curves represents the mean of single determinations in 7 animals. The points for saline injection (S) were similarly calculated. The mean and variation of undrugged controls (C) were calculated from 3 observations for each rat, made during the dose-response determination period. Vertical bars represent ±SEM.

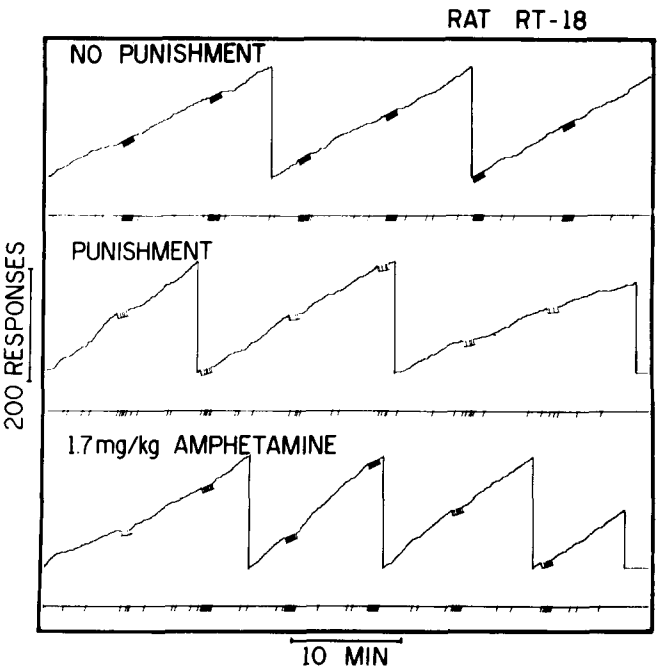

FIG. 3. Representative cumulative records of performance of one rat under the multiple VI 2 CRF schedule without punishment, when every response in the CRF component was also followed by electrical stimulation of the dorsal midbrain (punishment) and after a dose of amphetamine (IP) that released punished responding. Lever-pressing responses were cumulatively recorded by upward movements of the recording pen; resetting to base line occurred after 200 responses or at the end of the experimental session. Vertical deflection of the same pen indicates delivery of brain electrical stimuli (1 sec, 60 Hz, 42 μA r.m.s.). Vertical deflection of the lower pen indicates water reinforcement.

role in response suppression by DPAG punishment. Therefore, in this procedure, activation of the brain aversive system including the DPAG [6, 7, 39, 43, 49] seems to determine response withdrawal, as previously suggested [19].

The releasing effect of minor tranquilizers on behavior punished by DPAG electrical stimulation, shown by the present as well as by previous results [19], may be due to a depressant action of these drugs on the brain aversive system, since doses of CDP causing little sedation or ataxia have been shown to increase the latency of lever-pressing responses switching off electrical stimulation applied in the same region of the rat's brain [42]. In addition, local injection of diazepam inside the amygdala, also a part of the brain aversive system [7], has been reported to release food-rewarded behavior punished by foot-shock, in the same species [37]. The last result together with previously reported evidence showing that CDP increase foot-shock punished responding already released by septal lesion [19] suggest that the brain aversive system also participates in response suppression generated by peripheral punishment. In this instance, however, the septo-hippocampal system and its serotonergic input from the mesencephalon [1,23], may act together with the brain aversive system for producing response suppression, because either septo-hippocampal lesions [19,23] or manipulations that presumably decrease brain 5-HT activity [12, 15, 17, 20, 40, 45, 46, 47] release foot-shock punished responding, in the same way as minor tranquilizers.

The above hypothesis of a dual mechanism operating in peripheral punishment as opposed to a single mechanism in central punishment, may also explain the marked increase in DPAG punished responding following amphetamine administration, shown by the present results. While amphetamines increase low rates of responding in many situations [8], they do not generally increase low rates of responding suppressed by peripheral punishment and often further accentuate response suppression [3, 13, 25, 30, 34]. Although moderate increases in very low rates of punished responding occurring early in a fixed-interval schedule have been reported when shock intensity is low and response suppression is moderate [10,35], these increases are slight compared with those obtained with minor tranquilizers and 5-HT antagonists. It is true that under present or past contextual influences, large increases in punished responding have been reported following amphetamine [33]. However, such influences did not exist in the present experimental situation. Therefore, the marked facilitatory effect of amphetamine on DPAG punished responding shown by the present results may be attributed to the type of punisher used.

It is not yet clear why amphetamine does not generally increase responding punished by peripheral noxious stimulation. The facilitatory effects of amphetamine on behavior seem to be mainly due to increased release together with diminished neuronal reuptake of dopamine in brain systems mediating exploratory behavior and incentive-reward [5,28]. On the other hand, several reported results suggest that amphetamine also facilitates brain serotonergic systems exerting an inhibitory influence on behavior. Thus, PCPA [4, 27, 31], 5-HT antagonists [18, 27, 44] or brain lesions destroying ascending 5-HT pathways [16, 24, 38] potentiate the facilitatory effect of amphetamine on locomotor activity [4, 16, 27, 31, 38] and on operant lever-pressing maintained by either natural reinforcement [18,24] or rewarding brain electrical stimulation [44], in the rat. In addition, biochemical evidence shows that amphetamine can release 5-HT in several regions of the rat brain [2, 4, 11] and an electrophysiological study revealed that relatively low doses (0.1–1 mg/kg) of amphetamine increase the firing rate of midbrain raphe neurons [9].

Therefore, it may be suggested that in punishment tasks using peripheral noxious stimulation amphetamine does not release responding or may even increase response suppression, because the drug facilitates behavioral inhibitory 5-HT systems presumably activated by punishment [17, 20, 45, 46, 47, 48]. Since the latter do not seem to participate in the response suppression caused by central punishment, releasing effects of amphetamine on responding punished by brain stimulation, as shown by the present results may occur. Nevertheless, additional evidence is needed in order to test this hypothesis, since recently reported results suggest the mediation by brain dopamine rather than by 5-HT of the suppressant effect of amphetamine on punished responding [30].

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