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Pimozide and Amphetamine have Opposing Effects on the Reward Summation Function

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GALLISTEL, C. R. AND D. KARRAS. *Pimozide and amphetamine have opposing effects on the reward summation function.* PHARMACOL BIOCHEM BEHAV **20**(1) 73–77, 1984.—The reward summation function is the plot of self-stimulation performance as a function of the number of pulses in a train of fixed duration. It has previously been shown that drugs that impair performance compress this curve but do not shift it laterally; whereas when the reinforcing efficacy is reduced by reducing current intensity, the curve shifts laterally. The amount of the shift is a measure of the magnitude of a drug's effect upon reinforcing efficacy. We report here that pimozide shifts the curve to the right in a dose-dependent manner, indicating an impairment of reinforcing efficacy, while amphetamine shifts it to the left, indicating an enhancement of reinforcing efficacy. When the two drugs are given together their effects on the reward summation function cancel out. These results are consistent with the hypothesis that pimozide and amphetamine exert their effects on reinforcing efficacy via one and the same set of dopaminergic synapses.

Pimozide Amphetamine Reinforcement Dopamine synapses Reward-summation-functions

PIMOZIDE, a relatively specific blocker of dopamine receptors [10] has been shown to reduce or abolish the reinforcing effect of electrical stimulation of the MFB in the rat, using behavioral measures that discriminate between a drug's effect on reinforcement and its effects on the animal's capacity to perform [5, 6, 7]. Pimozide is a neuroleptic. The pharmacological profile of neuroleptic action on reinforcement correlates strongly with the profile for in vitro neuroleptic binding to the dopamine D_2 receptor [8]. Amphetamine, which among other things, both increases the release of dopamine and acts as a dopaminergic agonist, elevates the rate of self-stimulation and lowers the threshold [1,4]. However, neither the rate measure nor the threshold measure demonstrably distinguishes between effects on reinforcement and effects on performance factors. If amphetamine in fact increases the reinforcing efficacy of the electrical stimulation and not simply self-stimulation performance at any given level of stimulation, then amphetamine's effect on a specific measure of reinforcing efficacy should be the opposite of pimozide's effect. Furthermore, if amphetamine acts by increasing the amount of dopamine and/or dopaminergic agonists in the same synapses in which pimozide exerts its competitive blocking action, the effect of one drug on the reinforcing efficacy of the stimulation should cancel or reduce the effect of the other. The present paper reports a confirmatory test of these two predictions.

Our measure of the reinforcing efficacy of the stimulation is the locus of the reward summation function [2,3]. The reward summation function is the rate at which a rat performs as a function of the number of pulses in the reward. As the number of pulses, N, is increased in equal logarithmic steps (with the duration of the rewarding train held constant), the rate of performance rises abruptly from zero to asymptotic levels. The locus of this rise is defined as the value of N at one-half maximum performance (Fig. 1). While performance effects alter the maximum performance, the locus of the rise is affected only by a change in the rewarding value of the stimulation, as when the current intensity is altered [2].

Franklin [6] has shown that the neuroleptic pimozide, a competitive blocker of dopamine receptors, shifts the locus of rise of the reward summation function to the right towards greater values of N. Drugs that enhance transmission in relevant synapses should cause a shift in the locus of rise toward a lower number of pulses (leftward).

We first replicated the results of Franklin's experiment which found that pimozide caused a rightward shift in the locus of rise. The amount of shift as a function of drug dosage was examined. Next, amphetamine was administered to see if it would cause a leftward shift in the locus. Finally, the effect of concurrent administration of pimozide and amphetamine was examined. If the shift due to amphetamine proved reversible by pimozide, then the effect of amphetamine could more plausibly be attributed to its action on dopamine functioning rather than to its numerous other actions.

METHOD

Subjects

Twelve male albino rats from the Charles River Breeding Laboratory were used in this study. The rats were 100 to 150 days old and weighed 425 to 550 grams at the time of electrode implantation. They were individually housed throughout the study, with continuous access to food and water. Lights were kept off from 8:30 a.m. to 6:30 p.m., so that the rats could be run during their active period.

Each rat was implanted with a single monopolar stimulat-

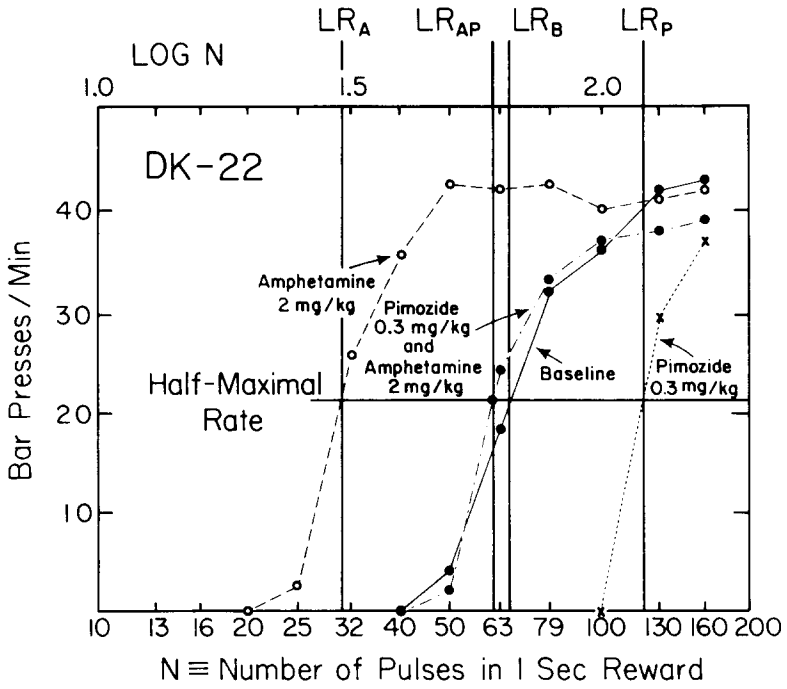

FIG. 1. An example of the shifts in the reward summation function produced by pimozide, by amphetamine, and by amphetamine plus pimozide. LR=locus of rise; subscripts signify: A=amphetamine; AP=amphetamine plus pimozide; B=baseline (undrugged); P=pimozide.

ing electrode, insulated with Formvar except at the tip. The tip was aimed for the posterior medial forebrain bundle, using horizontal skull coordinates: 4.0 mm behind Bregma, 1.5 mm lateral to the sagital suture, and 9.0 mm below the skull surface. An indifferent electrode was wound around three skull screws. Surgery was carried out under Chloropent anesthesia, with Atropine administered to prevent respiratory failure. Upon completion of the experiments, electrode placement was verified in 5 of the rats. The tip was consistently in the MFB at the level of the posterior hypothalamus (Levels 3290–3750 in [9]).

Apparatus

Animals were tested in a Skinner box, 26 cm on a side, 46 cm high and closed at the top except for a hole through which the stimulating wires passed. Each press on the lever caused a train of cathodal stimulating pulses to be delivered to the rat. Train duration and pulse duration were fixed at 1 second and 0.1 msec, respectively. The number of pulses in a train was varied to obtain reward summation functions. The current intensity for each rat was fixed in a manner described below so as to insure that the locus of rise under control conditions was approximately 50 pulses per second (pps) for each rat. Both the current and the voltage being applied were monitored with an oscilloscope.

Training

In three $^1/_2$-hour training sessions each rat learned to lever-press for a reward of 50 pps, at a current arbitrarily chosen to produce high response rates. Then, in three sessions on separate days the current was decreased in steps of 50 μA until responding extinguished, then increased in reverse sequence until responding reached asymptote. The average number of responses per minute was plotted against the log of the current intensity to obtain a rate-intensity function. The current which produced the half-maximal response rate was determined by interpolation. This was the standard current, the current used in all future tests on the rat. Choosing the current for each rat in this way ensured that the locus of rise would fall around 50 pps for each rat.

Data Collection

To determine the locus of rise, the rewarding stimulation was set at 160 pps and lowered in steps of 0.1 log units until responding extinguished. It was then increased in equal steps back up to 160 pps. The average number of responses per minute was plotted against the log of the pulse number. The number of pulses, N, producing half-maximum response rates was the control locus of rise. (Actually, we used log N in our computations—see Fig. 1.)

During drug sessions the locus of rise was determined in the same manner as during control sessions. To get the shift in locus of rise, the locus of rise during the drug session in log units was subtracted from the logarithmic mean locus of rise for all control sessions for that rat. This yields a difference with a positive sign when a drug enhances reinforcing efficacy and a negative sign when it reduces reinforcing efficacy.

A control locus of rise was determined before each treatment.

Drug Treatments

Each rat was treated with pimozide and amphetamine separately before being administered combinations of the

TABLE 1

PIMOZIDE- AND AMPHETAMINE-INDUCED SHIFTS IN THE REWARD SUMMATION FUNCTION (LOG_{10} UNITS)

	Pimozide		Amphetamine		Combinations			
	0.3 mg/kg	0.6 mg/kg	2 mg/kg	4 mg/kg	Pimozide 0.3 Amphetamine 2	Pimozide 0.3 Amphetamine 4	Pimozide 0.6 Amphetamine 2	Pimozide 0.6 Amphetamine 4
Dk-5	−0.34	Ext. (<−0.64)	+0.20	+0.24	+0.11			+0.02
DK-6	−0.10	−0.19	+0.33	No ext. (>+0.74)	+0.02	−0.01	−0.10	0.0
DK-11			+0.31					
DK-12	−0.58	Ext. (<−1.00)	+0.20	No ext. (>+0.60)	−0.18	+0.02	−0.13	−0.16
DK-14	−0.02			+0.11				
DK-19	−0.08	−0.16	+0.42	No ext. (>+0.60)	−0.05	−0.26	−0.13	−0.24
DK-20	−0.02	−0.10	+0.22	+0.54	+0.11	+0.20	−0.03	+0.08
DK-22	−0.25		+0.30		+0.03			
AR-2	−0.06	−0.16						
BM-1	−0.09	−0.30	+0.20	+0.25			+0.01	
BM-22			+0.42					
BM-3			+0.17					

A positive shift is a shift to the left (toward smaller values of N); a negative shift is to the right (toward larger values). "Ext."—extinction, means that we could not get responding at the highest value of N we tried (160). The value underneath in parentheses gives the maximum shift we could have detected. "No ext."—no extinction, means that the rat did not cease responding even at the smallest value of N we could generate (10). Again, the value in parentheses gives the maximum shift we could have detected.

two drugs in various dosages. A given dosage of a drug was occasionally given more than once to the same rat. At least seven days separated any two drug tests.

Pimozide. Rats were injected IP with either 0.3 or 0.6 mg/kg pimozide in 0.3% tartaric acid. Data collection began four hours after injection.

Amphetamine in saline was injected IP in either 2 or 4 mg/kg doses. Data collection began 1/2 hour after injection.

Amphetamine and pimozide. Rats were next injected with either dose of pimozide, followed 3 1/2 hours later by an injection of either dose of amphetamine. Data collection began 1/2 hour after the second injection.

RESULTS

Pimozide

Pimozide in doses of 0.3 and 0.6 mg/kg reliably shifted the curve of performance versus number of reward pulses to the right, so that higher numbers of pulses were required to sustain half-maximal performance (Fig. 1). The shift was dose-dependent in that a larger dose in a given animal reliably produced a larger shift (Fig. 2). However, it was difficult to get two significant shifts of different magnitude in the same animal. If the 3 mg/kg dose produced a shift greater than 0.1 log unit (or 25%), then the shift at a dose of 0.6 mg/kg was off-scale in the sense that we could not get any performance within the range of pulse frequencies we felt justified in using (160–10 pps). If 0.3 mg/kg produced a negligible (<0.1 log unit) shift, then 0.6 mg/kg produced a significant shift (see Table 1). The "off-scale" problem in combination with the between-animal variation in the reaction to the lower dose (shifts ranging from −0.02 to −0.58) make across-animal averages meaningless. The rightward shifts in the locus of rise were sometimes accompanied by reductions in maximal rate and sometimes not. We do not report maximal rate here because the upper limit of 160 pps often made it hard to estimate this with precision (see, for example, Fig. 1).

A 0.3 log unit shift means that reinforcing efficacy has been altered by a factor of 2. A −0.6 log unit shift means that reinforcing efficacy has been reduced by a factor of 4: 4 times as many pulses are required to attain a given level of reinforcement. A +0.3 unit shift means that 1/2 as many pulses are required, and so on.

Amphetamine

Amphetamine in doses of 2 and 4 mg/kg reliably shifted the curve of performance versus number of reward pulses to the left, so that fewer pulses were required to sustain half-maximal performance (Fig. 1). Again, there was an "off-scale" problem in that at larger doses, the animals sometimes fail to extinguish altogether, continuing to press for as long as 15 minutes with the stimulation turned entirely off. The 2 mg/kg dose produced a shift of +0.28±0.09 (n=10). The effect of 4 mg/kg could not be measured in 3 of the 7 animals tested and were quite variable (0.28±0.18) in the 4 that could. In the 3 rats where both doses produced measurable shifts, the larger dose always produced a larger shift. The shifts produced by amphetamine were about the same when one examined separately the curve obtained during the descending sequence of pulse numbers and the curve obtained during the ascending sequence. They were not, in other words, due to "hysteresis" on the downward sequence as one might suspect from the absence of extinction at larger doses of the drug.

Pimozide and Amphetamine Combined

Combining doses of pimozide in the 0.3–0.6 mg/kg range

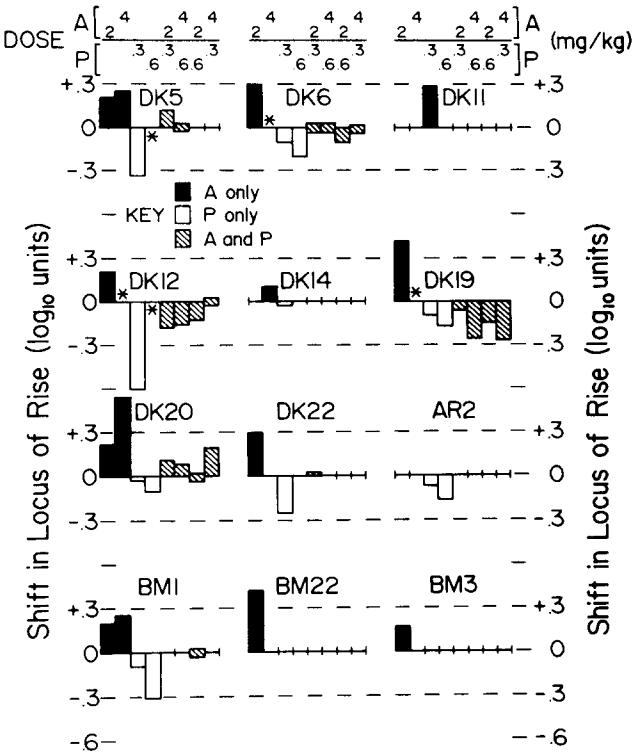

FIG. 2. Shifts in the locus of rise of the reward summation function produced by amphetamine and pimozide, alone and in combination. The subjects are identified by letter-number combinations above each plot. A=amphetamine dosage. P=pimozide dosage. Shifts lying within 0.02 log units of 0 are plotted straddling the 0 line. An asterisk above the 0 line means that no shift could be determined because there was no extinction; an asterisk below the line means that no shift could be determined because there was no performance.

with doses of amphetamine in the 2–4 mg range yielded reward summation curves that most commonly had a locus of rise within ±0.1 log unit of the control value (Fig. 1). Each possible combination of doses was tested in between 4–6 animals. Averaging across animals yields an average shift within 0.1 log unit of control for each combination. The data are noisy both within and between animals in that the combinations that should yield a relatively more negative or relatively more positive shift did not reliably do so. However, except for the data from DK-19, the effect of a pimozide-amphetamine combination was always intermediate between the effects of the constituents alone (Fig. 2).

DISCUSSION

Pimozide reliably caused a rightward shift in the locus of rise, indicating a reduction in the rewarding effect of brain stimulation. This is similar to the results obtained by Franklin [6], but differs in that the rate of lever-pressing in a Skinner box rather than the rate of alley running was used to measure performance. Also, in this study train duration was held constant and pulse frequency covaried with the number of pulses, while in Franklin's work, pulse frequency was constant and train duration covaried with the number of pulses. The methods used for this paper have the advantage of taking considerably less time, which is important when drugs with a brief period of potency are used. They have the disadvantage of not distinguishing between the priming and reinforcing effects, but earlier work has shown that pimozide does not affect priming [11].

Amphetamine clearly has the opposite effect of pimozide; it reliably caused a leftward shift in the locus of rise. It should be noted that while the direction of the shift with either drug was consistent, the magnitude of the shift varied widely between subjects.

When pimozide and amphetamine treatments were combined the net effect on reinforcing efficacy was intermediate to the effects of the two individual treatments. The fact that pimozide and amphetamine have opposing effects on reinforcing efficacy and that these opposing effects cancel when both drugs are given is consistent with the hypothesis that the two drugs are exerting their effects on the same synapses, and thus involve the same neurotransmitter. It has been shown that pimozide is a specific DA antagonist [10] and that neuroleptic capacity to block reinforcement correlates strongly within vitro affinity for the D_2 receptor [8]. The present results suggest therefore that it is the enhancement of dopaminergic transmission by amphetamine that is increasing reinforcing efficacy, rather than amphetamine's effects on NE, E, or 5-HT synaptic mechanisms. A simple explanation would be that while the pimozide continues to block DA receptors in the reward pathway, the amphetamine is enhancing the release and slowing the reuptake of DA in the same synapses. The net result is a response to the drug combination which is intermediate to the response to either drug alone. These data do not, of course, prove such an hypothesis; they are merely consistent with it.

The effect of the two drugs on the magnitude of the reinforcing effect generated by a given amount of stimulation may arise in at least two quite different ways. The dopaminergic synapses may form a stage or stages in the reward pathway, the pathway that conveys the signal generated by the stimulation to the place where that signal is converted to an enduring reinforcing effect. In that case the action of amphetamine amplifies the signal while the action of neuroleptics attenuates it. Alternatively, the dopamine synapses may form no part of the reward pathways; rather they may control a signal that can modulate (amplify or attenuate) the reinforcing effect of a signal in the reward pathway.

The data reported here demonstrate how the locus of rise of the reward summation function may be used to study quantitatively the manner in which drug combinations effect reinforcing efficacy. From the study of such combinations, we may gather a clearer picture of the pharmacology of diencephalic reinforcement mechanisms.

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