Pharmacology Biochemistry & Behavior, Vol. 26, pp. 731–741. © Pergamon Journals Ltd., 1987. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Quantitative Determination of the Effects of Catecholaminergic Agonists and Antagonists on the Rewarding Efficacy of Brain Stimulation

C. R. GALLISTEL AND GWEN FREYD[1]

Department of Psychology, University of Pennsylvania, Philadelphia, PA 19104

Received 28 October 1986

GALLISTEL, C. R. AND G. FREYD. *Quantitative determination of the effects of catecholaminergic agonists and antagonists on the rewarding efficacy of brain stimulation.* PHARMACOL BIOCHEM BEHAV 26(4) 731–741, 1987.—The effects of amphetamine, clonidine, molindone, pimozide and yohimbine on the rewarding efficacy of electrical stimulation of the medial forebrain bundle in the rat were determined from the effects of these drugs on the rate-frequency function, which is the plot of the rat's rate of pressing a lever against the frequency of the pulses in a rewarding train of fixed duration. These catecholaminergic agonists and antagonists produced dose-dependent alterations in the measurable rewarding efficacy, but only up to a factor of about 2, even though the method is capable of measuring 25–30-fold changes. At elevated doses, the effects on rewarding efficacy became unmeasurable, because the animals would not consistently self-stimulate at any parameters of stimulation. Amphetamine (0.5–3 mg/kg) enhanced rewarding efficacy. Clonidine (0.05–0.4 mg/kg), molindone (0.25–1 mg/kg) and pimozide (0.1–0.6 mg/kg) attenuated it. Pimozide and clonidine were equipotent despite their radically different receptor affinities. The effects of pimozide, clonidine and amphetamine were approximately additive (amphetamine cancelled the effects of pimozide and clonidine, while clonidine augmented the effect of pimozide). The α_2 antagonist yohimbine (0.05–10 mg/kg) had the same effect as the α_2 agonist clonidine (attenuation of rewarding efficacy), but these effects did not combine additively: yohimbine neither cancelled nor augmented the effect of clonidine. It is suggested that catecholaminergic agonists and antagonists do not alter the magnitude of the rewarding signal by acting on postsynaptic receptors in the reward pathway; rather, they may drive beyond functional limits a variable that is crucial to the proper recording of the magnitude of the rewarding signal.

Amphetamine Clonidine Molindone Pimozide Yohimbine Self-stimulation Rate-frequency

IT has been demonstrated in a variety of ways that catecholamine antagonists, particularly those with affinity for the D_1 or D_2 receptor, attenuate or abolish the rewarding effect of brain stimulation [12, 13, 16, 17, 27, 36]. The catecholamine agonist amphetamine augments the rewarding effect of the stimulation [10,30] and counteracts the effects of catecholaminergic antagonists [18,20]. The results so far obtained have been qualitative in nature: they show that the rewarding impact of the stimulation has been altered but they do not measure the magnitude of this alteration in a manner that places quantitative constraints on the underlying physiological effects of these drugs.

A method has recently been developed that measures changes in the rewarding efficacy of brain stimulation in such a way as to yield a physiologically interpretable quantitative estimate of the magnitude of the underlying change [5, 19, 25]. We have used the method to determine dose-response curves for catecholamine agonists and antagonists and to assess the nature of some of their interactions. The results suggest a new hypothesis regarding the mechanism of catecholaminergic action on the rewarding effect of brain stimulation.

The Method of Measurement

Early studies in the neuropharmacology of self-stimulation used changes in the rate of responding as an indication of change in the rewarding effect of the stimulation [29,34]. This behavioral measure does not have a quantitative physiological interpretation: a two-fold reduction in the rate of responding does not imply any specifiable reduction in the magnitude of any underlying physiological variable. This measure also does not distinguish performance effects from effects on reward, a point whose importance has

[1]Present address: Department of Biology, MIT, Cambridge, MA.

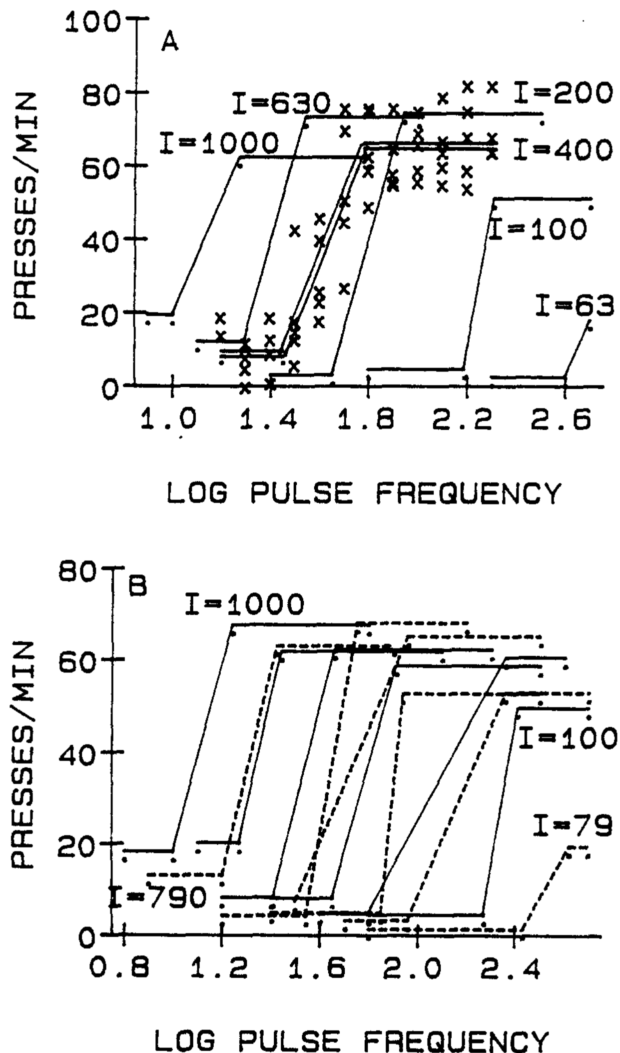

FIG. 1. Lateral shifts in rate-frequency functions measure 25–30-fold changes in the rewarding efficacy of the stimulation, produced by varying current intensity. The functions shown are the best-fitting broken-line functions. These were fit to rate-frequency data gathered at 0.1 log unit intervals on the frequency axis, over the range indicated by the horizontal extent of each function. (A) A selection from subject, R7, with the current intensity indicated by each function. The *x*'s are the data at 400 μA, to which the double-lined function was fit. (B) Twelve functions generated from subject CLO 11 by stepping the current in 0.1 log increments from 79 to 1000 μA.

repeatedly been emphasized [22,32]. Instead of measuring changes in the rewarding effect itself, we have measured changes in the rewarding efficacy of the stimulation, which is its capacity to produce some fixed level of reward.

Our measure derives from the effects of drugs on the rate-frequency function, a plot of the rat's rate of bar pressing against the logarithm of pulse frequency. We decompose the effect of a drug on the rate-frequency function into two components—a lateral shift and a change in shape. The lateral shift is the number of log units by which the half-maximal frequency of the new curve differs from the half-maximal frequency of the old. The half-maximal frequency is the frequency required to sustain performance half as fast as the upper performance asymptote for a given curve. The change in shape is the change in the upper and lower asymptotes of performance and in the slope of the transition between minimal performance and maximal performance.

The justification for the procedure rests on two findings: first, alterations in performance factors (produced by changes in task difficulty, by drugs, by illness, or by brain lesions) produce only small lateral shifts in the rate-frequency and rate-intensity functions (less than 0.1 log unit), even when the alteration in the performance factors changes the shape of these functions (see Fig. 2 of [5], and [24, 25, 31]). Second, changes in parameters of stimulation that alter the rewarding efficacy of the stimulation produce new psychometric functions that parallel the old ones (Fig. 1). The parallel functions obtained by changing current intensity are examples of pure lateral shifts. The parallelism means that the factor by which rewarding efficacy is altered is independent of the level of performance at which this factor is determined. These two findings motivate our decomposing any change in the rate-frequency function into a lateral shift (indicative of a change in rewarding efficacy) and a change in shape (indicative of a change in performance factors).

The size of the lateral shift in the rate-frequency function gives a physiologically interpretable measure of changes in rewarding efficacy, because the lateral shift is the change in the number of 0.1 msec cathodal pulses a train of fixed duration must contain to produce a mid-range level of reward. With brief cathodal pulses, it is likely that each pulse fires only one action potential in the reward-relevant axons within the radius of effective excitation (or, in any case, a fixed number of impulses in any particular axon). Since the number of reward-relevant action potentials is proportionate to the number of pulses, a two-fold change in the required number of pulses implies a two-fold change in the number of action potentials required to produce the same rewarding effect. The use of a physiologically interpretable behavioral measure lays the foundation for quantitative comparisons between behavioral findings and cellular findings. The neurophysiologically measured effect of a drug on the capacity of the stimulation to produce a fixed level of a cellular variable that constitutes either the rewarding signal or the ultimate rewarding effect itself must be quantitatively the same as its behaviorally measured effect on rewarding efficacy.

The Choice of Drugs

We determined the effects of the neuroleptic pimozide, because neuroleptics have repeatedly been shown to block the reinforcing effect of brain stimulation [12, 13, 16]. The neuroleptic dose required to produce extinction of self-stimulation correlates strongly with *in vitro* affinity for the D_2 receptor and not at all with affinity for any other aminergic receptor [17,34], including D_1. However, SCH 23390, which is thought from *in vivo* experiments to have low affinity for the D_2 receptor and high affinity for D_1, has recently been shown to attenuate the rewarding efficacy of stimulation, while sulpiride, which has the opposite pattern of dopamine receptor affinities, did not [27], so which dopaminergic receptor is crucial remains unresolved. We wanted to get dose-response data for a representative neuroleptic like pimozide, which has high affinity for the D_2 receptor [9,21] and has often been used in previous work.

As a check on the generalizability of our findings to other neuroleptics, we also tried molindone. Molindone appears to

have a two-fold dopaminergic action: in small doses, it blocks presynaptic autoreceptors, while in larger doses it acts like other neuroleptics to block the postsynaptic dopamine receptors [1].

We chose amphetamine, because it has been shown to enhance the rewarding effect [10]. It has also been shown to oppose the effects of the neuroleptic pimozide [18] and the alpha$_2$ agonist clonidine [20]. We wanted to explore this opposition in a more quantitative manner, to test whether the effects of the two drugs combined additively. Do doses of these drugs that by themselves shift the required number of pulses by equal factors in opposite directions cancel out when given concurrently?

The "dopaminergic hypothesis"—the hypothesis that a dopaminergic projection system forms a stage in the reward pathway—has been the dominant hypothesis regarding the pharmacological basis for self-stimulation for the last 10 years. It replaced the "noradrenergic hypothesis"—the hypothesis that an ascending noradrenergic projection system forms a stage in the reward pathway [30]—when it was shown that almost complete elimination of the noradrenergic projection to the forebrain left self-stimulation intact [6–8], whereas elimination of the dopaminergic projections prevented self-stimulation [28]. (For reviews, see [11, 35, 36].) However, the idea that noradrenergic systems are irrelevant to the rewarding effect of medial forebrain bundle stimulation is hard to reconcile with persistent findings regarding the effects of the alpha$_2$ agonist clònidine [2].

Clonidine was as effective as pimozide in laterally shifting the plot of running speed in an alley as a function of the number of pulses in a train of fixed frequency and variable duration [13]. This shift was reversed by piperoxane, an alpha$_2$ antagonist. The rewarding efficacy of a train of stimulating pulses varies with train duration [15], so the effects of the drug were confounded with the effects of train duration in this experiment. However, this does not invalidate the qualitative conclusion that clonidine reduces the rewarding efficacy. Clonidine has been shown to increase the latency to initiate rewarding stimulation in a shuttle box, and amphetamine reversed this effect [20]. Since clonidine has negligible affinity for the D$_2$ receptor ([9], p. 380), but has effects on the rewarding efficacy of stimulation similar to those of neuroleptics, we included it in our study, along with the alpha$_2$ antagonist yohimbine (piperoxane being no longer readily available). Yohimbine has been shown to inhibit the effects of clonidine at the alpha-adrenoreceptor in a variety of preparations, including flexor reflex activity and inhibition of alpha-methyltyrosine-induced disappearance of noradrenaline in the spinal cord and brain of rats [2]. Yohimbine attenuated the rate of response to hypothalamic stimulation in a two-way shuttle box, but this effect was thought to be a performance effect [20].

In sum, we measured the time course and dose-response characteristics of 5 drugs—pimozide, molindone, clonidine, amphetamine and yohimbine—and also the effects of combinations of these drugs—pimozide + amphetamine, pimozide + clonidine, clonidine + yohimbine, and clonidine + amphetamine.

METHOD

Subjects

The subjects were 10 male albino rats from the Charles River Breeding Laboratory, 90 to 120 days old and weighing 300 to 500 grams at the time they were implanted under ketamine anesthesia (150 mg/kg) with a single monopolar Formvar insulated stainless steel stimulating electrode (Plastic Products M303/1, 0.25 mm diameter, cross-section at tip uninsulated) aimed for the posterior medial forebrain bundle (4.0 mm behind bregma, 1.5 mm lateral to the sagittal suture, and 9.0 mm below the horizontal skull surface). The indifferent electrode was on the skull surface. The rats were individually housed in a reverse cycle room (lights off 8:30–18:30). All experimental procedures were carried out during their active period. The weights at the time of drug testing ranged from 300 to 800 grams. In 5 subjects, the location of the tip in the MFB at the level of the posterior hypothalamus was verified by standard histological procedures.

Apparatus

The rate-frequency functions were obtained in 4 Skinner boxes, 26 cm square and 46 cm high, with front walls of Plexiglas and the others of plywood. The floors were hardware cloth. A retractable rodent lever (BSR/LVE: RRL-015) extended from a side wall in each box, 5 cm above the floor. Stimulating leads were connected via a slip-ring. Trains of 0.1 msec cathodal pulses were generated by a constant current stimulator, whose output was shunted to the indifferent electrode between pulses, to prevent electrode polarization. Stimulating currents were monitored on a differential oscilloscope across a 1000 ohm resistor in series with the rat. Test sessions were controlled by the microcomputer-based system described in [5].

Testing Procedure

After a 7-day post-surgical recovery period, animals were shaped to press the lever for a 1 sec train at 50 pps. Only rats that learned within at most two $^1/_2$ hour shaping sessions were used. Current was rapidly increased from around 200 μA to between 400 and 700 μA during shaping, to promote more rapid stabilization of pressing at a high rate. After two stabilization sessions, rate-intensity functions were determined by varying current in 0.1 log unit steps. When the rate-intensity function was stable over three consecutive sessions, we estimated by interpolation the current required to produce half-maximal responding. The current thus selected was the current used in drug testing. We chose the current in this manner so that under baseline conditions the frequency required to sustain half-maximal responding in each rat would be within 0.1 log unit of 50 pps.

Before any drug test, we gathered at least four baseline rate-frequency functions in the following manner. The rat was connected to the stimulating leads, placed in a test box, and induced to self-stimulate, with the stimulating frequency set at 100 pulses per second (pps). After a 2 minute warm-up period, the stimulator was automatically switched off. The experimenter waited until the rat stopped pressing for at least 20 seconds, then initiated the data-gathering phase. During this phase, the lever first withdrew for 5 sec. As it extended back into the box, the rat received a single train at one of 16 pulse frequencies, ranging in 0.1 log steps from 10 pps to 320 pps (10, 13, 16, 25, 32 . . .). The system waited 15 seconds for the rat's rate of responding for this pulse frequency to stabilize, then counted the number of presses during the subsequent 60 seconds, at the end of which the lever withdrew for another 5 seconds. The pulse frequency in force for the next 75 seconds was indicated by a free train delivered as the lever extended back into the box; and so on,

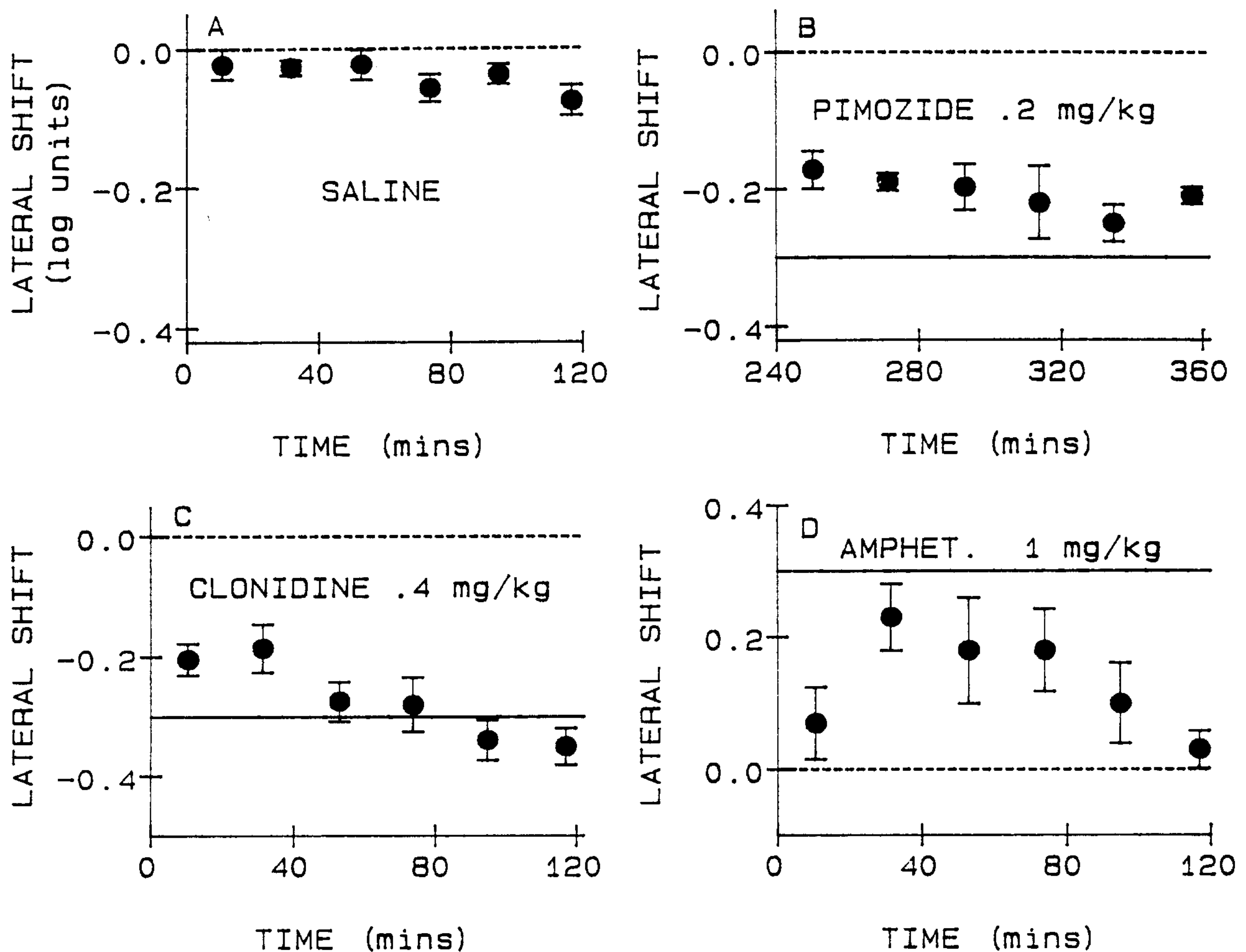

FIG. 2. Lateral shifts in the rate-frequency function as a function of time since injection. Error bars are ±1 sem. (A) Six passes following saline injection, N=6. (B) Six passes commencing four hours after an injection of pimozide. N=4. (C) Six passes following an injection of clonidine, N=4. (D) Six passes following an injection of amphetamine (1 mg/kg), N=7.

until all 16 frequencies were tested. The sequence of pulse frequencies was randomized at the beginning of each session. One complete "pass," during which all 16 frequencies were tested, took just over 21 minutes. In baseline sessions, there were always two complete passes.

When a session was complete, the computer calculated the "broken-line" function that best fit the rate-frequency data by the least squares criterion. The broken-line function is composed of three connected line segments: a horizontal lower asymptote, a horizontal upper asymptote, and a linear transition between the two (Fig. 1). The function is specified by the coordinates of its upper and lower break points. The half-maximal frequency for the function is the frequency at which the linear transition segment intersects a horizontal line half-way between the upper and lower asymptotes

On a drug testing day, testing began within minutes after the injections, except in the case of pimozide, where previous research had shown that its peak effect did not occur until 4–5 hours post-injection [3]. With pimozide, testing began four hours after injection. The testing procedure was identical to the baseline procedure, except that we ran 6 successive passes, one right after the other, in a session lasting just over two hours. Thus, we obtained 6 rate-frequency functions, one for each successive 21 minute period. In some cases, in order to define more fully the time-course of the drugs' effect, we initiated a further 6-pass, 2-hour session, half an hour after the completion of the first such session.

The animals were tested repeatedly, with different doses of the same drug, and with different drugs. Between drug testing days, there was always a recovery period of at least 48 hours, during which there were at least two baseline sessions. A drug test was not initiated unless the mean half-maximal frequency from a baseline session on the morning of testing was within 0.1 log unit of 50 pps. After weeks of testing, we sometimes observed abrupt and enduring shifts in the half-maximal frequencies obtained during baseline sessions. (This may have been due to small displacements of the electrode tip; such shifts are often, but not always, followed by loss of the electrode cap.) When this happened, we calculated the mean shift from 50 pps (in log units) and increased or decreased the current by this factor, in order to restore the baseline to the 50 pps value.

Drug Treatments

Saline. To provide the 0-dose data, we injected 1 cc of normal saline IP. This was the injection route and bolus vol-

ume for all the drugs. Normal saline was the vehicle for all the drugs except pimozide and yohimbine. Testing began within two minutes after the injection.

Pimozide (McNeil Pharmaceuticals) in doses of 0.1, 0.2, 0.4, and 0.6 mg/kg was dissolved in a 0.3% tartaric acid vehicle. Testing began 4 hours after the injection.

Molindone hydrochloride (Endo Pharmaceuticals) was administered in doses of 0.25, 0.5, and 1.0 mg/kg. Testing began within two minutes after the injection.

Amphetamine was administered in doses of 0.5, 1.0, and 3.0 mg/kg. Testing began within two minutes after the injection.

Clonidine hydrochloride (Sigma) was administered in doses of 0.03, 0.1, 0.2, 0.3, and 0.4 mg/kg. Testing began within two minutes after the injection.

Yohimbine hydrochloride (Sigma) was administered in doses of 0.5, 1, 5, and 10 mg/kg. Testing began immediately after the injection.

Pimozide and amphetamine. A 6-pass testing session began four hours after an injection of the 0.4 mg/kg dose of pimozide. At the end of the second pass (4 hours and 45 minutes after the pimozide injection), the rats were injected with either 1 or 3 mg/kg amphetamine and immediately replaced in the box, for Passes 3–6. After the larger dose, some animals were given a second 6-pass session, beginning half an hour after the completion of the first session.

Pimozide and clonidine. This treatment proceeded exactly as in the pimozide plus amphetamine treatment, except that clonidine in a dose of 0.2 mg/kg was injected following the second pass, rather than amphetamine.

Clonidine and yohimbine. Testing began 10 minutes after the injection of a 0.2 mg/kg dose of clonidine. At the end of the first pass (30 minutes after the clonidine injection), the rats were injected with a 0.5, 5, or 10 mg/kg dose of yohimbine and immediately replaced, for Passes 2–6.

Clonidine and amphetamine. This treatment proceeded exactly as in the clonidine plus yohimbine treatment, except that a 1 or 3 mg/kg dose of amphetamine was injected following the first pass, rather than yohimbine.

Determining the Range of Measurement

As testing progressed, it became clear that we were consistently failing to see alterations in rewarding efficacy of the size one would expect if the antagonists attenuated the rewarding signal by acting as competitive blockers of synaptic transmission at a synapse in the reward pathway. It became important to establish that the measurement method was capable of revealing much bigger effects than we were observing. Therefore, in two rats, we obtained rate-frequency functions at many different current intensities—from less than 100 μA to 1000 μA. We used the same procedure used in baseline testing, but we varied the range of pulse frequencies tested in a pass, so that this range spanned the dynamic interval of the rate-frequency function, the frequency range over which it rose from the lower to the upper asymptote. At most of the current intensities, we ran a single 2-pass session. However, at 400 μA we ran 5–6 passes in three sessions of one to two passes each, in order to assess how well the broken-line function fit the rate-frequency data.

RESULTS

The Range of Measurement

Figure 1 shows the shifts in the rate-frequency function produced by changing the current intensity of the stimulation from less than 100 μA to 1000 μA. For subject R7 (Fig. 1A), the half-maximal frequency ranged from log=1.14 (14 pps) at 1000 μA to log=2.65 (447 pps) at 63 μA. The lower value is 0.5 log units less than our baseline frequency of 50 pps (log=1.7), while the higher is more than 0.9 log units greater, altogether a 30-fold change in measured rewarding efficacy. For subject CLO 11 (Fig. 1B), the half-maximal frequency ranged from log=1.12 (13 pps) to log=2.52 (331 pps), a 25-fold change.

The broken-line functions fully represent the systematic variation in these data. The ×'s in Fig. 1A are the data at 400 μA and the double-lined function is the best-fitting function for these data. It accounts for 84% of the variance. (The other functions shown in Fig. 1 account for 72% to 97% of the variance in their respective data sets.) All of the residual variance is within-frequency (inherent) variance, because the ratio between the residual variance about the broken-line function and the within-frequency variance is slightly less than 1. Thus, no other function could account for significantly more of the variance in these data. A similar analysis on other sets of rate-frequency and speed-frequency data from different self-stimulation tasks yields the same result [33].

The transition segments of the rate-frequency functions remain parallel across the whole range of measurement. The mean slope of the transition for the two functions at the highest currents in Fig. 1A (630 and 1000 μA) is 20.1 presses/min per 0.1 log unit increment in pulse frequency, with a standard deviation of ±6; the mean for the lowest two currents (63 and 100 μA) is 27.2±16.1; and the slope at 400 μA is 17.2. Thus, the differences in the transition slopes for rate-frequency functions from opposite ends of this family of curves is less than the uncertainty regarding their true value, which is on the order of a factor of 2. Similarly, the mean slope for the highest two functions in Fig. 1B is 21.7, for the lowest two, 20.9 and for the curve at 400 μA, 21.6. It is evident from the variability in the slopes of the 12 functions plotted in Fig. 1B that these small differences in slope are much less than the variability in slopes from one determination to the next.

At very low current intensities, there is a decrease in the asymptotic rate of responding. We believe this reflects a decrease in the maximum possible reward, because there is no evident performance-hindering effect of the stimulation at these low currents and high frequencies.

Dose-Response and Time-Course Functions

Representative time course data for several of the treatments are given in Fig. 2. Negative shifts indicate a reduction of rewarding efficacy (a shift to the right in the rate-frequency function); positive shifts, an enhancement (a shift to the left). A shift of 0.3 log units represents a halving (in the case of negative shifts) or a doubling (in the case of positive shifts) of rewarding efficacy. A striking feature of the data is that none of the treatments produced alterations in rewarding efficacy substantially greater than this, even though the doses were increased to the point where the animals would no longer perform properly or where the side effects were so pronounced that testing with still higher doses did not seem advisable.

From plots like those in Fig. 2, we determined the passes during which the effects of the drug treatments appeared to be maximal. The dose-response data in Fig. 3 are from these

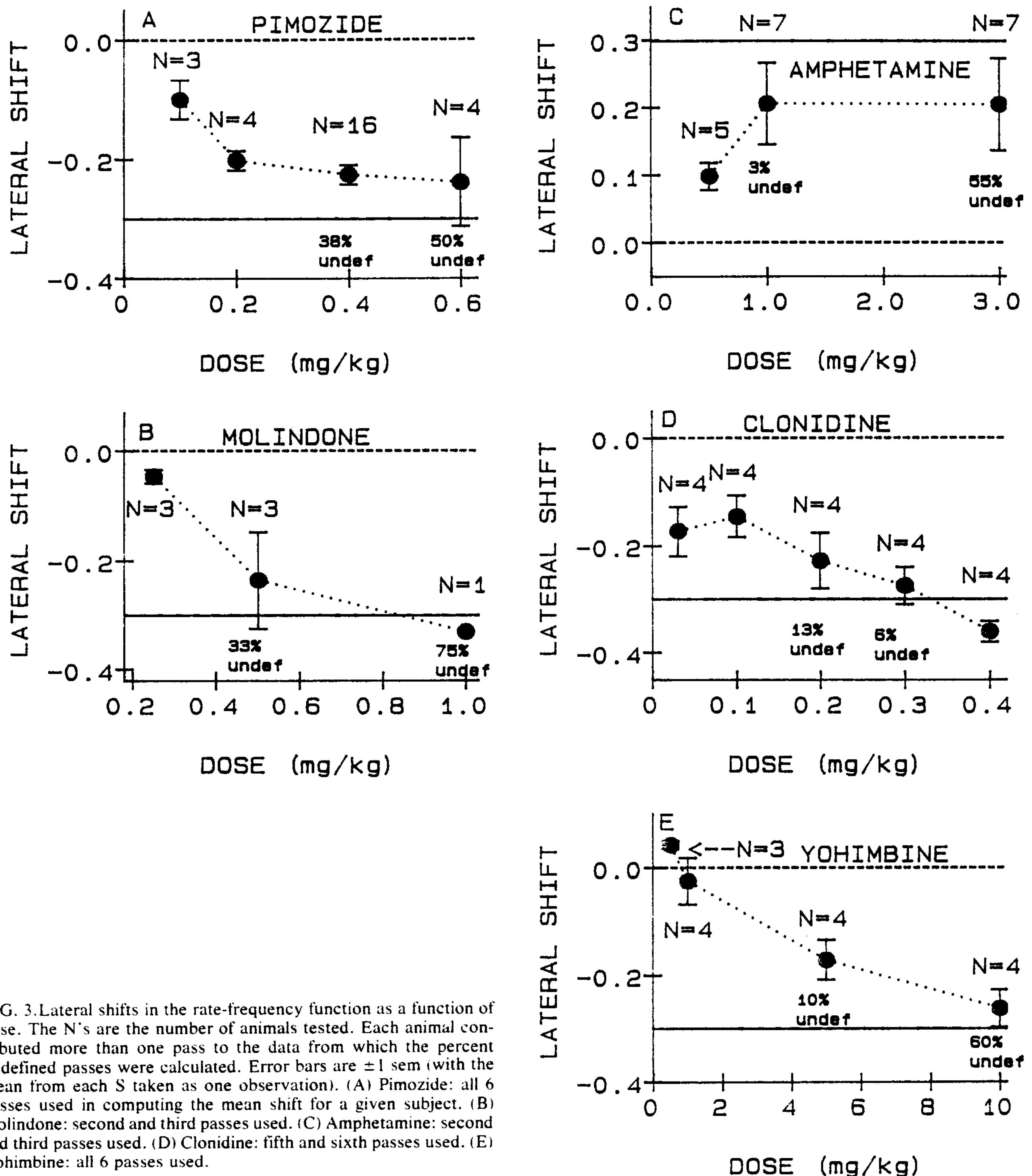

FIG. 3. Lateral shifts in the rate-frequency function as a function of dose. The N's are the number of animals tested. Each animal contributed more than one pass to the data from which the percent undefined passes were calculated. Error bars are ±1 sem (with the mean from each S taken as one observation). (A) Pimozide: all 6 passes used in computing the mean shift for a given subject. (B) Molindone: second and third passes used. (C) Amphetamine: second and third passes used. (D) Clonidine: fifth and sixth passes used. (E) Yohimbine: all 6 passes used.

passes. The 0-dose data are from the corresponding passes from the saline treatment. For example, we used data from Passes 5 and 6 to plot the maximum effects of the various doses of clonidine; therefore, the effect of a 0 dose is based on Passes 5 and 6 in the saline treatment. We present and briefly discuss the results from each type of drug treatment, then go on to the discussion of their overall implications.

Saline

The results with saline (Fig. 2A) indicate the temporal stability of the measure of rewarding efficacy, during the prolonged testing the rats received following treatment with the pharmacologically active agents. During the first six passes, the efficacy of the stimulation drifted slightly down-

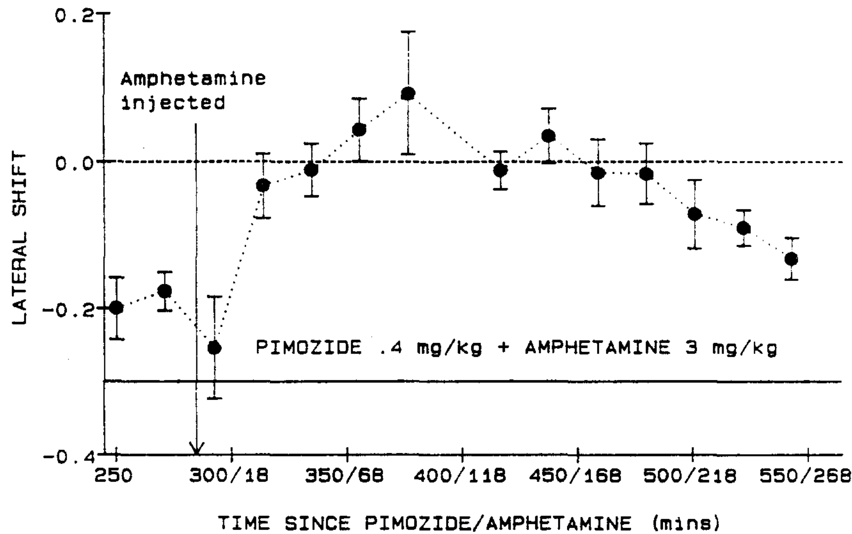

FIG. 4. The effects of pimozide and amphetamine cancel in a manner whose time course can be predicted from the time course of the individual effects. Error bars = ±1 sem. N=6 (animals, with one pass from each animal to each interval).

ward; on the 6th pass (2 hours after the onset of testing) the apparent efficacy of the stimulation was reduced by 0.07 log units (15%). When there was a subsequent 6-pass session, it did not further decline. This small negative shift is within the range of shifts that may be produced by performance factors, so one cannot say whether it reflects fatigue (a performance factor) or a decline in the efficiency of the reward pathway in consequence of repeated strong stimulation.

Pimozide

The effects of pimozide were constant throughout a 2-hour testing session that begins 4 hours after treatment. At 0.1 mg/kg, it produced a small (0.1 log unit=21%), statistically insignificant reduction in rewarding efficacy. At 0.2 mg/kg, there was a more substantial (0.2 log unit=37%) and statistically significant effect. Increasing the dose still further did not increase the size of the *measured* effect on rewarding efficacy (Fig. 3A). At 0.4 mg/kg, it was frequently not possible to obtain a rate frequency function; some animals did not respond consistently at any pulse frequency, although they commonly showed sporadic lever pressing at some of the higher pulse frequencies. Other animals responded consistently on some passes, but not on others. The result was that on about 40% of the passes, the frequency that produces half-maximal responding is undefined. The frequency was classified as undefined when either: (a) there was no variance in the rates of pressing, because there were no presses at any frequency, or (b) the broken-line function accounted for less than 50% of the variance, which happened when there were bursts of pressing at some but not all of the higher frequencies, producing a rate-frequency function with multiple rises and falls. On those passes that yielded a clear rate-frequency function, there was only a 0.23 log unit (41%) reduction in rewarding efficacy.

The failure to measure larger reductions in rewarding efficacy was not due to a frequency ceiling, an upper limit on the frequency, beyond which the reward relevant axons no longer respond to the stimulation. A 0.3 log unit negative shift from a 50 pps baseline means that the required frequency has increased from 50 to 100 pps. Much greater shifts may be obtained by varying current (Fig. 1). Also, negative shifts of 0.5–0.6 log units are produced by the direct injection of anticholinergics into the VTA [37]. Hence, the method is capable of measuring much larger negative shifts than are produced by pimozide.

It is possible, however, that pimozide reduces the capacity of the synapses in the reward pathway to respond to high frequency input. In that case, one might be able to get larger shifts by starting from a lower baseline. To test this, we reduced the baseline frequency from 50 to 25 pps in five animals (by increasing the current intensity) and tested them after treatment with 0.6 mg/kg of pimozide. Despite the reduction in baseline, more than 50% of the passes yielded undefined rate-frequency functions, and the half-maximal frequencies on the passes yielding a useable function were shifted only an average of 0.4 log units from the new baseline, that is, they were shifted only slightly beyond the old baseline frequency of 50 pps.

In sum, a dose of pimozide between 0.4 and 0.6 mg/kg reduces the rewarding efficacy of brain stimulation by a factor of approximately 2 and attempts to produce still greater reductions yield undefined rate-frequency functions, no matter what the baseline locus of rise. At doses of pimozide above 0.6 mg/kg, either its effects on performance factors become so severe that its effect on rewarding efficacy can no longer be measured, or it causes an abrupt failure in the reward system, so that no amount of stimulation can produce an acceptable rewarding effect.

Molindone

Molindone has a different time course: it achieves its peak effect about 20 minutes post-injection and its effect is noticeably reduced by 70 minutes post-injection. The dose-response results (from the period of peak effect, 20–80 minutes post-injection) parallel the results with pimozide (Fig. 3B): at 0.25 mg/kg, it had no significant effect; at 0.5 mg/kg, it produced a 0.23 log unit reduction in rewarding efficacy—and one began to see passes yielding undefined rate-

frequency functions. At 1 mg/kg, most of the passes yielded undefined functions. It appears that the quantitative effects of pimozide are representative of the quantitative effects of the D_2 antagonists.

We did not observe a biphasic action of molindone on rewarding efficacy within the dose range that we tried (0.25–1.0 mg/kg), nor did we see suggestions that such an effect might be present during the onset and offset of molindone's action.

Amphetamine

At 1 mg/kg, amphetamine rendered stimulation about 60% more effective (a 0.2 log unit increase, see Fig. 3C). The effect was maximal in the first or second pass (Fig. 2D) and was noticeably reduced by the 6th pass (2 hours post-injection). When we attempted to produce still greater enhancements by increasing the dose, the half-maximal frequency was no longer well defined in a large percentage of the passes, and the mean effect on those passes on which there was a definable half-maximal frequency was not increased. At doses in the 2–4 mg/kg range, the half-maximal frequency was undefined, because the lower asymptote rose to meet the upper asymptote, which is to say that the animal responded at a high rate no matter what the frequency. This is not because the half-maximal frequency was below 10 pps, the lowest frequency we used. Even when we shut the stimulator off altogether, the animals continued to press the lever for as long as we cared to observe them (15–30 minutes). At still higher doses (>4 mg/kg), amphetamine induced stereotypic behavior (repeated rearing) became so pronounced that the animals stopped pressing altogether.

In sum, the maximum alteration in rewarding efficacy that may be produced by amphetamine is comparable in magnitude but opposite in sign to the maximum effect produced by pimozide. In neither case can one change the efficacy by appreciably more than a factor or two.

Pimozide and Amphetamine

When the rewarding efficacy had been significantly reduced by a 0.4 mg/kg dose of pimozide, it was restored to the level seen in saline treated rats by the second pass after an injection of 1 mg/kg amphetamine. This is approximately the level predicted by summing the effects of the two drugs given separately (sum = −0.02, observed = −0.07). When 3 mg/kg of amphetamine was used, the net effect eventually became slightly positive (rewarding efficacy was higher than under baseline conditions). The rewarding efficacy was significantly greater than in saline treated animals over the interval from 75 to 160 minutes after the amphetamine injection (Fig. 4). This was a slightly (but statistically insignificant) greater reversal than would be predicted from the sum of the individual effects (sum = −0.03, observed = 0.05), but it should be borne in mind that the measured value of the amphetamine at this dose was distorted by the fact that many passes yielded undefined half-maximal frequencies. In short, the data provide no reason to reject the hypothesis that the opposing effects of the two drugs combine additively.

Amphetamine enhances the release of dopamine, among many other actions. The fact that its effect and the effect of the dopamine antagonist pimozide cancel each other out has been taken as evidence that both compounds acted via a dopaminergic projection system [18]. However, the results obtained with clonidine and amphetamine (see below) call this interpretation into question.

Clonidine

This $alpha_2$ agonist, with negligible affinity for the D_2 receptor, presents a somewhat different picture. Its action reached its peak about 90 minutes after injection and was attenuated at the end of 4 hours (Fig. 2C). With this drug, there were very few passes yielding undefined rate-frequency functions (Fig. 3D), even at the highest dose (0.4 mg/kg). The highest dose reduced rewarding efficacy by 0.36 log units (54%). The dose-response curve is shallow: the drug had a statistically significant effect at a dose as low as 0.03 mg/kg, but an increase of more than an order of magnitude in this dose reduced rewarding efficacy by only slightly more than a factor of 2. At this high dose, the side effects of the drug are pronounced. Fear of damaging the animals prevented our trying still higher doses.

At a dose of 0.2 mg/kg, there was no significant difference in the extent to which pimozide and clonidine reduced the rewarding efficacy of brain stimulation (mean reductions of 0.20 and 0.23 log units, respectively), despite the fact that their affinity for the D_2 receptor differs by at least 5 orders of magnitude. The strong correlation between neuroleptic affinity for the D_2 receptor and neuroleptic potency in blocking the rewarding effect of brain stimulation [17] suggests that the effect of neuroleptics on rewarding efficacy is mediated by their binding to this receptor. If this assumption is correct, then the effect of clonidine must have a different neurochemical basis than the effects of neuroleptics.

Yohimbine

The uncertainty regarding the neurochemical mechanism that mediates clonidine's effect on rewarding efficacy is deepened by the results of yohimbine treatment. Yohimbine, which is an $alpha_2$ antagonist, produced a slight but statistically significant enhancement of rewarding efficacy at the lowest dose we tried (0.5 mg/kg, see Fig. 3E). The effect was maximal during the first four passes (15–75 minutes post-injection) and was gone by the 6th pass (110 minutes post-injection). At 1 mg/kg, yohimbine had no significant effect; at 5 mg/kg, it significantly reduced rewarding efficacy (by 0.17 log units = 32%), without producing any undefined rate-frequency functions; while at 10 mg/kg, the reduction in rewarding efficacy was 0.26 log units (45%), but 60% of the passes yielded undefined functions. At the higher doses, where yohimbine reduced rewarding efficacy, the effect was maximal on the first pass and disappeared by the 12th pass (5 hours post-injection).

Clonidine and Yohimbine

Yohimbine did not counteract the effect of a 0.2 mg/kg dose of clonidine at any dose; at the lower doses (0.5 and 5 mg/kg), it neither counteracted nor enhanced clonidine's effect. In the case of the 5 mg/kg dose (Fig. 5B), there is a statistically significant failure of additivity (sum = −0.40, observed = −0.22). The frequency of undefined passes prevented our testing for additive combination at the highest dose of yohimbine. The finding that yohimbine does not counteract clonidine's effect on reward is consistent with previous findings [20]. The previously noted negative effect of yohimbine by itself on self-stimulation performance has been attributed to performance factors [20], but our findings show that there is an effect on reward as well, when the dose is raised to the 5–10 mg/kg range. In the 0.5–2 mg/kg range, where yohimbine has been reported to increase the latency

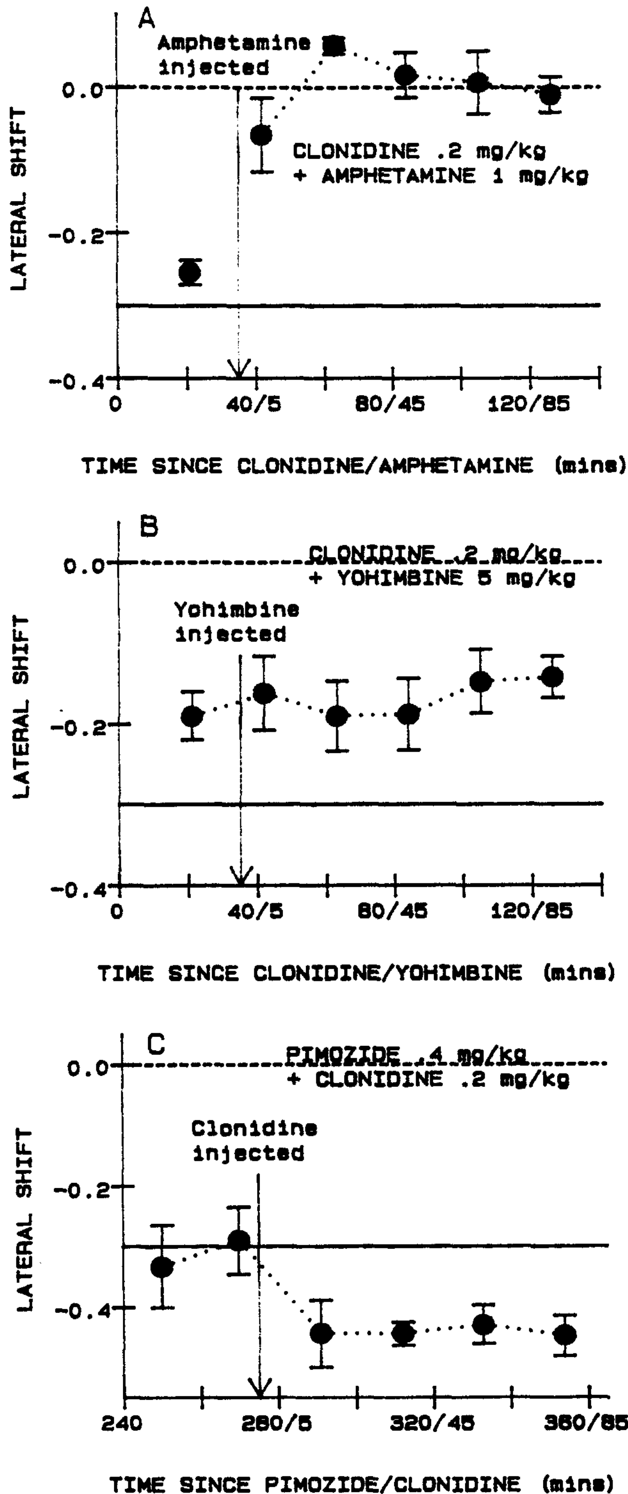

FIG. 5. The effects of pairwise drug combinations over time. Error bars=±1 sem. N=4 (animals, with one pass from each animal at each interval). (A) Amphetamine opposes the effect of clonidine. (B) The effects of clonidine and yohimbine do not combine additively; yohimbine, which by itself produces a −0.17 log unit shift at this dose, does not augment the effect of clonidine. (C) The effects of pimozide and clonidine do combine additively: the effect following the clonidine injection is very close to the sum of the individual effects of these drugs at these doses.

to initiate rewarding stimulation in a shuttle box [20], our data show a positive or negligible effect on rewarding efficacy itself. This highlights the importance of a measurement method that distinguishes effects on performance factors from effects on reward. Yohimbine neither counteracts nor supplements clonidine's effects, despite the fact that they are thought to bind to the same receptor. By contrast, as reported below, pimozide and clonidine combine additively in their effect on rewarding efficacy, even though there is no known receptor to which they both bind.

Clonidine and Amphetamine

Amphetamine (1 mg/kg) counteracted clonidine (0.2 mg/kg), with somewhat greater potency than it counteracted the effects of pimozide (0.4 mg/kg), as shown in Fig. 5A. However, there are no grounds for rejecting the hypothesis that the effects of clonidine and amphetamine are additive, just as are the effects of pimozide and amphetamine. Given the small range of measurable effects produced by any of the three drugs, it would require very precise data to reject the assumption of additive combination. Just as the counteracting effects of amphetamine and pimozide on rewarding efficacy have been interpreted in terms of the actions of both drugs at dopaminergic synapses, so the counteracting effects of amphetamine and clonidine have been interpreted in terms of amphetamine's capacity to block clonidine's inhibition of noradrenalin release [20]. Both interpretations focus on the action of amphetamine within the same synaptic system in which the countervailing drug is presumed to act. One difficulty with this interpretation of amphetamine's effect on clonidine is that the $alpha_2$ antagonist yohimbine does not have a comparable effect. If amphetamine antagonizes the effect of clonidine on rewarding efficacy by blocking the $alpha_2$-mediated action of clonidine, then yohimbine ought to have the same effect, but it doesn't. A second difficulty is that the same dose of amphetamine that antagonizes clonidine's effect also antagonizes pimozide's effect, even though pimozide is not an $alpha_2$ agonist, like clonidine, and clonidine is not a D_2 antagonist, like pimozide. It appears that the effects on rewarding efficacy from agents that act on catecholaminergic receptors cannot be understood in terms of their known effects on any one receptor.

Clonidine and Pimozide

When 0.2 mg/kg of clonidine was injected into rats that had received 0.4 mg/kg of pimozide approximately 5 hours earlier, the clonidine substantially reinforced pimozide's effect on rewarding efficacy. The resulting 0.44 log unit reduction in rewarding efficacy was very nearly the sum of the effects of the two drugs given individually (sum=−0.46, observed=−0.44). We are not aware of any receptor binding data that would explain these two drugs having essentially additive effects at approximately equivalent doses. The fact that in combination they produce a greater *measurable* attenuation of rewarding efficacy than pimozide alone can produce would suggest that they act on different receptors and that their effects are summated at a postreceptor stage. The summation of the effects of clonidine and pimozide are more remarkable in that the effects of clonidine and yohimbine do not summate.

DISCUSSION

The usual assumption in catecholaminergic theories of

reward is that a catecholaminergic projection system is a stage in the reward pathway, the pathway that transmits the rewarding signal from the site of stimulation to the point where it is converted into an enduring rewarding effect (a memory of past reward). If that were so, then one would expect that an appropriate catecholaminergic antagonist would produce a graded reduction in rewarding efficacy, with a dose-response curve analogous to that obtained when rewarding efficacy is reduced by reducing current intensity. This is not what one observes. The maximum reduction in rewarding efficacy that we could reliably produce with any of the drugs here tested was a reduction by a factor of 2. Similar results have recently been reported for the effect of pimozide on self-stimulation of the central grey [23]. The simplest explanation for this is that at high doses side effects of these drugs prevent performance, making it impossible to measure their effects on rewarding efficacy. However, we are inclined to reject this explanation.

In the case of clonidine, the side effects of the drug did prevent the testing of higher doses, but not because they prevented performance. The animals responded well at the highest doses tried, although the side effects of the drug were so extreme that we did not wish to try still higher doses. From the shallow slope of the dose-response function, it was clear that achieving a 0.6 log unit (75%) reduction in rewarding efficacy would require damaging or lethal doses of the drug.

The fact that the clonidine treated animals responded regularly despite the pronounced side effects of the drug should be borne in mind in evaluating the failure of animals to perform when treated with doses of pimozide greater than about 0.5 mg/kg, because the clonidine results are indicative of the robustness of self-stimulation performance. When a rat is treated with sub-anaesthetic doses of a general anaesthetic, it will self-stimulate so long as it can drag itself to the lever [16]. Doses of pimozide in the 0.5–5 mg/kg range produce some reduction in spontaneous activity (although this is hardly noticeable in many rats), but rats treated with doses in this range have repeatedly been shown to be capable of performing a variety of experimental tasks, including the lever-pressing tasks, and more demanding runway and running wheel tasks: doses of 0.75–1.5 mg/kg, which abolished sustained self-stimulation at all current intensities, had little or no effect on the latency with which the same animals pressed the same lever to turn the stimulation off, and vigorous responding to turn stimulation off continued throughout sessions equal in length to those used here [26]. Also, rats treated with as much as 5 mg/kg of pimozide (an order of magnitude greater dose) ran at normal rates on the first trial in a runway that they had learned to run for brain stimulation reward [16]. The decline in their running speed over subsequent trials paralleled the decline seen when the stimulator was turned off, suggesting that it was a failure of the stimulation to have a rewarding effect that led to the decline. This interpretation was confirmed in rats treated with 0.5 and 0.75 mg/kg, by showing that, after they refused to press any more in a Skinner box, they would transiently resume normal performance in a runway (and vice versa)—that is, the extinction was task specific. When pimozide treated animals were given stimulation-elicited running sessions in a running wheel prior to the runway testing, they did not slacken their running during several minutes in the running wheel, and their subsequent performance on the initial trials in the runway was normal. When, after 6–15 trials in the runway they refused to perform, they were returned to the running wheel, where they continued to run in response to stimulation for many minutes.

These findings and other similar findings [14,36] indicate that doses of pimozide below 5 mg/kg do not have motor side effects sufficient to prevent sustained performance of the lever pressing response. We therefore suggest that the failure for rats to show sustained performance in our measuring paradigm when the dose exceeds about 0.5 mg/kg indicates either a failure of effective transmission in the reward pathway or a failure in the process that converts the transmitted signal into a rewarding effect (a memory of the reward received). We suggest that neuroleptics modulate some physiological variable relevent to the proper operation of the reward pathway. The response of the rewarding process to the pimozide-induced change in the level of this variable is a modest reduction in rewarding efficacy, up to the point where the process abruptly fails altogether.

More generally, the pattern of our quantitative results with all of the catecholaminergic agents leads us to suggest that their effects on rewarding efficacy are indirect. We suggest that none of them acts directly on a receptor involved in the transmission of the rewarding signal. Instead, these agents may alter the values of one or more homeostatic parameters in the relevant neural circuitry. By homeostatic parameters, we mean variables whose values are actively maintained within narrow limits, because the circuitry cannot function properly when their values stray outside those limits. On this hypothesis, modest alterations in rewarding efficacy show up when the value of some crucial parameter is pushed to the limit of the normal range. The system fails altogether when it is pushed beyond that limit, so that the change in rewarding efficacy becomes unquantifiable.

ACKNOWLEDGEMENTS

This research was supported by National Science Foundation Grant BNS 82 11972 and by Biomedical Research Support subgrant 2-SO7-RR-07083-20 SUB 08. An initial draft of this report was written while C.R.G. was at the Center for Advanced Study in the Behavioral Sciences, with partial fellowship support from the Alfred P. Sloan Foundation.

REFERENCES

1. Alander, T., M. Grabowski-Anden and N. E. Anden. Physiological significance of dopamine autoreceptors as studied following their selective blockade by molindone. *J Pharmacol* 32: 780–782, 1980.
2. Anden, N.-E., M. Grabowski and U. Strombom. Different alpha-andrenoreceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Arch Pharmacol* 292: 43–52, 1976.
3. Atalay, J. and R. A. Wise. Time course of pimozide effects on brain stimulation reward. *Pharmacol Biochem Behav* 18: 655–658, 1983.
4. Breese, G. R. and B. R. Cooper. Relationship of dopamine neural systems to the maintenance of self-stimulation. In: *Neurotransmitter Balances Regulating Behavior*, edited by E. F. Dominao and J. M. Davis. Ann Arbor, MI: 1975.

5. Campbell, K. A., G. Evans and C. R. Gallistel. A microcomputer-based method for physiologically interpretable measurement of the rewarding efficacy of brain stimulation. *Physiol Behav* **35**: 395–403, 1985.
6. Clavier, R. M. and H. C. Fibiger. On the role of ascending catecholaminergic projections in intracranial self-stimulation of substantia nigra. *Brain Res* **131**: 271–286, 1977.
7. Cooper, B. R., J. M. Cott and G. R. Breese. Effects of catecholamine-depleting drugs and amphetamine on self-stimulation of brain following various 6-hydroxydopamine treatments. *Psychopharmacologia* **37**: 235–248, 1974.
8. Corbett, D., R. W. Skelton and R. A. Wise. Dorsal bundle lesions fail to disrupt self-stimulation from the region of locus coeruleus. *Brain Res* **133**: 37–44, 1977.
9. Creese, I., D. Burt and S. Snyder. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. *Science* **192**: 481–483, 1976.
10. Esposito, R. U., W. Perry and C. Kornetsky. Effect of d-amphetamine and naloxone on brain stimulation reward. *Psychopharmacology (Berlin)* **69**: 187–193, 1980.
11. Fibiger, H. C. Drugs and reinforcement mechanisms: A critical review of the catacholamine theory. *Annu Rev Pharmacol Toxicol* **18**: 37–56, 1978.
12. Fouriezos, G. and R. A. Wise. Pimozide-induced extinction of intracranial self-stimulation: Response patterns rule out motor performance deficits. *Brain Res* **103**: 377–380, 1976.
13. Franklin, K. B. J. Catecholamines and self-stimulation: Reward and performance deficits dissociated. *Pharmacol Biochem Behav* **9**: 813–820, 1978.
14. Franklin, K. B. J. and S. N. McCoy. Pimozide-induced extinction in rats: Stimulus control of responding rules out motor deficit. *Pharmacol Biochem Behav* **11**: 71–76, 1979.
15. Gallistel, C. R. Self-stimulation in the rat: Quantitative characteristics of the reward pathway. *J Comp Physiol Psychol* **92**: 977–998, 1978.
16. Gallistel, C. R., M. Boytim, Y. Gomita and I. Klebanoff. Does pimozide block the reinforcing effect of brain stimulation? *Pharmacol Biochem Behav* **17**: 769–781, 1982.
17. Gallistel, C. R. and A. J. Davis. Affinity for the dopamine D_2 receptor predicts neuroleptic potency in blocking the reinforcing effect of MFB stimulation. *Pharmacol Biochem Behav* **19**: 867–872, 1983.
18. Gallistel, C. R. and D. Karras. Pimozide and amphetamine have opposing effects on the reward summation function. *Pharmacol Biochem Behav* **20**: 73–77, 1984.
19. Gallistel, C. R., P. Shizgal and J. S. Yeomans. A portrait of the substrate for self-stimulation. *Psychol Rev* **88**: 228–273, 1981.
20. Hunt, G. E., D. M. Atrens, F. T. Becker and G. Paxinos. Alpha-adrenergic modulation of hypothalamic self-stimulation: effects of phenoxybenzamine, yohimbine, dexamphetamine and their interactions with clonidine. *Eur J Pharmacol* **53**: 1–8, 1978.
21. Leysen, J. W., W. Gommeren and P. Laduron. Spiperone: a ligand of choice for neuroleptic receptors. *Biochem Pharmacol* **27**: 307–316, 1978.
22. Liebman, J. M. Discriminating between reward and performance: A critical review of intracranial self-stimulation methodology. *Neurosci Biobehav Rev* **7**: 45–72, 1983.
23. Miliaresis, E., J. Malette and D. Coulombe. The effects of pimozide on the reinforcing efficacy of central grey stimulation in the rat. *Behav Brain Res* **21**: 95–100, 1986.
24. Miliaresis, E., P.-P. Rompre and A. Durivage. Psychophysical method for mapping behavioral substrates using a moveable electrode. *Brain Res Bull* **8**: 693–701, 1982.
25. Miliaresis, E., P.-P. Rompre, P. La Violette, L. Philippe and D. Coulombe. The curve-shift paradigm in self-stimulation. *Physiol Behav* **37**: 85–91, 1986.
26. Miller, P. An investigation of neural substrates of reward and aversion in rats. Ph.D. thesis, University of Pennsylvania, 1981.
27. Nakajima, S. and G. M. McKenzie. Reduction of the rewarding effect of brain stimulation by a blockade of dopamine D_1 receptor with SCH 23390. *Pharmacol Biochem Behav* **24**: 919–922, 1986.
28. Phillips, A. G. and H. C. Fibiger. Long term deficits in stimulation induced behaviors and self-stimulation after 6-hydroxydopamine administration in rats. *Behav Biol* **16**: 127–143, 1976.
29. Stein, L. Self-stimulation of the brain and the central stimulant action of amphetamine. *Fed Proc* **23**: 836–841, 1964.
30. Stein, L. Chemistry of purposive behavior. In: *Reinforcement and Behavior*, edited by J. T. Tapp. New York: Academic, 1969, pp. 328–355.
31. Stellar, J. R. and S. P. Neeley. Reward summation function measurements of lateral hypothalamic stimulation reward: effects of anterior and posterior medial forebrain bundle lesions. In: *Neuroscience of Feeding and Reward*, edited by B. G. Hoebel and D. Novin. Brunswick, ME: Frederick Haer, 1982.
32. Valenstein, E. S. Problems of measurement and interpretation with reinforcing brain stimulation. *Psychol Rev* **71**: 415–437, 1964.
33. Waraczynski, M., J. R. Stellar and C. R. Gallistel. Reward saturation in medial forebrain bundle self-stimulation. Submitted.
34. Wauquier, A. The influence of psychoactive drugs on brain self-stimulation in rats: A review. In: *Brain Stimulation Reward*, edited by A. Wauquier and E. T. Rolls. New York, Elsevier, 1976, pp. 123–170.
35. Wise, R. A. Catecholamine theories of reward: A critical review. *Brain Res* **152**: 215–247, 1978.
36. Wise, R. A. Neuroleptics and operant behavior: The anhedonia hypothesis. *Behav Brain Sci* **5**: 39–87, 1980.
37. Yeomans, J. S., O. Kofman and V. McFarlane. Cholinergic involvement in lateral hypothalamic rewarding brain stimulation. *Brain Res* **329**: 19–26, 1985.