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Differential Effects of *d*-Amphetamine, Pipradrol and Bupropion on Shuttlebox Self-Stimulation

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LIEBMAN, J. M., S. GERHARDT AND J. PROWSE. *Differential effects of* d-*amphetamine, pipradrol and bupropion on shuttlebox self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 16(5) 791–794, 1982.—The shuttlebox self-stimulation test is claimed by Atrens to differentiate drug effects on brain stimulation reward from those on performance variables. Thus, for example, drug-induced enhancement of the reward value of stimulation should be reflected in a selective reduction of the latency to initiate stimulation (the ON latency), as compared with the latency to terminate stimulation (the OFF latency). The effects of the psychostimulant drugs, *d*-amphetamine and pipradrol, and the antidepressant, bupropion, were evaluated in this procedure as well as in a bar-pressing test of self-stimulation. Pipradrol (3 and 10 mg/kg) and bupropion (54 mg/kg) reduced ON latencies by 40% or more but failed to shorten OFF latencies, indicating that performance variables were not involved in the ON latency decrements. Although *d*-amphetamine (0.3 and 1.0 mg/kg) shortened ON latencies, the 1.0 mg/kg dose also reduced OFF latencies. Drug doses that reduced ON latencies also increased bar-pressing self-stimulation. The shuttlebox self-stimulation test appears to be capable of discriminating drug-induced enhancement in brain stimulation reward from performance variables.

Self-stimulation Shuttlebox *d*-Amphetamine Bupropion Pipradrol

ACCORDING to Atrens and co-workers [1,10], the shuttlebox self-stimulation procedure can dissociate drug effects on brain stimulation reward from those on performance variables. For example, the ON latency (i.e., the latency to activate reinforcing brain stimulation) is elevated by optimal doses of drugs that disrupt noradrenergic and/or dopaminergic neurotransmission, while the OFF latency (the latency to terminate stimulation) remains unaltered [1, 6, 13]. If the OFF latency fails to increase, it is inferred that confounding performance variables cannot wholly account for the observed drug effects (but see [8]).

Conversely, according to this reasoning, drug-induced increases in the reward value of brain stimulation should be reflected in selective decrements in ON latencies. At the same time, the OFF latency should either remain unchanged or increase if the drug in question is claimed to have a selective effect on reward, apart from nonspecific psychomotor activation. Such a demonstration would extend the usefulness of the shuttlebox self-stimulation test, and strengthen interpretation of drug effects in this procedure. In fact, a low dose of *d*-amphetamine (0.5 mg/kg) has been reported to reduce the ON latency selectively [10]. Using various tests of self-stimulation, others have shown that *d*-amphetamine lowers the "reward" threshold and otherwise enhances self-stimulation [8,15]. At certain doses and electrode placements, however, decrements in the shuttlebox OFF latency also can be produced by *d*-amphetamine [2].

We have re-examined this question by comparing the effects of various doses of *d*-amphetamine, pipradrol and bupropion in both the shuttlebox and bar-pressing self-stimulation test procedures. The effects of bupropion were of particular interest as this novel antidepressant has stimulus properties similar to psychomotor stimulants in animals [11]. Bupropion shows little or no undesirable psychomotor stimulant activity in humans [7], in contrast to *d*-amphetamine and pipradrol [4,16].

METHOD

Animals and Surgical Procedures

Male Fischer (F-344, Charles River) rats (250–300 g) were anesthetized and bipolar electrodes were stereotaxically implanted in the lateral hypothalamus (see [13] for details).

Behavioral Procedures

The apparatus was similar to that used by Atrens and co-workers [1] and has been described elsewhere [13]. Brain stimulation was delivered by a Haer 4 bp stimulator according to the following parameters: pulse duration, 0.4 msec; pulse frequency, 100 Hz; current intensity 40 to 200 μA (biphasic square wave pulses). Task programming and training procedures are described in detail elsewhere [13]. Briefly, interruption of a photocell beam at one end of the shuttlebox caused continuous brain stimulation to be activated. Stimulation was terminated when the rat interrupted another photocell beam at the opposite end of the box.

A total of 17 rats were used for shuttlebox experiments. In these rats, current intensity was individually adjusted so as to yield between 35 and 80 crossing cycles per session.

Rats received drug treatments after at least two days of stable shuttlebox performance within these limits. An additional constraint on baseline performance was that drug data were not collected if either the baseline ON or OFF latency for a given rat was less than 2.0 sec. The reason for this additional criterion was that very short latencies were found in pilot studies to be relatively insensitive to drug-induced effects [13]. The rationale for these procedures is described in more detail elsewhere [13].

A separate group of rats (n=27) was used for the bar-pressing self-stimulation experiments. After initial training to bar-press for brief brain stimulation trains (train duration 100 msec, biphasic square wave pulses, pulse duration 0.05 msec) on a continuous reinforcement schedule, these rats were tested in 15 min sessions. Current intensity was progressively reduced in succeeding sessions until a submaximal response rate was consistently elicited, typically 300 to 800 bar-presses per session. Drug treatments were administered after at least three consecutive days of stable baseline responding occurred within these limits.

Drug Treatments

Drugs and sources were, respectively: bupropion hydrochloride (synthesized by CIBA-GEIGY chemists), *d*-amphetamine sulfate (Smith, Kline and French, Philadelphia, PA) and pipradrol hydrochloride (Merrell, Cincinnati, OH). Drug doses were expressed as the respective salts. Threefold dose increments were utilized for dose-response studies. In one case (bupropion), an additional dose (54 mg/kg) was logarithmically interpolated between the 30 and 100 mg/kg doses. All injections were intraperitoneal in normal saline solution, using a volume of 1 ml/kg body weight. At least five days elapsed between successive drug treatments. No evidence of tolerance to drug effects was seen under these conditions.

In the shuttlebox experiments, each rat received all doses of a given drug. An incomplete block design, in which each rat received only two doses of a given drug, was utilized for bar-pressing and self-stimulation experiments. In both procedures, drugs were administered 30 min before testing. A second bar-pressing session was also conducted 3 1/2 hr after pipradrol and bupropion. Since the facilitatory effects of these drugs on bar-pressing were always more prominent at the 30 min interval, the data for 3 1/2 hr are not reported.

Analysis of Data

The method of shuttlebox data analysis is described elsewhere [13]. Briefly, regression analyses were performed separately on log transforms of ON and OFF latency data. If a significant dose-response relationship was found, the trend test [3] was then employed to identify doses that significantly ($p<0.05$) increased latency over the pre-drug baseline. The percent change in the ON latency, relative to baseline, was also compared directly with that in the OFF latency in a separate analysis. Following ANOVA to determine whether significant main effects of type of latency and of dose were present, matched pair *t*-tests were performed to compare these percent changes from baseline at given drug doses.

Bar-pressing data were evaluated statistically by analysis of variance that included a test for significance of the linear dose-response component. Trend tests [3] were then performed to determine which doses produced a significant increase in responding from baseline control values.

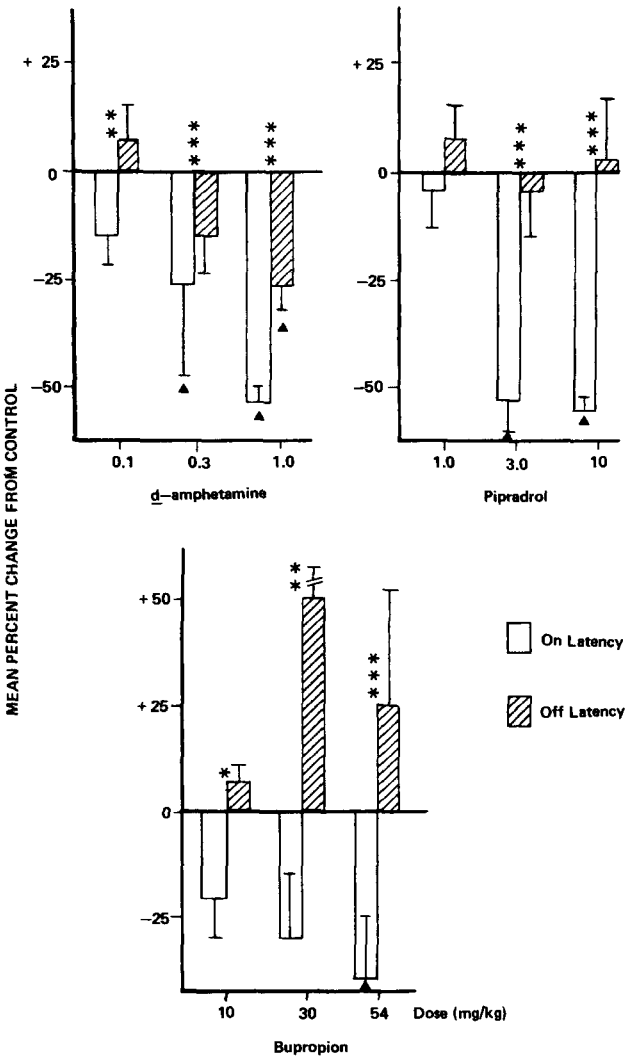

FIG. 1. Effects of *d*-amphetamine, pipradrol and bupropion on shuttlebox self-stimulation. Bars indicate mean (±S.E.) percent increase in latency from pre-drug baseline. ▲ Significantly different from pre-drug baseline by the trend test, $p<0.05$; *percent increase in ON latency significantly differed from that in the OFF latency, $p<0.05$; **$p<0.01$, ***$p<0.001$. See text for explanation of statistical analyses. Treatment group sizes: *d*-amphetamine, n=8; pipradrol, n=8; bupropion, n=7.

Histology

At the conclusion of experimentation, representative rats were sacrificed by overdose of anesthesia, followed by transcardial perfusion of 50 to 100 ml normal saline and 50 to 100 ml Formalin. Brains were removed, allowed to stand in Formalin for at least 24 hr, then were frozen and sectioned for histological examination using a cresyl violet stain.

RESULTS

Pre-Drug Baselines

The mean shuttlebox baseline latencies in each group

TABLE 1

EFFECTS OF *d*-AMPHETAMINE, PIPRADROL AND BUPROPION ON BAR-PRESSING SELF-STIMULATION

Treatment	Dose mg/kg IP	Percent Increase in Responses (mean ± S.E.)
d-Amphetamine	0.1	+ 24 ± 11
	0.3	+ 43 ± 19
	1.0	+107 ± 30*
	2.0	+101 ± 38*
Pipradrol	0.3	+ 12 ± 25
	1.0	+ 32 ± 13
	3.0	+ 97 ± 51*
Bupropion	3.0	+ 5 ± 7
	10	+ 45 ± 16
	30	+ 87 ± 24*

*$p<0.05$ for difference from pre-drug baseline by trend test. Treatment group sizes: *d*-amphetamine, n=8; pipradrol, n=6; bupropion, n=6.

prior to drug treatment ranged from 4.8 to 6.8 sec (ON latency) and from 5.8 to 7.6 sec (OFF latency). In no case did the group baseline ON and OFF latencies differ significantly prior to a given drug treatment ($p>0.10$ for all comparisons, matched pair *t*-test, two-tailed). The mean baseline bar-press rates prior to treatment with any given drug dose ranged from 433 to 632 per 15 min session.

d-Amphetamine

Administration of 0.3 or 1 mg/kg *d*-amphetamine significantly reduced mean ON latencies from pre-drug baseline values (Fig. 1). At these doses and at 0.1 mg/kg as well, the percent reduction in ON latencies significantly exceeded that in the corresponding OFF latencies. However, a small reduction in OFF latencies was also evident and this reduction reached statistical significance at the 1 mg/kg dose when compared with baseline.

The enhancement of bar-pressing self-stimulation by *d*-amphetamine was dose-related and reached statistical significance at 1 and 2 mg/kg with a strong trend evident at 0.3 mg/kg (Table 1).

Pipradrol

Pipradrol (3 and 10 mg/kg) also reduced ON latencies significantly by comparison with pre-drug baseline values, and the magnitude of these decrements was at least as great as those associated with *d*-amphetamine. Pipradrol failed, however, to reduce OFF latencies at any of these doses.

The dose-response relationship for the effects of pipradrol on bar-pressing self-stimulation approached significance ($p=0.06$) (Table 1). The trend test indicated a significant increase in bar-pressing at 3 mg/kg, the highest dose tested.

Bupropion

Bupropion reduced ON latencies at 10, 30 and 54 mg/kg, but OFF latencies actually increased at all of these doses (Fig. 1). Generally, bupropion's effects on shuttlebox performance were more variable than those of *d*-amphetamine or pipradrol, as indicated in the relatively large standard errors. The percent change in the ON latencies differed significantly from the OFF latencies at all of these doses; additionally, bupropion reduced ON latencies significantly as compared with baseline at the 54 mg/kg dose. The increases in the OFF latencies were highly variable and failed to reach significance at any of these doses.

A 100 mg/kg dose of bupropion was also tested in the shuttlebox procedure (data not shown). This dose produced a large increase in both the ON and OFF latencies and two of seven treated rats virtually ceased to respond. Thus, this dose seemed to be associated with nonspecific behavioral disruption.

In the bar-pressing test, bupropion increased self-stimulation in a dose-related fashion at doses from 3 to 30 mg/kg, and at 30 mg/kg responding was significantly elevated above baseline (Table 1).

Histology

Histological evaluations were completed in 9 of the 17 rats serving in the shuttlebox experiments, and in 15 of the 27 bar-pressing rats. With the exception of 4 placements located slightly dorsal or medial to the zona incerta, all placements were well within the lateral hypothalamus. Placements were bounded anteriorly by the +4620 section and posteriorly by the +3180 section in the König and Klippel atlas [12] and were from 0.6 to 1.8 mm lateral to the midline.

DISCUSSION

These results show that appropriate pharmacological treatments can reduce selectively the latency to initiate brain stimulation in the shuttlebox test. In particular, pipradrol and bupropion reduced ON latencies without causing OFF latencies to decrease, thus effectively dissociating the two measures. The dose ranges within which these drugs reduced ON latencies corresponded to those that were associated with enhancement of bar-pressing rats for self-stimulation. It is reasonable, then, to attribute these effects of pipradrol and bupropion to enhancement of the reward value of hypothalamic brain stimulation in the shuttlebox test, and not to nonspecific psychomotor stimulation.

The effects of *d*-amphetamine in the present experiments essentially confirm previous reports. Although 0.5 mg/kg of *d*-amphetamine reduced ON latencies without altering OFF latencies [10], both latencies were shortened by a 2 mg/kg dose administered to animals with medial hypothalamic electrodes [2]. It is also of interest that in a bar-pressing task measuring escape from rewarding brain stimulation, *d*-amphetamine again reduced escape latency [20].

One other drug, 5-methoxy-NN-dimethyltryptamine, has been reported to reduce ON latencies selectively while increasing OFF latencies simultaneously [18]. The magnitude of the reported reduction in ON latencies was relatively small (20% from baseline), compared to the observed effects of the stimulant drugs used in the present experiments. No indication is apparent that 5-methoxy-NN-dimethyltyryptamine is capable of enhancing self-stimulation in other test procedures such as bar-pressing.

In the present experiments, OFF latencies were unchanged by pipradrol at doses (3 and 10 mg/kg) that reduced ON latencies as markedly as did *d*-amphetamine at 1 mg/kg. Therefore, the differential effect of *d*-amphetamine and pip-

radrol on OFF latencies cannot be attributed to greater overall efficacy of *d*-amphetamine. Two alternative hypotheses can be offered for the ability of *d*-amphetamine to reduce OFF latencies. One possibility is that nonspecific facilitation of ongoing responding by *d*-amphetamine may be more prominent at this dose, and may co-exist with the evident enhancement in reward value of stimulation.

Alternatively, *d*-amphetamine may enhance the aversive properties of brain stimulation, thus reducing the maximum stimulation duration that can be tolerated by the treated rat. The OFF latency appears to reflect the accumulated aversive properties of stimulation [9, 17, 19] and is elevated by known anxiolytic drugs in animals from the same colony as those involved in the present experiments [9]. That *d*-amphetamine may have aversive properties under some conditions is independently suggested by its ability to produce a conditioned aversion to saccharin [21]. Such aversive properties could be mediated by the effects of high doses of *d*-amphetamine upon serotonergic neurons [14]. Moreover, *d*-amphetamine does, in fact, further suppress punished responding in the Cook/Davidson model of experimental conflict [5]. It remains to be seen whether pipradrol and/or bupropion share this action of *d*-amphetamine.

It would be tempting to correlate the reported absence of clinical psychomotor stimulant properties of bupropion [7] with its inability to reduce OFF latencies in the shuttlebox test. For the moment, this hypothetical correlation must be viewed with caution in view of pipradrol's resemblance to bupropion in the shuttlebox test. Nevertheless, the shuttlebox test may potentially discriminate among agents that increase bar-pressing self-stimulation in an apparently identical fashion. These results further emphasize the versatility of the shuttlebox self-stimulation procedure in evaluating various classes of psychoactive drugs.

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