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Differential Effects of Drug Treatments on Nose-Poke and Bar-Press Self-Stimulation

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GERHARDT, S. AND J. M. LIEBMAN. *Differential effects of drug treatments on nose-poke and bar-press self-stimulation.* PHARMAC. BIOCHEM. BEHAV. 15(5) 767–771, 1981.—To assess the possibility of dissociating drug-induced gross performance deficits from effects on brain stimulation reward, the nose-poke and bar-press operants were systematically compared. Pentobarbital and methocarbamol (a muscle relaxant) reduced bar-pressing more strongly than nose-poking. In contrast, clonidine and haloperidol, which disrupt noradrenergic and dopaminergic neurotransmission respectively, had no differential effect on these operants. The nose-poke operant appears less vulnerable to drug-induced gross motor impairment and may be more suitable for pharmacological studies of self-stimulation.

Self-stimulation Nose-poke Pentobarbital Methocarbamol Clonidine Haloperidol

TRADITIONALLY, the effects of drugs on brain stimulation reward have been assessed by measuring the rate at which a lever in an operant conditioning chamber is pressed to obtain brief stimulation trains. This operant is a relatively unnatural one for rats and involves some motor learning. Potentially, therefore, the use of the bar-press operant may confuse the issue [6, 19, 22] of whether drug-induced effects on brain stimulation reward reflect true changes in reward value of stimulation, as distinguished from performance deficits.

In contrast to bar-pressing, the "nose-poke" response only requires that the animal insert its snout briefly into an aperture. This operant is a natural one for the rat, taking advantage of this animal's propensity to investigate apertures in experimental chambers [16]. This method of measuring self-stimulation has recently been employed by various investigators [11,15].

Because it seems to be a less complex response for the rat, nose-poking might be hypothesized to be less vulnerable to drug-induced deficits in motor behavior than is bar-pressing. However, drug effects on the quality of brain stimulation reward would be expected to be comparable regardless of the operant. We have systematically evaluated this hypothesis by comparing the effects of a muscle relaxant, methocarbamol [1] and a barbiturate, pentobarbital, with those of haloperidol and clonidine. The latter two drugs are reported to impair brain stimulation reward under conditions where gross motor impairment is not detectable [3,9].

METHOD

Animals

Male Fischer (F-344, Charles River) rats were used, and weighed 250–300 g at the start of experimentation.

Apparatus

A modified operant conditioning chamber (BRS/LVE, Beltsville, MD), 30.5×25×27 cm high, was used. A standard aluminum lever, located 4 cm above the grid floor, protruded 2 cm from the wall and was 3 cm wide, 1 cm thick. A circular aperture (diameter=3.5 cm) was located on the same wall. The center of the aperture was 9 cm from the center of the lever and 3 cm above the grid floor. Each interruption of a photocell beam 1 cm inside the aperture activated solid-state programming equipment and resulted in the delivery of brain stimulation. The lever was coupled to a microswitch which independently activated the stimulation contingency. At no time were the photocell beam and the lever simultaneously operative.

In most cases, stimulation was delivered by a Grass stimulator (Model S-88, Grass Medical Instruments, Quincy, MA). Each stimulus train lasted 100 msec and consisted of bipolar square wave pulses (pulse width=0.5 msec). In several experiments, a Haer stimulator (Frederic Haer, New Brunswick, ME) was used. The stimulation parameters were similar except that the pulse width was 0.05 msec.

Procedure

Rats were anesthetized with approximately 40 mg ketamine HCl and were stereotaxically implanted with stainless steel bipolar electrodes pre-attached to plastic connectors (Plastic Products, Roanoke, VA). Electrodes were aimed at the later hypothalamus (AP=4.3 to 4.8; L=1.0 to 1.5; V=2.7 to 4.1). Acrylic cement was used to attach the implanted electrode-plug assembly to stainless steel screws that had been threaded into the skull.

One week after surgery, rats were trained to lever-press for rewarding brain stimulation. Rats that showed good re-

sponding (greater than 1000 bar-presses/30 min) were then allowed to acquire the nose-poke operant. No shaping was required for nose-poke learning. During nose-poke training and testing sessions the lever was removed from the chamber, and during bar-press sessions the aperture was covered.

In an initial group of nine rats, the two operants were compared at different stimulation parameter combinations that elicited overall responding ranging from low to maximal rates. In some rats, intensity was systematically varied from 0.05 to 0.15 mA while frequency was held constant at 500 pps. In other rats, the intensity threshold was below 0.05 mA. Since intensity could not be reduced below this value using the Grass stimulator, stimulation frequency was therefore varied systematically from 60 to 500 pps to obtain suitable responding. Only one combination and one operant was presented during each daily session, which lasted 30 min. Altogether, each rat received, in randomized order, six different combinations of frequency and intensity, including a zero current condition. Each combination was presented once in conjunction with each of the two operants; twelve test sessions therefore comprised this initial experiment.

These and additional experimental rats were then trained to perform in the schedule that was utilized for drug testing. In this schedule, rats performed each operant for 15 min within a single, 30 min-session. The order of the two operants was reversed on alternate days. At the end of the first 15-min interval, the experimenter intervened briefly to prepare the chamber for testing on the other operant. As rats were trained in this schedule, intensity and (as required) pulse frequency were reduced so as to yield submaximal response rates of 400 to 800 responses per 15 min. Rats were used for drug experiments only if response rates in both operants could be simultaneously held within these limits, using the same current parameters for each operant. Some rats which failed to meet this criterion had excessively rapid nose-poke rates relative to bar-pressing rates. However, in other rats that were also judged unsuitable, bar-pressing rather than nose-poke rates were excessive. Once response rates by an individual rat had stabilized within the required range for both operants, drug testing was initiated following three days of baseline responding. Drug treatments were separated by at least five days, during which baseline responding was reestablished.

Drugs

All drugs were administered intraperitoneally. Sodium pentobarbital (Ganes Chemical Co., New York, NY) was dissolved in saline and was administered 15 min before testing to rats that had been fasted overnight. Clonidine HCl (Boehringer-Ingelheim, Ridgefield, CT) was administered in saline 30 min before treatment. Methocarbamol (A. H. Robins, Richmond, VA) was also administered 30 min before testing but was prepared in a 3% colloidal cornstarch suspension containing 5% PEG-400 and 0.34% Tween 80. Haloperidol (McNeil. Fort Washington, PA) was administered in the cornstarch vehicle 3.5 hr before testing. The volume of injection was 1.0 ml/kg body weight except that the highest dose of methocarbamol administered (300 mg/kg) was diluted to 2.0 ml/kg to reduce the viscosity of the suspension.

Analysis of Data

In the experimental design, each rat received all three doses of a given drug in counterbalanced order. Several rats failed to complete this design (due to premature plug dislodgement or unstable baseline responding) and were not included in the analysis of data.

At each dose of a given drug, the percent change in bar-pressing from the preceding day's baseline was compared with the corresponding change in nose-poke responding. These comparisons were planned and orthogonal, and were therefore performed using Student's t-test (two-tailed, matched pair). Analysis of variance was additionally employed to evaluate the *a posteriori* observation that pentobarbital elevated responding at lower doses.

Histology

To verify the approximate location of the electrode placements, representative rats were sacrificed at the conclusion of experimentation. Altogether, histological evaluation was completed on 19 of the 29 rats used in these experiments. Following transcardial perfusion with saline and Formalin, the brains were removed, allowed to stand in Formalin for at least 24 hr, then sectioned and stained using the cresyl violet or Weil method.

RESULTS

Stimulation Parameters

In the absence of brain stimulation, nose-poke rates (mean=129 per 30 min; S.E.=18) significantly exceeded bar-pressing rates (mean=21 per 30 min; S.E.=8), $t(8)=5.47$, $p<0.001$. However, nose-poke rates under this condition remained well below pre-drug baseline rates (400–800 responses per 15-min session). At stimulation parameters that were apparently subthreshold and yielded low overall response rates, nose-poke rates still exceeded bar-press rates. Higher stimulation frequencies and/or intensities increased response rates (both bar-press and nose-poke) to maximal levels, sometimes exceeding 3000 responses per 30 min session. As responding was increased in this fashion, bar-pressing rates approached and (at high stimulation frequencies) surpassed nose-poke rates.

Drug Effects

Pentobarbital slightly elevated responding on both the nose-poke and bar-press operants at the lowest dose tested (5 mg/kg) (Fig. 1). At 10 mg/kg, nose-poke remained slightly elevated, but bar-pressing declined. A striking observation at this dose was that some animals actually increased nose-poking within the same session in which bar-pressing was decreased from baseline. Correspondingly, the two operants differed significantly from each other with respect to the percent change from baseline. The increase in nose-poking was not significant by comparison with baseline. Nonselective decrements in both operants occurred at 15 mg/kg, indicating a relatively narrow dose-response relationship for this drug.

Methocarbamol also attenuated both operants (Fig. 1), but an intermediate dose (100 mg/kg) caused a significantly greater reduction of bar-pressing than nose-poking. At the high dose of 300 mg/kg, responding was virtually abolished, concomitant with the appearance of marked muscle relaxation.

Haloperidol decreased response rates for both types of

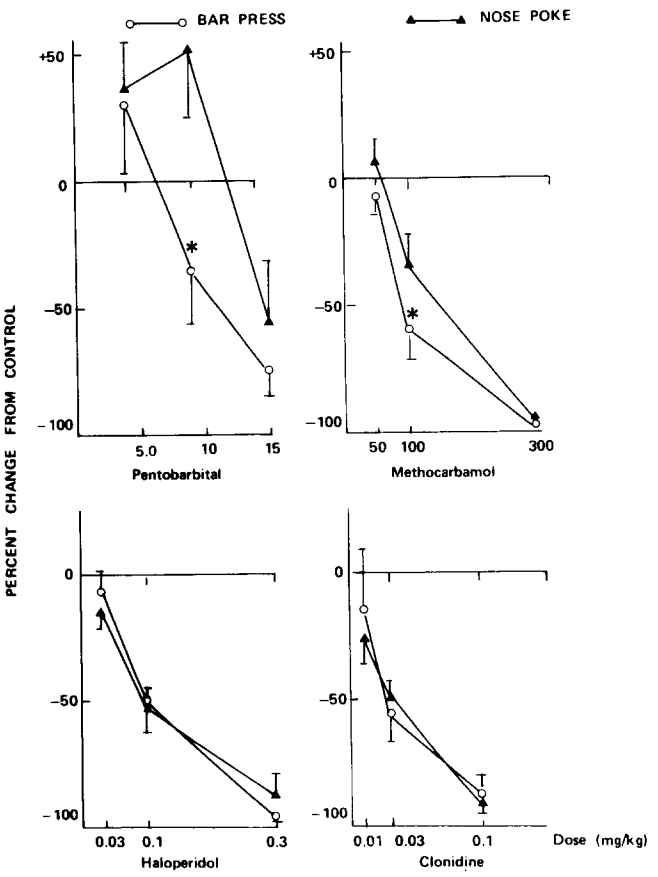

FIG. 1. Effects of pentobarbital, methocarbamol, haloperidol and clonidine on bar-press and nose-poke self-stimulation operant responses. Bar-pressing was significantly more strongly reduced than was nose-poking by pentobarbital at 10 mg/kg (*$p<0.02$ for the difference between the respective percent changes from baseline) and by methocarbamol at 100 mg/kg (*$p<0.02$). The group sizes were: pentobarbital (n=8), methocarbamol (n=7), haloperidol (n=7), and clonidine (n=8).

operants, but neither response was preferentially affected at any of the doses tested (Fig. 1). Similarly, clonidine also attenuated bar-pressing and nose-poking to an equivalent degree over the dose range examined.

In no case did the pre-drug baseline rate of nose-poke differ from that of bar-pressing prior to drug testing at any dose ($p>0.10$ for all comparisons by matched-pair two-tailed t-test). Pre-drug mean baseline rates for treatment groups ranged from 539 to 694 for nose-pokes and from 518 to 658 for bar-presses per 15-min sessions.

Histology

Histological examination revealed that all electrode tips were located in the lateral hypothalamus or slightly dorsal or medial to it, except for one placement in the medial lemniscus and two in the ventral area of the zona incerta. Within the limited sample of verified placements, no correlation was apparent between location and drug effects.

DISCUSSION

The primary finding of the present experiments was that bar-pressing and nose-poking self-stimulation are pharmacologically dissociable. Two drugs that would be expected to impair the motor aspects of performance, pentobarbital and methocarbamol, were found to reduce bar-pressing more strongly than nose-poking for brain stimulation reward. This differential effect of drugs on the two operants was demonstrable in animals performing both tasks within the same experimental session. Moreover, baseline bar-pressing and nose-poking rates were equivalent prior to each treatment. The importance of having equivalent baseline levels of performance whenever different operants are compared has been previously emphasized [6].

These results support the hypothesis that nose-poking is physically a simpler and more "natural" response for the laboratory rat than is bar-pressing. Further, in correspondence with previous reports [2], we noted informally that nose-poking was more readily acquired than was bar-pressing, even in naive animals. Baseline response rates also tended to be more stable in rats that only nose-poked for self-stimulation, as compared with animals that only bar-pressed (unpublished observations). These characteristics indicate that the nose-poke operant may be a more convenient method for pharmacological investigations.

Under no-stimulation conditions, nose-poke rates were higher than bar-press rates, suggesting higher rates of spontaneous (operant) responding. However, at intermediate baseline response rates (prior to drug testing), neither operant consistently occurred at higher rates. Further increases in stimulation frequency and/or intensity readily elevated nose-poke and bar-press rates to levels well in excess of the baselines that preceded drug treatment. Many animals were observed to grasp the bar with the teeth or paws and shake it perseveratively when presented with high stimulation frequencies or intensities. This "jiggling" may have accelerated the rate of bar-pressing to an artifactual extent, accounting for faster bar-pressing under these conditions.

In contrast to pentobarbital and methocarbamol, haloperidol did not exert a differential effect on nose-poking as compared with bar-pressing. It would be tempting to take this finding as further evidence that haloperidol has a selective action on "reward" as opposed to "performance" variables. Such an inference would be unwarranted, however. Certain findings concerning the effects of representative neuroleptics particularly pimozide, can be explained by suggesting that these drugs impair the animal's ability to sustain a motivated response for an appreciable period of time, in the absence of observable gross motor impairment [22]. In fact, it has recently been shown [4] that *unreinforced* nose-poking is attenuated by pimozide. At most, the present results permit the conclusion that at the doses administered, haloperidol's effects are not attributable to gross motoric incapacity paralleling that caused by pentobarbital or methocarbamol.

The effects of clonidine were similar to those of haloperidol. These results are consistent with the suggestion that clonidine has a selective effect on reward, as opposed to performance, aspects of self-stimulation [9,12]. However, the possibility that clonidine may cause subtle deficits in sustained responding, similar to haloperidol's reported effects, has not been evaluated.

The present results constitute the first systematic pharmacological comparison of nose-poke with bar-press measures of self-stimulation. Previous investigations have com-

pared bar-pressing with other types of operant responses [7, 14, 20]. Pentobarbital was reported to reduce "capacitance probe" touching, another simplified operant, less than it reduced bar-pressing for self-stimulation in gerbils [7]. In these experiments, spiperone, a neuroleptic closely related to haloperidol [19], had no differential effect. The baseline response rates in this experiment, however, were not equated over the two operants, leaving open potential alternative interpretations of the results (cf. [6]).

Two other groups independently compared "lick" responding with bar-pressing for self-stimulation [14,20]. In one experiment, spiroperidol was reported to attenuate both responses to an equivalent extent, but the comparative control bar-pressing rates were not indicated [14]. Suprisingly, another group indicated that "lick" responding was reduced more strongly by neuroleptics than was bar-pressing [20]. In these experiments, it was noted that response thresholds were higher for "lick" responding than for bar-pressing, whereas the reverse was the case for nose-poking in the present experiments. Unfortunately, the effects of non-neuroleptics were not investigated. These results nevertheless indicate that seemingly simple operants cannot be assumed to be equivalent. Rather, the pharmacological results may be influenced by methodological differences as well as by unsuspected complexities in the organization of response output systems in the rat.

Further evidence has been presented by White and coworkers [18,21] indicating that the neuronal elements subserving various self-stimulation operants may differ. For example, bar-pressing for self-stimulation was reported to depend on the integrity of both noradrenergic and dopaminergic neurotransmission in the brain, while runway performance and tail waving appeared to be mediated primarily by dopaminergic or noradrenergic systems, respectively [18,21].

The ability of clonidine to attenuate self-stimulation has been attributed to its agonist activity at brain noradrenergic autoreceptor [8, 9, 10], although clonidine has other actions as well [17]. In the present experiments, clonidine had effects very similar to those of haloperidol, a selective dopamine receptor antagonist. Thus, no evidence was available to indicate whether nose-poking or bar-pressing would be selectively mediated by noradrenergic as opposed to dopaminergic neurotransmission.

In agreement with previous investigations [7,13], we found that the two lower doses of pentobarbital (5.0 and 10 mk/kg) facilitated self-stimulation, although erratically. This facilitation was more readily apparent in the nose-poke operant, as compared with the bar-pressing operant. Various drugs of abuse are known to enhance self-stimulation responding [23] and the facilitatory effect of pentobarbital may therefore correlate with its abuse potential. The use of the nose-poke operant may constitute a further refinement in the use of self-stimulation to detect stimulant and abuse potential of psychoactive drugs.

NOTE ADDED

After this manuscript was submitted, Ettenberg *et al.* [5] reported that the neuroleptic, alpha-flupenthixol, decreased bar-pressing self-stimulation relatively more strongly than nose-poking self-stimulation. These results are at variance with our finding that haloperidol attenuated both operant responses equally. There are, however, several prominent methodological differences between these investigations and the present experiments. In their experiments, Ettenberg *et al.* [5] always tested nose-poking before bar-pressing, whereas the order of the operants was alternated in the present investigations. Their animals were tested in lengthy sessions at current intensity levels that evidently produced high baseline response rates. We used shorter test sessions and the current intensities were just above threshold, thus producing lower response rates. These or other methodological differences may account for the discrepant results.

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