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# Anxiolytic Drugs Selectively Increase Preferred Duration of Rewarding Brain Stimulation in a Shuttlebox Test

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GERHARDT, S., J. PROWSE AND J. M. LIEBMAN. *Anxiolytic drugs selectively increase preferred duration of rewarding brain stimulation in a shuttlebox test.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 795–799, 1982.—In the shuttlebox self-stimulation test described by Atrens, rewarding brain stimulation is independently initiated and terminated by rats. It has been hypothesized that gradually accumulating aversiveness of stimulation motivates the rat to terminate the rewarding stimulation train. In agreement with this view, optimal doses of the known anxiolytics, pentobarbital (5 and 10 mg/kg) diazepam (1 and 2.5 mg/kg), chlordiazepoxide (3 and 5.4 mg/kg) and CL 218,872 (3, 10 and 30 mg/kg) preferentially increased the latency to terminate stimulation (the OFF latency), as compared with the latency to initiate stimulation (the ON latency). Higher doses increased both latencies in a nonselective fashion, suggesting nonspecific performance impairment. The shuttlebox self-stimulation test constitutes a potentially useful measure of experimental approach-avoidance conflict, with the OFF latency indicating anticonflict activity and the ON latency providing a control for performance variables.

Self-stimulation Shuttlebox Diazepam Chlordiazepoxide Pentobarbital CL 218,872 Conflict

IN the shuttlebox procedure of Atrens [2], rats are permitted to self-regulate the duration of rewarding brain stimulation. By interrupting a photocell beam at one end of a shuttlebox, the rat initiates a train of electrical brain stimulation. This train is terminated when the animal crosses to the other side of the chamber and interrupts another photocell beam. The latency to initiate brain stimulation is termed the ON latency, and that to terminate stimulation is the OFF latency.

Two divergent hypotheses have been proposed to account for the voluntary termination by rats of self-initiated brain stimulation trains. According to one proposal, neuronal adaptation takes place to the rewarding qualities of stimulation, compelling the animal to terminate stimulation and go through a "time-out" period before the stimulation can again be rewarding [5, 6, 11]. Alternatively, evidence has been submitted in favor of the proposal that termination of brain stimulation may be motivated by a gradual build-up in the aversive properties of the initially rewarding stimulation [18, 22, 23]. According to this hypothesis, rats terminate stimulation when it becomes aversive, and not necessarily because the rewarding qualities per se have dissipated.

On the basis of the latter hypothesis, it may be speculated that the shuttlebox procedure constitutes, in part, a conflict task. The duration of brain stimulation (i.e., the OFF latency, also termed the "ON time" by some groups [3,7]), may represent the animal's attempts to balance the initially rewarding properties of stimulation against the gradually emerging aversive consequences [1]. That this may be especially true of placements bordering on the medial hypothalamus is also suggested by the observation that stimulation within the medial hypothalamus is aversive [18] and that placements closer to the medial hypothalamus yield shorter OFF latencies in a self-regulated stimulation duration task [21]. On the other hand, the ON latency is believed to be inversely related to the rewarding value of stimulation. For example, the ON latency is shortened by *d*-amphetamine and other stimulation drugs [14] and is lengthened by drugs that impair catecholaminergic neurotransmission [2,16].

These considerations lead to the prediction that anxiolytic drugs will selectively lengthen the OFF latency at doses that do not disrupt motor function. Under conditions where motor function is disturbed, however, both the ON and OFF latencies should increase nonselectively. We have examined in the shuttlebox self-stimulation procedure the effects of diazepam, chlordiazepoxide and pentobarbital, all of which have known antianxiety properties in addition to their other pharmacological actions [10]. In addition, CL 218,872, a novel anxiolytic [17], was also evaluated.

## METHOD

### *Animals and Surgical Procedures*

Male Fischer (F-344, Charles River) rats (250–300 g) were anesthetized and bipolar stimulation electrodes were stereotaxically implanted in the medial portion of the lateral hypothalamus (see [16] for details).

### *Behavioral Procedures*

The apparatus was similar to that used by Atrens and

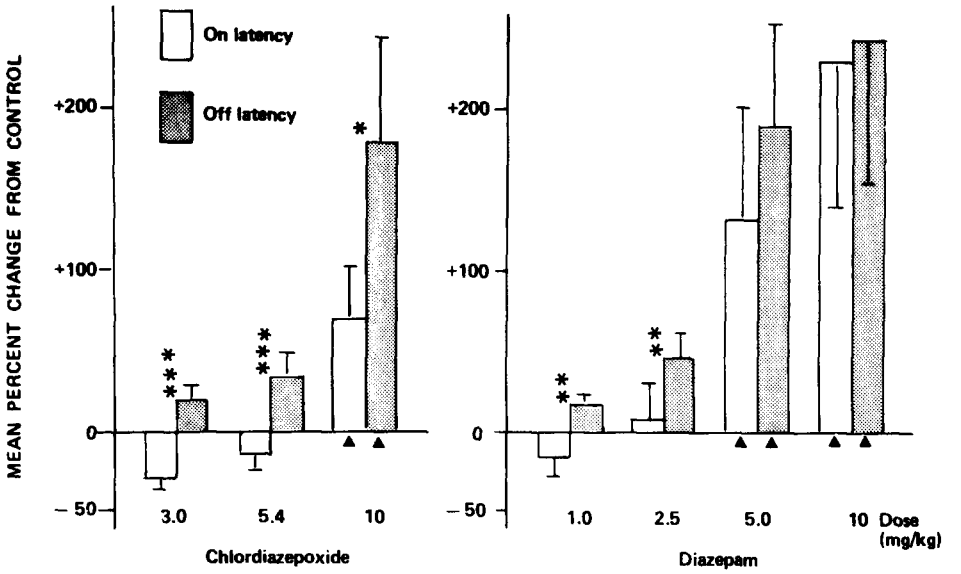


FIG. 1. Effects of chlordiazepoxide and diazepam on shuttlebox self-stimulation. Bars indicate mean (±S.E.) percent change in latency from baseline. ▲Significantly different from pre-drug baseline by the trend test, $p<0.05$. *Percent increase in OFF latency was significantly greater than that in the ON latency, $p<0.05$. **$p<0.01$. ***$p<0.001$. See text for explanation of statistical analysis. Treatment group sizes: chlordiazepoxide, n=8; diazepam, n=9.

co-workers [2] and is described elsewhere [16]. 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. Task programming and training procedures are described elsewhere [16].

A total of 43 rats were used for drug experiments. In these rats, current intensity was individually adjusted so as to yield between 35 and 80 crossing cycles per 10 min session. Rats received drug treatments after at least two days of stable 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 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 [16].

*Drug Treatments*

All drugs were administered intraperitoneally in a volume of 1 cc/kg body weight. Diazepam and chlordiazepoxide were supplied by Hoffmann-La Roche, Nutley, NJ; pentobarbital was obtained from Ganes, New York NY and CL 218,872 was synthesized by CIBA-GEIGY chemists. Pentobarbital and chlordiazepoxide were dissolved in normal saline. Diazepam and CL 218,872 were administered in a 3% colloidal cornstarch suspension containing 5% PEG-400 and 0.34% Tween 80. Drugs were given 30 min before testing except that pentobarbital was given at 15 min instead. Animals were fasted overnight before pentobarbital treatment to facilitate absorption of drug. To control for the effects of food deprivation, the effects of vehicle treatment were examined in the same rats after food deprivation. In all other cases, food was available ad lib. At least five days elapsed between successive doses of a given drug; no tolerance to drug effects was seen under these conditions. CL 218,872 was administered to a single group of rats at doses of 1, 3 and 10 mg/kg in counterbalanced order. Subsequently, higher doses of CL 218,872 (30 and 100 mg/kg) were examined in other groups of rats. In all other instances, a single group of animals received all doses of a given drug in counterbalanced order.

*Analysis of Data*

The method of data analysis is described elsewhere [16]. Briefly, regression analyses were performed separately on ON and OFF latency data to examine dose-response relationships. If a significant relationship was found, the trend test [4] was then employed to identify doses that significantly increased latency over the pre-drug baseline. The percent change in the on latency, relative to baseline, was also directly compared with that in the OFF latency. Following ANOVA to determine whether the main effects of dose and type of latency were significant, matched pair $t$-tests were performed to compare these percent changes from baseline at given drug doses.

*Histology*

At the end of experimentation, representative rats were sacrificed by overdose of anesthetic, followed by transcardial perfusion of 50 to 100 cc normal saline and 50 to 100 cc Formalin. Brains were removed, allowed to stand in Formalin for at least 24 hr, then were frozen; sectioned, and stained with cresyl violet stain for histological examination.

## RESULTS

The mean baseline ON latencies for each treatment group ranged from 4.5 to 6.3 sec, and the mean baseline OFF latencies ranged from 5.0 to 7.0 sec. The mean baseline ON and OFF latencies did not differ significantly prior to any treatment condition ($p>0.10$ for all comparisons, two-tailed matched pair $t$-test).

All four anxiolytics shared a common effect on response patterns in the shuttlebox test (Figs, 1, 2 and 3). At low drug doses (chlordiazepoxide, 3 mg/kg; diazepam, 1 mg/kg; pentobarbital, 5 mg/kg; CL 218,872, 1 mg/kg), the mean OFF latency increased while the mean ON latency showed a slight reduction. The percent change in the OFF latency

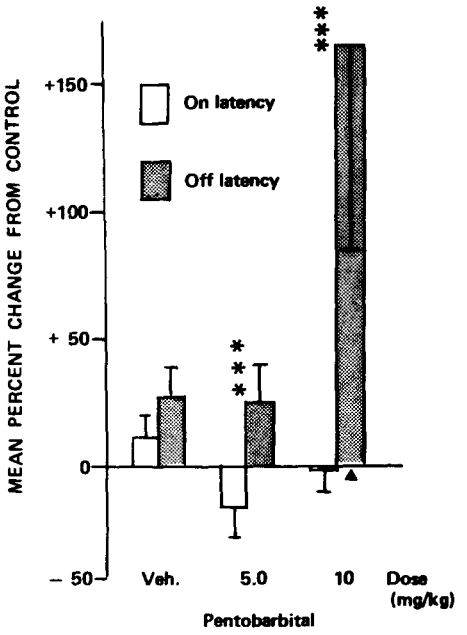


FIG. 2. Effects of pentobarbital on shuttlebox self-stimulation. See legend, Fig. 1, for explanation of format and symbols. N=8.

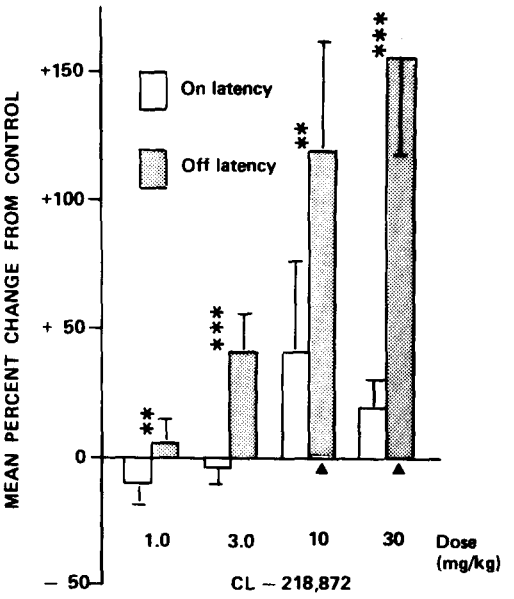


FIG. 3. Effects of CL 218,872 on shuttlebox self-stimulation. See legend, Fig. 1, for explanation of format and symbols. N=8.

from baseline differed significantly from that in the ON latency in these instances, reflecting a consistent shift within each animal in the relative duration of the two latencies. However, several individual rats failed to increase OFF latencies by comparison with baseline at these low drug doses. Thus, the increases in mean OFF latencies did not reach significance when directly compared with the pre-drug baselines.

At higher doses (chlordiazepoxide, 5.4 mg/kg; diazepam, 2.5 mg/kg; pentobarbital, 10 mg/kg; CL 218,872, 3, 10 and 30 mg/kg), larger increases in the OFF latencies were seen, while the ON latencies closely approximated baseline values. At these doses, the percent increases in the OFF latency were again significantly different from the very slight changes in the ON latency. The OFF latencies were also significantly elevated, relative to baseline, by these doses of pentobarbital and CL 218,872, although not by chlordiazepoxide or diazepam.

Further increases in the doses of diazepam and chlordiazepoxide elevated both the ON and OFF latencies significantly (Fig. 1). Higher doses of pentobarbital (20 mg/kg) and CL 218,872 (100 mg/kg) not only caused even more marked increases in both latencies, but produced a virtual cessation of responding in some animals (data not shown). At these high doses of each drug, the percent increases in ON and OFF latencies showed large variability and did not differ significantly from each other.

Histological evaluations were completed in 18 of 43 rats in these experiments. The placements that were sampled were all within the lateral hypothalamus, 0.6 to 1.6 mm from the midline and between the +3290 and +4640 frontal planes in the König and Klippel stereotaxic atlas [12].

## DISCUSSION

The most characteristic effect of anxiolytic drugs on shuttlebox self-stimulation responding was to increase OFF latencies preferentially as compared with ON latencies. This effect was induced by low to moderate doses of pentobarbital, CL 218,872, chlordiazepoxide and diazepam.

Overt muscle relaxation was evident at higher doses of these drugs, but the preferential increase in OFF latencies cannot be attributed to such an effect. Other muscle relaxants, such as baclofen and methocarbamol, actually increase ON latencies more strongly than OFF latencies in this test procedure [15,16]. Maximum separation between ON and OFF latencies occurred at intermediate doses at which muscle relaxation was less prominent, while high doses increased both latencies nonselectively.

The actual reductions in ON latencies at lower doses were small and failed to reach significance by comparison with pre-drug ON latency baselines. The disappearance of these effects on ON latencies at higher anxiolytic doses contrasts with the pronounced and dose-related shortening of ON latencies that results from treatment with stimulants such as pipradrol and *d*-amphetamine [14]. However, shortened ON latencies were consistently noted at low doses of the anxiolytic drugs examined. Pentobarbital and chlordiazepoxide have been reported to facilitate self-stimulation, albeit weakly, in other test procedures [9,20]. Possibly, low anxiolytic doses may have subtle, reward-enhancing effects that are superseded at higher doses by anxiolytic and, ultimately, motor disruptive effects.

Pentobarbital has been reported to increase preferred brain stimulation duration in a different test procedure that involved pressing and releasing a single operant lever [23]. The present results essentially confirm these findings and are consistent with the reported antianxiety effect of pentobarbital [10]. The steep dose-response relationship that was observed for pentobarbital may be explained by its well known hypnotic properties.

The non-benzodiazepine anxiolytic, CL 218,872, had a

profile similar to that of the other anxiolytics but with several important differences. In agreement with previous studies of receptor binding and anticonflict acitivty [17], CL 218,872 appeared to have a shallower dose-response slope in the shuttlebox test than did the benzodiazepines tested. Moreover, the percent increase in OFF latencies differed significantly from that in ON latencies over a 30-fold dose range of CL 218,872. In contrast, the comparable "selective" range of other anxiolytics was not more than threefold. This finding is anticipated by the claim [17] that CL 218,872 shows a greater separation between anxiolytic activity and motor impairment than do the benzodiazepines.

It was of interest that diazepam caused a relatively weak separation between OFF and ON latencies, although high levels of significance were achieved. In another set of experiments, a longer warm-up period (5 min) was utilized prior to the initiation of data collection to determine whether diazepam's effects would be more marked under such conditions. Although diazepam's lengthening of ON and OFF latencies was slightly more prominent under this condition, the separation between ON and OFF latencies was not enhanced (unpublished experiments).

The narcotic drugs, etorphine and morphine, have been reported by others to increase OFF latencies selectively [3,13]. The interpretation of these experiments may be questionable, however, because the mean baseline OFF latencies were much longer (10–12 sec) than the mean baseline ON latencies (2 sec). Latencies of 2 sec or less tend to be less readily altered by drugs, as indicated by the lack of drug effects on shuttlebox responding for high stimulation frequencies [7] and by our informal observations. In fact, the etorphine data presented by Baltzer *et al.* [3] suggest that at intermediate doses of etorphine (10 and 20 μg/kg), the percent increases in ON and OFF latencies from baseline were similar despite the greater magnitude of the increase in the OFF latency.

We have also noted that apomorphine, a drug lacking anxiolytic activity, produced a weak increase in OFF latencies (unpublished observations). However, this effect was not statistically significant nor dose-related over a wide range of doses (0.03–3 mg/kg) and its magnitude fell far short of the 500% increase over baseline that was previously reported [1]. Conceivably, this descrepancy could be accounted for by differences in baseline latencies, which were not given by Atrens *et al.* [1]. Alternatively, subtle differences in electrode placements may also be relevant as others have reported that the effects of apomorphine on self-stimulation differ markedly among placements [19].

It has been argued that termination of brain stimulation in the shuttlebox procedure represents a simple "respondent" that is not sensitive to drug effects [8]. As previously noted [1], this hypothesis is refuted by the demonstrated ability of selected drugs, particularly anxiolytics, to elevate the OFF latency selectively. Additionally, the OFF response cannot be a simple escape respondent because the initiation of stimulation gives the trained rat forewarning of impending aversiveness. Rather, the present results support the view [18, 22, 23] that termination of rewarding brain stimulation, at least among placements that support shuttlebox self-stimulation behavior, is motivated by accumulated aversiveness and not by diminishing reward value of prolonged brain stimulation. Thus, in addition to its other psychopharmacological applications [2, 14, 16], the shuttlebox self-stimulation test constitutes a potentially useful index of experimental conflict.

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