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# Catecholaminergic and Cholinergic Agents and Duration Regulation of ICSS in the Rat[1]

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EDWARDS, M., J. WISHIK AND H. M. SINNAMON. *Catecholaminergic and cholinergic agents and duration regulation of ICSS in the rat.* PHARMAC. BIOCHEM. BEHAV. **10**(5) 723–731, 1979.—ICSS in the diencephalon and midbrain was measured in a preferred duration situation as a function of pulse frequency and various pharmacological agents. Response measures were ON Time, the mean time ICSS was maintained; OFF Time, the mean time between the offset of a train and the initiation of the next; the log-log slopes of these two measures as a function of frequency; and Percentage Time ON. In both a shuttlebox (N=13) and in an operant chamber (N=10), Haloperidol (0.050 and 0.075 mg/kg) consistently decreased Percentage Time ON by elevating OFF times without consistent effects on ON Times. Haloperidol effects were greater at lower frequencies in the shuttlebox. Phentolamine (5 and 10 mg/kg), tested with 9 sites in the shuttlebox and 10 sites in the operant chamber, also differentially increased OFF Times with consistently greater effects at low frequencies. Evidence was not found for regional selectivity in the action of phentolamine or haloperidol among middle levels of the median forebrain bundle (MFB), the dorsal hypothalamus, the posterior MFB and the substantia nigra. Scopolamine (0.25, 0.50, and 1.00 mg/kg, six sites) and propranolol (10 and 15 mg/kg, seven sites) produced no consistent effects. FLA-63 (10 and 25 mg/kg, eight sites) produced some disruptions of performance at low frequencies but was without consistent effects. The results are consistent with a model of ICSS which includes a reward maintenance process, insensitive to catecholaminergic agents, and an approach-facilitation process which involves $\alpha$-noradrenergic and dopaminergic systems.

ICSS Haloperidol Phentolamine Scopolamine Propranolol FLA-63

MANIPULATIONS in the efficacy of catecholaminergic (CA) transmission generally produce powerful effects on intracranial self-stimulation (ICSS). In particular, the use of reversible blockers of CA receptors such as phentolamine and haloperidol which at low doses are relatively selective for noradrenergic $\alpha$-receptors and dopaminergic receptors [1], respectively, have been common and the resulting reductions in ICSS have usually been interpreted as supporting the involvement of one or both of the transmitters in some aspect of the behavior (e.g. [22, 29, 30, 37–39]). Controversy has attended the use of these drugs for a number of reasons (see [17,19]). A recurrent problem has been the distinction between effects on the essential process underlying ICSS, reward, and effects on the process responsible for the motoric expression of reward, performance. It has been approached by comparing pharmacological effects on ICSS with those on parallel measures of motor function [20,34], by analyzing barpressing patterns [18,19], and by determining wether otherwise diminished barpressing will reappear with increased intensities [29].

Another approach is to employ ICSS measures that are relatively insensitive to performance factors. One such measure is the locus of rise in the reward summation function [14–16]. A related approach is to use the preferred duration method in which the rat controls the duration of ICSS (ON Time) and the time between the offset of one train and the initiation of the next (OFF Time). It is generally agreed that OFF Times provide a measure of the behavior facilitatory effects of ICSS, i.e., incentive. However, the processes determining ON Times have been the subject of some controversy (see Discussion). In addition to providing two independent [3,7] measures of ICSS, the preferred duration method offers a number of possibilities for distinguishing effects of drugs. The two elemental measures of ON and OFF Times can be combined into the measure Percentage Time ON which indexes the overall preference for stimulation vs no stimulation. This measure is a ratio of two response measures and should not be influenced by simple motoric disturbances that affect both responses equally [5,25]. It should be noted, however, that the method as normally used does not allow the separation of effects on the incentive properties of stimulation from effects on response initiation when OFF Times are affected.

The preferred duration method was used here to determine the effects of several drugs on ICSS measured as a function of pulse frequency. Of principal concern were the effects of haloperidol and phentolamine but also studied were propranolol, a $\beta$-receptor blocker, FLA-63, a dopamine-$\beta$-hydroxylase inhibitor, and scopolamine, a muscarinic cholinergic receptor blocker. Most sites and drugs were tested in a condition using a shuttlebox. The most interesting effects were produced by the lower doses of

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haloperidol and phentolamine so these effects were tested at a wider range of frequencies in a subsequent experiment requiring different behavioral responses in an operant chamber to index preferred duration. Where permitted by the data, the sites were combined to test for regional differences in the effects of the two drugs. The data indicated that both haloperidol and phentolamine increased the absolute values of the OFF Time component of ICSS. Phentolamine had clearly greater effects on lower frequencies, haloperidol had a more equivocal frequency dependence. Neither drug had clear effects on the ON Time component or its response to pulse frequency. Regional differences or indications of nonspecific effects at the low doses were not found.

## METHOD

### *Animals*

The animals used were 22 adult male inbred DA agouti rats weighing 207–350 g and housed individually with free access to food.

### *Surgery*

Following sodium pentobarbital anesthetization (30–50 mg/kg), each rat was stereotaxically implanted with two bipolar electrodes, made by twisting 125 $\mu$m-diameter steel wires that were insulated with Teflon except for the cross-section of the tips. The tips were cut evenly and oriented so that the centers were aligned perpendicularly to the long axis of the brain. In 15 rats, the electrodes were implanted bilaterally, in seven they were implanted unilaterally.

The electrode leads were connected to Amphenol pins, which were inserted into a plastic strip during surgery. The strip was embedded in acrylic cement anchored to the skull with four stainless steel screws (size 0/80). At least 5 days postoperative recovery preceded any training sessions.

### *Apparatus*

For behavioral testing a Plexiglas shuttlebox (40 cm long, 24 cm wide, and 26 cm high) was used with 13 rats (21 sites) and a Coulbourn Instruments operant chamber was used with 9 rats (10 sites). Both apparatus were enclosed in a dimly illuminated, ventilated cubicle that also contained a speaker which delivered white noise.

In the shuttlebox one side of the wiremesh floor was counterbalanced and suspended on a pair of microswitches. Displacement of this floor by the animal's weight closed the microswitches, leading to the initiation of brain stimulation. In the operant chamber, depression of a lever at one end initiated stimulation. Stimulation was terminated when the rat moved to the opposite end and its presence was detected by a low current circuit through the floor grids. Preliminary unpublished work showed that the two apparatus yield measures of ICSS which respond similarly to pulse frequency. In the shuttlebox, brain stimulation was provided by biphasic square wave pulses of 0.3 msec duration per phase, produced by two coupled stimulators (Grass SD-5 Model, Grass Instrument Co., Quincy, MA). A 0.33 m$\Omega$ resistor in series with the rat served to maintain relatively constant current through the electrode. Current was monitored continuously by displaying on an oscilloscope the voltage drop across a one kilohm series resistor. The frequencies used were 160, 130, 100, 70, and 40 Hz. In the operant chamber, stimulation was provided by constant current monophasic pulses of 0.3 msec duration produced by a F. Haer Pulsar IV stimulator. Frequencies used in this case were 178, 132, 100, 77, 55 and 40 Hz. Connection with the stimulators was made via a commutator and a light, flexible cable.

### *ICSS Measures*

At each offset and onset of stimulation the preceding time interval was recorded to an accuracy of 0.01 sec on paper tape by a printing counter. These data are referred to as ON Time (time with stimulation on) and OFF Time (time with stimulation off), respectively. To characterize the frequency functions of these measures, the slope of the $\log_{10}$ time vs $\log_{10}$ frequency (Hz) was determined by the method of least squares. Both ON and OFF Times generally show linear decreases between 40 and 160 Hz when log-log plots are used. For data obtained in the shuttlebox frequencies between 40 and 160 Hz were used; for the operant chamber method, 40 to 132 Hz frequencies were used. The highest frequency of 178 Hz was not used because the function often showed nonlinear upturns at this point. The frequencies tested with the operant chamber represented approximately equal log intervals. To characterize the displacement of ON and OFF times the relatively extreme points at 40 and 130 Hz (132 Hz for the operant chamber) were used. The ratio of mean ON Time to the sum of the mean ON Time and the mean OFF, quantity multiplied by 100, was defined as the Percentage Time ON. This measure was derived at 40 Hz and 130 Hz and provided an overall index of the preference for stimulation versus no stimulation.

### *Procedure*

Each site was tested for ICSS using shaping procedures in at least three sessions. Of the 44 sites tested in 22 rats, 31 were positive for ICSS and produced no behavioral signs of seizures. For the nine rats with two positive sites, the order of testing was selected randomly once and maintained thereafter. Minimal intensities were selected such that the rats would perform onset and offset behaviors reliably at the lowest frequency of stimulation, 40 Hz. The intensities adopted ranged from 60–275 $\mu$A with a median of 125 $\mu$A. The rats were next tested in sessions in which frequency of stimulation was varied.

To stabilize performance the rats were tested in at least five sessions under a regimen identical to subsequent drug tests except that no injections were given. Rats were tested every 4 days in the shuttlebox and every 2 days in the operant chamber. All sessions began with a warmup period consisting of 10 self-stimulations at 100 Hz. Measures of ON Times and OFF Times were then taken at each frequency. Frequencies were presented in a descending-ascending series, starting at the highest frequency descending to 40 Hz, and then ascending to the highest frequency. In the shuttlebox, 20 ON and OFF Times were recorded at each step in frequency. In the operant chamber five ON and OFF Times were recorded at each step. The frequency changes were made without interruption, and the first 3–5 stimulations after each change were not recorded. Test sessions generally lasted 20–30 min. For animals with two electrodes, there was a 15–30 rest period before testing the second electrode site.

Drug tests were begun only after the rats' performance stabilized, i.e., the ON Times and OFF Times of two consecutive tests did not show intersession differences greater

than 15%. Most sites required at least 12 sessions to reach this level of consistency. Each test of a drug at a dose consisted of three sessions, the first with a control injection, the second with the drug, and the third with a control injection. Sufficient recovery time was allowed between drug tests. If the mean values from the postdrug control test were within 15% of the values from the pre-drug control test, the postdrug control session served as the pre-drug control session for the next drug test. Thus, a minimum of four days separated drug injections. In the shuttlebox condition in which all the higher doses were tested, a longer interval was more frequent (median=12 days).

If on a drug test (or, rarely, on a control test) a rat did not self-stimulate within 5 min, the experimenter manually administered noncontingent, or priming, stimulation trains in the form of three trains of 0.5-sec duration. If priming was ineffective, shaping of the onset response was attempted. Priming stimulation was also given if pauses of greater than 3 min occurred. If no response resulted the frequency was moved to the next step. The frequency was also changed if the rat had greater than four 30-sec OFF Times (or three 60-sec or two 180-sec OFF Times) within 10 stimulation cycles. Times longer than 30 sec were coded as 30 sec.

The principal focus of the study was on the effects of the catecholaminergic receptor blocking drugs haloperidol and phentolamine. The majority of the ICSS sites and drugs were tested in the shuttlebox. Additional sites were subsequently tested with the low doses of haloperidol and phentolamine using the operant chamber. All injections were intraperitoneal, had a total volume of 0.75 cc, and were given 30 min prior to testing except FLA-63 which was injected 3 hr before testing. All drugs were dissolved in 0.9% saline except FLA-63 which was dissolved in a solution of ethanol and 0.1N HCl in a 1:4 ratio brought to a pH of 7 by the addition of NaOH. The appropriate vehicle served for the control injections.

In the shuttlebox condition, when more than one drug was tested, the order of presentation was scopolamine, haloperidol, phentolamine, propranolol, and FLA-63. No single site, however, received all drugs. The most common combinations were scopolamine and haloperidol (2 sites); scopolamine and phentolamine (2 sites); haloperidol, phentolamine, propranolol (7 sites); four of the latter sites also received FLA-63. The lower doses of a drug were tested before the higher doses. In the operant chamber condition where only the low doses of haloperidol and phentolamine were tested, half of the 10 sites received haloperidol first and half received phentolamine first.

*Histology*

Each rat was injected with a lethal dose of sodium pentobarbital and perfused transcardially with 0.9% NaCl, followed by 10% Formalin. After further fixation for at least 2 days in 10% Formalin, the brain was sectioned on a freezing microtome and stained with cresyl violet. The electrode tracts were plotted on brain drawings adapted from Konig and Klippel [27].

The sites in the middle median forebrain bundle (MMFB) lay between 3.3 and 4.4 mm on the anterior-posterior (AP) axis of Konig and Klippel [27]. The five sites classified as dorsal hypothalamic (DH) were located in the zona incerta (N=3) and the subthalamic nucleus (N=2). The sites in the posterior median forebrain bundle (PMFB) were restricted to the AP level between 2.2 and 2.9 mm. Substantia nigral (SN) sites were located in the pars reticulata with the single exception of Site 20 which was located in pars compacta. Other sites were too infrequently sampled to make regional groups; they included the ventral central gray (Sites 30 and 31), the internal capsule (Sites 25 and 26), the medial mammillary nucleus (Site 28), the mammillotegmental tract at 2.6 mm AP (Site 29) and the ventromedial nucleus of the thalamus (Site 27).

The drugs and doses tested at the various sites with the two methods are listed in Table 1.

## RESULTS

To determine drug effects, the Percentage Time ON, ON and OFF Times, and the log-log slopes of the latter two measures for the pre- and postdrug sessions were averaged and compared to the corresponding values in the intervening drug session at each frequency. Drug effects at sites tested in a particular method were determined by two-tailed $t$-tests for paired observations. When sites from two methods were combined for regional comparisons, an analysis of variance with repeated measures over the two levels of the drug factor (control and drug) and independent groups over the regional factor was used.

*Haloperidol*

In the shuttlebox condition, haloperidol at the lower dose of 0.05 mg/kg was tested with 13 sites listed in Table 1. As shown in Fig. 1 the drug reduced the Percentage Time ON of stimulation at both frequencies of 40 Hz and 130 Hz ($p$'s$<0.02$). In both cases, the Percentage Time ON measure was decreased because haloperidol tended to differentially increase the OFF Times (40 Hz, $p<0.05$; 130 Hz, $p<0.10$) but had no effect on ON Times at either frequency ($p$'s$>0.57$). Self-stimulation at the lower frequency of 40 Hz was more sensitive to haloperidol's effects. At three sites (1, 27 and 29) shuttling was reduced by the drug to below criteria (see Methods) at the lowest frequency but not the higher frequencies. Therefore, for 10 sites the slopes of the frequency function could be calculated and examined for effects of haloperidol. Although the slope of the ON Times was unaffected ($p>0.10$) the drug made the slope of OFF Times more negative ($p<0.05$), thus indicating a proportionally greater effect at lower frequencies of stimulation.

In the operant chamber condition, haloperidol at a dose of 0.05 mg/kg was tested at 10 sites listed in Table 1 with findings generally similar to the shuttlebox condition. As shown in Fig. 2, the drug again reduced the Percentage Time ON at both 40 Hz ($p<0.05$) and at 130 Hz ($p<0.02$). Again this effect was due to a relatively greater increase in the OFF Time measure compared to the ON Time measure. At 40 Hz the ON Times were increased by an average of 26% which although reliable ($p<0.05$) was exceeded by the 59% increase in OFF Times ($p<0.01$). At 130 Hz the increase in ON Times was not significant ($p<0.20$) but the increase in OFF Times was ($p<0.02$). At one site (12) ICSS was abolished at only 40 Hz and at two other sites (13 and 24) it was abolished at all frequencies.

Slopes of the ON and OFF Time-frequency functions could, therefore, be calculated for seven sites. As with the sites tested in the shuttlebox, haloperidol at 0.05 mg/kg did not affect the response of ON Times to frequency ($p>0.50$). However, in contrast to the shuttlebox data, the drug also failed to change the slope of the OFF Time slopes ($p>0.50$).

TABLE 1

DRUGS AND DOSES (mg/kg) TESTED WITH ICSS SITES IN VARIOUS REGIONS

| ICSS Sites | Haloperidol | | Phentolamine | | Scopolamine | | | Propranolol | | FLA-63 | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0.05 | 0.075 | 5.0 | 10.0 | 0.25 | 0.50 | 1.00 | 10.0 | 15.0 | 10.0 | 25.0 |
| MMFB | | | | | | | | | | | |
| 1 | ○ | ○ | | | | | | | | | |
| 2 | | | | | ○ | | ○ | | | | |
| 3 | ○ | ○ | ○ | ○ | | ○ | | ○ | ○ | | |
| 4 | ○ | ○ | ○ | ○ | | | | ○ | ○ | ○ | |
| 5 | | | | | | | | | | ○ | ○ |
| 6 | ○ | ○ | | | | | | | | ○ | ○ |
| 7* | ○ | | ○ | | | | | | | | |
| 8* | ○ | | ○ | | | | | | | | |
| DH | | | | | | | | | | | |
| 9 | ○ | | | | ○ | ○ | ○ | | | | |
| 10 | ○ | ○ | ○ | ○ | | | | ○ | ○ | ○ | |
| 11 | ○ | ○ | ○ | ○ | | | | ○ | ○ | ○ | |
| 12* | ○ | | ○ | | | | | | | | |
| 13* | ○ | | ○ | | | | | | | | |
| PMFB | | | | | | | | | | | |
| 14 | | | ○ | ○ | | | | | | | |
| 15 | ○ | ○ | | | ○ | ○ | ○ | | | | |
| 16 | ○ | | ○ | ○ | | | | ○ | ○ | | |
| 17* | ○ | | ○ | | | | | | | | |
| 18* | ○ | | ○ | | | | | | | | |
| SN | | | | | | | | | | | |
| 19 | | | ○ | ○ | | | | | | | |
| 20 | | | | | ○ | ○ | ○ | | | | |
| 21* | ○ | | ○ | | | | | | | | |
| 22* | ○ | | ○ | | | | | | | | |
| 23* | ○ | | ○ | | | | | | | | |
| 24* | ○ | | ○ | | | | | | | | |
| Other | | | | | | | | | | | |
| 25 | ○ | ○ | | | | | | | | | |
| 26 | | | | | | | | | | ○ | |
| 27 | ○ | ○ | ○ | | | | | ○ | ○ | ○ | |
| 28 | | | | | | ○ | ○ | | | | |
| 29 | ○ | | | | | | | | | | |
| 30 | | | | | | ○ | ○ | | | | |
| 31 | ○ | ○ | ○ | ○ | | | | ○ | ○ | ○ | ○ |
| Total | 23 | 10 | 19 | 8 | 4 | 6 | 6 | 7 | 7 | 8 | 3 |

*Site tested in operant chamber, all other sites tested in shuttlebox.

Thus in the operant chamber, haloperidol increased the OFF Times proportionally across frequencies but in the shuttlebox the increases were greater at lower frequencies.

The similar findings regarding absolute values of ON and OFF Times with the two testing conditions permitted the pooling of data for inquiry into possible regional differences in the response to haloperidol. Sufficient data was available to form four regional groups, MMFB, DH, SN and PMFB as are listed in Table 1. Four scattered sites tested with shuttlebox (25, 27, 29 and 31) were not included in the analysis. Analyses of variance showed consistently large increases in OFF Times both at 40 Hz and at 130 Hz ($p<0.001$). In contrast, the effects on ON Times were relatively minor at the four regions at either frequency ($p$'s$>0.05$). The four regions were consistent in displaying these effects of haloperidol; the F-ratios for the interaction of drug effects and regions were all nonsignificant ($p>0.10$). The four regions did not differ in ON Times at either frequency ($p>0.10$) although the regional variation in OFF Times approached significance ($p$'s$<0.10$). Due to the differential increases in OFF Times over the four regions, the Percentage Time ON under the drug decreased at both frequencies ($p$'s$<0.005$), with no evidence of a differential effect on any region, a drug-region interaction ($p$'s$>0.10$). The regions did differ in the Percentage Time ON at 130 Hz ($p<0.01$). Although the regions differed in the ON Time-frequency slopes ($p<0.005$), haloperidol was consistently without effect on the slopes (p's $>0.20$). The sites could not

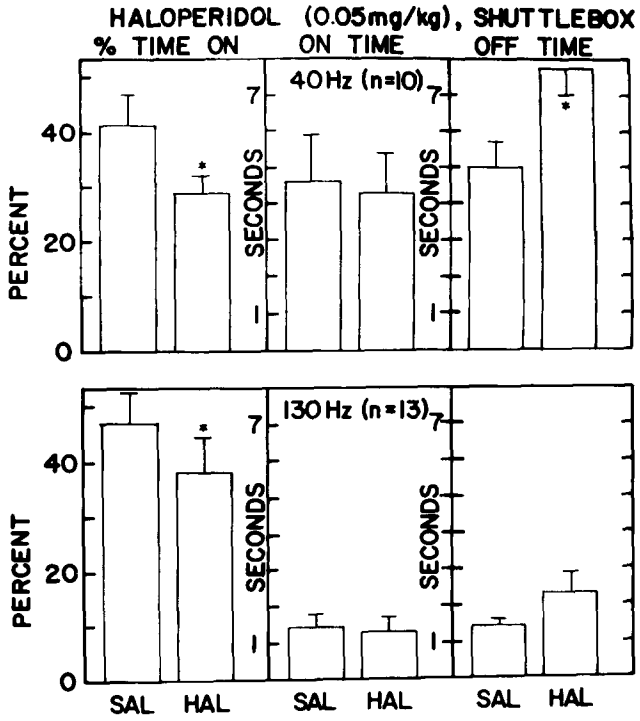


FIG. 1. Effect of haloperidol (0.05 mg/kg) on three measures of ICSS at low and high frequencies of stimulation in the shuttlebox. The lines on the histogram bars indicate the standard error of the mean. An asterisk indicates a significant ($p<0.05$) difference between saline (SAL) and haloperidol (HAL) means (paired $t$-test).

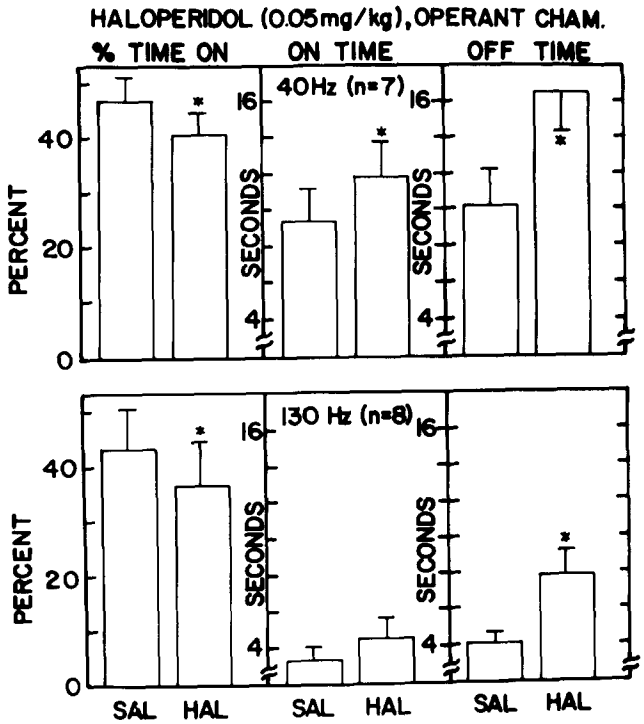


FIG. 2. Effect of haloperidol (0.05 mg/kg) on ICSS in the operant chamber.

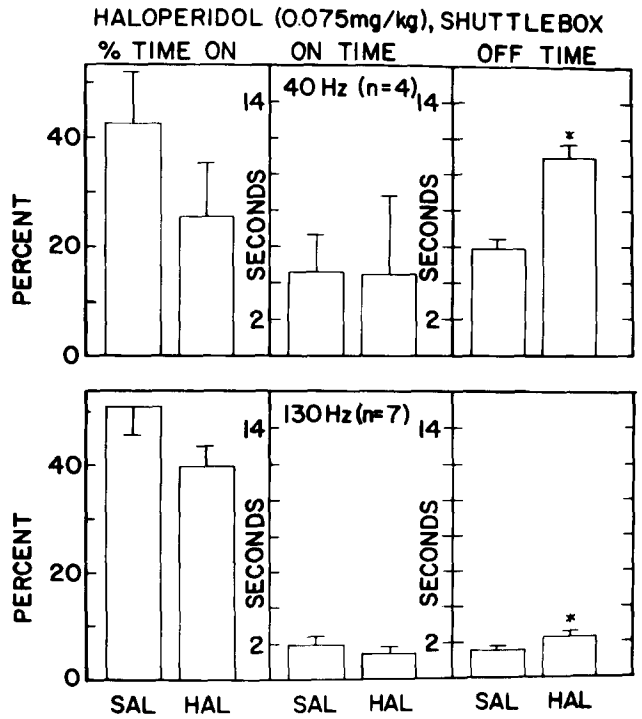


FIG. 3. Effect of haloperidol (0.075 mg/kg) on ICSS in the shuttlebox.

be examined for regional differences in haloperidol's effect on OFF Time-frequency slopes because of the differences in the two methods described above.

In interpreting the differential increases on OFF Times compared to ON Times it is important to note that effects on the ON Times were not artifactually constrained by high absolute values in the control sessions. Figures 1 and 2 are clear in this regard.

The higher dose of haloperidol, 0.075 mg/kg, was tested with 10 sites in the shuttlebox which are listed in Table 1. At three sites, ventromedial thalamus, central gray, and the DH, ICSS was reduced to the extent that no quantitative data were obtainable. In three other sites, MMFB, DH, and internal capsule, ICSS at 40 and 70 Hz but not at the higher frequencies, was abolished. These drastic effects of the 0.075 mg/kg dose which precluded quantitative analysis were the basis for the emphasis on the lower dose of 0.05 mg/kg. As shown in Fig. 3 the pattern of effects on ICSS in the sites yielding quantitative data were generally similar to those of the lower dose. At neither 40 nor 130 Hz were ON Times affected ($p$'s$>0.10$). In contrast, OFF Times were increased at both 40 Hz ($p<0.02$) and at 130 Hz ($p<0.05$). The decreases in Percentage Time ON approached the 5% significance level at both 40 Hz and at 130 Hz. The increased negativity of the OFF Time frequency slopes also approached significance but ON Time slopes showed no consistent effect ($p<0.50$).

*Phentolamine*

In the shuttlebox, phentolamine, 5.0 mg/kg, was tested with nine sites listed in Table 1. Overall the effects of the low dose of phentolamine resembled those of haloperidol. Figure 4 shows the results with the shuttlebox. Percentage Time ON was reduced significantly at 40 Hz ($p<0.01$) and to a level

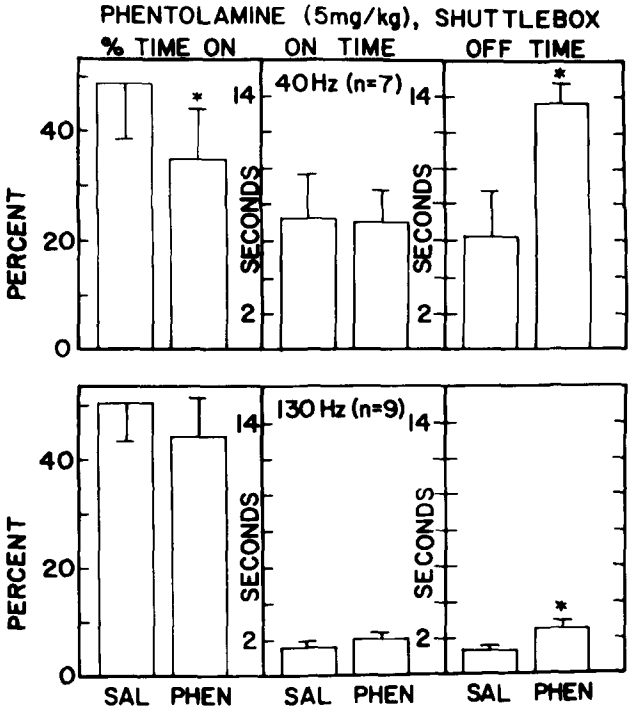


FIG. 4. Effect of phentolamine (5 mg/kg) on ICSS in the shuttlebox.

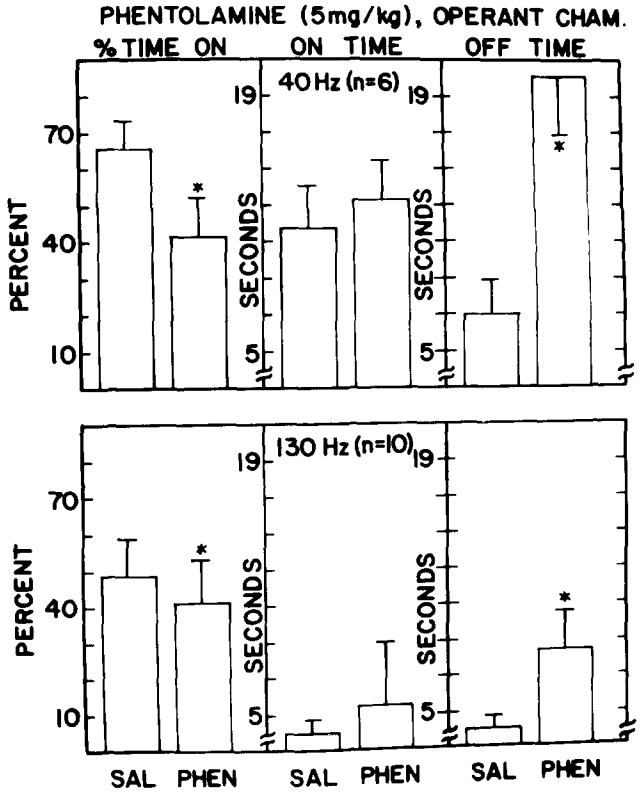


FIG. 5. Effect of phentolamine (5 mg/kg) on ICSS in the operant chamber.

that approached significance at 130 Hz. The reductions in Percentage Time ON were due to the large increases in OFF Times at the two frequencies ($p$'s$<0.02$) and the lack of any consistent effect on ON Times ($p$'s$>0.10$). With two electrodes, Site 10 in the DH and Site 27 in the ventromedial thalamus, ICSS was reduced to below criterion levels at 40 Hz but not at higher frequencies. The ON Time-frequency slopes in the seven remaining sites were not affected ($p>0.05$). Thus similar to haloperidol, phentolamine produced a greater increase in OFF Times at the lower frequencies.

With the operant chamber, 5.0 mg/kg phentolamine was tested at 10 sites. The results are shown in Fig. 5. With four electrodes, Site 12 in DH, Site 17 in the PMFB, and Sites 21 and 22 in the SN, ICSS was reduced to below criterion levels at 40 Hz. As with the sites tested in the shuttlebox, Percentage Time ON was decreased at 40 Hz ($p<0.01$) and at 130 Hz ($p<0.05$). Again large increases in OFF Times were found at both frequencies ($p$'s$<0.05$). Effects on ON Times were minimal at 40 Hz ($p>0.10$) while at 130 Hz the increase approached significance. The slopes of ON and OFF Times could be calculated for six of the ten sites that maintained performance at 40 Hz. The effect of phentolamine failed to achieve significance with either measure ($p>0.10$). However, it was noted that the increased negativity of OFF Time slopes found in the shuttlebox was also seen in five of the six available sites tested in the operant chamber. Considering also that at the four sites where slopes could not be calculated, the OFF Times at only 40 Hz exceeded criterion levels, the effects of phentolamine on OFF Times appeared greater at lower frequencies for both methods.

Pooling the observations from the shuttlebox and operant chamber conditions permitted the comparison of the MMFB, the DH, the SN and the PMFB for differential effects of phentolamine. Two isolated sites, one in the central gray and one in the ventromedial thalamus, were not used in this analysis. For none of the three measures was any regional difference significant ($p>0.10$). Nor was there evidence of differential drug effects in any of the four regions ($p$'s$>0.10$). All regions showed strong increases in OFF Times at both 40 Hz ($p<0.001$) and at 130 Hz ($p<0.005$). No region showed effects of phentolamine on ON Times at 40 Hz ($p>0.10$) but at 130 Hz, there was a consistent increase in ON Times across all regions ($p<0.05$). The large increases in OFF Times relative to ON Times produced reductions in Percentage Time ON across all regions at both 40 Hz ($p<0.001$) and at 130 Hz ($p<0.005$). The ON Time-frequency slopes did not differ across the regions ($p>0.05$), and were not influenced by phentolamine ($p>0.20$) with no indication of a drug-region interaction ($p>0.20$). The regions did not differ in OFF Time-frequency slopes. The drug, however, produced an increased negativity of the OFF Time slope ($p<0.025$) that was consistent across the four regions ($p>0.20$).

The higher dose of phentolamine, 10 mg/kg, was tested at eight sites in the shuttlebox condition with the results shown in Fig. 6. This dose of phentolamine reduced ICSS performance below criterion levels in five of the eight sites (Sites 10 and 11 in the DH, Site 14 in the PMFB, Site 19 in the SN, and Site 27 in the ventromedial thalamus). In all but Site 27 performance was seen at frequencies above 70 Hz. The loss of quantitative data at 40 Hz reduced the observations to 3 cases and the drug effects were not significant for any of the measures. Note, however, in Fig. 6 that the increase in OFF Times was large relative to the minor increase in ON Times. At 130 Hz where eight sites yielded quantitative data, the Percentage Time ON was significantly reduced ($p<0.10$) due to the relatively large increases in OFF Times which ap-

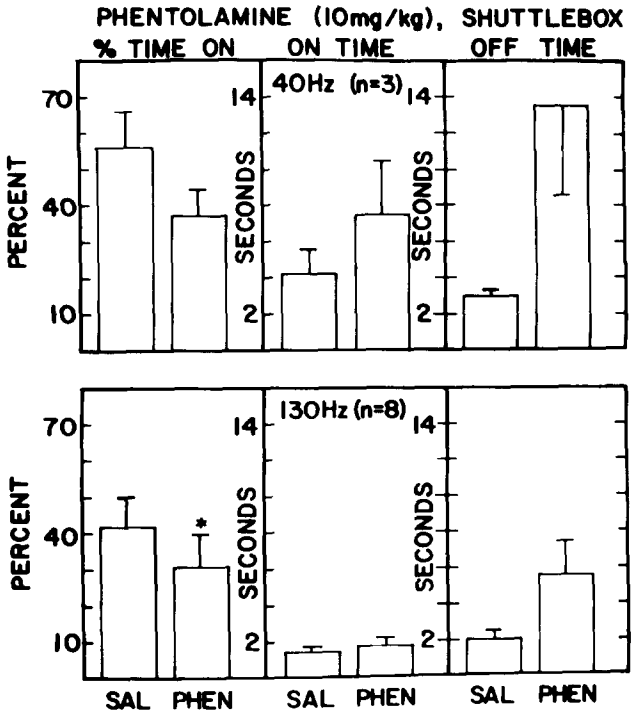


FIG. 6. Effect of phentolamine (10 mg/kg) on ICSS in the shuttlebox.

proached significance ($p<0.10$) compared to the negligible effects on ON Times ($p>0.10$). In the three sites at which slopes of the frequency functions could be determined, the high dose of phentolamine had no significant effect on the OFF Time slope ($p>0.50$) but made the ON Time slopes more negative ($p<0.01$).

*Scopolamine*

Scopolamine was tested at six sites in the shuttlebox condition at three doses, 0.25, 0.50, and 1.00 mg/kg. The lower dose was tested with only four of the sites. In contrast to the potent effects of the catecholaminergic antagonists, the cholinergic antagonist produced almost no significant changes in any measures. The single significant effect was a 33% reduction in ON Times at 130 Hz ($p<0.05$). Since 23 other $t$-tests were performed, the isolated case likely represents an $\alpha$-error, and scopolamine appears to have produced no convincing effect at the sites tested.

*Propranolol*

Propranolol was tested at seven sites in the shuttlebox condition at doses of 10 and 15 mg/kg. All sites were tested with both doses. Propranolol at neither dose produced effects on any of the three measures at either frequency or on slopes of the frequency functions ($p$'s$>0.10$).

*FLA-63*

Eight sites were tested with 10 mg/kg FLA-63 in the shuttlebox. Three of these sites were subsequently tested with a 25 mg/kg dose. Although the 10 mg/kg dose was effective to the extent of reducing performance at 40 Hz in two sites to below criterion levels, there were no significant effects on any of the three measures at either frequency or on frequency function slopes. At the higher dose of 25 mg/kg, one site showed disruption of performance at 40 Hz but no consistent effects were seen on the three measures at 130 Hz or on the frequency function slopes.

## DISCUSSION

The principal findings of this study are that the two CA receptor blockers, haloperidol and phentolamine lowered overall preference for ICSS by producing similar selective increases in the OFF Time index but no effects on the ON Time index. The pattern of results is inconsistent with the idea that the drugs at the doses used affected ICSS through a simple motoric impairment. In neither testing method were the onset and offset responses identical but they shared a common locomotor element in all cases and it is difficult to see how a simple motoric impairment would be manifest in one index and not the other. Moreover, the offset response in the operant chamber closely resembled the onset response in the shuttlebox. Both responses required locomotion to a specific side of the box yet only the latency of the response that initiated stimulation was affected. Thus, it appears that the drugs reduced the approach behavior to the rewarding brain stimulation without affecting topographically similar behaviors that terminate stimulation.

The deficits in approach to rewarding brain stimulation produced by the CA receptor blockers could result from either or both a reduction in the incentive properties of stimulation or an impairment in the initiation of operant behavior. Operant initiation deficits particularly have been emphasized by Fibiger [17] as a likely locus of effect in pharmacological studies of ICSS. By his interpretation selective effects on OFF Times as opposed to ON Times can not be considered as reward-specific since OFF Times index operant behavior whereas ON Times index respondent behavior which may be controlled by different neural processes. The validity of this behavioral classification remains to be supported but nevertheless Fibiger's position underscores the importance of determining the transmitters involved in the influence of reward on subsequent operant behavior.

No evidence was found for a regional specificity in the effects of haloperidol and phentolamine on OFF Times. It is possible that regions other than the caudal half of the hypothalamus and substantia nigra may be associated with OFF Times having differential sensitivity. The present data, however, indicate that the sensitivity of OFF Times to these drugs does not represent a process that is anatomically differentiated in the regions studied. Haloperidol and phentolamine showed a dose-dependent tendency to increase OFF Times at some ICSS sites to the point of complete cessation of performance at the lowest frequencies. These qualitative observations of the greater effectiveness of the two CA blockers at lower frequencies were supported by quantitative measures. At sites where performance was maintained under the drugs, the OFF Time slopes generally were made more negative than under control conditions. The data from the two testing methods were consonant in this regard for phentolamine, 5 mg/kg, but showed some discrepancy with haloperidol, 0.05 mg/kg, in that the sites tested in the operant chamber failed to show effects on OFF Time slopes. The discrepancy could reflect unintended differences between the two methods in the sites yielding quantitative data for slope determination. Specifically, the three DH sites were tested in the shuttlebox and the three SN sites were tested in the operant chamber. To adequately test for regional differences on the effects of haloperidol or OFF Time

slopes it will be necessary to evenly represent the regions across the methods and to show the equivalence of the two methods as was the case with phentolamine (see Table 1). Therefore, on the basis of the present data it is not possible to exclude the possibility of regional differences in the effects of haloperidol on slopes of the OFF Time frequency function.

In that performance factors are presumably held constant across frequency, the frequency dependence of haloperidol and phentolamine's effects provides further evidence that the drugs, at low doses at least, have effects on ICSS that are independent of simple motoric impairments. If the CA receptor blockers interfere with the coupling of reward and approach behavior the deficit could be mitigated by the higher reward produced by the higher frequencies of stimulation.

Neither scopolamine, a muscarinic receptor blocker, nor propranolol, a noradrenergic $\beta$-receptor blocker, had consistent effects on any measure of ICSS. These results extend earlier observations based on barpressing measures that indicate relatively minor involvement of muscarinic cholinergic and $\beta$-adrenergic systems in ICSS [22,33]. Although occasional disruption of performance was produced at low frequencies by the dopamine-$\beta$-hydroxylase inhibitor, FLA-63, no consistent effects were produced on the quantitative measures. In light of the clear effects of phentolamine, the lack of effect of FLA-63 can not be taken as strong evidence against norepinephrine's involvement in ICSS. The 3-hr interval between administration of FLA-63 and the ICSS tests may not have been optimal for depletion of releasable stores [9]. Moreover, other agents, which affect functional transmitter pools administered in conjunction with FLA-63, very well could have affected the ICSS measures.

The selective increases in OFF Times produced by haloperidol and phentolamine provide further evidence that ON and OFF Times reflect independent aspects of ICSS. A similar selectivity has been described for clonidine, $\alpha$-methyl-$p$-tyrosine [25] and clozapine [5]. However, Atrens *et al.* [5] have reported that haloperidol (0.05 and 0.10 mg/kg) increased both ON and OFF Times. The reason for this discrepancy with the present results is puzzling since the doses and methods were generally similar to the present study. Specific increases in ON Times without effects on OFF Times have been reported to follow both etorphine [8] and morphine [28] administration. These pharmacological dissociations of ON and OFF Times are consistent with data from other approaches. For example, food deprivation shortens OFF Times at posterior hypothalamic sites, increases OFF Times at anterior hypothalamic sites, but does not affect ON Times at either region [6]. The two measures also respond differently to variations in stimulation density. When stimulation is delivered in bursts of short trains separated by intervals of no stimulation, ON Times show greater increases than OFF Times [35]. Independence of the two measures is also indicated by their lack of correlation [3,7].

The selective effects of haloperidol and phentolamine on OFF Times as opposed to ON Times underscores the importance of determining what processes are reflected in these measures. The bulk of the evidence seems to indicate that the offset of ICSS in a preferred duration situation does not indicate the ascendance of an aversive process [24, 26, 36] but rather occurs because of adaptation of the reward effect [10–13]. Despite assertions that ON Times measure the latency to escape an aversive event [2, 4, 23, 31, 32, 35], there has been no direct evidence that an aversive state exists at offset. The occurrence of the offset response in itself is not sufficient evidence since it could be maintained by a subsequently increased availability of reward. If then, ON Times measure the duration over which reward is maintained with prolonged stimulation, it appears that none of the drugs used in this study affected this aspect of reward.

OFF Times reflect another aspect of ICSS reward, that which activates subsequent instrumental approach behavior. This process, in contrast to reward maintenance, appears to be strongly influenced by haloperidol and phentolamine. It seems likely that the facilitation of approach behavior indexed inversely by OFF Times has some features in common with the priming effect (or transient process) described by others [20]. This process may also predominate in situations where barpressing is used to index ICSS: a correlation has been reported between OFF Times and barpressing rates at the same sites [2]. Such relationships are consistent with the frequency by which CA drugs have been reported to affect ICSS in barpressing situations and the more recent concerns that simple interpretations are inadequate [17]. It is becoming increasingly clear that ICSS is multifaceted and these complexities must be addressed in order to adequately study the substrates of the phenomena.

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