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Measurement Issues in Curve-Shift Analysis of Apomorphine Effects on Rewarding Brain Stimulation

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HALL, F. S. AND J. R. STELLAR. *Measurement issues in curve-shift analysis of apomorphine effects on rewarding brain stimulation.* PHARMACOL BIOCHEM BEHAV **53**(2) 417–423, 1996.—The direct dopamine agonist apomorphine has been reported to reduce the rewarding efficacy of lateral hypothalamic (LH) self-stimulation. This effect has been claimed to support the notion that dopamine mediates the rewarding effects of LH self-stimulation. Using a standard rate-frequency curve-shift paradigm with ascending order of frequency presentation, we also found that apomorphine (0.1–0.8 mg/kg, SC) appeared to decrease LH self-stimulation reward. These apparent rightward curve shifts were exacerbated by shortening the test duration, which also produced a number of sessions in which the subjects did not respond at all. When the presentation order of stimulation frequencies was reversed, apomorphine did not produce large reward decreases. These results suggest that the previously reported effects of apomorphine on LH self-stimulation were the result of artifact, perhaps related to apomorphine-induced stereotypical behavior combined with rapid pharmacological recovery.

Dopamine Apomorphine Lateral hypothalamus Self-stimulation Reward

DOPAMINE (DA), particularly in the nucleus accumbens (NAc), has been shown by many researchers to be important to the rewarding effects of electrical stimulation of the lateral hypothalamus (LH), as well as to the rewarding effects of certain abused drugs (4,19,21,25,30,37,38,43–46). However, despite an extensive literature, it is not yet known whether DA systems actually carry the LH stimulation reward signal or whether DA systems more generally modulate the LH reward signal, which is carried by another pathway. Psychophysical studies of LH stimulation reward indicate that the LH and the ventral tegmental area (VTA) share common reward-relevant axons (34) and that some of these axons descend in the medial forebrain bundle (3). This descending direction of conduction study, in combination with refractory period (47), conduction velocity (34), and firing threshold studies (47) strongly suggest that DA neurons are not directly excited by the LH stimulation. However, DA neurons in the VTA may be a second stage of activation. VTA neurons may be excited transynaptically by LH stimulation (36,46). An in vivo voltammetry study (15), and some in vivo microdialysis studies (11,26,29), have demonstrated that square-wave stimulation pulses of standard charge in the lateral hypothalamus release DA in the NAc.

Administration of direct and indirect DA agonists in self-stimulation curve-shift paradigms has been investigated with respect to the relationship between DA and LH self-stimulation. If DA systems carry the self-stimulation reward signal, direct DA agonists, such as apomorphine, should elevate the background level of DA receptor activation and reduce the perceived signal-to-noise ratio generated by a DA-mediated LH reward signal. This would make the LH reward signal less effective and elevate thresholds in the curve-shift paradigm. Indirect agonists, such as amphetamine, which amplify the DA release seen with DA axonal activity, should make any DA signal more effective, decreasing reward thresholds. However, DA agonists are also known to produce behavioral stereotypy that might interfere with operant behavior by directly eliciting competing behavior.

Systemic administration of the indirect DA agonist, amphetamine, generally lowers LH stimulation reward thresholds in curve-shift measurement paradigms (10,14,35). Systemic

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administration of the direct DA agonist, apomorphine, has been studied most extensively using the nonspecific response rate measure that has been reported to increase (20), decrease (40), and both increase and decrease self-stimulation behavior (5,39) depending upon dose (42), stimulation site (8,18,28,31), current (24), or lesion state (41). Apomorphine has also been reported to increase stimulation initiation (9) and escape latencies (2,9). The inadequacies of these types of experiments have been discussed extensively elsewhere (36).

To a first approximation, the curve-shift paradigm is capable of measuring LH stimulation reward thresholds quantitatively and independently of effects upon performance (6,17, 23,27). Using methods of this type, apomorphine has been reported to increase or decrease (7,12,22) LH reward thresholds. In fact, these effects have been suggested to vary depending upon the dose administered (12,22). There were a number of methodological differences between these studies that may account for some of these differences, but one interesting point is that two of these studies found nonspecific responding under some conditions (12,22). LH stimulation reward measurement, even in a curve-shift paradigm, might be compromised by such effects. Thus, previously reported effects of apomorphine might be the result of inability to respond under apomorphine rather than changes in reward efficacy. The following experiments were conducted to determine whether varying the test parameters could influence the effects of apomorphine in a self-stimulation curve-shift paradigm, by shortening the duration of testing and reversing the order of frequency presentation. If apomorphine were truly affecting reward, such manipulations would be without effect.

METHODS

Subjects

All subjects were male Sprague–Dawley rats. Under Nembutal anaesthesia (55 mg/kg) and atropine sulphate (0.1 mg/kg) to reduce mucus formation, monopolar electrodes (350 μm diameter; Plastics One, Roanoke, VA) were implanted in the LH. The level-skull electrode coordinates were: AP − 3.0 from bregma, ML ± 1.7 from the midsagittal sinus, and DV − 7.5 from cortex. A ground wire was attached to stainless steel screws implanted in the skull and the entire construction was covered with dental acrylic anchoring the electrode to the skull. Subjects were housed in plastic tubs filled with wood shavings. The colony was temperature and humidity controlled with a 12 : 12 h light-dark cycle (light on at 8 PM). Food and water were available ad lib.

Apparatus and Training

Self-stimulation testing in four operant chambers was controlled by 4 ST-1000 Stimtek Co. (Acton, MA) microcontrollers with constant-current stimulator boards. The microcontrollers were linked to an IBM PC, which served as a terminal and enabled disk storage of programs and data. The operant chamber was equipped with a standard response lever mounted in the wall 2 cm above a wire rung floor, a house light situated in the ceiling, and a reinforcement light mounted next to the lever. During each trial, pressing the lever resulted in the delivery of stimulation under ST-1000 control. Stimulation was delivered through an electrical commutator and lead (Plastic Ones, Roanoke, VA). Stimulation always consisted of a 1.0 s burst of 0.1 ms square-wave, cathodal pulses of constant current selected by the experimenter. Between stimulating pulses, the ST-1000 electronically established a low resistance connection between the skull ground and the stimulating electrode to prevent interpulse accumulation of charge at the electrode tip. During each trial the house light was illuminated to signal that the lever was active. Between trials, when the lever was inactive, the house light was turned off. Delivery of stimulation was accompanied by the illumination of the reinforcement light.

Following established procedures (16), rats were first trained on a CRF schedule that was gradually shifted to a VI-3 s schedule of reinforcement. Behavioral testing was divided into trials during which all stimulation parameters were fixed except the stimulation frequency, which was varied systematically. Each trial consisted of 150 s of VI schedule time with an intertrial interval of 15 s. Within a trial, behavioral responding from the first 30 s was discarded to allow for adaptation to the new stimulation condition and the average response rate was calculated over the last 120 s. During the stimulation burst no behavioral data were collected and the VI schedule was temporarily paused to prevent stimulation-elicited motor effects (e.g., lever biting or stimulation-induced rearing) from distorting the measure of operant responding (17). Thus, the actual time elapsed for each trial was longer than 150 s by the total time of stimulation receipt, which at maximum rates of responding on a VI-3 s schedule could be 20 s for each minute of schedule time. At the beginning of training the stimulation current was adjusted so that high rates of responding (50–70 presses/min) were obtained for a 63 Hz stimulation burst with no signs of aversiveness (i.e., retreat from the lever, defecation, vocalization) or forced movements. After subjects were self-stimulating vigorously, they were exposed to a 10 trial sequence of alternation between 63 Hz and 1 Hz trails to insure that the rat would adjust between high and low rates of responding as stimulation levels changed dramatically.

Basic Rate-Frequency Testing and Analysis

Two warm-up conditions (63 Hz followed by 1 Hz) were followed by an ascending series of 8 pulse frequencies in the range of 1.0–2.4 log Hz (10–251 Hz). A 0.2 log step progression was used. The entire session lasted 45 min. The response-rate for each frequency was analyzed using a curve-fitting program that has been previously described (6). With stimulation frequency as the x-axis and response rate on the y-axis, the resulting rate-frequency curves were sigmoidal. According to standard procedures (6), two statistics were calculated from the rate-frequency curve: locus-of-rise (LOR) and the asymptotic, or maximum, response rate (MAX). The LOR is the stimulation pulse frequency required to maintain half-maximal responding and is similar to the ED_{50} in a drug dose–response curve. The MAX is the asymptotic rate of responding. These two statistics are believed to independently reflect stimulation reward efficacy and motor/performance capacity, respectively (6,29).

Rats were tested daily. Any final adjustments in current were made before behavioral stability was assessed. Stability was judged to occur when there were no trends in the LOR or MAX statistics (see data analysis section) and the LOR remained within 0.1 log units of baseline each day. After baseline stabilization, rats were tested in the experiments described below.

Experiment 1: Long Rate-Frequency Curve

Four subjects were given SC injections of apomorphine HCl (Sigma, St. Louis, MO) dissolved in isotonic saline with 10 mM ascorbate added to prevent oxidation. The drug was

prepared freshly before each administration and testing began 5 min after injection. The doses of apomorphine administered were 0.1, 0.2, 0.4, and 0.8 mg/kg, and were given in a counterbalanced order across subjects. The warm-up conditions, trial length, and the number of frequencies employed were as described above. After a drug test day, 2 or 3 no-drug baseline test days were assessed to verify that performance had returned to baseline. If a subject did not return to within 0.05 log Hz of LOR baseline, additional baseline days were run until the subject's performance returned to baseline levels.

Experiment 2: Short Test Procedure

For this experiment, subjects ($N = 6$) were tested on a shorter version of the rate-frequency test procedure used in Experiment 1. After a 1-week period of testing to restabilize LOR and MAX baselines, the subjects were given two doses of apomorphine (0.2 and 0.4 mg/kg, SC) separated by at least three nondrug sessions. More rapid testing was accomplished by changing the trial length from 150 to 90 s, reducing the intertrial interval from 15 to 5 s, and testing at fewer frequencies. The extinction condition was eliminated so that the frequency conditions consisted of a high warm-up condition (158 Hz) and seven ascending frequency conditions. Each rate-frequency curve took 13–15 min to complete. Three consecutive short rate-frequency sessions were made beginning at 0, 15, and 30 min after drug injection. All other procedures were as in Experiment 1.

Experiment 3: Short Test Procedure, Descending Frequencies

For this experiment, the shorter rate-frequency test format was used as in Experiment 2, but the pulse frequency conditions were presented in descending order. The warm-up condition of the previous experiments was eliminated because the first frequency ensured initially high rates of responding. Extinction was always observed at low test frequencies in baseline. Subjects were restabilized and four doses of apomorphine HCl were administered (0.1, 0.2, 0.4, and 0.8 mg/kg SC), with intervening baseline days, as before. The same subjects were used as in the previous experiment.

Data Analysis

For purposes of analysis LOR data from individual curves were converted into difference scores by subtracting the LOR on the test day from the baseline LOR. MAX scores were converted into percent differences from baseline MAX scores. Baseline was computed based on a 6-day baseline period, 3 days before and 3 days after each drug day. Only the difference scores are reported here, which were analyzed for significance by the modified method of confidence limits as described previously (35).

If the curve-fitting program did not account for more than 80% of the variance, the curve was examined visually and classified for the purpose of data presentation as: no response (NR), stereotypy high (STH), or stereotypy low (STL). The term "stereotypy" in these classifications is intended to describe the highly repetitive and unvaried behavior observed under apomorphine, which was unresponsive to changes in contingencies (like stimulation frequency). Thus, if the subject failed to respond under any frequency condition, this was termed NR. If the subject responded at a high rate (above the half-maximal baseline response rate) to all stimulation frequencies, resulting in a flat curve, this was termed STH. The same response pattern but at low response levels (below the half-maximal baseline response rate) resulted in an STL classification.

Histology

At the conclusion of the experiment, all animals were overdosed with nembutal and perfused transcardially with isotonic saline followed by 10% formalin. Brains were removed, stored for at least 1 week in 10% formalin, 1 day in 20% sucrose formalin, frozen and sectioned on a cryostat at −14°C. Forty micrometer sections were placed on gelatin-coated glass slides and stained with Cresyl violet for localization of the electrode.

RESULTS

Examination of brain sections revealed that the electrode tips for all subjects were localized in the lateral hypothalamus. The range of the actual placement coordinates were ML 1.6 to 1.9 from the midline, AP 2.8 to 3.1 from Bregma, and DV 7.4 to 7.6 from the surface of the brain.

In Experiment 1, apomorphine produced some modest increases in LOR (Table 1A) but there was substantial variability. Inspection of Table 1 reveals that occasional LOR increases were seen in the range of 0.30 log Hz, which could be interpreted as a 50% decrease in the effectiveness of stimulation pulses in producing reward. One animal (not shown) that was tested at 1.6 mg/kg, exhibited a 0.45 log unit increase in LOR, equivalent to a 65% decrease in stimulation reward effectiveness. Apomorphine also produced depressions in the MAX statistic (Table 1B).

In Experiment 2, with the faster, multiple rate-frequency test procedure, apomorphine produced larger and more consistent increases in LOR (Table 2A). These rightward curve-shifts occurred particularly in the second test coming 15 min after injection. At the 0.2 mg/kg dose, the average LOR shift for the 15 min test was 0.21 log units or about twice that observed in Experiment 1. An important observation from Table 2 is that most of the LOR increases were preceded by rate-frequency tests in which no responding (NR) occurred at all test frequencies and were followed by rate-frequency tests in which normal responding was observed. This pattern can be seen in Fig. 1, which presents the full rate-frequency curves

TABLE 1

A. APOMORPHINE–LOR (LONG R-F CURVE, ASCENDING)

Dose (mg/kg)	SH55	SH56	SH57	SH58
0.1	+0.17[b]	+0.00	−0.05	+0.03
0.2	+0.13[b]	+0.20[b]	+0.04	+0.05
0.4	+0.12[a]	−0.08[a]	+0.11[a]	+0.02
0.8	−0.06	−0.06[a]	+0.30[b]	+0.26[b]

B. APOMORPHINE–MAX (LONG R-F CURVE, ASCENDING)

Dose (mg/kg)	SH55	SH56	SH57	SH58
0.1	+8.9	−9.8	+2.1	−12.1[a]
0.2	+5.1	−31.0[a]	−3.4[a]	−12.1[a]
0.4	+0.0	−52.8[b]	−12.0[b]	−30.5[b]
0.8	+10.1	+22.9	−37.3[b]	−36.0[b]

LOR data presented as difference from baseline value, MAX data presented as percent differences from baseline value; [a]$p < 0.05$, [b]$p < 0.005$.

TABLE 2

A. APOMORPHINE–LOR (SHORT R-F CURVE, ASCENDING)

Dose (mg/kg)	Min	SH55	SH56	SH57	SH58	SH59	SH60
0.2	0	NR	NR	STL	NR	+0.12[b]	+0.10[a]
	15	+0.24[b]	+0.47[b]	+0.34[b]	+0.16[b]	−0.18[b]	STH
	30	−0.07[a]	−0.15[b]	+0.28[b]	+0.08[b]	−0.09[b]	+0.01
0.4	0	NR	NR	STL	NR	−0.08[b]	NR
	15	NR	NR	NR	+0.40[b]	+0.23[b]	NR
	30	NR	+0.50[b]	+0.63[b]	+0.13[b]	−0.07[b]	+0.50[b]

B. APOMORPHINE–MAX (SHORT R-F CURVE, ASCENDING)

Dose (mg/kg)	Min	SH55	SH56	SH57	SH58	SH59	SH60
0.2	0	NR	NR	STL	NR	−9.1[a]	−47.3[b]
	15	−43.3[b]	−59.8[b]	−71.9[b]	−27.0[b]	+8.4[a]	STH
	30	−26.1[b]	−32.0[b]	−12.3[b]	−0.4	+8.4[a]	−18.7[b]
0.4	0	NR	NR	STL	NR	+4.1[a]	NR
	15	NR	NR	NR	−28.2[b]	−27.0[b]	NR
	30	NR	−54.1[b]	−74.3[b]	−5.5	+27.9[b]	−48.0[b]

LOR data presented as difference from baseline value, MAX data presented as percent differences from baseline value; [a]$p < 0.05$, [b]$p < 0.005$, NR = no responding, STL = stereotypical responding low, STH = stereotypical responding high.

for subject SH58 at 0.4 mg/kg. Under the shorter test format, apomorphine also depressed MAX (Table 2B) and this effect appeared to be enhanced compared to Experiment 1. For example, a 38.7% average suppression of MAX at 0.2 mg/kg in the 15 min test vs. 10.3% average suppression of MAX in the previous experiment. These MAX decreases could have been the result of failing to examine high enough stimulation frequencies, so that the real asymptote was not actually measured. Although such curves were observed occasionally in Experiment 2, MAX decreases were also observed in Experiment 3 when LOR decreases were not observed.

In Experiment 3 (Table 3A), when the pulse frequencies were presented in a descending order in the short test procedure, the effects of apomorphine on LOR observed in the previous experiment disappeared. Although apomorphine had some effects upon LOR, they were equally distributed between reward-increasing and rewarding-decreasing effects. There was also a substantial increase in the number of conditions in which no responding occurred. Additionally there were more conditions with stereotypical responding (i.e., flat curves), particularly at high doses and/or on early tests in the session. At low doses, LOR shifts were minimal or the LOR decreased (reward increased). As before, rats that did not respond were observed to be stereotypically sniffing the corners or floor of the operant chamber. When the rats did respond, the MAX was almost always depressed (Table 3B) and the percent depression was comparable to that seen in the previous experiment.

To further examine the possibility that the reward-decreasing effects of apomorphine were artifactual, regressions were performed between the difference scores for LOR and the percent difference scores for MAX from all experiments. For Experiment 1 (long test, ascending frequency pattern), the correlation between log unit LOR shift and percent change in MAX was statistically significant [$r = 0.55$, $F(1,22) = 8.96$, $p < 0.01$]. For Experiment 2 (short test, ascending frequencies) the LOR-MAX correlation was also significant [$r = 0.76$, $F(1,20) = 25.42$, $p < 0.001$]. However, for Experiment 3, in which there were not large rightward shifts in LOR, the LOR-MAX correlation was nonsignificant [$r = 0.11$, $F(1,29) = 0.31$, ns].

DISCUSSION

The principal finding of this report is that systemic apomorphine does not necessarily degrade LH stimulation reward in a rate-frequency curve-shift paradigm despite the appearance, under some conditions, of large increases in LH self-stimulation half-maximal thresholds (LOR); for example, 0.47 log Hz (Table 2, SH56, 0.2 mg/kg dose). These LOR increases were observed only when stimulation frequencies were presented in ascending temporal order. On the basis of this and other aspects of the data discussed below, it is concluded that systemic apomorphine produces artifactual shifts in LOR by producing stereotypical behavior that is incompatible with operant responding. This observation is important on a practical level for studies of DA agonists where stereotypy competes with other behavior for expression (e.g., 30), and on the theoretical level because conclusions about the role of DA in self-stimulation (7,22) cannot be made on the basis of such data.

If apomorphine stereotypy abated precipitously, as suggested, then, as ascending rate-frequency tests proceeded, the subjects suddenly became able to respond just as higher stimulation frequencies were available. Such timing of recovery from response impairment created would create the artifactual rightward curve-shifts observed in Experiments 1 and 2. By testing the same subjects with the reversed pattern of frequency presentation (i.e., descending, Experiment 3), the recovery from apomorphine could not interact in this way with the frequency test pattern, and LOR increases were not generally observed. In this case the subjects recovered during low frequency conditions, or after testing, resulting in more NR ratings. If the LOR increases in Experiments 1 and 2 were actually the result of reward degradation, changing the order

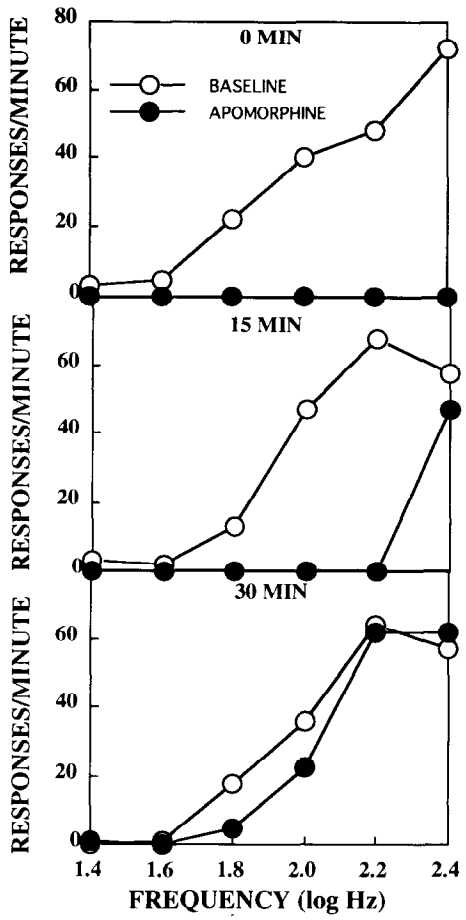

FIG. 1. Results for SH58 from Experiment 2 after administration of 0.4 mg/kg apomorphine SC. Note that the large rightward curve-shift in the middle graph occurs between an earlier test in which responding is completely suppressed and a later test in which responding is normal. LOR and MAX statistics are presented in Table 2 for the entire experimental group.

of frequency presentation should not have eliminated the effects of apomorphine. Furthermore, in Experiment 3 a number of significant reward increases were observed (Table 3A), although these changes lacked regularity and any group tendency toward reward-increasing effects was obscured by apomorphine-induced response impairments.

Further evidence for this pharmacokinetic hypothesis presented above is found in Experiment 2, where testing with a shorter format in three successive ascending rate-frequency tests often revealed a pattern of complete behavioral (i.e., operant) suppression in the first session, followed by LOR increases in the second session, and normal LOR scores in the third session. When this pattern was not observed, the animal either failed to respond completely or responded with a small LOR shift. Furthermore, as the dose of apomorphine was increased more NR ratings occurred during latter sessions. Another observation that supports this hypothesis is that rats that are self-stimulating usually sample the stimulation occasionally under low reward or no reward conditions. Under apomorphine this type of responding did not occur, again suggesting that the animals were incapable of normal responding. A final observation supporting the response impairment explanation is that the MAX statistic was suppressed in most of the rate-frequency tests for all apomorphine experiments, but MAX depression was correlated with LOR shift only in the two ascending frequency experiments, and not in the descending frequency experiment.

It could be suggested that some of the differences between the effects of apomorphine in Experiment 2 and Experiment 3 were due to sensitization. However, there were no indications in any of the experiments of sensitization to apomorphine, based on direct observation of the recovery from apomorphine-induced stereotypy. The recovery varied with dose occurring between 30 and 45 min at the two highest doses. However, this meant that recovery often occurred during high frequency conditions in Experiment 2, but during low frequency conditions during Experiment 3.

Previously reported effects of apomorphine on LH self-stimulation may have been the result of a type of artifact elucidated in the present experiments. Supporting this assertion, in one report Carey (7, page 60) suggested that apomorphine inhibition of self-stimulation was secondary to its effects on "competing motoric response patterns"; that is, stereotypical behavior interfered with operant responding. The present results are consistent with this conclusion. Leith (22) suggested that apomorphine-induced reward degradation was the result of presynaptic autoreceptor stimulation, whereas at higher doses there are differential effects on high and low current responding—lowering of high current responding and raising of low current responding. Furthermore, Leith also suggested that apomorphine mimics the effects of low current background stimulation. The implicit conclusion of her experiment was that direct dopaminergic stimulation produces a reward signal that is noncontingent—and therefore disruptive of reward. Implicit in this conclusion is the belief that dopamine carries the reward signal generated by LH stimulation. Fouriezos and Francis (12) found similar results, but interpreted high dose effects as a reward facilitation due to direct postsynaptic stimulation, and low dose effects as a reward reduction due to autoreceptor stimulation. Unlike these experiments (12,22), under no conditions in the present experiments did apomorphine produce consistent increases in responding under low reward conditions (although inconsistent effects of this type were observed).

The nature of the effect elucidated in these experiments is no doubt related to the well-known effects of apomorphine in different areas of the striatum. Apomorphine stereotypy is produced primarily within the dorsal striatum (1), whereas dopaminergic effects upon LH self-stimulation are thought to be mediated by the NAC (35,36). A microdialysis study (32) found that amphetamine-induced stereotyped head and forepaw movements were more closely correlated with DA release in the striatum, whereas locomotion (the primary behavioral effect of amphetamine at low doses) was more closely correlated with DA release in the NAC. Thus, with peripheral administration of dopaminergic agonists, the role of DA in LH self-stimulation cannot be assessed because the effects of apomorphine on postsynaptic activity in the dorsal striatum might obscure its effects upon the NAC. Direct intracranial administration of direct dopamine agonists might be able to avoid this problem by producing regionally specific reward effects.

In conclusion, previous demonstrations of LH self-stimulation reward decreases may have been erroneous, the result of response impairment. Additionally, the present experiments

TABLE 3

A. APOMORPHINE–LOR (SHORT R-F CURVE, DESCENDING)

Dose (mg/kg)	Min	SH55	SH56	SH57	SH58	SH59	SH60
0.1	0	+0.11[b]	NR	+0.03	−0.08[a]	NR	+0.11d
	15	−0.09[b]	−0.31[b]	−0.03[a]	−0.12[b]	−0.13[b]	+0.10[a]
	30	+0.01	−0.15[b]	−0.01	−0.02	+0.00	+0.14[b]
0.2	0	STL	NR	STH	NR	−0.08[b]	NR
	15	−0.40[b]	NR	STH	−0.08[a]	+0.22[b]	−0.11[a]
	30	−0.17[b]	−0.15[b]	+0.02[a]	+0.02	+0.16[b]	+0.01
0.4	0	NR	NR	STL	NR	STL	NR
	15	NR	NR	STL	NR	+0.38[b]	NR
	30	NR	NR	STL	+0.05	+0.12[b]	NR
0.8	0	NR	NR	STL	NR	STL	NR
	15	NR	NR	STL	NR	STL	NR
	30	NR	NR	NR	NR	NR	NR

B. APOMORPHINE–MAX (SHORT R-F CURVE, DESCENDING)

Dose (mg/kg)	Min	SH55	SH56	SH57	SH58	SH59	SH60
0.1	0	−50.9[b]	NR	−50.4[b]	−70.3[b]	NR	−52.6[b]
	15	−44.0[b]	−38.1[b]	−15.5[a]	−28.2[b]	−26.8[b]	−33.1[b]
	30	−25.5[a]	−28.2[a]	+4.7	−23.2[a]	−18.7	−18.8[a]
0.2	0	STL	NR	STH	NR	−68.4[b]	NR
	15	−48.6[b]	NR	STH	−28.6[b]	−24.0[b]	−52.6[b]
	30	−43.3[b]	−40.4[b]	−36.0[b]	+13.4[a]	−12.2	−23.1[a]
0.4	0	NR	NR	STL	NR	STL	NR
	15	NR	NR	STL	NR	−69.6[b]	NR
	30	NR	NR	STL	−47.2[b]	+6.5	NR
0.8	0	NR	NR	STL	NR	STL	NR
	15	NR	NR	STL	NR	STL	NR
	30	NR	NR	NR	NR	STL	NR

LOR data presented as difference from baseline value, MAX data presented as percent differences from baseline value; [a]$p < 0.05$, [b]$p < 0.005$, NR = no responding, STL = stereotypical responding low, STH = stereotypical responding high.

emphasize the general importance of considering subtle experimental parameters, such as the order of frequency presentation, that may interact with pharmacodynamics in self-stimulation.

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