Indian J Physiol Pharmacol 1996; 40(1): 15-22

DOSE-RESPONSE FUNCTIONS OF APOMORPHINE, SKF 38393, LY 171555, HALOPERIDOL AND CLONIDINE ON THE SELF-STIMULATION EVOKED FROM LATERAL HYPOTHALAMUS AND VENTRAL TEGMENTUM

JITENDRA SINGH, T. DESIRAJU AND T. R. RAJU*

Department of Neurophysiology,
National Institute of Mental Health and Neuro Sciences,
Bangalore - 560 029

(Received on June 25, 1995)

Abstract : The experimental animals were implanted with two bipolar electodes, one in the lateral hypothalamus including medial forebrain bundle (LH-MFB) and other in ipsilateral ventral tegmental area-substantia nigra (VTA-SN) and were trained to press a pedal for self-stimulation. This provided the scope to compare directly the effect of a given dose of a drug on the two reward regions in the same animal in the same testing situation. The current intensity was set to produce intracranial self-stimulation (ICSS) response rates of 50% less than the maximal shaping response rates for the respective animals (M_{50}). Following systemic (intraperitoneal) administration of apomorphine (a dopamine receptor D_1/D_2 mixed agonist), SKF 38393 ($D_1>D_3>D_2$ agonist), LY 17155 or quinpirole ($D_3>D_2$ and D_1) agonist), haloperidol (a DA-D_2 antagonist), and clonidine (noradrenaline receptor α_2 agonist), the ICSS response rates evoked from LH-MFB and VTA-SN were compared with vehicle or saline-treated animals on the basis of dose-response functions. A dose-dependent inhibitory effect at M_{50} was observed with apomorphine (0.01-1.00 mg/kg) and haloperidol (0.05-0.30 mg/kg) for both the sites of stimulation. These doses of haloperidol did not produce any motor deficits like catalepsy and muscular rigidity. The dose-response and time-effect functions of SKF 38393 and LY 171555 at M_{50} showed the facilitation and suppression of ICSS of VTA-SN and LH-MFB respectively. Clonidine (0.05-0.25 mg/kg) also produced inhibitory effect on ICSS rates, but this suppression was of different magnitude with respect to the site of stimulation. These doses of clonidine were in the range that did not prevent active pedal pressing responses. ED_{50} (the dose required to reduce the ICSS response rate 50% of the rate after administration of vehicle) for LY 171555 was 0.8 and 4.4 mg/kg for the ICSS of VTA-SN and LH-MFB respectively and thus statistically different. ED_{50} for apomorphine was 0.27 and 0.36 mg/kg; and for haloperidol was 0.75 and 0.90 mg/kg for LH-MFB and VTA-SN respectively and thus not different significantly. ED_{50} for clonidine was 0.25 and 0.08 mg/kg for VTA-SN and LH-MFB respectively and thus statistically different. The two-way analysis of variance (ANOVAR) of interaction of dose-response function of α_2 agonist with respect to LH-MFB and VTA-SN showed significant independence in their suppressive effects.

*Corresponding Author

Key words : intracranial self-stimulation apomorphine SKF 38393 LY 171555 haloperidol clonidine rat lateral hypothalamus medial forebrain bundle ventral tegmentum extinction

Abbreviations : ICSS = Intracranial self-stimulation;
LH-MFB = Lateral hypothalamus-medial forebrain bundle;
VTA-SN = Ventral tegmental area - substantia nigra.

INTRODUCTION

Quantitative as well as qualitative differences have been reported between the self-stimulation of lateral hypothalamus-medial forebrain bundle (LH-MFB) and the self-stimulation of ventral tegmental area-subtantia nigra (VTA-SN) and other brain areas including nucleus accumbens and medial prefrontal cortex (1, 2). This has been interpreted to suggest that brain structures subserving self-stimulation may be somewhat unique, despite their anatomical interconnections through the MFB (3, 4). Even forty years after its discovery, the debate over which neural system mediates brain reward continues: dopaminergic or noradrenergic? (5, 6). Moreover, response characteristics (1), rate-frequency (7, 8), reward saturation frequency (9), strength-duration characteristics (10), spatial summation, spatio-temporal integration (11), psychophysical measures of refractory period (12, 13), lesion studies (14), *in vivo* microdialysis studies of metabolites following stimulation of MFB or VTA (15), as well as, metabolic 2-(C^{14}) deoxyglucose (DG) mapping studies (16, 17) provide convincing evidences for separate neural systems underlying the self-stimulation of lateral hypothalamus and ventral tegmentum.

In order to further investigate the role of dopaminergic and noradrenergic receptors, we have examined the effects of neurotransmission modulators of dopaminergic (DA-D_1/D_2D_3) and noradrenergic (NA-α_2 receptors), assesed on the basis of dose-response functions, by testing a given drug on the ICSS of two brain regions viz LH-MFB and VTA-SN in the same animal and in the same testing conditions.

METHODS

Experimental subjects : Wistar adult male rats (n=74) weighing in the range of 260-320 g that were about 5-6½ months old at the time of surgery/implantation were used. The implanted animals were housed individually in polypropylene cages (22.5W x 35.5L x 15H cm) in temperature and humidity controlled conditions with access to 18% caesin-wheat-lipids-vitamins-minerals diet and water *ad libitum* except during the experimental sessions.

Surgery/Implantation : Rats were anaesthetised with sodium pentobarbitone anaesthesia (40 mg/kg, ip) and then given atropine sulphate (0.25 mg/kg, sc) to minimise any respiratory discomfort. A local anaesthetic lignocaine was used to minimise the discomfort in the incision site. In each rat, following standard stereotaxic procedures, the chronically indwelling two bipolar electrodes were implanted, one of them in LH-MFB and other in ipsilateral VTA-SN. The bipolar electrode was made from a pair of insulated stainless steel wire 220-250 μm and were fitted to the miniplastic collar. The final length of the bipolar electrode was kept 13 mm except the length of the connecting collar. The pre-determined flat skull stereotaxic co-ordinates were adapted from Watson (1992) rat atlas (18). LH-MFB: A-P(antero-posterior) (in mm referring to Bregma)=1.8 to —3.3; M-L (medio-lateral) (in mm referring to sagittal line)= 1.6 to 2.0; D-V (dorso-ventral) (in mm from dura) = 8.4 ± 0.2/0.3; VTA-SN : A-P=4.8 to —6.3; M-L=1.1 to 1.8 and D-V=8.6 ± 0.2/0.3. The implantations were done in the left or the right hemisphere. The probes and anchoring acrews were

secured with dental cement to obtain foundation to the skull. Penicillin G sodium (30,000 IU, im) was given at the end of operation to prevent infection.

Behavioural testing procedure : After 5-7 days of post-surgical recovery, the testing, screening and training for self-stimulation was conducted in a Skinner's operant chamber modified for ICSS that was constructed of plexiglass with inside dimensions 22Lx22Wx24H cm, and having a stainless steel pedal (4 cm wide with 2 cm projection length) positioned on one wall 6.5 cm above the grid floor. Depressing the pedal required a minimal force of 15 g of weight to ON/OFF the microswitch to trigger electrical stimulation. Contigent to the pedal pressing, electrical stimulations were delivered by a constant current stimulator to the animal through a light flexible cable, which permitted unrestricted movement. The bipolar electrode showing resistance >20 Kohms were excluded for further experimentation. The stimulation current was monitored on an oscilloscope and calculated by measuring voltage drop across a 14 Kohms precision resistor placed in series with the stimulating electrode of the lanimal. During initial training, each rat was allowed to press the pedal for ICSS to obtain reliable, sustainable and reproducible seizure-free rates of ICSS pedal press responses from the respective sites on a continuous reinforcement schedule (CRF=1:1). The stimulus obtained by the animal during each pedal pressing was 0.25s train of rectangular monophasic cathodal wave, 0.1 ms pulse duration (for MFB-LH stimulation), 0.3 ms pulse duration (for VTA-SN stimulation). The response/reinforcement data was recorded on an automated digital counter. The behavioural criterion rates were: a) a minimum of 60 pedal press responses/min for LH-MFB and b) a minimum of 90 pedal press responses/min of VTA-SN at the optimum current intensity and frequency. An optimal current was determined as one that yielded the highest rate of responding and lowest observable signs of aversiveness (e.g. retreat from pedal, vocalisation ..... etc.) (39).

Determination of M_{50} (50% of the maximum asymptotic response rate) : The current intensity and frequency was adjusted to such a level so as to get 50% of the maximum asymptotic pedal pressing rate designated as (M_{50}). The mean ($\pm$SEM) pedal pressing rates/min at M_{50} for LH-MFB and VTA-SN were 41.43 $\pm$ 5.72 and 57.96 $\pm$ 4.50 respectively.

Histology : After the completion of experiments, the sites of stimulation were examined. For this purpose, the animals were perfused intracardially with isotonic 0.9% saline followed by10% formalin. Frozen sections of 60 µm were cut coronally and stained with cresyl violet and mounted on gelatinized slides. The tip locations of the electrodes were examined and charted on plates from Paxinos and Watson rat atlas (18).

Statistical analysis : The results were analysed using 'F' ratio to find out the homogenity of variance and normality and if there were no statistical differences between the variance. Then accordingly comparisons of means of control (vehicle) and experimental (drug) were done, employing "matched paired" t-test (two-tailed). The two-way analysis of variance (ANOVAR) was applied to ascertain the effect of different doses of a given drug on the site of self-stimulation.

Materials : Apomorphine and clonidine from Sigma, U.S.A. and Haloperidol from Searle, U.S.A. were obtained. R(+) SKF 38393 and LY 171555 (Quinpirol-HCL) were from Smith Kline French Laboratories, U.S.A. and Lilly Research Laboratories, U.S.A. respectively as gift. All the chemicals were of analytical grade. Haloperidol was dissoloved in minimal quantity of glacial acetic acid and the pH was adjusted to 5.5 with 1M NaOH solution. Apomorphine, SKF 38393, LY 171555 and Clonidine were dissolved in isotonic physiological saline solution (0.9%).

RESULTS

Dose-response function of Apomorphine : With the stimulation parameters set in such a manner, so that a given animal will yield M_{50}

response rates, a control i.e. 0 dose data with vehicle injection was obtained for 15 min. Then each of the doses of apomorphine i.e. 0.1, 0.25, 0.5 and 1.0 mg/kg was administered. The percentage of reduction of ICSS response was 16.3%, 48.3%, 86.2% and 97.1% respectively for LH-MFB; and 10.2%, 29.9%, 69.3% and 89.2% respectively for VTA-SN. A dose-response function was plotted and the dose that would be just sufficient to suppress the response rate to 50% of the rate after administering vehicle (ED_{50}) was calculated. ED_{50} for LH-MFB and VTA-SN was 0.27 and 0.36 mg/kg respectively and thus not statistically different ($F_{1,22}$ =5, 46; P<0.01) (Fig.1a).

Dose-response function of SKF 38393 and LY 171555: SKF 38393 was administered in the dose of 0.10, 0.25, 0.50 and 1.00 mg/kg. The dose dependent facilitation was observed for the ICSS of VTA-SN as well as that of LH-MFB with 0.5 and 1.0 mg/kg doses only. 53% and 84% increase in the ICSS of VTA-SN and 38% and 59% that of LH-MFB was observed with the two doses 0.5 and 1.0 mg/kg respectively (Fig. 1c). The overall two-way ANOVAR showed that effect of the different doses of SKF 38393 on the site of ICSS was significant ($F_{1, 52}$=11.23; P<0.01). LY 171555 was administered in the dose of 0.1, 1.0, 2.5 and 5.0 mg/kg. The suppression was observed in the ICSS of VTA-SN and that of LH-MFB. ED_{50} was 0.8 and 4.4 mg/kg for the ICSS of VTA-SN and LH-MFB respectively (Fig. 1d). The two-way ANOVAR showed that the different doses of LY 171555 affect the ICSS of VTA-SN and LH-MFB differently ($F_{1,52}$ =16.33; P<0.01).

Time-effect function of SKF 38393 and LY 171555 : After 25 min of injection, SKF 38393 (0.5 mg/kg) showed the facilitatory effect on the ICSS of VTA-SN and lasted for more than 40 min in the representative case of rat JS 27/BE (Fig. 2). Similarly, in case of ICSS of LH-MFB, the effect was observed 35 min after the injection. The mean (±SEM) time for the facilitatory effect to appear was 22±4 min and 31±6 min for VTA-SN and LH-MFB respectively. After 10 min of injection of LY 171555 (2.5 mg/kg), the

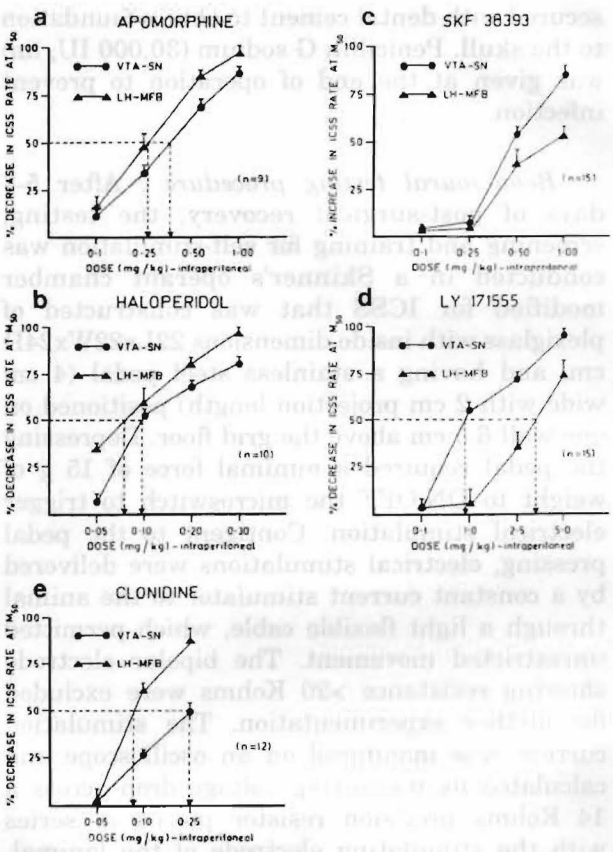

Fig. 1 : Dose-response functions following systemic administration of (a) apomorphine, (b) haloperidol, (c) SKF 38393, (d) LY 171555 and (e) clonidine. These drugs were administered 15 min prior to testing.

Note ED_{50} for apomorphine, haloperidol, clonidine and LY 171555 at M_{50}. The dose-dependent increase in the ICSS response rates with SKF 38393 at M_{50}. The number of animals tested (n) are indicated in each figure. Matched paired t-test (2-tailed).

Bar on each point represents ± SEM (standard error of mean).

attenuating effect on the ICSS of VTA-SN was observed. This effect lasted for 30 min till there was total cessation of responding. The mean (±SEM) time for appearance of the attenuating effect was 12±4 min and 18±3 min for VTA-SN and LH-MFB respectively.

Dose-response function of Haloperidol : Fig.1b summarises the dose-response data for

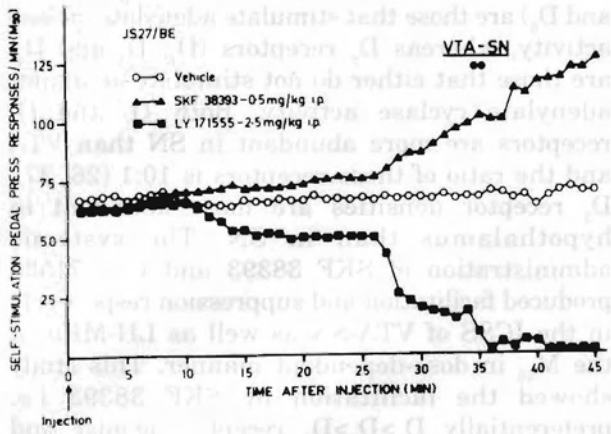

Fig. 2 : Time-effect function following systemic administration of SKF 38393 (0.5 mg/kg) and LY 171555 (2.5 mg/kg) on the ICSS of VTA-SN at M_{50} as compared to the vehicle injection. Such data was obtained for 7 rats for each of the site and each of the dose tested. For sake of clarity, only a representative example of rat JS 27/BE has been depicted.

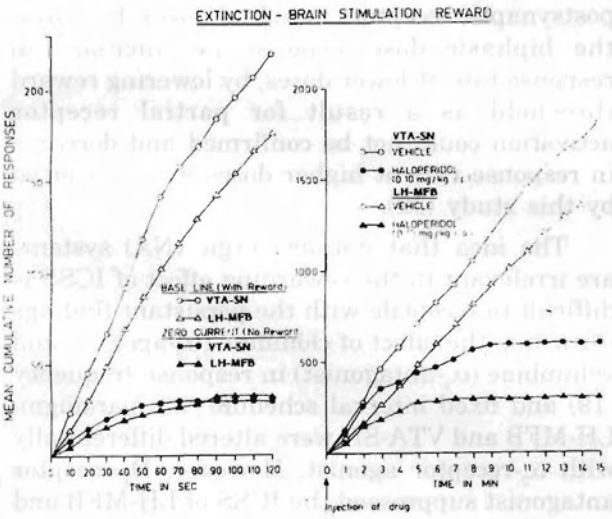

Fig. 3 : Comparison of extinction pattern with time-effect following the systemic administration of haloperidol (n=6). SEM has been avoided for clarity of depiction.

haloperidol (0.05, 0.10, 0.20 and 0.30 mg/kg). The percentage reduction of ICSS responses was 7.3%, 52.3%, 67.2% and 84.1% for VTA-SN and 34.5%, 58.5%, 79.7% and 97.2% for LH-MFB respectively and thus was not statistically different ($F_{1, 72}$ = 3.12; P<0.01).

Dose-response function of Clonidine: Fig. 1e summarises the dose-response data for clonidine (0.05, 0.10 and 0.25 mg/kg). The percentage of suppression of ICSS responses was 28.5% (with 0.10 mg) and 49.5% (with 0.25 mg) for VTA-SN; and 61.7% (with 0.10 mg) and 84.6% (with 0.25 mg) for LH-MFB. ED_{50} was 0.25 mg and 0.08 mg for VTA-SN and LH-MFB respectively (F 1, 24 = 9.37; P<0.01). The doses of clonidine were in the range that are not likely to prevent the

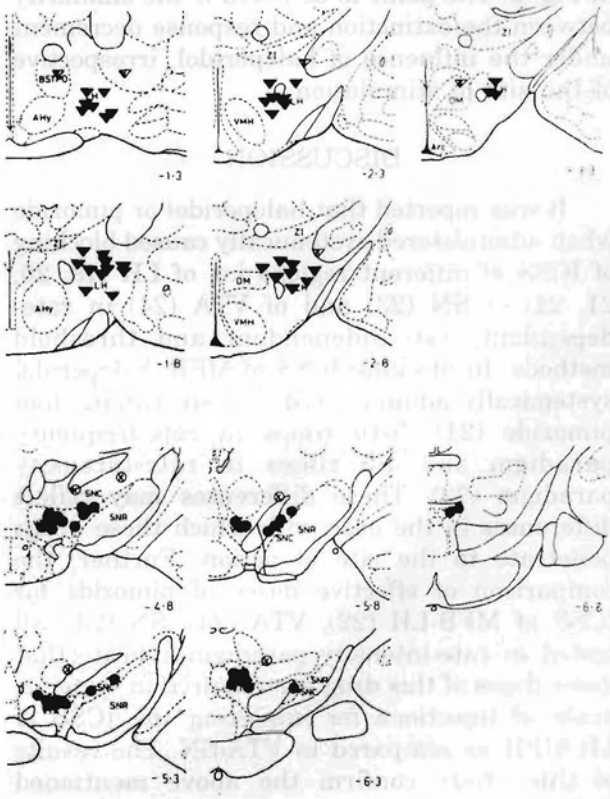

Fig. 4 : The location of positive site of self-stimulation electrode placements on the coronal section histological plates of Paxinos and Watson's rat atlas (1992).

The filled triangle (▼) for LH-MFB sites and circle (●) for VTA-SN sites indicates the tip of the bipolar electrode for only 36 out of 74 animals data for clarity of the figure. The crossed (x) triangle and circle indicates electrode sites negative for ICSS.

animal from actively responding to the pedal pressing task (19). The doses more than 0.25 mg/kg could not be tested as systemic administration of it caused jerking and circular movements.

Extinction vs response decrement pattern with haloperidol: The extinction curves are better represented by plotting cumulative response scores against time and comparing it with zero current (no reward) pattern. Following administration of haloperiodol (0.10 mg/kg) the response decrement pattern for VTA-SN and LH-MFB stimulating electrode has been shown in Fig. 3. The point to be noted is the similarity between the extinction and response decrement under the influence of haloperidol, irrespective of the site of stimulation.

DISCUSSION

It was reported that haloperidol or pimozide when administered systemically caused blocking of ICSS of different regions i.e. of LH (19, 20, 21, 22) of SN (23) and of VTA (24) in rate-dependent, rate-independent and threshold methods. In blocking ICSS of MFB, haloperidol systemically administered is more potent than pimozide (21), 5-10 times in rate-frequency paradigm and 1.5 times in rate-intensity paradigm (22). These differences may reflect differences in the ease with which these drugs penetrate to the site of action. Further, the comparison of effective doses of pimozide for ICSS of MFB-LH (22), VTA (24), SN (23), all tested in rate-intensity paradigm indicate that lower doses of this drug are required in systemic mode of injections for inhibiting the ICSS of LH-MFB as compared to VTA-SN. The results of this study confirm the above mentioned findings.

DA receptors can be divided into atleast five sub-types based on their molecular configuration (25) and are referred to as D_1 through D_5. However, these receptor sub-types can also be separated into two broader categories based on their relationship to the enzyme adenylate cyclase. In this categorisation, D_1 receptors (D_1 and D_5) are those that stimulate adenylate cyclase activity, whereas D_2 receptors (D_2, D_3 and D_4) are those that either do not stimulate or inhibit adenylate cyclase activity. Both D_1 and D_2 receptors are more abundant in SN than VTA and the ratio of these receptors is 10:1 (26, 27). D_3 receptor densities are more abundant in hypothalamus than in SN. The systemic administration of SKF 38393 and LY 171555 produced facilitation and suppression respectively in the ICSS of VTA-SN as well as LH-MFB at the M_{50} in dose-dependent manner. This study showed the facilitation by SKF 38393 i.e. preferentially $D_1 > D_3 > D_2$ receptor agonist and suppression by LY 171555 i.e. preferentially $D_3 > D_2$ and D_1 receptor agonist. Thus, the results of this study strengthens finding of blocking the ICSS of LH and VTA by D_1 receptor antagonist SCH 23390 and synergistic interactions (6, 28, 29, 30, 31), This also helps to explain the suppressive action of apomorphine which is a mixed (D_1/D_2) receptor agonist and binds to presynaptic receptors in lower doses and also to postsynaptic receptors in higher doses. However, the biphasic dose-response i.e. increase in response rate at lower doses, by lowering reward threshold as a result for partial receptor activation could not be confirmed and decrease in response rate at higher doses was supported by this study (32).

The idea that noradrenergic (NA) systems are irrelevant in the rewarding effect of ICSS is difficult to reconcile with the persistant findings regarding the effect of clonidine (α_2-agonist) and yohimbine (α_2-antagonist) in response-frequency (19) and fixed interval schedule (33) paradigm. LH-MFB and VTA-SN were altered differentially with α_2-receptor agonist. However, D_2-receptor antagonist suppressed the ICSS of LH-MFB and VTA-SN almost in similar manner. As both the sites were affected with haloperidol, a discrimination between the reduction in reward and the performance could not be derived unambigously. For this purpose, response decrement pattern was obtained from both the sites for a low dose of haloperidol (0.10 mg/kg, ip). But this experiment ruled that decrese in response rates were not due to the

deficits in the execution of motor performance (27, 34).

These results seem to suggest that it is difficult to discard either "ascending DA projection hypothesis" or "ascending NA projection hypothesis" of self-stimulation (35, 36, 37, 38, 39, 40). The comparative assessment of response rates of two regions, however, does not provide good co-relation with ascending projection hypothesis. It is consistent with descending projection hypothesis as supported by lesion studies (14) and 2-DG metabolism studies (16, 17).

Whatever may be the site of the action of these drugs, whether on the post-synaptic receptors of the above mentioned projections or on to the presynaptic receptors of the afferents that terminate in the VTA-SN and LH-MFB (26, 27, 41), they somehow after the reward signal (7). The most parsimonious interpretation of the data reported in this study, in the light of the other published reports, supports the view of multiple reward system as opposed to unitary reward system based on a single dopamine neurotransmission-gated mechanism (4, 42).

REFERENCES

1. Liebman JM. Drug effects on behaviors maintained by electrical brain stimulation. *In* Boulton AA, Baker GB and Greenshaw AJ eds. Neuromethods V. 13. Psychopharmacology. Clifton, N.J., the human press, 1989; 447-511.
2. Redgrave P, Dean P. Intracranial self-stimulation. *Br Med Bull* 1981; 32:141-146.
3. Geeraedts LMG, Nieuvenhuys R, Veening JG. Medial forebrain bundle of the rat : IV cytoarchitecture of the caudal (lateral hypothalamic) part of the medial forebrain bundle of bed nucleus. *J Comp Neurol* 1990; 294:537-568.
4. Phillips AG. Brain reward circuitry : a case for separate systems. *Brain Res Bull* 1984; 12:195-201.
5. Fibiger HC, Phillips AG, Role of catecholamine transmitters in brain reward systems implication for the neurobiology of affect. *In* Engle J and Oreland JL. eds. Brain reward system and abuse. New York, Raven, 1987; 61-74.
6. Hunt GE, Atrens DM. Reward summation and the effects of pimozide, clonidine and amphetamine on fixed-interval responding for brain stimulation. *Pharmacol Biochem Behav* 1992;42:563-577.
7. Anderson R, Miliaressis E. Does the MFB convey functionally different reward signals? *Behav Brain Res* 1994; 60: 55-61
8. Milliaressis E, Rompre PP, Laviolette LP, Phillipe L, Coulombe D. The curve-shift paradigm in self-stimulation. *Physiol Behav* 1986;37:85-91.
9. Waraczynski MEG, Stellar JR, Gallistel CR. Reward saturation in medial forbrain bundle self-stimulation. *Physiol Behav* 1987;41:585-593.
10. Hamilton AL, Stellar JR, Hart EB. Reward, performance and response strength method in self-stimulating rats: validation and neuroleptics. *Physiol Behav* 1985; 35: 897-904.
11. Schenk S, Shizgal P. The substrates for self-stimulation of the lateral hypothalamus and medial prefrontal cortex : A comparison of strength duration characteristics. *Physiol Behav* 1985;34:943-949.
12. Bielajew C, Shizgal P. Evidence implicating descending fibers in self-stimulation of the medial forebrain bundle. *J Neurosci* 1986;6:919-929.
13. Yeomans J. Mechanisms of brain-stimulation reward. *Prog Psychobiol Physiol Psychol* 1988;13:227-266.
14. Waraczynski MA. Basal forebrain knife cuts and medial forebrain bundle self-stimulation. *Brain Res* 1989;538:8-22.
15. Milliaressis E, Emond C, Merali Z. Re-evaluation of the role of dopamine in intracranial self-stimulation using *in vivo* microdialysis. *Behav Brain Res* 1991;46:43-48.
16. Gallistel CR, Gomita Y, Yadin E, Campbell KA. Forebrain origins and terminations of the medial forebrain bundle metabolitically activiated by rewarding stimulation or by reward blocking doses of pimozide. *J Neurosci* 1985;5:1246-1261.
17. Porrino LJ, Huston-Lyons D, Bain G, Sokoloff L, Korenetsky C. The distribution of changes in local cerebral metabolism associated with brain stimulation reward to the medial forebrain bundle of the rat. *Brain Res* 1990;511:1-6.
18. Paxinos G, Watson C. The Rat brain in stereotaxic co-ordinates, New York, Academic Press, 1992.
19. Gallistel CR, Freyd G. Quantitative determination of the catecholaminergic agonist and antagonists on the rewarding efficacy of brain stimulation. *Pharmacol Biochem Behav* 1987;26:731-742.
20. Ettenberg A, Durvanchelle CL. Haloperidol blocks the condition place preference induced by rewarding brain stimulation. *Behav Neurosci* 1988;102:687-691.
21. Gallistel CR, Davis AJ. Affinity for the dopamine D_2 receptor predicts neuroleptic potency in blocking the

reinforcing effect of MFB stimulation. *Pharmcol Biochem Behav* 1983;19:867-872.

22. Lynch MR, Wise RA. Relative effectiveness of pimozide, haloperidol and trifloperazine on self-stimulation rate-intensity functions. *Pharmacol Biochem Behav* 1985;23:777-780.
23. Bowers W. Hamilton M, Zacharko RM, Ansiman H. Differential effects of pimozide on response-rate and choice accuracy in a self-stimulation paradigm in mice. *Pharmacol Biochem Behav* 1985;22:521-526.
24. Phillips AG, LePiane FG. Effects of pimozide on positive and negative contrast with rewarding brain stimulation. *Pharmacol Biochem Behav* 1986; 24:1577-1582.
25. Gingrich JA, Caron MG. Recent advances in the molecular biology of dopamine receptors. *Ann Rev Neurosci* 1993; 16:299-321.
26. Boyson SJ, McGonigle P, Molinoff PB. Quantitative autoradiographic localization of the D_1 and D_2 subtypes of dopamine receptors in rat brain. *J Neurosci* 1986; 6:3177-3188.
27. Richfield EK, Pennay JB, Young AB. Anatomical and affinity state comparisons between dopamines D_1 and D_2 receptors in the rat central nervous system. *Neurosci* 1989; 30:767-777.
28. Nakajima S, Liu X, Lau CL. Synergistic interaction of D_1 and D_2 dopamine receptors in the modulation of the reinforcing effect of brain stimulation. *Behav Neurosci* 1993; 107:161-165.
29. Nakajima S, McKenzie GM. Reduction of the rewarding effect of brain stimulation by a blockade of dopamine D_1 receptor with SCH 23390. *Pharmacol Biochem Behav* 1986; 24:919-923.
30. Nakajima S, O'Regan NB. The effects of dopaminergic agonists and antagonists on the frequency-response function for the hypothalamic self-stimulation in the rat. *Pharmacol Biochem Behav* 1991; 39:465-468.
31. Ranaldi R, Beninger RJ. The effects of systemic injections of D_1 and D_2 agonists on brain stimulation reward. *Brain Res* 1994; 651: 283-292.
32. Fouriezos G, Francis S. Apomorphine and electrical self stimulation of rat brain. *Behav Brain Res* 1992; 52: 73-80.
33. Hunt GE, Atrens DM, Jackson DM. Reward summation and the effects of dopamine D_1 and D_2 agonists and antagonists on fixed-interval responding for brain stimulation. *Pharmacol Biochem Behav* 1994; 48:853-862.
34. Fenton HM, Liebman JM. Self-stimulation decrement patterns differentiate clonidine, baclofen, and dopamine antagonists from drugs causing performance deficit. *Pharmcol Biochem Behav* 1982; 17:1207-1212.
35. Ettenberg A. Dopamine, Neuroleptics and Reinforced behavior. *Neurosci Biobehav Res* 1989; 13:105-111.
36. Nakajima S. Subtypes of dopamine receptors involved in the mechanism of reinforcement. *Neurosci Biobehav Res* 1989; 13:123-128.
37. Phillips AG, Fibiger HC. Neuroanatomical basis of intracranial self-stimulation : untangling the Gordian Knot. *In* Liebman JM and Cooper SJ eds. The Neuropharmacological basis of reward. *Claredon. Oxford* 1989; 14-65.
38. Singh J, Desiraju T. Differential effects of opioid peptides administered intracerebrally in loci of self-stimulation reward of lateral hypothalamus and ventral tegmental area-substantia nigra. *In* Rapaka RS and Dhawan BN eds. Opioid peptides : An Update NIDA (National Institute on Drug Abuse) Research Monograpch # 87. Washington, DHEW publications. 1989; 180-191.
39. Singh J, Nagaraja TN, Desiraju T, Raju TR. Facilitation of self-stimulation of ventral tegmentum by microinjection of opioid receptor subtype agonists. *Physiol Behav* 1994; 55:627-631.
40. Wise RA, Rompre PP. Brain dopamine and reward. *Annu Rev Psychol* 1989;40: 191-225.
41. Leibowitz SF, Jhanwar-Uniyal M, Dvorkin B, Makman MH. Distribution of α-adrenergic, β-adrenergic and dopaminergic receptors in discrete hypothalamic areas of the rat. *Brain Res* 1982; 233:97-114.
42. Wise RA, Bozarth MA. Brain reward circuitry : four element "wired" in apparent series. *Brain Res Bull* 1984;12:203-208.
43. Fibiger HC, LePiane FG, Jakubonic A, Phillips AG. The role of dopamine in intracranial self-stimulation of the ventral tegmental area. *J Neurosci* 1987;7:3888-3896.
44. Hunt GE, Atrens DM, Johnson GFS. The tricyclic antidepressant mianserin : evaluation of its blockade of pre-synaptic α-adrenoreceptors in a self-stimulation model using clonidine. *Eur J Pharmacol* 1981; 70:69-63.
45. Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. *Science* 1988; 242:715-723.