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EFFECT OF LESIONS OF AREAS OF BRAIN - STIMULATION REWARD OF ONE REGION OF BRAIN ON OPERANT BEHAVIOUR FOR RECEIVING ELECTRICAL STIMULATION INTO SITES OF ANOTHER REGION AND ON OPERANT BEHAVIOUR FOR FOOD REWARD

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Abstract : It was aimed to study the effects of lesions of a self-stimulation (SS) area of one region of brain on the SS of another region, and on feeding behaviour in adult Wistar rats (males). The two regions proposed for study were the lateral hypothalamus (LH) and the substantia nigra - ventral tegmental area (SN-VTA). The objective was to elucidate whether each region had its own neural organization for SS behaviour or not, and whether the neural substrates of SS behaviour and feeding behaviour were one, or separate. Four bipolar electrodes were implanted bilaterally in LH and SN-VTA in each rat, and their SS pedal press rates for rewarding electrical stimulations were characterised. The rats were also trained in operant conditioning paradigm for receiving reward of food grains in FR-30 schedule. Their free-field food intake in home cages was measured. Later, electrolytic lesions of the four electrode sites were made one after another at 2-day intervals through the same biopolar electrodes. After each lesioning, the SS of the same and of the other electrode sites, and the operant performance of FR-30 food reward schedule, and daily free-field food intake (in home cage) were determined. Lesions of the LH SS site always disrupted SS-of contralateral LH but not of SN-VTA SS. Lesions of SN-VTA had not modified contralateral SN-VTA SS. A study of effects of ipsilateral lesions of LH SS site on SN-VTA SS, or of lesions of SN-VTA SS site on LH SS, revealed a range of changes, as were also effects on the FR-30 operant performance and daily food intake. Medium size lesions of SS area made in one region affected the SS of that area but not usually the SS of the other region. Large lesions of one region affected the SS of the other regions also. With large lesions, feeding behaviour also was affected, firstly of the operant type and secondly the free-field tpye.

A hypothetical scheme of regional organizations is provided to suggest the existence of both independent and interlinked neuronal substrates for SS and for feeding reward in the two regions of brain.

Key Words : lesions of self-stimulation areas, lateral hypothalamus, substantia nigra, ventral tegmental area, brain-stimulation reward, feeding behaviour

INTRODUCTION

There is as yet no framework of understanding on organization of neural subsystems underlying behavioural responding for electrical self-stimulation of brain regions, or the so-called self-stimulation (SS) of brain reward system. Rats and other species self-stimulate (1) a number of regions (eg., midbrain, hypothalamus, septum, medial prefrontal cortex), but it is not clear over the years whether the neural substrates of the different regions constitute one continuous neural substrate, or are

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independently organized in respective regions of brain (vide discussions in 1-6). Secondly, it is also not clear (1-6) as to whether the systems of brain-reward and of natural drives such as for food, sex and sleep, are the same or separate in each region (eg. hypothalamus, septum, brainstem, cortex). In earlier years, speculations on these issues were made in various ways (1-6). It can be argued that the brain-reward system is separate from those of natural drives and their rewards, as for example, the hunger drive cannot be satisfied either by self-stimulation of hypothalamus or by any other stimulation but by food. The overlapping effects noted in some studies were probably because of anatomical proximity and some secondary linkages possible in the neural networks in those regions of brain. Some influential relation between SS behaviour and drinking or feeding behaviours (7-10) observed as in hypothalamus has not been noted in other regions, and the question of what kind of relations exist between SS reward and natural drive-reward systems has remained a subject of varying speculation and many discussions (eg., vide 1, 5). In these probings, the methods of regional ablations of various kinds have been tried (6, 11-29). One way to further probe these questions is to study effects of lesions made at *identified SS* sites of one brain region and examine whether a change is caused in the SS of sites of another related brain region, and in feeding behaviours. Such experiments have not been previously reported, hence have been carried on by us and presented in this paper. In previous studies with lesions (6, 11, 12, 15-24), the sites were not firstly characterised for self-stimulation before being lesioned, and moreover, the results reported were contradictory and varying (as pointed out in the discussion section). To avoid doubts, it would be necessary to ensure by obeserving the prelesion SS that the lesioned area has brain-reward substrate, since different sites of a region differ in being participants in the brain-reward systems. In previous studies, interruption of neural pathways was done by method of electrolytic lesioning (6) or by knife cuts (25, 26), or by 6-OHDA injections (19, 27-29), or by entire forebrain ablations (13, 14). However, the conclusions based on 6-OHDA lesions which were thought to be specifically destroying only catecholaminergic pathways, became seriously suspect because of the nonspecific destructive effects of the large doses of 6-OHDA and vehicle (ascorbate) solutions employed in those studies, excepting very few (29). The electrolytic method has relatively less ambiguity as it destroys all the elements of neuropil and interpretations are made accordingly.

In view of above, the present experiments were conducted on rats, each of them having 4 bipolar electrodes implanted bilaterally in lateral hypothalamus and ventral midbrain for characterising self-stimulation and thereafter to assess the ipsilateral and contralateral effects of electrolytic lesions of SS sites of one region (i) on the SS performance of sites of the other regions, and (ii) on feeding behavior (both in operant conditioning paradigm, and under free-field food intake in home cage).

METHODS

In adult Wistar rats (males), bipolar electrodes of stainless steel were implanted stereotaxically (30) in lateral hypothalamus (LH) and substantia nigra — ventral tegmental area (SN-VTA) in both hemispheres of each rat. Bipolar electrodes were prefered to minimize diffuse spread of stimulus current and to narrow down focus of stimulation, as such conditions cannot be achieved by unipolar electrode stimulation across a distant anode. No problem of tissue destruction or performance distortion arose with bipolar electrodes to cause suspicion of metallic deposits, under the stimulus parameters used here. If proper precautions are not taken, electrical stimulation can lead to tissue destructions (31). The rat was shaped in Skinner box to operantly respond by pedal pressing for self-stimulation through each of the four electrodes (32-34). The electrical stimulus parameters provided with each pedal press were: 50 Hz sine wave train of about 0.25 sec duration. The stimulus current level was set optimally during the initial shaping and testing phase to obtain the maximum possible pedal press rate, but without having any accompanying motor or aversive side effects (21-23). In subsequent days this current level was not required to be changed but for small variations needed to

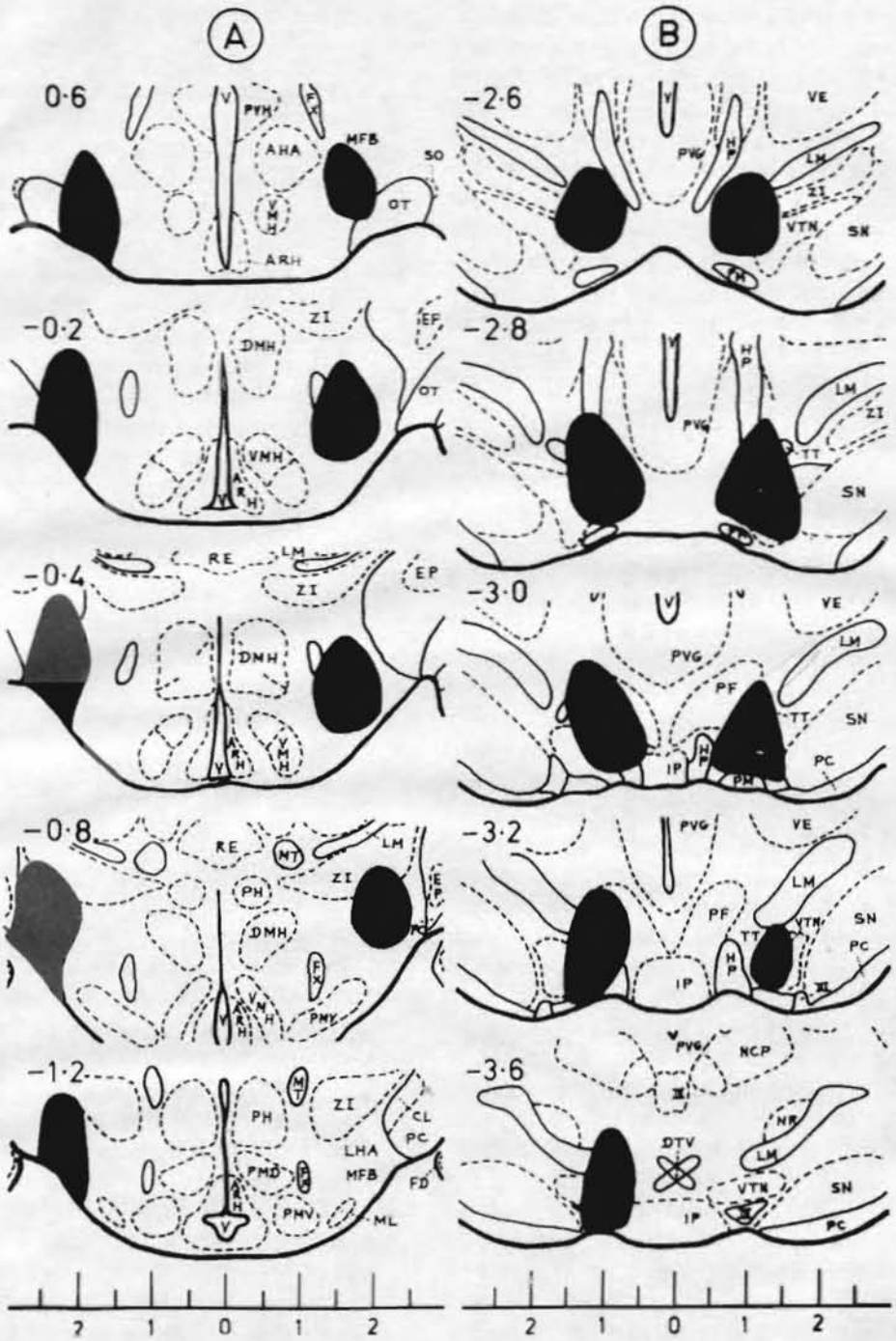

Fig. 1 : Illustration of the major extent of a medium size lesion of LH (A) and of SN-VTA (B). Numbers on frames indicated atlas (20) coordinates from bregma.

elicit the maximum possible pedal press responding. All test sessions of SS were of 15 min duration. Any rat having poor rate of SS (from even one out of the four electrodes) was excluded out of the study. This approach of testing of each of four electrode sites for SS, and study of two behaviors (SS and feeding) in each subject provided also a scope to resolve the possiblility in most of the experiments whether motor incapacitation could have been a factor underlying any reduction of SS at one or more sites by comparing with the other sites and with the second type of behavior in the same subject (35).

Lesioning of SS sites and testing the same and remaining sites: Before commencing sequential lesioning of SS sites in a subject, the stable SS rates of all the four electrode sites were recorded for comparing with data obtained after making lesions. Lesioning was done through the same SS electrodes by passing current of 2 mA of 1000 Hz of 0.7 msec square pulses or 700 Hz of 1 msec square pulses for about 6-10 sec through the bipolar electrode wires connected to the cathode, against a large buccal electrode or return path, to cause about 2 mm^3 size lesions (Fig. 1), or for about 20-30 sec to cause large lesions of about 4 mm^3. Lesioning of the 4 sites was done in sequence, one at a time at two-days intervals. Effects of the lesion on SS of the same site and of the other 3 sites were assessed on the day following each lesioning. One day after completing lesioning of the 4th site and assesment of SS, the subjects were sacrificed either for histological verification or for neurochemical estimations (done in a sample of subjects, data reported separately). They were not maintained for longer periods so as to avoid mixing up of effects of chronic reorganizational changes. No SS behavior could be elicited from an SS site after lesioning. To rule out that this lack of stimulation effect could be due to shunting of current through bipolar electrode tips by fluid of lesion cavity, monopolar mode (against a skull screw) of testing was also done and found no SS. Only by increasing the stimulus current strength to 2-3 times of pre-lesion level, about 20-30% of the pre-lesion level of SS rate as appropriate to bipolar or monopolar stimulation could be elicited in small lesion cases only.

Testing for operant behavior with food reward: Before making lesions, subjects were also additionlally trained in a different Skinner box (Coulbourn, USA or Stoelting, USA) on operant conditioning for food reward either on FR-5 or FR-30 reinforcement schedule using green gram grains soaked and half-boiled in water containing a little sugar and salt. Since the rats were intended for lesions in hypothalamus, dry food pellets were avoided, and the soaked gram grains worked very well in both control and lesioned conditions. In the home cages also, they were fed on prepared food. Their food intake in home cages was measured. After making each lesion, their operant feeding behavior, as well as free-field feeding in home cage were assessed.

Statistical analysis: Data were evaluated by using Student's t-test (two-tailed), or analysis of variance (ANOVA, treatments X subjects, data of at least 3 sessions averaged for each subject), or Wilcoxon Signed Rank test (36). p of 0.05 or less was considered significant.

RESULTS

General

SS at lesioned site: After lesioning the site of SS (with either medium or large lesion), there was no SS elicited with same stimulus from that electrodes site during the following week. By increasing the stimulus strength enormously (2 to 3 times the original), only about 30% of original SS rate could be elicited in small or medium size lesion sites but not in larger lesion sites. Monopolar stimulation against a distant electrode (large) in a normal subject caused a significantly lower SS rate than bipolar electrode, and monopolar mode required lower current than bipolar mode. After the medium size lesions, monopolar stimulation of that site yielded (with 2-3 times raised strength) only 20-30% of SS rate, and no SS with larger lesions. That was enough of functional proof that the substrate of the site that contributed to the SS behavior

was destroyed by the lesion at that area. Having created such a condition in the SS substrate of a region what consequence would it have on SS of another region?

TABLE I : Effects of lesions of SS sites of LH on SS of SN VTA, and vice versa, showing examples of the kind that lesions of one SS region do not significantly affect SS of the other region.

Lesion side	*After LH Lesion SS rates of SN VTA (n−10) Mean* ± SD	*After SN-VTA Lesion SS rates of LH (n=10)* Mean ±
Pre-lesion (N)	1631 ± 130	1382 ± 168
Ipsilateral (I)	1634 ± 163	1477 ± 227
Contralateral (C)	1665 ± 152	1406 ± 101
Both sides (B)	1613 ± 135	1409 ± 143
ANOVA F-test (N, I, C, B)	NS	NS
Wilcoxon test (N: I or C or B)	NS	NS
t-test (N: I or C or B)	NS	NS

SS rate is per 15 min. Stimulus parameters: 50 Hz, sine wave,, 0.25 sec train per pedal press, current level adjusted in each rat to obtain maximum possible SS rate. n = number of rats.
NS: not significant.
In every case, SS of the lesioned site was lost completely at stimulus parameters that caused maximal SS rates prior to lesioning. By increasing the stimulus strength 2-3 times (tested bipolarly or monopolarly) the SS could be produced at the most of about 25% of pre-lesioning rate.

SS at other sites: After the medium size lesions were made (through the SS electrode) either unilaterally or bilaterally in one of the two regions (LH or SN-VTA), usually no significant changes were caused in the SS of the other region (Table D). Hence, the question of such lesions causing motor incapacitation had not arisen.

When large lesions were made in one region, the SS of the other region was also affected. However, within reasonable limits, it was not the size alone that seemed to be important, as larger lesions also in a number of examples had not affected the SS of the other brain region. The differences in anatomical substrates that get damaged by different lesions will be more important than merely the size, in affecting, or not, the SS of the other region. The details of the effects are presented below.

LH lesions: Table II reveals one pattern of effects of LH lesions that caused significant reduction of midbrain SS, and also operant behavior for food reward and free-field food intake. Two individual examples of this type of effects are illustrated in Fig. 2. Another interesting type of effect is revealed in Table III, showing that some lesions of LH would not affect the SS of midbrain but affect the operant behavior for food reward

TABLE II : Effects of lesions of SS sites of LH on SS of SN-VTA: Data of examples of the kind that revealed that SS site lesions of one region affected SS of other region and also feeding behaviour.

Treatments	*SS rate of SN-VTA (per 15 min) (Replication in subjects)*						*Row Mean ± SD*	*Pedal press rate/15 min in FR-30 for food Mean ±SD (n=6)*	*Daily free food intake (gm) Mean* ±SD (n=6)
	1	*2*	*3*	*4*	*5*	*6*			
A. Normal before lesioning LH	2161	1559	2011	2572	2503	2449	2201 ± 386	379 ± 72.2	24 ± 3.4
B. After lesioning in one LH									
a) Ipsilateral lesioning	2367	175	1746	1735	2787	0	1468 ±1142	221 ± 63.6	15 ± 6.2
b) Contralateral lesioning	2326	1280	1856	1953	2857	2301	2095 ± 532		
C. After lesioning both LH	0	936	1275	1041	2959	0	1040 ± 1097	95 ± 46.3	6 ± 4.3
Column Mean ± SD	1713± 1445	987 ± 598	1722 ± 631	1825 ± 631	2784 ± 205	1187 ± 1372	Rows LSD 1202		

TABLE II : Contd.

Summary of statistical tests of behavioral changes after the LH lesions (bilateral) compared to pre-lesioning (Control) values

	Error	*Rows*	*Columns*	*Behaviour*	*ANOVA*	*t-test*	*Wilcoxon*
df	15	3	5	SS rates of SN-VTA	$P < 0.01$	$P < 0.01$	$P < 0.05$
SS	40641977	42812005	17927794				
MSS	2709465	14270668	3585558	FR-30 for food	$P < 0.05$	$P < 0.001$	$P < 0.05$
F		5.266	1.323	Daily free food	$P < 0.05$	$P < 0.05$	$P < 0.05$
P		<0.01	NS	intake			

FR-30: Operant behaviour for food reward on FR-30 schedule, NS: not significant. Stimulus parameters as in Table 1.

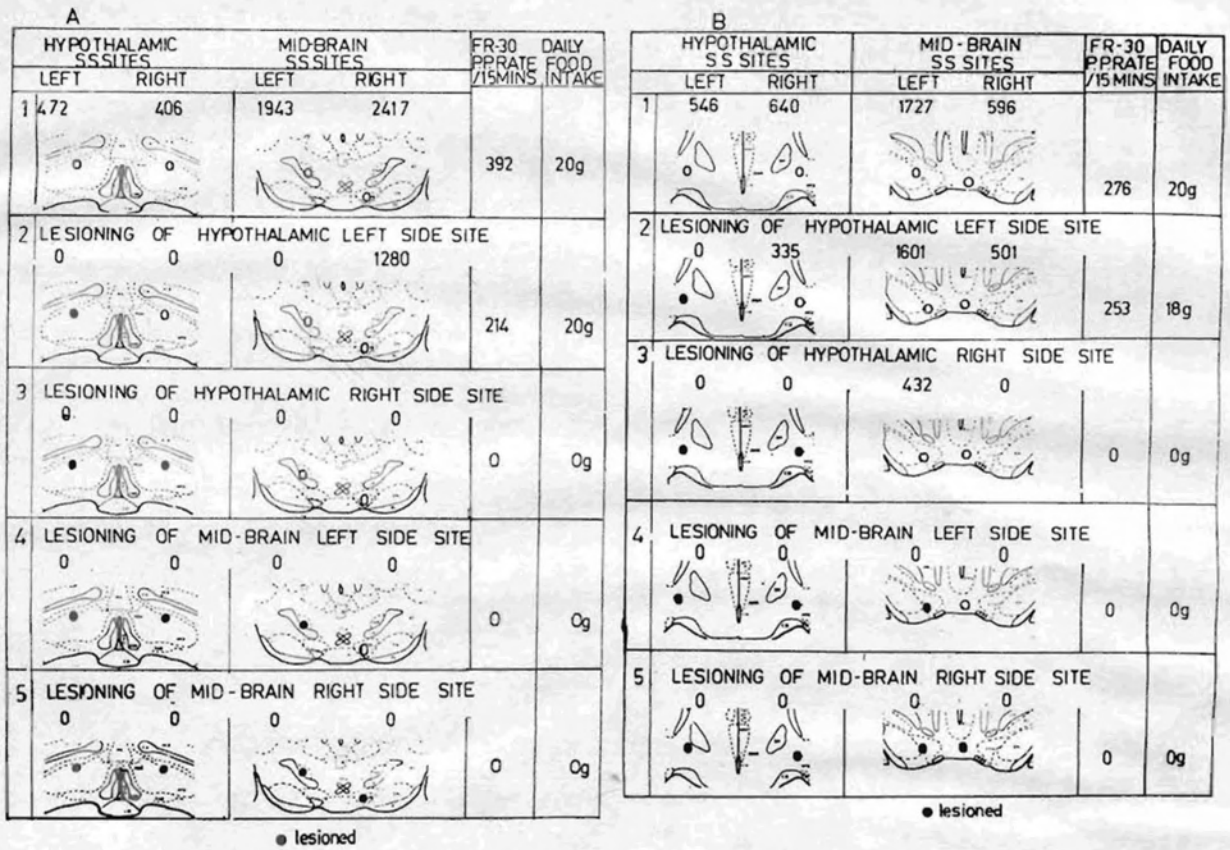

Fig. 2 : Schematic presentation (anatomico-functional) of two examples (rats A, B) of effects of large size lesions of the contralateral intact LH site, and also on the FR-30 pedal press operants for food reward as well as the daily free food intake in the home cage (rows 2,3). The anatomical loci of mid points of the tips of SS electrodes are represented by small open circles. Following lesioning of the sites through the electrodes, these tips are differentially indicated with filled circles. The sequence of lesioning and consequent changes in behaviors, are presented in rows 1-5. The number given above each histological figure represents the SS pedal press rates (per 15 min) of the electrode marked beneath in the intact or the lesioned sites tested at successive stages of lesioning in the experiment. The atlas (20) drawings of sections under the headings of hypothalamus and midbrain are at levels (in front or behind bregma) 2.2 and — 3.6 respectively in rat A, and at levels 1.0 and — 2.8 in rat B. Stimulus parameters of SS are as in Table 1.

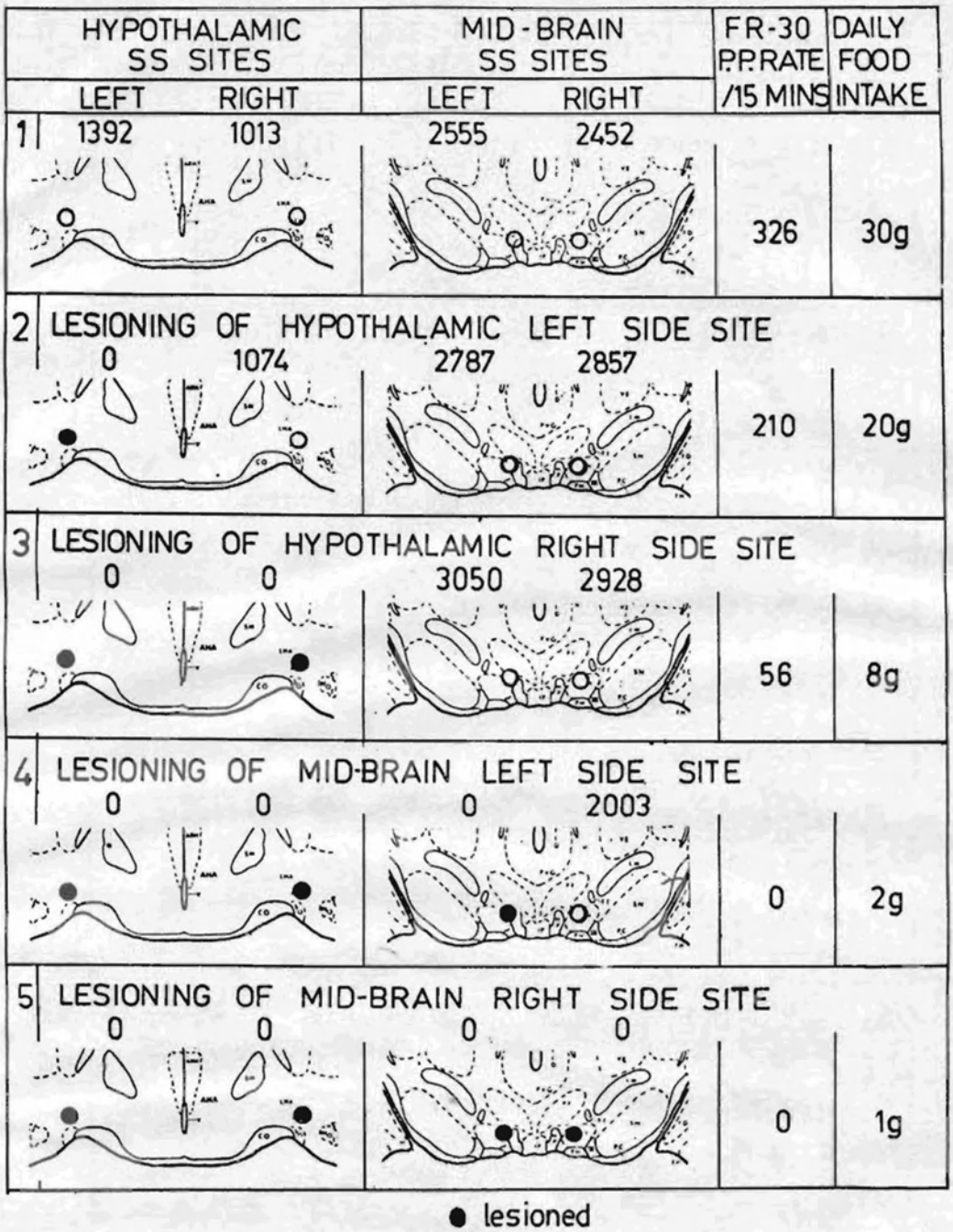

Fig. 3 : Schematic presentation of an example of the effects of medium size lesions of the SS sites of LH, causing no effect on the SS of the midbrain sites (rows 2, 3), and yet affecting markedly the responding for food reward under the FR-30 operant paradigm and also under the free field condition. The hypothalamus and midbrain drawings are at bregma coordinates 1.0 and −3.0 respectively. Rest of legend is as in Fig. 2.

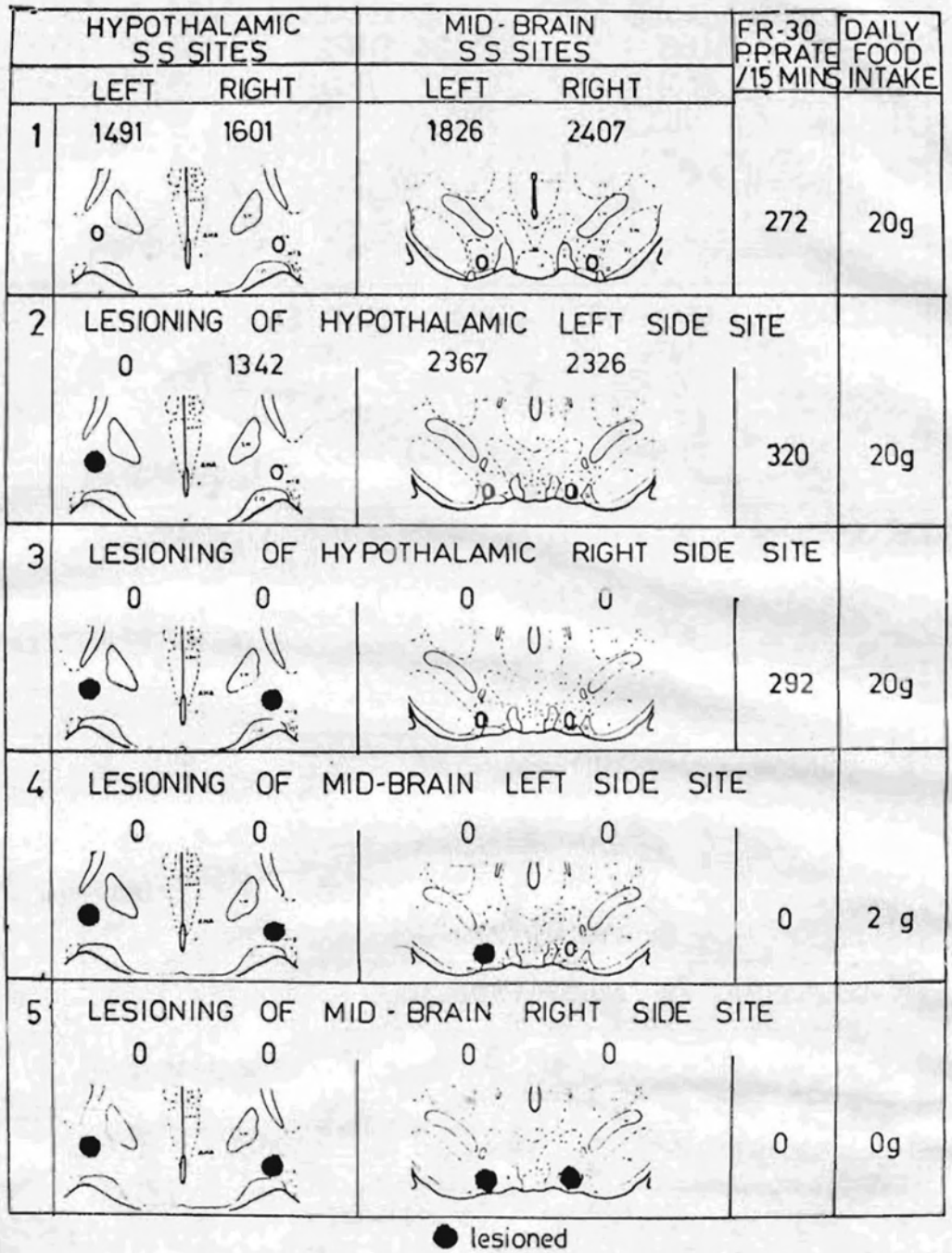

Fig. 4 : Schematic presentation of an example of effects of medium size lesions of the SS sites of LH affecting markedly the SS of midbrain, but not the responding for food reward under either operant of free-field conditions (rows 2, 3). The responding for food reward was affected only after the midbarin SS sites were lesioned (rows 4, 5 SN-VTA aphagia). The drawings of hypothalamus and midbrain are at bregma coordinates 1.0 and -3.2. Rest of legend is as in Fig. 2.

and the free-field food intake. Fig. 3 (row 3) illustrates an example of this pattern of effects. These results indicate that the lesions made in LH SS sites overlapped on the anatomical substrates of LH feeding mechanism, but not on any SS substrates that connect midbrain with LH. Moreover, the data revealed that the lesions have not depressed the motor ability, but affected differentially the motivational system of food reward, as the subject has been able (after the LH lesions) to do the SS of midbrain (Fig. 3, rows 3, 4) Another differential effect on behaviors is illustrated in Fig. 4 (row 3) in which lesions of SS sites of LH caused abolition of SS of midbrain, but not the operant behavior for food reward or the free-field food intake. Only after the additional lesions of the SS sites of midbrain, the feeding behavior also was disrupted (Fig. 4, rows 4, 5).

In summary, the above results revealed that lesions of SS sites of LH could cause four types of effects on behaviors : (i) lesions that spared SS of midbrain (Fig. 3), (ii) lesions that affected SS of midbrain (Fig. 4), (iii) lesions that spared operant behavior for food reward (Fig. 4 row 3), and (iv) lesions that affected operant behavior for food reward (Fig. 3, row 3). Hence the SS and feeding behaviors could be affected independetly, or all in combination (Table II Fig. 2).

Another observation was that lesions of SS site of one side of LH affected the SS of the site of opposite side, indicating interhypothalamic facilitatory linkage in the SS mechanism (row 2 in Fig. 2A, B; Fig. 4).

Midbrain lesions: Table IV presents one pattern

TABLE III : Effects of lesions of SS sites of LH on SS of SN-VTA: Data of examples of the kind that reveal that the SS site lesions of one region did not affect the SS of other region (LH) but affected the feeding behaviour only.

Treatments	*SS rate of SN-VTA (per 15 min) in subjects (replicated)*										*Row Mean ± SD (n=10)*	*Pedal press rate per 15 min. in FR-5 Mean ± SD (n=10)*	*Daily free food intake (gm) Mean ± SD (n=10)*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*			
A. Normal before lesioning SN-VTA	1936	1244	1286	1467	1339	1334	1332	1509	1193	1465	1460±208	85±9.3	17.4±2.2
B. After lesioning in one LH													
a) Ipsilateral lesioning	1991	1158	1312	1198	1182	1012	1803	1400	1213	1388	1366±290	68±6.3	15.8±1.7
b) Contalateral lesioning	1608	1056	1062	1486	1132	1211	1389	1308	1124	1424	1280±182		
C. After lesioning both LH	1736	1208	1166	1377	1298	4090	1655	1375	1086	1417	1335±213	41±7.8	11.2±1.8
Column Mean ± SD	1817± 153	1153± 66	1208± 97	1382± 113	1237± 83	1161± 122	1617± 176	1398± 72	1154± 51	1423± 27	Rows LSD 236	Rows LSD 19	Rows LSD 2.7

Summary of statistical tests of behavioral changes after the LH lesions (bilateral) compared to pre-lesioning (control) values

	Error	*Rows*	*Columns*
df	27	3	9
SS	2004520	105692	304466
MSS	74241	35230	33829
F		0.47	0.45
P		NS	NS

Behaviour	*ANOVA*	*LSD*	*t-test*	*Wilcoxon*
SS	NS	NS	NS	NS
FR-5 for food	$P < 0.001$	S	$P < 0.001$	$P < 0.001$
Daily free food intake	$P < 0.001$	NS	$P < 0.001$	$P < 0.001$

LSD: least significant difference, n: subjects, FR-5: operant behaviour for food reward on FR. 5 schedule, NS: not significant, S: significant, Stimulus parameters as in Table I.

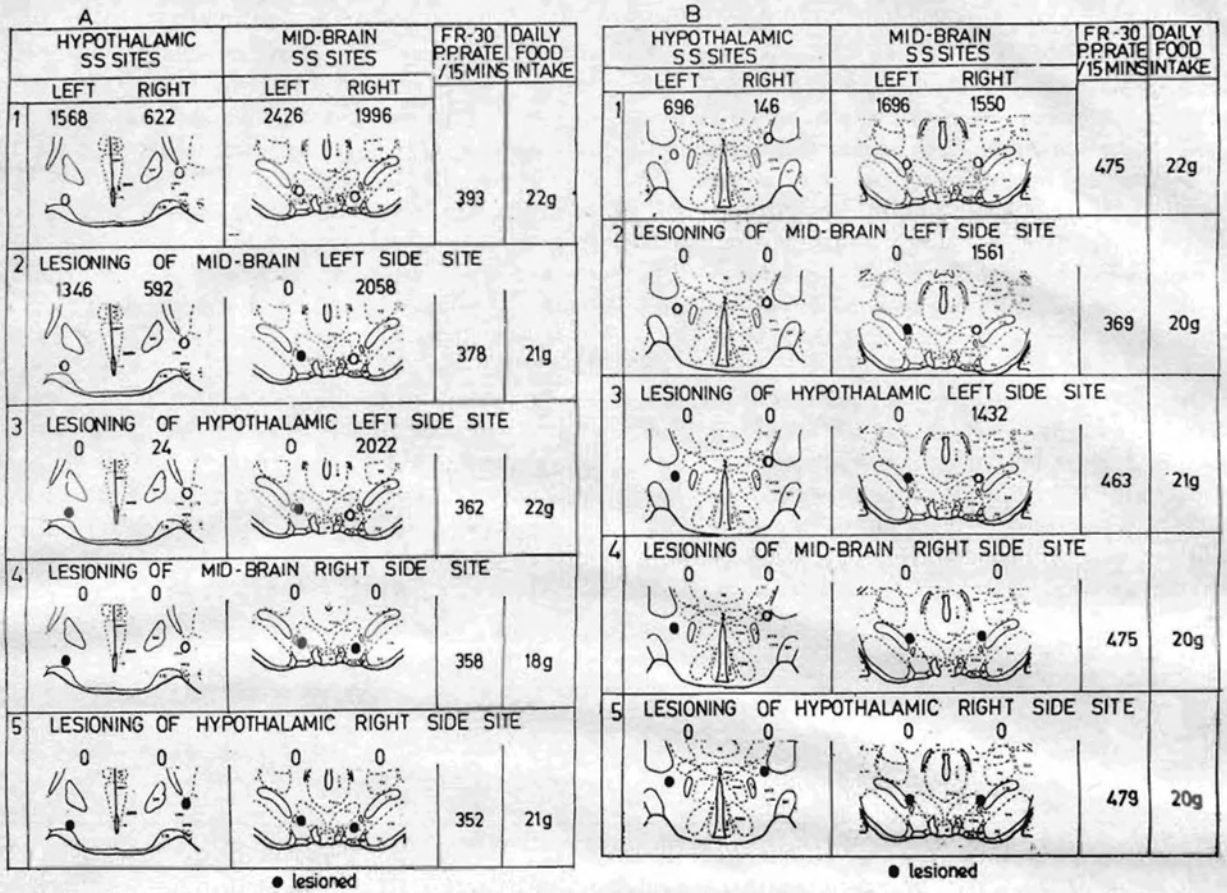

Fig. 5 : Schematic presentation of two examples (A, B) of medium size lesions of SS sites of midbrain causing attenuating effects on the SS of LH, but not affecting responding for food reward. The midbrain lesion had no significant effect on the SS of its contralateral intact site. Even after the lesions of all the four SS sites in the hypothalamus and midbrain, the responding for food reward was not significantly affected. Note that the effects establish the point of differential organization of regional subdivisions for the two types of rewards (electric stimulus, food). The drawings under hypothalamus and midbrain are at bregma levels 1.0 and -3.0 respectively in A, and at 0.2 and -3.0 in B. Rest of legend is as in Fig. 2.

TABLE IV : Effects of lesions of SS sites of SN-VTA on SS of LH, showing examples of the kind that show that the SS site lesions of one region affected the SS of other region and operant feeding behaviour but not free-feeding.

Treatments	*SS rates of LH (per 15 min) in subjects (replication)*										*Row Mean ± SD*	*Pedal press rate per 15 min FR-5 Mean ± SD (n=10)*	*Daily free food intake (gm) Mean ± SD (n=10)*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*			
A. Normal rate	1593	1472	1648	1701	1614	1000	1601	1588	1449	1233	1490±206	93±11.4	17.1±2.5
B. After lesioning in one SN-VTA													
a) Ipsilateral lesioning	968	909	1011	886	689	582	928	998	808	896	867±130	47±9.7	16.3±2.1
b) Contalateral lesioning	662	998	808	731	902	818	698	839	967	686	816±114		
C. After lesioning both SN-VTA	584	660	601	508	578	375	678	543	509	402	551±101	24±3.4	15.8±1.7
Column Mean ± SD	951± 397	1009± 294	1037± 371	956± 450	945± 402	693± 236	976± 373	1004± 376	933± 340	804± 304	Rows LSD 137	Rows LSD 29	Rows LSD 1.8

TABLE IV : Contd.

df	*Error* 27	*Rows* 3	*Columns* 9
SS	436701	4736191	393663
MSS	16174	1578730	43740
F		97.70	2.70
P		0.001	NS

Summary of statistical tests of behavioral changes after SN-VTA lesions (bilateral) compared to normal (pre-lesioning) values

Behaviour	*ANOVA*	*LSD*	*t-test*	*Wilcoxon*
SS	P 0.001	S	P 0.001	P 0.001
FR-5 for food	P 0.001	S	P 0.001	P 0.001
Daily free food intake	NS	NS	NS	NS

LSD: least significant difference, n: subjects, FR-5: operant behaviour for food reward on FR- 5 schedule, NS: not significant, S: significant, Stimulus parameters as in Table I.

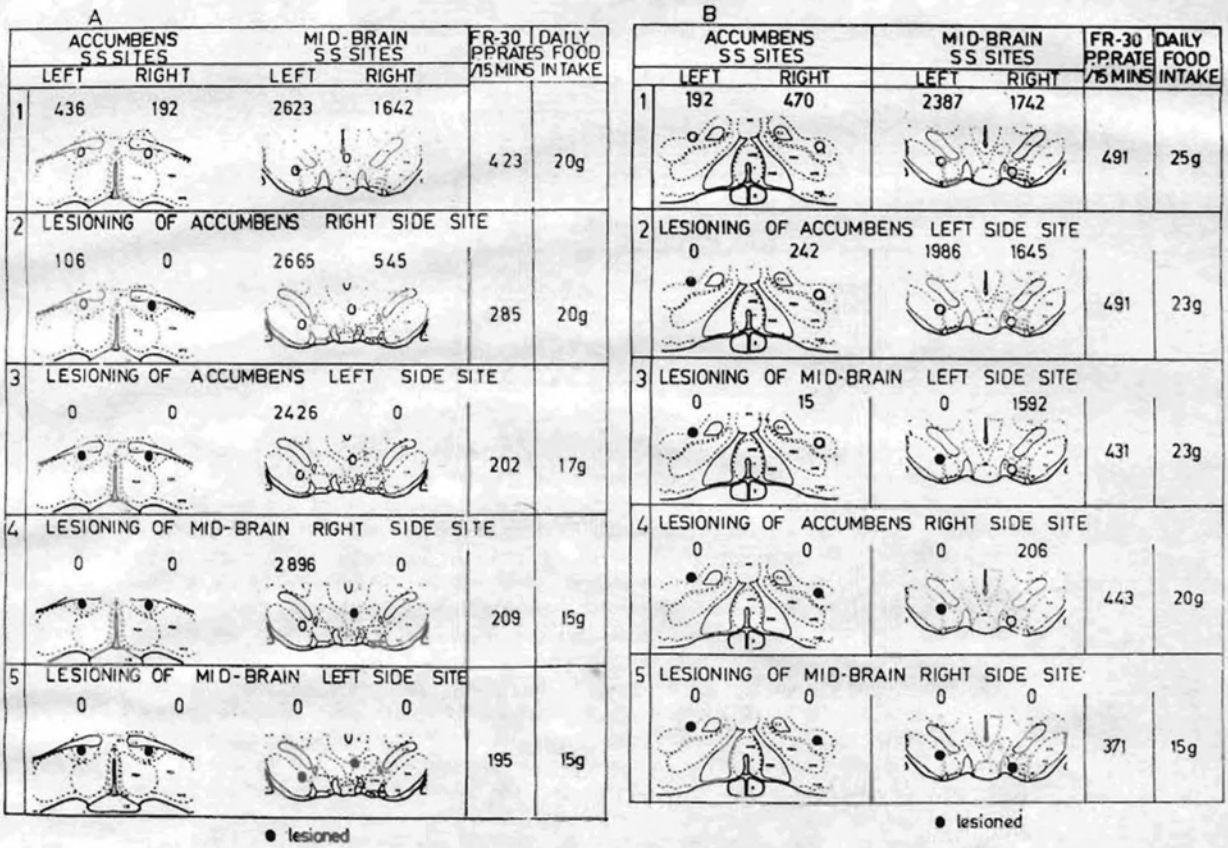

Fig. 6 : Schematic presentation of two examples (A, B) of medium size lesions of SS sites in nucleus accumbens affecting the ipsilateral midbrain SS, and also the SS of the contralateral intact accumbens site. In both the examples, after lesioning of all the four sites, the responding for food reward under the FR-30 operant paradigm as well as under the free-field paradigm were affected (row 5 in A, B). The drawings of accumbens and midbrain are at bregma levels 2.0 and -3.2 respectively in A, and at 2.4 and -3.2 in B. Rest of the legend is as in Fig. 2.

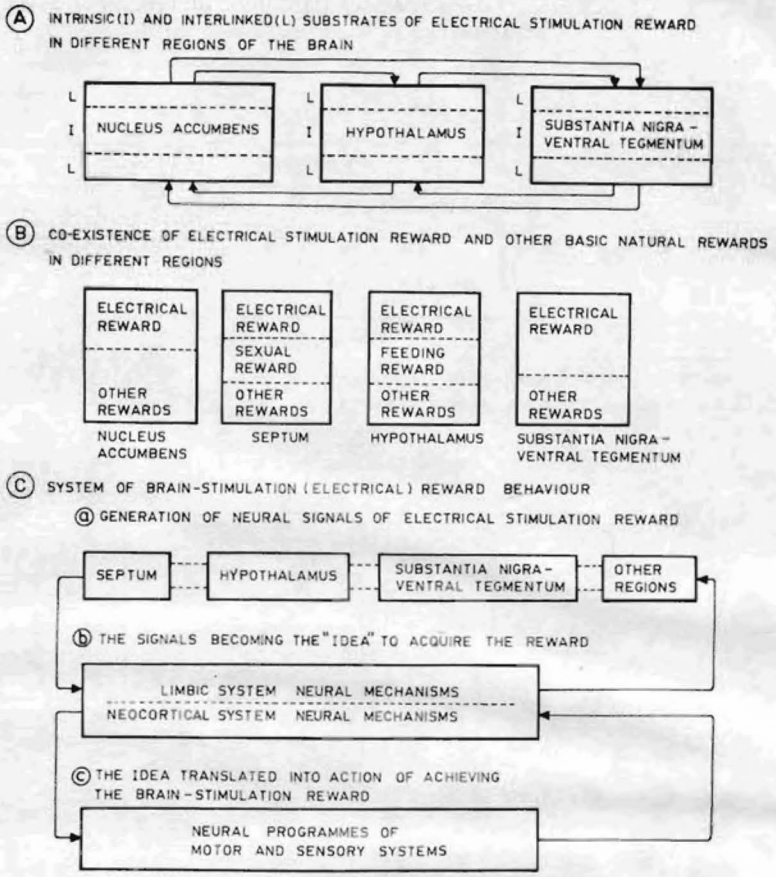

Fig. 7 : Hypothetical scheme on the neural organization of brain-stimulation (electrical) reward system in three regions (A), its coexistencial relation with substrates of basic natural rewards in each region (B), and consequences of self-stimulation of any one region in engaging limbic and neocortical neuronal processes of hedonia and reward that sustain the responding for SS without stop or satiation (C). In A, it is suggested that the electric reward substrate in each region contains two subdivions or components, one the intrinsic, and the other the interlinking. The word substrate is used to mean a mechanism that includes the anatomical network (hardware) that would be involved, and the range of operational process (programmes) of that network that contribute to the responding for SS. The scheme can account for the range of the different patterns of the effects of lesions observed in the present study, and also the apparently contradicting results reported in past literature on the effects of regional lesions on other regional SS performance. The scheme suggests that depending on the extent of invasion of the lesion in a region into intrinsic or interliked or into both subdivisions, the effect on SS would be limited to the same region or extend also on to other regions. In B, the different types of rewards are indicated to have their respective mechanisms independently operating but for coordinating linkages. Hence, again depending on the placement and extent of the lesion, responding for either only one or for more types of rewards would be affected. Such a scheme would reconcile also the differences reported in past studies on the effects of lesions on responding to SS and food reward. This scheme also implies that different regions may not be participating to the same extents in responding to SS and basic natural rewards.

of effects that lesions of SS sites of midbrain cause reduction of SS rates of LH, and also the operant behavior for food reward but not free-field food intake. There were also the other types of combinations of effects as noted with LH lesions. Fig. 5 presents two types of midbrain lesions that had not affected operant behavior for food reward (Fig. 5, A, B), while SS of LH was affected in one subject negligibly (Fig. 5, row 2), and in another significantly (Fig. 5, row 2).

Accumbens lesions: Lesions of SS sites in nucleus accumbens affected the SS of midbrain and also the operant behavior for food reward and free-field food intake (Fig. 6, row 3). In the examples of Fig. 6 B the effect was less on the operant behavior for food reward. Lesion effects between accumbens and midbrain were also like the independent as well as interacting patterns like those stated above between LH and midbrain.

DISCUSSION

Comments on previous studies on effects of lesioning of a region of brain on SS of another region: In previous studies, contradictory and varying results were reported on effects of lesions as recapitulated below.

It was reported (21) that lesions in rostral medial forebrain bundle (MFB) at the level of olfactory tubercle caused highly varying effects on SS of posterior part of lateral hypothalamus (LH), a highly varying effect of reduction being on an average of about 54% in some rats and in another rat an increase of 30%. One other study (2) reported a reduction of 50% but no such reduction effect was observed in yet another study (37). Large lesions placed in LH, either rostral or caudal to the LH SS electrode had no effect on SS of LH in other studies (6). Preoptic area lesions reduced only temporarily the LH self-stimulation (38). Basal forebrain knife-cuts (coronal) made anteriorly to hypothalamus have not affected SS of LH (26), except when perhaps the cuts passed through lateral preoptic area (25). It was reported (22) that with lesions of posterior LH the SS of the anterior LH was relatively more affected than when the experiment was the other way round. After ibotenic acid lesions of neurons in middle LH, the SS of anterior LH was more affected than SS of posterior LH implying role of descending projections of local neurons (39-41). Knife cuts along lateral border of LH abolished SS of LH (18).

LH self-stimulation was enhanced by septal lesions (16, 37). Locus coeruleus lesions were reported to have no effect on the SS of LH (20), although the lesions caused 42% reduction of noradrenaline. Damage of ascending noradrenergic neurons or small lesions of ventral tegmental area had only a negligible effect on the SS of LH (24). SN-VTA lesions caused about 50% reduction of the SS of LH (11). 6-OHDA induced lesions of SN-VTA also disrupted SS of LH (19). Effects of LH lesions on SS of SN-VTA were seldom studied in the past, and only a temporary depression of the SS of the VTA was reported in a study (17).

It was reported (13, 15) that if SS was elicited with other types of operants (head-turning or limb movements) instead of with lever pressing, SS of LH was found to be present even in the thalamic animal (i.e, after ablation of cortex, hippocampus, amygdala, septum and striatum), or in subjects after 6-OHDA lesions of substantia nigra, or after parasagittal knife-cuts at lateral border of LH. Unilateral ablation of entire forebrain had no effect on runway running of SS of LH (14). It appeared from ablations (13, 14) that LH SS is not dependent on presence of the entire forebrain (cortex, hippocampus, septum, amygdala, striatum).

Septal SS was lost after lesions in midbrain reticular formation in one study (23), whereas it was unaffected in another study (12).

The ambiguities and differences in the results of the above studies on effects of lesions was probably partly due to lack of accounting of relation of different sizes of lesions to differences in results, to locational differences of the lesions, to lack of SS functional characterisation (knowledge) of the areas lesioned, and to differences in time delays in testing after lesioning. Hence despite so many studies in the past, an unequivocal answer could

not emerge as to how much the SS of a brain region is dependent on another region.

Comments on present study of effects of lesions of characterised SS sites: The present results of lesions of characterised SS sites in the regions of LH and SN-VTA showed that different types of effects are caused by lesions of a region. Some lesions but not all may affect the SS of the other region also. Also, the SS of the lesioned sites could be affected alone, or in combination with the feeding behavior (operant or free-field). The differences in effects have been primarily corelated to size of lesion. Medium size lesions of the SS site area affected only its own SS, and not of SS of the other region. When lesion was large, the SS of the other region also affected, possibly due to invasion of the lesion over neuropil that contained either the elements that link the two regions, or the projection pathways of the other region passing through lesioned area. Since different regions of brain have their own network organizations and also have interconnections, the results on SS can be considered to suggest existence of separate intrinsic organizations in respective regions, but with some interlinking components, constituting the brain-reward neuronal subsystems that motivate volitional behaviors. The observations that lesions of one SS area may not affect SS of another region as shown by this study, that different regions have different patterns of SS (32-34), and that other behaviors associated with SS are different for different regions, and the like, gave a basis to the propositon that the mechanisms of SS in different regions are separately organized (see details in 1, 5) Philips (49) also argued for multiple and independently organized, parallel reward systems. Routtenberg (4) had changing views, and lately speculated that the SS system constituted a single one having its "head" in frontal cortex with its descending projection "perforating" through medial forebrain bundle, brainstem, locus coeruleus, and hence loss of SS in brainstem following lesions of lateral hypothalamus was due to interruption of those projections. He later on (48) added dopamine system into this for serving "memory consolidation". The present results, and of others on lesions (6, 11, 12, 15-29) and data of forebrain ablation (13, 14) as well as other data (see 1-5) cannot lend support to such a role to frontal cortex projections in brain reward system of SS. Since the SS behavior and feeding behavior were not always affected together, it was interpreted that their neural substrates also could not be one and same system but separate subsystems. Different regional intrinsic mechanisms differ in their drive-motivational impact for different kinds of rewards. Some interlinkages between them could be present to aid in achieving coordinations or adaptive modifications in homeostatic regulations of drive functions. Keeping all aspects mentioned above, Fig. 7 summarises such a hypothesized organization of networks of brain regions. A lesion falling in an intrinsic network would affect the SS functions mediated in that region and not of other regions, whereas a larger lesion that extends over both intrinsic and interlinked components would affect the SS functions of that and of the other regions. Under the same logic, different types of rewarding behaviors can also be affected separately or together according to the extent of invasion of the lesion over different neuronal mechanisms underlying the rewards.

Under the above kind of an organizational concept, some of the contradicting results on effects of lesions on SS reported in previous literature referred above could be reconciled. The contradicting reports on the effects of lesions of anterior LH on SS of posterior LH (11, 21, 37, 38) can be considered not contradictory if the lesions had extended into different subdivisioins (intrinsic or interlinked) in the studies of different workers. The contradiction (12, 23) about the effects of lesions of midbrain reticular formation on septal SS could also be reconciled with a similar explanation. The observations that ventral tegmental area (VTA) lesions can affect SS of LH (11), or that LH lesions may not affect SS of VTA (17), can now be considered under two of the four possibilities indicated by the results of the present study, and represented in the organizational schematic (Fig. 7).

Contralateral effects: The present results showed also that LH lesions of one side affected

the SS of opposite side LH, but such a contralateral effect was not observed for SN-VTA. It has been reported (42) that locus coeruleus projections decussate in hypothalamus at supra-optic level and also in anterior and posterior commisures. The contralateral effects of LH lesions observed in the present study may be due to injury to these and any other such decussating components.

Lesions and operant behavior for food: The hypothalamic lesions were relatively more effective in influencing feeding behavior than the midbrain lesions. With either regional lesion, the operant feeding behavior was firstly affected before the free-field feeding behavior.

It was interesting to note in the present study that feeding and SS behaviors could be independently affected. Such independent behaviors were not previously reported. Only the interacting effects between feeding or drinking drive state and SS of hypothalamus was noted (7-10, 43-46), but such an interaction between feeding and SS behaviors was not confirmed in VTA or in habenula (47). These reports can now be reconciled by the present results which suggested the possiblilities of both interacting and non-interacting mechanisms, as presented schematically in Fig. 7. This schematic also fits with the natural logic of imperatives that different drive states would require behavioral motivations and expressions in different modes according to needs at specified times and conditions, hence would have evolved separate neural mechanisms for separate rewards and appropriate behaviors that serve the needs of homeostatic regulations and adaptations for survival and propagation. The task then is to resolve the puzzle of what role the powerful substrate of SS is doing in the brain.

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