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COMPARATIVE ASSESSMENT OF PEDAL PRESSING RATES OF SELF-STIMULATION OF HYPOTHALAMUS AND MIDBRAIN WITH BOTH SQUARE WAVE AND SINE WAVE STIMULUS PARAMETERS

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Abstract : In Wistar rats, the regional differences of pedal pressing rates of self-stimulation (SS) of lateral hypothalamus (LH) and substantia nigra - ventral tegmental area (SN-VTA) were assessed with electrodes implanted in both regions in each subject. Average of SS rates of SN-VTA sites was significantly higher than that of LH sites, tested with both sine wave and square wave types of stimuli. There was no significant difference in SS rates between males and famles, and also in the females between different days of oestrus cycles. The high rates of robust SS observed in this study relative to SS rates reported in past literature were probably due not only to the placements of electrodes in the main substrates of SS, but also to the parameters of stimulus used (0.25 sec trains of sine waves through bipolar electrodes).

Key words : self-stimulation regional differences, self-stimulation stimulus parameters, self-stimulation gender differences, hypothalamus, ventral midbrain

INTRODUCTION

Electrical self-stimulation (SS) sites are distributed in regions of limbic system, extending from medial prefrontal cortex to ventral midbrain dopaminergic regions (1, 2). The rates of self-stimulation in a pedal press paradigm were low in septal region, and high in the midbrain sites. Hypothalamus also has a high rate of SS. However, there have been no systematic attempts to reveal the pattern of differences of pedal press rates in the different regions. Since the pedal pressing is a volitional act done for receiving electrical stimulation of a region by the subject, the rate of pressing is generally considered as an indicator of the magnitude of rewarding impact (intensity and duration) of that stimulation to the subject. Hence by studying the pattern of differences in the pedal press rates of different sites, one might obtain clues about the organization of the rewarding foci in the brain regions. For this purpose, the present study was carried out to reveal the self-stimulation rates of a number of sites in the regions of hypothalamus and ventral midbrain. Besides the above, two other aspects were also investigated. One was aimed to assess whether there would be any difference in the rewarding impact between the square wave and sine wave stimuli, since both these types of stimuli have been used in experiments reported in the literature but there is no knowledge about their comparative efficacy. The second was aimed to assess the difference between the two sexes with regard to the self-stimulation behavior, since only males have been commonly used; this practice is not based on any comparative study in the previous literature. Males have been used perhaps to avoid any modulations that might be imposed by the estrous cycles. However, it is scientifically imperative to assess whether there are really any cyclical variations of SS in the females in contrast to the males.

METHODS

Implantation of electrodes in two or more SS sites in each subject : Bipolar electrodes, each

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made of a pair of 350 μm dia insulated wires of stainless steel, tips having been separated by about 0.3 mm, were implanted stereotaxically (3) in Wistar rats under barbiturate anaesthesia. In each rat, two electrodes were implanted, one in lateral hypothalamus (LH), and another in ventral tegemental area - substantia nigra (VTA-SN), for comparison of SS rates in the same subject from two sites.

Several coordinates (3) were aimed at in lateral hypothalamus and substantia nigra - ventral tegmental area for electrode implantations for eliciting maps of rates of the SS sites. Typical coordinates for LH and SN-VTA were (bregma reference): LH: AP 0, L 1.5 to 2, H 8.5 to 8.7 mm; SN-VTA: AP -3.5 to -3.8, L 1.0 to 1.5, H 8.5 to 8.7 mm. After the experiments, the brains of subjects were fixed in formalin and studied either in free-hand cut sections or in freezing microtome cut sections stained with cresyl violet. The anatomical positions of the tips of electrode tracks were marked on to atlas charts (3) for synthesizing patterns of placements correlated to rates of SS behavior.

Stimulus delivery conditions of self-stimulation behavior : After 3-4 days of implantation of electrodes, the subject was shaped for pedal pressing for SS through each electrode in a Skinner box (size 30×30×38 cm) provided with a single pedal. The pedal was 5 cm in width and 3.2 cm in length projecting into the box, and was mounted 5 cm above the floor. A 14.5g weight was needed to press it down from resisting position to activate the microswitch of stimulator. Making the pedal movement heavier caused reduction in the SS rate, but making it lighter upto 9g caused no significant increase (15 min session). Also, changing the pedal height between 5 and 6.5 cm caused no sigificant alteration of the SS. Each pedal press triggered delivery of a 0.25 sec duration stimulus train of 50 Hz of either sine waves, or square pulses of 0.25 msec, according to the prior setting of stimulation. The self-stimulation stimulus currents were measured by a moving coil micro-ammeter which was calibrated (at several settings) with oscilloscope by monitoring voltage changes across known resistors (setting the parameters similar to SS) and calculating according to Ohm's law. For each electrode site in a rat, the optimal intensity of the SS stimulus was adjusted by increasing the current gradually in the first two training sessions to elicit the maximum possible of reproducible pedal press rate, without the current producing any motor handicaps or aversive signs. Subsequently, only small readjustments in the current level were required to elicit about the same type of maximal SS rate response. All sessions of SS were of 15 min duration each. Pedal press responses of 3 different sessions of rat were averaged and expressed as one value for that electrode site. The SS rate remained constant for each site under the stimulation conditions. The organ used by the rat for pedal pressing (left or right hand, or mouth) was also characteristic for each rat, and the same organ was used for SS of the other electrode that he had. A random check of data (of different experimenters) revealed that the majority of the rats were right paw type, the next in ranking were users of left paw. Only a minor proportion of rats (5-25%) used mouth, or both mouth and paw. The mouth usage caused no artifactual elevation of SS rate due to any "chattering" effect. A comparison of SS rates with stimulus delivery in bipolar mode and monopolar mode (using each wire of the same bipolar electrode against a large distant electrode on skull) revealed that monopolar mode evoked much less SS rate than bipolar mode and caused aversive effects at much less current strength than with bipolar mode. In the present paper, data of SS through bipolar electrodes are presented.

RESULTS

Differences of self-stimulation rates with square pulse and sine wave stimuli : Square pulse (duration of 0.25 msec) train stimuli always evoked significantly lower SS rates than with the sine wave train stimuli from any given site in either hypothalamus or midbrain (Fig. 1). The amount of stimulus charge flowing in the tissue during sine wave stimuli would be about 30 times more than that during square wave stimuli because of the difference of durations of the sine and square waves used. However, the difference in the SS rates did not

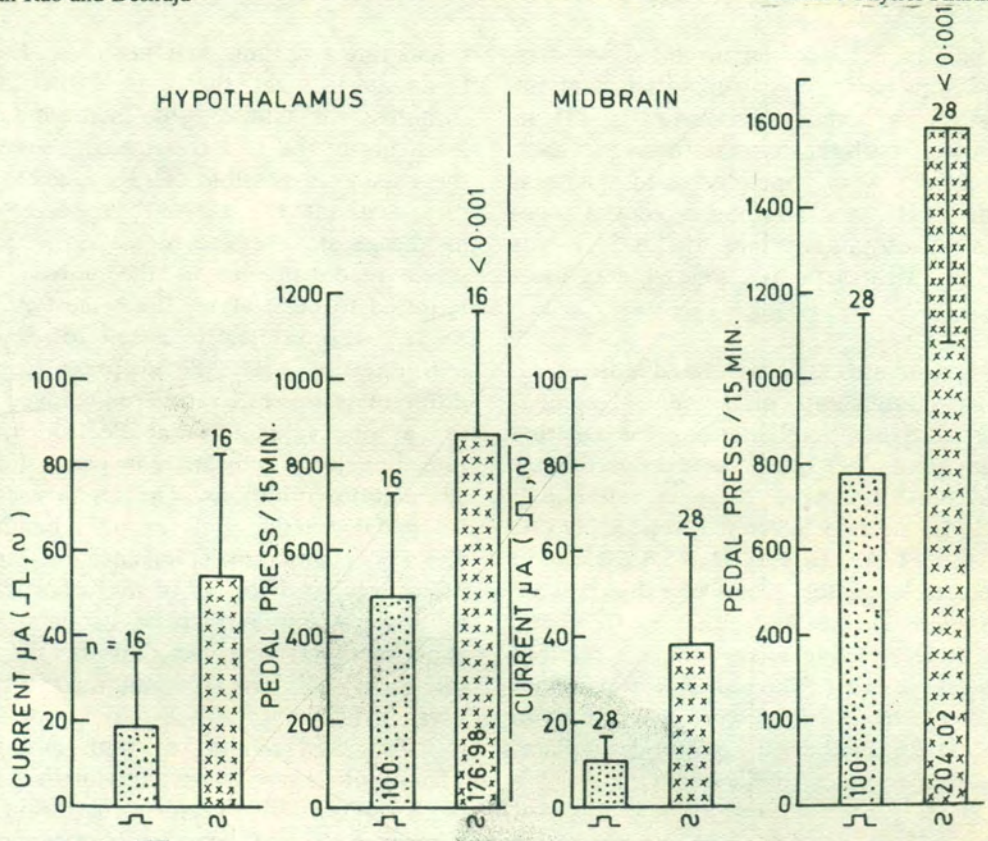

Fig. 1: Differences in pedal press rates elicited wih square wave and sine wave stimuli, in the SS through electrodes located in both hypothalamus and midbrain. Each bar is mean ± SD for data of the number of rats indicated. Note that the sine wave stimuli caused significantly higher rates of responding for SS of both brain regions.

seem to be due to difference in amount of stimulus charge, as increasing the strength of square wave stimuli was of no avail or evoked only inconveniencing motor side effects, or aversive behaviour, but not any higher SS rates. When the square wave duration was increased instead of current strength from 0.25 msec to 6 msec (keeping the stimulus train duration same as for sine wave stimulus) the SS rate of SN-VTA showed no significant increase but on the contrary showed a trend towards a decrease. Hence the results suggested that the effect of sine wave stimuli was due to the wave form but not due to differences of current strength (amount of charge) or wave duration. It is well known that different wave forms (square, ramp, sine, etc.) exert different types of effects on neuronal excitation or inhibition, hence the differences in SS are probably due to square and sine wave form of the stimuli.

Differences of SS rates of sites of midbrain and hypothalamus : Experiments conducted on 92 rats consistently revealed a significant difference in SS rates of hypothalamus and midbrain (Fig. 2). There were also some SS sites that gave similar rates of SS in both regions. The ranges of SS rates of the two regions are illustrated in Fig. 2. In summary, in the midbrain (Fig. 2a), 49.4% of the sites showed SS rates (per 15 min) in the range of 1001-2000, 25.3% in the range of 300-1000 (data of rates below 300 not included), and another 25.3% in the range of 2001-3000. The midbrain sites studied were distributed in the regions of substantia nigra, ventral tegmental area, retrorubral nucleus, interpeduncular nucleus, zona incerta and the tracts within. In hypothalamus, in summary

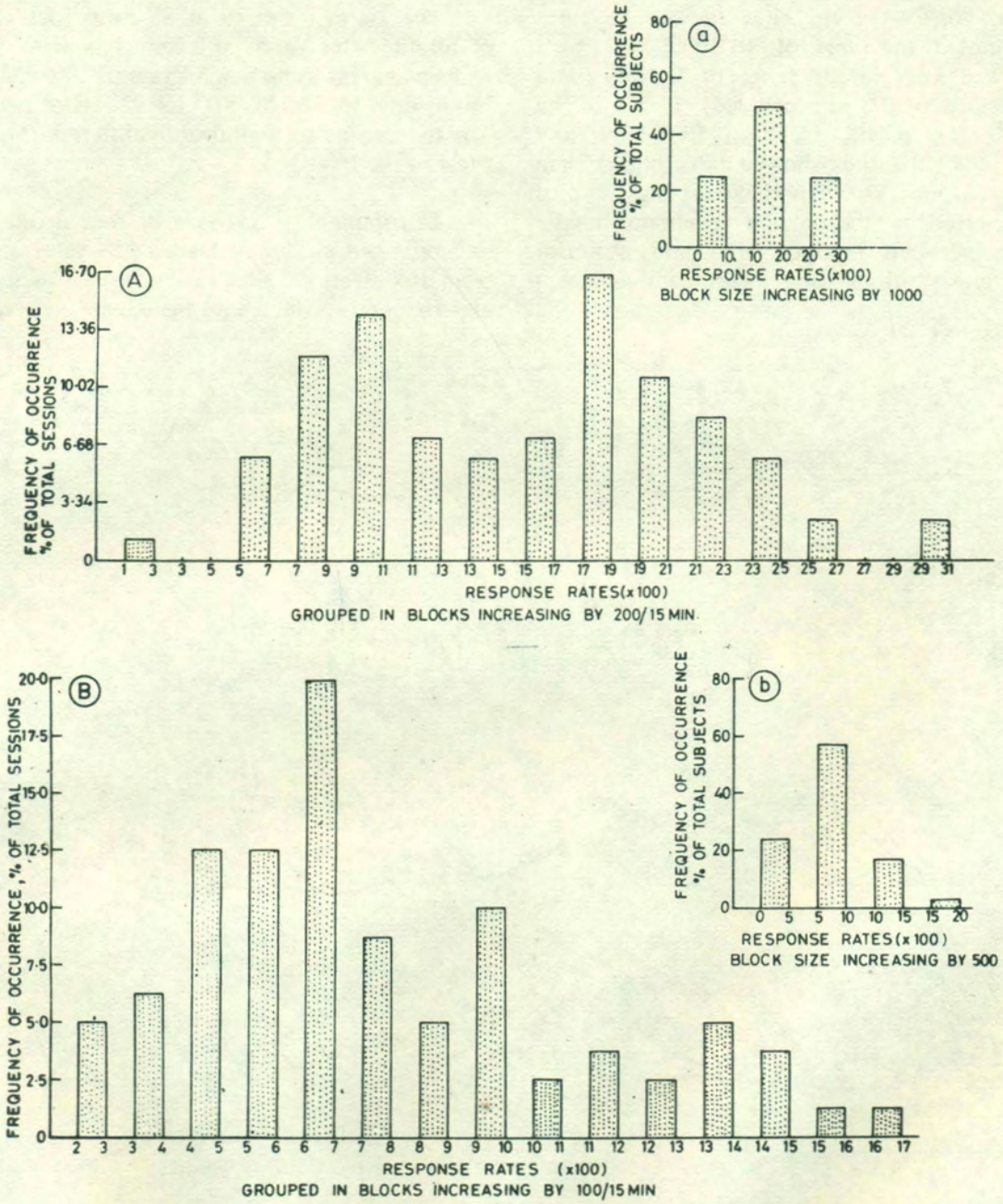

Fig. 2: SS rate spectra of the midbrain sites observed in a sample of 84 rats in 431 sessions (A), and of hypothalamus observed in 80 rats of same group in 343 sessions (B). The rates of midbrain sites (A) fall in two clusters, one in the range of 500-1300 (per 15 min), and the other in the range of 1500-2500. The grand average (a) showed that the majority of the sites had high SS rates in the range of 1000-2000 per 15 min. Majority of the rates of the hypothalamic sites (B) had rates in the range of 300-1100 per 15 min. The grand average (b) revealed that the SS of majority of sites was in the range of 500-1000 per 15 min. Stimulus: 50 Hz, sine wave, 0.25 sec train per pedal press.

(Fig. 2b), 56.25% of the sites showed SS rates (per 15 min) in the range of 501-1000, 23.75% of sites showed rates in the range of 200-500 (data of rates less than 200 not included), 17.5% of the sites had rates in the range of 1001-1500, and about 2.5% of the sites showed rates higher than 1686 per 15 min. The hypothalamic sites studied were dispersed in the medial forebrain bundle extending between preoptic area and posterior hypothalamus, and in zona incerta and adjacent regions.

The grand averages of SS rates (per 15 min) of all the sites of hypothalamus, and of ventral tegmental area—substantia nigra were: 850±322 (SD) for 76 rats, 1523±540 (SD) for 92 rats respectively. The two means are significantly different (Student's t-test, $P < 0.001$).

Distribution of SS rates of sites in the hypothalamus and midbrain: Data of SS rates obtained from 168 electrode sites, assessed in a total of 774 experimental sessions have been mapped in relation

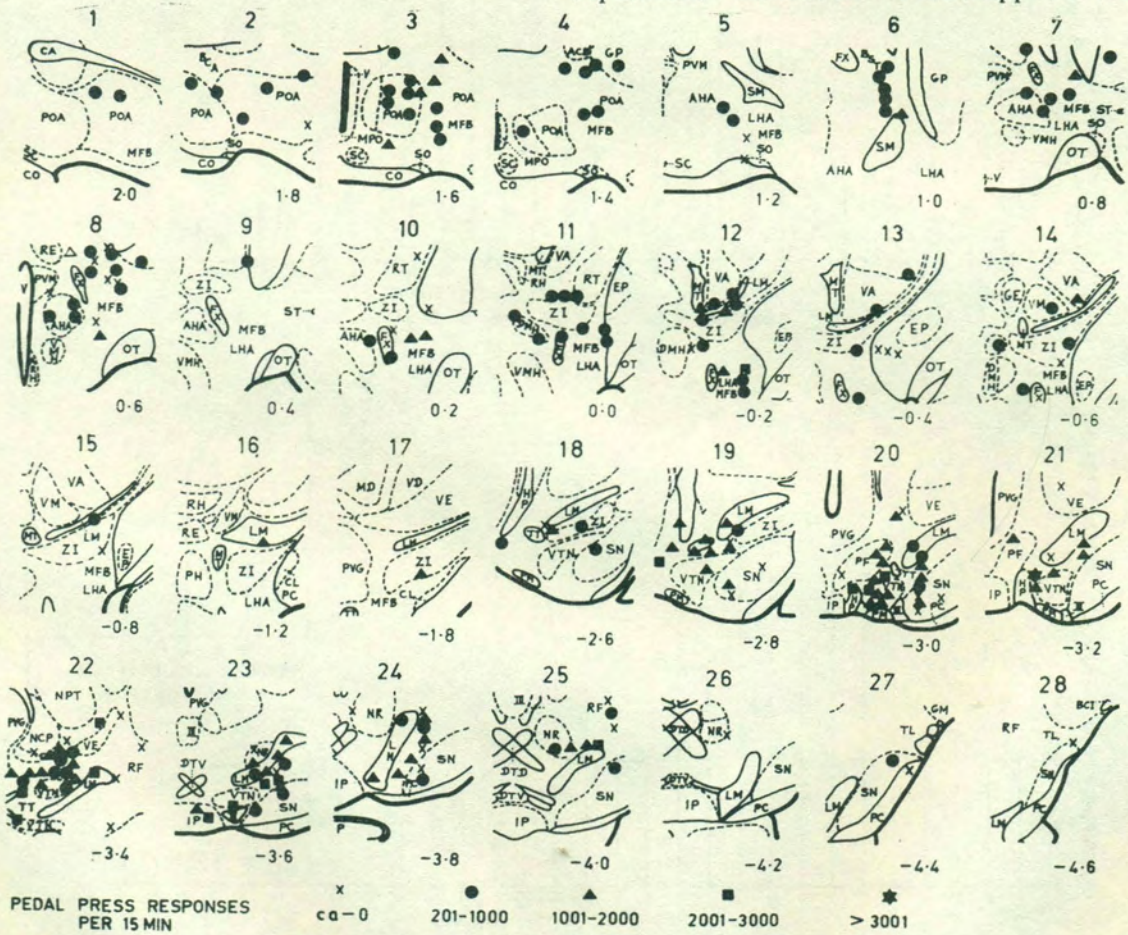

Fig. 3: Summary map of distribution of the sites of self-stimulation (data of Fig. 2) categorized according to the SS rates. Atlas coordinates are indicated beneath each inset. Insets 1-4 represent the preoptic area level, 5-15 represent anterior to posterior levels of hypothalamus, 16-28 represent rostral part of midbrain to caudal level of substantia nigra. The distribution map of SS rates revealed that the lateral preoptic area (inset 3), and dorsal parts of lateral hypothalamus (insets 8-12) had high rates, upto 2000 (per 15 min). In midbrain, the rates were mostly above 1000 (insets 19-25), several sites having rates above 2000. The figure reveals a clear difference in the overall pattern of SS rates of sites of the hypothalamus and the midbrain, alhough, within any region, the sites with differing rates were found intermingled.

to electrode sites to reveal patterns of regional difference in SS (Fig. 3).

Hypothalamus: The sites of very high SS rates (2001-3000 or more per 15 min) were very few and located in the far lateral and posterior hypothalamus (Fig. 3/12-14). The sites of high rates (1001-2000 per 15 min) were located in the rostral hypothalmus, at the level of the preoptic region (Fig. 3/3). A few sites of high rates were also in the dorsal margin of zona incerta (Fig. 3/12) where the catecholaminergic pathways traverse (4). The sites of moderate rates (201-1000 per 15 min) were commonly found to be distributed throughout (Fig. 3/1-17) in the lateral hypothalamus and in vicinity of nucleus accumbens, in the fiber pathways of noradrenergic (4), dopaminergic (5, 6) and other systems (7, 8, 9). Thus, different sites in the hypothalmus evoked different rates of SS. Each site gave similar maximal rate of SS within limits of narrow variation across sessions conducted on different days i.e., the rate was characteristic for each site. The further point of interest was that the sites of different rates were mingled, or SS rates of adjacent loci had marked differences.

Midbrain: Very high SS rates (over 2000 per 15 min) were observed in approximately the middle level of the midbrain in SN-VTA regions (Fig. 3/20-23). High rates of SS (1001-2000 per 15 min) were observed in sites rostral and caudal to the above region and also in sites dorsal to the SN-VTA (Fig. 3/19-25). The sites of the moderate rates (201-1000 per 15 min) mingled with the sites of the high rates, and were also observed in the more lateral regions of substantia nigra. Almost all the sites of very high rates were in the region of the vental tegmental area and in the medial nigra compacta. These regions correspond to areas known to contain DA and CCK-DA neurons (6). The nucleus accumbens projections go to the dorsal part of the medial nigra (8). These regions do not correspond to the noradrenergic fiber projection sites which are known to be more dorsally located and adjacent to the peri-aqueductal grey (4). Very few electrode placements fell in the region of the NA fiber pathways in the midbrain. On the whole, in the midbrain also, like in the hypothalamus, the sites of high and low rates of SS were mingled, and not seemed to be clustered along any identifiable projection fiber pathway.

General: The interesting difference between midbrain and hypothalamus is that in the lateral hypothalmus the sites in dopaminergic fiber pathway yielded only moderate SS rates, whereas in the midbrain the sites in DA neuronal regions showed very high SS rates. Conversely, all the midbrain dopaminergic neuronal sites have also not yielded the high rates. Thus there seems to be some other elements of neuropil playing important role in the SS behaviour.

Gender differences of SS: Previously, only one mention was made (10) of having used only two females along with 132 males, and no comment was made about any difference of SS of those two females from males. We studied comparatively 24 females and 26 males with electrodes implanted in midbrain sites. The results revealed that the SS rates of the two groups have not differed significantly. The mean of the female group was higher than that of the males by about 22.5%, but was not statistically significant. Three of the females were also assessed in detail during different phases of their estrous cycles, and no significant differences in the SS rates were oberved during the different phases of the cycles.

DISCUSSION

Usefulness of SS rate-measures elicited from two sites in a subject: SS pedal press rate-measure is generally viewed to serve (under proper circumstances) as an approximate indicator of the intensity and/or duration of impact on motivation and reward generated by successive stimuli of the self-stimulation (SS). SS rates may differ, depending on the placement of electrodes as different brain regions may differ in their role or involvement in SS behaviour. Hence a systematic study to correlate electrode place differences and SS rates could suggest clues to the manner of organization of the brain-reward system. When drugs that affect motor systems are not administered, the SS rate-measure has a straight forward utility as a fair index of

the rewarding impact of the brain region. However, when experimental conditions raise doubts of motor impairment, alternative testing procedures of SS have also to be devised (11-13). Since conditions of motor impairment were not expected to be prevailing in the present experiments, the SS rate data were elicited with one frequency setting (50 Hz) of stimulus delivered to different sites of electrode placements for comparison.

Differences in SS rates due to sine and square wave forms: Since different workers in the past have used either square or sine wave stimuli, a comparison of the impact of the two types of stimuli has been firstly made by eliciting SS with the two types of stimuli delivered to the same electrode site. Such a comparative study of stimulus-repsonse relation was not previously reported in the literature.

Sine wave stimuli evoked significantly higher SS rates than square wave stimuli from any given electrode placement in both hypothalmus and midbrain. The difference was not related to the difference of amount of electric charge, as by increasing stimulus current (thereby the charge) the square wave stimuli caused either no further increase of SS rate, or only caused disturbing motor side effects or aversive behaviour. Conversely, by bringing down the current (thereby the charge) of sine wave stimuli to the level of square wave stimuli, either very low or none of SS responses could be evoked. Alternatively, instead of increrasing the current strength, the duration of the square pulse was increased from 0.25 msec to 6 msec (train duration unaltered). In this case also the SS rate had not increased with increase in pulse duration. It was noted also in the past (1): "In square wave studies, lengthening the duration of the pulses was not so effective a way of adding to the peak amplitude...to get the most behaviour for the least charge, very brief pulses were by far the most effective." Hence the sine wave superiority in eliciting higher SS rates or rewarding behaviour is probably due to a different combination of effects of excitations and inhibitions evoked by this wave form in the underlying neural networks, as neuronal excitability is known to be highly influenced differently by different wave-forms (sine, ramp, square). There have been no attempts in the past to compare the effects of sine and square wave stimuli in SS behaviour.

High SS rates in the present study and stimulus parameters used: The SS rates of the present study are consistently higher than those reported previously. SS rates of only upto 1500 per 15 min (stimulus train of 0.25 sec, 100 Hz) were reported in ventral tegmentum (14-16), or only 468 per 15 min in substantia nigra compacta and 153 per 15 min in its reticulata area (17). In a moveable monopolar electrode survey (18), SS rate of 80 per min was observed, but it was not clarified as to how many sites showed this rate. In the present study, rates of about 200 per min were observed, i.e., 2½ times more than the rates reported previously (18). In lateral hypothalamus an average rate of about 328 per 15 min was reported (17). In the present study, most of the subjects showed rates as high as 900-1000 per 15 min or even more.

The high rates of SS observed in this study in comparison to the past studies cited above were probably due to differences in stimulus parameters and also perhaps due to differences in loci of SS, so that aversive responses would be least compounded. It was reported that a change from self-stimulation to experimenter-administered stimulation, though stimulus parameters and delivery pattern was kept identical to self-stimulation by replaying a prerecorded version of stimuli of an ealier SS session in the same rat, there was an escape response (19). It was known since a long time that stimulations in hypothalamus could cause either aversive or rewarding or mixed patterns of effects depending on the electrode sites and the parameters of stimulation (20-23). Opposite effects on other behaviours also could be evoked from many brain regions by changing the parameters of stimulation, and such shifts of responses would be due to changes occurring in synaptic activations and inhibitions in the regions linked to stimulated site, and due to stimulus currents spreading over to adjacent sites of opposing functional properties. Self-selection experiments revealed that short train duration are preferred for SS (24). Long train

durations probably contribute to build up of aversive effects counter to SS (1, 20).

The usage of short train duration (0.25 sec) of relatively low frequency (50 Hz), and sine wave (instead of steeply rising, highly excitatory square waves of high frequencies) could have contributed in this study to minimise the chances of compounding aversive neuronal processes with the positive processes of SS reward resulting in the generally higher SS rates than in past studies.

Differences of SS rates of different sites and regions: Since the magnitude and/or duration of reward and motivation for responding to SS will be reflected in the SS rate, it can be assumed that the significant difference observed in patterns of rates of LH and SN-VTA to be due to differences in rewarding properties of the two regions, because motor capacity is unimpaired during SS of both LH and SN-VTA. No other satisfactory explanation appears to be plausible to explain the significance of differences of SS rates of LH and SN-VTA. Furthermore, as there are also sites of different SS rates within each of the two regions, different subsets of neurons of local network and of projection fiber pathways might be stimulated by SS of the different sites.

LH and SN-VTA SS rates observed in this study are higher than those previously reported for other brain regions, including medial frontal cortex (25), entorhinal cortex (26), habenula (27), septum (28), amygdala (29), and thalamic intralaminar and medial nuclei (30). Most of these regions have low to moderate SS rates (below 500 per 15 min). Locus coeruleus SS rates have been contradictory, reported variously as low (500 per hour) (14), high (1100 to 8000 per hour) (31) and even as none (32,33). The above differences of SS rates on normal rats, though obtained in differing experimental methods, indicate generally that the differences might be due to differences in the capacities of their neuronal organizations in activating and sustaining the rewarding meachnisms related to electrical SS.

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