SUCCESS OF AUTONOMIC OPERANT CONDITIONING OF HEART RATE WITHOUT INVOLVING CONTRACTIONS OF SOMATIC SKELETAL MUSCLES

P. N. BINDU AND T. DESIRAJU

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

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Summary : In conscious Wistar rats neuromuscularly paralysed by gallamine, operantly conditioned reduction of heart rate was achieved under both negative and positive reinforcement schedules using the tail shock avoidance or the rewarding brain-stimulations in 20-min test sessions. The primary aim was to assess whether it would be possible to achieve operant conditioning of the heart rate, evoked not as a secondary reflex response of any voluntary skeletal muscular contractions of trunk but as a conditioned voluntary function of the central autonomic regulation of a visceral organ, since this entire subject was peculiarly left in confusion by Miller (5, 8) who wanted that others should independently study it. This study revealed interestingly that not every subject might be able to achieve the visceral learning in a given set of conditions, and suggested that this type of a special learning might be dependent on individual predisposition in the central nervous system. In the present study, 15 showed the learning, out of the 58 subjects assessed. It was also observed that there was a variation in the magnitude of the learning response among different learners, and, also, in the same subject in different sessions conducted on different days. This is considered as an indication that this type of conditioned autonomic function is probably not easily recruited into the long-term memory mechanisms. The overall average of the operant lowering of the heart rate progressively achieved by the end part of the learning session was about 10.5% from the basal average rate, and the score of reinforcement (per cent of painful tail shocks avoided, or of increase in number of brain shocks achieved) was over 80%. The extinction test confirmed the learning. Control experiments revealed that the conditioned heart rate changes were not due to any unconditioned stimulus effects. The learning observed under the brain-stimulation reinforcement was confirmed by losing the learning response after lesioning the site of the rewarding stimulation. The visceral operant learning occurring in state of somatomotor paralysis under both negative and positive types of reinforcement was blocked by haloperidol. Morphine delayed the onset of the pain avoidance operant learning, whereas it speeded up the hedonic brain-stimulation operant learning. The results, considered from all the above angles, dispell the doubt previously expressed about the occurrence of the operant conditioning of heart rate under a visceral learning paradigm.

Key words : visceral learning
operant conditioning of heart rate
cardiovascular conditioning operantly
psycho-visceral conditioning

INTRODUCTION

In recent years, the possibility of voluntarily influencing or controlling the visceral organs directly has been brought under the scope of experimental investigation by utilising the operant conditioning methods in unanaesthetised but curarized experimental preparations (10, 14). The curarization has been considered essential to avoid cardio-vascular reflex contribution of the somatic muscular manipulations made under volitional efforts while acquiring control on the visceral functions. Electrical stimulations of the neocortical areas in monkey (even in anaesthethetised state) can evoke clear changes in respiration and blood pressure, implying that higher cerebral functions can influence the autonomic functions (3). If a visceral activity like the heart rate can be altered according to the operant conditioning procedure in a subject under the skeletal muscular paralysis, it would establish a new central nervous system mechanism linking between the neural substrates of volition (in forebrain-limbic areas) and the neural substrates controlling the visceral organ (diencephalon and brainstem). If it is so established, the possibilities open up well to develop the volition based methodologies for making improvements of health or homeostasis, or correction of deviations in the functioning of visceral organs (6-9).

In 1967, Trowill (14) reported in a study on curarized rat the occurrence of conditioned changes of about 5% in heart rate under the reinforcement schedule of brain-stimulation reward. Miller and DiCara in 1967 (10) have also reported that the subjects could be shaped to improve the magnitude of their operant responses (upto about 20%) under the rewarding stimulation of medial fore-brain bundle, by shifting the reinforcement criterion level in successive steps. Subsequently, to the surprise of all, Miller and his colleagues reported (4, 5, 8, 11) that they could not replicate their own earlier experimental results, yet they could not negate the visceral operant learning. Miller (8) recognised that this research has some peculiarities and indcated that till independent experimental evidence is obtained from some other laboratories, the fact of visceral operant conditioning be left in open The studies of success and failure have been earlier reviewed (12). Further, Miller (8), and Miller and Brucker (9) have also provided strong indication from the results of conditioning of human patients paralysed due to spinal lesions, that volitional alterations of visceral functions could be strongly possible, independently of the consequences of the mechanical effects of the skeletal muscular contractions.

In view of the above puzzling background, it was felt important to design the basic experiment on operant visceral conditioning using the curarized rat and using both negative and positive reinforcement schedules, to firstly assess the possibility of the operant conditioning of visceral function, whatever the nature of the central nervous mechanism could be. The study was deliberately not intended to replicate Miller's original experiments (5), but to carry on in an independent and unusual manner to give a fresh assessment to the subject.

MATERIAL AND METHODS

The results described in this paper were based on experiments completed on 58 Wistar rats. Out of these experiments, learning was observed in 15 rats. This was an interesting observation in this special area of research, as it indicated that there were some yet unrecognised experimental factors which required to be satisfied carefully on an individual basis. The implied factors matched apparently to be satisfactory for only about 25% of the subjects used in the present experiments. Hence, the unconditioned stimulus (UCS) effects had to be assessed in the same subject, instead of in yoked controls as usually done in other areas of research.

The tail shock avoidance and also the brain-stimulation reward paradigms, in their basic designs of the operant conditioning have been used in this research (Fig. 1).

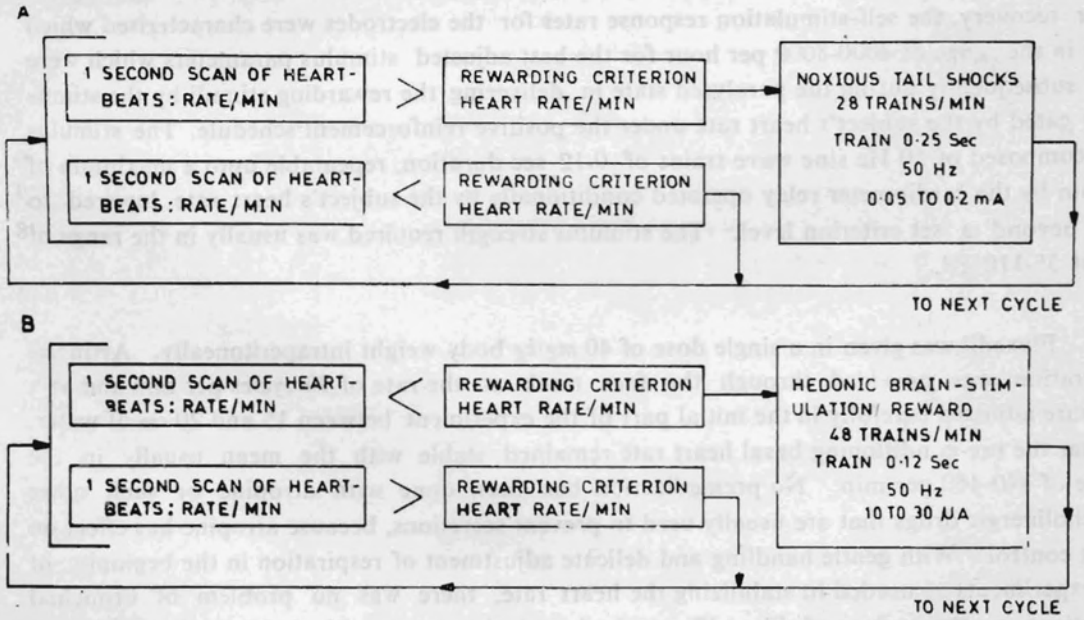

Fig. 1 : Operant visceral conditioning for the reward of tail shock avoidance (A), or for the reward of brain-stimulation (B), in a continuous reinforcement schedule. The maximum possible rewarding or painful trains of stimuli available in each schedule is indicated.

The animals were handled most carefully to avoid stimuli that evoke stress of fear and thereby affecting their psycho-somatic behaviour. Instead of curare, gallamine triethiodide (Flaxedil, May & Baker) was used in this study.

In each experimental session, the rat was first tested before paralysing it to find the correct noxious stimulus strength required to produce a moderate pain response (withdrawal of the tip of the tail by about 1 cm), without leading to any vocalization or a struggling behaviour. The electrodes and the tail skin were cleaned and electrode paste was applied to improve the contact. The stimulus strength required differed among the individuals, usually falling in the range between 0.05 and 0.2 mA. The current so determined was used during the subsequent part of the experiment under the paralysed condition. This pre-paralysis determination of the moderate stimulus strength was an important step of precaution, as some times stimuli could be of higher or lower than required, and go undiscovered due to paralysed state, but affect the outcome of the experiment by evoking either undesirable cardio-vascular responses when of high strength or ineffective for conditioning when of low strength.

In the rats prepared for brain-stimulation reward, bipolar electrodes were permanently implanted in the lateral hypothalamus or in the ventral tegmental area-substantia nigra region. After recovery, the self-stimulation response rates for the electrodes were characterised which were in the range of 4000-8000 per hour for the best adjusted stimulus parameters which were used subsequently during the paralysed state in delivering the rewarding stimuli by the stimulator gated by the subject's heart rate under the positive reinforcement schedule. The stimulus was composed of 50 Hz sine wave trains of 0.12 sec duration, repeatable upto a maximum of 48/min by the cardiometer relay operated conditionally by the subject's heart rate lowered to and beyond a set criterion level. The stimulus strength required was usually in the range of about 35-110 μA.

Flaxedil was given in a single dose of 40 *mg/kg* body weight intraperitoneally. Artificial respiration was provided through the face mask at the rate of 70 cycles per min and at a pressure adjusted carefully in the initial part of the experiment between 15 and 20 *cm* of water. so that the pre-conditioning basal heart rate remained stable with the mean usually in the range of 440-460 per min. No premedication has been done with atropine or such other anticholinergic drugs that are usually used to prevent secretions, because atropine has effect on heart control. With gentle handling and delicate adjustment of respiration in the beginnin of the experiments as needed in stabilizing the heart rate, there was no problem of bronchial secretions, no repeat dose of Flaxedil required, and the rat would maintain harmoniously during the few hours of the experimental session.

The EKG was monitored through lead II montage on the cardio-tachymeter which computed and displayed also the rate from the number of beats occurring in each sec. After the satisfactory adjustments of the respiratory parameters, and the electrodes having been connected to the tail or brain shockers through relays of the EKG monitor, the heart rate was

watched for its stabilization without the reinforcement relays activated. Under these basal conditions, it would usually stabilize between 440 and 460 beats per min, with only brief transient fluctuations within $\pm 5\%$ from the mean rate. The heart rate (per min) reading which was continuously monitored by the meter display, was noted at successive intervals of 15 sec throughout the experiment so that 4 samples of the rate were provided in each min for averaging and obtaining the mean rate over a min. After noting that the subjects had a stable heart rate and basal readings taken, the criterion for reinforcement availability was set at a level of 2-3% *lower* than that of the general (basal) *mean* of the heart rate level. This manner of setting the criterion level would enable the subject to experience reward quickly, without need of any other external cue, the reward initially triggered unexpectedly by the randomly occurring brief *transient* reductions (temporary fluctuations) normally occurring in the heart rate, and later leading to the development of the operant type of association of heart rate, to getting reward. If there was (i) a progressive shift in the heart rate towards the set criterion and beyond, and (ii) a related progressive increase in the earning of the quantity of reward, then the learning to obtain the reward by lowering the heart rate could be considered to be established, i.e. change in the behaviour of the heart rate attained through the conditioned reinforcement. The numbers of the rewards (tail shocks or the brain shocks) obtained by the subject were counted continuously during the experiment as their changes indicate as learning score. Heart rate reduction beyond the criterion level caused a corresponding decrease of the number of noxious shocks received or an increase in the number of hedonic brain shocks received. Thus, the scores of conditioned change could be quantified, and evaluated session-wise and subject-wise and also statistically. Extinction test also was done by disabling or shutting off the reinforcement schedule. Further, in the control experiments, the effects of the unconditioned delivery of the stimuli used in both the types of paradigm were also studied to find whether the delivery of stimuli without the conditioned reinforcement schedule would produce heart rate changes of lowering in any way comparable to those observed in the learning sessions. Only on the basis of all the above experiments and statistical analysis (ANOVA, t-test, 13) of the results, inference was made whether visceral learning could occur successfully or not.

In addition to the above types of experiments, the effects of the modulators of mono-aminergic synapses were also tested to find whether the above type of visceral learning would be influenced as was done in studies on analysis of brain-stimulation reward and other somatic operant behavioural mechanisms to provide another important type of information about occurrence of the visceral learning.

RESULTS

TAIL SHOCK AVOIDANCE SCHEDULE

Control experiments : Four types of control experiments were carried on, three types of these were without the delivery of the tail shocks, and the other type was with the continuous delivery of the tail shocks to find the effect of the unconditioned stimulation on the heart rate. In the first type of control experiments the time dependent changes on the heart rate were studied in the preparation that was kept paralysed, artificially respired and with all other connections made exactly as in the learning experiment but without the relays of shockers working. This type of control experiment revealed the magnitude of brief transient fluctuations occurring in the heart rate and also the degree of stability of the average or basal rate obtained under the experimental conditions of the research study. In two other control types of experiments also the shock was not delivered but the associated shocker relay sounds (clicks) were allowed to go on either continuously, or in relation to the fluctuations of heart rate relative to the criterion level which was set as in a learning experiment (Fig. 2A). These control experiments showed that the basal heart rate under the presently set experimental conditions had brief transient fluctuations of only about $\pm 5\%$ from the basal mean level which was well established.

In another type of control experiment, the tail shock was delivered continuously as an unconditioned stimulus at the maximum possible rate (about 24 shocks/min). The stimulus strength has been set at the moderately noxious level as described in the methods section and as is used in the conditioning experiments. The heart rate showed a bigger range of transient fluctuations on either side of the mean, but no significant change in any one direction occurred in the average rate (Fig. 2B).

Conditioned alterations : In the experiments with the operant reinforcement of tail shock avoidance, there was a progressive reduction in the number of shocks received by the subject, correlated to the reduction in the mean heart rate moving below the criterion level set for avoiding the shocks (Fig. 3A). It was also observed that by putting off the shocker, the response became extinct and the heart rate returned towards the pre-conditioned level (Fig. 3A). It was observed, on an average, that by the end part of the 20 min conditioning session the mean heart rate was decreased by about 10% and the reinforcement achieved was about 80% (Table 1). The magnitude of the reinforcement achieved (i.e., learning) differed among different subjects, and also in the same subject in different experimental sessions The starting level was not altered often. Hence, the visceral learning has to be judged session-wise, rather than on group statistics.

In the majority of the subjects, the same experimental paradigm has not produced the avoidance learning (Fig. 3B). ANOVA of the learning and non-learning types of subjects revealed their difference (Table II).

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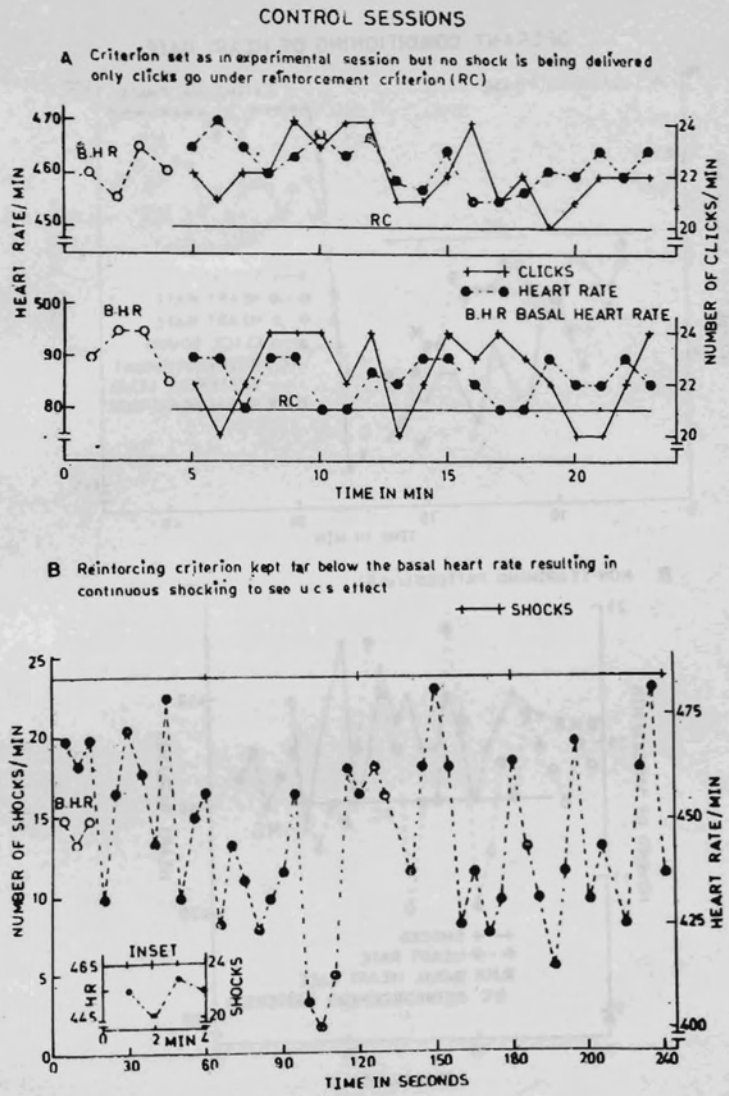

Fig. 2 : Control experiments with tail shocker off but with the heart rate-dependent relay trigger circuit clicking on as per the setting of reinforcement criterion (RC) (A), or with the tail shocker allowed to be on at its maximum possible rate continuously (disconnected from heart rate dependent relay-trigger) as an unconditioned stimulus (UCS) to find its effects (B). A click is a fine sound of the relay-trigger and is an indication of delivery of a shock, provided the schocker is on. Hence, in Fig. A, "Shocks" means only clicks. BHR : basal heart rate preceding the conditioning part of the experiment. B. shows the first 4 min of the UCS experiment in an expanded scale (inset drawn in standard scale). Note that even under the maximum shocking under the UCS the transient fluctuations increased by only about 5% on either side of the mean. The shock strength was determined and kept at a moderate level only, as specified in the methods.

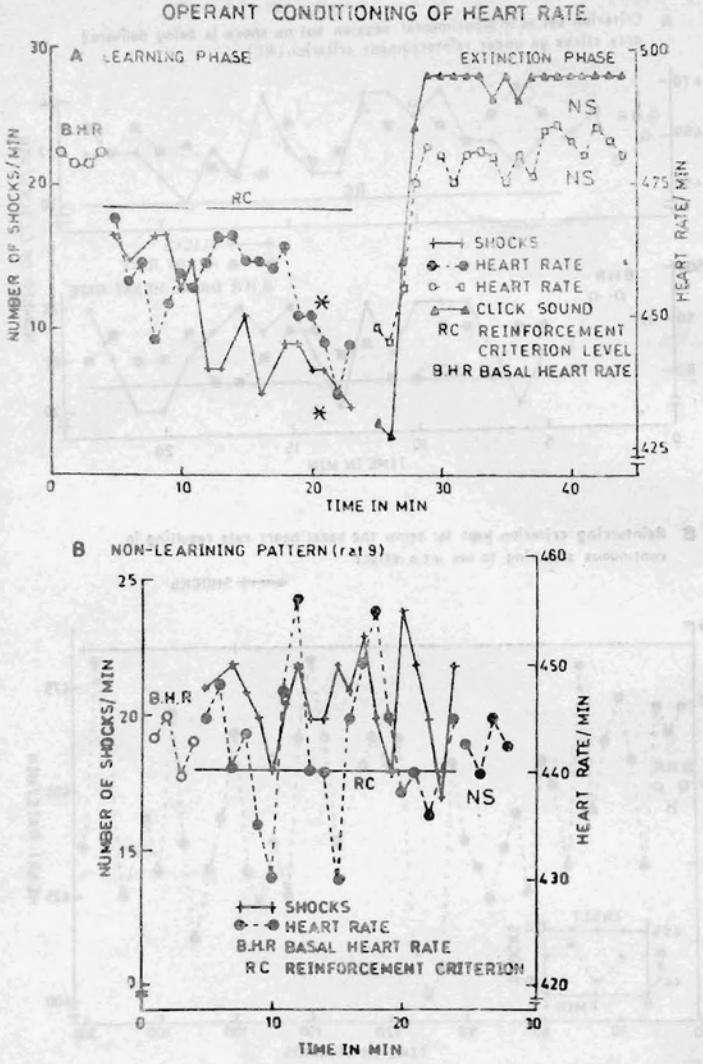

Fig. 3 : A : Illustration of a typical example of heart rate conditioning under the tail shock avoidance schedule, and also the results of the extinction test. Note the progressive improvement in the avoidance of the tail shocks and also the progressive reduction in the mean of the heart rate. Asterisk is an indication that the means of the first five and the last five values of heart rate under conditioned test period (RC) are significantly different in t-test ($P<0.005$), so also the values of the shocks ($P<0.005$). NS indicates no such significant difference from the basal level or from the first five values under conditioning. Note also that the disabling of the reinforcement criterion resulted in the returning back of the heart rate to the basal level (extinction of learning), while the delinked relay circuit clicking at its maximum as it should be. B : A typical example (a non-learning rat) which showed no progressive improvements in avoiding the shocks despite conditioning criteria, i.e., no learning pattern.

TABLE I : Analysis of variance (ANOVA, two-way) of data of visceral operant conditioning in negative reinfor schedule in learners group. C_1, mean of three values of the first part (3 min) of the conditioned sta mean of three values of the end part (3 min) of the conditioned state. NS, not statistically sign $P<0.05$ is considered significant.

(A) Conditioned heart rate change (operants)

Treatment	*Subjects (replication) : heart rate/min*									*Row Total*	*Row Mean±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	9				
C_1	396	432	435	460	475	424	458	491	492	4063	451±30.4	df	8
C_2	280	430	408	434	461	398	425	436	440	3712	412±49.8	SS	4434
												MSS	554
Column Total	676	862	843	894	936	822	883	927	932	7775		F	
												P	

(B) Tail shock avoidance (reinforcement)

Treatment	*Subjects (replication) : number of shocks/min*									*Row Total*	*Row Mean±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*	*7*	8	*9*				
C_1	18	19	21	15	20	13	19	16	19	160	17±2.4	df	8
C_2	0	15	0	6	0	5	0	0	3	29	32±4.4	SS	147
												MSS	18.3
Column Total	18	34	21	21	20	18	19	16	22	189		F	
												P	

TABLE II : Analysis of variance (ANOVA, two-way) of data of visceral operant conditioning in negative reinfo schedule in learners (L) and non-learners (NL). Mean of the four values of the end part (4 min) conditioning session is given for L and NL groups. NS, not statistically significant. $P<0,05$ is co significant.

(A) Conditioned heart rate change (operants)

Treat-ment	*Subjects (replication) : heart rate/min*									*Row Total*	*Raw Meau±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*				
L	278	429	409	441	464	398	421	428	440	3708	412±50	df	8
NL	440	440	483	485	465	463	485	438	400	4099	455±26.8	SS	13407
												MSS	1675
Column	718	869	892	926	929	861	906	866	840	7807		F	
Total												P	

(B) Tail shock avoidance (reinforcement)

Treat-ment	*Subjects (replication) : number of shocks/min*									*Row Total*	*Row Mean±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*				
L	0	16	0	7	0	5	0	0	4	32	3.5±5	df	8
NL	21	23	18	16	17	21	22	15	17	170	18.8±2.7	SS	101
												MSS	12.62
Column	21	39	18	23	17	26	22	15	21	202		F	
Total												P	

Only about 25% of the subjects showed clearly the results of learning. Fig. 4 provides the averaged data of 15 experimental sessions obtained in 9 rats, and the ANOVA in Table I shows unequivocally the significant change in the visceral response and the reward achieved under the tail shock avoidance (learning).

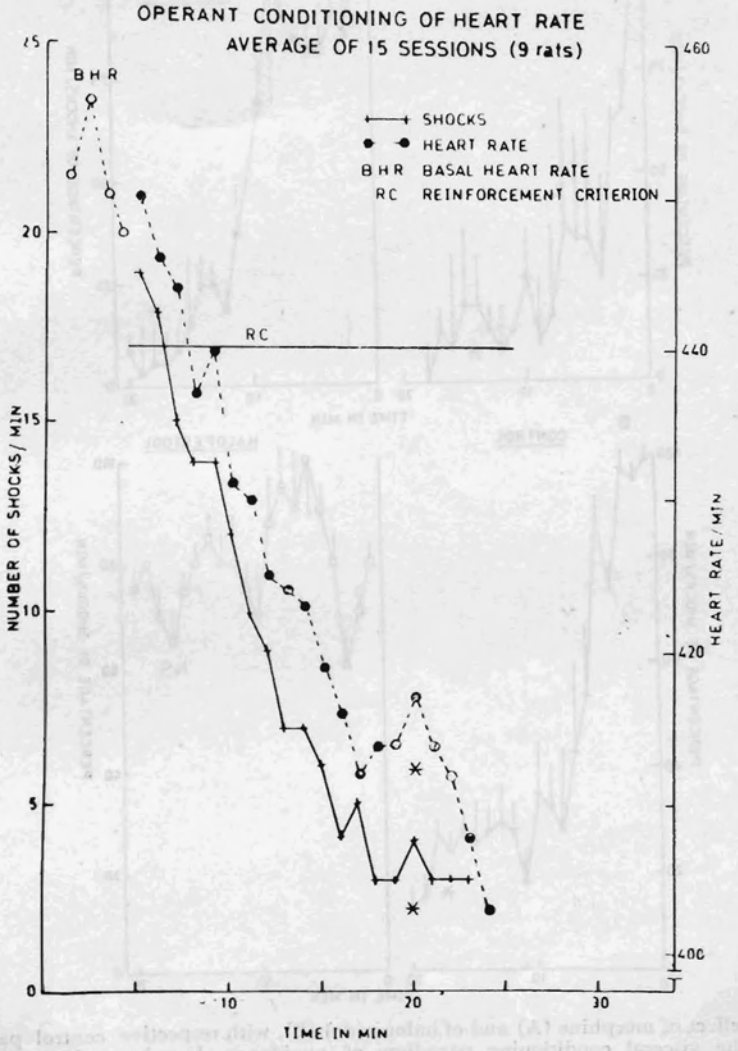

Fig. 4 : Average pattern of data of 15 experiments (in 9 learning rats) selected on the basis of their approximate similarity for the averaging purpose to provide the pattern of the learning curve obtained under the tail shock avoidance through visceral (heart rate) operant conditioning. Asterisk indicates as in Fig. 3.

Synaptic modulators on visceral learning

Morphine : As morphine is an opioid synapse modulator and an analgesic, its effect was tested by administering intraperitonially at a dose of 300 $\mu g/kg$ body weight and assessing

15 min later the conditioned response. The results revealed that it caused only a delay in the onset of avoidance learning in comparison to learning observed in the same subjects in control conditions (Fig. 5A).

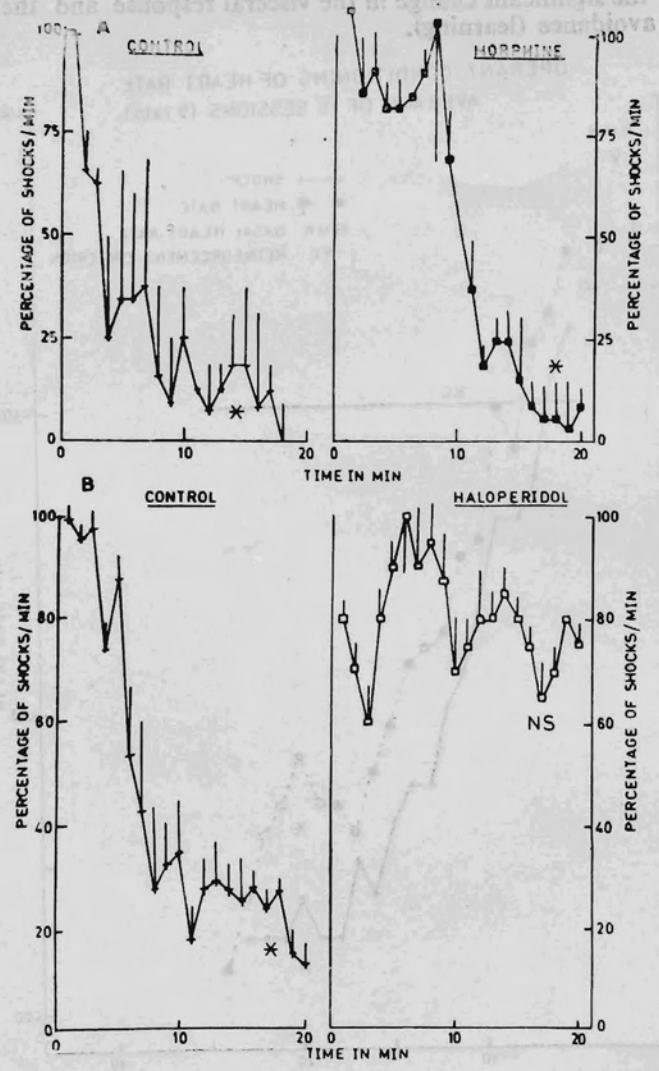

Fig. 5 : The effect of morphine (A) and of haloperidol (B), with respective control patterns, on the visceral conditioning paradigm of avoidance learning. Note that the morphine (300 $\mu g/kg$, i.p.) h s caused a delay in the onset but has not otherwise affected the occurrence of the learning, whereas the haloperidol (300 $\mu g/kg$, i.p.) blocked the occurrence of the visceral learning completely. Shocks number normalized as per cent value of the unconditioned rate of the shocker. Asterisk indicates comparison as in Fig. 3. NS indicates no such significant change occurred under haloperidol. Each graph in A was based on two experimental sessions (2 rats), and in B on 4 sessions (3 rats) for each graph. The means of first ten values of the control graph and of the morphine graph differed significantly in t-test ($P<0.001$).

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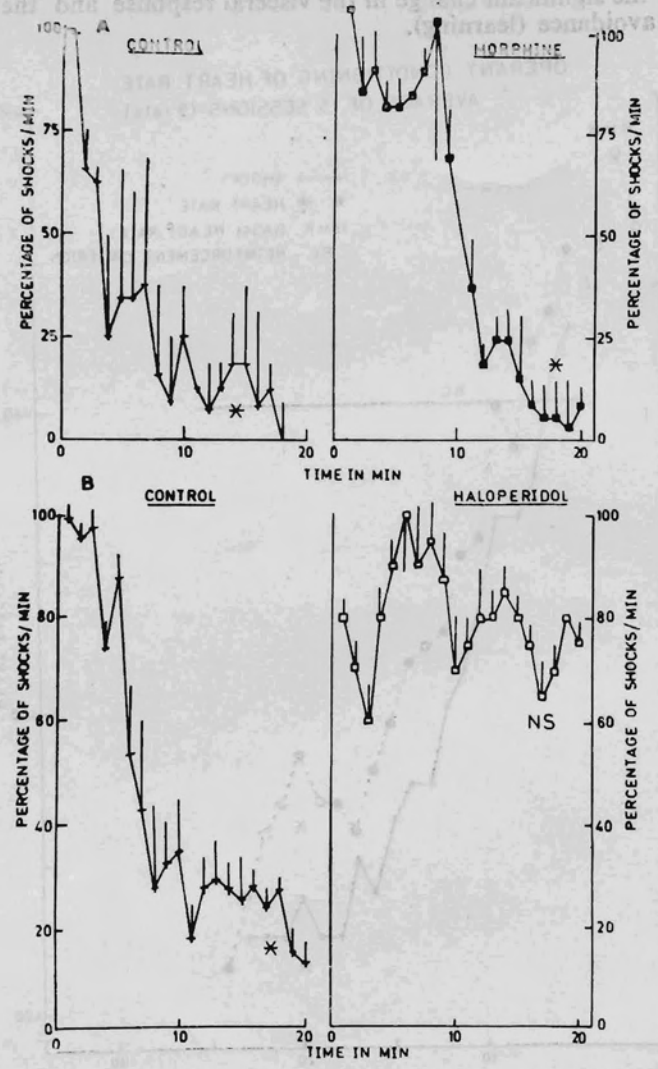

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Haloperidol : Haloperidol is a dopamine receptor blocker and also a cortical serotonergic receptor blocker. It was administered at a dase of 300 $\mu g/kg$ body weight intraperitoneally. Fifteen min later, the conditioned response was assessed. The results showed a complete failur of the occurrence of learning (Fig. 5B) in comparison to learning occurring in control state in the same subjects. This was an interesting result as it showed that visceral learning function could involve dopaminergic synaptic mechanisms. The usual doubts of motor incapacitation as a possible cause of haloperidol action is excluded, because in this conditioning there is no involvement of somato-motor system as the subject is paralysed.

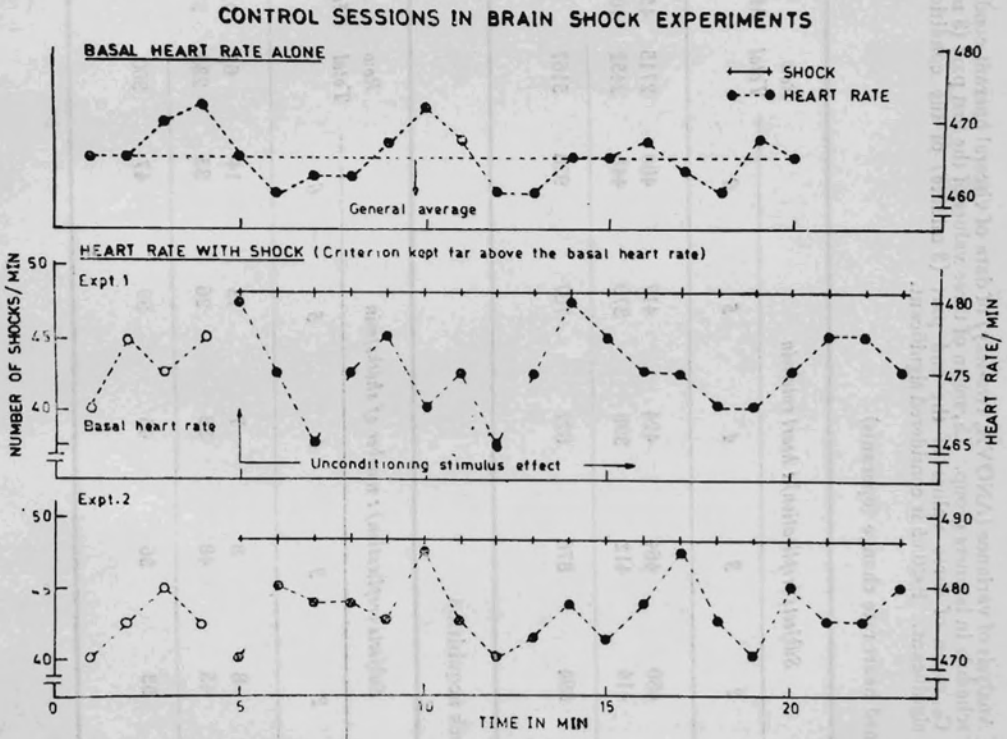

Fig 6 : Control experiments under the series of rewarding brain-shock experiments. Upper panel shows the range of change in the basal heart rate under the artificial conditions of neuro-muscular blockade, over the duration of an experiment if nothing else is done. Note that the transient variations are only about 5 beats or about 1% on either side of the average. In the lower two panels, the UCS effects are illustrated, with the brain shocks going on at maximum possible rate. Note that the heart rate continued during the UCS period as in basal control, except for a slight increase in the transient fluctuations from the mean level. It should be recalled that the stimulus strength has been kept in the optimum psychophysiological level as specified in the method so as to avoid or minimise the side-effects of the stimulations on cardiovascular system.

TABLE III : Analysis of variance (ANOVA, two-way) of data of visceral operant conditioning in positive reinfo
schedule in learners group. C_1, mean of three values of the first part (3 minute) of the conditione
C_2, mean of three values of the end part (3 minute) of the conditioned state. NS, not sta
significant. $P<0.05$ is considered significant.

(A) Conditioned heart rate change (operants)

Treat-ment	*Subjects (replication) : heart rate/min*						*Row Total*	*Row Mean±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*				
C_1	450	480	464	424	417	480	2715	452±25	df	5
C_2	410	414	412	398	370	448	2452	408±23	SS	513
									MSS	103
Column Total	860	894	876	822	787	928	5167		F	
									P	

(B) Brain shock acquisition

Treat-ment	*Subjects (replication) : number of shocks/min*						*Row Total*	*Row Mean±SD*		*Error*
	1	*2*	*3*	*4*	*5*	*6*				
C_1	15	8	8	7	13	14	65	10.8±3.2	df	5
C_2	45	45	48	38	26	33	235	39±7.7	SS	272
									MSS	54.4
Column Total	60	53	56	45	39	47	300		F	
									P	

BRAIN-STIMULATION REWARDING SCHEDULE

Control experiments : Like in the tail shock avoidance schedule, the control experiments were done in this schedule also to find the effects of the unconditioned stimulation of the hedonic brain-stimulation continuously at the maximum rate of 8/min on the heart rate (Fig. 6). The results showed that the average rate and the stability of the heart rate was not much altered by this type of the unconditioned stimulation at a strength which would be psychophysiological as was determined in the pre-paralysis stage of experiment by self-stimulation as described in the methods.

Conditioned experimental alterations : In the experiments set for providing the hedonic brain shocks under the condition of lowering the heart rate below the set criterion level, there was a progressive reduction in the heart rate and an increase in the brain shocks obtained by the subject, attaining over 80% score in about 20 min (Table III, Fig. 7A). In 43 nonlearning subjects (Fig. 7B), the conditioned lowering of heart rate was not observed despite the conditioning schedule.

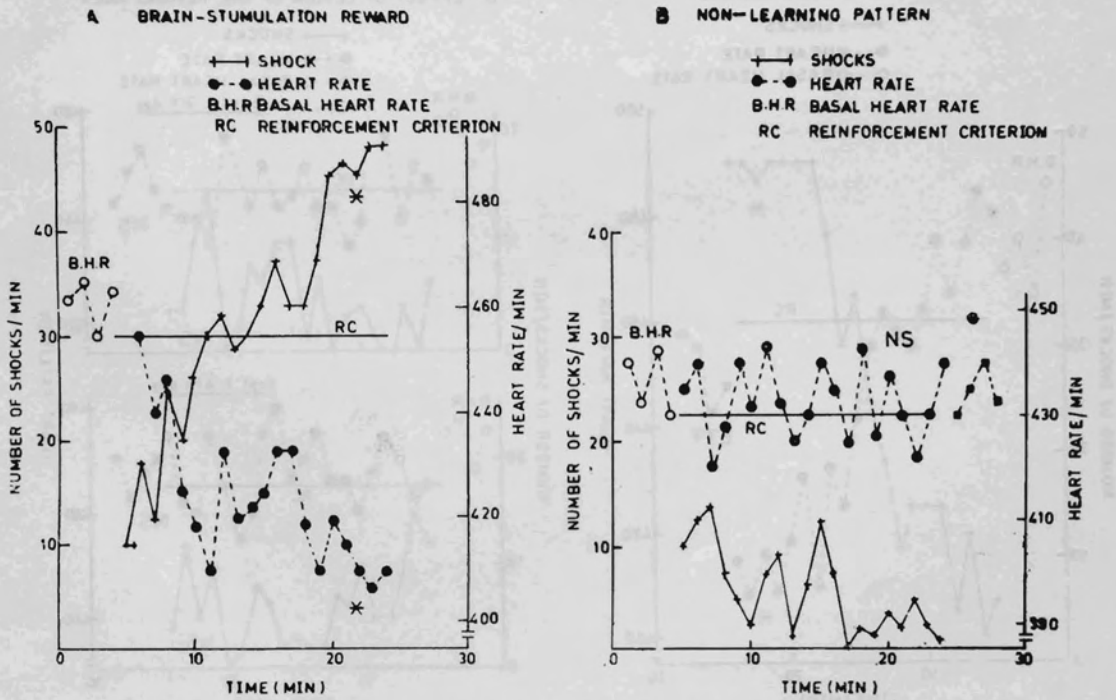

Fig. 7 : A : Learning of experiments with the reinforcement of rewarding brain-stimulation. Note the progressive increase in the number of rewarding brain shocks earned per min and the progressive reduction in the mean of the heart rate in a learning rat. B : An example of non-learning rat experiment in the same reinforcement schedule. Asterisk indicates comparison as in Fig. 3. It is not significant (NS) in B.

The results of the noxious tail shock experiment and of the hedonic brain shock experiments together show that the nonlearning or learning is probably not dependent on the type of the reinforcement but on some other cerebral states of the subjects.

Lesions of the rewarding stimulation site : After observing the learning behaviour under the brain-stimulation reward, the stimulation site of the subject was lesioned by passing current through the same electrode. The subject was retested after a few days for self-stimulation in normal behaving state, and also for the heart rate conditioning in the paralysed state under conditions identical to those set in that subject before the lesioning. It was observed that along with the loss of self-stimulation from lesioned site, the visceral learning also no longer occurred after the destruction of the brain-stimulation reward site (Fig. 8B). Hence, this is another important experimental confirmation indicating that the progressive changes in heart rate and number of rewards attained in the learning session are due to the reinforcement provided by the stimulation of the particular site.

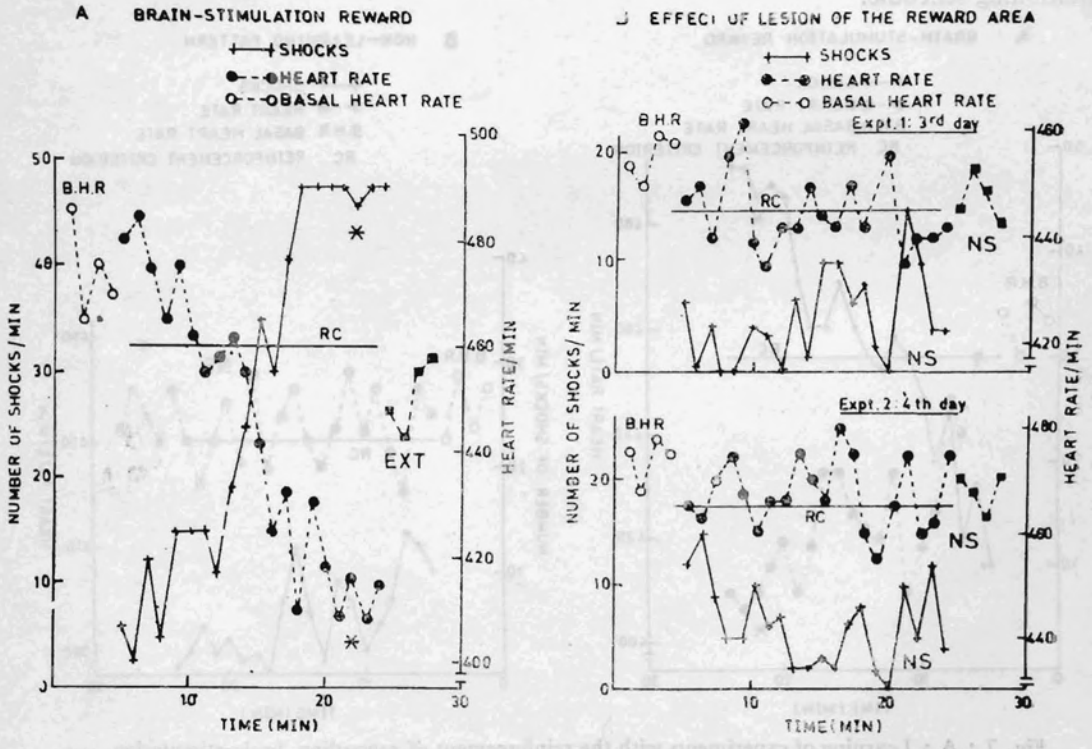

Fig. 8 : Another example of the learning curve obtained in the rewarding brain-stimulation schedule (A), and to show in the same rat how it became extinct after a localised lesion of the rewarding site (B). The testing was done on 3rd and 4th day after the lesioning. Asterisk and NS signify as in Fig. 3. EXT : extinction test result.

Synaptic modulation effects

Morphine : Morphine administered as stated before, caused a facilitation of this type of learning (Fig. 9A). Under morphine, the achievement of the 90% of the reward was

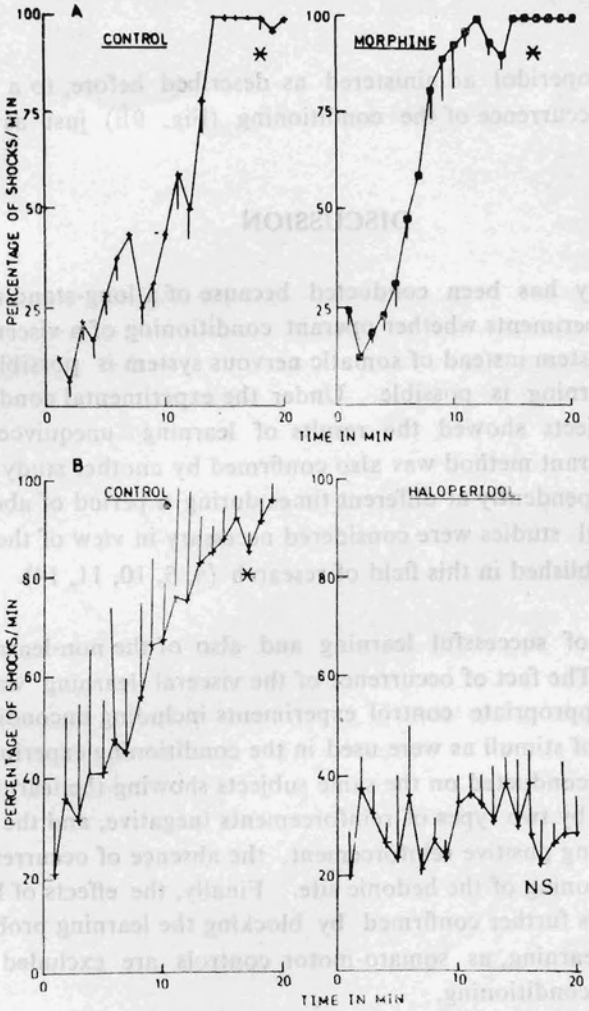

Fig. 9 : Effect of morphine (A) and haloperidol (B) on the conditioned acquisition of the rewarding brain-stimulations. Note by the comparing graph of number of rewards earned in control experiments, morphine (300 $\mu g/kg$, i.p.) speeded up the onset of learning, whereas haloperidol (300 $\mu g/kg$, i.p.) caused almost a complete failure of the occurrence of the visceral learning. Asterisk and NS signify as in Fig. 3. Also, the first ten values of control graph and of morphine graph differed significantly (1% level) in Wilcoxon matched pairs ranking test. Each graph in A was based on two experiments (2 rats) and in B on four experiments (3 rats, different from A). The rats used in (A) are same as in Fig. 5A other notations as in Fig. 5.

attained in only about 9 min, in contrast to about 12 min of the control experiments done on the same subjects (Fig. 9A). These and the tail shock experiments revealed that the effect of morphine on progression of the learning depended on the nature of the reward, the hedonically rewarded one occurring rapidly, in contrast to delaying of the pain avoidance rewarded one.

Haloperidol : Haloperidol administered as described before, to a learning subject has completely blocked the occurrence of the conditioning (Fig. 9B) just as in the tail shock avoidance experiments.

DISCUSSION

The present study has been conducted because of a long-standing necessity (11), to assess in independent experiments whether operant conditioning of a visceral organ controlled by autonomic nervous system instead of somatic nervous system is possible. The study has established that the learning is possible. Under the experimental conditions of this study, about 25% of the subjects showed the results of learning unequivocally. The visceral conditioning through operant method was also confirmed by another study (1, 2) done in the same laboratory but independently at different times during a period of about 5 years. These independent experimental studies were considered necessary in view of the specially unsettled and conflicting papers published in this field of research (4, 5, 10, 11, 14).

The experiments of successful learning and also of the non-learning type have been provided in the results. The fact of occurrence of the visceral learning was confirmed from different angles. The appropriate control experiments including unconditioned stimulations using the same strengths of stimuli as were used in the conditioning experiments, and also the extinction tests were also conducted on the same subjects showing the learning. The learning possibility was confirmed by two types of reinforcements (negative, and the positive). In the brain-stimulation rewarding positive reinforcement, the absence of occurrence of learning was also checked after the lesioning of the hedonic site. Finally, the effects of haloperidol in both types of learning schedules further confirmed by blocking the learning probably by actions on the synapses involved in learning, as somato-motor controls are excluded in the paralysed subject's visceral operant conditioning.

The purpose of this study was to firstly examine whether the operant conditioning of visceral function would be possible or not, in a subject under the state of skeletal muscular paralysis, as that has been in question (5, 11). The factors contributing to the large number of unsuccessful experiments have yet to be discovered. Comments on the possible causes of the present results are made below.

The present experiments have been carried on under Flaxedil (gallamine) instead of under curare which is stronger in action. The subject was handled as carefully and gently as possible to avoid fear. The stimulus strength of the noxious tail shock was carefully determind to be of only a moderate intensity for evoking a moderate degree of noxious response. The hedonic stimulus strength was determined in the self-stimulation experiments on the subject prior to paralization and the same optimal strength was used for the delivery of reinforcement during the heart rate conditioning session. These prior determinations of the strengths of the reinforcement stimuli could be of importance. The respiratory level was adjusted (rate and pressure) in the first 30 min of the observation period by taking the indication of the average besal heart rate to maintain it in a stable state usually settling in the range of about 440 to 460 beats per minute, with the transient fluctuations not exceeding $\pm 5\%$. If the preparation was not stable, the experiment was discontinued. The reinforcement criterion was set in the beginning at a fixed level to lower the heart rate, and the setting of the criterion level was chosen to be within the magnitude of the transient fluctuations so that the subject would be obtaining initially the experiencing of the reward by chance and thus aid in quickly developing an understanding of getting the reward voluntarily by heart rate alteration. No complexity of shaping was added, as the learning change was progressive and clear enough under the present paradigm itself. Further, no warning cue of the impending tail shock or brain shock was provided to the subject in the operant conditioning procedure, so as to exclude contamination of (i) any effects of frightened freezing out of helplessness to escape (being paralysed) from announced tail shock, or (ii) any kind of effects of unconditioned stimulus, or (iii) any orienting responses, or (iv) of any effects of classical conditioning. It is difficult to resolve whether or not such contaminations occurred in any learning experiments, hence the operant conditioning paradigm and the experimental procedures have been kept to be at the simplest possible level in the present study to firstly assess whether the learning of visceral organ control under paralysed condition is possible. Under the above experimental conditions, some subjects (about 25%) have shown the correlates of learning and the rest have not. This is the most important finding of this research. This also suggests that all subjects may not have equipotentiality for the operant visceral learning, or that some other cerebral factors have not been triggered in these non-learning subjects by the set of experimental conditions adapted here. By changing the experimental conditions they might also be conditioned to emit the operant responses The successful experiments not only established that the learning is not in doubt, but also revealed that the large percentage of non-learning experiments that one encounters in the way of getting the successful learning experiments could be the cause of frustration to the worker in this area of experimental research, demanding special efforts as in any difficult experimental research.

Another interesting observation was the session-wise, and non-cumulative changes produced in the conditioning. This is interesting in that the visceral learning may be of the

short term memory category, or that this type of experimentation mainly reveals only that much. Moreover, the voluntary controls on visceral functions have to be homeostatically integrated and harmonised with the other central cerebral mechanisms of the affect and the somatic sensory-motor system. It will not at all be of physiological advantage if the volitional controls are designed to directly influence easily and permanently the neural substrates of the vital visceral functions. In the course of phylogenetic development of brain, the visceral regulations have been insulated normally from volition, hence the difficulty of establishing their conditioning operantly in a large percentage of subjects. The lengthy discussions sometimes made in the psychophysiological literature arguing for the necessity of discovering completely independent play of the volitional influences on the visceral controls are not based on proper physiological considerations. Shifts in the factors like PaO_2, $PaCO_2$ and pH might not be the main causes that contribute to absence of visceral learning as was thought (5). The factor of individual predisposition in the central nervous system (CNS) for visceral operant learning appears to be probably most relevant. At the present time, there is no sure way of assessing or detecting the CNS factors, except assessing the learning feasibility on individual basis.

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