

THE INFLUENCE OF ELECTROSTIMULATION ON
HEXOBARBITAL INDUCED LOSS OF RIGHTING REFLEX IN RATS

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ABSTRACT Electrostimulation (ES) of slow (SF, 10 Hz) or fast (FF, 500 Hz) frequency decreases the sleeping time of rats anaesthetized by administration of acute doses of hexobarbital. When ES is applied via the ears, both SF and FF are equally efficient in reducing the loss of righting reflex (LRR), whereas if ES is applied peripherally via the paws, only FF decreases the acute narcosis time. Applied cranially, either continuous stimulation or administration of intermittent current (5 minutes on and off) were equally effective in reducing narcosis. A decreased period of 30 minutes' continuous stimulation will reduce sleeping time only if administered immediately after LRR. When restrained animals received ES for periods of up to 3 hours prior to administration of the barbiturate, the sleeping time of the stimulated and sham treated animals were not significantly different.

Key Words Electrostimulation, rats, hexobarbital, narcosis, duration.

INTRODUCTION

The possibility that therapeutic electrostimulation could ameliorate pathological conditions, including various forms of intractable pain (1-4) and those symptoms associated with the withdrawal of drugs of abuse in chronically addicted individuals (5-7), has stimulated experimental studies to investigate its mode of action (8, 9). Experimentally, determination of the efficacy of ES in the relief of pain is hindered by the absence of readily quantifiable biochemical parameters and the lack of objective determinants of analgesic response (10-14). Unlike ECT (electro-convulsive therapy), the low intensity

currents employed for therapeutic ES are normally applied for a prolonged period of time. Thus, investigation of the mode of action of ES in chronic addiction is complicated by the necessity to restrain experimental animals to prevent their removal of the electrodes during the period in which the stimulus is being applied. Restraining both stimulated or sham treated animals produces a considerable stressful response (15). It has been demonstrated that the neurohumoral responses to stress may be invoked by processes normally associated with animal husbandry, such as moving the cages (16). Thus, with restrained animals the effects of ES are being superimposed on an abnormal endocrinological situation.

The measurement of the duration of the loss of righting reflex after acute narcotic doses of anaesthetics, especially barbiturates, is frequently interpreted as an in vivo indicator of the hepatic metabolism (detoxication) of these substances. The mechanism of action of anaesthetics in the central nervous system (CNS) is uncertain (17) however, so that any observed alterations in sleeping-time may be the result of a number of interacting mechanisms. In previous experiments the LRR associated with the administration of various barbiturates was decreased by square wave ES of either SF or FF (18). This effect of SFES, but not FF, was inhibited by prior administration of the specific opioid peptide antagonist - naloxone.

Although the acute narcosed animal cannot be compared with the chronic or dependent state, measuring the LRR in such animals provides a quantifiable response which may be studied in a controlled relatively non-stressed situation. The present experiment was conducted to determine the mode of administration of ES which produces the maximal decrease in sleeping time and elucidate its mechanism of action.

MATERIALS AND METHODS

Chemicals Hexobarbital was obtained from the Sigma Chemical Company, Poole, Dorset, U.K.

Electrostimulators The instruments used were NET (Neuro-Electric Therapy) stimulators, developed by Dr. M.A. Patterson and manufactured by European Electronic Systems, Maldon, Essex, U.K. The instruments used in the present experiment were specially modified by EES to operate from a transformer connected to the mains supply. Under these conditions the output voltage (D.C. coupled) was adjustable from 0-4V and the output impedance approximately 150 Ω . The output pulse width was selectable in 6 ranges - 0.1, 0.22, 0.47, 0.82, 1.0 and 1.5 msec and the output frequency adjustable in 4 ranges - 1-10, 10-100, 100-1000, 1000-2000 Hz.

Animals Female CD rats of body weight 250 \pm 20g were purchased from Charles River, Nanston, Kent, U.K. These animals were maintained in groups of six on mineral bedding in high density polypropylene cages on a 12h 08.00-20.00h light : dark cycle. Food, R & M experimental No. 1 (BP Nutritional), and water were available ad libitum. All experiments were conducted between 09.00-12.00h.

Treatment Rats, in groups of six, were anaesthetized by intraperitoneal administration of hexobarbital (50mg/2ml/Kg) dissolved in sterile alkaline solution. Stainless steel Michel^R suture clips were inserted either by piercing the lower part of the concha just external to the auditory meatus in the manner described by Pert et al (19), or by clipping firmly into the plantar pads of the fore paws. The applied current was DC square (with respect to both the vertical axis and horizontal base line) wave of pulse width either 0.22msec or ranging from 0.1-1.0msec.

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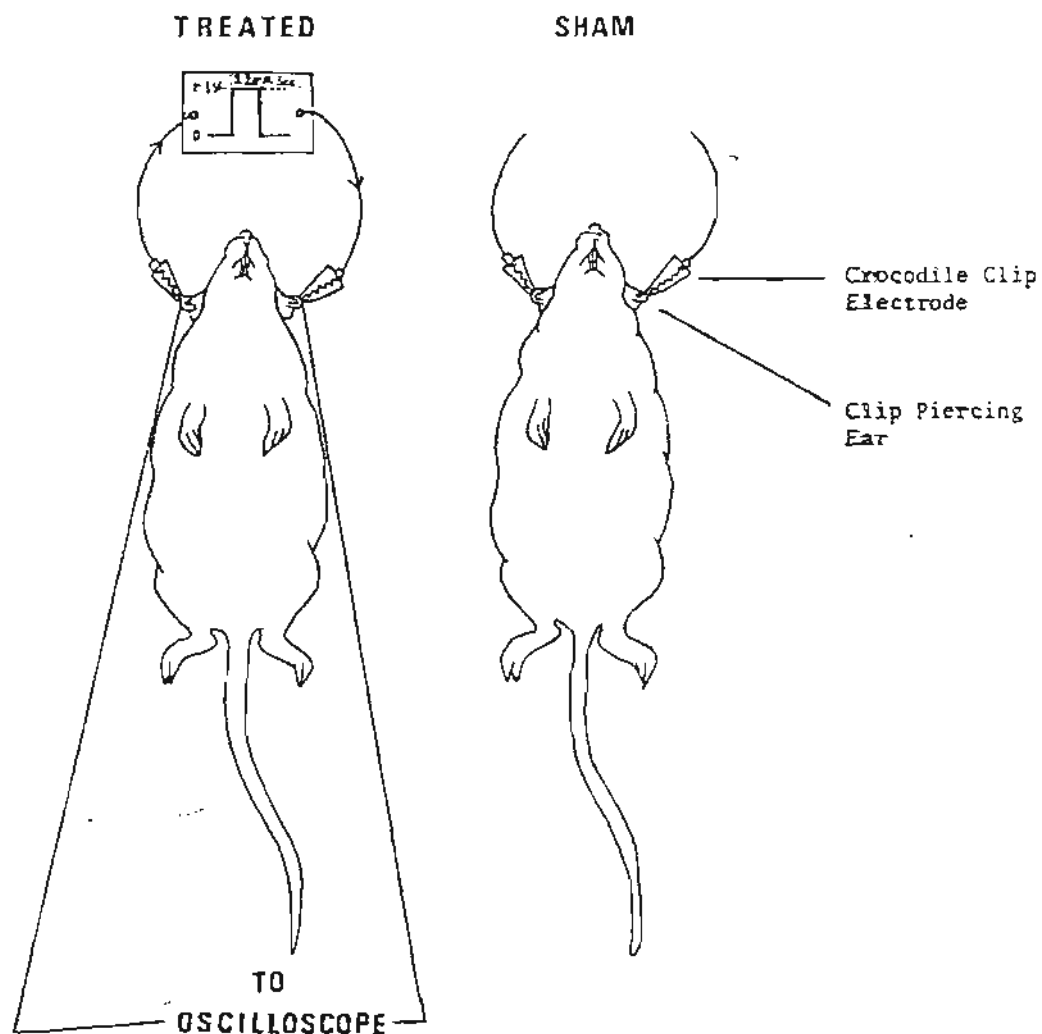


FIG 1. All animals were anaesthetized with hexobarbital (80mg/Kg). Electrostimulated and sham treated animals were connected to the crocodile clips at the electrode terminals via suture clips piercing the skin of their ears. The current at the suture clips was monitored via an oscilloscope. The current was passed continuously to the treated animals and none to the shams and the time taken for each rat to regain its righting reflex determined.

The current was monitored continuously with the recipient *in situ* using an oscilloscope (Advance Instruments, Hainault, Essex, U.K.), which was applied simultaneously at the stainless steel clips. Each NET instrument was adjusted until a constant intensity of 1V (measured at the electrodes of the pinna) registered on the calibrated oscilloscope and any necessary adjustments were made during the experiments to maintain this potential difference. Two frequencies of electrostimulation, either 10Hz or 500Hz, were applied in each experiment. These have previously been demonstrated to be the most effective frequencies for reducing barbiturate induced sleeping-times (9, 18).

The groups of rats were either stimulated continuously until they regained their righting reflex or for the first or second 30 minutes immediately after loss of consciousness. Another group of animals received trains (5mins on, 5mins off) of intermittent impulses until they regained their righting reflexes. Further groups of rats were restrained in cylindrical tubes while receiving continuous ES for periods of 1, 2 or 3h. After this time these animals were anaesthetized with hexobarbital and the times to regain righting reflex determined. In all experiments 6 treated animals were compared with 6 sham treated controls in which the rats were attached to the stimulators in the same manner as the ES animals, but no current was passed. The environmental temperature of the anaesthetized rats was maintained at $28^{\circ}\text{C} \pm 1^{\circ}\text{C}$.

The righting reflex was considered to have returned when the animal was able to rotate sufficiently to place an opposite hind paw on the surface of the bench.

Statistical Analyses The statistical significance of differences in results between groups of ES and sham treated animals was determined by unpaired Student's t test. Results were considered significant when $P < 0.05$.

RESULTS

FFES or SFES decreases the time taken to regain righting reflex when administered via the ears. Only FFES applied via the plantar pads was capable of producing this response. In all cases, the ES reduced the sleeping-time of the animals to approximately 60% of the control value, see Table 1.

The results in Table II indicate that 30mins continuous ES was sufficient to reduce the righting reflex to approximately 60% of the control value provided that the stimulus is applied immediately after LRR. When application of the current commenced 30mins after LRR, i.e. for the latter half of the time the animals were anaesthetized, there was no significant reduction in narcosis between sham treated and ES rats. Intermittent stimulation (5mins bursts administered at 5mins intervals) commencing immediately on narcosis and continuing until the rats regained their righting reflex was equally efficacious in decreasing sleeping-time.

Table III indicates that the "optimum" ES pulse widths to decrease the duration of hexobarbital-induced LRR were 0.22 and 0.50msec. The shortest pulse width examined (0.1msec) significantly increased narcosis time.

ES of restrained rats for periods of up to 3h prior to administration of hexobarbital did not significantly decrease the duration of LRR induced by the drug (Table IV).

TABLE I

The Influence of Cranial or Peripheral Slow or Fast Frequency Electrostimulation†
On the Hexobarbital-Induced Loss of Righting Reflex in Female Rats

Mode of Administration		Sleeping-time (mins)		% of control Sleeping-time
		Stimulated	Sham	
Cranial ¹	Slow (10Hz)	46 ± 8*	79 ± 11	58
	Fast (500Hz)	43 ± 5*		60
Peripheral ²	Slow (10Hz)	71 ± 11	81 ± 6	-
	Fast (500Hz)	51 ± 9*		63

† Voltage intensity 1V, pulse width 0.22msec. Results represent the mean ± S.D. for 6 animals in each group.

1 Cranial - electrodes applied via the pinna of the ears.

2 Peripheral - electrodes applied via plantar pads of the fore paws.

* Significantly different from controls P < 0.05.

TABLE II

The Effect of Electrostimulation† of Varying Duration on
The Hexobarbital-Induced Loss of Righting Reflex in Female Rats

Duration of Stimulation	Sleeping-time (mins)	% of control Sleeping-time
0 Sham treated	90 ± 10	
Continuous	55 ± 7*	61
30mins ¹	60 ± 8*	67
30mins ²	85 ± 13	-
Intermittent 5mins trains	61 ± 11*	63

† Voltage intensity 1V, frequency 500Hz, pulse width 0.22msec. Results represent the mean ± S.D. for 6 animals in each group.

1 First 30mins after loss of righting reflex.

2 Second 30mins after loss of righting reflex.

* Significantly different from the controls P < 0.05.

TABLE III

The Influence of Pulse Width of Electrostimulation† on
The Duration of Hexobarbital-Induced Sleeping-time in Female Rats

Pulse Width (msec)	Sleeping-time (mins)	% of control Sleeping-time
0.10	112 ± 13*	132
0.22	51 ± 9*	60
0.50	56 ± 9*	66
1.0	83 ± 15	100
Sham treated	85 ± 8	-

† Voltage intensity 1V, frequency 10Hz.

Results represent the mean ± S.D. for 6 animals in each group.

* Significantly different from the controls $P < 0.05$.

TABLE IV

The Effects of Varying Periods of Electrostimulation† Before
Hexobarbital Administration on the Duration of the Loss of Righting Reflex

Duration of Stimulation (h)	Sleeping-time	
	Stimulated	Sham
3	63 ± 15	66 ± 12
2	78 ± 14	50 ± 15
1	74 ± 12	77 ± 16
0 (Controls)	-	81 ± 8

† Voltage intensity 1V, pulse width 0.22msec,
frequency 10Hz. Results represent the mean ±
S.D. for 6 animals in each group.

These animals were restrained in cylindrical tubes
for electrostimulation or sham treatment.
Hexobarbital was administered immediately after the
rats were removed from the restraining cages.

DISCUSSION

Using the present behavioural model (LRR), the superiority of rectangular or square as opposed to sine wave ES was demonstrated (18). Similarly, rectangular wave stimulation has been demonstrated to be more efficacious than sine in producing analgesic responses, as dV/dt is much greater in the rising and falling phase of the pulse. In addition, pulse duration should be optimally minimal in order to avoid potential necrosis of tissue around the electrodes, due to electrolysis phenomena. Detailed experimental and theoretical analysis of these relationships, using basic electrical parameters as well as the effect of pulse repetition rate in the wide range between 0.1 Hz and 500 KHz, has been reported by Y. Omura in 1975 (20), and similar findings were reported by M. Janko et al in 1980 (33).

It is unlikely that comparisons between the LRR model and induction of auto-analgesia will elucidate the differing responses to SFES and FFES observed in the present experiment. Although the evidence is largely equivocal, a number of reports recommend FFES for autoanalgesia (2, 22, 33). Regardless of the frequency of ES, the treatment is most beneficial for the amelioration of cutaneous pain when applied locally (33). Possibly an additive effect between the pain and ES is conducted by ascending afferent fibres in the spinal cord (2). It is unlikely, however, that the weak low intensity ES would activate C or A δ fibres and the impulses generated by ES would mainly activate A δ fibres (23,33). It is probable that in the case of electroanalgesia continuous FFES produces local changes in excitation frequency and is thus efficacious in decreasing local pain at distal sites (33).

The ears are known to have sensory connections with several cranial nerves which have diffuse connections through the neuroaxis and it is possible that only transauricular application is responsive to SFES, whereas FFES is more generally applicable at sites other than the pinna or cranium.

SFES and FFES have been demonstrated to invoke differing biochemical responses, the former being associated with increased β -endorphin activity (24). It was previously demonstrated that the decrease in barbiturate-induced LRR duration resulting from SFES was impaired by the specific opiate receptor antagonist naloxone, whereas this drug enhanced the action of FFES (9).

FFES increases serotonin levels in certain regions of the brain (8,21,24). In vitro studies indicate FFES increases γ -glutamate receptors (25) and causes structural changes in the hippocampus, increasing synaptosomal connections (26). Further experimentation using this and other models is necessary to determine the relative importance of all the factors contributing to the differences between SFES and FFES.

It has been reasoned that the meanings of signals are conveyed in the impulse frequency rather than the duration or pulse width (27). The studies conducted with this (LRR) model confirm the importance of the frequency of ES but also provide some indication that the pulse width does influence the response, which is in accordance with another report (33). In addition, clinical use has established 0.2-0.3 msec as the optimum pulse duration for the treatment of addicted patients and other conditions, since excessive pulse duration can cause electrolysis in tissue around electrodes (5, 20, 21).

The intervals between stimulation are an essential determinant of sensitization or the development of tolerance (22). Kindling seizures are produced by repetitive intermittent sub-threshold stimuli to the limbic regions of the brain, especially the amygdala (22). It has recently been reported that SFES is even more potent in inducing kindling seizures than FFES, this form of ES corresponding more closely to the amygdaloid discharge (29). More frequent stimulation retards kindling seizures. It has been demonstrated that chronically administered ES of the amygdala, via implanted electrodes, increases blood prolactin and an amygdalohypothalamic control of neuroendocrine function has been postulated (30).

The symptoms associated with the withdrawal of many addictive drugs may be likened to the kindling seizure (28) and it is possible that the efficacy of NET

electrostimulation for drug addicts could be the result of amygdaloid stimulation in the absence of the drug of abuse. δ -Endorphin, previously implicated in the action of SFES (8, 9), may be released indirectly as a result of this stimulation of the limbic area of the brain or be another independent effect of ES. Recent evidence has shown that phenobarbital increases some opiate-antagonistic actions of naloxone (31) and it has also been suggested that a specific opiate peptide released from a separate part of the brain prolongs barbiturate-induced LRR (32). It is possible, therefore, that there is a complex interaction between opiate peptides and barbiturate-induced LRR which complicates the interpretation of the results of the present experiment. However, apart from potential applications in anaesthesia and detoxication in the acute narcosed state, this experiment could indicate a simple behavioural model by which the effects of differing forms of ES may be readily quantified.

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