# Electronarcosis Induced By Mixed Frequencies Electric Stimuli In Rats

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**Citation**

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**Abstract**

Three population of albino male Wistar rats were studied through immersion tail retreat test in order to evaluate the antinociceptive action of electric stimulation. Two populations (test) were treated with electric pulses trains of 166KHz carried by 100Hz waveforms with 1mA and 0.5mA. One population was not stimulated (control) but received fronto-occipital needles electrodes as well. In addition, residual post-effects were estimated measuring the elapsed time for recovering the cognition and for occurring movements on paws. Results showed a complete abolition the nociceptive sensibility in both test groups and a faster recovery after the 0.5 mA electric pulses treatment, as compared to control group.

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## INTRODUCTION

Historically, electric pulses has been used for pain inhibition ([1]). Mach (1875) anesthetized fishes using direct currents and fifteen years later and D'Ansorval discovered anesthesia produced by high frequency currents ([2]). Electronarcosis was induced through a alternating electrical currents (2) and later with mixed alternated and direct currents([3]). Following this technique a system for electroanesthesia ([4], [5], [6]) was developed and the actual parameters stated for the electronarcosis use in humans ([7], [8]). An equipment using low currents ( 4 to 10 uA ), for dental electroanesthesia ([9]) anesthesia in rats ([10]) and humans ([11],[12]) was developed. Recent studies on transcranial electrostimulation using low frequency (4Hz) electrical pulses and applied in rats ([13],[14]), obtained abolition of pain reflexes without influence on the circadian cycle ([15]). Electric stimulation has been used to induce a deep sleep ([17]), in human patients, as well.

In order to study the influence on pain sensibility of 166 KHz electrical pulses carried by square waveforms with low frequency (100 Hz), a stimulator was developed to furnish low and medium frequency stimulating pulses to be applied in occipto-frontal electrode arrangement in male albino rats. Intensities of 1 mA and 0.5 mA were applied and the nociceptive threshold was evaluated through immersion tail retreat reflex test.

## MATERIALS & METHODS

Sixty albino male Wistar rats, weighting from 140 to 160 g, were separated in 1 mA test group, 0.5 mA test group and control groups, each having 20 animals.

The rats received water and food "ad libitum", kept in collective cages under a 12 hours dark/light cycle at environmental temperature. The studied rats never had experienced electric stimulation or anesthetics before.

The stimulation was provided by a home made generator producing electric pulses with an optional intensity of 0.5 or 1 mA. It was compared with a FAC-300A Stimulator and calibrated with a digital oscilloscope. The emitted 100Hz electric pulses has a 166 KHz burst, 0.5 and 1 mA intensity and duration of 2ms has a balanced waveform. A constant

current circuit was added to maintain the same intensity whenever the skin-electrode interface impedance change.

A previous sedation was achieved by a 3 mg/Kg intra-peritoneal injection of sodium thiopental, to facilitate the electrode insert. After sedation the rats were, each by time, confined in a transparent pliant contention box which impaired deambulation but left free the head and tail. Thin and flexible cables connected the stimulator to inoxidable steel needle type electrodes, inserted in the forehead and in the occipital skin region. In fact, venous 25x7 needle were used to made up the electrodes, as previously used (17) Then the electrodes were inserted and the rats stimulated, in the tests groups. Control group had the electrodes placed in but did not receive electric stimulation. The pre-sedative effects persisted during 30 minutes, as shown the control group. Before to turn off the stimulator, the contention box was disclosed and the unconscious rats put in freedom.

To evaluate the resulting insensibility to noxious stimuli, the animals had the tail immersed, up to distal 4 cm from the tip, in 50 oC heated water existing in a constant temperature container. The time (seconds) required for the rats to "flick", or withdraw their tails (retreat reflex) after the immersion, was measured with a Casio chronometer in the immersion Tail Flick Latency test (TFL), as previously used ($_{18}$). So, the first tail movement imposed a time count interrupt. Whenever the tail retreat reflex did not arise until 20 seconds, the tail was manually withdrawn from water to avoid tissue lesions.

Each animal was submitted to 9 TFL test by assay, separated each other by a lapse of 15 minutes in order to allow the tail temperature recover from the precedent test. The time duration of each assay was 2 hours and 15 minutes, apart the time for pre-sedation and electrodes placement procedures (near 15 minutes) .

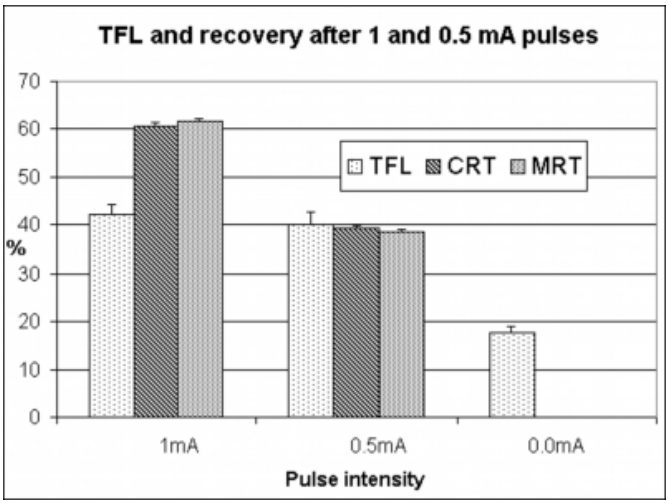
Furthermore, the time for recovery of perception and eye movements, here called Cognition Recovery Time (CRT) and the time for recovery of free limb movements or deambulation, here named Motion Recovery Time (MRT) were measured only in test groups. The time measurement for CRT was counted up to the first movement on muzzle or on the eyes, beginning at the stimulator turn off. For MRT, the procedure was the same but the time count was ever interrupted after the first paw movement observed. As a previous trial shown burned skin at the electrodes insertion point, because the used pulse waveform was unbalanced, the animals were treated with balanced current pulses, after modification in the pulse generator.

## RESULTS

A summary of the obtained data among the animals in the control groups are seen in Table 1 and agrees with the pertinent literature data concerning the physiological responses (7).Table 1 shows the time delay between immersion in 50 ºC water and the time spent for arising the tail reflex of retreat (Tail flick latency = TFL). The CRT and MRT, after 0.5 mA pulses were smaller than after 1 mA pulses, resulting a mean CRT 1.48 min and a 2.92 min reduction of MRT respectively.

### Figure 2

Figure 1– CRT, MRT and TFL averaged responses from experimental and control groups:




* = statistical significance (test versus control)

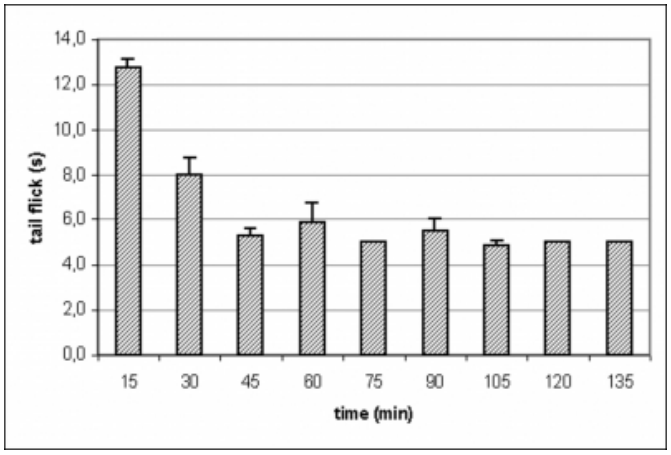
Even after a 34 minutes immersion, the pain reflex was absent. In view of the previous recommendation (13,14,15), in all the cases the tail was retreated from water by the assistant in 23 seconds mean time, to avoid tissue damages.

The time for the tail retreating of the last TFL (at 120 min) was deliberately prolonged up to 34 seconds average, without presence of the retreat reflex .

The statistical data analysis resulted highly significant as comparing the test and control groups TFL data. Chi-square analysis showed p = 2.4077E-134 for 1mA and p=1.9618E-120 for 0.5mA stimulation. Unpaired Student T test resulted p=7.72913E-14 and p=7.72913E-14 for 1 and 0.5 mA stimulation, respectively. TFL test data were obtained artificially but this does not invalidate the statistical analysis. Figure 1 displays the resulting TFL from control group so as CRT and MRT.

**Figure 3**

Figure 2 – Control group tail flick latency, showing the pre-sedative effect up to 30 minutes




CRT and MRT obviously was measured only the test group and resulted statistically significant, as well. Chi-square test showed $p<0.05$ on CRT 1 and 0.5mA stimulation comparison analysis but MRT 1 mA and 0.5mA stimulation resulted a probability inferior to 1% to be the same. Student test done $p<0.001$ for both CRT and MRT after 1 and 0.5 mA stimulus intensity

The control group shown a decrement in pain threshold up to 45 minutes despite the literature settle a 30 minutes period for Thiopental sedative action. However, 45 minutes after the control assay beginning, the TFL remained stable with value of approximately 5 seconds, until the end of the experiments (see Figure 2).

{image:3}

## DISCUSSION

All the animals submitted to transcranial eletroestimulation presented a statistically significant nociceptive threshold above the group control. These results are compatible to those previously obtained with 10 m A square wave pulses without high frequency bursts (14,15).

Thiopental is a barbiturate whose time of brain action for the used dose is inferior to 30 minutes`(18), however the normal retreat reflex time was reached only after 45 minutes. This pre-sedation simplified the experimental procedures and handling of the animals and it enhanced the anesthetic induction, despite the chemical character of the sedation utilized ([19,20,21,22]). The initial sedation values were 12 seconds in the first TFL (at 15min); this justify the initial depression observed in the control group, that progressively reached the physiological response time after 45 seconds. In the test group, an abolition of the reflex of retreat of the tail was observed since the first TFL. This high pain sensitive numbness may be due to the action of pre-sedation added to the effects of the applied electric neuro-stimulation and after this anesthetic induction the pain threshold remained high. TFL data from both 1 mA and 0.5 mA test groups (Table 1) were statistically similar each other, leading to think on a saturation of the inhibitory mechanisms of pain sensitiveness. Conversely, the recovery showed an earlier recuperation of mindfulness and movements after the lower intensity pulses, suggesting an inverse stimuli intensity correlation.

The neurobiological substratum of these effects remains ignored, up to now. One hypothesis points to a possible activation of endogenous opioids, emphasizing the use of transcranial stimulation for naloxone-reversible anesthesia (13, [23]). Moreover, TENS (transcutaneous electric nervous stimulation) was found to relieve the deprivation symptoms in morphine dependent animals ([24, 25]). On the other hand, an electrical hyperpolarizing action on medial thalamic nuclei can be hypothesized, based on Skolnick M. and cols ([26]) observations, reinforced by another finding ([27]) stating that naloxone does not antagonize general anesthesia in rats. It is known that square electric pulse without high frequency applied over the skin cannot induce opiate liberation. Thus, high frequency seems to be associated to naloxone-reversible anesthesia.

Curiously, anesthetic recovery time the groups stimulated with 0.5 mA showed a superior performance, despite they had presented similar sensitive numbness. The 0.5 mA intensity pulses revealed to be so effective as 1 mA ones, resulting a pain blockade at the level, needing a manual tail retreat due to the absence of tail retreat reflex response.

CRT data comparison showed 55.43% faster after 0.5 mA pulse than after 1 mA stimulation. In the same way, MRT after 0.5 mA electric stimulation was 53.41% smaller than with 1 mA. These findings point to a lower inhibition of superior nervous structures after a low intensity stimuli, leading to direct proportionality between cause and effect. This reasoning could support an enhancement of opioid production model rather thalamic inhibition as affirmed by Hosobuchi and cols. ([28]), after brain electrical stimulation.

The transcranial eletroestimulation has been proven to be an effective method for increase the nociceptive threshold as observed in the studied rats, however an important finding was the above proportionality of CRT and MRT to the

electric stimulation intensity.

The results obtained with the 100 Hz to 0.5 mA electric stimulation was shown fully satisfactory and the reached anesthetic level was high, although not provoking cardio-respiratory sequels or any other collateral effects in the animals, during nearly four and a half hours of anesthesia. In addition, the anesthetic effect has been confirmed through the absence of pain reflex despite the tail immersion surpass half a minute immersed in 50ºC water. Further investigation is to be done using lower pulse intensities and endogenous opioid blood levels so as the use of naloxone will be observed during and after electric stimulation as well.

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