Development of a neurostimulator for studies of epileptic crisis

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Abstract. This work describes the development and use of a multi-channel, programmable physiological stimulator. The device is intended for the detection of epileptic events and crisis prediction using encephalograms. The animals were followed by the kindling model. The system program accepts several inputs of stimulation parameters such as, pulse width, separation between pulses, pulse intensity and total number of pulses. The stimulator generates constant-current, bipolar pulses with a maximum amplitude of 5 mA and a resolution of 50µA. As regards voltage, the maximum amplitude is 150 V. The stimulator was constructed with a microcontroller (PIC18F4550), the latter version being controlled by a personal computer. Experiments of achieving the epileptic events were carried out on rats.

1. Introduction

The field of physiological stimulation by electric means is a well-known subject in medicine. However, such procedures have become considerably specific, depending on the operating conditions of the device that emits the stimulation signal.

Epilepsy is one of the most common neurological disorders and affects about 60 millions people around the world. It is characterized by the sudden development of hypersynchronous neural activity involving a high number of neural networks that disturbs the normal behavior of the brain.

Epilepsy is expressed through changes in behavior, and the diversity of its clinical manifestations depends on the cerebral cortex zone where the epileptic discharge originates, as well as on its propagation characteristics.

The induction of epilepsy by electrical stimulation to laboratory animals has proved to be an effective tool to study the underlying physiological mechanisms.

The kindling model [1] used in this investigation consists in a repeated and periodic stimulation of the limbic system (typically the amygdala or hippocampus), which induces partial epileptic crisis which eventually generalize.

During an initial step, the electrical stimulation induces postdischarges with little clinical manifestation. However, in subsequent sessions, postdischarges begin to be recorded in the EEG (see figure 1), with alterations in the morphology of tips and more complex behavioral disturbances.
The Kindling phenomenon can be produced by electrical stimulation in various brain sites, but not in everyone. For instance, cerebellar stimulation does not lead to Kindling. Moreover, the changes occurring during the Kindling process are evidenced by responses of behavioral, electroencephalographic and neuron-chemical nature. In principle, electrical stimulation induces epileptiform postdischarges of short duration. Gradually, postdischarges become longer and propagate to other areas, finally comprising the whole brain.

The Kindling model possesses a number of advantages such as:
1. It is able to delimit the epileptogenic zone, responsible for the discharges, in order to study it.
2. The starting time of the epileptic crisis can be controlled.
3. By using a continuous record after a long stimulation period, the spontaneous discharges can be captured.

The characteristics and advantages of the Kindling model mentioned above prompted the development of a programmable, encephalic stimulation system of use on laboratory animals.

The objective of the present investigation is the development of a prototype for electric stimulation as well as the tests carried out for its validation in animals.

2. Material and Methods

2.1. Initial Considerations

The neurostimulator for epileptic crisis investigation complies with the following design requirements:
1. The system generates bipolar pulses at constant current, allowing the user to introduce (program) the following parameters: pulse width (A), separation between successive pulses (S), number of pulses applied (test duration, N), maximum current amplitude (IMAX).

The parameter “frequency” was not included, since it does not provide the user enough information as compared with “separation between pulses”; however, with a simple mental exercise, it can be demonstrated that the sum $A + S$ gives, as a result, the signal period.

Figure 2 shows the schematics of the stimulus generated by the system, along with the parameters mentioned above.

![Figure 1. A characteristic Kindling EEG (Postdischarge can be observed to occur from 4 to 14 seconds)](image)

![Figure 2. Schematics of the stimulus and its parameters.](image)
Table 1. Parameters, range of variations and resolution

| Parameter                  | Range           | Resolution |
|----------------------------|-----------------|------------|
| Pulse width                | 100 µs – 500 µs | 50 µs      |
| Separation between pulses  | 10 ms – 1000 ms | 1 ms       |
| Nº of pulses applied       | 1 – 5000        | 1          |
| Maximum current amplitude  | 60 µA – 5000 µA | 20 µA      |

2. An output stage, capable of providing a voltage with maximum amplitude of 150 V.
3. A reliable and friendly system that can be easily changed by the operator in order to implement diverse tests.

The device thus developed, as seen in figure 3, is composed of several consecutive blocks.

2.2. Description of the consecutive blocks
The central element of the device is a microcontroller block (or control block) presented in figure 3. The chosen device, codified as 18F4550 [2] from Microchip company, carries out the following tasks:

![Figure 3. Block Diagram of the stimulator system.](image-url)
1. Generate the following parameters: width, separation between pulses and number of tests, from the information supplied from the PC (see figure 2).
2. Drives the selector switch, responsible for connecting the electrodes to the stimulator or the recorder.
3. Communicates with the PC: through an opto-isolated RS422 series normative.

The target signal generator (see figure 4) is composed mainly of an analogic switch CD4066BC plus a high speed analog-to-digital converter.

The working principle is based on a DAC that generates a direct current voltage with a level that is a linear function of the maximum amplitude chosen by the operator from the PC.

Two amplifying stages, the first of buffer type (\(A_v = +1\)) and a second in cascade (\(A_v = -1\)) input to an analog switch whose common outlet terminal provides the target voltage.

In this block, by commuting between two states, the switch is intended for the bipolar configuration of the excitation pulse.

![Figure 4. Schematics of the target signal generator.](image)

The development of the constant current generator satisfies the need of a high voltage output stage. Impedance in small laboratory animals is such that voltages of up to 150V are required.

The circuit configuration (see figure 5) of the generator is of the feedback type, whose sampling configuration is of current-inlet series connection [3].

The selector switches (Stimulation/recording) were developed with reed relays and, once the stimulation process is over, allows the system to liberate the electrodes in order to use them to record the response to the stimulation.

Finally, the PC program, performed in Visual Basic language, was developed to configure and program the stimulation tests in a friendly and flexible manner.
3. Results
Specific electronic measurements were made to verify that the stimulator developed here complied in a highly satisfactory manner the design requirements, before starting the tests on laboratory animals.

Wistar male rats, 200-250 g in weight, were the laboratory animals selected for these tests. A bipolar electrode, by stereotaxic implanting, was placed in the rat hypocampus.

Once the system was connected to the animal, the stimulation protocol began (see figure 6). Firstly, the postdischarge threshold was determined, defined as the minimum intensity that generates a postdischarge lasting 5 sec. By utilizing such a current intensity value, the electric stimuli were applied on a daily basis until reaching stage V of the Kindling model (the animal falls, loose posture control). The same electrodes are used to carry out the electroencephalographic recording [4].

In particular, the Kindling was studied in the CA1 region of the hippocampus, with the following stimulation parameters:

- Pulse width: 500 µs
- IMAX : (depends on the threshold determined for each animal)
- Duration of testing: 2 sec
- Separation between pulses: 19 msec (signal frequency: 50 Hz).

Figure 7 shows an EEG signal of a rat after being stimulated with the parameters described previously.
4. Discussion
Before developing this system, a first prototype of a neurostimulator was developed to work autonomously, i.e., not connected to a PC. That system was considerably useful in a first stage, allowing, through numerous experiments, to define the specific requirements that are found only by experimentation. However, the system was not sufficiently flexible, showing also limitations on the maximum output voltage (up to 24 V).

From the experiments made with the Kindling model, the following observations can be listed:

· The association between stimulation and electroencephalographic recording was evident.
· A rational study using various successive stimulations must have programmable rest stages.
· It is advisable that the commuting between stimulation and recording be reliably automatic (especially for stimulation pulses of up to 150 V in maximum amplitude).
· The necessity of having software fitted with a graphical environment in order to program the stimulation parameters, and thus superseding the first development (with buttons and LCD visualization).
· After due experimentation, the analog and digital electronics of the first system was made simpler and improved.

On these grounds, the new development was decided.

It is interesting for us to develop a stimulator-EEG recorder which, besides being capable of conducting successive stimulations, be also capable to program experiments for more than one animal at a time, as well as to apply an unlimited number of stimulus-rest processes, and to combine in stimulation and recording in a single system.

5. Conclusions
The performance of the developed system proved to be highly satisfactory for the expected uses.

The experience collected with the first stimulated animals allowed us to adjust diverse technical characteristics of the device so as to ensure its reliability for further use.

The integral design of the neurostimulator makes it possible to modify and or improve its technical characteristics, depending on the requirements of the research protocol to carry out.

References
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