electrodes E_1 and E_2 (Figure 1). The current is set by means of a variable resistor. Both switches are driven by built in drivers that, in the case of the CMOS Siliconix DG-200, are closed by a logic low. A simple way of driving these switch drivers with a conventional electrophysiological stimulator is to apply +2V to one input of a 7400 NAND, while the other input is tied to +5V. The resulting logic low output is then used to drive the switches. There are several other CMOS switches (like Analog Devices AD 7513) that can perform the same function with equally good results.

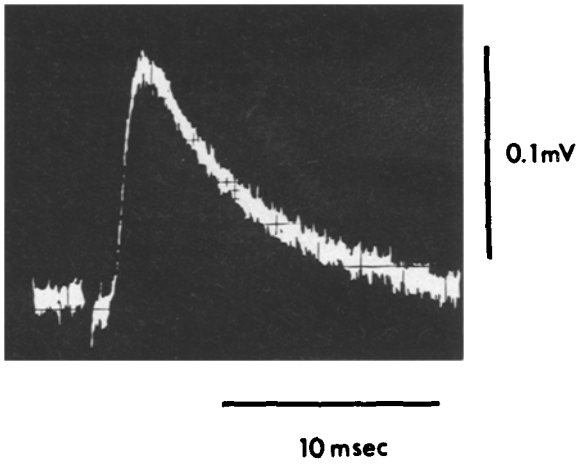

Fig. 2. End plate potential of a frog sartorius neuromuscular junction recorded using the fluid electrode technique[7]. The nerve was stimulated by the CMOS-switch SIU and the response was recorded with a DC amplifier with a band of 40 kHz. Notice the small size of the stimulus artifact, although the size of the response was 0.1 mV.

The ground isolation (as well as the resistance between the terminals during the off state) is $>10^9$ Ω. The capacitance to ground during the on state is ~25 pF and the frequency response goes from DC to ~100 kHz. The maximal voltage output is ± 16 V and the maximal current is 40 mA. The *t* off (delay between the 50% point of the positive edge of the control signal and the 10% output) increases by increasing the electrical resistance between the electrodes and ground: For this reason the CMOS SIU should be used with low resistance stimulating electrodes (<100 k) and the preparation should be grounded (Figure 2). However, the ground electrode should not be in direct contact with any of the stimulating electrodes, to avoid destroying the isolation. If these simple precautions are taken, *t* off will consistently be < 20 μsec.

Summary. A simple stimulus isolation unit for extracellular stimulation is described. The current is provided by a battery and is controlled by a dual CMOS switch and a variable resistor.

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A Rapid Method for the Verification of Drug Injection into the Cerebral Ventricular System of the Rat

The study of catecholamines metabolism in the brain is at present carried out mainly by the introduction of these substances into the cerebral ventricular system. Likewise, in order to investigate the effects of different drugs acting on the central nervous system, the injection of these substances into different regions of the cranial cerebro-spinal fluid system is often used. While some investigators have used stereotaxic techniques[1–4], others have injected the drugs according to several anatomical reference points[5–8]. In both cases, however, it is necessary to be sure that the drug was injected in the previously selected site, especially when difficult and prolonged experiments are carried out (i.e. electrophysiological studies on the action of some substances on the central nervous system). When these sorts of experiments are done in large animals like dogs, cats and rabbits, the proof that the drug was injected in the selected target is easily checked by the extraction of a small volume of cerebro-spinal fluid. However, when experiments are done in small animals like guinea-pigs, rats and mice, this approach is not possible and the usual way to check that the drug has been injected into the selected target is

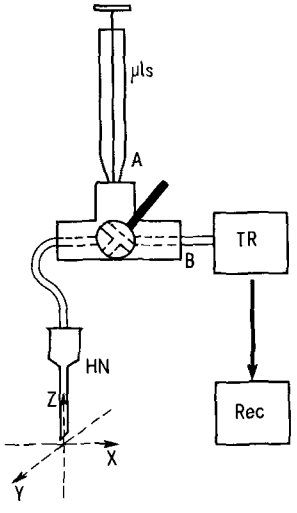

Fig. 1. HN, Hypodermic needle placed in stereotaxic coordinates (x, y, z); μlS, microliter syringe; TR, pressure to electrical signal transducer; Rec, single-channel recorder; A, position of the 3-way stopcock to connect HN to TR. B, position of the 3-way stopcock to connect HN to μlS.

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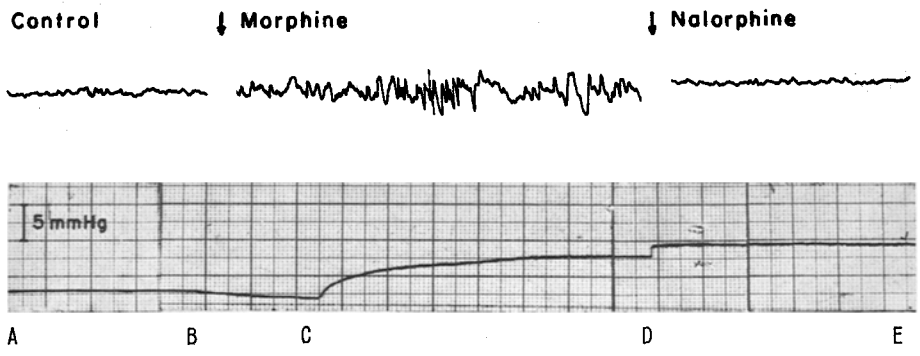

Fig. 2. Upper trace: Electrocorticographic modifications: control, 5 min. after 10 mg/kg morphine i.v. injection, 5 min after an intraventricular (4th ventricle) injection of 50 μg nalorphine. Lower trace: Pressure variations registered in the recorder system: A-B, level of the base line; B-C, negative pressure during the penetration of the needle into the brain; C-D, intraventricular positive pressure; D, instant of the intraventricular injection; D-E, intraventricular pressure after injection of nalorphine solution.

by means of histological studies. This is an imperative requirement, especially when anaesthetized animals are used, since overt behaviour effects are absent.

In this communication, we report a method that gives absolute certainty for the injection of a drug at a previously selected site on the cerebral ventricular system. Furthermore, the method described permits the graphic record of changes on the intraventricular pressure after the injection of the drug. With this technique, subsequent histological studies are avoided.

A 27-gauge hypodermic needle is placed in a stereotaxic apparatus (Horsley-Clarke type) and connected to a 3-way stopcock by a thin polyethylene tubing; the other 2 ways of the stopcock are connected to a 10 μl syringe and to a Sanborn 268 B pressure transducer, respectively. The electrical signals are recorded in a Sanborn 301 single-channel direct-writer apparatus (Figure 1). The system formed by hypodermic needle, tubing, stopcock, transducer and syringe is filled with the solution to be injected; the recorder is regulated to register the pressure in mm of Hg and centred to the zero base line. A conscious animal immobilized with D-tubocurarine or an anaesthetized animal under artificial ventilation is placed on the stereotaxic apparatus. A midsagittal incision is made from the eyes to the ears and the cranial bones are exposed. A 2–3 mm diameter hole is made with a dental engine on the selected site until reaching the duramatter; this perforation allows the introduction of the needle.

The 3-way stopcock is moved to the A position and the needle is slowly descended within the brain to the stereotaxic selected site. This slow descent is recorded like a negative pressure, and it corresponds to a hydrostatical pressure change in the chamber of the transducer. When the point of the needle contacts with the cerebrospinal fluid, the recorder detects a 5 to 10 mm of Hg positive pressure. Then, the 3-way stopcock is changed to position B and a desired quantity of the drug is injected. Moving the 3-way stopcock back to the A position, it is possible to record the intraventricular pressure attained after the injection of the drug during the whole experiment.

Figure 2 shows the synchronization of the cortex-EEG in a conscious rat after i.v injection of 10 mg/kg morphine, and the antagonistic effect produced by the injection of 10 μl of a 5% nalorphine solution into the 4th ventricle[9]. A 300 g white rat (strain UCh-A) was used, initially anaesthetized by ether and subsequently immobilized with D-tubocurarine and maintained under artificial ventilation. Morphine was injected 30 min after the recovery of the rat from the ether action, and the intraventricular injection of nalorphine was made 10 min later.

The stereotaxic coordinates (A: −3.0, L: 0.0, V: −4.0, in mm) corresponding to the 4th ventricle of the rat brain were taken from the PELLEGRINO and CUSHMAN atlas[10].

The electrocortical activity was continuously monitored using a Grass ink-writing multichannel EEG apparatus, by the application of 2 electrodes to the cortex surface, after the withdrawal of a portion of a parietal bone.

Figure 2 also shows the pressure changes recorded during the penetration of the hypodermic needle into the brain (line between B and C) and the sudden pressure increase registered when the hypodermic needle contacts with the cerebro-spinal fluid of the 4th ventricle (line between C and D); the rest of the record (line between D and E) corresponds to the intraventricular pressure level reached after the injection of nalorphine.

By means of this method, similar experiments were performed by injecting drug solutions into the aquaeductus, 3rd and lateral ventricles. In all cases solutions were injected without difficulty and the records of the intraventricular pressure proved that the injected drug reached the desired anatonical site.

Summary. A precise method for the injection of drugs into the cerebroventricular system of small animals used for experimentation is described. This method, based on the intraventricular pressure record, gives absolute certainty for the injection of a drug into a previously selected site and thus avoids the subsequent histological studies.

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