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A TECHNIQUE FOR QUANTITATIVE MEASUREMENT OF CLONIC CONVULSIONS IN RATS

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Abstract : A capacitance sensor which detects vibrations caused by the movements of animals can be used for measuring automatically the clonic convulsions induced by chemical convulsants. This knowledge has been utilized to devise an instrument which has satisfactorily measured the clonic convulsions induced by picrotoxin in rats.

Key words : picrotoxin capacitance sensors clonic convulsions rats

INTRODUCTION

The convulsive agents, picrotoxin and pentylenetetrazol are known to produce a sequence of myoclonic jerks, clonic convulsions and tonic seizures (extension of both fore- and hind-limbs) which invariably resulted in death. The convulsive responses were generally evaluated visually. The latency to the onset of the components of seizure activities was determined by measuring the time elapsed from the time of injection of the convulsant to the appearance of the first clonic movement, tonus and death. The number of animals in each group exhibiting clonus, tonus and death during the test period were also observed (1,2,3). Since an assessment of the clonic convulsive movements was required in anticonvulsant drug testing procedure, an approach to measure the severity of clonic convulsions was made by an arbitrary 0-4 scale scoring method (4,5). This method required a constant vigil of the observer on the animal throughout the test period which invariably lasted for about 60 min for both picrotoxin and pentylenetetrazol-induced convulsions in rats. A Varimex activity meter (Columbus Instrument Co., Columbus, U.S.A.) which measured only the gross movements of rats was used for an automatic recording of clonic convulsions (6). A much more sensitive method of measuring the violent as well as the fine movements of convulsing animals can be achieved by monitoring automatically the vibrations caused by their convulsive movements. Using this technique, an instrument was devised by the authors and it was tested by measuring picrotoxin-induced clonic convulsion in rats. The results were compared with the scoring data obtained from similarly treated animals. The description of the instrument, its advantages over the visual scoring method and its sensitivity to measure fine clonic movements of the rat have been illustrated here.

METHODS

The apparatus consisted of a transparent acrylic cabinet measuring 20 × 20 × 20 cm. The side walls were perforated for ventilation. The lid was provided with hinges to open the chamber for placing the animal.

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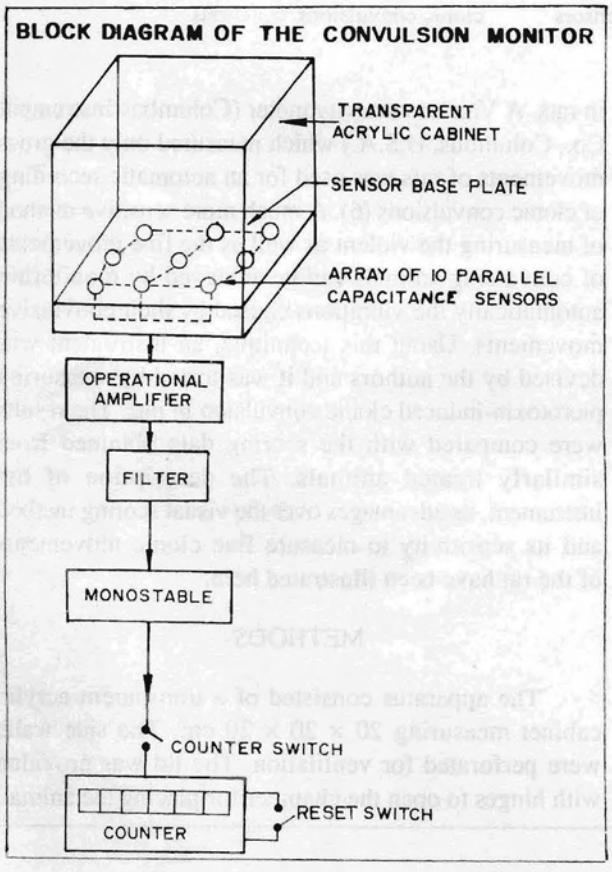
The floor was a laminated base-plate. Ten capacitance sensors were mounted parallely, as shown in the diagram beneath the base-plate. Each sensor was made of two metallised polyester films with air as dia-electric medium. Vibrations produced by the animal movement on the base-plate, caused the effective capacitance of the sensors to change. This change in the capacitance was converted to electrical voltage signals using an Operational Amplifier. The Amplified signals were processed by a Filter Circuit to eliminate unwanted disturbances from external radio-frequency interference and supply mains voltage variations. They were then converted into pulses of uniform width and amplitude by the Monostable Circuit. These pulses activated the Counter which recorded the vibration signals, adding one digit for every active pulse received. The maximum counting frequency of the counter was 60 counts per second. It was a 6 digit counter with a reset facility.

Colony bred adult Wistar male rats (120-150 g) were used for the convulsion test. The animals were divided randomly into 8 groups (n=10), housed in polypropylene cages (5 in each) in room temperature (30-32°C) and were allowed free access to a balanced diet (Gold Mohur) and tap water. They were acclimatized to the laboratory condition for 3 days. The tests were carried out between 10.30 and 12.00 h under the same light and temperature conditions as the housing.



A graded doses (3, 4.5 and 6 mg/kg) of picrotoxin (Sigma, U.S.A., dissolved in pyrogen-free distilled water) were injected intraperitoneally 15 min after pretreating 3 groups of animals with distilled water. The fourth group received 6 mg/kg of picrotoxin 15 min after an anticonvulsant dose (0.5 mg/kg, i.p.) of diazepam (Calmpose). After picrotoxin injection, the rat was placed in the Convulsion Monitor chamber and the latency to the appearance of the first clonic movement was measured using a stop watch. A sudden twitching of head or jerky movement of body indicated the onset of clonic convulsions. The instrument was activated immediately and the frequency of the clonic movements was recorded till normal activity was restored. Clonic convulsions occurred intermittently. Therefore, the instrument was switched off when convulsions disappeared; and it was operated immediately after the clonic movement recurred. Thus the spontaneous motor activity of the animal to which the instrument was sensitive, was eliminated. The counter was not reset to 0 during the test. The counting was allowed to accumulate till the end of the test period (60 min). The counts were noted down at 15 min interval (15, 30, 45 and 60 min) after picrotoxin injection. The number of animals exhibiting tonus and the occurrence of mortality during the test period were recorded in each group.

The other 4 groups were treated similarly and the clonus latency, tonus and mortality were observed as done in the previous group. The clonic convulsions of these animals were scored using a previously described 0-4 scale scoring method (5); No jerking = 0, weak occasional jerking of head = 1, mild jerking of head and forelimbs = 2, pronounced jerking of head and forelimbs = 3 and a short period of clonic convuslions involving whole body = 4. The convulsive episodes that occurred during every 15 min interval were scored and the average was determined for statistical analysis. The observer (scorer) was unaware of the groupings.

The time and counting data were analysed by Student's t-test. Mann-Whitney rank order test was used to analyze scoring data. Tonus and mortality data were analyzed using Chi-square method.

RESULTS AND DISCUSSION

A dose-dependent increase in the convulsive effect of picrotoxin was evident from the convulsion latency, tonus and mortality data. A corresponding increase was found in the convulsion countings which indicated the frequency of convulsions and in the scoring data which indicated the severity of convulsive movements. The data of both the methods clearly showed that the clonic convulsions were mild in the begining (0-15 min), increased to a maximum between 15 and 30 min and diminished thereafter. All parameters tested here demonstrated the anticonvulsant effect of diazepam (Table I).

TABLE I : Picrotoxin-induced convulsions in rats.

	Pre-treatment	*Dose of picro-toxin (mg/kg)*	*Clonic convul-sion latency (min)*	*Convulsion countings (min after picrotoxin)*				*Number exhibiting*	
				15	*30*	*45*	*60*	*Tonus*	*Death*
A.	Distilled water	3.0	8.4 ±0.8	37 ±6	107 ±17 (189)	132 ±27 (23)	132 ±27 (0)	0	0
	Distilled water	4.5	7.2 ±0.6	65 ±12	225 ±35 (246)	308 ±28 (36)	315 ±38 (2)	2	2
	Distilled water	6.0	6.0 ±0.4	105 ±15	385 ±36 (266)	461 ±42 (19)	471 ±45 (2)	6	6
	Diazepam (0.5 mg/kg)	6.0	11.1* ±1.2	52** ±8	146** ±14	195** ±13	195** ±13	0+	0+
				Convulsion scorings (min after picrotoxin))					
				0-15	15-30	30-45	45-60		
B.	Distilled water	3.0	9.2 ±0.8	0.62 ±0.04	1.26 ±0.12	0.84 ±0.06	0.0	0	0
	Distilled water	4.5	7.8 ±0.6	1.82 ±0.09	2.48 ±0.12	2.08 ±0.12	0.62 ±0.05	3	3
	Distilled water	6.0	6.4 ±0.4	2.42 ±0.24	3.82 ±0.26	3.12 ±0.14	1.82 ±0.12	6	5
	Diazepam (0.5 mg/kg)	6.0	12.2* ±1.4	1.84" ±0.08	2.42"" ±0.35	1.22"" ±0.08	0.0"	0+	0+

Each group (n=10) received picrotoxin intraperitonealy 15 min after distilled water or diazepam (0.5 mg/kg, i.p.). The counting and scoring data are mean ±SEM of animals surviving at the appropriate time. The percent difference from that measured 15 min previously is shown in parenthesis. Data of diazepam groups were compared with that of distilled water pretreated 6.0 mg/kg picrotoxin group.

*P < 0.05, **P < 0.01 (Student's t-test)

"P < 0.05, ""P < 0.01 (Mann-Whitney rank order test)

+P < 0.05, (Chi-square method).

Both the methods measured indistinguishably the sequence of the clonic convulsive effect of picrotoxin. However, a difference in the accuracy and other advantages between an automatic and an arbitrary method cannot be ruled out. In the former, the animals are to be watched only for the appearance of the jerky movements during the test period and the recording is done automatically; whereas in the latter, the occurrence as well as the severity of the clonic convulsions have to be watched and the latter has to be scored. Thus, it is fully depended on human participation. In addition, bias is likely to occur in scoring procedure even if the scorer is unaware of the groupings, since convulsive movements of all animals do not always adhere to the pattern described in the scoring method. Human error can be completely eliminated if a convulsion monitor is used. Since the frequency of convulsions is recorded throughout the test period, it helps in assessing the time course of the convulsive and anticonvulsive potential of test compounds. Thus the convulsion monitor described here is a valuable instrument in anticonvulsant drug testing studies where a quantitative measurement of clonic convulsions is required.

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