increase in 5-HT levels in mouse brain, reaching 188% of normal levels 45 min after an i.p. injection of pargyline.

The administration of methysergide to mice (Figure 3) resulted in a prolonged hyperthermia which followed the injection artifact. This hyperthermia did not, however, reach statistical significance. On the other hand, when methysergide was injected intraventricularly concomitantly with 5-HT (25 nmoles) it significantly attenuated the hypothermia produced by 5-HT (Figure 3). The block of the hypothermic response to 5-HT by methysergide, which has been shown to be a 5-HT receptor blocker in the CNS[13], again indicates that the 5-HT induced hypothermia is probably a direct effect of this amine on central serotonergic receptors. The data obtained with methysergide would also weaken the argument that the effects of intraventricularly injected 5-HT would be mediated by a displacement of other amines (e.g., catecholamines) from storage sites. The possibility remains that the location of various receptors for 5-HT may play a primary role in determining whether the response to intracerebral administration of 5-HT is hypo- or hyperthermia. Several studies[14,15] have indicated that 5-HT injected into cerebral ventricles, reaches only a limited distribution in tissue surrounding the ventricular system in brain. Although most of the 5-HT sensitive thermoregulatory areas have been found to reside in the area of the third ventricle the diffusion gradient produced after intraventricular administration of 5-HT may produce results differing from those produced by instilling 5-HT directly into particular areas of the hypothalamus.

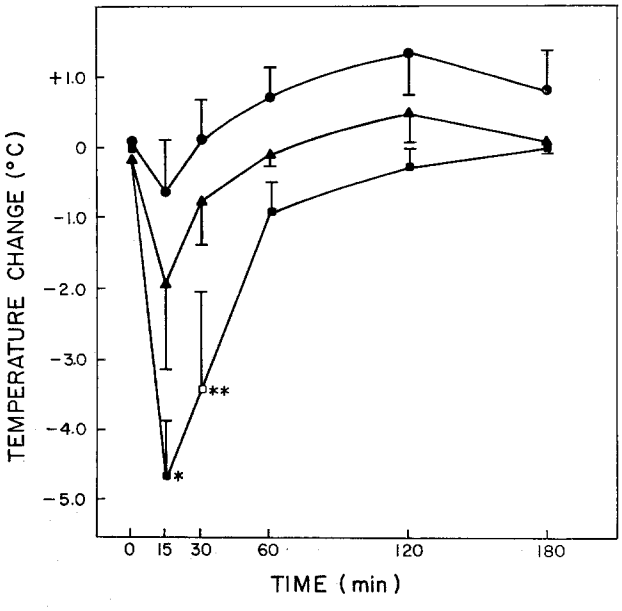

Fig. 3. Methysergide, 20 nmoles (n = 4, ●—●), or serotonin, 25 nmoles(n = 6, ■—■), or a solution containing both methysergide and serotonin at these same dose levels (n = ▲—▲) in artificial CSF, were injected intraventricularly in mice. Rectal temperature was recorded just prior to injection and at various times thereafter and results are expressed as change from preinjection temperature ± SD. **$p < 0.001$; *$p < 0.05$ as compared to animals receiving serotonin and methysergide.

The recent explanations[16,17] that changes in body temperature, after administration of drugs, are due to a block in the production of 5-HT metabolites in the CNS should, however, be reconsidered. The function of such metabolites (e.g., 5-HTOL) was postulated to be production of hypothermia. Our results do not support such a postulate, and indicate that these metabolites of 5-HT have no effect on body temperature even when administered into the CNS in doses well above the levels normally found in brain. Previous data[8] showing a hypothermic response to 5-hydroxytryptophol in doses of 0.8 or 1.2 g/kg may have been an expression of a more general phenomenon of alcohol induced lowering of body temperature[18]. Hydroxytryptophol, may have also been oxidized to 5-hydroxyindoleacetaldehyde which could have indirectly influenced body temperature. Our recent studies have indicated that this aldehyde inhibits brain ATPase activity[19].

Our current data strongly supports the direct involvement of 5-HT in mediating the temperature response produced after administration of this neurotransmitter into the CNS and indicates the presence of receptors for 5-HT in brain which, when activated, produce hypothermia in the mouse.

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Paroxysmal Discharges in the Electroencephalogram of the El Mouse

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Summary. The spike discharges in the EEG of the El mouse, a seizure-susceptible strain, were recorded during convulsive seizures. This fact provides evidence that those seizures are really epileptic convulsions.

Since an El mouse was found and developed by IMAIZUMI et al.[2,3], its seizure has been regarded as epileptiform and it has appeared to be a suitable model of a human genuine or hereditary epilepsy. Now it is available as a genetically pure strain; all the mice of this strain display epileptiform seizures, so far as the proper care is taken[2,4]. Whereas some neurochemical studies were carried out[4], neurophysiological efforts were not successful. There has been no evidence that an El mouse might be really epileptic, namely that it might have the disorders of paroxysmal cerebral dysrhythmia[5]. Recently, I was able to record the paroxysmal discharge patterns in the electro-

encephalogram (EEG) during seizures and inter-ictal periods of the animals. Accordingly, I can definitely establish that an El mouse is epileptic.

Material and methods. 32 adult mice were used. All of them were of F 56-58 generation and susceptible to seizures of tonic and clonic convulsions, running fits or other manifestations. After the ordinary methods developed by IMAIZUMI et al.[2,4], stimulations were given once a week from 3 weeks of age. The procedures of the stimulation were as follows: a mouse was taken out of the home cage and placed on the metal mesh of the cage and observed for 3 min, and then it was thrown up in the air about 15 cm, 30 times. In most cases, the mouse exhibited the first seizure at 7 or 8 weeks of age and became more susceptible to the seizures with fewer throwing-up procedures. In some litermates of the mice, seizures occurred during the period of the observation on the mesh after several weeks of stimulations.

The EEG was recorded through the thin stainless steel screws implanted in the calvarium as shown in the Figure. I carefully avoided inserting the screws into the cortex or deep structures, so that the brain might not be damaged. The reference electrode was fixed to the utmost caudal part of the skull. The electromyogram (EMG) and the electrocardiogram (ECG) were recorded from the neck muscles and the back respectively. These operations were carried out under ether anesthesia. Polygraphic recordings were done on the completely freely moving animals after recovery. For a technical reason, I show here the records of the seizures during the observation on the mesh without the procedure of throwing up.

Results and discussion. On arousal or sleep, no remarkable difference in the background activities was recognized between an El mouse and a control ddN one. The seizure presented in the Figure was typical both in clinical features and in the EEG. Until several prodromal clinical signs[2,4] of the seizure began, in the right central and slightly later in the contralateral occipital region, small negative monophasic spike discharges appeared at first. Soon they increased in amplitude and duration. The frequencies of the spikes were irregular at first and became regular at 1 Hz (Figure A and B). This prodromal stage continued for 62 sec.

Suddenly convulsions started with tonic flexions of limbs and the upright tail for a very short time. Soon after that, remarkable clonic dorso-ventral convulsions of the head followed and in the EEG very fat but smaller spikes occurred as bursts and spread to the frontal region,

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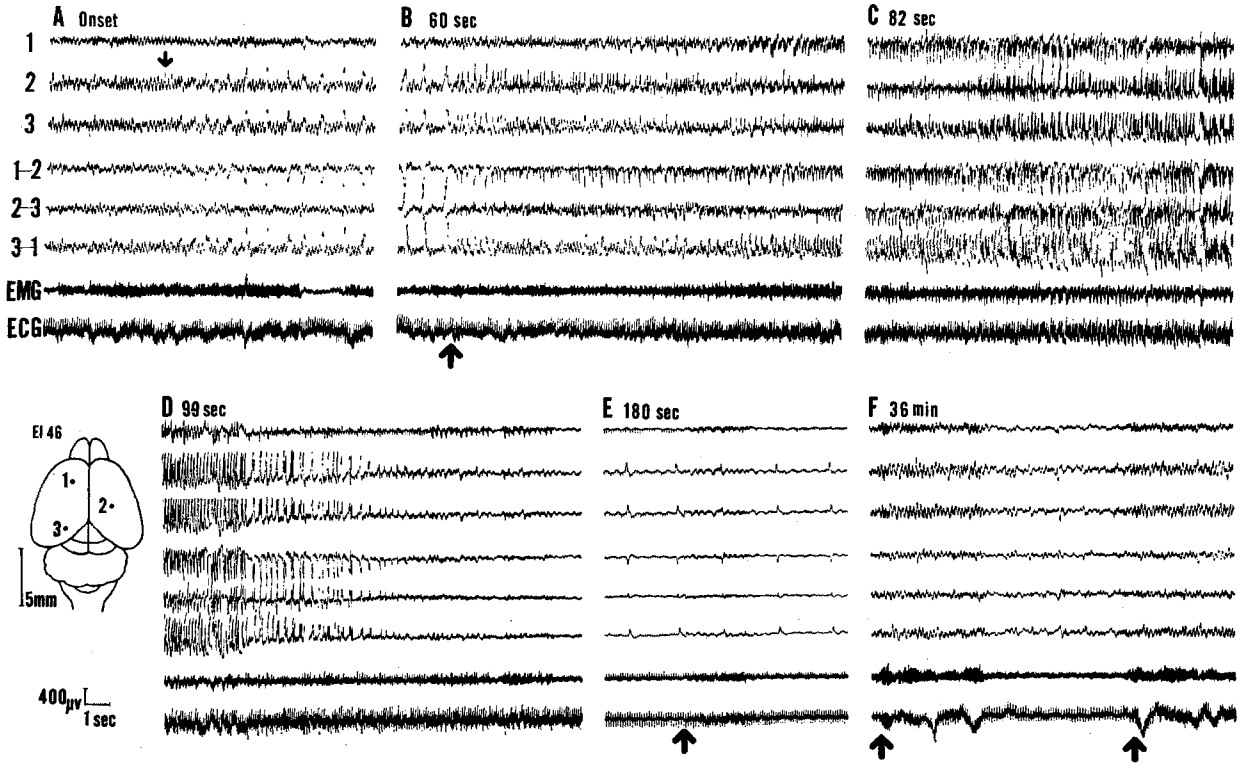

Polygraphic records of a seizure of an El mouse (El 46). In each column, upper 3 traces show monopolar EEG and middle 3 bipolar ones. The numbers of time represent time lapse after the onset of the seizure. A) Onset of the seizure and the spikes in the central region (2) (arrow). B) Clonic stage occurred (arrow) and continued for about 20 sec. Note the short but remarkable delay in the phase between the lead 2–3 and the lead 3–1. C) Catatonic stage began but the paroxysms reached the maximum. D) Depression of both paroxysms and the background activities followed the termination of the discharges. E) Stupor continued but adversive movements of the head to the left frequently took place (arrow). Regular continuous spiking lasted for a long time. F) Almost recovered to normal arousal pattern when the animal resumed grooming. Calibration: 400 μV for EEG and 200 μV for EMG and ECG.

varying in amplitude through this stage of 26 sec duration (Figure B). Successively, the spikes reached the maximum in size and a regular frequency within 15 sec, while the animal appeared catatonic (Figure C). Within 6 sec these rhythmic frequent spike discharges were retarded and depressed (Figure D). The cessation of the convulsion stage was followed by silence of all the electrical activities. After 20 sec small spikes were recognized again at the low frequency of 1 every 1–2 sec. The animal manifested stupor sometimes with a characteristic posture like a kangaroo and adversive movements of the head to the left (Figure E). Oral movements or resuming behavior were present at times simultaneously with the arousal pattern of the EEG for a brief duration (Figure F). This post-ictal stage continued for 6 min and recovery both in the EEG and behavior was seen. In the records of the El mouse, the paroxysmal discharges were mostly composed of spikes of duration within 120 msec with fewer slow waves.

In the inter-ictal period also paroxysmal discharges could be observed less frequently and in limited regions. Even during drowsiness, deep sleep or the REM sleep, the spikes occurred at the same regions with a short duration. In abortive seizures, which were only squeaking and/or an instantaneous catatonic posture and were often seen at the first few weeks after the commencement of the seizures, repetitive spike discharges occurred in same regions but were unlikely to spread and generalize.

The present paper reports for the first time the EEG of the hereditary epileptic mouse while freely moving. On the audiogenic seizure mouse, there was the EEG recording for a short duration only under anesthesia[6]. An El mouse is considered as a kind of the sensory precipitated epilepsy[7] comparable to similar types of epilepsy in man, a mouse with the audiogenic seizure[8], a baboon of photic sensitivity[9], a particular strain of a Mongolian Gerbil[10] and a domestic fowl[11], because of its susceptibility to changing position or environment. The paroxysmal discharges have a certain localized onset at the centro-parietal region of the hemisphere and spread to become general over the whole cortex and are followed by sudden depression in the El mouse. During the seizure, the features of the paroxysms are quite similar to those in a photogenic baboon, except for the focus. In the latter, the spikes started at the fronto-central region[9].

As the provoking conditions of seizures in El mice are rather complicated and obscure in nature, investigations on them should be made. So far the seizures seem likely to be caused by vestibular or proprioceptive stimulations.

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Preparation of Isolated Single Cardiac Cells from Adult Frog Atrial Tissue[1]

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Summary. Isolated cardiac cells from bullfrog atrial tissue can be readily prepared by digestion of intact fragments of atrial tissue with trypsin and collagenase. These isolated cells have dimensions of about 5 μm in width and range in length from 300 μm to over 500 μm. Such isolated cells may prove useful for the investigation of contractile activity of cardiac muscle at the single cell level and at the sarcomere level within the single cell.

The preparation of component cells from various tissues perfusion with a variety of proteolytic enzymes has been known for some time. However, the preparation of isolated cardiac cells has been primarily confined to the embryonic chick heart. Only recently have attempts been made to isolate viable cardiac cells from adult myocardial tissue and to date the majority of this work has been confined to mammalian cardiac muscle.

In 1970 VAHOUNY et al.[3] described a method for the preparation of isolated cardiac cells from adult male rats by incubation of the heart fragments in a saline solution containing trypsin and collagenase. Isolated cells obtained by this method were spontaneously active and remained active for several hours. Since then, several investigators have used VAHOUNY's technique to prepare single smooth muscle cells from the stomach muscle of *Bufo marinus*[4,5]. In the present study, we adapted the technique for the preparation of isolated cardiac cells from adult bullfrog atrial muscle.

Materials and methods. Bullfrog (*Rana catesbeiana*) atrial tissue was cleaned of as much noncardiac cell tissue as possible and then minced into coarse fragments. The fragments from one atrium were placed in 2 ml of a Ca^{++}-free Ringer's digestion solution having the following composition: 111 m*M* NaCl, 5.4 m*M* KCl, 10 m*M* *tris*

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