

The clinico-electroencephalographic  
correlate of Megimide-induced epileptic  
seizures in epilepsy prone El and its  
mother strain ddY mice.

遺伝性てんかんモデル鼠とその母系 ddY マウスにおける  
Megimide 誘発てんかん発作の臨床と脳波の相関

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## CHAPTER 1. Introduction

In order to obtain profound understanding on the function of central nervous system in relation to the epileptogenesis, the concept of focus and the propagation mechanism, appropriate animal models should be utilized.

A wide variety of experimental animal model has become available for the study of human epilepsy<sup>89</sup>. Animal models of epilepsy may be categorized into two: one in which seizures are induced in non-epileptic individuals and the other in which seizures are genetically determined in epilepsy prone individuals<sup>40,51,56,67,68</sup>.

For one of the former examples, kindling phenomena have been induced successfully in many species by electrical<sup>13,14,15,16</sup>, or chemical<sup>2,11</sup> stimulation. For the latter, epileptic phenomena similar to those of humans have been observed in a mutant strain of several species, and some of them have been successfully maintained inbred with the same phenotype.

Whatever the type of experimental model is, "how are we to judge the validity of a given experimental model for the study of human epilepsy?" Jasper<sup>31</sup> asked and gave a definite answer, after noting the importance of similarity in both electrical and behavioral manifestations to those known to characterize human epilepsy, that "one must depend more upon electrical manifestations than upon clinical or behavioral manifestations of epilepsy in judging the validity of a given model of epilepsy". In this context, Jasper's concern apparently resides in the fundamental understanding of epileptogenesis



and the propagation of discharge in human epilepsy as well as in animal models.

In this sense, the technical development of EEG recording in animal models has been the primary concern in the experimental epileptology. Jasper's concept has been well prevailed in many animal models with the possible high quality of EEG recordings.

However, in mice models no comparable EEG recordings have been accomplished enough to make any reference to human epilepsy. Seyfried<sup>67</sup> reviewed the genetic model of epilepsy in mouse mutants with only scanty descriptions on EEG analysis. Rarely kindling models of mouse have been reported with the precise description of EEG analysis. Nakano<sup>49</sup> reviewed the mouse EEG ( Table 1-1) which had been described in only limited reports<sup>3,6,12,20,28,34,37,41,42,53,56,62,64,65,77,78,79,85,87,88,90,96</sup> and noted the paucity and the unacceptable quality in mouse EEG.

Zornetzer<sup>96</sup> first described the detailed method of the chronic brain implantation technique for the referential derivation of epidural recording of EEG in mice. This method was modified by several authors afterwards<sup>34,37,41,42</sup>, however, they preferentially used bipolar recordings. Although there have appeared several atlases of mouse brain after 1970<sup>39,71,72</sup>, there have been no reports of intracerebral recording of EEG in mice with the precise coordination of stereotaxic guidance<sup>28,64</sup>. In this regard, Zornetzer had already pointed out the morphological variation of brain structures among different genetic strains of mice as well as the small size of deep brain structures.

The El mouse<sup>35</sup> came in my view for the research of epilepsy, since this strain was found and established as a well known epilepsy model in Japan<sup>26,27</sup>. I have observed carefully the seizures presented by this animal and referred to the published documents concerning the

electrophysiological studies. And I have noted two points which made me initiate the present research: some clinical manifestations have not been described completely in the prior documents and the ictal EEG recordings reported only in a limited articles <sup>28,77,78,79</sup> did not explain correctly the clinico-electroencephalographic correlates. I finally concluded that the determination of seizure type with the evidence of clinico- electroencephalographic correlates should be concerned in the first place before entering into the more detailed neurophysiological studies.

Therefore, the research had to be oriented to the development of the method for EEG recording with a view to the description of electroencephalographic correlates of the genuine ictal manifestations in El mouse.

This thesis, accordingly, begins with the description of the technical development for EEG recording in El and ddY mouse: Chapter 2 for the referential derivation of epidural EEG in restrained El mouse and Chapter 3 for the method of the implantation of electrode system for the EEG recording in freely moving El and ddY mice. In Chapter 4, this method is then applied to the overt manifestation of pharmaceutically ( Megimide- ) induced epileptic seizures in El and ddY mice. With the EEG thus obtained as well as with the clinical observations, the ictal clinico-electroencephalographic correlate will be described in two strains of mouse. The evidences will be presented to support the advantage of the present recording method for further application in the electrophysiological research in El mouse, of which the principal study should be about the genuine epileptic seizures.

Table 1-1. A review on the recording technique of mouse EEG

Author	Strain	Electrode	Placement	Anesthesia	Polarity (number)
Zornetzer (1970)	Swiss Webster	Silver bead Stainless screw (insulated)	Epidural Hippocampus	Ether	Mono(2)
Valatz et al. (1970) (1975)	C57,GBA (1970) Quaking(1975)	Gold-plating brass screw	External table of skull	Pentobarbital	Bip(2)
Pagel et al. (1973)	CBA,C57,DBA ,C3H, BALB, Swiss	Stainless steel	dural	Nembutal	Bip(3)
Eleftheriou et al. (1975)	DBA,C57	Stainless steel	Hippocampus	Chloralose Urethane	Mono?(2)
Maxon et al. * (1976)(1983)	C57,DBA,Rb, HAS(1976) C57(1983)	Silver bead	Cortex	Chloral hydrate	Bip(2)
Suzuki et al. (1976)(1977) (1982)	El	Stainless steel	Skull	Ether	Bip(4)
Tubone et al. (1977)	IDT	Silver bead	Epidural	?	Bip(4)
Kaplan et al. * (1979)	Tottering,C57	Silver bead	Epidural	Pentobarbital, Ether	Bip(4)
Noebels et al. (1979)(1990)	Tottering,C57 (1979) Stargazer,C57, CH3(1990)	Gold(1979) Silver wire (1990)	Epidural(1979) Subdural(1990)	Tribromoethan- ol	Bip(4)
Ryan et al. (1979)	DBA,C57,C3H	?	Intracerebral	Pentobarbital Chloral hydrate	Bip(6)
Chauvel et al. (1980)	Quaking	Silver ball	Epidural	Pentobarbital	Bip(4)

Table 1-1. ( continued )

Heller et al. (1983)	Tottering	Gold	Epidural	Tribromoethanol	Bip(4)
Sato ** (1985)	El,ddY	Stainless steel	Skull	Ether	Bip(4)
Ishida et al. (1987)	El	Shielded stainless steel	intracerebral	Ketamine Pentobarbital	Mono(6)
Kostopoulos et al. *	Tottering	Silver ball	Epidural	Chloral hydrate	Bip(4)
Krauss et al. (1989)	96CF1	25-gauge needle	Skull	?	Bip(6)
Gioanni et al. (1991)	Quaking	Silver ball (Teflon coated)	Cortex	Pentobarbital Halothane-N <sub>2</sub> O	Bip(2)

\* Modification of Zornetzer

\*\* Modification of Suzuki

## CHAPTER 2. The referential derivation of epidural EEG in restrained El mice<sup>48</sup>.

### Introduction

Several placements of the exploring electrode have been used for the extra-cerebral recording of EEG in animal models: scalp, subcutaneous, intraosseous, epidural and cortical. The epidural derivation has been preferentially employed in previously reported methods of EEG recording in mice ( Table 1-1 ). The same placement was employed in this experiment for it was considered preferable for the recording of eventual cortical activities that has been my first concern in view of the epileptic discharges.

All the investigators in mice EEG preferentially employed the bipolar derivation except Zornetzer<sup>96</sup> who placed the reference electrode in the nasal sinus. After several preliminary examinations, the reference electrode was placed subcutaneously near the nose. Although the positive proofs for its adequacy had not been promised at the beginning, the EEG data in restrained animals have proven its efficacy as for the discriminative ability of the localization-related eventual cortical activities.



## Materials and Methods

The EI mice used in this experiment were donated at 104th filial generation by Dr. Asano ( National Institute of Health, Tokyo ) and have been maintained by sister-brother matings since 1991 in the department of veterinary physiology, Nippon Veterinary and Animal Science University, Tokyo, Japan. The mice have been kept in a conventional animal room at 23-26°C in 60% of humidity under 12L12D lightening conditions ( on 6:00 and off 18:00 ). The animals fed commercial diet ( CE-2; Japan CLEA Co., Ltd., Tokyo ) and water ad libitum. The growth curve of 24 EI mice at 105th generation is demonstrated in Fig. 2-1. The mice used in the following experiments in Chapter 3 and 4 were of the same origin.

Twenty EI mice ( 10 males and 10 females ), 6 to 10 weeks of age, weighing 23-27g were used in the experiments. All the animals had been confirmed to be seizure prone secondary to passive movements such as transport from one cage to another. The cumulative incidence of epileptic seizure at 105th generation is presented in Fig. 2-2.

Silver wire of 0.5mm in diameter was melted in a gas flame to form ball electrodes which were connected with soft silicone coated 1.5mm-diameter copper cables. All of the silver ball electrode and the naked part of the cable were coated with epoxy resin ( 2086; Three bond Co., Ltd., Tokyo ) to make an approximate diameter of 1mm at the tip, which was placed in an incubator at 37°C for 1 day to complete the chemical reaction. The ball tips were denuded under microscope by 0.1mm in diameter for dural attachment ( Fig. 2-3 ). A stainless steel

needle was employed for the reference and another for the ground electrode.

Surgical procedures were carried out under Ketamine ( 40mg/kg, i.m. ) and Xylazine ( 18mg/kg, i.m. ) anesthesia. The animals were fixed on a dry wooden table in a prone position. Frontal and parietal bone were exposed and four burr holes of 1mm in diameter, two anterior to the coronal suture and two anterior to the lambdoid suture, were made with an electric drill. The care was needed to avoid damaging to the dura mater. Bleeding in the burr hole was easily controlled with cotton tampon unless the burr hole was placed too far anteriorly near the olfactory cortex. Electrodes were placed and attached to the dura mater in the burr holes and fixed with plastic glue ( GEL-10; Toa Gosei Kagaku Co., Ltd., Tokyo ). A stainless steel needle electrode was placed subcutaneously near the nose and a ground electrode near the rump. Fig. 2-4 shows a schematic illustration of the placement of the electrodes and the burr hole locations. The terms: frontal and occipital, were momentarily employed, although its precise topography is not determined. The same terms will be used in the following chapters.

All the operative procedure was undertaken according to the NIH guide for the care and use of laboratory animals ( the same discipline in the following experiments in Chapter 3 and Chapter 4 ).

EEG was recorded for one hour under the bandpass settings: TC=0.1sec, High cut=120Hz ( the recording apparatus: 1A72; NEC SAN-EI Co., Ltd., Tokyo ). ECG was recorded with the same bandpass settings and EMG of the proximal part of forelimb with TC=0.03sec, High cut=1500Hz.

## Results

Fig. 2-5 represents an example of EEG 30 minutes after Ketamine and Xylazine administration. Referential recordings show small spikes on the left frontal recording which precede the multiple spike complex rapidly synchronizing on both frontal recordings.

Eight animals out of 20 presented multiple spike complexes. Multiple spike complex synchronizing on both frontal lobes was observed in 8 mice. Among them, small spikes preceding the multiple spike complex was also observed on the left frontal lead in 7 mice and on the right in a mouse.

Occipital recordings were not affected by spikes and multiple spike complex, except positive deflections ( Fig. 2-5 \*\* ) seemingly due to an active reference electrode secondary to these paroxysms.

Fig. 2-6 shows that referential recordings are free from the spread of ECG and EMG.

In the present experiment, EEG was recorded in good quality. Although not all the animals had similar spikes, the analysis of the pharmaceutical effects on the EEG manifestation is beyond the scope of this thesis.

## Discussion

My initial concern has been about the possibility of referential derivation of EEG. Several preliminary tests excluded the noncephalic reference electrode due to the inevitable ECG contamination.

Suzuki <sup>77,78</sup> placed a stainless steel reference in the posterior part of the skull presumably because the cerebellum seems to be the least electrically vulnerable part of the epileptic brain. It is apparent that the nasal reference electrode is not an ideal one. Suzuki <sup>78</sup> pointed out the disadvantage of this placement for the contamination of respiratory movement, while Valatz <sup>87,88</sup> employed the placement profitably for its recording. It is so near the olfactory cortices that the nasal reference might receive occasional activities from them.

The referential derivation had to clear the two key points to have the proofs for the monopolar recording: 1) the coated electrodes must cut off the short circuit maximizing the impedance between reference and exploring electrodes and 2) the reference must be the least vulnerable to artifact contamination. As shown in Fig. 2-5, the exploring electrode recorded the synchronous spikes on the frontal derivations and the preceding isolated spikes of the left frontal derivation: the discriminating ability of the localization-related cortical discharges. It is concluded that the referential derivation played a role of the monopolar recording in this preliminary experiment.

The frontal spike discharges in Fig. 2-5 might spread to the reference electrode resulting in the small positive deflection on the

occipital derivations. I thought the spread was due to the active reference electrode inserted far behind near the anterior part of the brain and this kind contamination would be avoided with the reference electrode placed more anteriorly.

Dural attachment of 0.1 mm in diameter may correspond to less than 2 mm for the human being provided the length were proportionate to the cube root of the weight: the average weight of the brain being 1500g in human being and 0.39g in El, 0.46g in ddY mice. This scale is less than the diameter of electrodes commonly used for electrocorticogram for epilepsy surgery ( Ad-Tech Co., Ltd., U.S.A. ). The dura mater of a mouse is much thinner than that of human being, therefore, the epidural recording of mouse EEG under the conditions reported here comes much nearer to the electrocorticogram.

As shown in Fig. 2-6, the EEG recorded by this method was not influenced by ECG and EMG from the forelimb. This is definitely due to the successful blockade of the short circuit between electrodes and surrounding structures.



Fig. 2-1. Graph of current (24.8) versus 1025 (microamp).



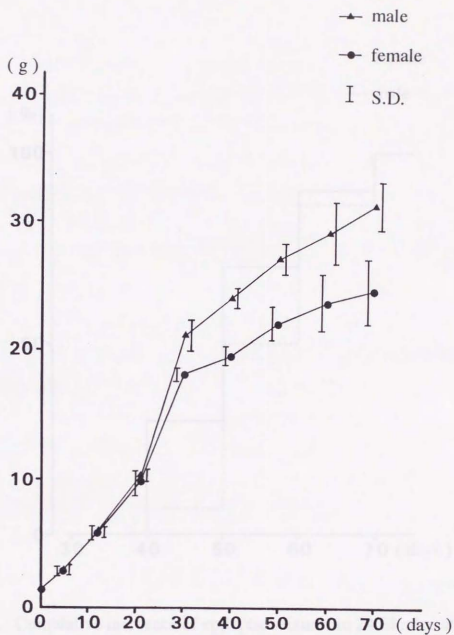


Fig. 2-1. Growth curve of 24 El mice at 105th generation.

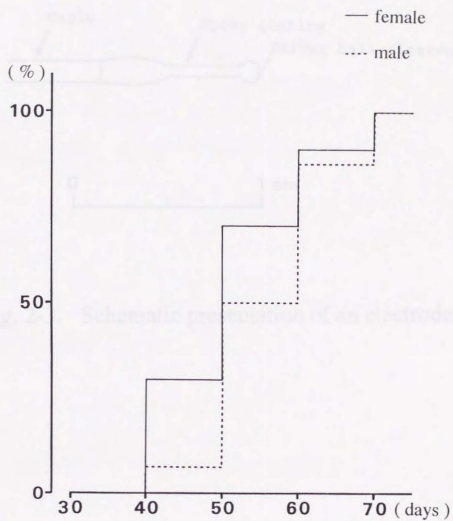


Fig. 2-2. Cumulative incidence of epileptic seizures in 24 El mice at 105th generation.

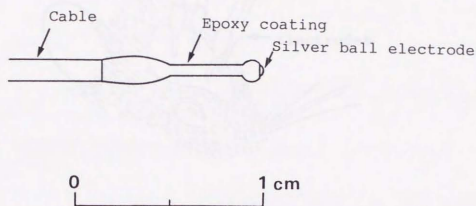


Fig. 2-3. Schematic presentation of an electrode.

Fig. 2-4. Upper: Schematic presentation of the placement of four electrodes in the skull of H. mause. Lower: Burr hole placement in the skull. R: right; L: left; F: frontal; O: occipital.

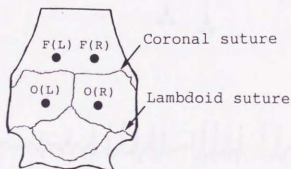
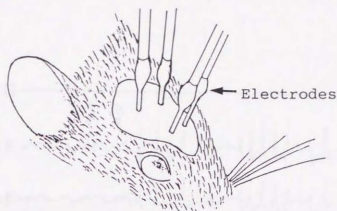


Fig. 2-4. Upper: Schematic presentation of the placement of four electrodes in the skull of El mouse. Lower: burr hole placement in the skull. R: right; L: left; F: frontal; O: occipital.

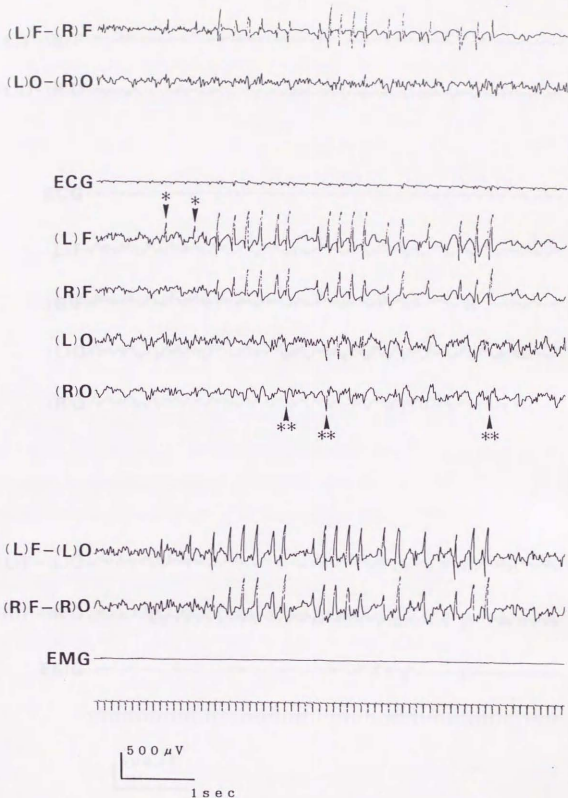


Fig. 2-5. An example of epidural EEG, ECG and EMG 30 minutes after Ketamine and Xylazine anesthesia. Note that small spikes(\*) precede multiple spike complex on both frontal recordings. Positive deflection on occipital recording(\*\*) may reflect an active reference electrode. R: right; L: left; F: frontal; O: occipital ( the same in Fig. 2-6 ).



(L)F-(R)F

(L)O-(R)O

ECG

(L)F

(R)F

(L)O

(R)O

(L)F-(L)O

(R)F-(R)O

EMG

|||||

500  $\mu$ V  
1 sec

Fig. 2-6. An example of epidural EEG, ECG and EMG 50 minutes after Ketamine and Xylazine anesthesia. The EMG was recorded in the left triceps muscle. The referential recording is free from the spread of EMG and ECG.

CHAPTER 3. The implantation technique for 4-channel epidural EEG monitoring in freely moving mice with a nasal subcutaneous reference and a cassette connector on the back<sup>49</sup>.

### Introduction

In the previous chapter, the referential derivation of epidural EEG was described with some proofs for the ability in topographical discrimination of spike discharges. In this chapter, the development for the method of implantation of electrodes is described for the EEG recording in freely moving El and ddY mice. Several difficulties encountered during the technical development had to be overcome. The method is, however, presented in a final complete form which could furnish the EEG recordings with acceptable quality for the description.

## Materials and Methods

### Mice

Six male ddY and 10 male El mice were used in this experiment.

All ddY mice aged 10 ( 4 mice ) and 11 ( 2 mice ) weeks, weighing approximately 30g were purchased from Saitama Experimental Animal Supplier Co., Ltd. ( Saitama, Japan ). El mice, weighing approximately 30g, were aged 14.5 weeks in average.

### Electrodes and cassette connector

In total, 8 electrodes were used; 4 for epidural EEG, 1 for reference, 1 for ground and 2 for EMG.

A silver ball electrode, 1mm in diameter, coated with epoxy resin ( 2082; Three Bond Co., Ltd., Tokyo ) and denuded by 0.1mm in diameter at the tip was used for dural attachment ( Fig. 3-1 ). The electrode was 5mm in length and bent at 1mm from the tip at right angle for stable placement in burr holes. Electrodes for reference, ground and EMG were non-coated 5mm length silver wires.

Eight polyurethane coated copper wires of 40 $\mu$ m in diameter ( Showa Electric Wire & Cable Co., Ltd., Tokyo ) were twisted, coated totally with silicone rubber ( Cemedine Co., Ltd., Tokyo ) as thin as possible and soldered to each electrode. These twisted wires for epidural electrodes and a reference were placed in two silicone tubes of 0.5mm in diameter; one for left side electrodes and the other

for right side electrodes with a reference electrode. The twisted wires for ground and EMG were also placed in silicone tubes.

A double row pin header with 8 pins, 10x5x6 in height x width x length, ( DF11-8DP-2DS; Hirose Electric Co., Ltd., Tokyo ) was used for a receptable connector ( schematically presented in Fig. 3-2, lower: A ). It was attached to a tetoron mesh of 8mm in length and 9mm in width ( Chiba Medical Co., Ltd., Tokyo ) infiltrated with silicone rubber ( Fig. 3-2, lower: B ). The twisted cables had been soldered underneath the mesh. Fig. 3-2 ( upper ) illustrates a complete ensemble of electrodes with the cassette connector ( in the photo, all cables are presented naked: without silicone tubes). Total weight of the implanted materials was about 0.8g. Currency test was done between all electrodes and output pin of the cassette prior to implantation. The ensemble was soaked in alcohol for about 30 minutes prior to use.

#### Implantation

After atropinization, the animals were anesthetized with Ketamine ( 40mg/kg, i.m. ) and Xylazine ( 18mg/kg, i.m. ). The animal's head and back were shaved and sterilized with alcohol. The animals were placed on the wooden operative table in a prone position. Midline skin incisions, 10mm on the head and 15mm on the back, were made and subcutaneous tissue was bluntly separated. The periosteum is scraped as widely as possible on the skull. Naked part of the skull was pretreated with Phosphoric acid ( Super-Bond C & B; Sun Medical Co., Ltd., Kyoto, Japan ) for 30 seconds to reinforce the osseous integration of the plastic glue.

Four burr holes ( Fig. 2-4, lower ), each 1mm in diameter, were symmetrically made at both anterior to the coronal and the lambdoid sutures, with an electric drill under stereomicroscope. Dural damage was carefully avoided. Hemorrhage in the burr holes was easily controlled with cotton tampon, unless the burr holes were made near the olfactory cortices where the fatal bleeding would occasionally occur.

The electrode was placed carefully to assure the mild contact at the tip with the dura mater and fixed to the skull by plastic glue ( GEL-10; Toa Gosei Kagaku, Co., Ltd., Tokyo ). A reference electrode was placed subcutaneously near the nose. A ground electrode was placed under the skin near the tail and bipolar electrodes for EMG were placed on the nuchal muscles except an El mouse in which bipolar electrodes for EMG were inserted in the right temporal muscle to verify occasional spike-like activities ( Fig. 3-7 ).

Before skin closure, EEG was monitored. There was occasional ECG contamination due to transient exudate or small hemorrhage around the electrodes. Thereafter, the animals were supplemented with 1ml of 5% glucose in physiological saline intraperitoneally. The skin was closed on the head and the receptable connector was sutured on four sides of the tetoron mesh with the skin. All the operative procedure required 20 to 40 minutes. The animals were kept warm for about 30 minutes and returned to the home cage. Fig. 3-3 demonstrates an El mouse implanted with the electrode system ( the plug connector of the connecting cable A is attached to the receptable connector ).



### Connecting cable

Between terminals of the plug connector and a joint box ( not presented ), a cable ( Fig. 3-3, A ) consisted of 8 twisted polyurethane coated copper wires of  $40\mu\text{m}$  in diameter was used. Eight cables from each terminal were placed together in a silicone tube of 1.5mm in diameter and 15cm in length. Solid attachment of the silicone tube to the plug connector was accomplished with epoxy resin. This assembly of cables was effective to prevent high amplitude spike-like artifact due to impedance mismatch between the cables. Furthermore, the cable had sufficient elasticity, so that the animals could move freely. The hand made joint box ( not presented ) and the preamplifier of the recording apparatus were connected by commercial flexible leads.

### Recording of EEG

EEG, ECG and EMG were recorded with the same bandpass setting and with the same apparatus as those described in Chapter 2. EEG was recorded from 4 days after operation up to 33 days. After several minutes of recording under the freely moving status, Megimide ( 15mg/kg ) was injected intraperitoneally in all mice to induce epileptic seizures and ictal-postictal EEG was monitored for 30 minutes. In total, 30 ictal recordings were obtained.

In one El mouse, EEG and respiratory movement were recorded under Ether anesthesia with a piezo-electric device placed tightly on the abdomen for monitoring of abdominal pressure ( Fig. 3-6 ). The piezo-electric device was made of membranous phosphor copper and barium titanate and used as a pressure transducer.

## Results

### Postoperative course

All mice recovered from surgery and survived in good health for more than 5 weeks except for a few mice which died immediately after the Megimide-induced seizure. In about 3 weeks, the skin on the back became necrotized around the implanted cassette connector. Occasional debridement and resuture were necessary for the revision of the implantation.

### EEG recordings

All examples of EEG ( Fig. 3-4,5,6,7,8,9,10 ) are those of EI mice.

EEG of freely moving EI mouse is presented in Fig. 3-4.

Megimide-induced ictal EEG was recorded with excellent quality. One example ( Fig. 3-5 ) showed the ictal EEG of general tonic clonic convulsion following several twitching movements ( rapid backward shrinkage of the neck ). Tonic phase of convulsion was evolving very rapidly and continued for several seconds during which EMG electrodes placed on the nuchal muscles were activated according to the tonic dorsiflexion of the neck ( arrow head A ). The tonic phase was followed by the clonic phase during which the animal was in a prone posture presenting clonic movements of the limbs or twitching movements of the neck. The rhythmic spikes on EEG seemed to be associated with these clonic movements. These spikes were

evidently recorded on the occipital leads. When the clonic movements were fading away, these spikes also disappeared and the animal became immobilized and stuporous. Postictal EEG demonstrates regular fluctuations of base line reflecting respiratory movements ( arrow head B ); this was verified by the following experiment.

Respiratory movements of an immobilized El mouse under Ether anesthesia were monitored by the piezo-electric device attached to the abdomen. Fig. 3-6 shows that slow regular baseline fluctuations of EEG corresponded to the abdominal respiratory movements. Analogously, the regular fluctuation of postictal EEG ( Fig. 3-5; arrow head B ) was due to respiratory movements while the animal stayed in drowsy status.

One El mouse was implanted with EMG electrodes in the right temporal muscle to verify the spike-like activities ( about 10Hz ) very often seen in freely moving status in both ddY and El strains which did not present biting behaviors. EEG ( Fig. 3-7 ) demonstrates that these activities exactly correspond to EMG of the biting muscle. Therefore, it is concluded that the major biting muscle discharges affected the reference electrode.

The initial quality of EEG was good except for several cases in which ECG contamination was observed in selected electrode(s). It might be due to imperfect contact with the dura mater of the electrode(s) in the case of continual contamination, or due to transient exudate or hemorrhage which would disappear in several days.

From 5 or 6 weeks on, ossification in the burr holes began and disrupted the contact of the electrodes with the dura mater. In this situation, ECG was recorded on that electrode(s) with base line instability ( Fig. 3-8 ). In one case, the reference electrode was

occasionally placed on the nasal bone instead of subcutaneous placement. In this situation, all referential derivations recorded ECG as well as abrupt baseline fluctuations presumably due to the unstable contact of the reference electrode with the nasal bone ( Fig. 3-9 ). These artifacts uniquely contaminate the referential derivations.

EEG in Fig. 3-10 shows spike-like activities. However, these activities were the results of the tapping on the cables connecting the joint box and the preamplifier. Bipolar recording of EEG mimics occasional paroxysms.

## Discussion

All the authors in Table 1-1, except Krauss<sup>38</sup>, Ryan<sup>64</sup> and Tubone<sup>85</sup> who did not use the implantation technique, employed the pin plug or the connector placed on the skull for chronic recording of EEG. This method has been well prevailed for much larger animals. There have been no reports on the cassette connector placed on the back of animal like the present one. It was apparent that the placement on the back was preferable for the chronic recording because it seems to be the only part of the body where the animal can not directly touch the connector with their limbs or teeth. In the present experiments, the cassette connectors remained intact during the recording period. The augmentation of the number of electrodes

appears to be more feasible with this method comparing to the cassette connector affixed to the skull. However, this placement necessitated the development for the implanted cables between the electrodes and the cassette connector. For the habitual violent movement of the neck would easily tear the cables. To assure the elasticity as well as the endurance of the cables, a single cable was made with 8 twisted fine wires (  $40\mu\text{m}$  in diameter ), then 2 to 3 single cables were further twisted and placed in the silicone tube of 0.5mm in diameter. These materials allowed the excessive movement of the cables without being damaged during the recording period. This also made it possible to match the impedance between the cables.

The connecting cable between the plug connector and the joint box also needed the elasticity as well as the endurance. The same method was applied as described for the implanted cables. There have been no artifact derived from the cables even while the animals were moving freely. Thus it was revealed not to be necessary to add any other devices like the mercury swivel as has been employed by Maxson<sup>41,42</sup> and Noebels<sup>56</sup>. This method demonstrated the complete blockade of the impedance mismatch between the cables placed altogether in a silicone tube of 1.5mm in diameter ( Fig. 3-3, A ). This kind of mismatch would otherwise have evoked paroxysmal-like activities as shown in Fig. 3-10.

Zornetzer <sup>96</sup> placed a stainless steel screw as a reference electrode in the nasal sinus. In the present study, the reference electrode was placed subcutaneously near the nose as far away as possible from the olfactory cortices. The EEG thus obtained after the Megimide administration demonstrates the spikes on the occipital electrodes during the clonic phase of convulsion ( Fig. 3-5 ), so that the



referential derivations could topographically discriminate the EEG activities.

It was necessary to differentiate the spike-like activities very often observed in referential recordings while the animals seemed irritative but did not present biting behaviors. The EMG electrodes placed in the temporal muscle showed this kind of activities were genuine muscle discharges spreading to the reference electrode ( Fig. 3-7 ).

Several physiological reactions to the implanted materials have been observed. Ossification in the burr holes beginning from about 5 weeks after operation was inevitable. Necrosis of the skin sutured on the tetoron mesh infiltrated with silicone rubber required the revision in 2 or 3 weeks after implantation. This skin reaction, however, did not affect the EEG quality. The necrosis might rather be due to ischemic insult than due to the reaction against foreign body, for the silicone is the well prevailed material used in the field of commercial instruments which necessitate the contact with human body.

The present method has several advantages over the previously reported techniques. The long stable recording period ( 4-5 weeks ) assured the repetitive monitoring of EEG in the same individual. The cassette connector placed on the back assured the long term implantation. The coated electrode with the attachment to the dura mater at the denuded tip ( 0.1mm in diameter ) could stably record the EEG in good quality during Megimide-induced seizures.

On the other hand, it is time consuming to manufacture the small and fine materials. However, only such effortfull procedures could bring the EEG of mice in acceptable quality that had never been accomplished with the methods summarized in Table 1-1.

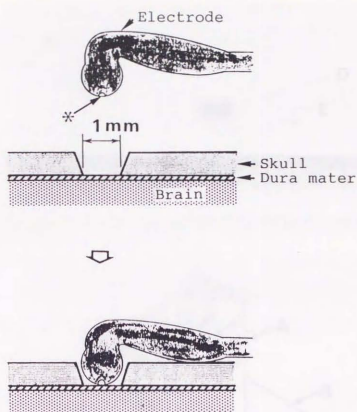


Fig. 3-1. Schematic presentation of a silver ball electrode placement on the dura mater. The electrode is coated with epoxy resin. \*: Denuded portion with 0.1 mm in diameter.

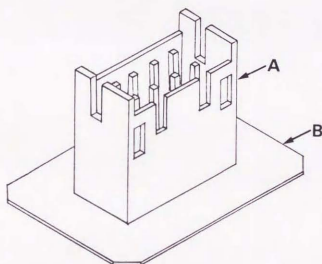
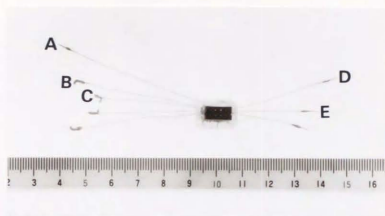


Fig. 3-2. Upper: The ensemble of the implanted electrode with the cassette connector. In this photo, cables are not placed in the silicone tubes ( see text ). The lead A is for the reference, B for frontal, C for occipital, D for the ground and E for EMG electrodes. Lower: Schematic presentation of the receptable cassette connector ( A ) and the tetoron mesh ( B ) attached under the cassette.

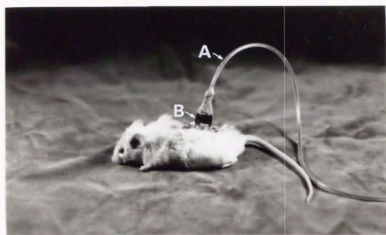


Fig. 3-3. An El mouse implanted with the ensemble of electrode and cassette connector. A is the connecting cable attached to the receptable connector ( B ).

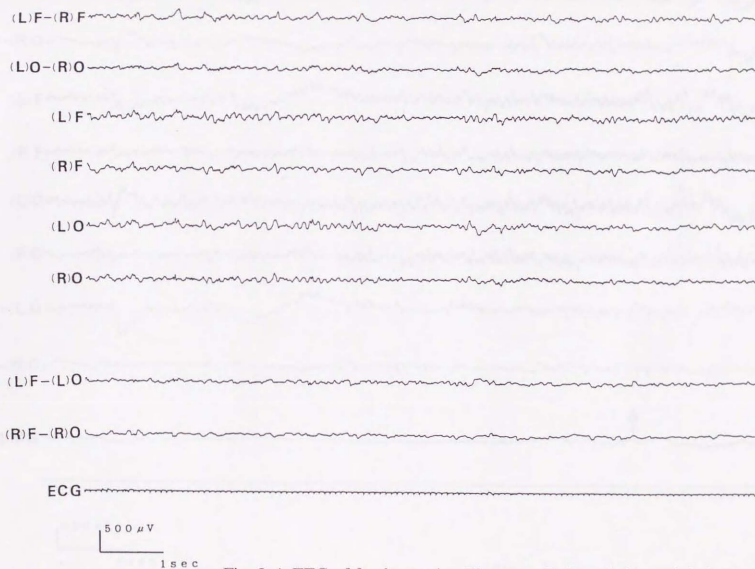


Fig. 3-4. EEG of freely moving El mouse. F: frontal; O: occipital; R: right; L: left. (L) signifies the referential derivation of the left frontal electrode. (L) F-(R) F signifies the bipolar recording between left and right frontal electrodes ( the same in the following EEG ).

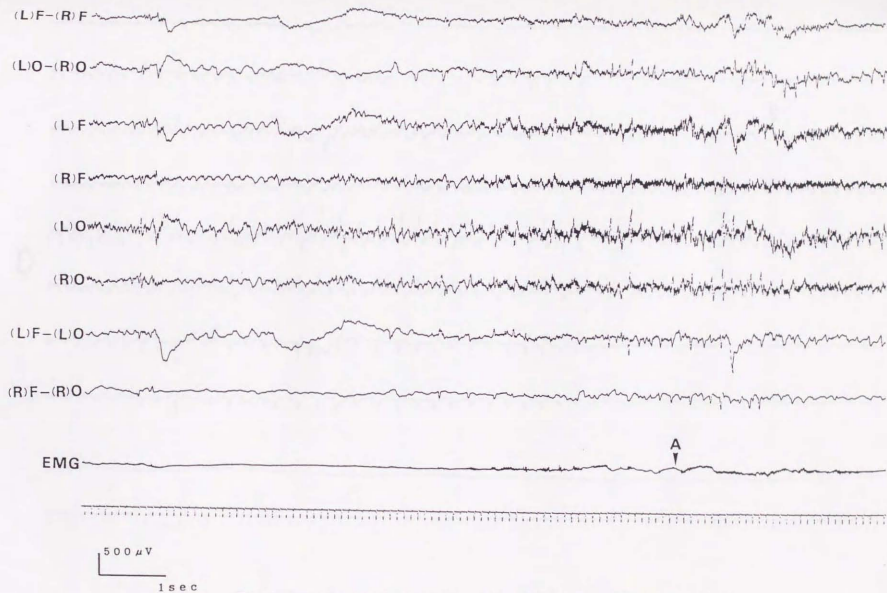


Fig. 3-5 (1). Megimide-induced ictal EEG of an EI mouse. EMG electrodes were placed on the nuchal muscles. EMG activities (A) during the tonic phase of convulsion represent the tonic dorsiflexion of the neck.



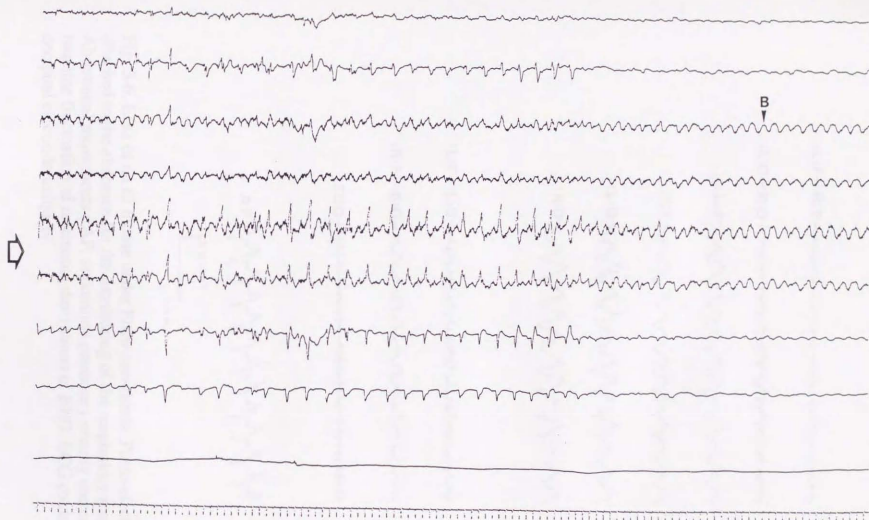


Fig. 3-5 (2: continued). Rhythmic spikes are seen on the occipital electrodes when tonic phase of convulsion progressed in the clonic phase. Rhythmic baseline fluctuations during the postictal period ( B ) are due to respiratory movements.

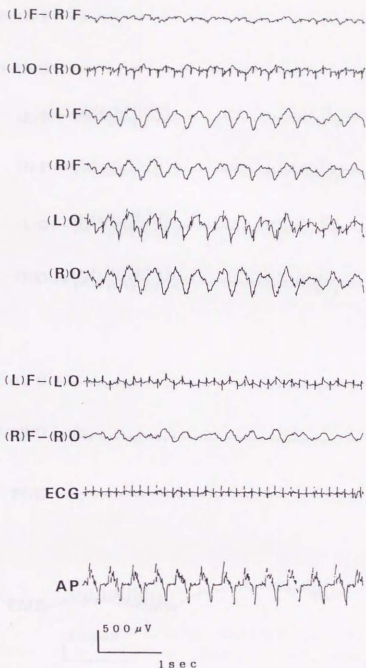


Fig. 3-6. EEG of an El mouse under Ether anesthesia. Piezo-electric device was attached to the abdomen for the monitoring of the respiratory movements. Abdominal movements ( AP: abdominal pressure ) exactly correspond to regular baseline fluctuation of referential derivations of EEG. ECG contaminates the left occipital electrode uniquely.

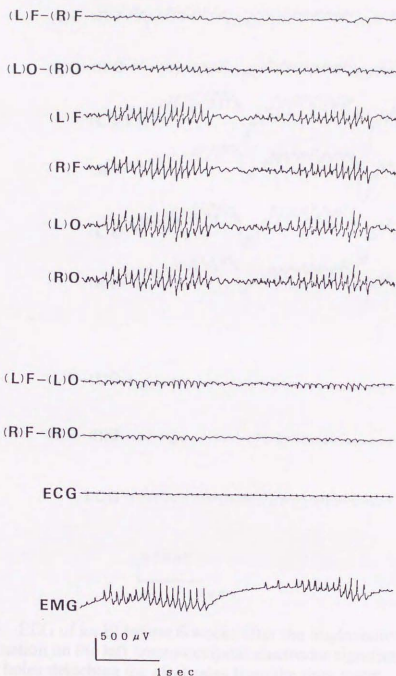


Fig. 3-7. Regular spike-like activities in an El mouse were verified with EMG electrodes placed in the right temporal muscle. These are evidently due to the temporal muscle discharges spreading to the reference electrode.

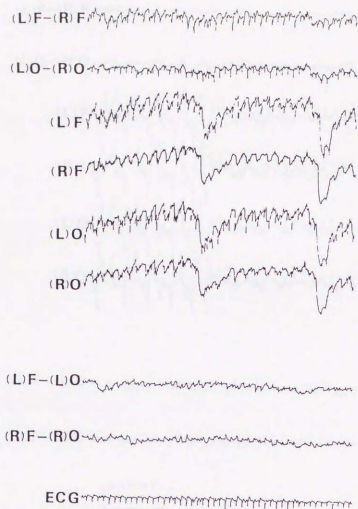


Fig. 3-8. EEG of an EI mouse 6 weeks after the implantation. ECG contamination on the left fronto-occipital electrodes signifies the ossification in the burr holes detaching the electrodes from the dura mater.

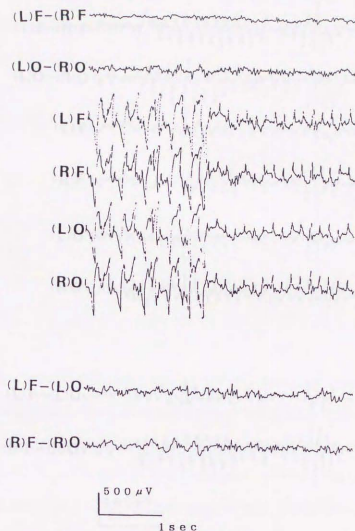


Fig. 3-9. EEG of an El mouse with the reference electrode misplaced on the nasal bone instead of subcutaneous placement. ECG is spreading in all referential derivations. High amplitude deflections may be due to the unstable contact of the reference with the bone.

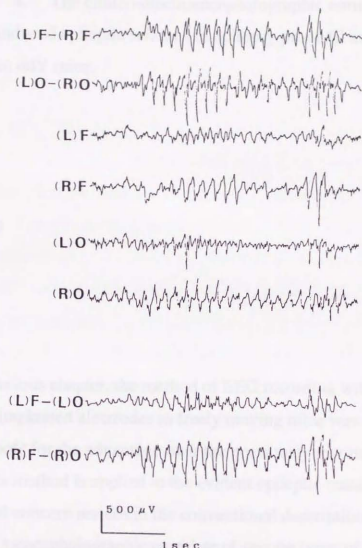


Fig. 3-10. Connecting cables between joint box and preamplifier were tapped with fingers. Rhythmic artificial activities of the bipolar recordings mimic the paroxysms, which were due to the impedance mismatch between cables.



#### CHAPTER 4. The clinico-electroencephalographic correlate of Megimide-induced epileptic seizures in epilepsy prone El and its mother strain ddY mice.

##### Introduction

In the previous chapter, the method of EEG recording with the chronically implanted electrodes in freely moving mice was described. With the proofs for the advantage over the previously reported methods, this method is applied to the evident epileptic manifestations. The principal concern resides in the conventional description of clinico-electroencephalographic correlate of seizure types of mice. For the model, Megimide-induced epileptic seizures were employed, for, in the preliminary tests ( data not presented ), very similar ictal manifestations had been induced by Megimide in ddY and El mice and the ictal EEG recording was considered as a good candidate for the comparative description of the clinico-electroencephalographic correlate in two strains of mice.

## Materials and Methods

Six male ddY and 9 male EI mice were used in the present research. The ddY mice were purchased from Saitama Experimental Animal Supplier Co., Ltd. ( Saitama, Japan ). The EI mice were used at 106th and 107th generations. The mean age was 13 (11-16 ) weeks on the date of 14 EEG recordings and 16.5 ( 14-20 ) on 15 recordings in ddY and EI mice, respectively.

Implantation of electrodes was performed according to the procedure described in Chapter 3.

EEG, ECG and EMG were recorded under the same conditions as those described in Chapter 2.

EEG recordings were performed between 3 and 32 days after the implantation of the electrodes. All animals were activated with Megimide ( 10-20mg/kg for EI and 20mg/kg for ddY, i.p. ) to induce epileptic seizures. The approximate sublethal maximum dose was determined by preliminary tests. EEG was recorded from preictal period until 30 minutes after epileptic seizures. In total, 14 seizures in ddY and 15 in EI were recorded.

The behaviors or the clinical manifestations of the animal were carefully observed and written on the recording paper of EEG.

## Results

### Clinical manifestations ( Table 4-1 ).

The three serial periods have been distinctly noted in both strains on the basis of clinical manifestations. They involved 1- twitching period, 2- general tonic clonic convulsion ( GTC ) and 3- postictal period. The GTC included three phases: 1) Tonic phase, 2) Tonic phase with clonic movement and 3) Clonic phase.

#### 1- Twitching period.

After intraperitoneal administration of Megimide, twitching movements were observed in all ddY and in 13 out of 15 seizures in El mice. This movement consists of a rapid backward shrinkage of the neck with a rapid withdrawal of the trunk ( Fig. 4-1). In some occasions it came suddenly while the animals were walking or smelling something with the neck stretched, whereas in others the animals seemed to prepare the precipitating twitching movement and dare not to move until it occurred, or it did not happen to occur and the animal began to walk. It did not seem to occur while the animals were doing any purposeful movements: eating, face washing, trying to find something in the tips or clinging on the wall of the cage. The movements were exactly the same in two strains. They were rarely

observed after GTC. However, in 3 occasions in El mice, the second GTC happened and twitchings were observed before the second one.

The twitchings never appeared with regular rhythm. While GTC was approaching, the frequency seemed to augment and in several occasions the animals no more walked and stayed in place manifesting the twitchings.

The animals were usually irritative to any stimuli between two successive twitchings, however, it was not possible to evaluate precisely the level of consciousness at the moment of this movement.

The salivation was usually observed during this period even before the twitchings became apparent.

So called running fits have never been observed during this period.

## 2- General tonic clonic convulsion ( GTC ).

### 1) Tonic phase.

The GTC was observed in all cases in both strains. The GTC had characteristic features symptomatically identical in both strains. It began always with tonic phase, however, during the very brief period ( less than 1sec ) at the onset of tonic phase, the animals often squeaked as a signal of onset and presented twitching-like movements more rapid and smaller than those observed during twitching period. Then the characteristic ictal manifestations followed during tonic phase of GTC.

The following symptom was the tonic dorsiflexion of the neck with the tail erected over the back. The animal might be in a prone position ( Fig. 4-2 ) ( 3 seizures in ddY and 2 in El ) presenting the tonic posture. However, in most occasions the tonic posture was

immediately followed by the violent turn over of the body on one side then on another with the torsion of trunk.

The tonic phase terminated when the animal became relaxed and immediately resumed the prone posture and stayed prone during the following clonic phase.

## 2) Tonic phase with clonic movements ( Table 4-2, 4-3 ).

During the tonic phase of GTC, in 10 cases among 14 seizures in ddY and 7 among 15 in El mice, clonic movements were superposed on the tonic posture. Table 4-2 ( for ddY ) and Table 4-3 ( for El ) summarize the clonic symptoms. I use the term clonic not for the small myoclonus but for the overt gross periodical movements of the limbs or the neck ( twitching ). The clonic movements were observed even while the neck was becoming dorsiflexed or while the animals turned over on the sides with the torsion of the trunk. When the clonic movements were observed in the same animal in different occasions, they were not always the same. In 5 cases in ddY, the same clonic manifestation continued in the following clonic phase even after the animals resumed the prone posture.

## 3) Clonic phase ( Table 4-2, 4-3 ).

When the tonic phase terminated, the animals resumed the prone posture and began the clonic phase. I defined this phase as clonic because the clonic symptoms were the only manifestations, although not all the animals presented clonic movements. The animals maintained the prone posture. Clonic movements were seen in 11 occasions in ddY ( Table 4-2 ) and in 7 in El mice ( Table 4-3 ). The



most frequently observed symptom was the twitchings in both strains. The twitchings were exactly the same as those of the twitching period except that the animals stayed in place and that the movements seemed to occur with regular rhythm.

Two different successive clonic movements were observed during the clonic phase in 4 cases in ddY mice ( Fig. 4-6 ). The clonic movements were never seen with the same manner in the other seizure(s) of the same animal.

The consciousness was deteriorated during this phase and the animals did not react to the touching stimuli, although the righting reflex was preserved.

The clonic phase terminated when spike discharges were no more observed and EEG activity was suppressed with regular baseline fluctuation due to respiratory movement.

### 3- Postictal phase.

When the clonic phase came to the end, some animals became suddenly stuporous and immobilized during several minutes while the others, after several seconds, began to walk erratically for less than a half minute before finally becoming immobilized.

During the early postictal period within 20 minutes, small jerks of temporal muscles were observed without any biting behavior in 1 ddY mouse and in 10 EI mice. The EEG correlate will be described later.

The animals began to move in several minutes but remained stuporous or drowsy during the following recording period of 30 minutes. It was obvious that ddY mice recovered the conscious activity earlier than EI mice did.

Second GTC was observed in 3 EI mice 340 seconds in average

( 160-480 ) after the first one.

Two El mice repeated twitchings and died in 140 and 320 seconds.

Clinico-electroencephalographic correlate.

#### 1- Twitching period.

When twitchings were observed, some of them were associated with focal spikes, generalized spikes or generalized spike & waves complex ( Fig. 4-4 for ddY and Fig. 4-5 for El ). Generalized spike & wave complex was observed in 6 cases ( Fig. 4-4 right ) and generalized spikes in 6 cases ( Fig. 4-5 left ) in ddY. Generalized spike & wave complex was observed in 1 case ( Fig. 4-5 right ), generalized spikes in 1 case ( Fig. 4-5 middle ) and focal spikes in 1 case ( Fig. 4-5 left ) in El mice. In other cases in both strains, any spikes could not be identified due to the EMG contamination.

Several spikes without the associated twitchings were observed in 10 cases in ddY ( Fig. 4-4 ) and in 6 cases in El mice. These spikes were generalized and/or focal and focal spikes were most often seen on occipital derivations ( Fig. 4-4 right ) in ddY while they were generalized in 2 and focal without topographical predominance in 4 cases of El mice.

This kind of spikes was not always the same in the same individuals in different occasions.

## 2- General tonic clonic convulsion ( GTC ).

### 1) Tonic phase.

All cases in ddY except one had generalized spikes from the onset ( Fig. 4-6,7 ). One case had spikes on the left frontal derivation from the onset.

In El mice, no obvious spike were determined at the onset except 2 cases in Fig 4-8 and Fig 4-10, in which small spikes were observed on occipital derivations, and on left frontal and occipital derivations, respectively.

### 2) Tonic phase with clonic movements ( Table 4-2, 4-3 ).

Whatever the clonic manifestations might be, in ddY ( Table 4-2 ), generalized spikes predominated during this phase, except one that presented left frontal and occipital spikes. Generalized spikes were seen in 3 cases and left frontal spikes in 1 without clonic movements.

In El mice ( Table 4-3 ), regardless of the presence or absence of clonic movements, spikes predominated on the occipital derivations in 11 cases: right and left in 6, left in 4 and right ( with right frontal spikes ) in 1. Left frontal spikes were observed in 2 cases. Spikes could not be identified in other 2 cases due to EMG contamination.

### 3) Clonic phase ( Table 4-2, 4-3 ).

During clonic phase, spike discharges were recorded in almost all animals.

Whatever the clonic manifestations might be, generalized spikes predominated in ddY ( Table 4-2 ), except 2 which presented right or left occipital spikes. No clonic movement was observed in 3 cases with generalized spikes in 2 cases and right occipital spikes in 1.

In El mice ( Table 4-3 ), regardless of the presence or absence of clonic movements, spikes predominated on occipital derivations in 13 cases: right and left in 7, right and left ( with left frontal spikes ) in 3, left in 2 and right ( with right frontal spikes ) in 1. The other 2 cases are omitted because of EMG contamination.

### 3- Postictal period.

When GTC terminated, EEG was suppressed with the regular fluctuation of base line corresponding to respiratory movement and the animals were immobilized. During early postictal period within 20 minutes, regular spike-like activities were recorded in 1 case of ddY and in 10 cases of El mice. This kind of EEG activities was proven to be the contamination of biting muscle discharges ( Fig. 3-7 ) .

The postictal EEG was suppressed while the animals became stuporous and immobilized. Postictal spikes were recorded on the occipital derivations in 11 cases of ddY: right and left in 8, right and left ( with right frontal spikes ) in 1, left in 1 and right in 1, and left frontal spikes were recorded in 1 case. Postictal spikes on occipital derivations were recorded in 5 cases of El mice: right and left in 4 and right in 1, and generalized spikes were recorded in 2 cases.

The second GTC was observed in 3 El mice. In all cases, twitchings with corresponding generalized spikes preceded the GTC. These twitchings were more numerous than those observed during the

twitching period. The pattern of the second ictal EEG was identical with that of the first GTC in 2 and different in 1 case.

In 2 El mice, in 140 and 320 seconds after the GTC, the violent behaviors: running, jumping and falling on one side and another with the torsion of trunk, began suddenly after twitchings and continued several seconds and the animals fell in a characteristic posture: tonic ventroflexion of the neck with tonic flexion of forelimbs and extension of hindlimbs. The same posture was reported with photograph in audiogenic seizure in a mouse of the inherently susceptible DBA/2J inbred strain <sup>4</sup> and in Pentylene-tetrazol-induced convulsion in El mice <sup>73</sup>. This posture once established subsided immediately and the animals looked relaxed without respiratory movements whereas the ECG activity was waning until final arrest. The clonic components could hardly be identified during the catastrophe. The EEG during this period was contaminated with EMG and any discharges could hardly be identified. So these dead cases could not be categorized as the second GTC.

## Discussion

Although the EEG analysis of pharmaceutically induced epileptic seizures in epilepsy prone El and its mother strain ddY mouse are beyond the scope of this thesis, it is important to note that some



comparative description of clinico-electroencephalographic correlates in two strains has become possible with the EEG recorded with the present method.

It is important to note that in the usual seizures presented by El mice, the twitching movements were almost always observed ( data not presented ). The twitchings might be the first symptom with the associated spike discharges in the Megimide-induced epileptic seizures and presumably in the non pharmaceutically induced seizures in El mice. The referential derivations demonstrated the focal or generalized spikes as well as the generalized spike & wave complex corresponding to the twitching movements. The diversity in the form of spikes corresponding to one type of clinical manifestation has to be noted<sup>17</sup>.

This kind of movement might be categorized into the myoclonus in general although the exact participating group(s) of muscle(s) could not be identified with the EMG electrodes placed on the nuchal muscle. It seems much likely that the paravertebral muscles at the neck participated grossly in the movements.

During the GTC, serial symptomatology was identical in both strains. The tonic phase came first and was followed by what I call the clonic phase with or without clonic manifestations. I have observed the variable clonic manifestations during the tonic phase of GTC and the predominant twitching movements during the clonic phase of GTC, whereas the electroencephalographic manifestations were different between two strains during these phases: generalized spikes in ddY ( Table 4-2 ) and occipital spikes in El mice ( Table 4-3 ). The description of the strain difference in EEG owed much the results of referential recordings.

The present results suggest that the quality of the referential derivations of the EEG was acceptable enough to describe the clinico-electroencephalographic correlates of epileptic seizures in mice. And it was also important to note that the best arguments for the reproducibility of the recording could be offered with the fact that the strain difference in the ictal EEG has become apparent.

	(1-17)	(1-40)
Mean latency to first switchings	79.2	56.4
Mean latency following last switching to GTC	6.1	3.2
	(1-25)	(3-17)
GTC		
Onset/switch	AG	AB
Mean latency to GTC	134.1	68.2
	(20-254)	(26-109)
Mean duration of GTC	28.4	27.4
	(8-32)	(8-29)
Mean duration of tonic phase	8	2.3
	(4-13.3)	(3.3-11)
Clonic movement during tonic phase	15 cases	7 cases
Clonic movement during clonic phase	21 cases	7 cases
Postictal period		15.3
Second GTC	0	3 cases

Numbers are (n sec)  
Parentheses: (total cases)

Table 4-1. Clinical manifestations

	ddY ( 14 Sz.)	El ( 15 Sz.)
<hr/>		
Twitching period		
Occurence of twitchings	All	13 cases
Mean twitching number	7.9 times (1-17)	3.5 times (1-6)
Mean latency to first twitchings	99.8	56.4
Mean latency between last twitching to GTC	6.1 (1-36)	5.2 (2-17)
GTC		
Occurence	All	All
Mean latency to GTC	154.1 (70-554)	98.2 (30-209)
Mean duration of GTC	20.1 (10-32)	17.1 (5-24)
Mean duration of tonic phase	6 (3-13.3)	7.3 (3.3-11)
Clonic movement during tonic phase	10 cases	7 cases
Clonic movement during clonic phase	11 cases	7 cases
Postictal period		
Second GTC	0	3 cases
<hr/>		

Numbers are in sec.  
 Parentheses: ( mini.-max. )

Table 4-2. Clinico-electroencephalographic correlate of clonic movements during GTC in ddY mice

	during tonic phase		during clonic phase	
A-1	-	( G )	-	( G )
A-2	l-F	( G )	l-F	( G )
B-1	-	( G )	r-F->Tw	( G )
B-2	Fs	( G )	l-H	( r-O )
B-3	l-F	( l-F,O )	l-F->Tw	( l-O )
C-1	Tw	( G )	Tw	( G )
C-2	-	( l-F )	-	( r-O )
D-1	r-H->r-F	( G )	-	( G )
D-2	r-F	( G )	Tw	( G )
D-3	r-H	( G )	r-H->Tw	( G )
E-1	l-F	( G )	Tw	( G )
F-1	Tw	( G )	Tw->r-F	( G )
F-2	-	( G )	Tw	( G )
F-3	l-F	( G )	Tw	( G )

A-F signify the individuals. The numbers are EEG recordings. l: left; r: right; F: forelimb; H: hindlimb; Tw: twitching movement; ( G ): generalized spikes; ( F ): spikes on frontal derivation; ( O ): spikes on occipital derivation.

Table 4-3. Clinico-electroencephalographic correlate of clonic movements during GTC in El mice

	during tonic phase		during clonic phase	
A-1	-	(?)	r-F	(?)
B-1	r-F	(?)	-	(?)
C-1	r-F	(l-O)	-	(l-O)
C-2	-	(r,l-O)	-	(r,l-O,l-F)
D-1	-	(r,l-O)	-	(r,l-O)
D-2	l-H	(r,l-O)	Tw	(r,l-O)
E-1	l-F	(l-F)	Tw	(r,l-O,l-F)
E-2	Fs	(l-F)	-	(r,l-O,l-F)
F-1	l-H	(l-O)	Tw	(r,l-O)
F-2	-	(l-O)	-	(r,l-O)
F-3	-	(r,l-O)	-	(r,l-O)
G-1	-	(r-F,O)	r-F	(r-F,O)
H-1	r-H	(r,l-O)	-	(r,l-O)
H-2	-	(r,l-O)	Tw	(r,l-O)
I-1	-	(l-O)	Tw	(l-O)

A-I signify the individuals. The numbers are EEG recordings. Abbreviations are the same as those in table 3.



Fig. 4-1. The twitching movement. The animal is briefly arrested just after the rapid shrinkage of the neck and body.



Fig. 4-2. The tonic dorsiflection of the neck with the tail coiled over the back during the tonic phase of GTC.



Fig. 4-3. The clonic movement of the right forelimb during the clonic phase of GTC. The animal is drowsy in a prone posture.



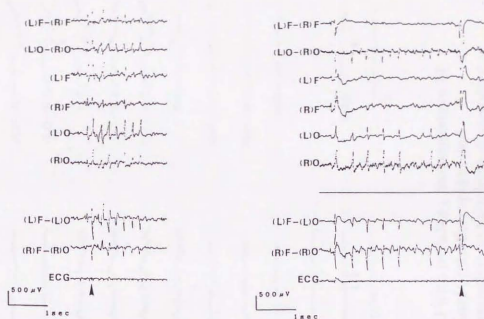


Fig. 4-4. Two cases of EEG associated with twitching movements in ddY mice. The polyspikes ( left ) and spike&wave complex ( right ) are corresponding to twitching movements ( arrow head ). EMG is demonstrated as contamination on ECG. L: left; R: right; F: frontal; O: occipital ( the same in the following EEG ).

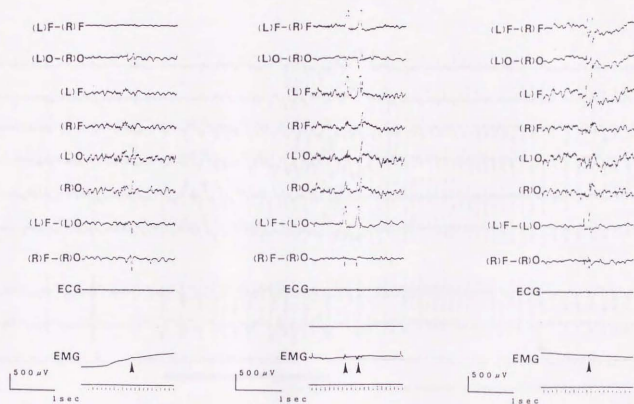


Fig. 4-5. Three cases of EEG associated with twitching movements in El mice. The occipital ( Left ) and generalized ( middle ) spikes and spike&wave complex ( right ) are corresponding to twitching movements. EMG electrodes are placed on the nuchal muscles.

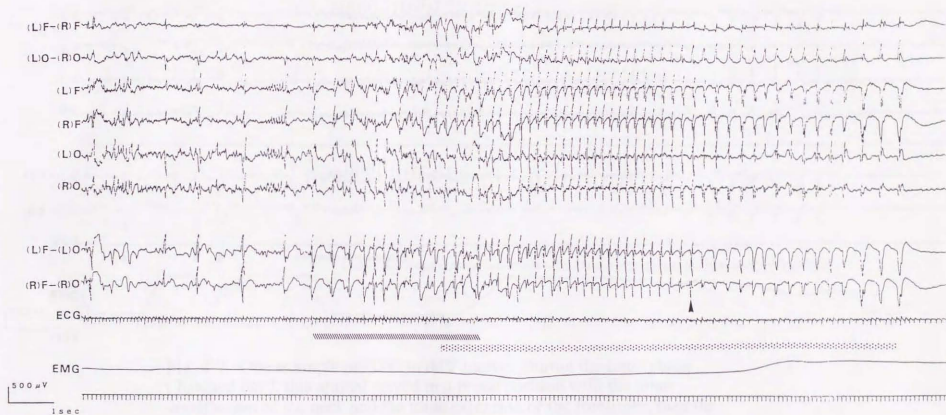


Fig. 4-6. One example of GTC in ddY mouse. Hatched bar represents the tonic phase of GTC, the overlapped portion with dotted bar is the tonic phase with clonic movement and the other dotted bar is the clonic phase of GTC ( the same in the following EEG ). During the tonic phase, twitching movements began and at the point ( arrow head ) were replaced by the clonic movement of right forelimb. Note that EEG manifestation changes at this point. Generalized spikes continue during GTC.

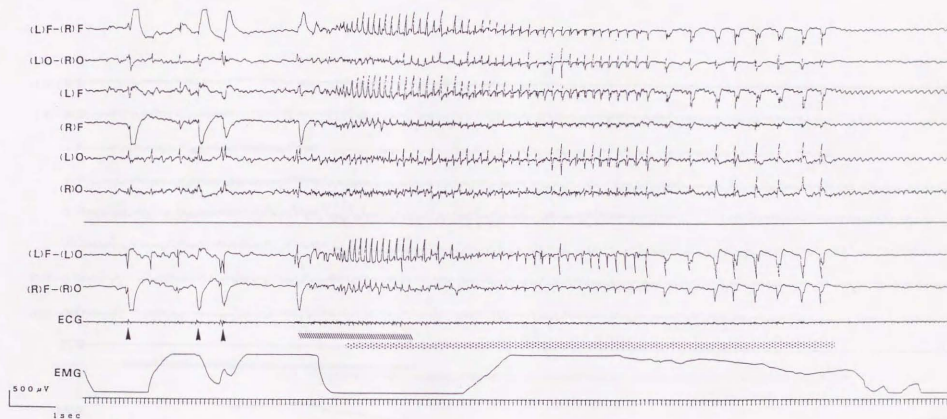


Fig. 4-7. One example of GTC in ddY mouse. During the tonic phase ( hatched bar ), this animal stayed in a prone position with the tonic dorsiflexion of the neck and the tonic extension of the forelimbs, then the twitching movements began with the neck in normal position while the tonic extension of the forelimbs continued ( overlapped bars ). After the animal became completely relaxed, the twitching movements continued in the following clonic phase of GTC. When GTC comes to end, EEG becomes suppressed with the regular fluctuation of the base line corresponding to respiratory movements. Note that several twitches ( arrow heads ) were observed before GTC.

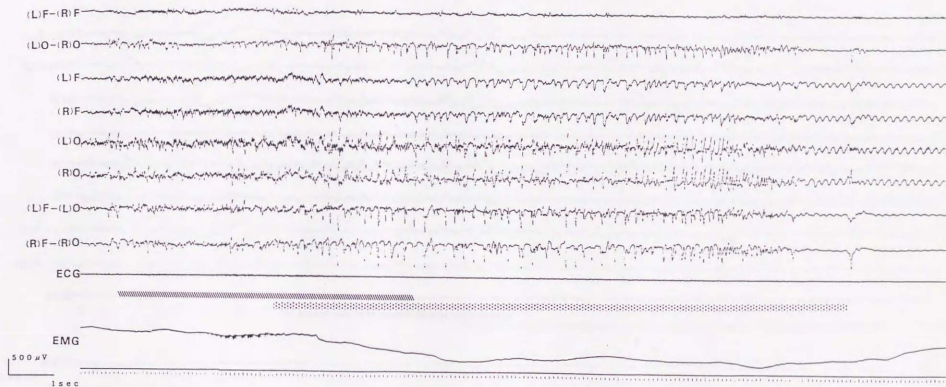


Fig. 4-8. One example of GTC in El mouse. During tonic phase with clonic movement ( overlapped bars ), the clonic movements of the left hindlimb were observed. When the tonic phase ( hatched bar ) terminated, the animal resumed the prone posture and the clonic movements of the left hindlimb were replaced by the twitching movements during the following clonic phase ( dotted bar ) of GTC. Note that no generalized spikes are recorded and occipital spikes predominate during GTC.



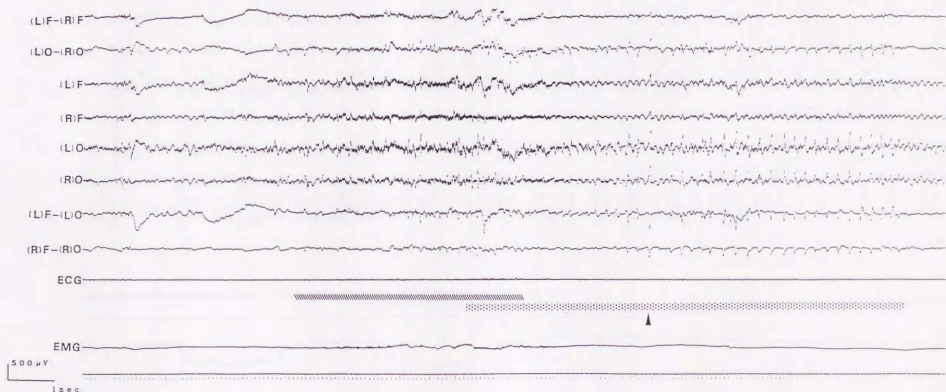


Fig. 4-9. One example of GTC in El mouse. During the tonic phase with clonic movement (overlapped bars), the clonic movements of the left hindlimb were observed, however they were no more obvious after the tonic phase terminated. During the following clonic phase (dotted bar), the twitching became apparent at the point (arrow head) and continued. Occipital spikes predominate during GTC.



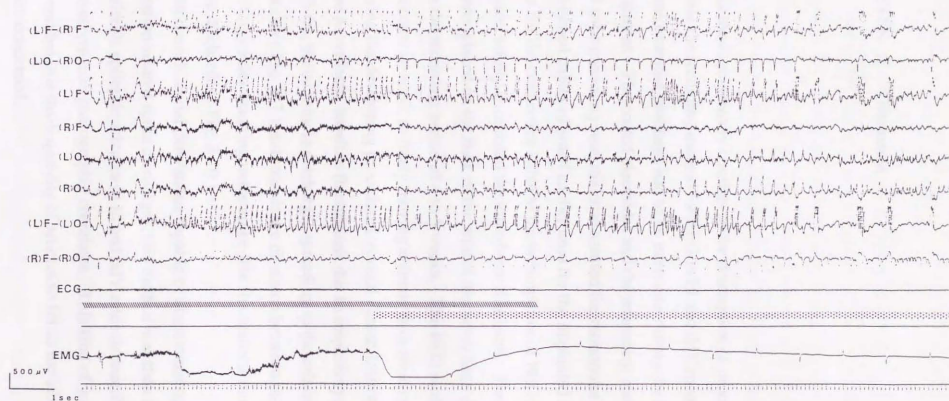


Fig. 4-10. One example of GTC in El mouse. During the tonic phase with clonic movement (overlapped bars), the clonic movements of the left forelimb were observed. After the tonic phase terminated, twitching movements became observed during the clonic phase of GTC. Left frontal spikes predominate during GTC while occipital spikes appear afterwards.

## CHAPTER 5. Discussion

This thesis principally consists of the description of procedures to complete the recording method for EEG in El and ddY mouse.

Some essential problems had to be overcome during the technical development. The coated electrodes and the referential electrode placed subcutaneously near the nose assured the monopolar recording. The twined cables of small caliber used for the implanted leads as well as for the connecting cable to the preamplifier of EEG apparatus have successfully eliminated the impedance mismatch. The cassette connector placed on the back of the animal has never been damaged during the recording period of 4 to 5 weeks. The EEG recording has thus become possible in freely moving animals with good quality.

During the developing procedures, several contamination had to be verified as artifacts: baseline fluctuations due to respiratory movement ( Fig. 3-6 ), biting muscle discharges spreading to the referential derivations ( Fig. 3-7 ) and impedance mismatch between the connecting cables resulting in the spike-like fluctuations mimicking the true discharges ( Fig. 3-10 ).

With the referential derivations capable of discriminating localization-related spike discharges, this method was then applied to the veritable epileptic seizures in El and ddY mice to describe the clinico-electroencephalographic correlates. This kind of work has been reported in a small quantity of articles so far as the laboratory mice are concerned.

" More than 300 strains of inbred lines of mammals are available today; over 250 of these are mouse strains." This phrase was written in the text: Inbred and genetically defined strains of laboratory animals published in 1979 <sup>1</sup>. The quantitative dominance of mice among laboratory animals derived from the historical background of the research in cancer and in immunology, and already in 1932 began the efforts to achieve a standardized nomenclature for the inbred mice. Nowadays, the genetic information of mice is the best available of any laboratory mammals.

There exist a number of inbred and neurological mutant strains of mice susceptible to convulsion. The mouse susceptible to audiogenic seizure <sup>41,42,91</sup>, the mouse Tottering <sup>20,34,37,53</sup>, the quaking mouse <sup>3,12,88</sup> and the El mouse <sup>26,27,77,78,79,80,81,82,83,84</sup> are the examples which have been destined to the study of epilepsy. The mouse Tottering and the quaking mouse present the spontaneous seizures whereas the audiogenic and the El mouse are considered as the model of reflex epilepsy.

Maxson <sup>41,42</sup> recorded the cortical EEG in audiogenic mice during the seizures induced by the sound and two drugs: Picrotoxin and Thiosemicarbazide. In this text Maxon presented only one trace of bipolar derivation in each examples of EEG.

As for the mouse Tottering, four investigators reported EEG with clinical correlate. Noebels <sup>53</sup> observed the bilaterally synchronous spike-wave bursts ( 6-7Hz ) accompanied by behavioral " absence" attacks with myoclonic head jerks. He presented only one trace of bipolar ( antero-posterior in each hemispheres ) EEG. Kaplan <sup>34</sup> recorded with the bipolar epidural electrodes the burst of 6Hz polyspikes correlated with the episode of behavioral immobility and

staring. Heller<sup>20</sup> and Kostopoulos<sup>37</sup> reported almost the same EEG pattern.

Gioanni<sup>12</sup>, Chauvel<sup>3</sup> and Valatx<sup>87,88</sup> reported independently the clinico-electroencephalographic correlate in quaking mice. The electrodes were placed differently for one from for the other investigators: cortex ( Gioanni ), epidural ( Chauvel ) and external table of the skull ( Valatx ). Gioanni presented only one trace of bipolar EEG demonstrating the flattening pattern during the tonic-clonic convulsions: occurring spontaneously or triggered by somesthetic and central electrical stimulation. On the other hand, Chauvel described precisely the several ictal manifestations and classified them into tonic, audiogenic and myoclonic seizure. Tonic seizure was associated with a bilateral rapid discharge, superimposed on faster and lower voltage back ground activity. Audiogenic seizures were composed of wild running followed by tonic-clonic generalized seizure. A wild running corresponded to a slackening of the background activity, whereas during the clonic phase, rhythmic high-amplitude waves of low frequency ( 3Hz ) became progressively faster. Myoclonic jerk corresponded to polyphasic spike that originated in one hemisphere and spread to the opposite side. In the description, Chauvel presented only one example of EEG correlated with myoclonus. Valatx described precisely the clinical manifestations of spontaneous seizures. And he showed with one trace of bipolar EEG the rapid and low amplitude cortical activity without paroxysmal discharge during the episode.

Although these three authors dealt with the same strain: quaking mouse, to establish the ictal clinico-electroencephalographic correlates, the paucity of the EEG data and no unanimous results are remarkable.

Since El mouse was discovered in 1954 by Imaizumi<sup>26</sup> and registered internationally in 1964 as a mutant inbred strain susceptible to epileptiform seizures<sup>27</sup>, this model has attracted many investigators in the field of chemistry<sup>7,9,10,23,29,43,46,57,58,60,61,70,86,93</sup>, metabolism<sup>18,21,22,24,30,32,33,47,50,52,75,95</sup>, pharmacology<sup>36,76</sup> or histo-pathology<sup>66,74</sup>; mostly outside the field of neurophysiology.

Suzuki<sup>77,78</sup> reported for the first time the ictal EEG in El mice. The seizures were induced by the vestibular or proprioceptive stimulus: tossing up maneuver, see-saw movement, etc. According to his observation, the course of seizure was in three stages: prodromal, tonic-clonic convulsive and postictal. The EEG was recorded through the stainless steel screws implanted in the calvarium, with a reference electrode fixed to the most caudal part of the skull. He described the electrical initiation of the seizures in the parietal cortex, the regular rhythmic slow spikes in the negative phase of high amplitude during the tonic phase and infrequent irregular fast spikes or wave-spike complexes during the clonic phase of convulsion. It is important to note that the EEG recorded with the present method during seizures induced by up and down movements of the animal demonstrated several different aspects from those of Suzuki's observation ( data not presented ).

When El mouse became the subject of research, I initiated the careful observations of ictal manifestations. I noted two points that must be clarified for the understanding of the epileptogenesis or for the determination of seizure type, but that had not been reported precisely by any authors. The details of these observations included: the fact that before the animal fell into the general tonic-clonic convulsion, twitching movements were observed and that the seizures were initiated with diverse stimulation. The twitching movements



have been observed by Imaizumi <sup>26</sup> as clonic movements of the trunk prior to the "epileptiform convulsions". Suzuki <sup>78</sup> later described the similar symptom during the "prodromal stage of seizures". As for the latter I have observed several triggering factors <sup>5,19</sup> including the squeaking of other mice precipitating to convulsion, environmental change, sniffing movement for unfamiliar objects or ultrasound exposure. El mouse is now considered as a model of reflex epilepsy, although the exact characters of sensory afferent participating in the seizure initiation are not identified even now. The reports on the clinico-electrophysiological correlate were mainly based rather on the stereotyped maneuver: tossing up <sup>77,78,79,83</sup>, and the observation is rather limited within the seizures induced by such maneuver.

The present report may give some orientations to the future investigation including these two points. As for the first point, the same twitching movements were observed during the Megimide-induced epilepsy in both El and ddY mice, corresponding to the apparent EEG discharges. Therefore, the twitching movements observed in the daily ictal manifestations may be the first epileptic symptoms, although the pharmaceutical mechanism must be taken in account. The present method clarified some strain difference in EEG manifestations during the Megimide-induced epileptic seizures. This kind of work will open the field of research on pharmaco-electrical relationship that has become much popular in other animal models.

The systemic observations have never been taken into consideration in the research of central nervous system ( CNS ) of this mouse. In the human medicine, the systemic investigations are inevitable in the study of every cases with congenital CNS anomaly. The pilot studies were accompanied with the present technical development ( precise data not presented ) and demonstrated several

strain differences between El and ddY mouse. The usual (interictal) behaviors differ in one from another. El mice are more irritative to stimuli. El mice walk erratically, suggesting additional anomaly in CNS. The weight of the brain is lighter than that of ddY mice. The testis of El mouse is about a half in weight that of ddY mouse. These differences have in part been reported. The life span is apparently shorter in male. Sometimes the male was discovered dead and autopsy did not reveal any gross anomaly in vital organs and in CNS. The growth curve and the seizure occurrence in 24 El mice are demonstrated in Fig. 2-3 and Fig. 2-4. The age dependent occurrence of epileptic seizures has been reported in several strains <sup>16,19,25,66,69</sup>.

In the history of epilepsy research, electrophysiological investigations have been the main concern in the laboratory as well as in the clinics. Recently, the development in the field of molecular biology is going to open the new field in the neuroscience. The epileptic phenomena should be regulated by certain genes <sup>8,25,44,45,54,55,59,63,92,94</sup>. This concepts will prevail and finally the epileptic phenomena will be explained with the "molecular" words. In this context, the genetically predisposed mouse models such as El mice must play an important role with the accumulated genetic information of mice in general. In this sense, the correct information of electrical phenomena in the genetically predisposed epilepsy models must be presented to be available for the most sophisticated scientific research as well as for the comparative study with the human epilepsy. This methodology is applicable for such purpose in various kind of genetically determined epilepsy model in mice and thus will contribute to the understanding on the human epilepsy mechanisms.

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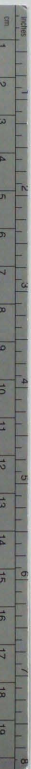
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## Kodak Gray Scale



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A      1      2      3      4      5      6      M      8      9      10      11      12      13      14      15      B      17      18      19

