BRAIN RESEARCH (2000) 881(2):152-158.

Convulsive seizures induced by N-methyl-D-aspartate microinjection into the mesencephalic reticular formation in rats

Ishimoto T, Omori N, Mutoh F, Chiba, S

Convulsive seizures induced by N-methyl-D-aspartate microinjection into the mesencephalic reticular formation in rats

Takahiro Ishimoto *, Nobuyuki Omori , Fukuyasu Mutoh ,

Shigeru Chiba

Department of Psychiatry and Neurology, Asahikawa Medical College,

Midorigaoka higashi 2-1-1-1, Asahikawa 078-8510, Japan

*, To whom correspondence should be addressed:

Takahiro Ishimoto, M.D., Department of Psychiatry and Neurology , Asahikawa Medical College, Midorigaoka higashi 2-1-1-1, Asahikawa 078-8510, Japan

Tel : +81-166-68-2473

Fax: +81-166-68-2479

E-mail : ishimoto@asahikawa-med.ac.jp

The number of text pages of the whole manuscript is 33 pages (including 4 Figures and 2 Tables).

Acknowledgements

The authors wish to thank Dr. Akihiko Nunomura for his advices in histological investigation and Prof. Yoshikatsu Mochizuki for statistical analyses. Part of material in the manuscript was presented at the 33rd Congress of the Japan Epilepsy Society in Sendai, Japan on October 22 1999.

Convulsive seizures induced by N-methyl-D-aspartate microinjection into the mesencephalic reticular formation in rats

Abstract

Effects of microinjections of a single 2 or 10 nmol dose of N-methyl-D-aspartate (NMDA) into the unilateral mesencephalic reticular formation (MRF) on behavior and electroencephalogram were examined in rats (n=18) during a 15 min period (Exp. 1), and subsequent effects of sound stimulation with key jingling applied at 15, 30, and 45 min after the injections were observed (Exp. 2). The microinjections of 2 nmol dose of NMDA (n=10) induced hyperactivity (9 of 10 rats) and running/circling (8 of 10 rats) in Exp. 1, and hyperactivity (3 of 10 rats) in Exp. 2. Moreover, the microinjections of 10 nmol dose of NMDA (n=8) induced not only hyperactivity (8 of 8 rats) and running/circling (7 of 8 rats) but also generalized tonic-clonic seizures (GTCS) (5 of 8 rats) in Exp. 1; these seizure patterns were also elicited by sound stimulation in Exp. 2. The seizure patterns were accompanied by electroencephalographic seizure

discharges in the MRF and the motor cortex. In contrast, the control group rats (n=10) which received a single dose of saline microinjection into the unilateral MRF showed no behavioral or electroencephalographic changes in both Exp. 1 and 2. These findings suggest that the MRF has an important role in the development of GTCS, which follows hyperactivity and running/circling, and that potentiation of excitatory neurotransmission in the MRF participates in the development of audiogenic seizures as well as GTCS.

Classification terms: Research Reports

Theme: DISORDERS OF THE NERVOUS SYSTEMS

Topic: Epilepsy : basic mechanisms

Keywords: Brainstem; N-methyl-D-aspartate; Convulsion; Epilepsy; Audiogenic seizures; Rats

1. Introduction

Previous experimental studies suggest that the brainstem reticular formation, particularly the MRF, is involved in the generation of primary generalized seizures [26,40] or in the secondary generalization of partial seizures originating from the forebrain [9,10,11,12,13]. Electrical stimulation of the unilateral MRF induces generalized convulsions in rats [6], rabbits [4], and cats [27]. The MRF can be kindled with the development of generalized convulsions in rats [10]. Local administration of bicuculline, a γ-aminobutyric acid (GABA) antagonist, to the unilateral MRF [9] induced generalized tonic seizures in rats. Moreover, propagation of seizure discharges from the inferior colliculus to the brainstem reticular formation (RF) is considered to be important for the development of audiogenic seizures in genetically epilepsy-prone rats (GEPR) [5, 21].

It has been suggested that an imbalance between inhibitory and excitatory neurotransmission participate in the generation and expression

of human ［16,17］ and animal epileptic seizures ［2,30,34,36］. With respect to inhibitory neurotransmission, repeated administration of a GABA receptor antagonist, picrotoxin ［7］ or bicuculline ［45］, to the unilateral amygdala induces the development of amygdala seizure in rats. Systemic administration of a GABA receptor agonist, muscimol , strongly suppresses amygdaloid kindled seizuresin rats ［35,36］. In contrast, in excitatory neurotransmission, systemic administration of excitatory amino acids such as NMDA ［33］ or kainic acid ［43］, or focal microinjection of NMDA into the unilateral amygdala ［14, 15］ or the massa intermedia ［24, 25］, produces generalized convulsive seizures. In the hippocampus and amygdala of the hippocampal kindled brain, increased release of glutamic acid during both ictal and interictal periods has been demonstrated in rats ［44］. Furthermore, dizocilpine (MK-801) ［42］ and 3-(2-carboxypiperazine-4-yl) propyl-1-phosphonic acid (CPP) ［38］, which is a NMDA receptor antagonist, have potent inhibitory effects on the development of amygdala kindling or kindled amygdala seizures in rats. Therefore, potentiation of excitatory neurotransmission by

excitatory amino acids seems to play a crucial role in the development of several experimental models of epilepsy.

To further clarify the role of MRF in the expression of epileptic seizures, we microinjected NMDA into the unilateral MRF in rats and observed the behavioral and EEG changes for 15 min (Exp. 1). Subsequently, we examined the effects of sound stimulation applied at 15, 30 and 45 min after NMDA microinjections on the rat behaviors and EEGs (Exp. 2) .

2. Materials and Methods

Experiment 1 : NMDA injection

Thirty male adult Sprague-Dawley rats (2-3 months of age, weighing 250-400 g) were used**.** The rats were randomly allocated to Group A (n=10), B (n=10), or C (n=10). Under pentobarbital anesthesia, chemitrodes, i.e., 24G guide cannulas with bipolar electrodes made of twisted stainless steel wire (200 μm in diameter), were implanted stereotaxically［39］ into the left MRF (the deep mesencephalic nucleus ;

5.8 mm posterior , 1.7 mm lateral from bregma, and 6.6 mm ventral from the skull). The tip of bipolar electrodes extended 1.0 mm beyond the ends of the guide cannulas. Two surface electrodes (stainless steel screws) were driven into the skull : one for recording from the unilateral sensorimotor cortex, and the other, over the unilateral olfactory bulb, as the reference electrode.

Seven days after operation, a single 10 nmol and 2 nmol dose of NMDA (Sigma, St Louis, MO, USA) was administered into the MRF in Groups A and B, respectively. The dose of NMDA was dissolved in saline and delivered in a volume of 1.0 μL at a rate of 1.0 μL/min by a microsyringe pump (Eicom corp. , Kyoto, Japan, EP-60). The microinjections were performed with 30G needles extending 1.0 mm beyond the ends of the guide cannulas. The Group C rats received a saline injection (1.0 μL) into the MRF in a manner identical to that carried out in Groups A and B. Behavioral and EEG changes were recorded for 15 min after the end of the NMDA microinjections in Exp. 1.

Experiment 2 : sound stimulation

Immediately after Exp. 1, we applied sound stimulation (for 60 sec) to the rats of Groups A, B, and C at 15, 30 and 45 min from the end of microinjections of NMDA or saline. The sound stimulation was provided by a short manual shake of a bunch of keys (6 metal door keys on a metal key-ring) held at 50 cm above the floor of an open-topped observation box (35 cm × 35 cm × 35 cm) ; the frequency and intensity of the sound was measured by sound level meters (Leader electronics corp., Yokohama, Japan, NL-05A) .

On completion of Exp. 2, the animals were deeply anesthetized and their brain were subjected to perfusion fixation with 10 % formalin, and then cut into frozen sections 10 μm thick to histologically confirm the position of the depth electrode. Statistical comparisons were made using Fisher's exact test.

3. Results

Histological examination revealed that the MRF electrodes were located

in the intended area (within 0.5 mm of the target site) in all the rats except for those in 2 of the Group A rats (Fig.1) ; results from these two rats were therefore excluded from data analysis. One of them, with MRF electrode located far dorsally, showed only mild hyperactivity and running/circling behavior while the other, with MRF electrode located ventrolaterally, showed mild hyperactivity without seizure discharges.

The peak frequency of sound stimulation was around 480 Hz , with a wide range of 300-2000 Hz. The intensity of the sound source ranged from 80 to 90 dB.

Experiment 1

The microinjections of 10 nmol dose of NMDA induced convulsive patterns in the following order : (1) hyperactivity (a state in which a rat is frequently moving or restlessly walking around in the observation box), (2) running/circling, and (3) GTCS. The microinjections of 2 nmol dose of NMDA induced only hyperactivity, and running/circling. As shown in Figs. 2 and 3, these seizure patterns were accompanied by electroencephalographic seizure discharges in the MRF and the motor

cortex. The Group C rats did not show any behavioral or electroencephalographic changes during the 15 min after the end of the saline injection. The incidence of the seizure patterns observed in each group is shown in Table 1. The incidences of hyperactivity and running/circling were significantly higher in Groups A and B than in Group C. The incidence of GTCS was significantly higher in Group A than in Groups B and C.

Experiment 2

Although NMDA-induced seizure patterns were not observed during the period from 15 to 45 min after the end of NMDA injections, sound stimulation applied at 15, 30 and 45 min after NMDA injections could induce hyperactivity, running/circling, and GTCS. These seizure patterns and their EEG findings observed in Exp. 2 were almost identical to those observed in Exp. 1 (Fig. 4). In contrast, no behavioral or EEG changes were elicited by sound stimulation in Group C in either Exp. 1 or 2. The incidences of the seizure patterns in Groups A, B, and C are summarized in Table 2. The incidence of each seizure pattern was

significantly higher in Group A than in Groups B and C. However, there was no significant difference in the incidence of each seizure between Groups B and C.

4 . Discussion

Previous experimental studies have suggested that generalized convulsions may have a MRF origin [4,6,9,10,27]. Electrical stimulation applied to the MRF triggers self-sustaining generalized tonic seizures in rats [6], rabbits [4], and cats [27]. Injection of bicuculline, a selective $GABA_A$ antagonist, into the unilateral MRF of rats results in fatal prolonged generalized tonic seizures, with the electroencephalographic seizure discharges predominant in the subcortical MRF or amygdala rather than in the motor cortex [9]. In addition, repeated electrical MRF stimulation can produce ultimately prolonged GTCS with afterdischarges in the MRF and the motor cortex [10]. In Exp. 1, we observed that GTCS was induced by NMDA injection into the unilateral MRF. The results

further support the view that generalized seizures can originate from the MRF.

In Exp. 1, the microinjection of 10 nmol dose of NMDA into the unilateral MRF induced varied seizure patterns, i.e., hyperactivity, running/circling, and GTCS, in that order. Injection of 2 nmol dose of NMDA also induced hyperactivity and running/circling, but did not produce GTCS. These findings suggest that potentiation of excitatory neurotransmission in the MRF participates in the generation of varied convulsive seizures including GTCS, and that the severity of the seizure symptoms depends on the level of potentiation of excitatory neurotransmission.

In Exp. 2**,** the results imply that the rats that received the injections of NMDA are susceptible to audiogenic seizures, suggesting that potentiation of excitatory neurotransmission in the MRF has a facilitory effect on the development of audiogenic seizures.

It is assumed that the brainstem RF participates in the development of audiogenic seizures. In GEPR, which shows running or generalized

tonic seizures with sound stimulations, elevated levels of glutamate and aspartate are observed in the inferior colliculus (IC) and the brainstem RF [8,28,41]. Bilateral microinjection into the IC or the pontine RF of exitatory amino acid receptor antagonists blocks audiogenic seizures in GEPR[22]. In addition , neuronal recordings using microwire electrodes in the IC and the pontine RF suggest that the IC serves as the initiation site of the audiogenic seizures, and that the influence of the IC on the pontine RF neurons is magnified in association with the susceptibility to audiogenic seizures, with the neuronal firing rate of the pontine RF increased markedly at the onset of audiogenic tonic seizure [21]. Therefore, it is assumed that the propagation of seizure discharges from the IC to the brainstem RF, particutarly to the pontine RF, is crucial for the development of audiogenic seizures, and that the brainstem RF neurons play a major role in generation of audiogenic seizures [21].

In our Exp. 2, the injected site of NMDA was the MRF, but not the pontine RF. The brainstem RF is caudally continuous with the intermediate zone of the spinal cord, and rostrally continuous with the

intralaminar nuclei of the thalamus and zona incerta of the ventral thalamus, occupying the central portion of the brainstem. In addition, complicated fiber connections are ventrically and horizontally present among neurons in the brainstem RF［37］. Therefore , it is speculated that within the brainstem RF epileptic discharges can easily propagate from one structure to the others. The fact that a GTCS induced by bucuculline microinjections into the MRF is almost identical to one induced by the bicuculline microinjections into the pontine RF［9］is not inconsistent with this possibility.

In order to induce audiogenic seizures, an electric bell ［5,18,19,20,21,22,31］ or key jingling ［1,23,29,32］have commonly been used as sound sources. Interestingly, in our preliminary study, key jingling induced the seizures more effectively than the electric bell. The reason for this remains to be clarified, but it is probably due to the difference in the peak of frequency between the electric bell and key jingling ; sound stimulation by the former bell has peaks at 1,000 and 1,600 Hz, while that by key jingling has a peak at around 480 Hz, with a

range from 300 to 2,500 Hz.

A recent experimental study using brain chimera technology on 12-somite stage chick embryos with hereditary reflex epilepsy (in response to either light or sound stimulations), demonstrated that the mesencephalon contains the generator of the epileptic manifestations of running and generalized convulsions [3]; the study suggested that the brainstem is important as a possible focus of epileptic seizures. Our present study revealed that NMDA microinjection into the rat MRF induces varied seizure patterns, including GTCS, and also results in increased seizure susceptibility to sound stimulation. Our findings provide an insight into the mechanism of epileptic seizures arising from the brainstem, suggesting that the potentiation of excitatory neurotransmission in the MRF may play a facilitory effect on the generation of epileptic seizures.

Reference

［1］ T. Asakura and J.A. Wada, Neurobiology of audiogenic seizure, Brain and Nerve (Tokyo) 24（1972）513-534 (in Japanese).

［2］ G.F. Ayala, M. Dichter, R.J. Gumnit, H. Matsumoto and W.A. Spencer, Genesis of epileptic interictal spikes. New knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms, Brain Res. 52（1973）1-17.

［3］ C. Batini, M-A. Teillet, R. Naquet and N.M. Le Douarin, Brain chimeras in birds：application to the study of a genetic form of reflex epilepsy, Trends Neurosci. 19（1996）246-252.

［4］ F. Bergmann, A. Costin and J. Gutman, A low threshold convulsive area in the rabbit's mesencephalon, Electroencephalogr. Clin. Neurophysiol. 15(1963) 683-690.

［5］ R.A. Browning, C. Wang and C.L. Faingold, Effect of norepinephrine depletion on audiogenic-like seizures elicited by microinfusion of an excitant amino acid into the inferior colliculus of normal rats, Exp. Neurol. 112(1991) 200-205.

［6］ W.M. Burnham, P. Albright, J. Schneiderman, P. Chiu and T. Ninchoji, “Centrencephalic” mechanisms in the kindling model, In : J.A. Wada (Ed), Kindling 2 , Raven Press, New York, 1981, pp. 161-178.

［7］D.P. Cain, Kindling by repeated intraperitoneal or intracerebral injection of picrotoxin transfers to electrical kindling, Exp. Neurol. 97 (1987) 243-254.

［8］A.G. Chapman, C.L. Faingold, G.P. Hart, H.M. Bowker and B.S. Meldrum, Brain regional amino acid levels in seizure susceptible rats : changes related to sound-induced seizures, Neurochem. Int. 8 (1986) 273-279.

［9］. Chiba, S. Kamata, A. Nunomura, F. Mutoh, M. Matsumoto and T. Miyagishi , Convulsive response induced by microinjections of bicuculline methiodide into the brainstem reticular formation in rats, Hokkaido Epilepsy Research 15（1994）39-44.

［10］S. Chiba, N. Omori, S. Kamata, A. Nunomura and F. Mutoh, Kindling of the mesencephalic reticular formation and its influence on

subsequent amygdala kindling in rats, Epilepsia 37 (Suppl.3) (1996) 116-117.

［11］ S. Chiba and J.A. Wada, Amygdala kindling in rats with brainstem bisection, Brain Res. 682 (1995) 50-54.

［12］ S. Chiba and J.A. Wada, Kindling of the interpeduncular nucleus and its influence on subsequent amygdala kindling in rats, Epilepsia 36 (1995) 410-415.

［13］ S. Chiba and J.A. Wada, The effect of electolytic lesioning of the midbrain prior to amygdala kindling in rats, Neurosci. Lett. 227 (1997) 83-86.

［14］ M.J. Croucher, K.L. Cotterell and H.F. Bradford, Amygdaloid kindling by repeated focal N-methyl-D-aspartate administration : comparison with electrical kindling, Eur. J. Pharmacol., 286(1995) 265-271.

［15］ M.J. Croucher, K.L. Ruffle and H.F. Bradford, The effects of focal N-methyl-D-aspartate pretreatment on the parameters of amygdaloid electrical kindling, Eur. J. Pharmacol. 319(1997) 207- 213.

［16］ M.J. During, K.M. Ryder and D.D. Spencer, Hippocampal GABA transporter function in temporal-lobe epilepsy, Nature 376 (1995) 174-177.

［17］ M.J. During and D.D.Spencer, Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain, Lancet 341(1993) 1607-1611.

［18］ C.L. Faingold, M.J. Marcinczyk, D.J. Casebeer, M.E. Randall, S.P. Arneric and R.A. Browning, GABA in the inferior colliculus plays a critical role in control of audiogenic seizures, Brain Res. 640（1994）40-47.

［19］ C.L. Faingold, M.H. Millan, C.A. Boersma Anderson and B.S. Meldrum, Induction of audiogenic seizures in normal and genetically epilepsy-prone rats following focal microinjection of an excitant amino acid into reticular formation and auditory nuclei, Epilepsy Res. 3（1989） 199-205.

［20］ C.L. Faingold, D.K. Naritoku, C.A. Copley, M.E. Randall, A. Riaz, C.A. Boersma Anderson and S.P. Arneric, Glutamate in the inferior colliculus plays a critical role in audiogenic seizure initiation, Epilepsy

Res. 13（1992）95-105.

［21］C.L. Faingold and M.E. Randall, Pontine reticular formation neurons exhibit a premature and precipitous increase in acoustic responses prior to audiogenic seizures in genetically epilepsy-prone rats, Brain Res. 704（1995）218-226.

［22］C.L. Faingold, M.E. Randall, D.K. Naritoku and C.A. Boersma Anderson, Noncompetitive and competitive NMDA antagonists exert anticonvulsant effects by actions on different sites within the neuronal network for audiogenic seizures, Exp. Neurol. 119（1993）198-204.

［23］W.J. Griffiths, Absence of audigenic seizures in wild Norway and Alexandrine rats, Science 99（1944）62-63.

［24］Y. Hirayasu and J.A. Wada, Convulsive seizures in rats induced by N-methyl-D-aspartate injection into the massa intermedia, Brain Res. 57（1992）36-40.

［25］Y. Hirayasu and J.A. Wada, N-methyl-D-aspartate injection into the massa intermedia facilitates development of limbic kindling in rats, Epilepsia 33（1992）965-970.

［26］ H.H. Jasper, Current evaluation of the concepts of centrencephalic and cortico-reticular seizures, Electroencephalogr. Clin. Neurophysiol. 78 （1991）2-11.

［27］ A. Kreindler, E. Zuckermann, M. Steriade and D. Chimion, Electroclinical features of convulsions induced by stimulation of brain stem, J. Neurophysiol. 21（1958）430-436.

［28］ S.M. Lasley, Roles of neurotransmitter amino acids in seizure severity and experience in the genetically epilepsy-prone rat , Brain Res. 560 (1991) 63-70.

［29］ N.R.F. Maier and N.M. Glaser, Studies of abnormal behavior in the rat. II. A comparison of some convulsion-producing situations, Comp. Psychol. Monographs 16（1940）1-30.

［30］ H. Matsumoto and C. Ajmone-Marsan, Cortical cellular phenomena in experimental epilepsy： ictal manifestations, Exp. Neurol. 9（1964）305-326.

［31］ M.H. Millan, B.S. Meldrum, C.A. Boersma Anderson and C.L. Faingold, Excitant amino acids and audgenic seizures in the genetically

epilepsy- prone rat. II. Efferent seizure propagating pathway, Exp. Neurol. 99 (1988) 687-698.

[32] Y. Mirovsky, G.C, Wagner, A. Sekowski, M. Goldberg and H. Fisher, Stimultaneous changes in striatal dopamine, serotonin, and metabolites after withdrawal seizures in rats from dependence on alcohol, Alcohol 12 (1995) 251-256.

[33] N. Mori and J.A. Wada, Bidirectional transfer between kindling induced by excitatory amino acids and electrical stimulation, Brain Res. 425 (1987) 45-48.

[34] K. Morimoto, S.E. Mason and G.V. Goddard, Kindling-induced changes in the EEG recorded during stimulation from the site of stimulation. II. comparison between spontaneous and evoked potentials, Exp. Neurol. 97(1987) 1-16.

[35] K. Morimoto, S.E. Mason and G.V. Goddard, Kindling-induced changes in the EEG recorded during stimulation from the site of stimulation. III. direct pharmacological manipulations of the kindled amygdala, Exp. Neurol. 97(1987) 17-34.

［36］K. Morimoto and N. Yamada, Abnormal neurotransmission in experimental models of epilepsy, Shinkei Kenkyu no Shinpo 38 (1994) 823-837 (in Japanese).

［37］R. Nieuwenhuys, J. Voogt and C. van. Huijzen(Eds.), The Human Nervous System, A Synopsis and Atlas, 3rd edn., Spring-Verlag, Berlin 1988.

［38］M. Osonoe, N. Mori, S. Hoshino, Y. Yamada, K. Osonoe, H. Kittaka and Y. Iwata, The effects of N-methyl-D-aspartate (NMDA) and its competitive antagonist, 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP), injected into caudate-putamen on kindled amygdaloid seizures in rats, Brain Res., 728(1996) 242-246.

［39］G. Paxinos and C. Watson (Eds.), The Rat Brain in Stereotaxic Coordinates, 2nd edn., Academic Press, Sydney, 1986.

［40］W. Penfield and H.H. Jasper (Eds.), Epilepsy and the Functional Anatomy of the Human Brain. Little, Brown and Company, Boston, 1954.

［41］C.E. Ribak, M.Y. Byun, G.T. Ruiz and R.J. Reiffenstein, Increased levels of amino acid neurotransmitters in the inferior colliculus of the

genetically epilepsy-prone rat, Epilepsy Res. 2(1988) 9-13.

［42］ K. Sato, M. Morimoto and M. Okamoto, Anticonvulsant action of a non-competitive antagonist of NMDA receptors（MK-801）in the kindling model of epilepsy，Brain Res. 463（1988）12-20.

［43］T. Tanaka，S. Tanaka, T. Fujita, K. Takano, H. Fukuda , K. Sako and Y. Yonemasu，Experimental complex partial seizures induced by a microinjection of kainic acid into limbic structures，Prog. Neurobiol. 38(1992) 317-334.

［44］ Y.Ueda and N.Tsuru, Sequential change of glutamate release in bilateral hippocampi in amygdaloid kindling rat. Jpn. J. Psychiat. Neurol. 47(1993) 225-228.

［45］ S. Uemura and H. Kimura, Amygdaloid kindling with bicuculline methiodide in rats，Exp. Neurol. 102(1988) 346-353.

Figure Legends

Fig.1. Distribution of MRF electrodes placement in Groups A, B and C. The triangles indicate the sites out of the intended area. Stereotaxic coordinates in mm from Paxinos and Watson［39］for the deep mesencephalic nucleus ; 5.8 mm posterior , 1.7 mm lateral from bregma , 6.6 mm from the skull.

Fig. 2. EEGs of hyperactivity and running／circling patterns in a Group A rat. From 62 sec after the NMDA injection, the rat displayed jumping , hyperactivity, and running／circling. NMDA, N-methyl-D-aspartate ; LMCO, left motor cortex ; LMRF, left mesencephalic reticular formation ; REF, reference electrode.

Fig. 3. EEGs of GTCS in a Group A rat. In the rat, GTCS appeared initially at 10 min 20 sec after NMDA microinjection. GTCS, generalized tonic-clonic seizures ; NMDA, N-methyl-D-aspartate ; LMCO, left motor cortex ; LMRF, left mesencephalic reticular formation ; REF, reference electrode.

Fig. 4. EEGs of GTCS elicited by sound stimulation at 15 min after NMDA injection. Sound stimulation applied at 15, 30 and 45 min after NMDA injections could induce hyperactivity, running/circling, and GTCS. These seizure patterns and their EEG findings observed in Exp. 2 were almost identical to those observed in Exp. 1. GTCS, generalized tonic-clonic seizures ; NMDA, N-methyl-D-aspartate ; LMCO, left motor cortex ; LMRF, left mesencephalic reticular formation ; Ref, reference electrode ; SS, sound stimulation.

Tables

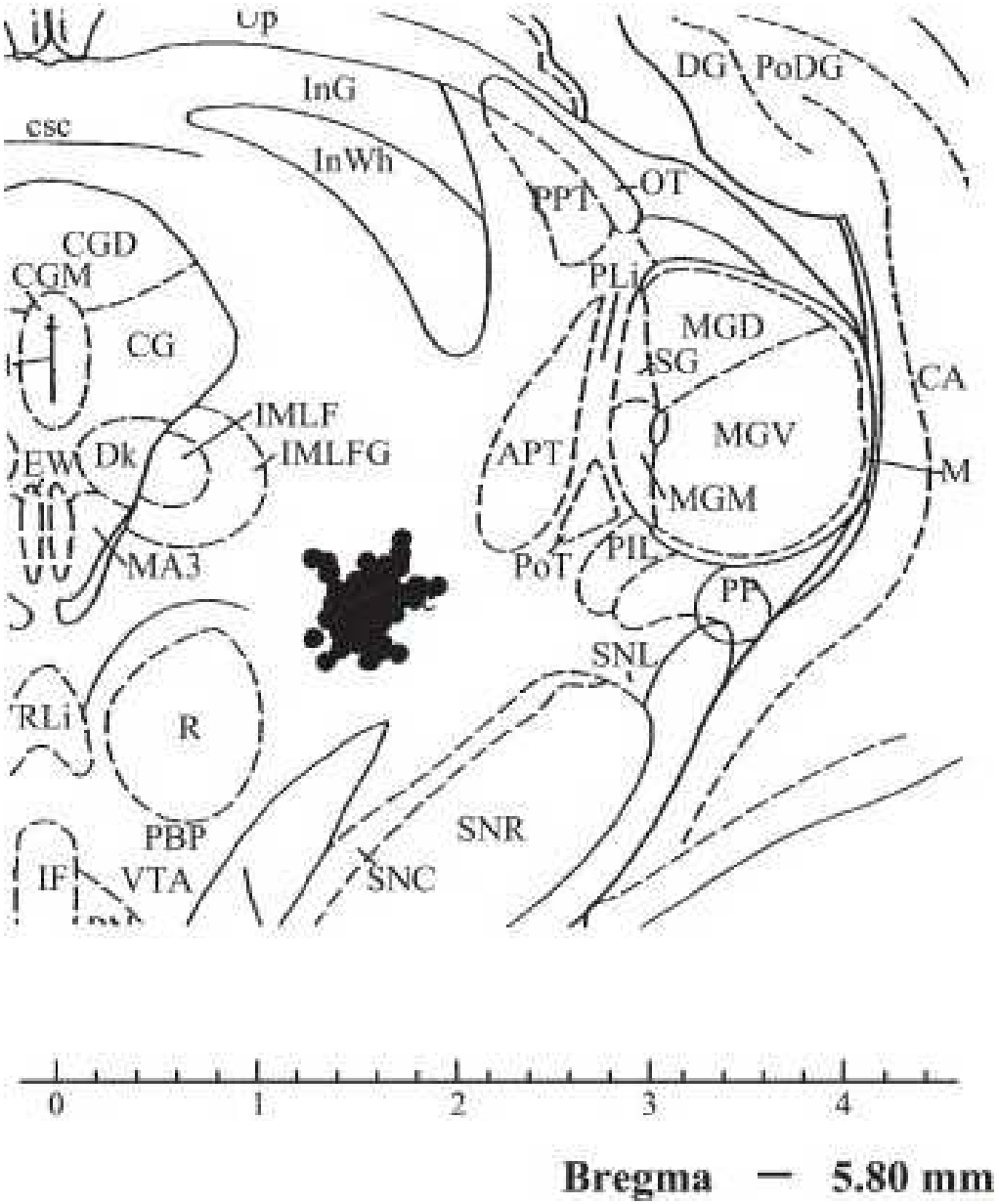
Table 1. The incidence of the seizure patterns induced by NMDA injections in each Group (Exp.1). Seizures were classified into 3 patterns: hyperactivity, running/circling and GTCS (generalized tonic-clonic seizures), and the incidence was analyzed using Fisher's exact probability test among Groups A , B and C . NMDA, N-methyl-D-aspartate ; GTCS, generalized tonic-clonic seizures. *P<0.05, **P<0.01 by Fisher's exact test.

	hyperactivity		running/circling		GTCS	
Group A	**8／8**	******	**8／8**	******	**5／8**	***** *****
（10 nmol, n=8）						
Group B	**9／10**	******	**8／10**	******	**0／10**	
（2 nmol, n=10）						
Group C	**0／10**		**0／10**		**0／10**	
(saline, n=10)						

Table 2. The incidence of the seizure patterns induced by sound stimulation in each Group (Exp.2). Sound stimulation induced seizures were classified into 3 patterns: hyperactivity, running/circling, and GTCS. For each figure, the numerator indicates the total number of rats which showed the seizure pattern induced by sound stimulation applied at either 15, 30, or 45 min after the end of NMDA injections. The incidence was analyzed using Fisher's exact probability test among the three groups. GTCS, generalized tonic-clonic seizures ; NMDA, N-methyl-D-aspartate.*P<0.05, **P<0.01 by Fisher's exact test.

	hyperactivity		**running/circling**		**GTCS**	
Group A	**8／8**		**7／8**		**5／8**	
（10 nmol, n=8）		******		******		*****
Group B	**3／10**	******	**0／10**	******	**0／10**	*****
（2 nmol, n=10）						
Group C	**0／10**		**0／10**		**0／10**	
(saline, n=10)						

Fig.1



Bregma — 5.80 mm

Fig. 2 *(NMDA microinjection)*

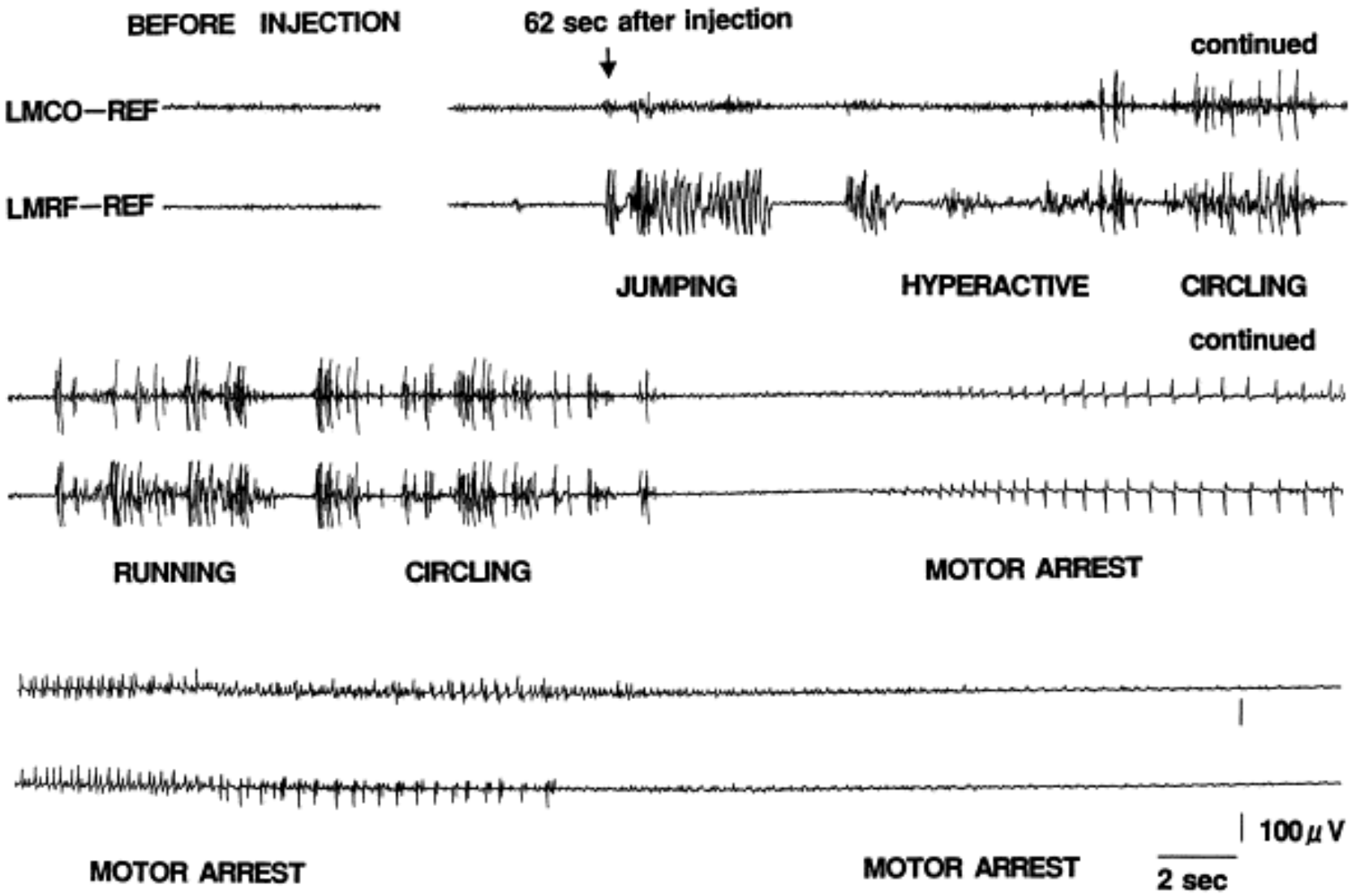


Fig. 3 *(NMDA microinjection)*

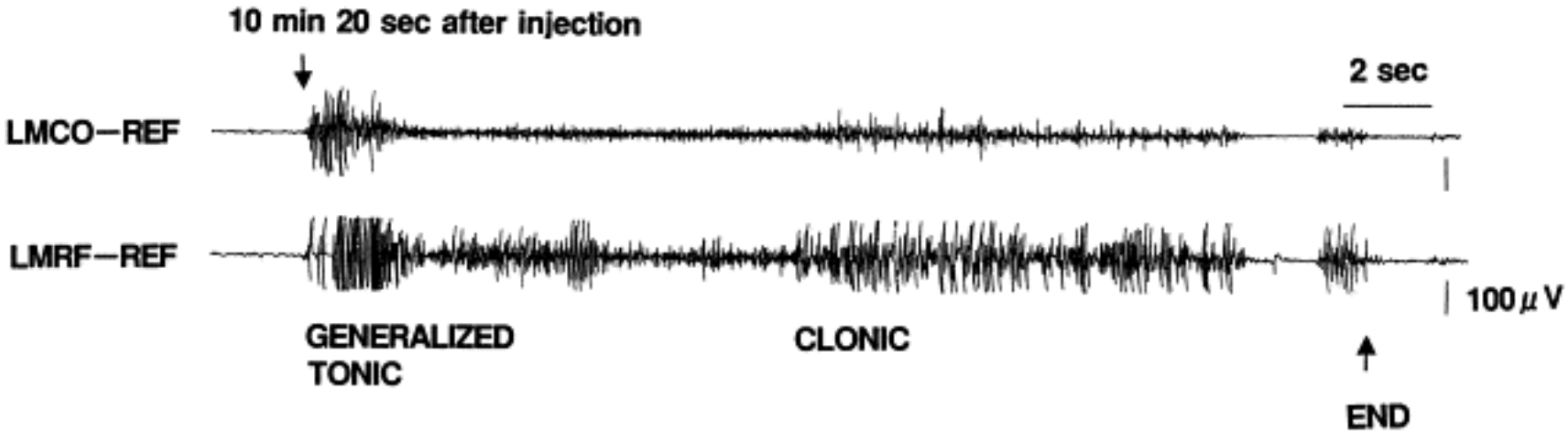
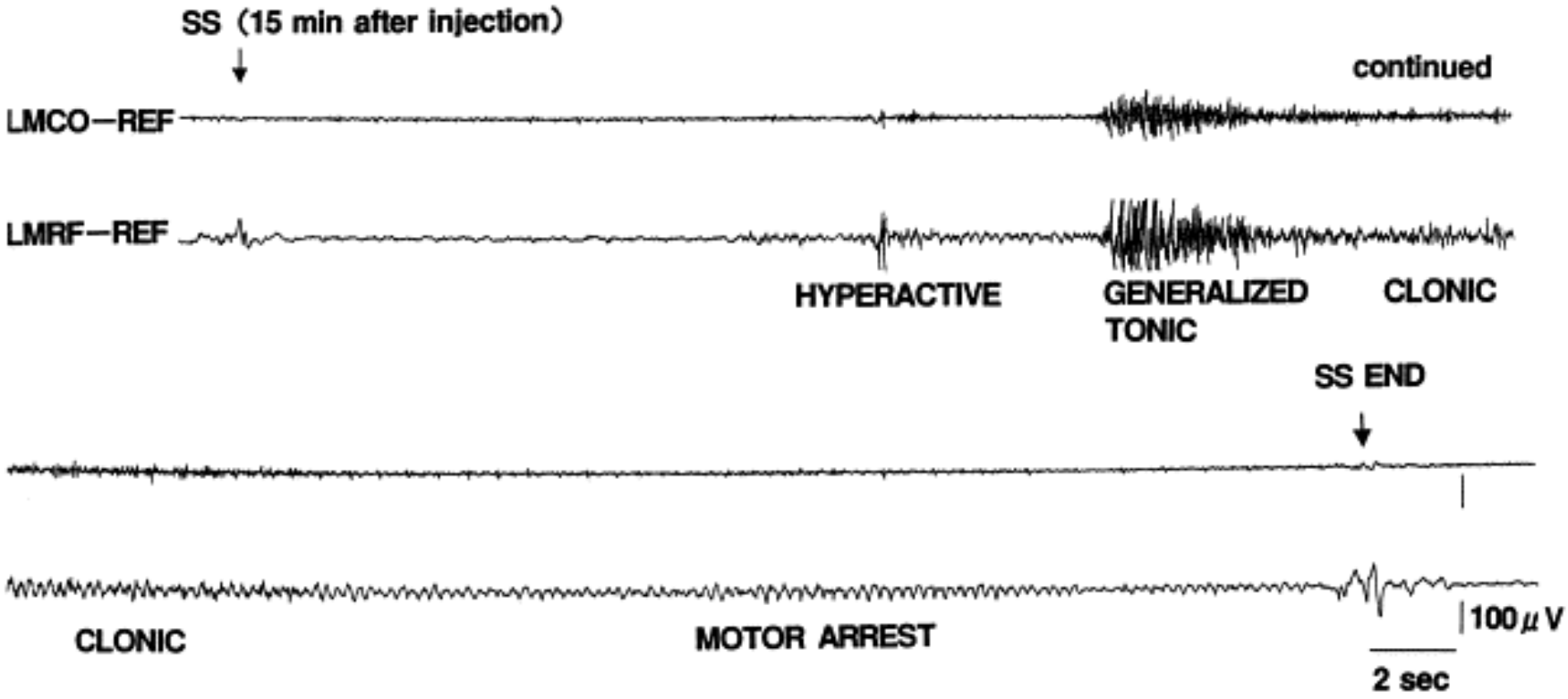


Fig. 4 ***(NMDA microinjection)***



SS, Sound Stimulations