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Abstract

Effects of microinjections of a single 2 or 10 nmol dose of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) into the unilateral mesencephalic reticular formation (MRF) on behavior and on the electroencephalogram were examined in rats (n= 30) over a 15 min period (Exp. 1); subsequent effects of sound stimulation with key jingling applied at 15, 30, and 45 min after the injection were observed (Exp. 2). The microinjections of a 2 nmol dose of AMPA (n=15) induced hyperactivity (15 of 15 rats) and running/circling (10 of 15 rats) in Exp. 1, and hyperactivity (5 of 15 rats) in Exp. 2. Moreover, the microinjections of a 10 nmol dose of AMPA (n=15) induced hyperactivity (15 of 15 rats), running/circling (13 of 15 rats), generalized tonic-clonic seizures (GTCS) (4 of 15 rats), and amygdala kindling-like seizures (AMKS) (8 of 15 rats) in Exp. 1; electroencephalographic seizure discharges were predominantly

observed in the MRF during hyperactivity, running/circling and GTCS, while those predominantly observed in the amygdala were during AMKS. In Exp. 2, hyperactivity (15 of 15 rats), running/circling (14 of 15 rats) and GTCS (6 of 15 rats) were elicited by sound stimulation, although AMKS were not. The control group rats (n=15) 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 potentiation of excitatory amino acid neurotransmission induced by AMPA injection into the MRF plays an important role not only in the development of hyperactivity, running/circling, GTCS and AMKS, but also in the development of audiogenic seizures.

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1. Introduction

An imbalance between inhibitory and excitatory neurotransmission is considered to be associated with the generation and expression of human ［18, 19］and animal epileptic seizures［2, 41, 52］. Regarding inhibitory neurotransmission, repeated administration of the γ-aminobutyric acid (GABA) receptor antagonist, picrotoxin［9］or bicuculline［53］, into the unilateral amygdala (AM) induces the development of AM seizure in rats. Systemic administration of the GABA receptor agonist, muscimol, strongly suppresses AM kindled seizures［41, 42］in rats. In contrast, in excitatory neurotransmission, systemic administration of N-metyl-D-aspartate (NMDA)［40］, or the focal administration of NMDA to the AM［15, 16, 17］, massa intermedia［31, 32］, or substantia nigra pars reticulata ［49,50］, produces convulsive seizures. The focal injection of kainic acid into the limbic structures［51］also produces convulsive seizures. Besides, 3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonic acid (CPP) ［45］, a competitive NMDA receptor antagonist, or dizocilpine (MK-801)

［48］,a non-competitive NMDA receptors antagonist, has potent inhibitory effects on the development of AM kindling in rats . α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) is known to be an excitatory amino acid. Some clinical studies suggest that AMPA-sensitive quisqualate receptor binding［33］or AMPA receptor mRNA［27］increased in the epileptogenic hippocampus in the human brain. 2,3-dihydroxy-6-amino-7-sulfamoyl-benzo(F)-quinoxaline (NBQX) ［43］, a selective AMPA receptor antagonist, was also shown to have potent inhibitory effects on the development of AM kindling in rats. Therefore, potentiation of excitatory neurotransmission seems to play a crucial role in the development of several experimental models of epilepsy.

Experimental studies suggest that the brainstem reticular formation (RF), particularly the mesencephalic RF (MRF), is involved not only in the secondary generalization of partial seizures originating from the forebrain ［13, 14, 34］ but also in the generation of primary generalized seizures［4, 8, 11, 12, 34, 35］. Electrical stimulation of the

unilateral MRF induces generalized convulsions in rodents［4, 8］ and cats［35］. The MRF can be kindled with the development of generalized convulsions in rats［12］. Local administration of bicuculline, a GABA antagonist, to the unilateral MRF induces generalized tonic seizures in rats［11］. Moreover, our previous study indicates that NMDA microinjection into the rat MRF induces varied seizure patterns, including generalized tonic clonic seizures (GTCS), and also results in increased seizure susceptibility to sound stimulation［34］. In genetically epilepsy-prone rats (GEPR-9s) the propagation of seizure discharges from the inferior colliculus to the brainstem RF is considered to be important for the development of audiogenic seizures［6, 22, 25］. Several brain chimera studies on 12-somite stage Fayoumi strain of chickens (FEpi) embryos with hereditary reflex epilepsy demonstrate that the mesencephalon contains the generator of the epileptic manifestations of running and generalized convulsions［3, 20, 29, 30, 44］. In addition, the mesencephalic neurons of FEpi are reported to be highly sensitive to high-frequency photic stimulation, capable of producing bursts during intermittent light stimulation [29,

30].

To further clarify the role of the MRF in the expression of epileptic seizures, we microinjected AMPA 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 AMPA microinjections on the behavioral and EEG changes in the animals (Exp. 2) .

2. Materials and Methods

Exp. 1 : AMPA injection

Forty-five male adult Sprague-Dawley rats (2-3 months of age, weighing 250-400 g) were used in this study. The rats were randomly assigned to Group A (n=15), B (n=15), or C (n=15). pentobarbital anesthesia, chemitrodes, i.e., 24G guide cannulas with bipolar electrodes made of twisted stainless steel wire (200 μm in diameter), were implanted stereotaxically [46] 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 the bipolar electrodes extended 1.0 mm beyond the ends of the guide cannulas. In addition, bipolar electrodes made of twisted stainless steel wire (200 μm in diameter), were implanted stereotaxically [46] into the left AM (2.8 mm posterior , 5.0 mm lateral from bregma, and 9.0 mm ventral from the skull) for AM recording electrode. Two surface electrodes (stainless steel screws) were driven into the skull; one for recording from the left sensorimotor cortex, and the other, over the left olfactory bulb, as the reference electrode.

Seven days after the operation, a single 10 nmol or 2 nmol dose of AMPA (Sigma, St Louis, MO, USA) was administered into the MRF in Groups A and B, respectively (Group A received 10 nmol and Group B received 2 nmol). The dose of AMPA 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 left 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 AMPA/saline microinjections in Exp. 1.

Exp. 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 the AMPA or saline microinjections in Exp. 1. Although there are several sound stimulation methods using key jingling [1, 27, 33, 36, 38], the sound stimulation in our Exp. 2 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) ［34］; the frequency and intensity of the sound was reported elsewhere ［34］.

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

3. Results

Histological examination revealed that the chemitrodes were located in the intended area (within 0.5 mm of the target site) in all the rats used (Fig.1).

Exp. 1

The microinjections of 2 nmol dose of AMPA induced only hyperactivity and running/circling. The microinjections of 10 nmol dose of AMPA induced the following seizure 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 which consisted of sudden running straightforward and circling in the open-topped observation box without their regular direction; (3) GTCS (which involved both forelimb and hindlimb extension); and (4) amygdala kindling-like seizures (AMKS) which consisted of facial movements, head nodding, bilateral forelimb clonus, and rearing. As shown in Figs. 2 and

3, electroencephalographic seizure discharges were predominantly observed in the MRF during hyperactivity, running/circling and GTCS, while those predominantly observed in the amygdala occurred during AMKS. AMKS was observed in 8 rats that exhibited hyperactivity and running/circling in the Group A, and AMKS was not observed earlier than hyperactivity and running/circling. Additionally, AMKS was usually observed within 15 min since the rats received AMPA microinjection. The Group C rats did not show any behavioral or electroencephalographic changes during the 15 min following 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 incidences of GTCS and AMKS were significantly higher in Group A than in Groups B and C.

Exp. 2

Although AMPA-induced seizures were not observed during the period from 15 to 45 min after the end of AMPA injections, sound stimulation

applied at 15, 30 and 45 min after AMPA injections induced hyperactivity (Groups A and B), running/circling (only Group A), and GTCS (only Group A), but did not produce AMKS. The EEG findings observed in Exp. 2 were similar to those observed in Exp. 1 (Fig. 4). In Group C, no behavioral or EEG changes were elicited by sound stimulation. The incidences of the seizure patterns in Groups A, B, and C are summarized in Table 2. The incidence of hyperactivity, running/circling and GTCS was significantly higher in Group A than in Groups B and C.

4 . Discussion

In Exp. 1, the microinjection of a 10 nmol dose of AMPA into the unilateral MRF could induce seizure patterns in the following general order: hyperactivity, running/circling, GTCS, and AMKS. These behavioral seizure patterns were accompanied by electrographic seizure discharges in the MRF, amygdala, and the motor cortex. Injection of a 2 nmol dose of AMPA also induced hyperactivity and running/circling, but did not produce GTCS or AMKS. These findings suggest that

potentiation of excitatory neurotransmission in the MRF contributes to 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 MRF.

In a separate study, we examined effects of the microinjection of a single 10 nmol dose of AMPA in two rats with guide cannulas positioned outside of the intended MRF area. One of these animals, with the guide cannula located 1.0 mm dorsally, exhibited only mild hyperactivity without electroencephalographic seizure changes, while the other, with its guide cannula located 0.5 mm ventrally and 0.4 mm laterally, showed mild hyperactivity and running/circling behavior, without electroencephalographic seizure discharges. These findings suggested that varied seizure patterns (hyperactivity, running/circling, GTCS and AMKS) observed in Exp. 1 were specifically derived from the activation of the intended MRF area by AMPA microinjection.

The results of Exp. 1 support the view that generalized seizures can originate from the MRF ［4, 8, 11, 12, 35］. However, the incidence of GTCS induced by AMPA (10 nmol) injection in Exp. 1 was lower

compared with that induced by NMDA (10 nmol) injection into the MRF in our previous study［34］(27% and 63%, respectively). Therefore, the development of GTCS in which the cortico-reticular system is considered to be involved seems to be easily produced by excitation of NMDA rather than AMPA neurotransmission. This speculation is not inconsistent with the fact ［54］that in audiogenic seizures after propylthiouracil administration in rats, the development of GTCS is mainly controlled by excitation of NMDA rather than AMPA neurotransmission. In contrast, AMKS was induced by the AMPA microinjection, not by NMDA ［34］, into the MRF, probably due to excitation of the limbic-brain stem connection.

In Exp. 2, sound stimulation applied at 15, 30 and 45 min after a 10 nmol of AMPA injection into the MRF could induce the seizure patterns (hyperactivity, running/circling, and GTCS) similar to those observed in Exp. 1, although similar sound stimulation after a 2 nmol of AMPA injection into the MRF induced only hyperactivity. These results imply that the rats which received the injections of AMPA were susceptible to varied audiogenic seizures, and suggest that potentiation of

excitatory neurotransmission induced by AMPA, as well as NMDA [34], in the MRF had a facilitatory effect on the development of audiogenic seizures. Genetically epilepsy prone rats (GEPR-9s) with audiogenic seizures (running and GTCS) show increased levels of glutamate and aspartate in the inferior colliculus (IC) and the brainstem RF［10, 21, 23, 24, 36, 38, 47］. Bilateral microinjections into the IC or the pontine RF of exitatory amino acid receptor antagonists block audiogenic seizures in GEPR-9s［26］. In addition, the initiation site of the audiogenic seizures is considered to be the IC; the IC activates brainstem RF neurons, with the neuronal firing rate of the brainstem RF being increased markedly at the onset of the audiogenic tonic seizure［21, 25］. Therefore, in the rats of Exp. 2, it is assumed that the propagation of seizure discharges from the IC to the brainstem RF is crucial for the development of audiogenic seizures and that the brainstem RF, especially MRF, neurons play a major role in the generation of audiogenic seizures.

In Exp.2, sound stimulation after AMPA injection did not induce AMKS, although it did induce the electroencephalographic seizure propagation from MRF to the limbic stuructures. This finding indicates

that audiogenic stimulation does not result in spontaneous epileptoginic excitation of the limbic structures, but merely exhibits electroencephalographic seizure propagation from MRF to the limbic structures. Some experimental researches indicated that facial and forelimb clonus with or without rearing depends on a forebrain network, while running and bouncing clonus as well as tonic extension depend on a brainstem circuitry, and transactions at precollicular level have been shown to separate these seizure behaviors whether produced by sound stimulation, electroshock or chemoconvulsants [5,7].

In conclusion, our study provides a further insight into the excitatory mechanism of epileptic seizures arising from the brainstem, suggesting that potentiation of excitatory neurotransmission in the MRF induced by AMPA microinjection plays an important role in the development of limbic seizures as well as GTCS, and results in an increased susceptibility to audiogenic running seizures and GTCS.

Reference

［1］ T. Asakura and J.A. Wada, Neurobiology of audiogenic seizure, No to Shinkei (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 and D.K. Nelson, Modification of electroshock and pentylenetetrazol seizure patterns in rats after precollicular transactions, Exp. Neurol. 93 (1986) 546-556.

［6］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.

[7] R.A. Browning, C. Wang, D.K. Nelson and P.C. Jobe, Effect of precollicular transection on audiogenic seizures in genetically epilepsy-prone rats, Exp. Neurol. 155 (1999) 295-301.

[8] R.A. 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.

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

[10] 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.

[11] 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.

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

［13］ 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.

［14］ 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.

［15］ 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.

［16］M.J. Croucher, K.L. Cotterell and H.F. Bradford, Characterisation of N-methyl-D-aspartate (NMDA)- induced kindling, Biochem. Soc. Trans. 24 (1996) 295S.

［17］ 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.

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

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

［20］ N. Fadlallah, N. Guy, M.A. Teillet, B. Schuler, N.M. Le Douarin, R. Naquet and C. Batini, Brain chimeras for the study of an avian model of genetic epilepsy : structures involved in sound and light-induced seizures, Brain Res. 675 (1995) 55-66.

［21］ C.L. Faingold, Neuronal network in the genetically epilepsy-prone rat, In: A.V. Delgado-Escueta, W.A. Wilson, R.W. Olsen, and R.J.Porter (Eds.) Advances in Neurology, vol.79, Lipppincott Willams & Wilkins, Philadelphia, 1999, pp. 311-321.

［22］ 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.

[23] 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.

[24] 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.

[25] 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.

[26] 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.

[27] F.J. Garcia-Ladona, J.M. Palacios, A. Probst, H.G. Wieser and G. Mengod, Exitatory amino acid AMPA receptor mRNA localization in several resions of normal and neurological disease affected human brain. An in situ hybridization histochemistry study, Brain Res. Mol. Brain Res. 21 (1994) 75-84.

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

[29] N.T. Guy, C. Batini, R. Naquet, and M.A. Teillet, Avian photogenic epilepsy and embryonic brain chimeras: neuronal activity of the adult prosencenphalon and mesencephalon, Exp. Brain Res 93 (1993) 196-204.

[30] N.T. Guy, N. Fadlallah, R. Naquet and C. Batini, Development of epileptic activity in embryos and newly hatched chicks of the Fayoumi mutant chicken, Epilepsia 36 (1995) 101-7.

[31] 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.

［32］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.

［33］D.A. Hosford, B.J. Crain, Z. Cao, D.W. Bonhaus, A.H. Friedman, M.M. Okazaki, J.V. Nadler, and J.O. Mcnamara, Increase AMPA-sensitive quisqualate receptor binding and reduced NMDA receptor binding in epileptic human hippocampus, J. Neurosci. 11 (1991) 428-34.

［34］T. Ishimoto, N. Omori, F. Mutoh and S.Chiba, Convulsive seizures induced by N-methyl-D-aspartate microinjection into the mesencephalic reticular formation in rats, Brain Res. 881 (2000) 152-158.

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

［36］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.

［37］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.

[38] 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.

[39] 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.

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

[41] 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.

[42] K. Morimoto and N. Yamada, Abnormal neurotransmission in

experimental models of epilepsy, Shinkei Kenkyu no Shinpo 38 (1994) 823-837 (in Japanese).

[43] T.Namba, K. Morimoto, K. Sato, N. Yamada and S. kuroda, Antiepileptogenic and anticonvulsant effects of NBQX, a selective AMPA receptor antagonist, in the rat kindling model of epilepsy, Brain Res. 638 (1994) 36-44.

[44] R. Naquet and A. Valin, Experimental models of reflex epilepsy, In: B.G. Zifkin, F. Andermann, A. Beaumanoir, and A.J. Rowan (Eds.) Advances in Neurology, vol.75, Lipppincott Willams & Wilkins, Philadelphia, 1998, pp. 311-321.

[45] 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.

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

[47] 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.

［48］ 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.

［49］ C. Shin, F.A. Scialabba and J.O. McNamara, Stimulation of substantia nigra pars reticulata enhances dentate granule cell excitability, Brain Res. 411 (1987) 21-7.

［50］ C. Shin, J.M. Silver, D.W. Bonhaus and J.O. Mcnamara, The role of substantia nigra in the development of kindling: pharmacologic and leision studies, Brain Res. 412 (1987) 311-7.

［51］ 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.

［52］ 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.

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

［54］ S. Yasuda, N. Ishida, A. Higashiyama and N.Kato, Running fit and generalized tonic-clonic seizure are differently controlled by different subtype receptors in the brainstem. Jpn. J. Psychopharmacol. 18 (1998) 123-126 (in Japanese).

Figure Legends

Fig.1. Distribution of chemirtodes placement in Groups A, B and C.

Stereotaxic coordinates for the deep mesencephalic nucleus are; 5.8 mm posterior , 1.7 mm lateral from bregma , 6.6 mm from the skull (according to Paxinos and Watson［45］)

Fig. 2. EEGs of hyperactivity, running/circling and GTCS patterns in a Group A rat. At 46 sec after the AMPA injection, the rat displayed hyperactivity and running/circling. GTCS appeared at 14 min and 16 sec after AMPA microinjection.

GTCS, generalized tonic-clonic seizures ; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; LMRF, left mesencephalic reticular formation ; LAM, left amygdala ; LMCO, left motor cortex ; REF, reference electrode.

Fig. 3. EEGs of AMKS in a Group A rat. AMKS, i.e., facial movements, head nodding and bilateral forelimb clonus with rearing appeared initially at 12 min and 10 sec after AMPA microinjection.

AMKS, amygdala kindling-like seizures ; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; LMRF, left mesencephalic reticular formation ; LAM, left amygdala ; LMCO, left motor cortex; REF, reference electrode.

Fig. 4. EEGs of hyperactivity and GTCS elicited by sound stimulation at 30 min after AMPA injection. Sound stimulation applied at 15, 30 and 45 min after AMPA injections induced hyperactivity and GTCS. The seizure patterns and EEG findings in these animals were similar to those observed in the animals in Exp. 1.

GTCS, generalized tonic-clonic seizures ; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; LMRF, left mesencephalic reticular formation ; LAM, left amygdala ; LMCO, left motor cortex ; Ref, reference electrode ; SS, sound stimulation.

Tables

Table 1. The incidence of the seizure patterns induced by AMPA injections in each Group (Exp. 1). Seizures were classified into 4 patterns; hyperactivity, running/circling, GTCS and AMKS.

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ; GTCS, generalized tonic-clonic seizures ; AMKS, amygdala kindling seizure patterns. *P<0.05, **P<0.01 by Fisher's probability exact test.

	hyperactivity	running/circling	GTCS	AMKS
Group A (10 nmol, n=15)	**15/15**	**13/15**	**4/15**	**8/15**
Group B (2 nmol, n=15)	**15/15**	**10/15**	**0/15**	**0/15**
Group C (saline, n=15)	**0/15**	**0/15**	**0/15**	**0/15**

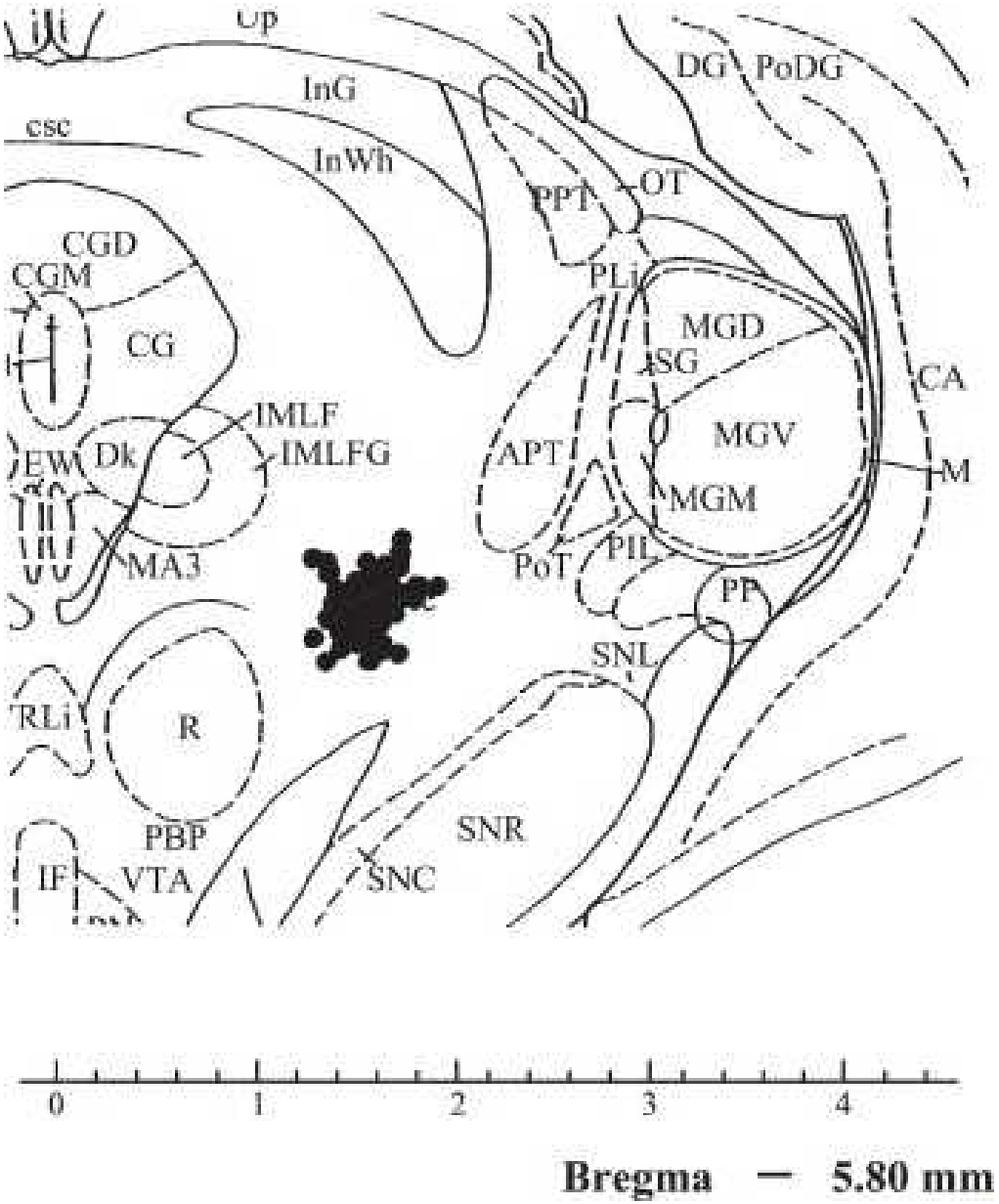
** ** * * ** **

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. In Group C, no behavioral or EEG changes were elicited by sound stimulationGTCS, generalized tonic-clonic seizures ; AMKS, amygdala kindling seizure patterns ; AMPA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid. **P<0.01 by Fisher's exact probability test.

	hyperactivity	**running/circling**	**GTCS**	**AMKS**
Group A (10 nmol, n=15)	**15/15**	**14/15**	**6/15**	**0/15**
Group B (2 nmol, n=15)	**5/15**	**0/15**	**0/15**	**0/15**
Group C (saline, n=15)	**0/15**	**0/15**	**0/15**	**0/15**

** ** ** ** ** **

Fig.1



Bregma — 5.80 mm

Fig.2 AMPA INJECTION (Group A)

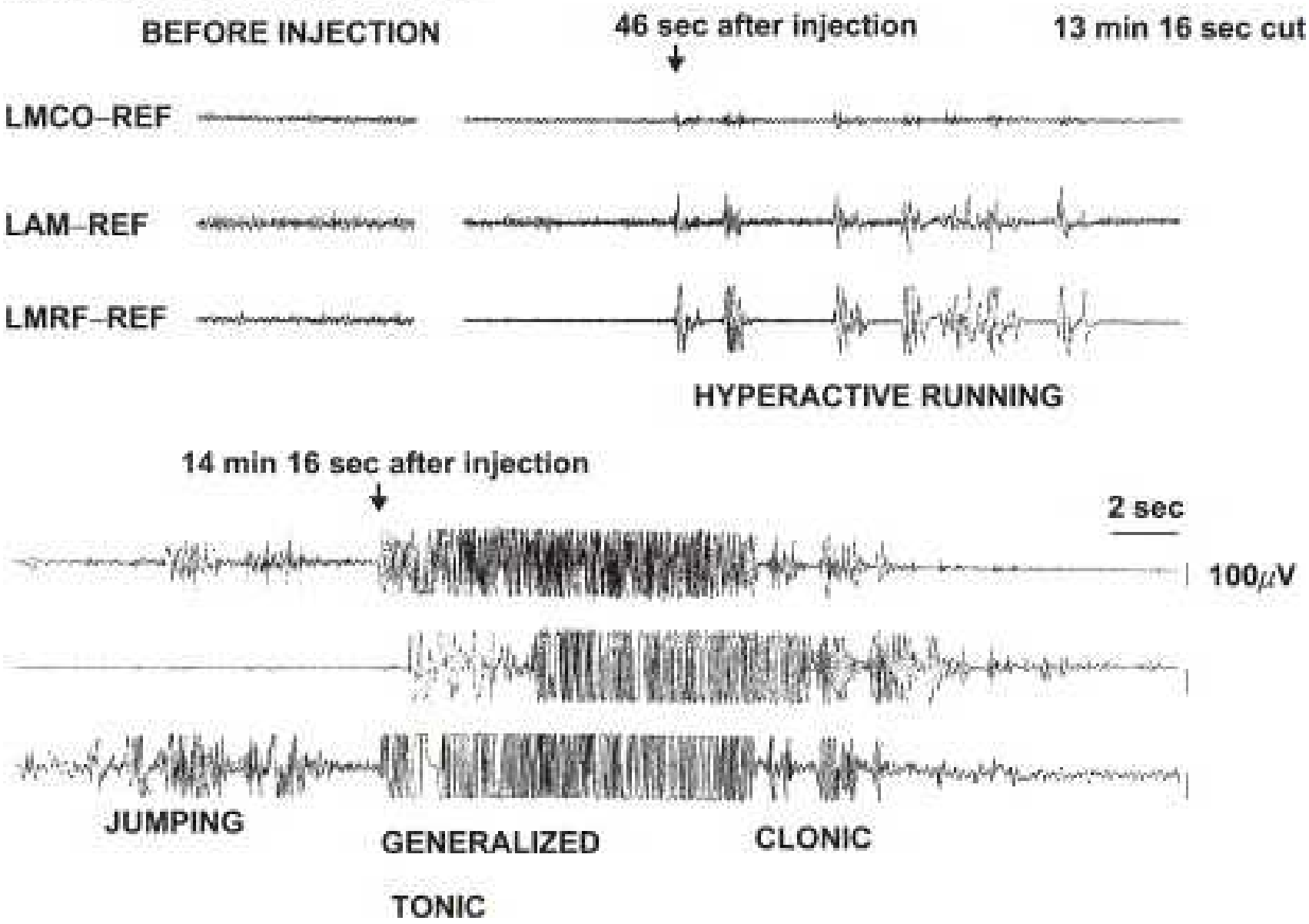


Fig.3

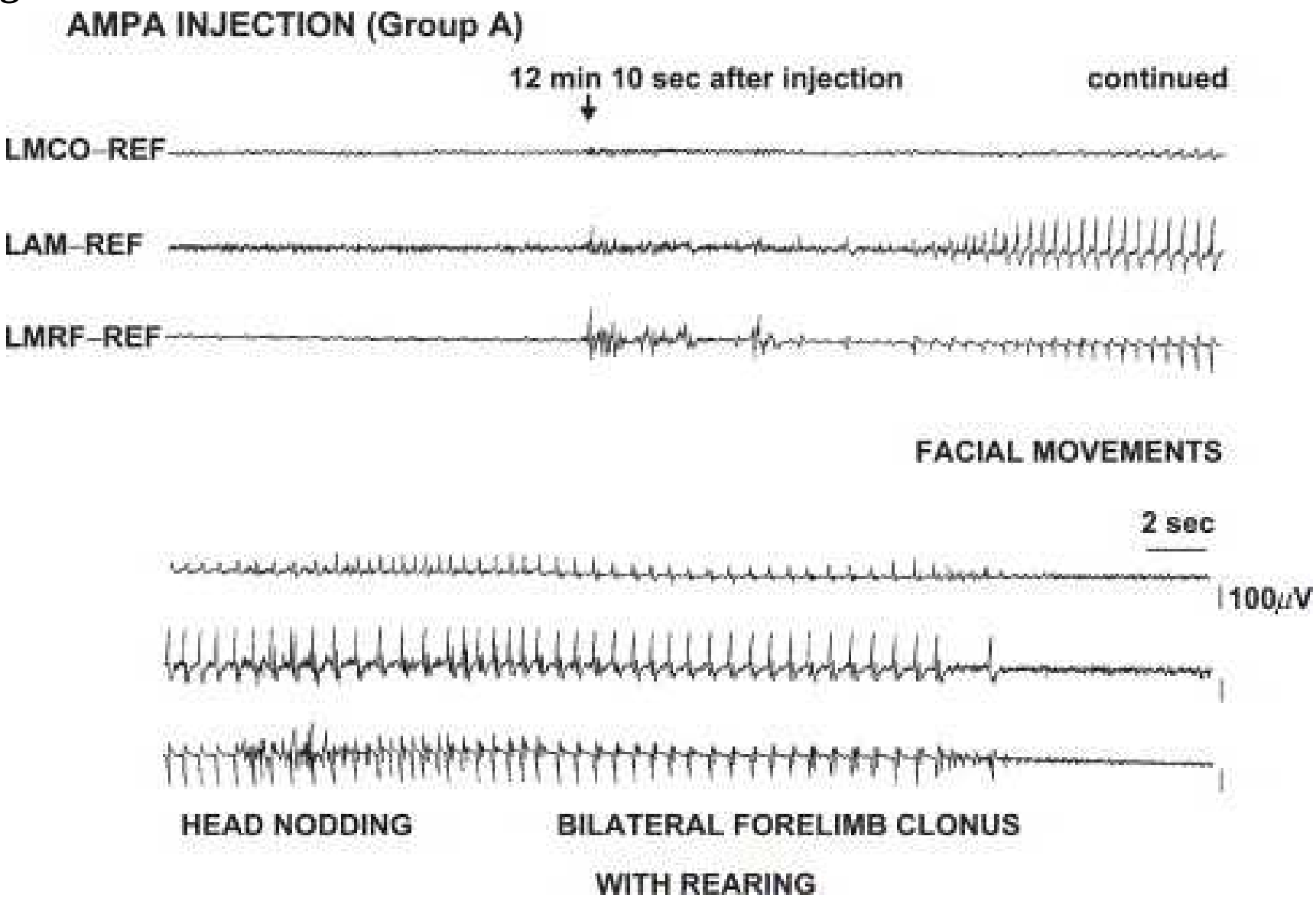

AMPA INJECTION (Group A)
12 min 10 sec after injection
continued
LMCO–REF
LAM–REF
LMRF–REF
FACIAL MOVEMENTS
2 sec
100μV
HEAD NODDING
BILATERAL FORELIMB CLONUS
WITH REARING

Fig.4

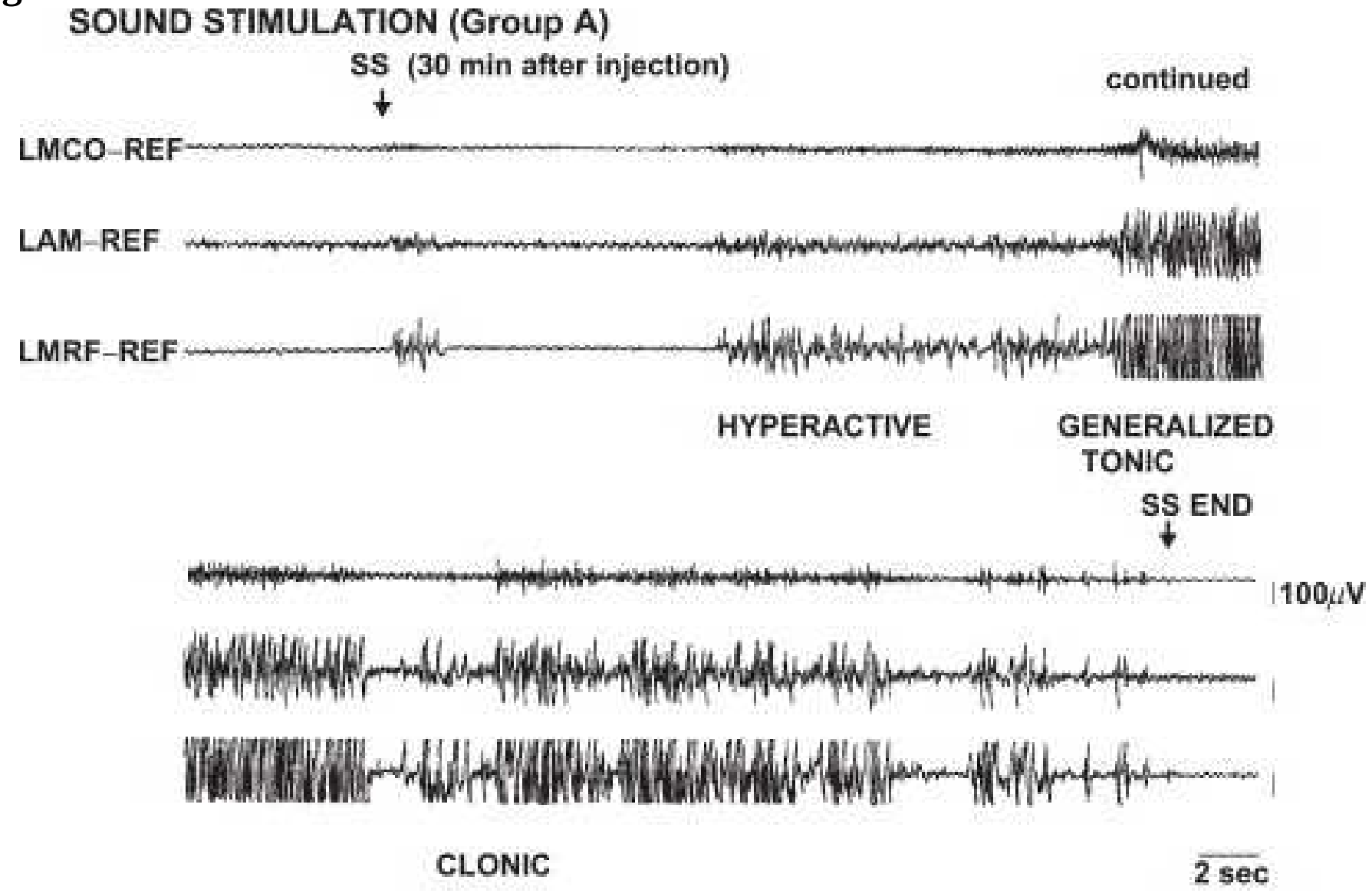
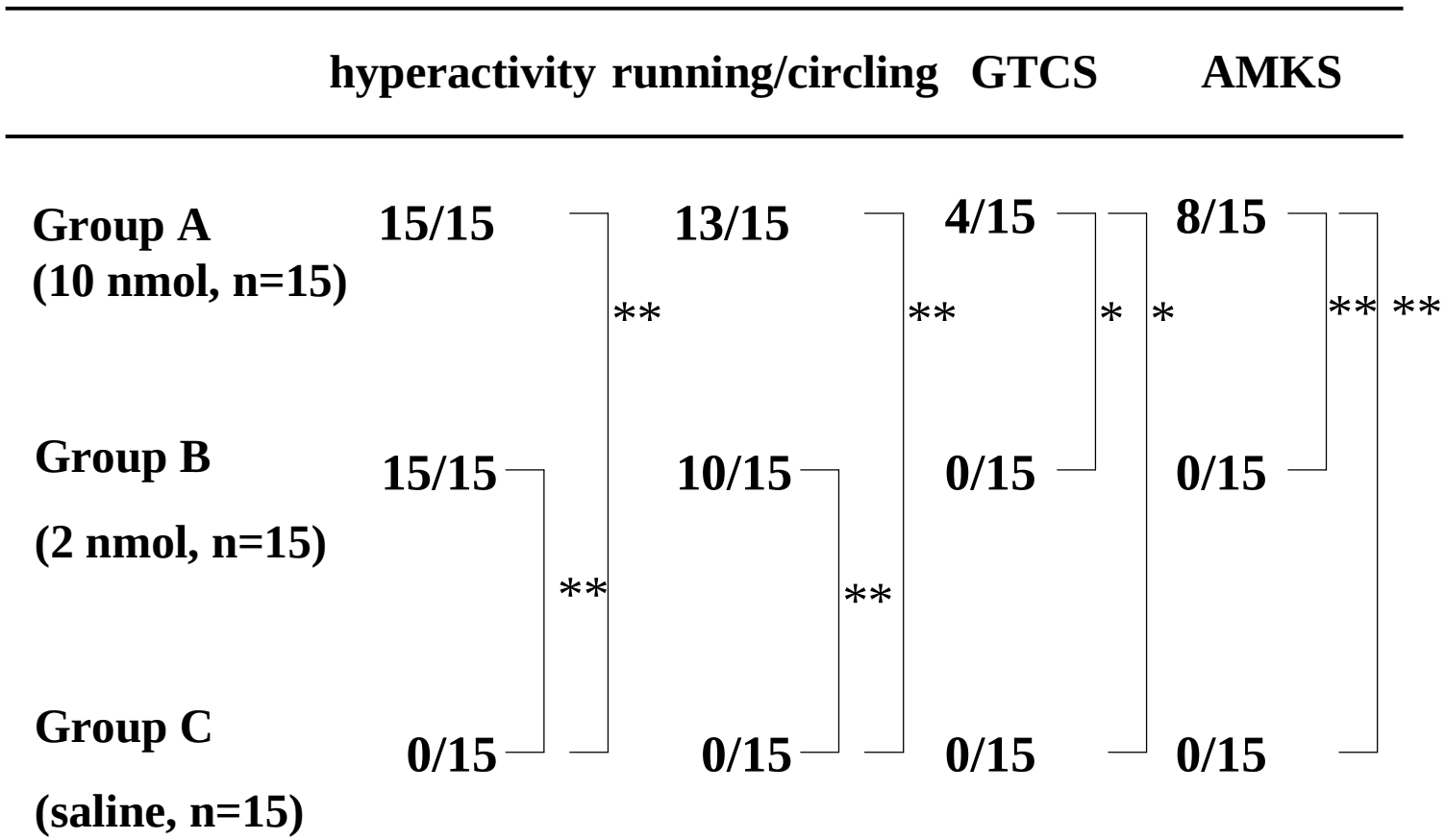

SOUND STIMULATION (Group A)
SS (30 min after injection)
continued
LMCO-REF
LAM-REF
LMRF-REF
HYPERACTIVE
GENERALIZED TONIC
SS END
100μV
CLONIC
2 sec

Table 1 *(AMPA microinjection; Experiment 1)*



AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid;
GTCS, generalized tonic-clonic seizures; AMKS, amygdala kindling-like seizures;
*P<0.05, **P<0.01 by Fisher's probability exact test.

Table 2 *(AMPA microinjection; Experiment 2)*

	hyperactivity	running/circling	GTCS	AMKS
Group A (10 nmol, n=15)	15/15 (6,7,2)	14/15 (6,7,1)	6/15 (3,3,0)	0/15 (0,0,0)
Group B (2 nmol, n=15)	5/15 (3,2,0)	0/15 (0,0,0)	0/15 (0,0,0)	0/15 (0,0,0)
Group C (saline, n=15)	0/15 (0,0,0)	0/15 (0,0,0)	0/15 (0,0,0)	0/15 (0,0,0)

** ** ** ** ** **

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid;
GTCS, generalized tonic-clonic seizures; AMKS, amygdala kindling-like seizures;
*P<0.05, **P<0.01 by Fisher's probability exact test.