



Short communication

Increased seizure susceptibility in adult rats with neuronal migration disorders

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Abstract

Recent data show that neuronal migration disorders (NMD) lower the seizure threshold in the immature brain. To assess if this is an age-related phenomenon, kainic acid (KA) was administered to induce status epilepticus in adult rats with NMD. Results of the present study demonstrate that adult rats with NMD had a shorter latency to seizures and longer duration of status epilepticus compared to age-related controls. Furthermore, in rats with NMD seizures were more severe and status epilepticus-induced mortality was worse than in age-matched controls. These data confirm that NMD lower the seizure threshold in the adult rat. The results of the present study combined with our previous studies in the immature rat, suggest that the facilitating effects of NMD on seizures are not age dependent. © 1997 Elsevier Science B.V.

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Neuronal migration disorders (NMD) are the result of abnormal neuronal migration during embryonic development. NMD are characterized by abnormal cortical architecture with both normal and dysplastic neurons in aberrant position. Recently, neuronal migration disorders have been diagnosed with increasingly higher frequency in patients with epilepsy refractory to medical treatment [23]. Furthermore, *in vitro* study of human neocortex with NMD reveal epileptogenic characteristics [2]. These observations suggest that NMD could be the cause of some types of epilepsies.

A significant limiting factor in understanding the link between NMD and seizures is the lack of well characterized experimental models that mimics behavioral and histological features seen in patients. Investigators have produced cortical dysgenesis with freeze or punctured lesions [8,25] and with the use of radiation and chemicals as teratogenic agents for altering normal developmental patterns in the central nervous system [5,6,12,17,20,26–28]. Transplacental exposure to methylazoxymethanol acetate

(MAM), an alkylating agent, has been used to induce experimental NMD [20,27,28]. MAM is an alkylating substance that is easily transported through the placenta and methylates the nitrogen in position 7 of the guanine of brain nucleic acids of cells preparing for, or undergoing mitotic division [20,22]. Previous reports mentioned neuronal depletion of the neocortex [4,18,19], striatum [3], and hippocampal neuronal ectopia [27] after transplacental exposure to MAM. These studies, however, lack systematic description of the effects of MAM on brain development and documentation of the incidence and severity of NMD after transplacental MAM.

Recently, we characterized the MAM model documenting that: (1) MAM induces NMD in 100% of the exposed rats with reliable patterns [10] and (2) MAM-induced NMD decrease threshold to seizures in the immature brain to hyperthermia-induced seizures [9] and to kainic acid induced status epilepticus [11].

The aim of this study is to assess if decreased seizure threshold secondary to NMD is present in adult rats. NMD were experimentally induced in offspring of Sprague–Dawley rats (Taconic, Germantown, NY) injected at pregnancy day 15 with methylazoxymethanol (MAM, 25 mg/kg *i.p.*, Sigma Chemical Co., St. Louis, MO). Pregnant control rats were injected with an equivalent volume

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of saline. Status epilepticus was induced in 60-day old rats ($N = 20$) by injection of kainic acid (KA, 15 mg/kg i.p., Sigma Chemical Co., St. Louis, MO). In this model [1], behavioral manifestations of seizure in the adult rat are graded in stages as following: ‘wet dog’ shakes (stage 1), scratching (stage 2), unilateral forelimb clonus (stage 3), bilateral forelimb clonus (stage 4), falling and rearing (stage 5), and tonic seizures (stage 6). Latency to clonic seizures (seizure onset), duration of status epilepticus, and mortality were recorded. Latency to seizure onset was the time interval between the administration of KA and the first behavioral clonic seizure. Duration of status epilepticus was considered the time from the first clonic seizure to the last clonic seizure. Two weeks after induction of seizures, rats were sacrificed, brain fixed in buffered paraformaldehyde 4% and embedded in paraffin. Five micron-thick sections were stained with cresyl violet to visualize cell morphology. Glial fibrillary acidic protein (GFAP) was visualized using standard peroxidase-antiperoxidase immunohistochemical techniques (GFAP Dako kit, Dako, CA) to assess astrocytic reaction.

Adult rats with NMD had a significantly shorter latency to seizure onset compared to age-related controls (Fig. 1). Furthermore, the severity of seizures in rats with NMD was greater than that observed in controls: 9 of 10 rats with NMD had tonic seizures (stage 6) compared to 4 of 10 controls. Additionally, tonic seizures occurred faster in rats with NMD than in controls (Student’s unpaired t -test, $t(18) = -3.5$, $P < 0.005$). Finally, kainic-acid-induced status epilepticus had a significantly longer duration in rats with NMD compared to controls (Fig. 2A). Status epilepticus induced mortality was also greater in adult rats with NMD. Eight of 10 rats with NMD died of status epilepticus compared to 2 of 10 controls (Fig. 2B). Histology of all rats exposed to MAM at PG 15 showed neocortical and hippocampal neuronal ectopia, as previously described [10].

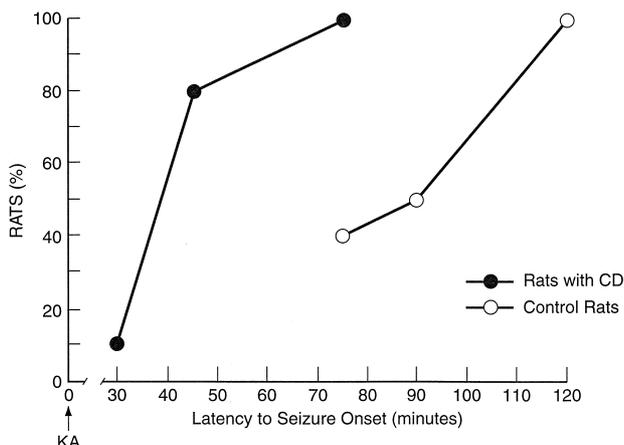


Fig. 1. Seizure threshold is decreased in adult rats with NMD. Adult rats with NMD had a shorter latency to seizure onset after administration of kainic acid compared to age-matched controls (*Student’s unpaired t -test, $t(18) = -5.9$, $P < 0.0001$).

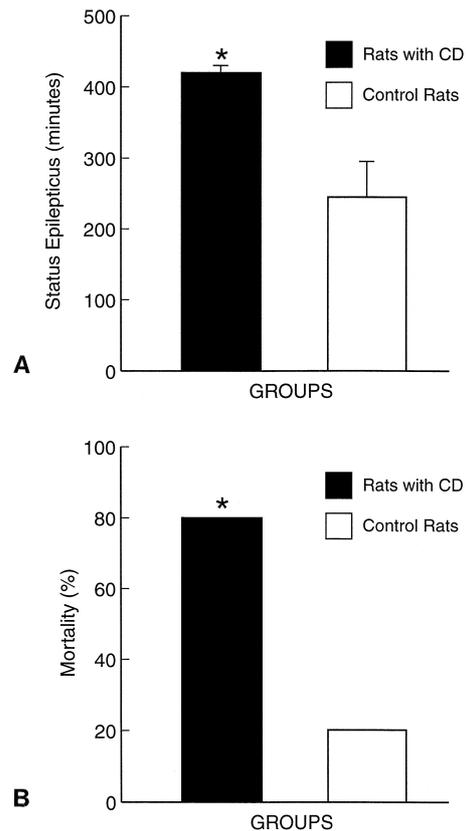


Fig. 2. A: status epilepticus is prolonged in adult rats with NMD. Adult rats with NMD had a longer duration of kainic-acid-induced status epilepticus compared to controls (*Student’s unpaired t -test, $t(18) = 3.4$, $P = 0.0027$). B: status epilepticus-induced mortality was increased in adult rats with NMD. Adult rats with NMD had a higher mortality than age-matched controls (* $\chi^2 = 7.2$, Fisher’s post-hoc $P = 0.023$).

The 2 rats with NMD that survived and were examined 2 weeks after the seizures showed neuronal hippocampal damage in pyramidal neurons of the hippocampus in the CA3/4 subfields. Immunohistochemistry showed increased GFAP expression compared to rats without seizures in the CA3/4 hippocampal subfields consistent with reactive astrocytosis. This pattern was similar to that observed in control rats (Fig. 3).

NMD were originally brought to the attention of epileptologists by Taylor, and his colleagues in 1971 [30]. Neocortical neuron connectivity and dendritic structural changes were studied over the years and abnormalities were demonstrated many years ago by Purpura [24]. However, it is only recently that stronger clinical [25], electrographic [13], electrophysiological [2], and experimental [9,10] evidence shows that NMD and epilepsy are linked. On the other hand, the mechanisms at the basis of NMD and increased seizure susceptibility are still being investigated.

Age-related differences in seizure susceptibility are well documented and it was recently reviewed [16]. In particular, the immature brain seem to be more susceptible to

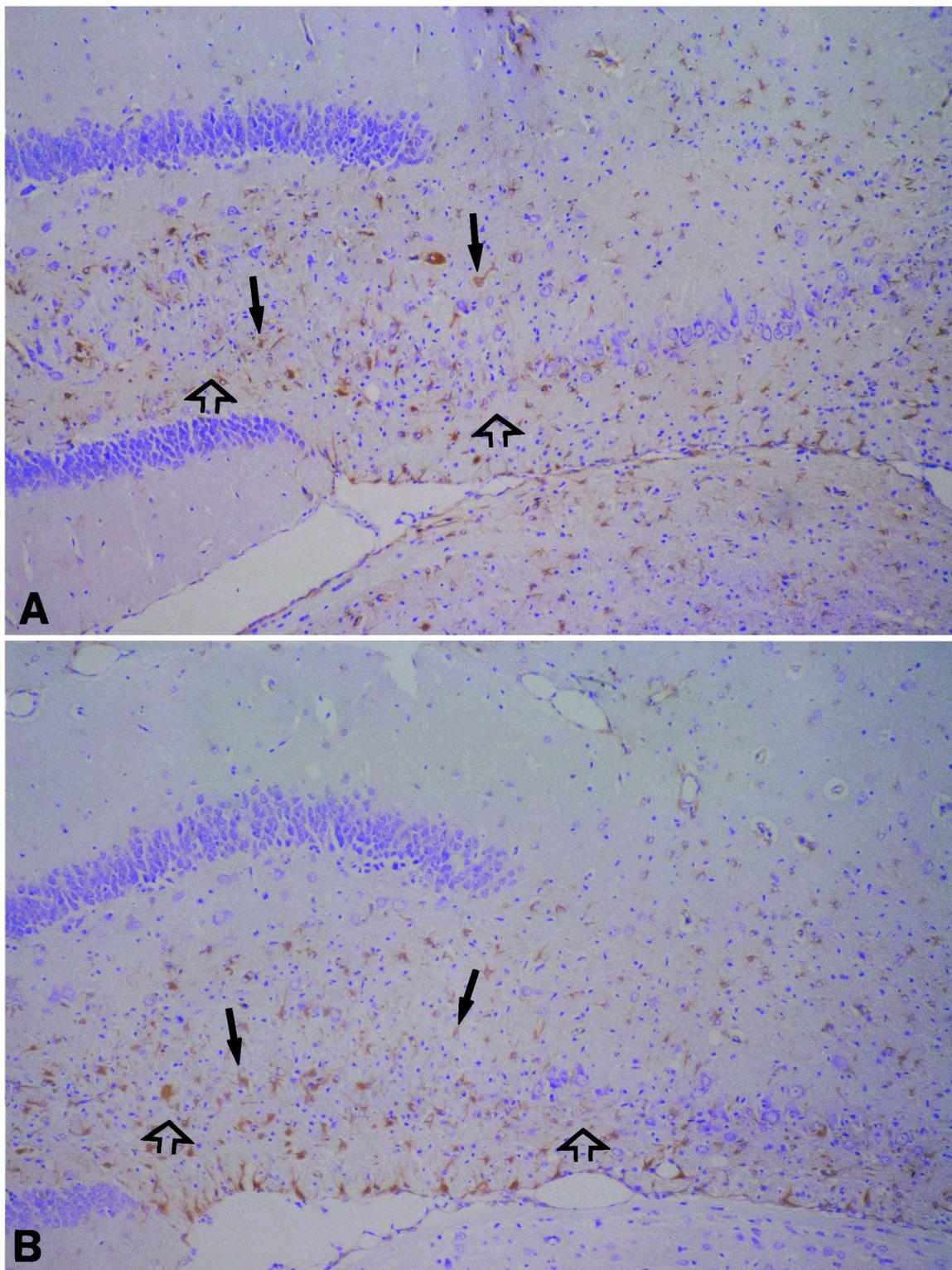


Fig. 3. Photomicrograph of coronal rat brain sections at the level of the CA3/4 hippocampal subfield. A: adult control rat. B: rat with NMD. Note that the seizure-induced hippocampal damage (open arrows) and increased number of GFAP-positive astrocytes (solid arrows) had a similar pattern in both animals.

seizures [14,15,21] and yet more resistant to seizure-induced hippocampal damage [29]. Recently, we showed that in the immature brain experimentally induced neuronal

migration disorders further increases threshold to hyperthermia-induced seizures [9]. Furthermore, in rat pups with NMD, kainic-acid-induced clonic and tonic seizures had a

faster onset than in controls [11]. These data corroborate a previous report showing that rat pups with NMD had significantly longer seizures and a greater number of seizures than controls [7].

The present study shows that the facilitatory effects of NMD on seizure susceptibility in adult rats. Adult rats with NMD have decreased seizure threshold to kainic-acid-induced clonic and tonic seizures. We have reported increased seizure susceptibility in the rat pup with two different seizure models: hyperthermia-induced [9] and kainic-acid-induced seizures [11]. Therefore, our present results taken together with our previous study suggest that in the rat increased seizure susceptibility in the presence of NMD is not age dependent.

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References

- [1] B.J. Albala, S.L. Moshe, R. Okada, Kainic-acid-induced seizures: A developmental study, *Dev. Brain Res.* 13 (1984) 139–148.
- [2] M. Avoli, A. Olivier, Electrophysiological properties and synaptic responses in the deep layers of the human epileptogenic neocortex maintained in vitro, *J. Neurophysiol.* 61 (1989) 589–606.
- [3] W. Balduini, M.P. Abbracchio, G. Lombardelli, F. Cattabeni, Loss of intrinsic striatal neurons after methylazoxymethanol acetate treatment in pregnant rats, *Dev. Brain Res.* 15 (1984) 133–136.
- [4] F. Cattabeni, M.P. Abbracchio, M. Cimino, D. Cocchi, M. Di Luca, L. Mennuni, F. Rosi, P. Zaratini, Methylazoxymethanol-induced microencephaly: persistent increase of cortical somatostatin-like immunoreactivity, *Dev. Brain Res.* 47 (1989) 156–159.
- [5] S. Ciaroni, T. Cecchini, P. Ambrogini, G. Minelli, P. Del Grande, Nerve cell rearrangement in neocortical layers of MAM treated fetal mice, *J. Hirnforsch.* 3 (1992) 303–310.
- [6] J.L. Diaz-Granados, P.L. Greene, A. Amsel, Memory-based learning in preweaning and adult rats after infantile X-irradiation-induced hippocampal granule cell hypoplasia, *Behav. Neurosci.* 106 (1992) 940–946.
- [7] M.R. DeFeo, O. Mecarelli, G.F. Ricci, Seizure susceptibility in immature rats with micrencephaly induced by prenatal exposure to methylazoxymethanol acetate, *Pharmacol. Res.* 31 (1995) 109–114.
- [8] P. Humphreys, G.D. Rosen, B.S. Press, G.F. Sherman, M.D. Galaburda, Freezing lesions of the developing rat brain: A model for cerebrocortical microgyria, *J. Neuropathol. Exp. Neurol.* 50 (1991) 145–160.
- [9] I.M. Germano, Y.F. Zhang, E.F. Sperber, S.L. Moshe, Neuronal migration disorders increase the susceptibility to hyperthermia-induced seizures in the rat, *Epilepsia* 37 (1996) 902–910.
- [10] I.M. Germano, E.F. Sperber, S.L. Moshe, Molecular and Experimental Aspects of Neuronal Migration Disorders, In: R. Guerrini, F. Anderman, R. Canapichi, J. Roger, P. Pfanner (Eds.), *Dysplasia of the Cerebral Cortex and Epilepsy*, Raven Press, New York, 1996, pp. 27–34.
- [11] I.M. Germano, E.F. Sperber, S.L. Moshe, Transplacentally induced neuronal migration disorders: an animal model for the study of the epilepsies, *J. Neurosci.*, (submitted).
- [12] K. Gillies, D.J. Price, The fate of cells in the developing cerebral cortex of normal and methylazoxymethanol acetate-lesioned mice, *Eur. J. Neurosci.* 5 (1993) 73–84.
- [13] R. Guerrini, C. Dravet, C. Raybaud, Epilepsy and focal gyral anomalies detected by MRI: electroclinico-morphological correlation and follow up, *Dev. Med. Child Neurol.* 34 (1992) 706–718.
- [14] W.A. Hauser, D.C. Hersdorffer, *Epilepsy: frequency, causes and consequences*, Demos, New York, 1990, pp. 486–496.
- [15] G.L. Holmes, J.L. Thompson, Effects of kainic acid on seizure susceptibility in the developing brain, *Dev. Brain Res.* 39 (1988) 51–59.
- [16] G.L. Holmes, Epilepsy in the developing brain: Lesson from the laboratory and clinic, *Epilepsia* 38 (1997) 12–30.
- [17] M.V. Johnson, J.T. Coyle, Histological and neurochemical effects of fetal treatment with methylazoxymethanol on rat neocortex in adulthood, *Brain Res.* 170 (1979) 135–155.
- [18] E.G. Jones, K.L. Valentino, J.W. Fleshman Jr., Adjustment of connectivity in rat neocortex after prenatal destruction of precursor cells of layers II–IV, *Dev. Brain Res.* 2 (1982) 425–431.
- [19] J. Majkowski, M.L. He, P.B. Kozlowski, R. Haddad, EEG and seizure threshold in normal and lissencephalic ferrets, *Brain Res.* 307 (1984) 29–38.
- [20] H. Matsumoto, M. Spatz, G.L. Laquer, Quantitative changes with age in the DNA content of methylazoxymethanol-induced microencephalic rat brain, *J. Neurochem.* 19 (1972) 297–306.
- [21] S.L. Moshe', B.J. Albala, R.F. Akerman, J. Engel Jr., Increased seizure susceptibility of the immature brain, *Dev. Brain Res.* 7 (1983) 81–85.
- [22] Y. Nagata, H. Matsumoto, Studies on methylazoxymethanol; methylation of nucleic acids in fetal rat brain, *Proc. Soc. Exp. Biol. Med.* 132 (1969) 383–385.
- [23] A. Palmi, F. Andermann, A. Olivier, Focal Neuronal migration disorder and intractable partial epilepsy: A study of 30 patients, *Ann. Neurol.* 30 (1991) 741–749.
- [24] D.P. Purpura, Dendritic differentiation in human cerebral cortex: normal and aberrant developmental patterns, *Adv. Neurol.* 12 (1975) 91–134.
- [25] G.D. Rosen, G.F. Sherman, J.M. Richman, L.V. Stone, A.M. Galaburda, Induction of molecular layer ectopias by puncture wounds in newborn rats and mice, *Dev. Brain Res.* 67 (1992) 285–291.
- [26] G.F. Sherman, L. Morrison, G.D. Rosen, P.O. Behan, A.M. Galaburda, Brain abnormalities in immune defective mice, *Brain Res.* 532 (1992) 25–33.
- [27] S.C. Singh, Ectopic neurones in the hippocampus of the postnatal rat exposed to methylazoxymethanol during fetal development, *Acta Neuropathol. (Berl.)* 40 (1977) 111–116.
- [28] M. Spatz, G.L. Laquer, Transplacental chemical induction of microencephaly in two strains of rats, *Proc. Soc. Exp. Biol. Med.* 1299 (1968) 705–710.
- [29] E.F. Sperber, K.Z. Haas, P. Stanton, S.L. Moshe', Resistance of the immature hippocampus to seizure-induced synaptic reorganization, *Dev. Brain Res.* 69 (1991) 88–93.
- [30] D.C. Taylor, M.A. Falconer, C.J. Bruton, J.A.N. Corsellis, Focal dysplasia of the cerebral cortex in epilepsy, *J. Neurol. Neurosurg. Psychiatry* 34 (1971) 369–387.