# Transplacentally Induced Neuronal Migration Disorders: An Animal Model for the Study of the Epilepsies

**Isabelle M. Germano**[1]* and **Ellen F. Sperber**[2]
[1]Department of Neurosurgery, Mount Sinai School of Medicine, New York, New York
[2]Departments of Neurology and Neuroscience, Albert Einstein College of Medicine, New York, New York

Recent clinical and laboratory data suggest that there is a link between neuronal migration disorders (NMD) and increased seizure threshold. To characterize an animal model with features similar to human NMD and to assess seizure susceptibility, NMD were induced in the rat at the time of neuroblastic division (PG15) and three other gestational ages (PG 13, PG14, PG16) by transplacental exposure to methylaxozymethanol (MAM, 25 mg/kg). Offspring pups were monitored for spontaneous and electrographic seizures. At postnatal day 14, randomly selected rat pups were sacrificed for histological examination. In other MAM-exposed pups and controls, status epilepticus was induced by intraperitoneal administration of kainic acid.

On histology, NMD were found in all PG 15 MAM-exposed rats, in comparison to 63% of PG 13, 70% of PG 14, 80% of PG16. Histological features included cortical laminar disorganization, ectopic neurons in the subcortical white matter and in cortical layer I, persistent granular layer, marginal glioneuronal heterotopia, and discrete areas of neuronal ectopia in the CA1 subfield of the hippocampus. Based on the severity of the neuronal migration abnormalities, rats were divided into three categories: severe, moderate, and mild. Severe and moderate NMD were only found in the PG 15 MAM-exposed rats. EEG recording in rats with NMD did not disclose spontaneous seizures; however, rats with severe NMD had higher slow wave activity compared to controls ($P < .05$). MAM-exposed rats with severe NMD were more susceptible to kainic-induced seizures compared to controls ($P < .05$). In rats with severe NMD, kainic acid-induced status epilepticus produced hippocampal damage in the CA3/4 region.

These results demonstrate that MAM-induced NMD have histological and electrographic characteristics similar to human NMD. The severity of neuronal abnormality depends on the time of transplacental exposure as the most severe NMD were found after exposure to MAM at the time of neuroblastic division. The degree of NMD positively correlates with seizure susceptibility, since only rats with severe NMD have decreased seizure threshold. The occurrence of status epilepticus-induced hippocampal damage in pups with severe NMD suggests that the severely compromised hippocampus is less resistant to seizure-induced injury than the normal developing brain. J. Neurosci. Res. 51:473–488, 1998. © 1998 Wiley-Liss, Inc.

Key words: epilepsy; seizures; neuronal migration disorders; methylazoxymethanol; status epilepticus; hippocampal damage; rat

## INTRODUCTION

The concept of ''idiopathic'' epilepsy is changing as advances in neuroimaging allow for the diagnosis of intracerebral pathologies previously not recognized (Germano et al., 1994). Recent technologies in high resolution magnetic resonance imaging (MRI) allow the identification of neuronal migration disorders (NMD) in humans (Guerrini et al., 1992; Lee et al., 1994). NMD have a wide range of clinical and histological features that reflect the presence of different disturbances in cortical development (Anderman and Palmini, 1992). Most patients with NMD suffer from varying degrees of mental retardation ranging from slight delay with associated learning problems to severe retardation with electrographic abnormalities (Anderman and Palmini, 1992). The histological features are characterized by disturbances in neuronal migration ranging from loss of cerebral cortical lamination to single or clustered ectopic neurons (Mischel et al., 1995).
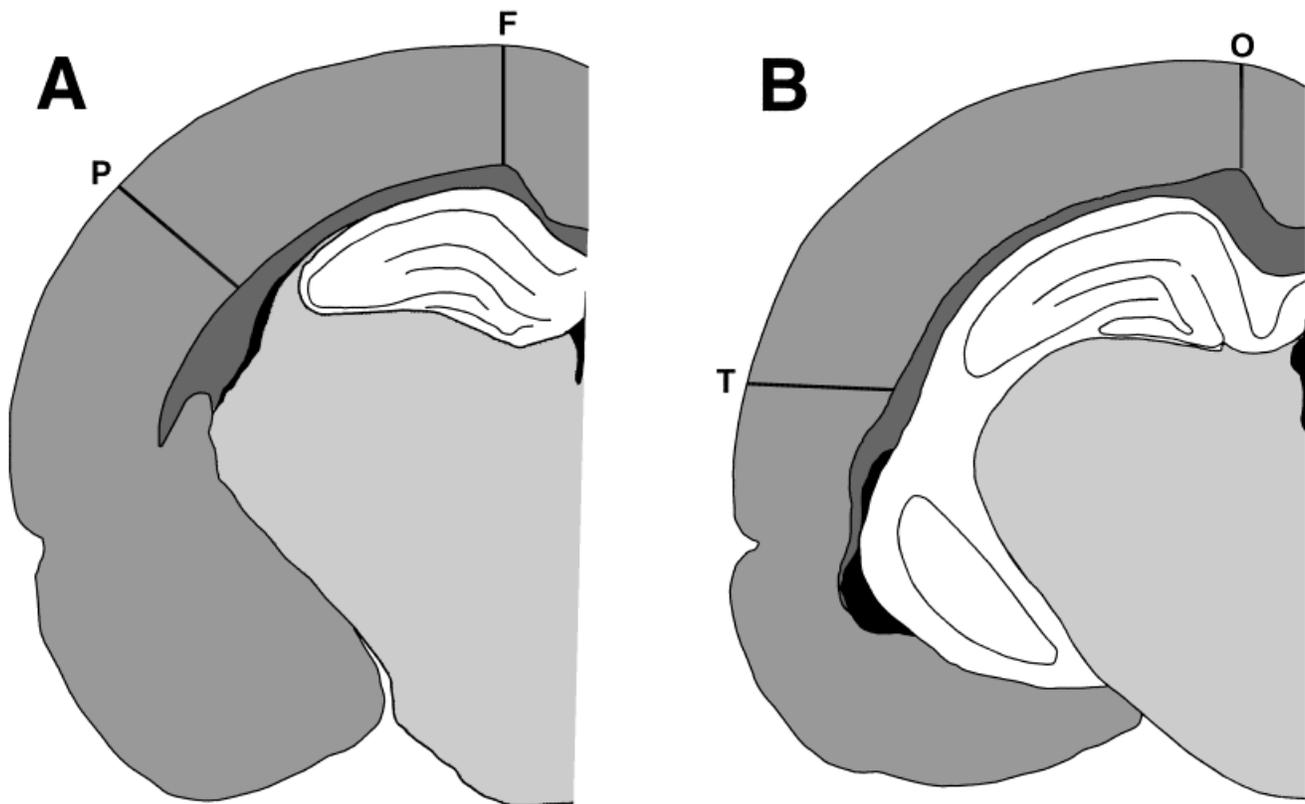
Fig. 1. Coronal diagrams of the rat brain indicating where the cortical, hippocampal, and thalamic measurement areas were obtained. **A:** Bregma −3.30 mm. **B:** −4.30 mm, according to Paxinos and Watson (1986); dark gray: cortex; light gray area: diencephalon; gray: corpus callosum; white: hippocampus; black: ventricular system. Further linear measurements were obtained where lines are drawn. F: frontal (2 mm from midline); T: temporal (90 angle from midline); P: parietal (perpendicular to the midpoint of the lateral ventricle); O: occipital (2 mm from midline).

Neuronal migration is a complex process. Genetic and/or environmental factors most likely cause multiple disturbances that interfere with neuron production, neuronal-glial interactions, growth factors and hormones. Genetic etiologies for NMD have been proposed (Rorke, 1994; Anderman et al., 1994; Jan and Jan, 1990). Alternatively, environmental factors such as destructive lesions, toxins, and radiation could be potential causes of NMD (Kabat et al., 1985; Richman and Steward, 1974; Spatz and Laqueur, 1968). There is evidence that NMD develop in the course of neuroblastic division and neuronal migration (Dvorak and Feit, 1977), which in humans occurs by the end of the 16th week of uterine life (Carpenter and Suitin, 1983). In the rat, neuroblastic division and neuronal migration toward cortical layers II–V occur at pregnancy day (PG) 15 (Angerine and Sidman, 1961). Other authors suggest that some types of NMD may be a postmigrational event, and therefore occur later in the gestational period which approximates the 5th and 6th month of intrauterine life (Richman et al., 1974).

The incidence of NMD in the human population is unknown, as some types of NMD may be minimally symptomatic. On the other hand, recent studies show that NMD are seen by MRI in an increasing number of patients with epilepsy (Brodkorb et al., 1992; Palmini et al., 1991). Furthermore, NMD are found in a high percentage of non-neoplastic epilepsy surgery specimens (Robitaille et al., 1992; Mischel et al., 1995). These data suggest that there may be a link between NMD and epilepsy; however, this has not been established.

Recent clinical data show that the risk of recurrent seizures in children is higher in the presence of an antecedent brain injury (Shinnar et al., 1990). This evidence suggests that a preexisting histological disturbance, such as NMD, may be necessary to increase the risk of epilepsy later in life. Laboratory and clinical observations show that the immature brain is resistant to seizure-induced hippocampal damage in certain seizure models and clinical situations (Moshe', 1987; Sperber et al., 1991; Represa et al., 1989). On the other hand, other laboratory data showed that seizure-induced hippocampal
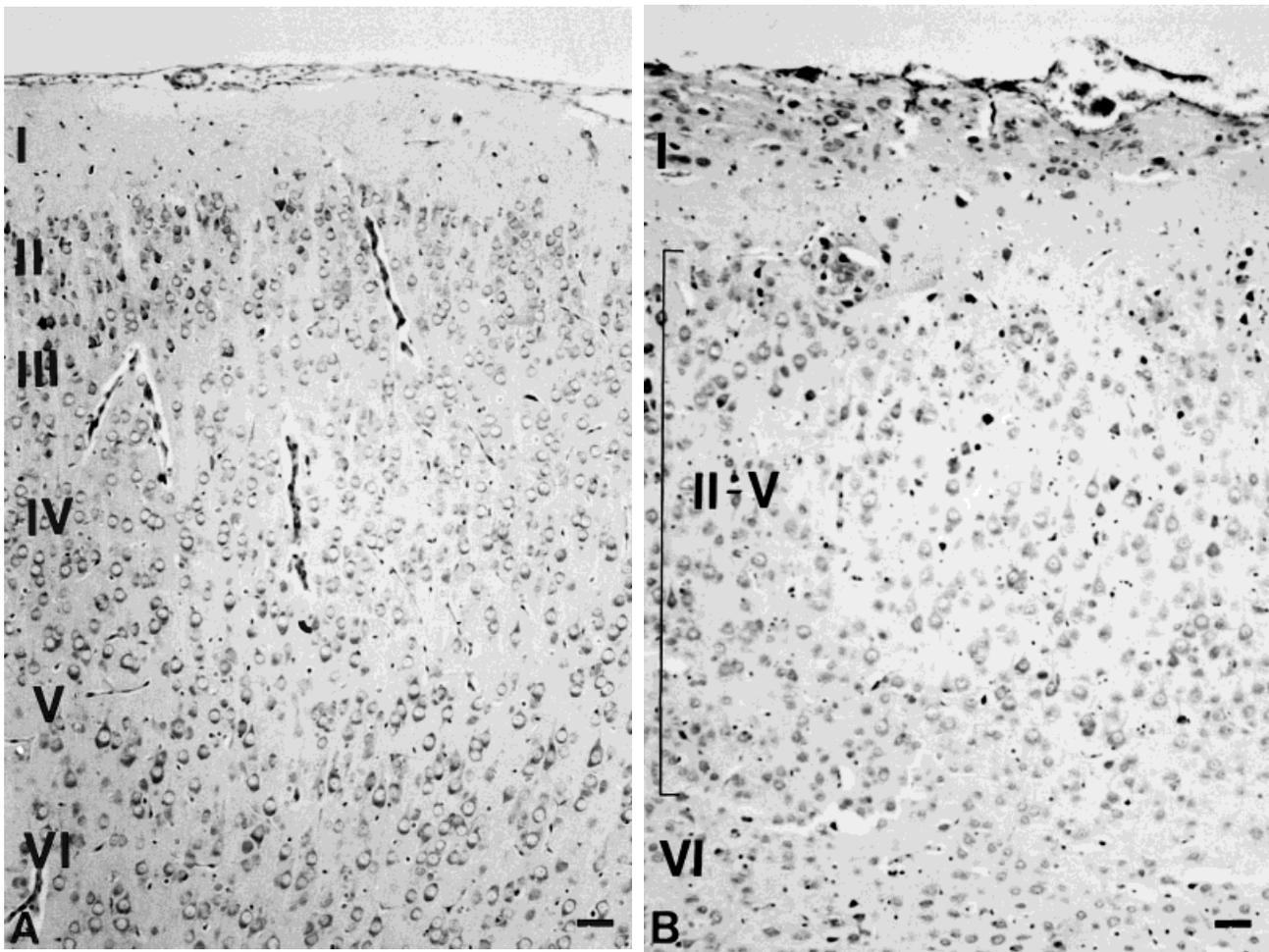
Fig. 2. Photomicrographs of coronal brain sections of a control (**A**) and a rat with NMD (**B**). Cortical layers I–VI are clearly seen in A. After exposure to methylazoxymethanol (MAM), fewer neurons and loss of lamination pattern are seen in cortical layers II–V (B). Nissl stain. Scale bars = 400 μm.

damage can be seen in the developing rabbit and rat (Frank and Schwartzkroin, 1984; Thompson and Wasterlain, 1997). The fact that the normal immature brain seems to be resistant to seizure-induced hippocampal damage is in agreement with the clinical observation that children with a preexisting pathology have a higher chance to develop epilepsy later in life. Thus, "a dual pathology" may be necessary to have seizure-induced hippocampal damage that in turn will lead to epilepsy (Levesque et al., 1991).

A significant limiting factor in understanding the link between NMD and seizures is the lack of an experimental model that mimics behavioral and histological features seen in patients. Altered neuronal migration after transplacental exposure to methylazoxymethanol (MAM) has previously been reported (Jones et al., 1982; Majkowsky et al., 1984; Singh, 1977, 1978; Spatz,

1968). 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, G1 and M phase of the cell cycle, respectively (Nagata and Matsumoto, 1969; Matsumoto et al., 1972). Previous reports mentioned neuronal depletion of the neocortex (Cattabeni et al., 1989; Johnson and Coyle, 1979; Jones et al., 1982), striatum (Balduini et al., 1984), and hippocampal neuronal ectopia (Sing, 1982) after transplacental exposure to MAM. These studies, however, lack systematic description of the effects of MAM on brain development. Furthermore, the incidence and severity of NMD after transplacental MAM is poorly documented.

The aim of the present study is to determine the incidence and severity of NMD after transplacental

exposure to MAM. In addition, the present study will focus on the effects of MAM-induced NMD on seizure susceptibility and seizure-induced neuronal damage. To assess the gestational age responsible for the most severe alterations of neuronal migration, pregnant rats were exposed to MAM before, during, and after neuroblastic division. The offspring brains were examined at PN 14 and based on histological criteria the severity of NMD was classified in a three-tier scale. Correlation between severity of NMD and seizure susceptibility showed that the most severe NMD are associated with a decrease in seizure threshold and hippocampal neuronal damage.

## MATERIALS AND METHODS

### Animals

The experimental procedures were conducted in 23 time-pregnant Sprague-Dawley rats (Taconic Farms, NY) and their litters; rat pups remained with their dam. Gestation day 1 (PG1) corresponded to the day of sperm cell detection by vaginal swab. All animals were maintained on a 12-hr light-dark cycle with an average room temperature of 23°C, humidity of 55%, with food and water ad libitum. Experiments were conducted according to Institutional Animal Care and Use Committee (IACUC) and Center for Laboratory Animal Science (CLAS) guidelines and approved protocol.

### Induction of NMD

The effects of transplacental exposure to MAM at four different gestational ages (PG 13, 14, 15, 16) were assessed in the offsprings of pregnant rats. Pregnant rats were intraperitoneally injected with 25 mg/kg MAM (Sigma, St. Louis, MO); MAM was dissolved in physiologic saline with a final concentration of 3 mg/cc. Control rats were injected with an equivalent volume of saline at the same gestational ages. A 25-gauge needle was used and careful aspiration was performed prior to the injection to rule out the presence of amniotic fluid. Rats that had intrauterine penetration with the needle or postinjection vaginal discharge were excluded from the study.

### Behavioral Observations and EEG Recording

The length of the gestational period and the number of pups per litter were documented in experimental and control dams. Body weight was recorded at birth, at postnatal (PN) day 7 and 14. Postnatal developmental milestones, such as eye opening, fur growth, sucking, ambulating, and grooming were recorded.

Rat pups were intermittently observed Monday–Friday (8 hour/day) for 2 weeks during their development
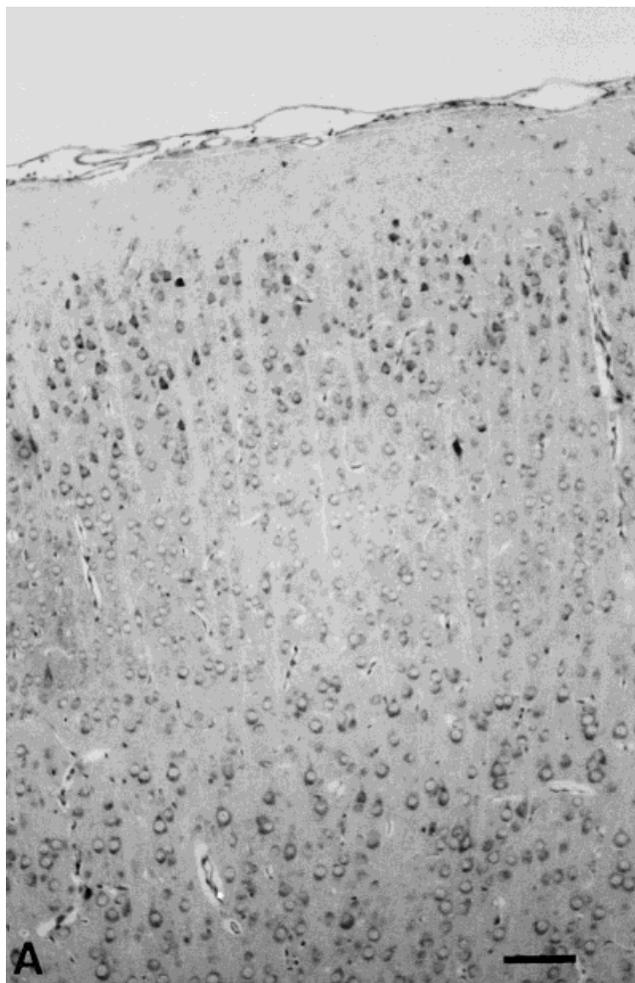


Fig. 3. Photomicrographs of coronal brain sections of a control (**A**) and a rat with NMD (**B** and **C** on facing page) showing cortical layers I–IV. A cluster of abnormally migrated neurons is seen in B; arrows. Several abnormally migrated neurons with irregular somata and abnormal orientation are observed. In addition, in rats with neural migration disorders (NMD), subpial excrescences of neuroglial tissue marginal glioneuronal heterotopia were often seen (C, arrows). Nissl stain. Scale bars = 200 μm.

for spontaneous seizures. Randomly selected pups (n = 30) underwent intermittent epidural EEG recording for 2 days prior to sacrifice. For this procedure, rats were anesthetized with xylazine and ketamine (15 mg/kg and 30 mg/kg i.m.) supplemented by lidocaine 1% s.q. A high speed drill was used to drill four holes 5 mm anterior and posterior to the coronal suture on either side of the skull. Microscrews were then implanted in the calvarium and stabilized with dental acrylic. The rats were allowed to recover from anesthesia on a heating pad and then returned to their dams. The following day, EEGs were obtained in our analog form (electroencephalograph 8–10, Grass, MA) to monitor for epileptic discharges. Each rat was monitored for 15 min every hour for 6 hours.
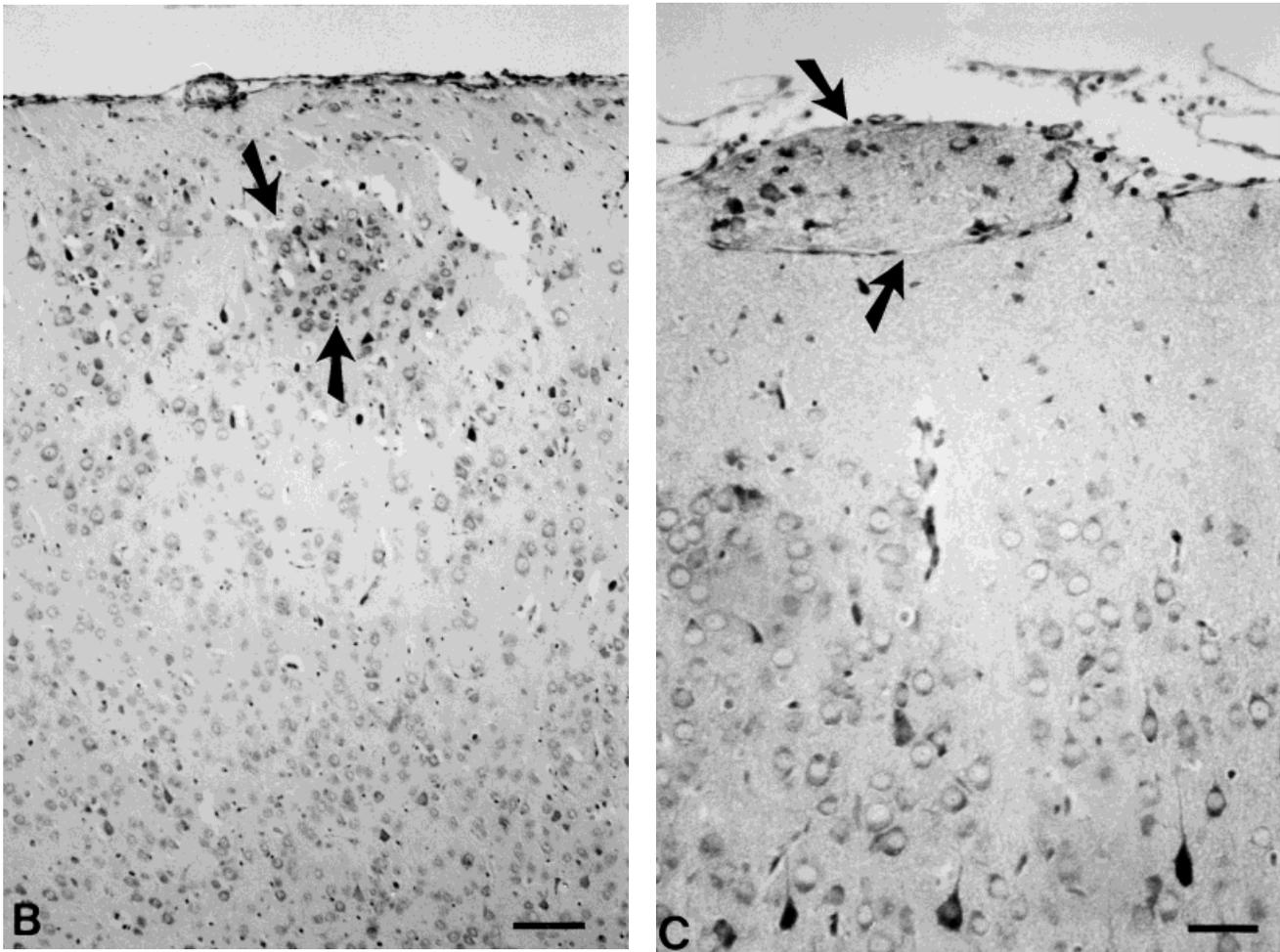
Figure 3 B and C.  (Legend appears on page 476.)

In addition, additional EEG segments (10 min every hour for 6 hours) were subjected to computerized analysis (Neurotrac, Interspec Medical, CA) to allow computation of beta, alpha, theta, and delta frequency. The 2-sec epochs obtained 5 min after the beginning of each recording were analyzed and averaged in each animal.

### Induction of Seizures

Status epilepticus was induced in 14-day-old pups by intraperitoneal injection of kainic acid (4 mg/kg); MAM-exposed rats n = 37; controls n = 10. In this model, behavioral manifestations of seizure consist of clonic and tonic seizures leading to status epilepticus (Cherubini et al., 1983; Albala et al., 1984). Latency to seizure onset is the time interval between the administration of kainic acid and the first clonic seizure. Latency to onset of tonic seizures is the time interval between the administration of kainic acid and the occurrence of tonus. Latency to onset of the first clonic movement (seizure onset), latency to tonic seizures, duration of status epilepticus, and mortality were recorded. After injection of kainic acid (KA), rats were observed for 6 hours and then returned to their cages and sacrificed 24 hours after status epilepticus.

### Pathology

Brain weight was measured using frozen brains after the forebrain was separated from the caudal structures. The forebrain included all structures rostral to the lower border of the inferior colliculi. Brain measurements, as described below, were performed on frozen 10-μm coronal sections stained with thionin using a computerized image analysis program (IPLab, Vienna, VA). The thickness of the frontal, temporal, occipital, and parietal cortices was measured on thionin-stained coronal brain sections at two levels posterior to bregma (Fig. 1): (I) −3.30 mm and (II) −4.30 mm (Paxinos and Watson, 1986). The frontal and occipital cortices were measured
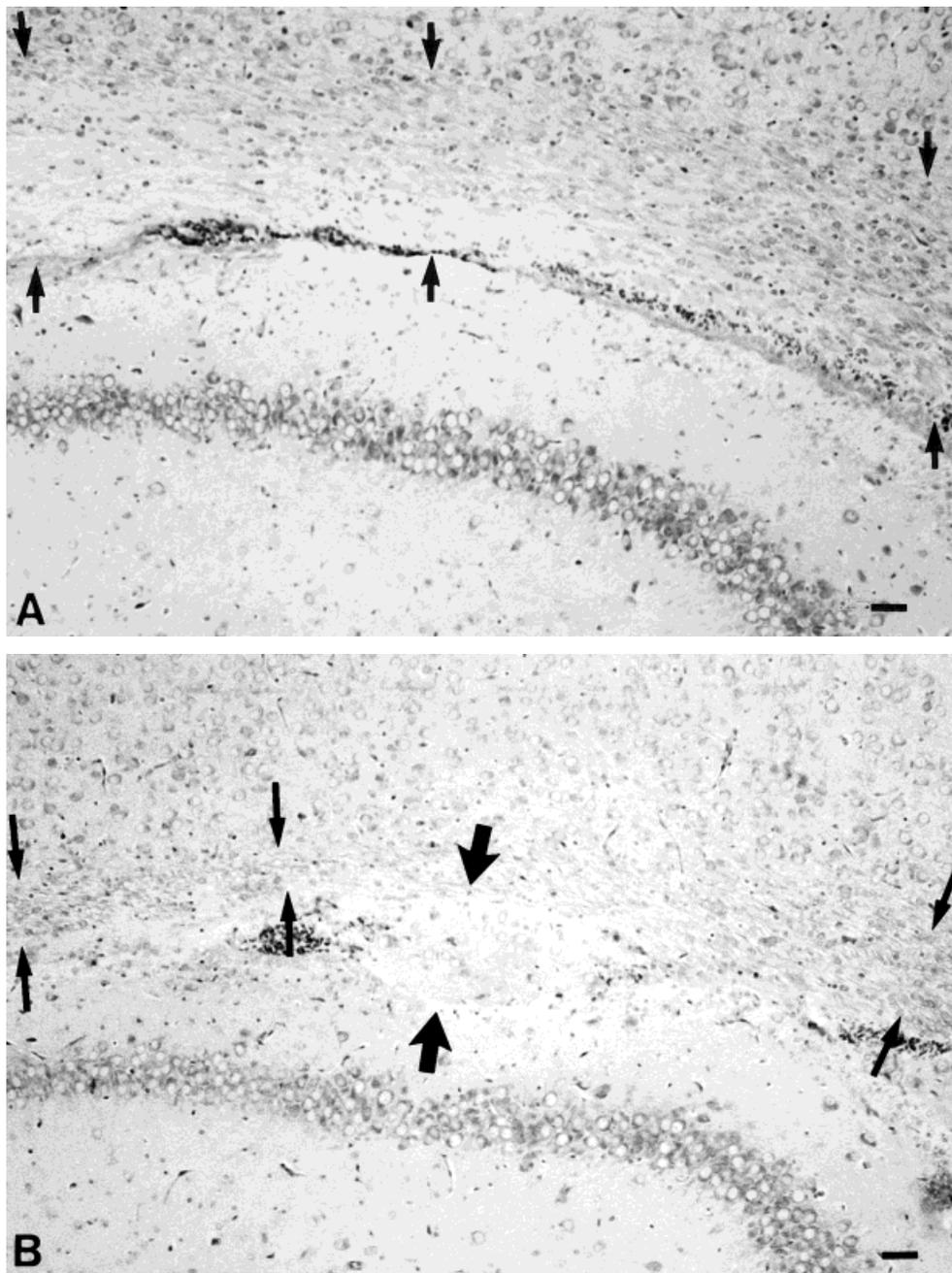
Fig. 4. Photomicrographs of coronal brain sections of a control (**A**) and a rat with NMD (**B**) showing cortical layer VI and subcortical white matter. Heterotopic neurons in the subcortical white matter were seen (large arrows) in rats with NMD. Furthermore, cortical neuronal depletion resulted in decreased thickness of the corpus callosum (small arrows indicate the inferior and superior extent of the corpus callosum). Nissl Luxol Blue stain. Scale bars = 200 μm.

2 mm from midline at levels I and II, respectively. The parietal cortex was measured at level I perpendicular to the midpoint of the lateral ventricle and the temporal cortex on level II at 90° angle from midline. Furthermore, surface areas of the hippocampus and diencephalon were measured at both levels. Measurements were performed on experimental and control rats from different litters at PN 14. The posterior aspect of the anterior dorsal hippocampus was 4 mm posterior to bregma (Paxinos and Watson, 1986).

## Histology and Histochemistry

Rats used for characterization of the effects of MAM on neuronal migration (n = 150) were sacrificed at PN 14 and randomized to be stained with Nissl stain and luxol blue to study neuronal and white matter morphology, or Timm-Haug silver sulfide stain (Timm stain) was used to assess if the presence of neuronal hippocampal ectopia induced mossy fiber synaptic reorganization after pyramidal neuron loss (Tauk and Nadler, 1985). Status epilepticus-induced early neuronal damage was studied 24 hours after induction of seizures.

Rats were anesthetized and perfused intracardially with buffered paraformaldehyde (PFA) 4%, pH 7.4. Brains were removed, postfixed in buffered PFA for 24–48 hr, embedded in paraffin, and cut coronally with a microtome (5-μm-thick sections); Nissl and luxol blue stains were performed according to standard techniques (Bloom and Fawcett, 1975). Count of damaged neurons 24 hours after status epilepticus was done in the cortex in the same areas where cortical measurements were obtained (Fig. 1) and in the hippocampal subfields (Lorente de No, 1934) by examining four Nissl-stained coronal sections per rat at 200× with a custom-made image analysis system interfaced with a Macintosh computer and IPLab software (Signal Analytics, Vienna, VA).

Rats used for Timm stain were anesthetized, decapitated, their brains removed and cut coronally in 3-mm-thick blocks. Blocks were perfused by immersion with a .4% sodium sulfide solution, fixed overnight in a solution of 1% PFA and 1.25% glutaraldehyde and kept in a 30% sucrose/fix solution for 24 hr before they were cut in a cryostat (10-μm) and mounted on glass slides. Slides were then immersed in a solution of 20% gum arabic, 5.6% hydroquinone, and citrate buffer with 1.5 ml of 17% silver solution for 45–60 min in the dark. The sections were then washed in distilled water and dehydrated in a series of alcohol. One slide per rat was double-stained with cresyl violet (5%) before coverslipping. The pattern and intensity of the mossy fiber terminal staining was rated using sprouting scores determined according to a modified Timm stain rating scale by Tauck and Nadler (1985).

## Statistical Analysis

Data were analyzed using analysis of variance test (ANOVA) with Bonferroni's ad hoc correction for intergroup analysis. EEG frequency data were expressed as percentage of the total power (μV) as indicated by the total power of each hemisphere. Linear cortical and surface hippocampal and diencephalic measurements of experimental rats are expressed as percentages of controls. A probability $P < .05$ was significant. Data are expressed as mean ± standard deviation (S.D.).

## RESULTS

To achieve a systematic description of the effects of MAM at different gestational ages on neuronal migration abnormalities and their impact on seizure susceptibility, we correlated histopathological features with behavioral, EEG and seizure threshold data in this experimental model.

## Histological Features of Experimentally Induced NMD

The gross anatomy of the brain of pups treated with MAM was characterized by exposure of the quadrigeminal plate. Brain measurements revealed that the occipital cortex had a 23% decrease in size compared to controls (ANOVA Bonferroni, $F(4,80) = 17.5$; $P < .001$) and was equally affected in all experimental groups. On the other hand, the degree of decrease in frontal, temporal, and parietal cortical thickness, hippocampal and diencephalon area varied among MAM-exposed rats ranging from 0% to 30% decrease compared to controls. MAM-exposed rats were initially divided into two groups. Animals with a decrease in cortical thickness by ≥25% were grouped as severe NMD and those with decrease in cortical thickness <25% rats were grouped as nonsevere NMD. In rats with severe NMD, hippocampus and diencephalon had a decrease in size of ≥20% compared to controls; in the nonsevere group, hippocampus and diencephalon had a <20% decrease in size compared to controls. Rats with severe NMD were only found in the PG 15 group; 54% of the rats had severe NMD. Furthermore, MAM-exposed rats at PG 15 had significant hippocampal asymmetry characterized by up to 18% difference in size between the two sides.

Histological features of experimentally induced NMD included neuronal migration abnormalities in the neocortex, hippocampus, and diencephalon.

*In the neocortex,* analysis of Nissl-stained histological slides showed abnormal neuronal migration resulting in loss of the normal lamination pattern in layers II–V and neuronal depletion (Fig. 2) more pronounced in rats with severe NMD. Furthermore, in rats with severe NMD, abnormally migrated neurons were often found in clusters in cortical layers II–V (Fig. 3B). Additionally, neurons often had large bodies, irregular shape, and abnormal orientation (Fig. 3B). Marginal glioneuronal heterotopia characterized by excrescence of neuroglial tissue extruding into the subarachnoid space was seen in most rats (Fig. 3C). Scattered ectopic neurons were found in cortical layer I, suggestive of persistent granular layer (Fig. 3B). Heterotopic neurons in the subcortical white matter were often seen (Fig. 4). The depletion of cortical neurons paralleled a decrease in size of the corpus callosum (Fig. 4).
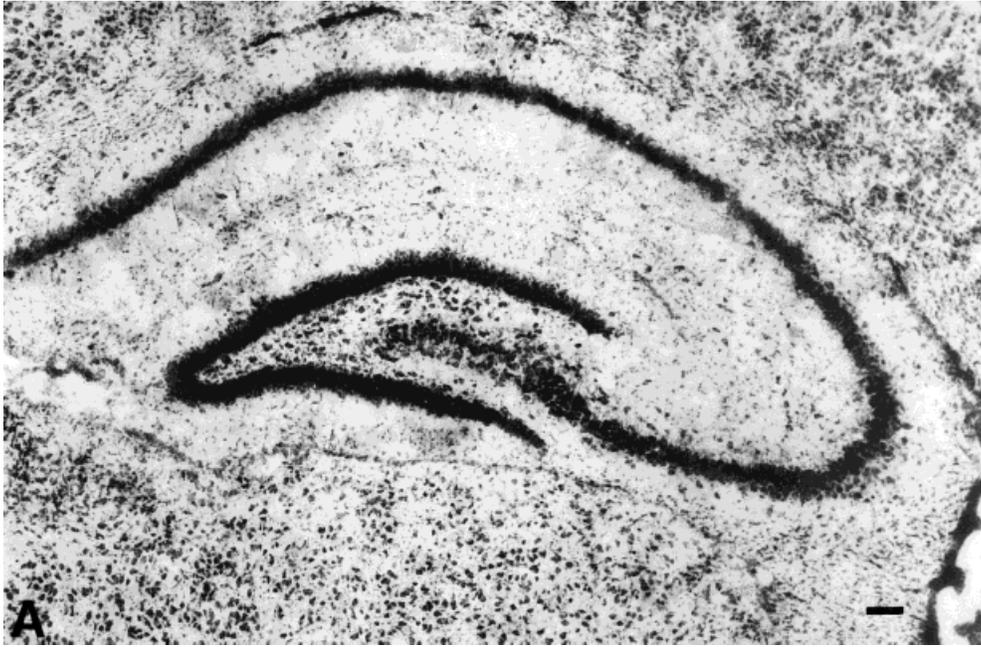
Fig. 5. Photomicrographs of frozen rat brain coronal sections at the level of the hippocampus showing histological features of experimentally induced NMD. **A:** Control rat. **B:** (See facing page.) Rat with severe NMD showing a large area of neuronal ectopia in the CA1 subfield (arrows). Ectopic neurons are scattered in the stratum oriens (so) and stratum radiatum (sr). Rats with moderate NMD had similar findings (data not shown). **C:** (See facing page.) Rat with mild NMD showing irregular cytoarchitecture of the pyramidal cell layer and presence of a small gap in the CA1 subfield (arrows). Thionin stain. Scale bar = 400 μm.

*In the hippocampus,* discrete areas of neuronal ectopia in the pyramidal cell layer of the CA1 subfield were found in PG 15 MAM-exposed rats only (Fig. 5B). These were present in 86% of rats exposed to MAM at PG 15 and were bilateral in 82% of cases. Discrete areas of neuronal ectopia were found in 100% of rats with severe NMD and in 32% of rats with nonsevere NMD. Areas of hippocampal neuronal ectopia were located most often in the anterior dorsal hippocampus (75% of the cases) and had dimensions varying from $100 \times 50$ μm to $500 \times 500$ μm. The ectopic neurons were scattered in the stratum radiatum and oriens in the CA1 subfield. In rats with ectopic CA1 pyramidal neurons, Timm stain indicated normal staining (not shown) suggesting that there is no aberrant organization of the mossy fibers.

Focal areas of hippocampal neuronal ectopia were not seen in rats exposed to MAM at the other gestational ages. However, the pyramidal cell layer of the CA1 subfield showed markedly disturbed cytoarchitecture in 63% of PG 13 rats, 70% of PG 14 rats, 14% of PG 15, and 85% of PG 16 rats (Fig. 5C). This cell layer was uneven in thickness with a ''wavy'' appearance and had numerous small 200-μm gaps (Fig. 5C). The remaining hippocampal subfields (CA2 and CA3/4) and the dentate gyrus were not affected in any of the rats examined.

*In the diencephalon,* neuronal loss was seen in all nuclei including the striatum and thalamus. This corresponded to a decrease in size of the white matter bundles.

## Effects of Transplacental Exposure to MAM at Different Gestational Ages on Experimentally Induced NMD

Based on these histological observations reported above, we concluded that transplacental exposure to MAM at PG 15 results in three phenomena: (1) severe cortical derangement with neuronal loss and decreased cortical thickness $\leq 25\%$; this was always found in combination with discrete areas of hippocampal neuronal ectopia in the CA1 subfield; (2) cortical derangement with neuronal loss and decreased cortical thickness $\leq 25\%$ with presence of discrete areas of hippocampal neuronal ectopia in the CA1; and (3) cortical derangement with neuronal loss and decreased cortical thickness $\leq 25\%$ without any areas of hippocampal neuronal ectopia in the CA1. Therefore, we finalized our classification of MAM-induced NMD as following: rats with severe NMD were those with cortical thickness $\geq 25\%$; rats with moderate NMD were rats with decreased cortical thickness $\leq 25\%$ and discrete areas of hippocampal neuronal
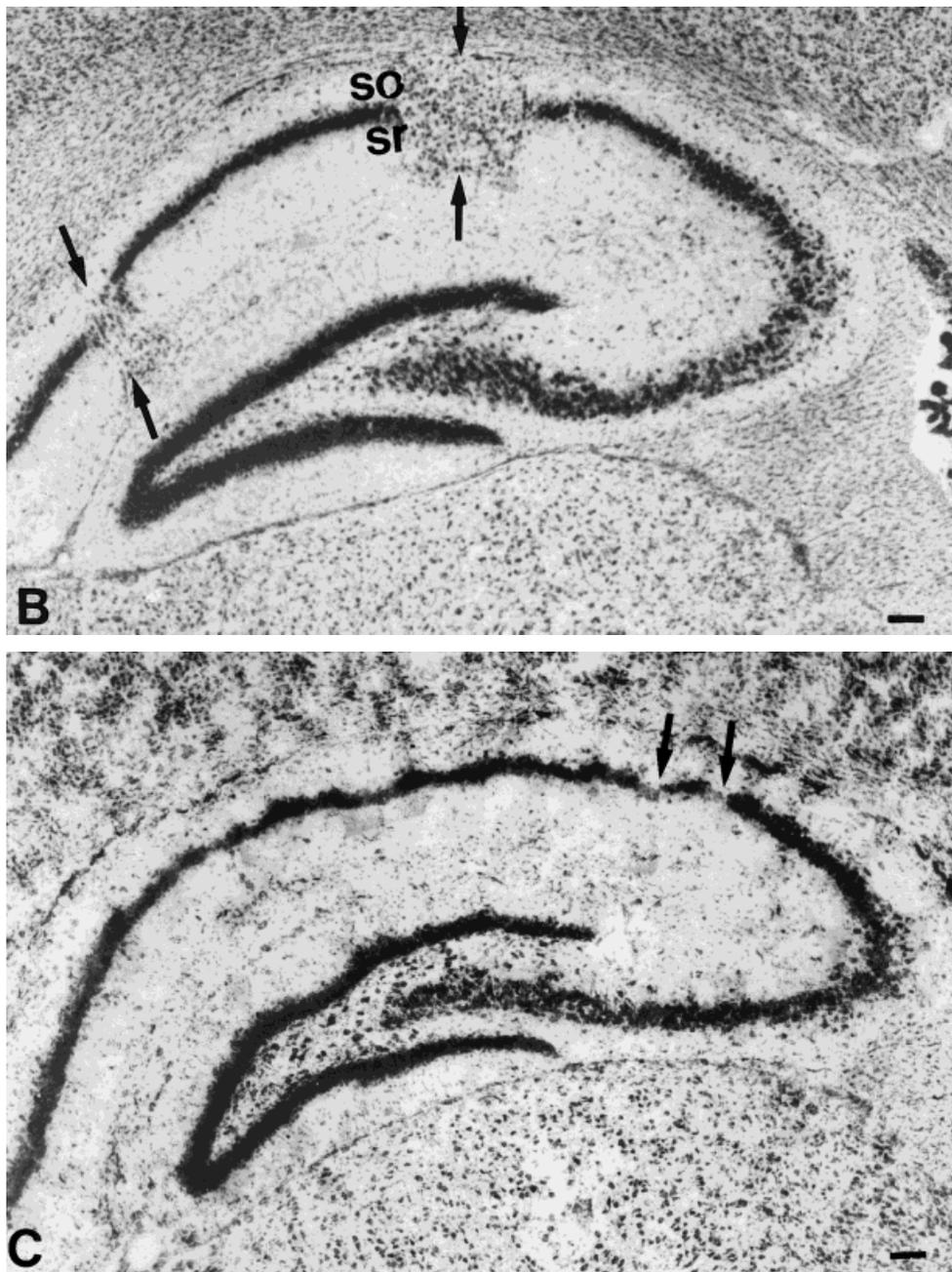
Figure 5 B and C.  (Legend appears on page 480.)

ectopia; rats with mild NMD had decreased cortical thickness ≤25% (Table I).

Incidence and severity of experimentally induced NMD were maximal in rats exposed to MAM at PG 15. NMD were found in all rats (n = 103) exposed to MAM at PG 15; lower percentages were found in the other three groups (Fig. 6). Furthermore, severe and moderate NMD grades were only found in rats exposed to MAM at PG 15. Thus, decrease in cortical thickness ≥25% and alteration of neuronal migration in the CA1 hippocampal subfield is specific for exposure to MAM at PG 15. In PG 15 rats, the histological type of NMD was homogeneously distributed within each litter: 80% to 100% of pups belonging to the same litter had the identical NMD grade. However, different litters exposed to MAM at the same gestational age were heterogeneously affected displaying histological features of the severe, moderate, or mild type NMD.

**TABLE I. Classification of Experimentally Induced Neuronal Migration Disorders (NMD)**

| Type | Histology |
|---|---|
| Severe | Decreased cortical thickness ($\geq$25%), hippocampal and diencephalon size ($\geq$20%) with defined areas of neuronal ectopia in the hippocampus and cortex. |
| Moderate | Decreased cortical (<25%), hippocampal size and diencephalon (<20%) with defined areas of neuronal ectopia in the hippocampus and cortex. |
| Mild | Decreased cortical (<23%), hippocampal and diencephalon size (<20%) with cytoarchitectural disturbances of hippocampal pyramidal cell layer. |

## Effects of Transplacental Exposure to MAM at Different Gestational Ages on Length of Gestation, Litter Size, Body and Brain Weight, Behavior, and Electrographic Activity

Length of gestation period, litter size, body and brain weight, behavior, and electrographic activity of rat pups exposed to MAM were compared to age-matched controls. Length of gestation and litter size did not vary among groups. Only pups exposed to MAM at PG 15 exhibited significant lower body (26 $\pm$ .5 g) and brain weight (1.3 + .1 g; controls: 30 $\pm$ .5 and 1.5 $\pm$ .1, respectively; $P < 0.05$). Differences in body and brain weight were not found in the other MAM-exposed groups (PG 13, 14, 16). Within the PG15 rats, pups with severe NMD did not have significantly smaller weights compared to those with moderate and mild NMD.

MAM treatment did not affect the general development of rat pups. Postnatal developmental milestones including eye opening, fur growth, sucking, ambulating, and grooming were reached at the same age in all experimental groups and controls.

EEG monitoring showed increased delta frequency in rats exposed to MAM at PG 15. In particular, rats with severe NMD had the most slowing on EEG ($P < .05$; Fig. 7). No differences in EEG were found in the other MAM-exposed groups. Spontaneous seizures and EEG changes indicating epileptic activity were not documented.
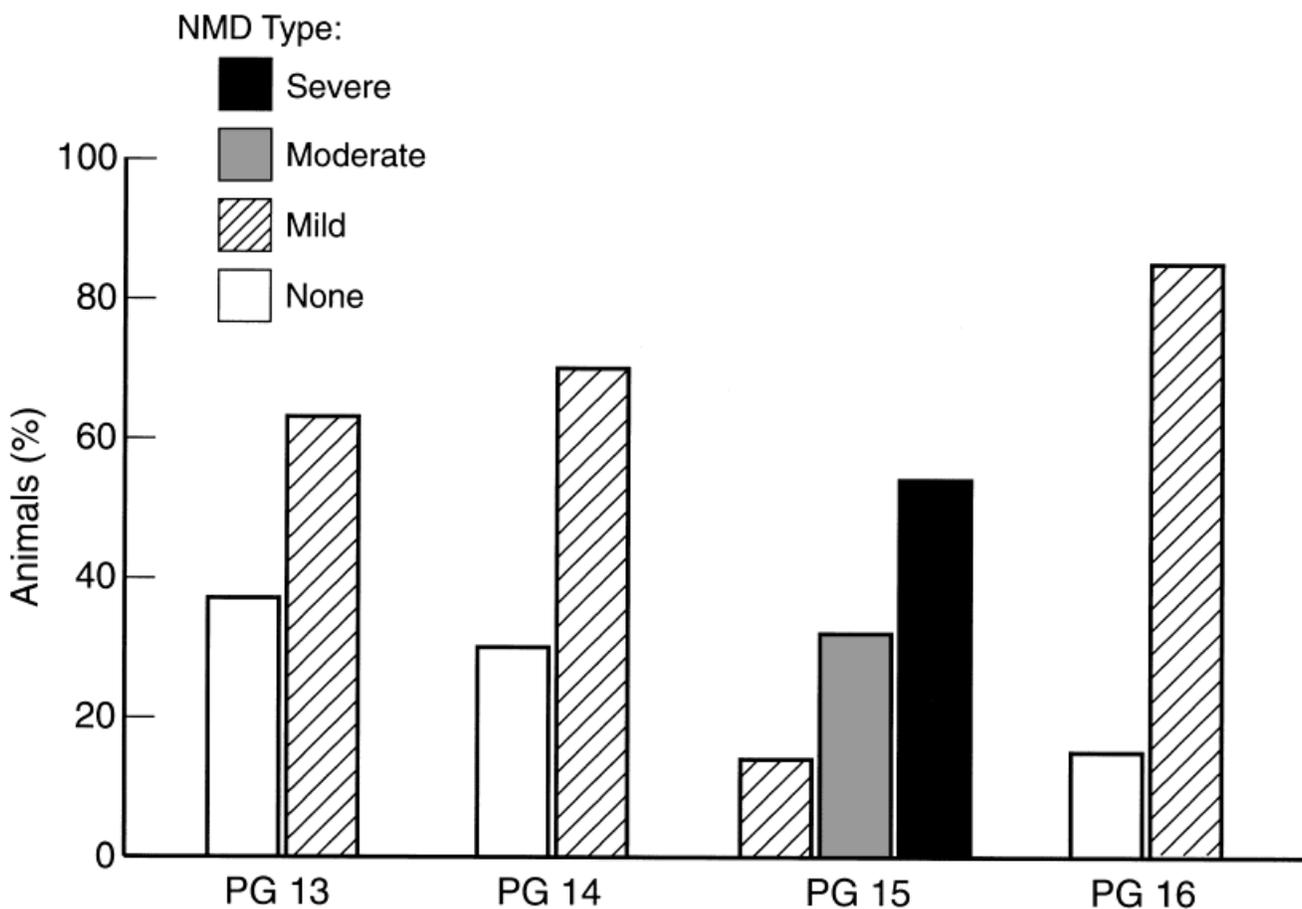


Fig. 6. Effects of transplacental exposure to MAM at different gestational days (PG) on the neuronal migration. NMD were found in 100% of rats exposed at PG 15. NMD types: see Table I.
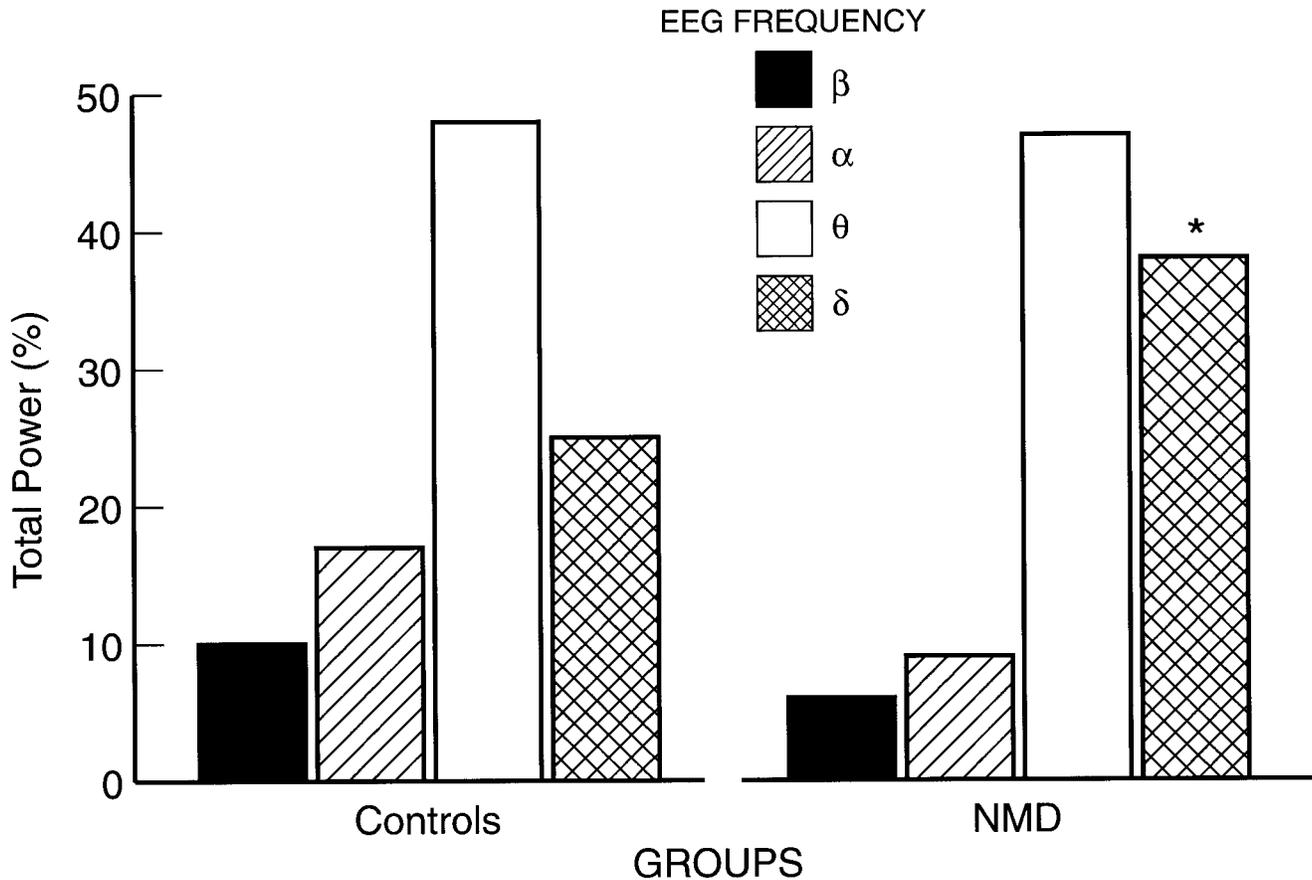
Fig. 7. Rats with NMD had electroencephalographic slowing. Baseline EEG of 14-day-old rats with NMD and age-matched controls. Rats with severe NMD (see Table I) had significant increase in delta frequency compared to controls (controls: n = 10; NMD: n = 10, *P* < .05, Student's t-test).

## Effects of Transplacental Exposure to MAM at Different Gestational Ages on Seizure Threshold and Seizure-Induced Acute Hippocampal Damage

Seizure threshold in the presence of NMD was assessed by analysis of seizure latency after administration of systemic kainic acid, severity of behavioral seizure pattern, duration of status epilepticus, and seizure-induced mortality in 14-day-old rats with NMD and controls. A lower seizure threshold was found in the PG 15 group comparing MAM-exposed rats with controls. In particular, latency to onset of clonic seizures was 53 ± 2 min in PG 15 MAM-exposed rats and 77 ± 8 min in controls (unpaired Student's t-test, t (35) = −4.048; *P* = .0003) and the latency to tonic seizures was 55 ± 2 min in PG15 MAM-exposed rats and 93 ± 10 in controls (unpaired Student's t-test, t (30) = −5.281; *P* < .0001). There were no differences among the PG 13, 14, and 16 MAM-exposed rats and controls, suggesting that both cortical and hippocampal dysplasia are necessary to increase seizure susceptibility in this experimental model.

Within the PG 15 group (Fig. 8), rats with severe NMD had a significantly shorter latency to onset of clonic and tonic seizures (ANOVA Bonferroni F(3,33) = 6.470; *P* = .0002). Furthermore, in rats with severe NMD (n = 10), the behavioral manifestation of seizures was significantly different compared to control ($\chi^2(3)$ = 9.6; *P* = .015). In particular, in 90% of control rats (n = 10) and rats with moderate (n = 6) and mild (n = 11) NMD, behavioral seizures manifested with clonic movements before the onset of tonic convulsions without preceding clonic seizures in 60% of the cases. There was no difference in duration of status epilepticus and seizure-induced mortality among groups and within the PG 15 group (PG 15 mortality: severe NMD: 5/10; moderate: 3/6; mild 4/11; controls: 5/10; in the other PG groups mortality was 50%).

Abundant "shrunken" and "dark" neurons (Fig. 9) were seen in the pyramidal cell layer of the CA3/4 hippocampal subfield 24 hours after kainate-induced status epilepticus in all surviving pups with severe NMD (n = 6, ANOVA Bonferroni F(3,20) = 163.9; *P* < .001). These
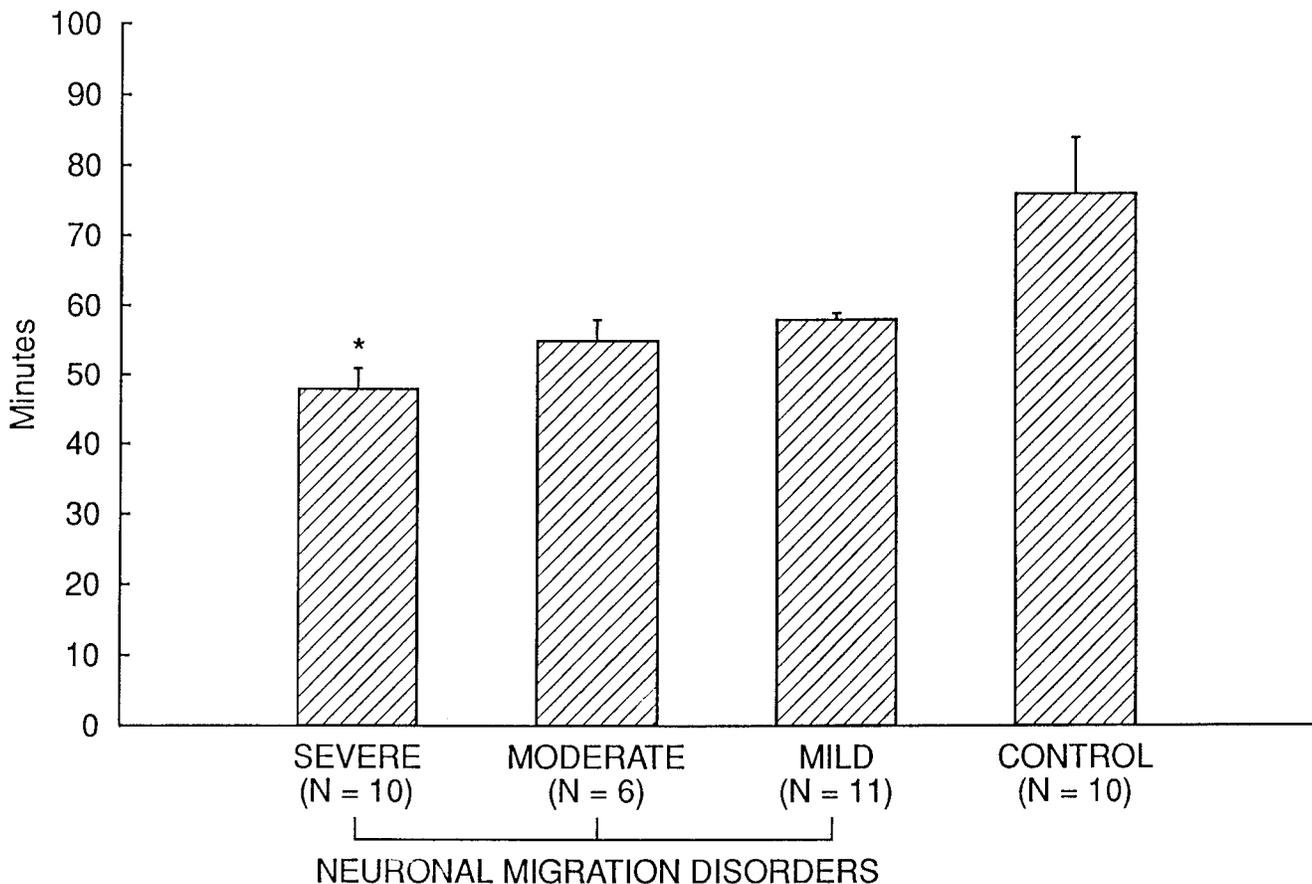
Fig. 8. Rats with NMD had a shorter latency-to-seizure onset. Seizure latency after kainic acid administration in rats with severe NMD (see Table I) had a shorter latency to onset of clonic seizures compared to controls. (controls: n = 10; NMD: n = 10) *$P < .05$ Student's t-test; data are expressed in mean ± S.E.

neurons represented seizure-induced acute cell damage. "Dark" neurons were not seen in cortical layers or other hippocampal neurons in the same rats, suggesting that the damage occurred only in "vulnerable" hippocampal neurons. In the other groups, kainic acid-induced status epilepticus did not produce any evidence of hippocampal neuronal damage.

## DISCUSSION

### Methylation of Nucleic Acid During Neuroblastic Division Induces Experimental NMD

Our study is the first to provide a classification and a systematic description of the histological features observed after transplacental exposure to MAM at different gestational ages in a large number of rats. In humans, NMD have an heterogeneous presentation and vary from lissencephaly to subcortical heretotopia (Anderman and Palmini, 1992). These differences probably depend on the severity of failure of the neuroblasts to migrate from germinal matrix to the loci where they are destined to reside. In our experimental model of NMD, we also observed a spectrum of neuronal migration abnormalities. However, severe and moderate disturbances of the neuronal migration occurred only in rats exposed to MAM at PG 15. These data demonstrate that the most vulnerable period for exogenous alteration of the nucleic acid inducing NMD is at the time of neuroblastic division. In the rat, neuroblastic division and migration of neuronal precursors to cortical layers II–V and pyramidal cell layer in the hippocampus occur at PG 15. MAM exposure at PG 15 targets these neuroblasts that are in the G1 and M phase. This can result in mitotic arrest and demise of the neuronal precursors or in the abnormal neuronal migration with the features described in this paper. The sparing of cortical layers I and VI (Jones et al., 1982) reflects the fact that neurons in these layers have completed their final division and migrated toward their target layers by PG 15 (Berry and Rogers, 1965).

The different grades of NMD that we observed may be caused by the severity of methylation occurring in each rat. Severe methylation may result in mitotic arrest
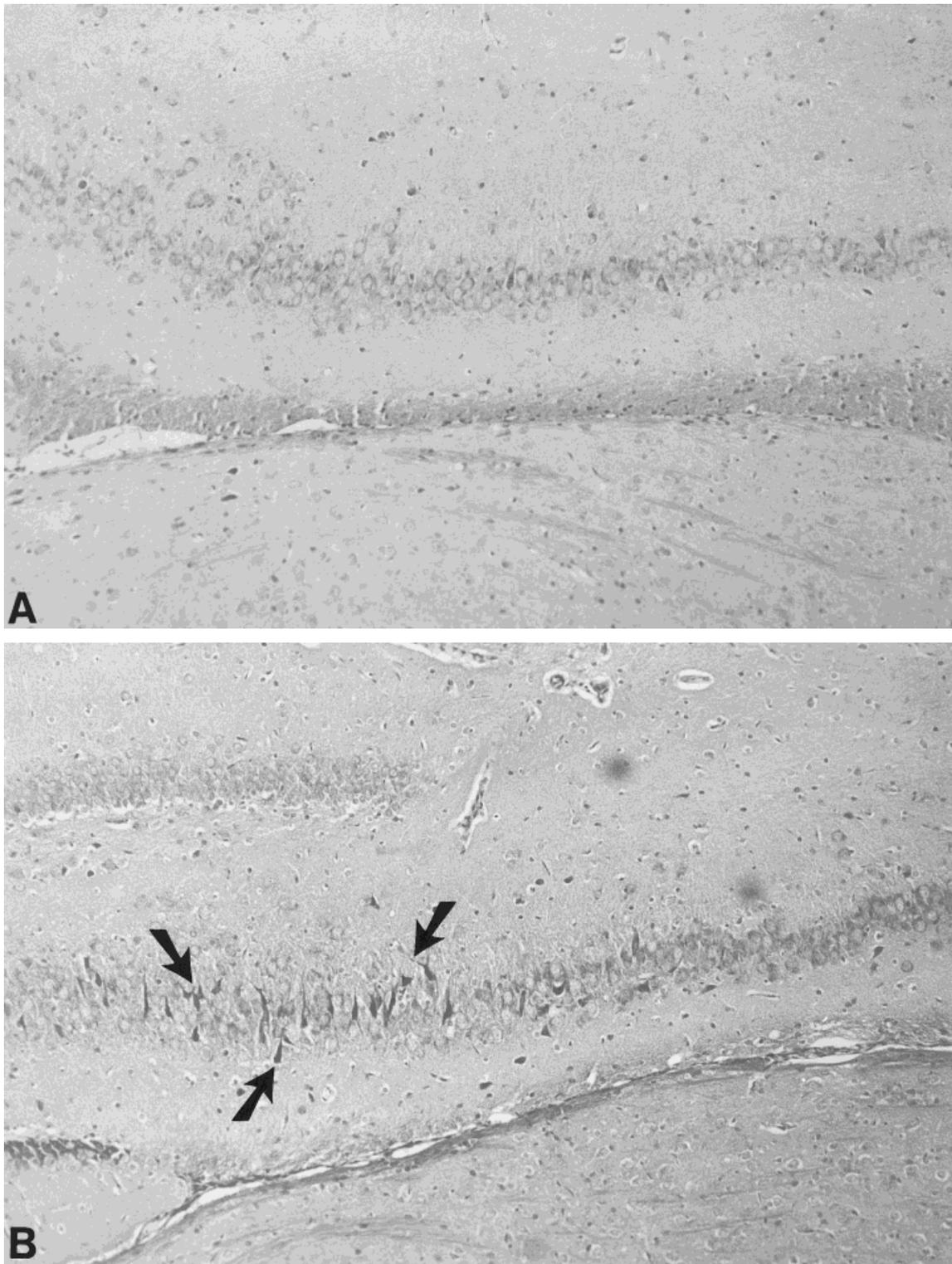
Fig. 9. Photomicrographs of rat brain coronal sections at the level of the hippocampus showing the CA3/4 subfield in a control (**A**) and a rat with NMD (**B**) 24 hours after kainate-induced status epilepticus. Dark and shrunken neurons consistent with seizure-induced early cell damage (arrows in B) were frequent in rats with NMD and were not seen in controls pups.

and immediate cell demise. Alternatively, severe methylation could cause changes in the cell genome leading to premature programmed cell death (apoptosis) and delayed neuronal demise. Both mechanisms are responsible for the significant neuronal loss seen in rats with severe NMD causing cortical atrophy. On the other hand, the heterogeneity in severity of neuronal migration disturbances observed in rats exposed to MAM at PG15 may rely on variability in the rate of MAM transported through the placenta (Matsumoto et al., 1972). Another explanation could be the relationship between the time of MAM injection and the actual time of pregnancy. Although the rats used in our study were time-pregnant, most likely dams were injected at different hours within the 24-hour period of the 15th day. This could have resulted in methylation of different amounts of neuroblasts in G1 and M phase, causing more severe effects when the latter cells were affected.

## MAM-Induced Experimental NMD Have Features Similar to Those Seen in Humans

NMD in humans encompass malformative processes of the cerebral cortex which exhibit a spectrum of pathological disturbances in the cortical development. Recently, Mischel et al. (1995) reviewed the histopathological features in 77 patients who underwent cortical resection for medically refractory epilepsy. Their histologic findings included cortical laminar disorganization, irregular neuronal clustering, neuronal cytomegaly, subcortical heterotopic neurons, and marginal glial neuronal heterotopia. All the above changes were found in MAM-induced NMD.

Mental retardation and EEG abnormalities are often present in children with NMD (Anderman and Palmini, 1992). Similarly, intellectual deficits in MAM-exposed rats are well documented in the literature (Balduini et al., 1991; Haddad et al., 1969; Vorhees et al., 1984; Rabe and Haddad, 1972). In our study, EEG recording showed increased slow wave activity in rats with severe NMD. These data further underline the similarity between human and our experimental model of NMD.

## Experimentally Induced NMD Lower Seizure Threshold

NMD are often found in patients with epilepsy, suggesting that there may be a link between the two pathologies (Palmini et al., 1991; Anderman et al., 1994). On the other hand, NMD may be an incidental finding in patients undergoing MRI for reasons unrelated to epilepsy. This observation raises the question that NMD alone may not consistently cause epilepsy. However, in vitro studies suggested that brain slices from MAM-exposed rats have increased epileptiform discharges in controlled medium (Baraban and Schwartzkroin, 1996).

We have recently shown than in the rat, severe NMD decrease the susceptibility to hyperthermia-induced seizures in the immature brain (Germano et al., 1996). In the present study, we showed that only severe alteration of the neuronal cytoarchitecture decreases the susceptibility to systemic kainic acid seizures in the rat pup. Thus, in the immature brain, various degrees of altered neuronal migration pattern may be associated with differences in seizure susceptibility. This experimental work might bring insights into the differences of seizure susceptibilities seen in the humans in the presence of NMD.

## Seizure-Induced Acute Hippocampal Neuronal Damage in the Immature Brain With NMD

Data from a variety of sources stress that the immature brain is more susceptible to seizures (Sperber et al., 1991; Albala et al., 1984; Moshe' et al., 1983; Hauser and Kurland, 1975). On the other hand, laboratory data on seizure-induced hippocampal damage in the immature brain are contradictory (Sperber et al., 1991; Franck and Schwartzkroin, 1984; Thompson and Westerlain, 1997). Recently, we showed that in rats with severe NMD, hyperthermia caused acute neuronal damage that seemed to be independent of a brief hyperthermia-induced seizure (Germano et al., 1996). In the present study, we showed that status epilepticus in the presence of severe NMD causes acute hippocampal neuronal damage. Thus, in the immature brain, seizure-induced neuronal damage seems to require status epilepticus with a preexisting hippocampal pathology, such as severe NMD. The seizure-induced neuronal damage observed in this model is localized to the CA3/4 pyramidal neurons. Transplacental exposure to MAM causes a broad spectrum of neuronal dysplasia as described in this paper. Thus, transplacental exposure to MAM seems to be a good experimental model to parallel the findings in children with hippocampal pathology, "dual lesion," and medically refractory seizures (Levesque et al., 1991). Further studies are necessary to ascertain the consequences of the acute hippocampal neuronal damage observed in this study. This could result in a "glial scar" weeks after status epilepticus, and could become the substrate for epilepsy later in life. Experiments are currently ongoing to confirm this hypothesis.

## Conclusions

Methylation of nucleic acids by MAM during neuroblastic division in the rat produces a range of disorders of cortical organization similar to that observed in humans. These data suggest that nucleic acid methylation may be one of the mechanisms responsible for neuronal migrational disorders. The severity of NMD is dependent on the gestational age targeted: the most severe neuronal migrational abnormalities are seen in rats ex-

posed to MAM at the time of neuroblastic division. Furthermore, in this model, only severe NMD increases susceptibility to seizures. In addition, in the presence of severe NMD, seizure-induced acute hippocampal neuronal damage is observed in the immature brain. We conclude that transplacental exposure to MAM at PG15 is a useful model to study the relationship between NMD and seizure disorders.

## ACKNOWLEDGMENTS

## REFERENCES

Albala BJ, Moshe SL, Okada R (1984): Kainic acid-induced seizures: A developmental study. Dev Brain Res 13:139–148.

Andermann E, Palmini A (1992): Neuronal migration disorder, tuberous sclerosis, and Sturge-Weber syndrome. In Lüders HO (ed): "Epilepsy Surgery." New York: Raven Press, pp 203–211.

Andermann E, Dobyns WB, Walsch C, Pinard J-M, Dulac O, Palmini A, Dubeau F, Tampieri D, Lee N, Andermann F (1994): X-linked inheritance in neuronal migration disorder (NMD): A clue to pathogenesis. Epilepsy 35:152.

Angerine JB, Sidman RL (1961): Autoradiographic study of cell migration during histogenesis of cerebral cortex in mouse. Nature 1192:766–768.

Balduini W, Abbracchio MP, Lombardelli G, Cattabeni F (1984): Loss of intrinsic striatal neurons after methylazoxymethanol acetate treatment in pregnant rats. Dev Brain Res 15:133–136.

Balduini W, Lombardelli G, Peruzzi G, Cattabeni F (1991): Treatment with methylazoxymethanol at different gestational days: Physical, reflex development and spontaneous activity in the offspring. Neurotoxicology 12:179–188.

Baraban SC, Schwartzkroin PA (1996): Electrophysiology of CA1 pyramidal neurons in an animal model of neuronal migration disorders: Prenatal methylazoxymethanol treatment. Epil Res 22:145–156.

Berry M, Rogers AW (1965): The migration of neuroblasts in developing cortex. J Anat (Lond) 99:691–709.

Bloom W, Fawcett DW (1975): "A Textbook of Histology." Philadelphia: Saunders Company, pp 73–74.

Brodtkorb E, Nilson G, Smevik O, Rinck PA (1992): Epilepsy and anomalies of neuronal migration: MRI and clinical aspects. Acta Neurol Scand 86:24–32.

Carpenter MB, Sutin J (1983): "Human Neuroanatomy." Baltimore: William & Wilkins, pp 61–84.

Cattabeni F, Abbracchio MP, Cimino M, Cocchi D, Di Luca M, Mennuni L, Rosi F, Zaratin P (1989): Methylazoxymethanol-induced microencephaly: Persistent increase of cortical somatostatin-like immunoreactivity. Dev Brain Res 47:156–159.

Cherubini E, De Feo Mr., Mecarelli O, Ricci GC (1983): Behavioral and electrographic patterns induced by systemic administration of kainic acid in developing rats. Dev Brain Res 9:69–77.

Dvorak K, Feit J (1977): Migration of neuroblasts through partial necrosis of the cerebral cortex in newborn rats. Contribution of the problems of morphological developmental period of cerebral microgyria. Acta Neuropathol 38:203–212.

Franck JE, Scwartzkroin PA (1984): Immature rabbit hippocampus is damaged by systemic but not intraventricular kainic acid. Brain Res 315:219–227.

Germano IM, Poulin N, Olivier A (1994): Reoperation for current temporal lobe epilepsy. J Neurosurg 81:31–36.

Germano IM, Zhang YF, Sperber EF, Moshe SL (1996): Neuronal migration disorders increase the susceptibility to hyperthermia-induced seizures in the rat. Epilepsia 37:902–910.

Guerrini R, Dravet C, Raybaud C (1992): Epilepsy and focal gyral anomalies detected by MRI: Electroclinico-morphological correlation and follow up. Dev Med Child Neurol 34:706–718.

Haddad RK, Rabe A, Laquer GL, Spatz M, Valsamis MP (1969): Intellectual deficit associated with transplacentally induced microcephaly in the rat. Science 163:88–90.

Hauser WA, Kurland LT (1975): The epidemiology of epilepsy in Rochester, Minnesota, 1935–1967. Epilepsia 16:1–66.

Jan YN, Jan LY (1990): Genes required for specifying cell fates in Drosophila embryonic sensory nervous system. Trends Neurosci 13:493–498.

Johnston MV, Coyle JT (1979): Histological and neurochemical effects of fetal treatment with methylazoxy-methanol on rat neocortex in adulthood. Brain Res 170:135–155.

Jones EG, Valentino KL, Fleshman JW, Jr (1982): Adjustment of connectivity in rat neocortex after prenatal destruction of precursor cells of layers II–IV. Dev Brain Res 2:425–431.

Kabat K, Buterbaugh GG, Eccles CU (1985): Methylazoxymethanol as a developmental model of neurotoxicity. Neurobehav Toxicol Teratol 7:519–525.

Lee N, Radtke RA, Gray L, Burger PC, Montine TJ, DeLong GR, Lewis DV, Oakes WJ, Friedman AH, Hoffman JM (1994): Neuronal migration disorders: Positron emission tomography correlation. Ann Neurol 35:290–297.

Levesque MF, Nakasato N, Vinters H, Babb TL (1991): Surgical treatment of limbic epilepsy associated with extrahippocampal lesions: The problem of dual pathology. J Neurosurg 75:364–370.

Lorente de No R (1934): Studies on the structure of the cerebral cortex II. Continuation of the study of ammonic system. J Psychol Neurol 45:113–177.

Majkowski J, He Lee M, Kozlowski PB, Haddad R (1984): EEG and seizure threshold in normal and lissencephalic ferrets. Brain Res 307:29–38.

Maytal J, Shinnar S (1990): Febrile status epilepticus. Pediatrics 86:611–616.

Matsumoto H, Spatz M, Laquer GL (1972): Quantitative changes with age in the DNA content of methylazoxymethanol-induced microencephalic rat brain. J Neurochem 19:297–306.

Mischel PS, Nguyen LP, Vinters HV (1995): Cerebral cortical dysplasia associated with pediatric epilepsy. Review of neuropathological features and proposal for a grading system. J Neuropath Appl Neurobiol 54:137–153.

Moshe' SL (1987): Epileptogenesis and the immature brain. Epilepsia 28:S3–S15.

Moshe' SL, Albala BJ, Ackermann RF, Engel JJ (1983): Increased seizure susceptibility of the immature brain. Dev Brain Res 7:81–85.

Nagata Y, Matsumoto H (1969): Studies on methylazoxymethanol; Methylation of nucleic acids in fetal rat brain. Proc Soc Exp Biol Med 132:383–385.

Nelson KB, Ellemberg JH (1976): Predictors of epilepsy in children who have experienced febrile seizures. N Engl J Med 295:1029–1033.

Palmini A, Andermann F, Olivier A (1991): Focal Neuronal migration disorder and intractable partial epilepsy: A study of 30 patients. Ann Neurol 30:741–749.

Paxinos G, Watson C (1986): "The Rat Brain in Stereotactic Coordinates." New York: Academic Press.

Rabe A, Haddad RK (1972): Methylazoxymethanol-induced microencephaly in rats; Behavioral studies. Fed Proc 31:1536–1539.

Represa A, Robain O, Tremlav E, Ben-Ari Y (1989): Hippocampal plasticity in childhood epilepsy. Neurosci Lett 99:351–355.

Richman DP, Stewart RM (1974): Cerebral microgyria in a 27-week fetus: An architectonic and topographic analysis. J Neuropathol Exp Neurol 33:374–384.

Robitaille Y, Rasmussen T, Dubeau F (1992): Histopathology on non-neoplastics lesions in frontal love epilepsy. In Chauvel P, Delgado-Escueta AV, Halgren E, Bancaud J (eds): "Advances in Neurology: Frontal Lobe Seizures and Epilepsies." New York: Raven Press, 57:499–513.

Rorke LB (1994): A perspective: The role of disordered genetic control of neurogenesis in the pathogenesis of migration disorders. J Neuropathol Exp Neurol 53:105–117.

Shinnar S, Berg AT, Moshe' SL, Petix M, Maytal J, Kang H, Goldensohn E, Hauser A (1990): Risk of seizure recurrence following a first unprovoked seizure in childhood: A prospective study. Pediatrics 85:1076–1085.

Singh SC (1977): Ectopic neurones in the hippocampus of the postnatal rat exposed to methylazoxymethanol during foetal development. Acta Neuropathol (Berl.) 40:111–116.

Singh SC (1978): Redirected perforant and commissural connections of eutopic and ectopic neurons in the hippocampus of methylazoxymethanol-acetate treated rats. Acta Neuropathol (Berl.) 44:197–202.

Singh SC (1980): Deformed dendrites and reduced spine numbers on ectopic neurones in the hippocampus of rats exposed to methylazoxymethanol-acetate. Acta Neuropathol (Berl.) 49:193–198.

Spatz M, Laqueur GL (1968): Transplacental chemical induction of microencephaly in two strains of rats. Proc Soc Exp Biol Med 1299:705–710.

Sperber EF, Haas KZ, Stanton P, Moshe' SL (1991): Resistance of the immature hippocampus to seizure-induced synaptic reorganization. Dev Brain Res 69:88–93.

Tauck DL, Nadler JV (1985): Evidence for functional mossy fiber sprouting in hippocampal formation of kainic acid-treated rats. J Neurosci 5:1016–1022.

Thompson K, Wasterlain C (1997): Partial protection of hippocampal neurons by MK-801 during perforant path stimulation in the immature brain. Brain Res 751:96–101.

Vorhees C, Fernandez K, Dumas R, Haddad RK (1984): Pervasive Hyperactivity and long-term learning impairments in rats with induced microencephaly from prenatal exposure to methylazoxymethanol. Dev Brain Res 15:1–10.