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Models of cortical malformation—Chemical and physical

Heiko J. Luhmann*

Institute of Physiology, University Medical Center of the Johannes Gutenberg University, Duesbergweg 6, D-55128 Mainz, Germany

HIGHLIGHTS

- Chemical and physical manipulations during early development induce cortical malformations.
- These malformations resemble cortical pathologies in humans with epilepsy.
- Animal models show cortical hyperexcitability due to excitatory–inhibitory imbalance.
- Animal models may uncover mechanisms of epileptic disorders.

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ABSTRACT

Pharmaco-resistant epilepsies, and also some neuropsychiatric disorders, are often associated with malformations in hippocampal and neocortical structures. The mechanisms leading to these cortical malformations causing an imbalance between the excitatory and inhibitory system are largely unknown. Animal models using chemical or physical manipulations reproduce different human pathologies by interfering with cell generation and neuronal migration. The model of in utero injection of methylazoxymethanol (MAM) acetate mimics periventricular nodular heterotopia. The freeze lesion model reproduces (poly)microgyria, focal heterotopia and schizencephaly. The in utero irradiation model causes microgyria and heterotopia. Intraperitoneal injections of carmustine 1-3-bis-chloroethyl-nitrosurea (BCNU) to pregnant rats produces laminar disorganization, heterotopias and cytomegalic neurons. The ibotenic acid model induces focal cortical malformations, which resemble human microgyria and ulegyria. Cortical dysplasia can be also observed following prenatal exposure to ethanol, cocaine or antiepileptic drugs.

All these models of cortical malformations are characterized by a pronounced hyperexcitability, few of them also produce spontaneous epileptic seizures. This dysfunction results from an impairment in GABAergic inhibition and/or an increase in glutamatergic synaptic transmission. The cortical region initiating or contributing to this hyperexcitability may not necessarily correspond to the site of the focal malformation. In some models wide-spread molecular and functional changes can be observed in remote regions of the brain, where they cause pathophysiological activities.

This paper gives an overview on different animal models of cortical malformations, which are mostly used in rodents and which mimic the pathology and to some extent the pathophysiology of neuronal migration disorders associated with epilepsy in humans.

1. Introduction

Cortical malformations are frequently the pathological substrate of epilepsy and can be often found in patients with pharmaco-resistant epilepsy. Models of cortical malformations, which are experimentally induced by chemical or physical manipulations of the developing cortex, resemble in many aspects the pathology and pathophysiology of epilepsy associated with malformations in human cortex. These chemical and physical manipulations interfere with different developmental processes occurring during prenatal and (depending on the species) early postnatal stages: (i) The generation of neurons (neurogenesis) and/or glial cells (gliogenesis) may be impaired. (ii) The insult may induce cell death or the process of programmed cell death (apoptosis) may be modified, e.g. cells destined to die may survive. (iii) Spontaneous activity patterns, which are important for the maturation of cortical networks may be altered. (iv) Most importantly, the tangential migration of inhibitory interneurons and the radial

* Tel.: +49 6131 39 26070; fax: +49 6131 39 26071.
E-mail address: luhmann@uni-mainz.de

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migration of pyramidal cells is often altered following these chemical or physical insults. The resulting malformations can be focal or diffuse extending over large cortical regions, resembling the spectrum of cortical pathologies described in tissue resected from patients with pharmaco-resistant epilepsy. The extent and type of the experimentally induced cortical malformation depends more on the time point of the insult in development and not so much on the cause. Therefore, in all animal models the timing of the experimental manipulation is most critical. The same protocol of chemical or physical insult will cause different pathologies when induced at different developmental stages.

It is mostly unclear how these structural lesions lead to seizure activity and animal models are required to understand the mechanisms of pathogenesis, epileptogenesis, and epileptogenicity of cortical malformations. An important and clinically unresolved issue relates to the question, whether the lesion itself is the main epileptogenic zone, or whether subtle molecular, structural and functional disturbances in the region surrounding the obvious lesion are the trigger for epileptic discharges (Luhmann et al., 2014).

This review will focus on different animal models, largely in rodents, of acquired cortical malformations (Table 1). For a review of genetic models of cortical malformations the reader is referred to the paper by S. Roper and R. Spreafico in this issue. This review will cover malformations in the hippocampus, an allocortical structure, and in the cerebral cortex, the neocortex.

2. Human pathology and pathophysiology of cortical malformations

One of the first reports on the pathology of cortical malformations associated with epilepsy goes back to Virchow (1867) who reported in the cerebral cortex of a 44 years old male patient local cell clusters and bulges ("Haufen und Wülste", p. 140) as an excellent example of heterotopia ("ausgezeichnetes Beispiel von Heterotopie"). A more recent overview on the pathology of cortical malformations in humans and some models has been published by Najm et al. (2007) and Takano (2011). Cortical malformations, especially focal cortical dysplasia, represent the most common pathological finding in pediatric epilepsy surgery patients, and range from mild to severe malformations including hippocampal sclerosis (Krsek et al., 2008). A number of molecular and cell physiological data have been obtained in cortical structures from human epileptic patients affected by periventricular nodular heterotopia, subcortical band beterotopia, or focal cortical dysplasia. Immunoblotting and immunoprecipitation analyses in human cortex resected from EEG-verified epileptic and distal nonepileptic areas demonstrated a higher expression of NMDA (NMDA) receptor subunits 1 and 2A/B in epileptic dysplastic cortex compared with the nonepileptic cortex, indicating that an increased NR1-NR2A/B coassembly may contribute to the epileptogenicity of the dysplastic cortex (Mikuni et al., 1999). These observations are supported by coimmunoprecipitation and immunoblotting studies in resected cortical tissues from patients with pharma-coresistant epilepsy associated with neocortical dysplasia, which showed an increased coassembly of NR1 and NR2B with PSD-95 when compared with non-epileptic tissue (Ying et al., 2004). A more distinct pattern of NMDA receptor modification has been reported by Finardi et al. (2006), who observed a selective increase in the NR2B subunit in all cortical dysplasia, but a reduced expression level of NR2A and NR2B subunits in all patients with heterotopia. These data suggest that different developmental malformations are associated with distinct alterations in NMDA receptor density and function. Differences in NMDA receptor function have been also reported at the cellular level with electrophysiological methods in pediatric cortical dysplasia. Cytomegalic neurons from human cortical dysplasia tissue showed NMDA currents with decreased Mg^{2+} sensitivity as compared to neurons from non-dysplastic tissue. Immunofluorescence analyses revealed a decrease in NR2B subunit expression in cytomegalic neurons and in a number of normal appearing pyramidal neurons from dysplastic tissue (André et al., 2004).

Beside these molecular and electrophysiological changes in the glutamatergic system, prominent alterations have been also documented in the structure and function of the GABAergic system in human cortical malformations. Using quantitative immunohistochemistry Thom et al. (2004) reported in cases of grey matter heterotopia in postmortem tissue from patients with epilepsy an overall normal density and distribution of GABA-containing interneurons, but morphologically these cells were less organized and more randomly orientated compared to control cortex. In contrast, a "scattering" of GABAergic interneurons has been demonstrated by Calcagnotto et al. (2005) in human dysplastic cortex. The same authors also showed with patch-clamp recordings from identified neurons a significant decrease in the frequency of spontaneous inhibitory synaptic currents (IPSCs), an increase in the decay-time constant of evoked and spontaneous IPSCs and a decrease in transporter-mediated GABA reuptake function. In slices from cortical tissue resected for the treatment of pharmacoresistant epilepsy in children (0.2–14 years), Cepeda et al. observed spontaneous GABA-mediated membrane depolarizations, which frequently elicited action potentials, indicating an excitatory role of GABA in pediatric cortical dysplasia (Cepeda et al., 2007). This assumption is supported by a report on changes in the expression of the chloride transporters KCC2 and NKCC1 in neocortical tissue resected in children with intractable focal epilepsy using quantitative western blot analyses (Jansen et al., 2010). A significant decrease in the mRNA and protein levels of the chloride outward transporter KCC2 has been demonstrated in human dysplastic tissue (Shimizu-Okabe et al., 2011), indicating that the chloride reversal potential is more depolarized, as reported by Cepeda et al. (2007). Finally, beside deficits in glutamatergic and GABAergic synaptic function contributing to the epileptogenicity of cortical malformations, abnormal intrinsic membrane properties have been also reported dysplastic cortex (Cepeda et al., 2003).

In the following paragraphs seven animal models of acquired cortical malformations associated with hyperexcitability or epileptic seizures will be reviewed.

3. The MAM model

The model of in utero injection of methylazoxymethanol (MAM) acetate into pregnant rats has been introduced by Spatz and Laqueur (1968). Singh (1977) documented in more detail the resulting hippocampal malformations, which are similar to periventricular nodular heterotopia in human patients with drug-resistant focal epilepsy. MAM is a potent cytotoxic agent, which induces a time-specific aberrant DNA methylation and alkylation leading to abnormal patterns of cell formation and migration. MAM seems to selectively affect developing brain structures and not other embryonic organs undergoing cell proliferation at the time of its administration. Neuronal precursors that are undergoing their final mitosis at the time of MAM exposure are specifically ablated, glial cells are only indirectly affected. Noctor et al. (1999) reported in the ferret neocortex, that injection of MAM during embryogenesis produces distorted radial glial cells and concluded that this interference with early cortical development causes premature differentiation of radial glial cells into astrocytes. Beside its antimitotic effects, MAM also seems to directly influence neuronal migration. The migration speed and the exploratory behavior of migrating neurons is significantly reduced after MAM treatment (Abbah and Juliano, 2014). MAM also modifies the migration pattern of interneurons, but this deficit is not intrinsic to the migrating