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Early susceptibility for epileptiform activity in malformed cortex

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Summary Despite early disruption of developmental processes, hyperexcitability is often delayed after the induction of cortical malformations. In the freeze-lesion model of microgyria, interictal activity cannot be evoked in vitro until postnatal day (P)12, despite the increased excitatory afferent input to the epileptogenic region by P10. In order to determine the most critical time period for assessment of epileptogenic mechanisms, here we have used low-Mg^{2+} aCSF as a second hit after the neonatal freeze lesion to examine whether there is an increased *susceptibility* prior to the overt expression of epileptiform activity. This two-hit model produced increased interictal activity in freeze-lesioned relative to control cortex. We quantified this with measures of incidence by sweep, time to first epileptiform event, and magnitude of late activity. The increase was present even in the P7—9 survival group, before increased excitatory afferents invade, as well as in the P10—11 and P12—15 groups. In our young adult group (P28—36), the amount of interictal activity did not differ, but only the lesioned cortices produced ictal activity. We conclude that epileptogenic processes begin early and continue beyond the expression of interictal activity, with different time courses for susceptibility for interictal and ictal activity.

Introduction

Cortical malformations, induced by errors in developmental steps or loss of neurons during the formation of the cortical plate, necessarily affect the subsequent course of maturation (Squier and Jansen, 2010). Interestingly, despite this early disruption of cellular processes and connections, hyperexcitability is often delayed in both experimental epilepsy models (Cobos et al., 2005; Jacobs et al., 1999a; Trotter et al., 2006; Zeng et al., 2011) and the clinical population (Bartolomei et al., 1999; Kobayashi et al., 2001; Park et al., 2006; Rademacher et al., 2000; Teixeira et al., 2007; Tezer et al., 2008; Tinuper et al., 2003; Widdess-Walsh et al., 2005). A waiting or latent period suggests that epileptogenic mechanisms are developing but not yet capable of producing epileptiform activity. This then makes the latent period a critical time for identification of epileptogenic mechanisms, particularly since seizures themselves can alter physiological processes (Mathern et al., 2002) and exacerbate hyperexcitability (Isaeva et al., 2010).

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The rodent neonatal freeze lesion model of microgyria replicates the histopathological features of human 4-layered polymicrogyria (Dvorak et al., 1978; Dvorak and Feit, 1977; Jacobs et al., 1996), with a focal four-layered malformed region missing deep layers present by P7 (Rosen et al., 1992). We have previously suggested that the absence of layer IV within the microgyrus triggers reorganization of afferent inputs, particularly thalamocortical ones that find the appropriate laminar targets in the cortex surrounding the malformation, the paramicrogyral region (PMR) (Jacobs et al., 1999b). This was subsequently supported by anatomical studies showing that afferents avoid the microgyrus and accumulate in the PMR (Rosen et al., 2000). The physiological evidence of increased excitatory input to this region further suggested that this remapping of afferents might contribute to the expression of epileptiform activity that occurs with an abrupt onset on P12 (Jacobs et al., 1999a). To confirm that excitatory hyperinnervation could be a cause rather than a result of hyperexcitability, we have previously examined whether it occurred prior to or after the onset of network hyperexcitability at P12. We found that there was an abrupt and consistent increase in the frequency of mEPSCs in PMR pyramidal neurons on P10 (Zsombok and Jacobs, 2007). When neonatally freeze-lesioned animals are given a second hit via hyperthermia on P10, they develop spontaneous seizures as adults (Scantlebury et al., 2005). Since many alterations of microgyral and PMR cortex other than the increased excitatory afferents are known, it is important to determine if it is indeed the presence of this increased afferent input that initiates the epileptogenesis.

To examine this we have asked if there is an increased susceptibility for epileptiform activity during the latent period, particularly coinciding with the hyperinnervation on P10. We have tested this in vitro with a two-hit model of neonatal freeze lesion followed 6 or more days later by the presence of low-Mg^{2+} aCSF. Low-Mg^{2+} aCSF has commonly been used to test for hyperexcitability and models a general overall increase in cortical excitability.

Methods

Freeze lesion

On postnatal day (P) one, Sprague Dawley rat pups were anesthetized in ice for ~4 min. When movement and response to tail pinch ceased, an incision was made through the scalp. With the skull exposed, a freezing (−50° C) rectangular probe (tip size = 2 mm × 5 mm) was placed over the somatosensory cortex for 5 s. The scalp was then sutured and the pup was placed under a heat lamp to warm, and ~10 min later returned to the dam. For a detailed description of procedure see (Jacobs et al., 1996).

Brain extraction and slice preparation

Between P7 and P38, rats were anesthetized with pentobarbital (55 mg/kg i.p.) or isoflurane exposure and decapitated for brain removal. Once the brain was removed it was immediately chilled in sucrose-modified artificial cerebral spinal fluid (aCSF) containing: (in mM) 2.5 KCl, 10$MgSO_4$, 3.4 $CaCl_2$, 1.25 NaH_2PO_4, 234 sucrose, 11 glucose, and 26 $NaHCO_3$. Coronal 400 μm thick slices were cut in modified aCSF with a 1000plus vibratome. Once cut, the slices were placed in an oxygenated *normal* aCSF containing: (in mM) 126 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 1.25 NaH_2PO_4, 10 glucose, and 26 $NaHCO_3$. The slices remained in this solution at 34° C for 30—45 min and at room temperature thereafter until placed in the recording chamber.

Electrophysiological recordings

Slices were placed in an oxygenated interface chamber with 34 °C normal aCSF flowing over the slice. Field potential recordings were made using glass micropipettes (2—8 MΩ, 1 M NaCl), placed within superficial layers (II/III) ~1 mm lateral to the microsulcus in PMR or in homotopic control (unlesioned) cortex. We have previously demonstrated that the PMR is the most sensitive region for epileptiform activity (Jacobs et al., 1999a). For extracellular stimulation, a concentric bipolar electrode was placed at the interface of white and gray matter directly beneath the recording site, such that these two electrodes were in a plane orthogonal to the pia. A single current pulse (20 μs long) of varying current intensity (0.30—10 mA, defined as threshold current) was applied to generate a short latency field potential with a peak negativity of 200 μV. To further assess slice health and to estimate intrinsic cortical excitability, a series of increasing stimuli (stimulus intensity protocol) were applied by successively increasing the duration of the pulse (1×, 2×, 4×, 8×, and 16×). Slices were deemed viable if they met two criteria: (1) the short latency response increased in a fashion that was graded with stimulus intensity; and (2) at a stimulus intensity of 16× threshold, the field potential negativity had a peak of at least 0.6 mV. Field potentials were amplified 1000× (AxoClamp 2B, Axon Instruments and FLA-01 amplifier, Cygnus Technologies) and digitized at 5—10 kHz with a Digidata 1322a (Axon Instruments) and recorded to hard drive with Clampex software (Axon Instruments). In order to test the susceptibility to epileptiform activity, before and after low-Mg^{2+} aCSF, 2 s of response to threshold current stimulation was recorded once a minute. After 10 min of recordings in normal aCSF, low-Mg^{2+} aCSF (normal aCSF without the $MgCl_2$ added) was applied for the remainder of the experiment (100 min). With our chamber, the solution required 5 min to reach the chamber.

Data analysis

Measures of the peak, area, and time to peak were made on three sweep averages of the short latency field potential negativity in response to the stimulus intensity protocol using IGOR software (Wavemetrics). Prior to measurement calculation, the responses were zeroed based on the time from zero to nine ms, with the stimulus occurring 10 ms into the sweep. Examination of the peak and area of responses began just after the stimulus artifact. Differences between control and PMR cortex were analyzed using 2-way ANOVAs (intensity versus experimental group). Differences within experimental group between age groups were also tested using 2-way ANOVAs. Epileptiform activity was detected and sorted into interictal and ictal-like (hereafter called ictal) activity by an automated epileptiform detection module