



Epilepsy in a Dish: An In Vitro Model of Epileptogenesis

Interictal Spikes Precede Ictal Discharges in an Organotypic Hippocampal Slice Culture Model of Epileptogenesis.

Dyhrfeld-Johnsen J, Berdichevsky Y, Swiercz W, Sabolek H, Staley KJ. *J Clin Neurophysiol* 2010;27:418–424.

In organotypic hippocampal slice cultures, principal neurons form aberrant excitatory connections with other principal cells in response to slicing induced deafferentation, similar to mechanisms underlying epileptogenesis in posttraumatic epilepsy. To investigate the consequences of this synaptogenesis, the authors recorded field-potential activity from area CA3 during perfusion with the complete growth medium used during incubation. At 7 days *in vitro*, slice cultures only displayed multiunit activity. At 14 days *in vitro*, the majority displayed population bursts reminiscent of interictal-like spikes, but sustained synchronous activity was rare. Band-pass filtering of interictal discharges revealed fast ripple-like complexes, similar to *in vivo* recordings. Spontaneous ictal-like activity became progressively more prevalent with age: at 21 days *in vitro*, 50% of organotypic hippocampal slice cultures displayed long-lasting, ictal-like discharges that could be suppressed by phenytoin, whereas interictal activity was not suppressed. The fraction of cultures displaying ictal events continually increased with incubation time. Quantification of population spike activity throughout epileptogenesis using automatic detection and clustering algorithms confirmed the appearance of interictal-like activity before ictal-like discharges and also revealed high-frequency pathologic multiunit activity in slice cultures at 14 to 17 days *in vitro*. These experiments indicate that interictal-like spikes precede the appearance of ictal-like activity in a reduced *in vitro* preparation. Epileptiform activity in cultures resembled *in vivo* epilepsy, including sensitivity to anticonvulsants and steadily increasing seizure incidence over time, although seizure frequency and rate of epileptogenesis were higher *in vitro*. Organotypic hippocampal slice cultures comprise a useful model system for investigating mechanisms of epileptogenesis as well as developing antiepileptic and antiepileptogenic drugs.

Commentary

Animal models of epilepsy have helped to promote investigations of underlying mechanisms of epileptogenesis and to facilitate the development and screening of novel treatments. A large number and variety of animal models have been created, involving pharmacologic (e.g., pilocarpine, kainate), electrical (e.g., kindling), genetic (e.g., knock-out mice), and other injurious (e.g., trauma, hypoxia, stroke) methods or stimuli, to match the equally numerous types and causes of epilepsy in people. Of course, *in vivo* models of epilepsy, in which animals exhibit actual behavioral and electroencephalographic seizures, most closely mimic the clinical features of human epilepsy. However, reduced biological systems, including brain slices, cell culture, and molecular assays, may also be advantageous in offering unique mechanistic insights into epilepsy. As there is obviously no perfect animal model of epilepsy, ultimately each model system must be carefully evaluated for its specific advantages and limitations in studying different aspects of epilepsy.

Given the complexity of epilepsy, there is increasing interest in developing simplified *in vitro* models of epilepsy that

allow more detailed investigations of cellular and molecular mechanisms of epileptogenesis while still preserving the critical network phenotypic features of epilepsy, particularly the development of spontaneous seizures. Intact hippocampal preparations or acute brain slices maintain much of the needed circuitry to generate electrographic seizures (1, 2). However, these preparations are typically only viable for several hours and thus are primarily useful only for studying acutely provoked seizures, not chronic epileptogenesis. Organotypic slice cultures, which can be maintained for weeks or longer, have been used to study a number of physiological and pathological processes in the brain, including circuit development, synaptic plasticity, and axonal sprouting (3). Slice cultures also afford the opportunity to investigate epilepsy. Most previous studies of organotypic slice cultures have focused on interictal spikes and electrographic seizures acutely provoked by convulsant drugs or other pharmacologic conditions over a relatively short time course (4–6). However, some studies have examined the development of spontaneous epileptiform activity over several weeks, which more closely mimics the process of epileptogenesis that occurs with *in vivo* animal models and human epilepsy (7, 8).

The recent study by Dyhrfeld-Johnsen et al. provides perhaps the most detailed characterization to date of the development and evolution of epileptiform activity within a chronic,



in vitro model system. Extracellular field potential recordings were made from the CA3 region of organotypic hippocampal slice cultures between 7 and 30 days in vitro (DIV). A clear progression in epileptiform activity was observed over this time period, starting with predominantly interictal spike discharges around 14–17 DIV and transitioning to mostly ictal-like electrographic activity by DIV 25–30. Ictal events could last greater than 3 minutes in duration and were quite robust, occurring in at least 80% of slices.

While obviously lacking a behavioral correlate to the electrographic seizures, this in vitro model exhibits a number of characteristics that resemble in vivo epilepsy. After the initial preparation of the slice cultures, there is a latent period of at least a couple of weeks, during which no seizures are apparent and potential antiepileptogenic therapies could be targeted, similar to many types of acquired epilepsy. The developmental sequence of the subsequent onset of interictal spikes and progressive transition to ictal events closely mimics a similar temporal progression observed in the kainate model of epilepsy (9). Furthermore, the standard anticonvulsant drug, phenytoin, could suppress the ictal events, but not the interictal spikes, as commonly observed in human epilepsy.

One intended application of this study was to address the mechanistic relationship between interictal spikes and seizures. In particular, while interictal spikes could simply represent a marker of the epileptic brain, one interesting hypothesis is that interictal spikes cause the subsequent development of seizures, possibly by promoting synaptic synchronization and axonal sprouting (10). While the observation that the emergence of interictal spikes temporally precedes the onset of seizures is consistent with spikes causing seizures, this evidence only demonstrates correlation, not causation. More definitive testing of this hypothesis would depend on the use of selective “anti-spike” agents, which currently do not exist.

The cellular and molecular mechanisms causing epileptogenesis in this in vitro slice culture model are not known and were not explored in this study. Presumably the initial act of cutting the hippocampal slices represents a precipitating traumatic injury, which instigates the process of epileptogenesis, and thus this model can be viewed as an in vitro model of posttraumatic epilepsy. As a result of the severing of synaptic connections at the cut surfaces, massive axonal sprouting and synaptic reorganization represents a rational mechanism that could promote epileptogenesis in this model. This hypothesis could be readily tested with pharmacologic interventions that inhibit axonal sprouting, such as rapamycin.

From a mechanistic standpoint, another remarkable feature of this model is that minimal additional interventions were necessary to trigger epileptogenesis. In most studies of acute slices or slice culture, a convulsant drug or other excitatory pharmacologic conditions are needed to induce epileptiform activity (2, 4–6, 8). In this study, no such additional provocations were used; however, there was an interesting dependence of the epileptiform activity on the presence of the normal growth medium, as epileptiform activity disappeared quickly after switching from the growth medium to

a standard artificial cerebrospinal fluid. The contribution of glutamine in the growth medium, with subsequent conversion to glutamate, was evaluated and did not appear to be critical in this study. Future studies are required to determine the necessary co-factors that seem to promote epileptogenesis in this model. In any case, this study has unexpected implications for all research using similarly maintained organotypic slice cultures for other applications independent of epilepsy—the current report suggests that seizures could be an unrecognized confounding factor in other studies that aren’t necessarily focusing on epileptogenesis.

Finally, this in vitro epilepsy model could have the largest impact in therapeutic applications. While in vivo acute seizure models, such as maximal electroshock or pentylenetetrazole, are convenient for screening effects of potential anticonvulsant drugs, testing for long-term antiepileptogenic or preventative actions of drugs in more chronic epilepsy models is much more cumbersome. Given the convenience of in vitro preparations and the relative compressed time frame of epileptogenesis in the slice culture model, this model could serve as a first-order, high-throughput screening of candidate anticonvulsant or antiepileptogenic drugs. The best candidates from the initial in vitro screen could then be tested further in traditional in vivo models of epilepsy.

by Michael Wong, MD, PhD

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