

Barrow Neurological Institute at St. Joseph's Hospital and Medical Center

## Barrow - St. Joseph's Scholarly Commons

---

Neurobiology

---

1-1-2015

### Mechanisms Of Intrinsic Epileptogenesis In Human Gelastic Seizures With Hypothalamic Hamartoma

Jie Wu

*Barrow Neurological Institute*, [jie.wu@dignityhealth.org](mailto:jie.wu@dignityhealth.org)

Ming Gao

Jian Xin Shen

Shen Feng Qiu

John F. Kerrigan

Follow this and additional works at: <https://scholar.barrowneuro.org/neurobiology>

---

#### Recommended Citation

Wu, Jie; Gao, Ming; Shen, Jian Xin; Qiu, Shen Feng; and Kerrigan, John F., "Mechanisms Of Intrinsic Epileptogenesis In Human Gelastic Seizures With Hypothalamic Hamartoma" (2015). *Neurobiology*. 449. <https://scholar.barrowneuro.org/neurobiology/449>

This Article is brought to you for free and open access by Barrow - St. Joseph's Scholarly Commons. It has been accepted for inclusion in Neurobiology by an authorized administrator of Barrow - St. Joseph's Scholarly Commons. For more information, please contact [molly.harrington@dignityhealth.org](mailto:molly.harrington@dignityhealth.org).

## Mechanisms of Intrinsic Epileptogenesis in Human Gelastic Seizures with Hypothalamic Hamartoma

Jie Wu,<sup>1,2,3</sup> Ming Gao,<sup>1</sup> Jian-Xin Shen,<sup>2</sup> Shen-Feng Qiu<sup>3</sup> & John F. Kerrigan<sup>4</sup>

1 Division of Neurology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA

2 Department of Physiology, Shantou University of Medical College, Shantou, Guangdong, China

3 Department of Basic Medical Sciences, University of Arizona College of Medicine-Phoenix, Phoenix, AZ, USA

4 Division of Pediatric Neurology, Barrow Neurological Institute at Phoenix Children's Hospital, Phoenix, AZ, USA

### Keywords

Epileptogenesis; Gelastic seizures; Human hypothalamic hamartoma.

### Correspondence

Jie Wu, M.D., Ph.D., Neural Physiology Laboratory, Division of Neurology, Barrow Neurological Institute, St. Joseph's Hospital and Medical Center, Phoenix, AZ 85013-4496, USA.

Tel.: +1-602-406-6376;

Fax: +1-602-406-7172;

E-mail: jie.wu@dignityhealth.org

Received 6 September 2014; revision 7

October 2014; accepted 8 October 2014

### SUMMARY

Human hypothalamic hamartoma (HH) is a rare developmental malformation often characterized by gelastic seizures, which are refractory to medical therapy. Ictal EEG recordings from the HH have demonstrated that the epileptic source of gelastic seizures lies within the HH lesion itself. Recent advances in surgical techniques targeting HH have led to dramatic improvements in seizure control, which further supports the hypothesis that gelastic seizures originate within the HH. However, the basic cellular and molecular mechanisms of epileptogenesis in this subcortical lesion are poorly understood. Since 2003, Barrow Neurological Institute has maintained a multidisciplinary clinical program to evaluate and treat patients with HH. This program has provided a unique opportunity to investigate the basic mechanisms of epileptogenesis using surgically resected HH tissue. The first report on the electrophysiological properties of HH neurons was published in 2005. Since then, ongoing research has provided additional insights into the mechanisms by which HH generate seizure activity. In this review, we summarize this progress and propose a cellular model that suggests that GABA-mediated excitation contributes to epileptogenesis in HH lesions.

doi: 10.1111/cns.12348

### Gelastic Seizures in Patients with HH

The hypothalamic hamartoma (HH) is a developmental malformation that occurs in the region of the tuber cinereum and inferior hypothalamus. This lesion is associated with a range of neurological and endocrine disorders, which may include intractable seizures, cognitive impairment, behavioral disturbances, and central precocious puberty [1–4]. The epileptic syndrome in HH patients is characterized by laughing (gelastic) seizures beginning in early infancy [1,5]. Gelastic seizures associated with HH are generally refractory to standard antiepileptic drugs as well as alternative therapies such as the ketogenic diet and vagus nerve stimulation [6,7]. Although the HH is a benign tumor and the accompanying gelastic seizures often present during the neonatal period, patients later develop additional seizure types [1,7]. The most effective treatment for epilepsy associated with HH is surgical resection of the lesion [7–9]. Gelastic seizures associated with HH represent the most common disease model of human subcortical epilepsy [10,11].

### Embryology and Neuroanatomy of HH

Although the embryological origin of the HH is unknown, high-resolution magnetic resonance (MR) imaging shows that HH associated with epilepsy consistently attach to the posterior hypothalamus in the immediate vicinity of the mammillary bodies [10,12,13]. This suggests that connections to limbic circuitry via the fornices and mammillothalamic tracts may play an important role in epileptogenesis in gelastic seizure patients with HH [10,14].

Surgical disconnection of HH from adjacent structures, including the mammillary bodies, can result in satisfactory control of gelastic seizures [5,8,9,15]. Based on a recent review by Wild and colleagues, the expression of laughter seems to depend upon two partially independent neuronal pathways [16]. Gelastic seizures likely express themselves through the “voluntary system” that originates in the premotor and frontal opercular areas. This system leads through the motor cortex and pyramidal tract to the ventral brainstem. Since ictal laughter associated with HH appears

mechanical and unnatural [1,17] and usually does not result in feeling positive emotions [10,14,18], it therefore seems possible that seizure activity originating within HH excites this system. Initiated by abnormal electrical activity spreading rostrally and dorsally to the areas in the neighboring limbic system, and caudally to the brainstem, the HH can induce physiological and psychophysiological manifestations of laugh attacks [15].

## Neuropathology of HH

Histological examination of surgically resected HH tissue from patients with treatment-resistant epilepsy shows clusters of small neurons intermixed with glia and relatively sparse large neurons [19,20]. Clusters of small HH neurons are highly variable with regard to their size and abundance within the tissue, but appear to be a universal feature of HH microarchitecture [19]. Approximately 90% of the total neuron population consists of small HH neurons which have an interneuron phenotype with small round soma and bipolar or unipolar morphology with largely unbranched and aspiny dendrites [19,21,22]. Small HH neurons express glutamic acid decarboxylase (GAD), the synthetic enzyme for GABA, and are therefore likely to be GABAergic interneurons with local network connections [23,24].

Large HH neurons make up approximately 10% of the total neuron population in HH tissue and are dispersed throughout the tissue, although often situated at the edges of the small neuron clusters [19,22]. These cells have features consistent with projection-type neurons, including pyramidal morphology, abundant Nissl substance, and multiple processes, often with a single large proximal dendrite [19,21,22]. Dendrites are more likely to be branched and spiny. At least some large HH neurons express markers consistent with excitatory neurotransmitters, such as VGLUT2.

## Epileptogenesis of Human Gelastic Seizures

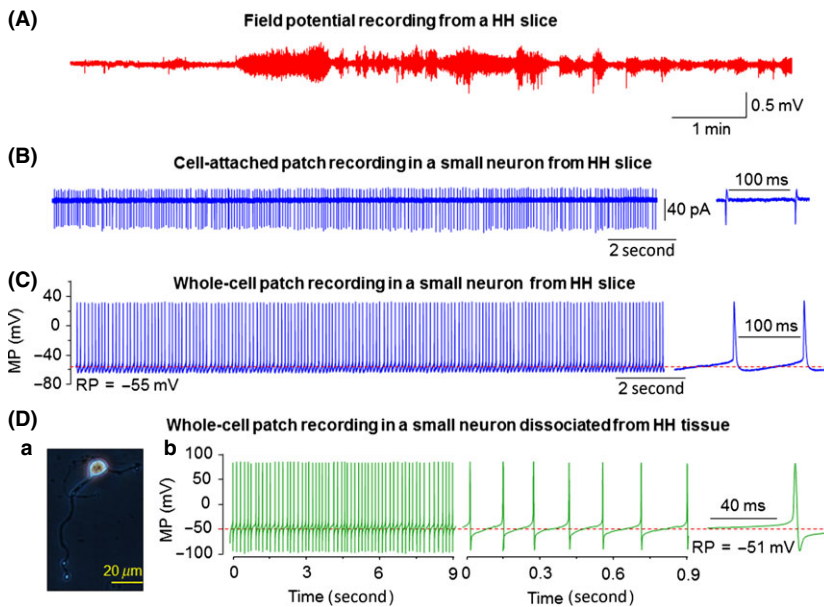
The mechanisms underlying the epileptogenesis of gelastic seizures are unknown. Several hypotheses relevant to epileptogenic mechanisms can be postulated. (1) *Local irritation of adjacent normal structures*: HH, especially large-size tumors, mechanically affect and distort local anatomy, including the mammillary body, which in turn may alter the excitability of hypothalamic networks. Anatomical analysis showed that HH lesions within the third ventricle are often associated with gelastic seizures [4,5,20,25], and larger lesions have the features of symptomatic generalized epilepsy [26]. However, the presence of epilepsy in patients with HH appears to correlate with the region of lesion attachment (posterior hypothalamus in the region of the mammillary body) rather than lesion size [13,27,28]. (2) *Abnormalities of hormone expression*: HH cells express neuropeptides which may contribute to seizures in other conditions [29,30]. Hormones secreted by the hypothalamus and pituitary have been identified during gelastic seizures [31]. However, experimental evidence supporting a role for endocrine mechanisms in seizure generation within HH tissue is lacking. (3) *HH is intrinsically epileptic*: Ictal EEG recordings from electrode contacts placed directly into HH tissue identify seizure

onset within the lesion itself [14,32]. Direct electrical stimulation of these electrodes within the HH can evoke typical gelastic seizures [15]. Functional imaging at the time of gelastic seizure activity demonstrates activation of the HH lesion with increased perfusion on single photon emission computed tomography (SPECT) or increased glucose utilization with fluorodeoxyglucose positron emission tomography (FDG-PET), consistent with the behavior of a seizure focus [15,17,33,34]. Perhaps most importantly, recent advances in surgical techniques, allowing for safe resection of the HH, can result in immediate disappearance of gelastic seizures in a majority of patients [8,9,35]. Taken together, these studies suggest that gelastic seizures originate within the HH itself, and it is now widely accepted that HH tissue is intrinsically epileptogenic [7,11,36].

## Roles of Intrinsic Neuron Firing in Seizure Genesis

Alterations in neuronal network input–output relationships underlie various nervous system disorders. An increase in neuronal network output is thought to underlie seizure genesis in areas such as the hippocampus. The input–output relationship of neuronal networks is dependent on both their synaptic connectivity and on the intrinsic properties of their neuronal elements [37]. Accumulating lines of evidence suggest that intrinsic neuronal properties play an important role in seizure genesis. It has long been postulated that the inherent firing properties of neurons can be linked to the epileptogenicity of brain tissue [38–40]. In most human epileptic tissue preparations *in vitro*, the prevalent neuronal firing pattern is that of spontaneous, regular action potential generation. Surprisingly, overt action potential bursts are rarely observed from human neocortical slices without injection of depolarizing current [41–43]. In cortical dysplasia (CD), cytomegalic neurons (but not balloon cells) act as epileptic “pacemakers” and likely contribute to seizure genesis [44–46].

Electrophysiological microelectrode recordings have demonstrated that small HH neurons have intrinsic pacemaker-like firing behavior [21,23,24,47]. Field potential recording shows the seizure-like discharges in most HH slices tested (Figure 1A). Patch-clamp recording in cell-attached mode shows spontaneous action potential (AP) firing (Figure 1B) in a small neuron from a HH slice (soma diameter <16  $\mu\text{m}$ ). In the same HH neuron, whole-cell patch-clamp recording (current-clamp) shows the similar intracellular AP firing pattern (Figure 1C). These small neurons in HH slices demonstrate diverse firing patterns with average firing frequency of  $10.5 \pm 0.8$  Hz at the resting membrane potential (RMP) of  $-53.2 \pm 1.1$  mV [23,24,47]. In 64 small HH neurons recorded from the tissue slice, derived from a total of 35 different patients, regular firing was observed in 63%, irregular firing in 28%, and burst firing in 9% of the recorded neurons [21]. These findings were verified with single-cell microelectrode recordings of small HH neurons in the freshly resected, perfused tissue slice, with RMP of  $53.9 \pm 0.9$  mV and spontaneous firing frequency of  $6.6 \pm 1.0$  Hz [21,48]. In acutely dissociated single HH neurons (small sized, Figure 1Da), there was spontaneous AP firing (Figure 1Db), which stopped in the presence of tetrodotoxin (TTX), a voltage-dependent sodium channel blocker, but show no change



**Figure 1** Seizure-like discharges and pacemaker-like AP firing in hypothalamic hamartoma (HH) neurons. (A) Field potential recording showed seizure-like discharges in a HH slice. In the same HH tissue (from another section), patch-clamp cell-attached recording showed a typical case of pacemaker-like AP firing (B). Patch-clamp whole-cell recording (current-clamp mode) demonstrated a similar spontaneous AP firing of 11 Hz at a resting membrane potential of  $-55$  mV that is indicated by horizontal dashed line (C). The same pacemaker behavior was also observed from a small HH neuron (Da), in which, this small HH neuron exhibited 7 Hz pacemaker-like firing at the resting potential of  $-51$  mV (Db).

in firing behavior when exposed to pharmacological blockade of GABA-A and ionotropic glutamate receptors, suggesting the intrinsic pacemaker-like firing [23]. Hyperpolarization of small HH neurons with current injection demonstrates “sag-like” membrane depolarization returning the cell to the RMP, consistent with an h-current as seen in other pacemaker cells [23]. As previously mentioned, small HH neurons express glutamic acid decarboxylase (GAD) with interneuron-like morphology, and therefore likely express GABA as their primary neurotransmitter [23,24]. Taken together, these findings suggest that spontaneous firing is an intrinsic membrane property of small HH neurons.

Conversely, in both the acutely dissociated single-cell preparation and in the tissue slice, microelectrode recordings from large HH neurons (soma diameter  $>16$   $\mu\text{m}$ ) showed that these neurons are usually firing at much lower frequency or even quiescent at rest with RMP of  $-60.8 \pm 1.7$  mV (dissociated cell) and  $-65.1 \pm 1.7$  mV (tissue slice) [21,47] (Figure 2A,B in right panel).

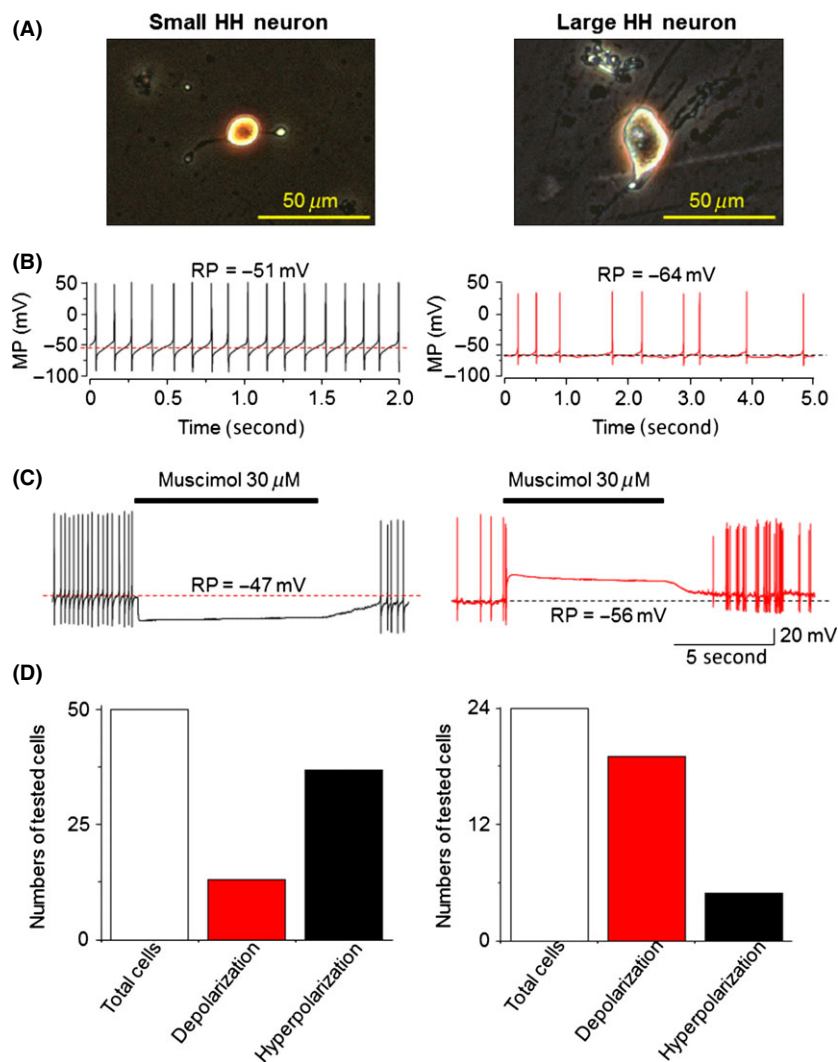
## GABA-Mediated Excitation during Normal Development and in Epilepsy

Gamma-aminobutyric acid (GABA) is the most important inhibitory neurotransmitter in the central nervous system of vertebrates, exhibiting binding to ligand-gated (GABA<sub>A</sub> and GABA<sub>C</sub>) and G-protein-coupled (GABA<sub>B</sub>) receptors [49]. The GABA<sub>A</sub> receptor is highly permeable to chloride ions, and its activation tends to hyperpolarize the membrane potential through the influx of these negative ions [50,51]. Deficits in GABA<sub>A</sub> receptor-mediated inhibition are a major mechanism contributing to seizure genesis in several forms of epilepsy [52].

In contrast to the hyperpolarizing (inhibitory) effect on normal mature neurons, GABA can exert an excitatory role on immature neurons [53,54]. Ben-Ari and colleagues reported that activation of GABA receptors on immature neurons in neonatal

hippocampal slices induced membrane depolarization [55]. In these immature neurons, the reversal potential for Cl<sup>-</sup> was more positive than the resting membrane potential, suggesting that the intracellular Cl<sup>-</sup> concentration was higher in these neonatal neurons compared to mature neurons. GABA produces depolarization and increased concentration of intracellular calcium in immature neurons in a variety of brain regions, including hippocampus, neocortex, and hypothalamus [53]. GABA-induced excitation likely plays an important role in activity-dependent neuronal development [56]. Notably, GABAergic excitation is also observed in several highly selected circumstances in normal mature neurons [54,57–67].

Recent studies, examining the functional alteration of GABA<sub>A</sub> receptors, have demonstrated that this functional switch can also been found in some epileptic conditions [61–63]. For instance, Cohen and colleagues reported that the interictal epileptic activity in slices prepared from surgical resection of human temporal lobe was substantially dependent on GABA<sub>A</sub> receptor-mediated depolarization. This was determined by observing cells with depolarizing IPSPs firing before and during interictal spikes that were effectively silenced by a GABA<sub>A</sub> receptor antagonist [61]. These authors postulated that the altered connectivity was associated with the abnormal temporal lobe. This resulted in neuronal deafferentation, causing neurons to switch to an immature state, in which GABA<sub>A</sub> receptors mediate remarkable depolarization [61]. Khalilov et al. [64], described a unique triple compartment chamber that accommodates two intact hippocampi and their commissures, allowing for application of a convulsive drug to one hippocampus to determine the consequences of the propagation of seizures on the other side of the control hippocampus. Using this novel preparation, they demonstrated that after establishing the propagation of 7–10 seizures (induced by chemical stimulation of one side of the hippocampus) and then interrupting the connections between the two hemispheres, the control hippocampus still maintained spontaneous epileptic activity, indicating that the



**Figure 2** Activation of GABA<sub>A</sub>Rs differently modulates small and large HH neuron activity. Typical small (A left panel) and large (A right panel) neurons acutely dissociated from human HH tissues are depicted using phase contrast photo picture. Both types of HH neurons showed spontaneous AP firing but large cell had much lower firing rate (B). Gramicidin-perforated patch-clamp recordings under current-clamp mode demonstrated that muscimol hyperpolarized small (C left panel) but depolarized large (C right panel) HH neurons. (D) Summary of the numbers of small and large HH neurons tested and their responses to the GABA<sub>A</sub>R agonist muscimol using gramicidin-perforated recordings.

“seizure-induced seizures”. Moreover, the application of a GABA<sub>A</sub> receptor antagonist before the formation of seizures induced an epileptogenic focus, which generated seizures, while the same procedure blocked ongoing seizures once the mirror focus had been established. This suggests that the secondary seizures were mediated by GABA<sub>A</sub> receptor-induced excitation [64]. Direct measurement of Cl<sup>-</sup> reversal potential indicated a shift toward a more positive value, causing GABA to depolarize and excite these neurons [64]. Therefore, seizures appear to reduce the ability of neurons to prevent excess chloride accumulation, which in turn results in a quasi-permanent accumulation of chloride intracellularly, leading to the excitatory effects of GABA. This mechanism has been suggested to operate in a variety of pathological conditions [62,65,66].

### Role of GABA<sub>A</sub> Receptor-Mediated Excitation in HH Epileptogenesis

Most large HH neurons have a functionally immature response with depolarization and increased firing when exposed to GABA

agonists [21,47,67]. Gramicidin-perforated patch microelectrode recordings of acutely dissociated single HH neurons were studied in both large and small HH neurons (total of 62 cells derived from 34 patients) [47]. Bath application of muscimol (selective GABA<sub>A</sub> receptor agonist) resulted in depolarization of 83% of large HH neurons and 24% of small HH neurons (Figure 2C,D).

The functional difference between large and small HH neurons correlates with differences in intracellular chloride concentration, consistent with reversal of the chloride gradient in large HH neurons. GABA-induced transmembrane currents were evaluated at different holding potentials, establishing a reversal potential of  $-36.2 \pm 1.9$  mV for large HH neurons (predicting an intracellular Cl<sup>-</sup> concentration of  $39.1 \pm 2.8$  mM) and  $-59.4 \pm 3.9$  mV for small HH neurons (predicted intracellular Cl<sup>-</sup> concentration  $19.2 \pm 3.7$  mM) [47].

These findings were confirmed in a complementary recording platform with single-cell patch-clamp recordings in perfused, surgically resected HH tissue slices [21,67]. Utilizing either whole-cell or gramicidin-perforated patch recordings, administration of muscimol resulted in a depolarizing response in 70%



of large HH neurons, whereas the mature hyperpolarizing response was observed in 100% of small HH neurons. The depolarizing response of large HH neurons was blocked with coadministration of bicuculline. Depolarizing behavior once again correlated with estimates of intracellular chloride concentration. Large HH neurons that depolarized to muscimol (70% of large HH neurons) had a reversal potential of  $-30 \pm 2$  mV (predicted intracellular  $\text{Cl}^-$  concentration  $40.2 \pm 4.3$  mM), whereas large HH neurons with a hyperpolarizing response (30%) had a reversal potential of  $-64 \pm 3$  mV (intracellular  $\text{Cl}^-$  concentration  $11.4 \pm 0.8$  mM) [21]. Bicarbonate efflux and activation of L-type calcium channels contribute to membrane depolarization of large HH neurons [67].

Reversal of the chloride potential in large HH neurons is associated with altered expression of the cation–chloride transporters NKCC1 and KCC2 [21,47]. Single-cell reverse transcriptase-polymerase chain reaction (RT-PCR) shows reduced expression of KCC2 mRNA in large HH neurons relative to small HH neurons and immunocytochemical staining for KCC2 protein shows lower levels in large versus small HH neurons [21,47]. Bumetanide (an NKCC1 inhibitor) partially blocks the depolarizing and firing behavior of large HH neurons exposed to GABA agonists [21]. Altered cation–chloride transporter expression may relate to constitutive activation of the tyrosine kinase B (trkB) receptor complex and related downstream intracellular signaling pathways based upon Western blot analysis of surgically resected HH tissue (see also Semaan et al., present issue).

## Network Activity within HH Tissue

Indirect evidence for functional network activity within HH is provided by high-density microelectrode arrays that provide two-dimensional field recordings of surgically resected HH tissue slices [68]. Perfused HH tissue slices generate spontaneous high-frequency oscillations (HFOs), including ripples (100–200 Hz) and fast ripples (200–600 Hz). Paired-pulse electrical stimulation shows potentiation (usually an expression of enhanced synaptic activity) with a 4-fold increase in single unit firing behavior. Pharmacological challenge with 4-aminopyridine (4-AP; a nonspecific potassium channel blocker) increases the abundance and burst duration of fast ripples [68]. Two-dimensional analysis of field potentials over brief time intervals (<200 mseconds) is consistent with spread of activity within small local regions, potentially correlating with the neuronal clustering characteristic of HH microanatomy.

In an effort to observe the activity of HH neurons within their completely intact (native) network, microelectrode field recordings were obtained from HH tissue via the working channel of a surgical endoscope immediately prior to surgical resection [69]. Analysis of single unit (single neuron) behavior confirmed spontaneous firing behavior *in situ*. For all neurons, the median firing frequency was 8.8 spikes/second, but at least two phenotypes were apparent with a slow-firing (median firing rate of 0.6 spikes/second) and fast-firing neuron populations (median firing rate 15.0 spikes/second). The fast-firing group of neurons was much more likely to have burst-firing features. Functional connectivity within a local network was examined by evaluating all possible neuron pairs (recorded within the same epoch)

for time-linked firing behavior with the finding that 51% of all possible neuron pairs had a significant level of firing synchrony [69].

## Mechanisms Responsible for Increased Synchrony

There are two basic cellular conditions required for every epileptic network; first, imbalance of excitatory and inhibitory influences in the direction of net excitation and second, hypersynchrony of neuronal firing [70]. The contribution of synchronized firing of inhibitory neurons within the reticular nucleus as a fundamental contributing factor to generalized spike-wave discharges in the thalamocortical circuit is well recognized [71].

GABA<sub>A</sub> receptor mediated synchronization also contributes to seizure-onset mechanisms in focal epilepsy, including mesial temporal sclerosis [72,73] and cortical dysplasia [74]. Cepeda and colleagues have recently reported postsynaptic evidence for pacemaker-like GABA-firing as a contributing factor for epileptic network synchronization in human surgically resected cortical dysplasia tissue [75]. With regard to synchronization of the ictogenic network in HH, GABA-mediated mechanisms are attractive candidates, in light of the high abundance of small HH interneurons, their spontaneous pacemaker-like firing behavior, and HH microanatomy, in which small HH neurons cluster in close proximity. These clusters, in association with large depolarizing pyramidal (projection-type) HH neurons, may be the “functional unit” for ictogenesis in HH [36]. As previously noted, there is indirect evidence for network activity within HH clusters as demonstrated by HFOs in HH tissue slices [68] and a high degree of firing synchrony of single units with *in situ* recordings from HH lesions [69].

A possible contributing mechanism to GABA-mediated synchrony is the phenomenon of GABA<sub>A</sub> current rundown, which is determined experimentally by observing decreasing transmembrane current responses to repetitive (use dependent) GABA-ligand exposure. GABA<sub>A</sub> current rundown has been related to temporal lobe epilepsy [76,77] and cortical dysplasia [78]. Functional rundown has also been described in GABA-related transmembrane currents in surgically resected HH tissue [79]. In two different experimental platforms derived from human HH, functional rundown was specific to GABA (not observed in response to repetitive exposure to glycine or glutamate) and was also specific to GABA<sub>A</sub> receptors expressed on the surface of small HH neurons and not large HH neurons [79]. We believe that rundown is likely to contribute to the entraining of synchronous firing of small HH neurons. Further testing of this hypothesis would be enhanced by developing a computational model.

Synchrony within small HH neuron clusters may also be enabled by nonsynaptic mechanisms [80]. Neuronal gap junctions (connexin-36) are highly expressed within small HH neuron clusters and microelectrode field recordings of seizure-like discharges in surgically resected HH tissues slices are significantly reduced by pharmacologic exposure to gap junction blockers. Taken together, these data suggest the hypothesis that synchrony of pacemaker-like GABAergic neurons play a critical role in HH ictogenesis.

## Summary of Cellular Model for Intrinsic Epileptogenesis of HH

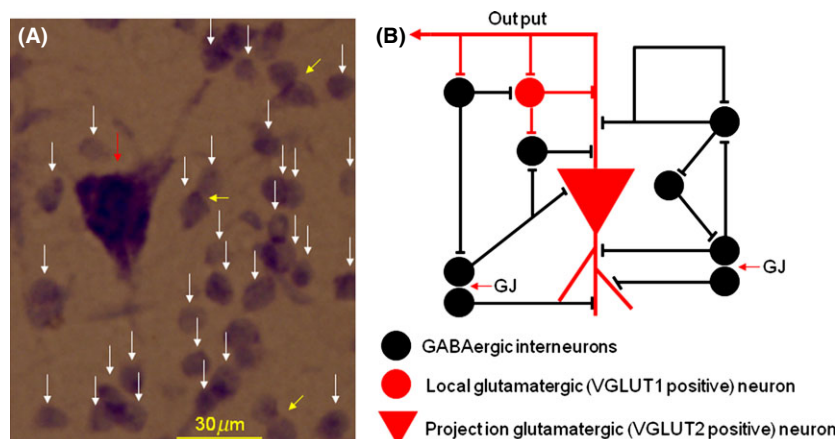
There are two types of HH neurons, small and large-sized neurons [21,23–34,47]. Small HH neurons are mature GABAergic interneurons and exhibit pacemaker-like action potential firing, while large HH neurons are immature, glutamatergic projection-type neurons [21,23,47]. These small and large HH neurons are synaptically connected [22,47]. The activation of GABA<sub>A</sub> receptors on small HH neurons inhibits these small neurons, while the activation of GABA<sub>A</sub> receptors on large HH neurons leads to excitation [21,47,67].

Based on these observations, we propose a cellular model to interpret a possible mechanism of epileptogenesis within the HH lesion [36]. Here, we update this cellular model with currently published and unpublished data [68,69,79]. The specific elements of this model are illustrated in Figure 3. Nissl staining of a HH tissue section (A) revealed one large (red arrow) and numerous small (white arrows) neurons. Some small neurons are closely clustered (yellow arrows). Based on our experimental data, a schematic representation is shown (B) of how these small, GABAergic interneurons (black), local glutamatergic neuron (small, red) and projection glutamatergic neuron (large, red) work together to generate seizure activity, in which, small GABAergic interneurons exhibit pacemaker-like firing, provide GABA to their target, the large neuron (red), which is functionally immature and thus excited by GABA, providing a substrate for hyperexcitation/epileptogenesis within the HH lesion. In addition, small HH neurons also may innervate other proximal, small HH neurons and/or themselves. Normally, this would inhibit firing activity of these small HH neurons. However, GABA<sub>A</sub> receptors on small HH neurons exhibit significant functional rundown during repetitive exposure to GABA compared to GABA<sub>A</sub> receptors on large HH neurons. Thus, periods of small,

GABAergic HH neuronal quiescence may contribute to episodic occurrence of excitatory output from the HH. In addition to small GABAergic neurons, we also found a small portion of glutamatergic small HH neurons (VGLUT1 positive small neurons), which may participate in glutamatergic synaptic connections to GABAergic small neurons. The large HH neurons excited by GABA may participate in feedback to small HH neurons, thereby also affecting excitability of these small neurons (red lines). Finally, the neuronal type of gap junctions (GJ) between small GABA neurons plays a role in synchronizing these GABAergic neurons and drives a GABAergic network to generate seizure activity.

## Methodological Issues

Investigational use of surgically resected human epileptic brain tissue is a powerful method for exploring the structural and electrophysiological properties of the neuronal networks responsible for human epilepsy [81,82]. Neuropharmacological probes can identify possible translational treatment strategies. Observations based upon human tissue can generate testable hypotheses that can be investigated with controlled experimentation in suitable animal models. However, the limitations of using surgically resected human material deserve mention. (1) The *in vitro* preparation (including slice, dissociated single neuron, or primary cell culture) using human brain tissue is a “reduced” system that does not possess the network connectivity present in the intact brain [83]. Thus, functional observations using any of these platforms cannot be expected to fully represent the behavior of epileptic tissue *in situ*. (2) The tools utilized to study surgically resected tissue usually differ from those available to study patients. For example, microelectrode field recordings derived from the surgically resected tissue slice have an uncertain relationship to the findings derived from scalp EEG. (3) There is generally an absence of



**Figure 3** Cellular model for intrinsic epileptogenesis of HH tissue. (A) Panel A consists of photomicrograph of human HH tissue stained with Nissl technique. A large HH neuron (red arrow) is visualized, with numerous surrounding small HH neurons (white arrows). Small GABAergic HH neurons exhibit intrinsic pacemaker-like firing and provide GABA to their synaptic targets, including the large, glutamatergic projection HH neuron (VGLUT2 positive). Small HH neurons often occur in closely juxtaposed pairs and clusters (yellow arrows). It is likely that some glutamatergic small HH neurons (VGLUT1 positive) also innervate large HH neurons (B). In addition, between GABAergic small neurons, there is gap junction (GJ) expression (red arrows), which contributes to the synchrony of these pacemaker-like GABAergic neurons. Together, these HH neuronal networks may underlie the cellular mechanisms of seizure-generation within HH lesion.

normal human control tissue. This is particularly true for HH research, where there is a very high premium on avoiding neurosurgical resection or injury to adjacent normal hypothalamus. Consequently, the use of “less abnormal” adjacent cortex as control tissue, a strategy utilized for experimentation on surgically resected cortical dysplasia tissue, is not available [82,84]. This lack of control tissue in HH research is particularly problematic for electrophysiological studies, whereas we have utilized age-matched human autopsy-derived hypothalamic tissue for selected neuropathological studies. We advocate utilizing the mammillary bodies for this purpose, as a large and generally recognizable hypothalamic nucleus within postmortem specimens that is

always in close proximity to HH lesions that result in epilepsy [13].

## Acknowledgments

This work was supported by an NIH grant (NS-056104, J.W.) and grants from the Barrow Neurological Foundation and Arizona Biomedical Research Commission (J.F.K., J.W.)

## Conflict of Interest

The authors declare no conflict of interest.

## References

- Berkovic SF, Andermann F, Melanson D, Ethier RE, Feindel W, Gloor P. Hypothalamic hamartomas and ictal laughter: Evolution of a characteristic epileptic syndrome and diagnostic value of magnetic resonance imaging. *Ann Neurol* 1988;**23**:429–439.
- Deonna T, Ziegler A-L. Hypothalamic hamartoma, precocious puberty and gelastic seizures: A special model of “epileptic” developmental disorder. *Epileptic Disord* 2000;**2**:33–37.
- Weissenberger AA, Dell ML, Liow K, et al. Aggression and psychiatric comorbidity in children with hypothalamic hamartomas and their unaffected siblings. *J Am Acad Child Adolesc Psychiatry* 2001;**40**:696–703.
- Freeman JL, Harvey AS, Rosenfeld JV, Wrennall JA, Bailey CA, Berkovic SF. Generalized epilepsy in hypothalamic hamartoma: Evolution and postoperative resolution. *Neurology* 2003;**60**:762–767.
- Valdúeja JM, Cristante L, Dammann O, et al. Hypothalamic hamartomas: With special reference to gelastic epilepsy and surgery. *Neurosurgery* 1994;**34**:949–958.
- Andermann F, Arzimanoglou A, Berkovic SF. Hypothalamic hamartoma and epilepsy: The pathway of discovery. *Epileptic Disord* 2003;**5**:173–175.
- Berkovic SF, Arzimanoglou A, Kuzniecky R, Harvey AS, Palmiini A, Andermann F. Hypothalamic hamartoma and seizures: A treatable epileptic encephalopathy. *Epilepsia* 2003;**44**:969–973.
- Rosenfeld JV, Harvey AS, Wrennall J, Zacharin M, Berkovic SF. Transcallosal resection of hypothalamic hamartomas, with control of seizures, in children with gelastic epilepsy. *Neurosurgery* 2001;**48**:108–118.
- Ng YT, Rekate HL, Prenger EC, et al. Transcallosal resection of hypothalamic hamartoma for intractable epilepsy. *Epilepsia* 2006;**47**:1192–1202.
- Berkovic SF, Kuzniecky RI, Andermann F. Human epileptogenesis and hypothalamic hamartomas: New lessons from an experiment of nature. *Epilepsia* 1997;**38**:1–3.
- Kerrigan JF, Ng Y-t, Chung SS, Rekate HR. The hypothalamic hamartoma: A model of subcortical epileptogenesis and encephalopathy. *Semin Pediatr Neurol* 2005;**12**:119–131.
- Diebler C, Ponsot G. Hamartomas of the tuber cinereum. *Neuroradiology* 1983;**25**:93–101.
- Parvizi J, Le S, Foster B, et al. Gelastic epilepsy and hypothalamic hamartomas: Neuroanatomical analysis of brain lesions in 100 patients. *Brain* 2011;**134**:2960–2968.
- Munari C, Kahane P, Francione S, et al. Role of the hypothalamic hamartoma in the genesis of gelastic fits (a video-stereo-EEG study). *Electroencephalogr Clin Neurophysiol* 1995;**95**:154–160.
- Kuzniecky R, Guthrie B, Mounitz J, et al. Intrinsic epileptogenesis of hypothalamic hamartomas in gelastic epilepsy. *Ann Neurol* 1997;**42**:60–67.
- Wild B, Rodden FA, Grodd W, Ruch W. Neural correlates of laughter and humour. *Brain* 2003;**126**:2121–2138.
- DiFazio MP, Davis RG. Utility of early single photon emission computed tomography (SPECT) in neonatal gelastic epilepsy associated with hypothalamic hamartoma. *J Child Neurol* 2000;**15**:414–417.
- Striano S, Meo R, Bilo L, et al. Gelastic epilepsy: Symptomatic and cryptogenic cases. *Epilepsia* 1999;**40**:294–302.
- Coons SW, Rekate HL, Prenger EC, et al. The histopathology of hypothalamic hamartomas: Study of 57 cases. *J Neuropathol Exp Neurol* 2007;**66**:131–141.
- Boyko OB, Curnes JT, Oakes WJ, Burger PC. Hamartomas of the tuber cinereum: CT, MR, and pathologic findings. *AJNR Am J Neuroradiol* 1991;**12**:309–314.
- Kim DY, Fenoglio KA, Simeone TA, et al. GABA<sub>A</sub> receptor-mediated activation of L-type calcium channels induces neuronal excitation in surgically resected human hypothalamic hamartomas. *Epilepsia* 2008;**49**:861–871.
- Beggs J, Nakada S, Fenoglio K, Wu J, Coons S, Kerrigan JF. Hypothalamic hamartomas associated with epilepsy: Ultrastructural features. *J Neuropathol Exp Neurol* 2008;**67**:657–668.
- Wu J, Xu L, Kim DY, et al. Electrophysiological properties of human hypothalamic hamartomas. *Ann Neurol* 2005;**58**:371–382.
- Wu J, Chang Y, Li G, et al. Electrophysiological properties and subunit composition of GABA<sub>A</sub> receptors in patients with gelastic seizures and hypothalamic hamartoma. *J Neurophysiol* 2007;**98**:5–15.
- Arita K, Ikawa F, Kurisu K, et al. The relationship between magnetic resonance imaging findings and clinical manifestations of hypothalamic hamartoma. *J Neurosurg* 1999;**91**:212–220.
- Castro LH, Ferreira LK, Teles LR, et al. Epilepsy syndromes associated with hypothalamic hamartoma. *Seizure* 2007;**16**:50–58.
- Sturm JW, Andermann F, Berkovic SF. “Pressure to laugh”: An unusual epileptic syndrome associated with small hypothalamic hamartomas. *Neurology* 2000;**54**:971–973.
- Striano S, Striano P, Sarappa C, Boccia P. The clinical spectrum and natural history of gelastic epilepsy-hypothalamic hamartoma syndrome. *Seizure* 2005;**14**:232–239.
- Bajorek JG, Lee RJ, Lomax P. Neuropeptides: A role as endogenous mediators or modulators of epileptic phenomena. *Ann Neurol* 1984;**16**(Suppl):S31–S38.
- Sato M, Ushio Y, Arita N, Mogami H. Hypothalamic hamartoma: Report of two cases. *Neurosurgery* 1985;**16**:198–206.
- Cerullo A, Tinuper P, Provini F, et al. Autonomic and hormonal ictal changes in gelastic seizures from hypothalamic hamartomas. *Electroencephalogr Clin Neurophysiol* 1998;**107**:317–322.
- Palmiini A, Chandler C, Andermann F, et al. Resection of the lesion in patients with hypothalamic hamartoma and catastrophic epilepsy. *Neurology* 2002;**58**:1338–1347.
- Palmiini A, Van Paesschen W, Dupont P, Van Laere K, Van Driel G. Status gelasticus after temporal lobectomy: Ictal FDG-PET findings and the question of dual pathology involving hypothalamic hamartomas. *Epilepsia* 2005;**46**:1313–1316.
- Shahar E, Kramer U, Mahajnah M, et al. Pediatric-onset gelastic seizures: Clinical data and outcome. *Pediatr Neurol* 2007;**37**:29–34.
- Rekate HL, Feiz-Erfan I, Ng Y-T, Gonzalez LF, Kerrigan JF. Endoscopic surgery for hypothalamic hamartomas causing medically refractory gelastic epilepsy. *Childs Nerv Syst* 2006;**22**:874–880.
- Fenoglio KA, Wu J, Kim DY, et al. Hypothalamic hamartoma: Basic mechanisms of intrinsic epileptogenesis. *Semin Pediatr Neurol* 2007;**14**:51–59.
- Beck H, Yaari Y. Plasticity of intrinsic neuronal properties in CNS disorders. *Nat Rev Neurosci* 2008;**9**:357–369.
- Gutnick MJ, Connors BW, Prince DA. Mechanisms of neocortical epileptogenesis *in vitro*. *J Neurophysiol* 1982;**48**:1321–1335.
- Chagnac-Amitai Y, Connors BW. Synchronized excitation and inhibition driven by intrinsically bursting neurons in neocortex. *J Neurophysiol* 1989;**62**:1149–1162.
- Sanabria ER, Su H, Yaari Y. Initiation of network bursts by Ca<sup>2+</sup>-dependent intrinsic bursting in the rat pilocarpine model of temporal lobe epilepsy. *J Physiol* 2001;**532**:205–216.
- Avoli M, Olivier A. Electrophysiological properties and synaptic responses in the deep layers of the human epileptogenic neocortex *in vitro*. *J Neurophysiol* 1989;**61**:589–606.
- Avoli M, Hwa GG, Lacaille JC, Olivier A, Villemure JG. Electrophysiological and repetitive firing properties of neurons in the superficial/middle layers of the human neocortex maintained *in vitro*. *Exp Brain Res* 1994;**98**:135–144.
- Foehring RC, Waters RS. Contributions of low-threshold calcium current and anomalous rectifier (I<sub>h</sub>) to slow depolarizations underlying burst firing in human neocortical neurons *in vitro*. *Neurosci Lett* 1991;**124**:17–21.
- Cepeda C, Andre VM, Levine MS, et al. Epileptogenesis in pediatric cortical dysplasia: The dysmature cerebral developmental hypothesis. *Epilepsy Behav* 2006;**9**:219–235.
- Cepeda C, Andre VM, Vinters HV, Levine MS, Mathern GW. Are cytomegalic neurons and balloon cells generators of epileptic activity in pediatric cortical dysplasia? *Epilepsia* 2005;**46**(Suppl 5):82–88.
- Cepeda C, Andre VM, Wu N, et al. Immature neurons and GABA networks may contribute to epileptogenesis in pediatric cortical dysplasia. *Epilepsia* 2007;**48**(Suppl 5):79–85.
- Wu J, DeChon J, Xue F, et al. GABA<sub>A</sub> receptor-mediated excitation in dissociated neurons from human hypothalamic hamartomas. *Exp Neurol* 2008;**213**:397–404.
- Chapman KE, Kim DY, Rho JM, Ng YT, Kerrigan JF. Ketogenic diet in the treatment of seizures associated



- with hypothalamic hamartomas. *Epilepsy Res* 2011;**94**:218–221.
49. Bormann J. The 'ABC' of GABA receptors. *Trends Pharmacol Sci* 2000;**21**:16–19.
  50. Macdonald RL, Olsen RW. GABA<sub>A</sub> receptor channels. *Annu Rev Neurosci* 1994;**17**:569–602.
  51. Farrant M, Nusser Z. Variations on an inhibitory theme: Phasic and tonic activation of GABA(A) receptors. *Nat Rev Neurosci* 2005;**6**:215–229.
  52. Benarroch EE. GABA<sub>A</sub> receptor heterogeneity, function, and implications for epilepsy. *Neurology* 2007;**68**:612–614.
  53. Ben-Ari Y. Excitatory actions of gaba during development: The nature of the nurture. *Nat Rev Neurosci* 2002;**3**:728–739.
  54. Stein V, Nicoll RA. GABA generates excitement. *Neuron* 2003;**37**:375–378.
  55. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL. Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* 1989;**416**:303–325.
  56. Represa A, Ben-Ari Y. Trophic actions of GABA on neuronal development. *Trends Neurosci* 2005;**28**:278–283.
  57. Fujiwara-Tsukamoto Y, Isomura Y, Nambu A, Takada M. Excitatory GABA input directly drives seizure-like rhythmic synchronization in mature hippocampal CA1 pyramidal cells. *Neuroscience* 2003;**119**:265–275.
  58. Gullledge AT, Stuart GJ. Excitatory actions of GABA in the cortex. *Neuron* 2003;**37**:299–309.
  59. DeFazio RA, Heger S, Ojeda SR, Moenter SM. Activation of A-type gamma-aminobutyric acid receptors excites gonadotropin-releasing hormone neurons. *Mol Endocrinol* 2002;**16**:2872–2891.
  60. Moenter SM, DeFazio RA. Endogenous gamma-aminobutyric acid can excite gonadotropin-releasing hormone neurons. *Endocrinology* 2005;**146**:5374–5379.
  61. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R. On the origin of interictal activity in human temporal lobe epilepsy *in vitro*. *Science* 2002;**298**:1418–1421.
  62. Nabekura J, Ueno T, Okabe A, et al. Reduction of KCC2 expression and GABA<sub>A</sub> receptor-mediated excitation after *in vivo* axonal injury. *J Neurosci* 2002;**22**:4412–4417.
  63. Cossart R, Bernard C, Ben-Ari Y. Multiple facets of GABAergic neurons and synapses: Multiple facets of GABA signalling in epilepsies. *Trends Neurosci* 2005;**28**:108–115.
  64. Khalilov I, Holmes GL, Ben-Ari Y. *In vitro* formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. *Nat Neurosci* 2003;**6**:1079–1085.
  65. Jin X, Huguenard JR, Prince DA. Impaired Cl<sup>-</sup> extrusion in layer V pyramidal neurons of chronically injured epileptogenic neocortex. *J Neurophysiol* 2005;**93**:2117–2126.
  66. Payne JA, Rivera C, Voipio J, Kaila K. Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci* 2003;**26**:199–206.
  67. Kim DY, Fenoglio KA, Kerrigan JF, Rho JM. Bicarbonate contributes to GABA<sub>A</sub> receptor-mediated neuronal excitation in surgically resected human hypothalamic hamartomas. *Epilepsy Res* 2009;**83**:89–93.
  68. Simeone KA, Sabesan S, Kim DY, Kerrigan JF, Rho JM, Simeone TA. L-type calcium channel blockade reduces network activity in human epileptic hypothalamic hamartoma tissue. *Epilepsia* 2011;**52**:531–540.
  69. Steinmetz PN, Wait SD, Lekovic GP, Rekate HL, Kerrigan JF. Firing behavior and network activity of single neurons in human epileptic hypothalamic hamartoma. *Front Neurol* 2013;**4**:210.
  70. Prince DA. Physiological mechanisms of focal epileptogenesis. *Epilepsia* 1985;**26**(Suppl 1):S3–S14.
  71. Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends Neurosci* 2007;**30**:350–357.
  72. Kohling R, Vreugdenhil M, Bracci E, Jeffreys JGR. Ictal epileptiform activity is facilitated by hippocampal GABA<sub>A</sub> receptor-mediated oscillations. *J Neurosci* 2000;**20**:6820–6829.
  73. Behr C, D'Antuono M, Hamidi S, et al. Limbic networks and epileptiform synchronization: The view from the experimental side. *Int Rev Neurobiol* 2014;**114**:63–87.
  74. D'Antuono M, Louvel J, Kohling R, et al. GABA<sub>A</sub> receptor-mediated synchronization leads to ictogenesis in the human dysplastic cortex. *Brain* 2004;**127**:1626–1640.
  75. Cepeda C, Chen JY, Wu JY, et al. Pacemaker GABA synaptic activity may contribute to network synchronization in pediatric cortical dysplasia. *Neurobiol Dis* 2014;**62**:208–217.
  76. Palma E, Roseti C, Maiolino F, et al. GABA(A)-current rundown of temporal lobe epilepsy is associated with repetitive activation of GABA(A) "phasic" receptors. *Proc Natl Acad Sci USA* 2007;**104**:20944–20948.
  77. Cifelli P, Palma E, Roseti C, Verlengia G, Simonato M. Changes in the sensitivity of GABA<sub>A</sub> current rundown to drug treatments in a model of temporal lobe epilepsy. *Front Cell Neurosci* 2013;**7**:108.
  78. Jansen LA, Peugh LD, Ojemann JG. GABA<sub>A</sub> receptor properties in catastrophic infantile epilepsy. *Epilepsy Res* 2008;**81**:188–197.
  79. Li G, Yang K, Zheng C, et al. Functional rundown of gamma-aminobutyric acid(A) receptors in human hypothalamic hamartomas. *Ann Neurol* 2011;**69**:664–672.
  80. Dudek FE, Yasumura T, Rash JE. "Non-synaptic" mechanisms in seizures and epileptogenesis. *Cell Biol Int* 1998;**22**:793–805.
  81. Avoli M, Williamson A. Functional and pharmacological properties of human neocortical neurons maintained *in vitro*. *Prog Neurobiol* 1996;**48**:519–554.
  82. Avoli M, Louvel J, Pumain R, Kohling R. Cellular and molecular mechanisms of epilepsy in the human brain. *Prog Neurobiol* 2005;**77**:166–200.
  83. Steriade M. To burst, or rather, not to burst. *Nat Neurosci* 2001;**4**:671.
  84. Kohling R, Avoli M. Methodological approaches to exploring epileptic disorders in the human brain *in vitro*. *J Neurosci Methods* 2006;**155**:1–19.