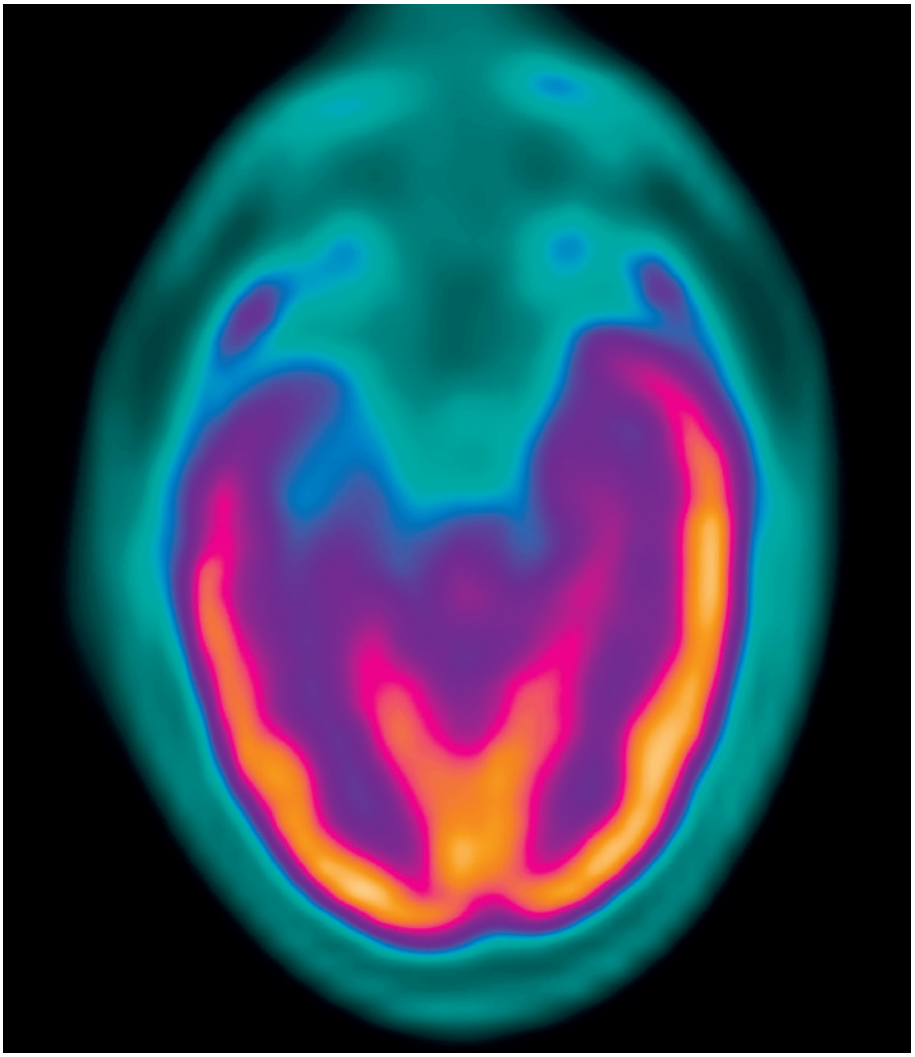

CENTRE JEAN PERRIN, ISM/SPL

A scan showing heightened activity on the right side of the brain (bright orange) during an epileptic seizure.

NEUROBIOLOGY

Unrestrained excitement

Epilepsy arises from natural mechanisms in the brain that go awry. Researchers are trying to unravel its complexities.

BY MICHAEL EISENSTEIN

"Saying 'epilepsy' is like saying 'sneezing'," says Douglas Coulter, director of the Epilepsy Research Laboratory at the University of Pennsylvania in Philadelphia. "It's a series of disorders with a common output — seizures — but even there, you have different kinds of seizures."

During a seizure, the brain's neurons are activated in synchronized, high-frequency patterns across broad populations, starting largely without warning and ending just as abruptly. Seizures share a common feature: a breakdown in the mechanisms that normally constrain neuronal activation, or firing. Indeed, findings from one type of epilepsy can potentially inform research on other types, but it remains unclear how deep or superficial those commonalities actually are. "My view is that there are going to be common pathways," says Coulter, "but many of the mechanisms that have been described to date are quite divergent."

Reports of epileptic seizures date back thousands of years, but scientists are still grappling with the fundamental nature of the events that take place in the epileptic brain. Research continually reveals added complexity, and the blanket term 'epilepsy' has largely become anachronistic — a clinical descriptor that has limited value.

THE LIMITS OF CONTROL

At the core of epilepsy is the delicate interplay between two types of brain cell: excitatory neurons and inhibitory interneurons. Excitatory neurons release the neurotransmitter glutamate, which binds to a receptor on other neurons and promotes firing (see 'Electric ups and downs'). Inhibitory interneurons release γ-aminobutyric acid (GABA), which binds a receptor on excitatory neurons that restrains firing. Neurotransmitters typically act across synapses — the spaces between the neurons that form a neuronal circuit. However, some neurons also have receptors outside the synapse that can detect any GABA floating about, and these act as an additional failsafe when synaptic inhibition is inadequate.

Both GABA and glutamate work by modulating the amounts of positively and negatively charged ions within neurons. Normally, neurons are negatively polarized relative to their surroundings. The binding of glutamate to its receptor triggers the opening of specialized protein-based pores in the membrane known as ion channels, which allows positively charged calcium and sodium ions to flood into the responding cell. This reduces the polarization of the cell and promotes neuronal firing. GABA signalling, on the other hand, opens other channels that allow negatively charged chloride ions to enter a cell — increasing the polarization and inhibiting firing. It's therefore no wonder, says neurologist Matthew Walker of University College London (UCL) that "for epilepsies in which a mutation in a gene has been identified, the majority of the mutations affect channels and receptors".

For example, the *SCN1A* gene encodes a channel that aids excitation of a cell by allowing positively charged sodium ions to enter it. This channel is predominantly active in inhibitory interneurons; mutations that impair its function can contribute to a paediatric epileptic disorder known as Dravet syndrome as well as to certain partial epilepsies because of its effects on the release of GABA.

Fortunately, because the brain has many safeguards, even profound defects in channel and receptor function rarely establish a permanent epileptic state. Nevertheless, defects mean that under certain conditions the 'dam' that keeps uncontrolled firing at bay breaks.

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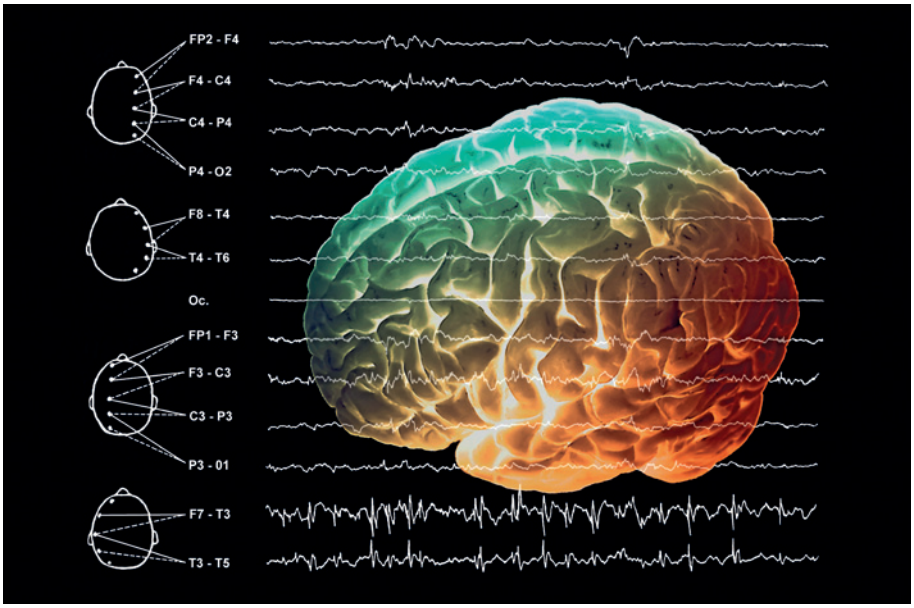

In this epileptic seizure, parts of the brain show erratic activity on an electroencephalogram (bottom traces).

"Compensatory mechanisms usually work very well until you push the system too far, and then a seizure occurs," explains Walker.

The features of various types of seizure emerge from the ordinary mechanisms of brain function. "The machinery that makes seizures happen is the same machinery that allows us to think or move," says Massimo Avoli, a neurologist at the Montreal Neurological Institute and Hospital in Canada. "It's only the balance between inhibition and excitation that is not right." Indeed, many epilepsies resemble a perverse imitation of normal activity for a given brain structure — and these lead to some of the distinctions between the pathology and activity associated with different epileptic disorders.

For example, a structure deep in the brain called the hippocampus plays a key role in learning and memory. It receives incoming data from the entorhinal cortex, which relays perceptual information to a class of excitatory neurons — granule cells — in a part of the hippocampus called the dentate gyrus. However, granule cells don't get excited easily: they are influenced by a network of inhibitory interneurons and only a tiny proportion of inflowing signals register in the hippocampus. This process of 'dentate gating' is thought to be important for processes such as pattern separation, which helps the brain distinguish between relatively similar stimuli.

But dentate gating seems to break down in temporal-lobe epilepsies affecting the hippocampus, resulting in unrestrained, synchronized firing of granule cells. The onset of such seizures may begin with the perception of 'auras', characterized by unusual emotional states or a sense of déjà vu, followed by a period of confusion and memory loss. Most temporal-lobe epilepsies arise from physical trauma, either from a direct injury to the brain or through damage arising from a stroke or tumour; according to Coulter, this leads to a large loss of interneurons as well as some loss of GABA receptors on the granule cells. Once the restrictions on excitatory signalling are lost, other disruptions may follow. "We're developing a 'domino theory' of epilepsy, where a transient failure of dentate function may disrupt downstream regulatory functions and give you a secondary failure of downstream circuits," says Coulter.

The brain patterns associated with absence seizures mirror those observed in sleep.

The mechanism underlying seizures known as absence epilepsies is rather different. Absence seizures can emanate from a single site in the brain, but then give rise to a distinctive 'spike wave' electroencephalogram (EEG) pattern that is synchronized across multiple regions on both sides of the brain. This process is driven by the interplay between the brain's outer layer, the cerebral cortex, and an inner structure called the thalamus. In contrast to many of the focal epilepsies, it is the inhibitory response of interneurons that fuels absence seizures. According to neurologist John Huguenard of Stanford School of Medicine in California, incoming signals from the cortex trigger a massive wave of GABA-driven inhibition of the excitatory neurons of the thalamus, deep in the brain. However, the inhibited excitatory neurons subsequently undergo a 'rebound' response — and react with a synchronized burst of activity. That activates cells that drive another wave of inhibition.

The thalamus is involved in wakefulness and awareness, and the hallmark symptom of the absence seizure is a loss of consciousness. Intriguingly, the brain patterns associated with absence seizures — coordinated cycles of thalamic and cortical activity — closely mirror those observed in sleep, particularly during 'spindle' EEG signatures that represent the brain's efforts to keep the sleeper unconscious, but on a far grander scale.

NETWORK NEWS

For all this knowledge, homing in on the faulty wiring involved in epilepsy remains difficult, confounding efforts to achieve lasting relief through surgery to remove the areas responsible. The success rate for patients with surgically treatable focal epilepsy is initially high, with around 80% becoming seizure-free post-surgery, and many experts endorse this form of treatment (see page S7). "But when you look 5, 10 or 20 years [post-surgery], it starts to drop to about 50% seizure-free," says Walker.

Two electrophysiology researchers, Andrew Trevelyan of Newcastle University, UK, and Catherine Schevon of Columbia University Medical Center in New York, have revealed a potential reason why: conventional EEG methods can encounter difficulties in accurately pinpointing the brain regions responsible for focal epilepsies. "As the seizure wave-front propagates, you end up with recruited territories that seem to involve every neuron in the network," says Trevelyan. But this in turn triggers a massive wave of inhibitory activity among the interneurons that surround this recruited zone. After identifying this phenomenon in rodent brain slices, Trevelyan and Schevon observed this same surrounding zone of inhibition, which they term the 'ictal penumbra', in humans by performing EEG using arrays of tiny electrodes implanted in the brains of epileptic patients[1]. EEG readings from electrodes placed on the scalp will detect strong synaptic activity within the seizure zone and in the ictal penumbra, even though most of those neurons are inhibited and not participating in the seizure.

Failure to accurately pinpoint the brain regions involved in a person's seizures can lead to the misidentification of the epileptic focus and increase the likelihood of failed surgery. Electrophysiology insights could guide more sophisticated monitoring of cortical epilepsies and possibly other focal epilepsies as well.

ON AND OFF SWITCHES

The discovery of light-responsive proteins that can directly switch neurons on or off could transform efforts to tease apart and modify the function of brain circuits in epilepsy. In 'optogenetics' experiments, researchers genetically modify specific subsets of neurons in animals to express these proteins and observe the extent to which different manipulations promote or prevent seizures. For example, Walker and UCL colleagues including Stephanie Schorge and Dimitri Kullmann reprogrammed neurons to express halorhodopsin,

a light-triggered 'off' switch[2]. They found that even limited restraint of neuronal firing in a region of the motor cortex markedly reduced the number of seizure events in a rat model of cortical epilepsy. Walker notes that only a few neurons in a small area were turned off in the experiment. "This shows that treating epilepsy might not be about cutting out large areas of the brain," he says. "Modifying the right areas might be enough to stabilize the network."

Similarly, a study[3] by Huguenard's team used halorhodopsin to show that forced inactivation of thalamocortical neurons can essentially halt seizures in rats with stroke-induced cortical epilepsy. This result was striking because these neurons were situated far from the injury site, in a region typically involved with generalized absence seizures. "It was thought that the primary reorganization is among regions adjacent to the cortical stroke," says Huguenard. "We were surprised to see such a strong thalamic involvement."

Coulter sees such findings as compelling evidence that epilepsy neuroscientists need to cast a wider net. "These optogenetics studies suggest a very distributed network," he says.

FROM SUPPORTING ROLE TO STAR

The nervous system is made up of two types of cells: neurons, which transmit nerve impulses, and glial cells, which play a supporting role. Glial cells perform essential functions such as providing a myelin sheath around the neurons to enable fast transmission of nerve signals and helping to maintain the correct ion concentration. Most epilepsy research to date has focused on the role of neurons. However, the brain contains at least as many glial cells. The past two decades of research have revealed surprising ways in which these once-underappreciated cells may contribute to both the onset and progression of epilepsy.

Increased proliferation and activation of glial cells is a well-established hallmark of epilepsy. Among the glial subtypes, astrocytes are known to contribute to the epileptic state due to erosion of their role in maintaining the chemical environment of the brain. For example, elevated potassium levels render neurons hyperexcitable, and numerous studies have found impaired potassium control by astrocytes in the epileptic brain. Astrocytes also help to clean up neurotransmitter molecules in the aftermath of neuronal activity, and disruptions in the absorption and recycling of glutamate and GABA by astrocytes can similarly lead to inappropriate neuronal sensitization or inhibition in certain contexts.

More recent findings suggest that astrocytes are not merely custodians, but can themselves secrete and respond to neurotransmitters. "We know that if you stimulate astrocytes from a human biopsy, these astrocytes exhibit calcium signalling and can also release glutamate," says glia researcher Giorgio Carmignoto of the University of Padova, Italy. This glutamate can in turn contribute to the excitation of adjacent neurons, and Carmignoto describes the formation of 'tripartite synapses' in which astrocytes actively participate in conversations once thought to occur exclusively between neurons. However, activated astrocytes can also help to reduce seizure intensity: they contribute to the production of adenosine, a signalling molecule that can counter the effects of glutamate. In short, adenosine turns down the neural signals that glutamate turns up, and that could reduce seizure activity, explains Carmignoto.

Huguenard has shown that astrocytes may also contribute to seizure control within the thalamus by producing endozepines[4], which are naturally occurring molecules that resemble the sedative benzodiazepine — better known as Valium. "Astrocytes might act as sensors for seizure activity or even pre-seizure activity, and respond by enhancing the release of endozepines, thereby suppressing activity in the reticular nucleus," he says.

The injuries that trigger epilepsy are often associated with an inflammatory response in the brain, and mounting evidence suggests that astrocytes and other glial cell types are central to this process. They secrete signalling factors that normally contribute to damage control and injury repair, but they can also create a feedback loop that promotes epileptic activity. "Pathological electrical activity per se is enough to activate the glial cells," says neuropharmacologist Annamaria Vezzani of the Mario Negri Institute for Pharmacological Research in Milan, Italy. Once activated, they secrete molecules such as interleukin-1β and these initiate a signalling cascade in neurons that renders them more sensitive to glutamate-induced excitation — and therefore, seizure activity. "So on the one hand you have this neuronal effect, and on the other you have a persistence of the inflammatory milieu because these neurons are activating the glia continuously," says Vezzani. The inflammatory response also seems to disrupt the blood–brain barrier, the tight cellular seal that limits entry of materials from the bloodstream to the brain. There is evidence that penetration of this compromised barrier by molecules such as the protein albumin can exacerbate both neuronal hyperexcitability and inflammation.

What remains unclear is the extent and the timing of these various astrocyte-mediated processes, and how they trigger or suppress epilepsy in humans. The evidence, which has mostly been obtained from either isolated brain slices or animal models, is intriguing but is not conclusive enough to form a robust disease narrative. "Any time you have an increase in excitability in a neuronal network, this, for sure, will involve astrocytes," says Carmignoto. "But what comes first — the neuronal defect or the astrocyte defect — is the issue."

For now, there is not enough evidence to map the natural history of epilepsy. Better disease models and more human data are needed before these individual findings can be linked into a satisfying narrative. "Understanding epilepsy," says Carmignoto, "means understanding how the brain works. It is as complex as that." ■

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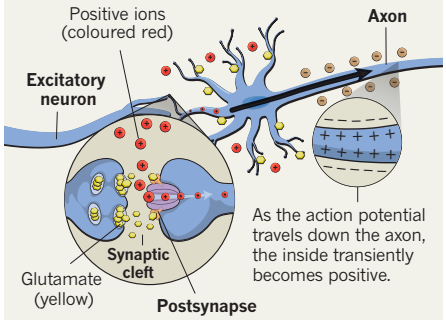
ELECTRIC UPS AND DOWNS

In a healthy brain, excitatory and inhibitory neuron dynamics are tightly balanced in a controlled way. In epilepsy, the mechanisms underlying this balance go awry, causing overly-excited neurons to fire uncontrollably.

EXCITATION

When a neuron becomes excited:
At excitatory synapses, the neurotransmitter glutamate is released. Glutamate crosses the synaptic cleft, binding to glutamate receptors at the postsynapse, initiating depolarization.

Na^+ ions enter the neuron, driving it to threshold and subsequent firing of an action potential, or nerve signal. In epilepsy, neurons are often very excitable, close to threshold and fire action potentials more readily.



INHIBITION

When a neuron becomes inhibited:
At inhibitory synapses, the neurotransmitter γ-aminobutyric acid (GABA) is released. GABA crosses the synaptic cleft, binding to GABA receptors at the postsynapse, initiating hyperpolarization.

Cl^- ions enter the neuron, driving it further from the action potential threshold, decreasing the probability that the neuron will fire. Increasing inhibition, in some cases, can assist in reducing the chance of unregulated seizure activity.