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Visions & Reflections

Dogma and dreams: experimental lessons for epilepsy mechanism chasers

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Abstract. Epilepsy mechanism chasers face one major difficulty. Since we don't know how the normal brain works, we can't start to understand how the diseased brain fails. Most of today's hypotheses are based on what we think about 'normal' brain function, which may lead to misconceptions, as will be developed here. Furthermore, since there are many different types of epilepsies, some mechanisms may only be relevant to some epilepsies. Here, I shall focus on temporal lobe epilepsy (TLE) the most common form of partial epilepsy in adults. TLE is often drug resistant, as are 30–40% of all forms of epilepsies. The failure of drug-treatments most likely reflects our lack of knowledge of the underlying mechanisms.

Key words. Epilepsy; GABA; interneuron; inhibition; excitation; hippocampus.

Perhaps the most useful approach to the problem is to start with the lessons taught by clinical studies. Investigations performed with electroencephalogram (EEG) or depth EEG show that TLE seizures are often preceded by large-amplitude field-potential spikes, which, at seizure onset, may increase in amplitude, become more rhythmic and display synchrony across different limbic structures. There is then a flattening of the EEG signal, and the seizure starts with a high-frequency discharge. The electrographic seizure typically consists of tonic rhythmic discharge and clonic bursting phases (rhythmic spikes that increase in amplitude and then decrease in frequency). It is important to note that patients diagnosed with TLE can display diverse seizure patterns that involve different cortical structures. Although the aetiology may be similar, the underlying mechanisms may be dissimilar, thus adding another degree of complexity. Between seizures, the EEG presents an abnormal activity pattern with the occurrence of paroxysmal interictal events (fast brief spikes) that can be recorded within and outside the epileptic zone. Chronic animal models of TLE usually display the same general patterns of activity before, during, after and between seizures. Ictal and interictal events are associated with the high-frequency firing of large populations of neurons [1–4]. Based on these observations, what kind of experimentably testable hypotheses can we propose? Cell firing results from a complex signal integration process. Synaptic inputs interact with ionic channels in the neuronal membrane and change membrane potential. If the membrane potential reaches firing threshold, an action potential is initiated. Any modification affecting synapses, ionic channels, membrane properties and so on will affect cell information processing. Some of these modifications might lead to hyperexcitability at the individual neuron or the neuronal network level. Gamma-amino butyric acid (GABA) is a ubiquitous neurotransmitter in the brain, and the consequences of the activation of GABAergic synapses can take multiple forms. Because the fate of GABAergic pathways plays such a central role in epilepsy research and the design of drug strategies, I shall focus on the dogma and dreams revolving around GABA.

In the adult brain, the common view of ionotropic GABAergic neurotransmission states that the opening of $GABA_A$ receptor channels leads to an influx of chloride ions into the cell and efflux of bicarbonate ions, ultimately producing hyperpolarization of the membrane and driving its potential away from the firing threshold [5]. Because of this hyperpolarizing action, GABAergic neurotransmission is inhibitory, i.e. antagonizing the excitatory, depolarizing action of glutamatergic neurotransmission. Classically, glutamatergic and GABAergic neurotransmission are presented as the yin and yang of brain activity, opposed in nature but necessarily complementary and balanced to ensure proper brain function. It has been proposed that the excitatory/inhibitory ratio is kept constant by homeostatic mechanisms to insure adequate neuronal computation [6–8]. Thus, 'normal' brain activity is assumed to result from balanced excitation and inhibition, the latter acting as a brake while the former acts as an accelerator. In keeping with this scheme, any alteration in this balance would lead to a pathological state. Seizures (or interictal events), which are usually presented as the result of runaway excitation, would thus be due to increased excitation and/or decreased inhibition. The favoured hypothesis in epilepsy research is that a deficit of inhibition underlies seizures. Two observations are at the core of this hypothesis: drugs that boost GABAergic neurotransmission control epilepsy in some patients, and blocking $GABA_A$ receptors with specific antagonists consistently produces seizures in vivo and epileptiform discharges in vitro [9]. However, if decreasing inhibition results in epilepsy, this does not mean that epilepsy is due to decreased inhibition. Yet, most studies considered a deficit of GABAergic inhibition as the Holy Grail. Over the years, the Holy Grail proved to be as unattainable as its medieval counterpart, but still fascinating to dream chasers. Legends tend to breed more myths, such as the 'dormant basket cell hypothesis' [10, 11], and then become dogma. Although it may mislead basic research, dogma may also delude pharmacological research, with a main focus on boosting GABAergic neurotransmission.

The following arguments strongly suggest that we may have been barking up the wrong tree. A car will not perform properly if you exert a balanced pressure on both the accelerator and the brakes. Clearly, this is not how the brain works. Perhaps the most misleading concept is that of the yin and yang nature of glutamatergic and GABAergic neurotransmission. First, one function of GABAergic networks in the central nervous system (CNS) is to synchronize activity and generate oscillations [12, 13]. Since the brain spends most of its time oscillating at different frequencies [14], how can GABAergic neurotransmission qualify simply as inhibitory? GABAergic neurons can drive the network as much as glutamatergic ones. Second, the inhibition concept stems from the observation that $GABA_A$ receptor activation leads to membrane hyperpolarization via an influx of negatively charged ions (mostly Cl^-) into the cell. However, the reversal potential of the GABAergic current (E_{GABA}, i.e. the potential at which there are as many ions flowing in and flowing out of the open receptor – with a net null current) is very close to the cell resting membrane potential (RMP). Accumulating evidence suggests that GABAergic neurotransmission is excitatory in many neurons (E_{GABA} > RMP, i.e. at RMP Cl^- flows out of the cell, thus creating depolarization of the membrane) not only in normal adult tissue [15, 16] but also in the epileptic brain [4]. Third, one very important aspect of GABAergic neurotransmission is to activate extrasynaptic $GABA_A$ receptors, thus shunting the postsynaptic membrane. Some $GABA_A$ receptors are located outside the synapse and can be activated by GABA spillover [17–19]. Although it is still unclear whether the activation of extrasynaptic $GABA_A$ receptors (also called tonic current) is always associated with classical GABAergic synaptic transmission (also called phasic current), the tonic background current generated by the opening of extrasynaptic receptors represents 90% of the combined extrasynaptic-synaptic current flowing through the membrane in vitro [20]. The opening of extrasynaptic receptors has a strong functional impact as it results in a decrease of the neuronal membrane resistance proportional to the amplitude of the tonic current, following Ohm's law. As a consequence, any synaptic current will create a lesser change in membrane potential. The tonic current thus acts as a filter, changing the input/output function of neurons [21]. The tonic current is physiologically relevant as it plays a direct role in information processing in vivo [19, 22].

These three arguments demonstrate that GABAergic 'inhibition' has no meaning per se. It is not the functional antonym of 'excitation'. It is safer to talk about GABAergic signalling rather than GABAergic inhibition.

The next argument brings another degree of complexity. GABAergic neurotransmission must be considered in its multiplicity. In the hippocampus, there exist many different classes of GABA-releasing interneurons, with different morphological, neurochemical and physiological properties [12, 23, 24]. The multiplicity of interneuron classes is physiologically relevant, as different classes of interneurons play different roles in hippocampal function [25–28]. For example, interneuron firing patterns are class dependent during oscillatory activities recorded in vivo [25, 26] or in vitro [29]. Interneurons that project to the dendritic tree or the soma have very different functional effects on information processing in their postsynaptic targets [30, 31]. Finally, GABAergic neurotransmission can be modulated by numerous factors, including extracellular ions and compounds such as Zn^{2+} or barbiturates, neurotransmitters such as dopamine or serotonine

and neuropeptides, again in a cell type- and brain region-dependent manner [12, 32].

The last argument that brings the final layer of complexity is dynamics. The physiological consequences of $GABA_A$ receptor activation critically depend upon the frequency at which each GABAergic pathway is activated. GABAergic neurotransmission can extinguish [33] or switch from one neuronal target zone to another [28] in a frequency-dependent manner.

These arguments clearly show that GABAergic neurotransmission is not a uniform concept. Brain state, history, dynamics, brain region and cell type provide many degrees of freedom. Accordingly, in pathological conditions, neuronal network behaviour will be differentially affected according to which type(s) of GABAergic pathway is modified and in which manner [5].

The Quest requires dream chasers to pinpoint loci where deficits in GABAergic inhibition are proven in epileptic tissue. What do experimental studies tell us? The first argument is morphological. It is now well established that epilepsy is associated with the death of some classes of GABAergic interneurons in both human and animal models [5]. Such a loss provides a morphological substrate for decreased GABAergic neurotransmission. However, in animal models, GABAergic interneurons are already lost soon after the initial status epilepticus well before the occurrence of spontaneous recurrent seizures [34]. This suggests that the partial loss of the GABAergic network does not constitute a sufficient condition to induce seizures. However, it may constitute a critical step for the constitution of an epileptic network.

The next argument is physiological. The fate of GABAergic neurotransmission proves to be very difficult to assess, as it depends upon the brain region under scrutiny and the class of GABAergic interneuron within a given brain region. It is region specific as, for example, the conductance of $GABA_A$ receptors and the number of postsynaptic receptors are increased in dentate gyrus granule cells [35, 36], whilst the conductance of $GABA_A$ receptors is decreased in CA1 pyramidal cells [35]. It depends upon the class of interneurons. Despite a decrease in the conductance of $GABA_A$ receptors and a deficit in GABA quantal release in CA1 pyramidal cells [35, 37], the GABAergic synaptic drive received by the soma of CA1 pyramidal cells is increased due to the hyperactivity of interneurons projecting to the soma [38]. In contrast, the GABAergic synaptic drive received by the dendrites of CA1 pyramidal cells is decreased as a result of the loss of a specific population of interneurons projecting to the dendrites [38]. Finally, since neuronal networks are subject to continuous remodelling during the course of the disease, one modification may operate only during a given time window. These arguments clearly show that changes in GABAergic networks are highly specific and that heterogeneity must be considered.

Do these experimental observations prove/disprove the hypothesis of a deficit of inhibition in epilepsy? There is no clear-cut answer to this question, if only because answers depend on how the question is considered. This is better illustrated by the three aspects of GABAergic neurotransmission developed above: oscillation, depolarization vs. hyperpolarization and shunt. First, if seizures (by analogy) can be classed as oscillations, increasing GABAergic neurotransmission may favour oscillating conditions, hence epileptogenicity, a proposal that remains to be tested. Decreasing GABAergic neurotransmission may be protective in this context. Interestingly, high-frequency oscillations or interictal activity, presumably under the control of interneurons [4, 13, 25, 26], appear after the initial insult and before the occurrence of spontaneous recurrent seizures [39, 40]. It is possible that the morpho-functional reorganizations that take place during epileptogenesis favour the emergence of transient interneuron-dependent bursts of high-frequency activities. Whether interictal activities/high-frequency oscillations play an active role in seizure genesis is still debated. Second, if GABA becomes depolarizing (Cl^- flows out of the cell as a result of Cl^- accumulation in the cell in chronic conditions), increasing GABAergic neurotransmission may have opposite effects, i.e. increasing excitation [4]. Interestingly, blocking $GABA_A$ receptors blocks interictal activity in human epileptic tissue in vitro, demonstrating that the latter is partly driven by GABAergic interneurons [4]. Third, if tonic GABAergic neurotransmission is more affected than phasic GABAergic neurotransmission, the major impact will be found on the input/output function of neurons [19, 21]. Increasing GABAergic neurotransmission (even if it becomes partly depolarizing) will increase the shunt of the membrane, hence decrease the firing probability in response to a given excitatory input.

Even if we manage to determine the functional impact of the alterations of GABAergic networks within a given region, an upstream (multi-regional) analysis will become necessary. Since seizures in the temporal pole involve several regions, it is important to consider how these regions interact. If area A functions to silence its target area B, and if area B is directly involved in ictogenesis, increasing excitability in area A may help keep area B under control. A deficit of inhibition in area A may be a protective mechanism in this context. The functional role of a given area within the overall network must be taken into consideration. The functional impact of area A onto area B is likely to be context dependent. For example, the synaptic drive coming from the dentate gyrus can switch from inhibition to excitation in its target CA3 region in a frequency-dependent manner [33]. Dynamics constitutes a critical factor.

Clearly much work remains to be done to establish the final functional impact of the modifications taking place

within the GABAergic circuitry in the different regions involved in seizure genesis and propagation.

Pharmacological studies in patients with epilepsy reflect the uncertainty regarding the fate and role of GABAergic neurotransmission. GABA boosters are known to worsen epilepsy in some patients, and powerful GABA boosters have been reported to be deleterious, inducing cell death [41, 42]. Yet GABA boosters do work in some patients [43]. Perhaps the underlying mechanism involves the shunt of the neuronal membrane [21]. Interestingly, Lamotrigin, an anti-epileptic drug that increases the activation of I_h, a nonspecific cationic channel, seems to operate via such a mechanism (shunt of the membrane) [44].

The concept of a deficit of GABAergic inhibition was so attractive that it became a dogma. Now it seems that the dogma must be relegated to the rank of hypothesis. Caution urges us now to speak about GABAergic signalling since 'inhibition' can be misleading. Considering the role of GABAergic interneurons in synchronizing neural networks, a deficit of GABAergic neurotransmission will still attract dream chasers, as alterations in synchronizing mechanisms may be epileptogenic. The fate of GABAergic neurotransmission is but one of the possible yet-to-be-discovered Grails. Other research avenues include the fates of glutamatergic neurotransmission and ionic channels that control membrane excitability [45–47]. In contrast to Indiana Jones's choice in 'The Last Crusade', the solution may not be the simplest one. It may reside in both its multiplicity and complexity.

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