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Review

Advances in understanding basic mechanisms of epilepsy and seizures

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ABSTRACT

Sixty years ago the clinical neurophysiology of epilepsy had progressed to the stage that it posed questions that could be addressed by major advances in cellular electrophysiology made around the that time. However, it took about 25–30 years to build up serious momentum in understanding the mechanisms of epileptic discharges. Over the past 2–3 decades developments in pharmacology and molecular biology have substantially increased the depth and complexity of our insights into the nervous system in general and the epileptic brain in particular. One of the biggest advances in our understanding of the brain is in its plasticity in the adult – that is its ability to modify its structure and function. The current state of play is that for most chronic epileptic foci it is possible to identify multiple differences from normal brain tissue in both the structure and function of neurons, neuronal networks and glia. This review will chart some of this progress to give an idea of the pace of advances over the decades.

Epilepsy is a condition in which neurons and neuronal networks malfunction to cause the sometimes dramatic symptoms of seizures. People, and other animals, without epilepsy can experience "symptomatic" seizures – the structure and function of the brain carries the risk of generating seizures if they are exposed to convulsant drugs or conditions. The epileptic brain generates seizures without any such trigger: this means that it has differences in structure and/or function that make it susceptible to spontaneous seizures, and that can also lead to altered behaviour and/or cognition between seizures, as reflected in the relatively recent concept of "Epilepsy Spectrum Disorder".

Seizures are essentially a malfunctioning of the brain, due to the "misfiring" of its neurons, so much of this review will centre on the essentially electrophysiological processes involved in generating seizures and other abnormal EEG activity. I will outline some aspects of clinical electrophysiology and of experimental approaches before considering how improvements in our understanding of the fundamentals of the nervous system over the past 60 years have impacted on epilepsy research. Space constraints, as well as my personal expertise, have led me to concentrate mainly on focal epilepsies.

1. Clinical electrophysiology of epilepsy

A great deal has happened in clinical neuroscience over the past 60 years. The electroencephalogram or EEG had been discovered much earlier, with Berger publishing his pivotal paper in 1929.[1] The EEG was becoming more routine during the late 1940s and early 1950s, especially in epilepsy diagnosis.[2,3] In one discussion paper Walter[2] outlined the use of the EEG in epilepsy in ways that are still relevant today (Fig. 1A). The key point from this work was that epileptic seizures were associated with abnormally synchronous brain activity, essentially a foundation for much of what follows.

Arguably the biggest breakthrough in clinical neurophysiology over the past 60 years was the advent of digital recording and the increasing power and availability of computers. For decades the mechanical constraints of the chart recorder limited bandwidth, or the range of signal frequencies, of the EEG to something under 100 Hz (Fig. 1B shows relevant components of the EEG spectrum, from[4] Ref. 4. Digital EEG recordings became available in the early 1990s, and in most cases simply emulated paper-based recordings but with less mess and bulk. However, two key papers did exploit the wider bandwidth to show that high-frequency activity (over 100 Hz) was associated with epileptic seizures.[5,6] Towards the end of 1990s even faster recordings demonstrated the presence of "ripples" (80–200 Hz) and "fast ripples" (>200 Hz), and particularly implicated fast ripples as markers for the epileptogenic zone.[4,7] Fast ripples were associated with epilepsy-related losses of neurons known as hippocampal sclerosis, both clinically and in experimental models[8] (Fig. 1D), though our own recent evidence argues that this lesion is not necessary for fast ripples,[9] suggesting that some more subtle pathophysiology is operating.

High frequency activity is best recorded with microwires inserted into the tissue of the epileptic focus, most likely because the synchronous activity that generates each cycle of the oscillations covers a limited volume of brain tissue.[4,10] Most epilepsy centres still use macro-electrodes with recording surfaces

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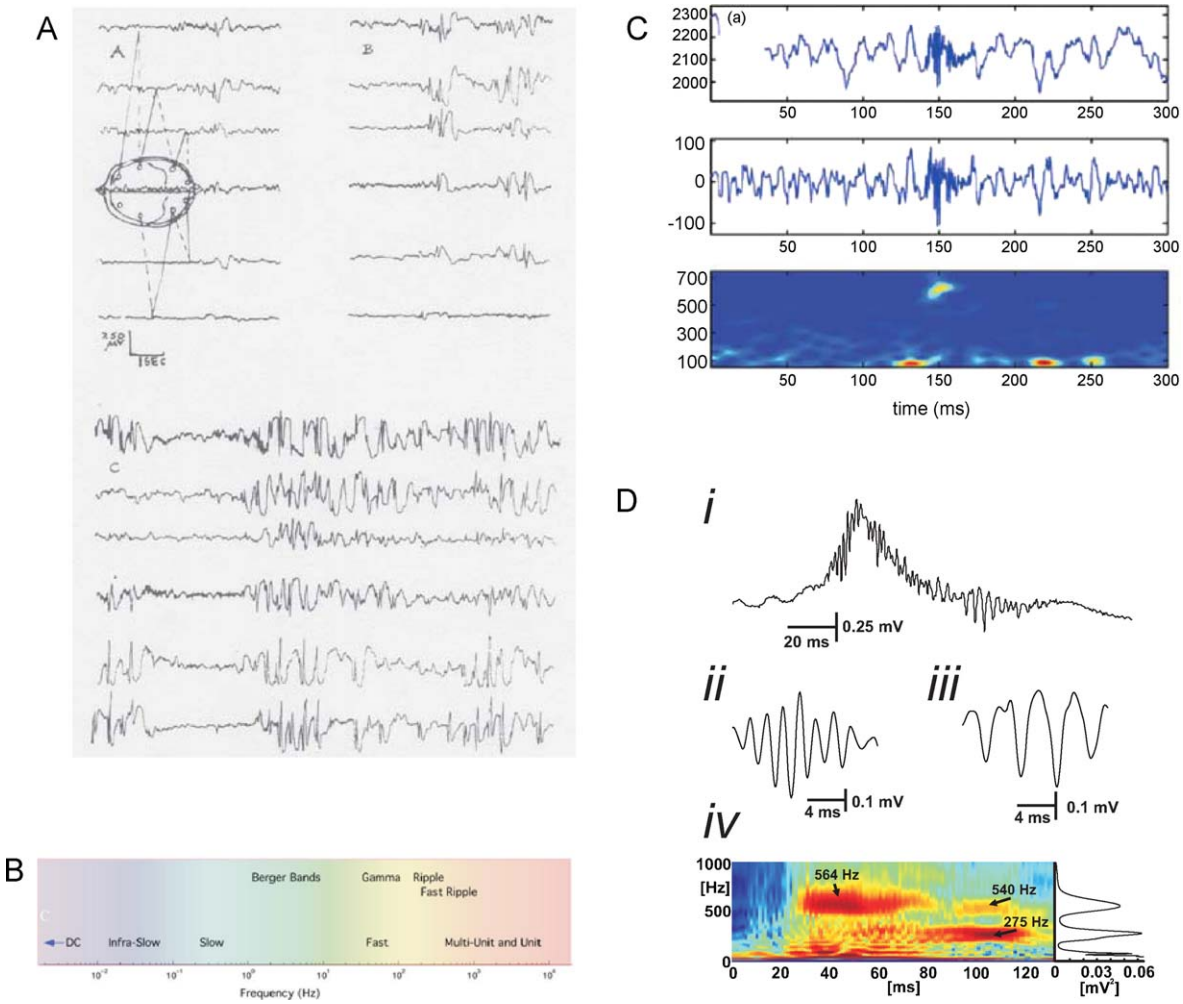

Fig. 1. Recordings of epileptic activity from 1951 to 2010. (A) Classical EEG recording of bilateral spike and wave discharges published in 1951. Note the slight curvature of some parts of the recording, which is due to the arc followed by the pens as they responded to the voltage. (B) The spectrum covered by the EEG. Chart recorders were limited to a few tens of Hz (the Berger Bands in the figure), perhaps reaching as fast as 100 Hz as the technology improved. (C) Interictal fast ripple recorded from a microelectrode implanted in the seizure onset zone of the medial temporal lobe of an epileptic patient. Top panel is the raw recording, middle is the signal band-pass filtered 80–1000 Hz and the bottom panel is a spectrogram with frequency on the *y*-axis and time (0–300 ms) on the *x*-axis, and higher power is indicated by hot colours (pale in monochrome). (D) Interictal discharge and fast ripples recorded from the hippocampus of an epileptic rat, with the raw recording (*i*), fast ripples isolated from early and late in the interictal discharge (*ii*, *iii*) and the corresponding spectrogram with areas of high power labelled (*iv*). The fast recordings and signal processing required for panels C and D are only possible with the advent of faster recording systems and modern computers. *Panel A* is from Walter et al.,[2] by permission of the Royal Society of Medicine Press, London. *Panels B and C* are taken from Worrell et al.[4] *Panel D* is from Jiruska et al.[9] *Panels B–D* by permission of the authors, Oxford University Press and the Guarantors of *Brain*.

of several square mm, often, but not always, limited to the subdural surface of the brain. These can record high-frequency activity reliably up to around 100 Hz and can detect some activity up to 200 Hz, while the much smaller microwires, tens of microns in diameter, can record epilepsy-related high-frequency activity over 500 Hz.[4] Two-dimensional high-density arrays of microelectrodes recently produced new surprises, revealing highly localized (hundreds of microns) areas of hypersynchronous activity, which have been termed "microseizures"[11] (Fig. 2). Along with the spatially restricted high-frequency interictal activity, these high resolution recordings reveal that the pathophysiology of the epileptic focus operates in volumes of cortex ~1 mm^2, which would contain neuronal populations of a few tens of thousands.

2. Experimental models of epilepsy

Experimental models of epilepsy go back much longer than 60 years. The freeze lesion was first reported by Openchowski[12] and intracerebral tetanus toxin by Roux and Borrel.[13] A substantial monograph has reviewed the range of experimental models of epilepsy recently,[14] but I will outline some of the major historic phases. The real growth in experimental epilepsy started with the use of strychnine in 1950s, and then a range of convulsants during 1960s including Metrazol, picrotoxin and penicillin. Depending on the way penicillin was administered it was possible to use it as a model of both focal and generalized epilepsies.[15] The 1950s also saw the use of metals and metal compounds to induce epileptic foci, including alumina (aluminium oxide) and cobalt, but interest has since moved on to other agents. Injection of iron salts into the brain (initially during 1980s) also establishes chronic epileptic foci, and can be considered as modelling iron salts left after the breakdown of red blood cells that had bled into brain tissue following head injury.

Genetic models of epilepsy go back to before 1950, when epileptic traits were identified in inbred strains of rats and mice. Selective breeding of both rats and mice, in particular for absence and tonic clonic seizures, grew from the late 1970s and is reviewed elsewhere.[14,16] The physiological phenotypes and the specific genes responsible for those epileptic traits are being unravelled at an increasing pace, particularly over the last 10–15 years.[17]

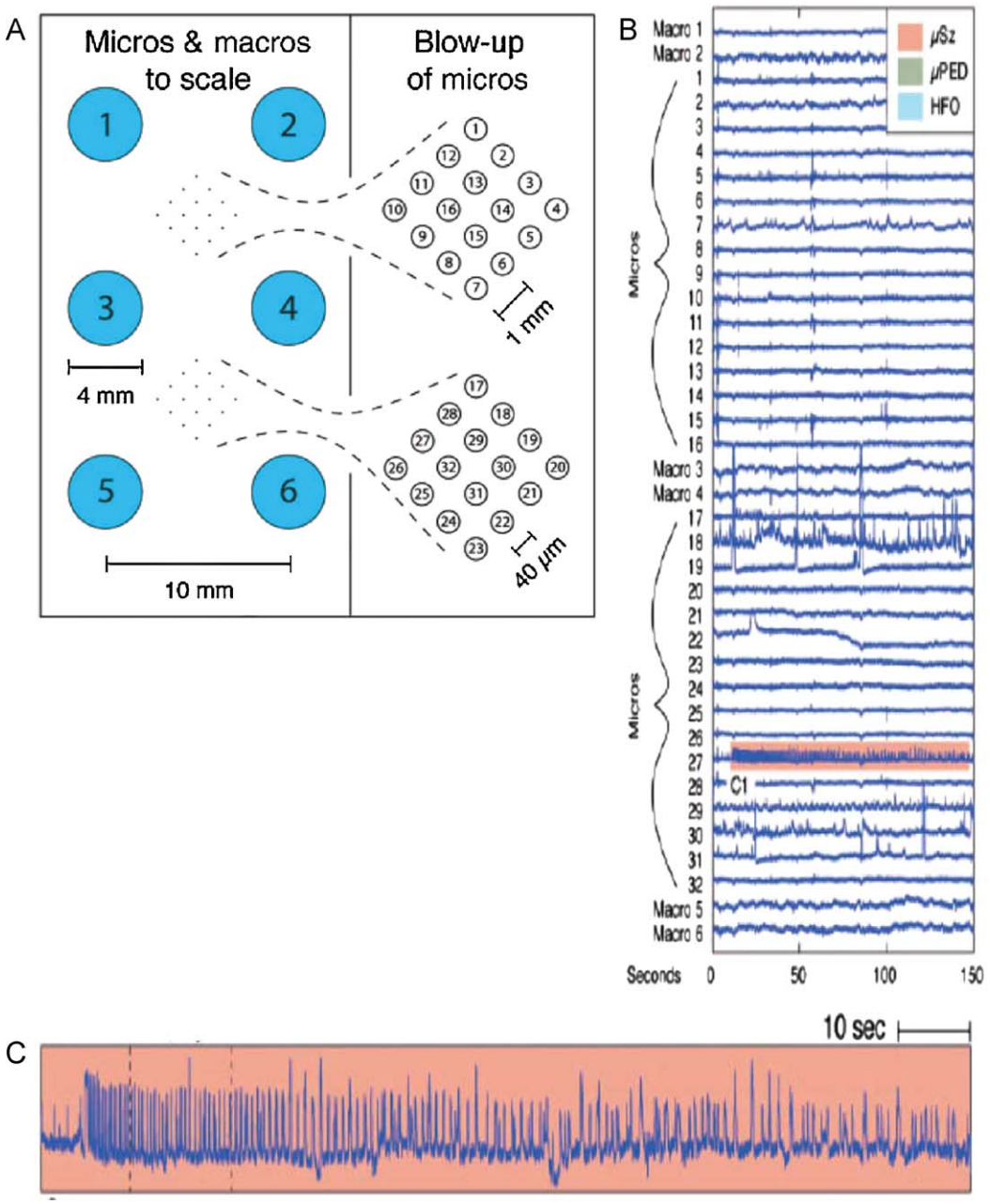

Fig. 2. Microseizures recorded in human brain using microelectrodes in subdural grid. (A) Novel subdural grids with microelectrodes (electrode surface areas 10^{-3} mm^2), as well as conventional macroelectrodes (~10 mm^2), produced new insights into activity in the epileptic brain. (B) Microseizure appears on a single microelectrode demonstrating its tight localization (pink/grey background). (C) Enlargement of microseizure. From Stead et al. (2010) "Microseizures and the spatiotemporal scales of human partial epilepsy", *Brain* **133**(9):2789–97 by permission of the authors, Oxford University Press and the Guarantors of *Brain*.

Brain slices cut a few hundred microns thick and maintained in an artificial cerebrospinal fluid played a pivotal role in experimental work on epilepsy. They had been used for biochemical studies of metabolism back in 1930s, but their advantages for epilepsy research[18] and particularly for cellular electrophysiology[19] became clear from the mid-1960s to early 1970s, when convulsant drugs, or changes in extracellular ions, were found to produce recognisably epileptic activity, in most cases resembling interictal discharges.[20] The brain slice preparation provided many of the advantages of the invertebrate and peripheral preparations, which were exploited in many of the critical early advances in fundamental neuroscience outlined below, but did so with mammalian brain tissue making it more directly relevant to epilepsy.

During the late 1960s the "kindling" model was identified[21]: electrical stimulation of certain brain regions, when repeated daily, leads to a progressive and permanent reduction in seizure threshold and increase in strength of seizure response. While it is hard to induce spontaneous seizures through kindling, it has been a very useful model of chronic epileptic foci, and of the process known as "epileptogenesis" that converts normal into epileptic brain tissue. Having models of chronic epilepsy is important when the goal is to treat epilepsy rather than symptomatic seizures in normal brain. One example of this is in the development of levetiracetam (Keppra), which failed in the standard anticonvulsant drug screening models in normal rodents but proved very effective in the chronic kindling model in rodents.[22]

Several experimental models of chronic temporal lobe epilepsy were developed during the late 1970s and early 1980s. The tetanus toxin model,[23,24] which we continue to use in my laboratory,[9,25] is characterised by a latent period of a week or two during which the neuronal circuits undergo epileptogenesis which leads to spontaneous seizures. In this model neuronal loss is minimal, except in a minority of cases which develop hippocampal sclerosis after the onset of spontaneous recurrent seizures. Several other chronic models rely on an initial very prolonged period of seizures known as "status epilepticus", which can be induced by one of: kainic acid,[26] pilocarpine[27] or prolonged electrical stimulation.[28] In all these cases epileptogenesis occurs during a latent period of a week or two, before spontaneous seizures start. The big difference in these models is the substantial neuronal loss caused by status epilepticus at the outset. Other models attempt to simulate clinical risk factors more directly, both acquired,[29] and inherited. However, genetic background is proving a major factor in finding epilepsy phenotypes in transgenic models of epilepsy-related mutations.[16]

The concept of the latent period in acquired, chronic models of epilepsy is fundamentally important. It models the clinical concept that acquired epilepsies usually occur many months or years after some kind of precipitating injury. Finding ways of treating the process of epileptogenesis, that links the initial injury to the development of seizures, is a major priority, as noted by an NIH Workshop in 2002.[30] Most antiepileptic drugs have minimal impact on epileptogenesis, so other classes of drugs are being tested.[31]

3. Fundamental neuroscience: advances in techniques

Our understanding of the fundamentals of the operation of the nervous system was at a critical stage 60 years ago. The field stood at the start of a rapid growth in insights into the functional properties of both individual neurons and the synapses through which they communicate. This progress went through a series of stages, reflecting advances in the technology available. Between the late 1940s and the mid-1990s electrophysiology was the technique that drove much of the progress. Improvements in electronics led to better amplifiers and to the control circuitry required for voltage clamp.[32] This recording method uses feedback electronics to clamp the membrane potential at a specific voltage, and measures the electric current needed to do so; these injected currents are equal and opposite to the currents flowing through ion channels in the membrane. Under normal conditions the voltage-dependent ion channels that are responsible for the special properties of neurons have permeabilities (or electric conductance) that depend both on voltage (obviously) and on time. Voltage clamp circuitry holds the membrane of the neuron at a set voltage to reveal the time dependence of the ion channels, while step changes of the controlled voltage reveals the voltage dependence. This technical advance led directly to the revolution in our understanding of the action potential developed by Hodgkin and Huxley at the start of this 60-year review period, a topic I will return to below.

The intracellular "sharp" glass microelectrode (filled with salt solution to provide electrical continuity between the inside of the cell and the recording apparatus) was first used to investigate synaptic transmission in work published in 1951.[33] This technical development made it possible to record membrane potentials from relatively small cellular components and has been a mainstay of cellular electrophysiology since then. Three decades later the "single-electrode voltage clamp" extended the voltage clamp method to cells that were too small to accommodate the two separate electrodes required for the original voltage clamp methods, by the trick of switching a single microelectrode rapidly between the two functions of recording voltage and injecting current to control that voltage.[34] The original intracellular glass microelectrode was made as sharp as possible, with a sub-micron tip in some cases, to make it possible to poke the tip through the cell membrane into the intracellular space without doing too much damage. The next major advance in cellular electrophysiology was made in 1970s and early 1980s with the advent of the patch clamp.[35] These larger ($\sim$1 μm tip diameter) glass pipettes were deliberately made smooth so that they could attach to the outside of the cell membrane, sticking so firmly that the leak resistance could be measured in giga-ohms. The relatively large diameter of the electrode provided low-resistance, low-noise recordings of the signal at the tip of the electrodes. Various tricks allowed recordings from inside cells ("whole-cell") or from the inside or outside of patches of membrane.[35]

Parallel advances in chemical and molecular sciences contributed to our growing understanding over the past 60 years. More specific drugs and toxins led to a deeper understanding of the diversity of both voltage-gated ion channels and synaptic receptors based on pharmacology. This approach had a long history with the classical distinction of the muscarinic and nicotinic classes of acetylcholine receptor in the autonomic nervous system, but when chemists started synthesizing derivatives of putative neurotransmitters the diversity of receptors made a considerable step change, for instance with the amino acids.[36] More recently molecular biology has impacted on neuroscience along with the other life sciences, perhaps starting with the cloning of nicotinic cholinergic receptors of the neuromuscular junction in the early 1980s,[37] an approach that rapidly expanded into other classes of channel and receptor and had a major impact on the classification of voltage dependent ion channels and synaptic receptors.[38,39]

Advances in imaging and molecular biology have led to the development of new methods to study and manipulate neurons, synapses and neuronal networks. Immunohistochemistry and in situ binding histochemistry have revolutionised our ability to localize specific proteins and RNA within neurons and other cells, allowing detection of molecular changes with subcellular precision, and of losses of specific classes of neuron in epileptic tissues (respectively starting starting late 1970s/early 1980s and 1990s). Confocal microscopy has greatly improved the resolution of microscopy of both fixed and living tissue (from 1990s). Progress in microscopy of the brain made a big step forward as a result of the discovery of green fluorescent protein and related compounds in luminescent jelly fish in 1960s and 1970s; now a huge range of these fluorescent proteins exists and can be used to label cells selectively, a great help in a tissue as complex as the brain. The new science of optogenetics (developed over the last 4–5 years) uses other specialised proteins, initially isolated from bacteria, which, when introduced into neuronal membranes, allows the use of light to control the excitability and activity of selected classes of neurons both in vivo and in vitro.[40] Non-invasive imaging, first computer tomography using X-rays and then Magnetic Resonance Imaging and fMRI became important clinical tools (respectively from 1970s to 1980s), and can be useful in experiments on chronic epilepsy models.

4. Intrinsic properties: voltage-gated ion channels

It was clear that neurons communicated through action potentials along their axons, and that those action potentials were electrical events (as suggested by the name) that depended on the movements of ions, particularly sodium and potassium, across the neuronal cell membrane. Technical advances, particularly in voltage clamp recordings, culminated in 1952 in the publication of four pivotal papers by Hodgkin, Huxley and Katz, back to back, in the *Journal of Physiology*, which defined in exquisite

detail the mechanisms of the action potential in the giant axon of the squid.[32,41–43] The precision of the quantitative analysis in these papers is remarkable; the use of mathematical methods and modelling, in conjunction with physiological measurement, anticipating the relatively recent growth in what has come to be called "systems biology" by many decades.

Advances in molecular biology have shown us in considerable detail the structure of ion channels, revealing an even greater complexity than could have been imagined 60 years ago. During 1980s ion channels started to be sequenced, with the electric eel's sodium channel published in 1984.[44] We now know of 9 molecularly distinct voltage gated sodium channels, four of which occur in the brain.[45] The genes for two of these channels have substantial numbers of mutations that have been linked to epilepsy: *SCN1A* has ~700 and *SCN2A* ~20.[45] (Epilepsy genetics is considered in more detail by M Rees elsewhere in this issue.) The sodium channel differs from many other voltage gated ion channels in that the pore that allows the ions to cross the membrane comprises a single large protein (the α subunit) instead of an assembly of several smaller subunits. It resembles some other ion channels in having an accessory protein, the β subunit, which plays a role in getting the sodium channels to the correct location on the neuronal surface also and controls the activity of the α subunit, especially the inactivation that follows each action potential.

The β subunit also has mutations that are linked with epilepsy, and was one of the first "epilepsy genes" identified, in 1998.[46] The GEFS+ (generalized epilepsy with febrile seizures+) was a new syndrome which describes families rather than individuals, and is based on successive generations having a variety of kinds of epilepsy, often including febrile seizures. This seems to represent a fundamental change in classifying epilepsy, away from diagnosing the specific epilepsy of the individual patient and towards identifying a genetic mutation shared by family members, but resulting in different clinical symptoms. This is one of the major changes over the past 60 years, and perhaps reflects our growing, although still modest, understanding of the causes of epilepsies.

Sodium channels can have abnormal epilepsy-related properties without mutations in their genes. Several acquired clinical and experimental epilepsies[47–49] have slower inactivation and weakened responses to antiepileptic drugs that normally target sodium channels, such as carbamazepine (sodium channels were identified as a target for certain AEDs ~1980).[50]

Voltage-gated calcium channels had long been known to be critical in cardiac function, and had been implicated in synaptic transmission, but were first studied in neurons in depth during 1970s.[51] Electrophysiology allowed the classification of calcium channels into low- and high-voltage activated, and together with pharmacology into 5 distinct types (L, P/Q, N, R, T); molecular biology revealed ten α (pore forming) subunits and four β subunits. The IUPHAR database on ion channels provides a comprehensive list of both voltage- and ligand-gated ion channels and gives some idea of the massive expansion of the numbers of ion channels identified in cell membranes (http://www.iuphar-db.org/DATABASE/).

The diversity of known voltage-gated potassium channels grew substantially in parallel to that for sodium and calcium, with over 40 distinct subunits on the IUPHAR database, and many more potassium channels in other classes including the Two-P, inwardly rectifying and calcium-activated. Mutations of some potassium channel genes are associated with epilepsy, for instance KCNQ2 and KCNQ3 (the "M-current") with benign familial neonatal seizures.[52] Other potassium channels change in chronic epileptic foci, for instance I_A which is down-regulated in experimental epileptic foci, allowing action potentials to invade further up dendrites.[53]

One final voltage-gated ion channel should be mentioned, I_h, which is an inward (depolarizing) current that was identified in the heart during 1970s but now is of growing interest in the brain.[54] It is altered in several experimental models of epilepsy and has an impact on neuronal excitability.[55,56]

5. Synaptic transmission

The term synapse was coined at the end of the 19th century by Sherrington. At the start of 1950s Fatt and Katz discovered the "quantal" mechanism of synaptic transmission at the neuromuscular junction,[33] laying the groundwork for the idea that transmitters are packaged into vesicles. Evidence for inhibitory synapses in the mammalian spinal cord first appeared in 1955,[57] but it was not clear at that stage that glycine was the transmitter.

Evidence for specific chemical neurotransmitters first appeared during 1920s and 1930s with the identification of acetylcholine and noradrenaline as neurotransmitters by Loewi, Dale, Feldberg and others. In 1950 acetylcholine was probably the only well-documented transmitter in the brain. The first papers on roles for noradrenaline and serotonin in the brain appeared in 1960s,[58,59] reflecting technical developments in recording from single neurons in the brain and in focal application of chemicals by microiontophoresis from micropipettes.

Evidence on the most common neurotransmitters in the brain, glutamate and GABA, did not start to accumulate until 1970s, following their identification in crustaceans during 1960s. Their effects on CNS neurons had been established in 1960.[36] The difficulty was demonstrating that the actions of the applied chemicals matched that of the endogenous neurotransmitter, which had to wait for the synthesis of agonist and antagonist drugs which really took off in 1970s.[60]

Now it is clear that there a large number of different neurotransmitters, including: acetylcholine, amino acids (glutamate, aspartate, GABA, glycine), monoamines (noradrenaline, dopamine, histamine, serotonin), purines such as adenosine, and an ever growing list of dozens of neuropeptides. Most of these neurotransmitters have multiple classes of receptor (as shown in the IUPHAR database). Acetylcholine receptors have been mentioned already: nicotinic receptors are ligand-gated ion channels, or ionotropic receptors, while muscarinic are metabotropic or G-protein coupled receptors, and so have slower electrophysiological actions because they depend on biochemical reactions to produce second messengers which open or close physically separate ion channels. The amino acids and some other classes of neurotransmitters also have both ionotropic and metabotropic receptors. The synthesis of selective agonists and antagonists let to subclassifications, in the case of ionotropic glutamate receptors into AMPA, kainate and NMDA. Molecular approaches further divided this classification into 18 different subunits that coassemble into complete glutamate receptors.[38] Ionotropic $GABA_A$ receptor subunits have a similar diversity.[61]

Two aspects of progress in neurotransmitters are particularly significant for epilepsy. The recognition of GABA as the major inhibitory transmitter of the brain led to attempts to synthesize GABA-enhancing drugs.[50] Benzodiazepine was known to be anticonvulsant before its effects on GABA receptors were identified. Gabapentin was developed as a drug acting at the $GABA_A$ receptor but turned out to act at a calcium channel subunit, and interestingly may be more effective in treating epileptogenesis than in preventing seizures in established epilepsy. Other GABA-targeted drugs include tiagabine, which slows GABA uptake and prolongs its synaptic action, and vigabatrin, which blocks the enzyme that degrades intracellular GABA. Both have some value as anticonvulsants, although vigabatrin can have serious complications on vision that need careful monitoring. The second aspect

was in the diversity of glutamate receptors.[38] NMDA receptor antagonists proved to be very effective anticonvulsants in animal models but proved to have undesirable cognitive side effects in humans (similar side effects occur in rodents but are hard to detect). One NMDA receptor antagonist, ketamine, has been used in treating the prolonged seizures of status epilepticus.[50] Drugs with selective actions on specific NMDA receptor subunits (e.g. felbamate) exist, and research continues on drugs that modulate the function of NMDA and other glutamate receptors.[50]

Space prevents inclusion of many other aspects of the basic mechanisms of epilepsies, but I will briefly mention two other issues. First, the extracellular environment is rather changeable, especially during hyperactivity, and it is clear that transient increases in extracellular potassium ions, and changes in other ions, and extracellular currents produced by neuronal activity, can all play roles in epileptic activity.[62] Second, evidence is accumulating that glia may play active roles in brain function and in epilepsy: their role in potassium homeostasis has been known since 1970s, in transmitter re-uptake since the mid 1970s, and in releasing glutamate and other "gliotransmitters" onto neurons since the mid 1990s.[63]

6. Circuits

The analysis of neuronal circuits starts in earnest with the classical work of Ramon y Cajal in the late 19th century and Sherrington in the early 20th. Eccles started to publish on the problem of relating cellular electrophysiology with the EEG in 1951, at the start of the review period,[64] progressing into dissecting the neuronal circuits of specific brain regions during 1960s.[65] Similar methods were soon applied to the pathophysiology of epileptic discharges, usually induced by convulsants such as penicillin.[66–69] An early outcome from this work was the discovery of the Paroxysmal Depolarization Shift (PDS; Fig. 3),[67] which is an abrupt, all-or-none depolarization which occurred during interictal discharges lasting a few hundred milliseconds (or tenths of seconds). Two alternative hypotheses emerged, that could broadly be categorized as the epileptic neuron versus the epileptic network. In practice it really is impossible to divorce the two: epilepsy is essentially a collective phenomenon that requires synchrony amongst large numbers of neurons, but the reason for the excessive synchrony and excitation can be abnormal intrinsic properties (the epileptic neuron), or abnormal circuitry (the epileptic network), or (in most cases?) both.

The epileptic neuron concept developed with the discovery that individual neurons could produce waveforms similar to the PDS, as a result of relatively slow voltage-dependent ion currents, such as calcium[70] or sodium currents that are much slower than typical action potentials.[71] While the PDS clearly had some properties of voltage-gated ion channels, it also had properties suggesting it was a giant excitatory postsynaptic potential (EPSP).[72–74] In practice it is impossible to produce a large EPSP without activating voltage-dependent inward (depolarizing) currents mediated by sodium and/or calcium ions, if those ion channels are present; in turn these relatively slow depolarizations can drive bursts of fast action potentials and thus amplify the synaptic outputs from each neuron.[75] The idea that the PDS is due to positive feedback through a synaptic network goes back to the early 1970s.[72] It took a combination of realistic computer simulations and experimental electrophysiology, mostly in brain slices in vitro, to demonstrate the importance in generating epileptic activity of both the intrinsic properties of individual neurons and the synaptic networks that connect them.[76–78] Essentially, recurrent collaterals between the pyramidal cells of the hippocampus allowed a "chain reaction" of excitation which recruited the whole population into the epileptic discharge. Even small networks of neurons have extremely

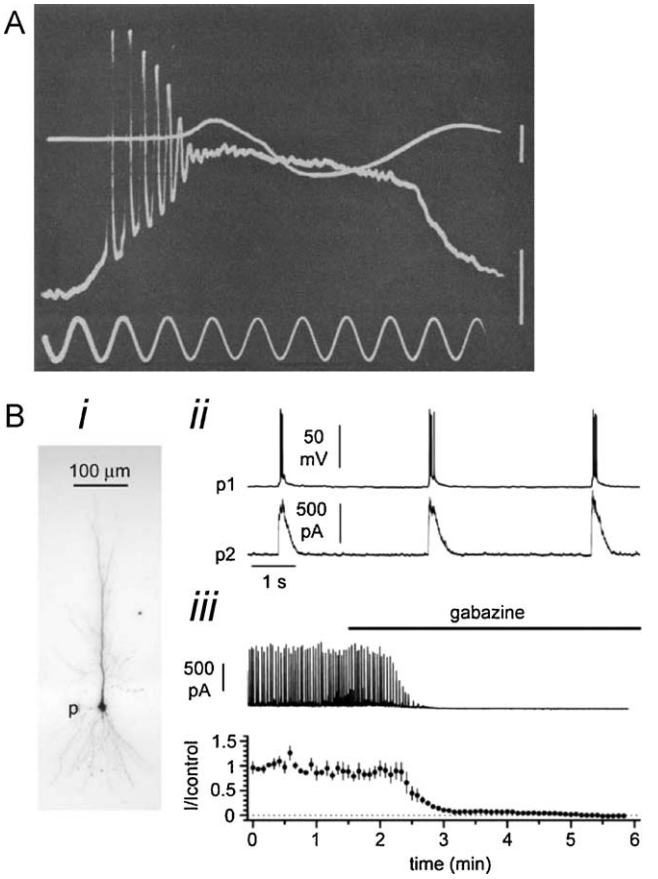

Fig. 3. Intracellular and whole cell recordings of epileptic activity. (A) One of the earliest published recordings of the PDS with superimposed brief action potentials recorded intracellularly from the cat neocortex in vivo following application of penicillin. The corresponding field potential (or depth EEG recording) is superimposed and a 100 Hz sinusoid provides a timescale. Voltage calibrations are 1 mV for the cortical surface recording (the smooth curve) and 10 mV for the intracellular recording. Recording was photographed from an oscilloscope screen, which can record much faster activity than a chart recorder, but remains an analogue method. (B) Whole cell recordings from a pair of hippocampal neurons in vitro in a slice exposed to 0 Mg^{2+} and 8.5 mM K^{+}. Pyramidal neurons are large and have extensive dendrites as shown by the cell labelled with biocytin during recording (*i*). Modern recording methods cope with the very fast depolarizations of action potentials during PDSs (*ii* p1), and allow the second pyramidal neuron to be voltage clamped to 0 mV to hide excitatory synaptic currents and to reveal inhibitory synaptic currents, which are prominent during the PDS in this model. These inhibitory synaptic currents were blocked by the $GABA_A$ receptor antagonist, gabazine (*iii*), confirming that they were IPSCs. *Panel A* is from Matsumoto and Ajmone-Marsan,[67] with permission, Elsevier Press. *Panel B* is from Marchionni and Maccaferri[80] with permission of the authors, John Wiley and Sons and The Physiological Society.

complex properties with many interdependent processes operating in parallel; simulating them quantitatively in synaptically connected model neurons provides a valuable tool to dissect the roles of specific ion channels or synaptic receptors, and most importantly to make experimentally testable predictions to determine whether the theoretical model is plausible.[20] An important result from this work was that epileptic discharges needed a critical mass, or size of neuronal population, to sustain epileptic activity: a few thousand pyramidal cells in the case of interictal discharges in vitro. The clinical equivalent may be the microseizures which are generated by a few tens of thousands of neurons.[11]

The networks responsible for epileptic activity have proved more a good deal more complex than the first cellular studies of epileptic activity suggested, for instance with the active participation of inhibitory interneurons in driving epileptic bursts.[79,80]

The cellular and local network properties of both clinical and experimental chronic epileptic foci can also be usefully studied in vitro, or rather ex vivo. While the in vitro preparation generally does not lend itself to the long range connections that participate in epileptic seizures in vivo, it does preserve the neurons and local circuits of the focus and can provide substantial insights into the cellular and synaptic mechanisms associated with the epileptogenic tissue. These studies have revealed complex changes in epileptic foci, which depend on the specific cell type and brain (sub-)region, and can include altered voltage-gated ion channels (see above), synaptic transmission, connectivity, selective neuronal losses and so on (reviewed in[20] Ref. 20). The multiplicity of changes found in most chronic epileptic foci will represent a complex mix of pro- and anti-epileptic mechanisms, and no doubt some epiphenomena, which are likely to progress over time, partly in response to repeated epileptic seizures.

7. Plasticity

Over the past few years it has become increasingly clear that the mature mammalian brain is a good deal more plastic, or modifiable, than was thought 60 years ago. The sprouting of new axons and synaptic connections in response to lesions in the mammalian forebrain was discovered in the late 1970s[81] and in response to epileptic activity in the late 1980s.[82] In these cases axons grow into territories that they do not normally innervate and create aberrant synaptic networks which may well play a role in epileptic foci. The production of new neurons in the mature mammalian brain, or ''adult neurogenesis'' was also considered impossible 60 years ago, and still meets resistance from some quarters. However there is good evidence for progenitor cells surviving in the subgranular zone of the hippocampal dentate gyrus and in the subventricular zone. The process of neurogenesis is complex, with multiple stages and fates for the cells, but at least some of them become new neurons. The rate of neurogenesis increases with epileptic activity, although the functional implications remain far from clear.[83]

The primary generalized epilepsy that is understood in some detail in terms of its basic mechanisms is absence. It differs from the focal epilepsies in the requirement for dynamic interaction between the thalamus and neocortex to produce the classic 3 per second (in humans) spike and wave discharge which is synchronized across most of the cortex. Conceptual models of the distributed circuits required to generate absence seizures have evolved over the 60 years covered by this review and are outlined in[84] Ref. 84. In short back in 1954 the idea was that a pacemaker in the thalamus projected diffusely to the cortex and thus produced the rhythmic spike and wave discharge, the ''centrencephalic'' theory. In the early 1990s the cellular mechanisms of the thalamic pacemaker were being resolved in detail. However the cortex is a necessary partner in absence epilepsy, and other teams postulated (in 1968) that it was an abnormal cortex which turned normal thalamic oscillations into absence seizures, the ''corticoreticular'' theory. This received support from an ingenious in vitro experiment in which thalamic slices were connected to an electronically simulated neocortex: changing the properties of the ''neocortex'' induced absence-like activity on the thalamus.[85] More recently (from 2002) several lines of evidence, both physiological and pharmacological,[84,86] lead to the theory that absence seizures are initiated by a cortical focus (perhaps complicating the idea that absence is a primary generalized epilepsy). The general idea is that the cortical focus drives the thalamus which produces a widespread drive to the cortex and that the two structures then both are necessary for sustaining the rhythmic discharge for the rest of the seizure.[84]

8. Conclusions

Sixty years ago many of the central features of the clinical electrophysiology of epilepsy and seizures were reasonably clear, and started to pose the questions briefly outlined in this review. At that time our understanding of the functional properties of neurons was accelerating rapidly with the development of improved recording methods. Since then advances in technology, first in electronics and computers, then in pharmacology and more recently in molecular biology, have revolutionised epilepsy research. The two fields of cellular and clinical electrophysiology have converged, in particular through the use of experimental models of epilepsy, validated where possible by evidence from clinical recordings or tissue samples. As in most fields, the more we learn the more we recognise the limits of our knowledge. Exciting new tools and concepts are coming on-line, for instance in: the optical control of specific neurons, the analysis of large datasets of neuronal recordings, and more refined recordings in vivo both clinically and experimentally.

The insights gained from studying basic mechanisms of epilepsy have contributed greatly to progress in brain research, but what does this progress mean for persons with epilepsy? Discoveries of the neurotransmitters of the brain led to targeted drug discovery, which has led to new drugs which have proved useful, if not always for the predicted reasons. Improvements in experimental models of epilepsies have helped discover treatments that have some selectivity for seizures generated by chronic epileptic tissue; they also have led to the recognition that the development of acquired epilepsies was a specific process, epileptogenesis, and that there is an unmet need for treatments to block or control it. Advances in recording techniques have led to more specific electrical signatures for epileptic foci, particularly fast oscillations, which have improved the precision of surgery. These are just a few examples of contributions of basic research on epilepsy over the past 60 years. They have not produced a ''magic bullet'' to cure epilepsy, but they have led to many significant improvements in treatment and diagnosis, and I am confident will continue to do so for many decades to come.

Conflict of interest statement

None declared.

References

1. Berger H. Über das elektroenkephalogramm des menschen. *Archives of Psychiatry* 1929;**87**:527–70.
2. Walter WG, Hodge RS, Last SL, Hutchinson JT. Discussion on recent advances in the EEG diagnosis of epilepsy. *Proceedings of the Royal Society of Medicine-London* 1951;**44**:315–21.
3. Flanigin H, Jasper H, Pertuiset B. A preliminary study of eeg and electrocorticographic findings in 104 patients with focal epilepsy. *Electroencephalography and Clinical Neurophysiology* 1950;**2**:229–30.
4. Worrell GA, Gardner AB, Stead SM, Hu SQ, Goerss S, Cascino GJ, et al. High-frequency oscillations in human temporal lobe: simultaneous microwire and clinical macroelectrode recordings. *Brain* 2008;**131**:928–37.
5. Allen PJ, Fish DR, Smith SJM. Very high-frequency rhythmic activity during SEEG suppression in frontal lobe epilepsy. *Electroencephalography and Clinical Neurophysiology* 1992;**82**:155–9.
6. Fisher RS, Webber WRS, Lesser RP, Arroyo S, Uematsu S. High-frequency EEG activity at the start of seizures. *Journal of Clinical Neurophysiology* 1992;**9**:441–8.
7. Bragin A, Engel JJ, Wilson CL, Fried I, Buzsáki G. High-frequency oscillations in human brain. *Hippocampus* 1999;**9**:137–42.
8. Bragin A, Engel JJ, Wilson CL, Fried I, Mathern GW. Hippocampal and entorhinal cortex high-frequency oscillations (100–500 Hz) in human epileptic brain and in kainic acid – treated rats with chronic seizures. *Epilepsia* 1999;**40**:127–37.
9. Jiruska P, Finnerty GT, Powell AD, Lofti N, Cmejla R, Jefferys JGR. High-frequency network activity in a model of non-lesional temporal lobe epilepsy. *Brain* 2010;**133**:1380–90.

10. Jiruska P, Csicsvari J, Powell AD, Fox JE, Chang WC, Vreugdenhil M, et al. High-frequency network activity, global increase in neuronal activity and synchrony expansion precede epileptic seizures in vitro. *Journal of Neuroscience* 2010;**30**:5690–701.
11. Schevon CA, Ng SK, Cappell J, Goodman RR, McKhann G, Waziri A, et al. Microphysiology of epileptiform activity in human neocortex. *Journal of Clinical Neurophysiology* 2008;**25**:321–30.
12. Openchowski P. Sur l'action localisée du froid, appliquée à la surface de la région corticale du cerveau. *Comptes Rendus des Séances et Mémoires de la Société de Biologie* 1883;**35**:38–43.
13. Roux E, Borrel A. Tétanos cérébral et immunité contre le tétanos. *Annals de l'Institut Pasteur* 1898;**4**:225–39.
14. Pitkänen A, Schwartzkroin PA, Moshé SL. *Models of seizures and epilepsy*. Elsevier; 2005.
15. Gloor P, Testa G. Generalized penicillin epilepsy in cat – effects of intracarotid and intravertebral pentylenetetrazol and amobarbital injections. *Electroencephalography and Clinical Neurophysiology* 1974;**36**:499–515.
16. Frankel WN. Genetics of complex neurological disease: challenges and opportunities for modeling epilepsy in mice and rats. *Trends in Genetics* 2009;**25**:361–7.
17. Noebels JL. The biology of epilepsy genes. *Annual Review of Neuroscience* 2003;**26**:599–625.
18. Yamamoto C. Intracellular study of seizure-like afterdischarges in thin hippocampal sections in vitro. *Experimental Neurology* 1972;**35**:154–64.
19. Yamamoto C, McIlwain H. Electrical activities in thin sections from mammalian brain maintained in chemically-defined media in vitro. *Journal of Neurochemistry* 1966;**13**:1333.
20. Jefferys JGR. Epilepsy in vitro: electrophysiology and computer modeling. In: Engel Jr J, Pedley TA, Aicardi J, Dichter MA, Moshé SL, editors. *Epilepsy: a comprehensive texbook*. Philadelphia: Lippincott, Willams & Wilkins; 2007 .
21. Goddard GV, McIntyre DC, Leech CK. A permanent change in brain function resulting from daily electrical stimulation. *Experimental Neurology* 1969;**25**:295–330.
22. Klitgaard H. Levetiracetam: the preclinical profile of a new class of antiepileptic drugs? *Epilepsia* 2001;**42**(Suppl. 4):13–8.
23. Mellanby J, George G, Robinson A, Thompson P. Epileptiform syndrome in rats produced by injecting tetanus toxin into the hippocampus. *Journal of Neurology Neurosurgery and Psychiatry* 1977;**40**:404–14.
24. Jefferys JGR, Walker MC. Tetanus toxin model of focal epilepsy. In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. *Models of seizures and epilepsy*. Amsterdam: Elsevier Academic Press; 2006. p. 407–14.
25. Vreugdenhil M, Hack SP, Draguhn A, Jefferys JGR. Tetanus toxin induces long-term changes in excitation and inhibition in the rat hippocampal CA1 area. *Neuroscience* 2002;**114**:983–94.
26. Dudek FE, Clark S, Williams PA, Grabenstatter HL. Kainate-induced status epilepticus: a chronic model of acquired epilepsy. In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. *Models of seizures and epilepsy*. Amsterdam: Elsevier; 2006. p. 415–32.
27. Cavalheiro EA, Naffah-Mazzacoratti MG, Mello LE, Leite JP. The pilocarpine model of seizures. In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. *Models of seizures and epilepsy*. Amsterdam: Elsevier; 2006. p. 433–48.
28. Mazarati AM, Thompson KW, Suchomelova L, Sankar R, Shirasaka Y, Nissinen J, et al. Staus epilepticus: electrical stimulation methods. In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. *Models of seizures and epilepsy*. Amsterdam: Elsevier; 2006. p. 449–64.
29. Pitkänen A, Kharatishvili I, Nissinen J, McIntosh TK. Posttraumatic epilepsy induced by lateral fluid-percussion brain injury in rats. In: Pitkänen A, Schwartzkroin PA, Moshé SL, editors. *Models of seizures and epilepsy*. Amsterdam: Elservier; 2006. p. 465–76.
30. Stables JP, Bertram EH, White H, Coulter DA, Dichter MA, Jacobs MP, et al. Models for epilepsy and epileptogenesis: report from the NIH workshop, Bethesda, Maryland. *Epilepsia* 2002;**43**:1410–20.
31. Pitkanen A. Therapeutic approaches to epileptogenesis-hope on the horizon. *Epilepsia* 2010;**51**:2–17.
32. Hodgkin AL, Huxley AF, Katz B. Measurement of current–voltage relations in the membrane of the giant axon of Loligo. *Journal of Physiology* 1952;**116**:424–48.
33. Fatt P, Katz B. An analysis of the end-plate potential recorded with an intracellular electrode. *Journal of Physiology-London* 1951;**115**:320–70.
34. Merickel M. Design of a single electrode voltage clamp. *Journal of Neuroscience Methods* 1980;**2**:87–96.
35. Sakmann B, Neher E. Patch clamp techniques for studying ionic channels in excitable-membranes. *Annual Review of Physiology* 1984;**46**:455–72.
36. Curtis DR, Watkins JC. The excitation and depression of spinal neurones by structurally related amino acids. *Journal of Neurochemistry* 1960;**6**:117–41.
37. Giraudat J, Devillersthiery A, Auffray C, Rougeon F, Changeux JP. Identification of a cDNA clone coding for the acetylcholine binding subunit of torpedo-marmorata acetylcholine-receptor. *EMBO Journal* 1982;**1**:713–7.
38. Traynelis SF, Wollmuth LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacological Reviews* 2010;**62**:405–96.
39. Lodge D. The history of the pharmacology and cloning of ionotropic glutamate receptors and the development of idiosyncratic nomenclature. *Neuropharmacology* 2009;**56**:6–21.
40. Szobota S, Isacoff EY. *Optical control of neuronal activity*. Palo alto: Annual Reviews; 2010.
41. Hodgkin AL, Huxley AF. Currents carried by sodium and potassium ions through the membrane of the giant axon of Loligo. *Journal of Physiology* 1952;**116**:449–72.
42. Hodgkin AL, Huxley AF. The components of membrane conductance in the giant axon of Loligo. *Journal of Physiology* 1952;**116**:473–96.
43. Hodgkin AL, Huxley AF. The dual effect of membrane potential on sodium conductance in the giant axon of Loligo. *Journal of Physiology* 1952;**116**:497–506.
44. Noda M, Shimizu S, Tanabe T, Takai T, Kayano T, Ikeda T, et al. Primary structure of *Electrophorus-electricus* sodium-channel deduced from cDNA sequence. *Nature* 1984;**312**:121–7.
45. Meisler MH, O'Brien JE, Sharkey LM. Sodium channel gene family: epilepsy mutations, gene interactions and modifier effects. *Journal of Physiology-London* 2010;**588**:1841–8.
46. Wallace RH, Wang DW, Singh R, Scheffer IE, George Jr AL, Phillips HA, et al. Febrile seizures and generalized epilepsy associated with a mutation in the Na^+-channel β1 subunit gene SCN1B. *Nature Genetics* 1998;**19**:366–70.
47. Vreugdenhil M, Wadman WJ. Modulation of sodium currents in rat CA1 neurons by carbamazepine and valproate after kindling epileptogenesis. *Epilepsia* 1999;**40**:1512–22.
48. Vreugdenhil M, Hoogland G, van Veelen CW, Wadman WJ. Persistent sodium current in subicular neurons isolated from patients with temporal lobe epilepsy. *European Journal of Neuroscience* 2004;**19**:2769–78.
49. Ellerkmann RK, Remy S, Chen J, Sochivko D, Elger CE, Urban BW, et al. Molecular and functional changes in voltage-dependent Na(+) channels following pilocarpine-induced status epilepticus in rat dentate granule cells. *Neuroscience* 2003;**119**:323–33.
50. Rogawski MA, Loscher W. The neurobiology of antiepileptic drugs. *Nature Reviews Neuroscience* 2004;**5**:553–64.
51. Standen NB. Properties of a calcium channel in snail neurons. *Nature* 1974;**250**:340–2.
52. Kullmann DM. The neuronal channelopathies. *Brain* 2002;**125**:1177–95.
53. Bernard C, Anderson A, Becker A, Poolos NP, Beck H, Johnston D. Acquired dendritic channelopathy in temporal lobe epilepsy. *Science* 2004;**305**:532–5.
54. Baruscotti M, Bottelli G, Milanesi R, DiFrancesco JC, DiFrancesco D. HCN-related channelopathies. *Pflugers Archiv European Journal of Physiology* 2010;**460**:405–15.
55. Huang Z, Walker MC, Shah MM. Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. *Journal of Neuroscience* 2009;**29**:10979–88.
56. Jung S, Jones TD, Lugo JN, Sheerin AH, Miller JW, D'Ambrosio R, et al. Progressive dendritic HCN channelopathy during epileptogenesis in the rat pilocarpine model of epilepsy. *Journal of Neuroscience* 2007;**27**:13012–21.
57. Coombs JS, Eccles JC, Fatt P. The specific ionic conductances and the ionic movements across the motoneuronal membrane that produce the inhibitory post-synaptic potential. *Journal of Physiology-London* 1955;**130**:326–73.
58. Bradley PB, Wolstencroft JH. Excitation and inhibition of brain-stem neurones by noradrenaline and acetylcholine. *Nature* 1962;**196**:840.
59. Bloom FE, Costa E, Salmoiraghi GC. Analysis of individual rabbit olfactory bulb neuron responses to microelectrophoresis of acetylcholine norepinephrine + -serotonin synergists + antagonists. *Journal of Pharmacology and Experimental Therapeutics* 1964;**146**:16.
60. Davies J, Watkins JC. Selective antagonism of amino acid-induced and synaptic excitation in the cat spinal cord. *Journal of Physiology* 1979;**297**:621–35.
61. Whiting PJ. The $GABA_A$ receptor gene family: new opportunities for drug development. *Current Opinion in Drug Discovery and Development* 2003;**6**:648–57.
62. Jefferys JGR. Non-synaptic modulation of neuronal activity in the brain: electric currents and extracellular ions. *Physiological Reviews* 1995;**75**:689–723.
63. Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annual Review of Physiology* 2010;**72**:335–55.
64. Eccles JC. Interpretation of action potentials evoked in the cerebral cortex. *Electroencephalography and Clinical Neurophysiology* 1951;**3**:449–64.
65. Andersen P, Eccles JC, Loyning Y. Recurrent inhibition in the hippocampus with identification of the inhibitory cell and its synapses. *Nature* 1963;**198**:540–2.
66. Kandel ER, Spencer WA. Excitation and inhibition of single pyramidal cells during hippocampal seizure. *Experimental Neurology* 1961;**4**:162.
67. Matsumoto H, Ajmone-Marsan C. Cortical cellular phenomena in experimental epilepsy: interictal manifestations. *Experimental Neurology* 1964;**9**:286–304.
68. Prince DA. The depolarization shift in "epileptic" neurons. *Experimental Neurology* 1968;**21**:467–85.
69. Schwartzkroin PA, Prince DA. Penicillin-induced epileptiform activity in the hippocampal in vitro preparation. *Annals of Neurology* 1977;**1**:463–9.
70. Schwartzkroin PA, Slawsky M. Probable calcium spikes in hippocampal neurons. *Brain Research* 1977;**133**:157–61.
71. Connors BW, Gutnick MJ, Prince DA. Electrophysiological properties of neocortical neurons in vitro. *Journal of Neurophysiology* 1982;**48**:1302–20.
72. Ayala GF, Dichter M, Gumnit RJ, Matsumoto H, Spencer WA. Genesis of epileptic interictal spikes. New knowledge of cortical feedback systems suggests a neurophysiological explanation of brief paroxysms. *Brain Research* 1973;**52**:1–17.
73. Johnston D, Brown TH. Giant synaptic potential hypothesis for epileptiform activity. *Science* 1981;**211**:294–7.
74. Gutnick MJ, Connors BW, Prince DA. Mechanisms of neocortical epileptogenesis in vitro. *Journal of Neurophysiology* 1982;**48**:1321–35.
75. Traub RD, Wong RKS. Cellular mechanism of neuronal synchronization in epilepsy. *Science* 1982;**216**:745–7.
76. Traub RD, Miles R, Jefferys JGR. Synaptic and intrinsic conductances shape picrotoxin-induced synchronized after-discharges in the guinea-pig hippocampal slice. *Journal of Physiology* 1993;**461**:525–47.

77. Traub RD, Jefferys JGR, Whittington MA. Enhanced NMDA conductance can account for epileptiform activity induced by low Mg^{2+} in the rat hippocampal slice. *Journal of Physiology* 1994;**478**:379–93.
78. Traub RD, Borck C, Colling SB, Jefferys JGR. On the structure of ictal events *in vitro*. *Epilepsia* 1996;**37**:879–91.
79. Avoli M, D'Antuono M, Louvel J, Kohling R, Biagini G, Pumain R, et al. Network and pharmacological mechanisms leading to epileptiform synchronization in the limbic system in vitro. *Progress in Neurobiology* 2002;**68**:167–207.
80. Marchionni I, Maccaferri G. Quantitative dynamics and spatial profile of perisomatic GABAergic input during epileptiform synchronization in the CA1 hippocampus. *Journal of Physiology-London* 2009;**587**:5691–708.
81. Lynch G, Gall C, Cotman C. Temporal parameters of axon sprouting in brain of adult rat. *Experimental Neurology* 1977;**54**:179–83.
82. Sutula T, He XX, Cavazos J, Scott G. Synaptic reorganization in the hippocampus induced by abnormal functional activity. *Science* 1988;**239**:1147–50.
83. Scharfman HE, McCloskey DP. Postnatal neurogenesis as a therapeutic target in temporal lobe epilepsy. *Epilepsy Research* 2009;**85**:150–61.
84. Meeren H, van Luijtelaar G, da Silva FL, Coenen A. Evolving concepts on the pathophysiology of absence seizures – the cortical focus theory. *Archives of Neurology* 2005;**62**:371–6.
85. Bal T, Debay D, Destexhe A. Cortical feedback controls the frequency and synchrony of oscillations in the visual thalamus. *Journal of Neuroscience* 2000;**20**:7478–88.
86. Manning JP, Richards DA, Leresche N, Crunelli V, Bowery NG. Cortical-area specific block of genetically determined absence seizures by ethosuximide. *Neuroscience* 2004;**123**:5–9.