OXFORD TEXTBOOKS IN CLINICAL NEUROLOGY

Oxford Textbook of Epilepsy and Epileptic Seizures

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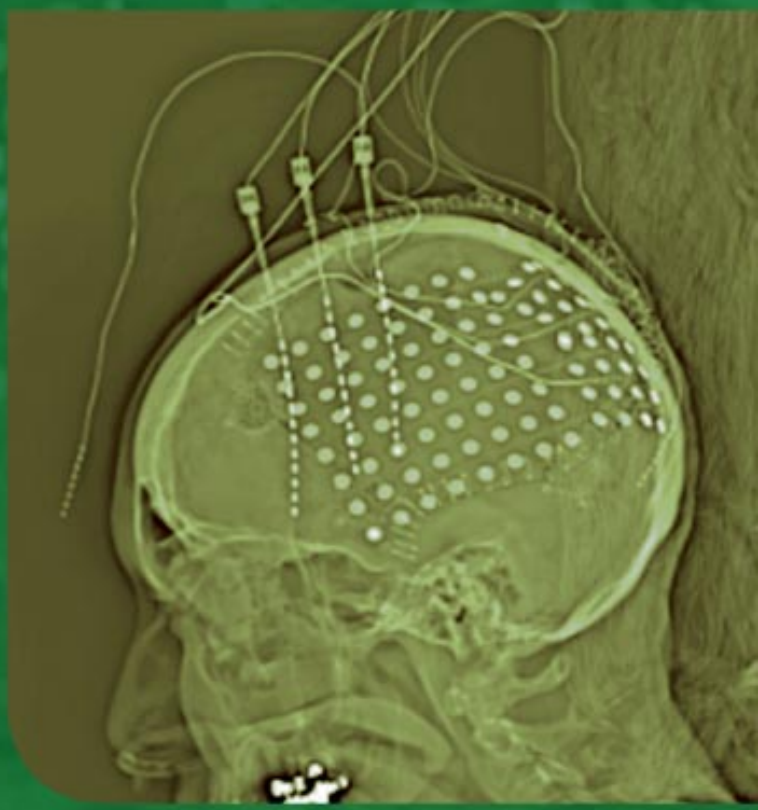

Series Editor
Christopher Kennard

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List of Abbreviations

5-HT	serotonin
AED	antiepileptic drug
ACRM	American Congress of Rehabilitation Medicine
ACTH	adrenocorticotropic hormone
ADA	acute drug administration
ADNFLE	autosomal dominant nocturnal frontal lobe epilepsy
ADTLE	autosomal dominant temporal lobe epilepsy
AMACR	alpha-methylacyl-CoA racemase
AMPA	alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
AMT	alpha-methyl-L-tryptophan
AN	anterior nucleus
AVM	arteriovenous malformation
BECTS	benign epilepsy of childhood with centrotemporal spikes
BFNC	benign familial neonatal convulsion
BFNIS	benign familial neonatal-infantile seizures
BFNS	benign familial neonatal seizures
BFPP	bilateral frontoparietal polymicrogyria
BIRD	brief ictal rhythmic electrographic discharge
BOLD	blood-oxygen level dependent
BPP	bilateral perisylvian polymicrogyria
Ca^{2+}	calcium
CAE	childhood absence epilepsy
CBZ	carbamazepine
CFR	case fatality rate
CGH	comparative genomic hybridization
CI	confidence interval
CK	creatine kinase
Cl^{-}	chloride
CM	centromedian
CNS	central nervous system
CNV	copy number variant
CPSE	complex partial status epilepticus
CRH	corticotropin-releasing hormone
CS	cortical stimulation
CSF	cerebrospinal fluid
CSH	carotid sinus hypersensitivity
CT	computed tomography
CVD	cerebrovascular disease
CYP	cytochrome P450
DALY	disability-adjusted life year
DAS	dialysis-associated seizures
DBS	deep brain stimulation
DDS	dialysis disequilibrium syndrome
DMPA	depot medroxyprogesterone acetate
DNET	dysembryoplastic neuroepithelial tumour
DTI	diffusion tensor imaging
EAAT	excitatory amino acid transporter
ECG	electrocardiography
ECoG	electrocortigogram
EDS	excessive daytime sleepiness
EE	epileptic encephalopathy
EEG	electroencephalogram
EIEE	early infantile epileptic encephalopathy
EME	early myoclonic epilepsy
EMG	electromyography
EPO	erythropoietin
EPSP	excitatory postsynaptic potential
ESES	electrical status epilepticus during sleep
ESI	electroencephalogram source imaging
ESM	ethosuximide
ESRD	end-stage renal disease
FCD	focal cortical dysplasia
FDA	Food and Drug Administration
FDG	fluro-2-deoxy-D-glucose
FER	facial emotion recognition
FLAIR	fluid attenuated inversion recovery
FLE	frontal lobe epilepsy
fMRI	functional magnetic resonance imaging
FS	febrile seizure
GABA	gamma-aminobutyric acid
GCD	granule cell dispersion
GCSE	generalized convulsive status epilepticus
GEFS+	generalized epilepsy with febrile seizure plus
GTCS	generalized tonic–clonic seizure
GVG	vigabatrin
H&E	haematoxylin and eosin
H^{+}	hydrogen
HD	haemodialysis
HFO	high-frequency oscillation
HIE	hypoxic-ischaemic encephalopathy

HP	hypothalamic–pituitary
HRF	haemodynamic response function
HS	hippocampal sclerosis
HSE	herpes simplex encephalitis
HSV-1	herpes simplex virus type-1
HUS	haemolytic uraemic syndrome
HWE	hot-water epilepsy
ICD-10	International Statistical Classification of Diseases and Related Health Problems, 10th revision
ICOE	idiopathic childhood occipital epilepsy
ICU	intensive care unit
IDD	interictal dysphoric disorder
IED	interictal epileptiform discharge
IGE	idiopathic generalized epilepsy
IHD	ischaemic heart disease
ILAE	International League Against Epilepsy
IPOLE	idiopathic photosensitive occipital lobe epilepsy
IPSP	inhibitory postsynaptic potential
IQ	intelligence quotient
IS	infantile spasms
ISSX	X-linked infantile spasms syndrome
JAE	juvenile absence epilepsy
JME	juvenile myoclonic epilepsy
K^+	potassium
LD	learning disability
LGS	Lennox–Gastaut syndrome
LIS	lissencephaly
LTG	lamotrigine
MCD	malformation of cortical development
MCM	major congenital malformation
MEG	magnetoencephalography
MERRF	myoclonus epilepsy with ragged red fibres
MHT	multiple hippocampal transection
MMIGS	multimodality image-guided surgery
MPRAGE	magnetization-prepared rapid acquisition with gradient echo
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MSI	magnetic source imaging
MST	multiple subpial transection
MTLE	mesial temporal lobe epilepsy
NAA	N-acetylaspartate
NALD	neonatal adrenoleucodystrophy
NCL	neuronal ceroid-lipofuscinosis
NES	non-epileptic seizures
NFLE	nocturnal frontal lobe epilepsy
NICE	National Institute for Health and Clinical Excellence
NMDA	N-methyl-D-aspartate
NREM	non-rapid eye movement
OR	odds ratio
pCO_2	partial pressure of carbon dioxide
PCOS	polycystic ovarian syndrome
PD	peritoneal dialysis
PDS	paroxysmal depolarizing shift
PEHO	progressive encephalopathy with oedema, hypsarrhythmia, and optic atrophy
PET	positron emission tomography
PHT	phenytoin
PMG	polymicrogyria
PMR	proportional mortality ratio
PNES	psychogenic non-epileptic seizure
PNH	periventricular nodular heterotopia
pO_2	partial pressure of oxygen
PRE	primary reading epilepsy
PRES	posterior reversible encephalopathy syndrome
PRIS	propofol infusion syndrome
PSW	polyspike wave
QOL	quality of life
RCDP	rhizomelic chondrodysplasia punctata
REM	rapid eye movement
RNS	responsive neurostimulator system
RR	relative risk
SBH	subcortical band heterotopia
SE	status epilepticus
SIGN	Scottish Intercollegiate Guidelines Network
SISCOM	subtraction ictal SPECT coregistered to MRI
SLE	systemic lupus erythematosus
SMA	supplementary motor area
SMEI	severe myoclonic epilepsy of infancy
SMR	standardized mortality ratio
SPC	Summary of Product Characteristics
SPECT	single-positron emission computed tomography
SpGR	spoiled gradient recalled
SSRI	selective serotonin reuptake inhibitor
SUDEP	sudden unexpected death in epilepsy
SW	spike wave
SWI	susceptibility-weighted image
TCI	tricyclic antidepressant drug
TEA	transient epileptic amnesia
TGA	transient global amnesia
TIA	transient ischaemic attack
TLE	temporal lobe epilepsy
TLOC	transient loss of consciousness
TLS	temporal lobe sclerosis
TPM	topiramate
TRH	thyrotropin-releasing hormone
TSC	tuberous sclerosis complex
UGT	uridine diphosphate glucuronyltransferase
VBM	voxel-based morphometry
VEM	video electroencephalography monitoring
VGLUT	vesicular glutamate transporter
VLCFA	very long chain fatty acid
VNS	vagus nerve stimulation
VPA	valproate
WoG	week of gestation
XLAG	X-linked lissencephaly with absent corpus callosum and ambiguous genitalia
XLMR	X-linked mental retardation

CHAPTER 1

Neurophysiology of Epilepsy

Florin Amzica

The epileptic syndrome encompasses a complex pathological reality with numerous and various aetiological origins. Each of these induces as many structural and functional alterations at the cellular and molecular level. Despite this rich diversity that eventually triggers epileptic or epileptic-like paroxysms, the electrical discharges, and ensuing behavioural correlates, during seizures share a surprisingly reduced variety of patterns, suggesting some common underlying mechanisms.

Both experimental and clinical evidence show that several types of epileptic seizures preferentially occur during slow-wave sleep. This is the case of absence (petit mal) seizures with spike-wave (SW) complexes at 2–4 Hz that appear during drowsiness or light sleep in children (1) and behaving monkeys (2), and in association with sleep spindles in stage 2 of human sleep (3). The Lennox–Gastaut syndrome (4–6), which is accompanied by SW and polyspike-wave (PSW) complexes that recur at relatively lower frequencies (generally 1.5–2.5 Hz) and are associated with episodes of fast runs (10–15 Hz), is also prevalently activated during slow-wave sleep (5, 7).

The behaviour of neurons during paroxysmal discharges has been extensively studied. However, for reasons that will become clear in this chapter, the understanding of the neurophysiological mechanisms of epilepsies has not progressed as much as one would have expected from the bulk of means that were invested in the sole study of neurons. Some pioneering studies have repeatedly acknowledged the abnormal activity of glial cells during epileptic events (8–11), and only few recent studies have addressed the interaction between neurons and glia. Therefore I will emphasize the contribution of glial cells to the triggering of the seizures and to the oscillating process accompanying them especially because the neuronal activity is modulated by several ionic species (mainly potassium (K^+), calcium (Ca^{2+}), and hydrogen (H^+)) of the extracellular space.

The analysis of the mechanisms underlying the occurrence of nocturnal SW seizures has to take into consideration the precipitating factors acting before and throughout the paroxysmal episode, as well as the (minimal) critical structures required to generate SW discharges. Interestingly, the same elements that rule and promote the behaviour of complex neuron, glia, and extracellular space loops during slow-wave sleep may also contribute to the triggering of pathological states such as seizures. The transition from normal (sleep) to abnormal (paroxysms) states may rely on a multitude of factors ranging from genetic causes (12) to imbalances of ionic concentrations (13) or the abnormal recruitment of cellular aggregates (14–16). As a precipitating factor, one may also consider the irregularity of respiration during the initial stages of sleep (17). Most of the time seizures result from a combination of factors. However, a basal condition that is already present in all sleeping patients is the presence of coherent oscillations during slow-wave sleep. Thus I will start with a brief survey of the mechanisms underlying slow sleep oscillations.

Mechanisms of slow sleep oscillations

The slow-wave sleep is dominated by a coherent cortical activity that imposes a slow (less than but close to 1 Hz) oscillation to both neurons (18) and glial cells (19) belonging to cortical networks (Fig. 1.1A). At the neuronal level, the slow oscillation is made of alternating steady depolarizations and hyperpolarizations. The depolarizing phase is made of synaptic events (excitatory as well as inhibitory) and intrinsic membrane currents (18). In parallel with the neuronal depolarization phase, glial cells are also depolarized, but at a slower pace that reflects the variation of extracellular K^+ concentration (20). The persistent neuronal depolarization is associated with a progressive depletion of extracellular Ca^{2+} (Fig. 1.1B) (21), which in turn initiates a disfacilitation process (22, 21) leading to the onset of the persistent hyperpolarizing phase of the slow oscillation.

During the hyperpolarizing phase, no neurons are active, their membrane potential reaching values much more hyperpolarized than the threshold for action potential generation. In parallel, glial cells slowly repolarize, as the extracellular K^+ concentrations return to control values (20). Extracellular Ca^{2+} is restored through ionic pumps and reaches concentrations compatible with efficient synaptic transmission (Fig. 1.1B) (21).

Several lines of evidence support the idea that the slow oscillation is generated within cortical networks. First, the slow oscillation survives in the cortex after extensive thalamic lesions and in preparations with high brainstem transactions (23). Second, the slow oscillation is absent from the thalamus of decorticated

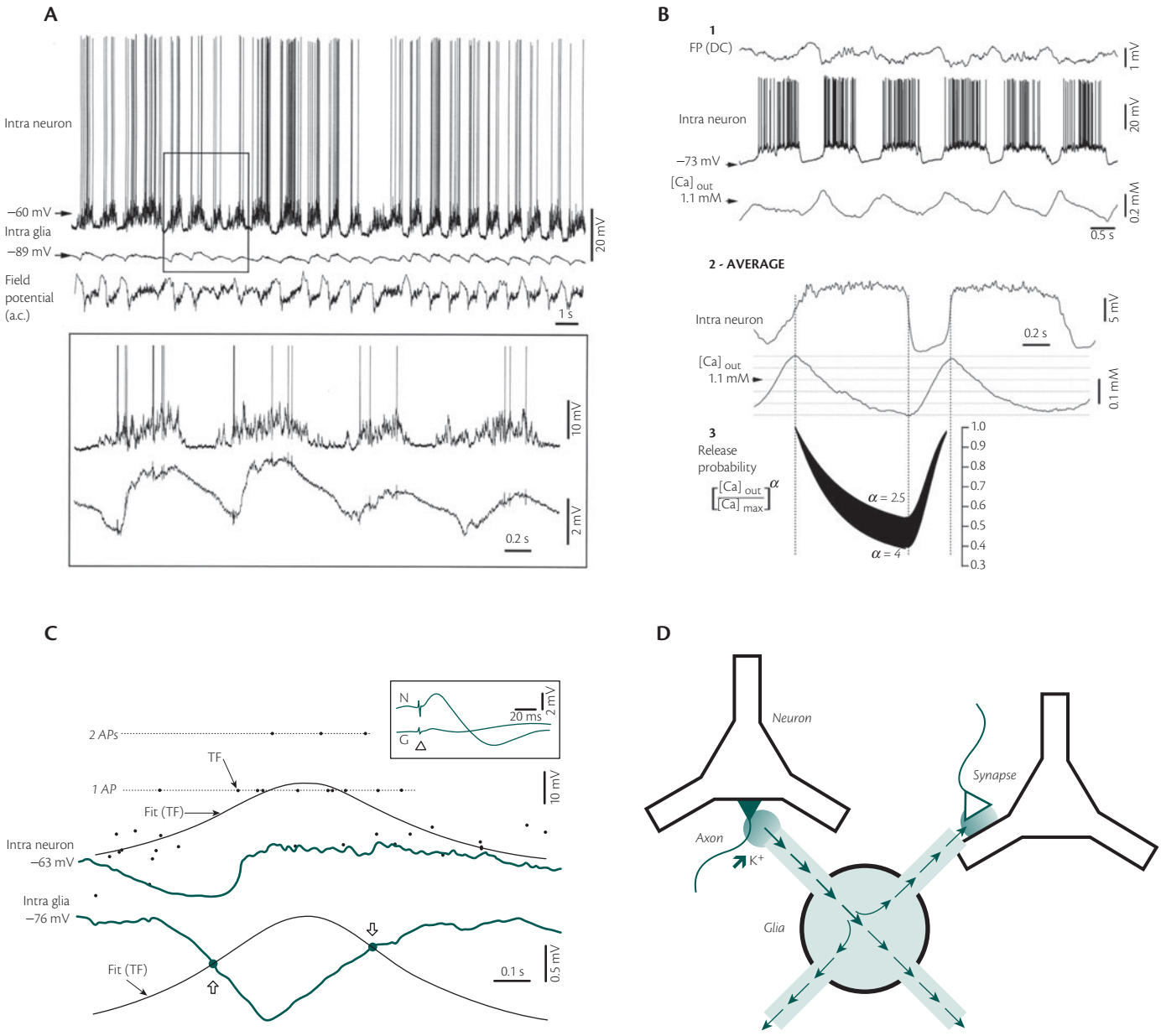

Fig. 1.1 Mechanisms of genesis of the slow (<1 Hz) sleep oscillation. A) Recordings from cortical areas 5 (neuron and depth field potential) and 7 (glia), displaying periodic depolarizations of the neuronal membrane, made of synaptic potentials and triggering somatic action potentials. Depolarizations are followed by hyperpolarizations with reduced synaptic noise. In synchrony with the onset of the neuronal depolarization, the glia depolarizes. The end of the depolarization coincides with the onset of the neuronal hyperpolarization. Note that the amplitude of the glial depolarization increases as the slow oscillation in the neuron becomes more rhythmic and of higher amplitude (*top right*; see also detail in box). In this and the following figures, polarities always are depicted with positivity upward. B) Relationships between intracellular membrane potential, extracellular Ca^{2+} ($(Ca)_{out}$) and estimated synaptic release probability. B1) Alternating neuronal depolarizations and hyperpolarization corresponding to negative and positive field potential waves, respectively. $(Ca)_{out}$ dropped by about 0.25 mM during the depolarizing phase reaching a minimum just before the onset of the hyperpolarization. Then, $(Ca)_{out}$ rose back until the beginning of the next cycle. B2) Thirty oscillatory cycles were averaged (neuronal spikes clipped) after being extracted around the onset of the neuronal depolarization. The vertical dotted lines tentatively indicate the boundaries of the two phases of the slow oscillation. B3) The transmitter release probability is contained within the black area between the estimations with α = 2.5 (90) and α = 4 (91) exponents. The release probability dropped to around 50% before the onset of the hyperpolarizing phase. C) Neuronal excitability during the oscillatory cycle of the slow oscillation. The *thick traces* represent the wave-triggered averages of a neuron–glia pair recorded intracellularly. The *dots* constitute the excitability curve and correspond to the maximum of the voltage values reached by the synaptic response elicited with a cortical volley. Artificial levels were attributed when the synaptic potential was crowned by 1 or 2 action potentials (*dotted horizontal lines* for *1AP* and *2APs*). The *thin curve* (*fit*) is for the Lorentzian fit of the excitability score. In the glial trace, the fitting curve is replicated in order to suggest the relative points of intersection with the intraglial potential (*open arrows*). Maximum neuronal excitability is achieved during the trough of the glial oscillation. The *inset* provides the shape of the intracellular responses evoked by the cortical shock (*triangle*) in the neuron (*N*) and glia (*G*). The former display and EPSP–IPSP sequence, while the latter contain a very slight initial depolarization (~0.3 mV). D) Schematic functioning of the spatial buffering during the slow oscillation. During the depolarizing phase of the slow oscillation, small and local increases of extracellular K^+ (*circle*) may occur in the proximity of the axon hillock. The neighbouring glial cells take up the K^+ and redistribute it at sites where the values of extracellular K^+ are normal. These locations may be close to a synapse, in which case the synaptic efficiency may be modulated, or close to a neuronal membrane such to modify the excitability of that membrane. Modified from: A (19), B (21), C (28), and D (20).

animals (24), while it is expressed by thalamic reticular and thalamocortical cells in intact animals (25). Moreover, the slow oscillation has been recently replicated in cortical slices (26). Thus, the slow oscillation is generated within cortical neurons.

The mechanisms underlying this oscillatory behaviour are partly elucidated at the neuronal level. It was shown that the slow oscillations were expressed by various cortical areas (18) and by virtually all types of neurons (27). The slow oscillation is marked by a variable neuronal responsiveness that is maximal during the first third of the depolarizing phase and progressively decays during the rest of the depolarizing phase (Fig. 1.1C) (28). Having demonstrated that the slow oscillation is synchronized between all cortical areas (29), we have sought for the mechanisms underlying this coherent behaviour and found that, beyond the essential role played by synaptic intracortical projections (30), glial cells, through local K^+ spatial buffering (31–33) and Ca^{2+}-dependent neurotransmitter release (34), might modulate the neuronal excitability and activity, respectively (Fig. 1.1D) (20). The presence of a large current sink in cortical layer III during the depolarizing phase (35) further strengthened the contribution of intracortical linkages to the propagation of the slow oscillation. Thus, the slow oscillation relies on widespread cortical networks.

The long-lasting hyperpolarization of the neuronal membrane often de-inactivates additional excitatory currents of the low-threshold Ca^{2+} spike type, which in turn will have a greater impact on the synchronization of the network. It has been therefore proposed that the slow oscillation acts as a precursor of nocturnal SW seizures (14, 16).

Electroencephalographic correlates of the slow oscillation

The synchronous nature of the slow oscillation has a direct impact on the electroencephalogram (EEG). Therefore the slow oscillation is detectable in the gross brain activity of the sleeping brain. First, the slow oscillation is expressed in the local field potentials reflecting the membrane currents generated by neurons and, possibly, glial cells. Since the depolarizing phase of the slow oscillation reflects the excitation of all neurons, the corresponding extracellular field potential consists of a negative deflection (27). The shape of this negative potential has been found similar to the one of the K-complex (36, 35). Besides disclosing the cellular bases of the K-complex, our studies have also established that this EEG event has a periodic and spontaneous recurrence (with the exception of occasional, sensory evoked K-complexes), and is generated in all sleep stages.

The hyperpolarizing phase of the slow oscillation corresponds to a round wave that has a negative polarity in the cortical depth (27) when measured with alternating current (AC) amplifiers (i.e. after removal of direct current (DC) and very slow components). Although AC recordings are the rule in routine EEG, it should be kept in mind that the procedure is altering the real shape of field potential and EEG waves by introducing some artificial components, like the negative wave during the hyperpolarizing phase of the slow oscillation. In fact, being the result of network disfacilitation, the field expression of the hyperpolarizing phase marks a return to resting extracellular potential (see comparison between DC and AC recordings in figure 3 from reference 37).

Transformation of the slow oscillation into SW seizures

The smooth transition from the physiological (slow oscillation) to the pathological (SW sequences) pattern suggests that the slow and the paroxysmal oscillations share common mechanisms. The electroencephalographic SW is made at the neuronal level by a paroxysmal depolarizing shift (PDS). The PDS is a giant excitatory postsynaptic potential (EPSP) (38–41) that also contains inhibitory postsynaptic potential (IPSPs) (42). Occasionally, the PDSs and their EEG counterparts, the 'spikes', are superimposed by faster oscillations at about 10–15 Hz that produce corresponding indentations on the EEG 'spike' with a polyspike shape (Fig. 1.2). This pattern of seizure is a quite typical signature for those occurring in the Lennox–Gastaut syndrome (5) and their frequency appears as a resonating phenomenon within the cortical network (43).

The wave component of the SW complex is not due to active inhibition because, as demonstrated with multiple intracellular recordings, virtually all neurons (local inhibitory interneurons included) are silent during this phase of the oscillation. In addition, the firing rate tends to diminish towards the end of the PDS due to the inactivation of action potentials. Therefore, the activity of local-circuit inhibitory cells tends to weaken towards the end of the PDS and undergoes the general disfacilitation affecting all neurons belonging to the cortical network. The somatic input resistance is lowest at the beginning of the PDS and continuous to increase up to the next PDS, assuming its largest value during the hyperpolarization (44). This behaviour would not be consistent with the possibility that gamma-aminobutyric acid (GABA)ergic currents induce the arrest of firing. The disfacilitation could be promoted, as in the case of the slow oscillation, by reduced synaptic efficacy consequent to depletion of extracellular Ca^{2+} ions (28). This reduced synaptic efficacy corroborates the relative refractoriness of cortically elicited PDSs (45).

Among the ions with a significant impact on the neuronal excitability, K^+ and Ca^{2+} were repeatedly investigated. The more synchronized the network, the higher the amplitude of its oscillations, with more K^+ being expelled in the extracellular space during action potentials and other currents. During SW seizures, the extracellular concentration of K^+ increases from resting values around 3–3.5 mM to pathological levels around 9–12 mM (46–50). An increase in the extracellular K^+ concentration has proved to favour SW seizures (13, 46, 51). Extracellular K^+ is regulated by glial cells uptake (32, 52, 53,) followed by spatial buffering through gap junctions connecting the glial syncytium (54, 55). The latter phenomenon evens out K^+ but may also contribute to the general depolarization of the neuronal membrane potential (Fig. 1.3) by positively shifting the Nernst equilibrium potential.

Cortical seizures developing in a circumscribed focus generate a local increase in extracellular K^+, which is then spatially buffered through the syncytium of glial cells at more distant locations. The neuronal population of the latter would thus become the target of synchronous synaptic bombardment from the seizure focus and would act in an environment with increased K^+ concentration. Hence, synchronization of neuronal activity, the striking feature of sleep activities, creates favourable premises for seizure genesis. The protective mechanism against this seems to rely on the ability of glial cells to regulate the extracellular K^+ concentration through

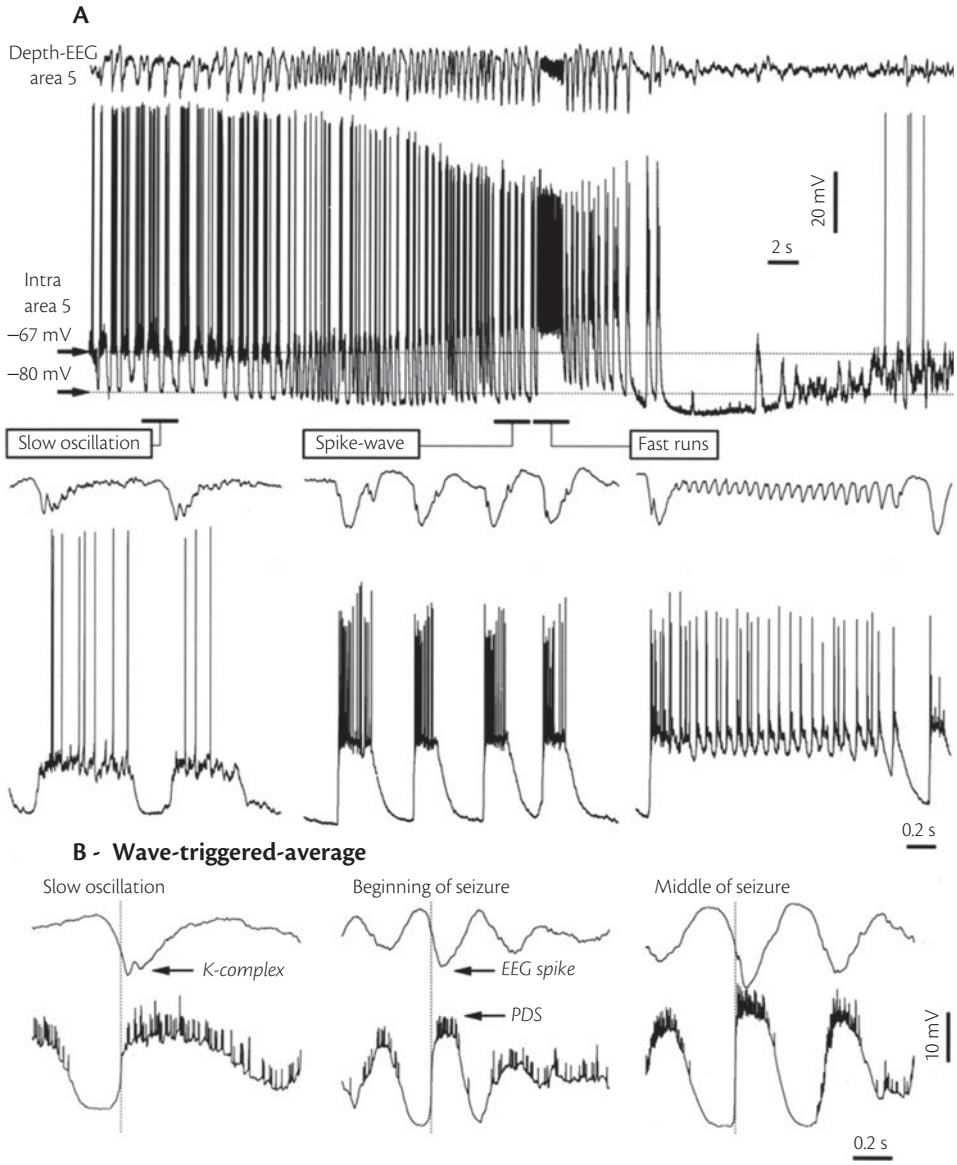

Fig. 1.2 Spontaneously occurring seizure, developing without discontinuity from slow sleep-like oscillation. Intracellular recording from regular-spiking area 5 neuron together with depth-EEG from the vicinity in area 5 in a cat under ketamine-xylazine anaesthesia. A) Smooth transition from slow oscillation to complex seizure consisting of SW complexes at ~2 Hz and fast runs at ~15 Hz. The seizure lasted for ~25 s. Epochs of slow oscillation preceding the seizure, SW complexes, and fast runs are indicated and expanded below. Note postictal depression (hyperpolarization) in the intracellularly recorded neuron (~6 s), associated with suppression of EEG slow oscillation (compare to left part of trace). B) Wave-triggered-average during the slow oscillation, at the beginning of seizure and during the middle part of seizure. Averaged activity was triggered by the steepest part of the depolarizing component in cortical neuron (dotted lines), during the three epochs. The depth-negative field component of the slow oscillation (associated with cell's depolarization) is termed *K-complex*. During the seizure, the depolarizing component reaches the level of a paroxysmal depolarizing shift (*PDS*), associated with an *EEG spike*. Modified from (16).

uptake, since impaired glial uptake of K^+ may cause epileptiform activity in the hippocampus (56).

Variations of the glial membrane potentials during sleep SW seizures

It has been well known for a long time that epileptic seizures are accompanied by significant increases in the extracellular K^+ concentration (46–48) and persistent depolarization of glial cells (8– 10). Simultaneous intracellular recordings of pairs of neurons and glia, together with extracellular ionic concentrations (mainly K^+ and Ca^{2+}) and the intracortical field activity during SW seizures (19, 20, 37) have allowed us to establish a pattern of correlation between these functional entities. Thus, it appeared that glial cells also express phasic depolarizations in relation to interictal and ictal PDSs (Fig. 1.3).

The estimate of neuronal and glial depolarizations during the phasic events building up the SW cycles led to high correlative values (>84%) (28). Since the neuronal phasic depolarizations are made of entry of Na^+ and Ca^{2+} and exit of chloride (Cl^-) and K^+, while the glial ones mostly reflect entry of K^+ (and possibly Ca^{2+}), the increased correlation between the depolarizations of the two

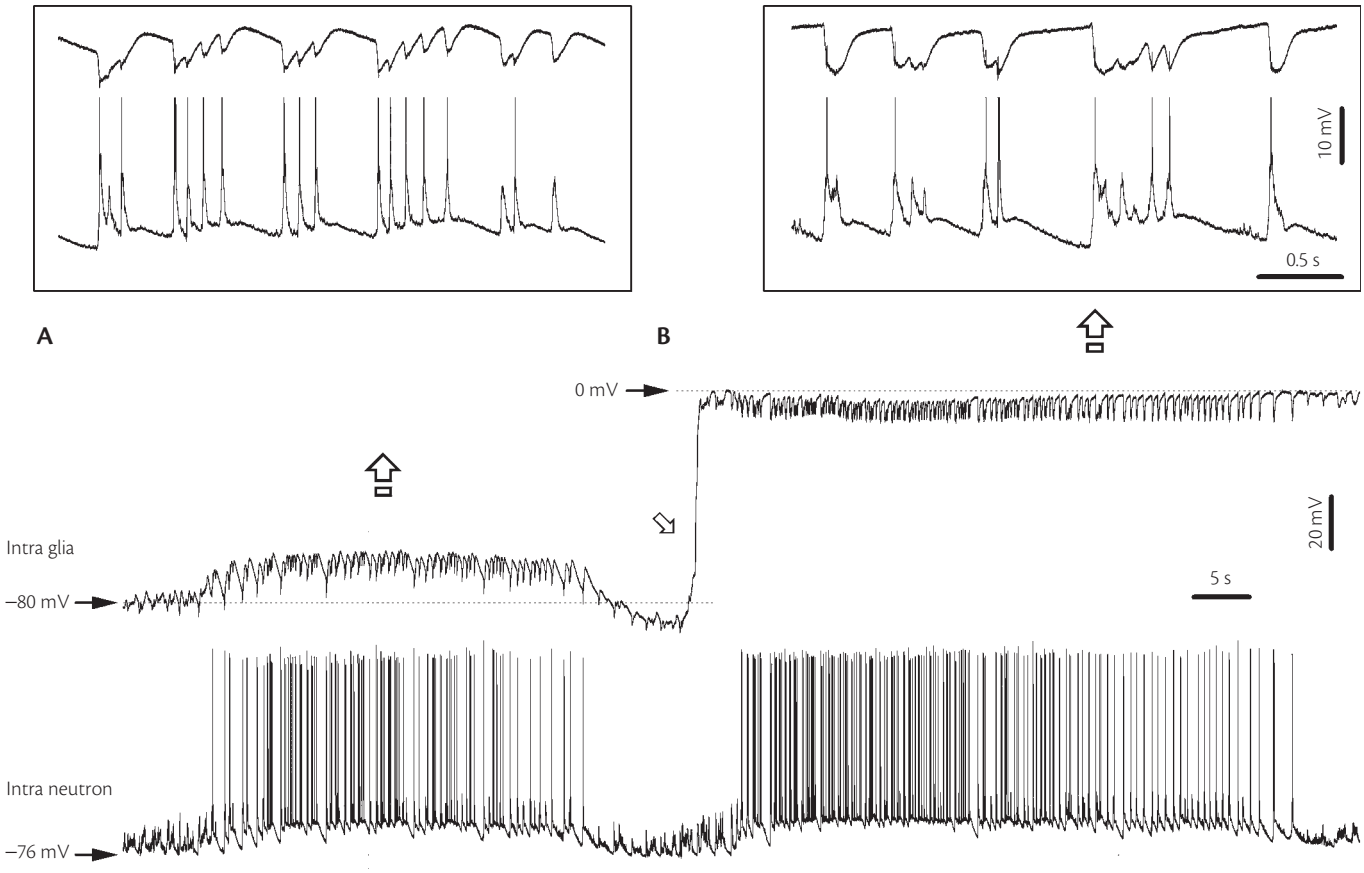

Fig. 1.3 Neuron–glia interaction during SW seizures. Continuous recording containing a double neuron–glia impalement (A), and neuron-field recording (B) in cortical association area 7. The two electrodes are separated by <1 mm. The transition from *A* to *B* is marked by the withdrawal of the pipette from the glia (*oblique open arrowhead*). Epochs within the squares are expanded above the respective panels. Modified from (37).

cell types suggests that the ionic activity of Na^+ and Ca^{2+} is proportional to that of K^+ and/or that the neuronal phasic depolarizations depend on the amount of extracellular K^+. Alternatively, it could be that SW seizures are accompanied by a more active glial uptake of extracellular K^+.

Measurements of membrane capacitance of both cortical glia and neurons during control sleep periods and SW seizures developing from the slow sleep oscillations suggests that the glial cells (not the neurons) undergo swelling and/or increased communication through gap junctions during paroxysmal discharges (Fig. 1.4) (19). The swelling of glial cells corroborates with previous experiments demonstrating shrinkage of the extracellular space during periods of increased extracellular K^+ (57). Therefore, in both cases, ephaptic transmission through the cortex might be favoured during SW discharges, as previously observed in the hippocampus (58) and could account, at least in part, for the increased synchronization recorded during epileptic seizures (14).

Correlates of paroxysmal glial activities with extracellular ionic (K^+ and/or Ca^{2+}) concentrations

Compared with the slow sleep oscillation, SW seizures appear as hypersynchronous phenomena. The progressive reduction of time lags between the activities of pairs of neurons in parallel with the development of the seizure might suggest increased synaptic coupling (14, 59). On the other hand, numerous studies have demonstrated that the extracellular Ca^{2+} concentrations decrease during SW seizures (Fig. 1.5A) (20, 60–63). Knowing that synaptic efficacy critically depends on extracellular Ca^{2+} levels (64), and assuming that the observed depletion is due to postsynaptic Ca^{2+} entry, these data challenge the classical view of the synaptic synchronization during SW seizures. Although Ca^{2+} uptake may also occur at the presynaptic level (65, 66), several lines of evidence suggest the preponderance of the postsynaptic uptake (67–71). This phenomenon alone could produce a steady decoupling of the neuronal networks during seizures.

Nevertheless, ictal extracellular Ca^{2+} levels display phasic variations in relation to rhythmic occurring PDSs (Fig. 1.5B) (20). A locally increased extracellular Ca^{2+} concentration coincides with the onset of a PDS and, although the overall variations have relatively low amplitude (~0.1 mM), they might explain the relative increased network excitability at the onset of a PDS (see figure 10 in reference 28). These data seem to confirm a previous report (45) in which the ability of the seizure-prone neocortical network to respond to electrical stimuli with interictal PDSs depended on the time lag between the end of the previous spontaneous PDS and the moment at which the stimulus was presented (Fig. 1.5C).

Intraglial as well as extracellular K^+ recordings during SW discharges show, in parallel with the Ca^{2+} depletion, a progressive

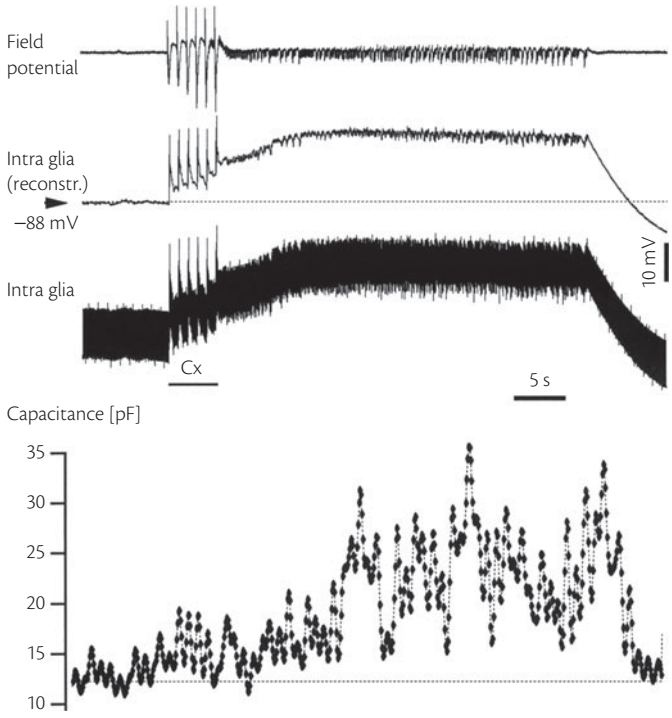

Fig. 1.4 Glial capacitance measurements during SW seizures elicited by electrical stimulation. The seizure was evoked by six trains of cortical stimuli at 1 Hz (every train contains 10 stimuli at 100 Hz). The second trace (*reconstr.*) represents the glial membrane potential after artificially eliminating the hyperpolarizing pulses (third trace) applied in order to calculate the membrane capacitance from the charging curve of the membrane. The capacitance increase reflects swelling and/or enhanced gap junction communication. Modified from (19).

depolarization and, respectively, an increase in extracellular K^+. Ca^{2+} and K^+ dynamics are clearly different (Fig. 1.5D). The epileptic tissue benefits from an enhanced gap junction communication (19, 72, 73), thus supporting the hypothesis that large cortical territories may be synchronized during SW seizures by means of K^+ waves travelling through a functional gap junction syncytium. This observation gains more weight in the presence of depleted extracellular Ca^{2+} at levels that are incompatible with synaptic functionality. Indeed, simultaneous extracellular K^+ concentrations at cortical sites separated by more than 2 mm, together with an intracellular recording close to one of the K^+ electrodes (Fig. 1.6A) have shown that, at some distance from a presumed epileptic focus, the estimated intracellular K^+ concentration increases faster than the K^+ concentration of its extracellular environment (20). These data suggest that the propagation of the epileptic activity in the cortex may use spatial buffering through the glial syncytium rather than the simple diffusion through the extracellular space.

This hypothesis gains corroborative support from the fact that isoflurane and other volatile anaesthetics, known to block gap junctions communication (74), are widely used to prevent status epilepticus (75, 76). Thus, in the absence of an efficient synaptic pathway to synchronize networks of neurons, the alternative pathway using the spatial buffering of K^+ appears as a compensatory mechanism contributing to the spreading of the seizures and to the synchronization of large cortical territories (Fig. 1.6B). Two ions could be involved in this process. First, glial cells take up Ca^{2+} through voltage-dependent channels (77), further contributing to the extracellular depletion of this ion. The subsequent increase of the intraglial Ca^{2+}, together with the increase resulting from the glutamate-mediated neuronal activity (78), may result in glutamate release from glia (79–81) and excitation of the neighbouring neurons. Such an effect may be enhanced by the increased excitability of axons resulting from the extracellular Ca^{2+} depletion (82).

It appears, therefore, that the tight homeostasis of the extracellular potassium plays a pivotal role in keeping the normal brain oscillations from evolving into paroxysmal ones. Glial cells normally fulfil this function. However, once the potassium regulation is compromised, the same cells also participate in the propagation/generalization of the ictal activities. Several studies have drawn the attention to the fact that glial dysfunction is intimately associated with the genesis of epileptic behaviour. Among others, the opening of the blood–brain barrier and the ensuing extravasation of blood proteins entrain a cascade of events beginning with astrocytic activation (83), downregulation of inward-rectifying potassium (Kir 4.1) channels in astrocytes, and impaired interstitial potassium buffering, ultimately leading to the genesis of epileptogenic foci (84).

Are nocturnal SW seizures generated in the cortex or in the thalamus?

The site of genesis of SW seizures has been hotly debated during the past decades. The hypothesis that SW seizures are generated within thalamic networks stemmed from old studies in which, however, no self-sustained activity outlasted SW-like cortical responses to medial thalamic stimulation (85). This hypothesis was replaced by the more reasonable idea that sleep spindles develop into SW seizures because of an enhanced excitability of neocortical neurons (86). Marcus and Watson (87) and Steriade (2) had emphasized the leading role of the cortex. More recently, intracellular studies in our laboratory have demonstrated that the cortically generated slow oscillation is the seed for paroxysmal developments such as SW seizures (14, 15, 59). Moreover, the thalamus of decorticated cats was unable to trigger seizures even after systemic or local injection of bicuculline (88). In contrast, local microinjections of bicuculline in the cortex of athalamic cats produced widespread synchronous seizures (88). It was thus concluded that the minimal substrate for the generation of such SW seizures is the neocortex, although the active participation of some thalamocortical cells was not precluded.

The minor role played by the thalamus in the genesis of such SW seizures is in agreement with recordings from a majority (at least 60%) of thalamic neurons in intact animals showing a tonic hyperpolarization, superimposed by phasic IPSPs, during seizures (Fig. 1.7) (15, 89). The behaviour of such thalamocortical cells further precludes their active implication in reinforcing the oscillations during seizures. It was suggested that the tonic hyperpolarization was induced by the corticofugal projections of GABAergic thalamic reticular neurons that faithfully follow cortical PDSs. A minority of thalamocortical cells was, however, discharging spike-bursts or spike-trains at 2–4 Hz, in phase with the depolarizing components of the cortical seizure. Therefore, during paroxysmal attacks the thalamic activity undergoes the antagonist influence of the steady excitatory cortex and inhibitory thalamic reticular neurons. This balance may evolve during the seizure itself,

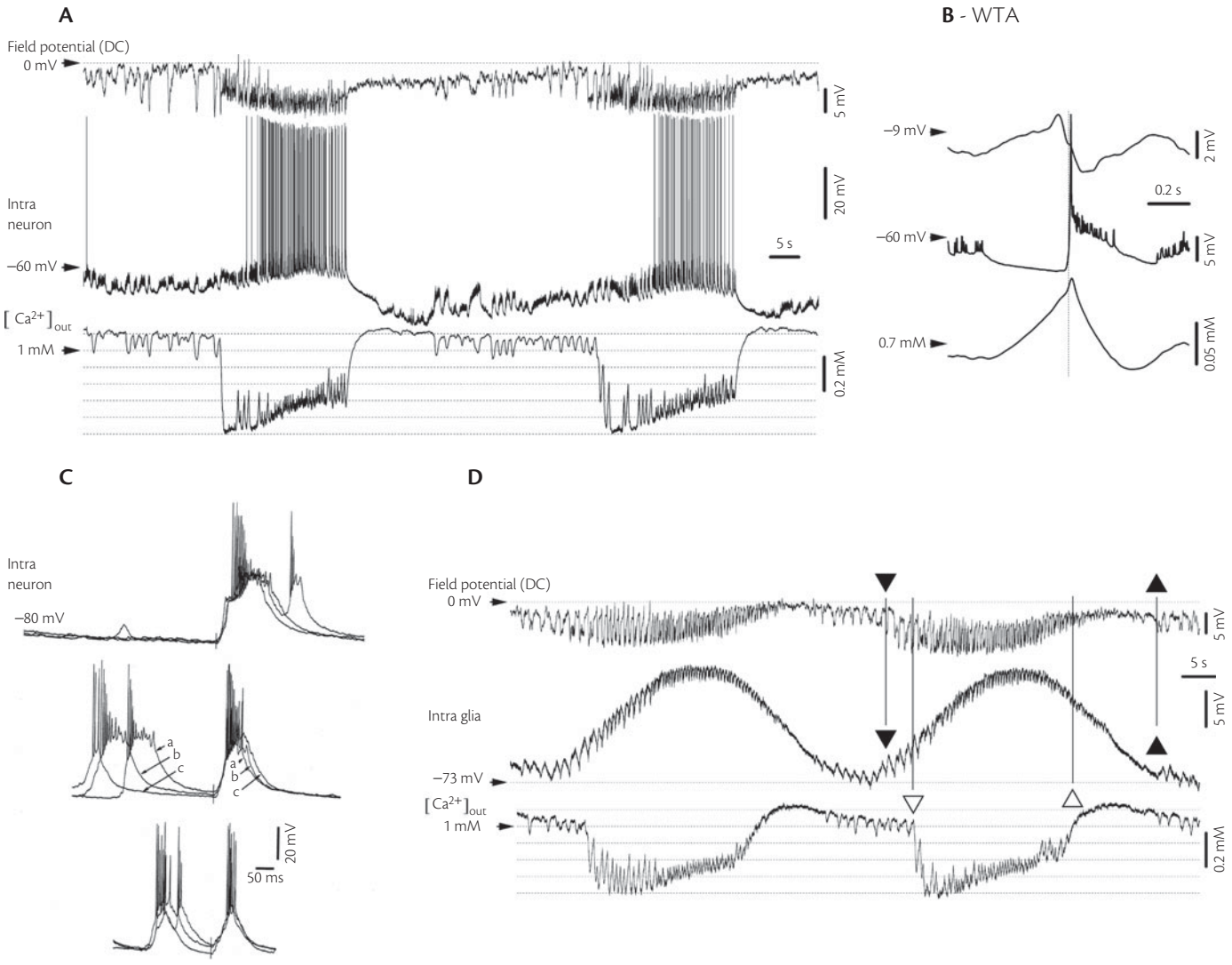

Fig. 1.5 A) Decrease of extracellular Ca^{2+} concentrations ($(Ca^{2+})_{out}$) during SW seizures. Simultaneous intracellular recording of a neuron in the suprasylvian gyrus and of neighbouring DC field potentials and $(Ca^{2+})_{out}$. SW seizures are accompanied by a persistent drop of ~0.6 mM of the extracellular Ca^{2+} concentration and by phasic oscillations of the $(Ca^{2+})_{out}$ during the SW complexes B) The wave-triggered averages (n = 40) were aligned with the maximum slope of the neuronal depolarization (*vertical dotted line*), and depict the relationship between the neuronal paroxysmal depolarization, the field potential and the Ca^{2+} concentration. Extracellular Ca^{2+} increases during the hyperpolarizing phase foregoing the onset of the ictal discharge, and decreases during the subsequent neuronal depolarization.
C) Relative refractoriness of cortically-elicited PDSs. Intracellular recording of a neuron in area 7. Three PDSs elicited by cortical stimuli to area 5 were superimposed and aligned; see stimuli artefacts from top to bottom. The amplitude and duration of cortically-evoked PDS depended on their 'time-distance' from the preceding (spontaneously occurring) PDSs: PDSs decreased both in duration and amplitude as the stimulus was closer to the spontaneous PDS. D) Relationship between $(Ca^{2+})_{out}$ and glial activities during recurrent SW seizures. Intraglial, DC field potentials, and Ca^{2+} concentrations were measured at short distance (<1 mm) in the suprasylvian gyrus. During seizures, the glial steady depolarization and presumably the $(K^{+})_{out}$, had a different time course from the $(Ca^{2+})_{out}$: the onset of the seizure was simultaneous in glia and field potential (black arrowheads oriented downward) and before the Ca^{2+} started to decrease (empty arrowhead downward). The end of the seizure was marked first by a rapid return of Ca^{2+} to normal values (empty arrowhead upward), followed by a much slower return of the glia and field potential to control potentials (black arrowhead upward). Modified from: (A), (B), and (D) (20), and (C) (45).

as suggested by the progression of the overall synchronization in corticothalamic networks, which becomes stronger as the seizure develops and more territories are recruited (59).

Conclusions

The developing of SW seizures is far from exclusively being a neuronal phenomenon. From the data presented here, we believe that glial cells and the extracellular environment, mainly through the traffic of ions, have an active role in modulating the neuronal behaviour. In the absence of reliable neuron-to-neuron synaptic transmission, alternative pathways may be used by the extraneuronal partners. Further investigation of the blood–brain barrier and the implication of its transporters may bring new insights as to their contribution to paroxysmal discharges. From this perspective, intractability may well be the reflection of our limited knowledge of the ensemble of mechanisms underlying epileptic syndromes.

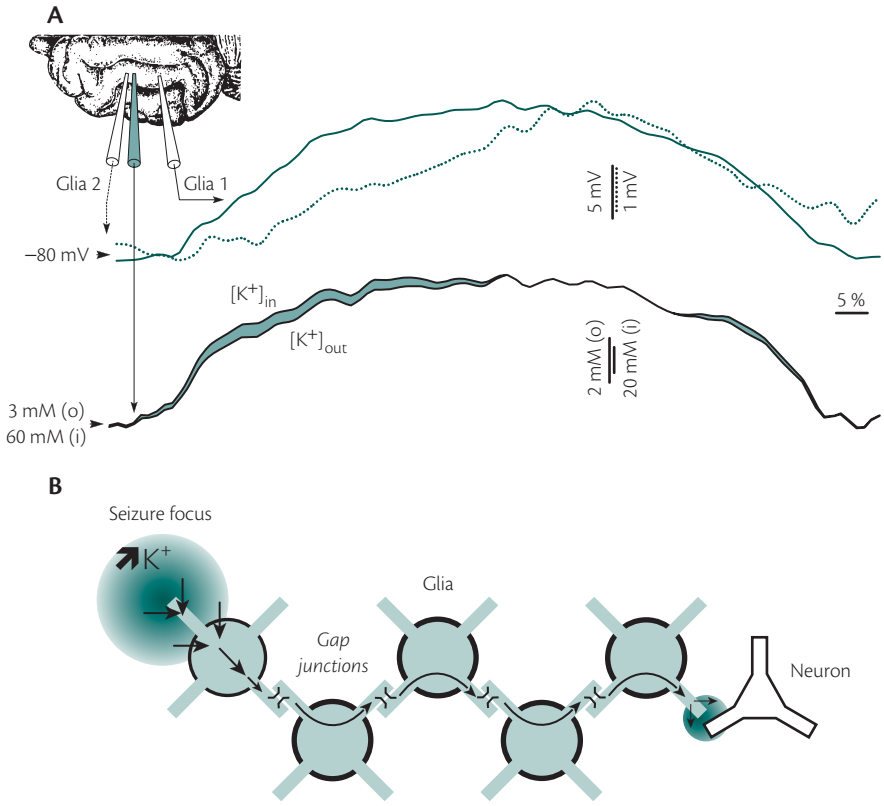

Fig. 1.6 Propagation of K^+ waves during SW seizures. A) Dual intraglial recording together with the $(K^+)_{out}$. The disposition of the recording electrodes in the suprasylvian gyrus is shown in the inset. The traces represent the average of 20 normalized seizure envelopes. The *upper* superimposition contains the intracellular seizures in the pair of glial cells expanded at their maximum amplitude (see different voltage calibrations—*continuous line* for *cell 1* and *dotted line* for *cell 2*, also corresponding to the envelope traces). From the higher amplitude of the signal, it may be inferred that *cell 1* is closer to a presumed seizure focus. The *lower* panel displays the intra- and extracellular K^+ concentrations superimposed and expanded at their maximum amplitude. The $(K^+)_{in}$ was calculated from the Nernst equilibrium potential in relation with the $(K^+)_{out}$ and the intracellular trace that was recorded closely to the K^+ microelectrode (2). Toward the beginning of the seizure, the estimated $(K^+)_{in}$ rose faster than the $(K^+)_{out}$ (*grey area* between the two traces). B) Schematic functioning of the spatial buffering during SW seizures. Important increases in the $(K^+)_{out}$ may not be buffered at short distances, in which case the internalized K^+ may travel through the glial syncytium and is externalized at a location with lower $(K^+)_{out}$ values, where it would modulate the activity of nearby neurons. Modified from (20).

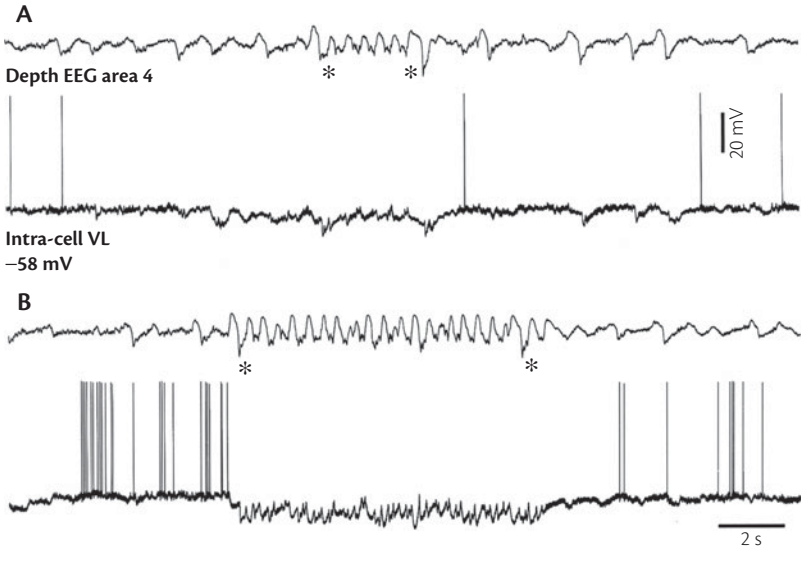

Fig. 1.7 Hyperpolarization of a thalamocortical neuron during cortical SW seizures. Cat under ketamine-xylazine anesthesia. Intracellular recording from a thalamocortical neuron in the ventrolateral (VL) nucleus, together with depth-EEG from cortical area 4. Development from embryonic SW activity (A, between asterisks) to longer-lasting seizures with SW complexes at about 2.5 Hz (B, between asterisks). Modified from (15).

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CHAPTER 2

Neurogenetics of Epilepsy

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Epilepsy can be caused by genetic or acquired factors, although, often, both contribute to its determinism. Epilepsies of unknown cause are classically divided into two main aetiological categories: 'presumed symptomatic' epilepsies are those whose cause is suspected to be induced by a pathology that is below the limit of detection of the available diagnostic tests; 'idiopathic' epilepsies are instead thought to be caused by a genetic predisposition. Symptomatic epilepsies are caused by an obvious brain abnormality, which can in turn be genetically determined or by external factors acting prenatally or after birth. Defining as 'symptomatic' a genetically determined epilepsy is easy, for example, in front of a neuronal migration disorder, or a neurocutaneous disease such as tuberous sclerosis or a syndromic form of mental retardation. The same applies to many forms of progressive myoclonus epilepsy, whose clinical expression is relatively homogeneous in relation to the genetically determined degenerative or metabolic causes. The task is much more complex when the genetic cause is hidden and will only be recognizable or hypothesizable later during the course, when a given electroclinical pattern or syndrome will become obvious. This is, for example, the case of several early-onset epileptic encephalopathies such as those caused by alterations of the *CDKL5*, *STXBP1*, or *ARX* genes. For this reason, the category of 'genetic epilepsy' which has recently been proposed is controversial and, perhaps, somewhat uncertain as what is presumed to be symptomatic today, may become genetic after a molecular screening, or remain as such if screening is unavailable.

Genetic research in epilepsy represents an area of great interest for both clinical purposes and for understanding of the mechanisms underlying epilepsy. In the past, genetic studies on families and twins contributed to the definition of genetic epilepsy, and especially to the evaluation of the risk of familial occurrence. After the first report in 1988 of the chromosomal mapping of juvenile myoclonic epilepsy (1), several additional loci were demonstrated to be implicated in syndromic epilepsies. In 1995, the first mutation in the gene coding for the nicotinic acetylcholine receptor was identified in a family with autosomal dominant nocturnal frontal lobe epilepsy (2). The subsequent identification of new epilepsy genes has greatly improved our understanding of the pathophysiological mechanisms underlying epilepsy and has favoured research into experimental models and new therapeutic strategies. A targeted molecular diagnosis is now available for different forms of epilepsy. However, ethical problems may arise especially in asymptomatic mutation carriers or in individuals and families in which although mutations of specific genes have been identified, severity of the associated phenotype is unpredictable.

Genetic epilepsy syndromes without structural brain abnormalities

About 30% of all epilepsies are idiopathic (3). The idiopathic epilepsies are characterized by age-related onset, normal neurological and cognitive development, and absence of brain damage. The study of electroencephalogram (EEG) anomalies while awake and during sleep is useful for characterizing the different forms. An underlying genetic defect has been identified for some forms with Mendelian inheritance. Most idiopathic epilepsies, however, do not follow a simple Mendelian pattern of inheritance. Even in families where involvement of a single gene is suspected, a high degree of complexity can actually be present. The phenotypic variability in some families has been ascribed to genetic modifiers (polymorphisms) or environmental factors that influence phenotypic expression (4).

The genetic alterations identified so far are responsible for rare forms of idiopathic epilepsies with a dominant pattern of inheritance, which occur with repeated seizures in the neonatal or early infantile period, and with febrile, generalized, or partial seizures that persist into adulthood. These forms are observed in a limited number of patients, but they are of great interest as they are caused by alterations in genes that encode voltage-gated ion channel subunits or receptor subunits. The identified mutations are located in the neuronal nicotinic acetylcholine receptor in familial forms of frontal epilepsy, the K^+ channels in benign familial neonatal seizures, the Na^+ channels in a particular form of generalized epilepsy with febrile seizures, and the gamma aminobutyric acid (GABA) receptor in a variant of juvenile myoclonic epilepsy (5). For the most common forms of idiopathic epilepsy, however, the molecular bases have not yet been defined; it is possible that different mutations can cause similar phenotypes in different families or in patients from different geographical areas (Table 2.1).

Table 2.1 Epilepsy syndromes with known genetic basis

Epilepsy syndromes	Type of seizures	Gene	Protein	Locus	OMIM
Generalized epilepsy with febrile seizures plus (GEFS+)	Febrile and afebrile, complex partial, generalized tonic–clonic, absences, myoclonic	*SCN1A*	Sodium channel, neuronal type 1, α subunit	2q24.3	*182389
		SCN1B	Sodium channel, neuronal type 1, β subunit	19q13.1	*600235
		GABRG2	Gamma-aminobutyric acid receptor, γ-2	5q31.1–q33.1	*137164
Severe myoclonic epilepsy of infancy (SMEI)	Febrile, partial, absences, myoclonic, generalized tonic–clonic	*SCN1A*	Sodium channel, neuronal type 1, α subunit	2q24.3	*182389
Benign familial neonatal seizures (BFNS)	Multifocal neonatal convulsions, generalized tonic–clonic	*KCNQ2*	Potassium channel, voltage-gated, KQT-like subfamily, member 2	20q13.3	*602235
		KCNQ3	Potassium channel, voltage-gated, KQT-like subfamily, member 3	8q24	*602232
Benign familial neonatal/infantile seizures (BFNIS)	Multifocal neonatal convulsions, generalized tonic–clonic	*SCN2A*	Sodium channel, voltage-gated, type 2, α subunit	2q23–q24.3	*182390
Benign familial infantile seizures (BFIS) with familial hemiplegic migraine	Multifocal neonatal convulsions, generalized tonic–clonic, hemiplegic migraine	*ATP1A2*	ATPase, Na^+/K^+ Transporting, α-2 polypeptide	1q21–q23	*182340
Autosomal dominant nocturnal frontal lobe epilepsy (ADNLFE)	Partial nocturnal motor seizures with hyperkinetic or tonic manifestations	*CHRNA4*	Cholinergic receptor, neuronal nicotinic, α polypeptide 4	20q13.2–q13.3	*118504
		CHRNB2	Cholinergic receptor, neuronal nicotinic, β polypeptide 2	1q21	*118507
		CHRNA2	Cholinergic receptor, neuronal nicotinic, α polypeptide 2	8p21	*118502
Autosomal dominant temporal lobe epilepsy (ADTLE)	Partial seizures with auditory or visual hallucinations	*LGI1*	Leucine-rich, glioma-inactivated 1	10q24	*604619
Childhood absence epilepsy (CAE)	Absences, tonic–clonic	*GABRG2*	Gamma-aminobutyric acid receptor, γ-2	5q31.1–q33.1	*137164
		CLCN2	Chloride channel 2	3q26	*600570
Juvenile myoclonic epilepsy (JME)	Myoclonic, tonic–clonic	*GABRA1*	Gamma-aminobutyric acid receptor, α-1	5q34–q35	*137160
Juvenile myoclonic epilepsy (JME)	Myoclonic, tonic–clonic	*EFHC1*	EF-hand domain (C-terminal)-containing protein 1	6p12.2	*608815
Infantile spasms, West syndrome	Infantile spasms, hypsarrhythmia	*ARX*	Gene homeobox aristaless related	Xq22.13	*300382
Early infantile epileptic encephalopathy	Myoclonic, infantile spasms	*CDKL5*	Cyclin-dependent kinase 5	Xp22X	*300203
Epilepsy and mental retardation restricted to females	Febrile, partial, absences, myoclonic, generalized tonic–clonic	*PCDH19*	Protocadherin 19	Xq22	*300460
Epileptic encephalopathy (Ohtahara syndrome)	Tonic, infantile spasms	*STXBP1*	Syntaxin binding protein 1	9q34.1	*602926

Severe myoclonic epilepsy of infancy or Dravet syndrome

Dravet syndrome starts at about 6 months of age in previously healthy infants, typically with prolonged generalized or hemiclonic febrile seizures. Between 1 and 4 years, other types of seizures appear, including myoclonic, partial, and absence seizures. Hyperthermia, such as fever or a warm bath, often precipitates seizures (6). Development in the first year of life is normal but subsequently slows and may regress. The EEG may be normal until age 2 years when generalized spike wave activity is seen; approximately 10% of patients are photosensitive. The magnetic resonance imaging (MRI) scan is either normal or shows non-specific features (7, 8).

Dravet syndrome is related to *SCN1A* mutations in at least 85% of cases. The majority of patients exhibiting mutations of this gene carry *de novo* mutations (90%); about 40% of these mutations are truncation and 40% are missense mutations. 10% of patients who are negative on sequence-based mutational analysis, have copy number variations including exonic deletions or duplications that can involve several exons or the whole gene (9, 10). Some rare patients have a mutation in the *GABRG2* gene (11). Germline mosaicism may result in siblings with Dravet syndrome born from an unaffected or mildly unaffected parent carrying a low level of mosaicism for the mutation (12, 13).

SCN1A mutations are also commonly found in the borderline variant of severe myoclonic epilepsy of infancy (SMEB), whose separation from Dravet syndrome may be arbitrary (14). Mutations are less commonly found in patients that have been categorized within different subgroups exhibiting various elements of Dravet syndrome (15–17).

Generalized epilepsy with febrile seizures plus

Generalized epilepsy with febrile seizures plus (GEFS+) is a familial epilepsy syndrome, diagnosed on the basis of at least two individuals with GEFS+ phenotypes in a family. The GEFS+ spectrum denotes the phenotypic heterogeneity observed in families including febrile seizures (FS) and febrile seizures plus (FS+). Overlap with classical idiopathic generalized epilepsy (IGE) is also seen. The course and response to antiepileptic drugs may be considerably variable within the same family: in some patients FS are rare, or disappear after a few years, while in other individuals within the same family, epilepsy is severe and drug resistant. GEFS+ was originally recognized because of remarkable large autosomal dominant pedigrees with 60–70% penetrance. It is likely that most cases, however, occur in small families or are sporadic (18).

Mutations in genes that encode alpha and beta subunits of the voltage-gated Na^+ channel (*SCN1A* and *SCN1B*) have been associated with GEFS + (19, 20). Missense mutations in *SCN1A* are the commonest identified molecular abnormalities and are found in about 10% of families (21). In some families, mutations in the gene encoding the gamma 2 subunit of the $GABA_A$ receptor (*GABRG2*) have been identified (22, 23). The phenotypic variability observed in GEFS + could be linked to the combined action of mutations in different genes.

The neuronal sodium (Na^+) channels consist of two subunits: alpha and beta (Fig. 2.1). *In vitro* functional studies have shown that mutations in the gene that encode the alpha 1 subunit determine a persistent depolarization of the Na^+ current resulting in neuronal hyperexcitability (24). The expression of many *SCN1A* mutations in human embryonic kidney cells or *Xenopus* oocytes has revealed both gain- and loss-of-function mechanisms. However, loss-of function seems to be the predominant mechanism of action causing FS and GEFS+, which is in agreement with genetic and functional studies in Dravet syndrome (25). Two recently published mouse models for SCN1A, in which loss of function mutations were introduced into the endogenous mouse gene, exhibited spontaneous seizures and reduced sodium currents with decreased sodium-channel expression selectively affecting inhibitory interneurons (26, 27). These findings suggest that Dravet syndrome, and maybe the other *SCN1A*-linked seizure disorders, are caused by a decreased excitability of GABAergic interneurons owing to *SCN1A* haploinsufficiency (26, 27). GABA receptors were long suspected to be involved in epileptogenesis. Functional expression of some *GABRG2* mutations, identified in patients with GEFS+, revealed a pronounced loss-of-function by altered gating or defective trafficking and reduced surface expression as a common pathogenic mechanism (25). Hence, these mutations reduce the main mechanism for neuronal inhibition in the brain, which can explain the occurrence of seizures.

Benign epilepsies of the first year of life

Benign epilepsies of the first year of life represent a group of syndromes that are defined as 'benign' because their clinical manifestations, which occur in otherwise asymptomatic babies, regress and eventually disappear spontaneously. These forms are quite rare and are transmitted with an autosomal dominant pattern of inheritance. Molecular diagnosis, where possible, is important in order to avoid unnecessary invasive testing and support genetic counselling. The clinical manifestations of these forms of epilepsy are rather similar, while age of onset is variable.

Benign familial neonatal seizures

Benign familial neonatal seizures (BFNS) are characterized by clusters of seizures that appear from the first days of life up to the 3rd month, and disappear spontaneously after weeks to months. Seizures have a focal onset, often with hemitonic or hemiclonic symptoms or with apnoeic spells, or can clinically appear as generalized. Interictal EEG is usually normal. The rare available ictal EEGs show focal and generalized discharges. The risk of seizures recurring later in life is about 15% (28). Although psychomotor development is usually normal, an increasing number of cases with learning disability have recently been described (29). BFNS are autosomal dominantly inherited with a penetrance of 85%. Most patients have mutations in the gene encoding the K^+ channel voltage-dependent, KQT-like subtype member (*KCNQ2*), and deletions/duplications involving one or more exons of *KCNQ2* (30–32). A small proportion of families carry mutations in the associated gene voltage-dependent K^+ channel, KQT-like subtype, member 3 (*KCNQ3*) (33). *KCNQ2* and *KCNQ3* form a heteromeric K^+ channel, which determines the M-current, influencing the membrane potential at rest (34). Co-expression of the heteromeric wild-type and mutant KCNQ2/3 channels usually revealed a reduction of about 20–30% in the resulting potassium current, which is apparently sufficient to cause BFNS (35). Although the reduction of the potassium current can cause epileptic seizures by a subthreshold membrane depolarization, which increases neuronal firing, it is not fully understood why seizures preferentially occur in neonates (36). It is possible that the neonatal brain be more

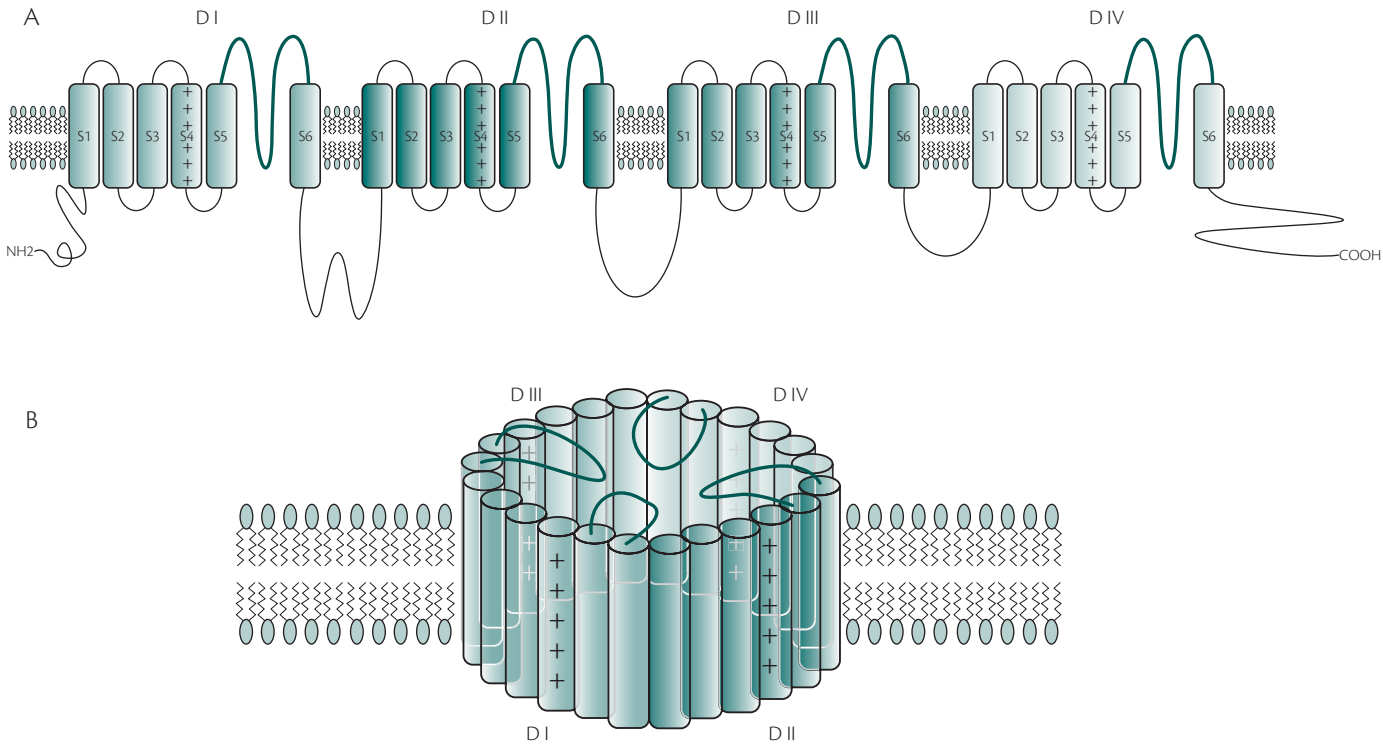

Fig. 2.1 A) Scheme of the structure of the linearized voltage-gated sodium channel α-subunit. The α-subunit consists of four repeated domains (DI–DIV), each of which is in turn composed of 6 α-helix transmembrane segments (S1–S6). Segment S4 is the voltage sensor. The S5 and S6 segments and the linkers form the pore that acts as ion selectivity filter. β-subunits, not represented here, modulate the kinetic and the voltage-dependence of the channel. B) Three-dimensional configuration of the sodium channel.

vulnerable to changes, even small, of neuronal excitability. An alternative is that KCNQ2 and KCNQ3 channels, when mutated, are replaced by other K^+ channels that become functional after the first months of life.

Benign familial neonatal-infantile seizures

Benign familial neonatal-infantile seizures (BFNIS) are characterized by seizures similar to those observed in children with BFNS. However, age at onset of seizures ranges from the neonatal period to infancy in different family members, with a mean onset age of 3 months. Remission occurs by 12 months with a very low risk of later seizures (37, 38). Mutations in *SCN2A* have been identified in most families with BFNS (39).

Benign familial infantile seizures

Benign familial infantile seizures (BFIS) are characterized by seizures similar to those observed in BNFS with an onset at around 6 months (40). Linkage studies have identified two loci for this type of epilepsy, one in the pericentromeric region of chromosome 16 and another on chromosome 19 (41, 42). However, the disease gene has not yet been identified. Some families also have paroxysmal dyskinesia beginning in later childhood (infantile convulsions and choreoathetosis) (41). Long-term prognosis for seizure remission is excellent. Several families with mutations in the *ATP1A2* gene have benign infantile seizures in conjunction with hemiplegic migraine (43). Rare families with mutations in *SCN2A* or *KCNQ2* have also been described in which only infantile seizures occur (44, 45).

Autosomal dominant nocturnal frontal lobe epilepsy

Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) includes frequent brief seizures that occur from childhood, with hyperkinetic or tonic manifestations, typically in clusters at night (46). Ictal video-EEG studies have revealed partial seizures originating from the frontal lobe but also from parts of the insula and temporal lobe suggesting a defect of a broader network (47, 48). Penetrance is estimated at approximately 70–80%. A mutation was identified in the gene *CHRNA4* encoding the a4-subunit of a neuronal nicotinic acetylcholine receptor as the first ion channel mutation found in an inherited form of epilepsy (2). Altogether, six mutations in *CHRNA4*, three in *CHRNB2*, which encodes the beta2-subunit of neuronal nicotinic acetylcholine receptor, and one in *CHRNA2*, encoding the neuronal nicotinic acetylcholine receptor alpha2-subunit, have been reported so far (25, 49–52). These receptors are heteropentamers consisting of various combinations of subunits. The alpha4-beta2 combination is the most represented in the thalamus and cerebral cortex (Fig. 2.2). All the identified mutations reside in the pore-forming M2 transmembrane segments. The exact pathomechanism is not fully understood, but an increased acetylcholine sensitivity could be the main common gating defect of the mutations.

Autosomal dominant temporal lobe epilepsy

Autosomal dominant temporal lobe epilepsy (ADTLE) is a form of autosomal dominant partial epilepsy associated to auditory symptoms. The first clinical manifestations, usually occurring during

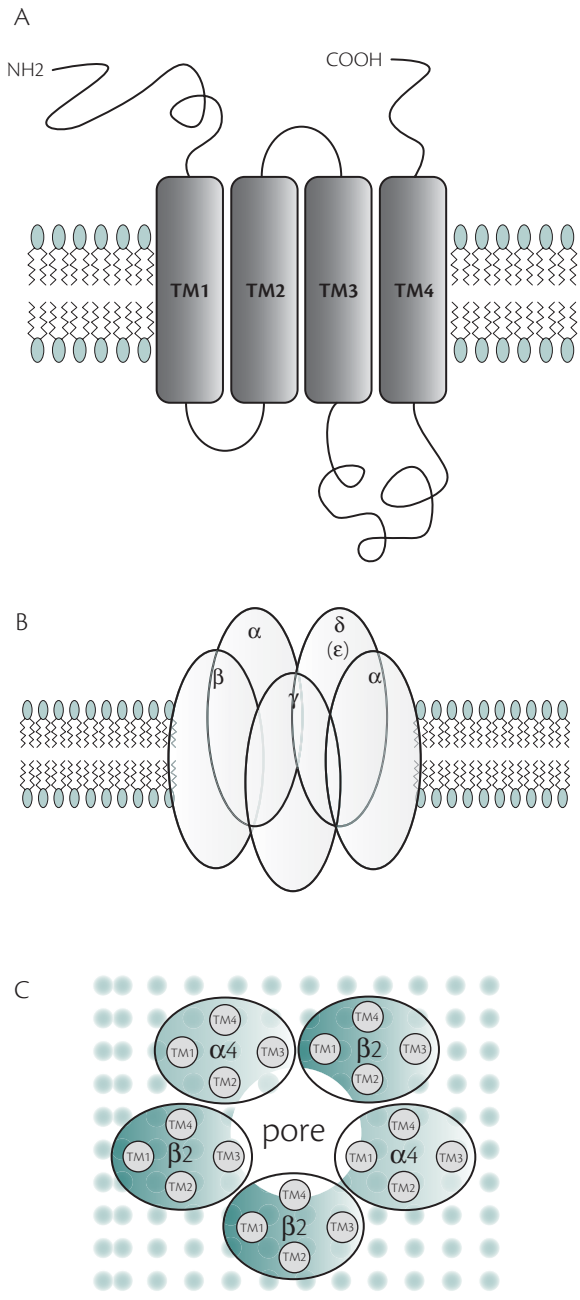

Fig. 2.2 A) General structure of a single subunit of the nicotinic receptor. B) Nicotinic receptors are formed by five subunits (pentamers), symmetrically arranged to delimit a pore through which cations flow, with Na^+ and Ca 2+ incoming, and K^+ outgoing. Nine different types of α-subunits and four different types of β-subunits are known. Isoforms for subunits δ, ε, and γ are not yet known. Numerous receptor subtypes that have specific anatomical locations (e.g. muscle or neuronal) are generated by multiple combinations of different types of these subunits. In the central nervous system, the pentameric structures of the receptor are composed of α-subunit homodimers or of α-subunits/β-subunits combinations. C) Top view of the structure of a neuronal nicotinic receptor composed by two α4-subunits (CHRNA4) and three β2-subunits (CHRNB2). Abnormalities in this receptor, caused by mutations of its subunits, cause nocturnal frontal lobe epilepsy.

childhood or adolescence, are auditory hallucinations, sometimes accompanied by vision or olfactory manifestations, or dizziness (53, 54). Mutations in the *LGI1* gene (leucine-rich, glioma-inactivated 1) have been associated with ADTLE in several families (55, 56). The *LGI1* pathogenetic mechanism remains to be clarified. Functional inactivation of one allele leads to ADTLE, whereas silencing of both alleles has been observed in several high-grade gliomas (57). The LGI1 protein harbours a domain consisting of a sevenfold repeat of 44 amino acids, the EAR (epilepsy-associated repeat) domain (58). This domain is common to MASS1, a large G-protein coupled receptor, mutated in the Frings murine model of audiogenic epilepsy (59).

Febrile seizures

FS are classified as a nosological entity distinct from epilepsy. FS affect 2–4% of children between the ages of 3 months and 6 years (60). The differential diagnosis includes seizures with fever in a child with epilepsy, seizures with central nervous system infection, or an acute metabolic disturbance (60). A positive family history is one of the few known risk factors for febrile seizures, with recurrence risk ratios of 3–5 in first-degree relatives (61). About 3–6% of children with FS will have epilepsy later in life, including idiopathic generalized epilepsy and temporal lobe epilepsy with hippocampal sclerosis that might be related to prolonged FS (62). Segregation analysis of a population-based sample showed that most FS have polygenic inheritance, although a small proportion have autosomal-dominant inheritance (63). Using large, multigeneration families with presumed autosomal dominant inheritance, four loci for FS have been identified (FEB1–FEB4) but no gene defect has yet been identified (64).

Single gene severe epilepsies and epileptic encephalopathies

Some types of severe epilepsies have a genetic basis. Patients often have severe epilepsy with early onset and infantile spasms, delayed neurological and cognitive development, and behavioural problems. Some examples of genes in which, in recent years, mutations have been identified in patients with particular forms of severe epilepsy are discussed in this section.

PCDH19 (protocadherin 19)

PCDH19 mutations have been associated to epilepsy and mental retardation limited to females (EFMR) (65), an X-linked disorder surprisingly affecting only females and sparing transmitting males, but also often appearing *de novo* (66). It is characterized by a variable clinical presentation, including slow development from birth, normal early development followed by regression starting at seizure onset, and normal development without regression (65, 67). The epilepsy spectrum associated with *PCDH19* mutations is in turn variable and includes mild focal epilepsy starting in infancy or epilepsy with recurrent episodes of focal or generalized seizures in series or status epilepticus triggered by fever (66). Protocadherin 19 is part of the protocadherin delta-2 subclass of the cadherin superfamily. Protocadherin members are expressed predominantly in the nervous system, where they have a role in establishing neuronal connections and in signal transduction at synaptic membranes (65). Males which are hemizygous for *PCDH19* mutations have normal cognitive level and no epilepsy. Cellular interference has been proposed to explain the discrepancy between the clinical

manifestations of heterozygous females and hemizygous males (68). This model suggests that if an individual has two populations of protocadherin cells (mutated and non-mutated) then a pathological phenotype occurs. A normal female or a transmitting male have only one protocadherin population of cells, protocadherin-wildtype or protocadherin-mutant cells, respectively, then they do not present a pathological phenotype. The development of genetically modified animal models will allow this puzzling disease mechanism to be better explored.

ARX (aristaless-related homeobox)

The *ARX* gene, on chromosome Xp22, is a transcription factor that belongs to a family of paired class homeobox genes, and plays an important role in embryogenesis, especially in the development of the central nervous system (69). To date, *ARX* mutations have been identified in about 10 different clinical conditions, with or without brain malformations (70–72). Malformation phenotypes, including X-linked lissencephaly with abnormal genitalia (XLAG), XLAG with severe hydrocephalus, and Proud syndrome (agenesis of the corpus callosum with abnormal genitalia), are associated with protein truncation mutations and missense mutations in the homeobox (70, 73). Non-malformation phenotypes are associated with missense mutations outside of the homeobox or expansion of the second polyA tract and include X-linked infantile spasms (ISS)/West syndrome, Partington syndrome (mental retardation with mild distal dystonia), and non-specific X-linked mental retardation (XLMR) (70, 73). Expansions in the first polyA tract cause X-linked infantile spasms (ISSX)/West syndrome (tonic spasms with clustering, severe psychomotor delay and hypsarrhythmia on the EEG) (74, 75), a severe epileptic-dyskinetic encephalopathy (71), tonic seizures and dystonia without infantile spasms (76), and Ohtahara syndrome (77).

CDKL5 (cyclin-dependent, kinase-like 5)

Mutations in the X-linked gene *CDKL5* cause early onset intractable seizures, severe developmental delay and, often, subsequent appearance of Rett syndrome (RTT)–like features (78). The phenotypic spectrum associated with *CDKL5* mutations also includes X-linked infantile spasms syndrome (ISSX) (79), a form of myoclonic encephalopathy (80), and severe encephalopathy with refractory seizures (81). Mutations in *CDKL5* are mainly found in females, suggesting gestational lethality in males (82). Parental germline mosaicism has been reported (83). In affected females, a seemingly normal early development, followed by onset of intractable seizures between the first days and 4th month of life are early key diagnostic criteria. Severe developmental delay with regression become apparent after seizures onset. Seizures are usually manifested as infantile spasms, or prolonged tonic seizures followed by spasms and myoclonus, with a peculiar electroclinical pattern (84) variably associated with, migrating focal seizures during the course. Overall, 16.3% of girls with early-onset intractable epilepsy, with or without infantile spasms, exhibit either mutations or genomic deletions involving *CDKL5* (85).

STXBP1 (syntaxin binding protein 1)

Mutations in the *STXBP1* gene have been associated with Ohtahara syndrome or early infantile epileptic encephalopathy (EIEE), characterized by early onset of tonic spasms, seizure intractability, a characteristic suppression-burst pattern on the EEG and poor outcome with severe psychomotor retardation (86, 87). *STXBP1* mutations have also been found in some children with infantile spasms (88, 89). The transition from EIEE to West syndrome occurs in 75% of individuals with EIEE (86, 87). STXBP1 protein plays an essential role in synaptic vesicle release and secretion of neurotransmitters (90). The Ohtahara syndrome–infantile spasms spectrum can also be caused by polyalanine expansions of the *ARX* gene (71, 77). Available information suggests a mutation rate of about 6% for *ARX* in boys with isolated or X-linked infantile spasms and no brain lesions (71) and up to 37% for *STXBP1* in infants with Ohtahara syndrome (90). However, larger series are needed to fully elucidate the phenotypic spectrum and better understand the causative role of this gene in epilepsy.

Progressive myoclonus epilepsies

These genetically heterogeneous disorders (Table 2.2) share clinical features that include action myoclonus, myoclonic jerks that are segmental and arrhythmic, appearing both at rest and as stimulus sensitive manifestations, epileptic seizures, predominantly generalized tonic–clonic. Progressive mental deterioration, cerebellar and extrapyramidal signs are also present in a variable proportion of patients (91, 92). Progressive myoclonus epilepsies (PMEs) represent less than 1% of all epilepsy cases and have a variable geographic and ethnic distribution. Many gene defects underlying PMEs have been identified (Table 2.3) but in a considerable proportion of patients the cause remains unknown. Most PMEs result from the intracellular accumulation of abnormal deposit material (93). The age onset, the rapid progression of the symptoms, and the prognosis are different and depend on the specific aetiology and on the type of causative mutations. The diagnostic approach varies in relation to the suspected form (Table 2.4). While the Unverricht–Lundborg disease, although disabling, can reach a degree of stabilization, all other forms progress relentlessly, leading to severe disability or death in matter of years.

Idiopathic generalized epilepsies with complex inheritance

Mode of inheritance

The idiopathic generalized epilepsies (IGEs) represent 20–30% of all epilepsies and include a group of syndromes characterized by

Table 2.2 Clinical classification of progressive myoclonic epilepsies

PMEs type	Age of onset
Unverricht–Lundborg disease (ULD)	7–16 years
Lafora disease (LD)	6–19 years
Neuronal ceroid-lipofuscinoses (NCLs):	
◆ Infantile (Haltia–Santavuori)	6 months–2 years
◆ Late infantile (Jansky–Bielschowsky)	1–4 years
◆ Intermediate (Lake or Cavanagh)	5–8 years
◆ Juvenile (Spielmeyer–Vogt)	4–14 years
◆ Adult (Kufs)	15–50 years
Myoclonic epilepsy with ragged-red fibres (MERRF)	3–65 years
Sialidosis:	8–15 years
◆ Type 1	
◆ Galactosialidosis (type 2)	
Dentato-rubro-pallido-luysian atrophy (DRPLA)	Childhood
Gaucher disease (type III)	Variable

Table 2.3 Molecular genetics of progressive myoclonic epilepsies

PMEs type	Pattern of inheritance	Locus	Gene
Unverricht–Lundborg disease (ULD)	AR	21q22.3	*CSTB*
Lafora disease (LD)	AR	6q 24.3 6p22.3	*EPM2A* *NHLRC1*
Neuronal ceroid-lipofuscinoses (NCLs):			
Infantile	AR	1p34.2	*PPT1*
Late infantile	AR	11p15.4	*TPP1*
Finnish variant	AR	13q22.3	*CLN5*
Gipsy variant	AR	15q23	*CLN6*
Turkish variant	AR	4q28.2	*MFSD8*
Juvenile	AR	16p12.1	*CLN3*
Juvenile variant	AR	8p23.3	*CLN8*
Juvenile variant	AR	–	*CLN9*
Adult	AR	15q23	*CLN4*
Congenital	AD	20q13.33	*DNAJC5*
	AR	11p15.5	*CTSD*
Myoclonic epilepsy with ragged-red fibres (MERRF)	Mitochondrial	Mt DNA	$tRNA^{Lys}$
Sialidosis:			
Type I	AR	6p21.33	*NEU1*
Galactosialidosis	AR	20q13.12	*CTSA*
Gaucher disease, type III	AR	1q22	*GBA*
Dentato-rubro-pallido-luysian atrophy (DRPLA)	AD	12p13.31	*ATN1*

AD, autosomal dominant; AR, autosomal recessive; Mt, mitochondrial.

absence seizures, myoclonus, and generalized tonic–clonic seizures. There is a partial overlap in age of onset, type, and frequency of seizures, prognosis, and response to treatment. Among these disorders there are several subsyndromes, including childhood absence epilepsy (CAE), juvenile absence epilepsy (JAE), juvenile myoclonic epilepsy (JME), and epilepsy with generalized tonic–clonic seizures alone. Generalized spike-wave activity and normal background are observed in the EEG. Among epilepsies with complex inheritance, IGEs seem very suitable for genetic studies because they are common, have a relatively well-defined phenotype and occur in familial clusters. Close relatives of IGE probands have 4–10% risk of developing epilepsy (94). Higher risk is seen in siblings and offspring, and is lower in second-degree relatives. In contrast to monogenic inheritance, polygenic inheritance leads to a more rapid decrease of the risk in relatives as the distance from the affected individuals increases (95). Twin studies have shown higher concordance for IGE in monozygotic than in dizygotic twins (0.76 versus 0.33), which is consistent with polygenic inheritance (96). IGEs have a genetic aetiology with complex inheritance. It has been suggested that they might result from the interaction of two or more genes (97). However a high degree of complexity is operating as large-scale exome sequencing of ion channels reveals that rare missense variation in known Mendelian disease genes are equally prevalent in healthy individuals and in those with idiopathic generalized epilepsy, revealing that even deleterious ion channel mutations confer an uncertain risk to an individual depending on the other variants with which they are combined (98).

Table 2.4 Diagnostic tools for progressive myoclonic epilepsies

PMEs type	Methods	Marker
Unverricht–Lundborg disease (ULD)	Molecular biology	*CSTB*
Lafora disease (LD)	Axillar biopsy Molecular biology	Lafora bodies *EPM2A* and *NHLRC1*
Neuronal ceroid-lipofuscinoses (NCLs)	Biopsy Molecular biology	Storage lipopigment in lysosomes *PPT1*, *TPP1*, *CLN3*, *CLN4*, *CLN5*, *CLN6*, *CLN8*, *CLN9*, *MFSD8*, *DNAJC5*, *CTSD*
Myoclonic epilepsy with ragged-red fibres (MERRF)	Muscle biopsy Molecular biology	Ragged-red fibres A8344G substitution (90%) (mitochondrial DNA)
Sialidosis	Urine Lymphocytes, fibroblasts Molecular biology	↑Urinary oligosaccharides α-N-acetyl neuroaminidase deficit *NEU1*
Galactosialidosis	Urine, leukocytes, and fibroblasts Molecular biology	β-galactosidase deficit *CTSA*
Gaucher disease, type III	Lymphocytes, fibroblasts Molecular biology	β-glucocerebrosidase deficit *GBA*
Dentato-rubro-pallido-luysian atrophy (DRPLA)	Molecular biology	*ATN1*

Molecular basis

Although several chromosomal loci for different forms of IGEs have been identified, they have not often been replicated. Linkage studies on a large number of families with IGE led to the identification of several susceptibility loci (18q, 2q, 3q, and 14q) (99, 100). In rare families pathogenic mutations in single genes have been reported. Mutations in the *GABRG2* gene were identified in families with febrile seizures and childhood absence epilepsy (22, 23), a mutation in the *GABRA1* gene was identified in a family with dominantly inherited juvenile myoclonic epilepsy (101). Mutations in *CLCN2* have been identified in families with heterogeneous IGE phenotypes, including CAE (102). Rare variants in *CACNA1H* have been identified in CAE and other generalized epilepsy phenotypes (103, 104). Finally, the study of variants has provided some evidence that *GABRD* (105), *ME2* (106), *BRD2* (107), and *NEDD4L* (108) are susceptibility genes for IGE.

Symptomatic epilepsy

Epilepsies are defined symptomatic when an external cause can be identified. Often, these epilepsies persist over time and are resistant to drug treatment. Advances in diagnostics, especially the use of brain MRI, has brought to attention how often structural brain anomalies, even discrete, are the cause of epilepsy. Developmental abnormalities of the cerebral cortex are a frequent cause of epilepsy. They occur as isolated manifestations or may be one expression of neurocutaneous diseases, vascular malformations, structural abnormalities associated with chromosomal disorders (Down syndrome, Angelman and Prader–Willi syndromes, Ring 20 syndrome, Wolff–Hirschhorn syndrome and many pathogenic copy number variations), mitochondrial encephalomyopathies, the organic acidurias, and the peroxisomal diseases. All these disorders have usually an early onset. However, even a relatively late onset of seizures (2nd to 3rd decade of life) may occur when a discrete developmental abnormality of the brain is the cause of seizures.

Malformations of the cerebral cortex

It has been estimated that at least 40% of children with drug-resistant epilepsy have a malformation of the cerebral cortex (109). These malformations consist of an abnormal distribution, organization, or sometimes differentiation of the neuronal components (110). Several cortical malformations associated with epilepsy are caused by alterations that occur during embryonic development, especially during the migration of neurons to form the cerebral cortex in its definitive shape. In recent years, several genes have been identified that, when mutated, cause abnormalities of cortical development. In the following sections, the most frequent cortical malformations associated with epilepsy (lissencephaly, periventricular heterotopia, and polymicrogyria) and those whose disease genes have been identified will be presented (Table 2.5).

Lissencephaly and subcortical band heterotopia

Lissencephaly (LIS) and subcortical band heterotopia (SBH) are malformations resulting from anomalies in cortical neuronal migration. LIS is characterized by absent (agyria) or decreased (pachygyria) convolutions, producing cortical thickening and a smooth cerebral surface (111). SBH is a related disorder in which bands of grey matter are interposed in the white matter between the cortex and the lateral ventricles (110).

Classical LIS is rare, with a prevalence of about 12 per million births. Patients with severe LIS have early developmental delay, early diffuse hypotonia, later spastic quadriplegia, and eventual severe or profound mental retardation. Seizures occur in over 90% of LIS children (112), with onset before 6 months in about 75% of cases. About 80% of children have infantile spasms, although EEG does not show typical hypsarrhythmia. Most LIS children subsequently have multiple seizure types. Epilepsy is present in almost all patients with SBH and is intractable in about 65% of them. About 50% of patients with epilepsy have focal seizures, and the remaining 50% have generalized epilepsy, often within the spectrum of Lennox–Gastaut syndrome (109).

Two major genes have been associated with classical LIS and SBH. The *LIS1* gene is responsible for the autosomal form of LIS (113), while the *doublecortin* gene (*DCX*) is X-linked (114, 115). Although either gene can result in either LIS or SBH, most cases of classical LIS are due to deletions or mutations of *LIS1* (116), whereas most cases of SBH are due to mutations of *DCX* (117). *LIS1*-related *LIS* is more severe in the posterior brain regions (p>a gradient) (Fig. 2.3A), whereas *DCX*-related LIS is more severe in the anterior brain (a>p gradient) (Fig. 2.3B). About 60% of patients with p>a isolated lissencephaly (ILS) carry genomic alterations or mutations involving *LIS1* (116). Most mutations (84%) are truncating (118). Small genomic deletions and duplications of *LIS1* occur in almost 50% of patients (116). A simplified gyral pattern in the posterior brain, with underlying SBH, has been associated with mosaic mutations of *LIS1* (119). Miller–Dieker syndrome (MDS) is caused by deletion of *LIS1* and contiguous genes and features severe p>a LIS (Fig. 2.3C), accompanied by distinct dysmorphic facial features and additional malformations. Most *DCX* mutations cause a>p SBH/pachygyria. Mutations of *DCX* have been found in all reported pedigrees and in 80% of sporadic females and 25% of sporadic males with SBH (117). Genomic deletions of *DCX* are a rare cause of SBH or X-linked lissencephaly in patients who are mutation negative after Sanger sequencing of *DCX* (Fig. 2.3D) (120). Maternal germline or mosaic *DCX* mutations may occur in about 10% of cases of either SBH or X-linked LIS (121).

Three rarer forms of LIS-pachygyria have been identified in recent years. One form, X-linked LIS with absent corpus callosum and ambiguous genitalia (XLAG), results from mutations of the *ARX* gene. XLAG is a severe condition that is only observed in boys. Female carriers have normal brain MRI scan or partial to complete agenesis of the corpus callosum, with normal cognition to mild retardation (73) (Fig. 2.3, E and F). A second form, due to mutations of the *TUBA1A* gene, exhibits characteristics that partially overlap with the LIS-SBH spectrum but is often accompanied by cerebellar hypoplasia (Fig. 2.3G) (122). A third, recessive form, results from homozygous mutations of the *RELN* gene and is characterized by moderate lissencephaly, severe cerebellar hypoplasia, dysmorphic facial features, developmental delay and epilepsy (123).

Periventricular nodular heterotopia

Periventricular nodular heterotopia (PNH) consists of nodules of grey matter located along the lateral ventricles with a total failure of migration of a subset of neurons (110); it ranges from isolated, single, to confluent bilateral nodules (Fig. 2.3H). Many patients

Table 2.5 Genes and chromosomal loci associated with cortical malformations

Cortical malformations	Pattern of inheritance	Gene	Locus	OMIM
Malformations from abnormal proliferation				
Tuberous sclerosis	AD	*TSC1*	9q34.13	* 605284
Tuberous sclerosis	AD	*TSC2*	16p13.3	* 191092
Malformations from abnormal migration				
Lissencephaly (LIS)				
Miller–Dieker syndrome (MDS)	AD	*LIS1+YWHAE*	17p13.3	*601545
Isolated lissencephaly sequence (ILS) or subcortical band heterotopia (SBH)	AD	*LIS1*	17p13.3	*601545
ILS or SBH	X-linked	*DCX*	Xq22.3–q23	*300121
ILS or SBH	AD	*TUBA1A*	12q12–q14	*602529
X-linked lissencephaly with abnormal genitalia (XLAG)	X-linked	*ARX*	Xp22.13	*300382
LIS with cerebellar hypoplasia (LCH)	AR	*RELN*	7q22	*600514
LCH group B	AR	*VLDLR*	9p24.2	
Periventricular nodular heterotopia (PNH)				
Classical bilateral PNH	X-linked	*FLNA*	Xq28	*300017
Ehlers–Danlos syndrome and PNH	X-linked	*FLNA*	Xq28	*300017
Facial dysmorphisms, severe constipation and PNH	X-linked	*FLNA*	Xq28	*300017
Fragile-X syndrome and PNH	X-linked	*FMR1*	Xq27.3	*309550
PNH with limb abnormalities (limb reduction abnormality or syndactyly)	X-linked	. . .	Xq28	
Williams syndrome and PNH	AD	. . .	7q11.23	
PH	AD	. . .	5p15.1	
PH	AD	. . .	5p15.33	
Agenesis of the corpus callosum, polymicrogyria and PNH	AD	. . .	6q26–qter	
PH	AD		4p15	
PH	AD	. . .	5q14.3–q15	
Agenesis of the corpus callosum and PNH	AD	. . .	1p36.22–pter	
Microcephaly and PNH	AR	*ARFGEF2*	20q13.13	*605371
Donnai–Barrow syndrome and PNH	AR	*LRP2*	2q24–q31	*600073
Cobblestone cortical malformations				
Fukuyama congenital muscular dystrophy or Walker–Warburg syndrome (WWS)	AR	*FKTN*	9q31.2	*607440
Muscle–eye–brain disease (MEB) or WWS	AR	*FKRP*	19q13.32	*606596
MEB	AR	*LARGE*	22q12.3	*603590
MEB	AR	*POMGnT1*	1p34.1	*606822
MEB or WWS	AR	*POMT1*	9q34.13	*607423
MEB or WWS	AR	*POMT2*	14q24.3	*607439
CEDNIK syndrome	AR	*SNAP29*	22q11.2	*604202
Malformations from abnormal cortical organization				
Polymicrogyria (PMG)				
Bilateral frontoparietal PMG	AR	*GPR56*	16q13	*604110
Asymmetric PMG	AD	*TUBB2B*	6p25.2	*612850
PMG and rolandic seizures, oromotor dyspraxia	X-linked	*SRPX2*	Xq21.33–q23	*300642
PMG and Agenesis of the corpus callosum (ACC), microcephaly	AD	*TBR2*	3p21.3–p21.2	*604615
PMG and aniridia	AD	*PAX6*	11p13	*607108
PMG	AD	. . .	1p36.3–pter	
PMG and microcephaly	AD	. . .	1q44–qter	
PMG and facial dysmorphisms	AD	. . .	2p16.1–p23	
PMG and microcephaly, hydrocephalus	AD		4q21–q22	
PMG	AD	. . .	21q2	
PMG and DiGeorge syndrome	AD	. . .	22q11.2	
PMG and Warburg Micro syndrome	AR	RAB3GAP1	2q21.3	*602536
PMG and Goldberg–Shprintzen syndrome	AR	KIAA1279	10q21.3	*609367

AD, autosomal dominant; AR, autosomal recessive.

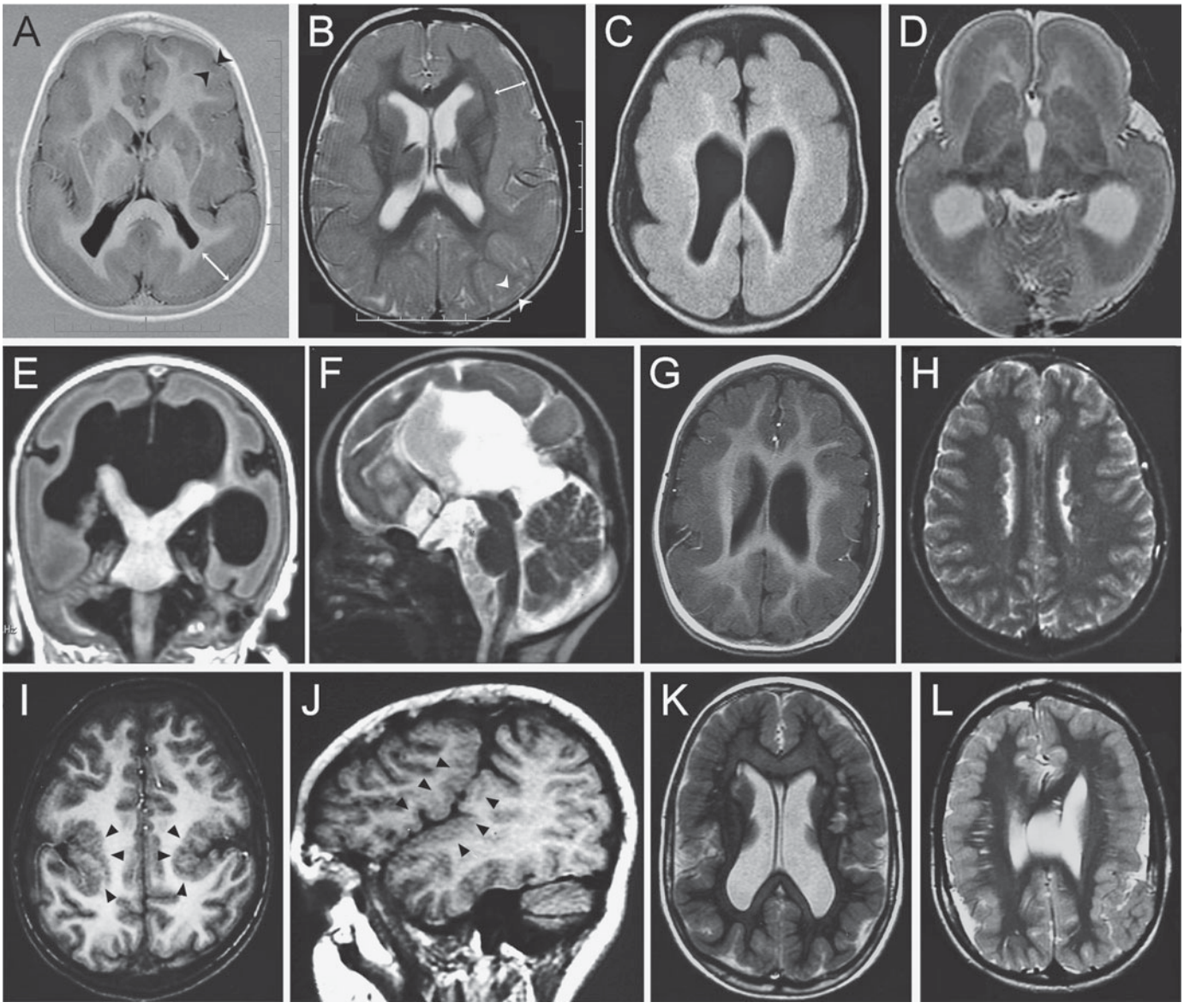

Fig. 2.3 Brain MRI of patients with different malformations of the cerebral cortex. A) Axial section. Classical lissencephaly in a boy with *LIS1* mutation. B) Axial section. Lissencephaly in a girl with *DCX* mutation. C) Axial section. Lissencephaly in a patient with Miller–Dieker syndrome. D) Axial section. Severe diffuse lissencephaly with relatively small frontal lobes in a boy with *DCX* mutation. E) and F) Coronal section and sagittal section of a boy with X-linked lissencephaly, complete corpus callosum agenesis, and ambiguous genitalia due to an *ARX* mutation. G) Axial section. Thickened cortex with simplified gyral pattern in a girl with a *TUBA1A* gene mutation. H) Axial section. Classical bilateral periventricular nodular heterotopia in a woman with a *FLNA* mutation. I) and J) belong to the same patient. I) Axial section. Bilateral perisylvian polymicrogyria. The sylvian fissures are open and the perisylvian cortex is thickened and irregular (black arrows). J) Sagittal section. Note the abnormally vertical orientation of the sylvian fissure, which appears to be fused with the rolandic fissure. K) Axial section. Bilateral frontoparietal polymicrogyria in a girl with a *GPR56* mutation and Lennox–Gastaut syndrome. L) Axial section. Polymicrogyria in a patient with *TUBB2B* mutation. The cortical mantle is diffusely polymicrogyric, with prominent infoldings in the posterior aspect of the abnormally oriented sylvian fissures, more on the left.

have epilepsy, with normal or borderline cognitive level. However, a wide spectrum of clinical presentations and associated features is possible, with a loose correlation between size of PNH, structural abnormality of the cortex, and clinical severity (124). PNH occurs most frequently in women as an X-linked trait (125) defined as 'classical bilateral PNH', associated with high rates of prenatal lethality in male fetuses, and 50% recurrence risk in the female offspring. Almost 100% of families and 26% of sporadic patients, harbour mutations of the *FLNA* gene (124), which also cause coagulopathy and cardiovascular abnormalities in some patients. Only a few living male patients with PNH owing to *FLNA* mutations have been reported (126, 127). Mild missense mutations or mosaic mutations account for survival of affected males, who can, in turn, pass their genetic defect to their daughters. A rare recessive form of PNH owing to mutations of the *ARFGEF2* gene was described in two consanguineous pedigrees in which affected children had microcephaly, severe delay, and early-onset seizures (128). PNH has been described in association with known genetic syndromes and a number of copy number variants (CNVs) in patients with variably impaired cognitive skills (129) (Table 2.5).

Polymicrogyria

The term 'polymicrogyria' (PMG) defines an excessive number of abnormally small gyri that produce an irregular cortical surface with lumpy aspect (110). PMG can be localized to a single gyrus, involve a portion of one hemisphere, be bilateral and asymmetrical, bilateral and symmetrical, or diffuse. The imaging appearance of PMG varies with the patient's age. In newborns and young infants, the malformed cortex is very thin with multiple, very small undulations. After myelination, PMG appears as thickened cortex with irregular cortex–white matter junction (130). Polymicrogyria is associated with a wide number of patterns and syndromes and with mutations in several genes (Table 2.5). Various PMG syndromes have been described, which have been designated according to their lobar topography (130). Bilateral perisylvian polymicrogyria (BPP) (Fig. 2.3I, J) is the most frequent form. It is associated with mild to moderate mental retardation, epilepsy, and impaired oromotor skills. Most cases are sporadic but genetic heterogeneity is apparent (130, 131). A missense mutation in the Xq22 gene *SRPX2* was found in an affected male (131). However, *SRPX2* does not account for X-linked pedigrees. BPP, frequently asymmetric and with a striking predisposition for the right hemisphere, has also been reported in association with 22q11.2 deletion (132). Bilateral frontoparietal polymicrogyria (BFPP) (Fig. 2.3K) has been reported in families with recessive pedigrees and has been associated with mutations of the *GPR56* gene (133). The imaging characteristics of BFPP resemble those of the cobblestone malformative spectrum (muscle-eye-brain disease and Fukuyama congenital muscular dystrophy) (130). Mutations of the *TUBB2B* gene have been associated with asymmetric polymicrogyria (134) but genotype–phenotype correlations need to be clarified (Fig. 2.3L). Different types of PMG as part of complex syndromes have been associated with several different pathogenic CNVs (Table 2.5) (135).

Metabolic disorders

Epilepsy is part of the clinical spectrum of a large number of inherited metabolic disorders (136, 137), often within the context of a complex neurological syndrome. Sometimes epilepsy is a prominent, or presenting, symptom, and, in a minority of patients can be cured by an appropriate dietary supplementation or regimen. Pyridoxine-dependent seizures, a recessive disorder due to mutations of the *antiquitin* (*ALDH7A1*) gene, respond dramatically to the intravenous injection of pyridoxine and recur within a few days if maintenance therapy is discontinued. The disorder is caused by a defect of alpha-amino adipic semialdehyde (α-AASA) dehydrogenase (antiquitin) in the cerebral lysine degradation pathway (138). The possibility of dosing specific metabolites such as α-AASA (139) and of mutation analysis has meant that pyridoxine withdrawal is no longer needed to establish the diagnosis. Epilepsy related to glucose transporter type 1 deficiency syndrome (GLUT1-DS) provides a paradigmatic example of how once the core phenotypic spectrum of a genetically determined inherited neurometabolic disorder has been defined, availability of biological and molecular markers allows progressive delineation of the syndrome spectrum. The cause of GLUT1-DS is heterozygous mutations of the *SLC2A1* gene encoding GLUT1, the molecule that transports glucose across the blood–brain barrier (140). Hypoglycorrachia is a key diagnostic laboratory feature. Clinical features classically comprise a combination of infantile-onset seizures, complex movement disorders, ataxia, cognitive impairment, and, in some children, microcephaly (141). Generalized spike-and-wave discharges and a combination of absence, myoclonic, and tonic–clonic seizures have been reported (142). A syndrome of paroxysmal exercise-induced dyskinesia and epilepsy (143) and recently an IGE phenotype (144) and more specifically early-onset absence epilepsy (145) and myoclonic-astatic epilepsy have been associated to *SLC2A1* mutations (146).

Chromosomal abnormalities

Chromosomal abnormalities are relatively common genetically determined conditions that increase the risk of epilepsy. Epilepsy has been associated with over 400 different chromosomal imbalances (147, 148). Among patients with epilepsy and intellectual disability, about 6% have chromosomal abnormalities, but this figure climbs to 50% if multiple congenital abnormalities are also present (147). The use of such current techniques as high-resolution chromosome banding, fluorescent *in situ* hybridization (FISH), comparative genomic hybridization (array CGH), and multiplex ligand-dependent probe amplification (MLPA) would certainly increase these detection rates. However, the likelihood of developing seizures varies greatly among the different chromosomal disorders. Chromosomal abnormalities almost constantly result from rearrangements or deletions/duplications that affect the function of more than one gene. As a consequence, even when the chromosomal imbalance can only be detected using techniques with the highest resolution, such as array CGH, affected patients have a combination of clinical features and only exceptionally have isolated epilepsy. Most often cognitive impairment and dysmorphic features, even subtle, co-occur with epilepsy. The ring chromosome 20 syndrome represents the most striking example in which a highly specific epilepsy phenotype can be, at least in some patients, the only expression of the chromosomal disorder.

Most pathogenic CNVs detected by array-CGH are rare or unique, making it difficult to collect a sufficient number of patients to identify characteristic features of epilepsy for each chromosomal syndrome. However, at the research level on the causes of epilepsy, the association between cryptic deletions/duplications and epilepsy is improving our ability to clone new critical genes, translating in turn in improved diagnosis. For example, heterozygous missense mutations in *STXBP1* in patients with infantile epileptic encephalopathy with suppression bursts (Ohtahara syndrome) were found after a *de novo* 2.0-Mb microdeletion at 9q33.3–q34.11 in an affected girl prompted analysis of candidate genes mapping to the deleted region in patients with similar phenotypes (90).

Array CGH has greatly improved the diagnostic yield of chromosomal imbalances, allowing the identification of new syndromes caused by imbalances as small as a few dozen kilobases (149). However, little information is available on genome-wide cytogenetic array screening in patients specifically selected for epilepsy. Engels et al. (150) analysed 60 patients with mental retardation combined with congenital anomalies, 25% of whom also had epilepsy. Novel imbalances were found in six patients (10%), two of whom had epilepsy. Kim et al. (151) reported various CNVs in patients with idiopathic generalized or partial epilepsies and FS. A study investigating the impact of these five microdeletions on the genetic risk for common IGE syndromes, using high-density single nucleotide polymorphism arrays, in a large cohort, found significant associations with microdeletions at 15q11.2 and 16p13.11 (152).

Genetic counselling

Genetic testing can be offered for single-gene or Mendelian epilepsy syndromes, or epilepsy-associated disorders, if the gene has been identified. If not, empirical counselling can be offered, based on the type of epilepsy, mode of inheritance, and penetrance. Although we can now carry out preclinical and prenatal diagnosis in many cases, the severity and prognosis of the epilepsy in specific individuals, particularly in those with idiopathic epilepsies, is difficult to predict. However, in symptomatic monogenic epilepsies, such as the progressive myoclonus epilepsies, phakomatoses, and malformations of cortical development, carrier detection, prenatal diagnosis, and presymptomatic testing may lead to prevention. Unfortunately, no curative treatment has emerged from any genetic finding in epilepsy to date (with the possible exception of the ketogenic diet in *GLUT1* deficiency syndrome), nor have the many pharmacogenomic studies in epilepsy yielded any widely applicable treatment advances. This is a disappointing lack of progress, reflecting as it does on the complex nature of epilepsy and its treatment. Counselling needs to consider these issues.

Future perspectives

The next steps include identifying additional genes both in monogenic epilepsies and epilepsies with complex inheritance, genotype–phenotype correlations, and functional studies of the abnormal proteins. These studies may have practical applications for diagnosis, genetic counselling, and possible treatment. Most of the epilepsy syndromes listed in this chapter are characterized by marked clinical and genetic heterogeneity. This may be explained by pleiotropic expression of a single-gene mutation, modifying genes, or by several genes producing a similar phenotype, at times because they affect the same developmental or metabolic pathway. It is hoped that a constantly updated database will be established for all the known gene mutations and polymorphisms and their clinical correlates, so that genotype–phenotype correlations can be determined. This is the objective of the Human Variome project (153). For example, over 600 mutations in *SCN1A* associated with Dravet syndrome have now been identified and the severity of the related phenotype can be predicted early, with reasonable accuracy for those recurrent mutations in which sufficient numbers of clinical observations are available.

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CHAPTER 3

Neurochemistry of Epilepsy

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Introduction

Epileptic seizures emerge from complex biochemical processes in the human brain. Physicians and scientists engaged in the study of this disease process have gained significant knowledge about the neurochemical basis for epilepsy. The earliest studies at the turn of the 20th century focused attention and subsequent theories on changes in chemicals detected in the urine, serum, and cerebrospinal fluid, many of which we today recognize as non-specific associated biochemical changes (1). However, with advances in studies of the human brain on cellular and molecular levels and the evolution of the pharmacology of epilepsy, the early idea that excitatory and inhibitory influences in the brain existed in balance that could be tipped toward excitation or away from inhibition to produce seizures was supported in numerous types of epilepsies and seizure-related conditions. With this simple yet elegant view in mind, studies advancing our knowledge of the neurochemistry of epilepsy continue to aid our understanding of the pathophysiology of epileptic disorders as well as our approach to their diagnosis and treatment.

The focus of this chapter will be on synaptic neurotransmission and its role in epilepsy. The main excitatory and inhibitory neurochemicals in the human brain are glutamate and gamma-aminobutyric acid (GABA), respectively, which have gained wide acceptance as the major neurochemical influences in epilepsy. However, other neurotransmitters such as aspartate and glycine remain chemicals of interest as excitatory and inhibitory amino acids. Also, the roles of acetylcholine, serotonin, and the catecholamines (dopamine and noradrenaline) in epilepsy have been studied. We will review the basic chemistry of neurotransmission for the major neurotransmitters glutamate and GABA to provide a substrate for further discussion. Next, we briefly consider the contribution of selective neuronal circuits to seizure generation. Animal models of seizures and epilepsy have been a critical source of knowledge of the neurochemistry of epilepsy, and we briefly review relevant data from several such models with a focus on microdialysis experiments. We turn back to human studies, again focusing on microdialysis experiments. We also review magnetic resonance spectroscopy and the emerging technology of microelectrode arrays as newer methods that are complementary to microdialysis. We then address potential contributions of the other neurotransmitter systems noted earlier. Having reviewed the neurochemistry of the neurotransmitter systems, we next turn to the neurochemistry of antiepileptic drugs (AEDs). Finally, we conclude with a review of a clinical trial of a drug specifically designed to modulate the GABA system that highlights some of the complexities of applying our knowledge of neurochemistry to patient care.

GABA

GABA is the major inhibitory molecule in the mammalian brain. GABA was recognized as a neurochemical in 1950 and as the amino acid in the inhibitory nerves of crustaceans by 1963 (2). It was later accepted as satisfying criteria as a neurotransmitter in the mammalian brain in 1973 (3). The idea that reduction of GABAergic inhibition results in epilepsy while its potentiation is antiepileptic became known as the GABA hypothesis of epilepsy and continues to withstand experimental scrutiny (4, 5). As discussed in more detail later, its role in epilepsy became viewed as so important that designer drugs (including vigabatrin, tiagabine, and gabapentin) were created in hopes of mimicking the effects of GABA.

GABA is biochemically synthesized in presynaptic terminals with the removal of a carboxyl group from the alpha carbon of L-glutamate by the enzyme glutamic acid decarboxylase (GAD) (6). Depolarization of GABA-producing presynaptic terminals leads to voltage-gated calcium channel activation. Calcium entering the terminals facilitates vesicular docking and release of GABA into the extracellular space at the synaptic cleft where it is able to interact with two recognized receptor types, $GABA_A$ and $GABA_B$. $GABA_A$ receptors are ligand-gated ion channels. When GABA binds $GABA_A$ receptors in the adult brain, a conformational change in the shape of the receptor allows chloride to flow along its concentration gradient into the postsynaptic cell increasing the negative membrane potential and hyperpolarizing the cell. $GABA_B$ receptors are metabotropic receptors that are coupled to G-proteins which activate adenylate cyclase leading to changes in cyclic adenosine monophosphate (cAMP) signalling to alter the conductance of a potassium channel. This, in turn, releases potassium from within the cell leading again to hyperpolarization. Both GABA receptor types make it more difficult for excitatory influences to depolarize the cell body to the point of creating new action potentials. GABA is actively removed from the extracellular space by

GABA transporters (GAT-1 through GAT-4). GAT is present on perisynaptic glia and the presynaptic cell. Coupled to the uptake of one GABA molecule is the influx of two sodium ions and one chloride ion (7, 8). The reversal of GATs may serve as a non-synaptic mechanism for GABA receptor activation as well (9).

Glutamate

Glutamate is the major excitatory neurotransmitter in the mammalian brain. It is also one of the 20 amino acids used to synthesize proteins in humans and is readily available through dietary consumption of L-glutamic acid salts (such as monosodium glutamate) and protein. However, most intracellular glutamate originates from glucose through glycolysis and the Krebs cycle. The Krebs cycle intermediate α-ketoglutarate is converted to glutamate through transamination. Because of its ubiquitous nature in the body and very high concentrations in the central nervous system, its role as a neurotransmitter was doubted for many decades. However, by the mid to late 1970s, investigators were demonstrating biochemical pathways of glutamate synthesis, specialized uptake by glia, and pharmacological antagonism of neuronal excitation by compounds which prevented glutamate binding (10–13).

Depolarization at glutamatergic presynaptic terminals leads to vesicular release of glutamate at the synaptic cleft where it can act on multiple receptor types, both ionotropic and metabotropic. Ligand-gated ion channels activated by glutamate include alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA), N-methyl-D-aspartate (NMDA), and kainate receptors. AMPA receptors permit sodium to rapidly enter the postsynaptic cell accompanied by a smaller efflux of intracellular potassium, with the net result being depolarization. Kainate receptors act very similarly to AMPA receptors although the duration of the open ion channel is shorter. Kainate receptors are also found presynaptically on some GABAergic terminals facilitating the release of GABA and, thus, can have an inhibitory effect at times. NMDA receptors require glycine as a co-ligand for activation and also have divalent magnesium ions blockading the channels. The depolarization of the cell membrane by AMPA activation leads to expulsion of magnesium ions, so that movement of sodium and potassium can occur. Although there is also a small calcium conductance through AMPA and kainate receptors, NMDA receptors are the main glutamate ionotropic receptors that allow the passage of calcium into the cell. The overall result is depolarization of the postsynaptic cell (14).

Metabotropic glutamate receptors are G-protein coupled receptors that can be divided into three groups: Group I (mGluR1 and 5), Group II (mGluR2 and 3), and Group III (mGluR4, 6, 7, and 8). Group I receptors activate phospholipase C via G_q proteins leading to phospholipid-related cell signalling through inositol 1,4,5-triphosphate (IP3) and diacyl glycerol (DAG), the latter of which can increase protein kinase C activity. Though they can be found presynaptically, Group I receptors are primarily postsynaptic. Experimentally, Group I receptor activation is both excitatory and inhibitory with capacity to modulate sodium, potassium, and voltage-gated calcium channels, inhibit glutamate release, and enhance NMDA receptor activity. Group II and III receptors coupled to G_0 proteins inhibit adenylate cyclase activity and decrease cAMP concentrations. These receptors are primarily presynaptic and inhibit further release of glutamate. Postsynaptic ligand-bound Group II and III receptors do not directly change the membrane potential but reduce the activities of other excitatory and inhibitory ion channels. Overall, in addition to an important role in positive feedback inhibiting further glutamate release, mGluRs modulate the postsynaptic cell's response to both excitatory and inhibitory influences (14, 15).

Glutamate is actively removed from the extracellular space by glial and postsynaptic neuronal uptake through five subtypes of excitatory amino acid transporters (EAATs) each with a predominant neuroanatomical distribution: EAAT1 (cerebellar glia), EAAT2 (forebrain glia), EAAT3 (cortical neurons), EAAT4 (cerebellar Purkinje neurons), and EAAT5 (retina) (16, 17). Glutamate is not taken back up into the presynaptic terminal, so to replenish vesicles, the glutamate must be shuttled back to the original cell. This is accomplished through the glutamate-glutamine cycle in which glial intracellular glutamate is converted to glutamine by glutamine synthetase. Glutamine is released by the glia and taken up by the presynaptic cell where it is converted back to glutamate by the enzyme glutaminase. Glutamate is actively transported along with protons into vesicles by the vesicular glutamate transporters (VGLUTs). Whereas EAATs will transport aspartate, VGLUTs will not (16). Reversal of glutamate uptake may also contribute to extracellular release with both potential physiological and pathologically effects (18).

GABA, glutamate, and epileptic circuitry

GABA and glutamate neurotransmission are integral components of how seizures emerge, spread, and recur, and modulation of their effects is one of the mechanisms by which many of our current AEDs prevent and abort seizures. In localization-related epilepsy, prolonged depolarization of neurons in the seizure focus leads to firing of multiple sodium-dependent action potentials, a phenomenon referred to as the paroxysmal depolarization shift (PDS). The PDS is also commonly referred to as the intracellular correlate of the epileptiform discharges seen with electroencephalographic recordings. The PDS is typically followed by GABA-mediated hyperpolarization. If GABAergic inhibition fails, the bursting behaviour of the PDS may precipitate continuous high-frequency neuronal firing which can propagate locally, spread through longer circuits to affect larger brain regions, or generalize to the entire brain (19). A typical seizure will end when the affected brain repolarizes, and the postictal state is characterized by hyperpolarization. In generalized epilepsies, where many or all brain areas begin firing abnormally simultaneously, the normal oscillatory rhythms of the thalamocortical loops are dysfunctional. The thalamic nucleus reticularis controls GABAergic thalamic relay neurons via T-type calcium channels. The thalamic relay neurons influence the activity of glutamatergic pyramidal cortical neurons (20, 21). Seizure creation results from an imbalance in excitatory and inhibitory influences within these focal and generalized networks with glutamate and GABA playing dominant roles.

The hippocampus within the temporal lobe is a region of extraordinary importance in epilepsy research and clinical practice. Temporal lobe epilepsy (TLE) represents about 40% of adult onset epilepsies. The hippocampus has been extensively studied microstructurally dating back to the initial studies of Ramón Y Cajal in the late 1800s, and with more advanced techniques, we now also understand much of the neurochemistry complementing the neuroanatomy. The major circuit within the hippocampus is the

trisynaptic pathway. Glutamatergic projections from the entorhinal cortex perforate across a fold of the spiral hippocampal structure to synapse on the dendrites of the granule cells of the dentate gyrus creating the perforant pathway. Glutamatergic projections from the granule cells project to the CA3 region. These axons are called mossy fibres due to their microstructural appearance. The third type of synapse of the pathway arises from glutamatergic projections from CA3 to CA1 pyramidal neurons called Schaffer collaterals. There are also recurrent pathways including dentate gyrus and CA1 to entorhinal cortex which are excitatory. Inhibitory control of this feedforward positive loop is provided by GABAergic interneurons found in the hilum (22). Finally, there is also subcortical modulation of the system along the septohippocampal pathway which broadly projects cholinergic connections throughout the hippocampus and well as specific GABAergic projections onto the GABA interneurons which would disinhibit the trisynaptic loop (23). More recently, glutamatergic projections have also been identified in the septohippocampal pathway with a lesser known role (24).

The pathological structural correlate of mesial temporal lobe epilepsy (MTLE) is mesial temporal sclerosis (MTS). MTS is the most common postsurgical histopathological finding from patients with refractory epilepsy who undergo epilepsy surgery (25, 26). It was first grossly recognized in the early 1800s followed by extensive study by Sommer in 1880 (27). MTS became widely recognized as a common correlate of temporal lobe epilepsy in the 1970s (28) and was histopathologically defined in a modern sense in the 1980s (29, 30). MTS has the same pathological findings as the very closely related Ammon's horn sclerosis but extends into other neighbouring regions including the entorhinal cortex and amygdala. Pathologically there is significant segmental neuronal loss accompanied by astrogliosis. Specifically, this damage is at minimum observed in Sommer's sector made up of the CA1 and presubiculum. Grossly, the tissue appears shrunken and hardened which can be appreciated with brain magnetic resonance imaging (MRI) and the corresponding metabolic dysfunction can be observed with positron emission tomography and single-positron emission computed tomography (31, 32). Additional histopathological changes in MTLE include loss of hilar mossy cells, hilar somatostatin-containing GABAergic interneurons, and CA1 pyramidal neurons with associated dispersion of the granule cell layer and aberrant sprouting from the granule cells, spared GABAergic interneurons, and remaining CA1 pyramidal neurons (26). The resulting alterations in glutamate and GABA transmission are widely assumed to contribute to the pathophysiology of MTLE and remain areas of intense study.

A lesser known epileptic region in which glutamate and GABA are important is the area tempestas, a structure in the forebrain's deep prepiriform cortex. It has been identified as a particularly epileptogenic region with connections to the temporal lobes. Initial studies showed that injections directly into this region with chemicals such as kainate, glutamate, aspartate, and NMDA as well as bicuculline produce seizures. At the same time, treatment with the NMDA receptor antagonist 2-amino-phosphonoheptanoic acid (2APH) or the $GABA_A$ agonist muscimol was found to prevent seizures (33, 34). Further studies attempted to elucidate specific neurotransmitter-receptor pathways through which seizures could propagate from the area tempestas into temporal regions. They found that GABA inhibition was sufficiently antiepileptic throughout the system, while NMDA receptor activation is needed to initiate seizures in the area tempestas and non-NMDA receptors (AMPA and KA) are required for the seizures to spread into temporal regions (35).

Lessons from animal models of epilepsy

In 1989, R.S. Fisher (36) published a review which described over 50 different animal models of epilepsy, several of which are still used today. Many of the neuroactive chemicals that were found to produce seizures have both historical relevance and academic importance in epilepsy. For example, penicillin was found to induce seizures during neurosurgical procedures when it was applied directly to the cortex for antibiotic prophylaxis. Later, while conducting intracellular recordings of cat neocortex following focal penicillin administration, the PDS was first described (37, 38). The chemical pentylenetetrazol (PTZ) was so reliable that it was used in British mental hospitals to provoke seizures to treat psychiatric illness until 1939 when it was supplanted by electroconvulsive therapy. For a time, it was also thought of as the prototypical epilepsy-invoking agent because of its ability to induce spike-wave or polyspike discharges on EEG. It even became an industry standard in AED screening; the ability to prevent PTZ-provoked seizures was associated with a higher chance of preventing primary generalized seizures, especially absence seizures. Much later, PTZ was found to work in part through the $GABA_A$ receptor although the exact mechanism by which it provokes seizures remains unclear. In fact, with the exception of the bromides and phenobarbital, every AED used today was first studied using animal models of epilepsy (36). Of special note, use of the maximal electroshock model to screen barbiturate derivatives led to the discovery of phenytoin in the late 1930s (39).

Fortunately, interest in developing animal models of epilepsy over decades has provided further insight into the role of GABA in epilepsy. In addition to PTZ, there are numerous additional GABA antagonists that have been shown to be effective in producing recurrent seizures in animals, including bemegride ($GABA_A$ antagonist), picrotoxin (non-competitive $GABA_A$ antagonist that acts by blocking the ion channel itself and not the receptor binding site), and bicuculline (competitive $GABA_A$ antagonist). In 1974, Meldrum et al. (40) showed that allylglycine, which inhibits glutamate decarboxylase (GAD) and prevents GABA synthesis, caused prolonged status epilepticus in monkeys. Treiman (41) outlined multiple GABAergic mechanisms found in animal models of epilepsy including alterations in GABA levels, receptor binding, and uptake as well as the antiepileptic properties of drugs which enhance GABA neurotransmission.

The animal models also support a significant role for glutamate in epilepsy. Most animal models of complex partial seizures utilize enhanced excitation either through genetic manipulation, kindling, or the administration of excitatory chemicals. Both glutamate and its structural analogue kainate, first discovered in red algae in 1953 (42), were known to be potent excitotoxins. Kainate became one of the most widely used neuroactive chemicals to induce seizures in animals starting in the late 1970s and 1980s (43). These animals also show structural changes in the brain which are very similar to human MTS. Studies show that mice genetically designed to be deficient in glutamate uptake are susceptible to seizures (44). Those strains without the ability to post-transcriptionally edit glutamate receptor subunits (Q/R GluR-B subunit), causing poorly regulated