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CHLORIDE-MEDIATED INHIBITION OF THE ICTOGENIC NEURONES INITIATING GENETICALLY-DETERMINED ABSENCE SEIZURES

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Abstract—Electroclinical investigations in human patients and experimental studies from genetic models demonstrated that spike-and-wave discharges (SWDs) associated with absence seizures have a cortical onset. In the Genetic Absence Epilepsy Rat from Strasbourg (GAERS), SWDs are initiated by the paroxysmal discharges of ictogenic pyramidal neurones located in the deep layers of the somatosensory cortex. However, the cellular and synaptic mechanisms that control the ictal discharges of seizure-initiating neurones remain unclear. Here, by the means of *in vivo* paired electroencephalographic (EEG) and intracellular recordings in the GAERS cortical focus, we explored the participation of the intracortical inhibitory system in the control of paroxysmal activities in ictogenic neurones. We found that their firing during EEG paroxysms was interrupted by the occurrence of hyperpolarizing synaptic events that reversed in polarity below action potential threshold. Intracellular injection of Cl^- dramatically increased the amplitude of the paroxysmal depolarizations and the number of generated action potentials, strongly suggesting that the inhibitory synaptic potentials were mediated by $GABA_A$ receptors. Consistently, we showed that intracellularly recorded GABAergic interneurones fired, during seizures, shortly after (~+8 ms) the discharge of ictogenic neurones and displayed a rhythmic bursting that coincided with the inhibitory synaptic events in neighbouring pyramidal ictogenic cells. In contrast with other forms of epilepsy, our findings suggest that paroxysmal activities in the cortical pyramidal cells initiating absence seizures are negatively controlled by a feedback Cl^--mediated inhibition likely resulting from the fast recurrent activation of intracortical GABAergic interneurones by the ictogenic cells themselves.

Key words: absence epilepsy, inhibition, epileptic focus, cortex, interneurones, GABA.

Absence seizures, mainly occurring in children of school age, result in transient impairments of consciousness, without convulsion, due to the abrupt appearance of spike-and-wave discharges (SWDs) over the cortical mantle and thalamic nuclei (Panayiotopoulos, 1997; Williams, 1953). Although the origin of SWDs was first attributed to functional disturbances in the intrinsic circuitry of the thalamus (Bal et al., 1995; Buzsáki, 1991), the most recent electroclinical studies in epileptic patients indicate that the onset of absence seizures is correlated with early activation of discrete cortical areas (Holmes et al., 2004; Sadleir et al., 2006; Westmijse et al., 2009). Cortical foci initiating SWDs have also been found in various rodent genetic models of absence epilepsy (Meeren et al., 2002; Polack et al., 2007, 2009). In the Genetic Absence Epilepsy Rat from Strasbourg (GAERS; Danober et al., 1998), the paroxysmal electrical activities first appear in a focus located within the facial somatosensory cortex (Polack et al., 2007). The functional inactivation of this ictogenic region prevents the occurrence of SWDs, whereas the inhibition of its related thalamic nuclei or remote cortical areas does not affect its endogenous ability to generate seizure activity (Polack et al., 2009).

Intracellular recordings in the GAERS revealed that SWDs are initiated in layer 5-6 pyramidal neurones of the cortical focus, which fire action potentials systematically before distant cortical and thalamo-cortical neurones (Polack et al., 2007, 2009). These ictogenic neurones display in between SWDs a distinctive sustained membrane depolarization and elevated tonic firing rate. This pattern of activity is converted during seizures into oscillatory-like paroxysmal depolarizations, leading to repeated brisk firing correlated with the electroencephalogram (EEG) spikes, superimposed on a tonic hyperpolarization lasting for the entire SWD (Polack et al., 2007, 2009; Polack and Charpier, 2009; see also Figs. 1B and 2A3). Moreover, intracortical or systemic injection in the GAERS of ethosuximide, a first choice anti-absence drug, has an anti-epileptic effect (Manning et al., 2004; Polack and Charpier, 2009) that is correlated with the conversion of the hyperactive ictogenic cortical neurones into cortical neurones having normal electrophysiological properties (Polack and Charpier, 2009).

The cellular and network mechanisms controlling the paroxysmal discharges in the cortical ictogenic neurones remain unclear. Investigations in rodent genetic models of absence epilepsy, mostly conducted in slice preparations where seizure activity is absent, pointed out dysregulations in intrinsic and/or synaptic properties that could account for the propensity of somatosensory cortical neurones to gen-

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Abbreviations: EEG, electroencephalogram; FS, fast-spiking; GAERS, Genetic Absence Epilepsy Rat from Strasbourg; IPSPs, inhibitory postsynaptic potentials; LTS, low-threshold spiking; SWD, spike-and-wave discharge.

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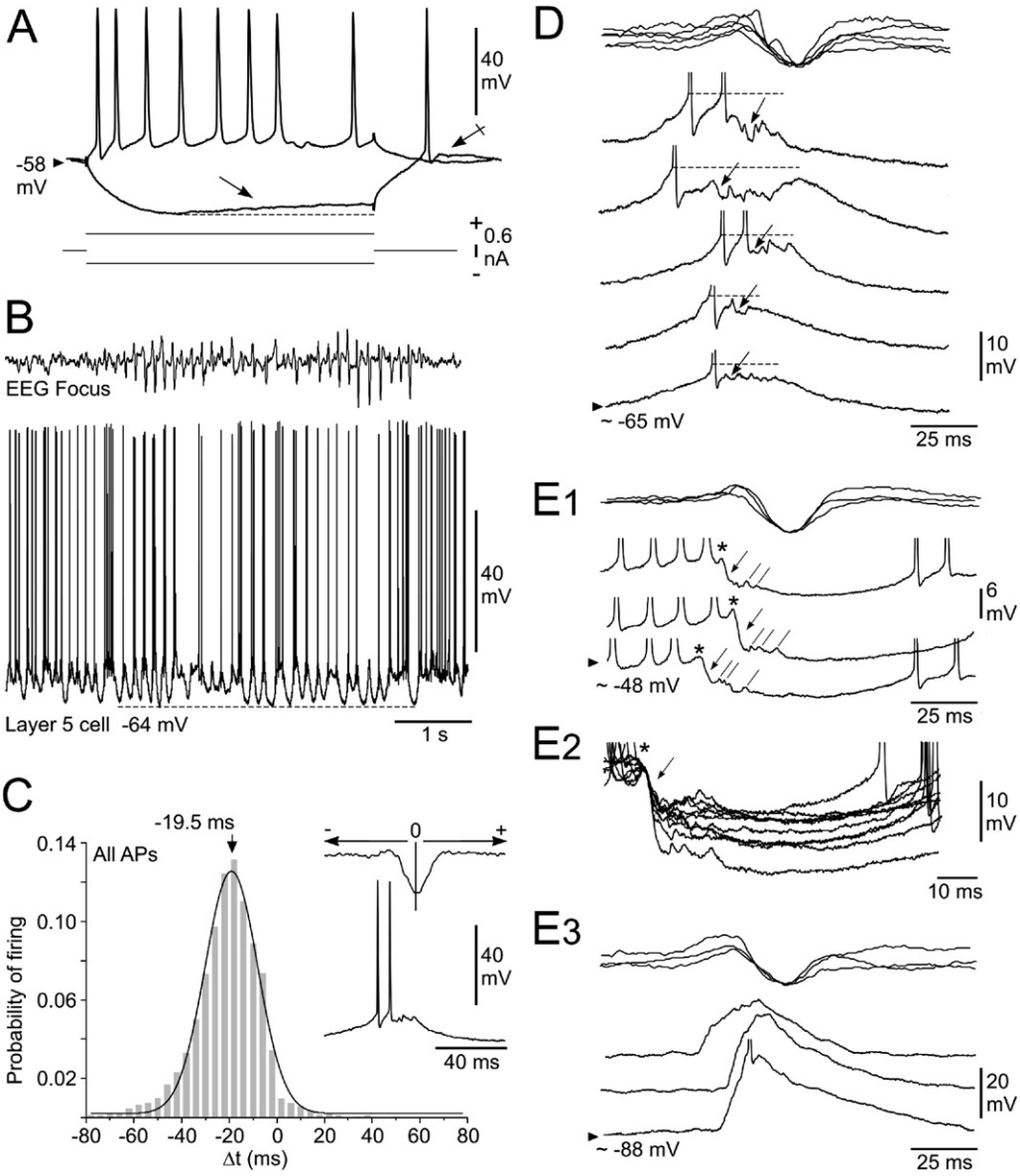

Fig. 1. Evidence for an inhibitory component during paroxysmal depolarizations in ictogenic neurones. (A) Intracellular responses (top records) to depolarizing and hyperpolarizing 200-ms current pulses (bottom traces) from a regular spiking neurone recorded in the layer 5 of the cortical focus. Note the sag in membrane voltage (arrow) during current-induced hyperpolarization and the rebound response at the break of the current pulse (crossed arrow). (B) Intracellular activity of the neurone shown in (A) (bottom traces) simultaneously recorded with the focus EEG (top traces). The occurrence of an SWD in the EEG was accompanied in the ictogenic neurone by rhythmic paroxysmal depolarizations, which were superimposed on a tonic membrane hyperpolarization (dashed line). (C) Pooled distribution of the timing (Δt) of all action potentials (APs; bin size, 4 ms) in ictogenic neurones ($n=12$), using the peak of the EEG spike as the zero-time reference (inset). The distribution was best fitted by a single Gauss-Laplace curve ($r^2=0.99$). Here and in the following figures, the mean value of action potentials timing is indicated at the top of the histogram. (D) Expanded dual records from the experiment shown in (B). The peak of the EEG spike was used to align the intracellular (bottom traces) and EEG records (superimposed top traces). The initial firing on the paroxysmal depolarizations was followed by near threshold (dashed lines) excitatory and inhibitory (arrows) synaptic events. (E) Voltage-dependency of paroxysmal depolarizations. (E1) Three successive intracellular records (bottom traces) from an ictogenic cell, obtained during DC injection of +1 nA, and the corresponding EEG spikes (superimposed top traces). Note the early depolarizing event (asterisks) followed by a brisk hyperpolarization (arrows) and subsequent bunches of small depolarizations (oblique lines). (E2) Superimposition ($n=10$) of the complex synaptic sequences during DC depolarization. The onset of the hyperpolarizing breaks (arrow), which disrupted the initial depolarization (asterisk), was used to align the records. (E3) Same representation as in (E1). The DC (−1 nA) hyperpolarization abolished the hyperpolarizing events and made emerge a large, up to 30 mV, depolarizing shift. In (D, E), action potentials are truncated for commodity. Here and in the following figures, the value of membrane potential is indicated by the arrowhead at the left of the intracellular record.

erate epileptic discharges. Specifically, the exacerbated activity of deep-layer neurones of the cortical focus could result from an increased expression of voltage-gated sodium channels (Klein et al., 2004) and/or a reduction in the dendritic hyperpolarization-activated inward cationic current (I_h) (Strauss et al., 2004). An increase in glutamatergic