Building Up Absence Seizures in the Somatosensory Cortex: From Network to Cellular Epileptogenic Processes

Guillaume Jarre1,2, Tristan Altwegg-Boussac3, Mark S. Williams3, Florian Studer1,2, Mathilde Chipaux4, Olivier David1,2,5, Stéphane Charpier3,6, Antoine Depaulis1,2,5, Séverine Mahon3 and Isabelle Guillemain1,2

1Univ. Grenoble Alpes, Grenoble Institut des Neurosciences, GIN, F-38000 Grenoble, France, 2Inserm, U1216, F-38000 Grenoble, France, 3Inserm U 1127, CNRS UMR 7225, Sorbonne Universités, UPMC Univ Paris 06 UMR S 1127, Institut du Cerveau et de la Moelle épinière, ICM, F-75013, Paris, France, 4Pediatric Neurosurgery Department, Fondation Ophtalmologique A. de Rothschild, 75019 Paris, France, 5CHU de Grenoble, F-38000 Grenoble, France and 6UPMC Univ Paris 06, F-75005, Paris, France

Address correspondence to Isabelle Guillemain, Grenoble-Institut des Neurosciences, Université Grenoble-Alpes – Faculté de Médecine, Chemin Fortuné Ferrini, BP170, 38042 Grenoble cedex 9, France. Email: isabelle.guillemain@univ-grenoble-alpes.fr; Séverine Mahon, Institut du Cerveau et de la Moelle épinière, Hôpital Pitié-Salpêtrière, 47 Boulevard de l’hôpital, F-75013, Paris, France. Email: severine.mahon@upmc.fr

Guillaume Jarre and Tristan Altwegg-Boussac equally contributed to this study
Séverine Mahon and Isabelle Guillemain co-supervised the study

Abstract

The epileptogenic processes leading to recurrent seizures in Genetic Epilepsies are largely unknown. Using the Genetic Absence Epilepsy Rat from Strasbourg, we investigated in vivo the network and single neuron mechanisms responsible for the early emergence of epileptic activity. Local field potential recordings in the primary somatosensory cortex (SoCx), from the second post-natal week to adulthood, showed that immature cortical discharges progressively evolved into typical spike-and-wave discharges following a 3-step maturation process. Intracellular recordings from deep-layer SoCx neurons revealed that this maturation was associated with an age-dependent increase in cortical neurons intrinsic excitability, combining a membrane depolarization and an enhancement of spontaneous firing rate with a leftward shift in their input-output relation. These cellular changes were accompanied by a progressive increase in the strength of the local synaptic activity associated with a growing propensity of neurons to generate synchronized oscillations. Chronic anti-absence treatment before the occurrence of mature cortical discharges did not alter epileptogenesis or the drug efficacy at adulthood. These findings demonstrate that recurrent absence seizures originate from the progressive acquisition of pro-ictogenic properties in SoCx neurons and networks during the post-natal period and that these processes cannot be interrupted by early anti-absence treatment.

Key words: absence epilepsy, early anti-epileptic treatment, epileptogenesis, GAERS, in vivo intracellular recordings, somatosensory cortex

© The Author 2017. Published by Oxford University Press. This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/4.0/), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact journals.permissions@oup.com.
**Introduction**

Idiopathic Generalized Epilepsies, now called Genetic Epilepsies (GE) by the International league against Epilepsy (ILAE) (Berg and Scheffer 2011), represent one-third of all epilepsies (Panayiotopoulos 2005). These epilepsies, most often diagnosed during childhood, are not associated with clear-cut inciting events or detectable structural brain abnormalities (Shorvon 2011) and their early onset suggests that ictogenic processes take place during brain development and maturation (Sanchez and Jensen 2001; Ben-Ari and Holmes 2006, Russo et al. 2016). While understanding the epileptogenic mechanisms occurring during this critical period is 1 of the major challenges in epilepsy research to develop strategies for preventive treatments (Baulac and Pitkänen 2009; Kelley et al. 2009), the cellular and network dysfunctions leading to the emergence of recurrent generalized seizures remain poorly understood (Pitkänen and Engel 2014). The current clinical investigations of genetic epilepsy ontogenesis using electroencephalographic (EEG) or brain imaging approaches are limited due to 1) the early age of seizures onset, 2) the fact that anti-epileptic drug therapy, generally initiated soon after the diagnosis, represents a confounding factor, and 3) the difficulty to set up longitudinal EEG recordings for several years (Grosso et al. 2005; Hirsch and Panayiotopoulos 2005).

Genetic animal models provide a first-choice alternative to study the mechanisms underlying GE's ictogenesis (Consorte et al. 1980; Frankel 2005; Löschner 2011; Guillemain et al. 2012). In particular, rodent models of absence epilepsy (AE), such as GAERS (Genetic Absence Epilepsy Rat from Strasbourg) and WAG/Rij rats (Wistar Albino Glaxo/Rijswijk), have proven to be highly relevant for the study of the pathophysiology of this prototypic form of GE (Danobe et al. 1998; Depaulis and Van Luijtenaar 2006; Depaulis et al. 2016). In both rat strains, spike-and-wave discharges (SWDs), the EEG hallmark of absence seizures, are initiated in adults within the deep layers of the primary somatosensory cortex (SoCx) (Meeren et al. 2002; Polack et al. 2007, 2009; David et al. 2008) by a population of pyramidal neurons exhibiting specific pro-ictogenic electrophysiological properties, combining a depolarized membrane potential and an elevated spontaneous firing rate with an increased intrinsic excitability (Polack et al. 2007, 2009; Billama et al. 2016). Previous ontogenetic studies in GAERS and WAG/Rij rats reported age-dependent changes in the number and duration of SWDs in mature animals (Vergnes et al. 1986; Coenen and Van Luijtenaar 2003), accompanied by modifications in the density of various voltage-gated ion channels in the SoCx (Klein et al. 2004; Kole et al. 2007). Moreover, recent works showed that several months of chronic treatment with anti-epileptic drugs, administered before and after the known onset of mature SWDs, could reduce the number of seizures and prevent changes in ion channels expression (Blumenfeld et al. 2008; Russo et al. 2010, 2011; Sarkisova et al. 2010; Van Luijtenaar et al. 2013; Dezsi et al. 2013; for review, see Russo et al. 2016). Yet, it remains unclear whether anti-epileptic medications restricted to the period preceding the occurrence of SWDs are sufficient to alter the epileptogenic processes.

To investigate the sequential changes taking place in SoCx neurons and networks during epileptogenesis, we performed local field potentials (LFPs) in GAERS and control Wistar rats at different ages, from the 10th (P10) to the 90th (P90) post-natal day, and characterized the electrical membrane properties and synaptic drive of deep-layer pyramidal neurons using in vivo intracellular recordings. Our findings establish that the first epileptiform events were detected in GAERS at the beginning of the third week of life, starting with cortical oscillatory discharges that progressively evolve with age into mature SWDs. Maturation of epileptic activities was associated with dynamical changes in the intrinsic excitability of SoCx neurons together with an augmented synchrony within local synaptic networks. We finally demonstrated that a chronic treatment with anti-absence drugs before the emergence of SWDs was ineffective for preventing epileptogenic processes and does not affect drug efficiency at adulthood.

**Materials and Methods**

**Ethics Approval**

The care and experimental manipulation of the animals were carried out in accordance with the guidelines of the European Union (directive 2010/63/EU) and approved by the Ethical Committees on Animal Experimentation of the “Grenoble-Institut des Neurosciences” and the “Institut du Cerveau et de la Moelle épineuse”.

**Local Field Potential Recordings in Freely Moving Rats**

**Animals**

LFP recordings were performed on GAERS (Grenoble Alpes University, France) aged from P10 to P90 (P10–P14, n = 9; P14–P18, n = 8; P17–P21, n = 2; P20–P24, n = 9; P23–P27, n = 8; P26–P30, n = 7; P40–P60, n = 4; P90, n = 19), and age-matched control Wistar rats (P10–14, n = 8; P17–21, n = 4; P23–27, n = 7) of either sex. Animals were maintained in cages with food and drink *ad libitum* with controlled temperature (21 to 23 °C) and light cycling (12 h/12 h; light period from 7 a.m. to 7 p.m.). All pups were housed with their mothers until weaning at P30.

**Electrodes implantation**

GAERS and Wistar rats were stereotaxically implanted in the primary somatosensory cortex (SoCx) and/or motor cortex (MoCx) with bipolar electrodes (enameled copper wire, Ø 220 µm, Block Germany), under generalized anesthesia. For pups <P20, anesthesia was induced with 4% isoflurane in air and maintained with 1% isoflurane during surgery. P20–P60 rats were anesthetized using a mixture of xylazine (5–10 mg/kg i.p.; Rompun®, Centravet) and ketamine (40–100 mg/kg i.p.; Clorektam1000®, Vetquotinol, Centravet). Incisions and compression points were repeatedly infiltrated with lidocaine (2%; Centravet). The stereotaxic coordinates for SoCx in adult rats were antero-posterior from bregma (AP): −1.3 mm; medio-lateral (ML): 5.0 mm; dorso-ventral (DV): −3.0 mm, and for MoCx, AP: +1.0 mm; ML: 2.0 mm; DV: −1.0 mm (Paxinos and Watson 1986). For pups, these coordinates were individually adapted from the adult ones using the bregma-lambda distance to determine the adult/pup ratio. All the electrodes were soldered to a female micro-connector (BLR150Z; Fischer Elektronik). After surgery, the pups were returned to their litter and mothers were observed to resume normal feeding and nesting behaviors. Animals under chronic pharmacological treatment were implanted at P32–33 with 4 single contact epidural stainless-steel electrodes placed bilaterally over the frontal and parietal cortices. An additional single contact electrode was positioned over the right cerebellum and served as the reference. Animals were allowed to recover for 2 days and were then recorded 1 h twice a week during 1 month and once per week thereafter. At the end of experiments, animals were euthanized by an overdose of pentobarbital (200 mg/kg, i.p.) and brains were removed, frozen at −50 °C in isopentane and cut in coronal sections for histological
processing. Brain sections were then stained with cresyl violet to localize each electrode tip.

**Video-LFP Recordings**

After the post-surgery recovery period, rats were connected to a computer-assisted video-EEG system (System-Flus Evolution™, Micromed). LFP recordings were acquired at 1024 Hz and band-pass filtered between 1–200 Hz. LFP signals, synchronized with the video, were recorded during 1 h in freely moving conditions. The occurrence of cortical discharges in the LFP was always confirmed with the video.

**Detection of Cortical Discharges**

The onset (or end) of a cortical discharge at Stages 2 and 3 was defined as the first (or last) epileptiform event with an amplitude of at least 3 times the standard deviation (SD) of the baseline LFP signal. In agreement with previous studies on AE epileptogenesis (Carçak et al. 2008; Ellens et al. 2009; Akin et al. 2011), this detection criterion was reduced to ≥2 times the SD of the baseline activity for Stage 1 cortical discharges because of their relatively lower amplitude. Only cortical discharges that lasted more than 1 s were included in the analysis and, to avoid selecting sleep-related oscillations, detection was limited to periods of wakefulness. Oscillatory discharges at Stage 1 could be easily distinguished from the 7–14 Hz sleep spindles/spindle bursts (Pinault et al. 2001, 2006; Yang et al. 2009) by their lower internal frequency that ranged from 4 to 6 Hz. Regardless of the stage of epileptogenesis, detection of each cortical discharge was validated using the video to differentiate epileptiform events from physiological activity patterns associated with sleep, exploratory behavior, or grooming (for review, see Danober et al. 1998; Depaulis et al. 2016).

**LFP Signals Analysis**

Time-frequency and spectral analyses of cortical discharges were performed using an in-house developed Matlab code for dynamical analysis of intra-cortical LFPs. For each cortical discharge, the amplitude (square-root of power) of oscillatory activity between 1 and 30 Hz, from 4 s before the onset and up to 4 s thereafter, was obtained using standard methods based on Hanning taper analysis with fixed time-window length set at 2.5 s (Percival and Walden 1993). Time-frequency sampling of the time-frequency plane was 0.1 s/0.2 Hz. The time-frequency plane was averaged over events having linearly adjusted the duration of the discharges to the same arbitrary value. Finally, the amplitude spectra of the cortical discharges were obtained by averaging the amplitude values over time during the whole duration of the discharges. The median of the amplitude spectra over events was chosen to summarize the spectral properties of each stage. For unitary epileptiform event analysis (Fig. 3), only discharges with an amplitude ≥3 times (Stages 2 and 3) and 2 times (Stage 1) the SD of the baseline LFP signal were considered.

**Pharmacological Experiments**

**Acute Injection of Anti-epileptic Drugs in Young Pups**

After a baseline recording period of 30 min, animals aged between P25 and P30 received either ethosuximide (ETHX, 200 mg/kg, i.p.; Sigma-Aldrich), valproate (VPA, 200 mg/kg, i.p.; Sigma-Aldrich) or saline injection in a counter-balanced order to test their sensitivity to these drugs at adulthood (Micheletti et al. 1985) (Fig. 7A). A delay >72 h was observed between the 2 injections to ensure drug elimination/clearance. LFP recordings were performed 30 min before (baseline) and during 1 h after drug injection. Then, the number, cumulated and mean duration of cortical discharges were compared between conditions.

**Chronic Treatment with Anti-epileptic Drug in Young Pups**

Twenty-one GAERS pups from 2 different litters were divided into 3 groups of 7 pups and were injected daily from P5 to P25 with either ethosuximide (ETHX group, 200 mg/kg/day, i.p., n = 7; Blumenfeld et al. 2008), valproate (VPA group, 200 mg/kg/day, i.p., n = 7) or saline (Saline group, n = 7) (Fig. 7A). All pups were housed with their mother until P30 and their weight and general body condition were controlled each day to detect any drug-induced side effects on animal’s development. Particular attention was paid to the possible appearance of lethargic behavior, stirring or pain. We did not observe any weight differences between the 3 groups throughout the treatment period (data not shown).

**Sensitivity to Acute Anti-epileptic Drugs Injection in Pre-treated Adult Animals**

Each group of pre-treated animals (11 weeks old) received 2 doses of ethosuximide (25 mg/kg and 200 mg/kg, i.p.), valproate (75 mg/kg and 200 mg/kg, i.p.) and saline in a counter-balanced order to test their sensitivity to these drugs at adulthood (Franceschetti et al. 1998). Injections were performed 30 min before (baseline) and after (48 h) drug treatment. The onset (or end) of a cortical discharge at Stages 2 and 3 was validated using the video to differentiate each epileptiform event with an amplitude of at least 3 times the standard deviation (SD) of the baseline activity for Stage 1 cortical discharges because of their relatively lower amplitude. Only cortical discharges that lasted more than 1 s were included in the analysis and, to avoid selecting sleep-related oscillations, detection was limited to periods of wakefulness. Oscillatory discharges at Stage 1 could be easily distinguished from the 7–14 Hz sleep spindles/spindle bursts (Pinault et al. 2001, 2006; Yang et al. 2009). Regardless of the stage of epileptogenesis, detection of each cortical discharge was validated using the video to differentiate epileptiform events from physiological activity patterns associated with sleep, exploratory behavior, or grooming (for review, see Danober et al. 1998; Depaulis et al. 2016).
and 10 Wistar rats). Pyramidal neurons were identified by their action potential (AP) properties and firing responses to suprathreshold current pulses. AP half-width was >0.5 ms, a value typically larger than that reported for GABAergic interneurons (Goldberg et al. 2011). As classically described for pyramidal cortical neurons (Connors and Gutnick 1990), recorded cells in adult animals were either regular spiking or intrinsically bursting (Fig. 5A).

**ECoG and Intracellular Signal Analysis**

Spontaneous firing rate of cortical neurons was measured from recording periods (n = 2–5/neuron) of 10–60 s. The coefficient of variation (CV2) of inter-spike intervals (ISIs), which compares adjacent intervals and is independent of firing rate variation (Holt et al. 1996), was calculated as follows: CV2 = (2 * Δτᵢ₋₁ – Δτᵢ)/(Δτᵢ₋₁ + Δτᵢ), where Δτᵢ defined the ISIᵢ. Average membrane potential (Vm) values and the magnitude ofVm fluctuations (Vm SD) were assessed, in between cortical discharges, from recording segments (n = 2–5/neuron) of intracellular activity lasting 10–60 s after removal of APs. AP voltage threshold was defined as the membrane potential at which dV/dt first exceeds 10 V/s (Mahon et al. 2003). The amplitude of APs was calculated as the potential difference between the voltage threshold and the peak after averaging at least 10 waveforms and their duration was measured as the width at half-maximal amplitude. Membrane input resistance (Rm) was calculated during baseline periods, from averaged (n ≥ 10) voltage deflections induced by low intensity hyperpolarizing current pulses (–0.4 nA, 100–200 ms duration, every 1.25 s). The membrane time constant (τr) was derived from an exponential decay fit applied to the current-evoked hyperpolarization. To perform cross-correlations between ECoG and Vm recordings, intracellular voltage traces were median filtered and both signals were downsampled at 0.5–1 kHz after removal of the DC component.

To quantify the transfer function of cortical neurons, we generated firing rate versus injected current (F–I) relationships. The firing rate was measured in response to depolarizing current pulses of increasing intensity (0.1–1.2 nA, 100–200 ms, every 2.25–3.25 s). Since the current-evoked firing could exhibit a trial-to-trial variability due to collisions with the background synaptic activity inherent to in vivo preparations, current pulses of a given intensity were applied 15–25 times and the corresponding firing responses were averaged. As previously described (Mahon and Charpier 2012), we applied linear regressions to F–I curves and determined the threshold current for AP generation, extrapolated as the x-intercept of the linear fit, and the neuronal gain, defined as the slope (γ) of the F–I curve. All data were analyzed using Spike2 version 7.06 (Cambridge Electronic Design) and Origin version 8.1 (OriginLab Corporation) softwares.

**Statistical Analysis**

Numerical values are given as mean ± s.e.m and statistical analyses were done with Prism version 6.05 (Graphpad software Inc.) or SigmaStat version 3.5 (Systat Software Inc.). The different tests and post hoc corrections used for each experiment are mentioned in each figure legend.

**Results**

**Post-natal Evolution of Epileptiform Discharges in the Somatosensory Cortex of Freely Moving GAERS**

We first explored the ontogenesis of absence seizures by performing on freely moving GAERS from P10 to P90, LFP recordings in the primary somatosensory cortex (SoCx), previously identified as the cortical region initiating absence seizures in the adult (Polack et al. 2007; 2009; David et al. 2008). Between P10 and P14, no epileptiform activities could be detected and the comparison of LFP frequency content (1–30 Hz) between GAERS and age-matched control Wistar did not reveal significant differences (data not shown, n = 9 GAERS and 8 Wistar; P > 0.05, 2-way ANOVA with Sidak’s correction). Recurrent oscillatory discharges first appeared in the GAERS SoCx at P15 (Fig. 1A1). We then observed a progressive evolution of these early epileptiform activities into SWDs as the animals matured (Fig. 1A, insets). According to the morphology of the individual epileptiform activities, their proportion and internal frequency, we identified 3 distinct stages in the maturation of cortical discharges (Fig. 1A–D).

**Stage 1**

From P15 to the beginning of the fourth post-natal week (P22), cortical discharges were devoid of classical spike-and-wave (SW) complexes and exclusively composed of oscillatory-like waves (Fig. 1A1,B1, inset). These oscillatory discharges had a fundamental internal frequency of 5.5 ± 0.1 Hz (n = 50 cortical discharges from 6 GAERS) (Fig. 1A1,B1) and were present in 62.5% of GAERS at P15 (n = 5 out of 8 GAERS) and in all animals at P20 (n = 11 GAERS). Long-term duration (≥1 h) spectral analysis of LFP recordings from GAERS and Wistar rats at P21 confirmed that 5-Hz oscillations were only present in epileptic animals (n = 6 GAERS and 4 Wistar; P < 0.05) (Fig. 1C, top panels). Moreover, as soon as Stage 1, oscillatory discharges were associated with behavioral arrest, often accompanied by chewing or slight hypotonia of the neck muscles (see Supplementary Video 1). Thus, the 5-Hz oscillatory discharges, characteristic of GAERS between P15 and P22, likely represent activities that can be considered as an electrical signature of the first stage of epileptogenesis.

**Stage 2**

From P25 to P40, the oscillatory cortical discharges were replaced by epileptiform activities composed of oscillations intermingled with sharp and narrow events closely resembling SW complexes (Fig. 1A2,B2, inset). Oscillations were defined by negative, nearly symmetrical, LFP deflections that were not followed by a consecutive wave, contrasting with the dual profile of SW complexes (Fig. 1B2, inset). During Stage 2, oscillations predominated over SW but their proportion progressively decreased with age (71.4 ± 2.9% at P25, 70.4 ± 4.2% at P30 and 53.4 ± 3.9% at P40) (Fig. 1D). Spectral analysis revealed that these mixed cortical discharges had a fundamental frequency of 5.2 ± 0.08 Hz, followed by a second harmonic at 10.4 ± 0.1 Hz that was absent during Stage 1 (n = 50 mixed cortical discharges from 8 GAERS) (Fig. 1B2). Cortical discharges at Stage 2 were also specific of epileptic animals as evidenced by the increased power of the 5–6 Hz frequency band in GAERS at P25 compared to age-matched control Wistar rats (n = 7 GAERS and 7 Wistar; P < 0.001) (Fig. 1C, lower panels). As for Stage 1, behavioral arrest, chewing and/or slight neck muscles hypotonia were observed during the mixed cortical discharges (see Supplementary Video 2).

**Stage 3**

We defined the beginning of Stage 3 as the post-natal time at which cortical discharges were composed of more than 50% of SW complexes (Fig. 1A3,B3,D). This took place after P40 since the proportion of SW pattern reached 75.4 ± 1.1% at P60 (n = 4 GAERS and 78.7 ± 1.8% at P90 (n = 19 GAERS) (Fig. 1D). Stage 3 discharges had a preferential internal frequency of 7.3 ± 0.05 Hz,
similar to that of SWDs in adult GAERS (Polack et al. 2007; Depaulis et al. 2016) together with a second and third harmonic at 14.6 ± 0.1 and 21.9 ± 0.1 Hz, respectively (n = 50 SW cortical discharges from 8 GAERS) (Fig. 1A3). Concomitantly with these mature cortical discharges, animals displayed behavioral arrest accompanied with whisker twitching and/or chewing and, in most animals, hypotonia of the neck muscles. These behavioral correlates were more stereotyped compared to Stages 1–2
and similar to those classically described in adult GAERS (Danover et al. 1998) (see Supplementary Video 3).

Because absence seizures in adult GAERS are known to be suppressed by specific anti-epileptic drugs (AEDs) (Micheletti et al. 1985; Danover et al. 1998), we tested the effects of 2 of these compounds, ETHX and VPA, on the mixed cortical discharges in P25–P30 GAERS. Injection of ETHX in Stage 2 GAERS (200 mg/kg, i.p.; n = 4) induced a strong sedative effect that precluded any reliable quantification of cortical discharges (data not shown). Injection of VPA (200 mg/kg, i.p.; n = 4) had a less pronounced sedative effect and caused a significant decrease in the cumulated duration (data not shown) and number (Fig. 1E) of cortical discharges compared to saline injection (20 min post-injection, P < 0.001; 40 min post-injection, P < 0.05). The few cortical discharges that persisted 40 min after drug injection were composed of both oscillations and SW, suggesting that VPA affected both LFP patterns. Beyond 1 h post-injection, GAERS pups tended to fall asleep, leading to a decrease of cortical discharges number even after administration of saline. This non-specific change in the number of epileptic discharges likely results from the combined effect of long-duration recordings (>1 h30) and the young age of the animals as this was not observed in our previous pharmacological studies in adults (Deransart et al. 1999, 2000).

Altogether, these data suggest that the recurrent oscillatory activities that emerged in GAERS at P15 represent an early signature of AE as 1) they were not found in age-matched non-epileptic animals, 2) they progressively evolved into SWDs with age, 3) they were associated with behavioral arrest, and 4) they were suppressed by AEDs. These early cortical paroxysms may thus reflect the very first sign of the pro-ictogenic dysfunctions emerging in the SoCx neural network in GAERS.

Leading Role and Maturation of the Somatosensory Cortical Discharges During Epileptogenesis

To determine whether the SoCx exerts a leading role in the occurrence of cortical discharges during the first stages of GAERS epileptogenesis, we performed simultaneous LFP recordings in the SoCx and ipsilateral MoCx and measured the onset delay between the 2 cortical regions at the 3 stages. Regardless of the stage of epileptogenesis, cortical discharges were systematically detected first in the SoCx (Fig. 2A), with a progressive increase in the onset delay from Stage 1 to Stage 3 (Stage 1, ΔMoCx-SoCx = 0.45 ± 0.04 s; Stage 2, ΔMoCx-SoCx = 0.92 ± 0.12 s; Stage 3, ΔMoCx-SoCx = 1.1 ± 0.08 s, n = 50 cortical discharges/stage from n ≥ 4 GAERS; P < 0.01) (Fig. 2B).

We further characterized the progressive maturation of oscillatory activities into SWDs by quantifying the number and mean duration of cortical discharges as a function of the developmental stage. During Stage 1, we found a gradual increase in the number of cortical discharges from P15 to P20 (P15, 1.3 ± 0.4 cortical discharges/h, n = 8 GAERS vs. P20, 13.8 ± 2.5 cortical discharges/h, n = 11 GAERS) together with an increase in their duration (P15, 2.2 ± 0.8 s, n = 8 GAERS vs. P20, 3.4 ± 0.2 s, n = 11 GAERS) (Fig. 2C,D). Similar increases in both number (P25, 46.0 ± 5.5 cortical discharges/h, n = 8 GAERS vs. P40, 71 ± 3.9 cortical discharges/h, n = 4 GAERS) and duration (P25, 5.1 ± 1.1 s, n = 8 GAERS vs. P40, 9.0 ± 0.3 s, n = 4 GAERS) of mixed cortical discharges occurred in the course of Stage 2 (Fig. 2C,D). While the number of discharges at the transition between Stage 2 and 3 still progressed, it rapidly reached a plateau as evidenced by the similar incidence of epileptiform activities at P60 and P90 (P60 = 103.3 ± 9.2 cortical discharges/h, n = 4 GAERS vs. P90, 91.6 ± 6.1 cortical discharges/h, n = 19 GAERS, P > 0.9) (Fig. 2C). However, the duration of SWDs during Stage 3 continued to increase linearly, from 11.8 ± 2.2 s at P60 to 21.7 ± 1.8 s at P90 (Fig. 2D). Noticeably, the number of cortical discharges calculated at P60 and P90 was very close to that previously estimated in P120 GAERS (Pouyatos et al. 2013), suggesting a stabilization of the propensity of SoCx networks to generate seizures from the first part of Stage 3.

These results indicate a rapid progression of epileptogenic processes during the third and fourth post-natal weeks, followed by a period of relative stability. Stage 1 may thus be considered as a “latent” period, Stage 2 would represent the development of the epileptogenesis due to rapid and pronounced modifications in SoCx neurons and networks, and Stage 3 the maturity of the underlying pro-ictogenic processes.

Progressive Shaping of Absence Seizure Electrical Signature: From Oscillation to SW Complex

We further characterized the age-dependent changes in the morphology of cortical discharges by comparing the average LFP waveform between the different stages of epileptogenesis (Fig. 3A). During Stage 1, LFP activity was dominated by nearly symmetrical oscillations of relatively small amplitude (Fig. 3A, left). The negative part of these oscillations had a mean initial slope of −6.4 ± 0.5 mV/s, a half-duration of 69.4 ± 2.1 ms and a total duration of 84.9 ± 1.9 ms (n = 19 cortical discharges, n = 4 GAERS) (Fig. 3A, left and B). Marked changes arose during the transition from Stage 1 to Stage 2. Averaged unitary epileptiform pattern became more variable in shape and amplitude and a pronounced narrowing of the negative deflection was observed (Fig. 3A, middle). This was reflected by an increase in the negative slope (−19.8 ± 1 mV/s, n = 29 cortical discharges from 4 GAERS; P < 0.001) associated with a decrease in the half (28.6 ± 1.4 ms, n = 29 cortical discharges from 4 GAERS; P < 0.001) and total (56.2 ± 3.5 ms, n = 29 cortical discharges from 4 GAERS; P < 0.01) duration of the negative deflection (Fig. 3A, middle and B). On average, we observed a 3-fold increase of the negative slope value between Stages 1 and 2, together with a 2.4-fold and 1.5-fold decrease in its half-duration and total duration, respectively (Fig. 3B). During Stage 2, we noticed the sporadic occurrence of a small positive deflection following the negative component, which exhibited, however, a large variability in shape and duration (arrow in Fig. 3A, middle). At Stage 3, as typically observed in adult GAERS (Depaulis et al. 2016), averaged individual epileptiform pattern was relatively stereotyped and composed of a large and narrow negative spike followed by a positive wave lasting 50–70 ms (Fig. 3A, right). The duration of the negative deflection was considerably reduced compared to Stage 2 (half-duration = 17 ± 0.6 ms, total duration = 33 ± 1.5 ms, n = 23 cortical discharges, n = 4 GAERS; P < 0.001 and P < 0.01) whereas its negative slope was enhanced (36 ± 1.6 mV/s, n = 23 cortical discharges, n = 4 GAERS; P < 0.001) (Fig. 3A,B). The average duration of the negative spike at Stage 3 was consistent with that previously measured in adult WAG/Rij rats (Sitnikova and van Luijtelaar 2007) and conforms the standard criteria of spike detection used by clinicians (Chatrian et al. 1974).

These findings demonstrate that epileptogenesis in GAERS is associated with a progressive tightening of the negative part of LFP waveforms coupled with the emergence of a subsequent positive wave, finally leading to a typical SW complex. Previous in vivo investigations from adult GAERS reported that the spike
component of the SW complex is associated with synaptic depolarization and brisk firing in cortical pyramidal neurons while neurons are hyperpolarized and silent during the wave component (Depaulis et al. 2016; see also Fig. 6A). The shaping of SW activity during the development of absence seizures may thus reflect the progressive establishment of synchronized oscillatory activities in SoCx synaptic networks.

**Age-dependent Alterations in Background Activity and Intrinsic Excitability of GAERS Somatosensory Cortex Neurons**

The post-natal transformation of oscillatory discharges into mature SWDs described from LFP recordings in freely moving GAERS could result from progressive functional alterations in SoCx neurons and networks, including changes in the excitability of individual neurons, the strength of network synaptic activity and/or the propensity of interconnected cortical neurons to generate synchronized oscillations. To explore these potential epileptogenic processes, we performed in vivo intracellular recordings of deep-layer pyramidal SoCx neurons, simultaneously with the corresponding surface ECoG, in GAERS under sedation and analgesia at the 3 developmental stages, and in age-matched control Wistar rats (see Materials and Methods). We found that most of the pro-ictogenic properties of deep-layer SoCx pyramidal neurons, supposed to promote SW activity in adult GAERS (Polack et al. 2007, 2009; Chipaux et al. 2011; Williams et al. 2016), progressively developed during the post-natal period.

Regardless of the age period examined, background intracellular activity in between cortical discharges in GAERS (Fig. 4A1) and in control animals (Fig. 4A2) was characterized by a continuous barrage of intermingled high-frequency depolarizing and hyperpolarizing synaptic potentials that resulted in unimodal distribution of membrane potential (V_m) values (Fig. 4B1, 4B2, left). While the mean V_m of cortical neurons was stable across age in control animals (P > 0.6), we observed a gradual membrane depolarization during the post-natal period in GAERS. The mean level of membrane polarization at Stage 1 (V_m = −64.4 ± 0.6 mV, n = 12 neurons from 5 rats), which was similar to that measured in age-matched control animals (Wistar P17, V_m = −65.3 ± 0.7 mV, n = 11 neurons from 4 rats; P > 0.3), shifted to a more depolarized value during Stage 2 (V_m = −62.3 ± 0.8 mV, n = 10 neurons from 5 rats; P < 0.05) and reached −59.4 ± 0.6 mV (n = 11 neurons from 8 rats).
at Stage 3 (P < 0.001 vs. Stage 1 and P < 0.01 vs. Stage 2) (Fig. 4A,B). Despite the relative membrane hyperpolarization of cortical neurons in younger GAERS, which could have amplified the amplitude of depolarizing synaptic potentials via an increase in the driving force of synaptic currents, the amplitude of spontaneous Vm fluctuations at Stage 1 (Vm SD = 1.8 ± 0.2 mV, n = 12 neurons from 5 rats) was significantly smaller than that measured at Stage 2 (Vm SD = 2.3 ± 0.1 mV, n = 10 neurons from 5 rats; P < 0.05) and in adult GAERS (Vm SD = 2.9 ± 0.2 mV, n = 11 neurons from 8 rats; P < 0.001 vs. Stage 1 and P < 0.05 vs. Stage 2) (Fig. 4A1). The increase in spontaneous Vm fluctuations could result from a progressive enhancement of the ongoing synaptic drive and/or an increase in neuronal membrane input resistance (Rm) and time constant (rm) during cortical maturation in GAERS. An increase in the resting membrane excitability could be eliminated. Indeed, averaged values of Rm (Stage 1, Rm = 24.8 ± 2.0 MΩ, n = 12 neurons from 5 rats; Stage 2, Rm = 24.2 ± 2.3 MΩ, n = 10 neurons from 5 rats; Stage 3, Rm = 21.5 ± 2.5 MΩ, n = 11 neurons from 8 rats; P > 0.5) and rm (Stage 1, rm = 9.5 ± 1.1 ms, n = 11 neurons from 5 rats; Stage 2, rm = 7.4 ± 1.0 ms, n = 10 neurons from 5 rats; Stage 3, rm = 8.7 ± 1.0 ms, n = 11 neurons from 8 rats; P > 0.3) were similar at the different developmental stages and did not differ from Rm and rm values measured in control neurons (P > 0.2 for both parameters) (Fig. 5A, lowest records and C).

The age-dependent depolarizing shift in GAERS neurons was associated with a significant increase in the spontaneous firing frequency from Stage 1 (0.2 ± 0.1 Hz, n = 11 neurons from 5 rats) to Stage 2 (2.0 ± 0.8 Hz, n = 10 neurons from 5 rats; P < 0.05). The rise in firing rate was also important between Stages 2 and 3 (15.5 ± 2.3 Hz, n = 11 neurons from 8 rats; P < 0.001) with, however, a high cell-to-cell variability at adult age (Fig. 4A1,B1). Consistent with the lack of changes in the mean Vm or Rm values of control neurons during post-natal development, their spontaneous firing was found similar in the different age groups (Wistar P17, 2.3 ± 0.9 Hz, n = 11 neurons from 4 rats; Wistar P30, 2.2 ± 1.4 Hz, n = 6 neurons from 4 rats; Wistar P ≥ 90, 2.9 ± 1.0 Hz, n = 10 neurons from 10 rats; P > 0.6) (Fig. 4B2). The firing rate in Stage 1 GAERS was too low (<0.5 Hz) to allow a reliable quantification of its regularity. However, we found that the CV2 of ISIs at Stage 2 (1.0 ± 0.1, n = 5 neurons from 3 rats) was higher than that calculated in adult GAERS (0.72 ± 0.02, n = 10 neurons from 7 rats; P < 0.01) and comparable to that of control neurons (P > 0.1) (Fig. 4B1,B2). The enhanced firing frequency of adult GAERS neurons was associated with a shorter AP half-width (Stage 1, 1.07 ± 0.09 ms, n = 12 neurons from 5 rats; Stage 3, 0.57 ± 0.02 ms, n = 11 neurons from 8 rats; P < 0.001) and a lower voltage firing threshold (Stage 1, −48.7 ± 1.1 mV, n = 11 neurons from 5 rats; Stage 3, −52.4 ± 0.8 mV, n = 11 neurons from 8 rats; P < 0.01).

These results indicate that epileptogenesis in GAERS is associated with an increase in the rate and rhythmicity of spontaneous firing in SoCx deep-layer neurons, a phenomenon that was not observed in control Wistar rats. In line with these observations, most of GAERS cortical neurons at Stage 3 (n = 6 out of 11)
could generate intrinsic bursts of APs in response to depolarizing current pulses (Fig. 5A1, right), whereas this intrinsic firing profile was absent during Stages 1 and 2 (Fig. 5A1, left).

We next examined whether these age-dependent cellular changes were associated with modifications in the transfer function of cortical neurons, which describes the relation between excitatory inputs of varying amplitude and neuronal output defined as AP generation (Silver 2010). We thus generated F–I relationships in GAERS neurons from Stage 1 (n = 9 neurons from 5 rats) and Stage 3 (n = 6 neurons from 5 rats), the 2 extremes stages of epileptogenesis, and in age-matched control Wistar rats (Wistar P17, n = 10 neurons from 4 rats; Wistar P30, n = 6 neurons; and P ≥ 90, n = 12 neurons). Dashed lines indicate −60 mV.

**Figure 4.** Age-dependent changes in membrane potential and spontaneous firing of GAERS somatosensory cortex neurons. (A) Electroencephalographic (ECoG) activity (top traces) and corresponding intracellular recordings (V_m, bottom traces) obtained, in between cortical discharges, from layer 5 somatosensory cortex pyramidal neurons of GAERS at Stages 1, 2, and 3 (A1), and in age-matched (P17, P30, and P ≥ 90) control Wistar rats (A2). Dashed lines indicate −60 mV. (B1) Left, V_m distribution histograms (1 mV bin) computed from the cells illustrated in (A1). Right, population data showing that the post-natal development of GAERS neurons is associated with a progressive depolarization of their mean V_m (Stage 1, n = 12 neurons; Stage 2, n = 10 neurons; Stage 3, n = 11 neurons), an increase in their spontaneous firing rate (Stage 1, n = 11 neurons; Stage 2, n = 10 neurons; Stage 3, n = 11 neurons) and in the regularity of AP discharge (CV2 ISI; Stage 2, n = 5 neurons; Stage 3, n = 10 neurons). (B2) Left, V_m distribution histograms (1 mV bin) computed from the cells illustrated in (A2). Right, population data comparing the mean V_m (P17, n = 11 neurons; P30, n = 6 neurons; and P ≥ 90, n = 12 neurons), the spontaneous firing rate (P17, n = 11 neurons; P30, n = 6 neurons; P ≥ 90, n = 12 neurons) and the firing regularity (CV2 ISI; P17, n = 7 neurons; P30, n = 4 neurons; P ≥ 90, n = 10 neurons) across the different groups of control Wistar rats. Here and in similar population graphs, each open circle represents an individual neuron and filled circles represent the corresponding mean value ± s.e.m. Significance was assessed using ANOVA (V_m and CV2 ISI) or Kruskal–Wallis ANOVA on ranks (firing rate). *P < 0.05; **P < 0.01; ***P < 0.001; ns, non-significant.
Figure 5. Developmental changes in membrane excitability and transfer function of GAERS cortical neurons. (A) Typical voltage responses of deep-layer somatosensory cortical neurons (top records), recorded during Stage 1 and Stage 3 in GAERS (A1) and in age-matched control Wistar rats (A2), to injection of depolarizing (+0.2 and +0.4 nA) and hyperpolarizing (−0.4 nA) current pulses (bottom traces). The responses induced by the negative current pulses were averaged from at least 10 successive trials. (B) Left, Corresponding F–I curves computed from the neurons shown in (A1) (blue circles, Stage 1; red circles, Stage 3) and (A2) (right gray circles, P17; black circles, P ≥ 90). The dashed lines represent the best linear fit after subtraction of the baseline firing rates. Right, Summary plot comparing the intensity of threshold current (Ith) and neuronal gain (γ) values across the different experimental groups. (GAERS Stage 1, n = 9; GAERS Stage 3, n = 6; Wistar P17, n = 10; Wistar P ≥ 90, n = 10). (C) Population data showing the constancy of Rm values in the 2 rat strains. (GAERS Stage 1, n = 12; GAERS Stage 3, n = 11; Wistar P17, n = 11; Wistar P ≥ 90, n = 12). (D) The increased firing rate in response to current pulses of +0.2 nA in GAERS cortical neurons at Stage 3 (left) was associated with a decrease in the first spike voltage threshold (middle) and first spike latency (right). Pooled data depicted in (D) are from 9 neurons recorded at Stage 1 and 6 neurons recorded at Stage 3. Significance was assessed using ANOVA (B–C) or unpaired t-test (D). **P < 0.01; ***P < 0.001; ns, non-significant.

Wistar P ≥ 90, n = 10 neurons from 8 rats) (Fig. 5A1,A2,B). The extrapolated values of threshold current (Ith) calculated at Stage 1 in GAERS (Ith = 0.08 ± 0.05 nA, n = 9 neurons), were similar to those measured in control neurons from P17 and adult animals (Wistar P17, Ith = −0.04 ± 0.03 nA, n = 10 neurons; Wistar P ≥ 90, Ith = 0.10 ± 0.07 nA, n = 10 neurons; P > 0.1), but significantly dropped in older GAERS (Ith = −0.34 ± 0.09 nA, n = 6 neurons; P < 0.001) (Fig. 5B). The lower firing threshold in Stage 3 GAERS neurons was likely influenced by their high background firing frequency. However, even after subtracting the baseline firing rate from F–I curves (dashed lines in Fig. 5B, left), the current-evoked firing threshold at Stage 3 remained lower than that calculated during Stage 1 (Stage 1, Ith = 0.08 ± 0.05 nA, n = 9 neurons; Ith = −0.10 ± 0.06 nA, n = 6 neurons; P < 0.05). This leftward sliding of F–I relation with age in GAERS was accompanied by a strong increase of firing responses to weak inputs (+0.2 nA) (Stage 1, 8.9 ± 1.8 Hz, n = 9 neurons vs. Stage 3, 34.7 ± 5.6 Hz, n = 6 neurons; P < 0.001), a lowering in the voltage threshold for the first AP (Stage 1, −47.1 ± 1.1 mV, n = 9 neurons vs. Stage 3, −53.6 ± 0.6 mV, n = 6 neurons; P < 0.001) and a reduction of its latency (Stage 1, 24.1 ± 3.2 ms, n = 6 neurons vs. Stage 3, 12.1 ± 1.1 ms, n = 6 neurons; P < 0.01) (Fig. 5D). Consistent with the stability of Rm values across the different experimental groups (Fig. 5C), the functional gain of GAERS neurons, measured as the slope (γ) of F–I curves, was not affected between Stage 1 and Stage 3 (Stage 1, γ = 69.4 ± 9.6 Hz.nA−1, n = 9 neurons; Stage 3, γ = 74.7 ± 15.4 Hz.nA−1, n = 6 neurons; P > 0.7) and was similar to that of control neurons (Wistar P17, γ = 62.7 ± 3.7 Hz.nA−1, n = 10 neurons; Wistar P ≥ 90, γ = 77.3 ± 8.3 Hz.nA−1, n = 10 neurons; P > 0.5) (Fig. 5B). These findings demonstrate that the sensitivity to weak inputs of SoCx deep-layer neurons in GAERS increases with age but that the dynamic change of their responsiveness over a wide range of inputs is not affected.
Neuronal Correlates of Developing Epileptiform Activities

To unveil the neuronal mechanisms underlying the progressive maturation of epileptiform activity, from 5-Hz oscillatory discharges to 7–8 Hz SWDs, and the sharpening of unitary epileptiform pattern (Fig. 3), we examined the intracellular activities of SoCx deep-layer neurons during the cortical discharges at the 3 developmental stages.

At the occurrence of oscillatory discharges (Stage 1), mixed discharges (Stage 2), and SWDs (Stage 3), the disorganized membrane potential fluctuations in pyramidal cortical neurons were replaced by clusters of rhythmic depolarizing synaptic potentials (Fig. 6A, left). At Stages 1 and 2, the individual
intracellular events, concomitant with the EcoG waveforms, were sculpted by the temporal summation of small depolarizing synaptic potentials (Fig. 6A, right). They increased in amplitude and reduced in duration with age, leading to relatively smooth and large (15–20 mV) synaptic depolarizations in the adult GAERS (Fig. 6A, Stage 3 in the right column). This suggests a progressive reinforcement of synaptic synchronization within SoCx networks during epileptogenesis, in agreement with the age-dependent tightening and increase in amplitude of the LFP/ECoG components (Figs 4 and 6A, right). An increasing coherence in the collective activity of cortical neurons is further supported by the enhancement of the correlation level (Fig. 6B, C, left) and the decrease in the time delay (Stage 1, −34.1 ± 3.8, n = 6 neurons from 3 rats; Stage 2, −24.5 ± 6.7, n = 5 neurons from 4 rats; Stage 3, −13.9 ± 2.4, n = 9 neurons from 6 rats; P < 0.01 vs. Stage 1, P = 0.06 vs. Stage 2) between intracellular and ECoG oscillatory waveforms (Fig. 6A right and B), which paralleled the increase in the internal frequency of cortical oscillations with age (Figs 1B and 6C, right).

The age-dependent increase in the amplitude of rhythmic synaptic depolarizations led to a progressive enhancement of the neuronal firing rate during cortical discharges. Indeed, the mean firing rate during SWDs at Stage 3 (10.1 ± 1.5 Hz, n = 230 SWDs from 10 neurons) was significantly higher than during oscillatory discharges at Stage 1 (1.0 ± 0.6 Hz, n = 225 oscillatory discharges from 6 neurons; P < 0.001) and mixed discharges at Stage 2 (1.6 ± 1.0 Hz, n = 280 mixed discharges from 5 neurons; P < 0.01) (Fig. 6A,D, right). As previously described (Charpier et al. 1999; Polack et al. 2007; Williams et al. 2016), ictal intracortical oscillations in adult GAERS were superimposed on a tonic membrane hyperpolarization that was maintained throughout the SWD and had an amplitude of 9.3 ± 1.0 mV (n = 230 SWDs from 10 neurons) (Fig. 6A, lowest record and D, left). This sustained polarization of neurons was significantly smaller during the early stages (Stage 1, 3.7 ± 0.9 mV, n = 225 oscillatory discharges from 5 neurons; Stage 2, 4.8 ± 0.9 Hz, n = 280 mixed discharges from 5 neurons; P < 0.01 compared to Stage 3) (Fig. 6A,D, left). The maximal level of membrane polarization reached during cortical oscillations, which was similar at the 3 stages (Stage 1, −67.0 ± 1.7 mV, n = 225 oscillatory discharges from 5 neurons; Stage 2, −69.0 ± 1.5 mV, n = 280 mixed discharges from 5 neurons; Stage 3, −69.4 ± 0.8 mV, n = 230 SWDs from 10 neurons; P > 0.4), was significantly lower compared to the corresponding baseline Vm (arrowheads in Fig. 6A) (P < 0.05 for each paired comparison). This finding is consistent with our initial hypothesis that the sustained hyperpolarization of GAERS neurons during SWDs probably results from a process of synaptic disfacilitation, that is, a transient interruption in the ongoing synaptic drive, setting passively the cell to its resting potential (Charpier et al. 1999; Depaulis et al. 2016).

Altogether, our findings indicate that the post-natal maturation of cortical epileptic activities in the GAERS is correlated with a progressive increase in the magnitude and level of synchronization of neuronal oscillations in the deep layers of the SoCx.

Effect of Chronic Treatments with Anti-epileptic Drugs Before SWDs Onset

Based on our results showing a progressive evolution of epileptic cortical discharges during the post-natal period, associated with age-dependent changes in the electrophysiological features of SoCx neurons and networks, we hypothesized that a chronic anti-epileptic treatment throughout the period preceding the occurrence of SWDs could alter the evolution of the disease.

GAERS pups were injected daily from P5 to P25 with saline or first-choice AEDs (i.e., ETHX or VPA), at doses known to suppress SWDs in adult GAERS (200 mg/kg/day, i.p., Micheletti et al. 1985). Changes in the properties of cortical discharges were assessed from P35 to P90 using long-term monitoring of LFP activity (Fig. 7A). At P35, cortical discharges were still observed in the 3 groups and their number (Saline, 78.7 ± 7.8 cortical discharges/h; VPA, 65.1 ± 15.5 cortical discharges/h, P = 0.43 vs. Saline, ETHX: 102.7 ± 11.6 cortical discharges/h, P > 0.30 vs. Saline, P > 0.08 vs. VPA) as well as their cumulated duration (Saline, 474.9 ± 69.2 s/h; VPA, 416.7 ± 77.1 s/h, P = 0.73 vs. Saline; ETHX: 610.1 ± 57.1 s/h, P > 0.64 vs. Saline, P > 0.58 vs. VPA) were similar (Fig. 7B,C). This lack of changes in the properties of cortical discharges between the different cohorts persisted until P90, although animals treated with ETHX showed a tendency to display more numerous and longer epileptic activities (Fig. 7B,C). In addition, we found that the composition of cortical discharges (proportion of oscillation vs. SW) was also not affected by the pharmacological procedures (data not shown).

Seven weeks after the end of the chronic anti-epileptic treatment, pre-treated animals from the 3 groups were challenged with acute injections of low and high concentrations of ETHX (25 and 200 mg/kg) or VPA (75 and 200 mg/kg). The effects of drug injections on the amount of SWDs were compared with injections of saline, applied in a counter-balanced order (see Materials and Methods). Regardless of the experimental group, the number of cortical discharges was decreased by 2-fold following the injection of ETHX at low concentration and completely suppressed after administration of larger doses (Fig. 7D). A similar dose-dependent decrease was obtained after VPA injection, with a slight diminution and a quasi-suppression of cortical discharges for low and large drug concentrations, respectively (Fig. 7E). This dose-dependent attenuation of SWDs was analogous to that previously reported in adult GAERS that did not undergo the chronic drug treatment (Micheletti et al. 1985).

Our results indicate that an early treatment with ETHX or VPA does not alter the occurrence of SWDs or the efficiency of AEDs in adult GAERS.

Discussion

To our knowledge, the present study provides the first in vivo description of the developmental alterations occurring in cortical networks and neurons during GE’s epileptogenesis. In a well-established animal model of AE, we found that epileptiform activity progressively developed in the SoCx, from 5-Hz oscillatory discharges at the beginning of the third post-natal week to 7–8 Hz SWDs in adults. This was accompanied by an increase in the number and duration of cortical discharges and by a progressive sharpening of the individual cortical epileptiform event composing the discharges. Intracellular recordings of SoCx deep-layer pyramidal neurons demonstrated that maturation of cortical discharges in GAERS was accompanied by a progressive increase in the intrinsic excitability of cortical neurons, a strengthening of the local synaptic activity and an enhanced ability of cortical networks to generate synchronized oscillations. Finally, while acute injections of AEDs were found to be effective in reducing the number of both immature and mature cortical discharges, a chronic anti-epileptic treatment prior to seizures onset failed to prevent the development of the disease.
Evolution of Cortical Discharges During Post-natal Brain Maturation in GAERS

Our findings obtained from freely moving animals show that the early oscillatory discharges that emerge in the GAERS SoCx around P15 progressively evolve into SWDs with age. Based on cortical discharges properties, we propose that the development of epileptic activity in GAERS follows 3 different stages of maturation. At first stage, between P15 and P22, cortical discharges were of short duration and relatively rare. They were exclusively composed of rhythmic, nearly symmetrical, oscillations with a fundamental frequency around 5 Hz. During Stage 2, between P25 and P40, epileptiform discharges were composed of both oscillations and mixed events closely resembling SW complexes, and their number (45–70/h) and duration (5–9 s) increased. After P40, maturation processes reached an ultimate stage where cortical discharges contained a majority of SW and occurred ~90 times per hour. Remarkably, the internal frequency of mature cortical discharges was shifted towards the typical frequency range (around 7–8 Hz) of SWDs in adult GAERS (Danober et al. 1998; Depaulis et al. 2016). They had longer duration (10–25 s) and exhibited an increased rhythmicity as evidenced by the appearance of second and third harmonics in power spectra. Our data further suggest that the 5-Hz oscillatory cortical discharges observed during the third and the fourth post-natal weeks represent an early signature of AE. Indeed, such cortical activities, which could not be detected in age-matched control animals, correlated with a behavioral arrest and were suppressed by VPA, an AED classically used in the clinic to suppress absence seizures. Interestingly, the presence of immature, transitional and mature epileptic discharges has also been reported in a mouse model of familial childhood epilepsy, the GABA_Aγ 2( R 4 3 Q)mutant mice (Tan et al. 2007) as well as in C3H/HeJ mice, another genetic model of AE (Ellens et al. 2009). Although the emergence of immature SWDs occurred earlier in C3H/HeJ compared to GAERS, similar age-dependent changes in the number, duration and morphology of cortical discharges were observed (Carçak et al. 2008; Ellens et al. 2009). In the WAG/Rij rat model, SWDs also evolve with age although the onset of seizures occurs at adulthood.
around 2–3 months after birth (Coenen and van Luijeltaar 1987; Kole et al. 2007).

Noticeably, we found that epileptic discharges start to appear in GAERS just after the end of the second post-natal week (≥P15), which corresponds to a key period of SoCx maturation in normal rats. During the early post-natal period, neocortical sensory networks generate successively different types of highly coherent activities (early network oscillations, spindle bursts, gamma bursts, giant depolarizing potentials) (Adelsberger et al. 2005; Allene et al. 2008; Yang et al. 2009; Minlebaev et al. 2011 for review see Luhmann et al. 2016) that are replaced by more sparse and less correlated firing patterns at the end of the second post-natal week (Golshani et al. 2009; Rochfort et al. 2009). These early synchronous network activities, which are associated with a number of structural changes such as axonal growth, increase in the complexity of dendrites and in the number of synapses (Bureau et al. 2004; Feldmeyer 2012; for review see Luhmann et al. 2016 and Hangauer-Opatz 2010), are known to be critical for the refinement of circuits and the establishment of sensory cortical maps (Mitrukhina et al. 2015). Thus, it seems likely that subtle alterations in these fundamental maturation processes may lead to dysrhythmic activities such as epileptic discharges.

Our multisite LFP recordings from the primary somatosensory and motor cortical regions indicate that cortical discharges first appeared in the SoCx even at the first stage of epileptogenesis, supporting the critical role of the SoCx in the initiation of cortical discharges. The reasons for the age-dependent increase in the propagation delay between both cortices remain unclear. It could reflect maturation of the sensorimotor circuits, including an extension of brain size that would increase the distance of propagation and/or a synaptic refinement. Moreover, the flow of epileptic discharges within the cortico-thalamic-cortical networks has been shown to follow complex spatiotemporal dynamics in rodent models of AE (Meeren et al. 2002; Polack et al. 2009). It is thus plausible that the maturation of epileptic networks be associated with alternate changes in the directionality of propagated activities between intra-cortical and long-range thalamo-cortical loops, a dynamic process that could extend the delay of activation between SoCx and MoCx.

**Cellular and Synaptic Changes in the GAERS Somatosensory Cortex During Epileptogenesis**

Our combined intracellular and ECoG recordings in GAERS and control Wistar rats have established that deep-layer neurons of the GAERS SoCx display distinctive post-natal changes in their electrophysiological properties that parallel the maturation of cortical discharges. The absolute value of their Vm was gradually reduced, reaching a mean value at adulthood significantly more depolarized compared to homologous neurons in control rats. These results extend our previous findings showing that the depolarized Vm of GAERS neurons in adult animals was specific to the SoCx and to the layers 5–6 of this cortical region (Polack et al. 2007, 2009; Polack and Charpier 2009; Williams et al. 2016). The different maturation profile of resting membrane properties in epileptic and control neurons is further supported by former in vitro studies describing either a progressive membrane hyperpolarization (Kriegstein et al. 1987; Zhu 2000; Frick et al. 2007) or nearly unchanged Vm values (Franceschetti et al. 1998; Stern et al. 2001) in SoCx pyramidal neurons of normal rats during post-natal development. Altogether, these results suggest that the age-dependent depolarization of GAERS SoCx neurons is a specific feature of absence epileptogenesis. The progressive change in Vm and the associated increase in the spontaneous firing could originate from alteration in the density of voltage-gated ion channels and/or an increase of the depolarizing synaptic drive. Consistently, mRNA and protein expression of sodium channel genes Nav1.1 and Nav1.6 is up-regulated selectively within pyramidal neurons in the SoCx of WAG/Rij rats and follows the age-dependent increase in seizures number and duration (Klein et al. 2004). An overexpression of sodium channels could be responsible, at least in part, for the age-dependent increase in tonic and bursting firing activities of SoCx deep-layer neurons. The enhanced propensity of cortical neurons to generate burst firing at adulthood (present study; Polack et al. 2007) could also be promoted by an age-dependent reduction of the hyperpolarization-activated cationic current Ih, like in WAG/Rij rats (Strauss et al. 2004; Kole et al. 2007).

The depolarization of SoCx deep-layer neurons was accompanied by a gradual increase in Vm fluctuations, suggesting a strengthening of the network synaptic activity. This could be caused by the rapid development of neocortical networks during the first weeks of life, including an increased synaptic connectivity between pyramidal neurons and a refinement of sensory maps, which results in an amplification of sensory-evoked depolarizing synaptic responses (Micheva and Beaulieu 1996; Stern et al. 2001; Bureau et al. 2004; Feldmeyer et al. 2013). The physiological development of SoCx networks could operate in synergy with pro-epileptogenic alterations in local synaptic transmission. As observed in WAG/Rij rats, an alteration in glutamatergic NMDA, AMPA or mGlu receptor-dependent synaptic transmission (van de Boovenkamp-Janssen et al. 2006; Ngomb a et al. 2005; D’Amore et al. 2013; Russo et al. 2016) could further facilitate the development of paroxysmal activities within the SoCx. Such changes remain to be confirmed in GAERS.

Pyramidal neurons from the GAERS SoCx also exhibited developmental intrinsic changes that may promote synchronized collective oscillations and participate to the progressive transformation of relatively slow cortical oscillations into SW activity. The comparison of F–I relations between epileptic and control neurons during post-natal development indicates that absence epileptogenesis is associated with a marked reduction in the minimal stimulus intensity required to induce firing in cortical neurons, a lowering of the voltage threshold for evoked APs and a shortening of their latency. This cortical hyperexcitability could result from the steady depolarization of neurons with age (Altwegg-Boussac et al. 2014) or from a reduction in dendritic Ih (Strauss et al. 2004; Kole et al. 2007). This should facilitate the synaptic excitation of cortical neurons embedded in an over-activated network and increase the reliability and temporal coherence of firing, as expected for ictogenic activity. Finally, changes in the pro-ictogenic properties of SoCx networks are likely influenced by developmental alterations in the synaptic interactions within thalamic circuits (Bessaih et al. 2006; Töth et al. 2007) or by the basal ganglia system, known to play a major role in controlling the expression of absence seizures (Deransart and Depaulis 2002; Paz et al. 2007).

**Lack of Changes in SWDs After Early Chronic Treatment with Anti-absence Drugs**

The finding of a critical period during which immature oscillatory discharges evolve into SWDs led us to hypothesize that AEDs treatment during this temporal window could perturb the epileptogenic processes and prevent the development of recurrent generalized seizures. However, our chronic treatment with either VPA or ETHX between P5 and P25 remained ineffective in
altering the number, the mean and cumulated duration as well as the EEG profile of cortical discharges from the 10th to the 80th day after the cessation of the treatment. This contrasts with previous studies showing that chronic administration of ETHX just before SWDs onset in different animal models of AE led to a quasi-suppression of SWDs, up to 1 month after treatment arrest (Blumenfeld et al. 2008, Russo et al. 2010, 2011; Sarkisova et al. 2010; Dezsi et al. 2013; for review, see Russo et al. 2016). The reasons for this discrepancy are still unclear. This may reflect heterogeneity in the duration of the antiepileptic treatment that was much longer (≥4 months) in the aforementioned reports (see also Van Luijten et al. 2013) and could, in some cases, exceed the period of epileptogenesis. This suggests that the observed decrease in seizure activity could have been the result of combined anti-epileptogenic and anti-epileptic effects. The lack of changes in SWD number and duration described in the present study, after a chronic treatment strictly targeting the latent period devoid of SW activity, indicates that such short treatment with current AEDs is not ineffective for targeting the cellular and network defects emerging in GAERS during the epileptogenesis period and highlights the need to develop new and specific medications (Lösch et al. 2013; Pitkänen and Engel 2014; Schmidt and Sillanpää 2016).

In conclusion, our results show that absence epileptogenesis in GAERS is associated with a gradual maturation of SoCx-generated epileptic activities together with progressive alterations in the membrane properties of deep-layer neurons and in their afferent synaptic networks. This suggests that the heterogeneity in the electrical features of seizures in untreated newly diagnosed patients with childhood AE (Sadleir et al. 2006) could reflect differences in the stage of maturation at the time of diagnosis. The precise characterization of the epileptogenic processes is of crucial importance to better define an appropriate therapeutic window and specific treatments. Our findings indicate that current AEDs, which are highly effective for SWDs suppression, are not good candidates to alter or reverse epileptogenesis in GAERS. Given the early onset of epileptic discharges in GEs, drugs specifically targeting the changes occurring during cortical networks development should be able to prevent, reverse, or interrupt the epileptogenic processes.

## Supplementary Material

Supplementary data are available at Cerebral Cortex online.

## Funding

This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Agence Nationale de la Recherche (ANR, “GlEpi” # R09131CS 2009 and “SoAbsence” # ANR-16-CE37-0011 2016), the Fédération pour la Recherche sur le Cerveau (FRC-2009), the Université Pierre et Marie Curie (UPMC) and the program “Investissements d’avenir” ANR-10-IAIHU-06. We also received supports from the French Ministry of Research (G), TAB and MSW, the Fondation Française pour la Recherche sur l’Épilepsie (GJ) and the Fondation Chamaillard (GJ).

## Notes

We thank Sylvain Andrieu and Cyrielle Colomb for the GAERS breeding, Claire Beaup for her technical assistance, Benoît Pouyatos for his advice for epileptiform event analysis as well as Laurent Vercueil and Colin Deransart for their suggestions on the manuscript and their advices. Conflict of Interest: None declared.

## References

Adelsberger H, Garaschuk O, Konnerth A. 2005. Cortical calcium waves in resting newborn mice. Nat Neurosci. 8(8):988–990.

Allene C, Cattani A, Ackman JB, Bonifazi P, Aniksztelj N, Ben-Ari Y, Cossart R. 2008. Sequential generation of two distinct synapse-driven network patterns in developing neocortex. J Neurosci. 28:12851–12863.

Akin D, Ravizza T, Maroso M, Carcak N, Eryigit T, Vanzulli I, Aker RG, Vezzani A, Onat FY. 2011. IL-1β is induced in reactive astrocytes in the somatosensory cortex of rats with genetic absence epilepsy at the onset of spike-wave discharges, and contributes to their occurrence. Neurobiol Dis. 44(3):259–269.

Alteweg-Boussac T, Chavez M, Mahon S, Charpier S. 2014. Excitability and responsiveness of rat barrel cortex neurons in the presence and absence of spontaneous synaptic activity in vivo. J Physiol (Lond). 592:3577–3595.

Baulac M, Pitkänen A. 2009. Research priorities in epilepsy for the next decade—a representative view of the European scientific community: summary of the ILAE Epilepsy Research Workshop, Brussels, 17–18 January 2008. Epilepsia. 50: 571–578.

Ben-Ari Y, Holmes GL. 2006. Effects of seizures on developmental processes in the immature brain. Lancet Neurol. 5: 1055–1063.

Berg AT, Scheffer IE. 2011. New concepts in classification of the epilepsies: entering the 21st century. Epilepsia. 52(6):1058–1062.

Bessaih T, Bourgeois L, Radiu CI, Carter DA, Toth TJ, Ruano D, Lambolez B, Crunelli V, Leresche N. 2006. Nucleus-specific abnormalities of GABAergic synaptic transmission in a genetic model of absence seizures. J Neurophysiol. 96: 3074–3081.

Blumenfeld H, Klein JP, Schridde U, Vestal M, Rice T, Khera DS, Bashyal C, Giblin K, Paul-Laughthouse C, Wang F, et al. 2008. Early treatment suppresses the development of spike-wave epilepsy in a rat model. Epilepsia. 49:400–409.

Bureau I, Shepherd GMG, Svoboda K. 2004. Precise development of functional and anatomical columns in the neocortex. Neuron. 42:789–801.

Carcak N, Aker RG, Ozdemir O, Demiralp T, Onat FY. 2008. The relationship between age-related development of spike-and-wave discharges and the resistance to amygdaloid kindling in rats with genetic absence epilepsy. Neurobiol Dis. 32(3):355–363.

Charpier S, Leresche N, Deniau JM, Mahon S, Hughes SW, Crunelli V. 1999. On the putative contribution of GABA(B) receptors to the electrical events occurring during spontaneous spike and wave discharges. Neuropharmacology. 38: 1699–1706.

Chatrian GE, Bergamini I, Dondey M, Klass DW, Lennox-Buchthal M, Petersen I. 1974. A glossary of terms most commonly used by clinical electroencephalographers. Electroenceph Clin Neurophysiol. 37:538–548.

Chipaux M, Charpier S, Polack P-O. 2011. Chloride-mediated inhibition of the icotogenic neurones initiating genetically-determined absence seizures. Neuroscience. 192:642–651.

Coenen AM, van Luijten EL. 1987. The WAG/Rij rat model for absence epilepsy: age and sex factors. Epilepsy Res. 1: 297–301.
Coenen AM, van Luijtelaar EL. 2003. Genetic animal models for absence epilepsy: a review of the WAG/Rij strain of rats. Behav Genet. 33:635–655.

Connors BW, Gutnick MJ. 1990. Intrinsic firing patterns of diverse neocortical neurons. Trends Neurosci. 13:99–104.

Consorto PV, Kudrya K, Schmitz R. 1980. Acute and chronic antiepileptic drug effects in audiogenic seizure-susceptible rats. Exp Neurol. 70:626–637.

D’Amore V, Santolini I, van Rijn CM, Biagini F, Molinaro G, Frate A, Conn PJ, Lindsay CW, Zhou Y, Vinson PN, et al. 2013. Potentiation of mGlu5 receptors with the novel enhancer, VU0201726, reduces spontaneous absence seizures in WAG/Rij rats. Neuropharmacology. 66:330–338.

Danöber L, Deransart C, Depaulis A, Vergnes M, Marescaux C. 1998. Pathophysiological mechanisms of genetic absence epilepsy in the rat. Prog Neurobiol. 55:27–57.

David O, Guillemin A, Saillet S, Reyt S, Deransart C, Segebarth C, Depaulis A. 2006. Genetic models of absence epilepsy in the rat. In: Pritkäne A, Schwartzkoarn PA, Mossè ML, editors. Models of seizures and epilepsy. San Diego: Elsevier Academic press. p. 233–248.

David O, Abaz Milan, Charrier S. 2016. The genetic absence epilepsy rat from Strasbourg as a model to decipher the neuronal and network mechanisms of generalized idiopathic epilepsies. J Neurosci Methods. 260:159–174.

Deransart C, Depaulis A. 2002. The control of seizures by the basal ganglia? A review of experimental data. Epileptic Disord. 4(Suppl 3):S61–S72.

Deransart C, Riban V, Lè BT, Hechler V, Marescaux C, Depaulis A. 1999. Evidence for the involvement of the pallidum in the modulation of seizures in a genetic model of absence epilepsy in the rat. Neurosci Lett. 265:131–134.

Deransart C, Riban V, Lè BT, Marescaux C, Depaulis A. 2000. Dopamine in the striatum modulates seizures in a genetic model of absence epilepsy in the rat. Neuroscience. 100: 335–344.

Dezsi G, Ozturk E, Stanic D, Powell KL, Blumenfeld H, O’Brien TJ, Jones NC. 2013. Ethosuximide reduces epileptogenesis and behavioral comorbidity in the GAERS model of genetic epilepsy rat. Exp Neurol. 70:626

Ellens DJ, Hong E, Giblin K, Singleton MJ, Bashyal C, Englot DJ, Frankel WN. 2005. Development of a new genetic model for generalized epilepsy. Epilepsia. 54:635

Feldmeyer D. 2012. Excitatory neuronal connectivity in the barrel cortex. Front Neuroanat. 6:1

Feldmeyer D, Brecht M, Helmchen F, Petersen CCH, Poulet JFA, Staiger JF, Luhmann HJ, Schwarz C. 2013. Barrel cortex function. Prog Neurobiol. 103:3–27.

Frankel WN. 2005. Development of a new genetic model for absence epilepsy: spike-wave seizures in C3H/He and backcross mice. J Neurosci. 25:3452–3458.

Frick A, Feldmeyer D, Sakmann B. 2007. Postnatal development of synaptic transmission in local networks of L5A pyramidal neurons in rat somatosensory cortex. J Physiol (Lond). 585: 103–116.

Goldberg EM, Jeong HY, Kruglikov I, Tremblay R, Lazarenko RM, Rudy B. 2011. Rapid developmental maturation of neocortical FS cell intrinsic excitability. Cereb Cortex. 21:666–682.

Golshani P, Gonçalves JT, Khoshkhooh S, Mostany R, Smirnaks S, Portera-Caillau C. 2009. Internally mediated developmental desynchronization of neocortical network activity. J Neurosci. 29:10890–10899.

Grosso S, Galimberti D, Gobbi G, Farnetani M, Di Bartolo RM, Morgese G, Balestari P. 2005. Typical absence seizures associated with localization-related epilepsy: a clinical and electroencephalographic characterization. Epilepsie Rev. 66:13–21.

Guillemin I, Kahane P, Depaulis A. 2012. Animal models to study aetiopathology of epilepsy: what are the features to model? Epilept Disord. 14:217–225.

Hanganu-Opatz II. 2010. Between molecules and experience: role of early patterns of coordinated activity for the development of cortical maps and sensory abilities. Brain Res Rev. 64(1):160–176.

Hirsch E, Panayiotopoulos CP. 2005. Childhood absence epilepsy and related syndromes. In: Roger J, Bureau M, Dravet C, Genton P, Tassinari CA, Wolf P, editors. Epileptic syndromes in infancy, childhood and adolescence. 4th ed.. Montrouge: John Libby Eurotext. p. 315–335.

Holt CR, Softky WR, Koch C, Douglas RI. 1996. Comparison of discharge variability in vitro and in vivo in cat visual cortex neurons. J Neurophysiol. 75:1806–1814.

Kelley MS, Jacobs MP, Lowenstein DH, for the NINDS Epilepsy Benchmark Stewards. 2009. The NINDS epilepsy research benchmarks. Epilepsia. 50:579–582.

Klein JP, Khera DS, Nercessyan H, Kimchi EY, Waxman SG, Blumenfeld H. 2004. Dysregulation of sodium channel expression in cortical neurons in a rodent model of absence epilepsy. Brain Res. 1000:102–109.

Kole MHP, Bräuer AU, Stuart GJ. 2007. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogensis and burst firing in a rat absence epilepsy model. J Physiol (Lond). 578:507–525.

Kriegstein AR, Suppes T, Prince DA. 1987. Cellular and synaptic physiology and epileptogenesis of developing rat neocortical neurons in vitro. Brain Res. 431:161–171.

Löschler W. 2011. Critical review of current animal models of seizures and epilepsy used in the discovery and development of new antiepileptic drugs. Seizure. 20:359–368.

Löschler W, Klitgaard H, Twyman RE, Schmidt D. 2013. New avenues for anti-epileptic drug discovery and development. Nat Rev Drug Discov. 12:757–776.

Luhmann HJ, Sinning A, Yang JW, Reyes-Puerta V, Stüttgen MC, Kirischuk S, Kilb W. 2016. Spontaneous neuronal activity in developing neocortical networks: from single cells to large-scale interactions. Front Neural Circuits. 10:40.

Mahon S, Casassus G, Muller C, Charrier S. 2003. Spike-dependent intrinsic plasticity increases firing probability in rat striatal neurons in vivo. J Physiol (Lond). 550:947–959.

Mahon S, Charrier S. 2012. Bidirectional plasticity of intrinsic excitability controls sensory inputs efficiency in layer 5 barrel cortex neurons in vivo. J Neurosci. 32:11377–11389.

Meeren HK, Pijn JP, Van Luijtelaar EL, Coenen AM, Lopes da Silva FH. 2002. Cortical focus drives widespread corticothalamic networks during spontaneous absence seizures in rats. J Neurosci. 22:1480–1495.

Micheletti G, Vergnes M, Marescaux C, Reis J, Depaulis A, Rumbach L, Warter JM. 1985. Antiepileptic drug evaluation and comparison of drug effects in audiogenic seizure-susceptible rats. Behav Genet. 15:843–852.

Micheletti G, Vergnes M, Marescaux C, Reis J, Depaulis A, Rumbach L, Warter JM. 1985. Antiepileptic drug evaluation and comparison of drug effects in audiogenic seizure-susceptible rats. Behav Genet. 15:843–852.

Micheletti G, Vergnes M, Marescaux C, Reis J, Depaulis A, Rumbach L, Warter JM. 1985. Antiepileptic drug evaluation and comparison of drug effects in audiogenic seizure-susceptible rats. Behav Genet. 15:843–852.

Micheletti G, Vergnes M, Marescaux C, Reis J, Depaulis A, Rumbach L, Warter JM. 1985. Antiepileptic drug evaluation and comparison of drug effects in audiogenic seizure-susceptible rats. Behav Genet. 15:843–852.
Absence Epileptogenesis in the GAERS

Jarre et al.

Minlebaev M, Colonnese M, Tsintsadze T, Sirota A, Khazipov R. 2011. Early γ oscillations synchronize developing thalamus and cortex. Science. 334:226–229.

Mitrukhina O, Suchkov D, Khazipov R, Minlebaev M. 2015. Imprecise whisker map in the neonatal rat barrel cortex. Cereb Cortex. 25:3458–3467.

Ngomba RT, Biagioni F, Casciato S, Willems-van Bree E, Battaglia G, Bruno V, Nicoletti F, van Luijteelaar EL. 2005. The preferential mGlu2/3 receptor antagonist, LY341495, reduces the frequency of spike-wave discharges in the WAG/Rij rat model of absence epilepsy. Neuropharmacology. 49(Suppl. 1):89–103.

Panayiotopoulos CF. 2005. Idiopathic generalized epilepsies: a review and modern approach. Epilepsia. 46:1–6.

Paxinos G, Watson C. 1986. The brain in stereotaxic coordinates. Sydney: Academic Press.

Paz JT, Chavez M, Sailer S, Deniau JM, Charpier S. 2007. Activity of ventral medial thalamic neurons during absence seizures and modulation of cortical paroxysms by the nigrothalamic pathway. J Neurosci. 27:929–941.

Percival DB, Walden AT. 1993. Spectral analysis for physical applications: multitaper and conventional univariate techniques. Cambridge: Cambridge University Press.

Pinault D, Vergnes M, Marescaux C. 2001. Medium-voltage 5-9 Hz oscillations give rise to spike-and-wave discharges in a genetic model of absence epilepsy: in vivo dual extracellular recording of thalamic relay and reticular neurons. Neuroscience. 105(1):181–201.

Pinault D, Slezia A, Acsády L. 2006. Corticalhemic 5-9 Hz oscillations are more pro-epileptogenic than sleep spindles in rats. J Physiol. 574:209–227.

Pitkänen A, Engel J. 2014. Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics. 11:231–241.

Polack P-O, Charpier S. 2009. Ethosuximide converts ictogenic neurons initiating absence seizures into normal neurons in a genetic model. Epilepsia. 50:1816–1820.

Polack P-O, Guillemin M, Hu E, Deransart C, Depaulis A, Charpier S. 2007. Deep layer somatosensory cortical neurons initiate spike-and-wave discharges in a genetic model of absence seizures. J Neurosci. 27:6590–6599.

Polack P-O, Mahon S, Chavez M, Charpier S. 2009. Inactivation of the somatosensory cortex prevents paroxysmal oscillations in cortical and related thalamic neurons in a genetic model of absence epilepsy. Cereb Cortex. 19:2078–2091.

Pouyatos B, Serduc R, Chipaux M, Chabrol T, Bräuer-Krisch E, Nemoz C, Mathieu H, David O, Renaud L, Prezado Y, et al. 2013. Synchronotron X-ray interlaced microbeams suppress paroxysmal oscillations in neuronal networks initiating generalized epilepsy. Neurobiol Dis. 51:152–160.

Rochefort NL, Garaschuk O, Milos RI, Narushima M, Marandi N, Pichler B, Kovalchuk Y, Konnerth A. 2008. Sparing of neuronal activity in the visual cortex at eye-opening. Proc Natl Acad Sci. 106(35):15049–15054.

Russo E, Citraro R, Scicchitano F, De Fazio S, Di Paola ED, Constanti A, De Sarro G. 2010. Comparison of the antiepileptogenic effects of an early long-term treatment with ethosuximide or levetiracetam in a genetic animal model of absence epilepsy. Epilepsia. 51:1560–1569.

Russo E, Citraro R, Scicchitano F, De Fazio S, Perrotta I, Di Paola ED, Constanti A, De Sarro G. 2011. Effects of early long-term treatment with antiepileptic drugs on development of seizures and depressive-like behavior in a rat genetic absence epilepsy model. Epilepsia. 52(7):1341–1350.

Russo E, Citraro R, Scicchitano F, De Fazio S, Di Paola ED, Constanti A, Leo A, Lüttjohann A, Van Luijteelaar G, de Sarro G. 2016. Upholding WAG/Rij rats as a model of absence epileptogenesis: Hidden mechanisms and a new theory on seizure development. Neurosci Biobehav Rev. 71:388–408.

Sadilek LG, Farrell K, Smith S, Connolly MB, Scheffer IE. 2006. Electroclinical features of absence seizures in childhood absence epilepsy. Neurology. 67:413–418.

Sanchez RM, Jensen FE. 2001. Maturational aspects of epilepsy mechanisms and consequences for the immature brain. Epilepsia. 42:577–585.

Sarkissova KY, Kuznetsova GD, Kulikov MA, van Luijteelaar G. 2010. Spike and wave discharges are necessary for the expression of behavioral depression-like symptoms. Epilepsia. 51:146–160.

Schmidt D, Sillanpää M. 2016. Prevention of epilepsy: issues and innovations. Curr Neurol Neurosci Rep. 16:95.

Shorvon SD. 2011. The etiologic classification of epilepsy. Epilepsia. 52:1052–1057.

Silver RA. 2010. Neuronal arithmetic. Nat Rev Neurosci. 11:474–489.

Sitnikova E, van Luijteelaar G. 2007. Electroencephalographic characterization of spike-wave discharges in cortex and thalamus in WAG/Rij rats. Epilepsia. 48(12):2296–2311.

Stern EA, Maravall M, Svoboda K. 2001. Rapid development and plasticity of layer 2/3 maps in rat barrel cortex in vivo. Neuron. 31:305–315.

Stauss U, Kole MHP, Bräuer AU, Pahnke J, Bajorat R, Rolfs A, Nitsch R, Deisz RA. 2004. An impaired neocortical Ih is associated with enhanced excitability and absence epilepsy. Eur J Neurosci. 19:3048–3058.

Tan HO, Reid CA, Single FN, Davies PJ, Chiu C, Murphy S, Clarke AL, Dibbens L, Krestel H, Mulley JC, et al. 2007. Reduced cortical inhibition in a mouse model of familial childhood absence epilepsy. Proc Natl Acad Sci USA. 104:17536–17541.

Tóth TI, Bessaih T, Leresche N, Crunelli V. 2007. The properties of reticular thalamic neuron GABAA IPSCs of absence epilepsy rats lead to enhanced network excitability. Eur J Neurosci. 26:1832–1844.

van de Bovenkamp-Janssen MC, van der Kloet JC, van Luijteelaar G, Roubos EW. 2006. NMDA-NR1 and AMPA-GluR4 receptor subunit immunoreactivities in the absence epileptic WAG/Rij rat. Epilepsy Res. 69(2):119–128.

Van Luijteelaar G, Mishra AM, Edelbrook F, Coman D, Frankenmolen N, Schaapsmeerders P, Covolet G, Daniellsen N, Niermann H, Janeczko K, et al. 2013. Anti-epileptogenesis: electrophysiology, diffusion tensor imaging and behavior in a genetic absence model. Neurobiol Dis. 60:126–138.

Vergnes M, Marescaux C, Depaulis A, Micheletti G, Warter JM. 1986. Ontogeny of spontaneous petit mal-like seizures in Wistar rats. Brain Res. 395:85–87.

Williams MS, Altwegg-Boussac T, Chavez M, Lecas S, Mahon S, Charpier S. 2016. Integrative properties and transfer function of cortical neurons initiating absence seizures in a rat genetic model. J Physiol. 594(22):6733–6751.

Yang JW, Hangaru-Opatz IL, Sun JJ, Luhmann HJ. 2009. Three patterns of oscillatory activity differentially synchronize developing neocortical networks in vivo. J Neurosci. 29:9011–9025.

Zhu JJ. 2000. Maturation of layer 5 neocortical pyramidal neurons: amplifying salient layer 1 and layer 4 inputs by Ca2+ action potentials in adult rat tuft dendrites. J Physiol (Lond). 526(Pt 3):571–587.