

Synaptic hyperexcitability of deep layer neocortical cells in a genetic model of absence seizures

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We used sharp-electrode, intracellular recordings in an *in vitro* brain slice preparation to study the excitability of neocortical neurons located in the deep layers (>900 μm from the pia) of epileptic (180–210-days old) Wistar Albino Glaxo/Rijswijk (WAG/Rij) and age-matched, non-epileptic control (NEC) rats. Wistar Albino Glaxo/Rijswijk rats represent a genetic model of absence seizures associated with generalized spike and wave (SW) discharges *in vivo*. When filled with neurobiotin, these neurons had a typical pyramidal shape with extensive apical and basal dendritic trees; moreover, WAG/Rij and NEC cells had similar fundamental electrophysiological and repetitive firing properties. Sequences of excitatory postsynaptic potentials (EPSPs) and hyperpolarizing inhibitory postsynaptic potentials (IPSPs) were induced in both the strains by electrical stimuli delivered to the underlying white matter or within the neocortex; however, in 24 of 55 regularly firing WAG/Rij cells but only in 2 of 25 NEC neurons, we identified a late EPSP that (1) led to action potential discharge and (2) was abolished by the *N*-methyl-D-aspartate (NMDA) receptor antagonist 3,3-(2-carboxypiperazine-4-yl)-propyl-1-phosphonate (20 μM; n = 8/8 WAG/Rij cells). Finally, we found that the fast and slow components of the stimulus-induced IPSPs recorded during the application of glutamatergic receptor antagonists had similar reversal potentials in the two strains, while the peak conductance of the fast IPSP was significantly reduced in WAG/Rij cells. These findings document an increase in synaptic excitability that is mediated by NMDA receptors, in epileptic WAG/Rij rat neurons located in neocortical deep layers. We propose that this mechanism may be instrumental for initiating and maintaining generalized SW discharges *in vivo*.

Keywords: Absence epilepsy, neocortex, NMDA receptors, pyramidal cells, WAG/Rij rat

Received 26 November 2004, revised 23 March 2005, accepted for publication 25 March 2005

Generalized spike and wave (SW) discharges at 2.5–4 Hz are the hallmark of primary generalized epilepsies in humans. This neurological disorder is characterized by brief periods of impaired consciousness, commonly referred to as absence seizures (Panayiotopoulos 1999). The pathophysiology of generalized SW discharges has been extensively analyzed by using normal animal brains that were acutely treated with convulsants or with low-frequency electrical stimuli (see for review: Avoli *et al*. 2001; Crunelli & Leresche 2002). According to these studies, SW discharges appear to originate from oscillatory thalamocortical interactions that are similar to those involved in sleep spindles. Moreover, it has been proposed that a hyperexcitable neocortex may play a major role in SW discharge generation (Avoli *et al*. 1983; Neckelmann *et al*. 1998; Steriade & Contreras 1998; Steriade *et al*. 1998; Timofeev *et al*. 1998). In line with this view, corticothalamic inputs (Deschênes *et al*. 1998) entrain thalamic networks into the SW rhythm by exciting thalamic reticular GABAergic cells which, in turn, are known to inhibit thalamocortical neurons (Contreras & Steriade 1995).

Absence seizures in humans occur spontaneously and have a genetic origin (Lennox & Lennox 1960; Metrakos & Metrakos 1961; Noebels 2003). Therefore, studies performed in naturally occurring genetic models such as the genetic absence epilepsy in rats from Strasbourg (GAERS) (Vergnes *et al*. 1990) or the Wistar Albino Glaxo/Rijswijk (WAG/Rij) rat (van Luijtelaar & Coenen 1986; Coenen & van Luijtelaar 2003) should provide new insights to the pathophysiology of absence seizures. These rodent strains generate spontaneously generalized SW discharges at 7–11 Hz and respond to antiepileptic drugs in a manner similar to that reported for patients with primary generalized epilepsy (Coenen & van Luijtelaar 2003; Drinkenburg *et al*. 1993; Midzianovskaia *et al*. 2001).

Electrophysiological analysis of SW discharges in either GAERS or WAG/Rij rats have identified a primary role of the cortex in the initiation of generalized SW discharges (Pinault 2003; Seidenbecher *et al*. 1998; Vergnes *et al*. 1990). In particular, they have pointed to a "consistent cortical focus" within the perioral area in the somatosensory cortex (Manning *et al*.

doi: 10.1111/j.1601-183X.2005.00146.x

2003; Meeren *et al*. 2002). Moreover, by using the thalamocortical slice preparation originally described by Agmon and Connors (1991), we have reported that the ability of epileptic WAG/Rij rat slices to generate slow rhythmic oscillations in the presence of low concentrations of 4-aminopyridine depends on the functional integrity of neocortical networks (D'Arcangelo *et al*. 2002). This slow *in vitro* synchronous oscillatory activity, which was not recorded in slices obtained from age-matched, non-epileptic control (NEC) Wistar rats, may be analogous to the SW rhythm seen *in vivo*.

Experiments performed *in vivo* in these genetic models of absence seizures indicate that the leading role of the neocortex in the generation of SW discharges may depend on *N*-methyl-D-aspartate (NMDA) receptor-mediated glutamatergic transmission (Peeters *et al*. 1989; 1990; Pumain *et al*. 1992). In addition, we have found in epileptic WAG/Rij thalamocortical slices that local application of an NMDA receptor antagonist to the somatosensory cortex abolishes the slow synchronous oscillatory activity disclosed by 4-aminopyridine; at variance, these effects were not produced by applying the antagonist to the ventrobasal thalamus (D'Arcangelo *et al*. 2002). This evidence has led us to investigate with sharp-electrode, intracellular recordings the intrinsic and synaptic properties of neurons located in the deep layers of neocortical slices that were obtained from epileptic (180–210-days old) WAG/Rij and age-matched NEC rats. Previous studies have indeed shown that neocortical projections to the thalamus originate from the deep layers (cf. Deschênes *et al*. 1998; Veinante *et al*. 2000).

Materials and methods

Over 30 epileptic WAG/Rij rats (180–210-days old; Harlan, Horst, the Netherlands) and 23 age-matched, NEC rats (strain: Crl(WI)BR Wistar; obtained from either Charles River, St. Constant, PQ, Canada, or Harlan) were used according to the procedures established by the Canadian and the European Union Councils of Animal Care. All efforts were made to minimize the number of animals used and their suffering. By analyzing the behaviour of NEC and WAG/Rij rats housed before the day of the *in vitro* experiment, we could document that WAG/Rij animals displayed frequent episodes (duration: up to 15 seconds) of behavioural arrest accompanied by mild myoclonic twitches. Previous *in vivo* studies have demonstrated that these clinical events correspond to bilaterally generalized SW discharges at 7–11 Hz (Drinkenburg *et al*. 1993; Midzianovskaia *et al*. 2001). By contrast, similar episodes were rarely observed in Wistar rats (Gallitto *et al*. 1987; Marescaux *et al*. 1985); these latter animals were not used for the experiments reported here.

Epileptic WAG/Rij and NEC rats were anesthetized with enfluorane and decapitated. Their brains were quickly removed and placed in cold, oxygenated artificial cerebral spinal fluid (ACSF). Neocortical slices (450 μm) were cut coronally with a vibratome from a region corresponding to the somatosensory cortex that matched the plates reported by Paxinos and Watson (1998) at −0.3 to +0.7 mm from the bregma (cf. also, Manning *et al*. 2003; Pinault 2003). Slices were then transferred to a tissue chamber where they lay in an interface between oxygenated ACSF and humidified gas (95% O_2/5% CO_2) at 32–34 °C (pH = 7.4). Artificial cerebral spinal fluid composition was in mM: NaCl 124, KCl 2, KH_2PO_4 1.25, $MgSO_4$ 1, $CaCl_2$ 2, $NaHCO_3$ 26 and glucose 10. Artificial cerebral spinal fluid could also contain 3-[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate (CPP, 10–20 μM) or 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX, 10 μM).

Conventional intracellular recordings were obtained with glass electrodes filled with 2 M K-acetate (resistance = 80–120 MΩ) from cells located at depths >900 μm from the pia. This location could be visualized in tangentially lit slices as a band of tissue that was comprised between the granular layer and the white matter. Signals were fed to a high-impedance amplifier with an internal bridge circuit for passing intracellular current (Axoclamp 2 A, Axon Instruments, Union City, CA). The bridge balance was routinely checked. Signals were displayed on an oscilloscope and/or on a Gould recorder (Cleveland, OH) or fed to a computer interface (Digidata 1200B, Axon Instruments) for subsequent analysis with the software pCLAMP8 (Axon Instruments). Extracellular stimuli (50–150 microseconds; <300 μA) were delivered through bipolar stainless steel electrodes that were placed in the underlying white matter or within the neocortical layers, at a distance <500 μm from the intracellularly recorded neuron.

The resting membrane potential (RMP) was measured after withdrawal from the cell, while the apparent input resistance (R_i) of the membrane was calculated from the peak of voltage responses to hyperpolarizing current pulses (amplitude = <0.5 nA, duration = 150 milliseconds). Action potential amplitude and duration were measured from baseline-to-peak and at half-amplitude, respectively. We included in this study, neurons that displayed $R_i \geq 20$ MΩ, RMP more negative than −65 mV and overshooting action potentials. Intracellular injection of depolarizing current pulses (>200 milliseconds, 0.3–1.8 nA) was used to classify neocortical neurons as regularly firing or bursting cells (cf. McCormick *et al*. 1985). The reversal potential of the fast and slow components of the stimulus-induced inhibitory postsynaptic potentials (IPSPs) was determined by linear regression from the plot of the IPSP amplitude vs. membrane potential. The amplitude values were calculated at latencies of 15 milliseconds and of 180 milliseconds from the extracellular stimulus for the fast and slow IPSP components, respectively. The peak conductances of the fast and slow IPSP components were calculated by linear regression analysis from the plot of the relation between injected current and membrane potential deflections before and after the

extracellular stimulus at latencies of 15 milliseconds and of 180 milliseconds, respectively (cf. Luhmann *et al*. 1995).

For intracellular labeling, electrodes were filled with 2% neurobiotin (Kita & Armstrong 1991) dissolved in 2 M K-acetate. Intracellular injection of neurobiotin was accomplished by passing pulses of depolarizing current (0.5–1 nA, 3.3 Hz, 150 milliseconds) through the recording electrode for approximately 10 min. Only one neuron was filled in each slice. At the end of the experiment, slices were fixed in 4% paraformaldehyde, 100 mM phosphate-buffered solution (pH 7.2) overnight at 4 °C. Slices were then rinsed in phosphate-buffered saline (PBS) and the endogenous peroxidase activity extinguished by incubating them in 0.1% phenylhydrazine for 20 min. After several rinses in PBS, the slices were incubated for 2 h in 1% Triton X-100 and then in Vectastain ABC reagent comprising the avidin-biotinylated horseradish peroxidase complex in PBS for at least 4 h. After wash in PBS, the sections were reacted with 0.5% 3,3′-diaminobenzidine tetrahydrochloride and 0.03% hydrogen peroxide in PBS, mounted on slides, dehydrated and covered (Kita & Armstrong 1991). Stained neurons were analyzed using a Zeiss Axiophot microscope with the KS300 software (Zeiss-Kontron, Munich, Germany) (cf. D'Antuono *et al*. 2001) that automatically calculated the area and diameter of the neuronal soma. Chemicals were obtained from Sigma (St. Louis, MO) with the exception of CNQX and CPP (Tocris Cookson, Langford, UK) and neurobiotin and Vectastain ABC (Vector Laboratories, Burlingame, CA).

Our electrophysiological work is based on 68 NEC and 103 WAG/Rij neocortical cells that were recorded in 34 and 48 slices, respectively. In addition, we obtained intracellular recordings from eight NEC and nine WAG/Rij neocortical cells that were recorded with K-acetate + neurobiotin-filled electrodes for morphological identification. Measurements throughout the text are expressed as mean ± SD and n indicates the neuron number. Data were statistically analyzed with the Student's t-test and considered significantly different if $P < 0.05$.

Results

Morphological and fundamental electrophysiological characteristics of NEC and WAG/Rij neocortical neurons

Neurons filled with neurobiotin in the deep layers of WAG/Rij neocortical slices displayed a pyramidal shape with a distinct apical dendrite that was directed toward the superficial layers. These features were encountered both in regularly firing (panel A, $n = 6$) and bursting (panel B; $n = 3$) cells (Fig. 1). In addition, we did not find any difference in the depth location (1032 ± 129 μm vs. 1012 ± 115 μm from the pia, respectively) and diameter (15.3 ± 1.1 μm vs. 16.9 ± 1.8 μm, respectively) of their soma. Non-epileptic control cells labeled with neurobiotin had also pyramidal shape (not shown). In these experiments, the soma of regularly firing cells ($n = 5$) were located at 1253 ± 160 μm from the pia and had diameter 14.1 ± 0.7 μm, while bursting neurons ($n = 3$) were found at 1245 ± 240 μm and had diameter 15.2 ± 0.5 μm. These values were not significantly different when compared between the two types of tissue.

The electrophysiological properties of these four groups of neurobiotin-filled cells were within the range of those measured in a larger number of non-labeled neocortical neurons, thus suggesting that labeled and non-labeled neurons presumably represented homogenous populations. In particular, we found that intracellular injection of depolarizing current pulses caused regularly firing of fast action potentials in 58 of 68 and 88 of 103 non-labeled neurons recorded in NEC and WAG/Rij neocortical slices, respectively, while the remaining NEC ($n = 10$) or WAG/Rij ($n = 15$) cells responded with action potential bursts. Table 1 summarizes some of the intrinsic membrane properties measured in the four groups of non-labeled neurons; these values were not significantly different among them.

Stimulus-induced synaptic responses in NEC and WAG/Rij neocortical neurons

Next, we analyzed the intracellular responses generated by cells in the deep layers of NEC and epileptic WAG/Rij neocortical slices following focal electrical stimuli that were delivered in the underlying white matter or within the neocortical layers. As shown in Fig. 2(a) (control), increasing the stimulus strength made the majority (92%) of NEC regularly firing cells ($n = 23/25$) produce larger excitatory postsynaptic potentials (EPSPs) that eventually triggered single action potentials. The amplitude and duration of these excitatory responses were not significantly changed by bath applying the NMDA receptor antagonist CPP (10–20 μM; $n = 7$; $P = 0.8$) (Fig. 2a,c), although a small reduction in EPSP amplitude and repolarizing component were seen in three neurons (Fig. 2a, arrow-heads). Similar findings, including the lack of changes induced by CPP (20 μM; $n = 9$), were obtained in 31 of 55 (56%) epileptic WAG/Rij regularly firing cells analyzed under control conditions (Fig. 2b,d). Details on the synaptic responses generated by the rest of NEC and WAG/Rij neurons are provided below.

Under appropriate conditions (i.e. RMP or stimulus strength), NEC or WAG/Rij neocortical neurons generated stimulus-induced hyperpolarizing IPSPs that lasted up to 400 milliseconds and were characterized by fast and slow components. Injection of depolarizing or hyperpolarizing pulses of current modified the amplitude of these responses recorded in NEC (Fig. 3a) and WAG/Rij (Fig. 3b) slices and revealed in both types of tissue similar reversal potentials for the IPSP fast and slow components. Moreover, application of medium containing both the CPP (20 μM) and the non-NMDA glutamatergic receptor antagonist CNQX (10 μM) abolished white matter-induced synaptic responses in both

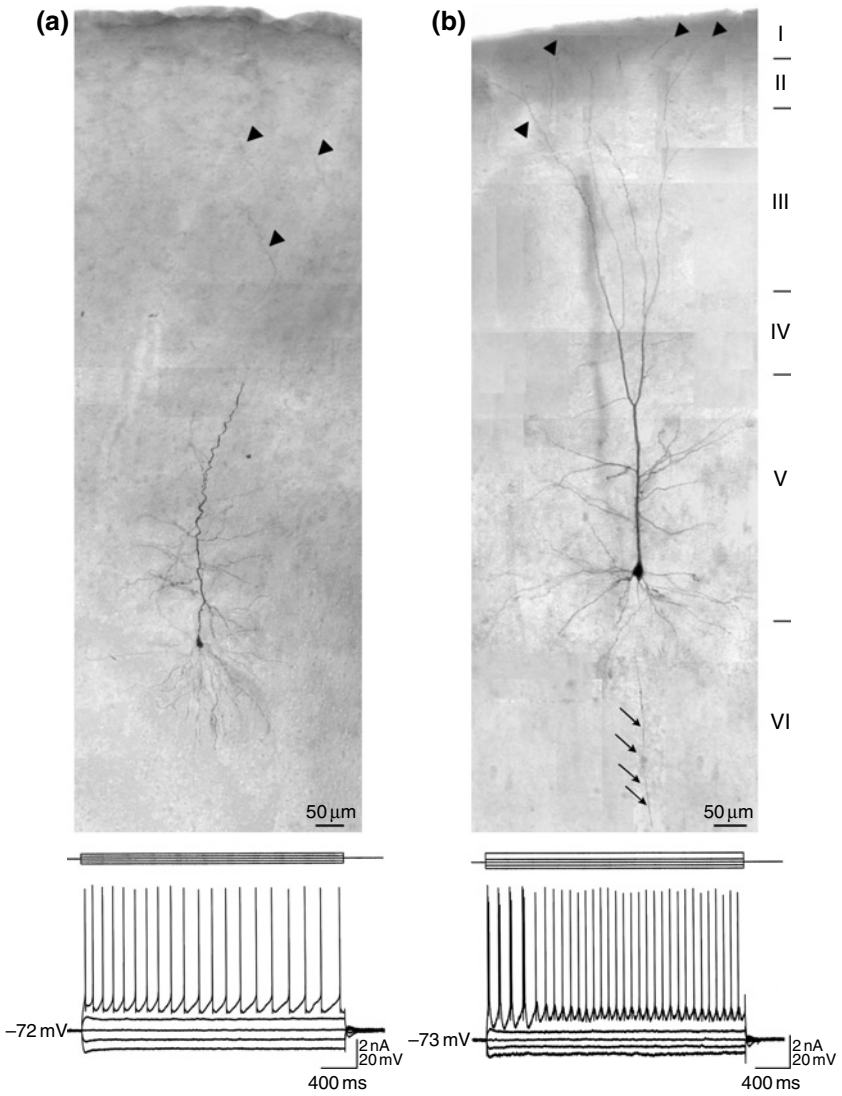

Figure 1: Photomicrographs of regular firing (a) and bursting (b) neurons in the somatosensory cortex of Wistar Albino Glaxo/ Rijswijk rat slices. Both the cells were injected with neurobiotin during the electrophysiological recordings that are shown below. Note that both the neurons present with pyramidally shaped bodies; however, in this experiment, the regularly firing cell displays a thinner, poorly ramified, apical dendrite terminating in layer III-II (arrowheads). By contrast, the bursting neuron is characterized by spiny basal dendrites and an axon travelling deeply in the neocortex (arrows); a thick apical dendrite, emerging gradually from the soma, gives rise to several branches and ends in layer I (arrowheads).

Table 1: Intrinsic membrane properties of neurons recorded in the deep layers of neocortical slices obtained from non-epileptic control (NEC) and Wistar Albino Glaxo/Rijswijk (WAG/Rij) rats

	Regularly firing cells			Bursting cells
	NEC ($n = 58$)	WAG/Rij ($n = 85$)	NEC ($n = 10$)	WAG/Rij ($n = 15$)
RMP	−72.4 ± 5.5	−70.7 ± 4.9	−68.5 ± 6.2	−72.4 ± 5.5
APA	88.5 ± 7.9	89.1 ± 6.5	79.9 ± 11.6	87.6 ± 6.2
APD	1.01 ± 0.23	0.98 ± 0.24	1.04 ± 0.17	1.04 ± 0.23
R_i	36.0 ± 8.2	39.6 ± 11.7	36.0 ± 5.7	37.4 ± 8.9
TC	8.4 ± 2.1	9.2 ± 2.7	8.3 ± 1.8	9.9 ± 2.7

APA, action potential amplitude (mV); APD, action potential duration (ms); R_i, input resistance (MΩ); RMP, resting membrane potential (mV); TC, time constant (ms).
Values are means ± SD.

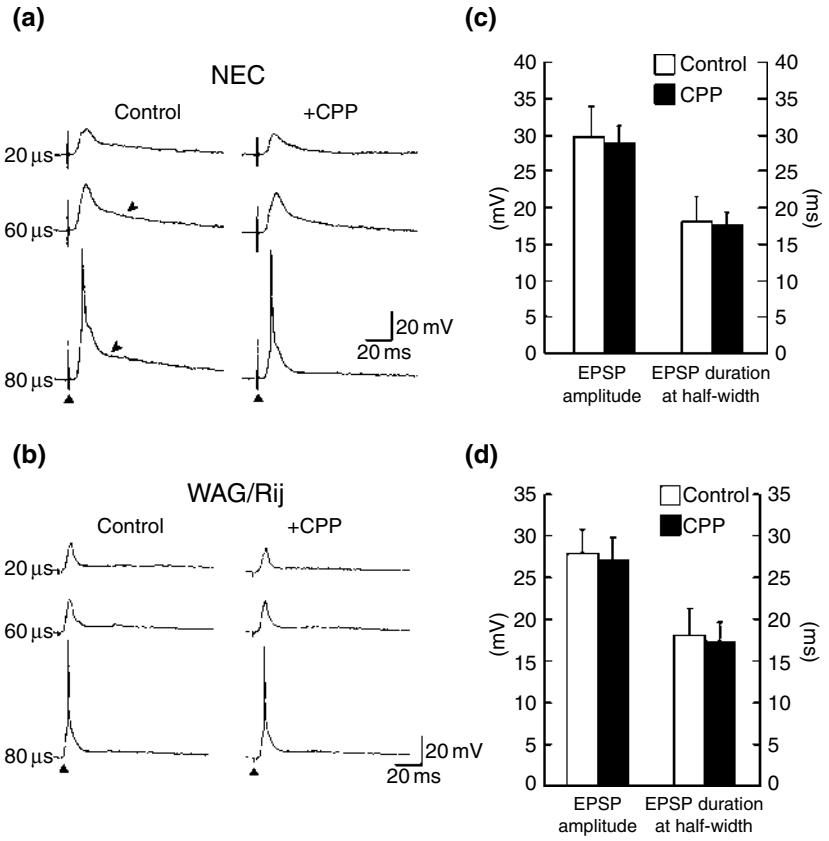

Figure 2: Intracellular potentials generated by non-epileptic control (NEC) (a and c) and Wistar Albino Glaxo/Rijswijk (WAG/Rij) (b and d) regularly firing neurons in response to single-shock stimuli delivered in the white matter at progressively higher strengths. (a) Excitatory postsynaptic potential (EPSP) recorded at resting membrane potential (−68 mV) from this NEC cell increases in amplitude and eventually triggers a single action potential in response to stimuli of increasing strength (Control panel). Similar synaptic responses are recorded following bath application of the *N*-methyl-D-aspartate receptor antagonist 3-[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate (CPP) (20 μM); however, in this experiment, CPP is capable of reducing EPSP peak amplitude as well as its repolarizing component (arrowhead in Control panel). (c) Summary of the effects induced by CPP on the amplitude and duration of the monophasic EPSPs recorded from seven NEC neurons; values were obtained from the intracellular responses to stimuli that were subthreshold for action potential discharge. (b) Synaptic responses generated by a neuron recorded in a WAG/Rij slice following focal stimuli delivered in the white matter at increasing strength under control conditions and during CPP (20 μM) application; note that as seen with the NEC cell, this procedure makes the EPSP amplitude increase eventually leading to single action potential discharge; note also that CPP does not modify the stimulus-induced synaptic responses. (d) Summary of the effects induced by CPP on the amplitude and duration of the monophasic EPSPs generated by nine WAG/Rij cells in response to stimuli that were subthreshold for action potential discharge. In this and following figures, the time of extracellular stimulus is indicated by a triangle.

NEC and epileptic WAG/Rij cells (not shown). However, stimuli delivered in the neocortical layers at locations close to the recorded neuron could elicit "monosynaptic" IPSPs that included fast and slow components in both the tissue types (Fig. 3c,d). The reversal potentials of the fast component of these glutamatergic-independent IPSPs were -66.6 ± 2.9 mV and -69.3 ± 1.7 mV in NEC ($n = 7$) and WAG/Rij ($n = 10$) neurons, respectively, while the slow components had reversal potential of -81.5 ± 1.7 mV in NEC and -85.6 ± 2.4 mV in WAG/Rij cells. Statistical analysis of these values revealed no significant difference when NEC and WAG/Rij neurons were compared. In addition, we found that the peak conductance associated with the "monosynaptic" IPSPs in NEC and WAG/Rij cells was 35.7 ± 9.4 nS and 20.9 ± 7.7 nS for the fast component ($P < 0.03$) and 7.5 ± 4.2 nS and 5.4 ± 2.4 nS (not significant) for the slow component, respectively.

In the remaining WAG/Rij ($n = 24$) as well as in two NEC regularly firing cells, low strength, focal stimulation of the white matter caused an early EPSP that could be followed by a series of small amplitude depolarizing events (Fig. 4a, asterisk and arrows in panel 0.01 mA, respectively). Moreover, when the stimulus strength was increased, these neurons generated a late depolarizing event (half-width duration up to 30 milliseconds). As shown in Fig. 4(a), the latency and the amplitude of this late

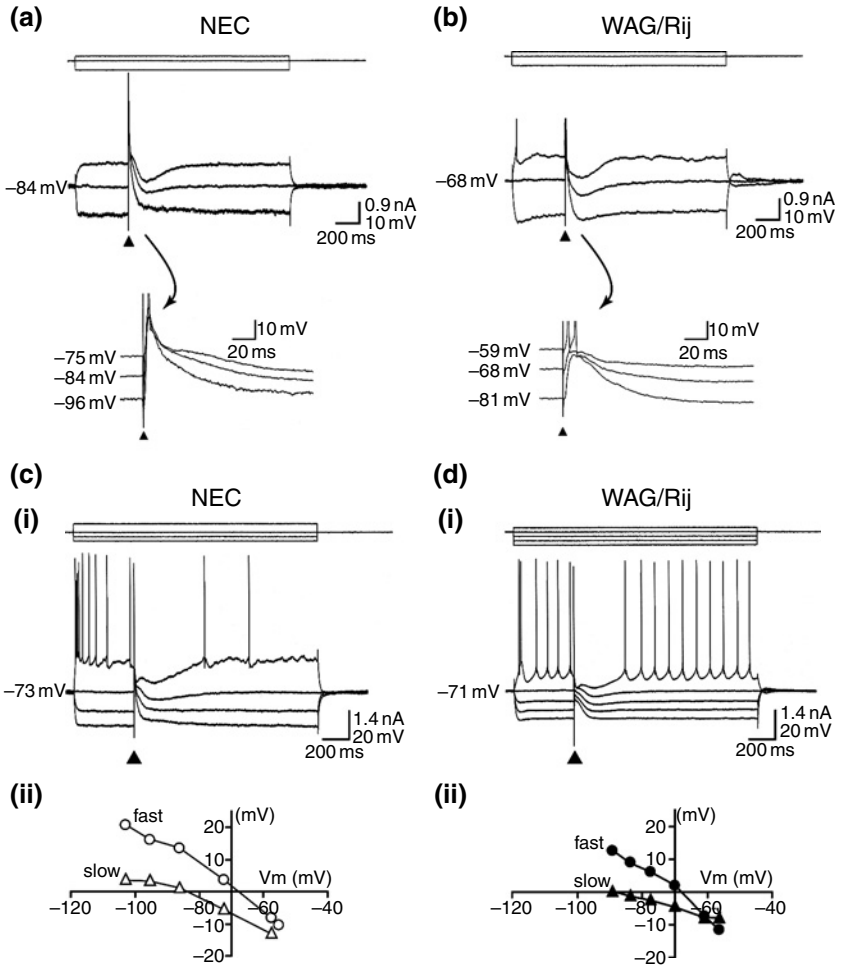

Figure 3: (a and b) Synaptic responses generated by non-epileptic control (NEC) and Wistar Albino Glaxo/Rijswijk (WAG/Rij) neocortical neurons following single-shock stimulation of the white matter in the presence of normal medium. Note that the intracellular responses were recorded at different membrane potentials by injecting depolarizing and hyperpolarizing current pulses as well as that in both NEC and WAG/Rij neurons the stimulus-induced responses recorded at depolarized membrane values are characterized by a robust hyperpolarization with duration >300 milliseconds. Lower panels represent a portion of the same recordings displayed at faster time base. (c and d) Synaptic responses generated by NEC and WAG/Rij neocortical neurons following single-shock stimulation delivered in the neocortical layers during superfusion with artificial cerebral spinal fluid containing antagonist 3-[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate + 6-cyano-7-nitroquino-xaline-2,3-dione. In both the panels, the intracellular responses were recorded at different membrane potentials by injecting depolarizing and hyperpolarizing current pulses, while the (ii) panels show the plots of the response amplitudes obtained 20 milliseconds (circles, fast) and 180 milliseconds (triangles, slow) after the focal stimulus vs. the membrane potential values.

depolarization were decreased and increased, respectively, when the stimulus strength was augmented by small steps. However, these minimal changes in stimulus strength did not modify the early EPSP. The late depolarization disappeared during repetitive stimuli at frequency 0.5 Hz (Fig. 4b), suggesting that it represented a polysynaptic event. In addition, by further increasing the stimulus intensity, we found that this late depolarization fused with the early EPSP, thus causing the appearance of action potential discharge (Fig. 4c, arrows), followed by a long-lasting (up to 500 milliseconds) hyperpolarization that reversed in polarity at -86.8 ± 2.5 mV ($n = 6$) (Fig. 4c, asterisk; Fig. 4d). The action potential discharge identified in WAG/Rij neocortical neurons generating the late stimulus-induced depolarization most often consisted of a doublet of action potentials with interspike intervals ranging 5–12 milliseconds (Fig. 4); however, overt action potential bursts could be recorded (Fig. 5, inset).

Application of the NMDA receptor antagonist CPP (20 μM) reversibly abolished the late, stimulus-induced depolarization and the associated action potentials without influencing the early EPSP ($n = 8$; Fig. 4e). Moreover, in the presence of

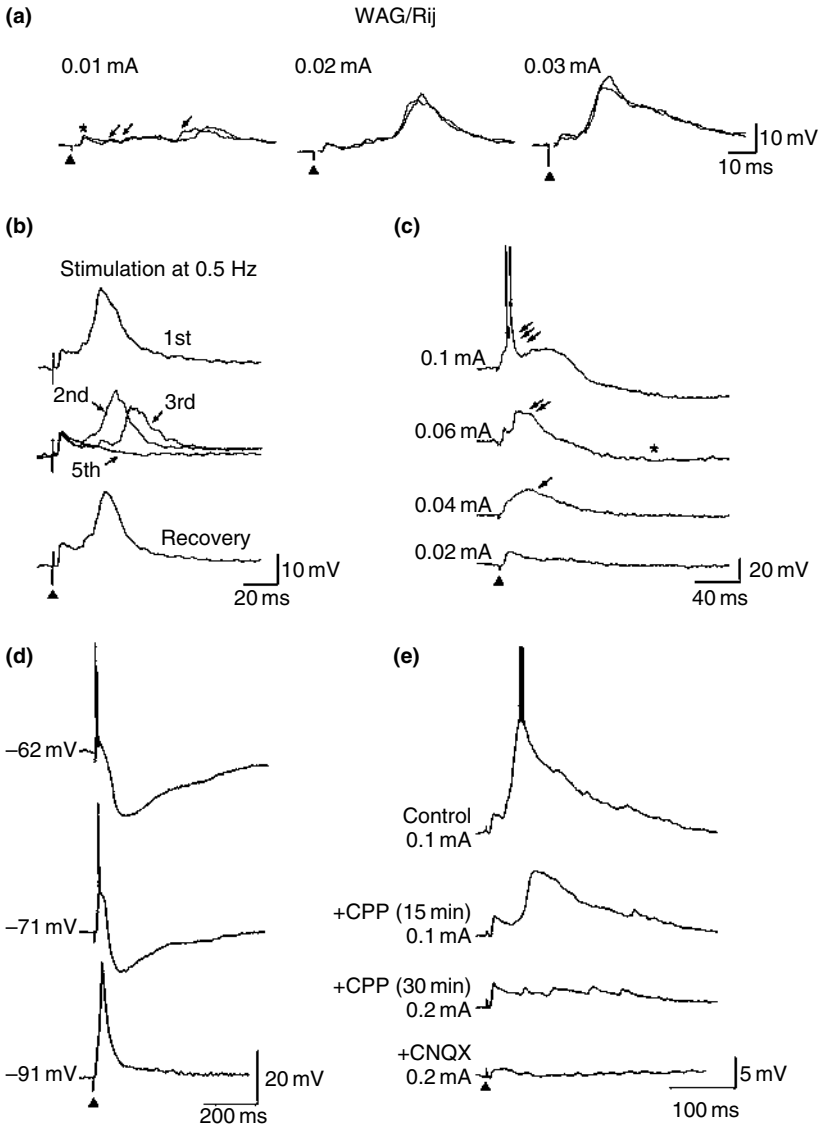

Figure 4: Stimulus-induced intracellular potentials recorded from regularly firing neurons in epileptic Wistar Albino Glaxo/Rijswijk (WAG/Rij) slices. (a) Low strength, focal stimulation of the white matter (0.01 mA) causes an early excitatory postsynaptic potential (EPSP) (asterisk) that is followed by a series of small amplitude depolarizing events (arrows). Increasing the stimulus strength leads to the appearance of a late depolarizing event (0.02 mA) that increases in amplitude and decreases its latency of appearance with a further increase of the stimulus intensity. Note that these changes are not accompanied by any noticeable increase in the size of the early EPSP. (b) Repetitive stimuli delivered in the white matter at 0.5 Hz decrease and abolish the late depolarizing event without affecting the early EPSP; the sample identified as "recovery" was obtained 10 seconds after termination of repetitive stimulation. (c) Increasing the stimulus strength decreases the latency of the late depolarizing event and causes action potential discharge. Note that the response induced by the 0.06 mA stimulus includes a long-lasting hyperpolarization (asterisk). Resting membrane potential (RMP) of this cell was −68 mV. (d) Effects induced by membrane potential changes on the long-lasting hyperpolarization generated by an epileptic WAG/Rij cell (RMP = −71 mV) following stimuli delivered in the white matter. Note that the long lasting hyperpolarization seen at −71 mV increases in amplitude during steady depolarization of the membrane to −62 mV and is reversed by steady hyperpolarization to −91 mV. (e) Application of the *N*-methyl-D-aspartate receptor antagonist 3-[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate (CPP) progressively abolishes the stimulus-induced, late depolarizing event without influencing the early EPSP that is blocked by further addition of 6-cyano-7-nitroquino-xaline-2,3-dione (CNQX). Note that small amplitude depolarizing events are seen after the initial EPSP in the presence of CPP.

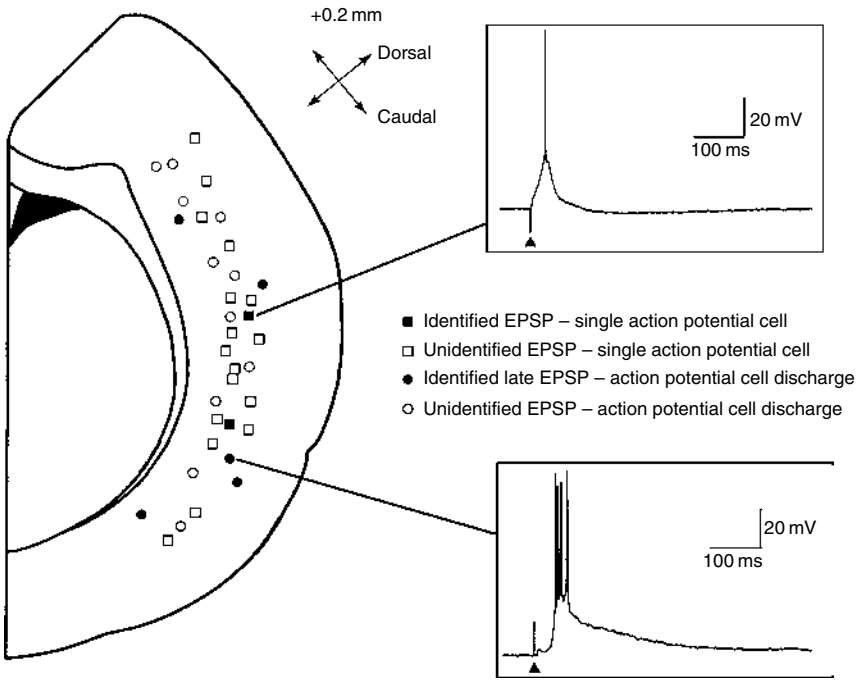

Figure 5: Distribution of regularly firing cells that generated either an excitatory postsynaptic potential (EPSP)/single action potential or a late EPSP/action potential discharge following electrical stimuli delivered in the white matter. The slice drawing, which was made by using an actual Wistar Albino Glaxo/Rijswijk rat neocortical slice, corresponds to the plate reported at +0.2 mm from the bregma in the atlas of Paxinos and Watson (1998). Square and circles indicate the location of neurons that produced EPSP/single action potential and late EPSP/action potential discharge, respectively, following white matter stimulation. Open symbols correspond to unidentified cells while filled symbols matched the location of neurons that were injected with neurobiotin; in the former cases, the neuron position was inferred from that of the intracellular electrode. Insets reproduce two typical stimulus-induced intracellular responses.

CPP, small amplitude depolarizing events could follow the early EPSP (Fig. 4e, +CPP 30 min). Further addition of CNQX blocked all the intracellular potentials induced by stimuli delivered in the underlying white matter (Fig. 4e, +CNQX). In contrast, stimuli delivered within the neocortical layers at locations close to the recorded neuron elicited "monosynaptic" IPSPs that displayed fast and slow components in four WAG/Rij neurons that were capable of generating synaptic bursting under control conditions (not illustrated). The reversal potentials for the fast and slow components of these IPSPs were -66.7 ± 1.5 mV and -85.2 ± 1.7 mV ($n = 4$), respectively.

Figure 5 summarizes in a diagrammatic fashion the distribution in the neocortex of WAG/Rij regularly firing cells that generated either an EPSP/single action potential (open and filled squares) or a late EPSP/action potential discharge (open and filled circles) following electrical stimuli in the white matter. The slice drawing, which was made by using an actual WAG/Rij rat neocortical slice, corresponds to the plate reported at +0.2 mm from the bregma in the atlas of Paxinos and Watson (1998).

Finally, we analyzed the synaptic responses recorded in "all-or-none" bursting cells. In both NEC ($n = 6$) and epileptic WAG/Rij slices ($n = 7$), white matter stimuli that were suprathreshold for eliciting an EPSP caused action potential bursts [Fig. 6a,b(i), control]. In epileptic WAG/Rij neurons, we tested the effects induced by CPP (10 μM, $n = 3$) on these stimulus-induced responses. As shown in Fig. 6(b), this NMDA receptor antagonist abolished the stimulus-induced bursting responses that could, however, be re-established in the presence of CPP by increasing the stimulus strength [Fig. 6b(i), compare 60 and 110 microseconds frames in +CPP panel]. 3-[(±)-2-Carboxy-piperazin-4-yl]-propyl-1-phosphonate did not influence the ability of these neurons to generate action-potential bursts during the injection of depolarizing current pulses [Fig. 6b(ii)].

Discussion

Neurons recorded intracellularly in the deep layers of somatosensory neocortical slices obtained from NEC and WAG/Rij rats are characterized by similar electrophysiological and firing properties including the ability of some cells to generate 'all-or-none' action potential bursts. These data are in line with those reported by Luhmann *et al.* (1995) who analyzed

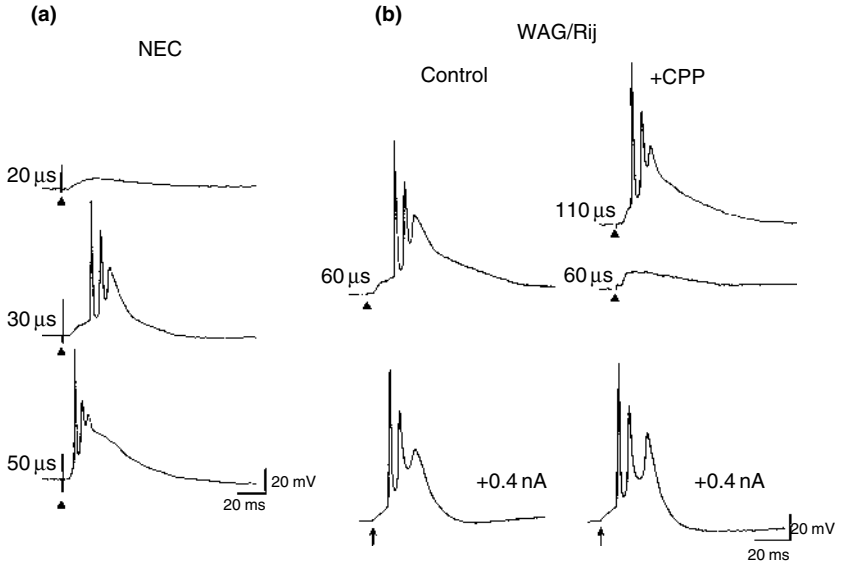

Figure 6: Synaptic responses recorded from bursting cells in non-epileptic control (NEC) and epileptic Wistar Albino Glaxo/Rijswijk (WAG/Rij) slice following white matter stimulation. (a) "All-or-none" characteristic of the bursting response that lasts less than 100 millseconds. (b) Effects induced by 3-[(±)-2-carboxy-piperazin-4-yl]-propyl-1-phosphonate (CPP) on the stimulus-induced bursting responses. Note that *N*-methyl-D-aspartate receptor blockade abolishes the bursting response induced by a stimulus with duration of 60 microseconds (middle row) without altering the intrinsic ability of the cell to generate action potential bursts (lower row, arrows indicate the onset of the depolarizing pulse, 0.4 nA). Note also that synaptic bursting responses can be elicited in the presence of CPP by increasing the stimulus strength (upper row).

the electrophysiological characteristics of WAG/Rij cells located in layers II/III and V of frontoparietal neocortical slices obtained from 180-day-old rats (i.e. an age that was similar to what used by us). By using intracellular injection of neurobiotin into some NEC and WAG/Rij neurons, we have also established that regularly firing and bursting cells in the two strains have similar pyramidal shape with an apical dendrite extending to the superficial layers. Regularly firing and bursting cells recorded in deep neocortical layers have been previously described as pyramidal (Chagnac-Amitai *et al*. 1990; Larkman & Mason 1990; Mason & Larkman 1990).

We have also found that the WAG/Rij neocortical tissue maintained *in vitro* is characterized by synaptic hyperexcitability that is presumably contributed by NMDA receptor-mediated conductances. In line with this conclusion, more than 43% of the WAG/Rij regularly firing neurons responded to focal stimuli delivered in the underlying white matter with a late synaptic depolarization that could cause action potential discharge. Similar long-lasting depolarizing responses have been previously reported in some WAG/Rij cells recorded intracellularly in the frontoparietal cortex (Luhmann *et al*. 1995). In contrast, this type of stimulating protocol (which is expected to mimic the activation of thalamocortical inputs, Agmon & Connors 1991) elicited in nearly all NEC regularly firing neurons monophasic EPSPs that triggered a single action potential; this type of synaptic response was also identified in the rest of the WAG/Rij regularly firing cells. Moreover, the late synaptic depolarization/burst discharge recorded from WAG/Rij regularly firing cells was readily abolished by bath application of the NMDA receptor antagonist CPP. These NMDA receptor-mediated synaptic responses were followed by a long-lasting hyperpolarization. Although we did not test the pharmacological nature of this hyperpolarizing event, its reversal potential suggests an underlying K^+ conductance. It is tempting to speculate that this hyperpolarization may contribute to the "slow wave" component of the SW discharge *in vivo*.

The overexpression of NMDA receptor-mediated synaptic responses in WAG/Rij neocortical tissue as well as their potential role in making these neuronal network hyperexcitable is in line with data obtained *in vivo* in this model of absence seizures by Peeters *et al*. (1989; 1990) who have reported that NMDA receptor antagonism reduces the number of SW discharges and their mean duration. In addition, Pumain *et al*. (1992) have found that the responses induced by local NMDA application are more widely distributed in the neocortex of GAERS (which are also genetically predisposed to generate generalized SW discharges) than in control animals. Finally, we have described a region-specific contribution of neocortical NMDA receptors to slow field oscillations that are induced by 4-aminopyridine in WAG/Rij but not in NEC thalamocortical slices (D'Arcangelo *et al*. 2002). We found in these experiments that slow field oscillatory activity disappears in WAG/Rij thalamocortical slices when CPP is locally applied to the neocortex, but it continues to occur when this NMDA receptor antagonist is delivered within

the thalamic region. In both NEC and WAG/Rij neocortical cells, we have also identified the presence of non-NMDA glutamatergic receptor-mediated potentials that can lead to stimulus-induced synaptic bursting in neocortical cells that are intrinsic bursters during the injection of depolarizing current pulses. These synaptic mechanisms may play an important role in implementing *in vivo* the spread of SW discharges throughout the neocortex by recurrent excitatory transmission that recruits superficial layer neurons.

A decreased function of GABA receptor-mediated inhibition is an obvious candidate for facilitating the expression of NMDA receptor-mediated mechanisms (cf. Thomson & West 1986) and thus synaptic hyperexcitability within the WAG/Rij neocortical network. Indeed, Luhmann *et al.* (1995) have reported that WAG/Rij neocortical neurons located in layers II and III generated fast and slow IPSP that were characterized by less negative reversal potentials than in NEC cells; in addition, they found that both the IPSP components were associated in WAG/Rij neurons with peak conductances that were significantly smaller than in NEC cells. Although we have found that the peak conductance of the fast IPSP component is significantly smaller in WAG/Rij deep layer cells, the other parameters analyzed in this study had similar values in the two strains. Hence, our findings suggest that the efficacy of GABAergic postsynaptic inhibition may be more preserved within the deep layers of the WAG/Rij somatosensory cortex and suggest that intracortical inhibition in this genetic model of absence seizure may be weakened in a layer-specific manner. Indeed, the preservation of some form of postsynaptic inhibition in WAG/Rij animals is well in keeping with the view that GABA receptor-mediated inhibition is critical to the generation of the generalized SW discharge as well as that a full-blown breakdown of inhibition in a cat model of absence seizures is mirrored by the appearance of electrographic tonic-clonic seizures (Avoli *et al.* 2001; Kostopoulos *et al.* 1983).

Our findings indicate that the hyperexcitable condition that characterizes neocortical networks in WAG/Rij rats presenting with absence seizures *in vivo* results from the abnormal function of ligand-dependent mechanisms. In WAG/Rij rats – known to transmit absence seizures according to an autosomal-dominant monogenetic inheritance (Peeters *et al.* 1992) – two different loci (*T1swd/wag* and *T2swd/wag* located on chromosomes 9 and 5, respectively) have recently been shown to be linked to SW discharges (Gauguier *et al.* 2004). In particular, *T1swd/wag* appears to be involved in seizures generated in the somatosensory cortex, the most frequent type of SW discharges found in epileptic WAG/Rij rats. Although several genes encoding ion channels, transporters or metabolic enzymes are found in these two loci, it is presently unknown which one may be altered in WAG/Rij rats. However, NMDA receptor subunits have been localized in the human chromosome 9 (Andersson *et al.* 2001), which is homologous to rat chromosome 5 (Szpirer *et al.* 1990). In addition, the key-enzyme glutaminase has been found in the rat chromosome 9 (Mock *et al.* 1989), suggesting the possibility that changes in glutamate synthesis and release could be associated to SW discharges in WAG/Rij rats. Hence, these genetic data, along with the evidence of functional overexpression of NMDA receptor-mediated depolarization, suggest that glutamate receptors could be good candidates for the identification of new pathophysiogenetic mechanisms in absence seizures.

Tolmacheva *et al.* (2004) have recently reported an increase in the duration of afterdischarges generated *in vivo* by epileptic WAG/Rij rats when compared with Wistar animals. In addition, these authors have also found hyperexcitable characteristics in the limbic system of these animals. Indeed, these data are in line with a lower threshold to pentylenetetrazole-induced seizures that was identified in epileptic WAG/Rij rats by Kliouéva *et al.* (2001). However, similar hyperexcitable patterns also occur in August x Copenhagen Irish (ACI) rats, which rarely present with SW discharges and absence seizures (Tolmacheva *et al.* 2004). Hence, these data may suggest that the functional overexpression of NMDA receptor-mediated potentials identified by us in WAG/Rij rat neocortical slices may reflect a mere strain difference that is unrelated to the presence of SW discharges *in vivo*. Although further studies are needed to clarify this issue, yet the ability of the NMDA receptor antagonist CPP to abolish slow field oscillations that are recorded in epileptic WAG/Rij rat slices (but not in those obtained from Wistar animals) (D'Arcangelo *et al.* 2002) supports the view that NMDA receptor-mediated mechanisms may represent a good candidate for the occurrence of absence seizures *in vivo*. At the same time, our findings highlight the role of neocortex in the genesis of generalized SW discharges *in vivo* as proposed in earlier studies in normal cats that were subjected to pharmacological manipulations or electrical stimulation (Avoli *et al.* 1983; Neckelmann *et al.* 1998; Steriade & Contreras 1998; Steriade *et al.* 1998; Timofeev *et al.* 1998) and in human studies (Gloor 1969).

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Acknowledgments

This study was supported by the Canadian Institutes of Health Research, the 'Ministero dell'Istruzione, Università e Ricerca' of Italy (MIUR), the 'Fondo per gli Investimenti della Ricerca di Base' (FIRB) and the Savoy Foundation. M. D'Antuono was recipient of a Fellowship from the Fragile X Foundation of Canada. We thank Dr J. Robson for reading an early draft of this paper and Ms T. Papadopoulos for secretarial assistance.