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Generation of Slow Network Oscillations in the Developing Rat Hippocampus After Blockade of Glutamate Uptake

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Cattani AA, Bonfardin VD, Represa A, Ben-Ari Y, Aniksztejn L. Generation of slow network oscillations in the developing rat hippocampus after blockade of glutamate uptake. *J Neurophysiol* 98: 2324–2336, 2007. First published August 8, 2007; doi:10.1152/jn.00378.2007. Cell-surface glutamate transporters are essential for the proper function of early cortical networks because their dysfunction induces seizures in the newborn rat in vivo. We have now analyzed the consequences of their inhibition by DL-TBOA on the activity of the developing CA1 rat hippocampal network in vitro. DL-TBOA generated a pattern of recurrent depolarization with an onset and decay of several seconds' duration in interneurons and pyramidal cells. These slow network oscillations (SNOs) were mostly mediated by γ-aminobutyric acid (GABA) in pyramidal cells and by GABA and *N*-methyl-D-aspartate (NMDA) receptors in interneurons. However, in both cell types SNOs were blocked by NMDA receptor antagonists, suggesting that their generation requires a glutamatergic drive. Moreover, in interneurons, SNOs were still generated after the blockade of NMDA-mediated synaptic currents with MK-801, suggesting that SNOs are expressed by the activation of extrasynaptic NMDA receptors. Long-lasting bath application of glutamate or NMDA failed to induce SNOs, indicating that they are generated by periodic but not sustained activation of NMDA receptors. In addition, SNOs were observed in interneurons recorded in slices with or without the strata pyramidale and oriens, suggesting that the glutamatergic drive may originate from the radiatum and pyramidale strata. We propose that in the absence of an efficient transport of glutamate, the transmitter diffuses in the extracellular space to activate extrasynaptic NMDA receptors preferentially present on interneurons that in turn activate other interneurons and pyramidal cells. This periodic neuronal coactivation may contribute to the generation of seizures when glutamate transport dysfunction is present.

INTRODUCTION

Dysregulation of glutamate homeostasis has been suggested to underlie several neurological disorders including amyotrophic lateral sclerosis, Alzheimer disease, and epilepsies (Kelly and Staley 2001; Maragakis and Rothstein 2004). This dysregulation may originate from an alteration in glutamate metabolism and also from a dysfunction of cell-surface glutamate transporters. Factors that affect transporters function (such as mutation, pH, and phosphorylation) have been extensively reported (Danbolt 2001; Gegelashvili and Schousboe 1997; Kalandadze et al. 2004; Maragakis and Rothstein 2004; Takahashi et al. 1997). Glutamate transporters are present at the surface of glial cells (i.e., GLT1 and GLAST) and neuronal cells (i.e., EAAC1, EAAT4, EAAT5), where they bind and transport glutamate into cells from the extracellular space (Danbolt 2001). Thus glutamate transporters play a key role in the clearance of extracellular glutamate and the prevention of glutamate excitotoxicity, receptor desensitization, and neuronal coactivation (Arnth-Jensen et al. 2002; Danbolt 2001).

There is now compelling evidence that glutamate transporters play an important role during cortical development before and during the formation of synapses. For example, glutamate transporters are expressed at fetal stages in the human and rodent brains (Bar-Peled et al. 1997; Furuta et al. 1997; Ullensvang et al. 1997), functioning during cell proliferation, migration, and differentiation because the deletion of genes encoding for GLT1 and GLAST strongly compromises the development of the cortex (Matsugami et al. 2006). Glutamate transporters are also essential for the proper function of early cortical networks because their inhibition generates partial seizures and recurrent paroxysmal activity in the electroencephalogram (EEG) of newborn rats (Milh et al. 2007), a cortical pattern reminiscent of a "suppression burst" observed in some severe forms of neonatal epilepsy (Aicardi 1985; Ohtahara and Yamatogi 2003; Schlumberger et al. 1992). Because of the clinical relevance of all these studies, it is important to clarify the role of these proteins during maturing cortical network activity. We previously showed that inhibition of glutamate transporters by DL-threo-β-benzyloxyaspartate (DL-TBOA) generated slow recurrent depolarization and burst of action potentials in developing neocortical pyramidal cells in vitro (Demarque et al. 2004). To evaluate whether this type of activity can also be generated in other brain regions and get more insights on the mechanism of this pattern, we focused our attention now on the CA1 region of the rat hippocampus, an area in which the development of synaptic and network activities are well documented. Studies have shown that glutamate play a major role in the generation of network-driven events, i.e., the giant depolarizing potential (GDP; Ben-Ari 2001; Cherubini et al. 1991). However, its action is powerfully controlled by pre- and postsynaptic mechanisms (Durand et al. 1996; Gasparini et al. 2000; Groc et al. 2002; Lauri et al. 2006) and glutamate transporters that prevent its diffusion in the extracellular space (Demarque et al. 2002; see also Marchionni et al. 2007). It is likely that this control is important for the maturation of hippocampal networks and that a deficiency of glutamate uptake would significantly alter network activities.

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In the present study, we show that in developing hippocampus, as in neocortex, the inhibition of glutamate uptake dramatically modifies the pattern of activity and generates network oscillations of several seconds' duration, in both interneurons and pyramidal cells—a process that involves the activation of extrasynaptic *N*-methyl-D-aspartate (NMDA) receptors.

METHODS

All the experiments performed in this study conformed to the French animal use legislation.

Electrophysiology

Experiments were performed on CA1 pyramidal neurons in hippocampal slices from Wistar rats obtained from the day of birth (P0) to postnatal day 20 (P20). Wistar rats were decapitated under chloral hydrate anesthesia (20–40 mg/kg). Brains were rapidly removed and placed in oxygenated ice-cooled artificial cerebrospinal fluid (ACSF) with the following composition (in mM): 126 NaCl, 3.5 KCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 25 $NaHCO_3$, 1.2 $NaHPO_4$, and 10 glucose (95% O_2-5% CO_2, pH 7.4). Coronal hippocampal slices (350–400 μm) were obtained with a vibratome and kept in oxygenated ACSF at room temperature at least 1 h before use. Individual slices were then transferred to the recording chamber where they were fully submerged and superfused with ACSF at 32–34°C at a rate of 2–3 ml/min. Neurons were recorded under visual control with a Zeiss Axioscope microscope using patch-clamp technique in the whole cell configuration. Microelectrodes had a resistance of 5–10 MΩ and were filled with a solution containing (in mM): 100 KM_2SO_4 or KGlu; 40 KCl, 0.1 $CaCl_2$, 1.1 EGTA, 10 HEPES, 4 Mg^{2+}ATP, and 0.3 Na^+GTP (pH 7.25; 270–280 mOsm), sometimes with *N*-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314) to block voltage-dependent Na^+ channels in the recorded cell. Biocytin was also added to the pipette solution for post hoc reconstruction of the recorded cells (see Tyzio et al. 1999). Some experiments have been performed with a CsGlu-filled pipette solution (in mM): 100 CsGlu, 40 CsCl, 0.1 $CaCl_2$, 1.1 EGTA, 10 HEPES, 4 Mg^{2+}ATP, and 0.3 Na^+GTP. Whole cell measurements in voltage-clamp or current-clamp mode were filtered at 3 kHz using an EPC-9 amplifier (HEKA Electronik). All electrophysiological data were digitized (1–2 kHz) with a digidata (Axon Instruments) interface card to a personal computer and analyzed with MiniAnalysis program (Synaptosoft). To obtain current–voltage (*I–V*) curves of slow network oscillations (SNOs), voltage ramps were applied for 1 s from the resting membrane potential to 20 mV and performed with patch pipettes containing QX-314. In some experiments, SNOs were recorded at +30 mV with CsGlu-filled pipettes and voltage ramps were applied from this membrane potential to −100 mV. *I–V* curves were constructed by subtracting the ramp response in control from the maximal current response. The *I–V* relation of NMDA-receptor–mediated response was performed with CsGlu-filled pipettes with a similar ramp protocol. The response was evoked by the pressure ejection of NMDA (30–100 μM) over a period of 2 s with a patch pipette connected to a picospritzer (General Valve, Fairfield, NJ). NMDA was applied four to five times per cell and the *I–V* relations obtained in individual cells were averaged and expressed as a percentage of the mean value obtained at 30 mV and then pooled for each cell type (interneurons and pyramidal cells). Extracellular recordings were performed with a glass pipette (<1 MΩ) filled with ACSF to record multiple-unit activity (MUA) and the signal was recorded with a DAM80 amplifier (WPI).

Morphological analysis

Sections used for electrophysiological analysis were fixed for 24 h (4% paraformaldehyde plus 0.5% glutaraldehyde) and subsequently stained with cresyl violet or with a Nissl fluorescent dye (NeuroTrace; Invitrogen/Molecular Probes) according to the manufacturer's instructions. Slices were analyzed using a confocal microscope (Zeiss LSM 510) to evaluate the efficacy of the dissection of hippocampal subfields and layers.

Statistics

Data are expressed as means ± SE. Statistical significance of difference between means was assessed with ANOVA test and the level of significance was set at $P < 0.05$.

Drugs

DL-TBOA, 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), bicuculline, D-2-amino-5-phosphonovaleric acid (D-APV), (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801), NMDA, and γ-aminobutyric acid (GABA) were purchased from Tocris (Bristol, UK). Tetrodotoxin (TTX) and QX-314 were purchased from Sigma–Aldrich (Strasbourg, France).

RESULTS

We used DL-TBOA, a drug known to inhibit glial GLT1 and GLAST and neuronal EAAC1 glutamate transporters (Shimamoto et al. 1998), the main glutamate transporters expressed in the rat hippocampus (Furuta et al. 1997; Ullensvang et al. 1997). DL-TBOA is a nontransportable inhibitor that does not induce an artificial release of glutamate through hetero-exchange and it does not act as partial agonist of glutamate receptors (Demarque et al. 2004; Jabaudon et al. 1999; Shimamoto et al. 1998; also see Anderson et al. 2001).

Blocking glutamate transporters generates slow network oscillations in the hippocampus

In a first series of experiments we determined the consequence of glutamate transporter inhibition on the whole developing hippocampal network activity from the day of birth (P0) to postnatal day 6 (P6). To this end, CA1 pyramidal cells were recorded using whole cell patch clamp technique in current clamp mode together with field recording of multiple-unit activity (MUA) in CA1 ($n = 7$ slices) or CA3 ($n = 17$ slices) using an extracellular electrode placed in the stratum pyramidale. In addition, we performed dual-field and whole cell recordings in CA3 ($n = 4$ slices). As already described in several studies the pattern of electrophysiological activity of the developing rat hippocampus during the firsts postnatal days of life is composed by fast synaptic events and also by the endogenous network-driven events, the so-called giant (GABAergic) depolarizing potentials (GDPs) (for reviews see Ben-Ari 2001; Cherubini et al. 1991). These rhythmic activities were reflected at the cellular level by a recurrent depolarization of duration 300 ms to 1 s, associated with MUA, and they occurred with a frequency of one GDP every 2 to 20 s. In CA1, GDPs were observed in 15/24 slices, whereas in CA3 they were observed in 20/21 slices. When CA1 and CA3 were recorded together, GDPs were observed concomitantly in both layers in 11/17 slices and were observed only in CA3 but not in CA1 in six slices. Bath application of DL-TBOA (50–100 μM) modified the pattern of activity and generated, after 3–5 min of application, recurrent slow and long-lasting depolarization in CA1 and CA3 lasting 32 ± 4 s ($n = 28$ slices)

associated with long-lasting recurrent MUA (mean 38 ± 13 s) (Fig. 1, *A* and *B*). Dual patch-clamp of CA1 pyramidal cells and field recordings in CA3 showed that MUA occurred in the same time window as the cellular depolarization (Fig. 1*C*). These recurrent activities that we shall refer to as *slow network oscillations* (SNOs) occurred with a mean frequency of one oscillation every 136 ± 10 s (n = 17 slices). SNOs in both layers were no longer observed after the washout of TBOA and they were always blocked by NBQX (10 μM), the antagonist of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors, and APV (80 μM) or MK-801 (10 μM), the antagonists of NMDA receptor (n = 5/5 slices; three with APV and two with MK-801), indicating that SNOs involved the activation of ionotropic glutamate receptors (data not shown).

Because the CA3 region is thought to drive most of the physiological and pathological network-driven activity in the hippocampus (Ben-Ari 2001; Ben-Ari et al. 1989; De la Prida et al. 2006; Menendez de la Prida et al. 1998), we next wondered whether the generation of SNOs in CA1 originated from CA3. We therefore disconnected CA1 from CA3 by a knife cut and tested the action of TBOA. GDPs were observed in both layers (n = 5/8 slices and n = 7/8 slices in CA1 and CA3, respectively) as already described in other studies (Garaschuk et al. 1998; Menendez de la Prida et al. 1998). DL-TBOA still generated SNOs in both regions (n = 8/8 slices, Fig. 1*D*). In CA1 the disconnection from CA3 did not significantly change the frequency of SNOs (one oscillation every 138 ± 6 s). In CA3 the oscillations occurred at a frequency rate of one burst every 118 ± 6 s. In the disconnected slices SNOs did not always occur concomitantly [i.e., SNOs may be observed in CA3 but not in CA1 and vice versa (see Fig. 1*D*)]. Together, these data indicated that *1*) the CA1 region can generate rhythmic activities independently of the CA3 region and *2*) the connection between the two areas allowed a better coherence of SNOs.

We then decided to focus our attention on the CA1 region to understand how SNOs may be generated specifically in this network. To this aim we conducted our experiments in CA1 minislices in which CA3 and the dentate gyrus were completely removed.

In CA1 pyramidal cells, SNOs were mediated mostly by the activation of $GABA_A$ receptors

SNOs were generated in 49/53 cells recorded in the minislices independently of the presence or the absence of GDPs before the application of DL-TBOA (see Fig. 2). SNOs had the following characteristics. *1*) In voltage-clamp mode, SNOs were characterized by periodic slow inward currents that reach

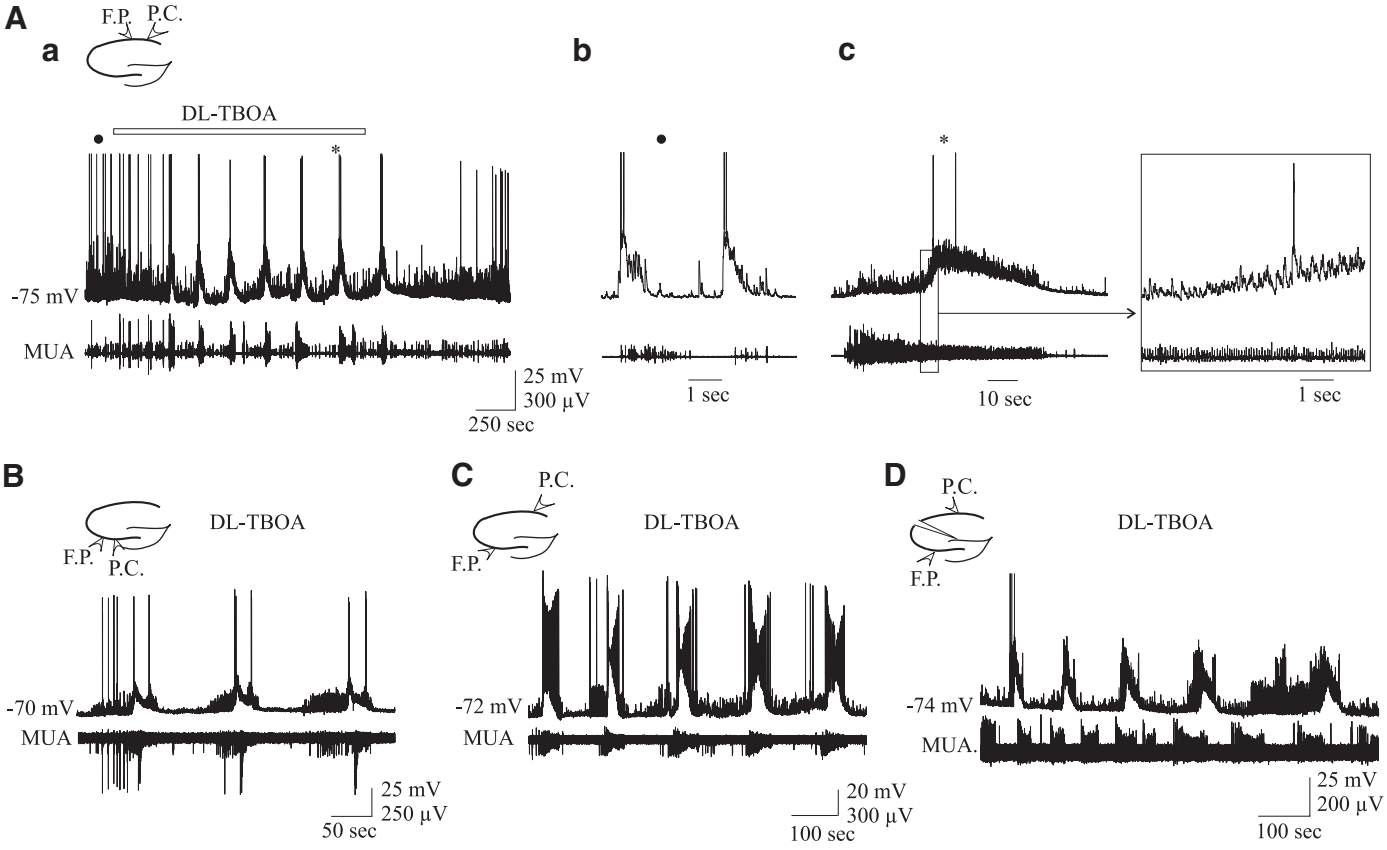

FIG. 1. Inhibition of glutamate transporters generates slow network oscillations (SNOs) in the hippocampal network. *Aa*: scheme shows the positions of both the extracellular field pipette (F.P.) to record multiple-unit activity (MUA) and patch-clamp (P.C.) electrodes in the CA1 region of the hippocampus. Control activity was composed by giant depolarizing potential (GDPs; see at expanded timescale in *b*, black dot). DL-Threo-β-benzyloxyaspartate (DL-TBOA, 100 μM) produces slow recurrent depolarizations associated with MUA. GDPs recovered after the washout of TBOA. One of the depolarization (indicated by an asterisk) is shown in *c* at an expanded timescale. Note the slow onset of the oscillation shown at the same timescale as that of the GDPs. *B*: example trace showing that SNOs are also generated in CA3 by DL-TBOA (100 μM). *C*: traces showing that the recurrent depolarizations in CA1 occurred concomitantly with MUA in CA3. *D*: slow depolarizations recorded in CA1 and MUA in CA3 in a slice after the cut of the Schaffer collaterals. SNOs are no longer coordinated in the 2 regions.

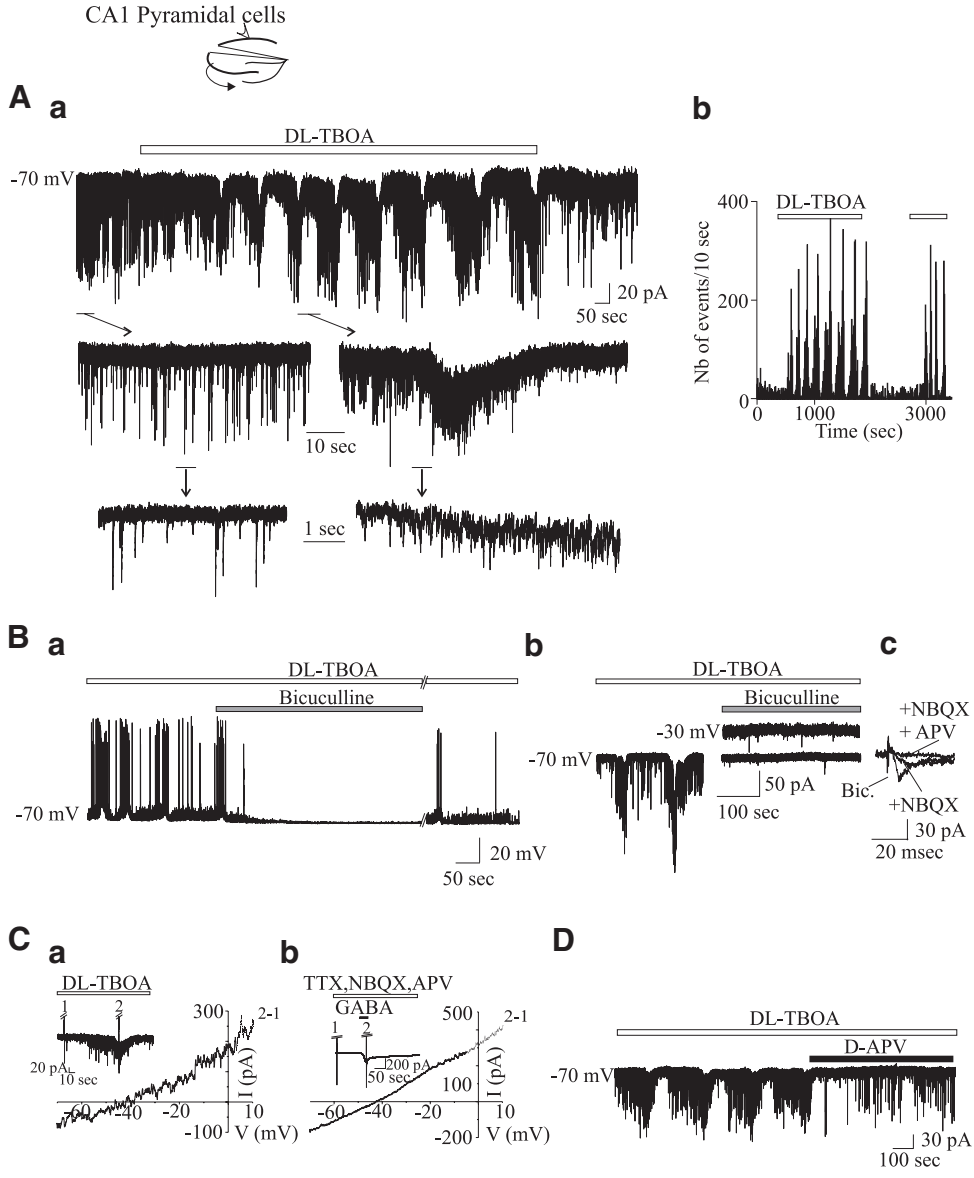

FIG. 2. Inhibition of glutamate transporters generates γ-aminobutyric acid type A ($GABA_A$)–receptor-mediated SNOs in pyramidal cells. *Aa*: pyramidal cell recorded in CA1 minislice. Trace shows SNOs recorded in voltage clamp in a pyramidal cell from a postnatal day 1–old (P1) rat. Some events in control and one oscillation are shown at 2 expanded timescales below. No GDPs were observed in this cell before the application of DL-TBOA. Note also the progressive growing of the oscillation. *b*: graph showing the effects of DL-TBOA on spontaneous events vs. time (bin size = 10 s) and corresponding to the full experiment shown in *a*. Each SNO is associated with a large increase in the number of spontaneous events. This effect is reversible after the washout of DL-TBOA. SNOs are reinstalled after a second application of TBOA. *Ba*: pyramidal cell from P6 rat recorded in current-clamp mode. SNOs are fully blocked by bicuculline (20 μM). *b*: same cell recorded in voltage clamp after the washout of bicuculline and the recovery of SNOs. Bicuculline fully abolished SNOs at −70 and −30 mV. This cell has spontaneous and evoked excitatory postsynaptic currents (EPSCs) mediated by glutamate receptors. *c*: in the presence of bicuculline, stimulation of the Schaffer collaterals evoked at −30 mV an EPSC that is not fully blocked by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX, 10 μM) and requires D-2-amino-5-phosphonovaleric acid (D-APV, 80 μM) to be fully abolished. *C*: current–voltage (*I–V*) relationship of SNOs (*a*) and of GABA-mediated current (*b*) in 2 different cells. GABA (10 μM was bath applied for 20 s). Cells were recorded with a pipette filled with KGlu solution containing *N*-(2,6-dimethylphenyl carbamoylmethyl)triethylammonium bromide (QX-314) to block the voltage-dependent Na^+ channels. *I–V* relationship was constructed by subtracting the ramp response in control (1) from that during the maximal current response (2) (see METHODS). *D*: pyramidal cell from P1 rat recorded in voltage-clamp mode. SNOs are fully blocked by D-APV (80 μM).

a maximum between 3 and 15 s (mean 7 ± 2 s) and with a duration that ranged between 10 and 60 s (mean 28 ± 1 s). They were associated with a three- to 20-fold increase in the number of spontaneous synaptic events (Fig. 2*Ab*, mean frequency rate of 18 ± 1 Hz). *2*) SNOs reversed polarity at the same membrane potential as that evoked by bath application of GABA (−34 ± 2 mV, $n = 16$ and −37 ± 2 mV, $n = 6$, for SNOs and GABA, respectively; Fig. 2*C*). *3*) In the large majority of pyramidal cells, SNOs were fully abolished by bicuculline (20 μM, $n = 13/18$ cells, Fig. 2*B*) or by gabazine (0.5 μM, $n = 3/5$ cells). Although $GABA_A$-receptor antagonists failed to block SNOs in seven cells, the antagonist strongly reduced their amplitude by 70–90% and in three of these cells it also reduced their occurrence (two to three oscillations in 15 min). In all cases, subsequent addition of APV blocked them (not shown). *4*) In the absence of bicuculline, D-APV (80 μM) or MK-801 fully abolished them ($n = 5/5$ cells, four cells with APV, one cell with MK-801; Fig. 2*D*). *5*) They were blocked by TTX (1 μM, $n = 3/3$ cells; not shown), a blocker of voltage-dependent Na^+ channels indicating that SNOs were mediated by an action potential–dependent release of transmitters.

Altogether, these data indicated that in pyramidal cells SNOs were mediated mostly by $GABA_A$ receptors and required the activation of NMDA receptors for their generation. This suggests that the initiation of SNOs might take place in GABAergic interneurons.

In interneurons, SNOs are mediated by the activation of both $GABA_A$ and NMDA receptors

We have recorded interneurons in the oriens or radiatum strata of the CA1 minislices. SNOs were generated after bath application of DL-TBOA ($n = 32/38$ cells). In interneurons, SNOs were associated with MUA in the stratum pyramidale, indicating that SNOs concerned at the same time pyramidal cells and interneurons of the CA1 region (Fig. 3*A*). SNOs in interneurons have similar duration (33 ± 3 s in current clamp and 31 ± 2 s in voltage clamp), frequency (one oscillation

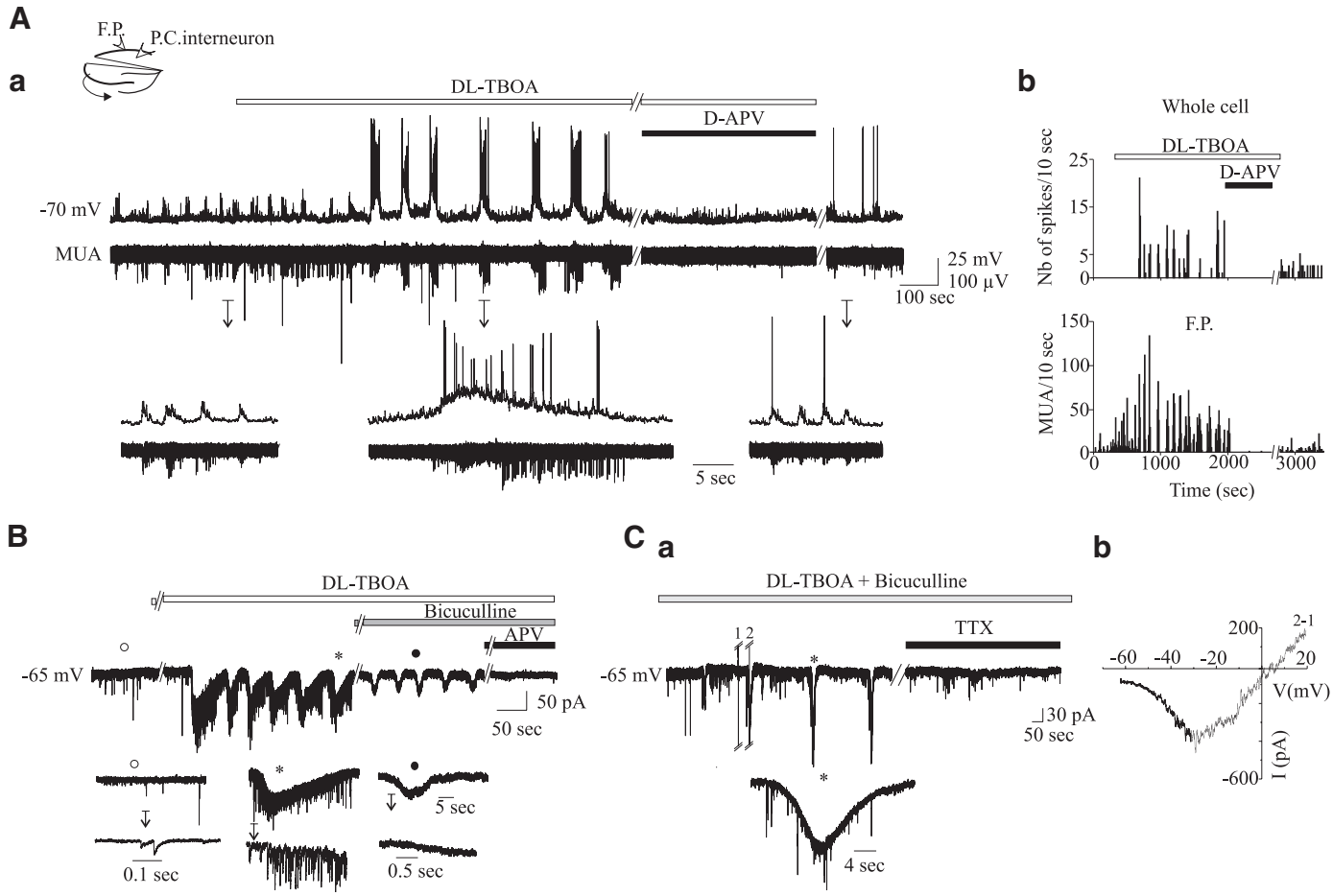

FIG. 3. SNOs in interneurons are mediated by $GABA_A$ and *N*-methyl-D-aspartate (NMDA) receptors. *Aa*: interneuron from P0 recorded in the stratum radiatum (*top trace*) with a KM_2SO_4-filled pipette solution and combined with the extracellular recording of MUA with a pipette located in the stratum pyramidale (*bottom trace*). Activity of the CA1 region is composed by GDPs and associated with MUA in the stratum pyramidale (shown at an expanded timescale below the 2 traces). Bath application of DL-TBOA generates SNOs. All the SNOs generated in this experiment are not shown in this figure. This pattern is blocked by D-APV (80 μM). Trace is shown 5 min after the application of D-APV. GDPs recover 15 min after the washout of the drugs. *b*: graph corresponding to the full experiment depicted in *a* and showing the effects of DL-TBOA on the number spikes in the interneuron and MUA in pyramidal cell layer (bin size = 10 s). *B*: interneuron from P6 rat recorded in the stratum oriens in voltage-clamp mode GDPs are absent during the control period. DL-TBOA generates SNOs that are not abolished by bicuculline (trace depicted is shown 10 min after the application of the antagonist). Remaining component is blocked by D-APV. SNOs are shown at expanded timescales below. Note the absence of EPSCs in the bicuculline-insensitive component of SNOs and the slow onset. *Ca*: tetrodotoxin (TTX) sensitivity of the isolated NMDA component of SNOs recorded in the presence of bicuculline in an interneuron of the stratum radiatum from P4 rat. Few EPSCs are observed during this slow current. *b*: *I–V* relationship of SNOs constructed by subtracting the ramp response in control (1) from that during the maximal current response (2).

every 121 ± 12 s), and time to peak (8 ± 1 s measured in voltage clamp) as in pyramidal cells.

We then determined which receptors mediated SNOs in interneurons. Bicuculline failed to block SNOs in a large majority of cells (n = 15/18 cells, Fig. 3*B*). The action of the antagonist was complex because from one cell to another it either decreased or increased or did not change the duration, the frequency, and the amplitude of SNOs. However, on average, bicuculline significantly reduced the duration of SNOs (in current clamp: 33 ± 3 vs. 19 ± 2 s in the absence and in the presence of bicuculline, respectively; in voltage clamp: 31 ± 2 vs. 21 ± 1 s in the absence and in the presence of bicuculline, respectively) but did not significantly affect their amplitude (−70 ± 12 and −57 ± 7 pA before and after bicuculline, respectively) or frequency (one oscillation every 121 ± 12 vs. 119 ± 20 s before and after bicuculline, respectively). The bicuculline-insensitive component had the following characteristics: *1*) it also had a slow onset with the peak of the current/depolarization being reached in 7 ± 2 s and blocked by D-APV (Fig. 3*B*); *2*) it may also be devoid of EPSCs (see Fig. 3*B* for example); *3*) the *I–V* relationship displayed a region of negative slope at membrane potential more negative than −20/−30 mV and the current reversed polarity at 5 ± 2 mV (n = 5; Fig. 3*Cb*); and *4*) it was blocked by TTX (n = 4/4 cells, Fig. 3*Ca*). Therefore this component was mediated by NMDA receptors that were activated by an action potential–dependent release of glutamate. In addition, application of APV alone, in the absence of bicuculline, also fully abolished SNOs (n = 5/5 cells, Fig. 3*A*).

These data suggest that *1*) in interneurons the activation of NMDA receptors is sufficient to generate SNOs; GABA-receptor activation amplifies the oscillations; and *2*) in pyramidal cells both NMDA- and $GABA_A$-receptor activation are necessary for expression of SNOs. Taken altogether, these data suggested that SNOs were initiated by the activation of NMDA receptors in interneurons that led to the

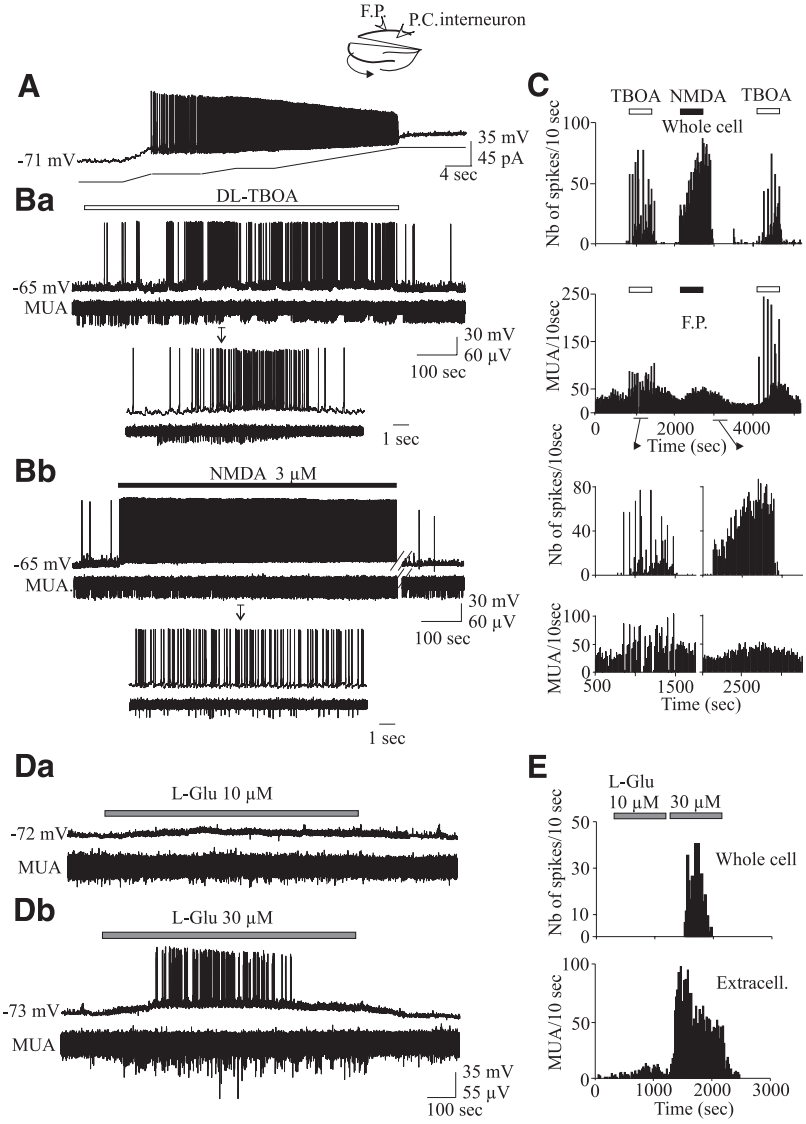

FIG. 4. SNOs are not generated by glutamate receptor agonists in interneurons. *A*: interneuron of P5 recorded in the stratum radiatum in current-clamp mode with a KM_2SO_4-filled pipette solution. Cell was progressively depolarized by injection of current (*bottom trace*). This leads to a sustained discharge of action potentials (*top trace*). *Ba*: DL-TBOA induces recurrent depolarizations and bursts of action potentials in interneurons and MUA in the stratum pyramidale. *Bb*: same recordings as in *Ba*. After the washout of DL-TBOA, NMDA was applied and generates a sustained cellular depolarization and action potential and unit discharges. *C*: graphs of the full experiment partially shown in *B* representing the number of spikes in the interneuron and MUA in the stratum pyramidale vs. time (bin size = 10 s). Two *bottom graphs* show at an expanded timescale the discharge during application of the 2 drugs. Note the recurrent increase of the discharge during DL-TBOA and the continuous increase during NMDA. *Da*: interneuron of P4 recorded in the stratum radiatum in current-clamp mode with a KM_2SO_4-filled pipette solution combined with the extracellular recording of MUA in the stratum pyramidale. Both glutamate concentrations, 10 and 30 μM (*a* and *b*, respectively) failed to generate oscillations. *E*: graphs corresponding to the experiment shown in *D*.

firing of interneurons, the release of GABA, and the activation of $GABA_A$ receptors of both interneurons and pyramidal cells.

SNOs are not generated by applications of glutamate agonists

How are SNOs initiated? Because blockade of glutamate transporters elevates the extracellular concentration of glutamate ($[glutamate]_o$) (Danbolt 2001; O'Shea et al. 2002), a sustained increase of $[glutamate]_o$ may directly trigger membrane oscillations in interneurons due to the voltage dependence of NMDA receptors and activation of K^+ channels as shown in several structures including the supraoptic nucleus, the spinal chord, and the tractus solitarii (Hochmann et al. 1994; Hu and Bourque 1992; Tell and Jean 1993). A sustained elevation of $[glutamate]_o$ could also provide a sufficient depolarization of interneurons to generate recurrent bursts of action potentials if these neurons have intrinsic rhythmic bursting properties, as recently shown for some CA3 pyramidal neurons (Sipilä et al. 2005). An alternative hypothesis is that the elevation of glutamate in the extracellular space during SNOs is transient and not sustained.

To discriminate between these possibilities, we have analyzed the behavior of interneurons in response to depolarizing current injection to mimic the depolarization produced by glutamate, or to long-lasting bath application of NMDA, nontransportable agonist, or glutamate. As shown in Fig. 4*A* current injections generated a continuous firing of action potentials but failed to generate rhythmic bursts. None of the interneurons recorded for this study ($n = 18$) displayed intrinsic rhythmic activity irrespective of the membrane potential. Additionally, in contrast to DL-TBOA, long-lasting bath application of NMDA for a period of 15 to 20 min depolarized the cell and generated a sustained firing of the cell and MUA in the stratum pyramidale but not SNOs (Fig. 4, *B* and *C*). Increasing NMDA concentration to 30 μM ($n = 3$) or decreasing the concentration of NMDA to 1 μM ($n = 4$) failed to produce SNOs (data not shown). Glutamate (10–100 μM) also produced a continuous cellular depolarization and an increase in the number of MUA but not SNOs ($n = 4$, Fig. 4, *D* and *E*). A similar protocol was applied for pyramidal neurons and they

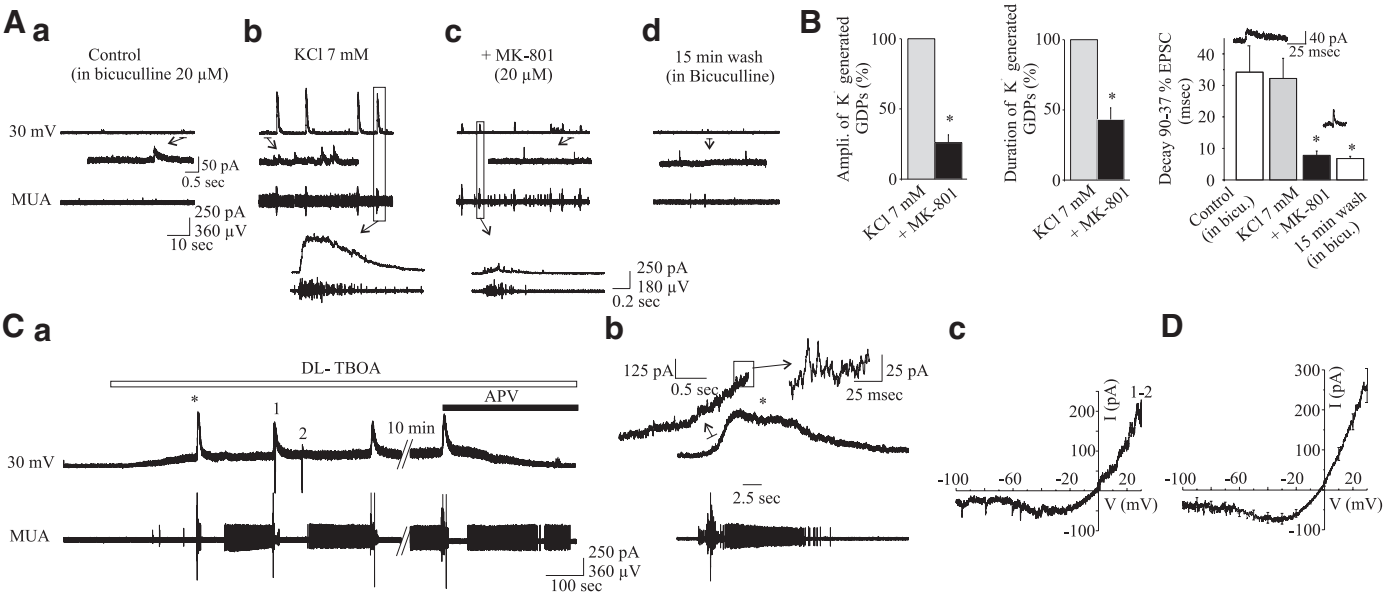

FIG. 5. NMDA extrasynaptic receptors contribute to the expression of SNOs. Concomitant recording of an interneuron of P6 located in the stratum oriens with a pipette filled with CsGlu solution at 30 mV in voltage-clamp mode and of MUA with an extracellular electrode placed in the stratum radiatum. Experiments were performed in the continuous presence of bicuculline (20 μM). *Aa–Ad*: 1-min recording in control (*a*), 8 min after application of KCl 7 mM (*b*), 9 min after the addition of (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine maleate (MK-801, 20 μM) (*c*), and 15 min after the reperfusion of the slice with control solution (KCl 3.5 mM and without MK-801) (*d*). Synaptic activity recorded during 5 s in the different conditions is shown at higher magnification between the 2 continuous traces. GDP-like events recorded during KCl 7 mM and KCl + MK-801 are shown at a higher timescale below the continuous traces. *B*: summary of the effects of MK-801 on both the amplitude (first histogram) and the duration of GDP-like events (second histogram) generated by KCl 7 mM in 5 cells. Third histogram shows the mean decay of EPSCs recorded in the 4 conditions in 6 cells. Two representative EPSCs recorded before and after MK-801 are also shown. *C*: DL-TBOA generates a tonic current and SNOs after MK-801. Both are abolished by D-APV. Slow current and the associated field activity indicated with an asterisk are shown at a higher timescale in *b*; beginning of the onset is also magnified. Rare EPSCs are present during the onset. They have kinetics of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)–mediated synaptic current. *I–V* relationship of the current constructed by subtracting the ramp response in control (2) from that during the maximal current response (1) is shown in *c*. *D*: mean *I–V* relationships (9 *I–V*s) of SNOs recorded in 5 cells.

did not display any rhythmic oscillations in response to current injection ($n = 5$; see supplemental Fig. 1*A*[1]) and SNOs were not generated after bath application of NMDA ($n = 5$) or glutamate ($n = 4$) (supplemental Fig. 1, *B–E*).

Therefore a sustained elevation of glutamate in the extracellular space and tonic activation of glutamate receptors did not generate SNOs. These results suggested that SNOs are initiated by a periodic increase of $[\text{glutamate}]_o$ and recurrent activation of NMDA receptors located in interneurons.

Extrasynaptic NMDA receptors contribute to SNOs

The very slow kinetics of the NMDA component of SNOs (in the presence of bicuculline) and the progressive growth of the current occurring sometimes in the absence of EPSCs together suggested that synaptic NMDA receptors may not fully account for the development of these oscillations. If this hypothesis is true then SNOs should still be produced in a neuron in which NMDA-mediated synaptic currents were abolished. To test this hypothesis, we used MK-801 (20 μM), the noncompetitive and irreversible NMDA-receptor antagonist that blocked the NMDA channel only when it is open. EPSCs were recorded in interneurons in the presence of bicuculline and at 30 mV, where Mg^{2+} did not block the NMDA channel. In addition an extracellular electrode was placed in the stratum radiatum to record MUA in this layer. The mean decay time constant of glutamate-mediated synaptic currents recorded in six cells in this condition is shown in Fig. 5*B*. To eliminate as much as possible NMDA-mediated EPSCs, the synaptic activity was enhanced by increasing the external concentration of KCl from 3.5 to 7 mM. This protocol augmented the synaptic activity two- to fivefold and MUA (Fig. 5*A*). Moreover, in keeping with another study (Sipilä et al. 2005), a rise in $[K^+]_o$ generated GDP-like events, as reflected by large outward currents associated with burst MUA (Fig. 5*A*, five of six cells). After 10 min, MK-801 was also bath applied for 10 min. This progressively diminished both the amplitude and the duration of the GDPs to a stable level that was reached after 7–9 min of application (from 274 ± 100 to 70 ± 35 pA, for the amplitude before and after the application of MK-801, respectively, and from 1.8 ± 0.3 to 0.7 ± 0.08 s for the duration before and during MK-801 application, respectively; Fig. 5, *A* and *B*). This also largely decreased the mean decay time of EPSCs to a value that corresponded to pure AMPA-mediated synaptic events (Fig. 5*B*) (see e.g. Cossart et al. 2002; Groc et al. 2002). DL-TBOA was applied 10–15 min after the reperfusion of slices with the control ACSF (in bicuculline). At this time GDP-like events were no longer present (Fig. 5*Ad*), whereas the mean decay time of EPSCs was similar to that during the application of MK-801 (Fig. 5*B*). This indicated that synaptic events were exclusively mediated by AMPA receptor, whereas NMDA-mediated EPSCs were still blocked by MK-801. In spite of this blockade, TBOA generated SNOs and recurrent bursts of units in five of six cells (Fig. 5*C*), occurring on a tonic outward current (10- to 200-pA range), as described in several

[1] The online version of this article contains supplemental data.

studies (Arnth-Jensen et al. 2002; Cavelier et al. 2002; Demarque et al. 2002; Jabaudon et al. 1999; Le Meur et al. 2007). SNOs had duration of 32 ± 2 s and occurred at a frequency rate of one oscillation every 215 ± 29 s. SNOs were blocked by APV and displayed an *I–V* relation characteristic of an NMDA-receptor–mediated component (Fig. 5, *C* and *D*). The mean frequency of SNOs was significantly lower than that measured with functional synaptic NMDA receptor (see previous text). In contrast the duration was similar to the isolated NMDA component of SNOs measured at 30 mV in separate experiments (34 ± 1 s, $n = 4$ cells, data not shown; also see following text).

These data suggested that the oscillations were expressed by the activation of extrasynaptic NMDA receptors.

NMDA activates interneurons more efficiently than pyramidal cells

The contribution of extrasynaptic NMDA receptors to SNOs suggested that the glutamate released periodically diffused in the extracellular space. Why did it preferentially activate interneurons compared with pyramidal cells? Are both cell types activated with the same efficacy by NMDA? To answer these questions we compared the effects of exogenous application of NMDA to generate current or depolarization in pyramidal cells and interneurons. First, we analyzed the *I–V* relationship of the NMDA-receptor–mediated response evoked by pressure ejection of NMDA (60–100 μM). These experiments were performed in the presence of NBQX (10 μM) and bicuculline (20 μM) to block respectively the AMPA/kainate receptors and $GABA_A$ receptors as well as TTX (1 μM) and Cd^{2+} (100 μM) to block respectively the voltage-dependent Na^+ and Ca^{2+} channels. In addition, voltage-dependent K^+ channels were blocked by tetraethylammonium (TEA, 20 mM) and CsCl (2 mM) and neurons recorded with a CsGlu solution–filled electrode. In both interneurons and pyramidal cells, NMDA was ejected close to the soma to reduce space-clamp errors and with the same pressure and duration. As shown in Fig. 6*A*, there was a significant difference in *I–V* curves of both interneurons and pyramidal cells at a membrane potential more negative than −55 mV. NMDA receptors of pyramidal cells were less activated at more hyperpolarized membrane potentials than those of interneurons. Second, to confirm this result under more physiological conditions, neurons were recorded in current-clamp mode with KM_2SO_4-filled pipette. We measured the input resistance and membrane capacitance of interneurons and pyramidal cells. Both cell types had the same capacitance (Fig. 6*F*) but showed a significant difference in their input resistance, which was significantly higher in interneurons than in pyramidal cells (Fig. 6*E*).

Next, we have analyzed the depolarization produced by NMDA application in interneurons and pyramidal cells in the presence of bicuculline and NBQX at the same membrane potential. To ensure that the depolarization evoked by pressure ejection of NMDA was mediated by NMDA receptors, the response was also evoked at the peak of the negative slope

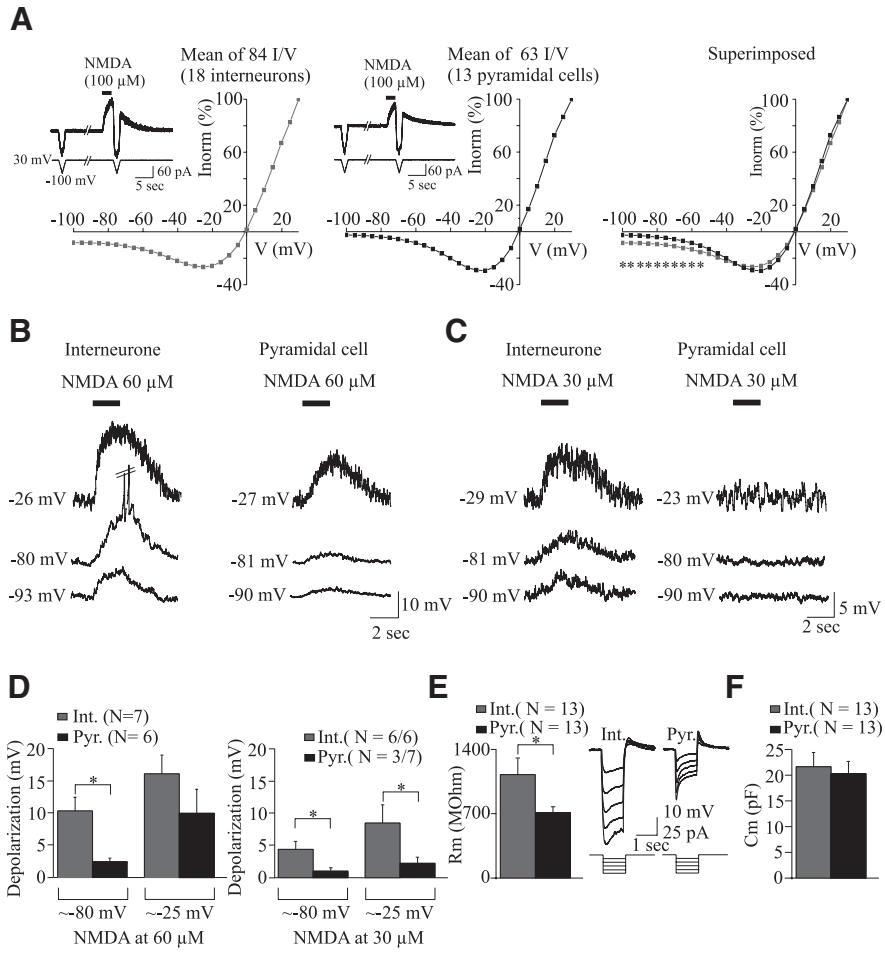

FIG. 6. Characteristics of NMDA-receptor–mediated responses in interneurons and pyramidal cells. *A*: *I–V* relationships of NMDA-receptor–mediated current in interneurons (grey) and pyramidal cells (black). *Insets*: protocol used to generate the *I–V* curves. Neurons have been recorded with pipette filled with CsGlu solution. Current was evoked by pressure ejection of NMDA. NMDA current is significantly larger in interneurons than in pyramidal cells at membrane potential more negative than −55 mV. *B* and *C*: NMDA-receptor–mediated response in interneurons and pyramidal cells recorded in current-clamp mode in the presence of NBQX and bicuculline with patch pipette filled with KM_2SO_4 solution. Depolarization was evoked by pressure ejection of NMDA during 2 s close to the soma at 60 μM (*B*) and at 30 μM (*C*) with the same pressure in both cell types (6 psi). *D*: histograms representing the mean depolarization produced by NMDA applied at 60 and 30 μM in interneurons and pyramidal cells at −80 and −25 mV. Depolarization is always significantly stronger in interneurons than in pyramidal cells. *E*: histogram representing the mean input resistance (R_m) of interneurons and pyramidal cells. *Left traces*: representative responses of an interneuron (Int.) and a pyramidal cell (Pyr.) following incremental current steps of −5 pA. *F*: histogram representing the mean capacitance (C_m) of interneurons and pyramidal cells.

(about −25 mV) and in some cases D-APV or MK-801 was applied at the end of the experiments. As shown in the Fig. 6, *B* and *D*, NMDA (60 μM) evoked a depolarization in interneurons and pyramidal cells that was stronger at −25 mV than that at −80 or −90 mV, in keeping with the voltage dependence of the NMDA-receptor–mediated response. However, the depolarization was significantly larger around −80 mV in interneurons than in pyramidal cells. Third, the concentration of NMDA in the pipette of ejection was decreased to determine whether the threshold concentration to generate a depolarization was the same in both cell types. As shown in Fig. 6*C* decreasing the concentration of NMDA to 30 μM in the pipette consistently evoked a depolarization in interneurons, whereas it failed to depolarize pyramidal cells in >50% of the cells. In the other cells, the depolarization was significantly lower than that in the interneurons (Fig. 6*D*). At 10 μM, NMDA failed to activate interneurons ($n = 5$ cells). Taken together, these data indicate that exogenous NMDA is more efficient to activate interneurons than pyramidal cells.

SNOs do not require the presence of pyramidal neurons

What is the source of glutamate release inducing SNOs? Blockade of the glutamate component of SNOs by TTX suggested that the release of glutamate had a neuronal rather than a glial origin because it required the generation of sodium action potentials. In addition, the fact that SNOs in pyramidal cells were largely blocked by bicuculline but still present in interneurons led us to determine whether pyramidal neurons were necessary for the generation of SNOs.

To test this hypothesis, the stratum radiatum was surgically isolated from the rest of the hippocampus and interneurons were recorded. To ensure the total ablation of pyramidal neurons, slices were analyzed post hoc with fluorescent Nissl staining or with cresyl violet. In this situation, DL-TBOA still generated SNOs (see supplemental Fig. 2*A*). The SNOs had the same properties as those recorded in control nonisolated minislices: *1*) they occurred at a frequency rate of one oscillation every 108 ± 10 s and their duration was 31 ± 2 s; *2*) they were fully blocked by APV; and *3*) they persisted in the presence of bicuculline and were subsequently blocked by TTX. To ensure that the APV sensitivity of SNOs reflected the presence of an NMDA component in the oscillations, we recorded in three different slices interneurons at 30 mV with CsGlu-filled pipette and analyzed the *I–V* relation of SNOs. In addition, to confirm that these oscillations are observed in this restricted network, we also recorded MUA in the stratum radiatum. As shown in Fig. 7, GDPs in control and SNOs during DL-TBOA were observed at both cellular and field level, indicating that these two rhythmic activities occurred concomitantly in a population of cells of this isolated interneuronal network. In the absence of bicuculline, SNOs had a duration of 41 ± 3 s and they occurred at a frequency rate of one oscillation every 97 ± 12 s. The current reversed polarity around −10 mV, a value more depolarized than E_{GABA} (Fig. 7, *C* and *D*). In the presence of bicuculline, SNOs occurred at a frequency rate of one oscillation every 90 ± 7 s and they had a duration of 33 ± 3 s. SNOs reversed polarity around 0 mV, the *I–V* relation displayed a region of negative slope, and this component was blocked by APV, indicating that SNOs were mediated by NMDA receptors (Fig. 7, *C* and *D*).

SNOs were also observed in interneurons recorded in the stratum oriens from slices containing both the strata pyramidale and oriens but not the strata radiatum ($n = 3/3$ slices; see supplemental Fig. 2*B*). In contrast, SNOs were not generated in interneurons recorded in isolated stratum oriens slices ($n = 3/3$ slices; not shown).

Taken together these data support the idea that SNOs may be initiated in the absence of the main glutamatergic pyramidal cells and suggested that an additional glutamatergic drive is present in the stratum radiatum that provides a source of glutamate necessary for the generation of SNOs.

The generation of SNOs is developmentally regulated

Finally, we wondered whether the generation of SNOs was restricted to immature networks or could be generated at adult

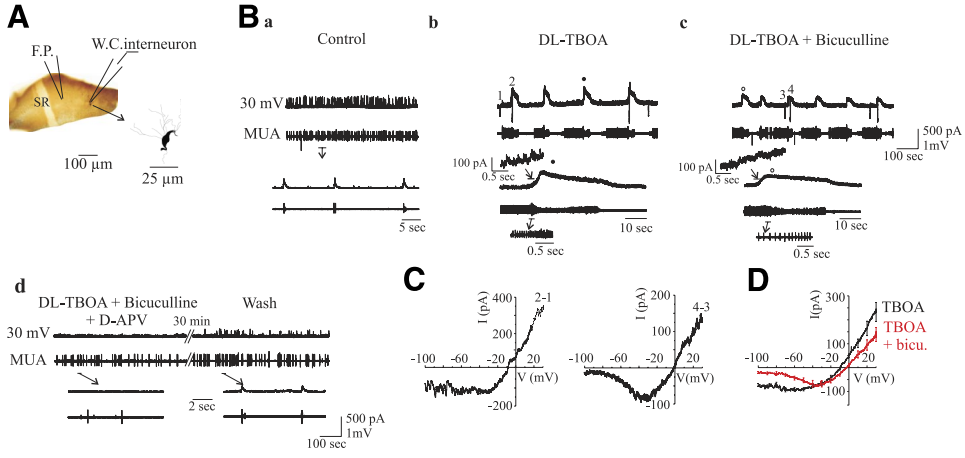

FIG. 7. SNOs occur in the absence of pyramidal cells. *A*: picture showing the isolated stratum radiatum of P5 stained with cresyl violet and the interneuron recorded with a CsGlu-filled pipette solution containing biocytin and reconstructed post hoc. An extracellular electrode was also placed in the stratum radiatum to record MUA. *Ba–Bd*: interneuron is recorded in voltage-clamp mode at 30 mV. GDPs are present in control (*a*) and DL-TBOA generates SNOs (*b*). They persist in the presence of bicuculline (*c*) and are fully abolished by APV (*d*). GDPs and SNOs are shown at a higher timescale below the continuous traces. Tonic current is not shown. *C*: *I–V* relationships of one of the slow current recorded in this experiment in the absence and in the presence of bicuculline. Ramps are indicated by numbers. *D*: mean *I–V* values of SNOs recorded in the absence (6 *I–V*s) and the presence of bicuculline (6 *I–V*s) in 3 different isolated stratum radiatum slices.

stages. To this aim, we first performed extracellular recordings in the stratum pyramidale to analyze the pattern of MUA in these populations of cells at different ages (P0–P20). At the beginning of the second postnatal week of life (P8–P9) DL-TBOA consistently generated recurrent long-lasting bursts of units characteristic of SNOs (4/4 slices). GDPs were also observed in these slices in control (Fig. 8*A*). After P12, GDPs were no longer observed and DL-TBOA failed to generate SNOs (14/14 slices) (Fig. 8*B*). Instead, the inhibition of glutamate transporters led to a continuous firing of units in the stratum pyramidale and a sustained depolarization and action potential discharge in the interneurons (3/3 cells, Fig. 8*C*). Together, these data indicated that SNOs were initiated during a restricted period of development.

DISCUSSION

The present study shows that during development, cell-surface glutamate transporters exert a powerful control of the hippocampal network activity preventing the occurrence of long-lasting bursts of activity due to the co-activation of a large neuronal population in both CA3 and CA1 areas. A deficiency in the transport of glutamate leads to the replacement of GDPs by slow network oscillations (SNOs), including most of the ongoing synaptic activity. We also show that the CA1 area can generate SNOs even when disconnected from CA3, which plays an important role in the generation of most of physiological and pathological network-driven events in the hippocampus (Ben-Ari 2001; Ben-Ari et al. 1989; De la Prida et al. 2006; Menendez de la Prida et al. 1998). The generation of SNOs is mediated by a periodic activation of extrasynaptic NMDA receptors primarily located on GABAergic interneurons. The interneurons then release GABA that is excitatory at that developmental stage (Ben-Ari 2002; Ben-Ari et al. 1989; Cherubini et al. 1991; Tyzio et al. 2006), leading to the activation of large populations of interneurons and pyramidal cells. Interestingly, our isolation experiments strongly suggest that the glutamatergic drive generating SNOs originates from radiatum and pyramidale strata. We propose that the coactivation of neuronal ensembles occurring during SNOs may contribute to the generation of seizures that are observed when glutamate transport is reduced (Milh et al. 2007).

Comparison of SNOs and GDPs

The mechanisms that generate GDPs have been extensively investigated in the developing hippocampus (for reviews see Ben-Ari 2001, 2002; Cherubini et al. 1991). The global picture that emerges from these studies is that a glutamatergic drive contributes to synchronize GABAergic interneurons that in turn generate the polysynaptic GDPs. Like GDPs, SNOs also require the activation of GABAergic interneurons by a glutamatergic neuronal source. Also, both patterns are observed in the disconnected CA1 and they do not require the presence of

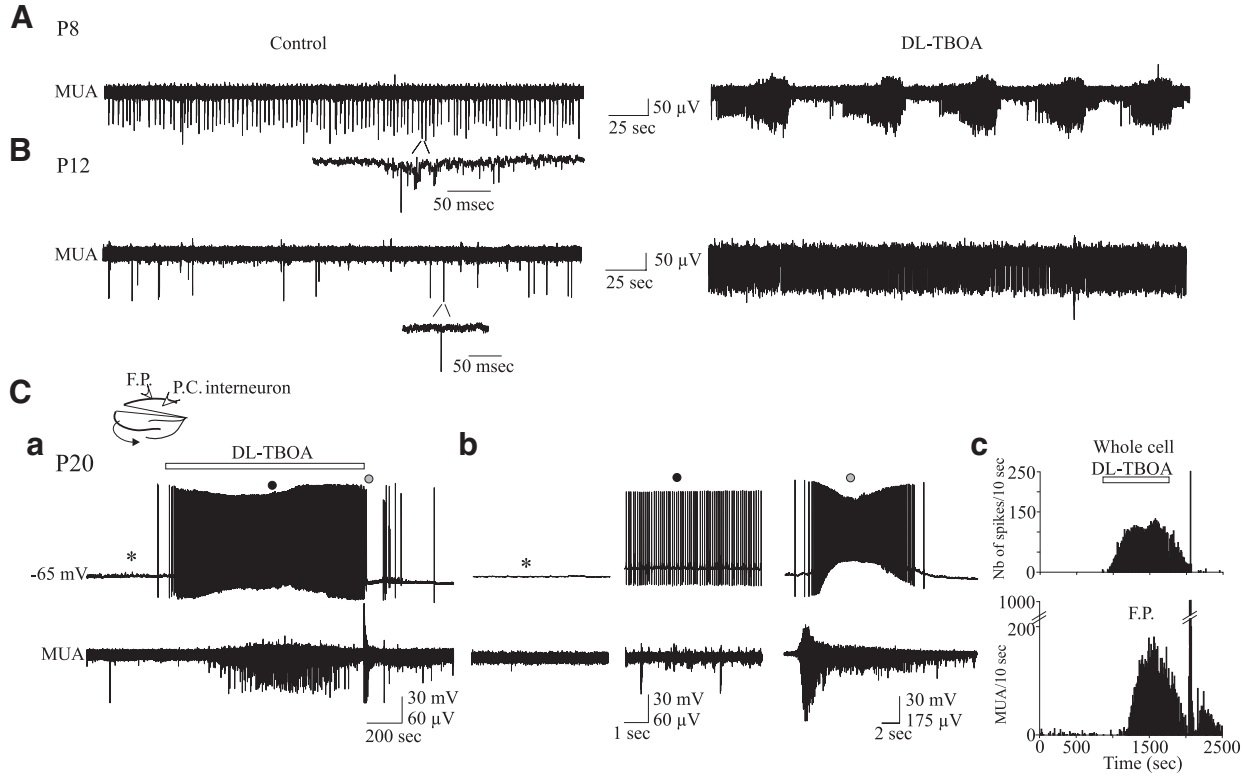

FIG. 8. Generation of SNOs is developmentally regulated. *A*: extracellular recording of MUA at P8 with a pipette located in the stratum pyramidale. Control activity is composed of GDPs (an example is shown at an expanded timescale below the continuous trace). Bath application of DL-TBOA generates recurrent bursts of MUA. *B*: same type of recording performed at P12. There are no more GDPs in control and bath application DL-TBOA (100 μM) induces a sustained discharge of MUA. *Ca*: interneuron of P20 recorded in the stratum radiatum combined with the extracellular recording (F.P.) of MUA in the stratum pyramidale. DL-TBOA induces a depolarization and a sustained discharge of action potentials in the interneuron and units in the stratum pyramidale. Note the presence of a burst at the end of DL-TBOA application. Traces indicated with symbols are shown at an expanded timescale in *Cb*. *Cc*: graph corresponding to the experiment shown in *Ca*. *Top graph*: number of spikes in the interneuron vs. time. *Bottom graph*: MUA recorded in the stratum pyramidale vs. time (bin size = 10 s).

pyramidal cells. Finally, they are restricted to a limited developmental stage because they are not observed after the second postnatal week of life.

However, SNOs differ from the GDPs by their very slow kinetics, i.e., the onset and the decay are of several seconds' duration for SNOs, whereas they are of only a few hundred milliseconds for GDPs. Several factors have been shown to modulate the frequency and/or the kinetics of GDPs (Bernard et al. 2005; McLean et al. 1996; Safiulina et al. 2005; Sipilä et al. 2004; Strata et al. 1995). At least in CA3, the blockade of $GABA_B$ receptors (McLean et al. 1996), GABA transporters (Sipilä et al. 2004), or cannabinoid receptors (Bernard et al. 2005) increased the duration of the GDPs for only few hundred milliseconds. However, none of these inhibitors produced modifications in the onset and duration of GDPs with the same magnitude as those generated after blockade of glutamate transporters. The time course of SNOs is reminiscent of that of the "paracrine" current that we previously described in CA1 during the perinatal period and is evoked by the release and diffusion of glutamate and GABA into the extracellular space and subsequent activation of extrasynaptic receptors (Demarque et al. 2002). Our data provide evidences that SNOs are also built or expressed by the activation of extrasynaptic NMDA receptors. Particularly, we found that in the presence of bicuculline, SNOs were still present after the blockade of NMDA-mediated EPSCs by MK-801 and with a duration similar to that observed with functional synaptic NMDA receptors. This procedure, however, decreases the occurrence of SNOs, suggesting that synaptic NMDA receptors may also play an important role in the initiation of the slow network oscillations. Because MK-801 was applied in the presence of 7 mM KCl that depolarizes the network, it is likely that synaptic NMDA receptors were affected not exclusively in the recorded cell but also in other cells of the network. This probably will alter the occurrence of the network oscillations. We propose that GDPs and SNOs may be initiated by the same glutamatergic drive but that the deficiency in the transport of glutamate enlarges the range of action of this drive in the network due to the diffusion of the glutamate released and extrasynaptic activation of receptors. This "spillover" may engage large populations of neurons in rhythmic activities including cells in which GDPs were absent.

SNOs are generated by a periodic release of glutamate

Blocking glutamate transporters enhances the extracellular concentration of glutamate (Danbolt 2001; O'Shea et al. 2002) and a recent study strongly suggests that this rise may originate from glial cells (Le Meur et al. 2007). The consequence is a tonic activation of glutamate receptors (mostly NMDA) of neurons that is revealed at positive membrane potential by a sustained outward current (Arnth-Jensen et al. 2002; Cavelier et al. 2002; Demarque et al. 2002; Jabaudon et al. 1999; Le Meur et al. 2007). Our data indicate that this tonic activation is insufficient to generate SNOs. Indeed, we showed that the long-lasting application of NMDA or glutamate induces a sustained activation of both interneurons and pyramidal cells but does not lead to the generation of slow oscillations. Therefore our data are better explained if, in addition to the persistent enhancement of $[glutamate]_o$, there is a periodic release of glutamate that diffuses in the extracellular space. However, we cannot exclude a contribution of tonic activation of glutamate receptors in the generation of SNOs. A recent study performed with dynamic two-photon microscopy and Ca^{2+} imaging described that the CA1 stratum pyramidale of neonatal mice hippocampus contains some neurons displaying intrinsic periodic bursts of action potential (Crepel et al. 2007). Because these recurrent bursts were observed in a particular range of membrane potential, a tonic depolarization produced by the activation of NMDA receptors may help these cells in generating this pattern and generate SNOs when glutamate transport fails.

Pyramidal cells are not required for the generation of SNOs

An intriguing observation is that SNOs can be generated in the absence of pyramidal cells. These SNOs generated by GABAergic interneurons have properties identical to those of the SNOs recorded in the minislices, i.e., they are blocked by APV and TTX but not by bicuculline. Moreover, their *I–V* relationship includes an NMDA-mediated component. This suggests that an action potential–dependent release of glutamate occurs in spite of the absence of strata pyramidale/oriens. It is unlikely that this release comes from the Schaffer collaterals, perforant path, or commissural fibers that innervate interneurons and pyramidal cells in these layers; otherwise, SNOs in pyramidal cells should often contain a glutamate component. In addition, it is difficult to conceive that these axons generated spontaneous action potentials and released glutamate with the periodicity of SNOs while they are disconnected from their soma. Our observations are therefore best explained by the presence in stratum radiatum of intrinsically active neurons that periodically release glutamate. Glutamatergic neurons have been identified in the stratum radiatum of the CA1 region of the adult rat hippocampus and they were called "giant cells" (Gulyás et al. 1998). Whether such cells are present in the developing hippocampus and constitute one source of the glutamatergic drive necessary for the initiation SNOs remain to be established.

Interneurons and pyramidal cells are not activated by NMDA with the same efficacy

Our data show preferential activation of extrasynaptic NMDA receptors of interneurons by glutamate during SNOs. The analysis of the sensitivity of interneurons and pyramidal cells to exogenous application of NMDA may provide some clues. First, in these cells, NMDA receptors display significantly less voltage sensitivity to Mg^{2+} block compared with that of pyramidal cells. As a consequence, in interneurons, unlike pyramidal cells (Leinekugel et al. 1997), NMDA receptors can be activated at hyperpolarized membrane potentials without the requirement of concomitant depolarizing action of GABA. Consistent with this, we found that SNOs in interneurons present an NMDA component in the presence of bicuculline. However, a difference in the Mg^{2+} block cannot solely explain the absence in pyramidal neurons of an NMDA-receptor–mediated component in SNOs. Indeed, in a large majority of these neurons, SNOs were mediated only by GABA and fully blocked by the $GABA_A$-receptor antagonist (bicuculline) even when the pyramidal cells were depolarized at -30 mV, where the Mg^{2+} block is alleviated. There are at

least three possible explanations for this: *1*) The concentration of glutamate near pyramidal cells is lower than that near interneurons and is insufficient to activate the NMDA receptors; glial cells could preferentially sheath pyramidal cells constituting a physical barrier for glutamate diffusion. *2*) NMDA receptors located on pyramidal cells do not have the same sensitivity for glutamate than the receptors located on interneurons. This could result from a possibly different composition of subunits of the NMDA receptor in the two populations of cells (see Monyer et al. 1994; Mori and Mishina 1995). *3*) Another element that has to be taken into account is the higher input resistance of the interneurons compared with pyramidal cells. This may favor their depolarization and contribute to make these cells the main sensors of variation of glutamate in the extracellular space.

The generation of SNOs is developmentally regulated

We show that SNOs could not be induced after the second postnatal week of life. After this period, DL-TBOA generated a sustained activation of the neurons. A number of parameters could contribute to the change of the pattern, which include: *1*) disappearance or alteration of the properties of the pacemaker cellular elements that provide the glutamatergic drive, *2*) a massive tonic release of glutamate that masks the periodic activation of NMDA receptors, and *3*) the formation of a high density of synapses that may be associated with a reduced density of extrasynaptic receptors. In addition, a switch in GABAergic signaling (from depolarizing to hyperpolarizing action) that occurred after the second postnatal week of life (see Khazipov et al. 2004) may also participate in the inability to generate SNOs at this period of time. Because GDPs disappear at the same time as SNOs, the change of pattern may have a common origin that remains to be clarified in further studies.

In conclusion, recent observations suggest that glutamate transporters may exert a powerful control of the developing spinal chord and of neocortical networks activity. These two structures that display GDP-like events (Garaschuk et al. 2000; O'Donovan et al. 1998) respond to DL-TBOA by glutamate-dependent slow recurrent depolarizations/inward currents with duration and frequency similar to those described in the present study (Demarque et al. 2004; Sharifullina and Nistri 2006). Therefore the modulating actions of glutamate transporters could be a universal rule for developing networks, suggesting that if glutamate transporters are not fully operational during development (see Maragakis and Rothstein 2004) this will similarly affect the activity of several brain structures and transform the ongoing physiological patterns (GDPs) to slow and long-lasting membrane oscillations. We propose that one important role of glutamate transporters during development is to reduce the impact of putative "generators" of rhythmic activity on the network. By preventing or reducing the spread of glutamate in the extracellular space, glutamate transporters act to limit the coactivation of the neurons and the number of cells that participate in network-driven activity in immature brain. This regulation is fundamental because it prevents the generation of pathological activities and seizures. Indeed, large coactivation of neuronal activities is the hallmark of epilepsy. The recent observations that the inhibition of glutamate transporters generates in vivo NMDA-receptor–dependent recurrent paroxysmal bursts and partial seizures (Milh et al. 2007) support this hypothesis. We propose that the mechanism described here for the generation of SNOs may be similar to that leading to the "suppression burst" pattern observed in vivo in the rat pups after intracerebroventricular injection of DL-TBOA (Milh et al. 2007).

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