Synaptic GABA$_A$ Activation Inhibits AMPA-Kainate Receptor–Mediated Bursting in the Newborn (P0–P2) Rat Hippocampus

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INTRODUCTION

During development, neuronal activity shapes, and is itself shaped, by emerging synaptic contacts. In the CA3-CA1 circuitry of the neonatal rat hippocampus, AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) synapses are proposed to be driven by spontaneous long-lasting GABA$_A$-R–mediated depolarizations (Durand et al. 1996; Hanse et al. 1997). Repetitive stimulation of afferents combined to postsynaptic depolarization has indeed been found to induce functional connections from initially silent AMPA-synapses in CA1 (Durand et al. 1996), yet no synaptic drives mimicked by the afferent stimulation in this scheme have been reported in the newborn hippocampus. The build-up of excitatory AMPA-R–mediated circuitry toward the end of the first postnatal week is paralleled by a graded ontogenetic switch from depolarizing GABA$_A$ responses to hyperpolarization (Rivera et al. 1999).

The idea of dormant fast glutamatergic transmission in the newborn rat hippocampus was initially based on morphological studies demonstrating a scarcity of identifiable dendritic synaptic contacts between pyramidal cells during the first postnatal days. Electrophysiological recordings using single-pulse stimulation protocols also speak for purely N-methyl-D-aspartate receptor (NMDA-R)–based glutamatergic transmission in rat hippocampal slices before P2–P3 (Durand et al. 1996; Hsia et al. 1998). However, it has been pointed out that anatomic criteria may be misleading in judging the functional status of developing synaptic contacts (Durand et al. 1996; Katz and Shatz 1996). Further, because spike bursts probably represent a basic element of the neural code (Lisman 1997), adequate activation of hippocampal synapses may also require natural, bursting-type firing pattern of neurons (Dobrunz and Stevens 1999; Selig et al. 1999). In particular, this may be the case in the immature hippocampus, where the AMPA-R–mediated postsynaptic currents (PSCs) show a large variability in their quantal responses and thus in the overall reliability of synaptic transmission (Hsia et al. 1998).

Because of the proposed absence of effective fast glutamatergic transmission, the absence of inhibitory GABA$_A$ action has not been considered physiologically enigmatic, and GABA in fact has frequently been suggested to be the major excitatory transmitter in the newborn rat hippocampus (Ben-Ari et al. 1997; Holmes and Ben-Ari 1998; but see Dailey and Smith 1994). It is, however, known from other brain areas that postsynaptic inhibition is required for developmental refinement of neuronal contacts (Hensch et al. 1998). Yet, the possible inhibitory aspects of GABA$_A$-R–mediated transmission in the newborn hippocampus have received little attention.

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Functional inhibition may still prevail despite depolarizing GABA<sub>Α</sub>-R–mediated responses (Jackson et al. 1999; Su and Chai 1998; see also Kaila et al. 1993; Taira et al. 1997). GABAergic shunting conductances exert effective inhibition at postsynaptic sites (e.g., Furshpan and Potter 1959) and desynchronize the discharge of pyramidal cells in the mature hippocampus (Jackson et al. 1999). Thus GABA<sub>Α</sub>-R–mediated conductances resulting from endogenous activity in the neocortical hippocampus are likely to affect membrane excitability and cable properties of individual neurons (see Häusser and Clark 1997; Staley and Mody 1992).

The purpose of the present work was twofold. First, we wanted to elucidate the role of synchronous discharge of pyramidal cell ensembles in hippocampal transmission at P0–P2. Instead of using traditional stimulation protocols, we chose to study spontaneous neuronal bursts, which are likely to represent the pattern of activity occurring in the newborn rat hippocampus in vivo (Lahtinen et al. 1999). Second, we examined the inhibitory aspects of GABA<sub>Α</sub>-R–mediated transmission in the P0–P2 rat hippocampus.

**METHODS**

**Tissue preparation**

P0–P2 Wistar rat pups were anesthetized by hypothermia. Animals were decapitated, and brains were quickly removed and dissected in iced (0–4°C), oxygenated (95% O<sub>2</sub>-5% CO<sub>2</sub>) standard solution containing (in mM) NaCl, 3.5 KCl, 10 HEPES, 2.0 CaCl<sub>2</sub>, 2.0 MgSO<sub>4</sub>, and 11 glucose. After removing the cerebellum and the most anterior part of the neocortex, the cerebral hemispheres were split. Hippocampi were then dissected from the separated hemispheres, as described previously (Khalilov et al. 1997). Isolated hippocampal structures were cut into transverse slices (600–μm) using a McIlwain tissue chopper. For some experiments intact structures were not sliced but were preserved as whole hippocampus preparations (Khalilov et al. 1997). Preparations were kept in oxygenated standard solution at room temperature (20–22°C) for ≥1 h before use.

**Electrophysiological recordings**

For experimental procedures, preparations were transferred to a conventional submerged chamber (0.4 ml), laid on nylon mesh, and anchored with platinum bars. Slices were superfused with oxygenated standard solution (32–33°C) at a rate of 3 ml/min and intact P0–P1 hippocampal structures at a rate of 5–6 ml/min. Extracellular field potential recordings were performed with conventional NaCl-filled (150 mM) glass capillary microelectrodes (5–6 MΩ). K<sup>+</sup>-selective microelectrodes were made from double-barreled borosilicate glass pipettes, according to techniques described in Voipio et al. (1994). The reference barrel was filled with 150 mM NaCl, and the filling solution in the silanized nonfilamented barrel was 150 mM NaCl and 3 mM KCl. A short column of the ion sensor (60938, Fluka, Neu-Ulm, Germany) was taken into the tip using slight suction. The resistance of the two barrels was 5–10 MΩ and 5–10 GΩ, respectively. The electrode responses were calibrated in terms of free concentration, and they had a slope of 56–59 mV for a 10-fold change in [K<sup>+</sup>]. Whole cell recordings were obtained by using the method of Blanton et al. (1989). An Axoclamp 2B amplifier (Axon Instruments, Foster City, CA) was used in continuous voltage-clamp mode. Cells were patched from CA1-CA3 area with 5–7 MΩ pipettes filled with a solution containing (in mM) 135 K-gluconate, 1–3 KCl, 10 HEPES, 2 Ca(OH)<sub>2</sub>, 5 EGTA, 2 Mg-ATP (pH 7.0 with NaOH). Gramicidin was used in experiments for perforated patch recording (Ebihara et al. 1995; Kyrozis and Reichling 1995). Gramicidin (Sigma, Deisenhofen, Germany) stock solution was prepared in DMSO by dissolving 20 mg/ml. The tip of the filamented borosilicate glass pipette (6–8 MΩ) was initially filled with gramicidin-free pipette solution containing (in mM) 135 KCl, 10 HEPES, 2 CaCl<sub>2</sub>, 5 EGTA (pH 7.0 with NaOH). Gramicidin was diluted in the solution of the same composition to a final concentration of 20 μg/ml, and thereafter it was sonicated for 1 min in 10-s periods. No filtering of the solution was performed after dissolving gramicidin. The remainder of the pipette was then back-filled with the gramicidin-containing solution. New gramicidin solutions were made every 2 h. After the cell membrane was patched, the series resistance was routinely monitored throughout the experiment. Measurements were started when a series resistance of 300 MΩ or lower was reached (approximately 20–30 min after cell attachment). After 1 h, the series resistance stabilized to 60–80 MΩ. Cell input resistance was measured by -50 pA current steps (500 ms). Liquid junction potential correction (~3 mV) was performed in all membrane potential values reported in perforated-patch recordings. Electrical stimuli (5–10 V, 0.1 ms) were delivered by a bipolar electrode placed in the area CA1.

**Drugs**

Muscimol, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 6-nitro-7-sulfamoylbenzoquinoxaline-2,3-dione (NBQX), and 2-amino-5-phosphonopentanoic acid (AP-5) were purchased from Tocris Cookson (Bristol, UK); bicuculline methiodide was obtained from Sigma. Drugs were dissolved directly to standard solution for bath application.

**Data analysis**

Spontaneous neuronal activity and stimulus evoked responses were recorded with a TEAC SR-31 tape recorder (data were low-pass filtered at 700 Hz), and digitized off-line at 2 kHz with a National Instruments AT-MIO-16-E-2 A/D board and LabView software (National Instruments). The spike latencies and amplitudes were determined from the field potential bursts with a peak detection algorithm (LabView) and verified visually. Spikes with an amplitude greater than three times the peak was determined and used for the spike detection algorithm (LabView). The preburst standard deviation were accepted. Decay time constants of fast synaptic currents were estimated by a least-squares exponential fit. Currents appearing as monosynaptic were selected for the spike constant estimation from late parts of the polysynaptic bursts. Data are given as mean ± SE. Care and use of animals conformed to the guidelines of the Helsinki University Animal Care Committee.

**RESULTS**

**AMPAR-mediated CA3-CA1 transmission in P0–P2 hippocampus**

Field potential recordings from stratum pyramidale in the area CA3 of P0–P2 rat hippocampal slices revealed negative-going shifts (interval 16 ± 3 s, n = 24 slices) that were accompanied by bursts of 2.9 ± 0.1 spike-like deflections (n = 106 bursts). In recordings from the stratum radiatum the extracellular spikes were typically reversed to positive-going changes, indicating that they were presumably generated by a discharge of a subset of CA3 pyramidal cells (Fig. 1, A and B). To determine whether the extracellular spikes reflect unit activity (action potentials of single neurons) or synchronous discharges in a neuronal ensemble, we performed paired field potential recordings using 100-μm interelectrode distance in CA3 s. pyramidale (n = 3 slices). Cross-correlation analysis showed a peak with ±1-ms phase lag indicating coincident spikes (c.f. Draguhn et al. 1998) (n = 43 averages; Fig. 1C).
These results indicate periodic bursts of synchronous firing in P0–P2 rat CA3 pyramidal cell population.

To examine the synaptic input to CA3 and CA1 neurons, we used the whole cell voltage-clamp technique with a low concentration of chloride (1–3 mM) in the pipette filling solution. The glutamate AMPA-kainate-type (hereafter referred to as AMPA) inward currents were studied in cells clamped close to the reversal potential of GABA_A-R–mediated currents (E_{GABA_A} ≈ 80 mV) (c.f. Khazipov et al. 1997). Inward unitary AMPA-R PSCs had a decay time constant (τ_{decoy}) < 3 ms and they were blocked by CNQX (20 μM) or NBQX (10 μM; n = 10 and 4, respectively), as illustrated in Fig. 2, A and B. Recordings close to E_{GABA_A} showed that AMPA-R PSCs in CA3 neurons were infrequent (0.20 ± 0.13 unitary PSCs/s/μM, range 0.05–0.55 s–1) between the CA3 field potential bursts but that they became strongly augmented when the bursts occurred. These short periods (<500 ms) of coherent discharge in the pyramidal layer were accompanied by a barrage of 7.6 ± 0.2 unitary AMPA-R PSCs in CA3 neurons (n = 260 bursts, 10 cells). On the average, 73 ± 5% of the total AMPA-R–mediated input consisted of the PSCs during the bursts (n = 10; Fig. 2B). CNQX (20 μM) as well as NBQX (10 μM; 10 min; n = 10 and n = 4, respectively) abolished the CA3 field potential bursts and strongly attenuated spontaneous GABA_A-R–mediated PSCs (see Bolea et al. 1999; Gaiarsa et al. 1991; Garaschuk et al. 1998; Hollrigel et al. 1998; Strata et al. 1997), thus speaking for an active role of AMPA-R–mediated recurrent excitation and recruitment of GABAergic interneurons at P0–P2 (see Fig. 2A).

To examine spontaneous activation of the excitatory synapses from CA3 pyramidal cells to CA1, we made simultaneous recordings of CA3 field potentials and of postsynaptic currents in whole cell clamped CA1 neurons (n = 4). As illustrated in Fig. 2C, CA1 neurons displayed a barrage of AMPA-R–mediated PSCs during the CA3 field potential burst. The bursting activity in CA1 is generated in CA3, as demonstrated by the disappearance of CA1 bursts after surgical isolation of CA3 from CA1 (Fig. 2D). The autocorrelation analysis from 72 barrages showed that AMPA-R PSCs were imposed to CA1 neurons with 10–50-ms intervals (Fig. 2E).

Interneurons inhibit CA3 pyramidal cells via GABA_A-Rs in the P0–P2 hippocampus

On antidromic stimulation of the CA3-CA1 Schaffer collateral pathway in P0–P1 hippocampal slices, a single-pulse stimulus elicited in the CA3 field potential a response similar to the spontaneous bursts (2.2 ± 0.3 spikes; n = 46 bursts, 6 slices). As shown in Fig. 3A, after blockade of GABA_A-R by bicuculline for >5 min (10 μM), the same stimulus generated a burst with 4.8 ± 0.5 spikes (P < 0.01, t-test). After a 15-min wash out of bicuculline and restoration of GABA_A-R–mediated transmission, the number of extracellular spikes was reestablished to the control value of 1.9 ± 0.2 (n = 41 bursts, 5 slices).

Gramicidin perforated-patch recordings demonstrated a network-driven 13.0 ± 1.0 mV (n = 30 cells) depolarization of the CA3 neurons from the resting membrane potential (E_m = −67.5 ± 0.7 mV) during the spontaneous field potential burst. A single-pulse antidromic stimulation of Schaffer collaterals elicited a similar depolarization (13.5 ± 2.0 mV; E_m = −69 ± 1.0 mV) with 1.6 ± 0.3 (range 0–4) action potentials (n = 12 cells). In six neurons the response was strongly intensified in bicuculline (10 μM) yielding a 28.0 ± 2.5-mV depolarization and generating 3.7 ± 0.7 (range 2–7) action potentials. In three cells the network activity was abolished, and only a monosynaptic response was elicited (16.0 ± 2.5 mV, 0–1 action potentials). In three cells no response was evoked in bicuculline. These results demonstrate that a powerful inhibitory effect of GABA_A-R inputs on the glutamatergic CA3 recurrent excitation can frequently be observed in the hippocampus as early as P0–P1. Figure 3B illustrates augmentation of network-driven
FIG. 2. CA3 field potential bursts coincided with barrages of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R)–mediated currents in whole-cell clamped CA1-CA3 neurons of P0–P2 slices. A: spontaneous bursts, revealed by field potential deflections (low-pass filtered 100 Hz), involved activation of glutamate (inward) currents and GABA_{A} (outward) currents in P0 hippocampus. 6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX) was an effective blocker of inward postsynaptic currents (PSCs) and the CA3 field potential bursts. It also inhibited the synchronous GABA_{A}R–mediated currents. B: barrages of AMPA-R currents emerged from bursting of CA3 recurrent glutamatergic circuitry. B1: typical spontaneous epoch of AMPA-R–mediated input in a CA3 neuron (V_h = −75 mV, slightly positive to E_{GABA_A}). After wash in of CNQX a single-pulse stimulus close to the recording site (200-μm inter-electrode distance) evoked only GABA_{A}R–mediated outward currents. B2: periodic bursts of CA3 recurrent glutamatergic circuitry provided AMPA-R–mediated transmission in P0–P1 hippocampus. Occurrence of unitary AMPA-R currents in CA3 neurons (mean ± SE; n = 260 barrages in 10 cells). C: simultaneous recording of CA3 field potential (low-pass filtered at 100 Hz) and AMPA-R PSCs in a CA1 neuron. C1: spontaneous field potential bursts in the area CA3 occurred with activation of AMPA-type synapses in a CA1 neuron (V_h = −82 mV). C2: AMPA-R–mediated (inward) currents in a CA1 neuron (V_h = −75 mV). D1: isolation of CA3 from CA1 results in a disappearance of the synchronous bursting activity in the latter. Only occasional inward currents were left in CA1 (D2). E1: activation of AMPA-type synapses in the CA1 neurons displayed a rhythmic pattern. Autocorrelogram from the same barrage of PSCs reveals peaks at 10–50 ms. E2: amplitude spectrum computed from the correlograms of successive barrages demonstrates prominent peaks at 20–80 Hz (n = 7).
excitation of CA3 neurons by the GABA A-R antagonist bicuculline in P0–P1 hippocampal slices.

AMPA-R–mediated transmission is better preserved in the neonate rat whole hippocampus preparation when compared with slices (Khalilov et al. 1997). Further, slices from septal and temporal sites may differ in their properties of network activity (Leinekugel et al. 1998). Thus, to better appreciate the contribution of AMPA-R–mediated transmission to the spontaneous network activity, some of the experiments were performed using the intact hippocampus in vitro preparation. Field potential recordings from CA3 area showed that the spontaneous events were similar to those occurring in slices. In P0 (n = 4) as well as in P1 (n = 7) preparations, exposure to bicuculline (10 μM) strongly augmented the spontaneous field potential responses during a 10-min exposure (see Fig. 4, A and B). In the presence of bicuculline, the occurrence of the bursts (at 96 ± 40-s interval) was not seemingly influenced by the NMDA-R antagonist AP-5 (40 μM) but became readily blocked by the AMPA-R antagonist CNQX (20 μM; n = 3; data not shown). Activity-induced accumulation of extracellular potassium ([K+]o) serves as useful indicator of epileptiform discharge (e.g., Swann et al. 1986). Therefore, we performed some experiments with bicuculline using ion-selective double-barrel microelectrode recording of field potential and [K+]o. These experiments revealed that periods of synchronous activity in control conditions were linked to a small increase in [K+]o from the basal 3.5 mM to ~3.7 mM (n = 6 preparations), but epochs in bicuculline were accompanied by prominent accumulation of [K+]o up to 6.0 mM (n = 6 preparations), as depicted in Fig. 4A.

After blockage of GABA A-Rs, whole cell recordings demonstrated a strong increase in the amplitude of an average AMPA-R PSC in the CA3 neurons from 27.0 ± 2.0 pA in control to 68.0 ± 3.5 pA in bicuculline (n = 112 and 182

![Image](https://example.com/image1.png)

**FIG. 3.** Bicuculline accentuated discharges in CA3 in P0–P1 hippocampal slices. A: antidromic stimulation of CA3-CA1 pathway (a single-pulse at 5–10 V) evoked a burst of population spikes in CA3. A1: CA3 field potential responses to an antidromic stimulus (low-pass filtered 700 Hz). Exposure to bicuculline >5 min, wash out >15 min. A2: increase in the number of population spikes during CA3 field potential bursts on exposure to bicuculline (mean ± SE, P < 0.01, t-test). Data were calculated from 46 bursts obtained from 6 slices. B: gramicidin perforated-patch recording of the membrane potential in a CA3 neuron. Resting E_m is depicted below the traces. Duration of the exposure and the wash out as above. Locations of the stimulation (s, at CA1) and recording (rec., at CA3) electrodes are schematically depicted in A1. Arrow: timing of the stimuli.

![Image](https://example.com/image2.png)

**FIG. 4.** Exposure to bicuculline induced epileptiform activity in the CA3 area of the intact P0–P1 rat hippocampus. A: combined field potential and [K+]o recording demonstrates augmentation of the spontaneous bursting in CA3. B: AMPA-R–mediated input to a CA3 neuron during the spontaneous bursts (V_h = −78 mV) in control and in bicuculline. Note the different vertical scaling of the traces. C: bicuculline induced an increase in the amplitude of AMPA PSCs during network-driven bursts (mean ± SE, P < 0.01, t-test). The data were calculated from 112 and 182 spontaneous unitary PSCs without and with bicuculline, respectively. Sample recordings from a CA3 neuron of P1 intact hippocampus in control (a) and bicuculline (b) are shown on the right.
PSCs, respectively, $P < 0.01$, t-test). However, the temporal properties of unitary PSCs were similar to control ($\tau_{\text{decay}} < 3 \text{ ms}$). This indicates that blockage of $\text{GABA}_A$-R–mediated transmission strongly enlarged the population of glutamatergic neurons firing synchronously in the bursts (Fig. 4, B and C). Thus, the results presented here suggest also that, as in the adult (Miles and Wong 1987), $\text{GABA}_A$-R–mediated activity plays a critical role in limiting network excitability in the CA3 area of newborn rat hippocampus (see Swann et al. 1989).

**Activation of $\text{GABA}_A$-R by muscimol inhibits CA3 pyramidal cell firing while depolarizing postsynaptic cells**

As shown by Fiszman et al. (1990), submicromolar concentrations of the agonist, muscimol, induce a bicuculline-sensitive depolarization in embryonic and early postnatal hippocampal neurons. Field potential deflections and the accompanying spikes were abolished by bath-applied muscimol (0.1–5 $\mu$M), as illustrated in Fig. 5A ($n = 14$). However, during the early wash in of the agonist, the frequency of the bursts was transiently increased. With a low concentration (10 nM) of muscimol, the rate of the field potential bursts was tonically increased to 155 ± 11% from control during a 10-min exposure (mean occurrence in control 9.5 ± 0.6 bursts/min, $n = 5$). After a >15-min wash out, the occurrence of the bursts was restored to 90 ± 10% ($n = 5$). The effect of muscimol was reversible at higher concentrations as well, and the field potential deflections reappeared after a 10-min wash out.

Whole cell recordings from CA3 neurons showed that bath-applied 100 nM muscimol (5 min) induced a steady outward current at clamping potentials $-20 \text{ mV}$ and increase in occurrence of unitary $\text{GABA}_A$-R PSCs in CA3 neurons (mean ± SE, $P < 0.05$, t-test, $n = 4$ slices). Bicucullin inhibited the increase in spontaneous activity of $\text{GABA}_A$ergic synapses ($n = 4$ slices; $P < 0.05$, t-test). These results are illustrated in Fig. 5, B and C. Gramicidin perforated-patch recordings showed that similar (5-min) exposure to 100–200 nM muscimol induced 8.0 ± 0.8 mV (range 2–13 mV) depolarization of CA3 neurons from their resting membrane potential $-67.0 ± 1.1 \text{ mV}$ ($n = 16$; Fig. 5D). In the presence of bicuculline (10 $\mu$M) the effect of muscimol was blocked ($n = 3$).

**DISCUSSION**

It is generally accepted that endogenous activity plays a crucial role in the formation of developing neuronal networks (see Goodman and Shatz 1993). In this context, the spontaneous network activity in the newborn rat hippocampus has received much attention (c.f. Hanse et al. 1997; Traub et al. 1998). Yet, mechanisms underlying the early network activity in the hippocampus have remained poorly understood (c.f.
O’Donovan 1999). In the current study, we first addressed the question of whether the fast spontaneous spiking seen in CA3 represents synchronous firing of pyramidal neurons. Having established that this is the case, we then examined the synaptic mechanisms driving these spontaneous events in CA3. In parallel, we studied the synaptic inputs to CA1 to reveal the functional connectivity between CA3-CA1 at birth. Finally, we investigated the nature of GABAergic transmission in the endogenous activity of the newborn rat hippocampus.

The following are our central findings. 1) As early as P0, periodic activation of glutamate AMPA-Rs gives rise to synchronous recurrent bursting of CA3 pyramidal cells. The occurrence of AMPA PSCs is mainly restricted to these bursts. 2) During the coherent discharging of CA3, AMPA PSCs are also seen in CA1 pyramidal cells, where the PSCs display a barrage at high (>20-Hz) frequency. 3) GABA A-R–mediated transmission, despite its depolarizing postsynaptic action, is already a major inhibitory mechanism of the synchronous pyramidal cell activity at birth in the rat hippocampus.

AMPA-R-activation underlies the synchronous bursts of CA3 pyramidal cells and conveys the excitation to CA1 at P0–P2

Notably, most of the studies investigating AMPA-R–mediated transmission in the newborn brain have been performed at room temperature using a single-pulse stimulation paradigm (c.f. Bolshakov and Siegelbaum 1995; Durand et al. 1996; Hsia et al. 1998). However, lowering the experimental temperature is known to effectively attenuate evoked responses in CA3-CA1 synapses (Igelmund and Heinemann 1995). Also, glutamate spillover and the consequent extrasynaptic activation of NMDA-Rs is critically dependent on temperature, the lowered temperature thus giving a biased view of the relative contributions of AMPA and NMDA-Rs to synaptic transmission (Asztely et al. 1997). In this study, we decided to investigate the synaptic inputs in immature CA3-CA1 circuitry during spontaneous bursts. Interestingly, it was very recently pointed out that a relevant way to study synaptic functioning in a neuronal network is to use stimulation patterns mimicking the spike trains driving synapses in vivo (Doeben and Stevens 1999), thus making the current approach even more appropriate.

In the light of the existing literature, the presence of strong AMPA-R–mediated inputs to pyramidal cells at P0–P2 was rather surprising. Intriguingly, the AMPA PSCs were temporally restricted to the spontaneous bursts, and only rarely did AMPA PSCs occur outside these events. Furthermore, the synaptic currents in CA1 showed a rhythmicity at gamma (20–100 Hz) frequencies reflecting a synchronized output of the neonate CA3 circuitry (see Palva et al. 1999). In the developing brain the spontaneous activity may be critical for the formation of synaptic contacts (Katz and Shatz 1996). Neuronal bursts capable of inducing synaptic modifications would thus be an effective way to convey synaptic transmission in the emerging CA3-CA1 circuitry. CA3 population activity gives rise to long-lasting GABAergic conductance in CA1 neurons, yet with a tens- or hundreds-of-millisecond delay (Strata et al. 1997). In terms of synapse induction, the sharply synchronous AMPA-R–driven bursts could allow for finer tuning of neuronal connections.

GABA A-R–mediated inhibition in the newborn rat hippocampus

In the immature rat hippocampus GABA A-R activation by afferent stimulation may evoke spiking in the postsynaptic cell (Khazipov et al. 1997). Also, the GABAergic depolarization in the neonate hippocampal neurons is well established (Ben-Ari et al. 1989; Rivera et al. 1999). Our current experimental approaches showed that although depolarizing in nature, GABA A-R activation can be strongly inhibitory also in the newborn rat pyramidal cells.

This conclusion is based on several findings. 1) The amplitude of the AMPA-R PSC peak, which is determined by integration of the synaptic inputs and therefore represents a good measure of synchronously activated presynaptic glutamatergic neurons, was increased in bicuculline. Also, the duration of the spontaneous CA3 bursts was much longer in the absence of GABAergic inhibition. 2) Experiments with antidromic stimulation of CA3 recurrent excitatory loop showed that at P0–P1 GABAergic transmission already restricts the network-driven excitatory input to CA3 neurons. 3) Simultaneous recordings of extracellular potential and [K+]o demonstrated that inhibition of GABA A-R–mediated transmission gave rise to epileptiform synchronous activity. The effect of the GABA A-R antagonist was even more accentuated in the intact hippocampus preparation, probably because of better preservation of the glutamatergic circuitry (Khalirov et al. 1997). This finding is in perfect agreement with the observation that bicuculline induces seizures in the newborn rat in vivo (Daval and Sarfati 1987). 4) AMPA-R–mediated transmission and the accompanying spike bursts were abolished on activation of GABA A-Rs by muscimol. However, whole cell recordings revealed that on muscimol application the occurrence of GABA A-R PSCs was increased, probably because of muscimol-induced depolarization and a consequent increase of GABA release from interneurons.

Regarding the mechanisms of GABA A-R–mediated inhibition in the neonate neurons, in all experiments in area CA3 gramicidin-perforated recordings showed only depolarizing responses to the GABA A-R agonist muscimol. Therefore the possibility that inhibition of the network would be mediated by a subpopulation of the neurons in which GABA already induces hyperpolarization seems unlikely. GABAergic inhibition by depolarizing GABA A-R conductances is known to occur in neonate and adult rat dentate granular cells (Hollriegel et al. 1998; Staley and Mody 1992). Thus, although sometimes capable of triggering a spike, the GABA A-R activation would not easily allow for tight long-range synchronization, and thus coherent population firing, to ensue because of the effective shunting of the postsynaptic membrane. Further, the GABA-induced Ca2+ influx could uncouple gap junctions and attenuate the fast network oscillations requiring electrical coupling in neonatal hippocampus (Draguhn et al. 1998; Palva et al. 1999; Strata et al. 1997). The high occurrence of GABA A-R PSCs in P0–P2 neurons indicates that spontaneous firing and thus “tonic” inhibition (Häusser and Clark 1997) is already a common feature of GABAergic neurons at this age. Similar strategies for the control of synaptic integration may thus be used in the newborn and in the adult brain.

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