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Acute Changes in GABAergic Function

Timing of the Developmental Switch in $GABA_A$ Mediated Signaling from Excitation to Inhibition in CA3 Rat Hippocampus Using Gramicidin Perforated Patch and Extracellular Recordings

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Summary: The timing of the developmental switch in the $GABA_A$ mediated responses from excitatory to inhibitory was studied in Wistar rat CA3 hippocampal pyramidal cells using gramicidin perforated patch-clamp and extracellular recordings. Gramicidin perforated patch recordings revealed a gradual developmental shift in the reversal potential of synaptic and isoguvacine-induced $GABA_A$ mediated responses from -55 ± 4 mV at postnatal days P0–2 to -74 ± 3 mV at P13–15 with a midpoint of disappearance of the excitatory effects of GABA at around P8. Extracellular recordings in CA3 pyramidal cell layer revealed that the effect of isoguvacine on multiple unit activity (MUA) switched from an increase to a decrease at around P10. The effect of synaptic $GABA_A$ mediated responses on MUA switched from an increase to a decrease at around P8. It is concluded that the developmental switch in the action of GABA via $GABA_A$ receptors from excitatory to inhibitory occurs in Wistar rat CA3 pyramidal cells at around P8–10, an age that coincides with the transition from immature to mature hippocampal rhythms. We propose that excitatory GABA contributes to enhanced excitability and ictogenesis in the neonatal rat hippocampus. **Key Words:** GABA—Development—Neonate—Hippocampus—Gramicidin.

GABA is the main inhibitory neurotransmitter in the adult brain. However, in the developing central nervous system, GABA, acting via $GABA_A$ receptors, exerts paradoxical depolarizing and excitatory action on the immature neurons (Cherubini et al., 1991; Ben Ari, 2002). In the immature hippocampus, $GABA_A$ mediated depolarization triggers sodium action potentials, activates voltage-gated calcium channels and increases intracellular calcium concentration ($[Ca^{2+}]_i$), and allows activation and influx of Ca^{2+} via NMDA channels due to attenuation of their voltage-dependent magnesium block (Mueller et al., 1983; Ben-Ari et al., 1989; Fiszman et al., 1990; Leinekugel et al., 1997; Garaschuk et al., 1998; Ganguly et al., 2001). The depolarizing and excitatory effects of GABA are due to elevated intracellular chloride concentration ($[Cl^-]_i$) and therefore depolarized values of the reversal potential of the $GABA_A$ mediated responses (E_{GABA}) in the immature hippocampal neurons caused by developmental changes in the chloride-homeostasis (Rivera et al., 1999; Dzhala et al., 2005). Excitatory GABA is instrumental in the generation of the immature patterns of activity in the neonatal hippocampus, the so-called Giant Depolarizing Potentials (GDPs) in slices (Ben-Ari et al., 1989; Garaschuk et al., 1998; Khazipov et al., 2004; Sipila et al., 2005) and intact hippocampus in vitro (Khalilov et al., 1997; Leinekugel et al., 1998), and spontaneous population bursts in vivo (Leinekugel et al., 2002). Growing evidence indicates that excitatory GABA participates in neurotrophin signaling and induction of synaptic plasticity in the developing hippocampus (for reviews, Ben-Ari et al., 1997; Leinekugel et al., 1999; Ben Ari, 2001, 2002). Excitatory GABA also contributes to enhanced excitability and ictogenesis during the critical developmental period—the second postnatal week in the rat hippocampus (Dzhala and Staley, 2003; Khazipov et al., 2004; Dzhala et al., 2005).

In keeping with the pivotal role of GABA in patterning the physiological and paroxysmal neuronal network activities and its trophic actions, it is of foremost importance to determine the timing of the developmental changes in the GABAergic signaling. However, the time course of the developmental changes in the $GABA_A$ mediated

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actions in the hippocampus remains a subject of controversy. Intracellular recordings using sharp electrodes suggested that the developmental switch in the action of GABA from excitatory to inhibitory occurs in the rat CA3 pyramidal cells at around P5 (Ben-Ari et al., 1989). In agreement with these results, inhibitory GABAergic PSPs were observed in the majority of CA3 pyramidal cell by P5–6 and using similar intracellular recordings by P9 in CA1 pyramidal cells (Swann et al., 1989). However, whole-cell recordings from CA1 pyramidal cells failed to reveal any excitatory action of GABA in P2–5 rat CA1 pyramidal cells with the inhibitory $GABA_A$-mediated responses emerging from P8 onwards (Zhang et al., 1991). It has been also demonstrated that the $GABA_A$-mediated responses in the immature hippocampal neurons strongly depend on the concentration of chloride ions in the recording patch pipette solution indicating that conventional whole-cell recordings are not appropriate to study the excitation-to-inhibition switch of the chloride-dependent $GABA_A$-mediated responses (Khazipov et al., 1997; Leinekugel et al., 1997; Garaschuk et al., 1998). Monitoring of $[Ca^{2+}]_i$ using fluorescent calcium-sensitive dyes revealed that activation of $GABA_A$ receptors causes influx of calcium ions via voltage-gated calcium channels in P2–5 CA3 and CA1 pyramidal cells and interneurons but does not affect $[Ca^{2+}]_i$ at P12–13 (Leinekugel et al., 1995). Using similar approach, the midpoint of disappearance of the excitatory effect of GABA in CA1 pyramidal cells was estimated at around P5–6; at P7–10, about 20% of cells were still excited by GABA and only little or no increase in the somatic $[Ca^{2+}]_i$ was produced by $GABA_A$ agonist from the beginning of the third postnatal week onwards (Garaschuk et al., 1998). In cultures of hippocampal neurons the midpoint of disappearance of the $GABA_A$-mediated increase in $[Ca^{2+}]_i$ occurred at around 11 days in vitro (Ganguly et al., 2001). A recent study using extracellular and cell attached recordings revealed a developmental switch in the effect of $GABA_A$ agonist isoguvacine on unit activity in CA3 Sprague Dawley rat hippocampal slices at around P13 (Khazipov et al., 2004). The $GABA_A$ antagonist bicuculline switched from decreasing to increasing activity of CA3 pyramidal cells at around P12 (Dzhala and Staley, 2003). Thus, estimations of switch in the action of GABA on hippocampal neurons vary from P5 to P13.

Large variability also exists in the estimations of the underlying developmental changes in the reversal potential of the $GABA_A$-mediated responses. Using intracellular recordings, E_{GABA} in the neonatal CA3 pyramidal cells at P2–5 was estimated at −25 mV with KCl-filled electrodes and at −51 mV with potassium methylsulfate-filled electrodes (Ben-Ari et al., 1989). With whole-cell recordings, E_{GABA} was found to be equal to the resting membrane potential in P2–5 CA1 pyramidal cells and became hyperpolarizing at P8–13 (Zhang et al., 1991). In cultures of hippocampal neurons, E_{GABA} was estimated using perforated patch recordings as −44 mV and −61 mV at 6–7 and 13–14 days in vitro, respectively (Ganguly et al., 2001). Using gramicidin recordings from organotypic slices of >6 weeks in culture, E_{GABA} in CA3 pyramidal cells was −56 mV (Mohajerani and Cherubini, 2005). A significantly more depolarized estimation of E_{GABA} was deduced from the measurement of $[Cl^-]_i$ in cultured hippocampal neurons using clomeleon: about −25 mV at P0–2 gradually shifting to −52 mV at P14 (−75 mV in the selected pyramidal cells) (Kuner and Augustine, 2000).

Thus, there is high variability in the estimation of the timing of the developmental switch of the $GABA_A$-mediated responses from excitatory to inhibitory and of the underlying changes in E_{GABA} in the hippocampal neurons. This variability can partly account for the errors introduced by the recording approaches. Therefore, in the present study, we estimated the time course of the developmental switch of $GABA_A$-mediated signaling from excitatory to inhibitory and the underlying changes in E_{GABA} in CA3 hippocampal neurons using the noninvasive technique of gramicidin perforated patch and extracellular recordings in acute hippocampal slices from Wistar rats. We provide evidence that the excitation-to-inhibition switch in the action of GABA occurs at around P8–10, and that the underlying negative shift of E_{GABA} occurs gradually during the two first postnatal weeks.

METHODS

Experimental system

Hippocampal slices were prepared from Wistar rats of both sexes. All animal use protocols conformed to the INSERM guidelines on the use of laboratory animals and with approval of the Animal Care and Use Committees of both Harvard Medical School and Dartmouth Medical School. Animals were anaesthetized with chloral hydrate (350 mg/kg, intraperitoneally) and decapitated. Brain was removed and transverse slices (350–500 μm) were cut from the middle third of hippocampus using Vibratome (VT 1000E; Leica, Nussloch, Germany). Slices were kept in oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) of the following composition (in mM): NaCl 126, KCl 3.5, $CaCl_2$ 2.0, $MgCl_2$ 1.3, $NaHCO_3$ 25, NaH_2PO_4 1.2, and glucose 11 (pH 7.4) at room temperature (20–22°C) at least 1 h before use. For recordings, slices were placed into a conventional fully submerged chamber superfused with ACSF (30–32°C) at a rate of 2–3 ml/minute.

Electrophysiological recordings and data analysis

Extracellular field potentials were recorded using electrodes made from tungsten wire of diameter 50 μm (California Fine Wire, Grover Beach, CA, U.S.A.). Electrodes were positioned in a pyramidal cell layer of CA3a subfield and signals were amplified using a custom made

amplifier (bandpass 0.1 Hz–4 kHz; ×1,000). For single action potentials detection, records were filtered with RC (single pole) high pass filter at >200 Hz. Patch-clamp recordings were performed using Axopatch 200A (Axon Instruments, Union City, CA, U.S.A.) and EPC-9 (HEKA Elektronik Dr. Schulze GmbH, Lambrecht/Pfalz, Germany) amplifiers. Patch electrodes were made from borosilicate glass capillaries (GC150F-15, Clark Electromedical Instruments). Patch pipette solution for gramicidin perforated patch recording contained (in mM): KCl, 150 and HEPES, 10, buffered to pH 7.2 with Tris-OH. Gramicidin was first dissolved in DMSO to prepare a stock solution of 10–40 mg/ml and then diluted to a final concentration of 80 μg/ml in the pipette solution. The gramicidin-containing solution was prepared and sonicated <1 h before the experiment. To facilitate cell-attached formation (4–10 GOhm), patch pipettes were backfilled with a gramicidin-containing solution, then the tip of pipettes was dipped into and filled with a gramicidin—free solution by applying a negative pressure for 20–30 s. Following 20–30 minutes after cell-attach formation, series resistance (*Rs*) decreased and stabilized at 8–60 MOhm. Series resistance was monitored during all recording session. At the end of each recording, negative pressure applied to break the membrane and establish whole-cell configuration. This was associated with a shift of the reversal potential of the $GABA_A$-mediated responses to near 0 mV. The membrane potential values were corrected for series resistance off-line as V (corrected) = V (holding)—*IRs*. In the study of the excitatory actions of GABA, membrane potential in current-clamp recordings was kept at −80 mV by injecting negative current. A picospritzer (General Valve Corporation, Fairfield, NJ, U.S.A.) was used to puff-apply isoguvacine (10–100 μM in ACSF) from a glass pipette in stratum radiatum at a distance of about 100 μm from soma in gramicidin perforated patch recordings. The pressure was from 10 to 20 kPa, and the duration of the puff varied from 50–200 ms. Synaptic responses were evoked by electrical stimulation in stratum radiatum via a glass electrode or via a bipolar nickel–chrome electrode. Recordings were digitised (10 kHz) online with Digidata 1200 intreface card (Axon Instruments) and analyzed offline with Axon package, mini-Analysis (Synaptsoft), and Origin 5.0 (Microcal Software, Northampton, MA, U.S.A.). Group measures are expressed as means ±SE; error bars also indicate SE. The statistical significance of differences was assessed with the Students t – test. The level of significance was set at $p < 0.05$.

RESULTS

Patch-clamp recordings were used to determine the developmental changes in the reversal potential of the $GABA_A$-mediated responses (E_{GABA_A}) in CA3 pyramidal cells. To avoid alterations in the $[Cl^-]_i$ concentration during these recordings, we used the perforated-patch technique (Marty and Finkelstein, 1975; Horn and Marty, 1988) with gramicidin D as the ionophore (Spruston and Johnston, 1992; Abe et al., 1994; Wang et al., 1994; Ebihara et al., 1995; Brickley et al., 1996; Owens et al., 1996; and Ulrich and Huguenard, 1997).

Perforated patch-recordings were obtained from 108 pyramidal cells with series resistance 48 ± 3 MOhm (n = 108) in slices from P0–26 rats. Two types of the $GABA_A$-mediated responses were studied: (1) responses induced by local brief application of isoguvacine and (2) $GABA_A$-PSCs evoked by local electrical stimulation in the presence of the glutamate ionotropic receptor antagonists CNQX (10 μM) and D-APV (40 μM). Both isoguvacine-induced and synaptic responses were blocked by bicuculline (20 μM). $GABA_B$-mediated PSCs were rarely observed with the low-amplitude stimulation used in this experiment.

Typical examples of the $GABA_A$-mediated responses recorded in CA3 pyramidal cells at P2 and P15 are shown on Figs. 1 and 2 respectively. The reversal potential of the isoguvacine-induced responses and $GABA_A$-PSCs was significantly more depolarised in the immature neurons. Fig. 3 summarizes the estimates of E_{GABA} measured in all 108 neurons from P0 to P26. We did not find any significant difference (paired t-test: $p = 0.53$; $n = 62$) in the estimates of E_{GABA} obtained from study of current-voltage relationships of isoguvacine-induced responses and pharmacologically isolated $GABA_A$-PSCs in neurons in which both estimates have been obtained. Therefore, the results obtained using both approaches were pooled together. At P0–2, averaged E_{GABA} was of −55 ± 4 mV (n = 17) and it negatively shifted with age by about −1.5 mV per day to attain −68 ± 3 mV (n = 16) at P8–10 and −74 ± 3 mV at P13–15 (n = 18).

Both isoguvacine-induced and synaptic $GABA_A$-mediated responses often triggered action potentials in the immature CA3 pyramidal cells (Fig. 1C,D). In the neurons in which were recorded both the responses to isoguvacine and synaptic $GABA_A$-PSPs (n = 62 cells), the results of the two approaches matched in 55 cells; in the remaining neurons, excitatory effects had only synaptic $GABA_A$-PSPs in 5 cells and isoguvacine-induced responses in 2 cells. The average rate of the excitatory effects at P0–5 was 76 ± 7% (n = 37; Fig. 4). From P6 to P11 the occurrence of the excitatory effect of $GABA_A$ receptor activation decreased and no excitatory effect was observed from P12 onwards. Boltzman fit of the age-dependence of the excitatory effect of $GABA_A$ receptor activation estimated the midpoint of the disappearance of excitatory effects at postnatal day 8.1 ± 0.9 (n = 99).

We further used extracellular field potential recordings in the CA3 pyramidal cell layer using metal electrodes that provided recordings of action potentials from tens to

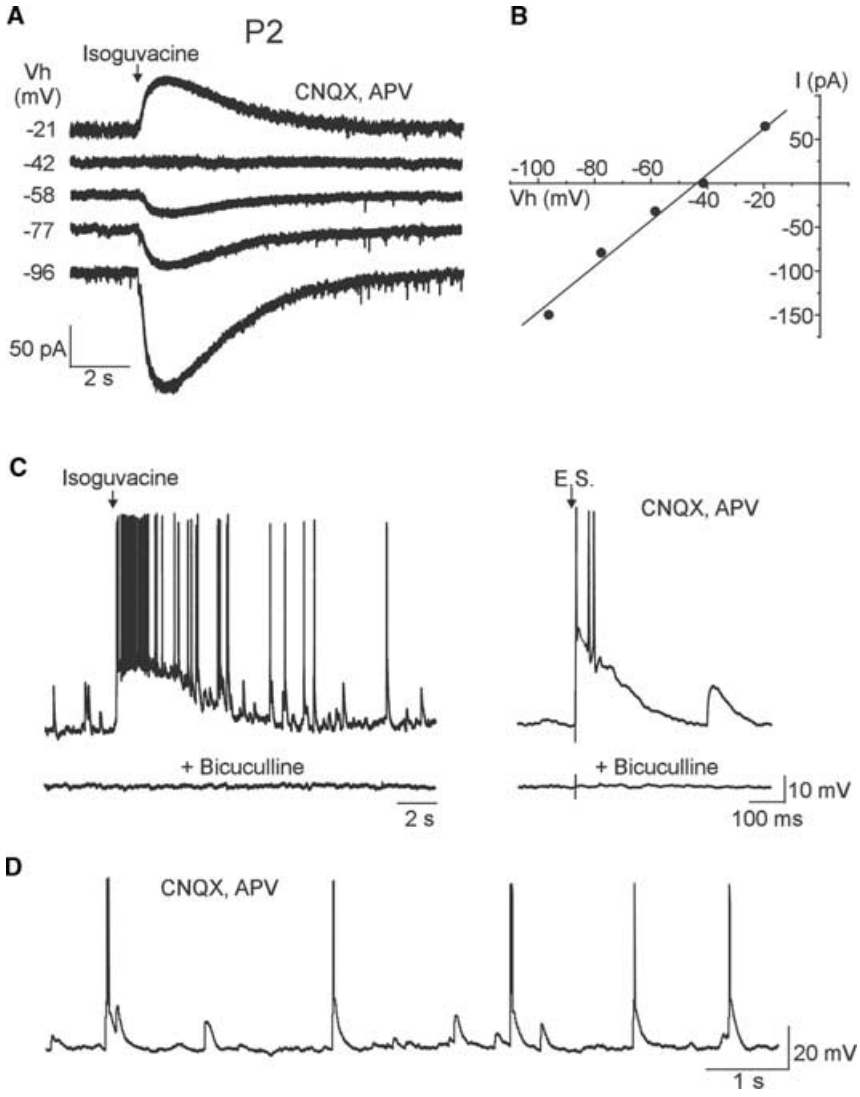

FIG. 1. $GABA_A$-mediated postsynaptic responses in a P2 CA3 pyramidal cell with gramicidin perforated patch. (**A**), Responses evoked by puff of the $GABA_A$ agonist isoguvacine in voltage-clamp mode at different holding potentials. (**B**), Dependence of the peak of the isoguvacine-induced responses on the membrane potential. Note that the responses reverse at −44 mV. (**C**), Current-clamp recordings of the same neuron. Brief application of isoguvacine (left) and electrical stimulation (E.S.) of slice in the presence of the ionotropic glutamate receptors antagonists CNQX and d-APV (right) evoke depolarization and action potentials. The responses are blocked by bicuculline (20 μM, traces below). (**D**), In the presence of the glutamate receptors antagonists CNQX and d-APV, spontaneous $GABA_A$-mediated postsynaptic potentials often result in action potentials. In (**C**) and (**D**) the membrane potential is hold at −80 mV by injection of negative current; the apparent "resting" membrane potential in this cell was of −49 mV (see also Tyzio et al., 2003).

hundreds of neurons nearby the electrode multiple unit activity (MUA) (Cohen and Miles, 2000). In addition to the convenience of recordings from the large neuronal population, extracellular recordings also affect neither membrane potential nor $[Cl^-]_i$ of the recorded neurons.

In the first experiment, we studied the effect of brief bath application of selective agonist of $GABA_A$ receptors isoguvacine (10 μM for 1 min) on MUA frequency. In the slices from young rats (P0–9), isoguvacine invariably induced an increase of MUA frequency that was also associated with an increase in frequency of GDPs (Fig. 5). At P10, both increases and decreases of MUA were observed in different slices. In slices from older animals, isoguvacine induced reduction of MUA frequency. Effects of isoguvacine on MUA frequency at different ages are summarized on Fig. 5C. Boltzman fit of the age-dependent change in the effect of isoguvacine on MUA revealed that the switch from excitation to inhibition occurs at postnatal day 10.0 ± 0.1 ($n = 28$).

In the second experiment, we examined the effect of synaptic activation of $GABA_A$ receptors on MUA. $GABA_A$-mediated postsynaptic responses ($GABA_A$-PSPs) were evoked by electrical stimulation of the slice in the presence of the antagonists of the ionotropic glutamate (10 μM CNQX and 50 μM D-APV) and $GABA_B$ (0.5 mM CGP35348) receptors. $GABA_A$-PSPs significantly affected the frequency of MUA in an age-dependent manner (Fig. 6), increasing MUA in slices from immature rats and decreasing it in slices from adult rats. Cross-correlation of action potentials with stimulus revealed that an alteration of MUA lasted tens of milliseconds and had a time course similar to that of $GABA_A$-PSPs. Both excitatory and inhibitory effects of stimulation were suppressed by the $GABA_A$ receptor antagonist bicuculline. As illustrated by Fig. 6B, the effect of $GABA_A$-PSPs on MUA switched from excitation to inhibition around postnatal day 8.0 ± 0.8 ($n = 21$).

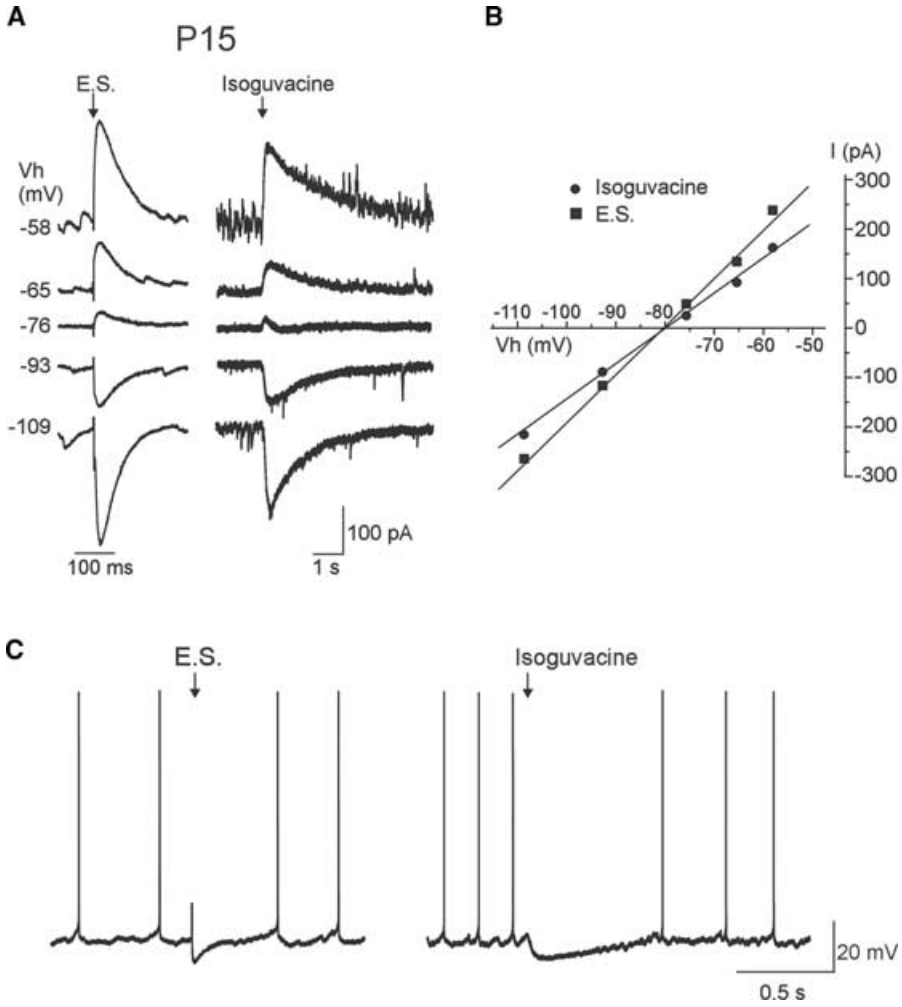

FIG. 2. $GABA_A$ mediated postsynaptic responses in a P15 CA3 pyramidal cell with gramicidin perforated patch. (**A**) Responses evoked by electrical stimulation (E.S.) in the presence of CNQX and APV and application of isoguvacine in voltage-clamp mode at different membrane potentials. (**B**) Dependence of the peak of the $GABA_A$ mediated synaptic responses (squares) and isoguvacine-induced responses (circles) on the membrane potential. Note that the responses reverse at −80 mV. (**C**) In current-clamp mode, both synaptic (left) and isoguvacine-evoked (right) $GABA_A$ mediated responses inhibit spontaneous firing. Membrane potential is hold at −76 mV by injection of negative current; the apparent "resting" membrane potential in this cell was of −69 mV.

DISCUSSION

The principal conclusions of the present study are that: (1) the developmental switch in $GABA_A$ signals from excitatory to inhibitory occurs in Wistar CA3 pyramidal cells during the second postnatal week, at around P8–10; and that (2) a gradual negative shift of E_{GABA}, which occurs during the two postnatal weeks underlies the developmental changes in $GABA_A$ signaling.

Performed in the present study day-by-day analysis of the $GABA_A$-mediated signals using the gramicidin perforated patch and extracellular recordings suggests that the developmental switch in the action of GABA in the Wistar rat CA3 pyramidal cells occurs at P8–10. While the results of two tests (gramicidin perforated patch and synaptic $GABA_A$-PSPs on multiple unit activity) provided close estimates of the switch at P8, isoguvacine test on MUA gave estimate of the switch at around P10. It should be noted that isoguvacine test on MUA was performed in the absence of any antagonists and isoguvacine-mediated excitation of relatively small neuronal population could be amplified in the network and result in a net increase in average unit activity. Indeed, increase in multiple unit frequency was associated with an increase in the frequency of GDPs (Fig. 5).

Values of the excitatory-to-inhibitory switch in the action of GABA at P8–10 obtained in the present study are within the range of the previous estimations (from P5 to P12) that have been obtained using various electrophysiological and imaging approaches (Ben-Ari et al., 1989; Garaschuk et al., 1998; Ganguly et al., 2001; Dzhala and Staley, 2003) (see also Introduction). The high variability of the previous estimates is probably due to a sex, species and strain differences (Galanopoulou et al., 2003; Galanopoulou, 2005), difference in the recordings techniques, preparations and the mode of activation of $GABA_A$ receptors. Thus, intracellular recordings introduce leak conductance, which results in neuronal depolarization (Barry and Lynch, 1991; Tyzio et al., 2003). In addition, the intracellular milieu is affected because of the

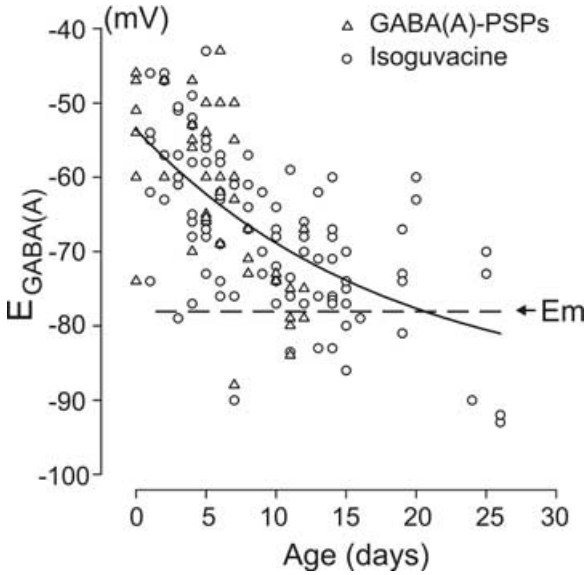

FIG. 3. Developmental change of the $GABA_A$ reversal potential in CA3 pyramidal cells measured using gramicidin perforated patch recordings. The values of the reversal potential of the pharmacologically isolated $GABA_A$-mediated postsynaptic potentials ($GABA_A$-PSPs, triangles) and isoguvacine-induced responses (circles). The line is an exponential fit. Note a gradual negative shift of the reversal potential of the $GABA_A$-mediated responses during the two postnatal weeks. Dashed line indicates the resting membrane potential (E_m) measured using cell-attached recordings of NMDA channels as voltage sensors (Ref. 39). Membrane potential values obtained using gramicidin perforated patch for the same set of cells is presented at the Fig. 2 of Tyzio et al., 2003.

exchange of intracellular and extracellular solutions via leak conductance and dialysis with the pipette solution, both errors being most important in small immature neurons. Thus, in a previous study using intracellular recordings, E_{GABA} in CA3 pyramidal cells at P2–5 was estimated at −25 mV with KCl-filled electrodes and at −51 mV with potassium methylsulfate-filled electrodes (Ben-Ari et al., 1989). Our perforated patch recordings in the present study gave a significantly more negative value of E_{GABA}, -59 ± 2 mV at P2–5. A plausible explanation for depolarized value of E_{GABA} with the intracellular approach is that cells are loaded by chloride from the KCl-filled recording electrode in which the concentration of chloride ions is about 3 M and from the extracellular solution through the leak conductance. Similar errors probably occur in measurement of E_{GABA} using intracellular electrodes in older neurons producing 8 mV difference in the value of E_{GABA} of −66 mV obtained using intracellular recordings (Ben-Ari et al., 1989) compared with -74 ± 3 mV obtained in the present study with gramicidin perforated patch at P13–15. Using the Nernst equation, from E_{GABA} we estimated the value of $[Cl^-]_i$ in the CA3 pyramidal cells at P0–2 as 16 mM reducing to 7 mM at P13–15. This is in keeping with the delay in the development expression of chloride extruding co-transporter KCC2 (Rivera et al., 1999) and early expression of the transporters that actively accumulate chloride including NKCC1 (Rohrbough and Spitzer, 1996; Clayton et al., 1998; Kakazu et al., 1999; Marty et al., 2002; Dzhala et al., 2005). Interestingly, the values of $[Cl^-]_i$ obtained in the present study are significantly lower than estimates of around 40–70 mM at P0–2 but they are close to the estimate of 7.9 mM at P14 obtained using the chloride-sensitive dye clomeleon (Kunerand and Augustine, 2000).

The time course of the developmental changes in GABAergic signaling that has been determined in the present study provides essential information for understanding of the ontogeny of CA3 neuronal network activity as well as the temporal frame for the trophic actions of GABA in the developing hippocampus. In particular, our results provide a simple solution of a "paradox" that GDPs that are dependent on excitatory GABA cease not at P5, when GABA was supposed to become inhibitory, but later, at around P10 (Ben-Ari et al., 1989). This timeline matches the switch in the action of GABA obtained in the present study. Interestingly, in Sprague Dawley rats, GABA switches its action from excitatory to inhibitory later than in Wistar rats, at around P13 (Khazipov et al., 2004) and that disappearance of the immature pattern of GDPs is also delayed by about three days. These results suggest that the immature pattern of GDPs is linked to the excitatory action of GABA. On the other hand, the $GABA_A$ inhibition dependent-adult pattern of theta oscillations emerges in the hippocampus starting from P8 (Leblanc and Bland, 1979; Leinekugel et al., 2002) (although brief episodes of theta activity can be detected

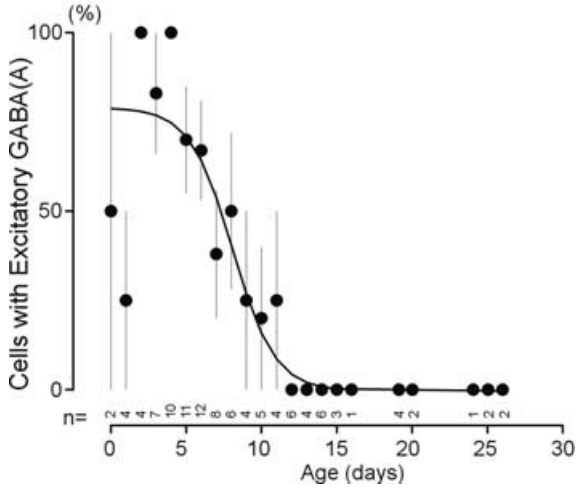

FIG. 4. Developmental change in the occurrence of the excitatory $GABA_A$ mediated responses with gramicidin perforated patch. Age dependence of the proportion of CA3 pyramidal cells with the excitatory responses to activation of the $GABA_A$ receptors. Responses were considered as excitatory if evoked synaptic $GABA_A$ mediated responses or application of isoguvacine triggered action potentials. Membrane potential of cells was maintained at −75 to −80 mV by injection of the negative current. Pooled data from 108 cells recorded using gramicidin perforated patch.

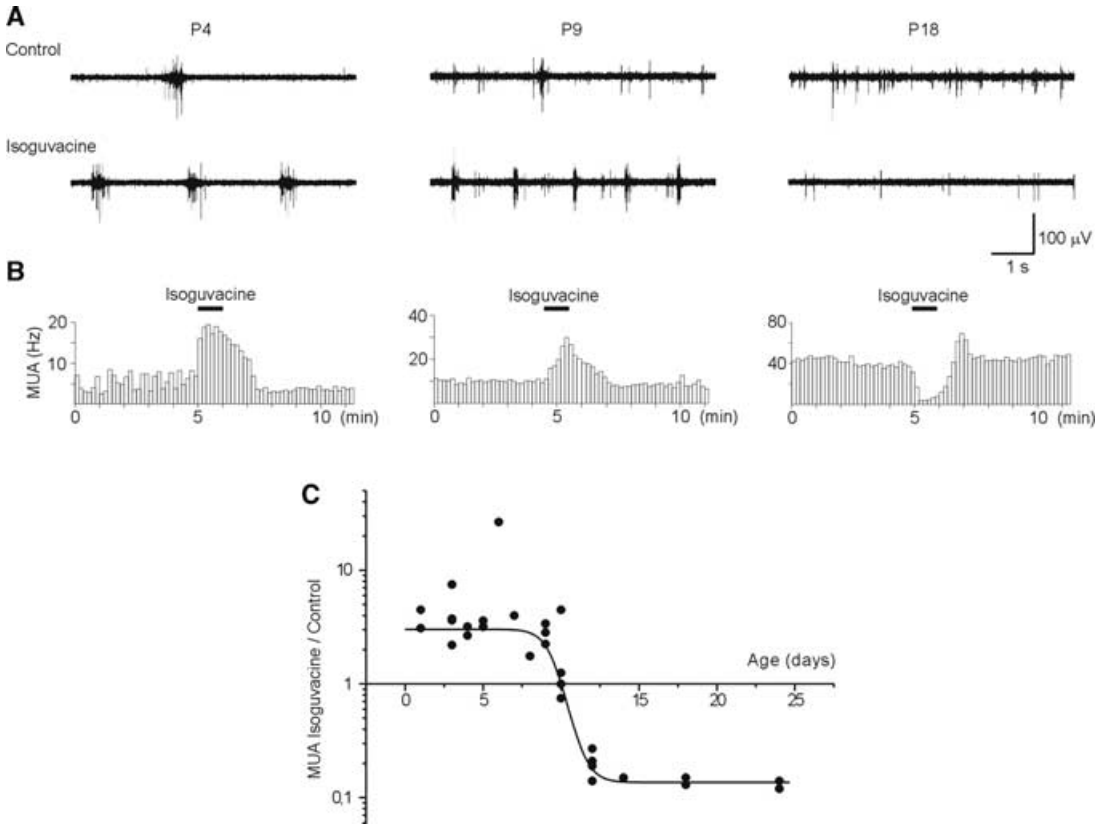

FIG. 5. Developmental changes in the effect of the $GABA_A$ receptor agonist isoguvacine on the multiple unit activity (MUA). (**A**) Bath application of isoguvacine (10 μM for 1 min) increases the frequency of extracellularly recorded action currents in CA3 pyramidal cell layer at postnatal days P4 and P9 but decreases it at P18. (**B**) The effect of isoguvacine on MUA frequency for the experiments shown on panel ***A***. (**C**) Age-dependence of the effect of isoguvacine on MUA frequency. *Ordinates*: MUA frequency in isoguvacine normalized to that in control; *abscissas*: age. Note that the effect of isoguvacine shifts from potentiation to inhibition at around the postnatal day P10.

already at P2 [Karlsson and Blumberg, 2003]). The hippocampal pattern of the high-frequency oscillations associated with the sharp waves (ripples [Buzsaki et al., 1992]) also emerges during the second postnatal week (Leinekugel et al., 2002; Buhl and Buzsaki, 2005). Therefore, the switch in the GABAergic signaling from excitation to inhibition can be a critical factor determining the transition from the immature to adult mode of function of the hippocampal network.

Developmental changes in $GABA_A$ signaling may contribute to the developmental changes in excitability and seizures. Indeed, the critical period of enhanced excitability that takes place in the rat during the second postnatal week is within the temporal window of excitatory action of GABA (Dzhala and Staley, 2003; Khazipov et al., 2004). Blockade of $GABA_A$ receptors during this period decreases the frequency or completely suppresses seizure like events (Dzhala and Staley, 2003; Khazipov et al., 2004) while barbiturates do not affect seizure-like activity (Dzhala et al., 2005). Blockade of NKCC1 by bumetanide causes a negative shift in E_{GABA} and suppresses seizures induced by high-potassium in vitro and kainate-induced seizures in vivo (Dzhala et al., 2005). However, $GABA_A$ contributions to the ictal-like activity in the neonatal rat hippocampus are more complex because in addition to an excitatory actions, GABA also exerts inhibitory actions via a shunting mechanism. Indeed, blockade of $GABA_A$ receptors by itself causes interictal-like activity starting from P0 and Ictal-like activity starting from P2 in the intact hippocampus in vitro (Khalilov et al., 1997a, 1997b, 1999), interictal-like activity in the hippocampal slices starting from P0 (Khalilov et al., 1999; Lamsa et al., 2000; Wells et al., 2000) and seizure-like events in the slices at P9–19 (Swann and Brady, 1984; Gomez-Di Cesare et al., 1997). Furthermore, the positive allosteric $GABA_A$ modulators (barbiturates, benzodiazepines) are efficient in suppressing neonatal seizures in several animal models (Smythe et al., 1988; Kubova and Mares, 1991; Velisek et al., 1995; Kubova et al., 1999; Isaev et al., 2005). Thus, GABAergic contributions to ictogenesis in the neonatal hippocampus are complex and further studies are required for better understanding of the role of the developmental changes in $GABA_A$ signaling in the neonatal ictogenesis.

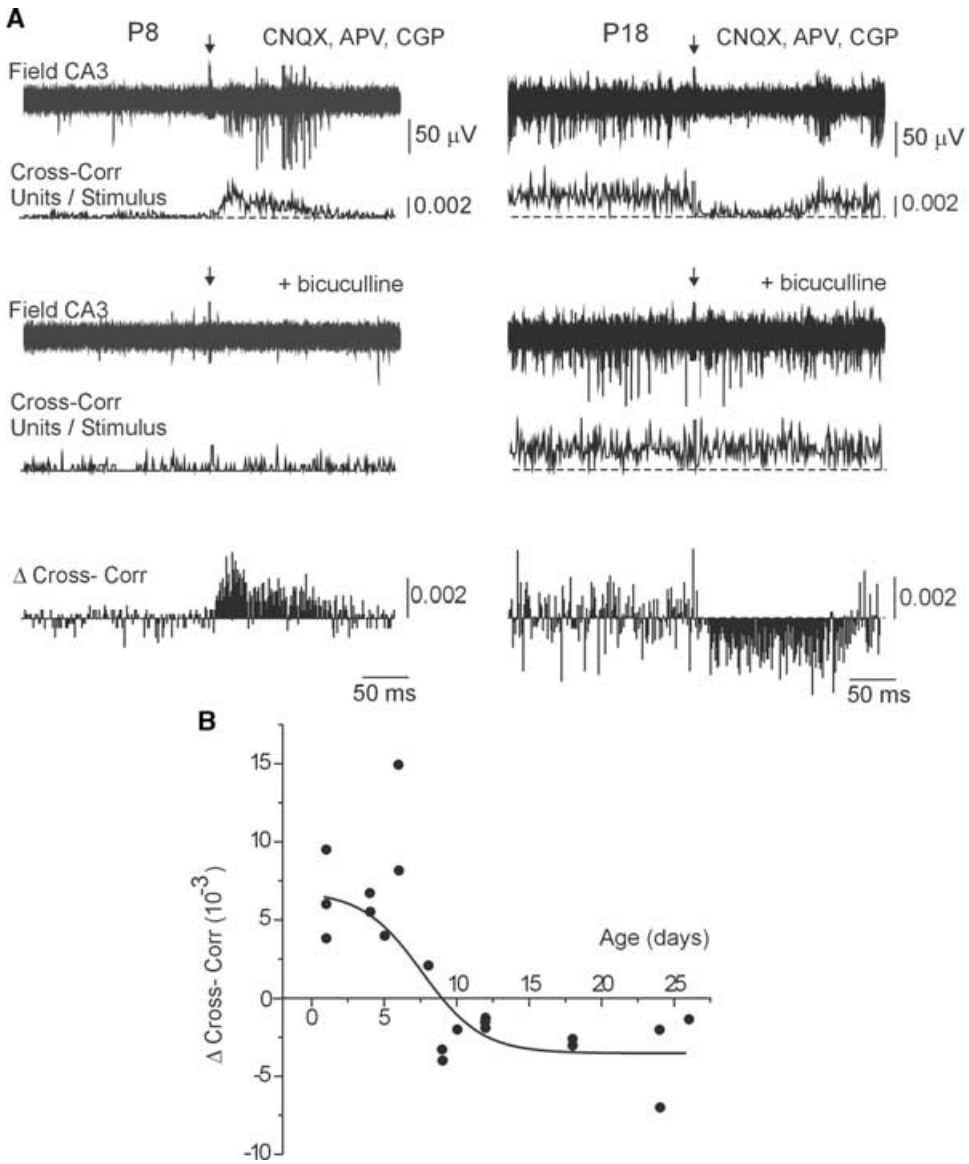

FIG. 6. Developmental changes in the effect of synaptic activation of $GABA_A$ receptors on MUA. (**A**) Activation of synaptic $GABA_A$ receptors by electrical stimulus (*arrow*) in the presence of the ionotropic glutamate and $GABA_B$ receptor antagonists (10 μM CNQX, 50 μM D-APV and 0.5 mM CGP35348) transiently increases the frequency of extracellularly recorded action currents in the CA3 pyramidal cell layer at P8 and decreases at P18. Ten traces are superimposed. *Below*, cross-correlation of unitary action currents versus stimulus; note positive correlation at P8 and negative at P18. Traces below: the effect of stimulation on units frequency is blocked by addition of the $GABA_A$ receptor antagonist bicuculline. Δ *cross-corr* is a result of subtraction of the cross-correlations between action currents and stimulus before and after addition of bicuculline. (**B**) Age-dependence of the effect of synaptic activation of the $GABA_A$ receptors on neuronal firing. Note a positive cross-correlation (excitation) at the youngest stages that shifts to a negative cross-correlation (inhibition) around P9.

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