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Activity- and age-dependent GABAergic synaptic plasticity in the developing rat hippocampus

Paolo Gubellini,[1,2] Yehezkel Ben-Ari[1] and Jean-Luc Gaïarsa[1]
[1]Institut de Neurobiologie de la Mediterranée (INMED), Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 29, Avenue de Luminy, BP 13, 13273 Marseille, Cedex 09, France
[2]Istituto di Neurobiologia e Medicina Molecolare, Consiglio Nazionale delle Ricerche (CNR), Viale Marx 15, 00137, Roma, Italy

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Abstract

Activity-dependent plasticity of GABAergic synaptic transmission was investigated in rat hippocampal slices obtained between postnatal day (P) 0–15 using the whole-cell patch-clamp recording technique. Spontaneous $GABA_A$ receptor-mediated postsynaptic currents ($sGABA_A$-PSCs) were isolated in the presence of ionotropic glutamate receptor antagonists. A conditioning protocol relevant to the physiological condition, consisting of repetitive depolarizing pulses (DPs) at 0.1 Hz, was able to induce long-lasting changes in both frequency and amplitude of $sGABA_A$-PSCs between P0 and P8. Starting from P12, DPs were unable to induce any form of synaptic plasticity. The effects of DPs were tightly keyed to the frequency at which they were delivered. When delivered at a lower (0.05 Hz) or higher (1 Hz) frequency, DPs failed to induce any long-lasting change in the frequency or amplitude of $sGABA_A$-PSCs. In two cases, DPs were able to activate $sGABA_A$-PSCs in previously synaptically silent cells at P0–1. These results show that long-term changes in GABAergic synaptic activity can be induced during a restricted period of development by a conditioning protocol relevant to the physiological condition. It is suggested that such activity-induced modifications may represent a physiological mechanism for the functional maturation of GABAergic synaptic transmission.

Introduction

Long-term potentiation (LTP) and long-term depression (LTD) are, respectively, persistent activity-dependent increases and decreases in synaptic efficacy induced by various experimental protocols. Initially studied as a model of learning and memory, several investigations have led to the hypothesis that these forms of synaptic plasticity may be instrumental to the functional maturation of developing excitatory synapses. In support of this hypothesis, LTP at cortical glutamatergic synapses shows an age dependency that closely matches the critical period during which the construction of the cortical network can be modified by sensory perturbations (Crair & Malenka, 1995; Kirkwood *et al*., 1995; Kirkwood *et al*., 1996). Moreover, activity-dependent competition between developing excitatory neuromuscular synapses may rely on Ca^{2+}-dependent LTD (Lo & Poo, 1991; Lo & Poo, 1994).

The maturation of the inhibitory synaptic transmission is also modulated by activity (Seil & Drake-Baumann, 1994; Aamodt *et al*., 2000; Marty *et al*., 2000). In the auditory system, for instance, the topographic organization of glycinergic inhibitory projections is achieved through synapses elimination (Sanes & Siverls, 1991) that involves an activity-dependent mechanism (Sanes & Tackacs, 1993). Moreover, an activity-dependent modulation of the inhibitory synaptic strength has been reported in several developing brain structures (Komatsu, 1994; McLean *et al*., 1996; Kotak & Sanes, 2000). All these findings lead to the hypothesis that LTP- and LTD-like mechanisms are involved in the establishment of functional inhibitory connections.

However, the validity of this hypothesis depends on the assumptions that (i) long-term changes in inhibitory synaptic strength are induced during a restricted period of development, which closely matches the window of the functional maturation, and that (ii) stimuli relevant to the physiological condition are able to induce these forms of plasticity. Most studies investigating LTP or LTD of γ-aminobutyric acid (GABA)ergic synaptic transmission in the developing brain have used tetanic stimulation (TS) as conditioning protocol (Komatsu, 1994; McLean *et al*., 1996; Kotak & Sanes, 2000). While the TS is a useful tool to study the mechanisms underlying the induction and expression of LTP and LTD (Caillard *et al*., 1999b; Komatsu, 1996; Caillard *et al*., 2000; Kotak & Sanes, 2000), it does not prove that GABAergic synapses naturally express these forms of plasticity.

In a previous study in the neonatal rat hippocampus (Caillard *et al*., 1999a), we reported that GABAergic synaptic transmission undergoes a Ca^{2+}-dependent LTP following the application of repetitive depolarizing pulses (DPs), between postnatal day (P) 3 and 6. In the present study, to gain insight into the functional significance of this form of synaptic plasticity, we explored its age dependency and investigated the minimal requirement for its induction. We report that long-term changes in GABAergic synaptic transmission can be induced during a restricted period of the postnatal development by a conditioning protocol relevant to the physiological condition. We also provide evidences that this form of plasticity may play a role in the functional maturation of the GABAergic synaptic transmission.

Correspondence: Dr Jean-Luc Gaïarsa, as above.
E-mail: gaiarsa@inmed.University-mrs.fr

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Materials and methods

Brain slice preparation

Experiments were performed on hippocampal CA3 neurons obtained from neonatal male Wistar rats, at P0–1, P4–8, P11–12 and P15. Brains were removed from cryo-anaesthetized rats and submerged in artificial cerebrospinal fluid (ACSF) (in mM): NaCl, 126; KCl, 3.5; $CaCl_2$, 2; $MgCl_2$, 1.3; NaH_2PO_4, 1.2; $NaHCO_3$, 25; and glucose, 11; pH 7.4, equilibrated with 95% O_2 and 5% CO_2. Hippocampal slices, 600 μM thick, were cut with a McIlwain tissue chopper and incubated in ACSF at room temperature for at least 60 min before use. Individual slices were then transferred to a submerged recording chamber and superfused with ACSF at 2.5–3 mL/min at 34 °C.

Whole-cell recordings

Whole-cell patch-clamp recordings were performed with an Axopatch 200B (Axon Instruments, Foster City, CA, USA) amplifier. Borosilicate microelectrodes (5–8 MΩ) were filled with internal solutions of the following composition (in mM). CsCl solution: CsCl, 110; K-gluconate, 30; ethylene glycol-bis (β-aminoethyl ether)-N,N,N′,N′-tetra-acetic acid (EGTA), 1.1; N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid (HEPES), 10; $CaCl_2$, 0.1; MgATP, 4; NaGTP, 0.3. K-gluconate solution: K-gluconate, 135; $MgCl_2$, 2; $CaCl_2$, 0.1; EGTA, 1; Na_2ATP, 2; HEPES, 10; pH 7.25, osmolarity 275 mOsmol. During experiments, prior to each stimulation, series resistances (R_s), capacitance and membrane resistance (R_m) were determined by an online fitting analysis of the transient currents in response to a 5-mV pulse with Acquis 4.0 software (ACQUIS1, G. Sadoc, Bio-logic, Orsay, France). Cells recorded with unstable R_m or R_s (> 20% change) were discarded. The R_s ranged between 10 and 50 MΩ.

Data acquisition and analysis

The spontaneous $GABA_A$ receptor-mediated postsynaptic currents ($sGABA_A$-PSCs) were stored on a digital tape recorder (Biologic, Claix, France) and analysed offline on a personal computer with Mini Analysis 5.1 (Synaptosoft, Leonia, NJ, USA) software. The detection threshold was set at twice the baseline noise. The fact that no false events would be identified was confirmed by visual inspection for each experiment. Offline analysis was performed on $sGABA_A$-PSCs recorded during a fixed time epoch (3–5 min), sampled every 5 or 10 min before (two samplings) and after (3–6 samplings) the conditioning protocol. Only cells that exhibited a stable $sGABA_A$-PSC frequency in control (< 20% changes during the two control samplings) were taken into account. We have chosen arbitrarily, as the defining criterion for maintenance of long-lasting changes in the frequency or amplitude of $sGABA_A$-PSCs, changes of $\geqslant$ 20% 30 min after the conditioning protocol. Averaged cumulative histograms were obtained by normalizing each distribution to the corresponding maximal value obtained in control. For data presented as the mean ± SEM, statistical analysis was performed using a paired or unpaired Student's *t*-test. When comparing differences between two cumulative distributions, the Kolmogorov–Smirnov (K–S-test) was used. The significance level was established at $P < 0.05$ for both tests.

Drugs

The 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and DL-2-amino-5-phosphovaleric acid (DL-APV) were purchased from Tocris Cookson (Bristol, UK). CNQX and DL-AP5 were dissolved in the ACSF and applied by bath.

Results

In the present study we determine the developmental period during which long-lasting modifications of GABAergic synaptic efficacy can be induced by depolarizing pulses (DPs), the minimal requirement for their induction and the functional significance that this plasticity may serve in the developing hippocampus.

Effects of DPs on spontaneous GABAergic synaptic activity

The $sGABA_A$-PSCs were recorded from CA3 pyramidal neurons in the presence of ionotropic glutamate receptor antagonists (10 μM CNQX and 50 μM DL-APV). Membrane potential was held at –60 to –70 mV during the sampling periods. In these conditions, $sGABA_A$-PSCs were resolved as inward currents. The DP conditioning protocol was applied after a 15-min control period and consisted of 20 DPs (500 ms each, from –80/–90 to 0 mV) delivered at 0.1 Hz. Each DP induced an inward Ca^{2+} current (see Figs 1A and 2A, centre).

The DP conditioning protocol given between P0 and P8 led to either an increase or a decrease in the frequency of $sGABA_A$-PSCs. Figure 1 shows the traces recorded from a neuron at P1 that exhibited a decrease in frequency following the application of the DP conditioning protocol. The average inter-event interval (IEI) increased from 0.094 ± 0.026 s in control to 0.148 ± 0.005 s 30 min after the DPs (896 events for both control and after the DPs; $P < 0.01$; K–S-test; Fig. 1A and B). The decrease in the frequency of $sGABA_A$-PSCs was associated with a significant increase in their mean amplitude (from –37.07 ± 0.67 pA in control to –57.60 ± 1.49 pA 30 min after the DPs; $P < 0.01$; K–S-test; Fig. 1A, C and D). Fig. 2 illustrates a single experiment at P5 in which DPs resulted in a long-lasting increase in the frequency of $sGABA_A$-PSCs. The average IEI decreased from 0.38 ± 0.02 s (193 events) to 0.11 ± 0.03 s (543 events) 30 min after the DPs ($P < 0.01$; K–S-test; Fig. 2A and B). The conditioning protocol also induced an increase in the mean amplitude of $sGABA_A$-PSCs, which passed from –28.5 ± 0.5 pA (165 events) to –39.8 ± 0.6 pA (165 events) 30 min after the DPs ($P < 0.05$; K–S-test; Fig. 2A, C and D).

At all developmental stages the frequency and amplitude of $sGABA_A$-PSCs remained increased for at least 30 min after the DPs when experiments were usually terminated (Fig. 2E, pooled data from all experiments including those with increase, decrease and no change in frequency). In control experiments, cells were recorded in similar conditions but the DP conditioning protocol was not delivered. In these experiments, the IEI of $sGABA_A$-PSCs was 0.75 ± 0.21 s and 0.82 ± 0.24 s 10 and 40 min after going into whole cell, respectively (Fig. 3A; $n = 6$; $P = 0.4$; paired *t*-test) and the average amplitude of $sGABA_A$-PSCs was –42 ± 5 and –35 ± 6 pA, 10 and 40 min after going into whole cell, respectively ($n = 6$; $P = 0.13$; paired *t*-test).

DP-induced changes in spontaneous GABAergic synaptic activity as a function of postnatal age

The Fig. 3A and Table 1 summarize the results obtained from all experiments performed between P0 and P15. The graph in Fig. 3A shows the change of $sGABA_A$-PSC IEI 30 min after the DPs at P0–1 ($n = 22$), P4–8 ($n = 24$) and P12–15 ($n = 12$). Some points overlap, so it can appear that there are fewer experiments than those stated above.

At P0–1, among the 22 cells recorded, the DP conditioning protocol induced a long-lasting increase in the frequency of $sGABA_A$-PSCs in 10 cells, a long-lasting decrease in five other cells and no significant effect in the seven remaining cells (Fig. 3A

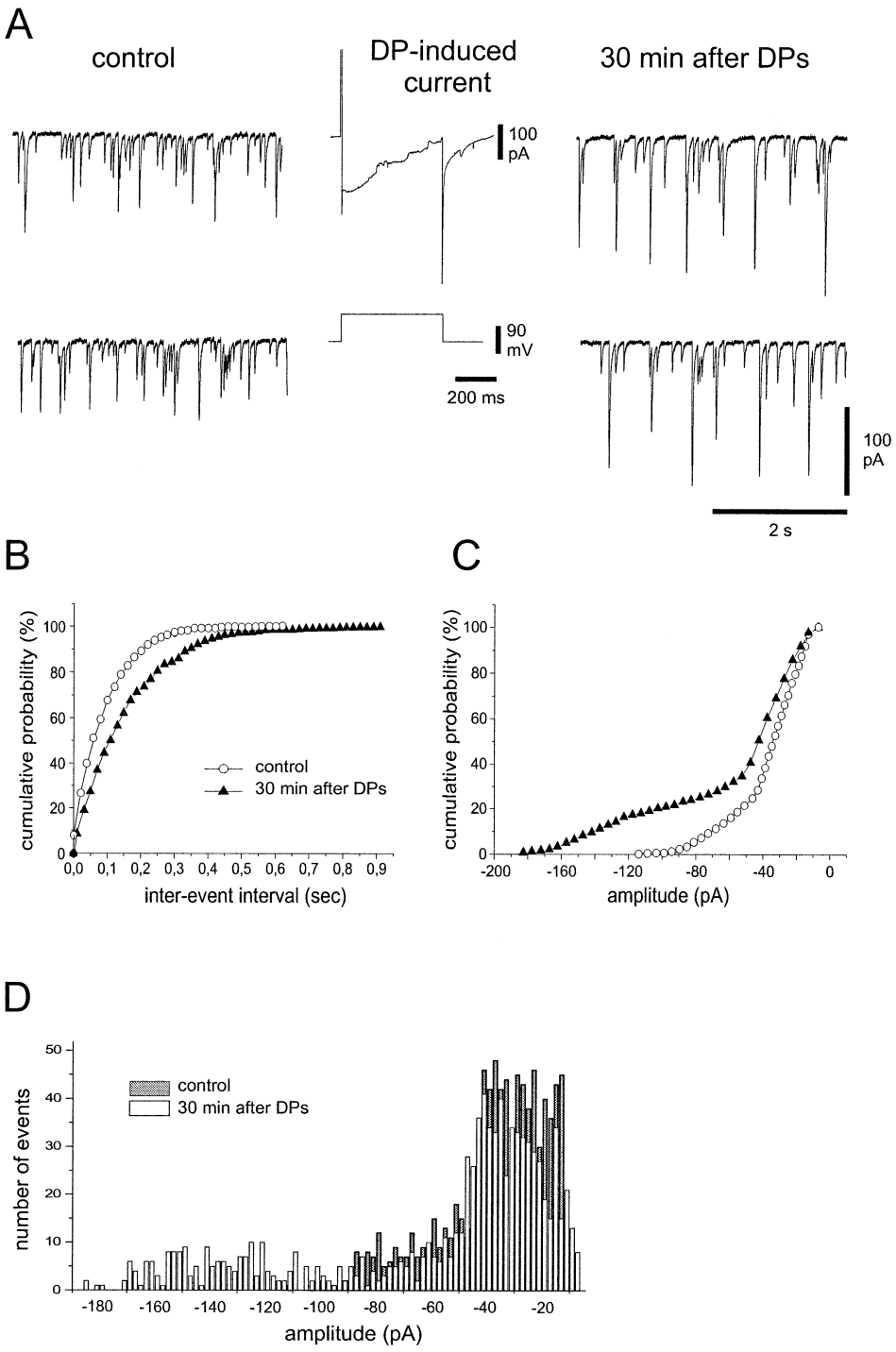

FIG. 1. DPs induced long-lasting decrease in the frequency of sGABA$_A$-PSCs. (A) Traces show sGABA$_A$-PSCs (holding potential –60 mV) recorded (left) before and (right) 30 min after the DP conditioning protocol (centre shows a sample DP) at P1. Recordings were performed with a CsCl-filled electrode in the presence of 10 μM CNQX and 50 μM DL-APV. (B) Cumulative probability plot of the sGABA$_A$-PSC IEI, (open circles) before and (filled triangles) 30 min after the DPs. (C) Cumulative probability plot of the sGABA$_A$-PSC amplitude, (open circles) before and (filled triangles) 30 min after the DPs. (D) Superimposed amplitude histograms (grey bars) before and (white bars) 30 min after the DPs. Traces in A and graphs in B, C and D are from the same cell.

and Table 1). Pooling together the results obtained from all cells, the average IEI decreased from 1.59 ± 0.35 to 1.19 ± 0.32 s 30 min after the DPs (n = 22; $P < 0.05$; paired t-test).

Between P4 and P8, the DP conditioning protocol led to a long-lasting increase in the frequency of sGABA$_A$-PSCs in 14 out of 24 cells, a long-lasting decrease in four out of 24 cells and no significant effect in the six remaining cells (Fig. 3A and Table 1). When the results from the 24 cells were pooled together, the average IEI decreased from 1.06 ± 0.28 to 0.43 ± 0.06 s 30 min after the DPs (n = 24; $P < 0.05$; paired t-test).

From P12, the probability that DPs led to significant long-lasting changes in the frequency of sGABA$_A$-PSCs clearly decreased. Among the 12 cells recorded between P12 and P15, the DP conditioning protocol induced a long-lasting increase in the frequency of sGABA$_A$-PSCs in two cells, a long-lasting decrease in one cell and no significant effect in the nine remaining cells (Fig. 3A and Table 1). On average the IEI was 0.039 ± 0.006 before and 0.04 ± 0.007 s 30 min after the DPs (n = 12; P = 0.37; paired t-test).

As pointed out above, whatever the polarity of the DP-induced changes in the frequency of sGABA$_A$-PSCs, this effect was always associated with an increase in their mean amplitude. Examination of the cumulative amplitude distributions and amplitude histograms (Figs 1C and D, and 2C and D) reveals that this increase is not

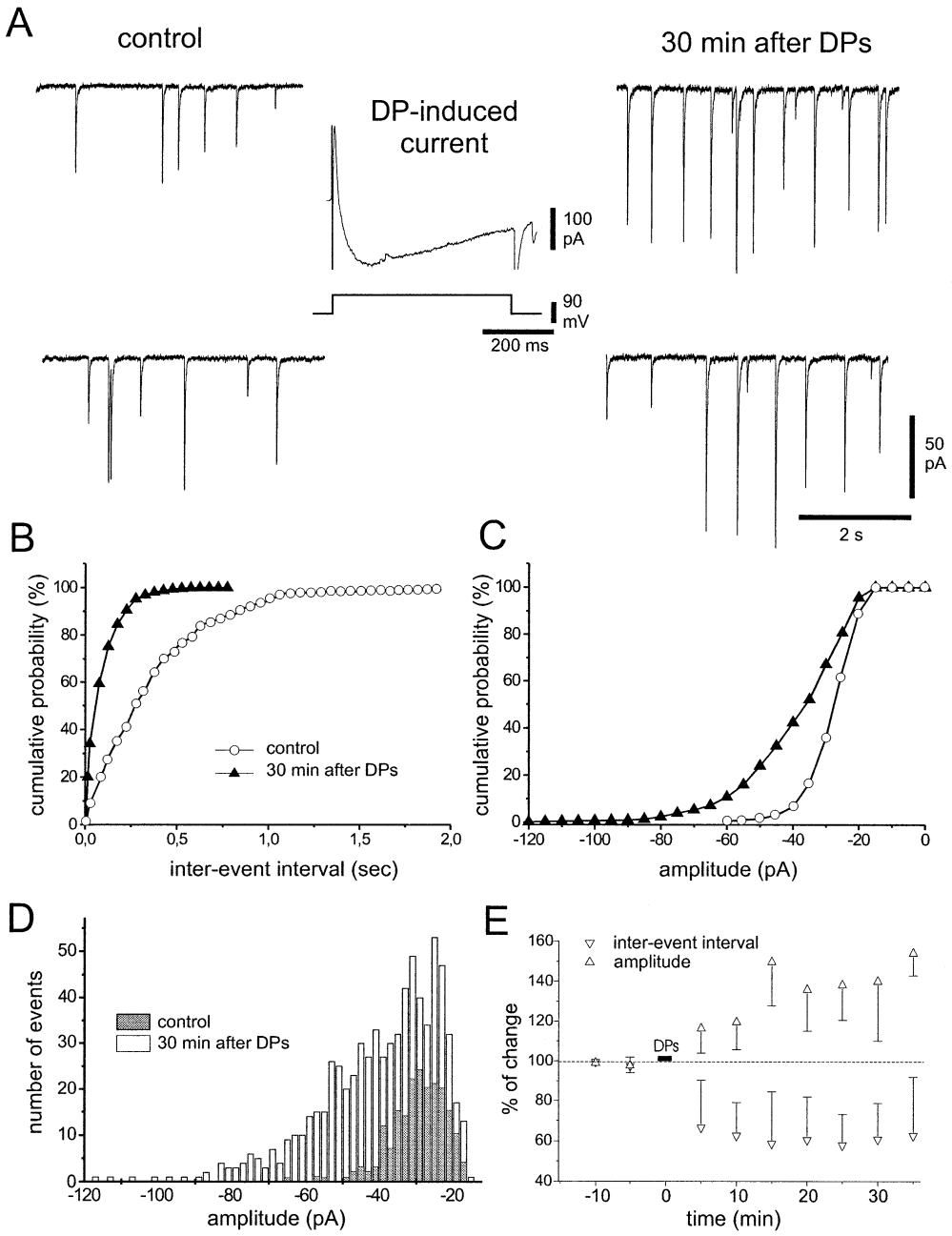

FIG. 2. DPs induced long-lasting increase in the frequency of sGABA$_A$-PSCs. (A) Traces show sGABA$_A$-PSCs (holding potential –60 mV) recorded (left) before and (right) 30 min after the DP conditioning protocol (centre shows a sample DP) at P5. Recordings were performed with a CsCl-filled electrode in the presence of 10 μM CNQX and 50 μM DL-APV. (B) Cumulative probability plot of the sGABA$_A$-PSC IEI, (open circles) before and (filled triangles) 30 min after the DPs. (C) Cumulative probability plot of the sGABA$_A$-PSC amplitude, (open circles) before and (filled triangles) 30 min after the DPs. (D) Superimposed amplitude histograms, (grey bars) before and (white bars) 30 min after the DPs. Traces in A and graphs in B, C and D are from the same cell. (E) Time course of the long-lasting effects of DPs on (up triangles) the amplitude and (down triangles) inter-event interval of sGABA$_A$-PSCs.

uniform and results from an increase in the number of large-amplitude events, and even from the appearance of events larger than any one observed in control (see also Fig. 4B). On average, the amplitude of sGABA$_A$-PSCs increased from –35.5 ± 4.4 to –46.2 ± 5.4 pA at P0–1 (n = 18; P = 0.01; paired t-test), and from –53.6 ± 8.8 to –72.7 ± 14.9 pA between P4 and P8 (n = 20; P = 0.04; paired t-test). From P12, DPs induced a significant increase in the amplitude of sGABA$_A$-PSCs in only one case. Interestingly, in cells that exhibited a decrease in the frequency of sGABA$_A$-PSCs after the DPs, we observed an increase in the total charge transferred per fixed time (i.e. total area of events/min), as a result of the increase in the proportion of large amplitude events. The amount of the increase 30 min after the DPs was 172 ± 25% of control (n = 9). Therefore in all cells, irrespective of the polarity of DP-induced changes in the frequency of sGABA$_A$-PSCs, the overall effect of the DP conditioning protocol was an increase of the GABAergic synaptic drive onto the target cells.

These data show that DPs induce long-lasting changes in the frequency and amplitude of sGABA$_A$-PSCs that are restricted to the first postnatal week of life. Although DPs could lead to either an increase or a decrease in the frequency of sGABA$_A$-PSCs, the

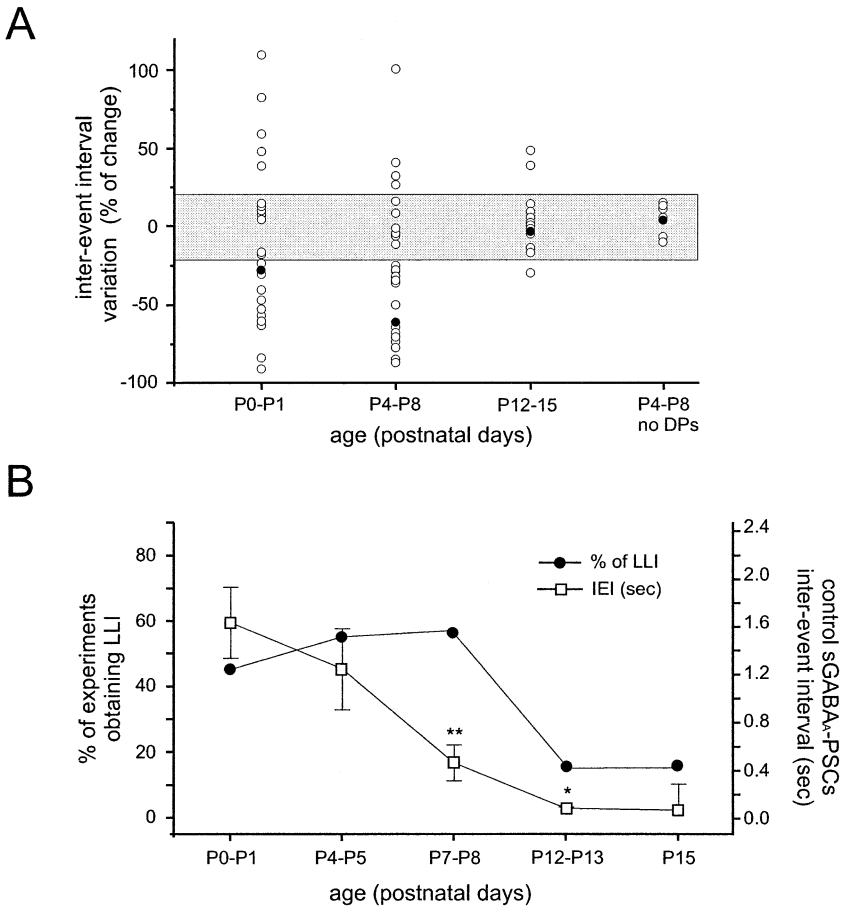

FIG. 3. DP-induced long-lasting changes in the frequency of sGABA$_A$-PSCs as a function of age and control frequency. (A) Change of the IEI of sGABA$_A$-PSCs 30 min after the DPs at P0–1 (n = 22), P4–8 (n = 24) and P12–15 (n = 12). On the same graph are represented control P4–8 experiments in which no DPs were delivered. For these cells, the IEI of sGABA$_A$-PSCs 10 and 40 min after going into the whole cell was compared. Each open circle represents the result from a single experiment and the filled circles show the average of all experiments at the corresponding developmental stage. The grey bar depicts the range of non-significant modifications. (B) The age-dependency of long-lasting increase in the frequency of sGABA$_A$-PSCs is summarized in terms of (filled circles) the percentage of neurons showing a long-lasting increase (LLI) in the frequency of sGABA$_A$-PSCs. On the same graph the average control IEI of sGABA$_A$-PSCs is plotted as a function of age (open squares). The IEI of sGABA$_A$-PSCs decreases as a function of age, reaching a plateau between P12 and P15. **$P < 0.01$; unpaired t-test, comparing P7–8 (n = 22) with P4–5 (n = 18); *$P < 0.01$; unpaired t-test, comparing P12–13 (n = 6) with P7–8 (n = 7).

probability that DPs induced a long-lasting increase is higher during the first postnatal week of life. Moreover, when a decrease in the frequency of sGABA$_A$-PSCs was induced, this decrease was compensated by a concomitant increase in their mean amplitude. Therefore, the overall effect of DPs during the first postnatal week of life was an increase of the GABAergic synaptic drive onto hippocampal cells.

DP-induced changes in GABAergic synaptic activity closely matched the period of functional maturation of GABAergic synaptic transmission

We next wanted to determine whether the developmental stage, during which the synaptic plasticity can be induced, correlates with the period of functional maturation of the GABAergic synaptic transmission. As an index for functional maturation, we measured the control IEI of sGABA$_A$-PSCs at different developmental stages.

As illustrated in Fig. 3B, the average control IEI of sGABA$_A$-PSCs decreased from P0–P12 to reach a plateau between P12 and P15. All experiments for each age window were included to calculate the average control IEI, which was 1.59 ± 0.35 s at P0–1 (n = 22), 1.2 ± 0.34 s at P4–5 (n = 18; P = 0.27 when compared with P0–1; unpaired t-test), 0.42 ± 0.14 s at P7–8 (n = 7; $P < 0.01$ when compared with P4–5; unpaired t-test), 0.039 ± 0.01 s at P12–13 (n = 6; $P < 0.05$ when compared with P7–8; unpaired t-test), and 0.037 ± 0.009 s at P15 (n = 6; P = 0.2 when compared with P12–13; unpaired t-test). On the same graph, we plotted the percentage of the experiments in which a long-lasting increase in the frequency of sGABA$_A$-PSCs was obtained at P0–1 (n = 10 out of 22), P4–5 (n = 10 out of 18), P7–8 (n = 4 out of 7), P12–13 (n = 1 out of 6) and P15 (n = 1 out of 6).

TABLE 1. DP-induced changes in inter-event interval (IEI) of sGABA$_A$-PSCs as a function of age

	Control IEI (s)	IEI 30 min after DPs	Cells (n)	Change (%)
All cells				
P0–1	1.59 ± 0.35	1.19 ± 0.3 *	22	–25
P4–8	1.06 ± 0.28	0.43 ± 0.06 *	24	–59
P12–15	0.039 ± 0.006	0.04 ± 0.007	12	2.5
DP-induced potentiation				
P0–1	1.67 ± 0.47	0.93 ± 0.44 *	10	–44
P4–8	1.6 ± 0.4	0.5 ± 0.096 *	14	–68
P12–15	0.05 ± 0.02	0.037 ± 0.01	2	–26
DP-induced depression				
P0–1	0.22 ± 0.05**	0.35 ± 0.07*	5	59
P4–8	0.17 ± 0.07**	0.24 ± 0.09 *	4	41
P12–15	0.056	0.083	1	48
DP-induced no effect				
P0–1	2.4 ± 0.75	2.2 ± 0.66	7	–8
P4–8	0.35 ± 0.11	0.37 ± 0.12	6	5
P12–15	0.034 ± 0.07	0.035 ± 0.07	9	2

Data are expressed as mean ± SEM. *$P < 0.05$ when compared to control, paired t-test. **$P < 0.01$ when compared with DP-induced potentiation at the same postnatal age, unpaired t-test.

Therefore there is an age window, between P0 and P8, when the long-lasting increase in the frequency of sGABA$_A$-PSCs could be

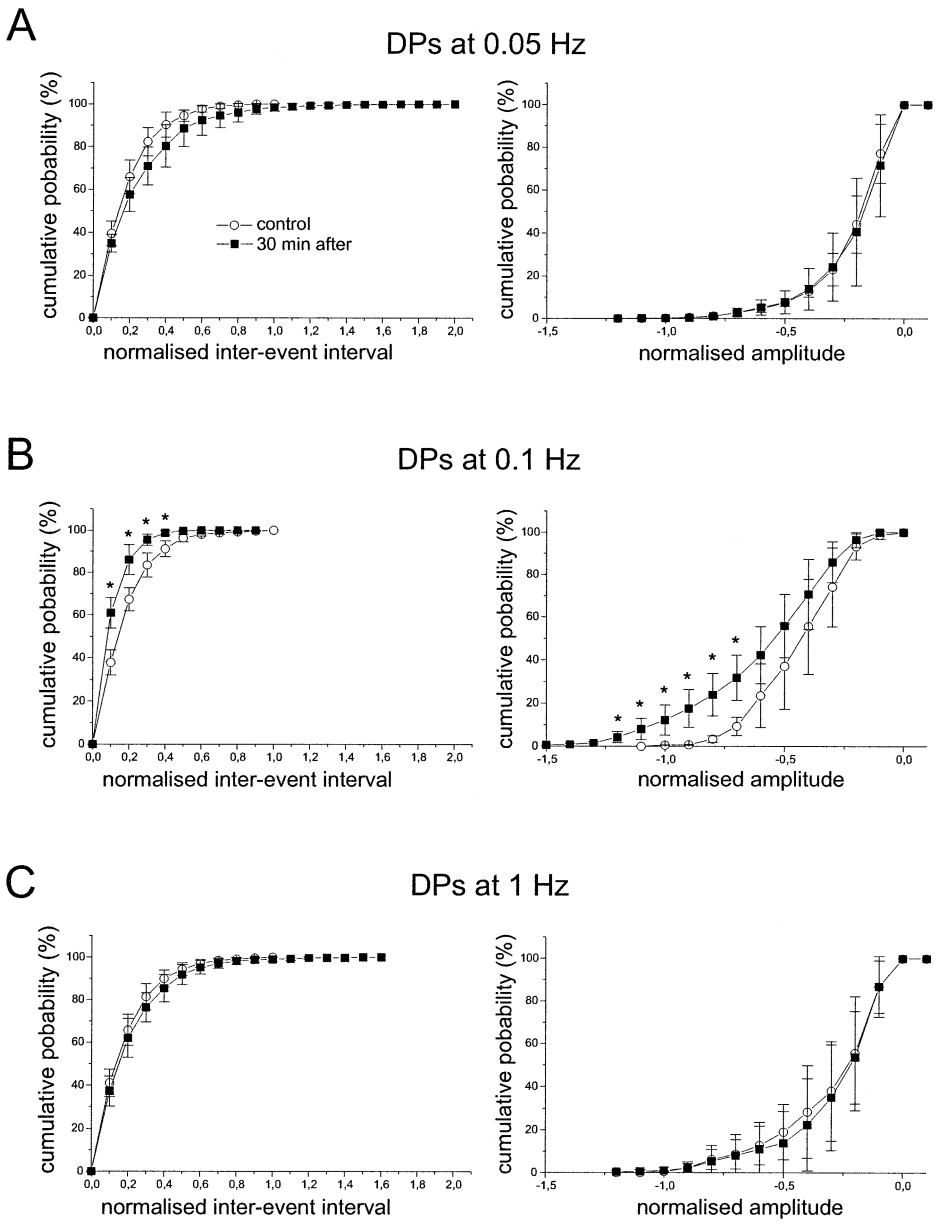

FIG. 4. DP-induced long-lasting changes in sGABA$_A$-PSC frequency and amplitude as a function of DP frequency. (A) DPs delivered at 0.05 Hz are not able to induce significant changes either on (left) average IEI or (right) amplitude of sGABA$_A$-PSCs (n = 6). (B) DPs at 0.1 Hz induce (left) a long-lasting decrease in sGABA$_A$-PSC IEI and (right) a long-lasting increase in sGABA$_A$-PSC amplitude (n = 6). (C) When applied at a frequency of 1Hz, DPs failed to induce significant changes in sGABA$_A$-PSC IEI (left) and amplitude (right) (n = 6). The graphs summarize overall experiments before (open circles) and 30 min after (filled squares) the DPs. Values are normalized to the maximum of control. All experiments were performed between P6 and P8. *P < 0.05; unpaired t-test.

induced by DPs. This age-window closely matches the period during which the GABAergic synaptic transmission undergoes the functional maturation.

DP-induced changes in GABAergic synaptic activity as a function of the conditioning protocol

We next wanted to determine the minimal requirement to induce the above described long-lasting changes in GABAergic synaptic activity. We therefore changed (i) the frequency at which the DPs were delivered in order to determine whether this parameter was important for the induction of GABAergic synaptic plasticity, and (ii) the recording conditions (i.e. intracellular and perfusing solutions) in order to obtain a more physiological experimental model. All experiments described in this section were performed between P6–8.

Changing the DP frequency

In the first set of experiments, DPs were delivered at 0.05 Hz or at 1 Hz and the results were compared to those obtained from interleaved experiments at 0.1 Hz. The number (20), duration (500 ms) and range (from –80/–90 to 0 mV) of each DP were not changed.

As shown in Fig. 4A, DPs delivered at 0.05 Hz (n = 6) did not significantly change either sGABA$_A$-PSC IEI (from 0.29 ± 0.07 s before to 0.31 ± 0.12 s 30 min after the DPs; P = 0.11; paired t-test) or amplitude (from –59.6 ± 4 pA before and –55.4 ± 3 pA 30 min after the DPs; P = 0.28; paired t-test).

Graphs in Fig. 4B show that at 0.1 Hz (n = 6) both IEI and amplitude of sGABA$_A$-PSCs are potentiated. On average, sGABA$_A$-PSC IEI decreased from 0.38 ± 0.13 to 0.22 ± 0.09 s, 30 min after the DPs (P < 0.01; paired t-test), while amplitude increased from –65 ± 13.9 to –90.4 ± 24.2 pA (P < 0.05; paired t-test). Note that the average amplitude increase was due to the augmented proportion of large events and to currents larger than any one observed before the DPs, rather than to a shift of the curve.

A shown in Fig. 4C, DPs at 1 Hz (n = 6) were unable to affect either average sGABA$_A$-PSC IEI, which was 0.32 ± 0.11 before and

0.34 ± 0.11 s 30 min after the DPs ($P = 0.13$; paired t-test) or amplitude that was –56.2 ± 13.1 and –55.2 ± 13.9 pA ($P = 0.40$; paired t-test).

Thus, a 0.1-Hz frequency at which DPs were delivered seemed to be necessary for the induction of long-lasting changes in the frequency of sGABA$_A$-PSCs.

Changing the recording conditions

In the second set of experiments, CA3 pyramidal neurons were recorded at P6–8 with K-gluconate-filled electrodes, and both CNQX and DL-APV were omitted from the perfusing solution. Under these conditions, DPs triggered action potential currents rather than large calcium currents, and the neuronal network activity was preserved. In these experiments, the cells were recorded at a holding potential ranging from –50 to –30 mV during the samplings period. At this potential, sGABA$_A$-PSCs were resolved as outward currents. We used two different DP conditioning protocols. The first DP conditioning protocol, delivered to 11 cells, was similar to that previously described: 20 DPs between –80/–90 and –30/0 mV, 500 ms duration, at 0.1 Hz (each DP was able to generate between 1 and 10 spike currents) (see Fig. 5A, centre). The second DP conditioning protocol, delivered to seven cells, consisted of bursts (5–12 Hz) of 4–6 brief DPs (30 ms each), each one evoking a single spike current. Each burst was repeated 20 times at 0.1 Hz at a holding potential of –60 mV (see Fig. 6, centre). As the two conditioning protocols led to similar effects, the results obtained from the 18 cells were pooled.

An example of such an experiment is illustrated in Fig. 5A–C. In this cell, each DP triggered 4.6 ± 0.9 spike currents (Fig. 5A, centre). Following the application of the DP conditioning protocol, the average IEI of sGABA$_A$-PSCs decreased from 0.77 ± 0.06 s (171 events) to 0.55 ± 0.04 s (238 events) 30 min after the DPs (Fig. 5B; $P < 0.01$; K–S-test). Moreover, the amplitude increased from 52.3 ± 2.3 pA (160 events) to 82 ± 4.3 pA (166 events) 30 min after the DPs (Fig. 5C; $P < 0.01$; K–S-test). As observed before, the increase in the mean amplitude resulted mostly from an increase in the proportion of large events and from the appearance of larger ones (Fig. 5C). Among the 18 cells recorded under these conditions, the DP conditioning protocol induced a long-lasting increase in the frequency of sGABA$_A$-PSCs in 12 cells, a long-lasting decrease in the frequency of sGABA$_A$-PSCs in two cells and no significant effect in the four remaining cells. When all data were pooled ($n = 18$), the average sGABA$_A$-PSC IEI decreased from 1.15 ± 0.31 s to 0.51 ± 0.09 s 30 min after the DPs ($P < 0.04$; paired t-test). The frequency of sGABA$_A$-PSCs remained increased for at least 30 min after the DPs, when experiments were usually terminated (Fig. 5D, pooled data from all experiments). In all these experiments, the increase in the frequency of sGABA$_A$-PSCs was associated with an increased proportion of large events and the appearance of larger ones. Although it did not reach significance ($P = 0.29$; paired t-test), the average amplitude of sGABA$_A$-PSC increased from 28.9 ± 4.1 to 31.5 ± 7.7 pA ($n = 18$).

Thus, a more physiological conditioning protocol is also able to induce a significant increase of spontaneous GABAergic synaptic activity onto hippocampal cells.

Repetitive DPs could trigger maturation of P0–1 silent cells

Among the 27 neurons recorded at P0–1 in the present study, five of them were synaptically silent. Cells were defined as silent when there was no synaptic activity recorded during a 15-min control period. In the experiments previously described, we have included only those cells that exhibited a spontaneous GABAergic activity. However, we also applied the conditioning protocol to the 'silent cells'. Among these five cells, in which DPs induced a Ca^{2+} or a spike current, the conditioning protocol led to the appearance of sGABA$_A$-PSCs in two cases. One of the two cells was recorded with a CsCl-filled electrode, in the presence of 10 μM CNQX and 50 μM DL-APV. The other (Fig. 6) was recorded with a K-gluconate-filled electrode in the absence of the glutamate receptor antagonists. In this cell, there was not any detectable sGABA$_A$-PSC before the conditioning protocol (Fig. 6, left); thereafter, sGABA$_A$-PSCs of variable amplitude appeared (Fig. 6, right). The mean IEI and amplitude of the sGABA$_A$-PSCs after the DPs were, respectively, 8.67 ± 5.6 s ($n = 137$) and 32.3 ± 2.2 pA ($n = 119$).

Discussion

In the present study we attempted to correlate the long-term changes in GABAergic synaptic activity with the functional maturation of GABAergic synapses. We therefore examined, in the neonatal rat hippocampus, the age-dependency of synaptic plasticity and the relevant conditioning protocol leading to changes in the GABAergic synaptic activity. We found that long-lasting changes in the frequency and amplitude of sGABA$_A$-PSCs were inducible, by a conditioning protocol relevant to the physiological conditions, during an age window restricted to the first postnatal week of life, one that closely matches the period of functional maturation.

DPs induced an increase of the GABAergic synaptic drive during the first postnatal week of life

Although the long-term changes in GABAergic synaptic activity induced by DPs were restricted to the early postnatal period, we found that DPs could lead to either an increase or a decrease in the frequency of sGABA$_A$-PSCs. At early stages of development, pyramidal neurons are extremely heterogeneous. For instance, at birth some have no functional synapses, others have only functional GABAergic synapses, and a last group has both GABAergic and glutamatergic functional synapses (Tyzio *et al*., 1999). At later stages, the morpho-functional heterogeneity is less pronounced, so pyramidal cells constitute a more homogenous population. We therefore suggest that the observed heterogeneity in the polarity of DP-induced changes of sGABA$_A$-PSC frequency is somehow related to the heterogeneity in the maturational stage of developing pyramidal cells. Postsynaptic Ca^{2+} influx through the activation of voltage-dependent calcium channels (VDCCs) has been demonstrated to be necessary for the induction of DP-induced plasticity at GABAergic synapses (Caillard *et al*., 1999a). Moreover, the polarity of the change in GABAergic synaptic strength appears to be correlated to the amount of spike-driven Ca^{2+} influx in the rat cerebellum (Aizenman *et al*., 1998). Therefore, the maturational stage of the cells could influence the polarity of the changes in sGABA$_A$-PSC frequency through, for instance, differences in either the density or the type of VDCCs as well as differences in Ca^{2+} buffering capability. However, it should be emphasized that the decrease in the frequency of sGABA$_A$-PSCs is compensated for by the increase in their amplitude, as shown by the augmentation of the total charge transferred per time unit. Therefore, even when a decrease in the frequency of sGABA$_A$-PSCs was induced, the net effect of DPs was an increase in the GABAergic drive during the first postnatal week of life.

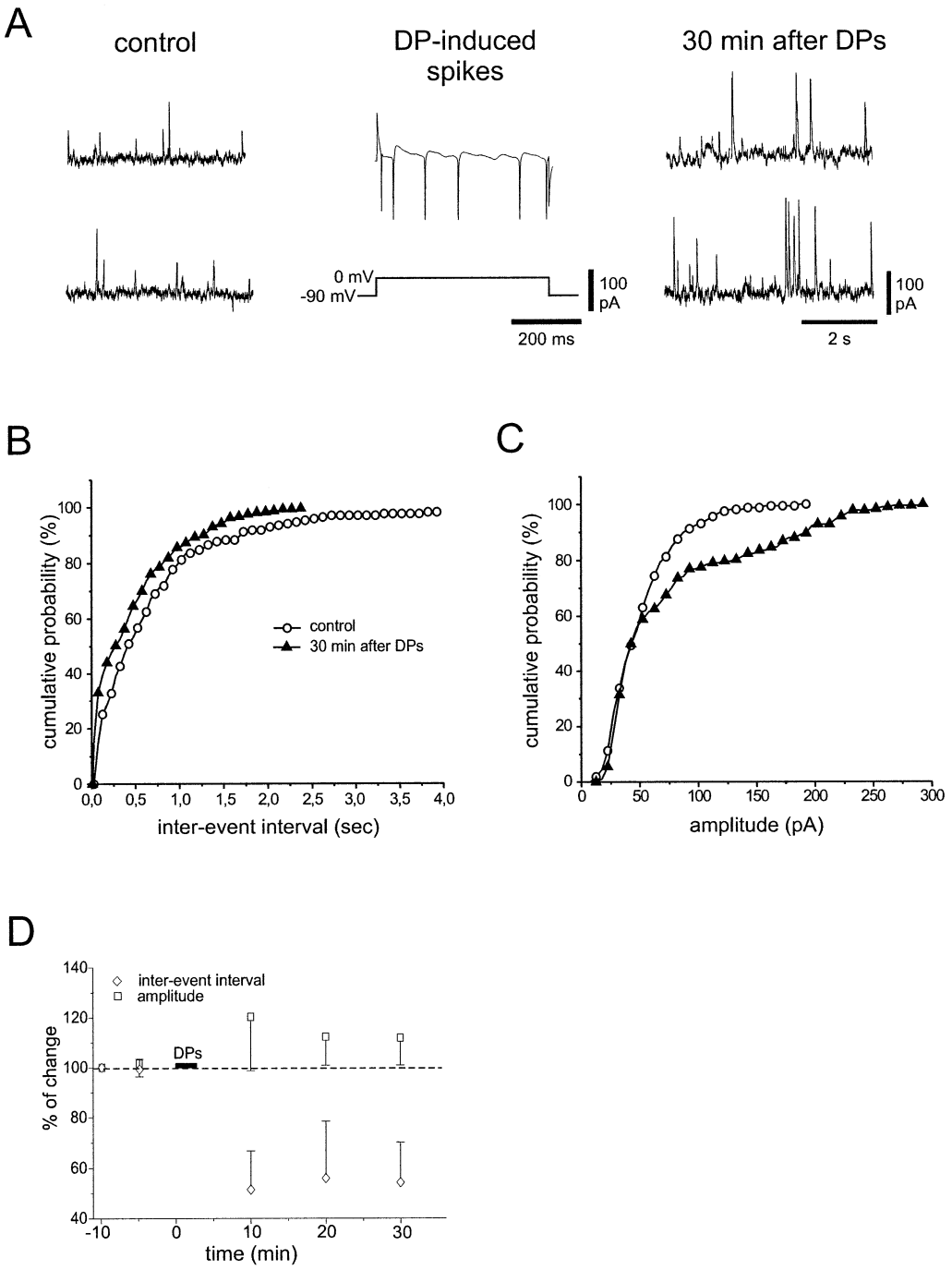

FIG. 5. DP-induced long-lasting changes in sGABA$_A$-PSC amplitude and frequency under more physiological conditions. (A) Traces show sGABA$_A$-PSCs (holding potential –40 mV) recorded (left) before and (right) 30 min after the DPs (centre shows a sample DP). Recordings were performed at P7 with a K-gluconate electrode in the absence of both CNQX and DL-APV. (B) Cumulative probability plot of the sGABA$_A$-PSC IEI (open circles) before and (filled triangles) 30 min after the DPs. (C) Cumulative probability plot of the sGABA$_A$-PSC amplitude (open circles) before and (filled triangles) 30 min after the DPs. Traces in A and graphs in B and C are from the same cell. (D) The potentiation of GABA$_A$-PSC IEI and amplitude is long-lasting (pooled data from 18 cells recorded between P6 and P8).

Mechanisms underlying the DP-induced changes in GABAergic synaptic activity

The questions that remain to be elucidated concern the mechanisms underlying the DP-induced long-term changes in frequency of sGABA$_A$-PSCs and the appearance of large amplitude events. We have previously shown (Caillard *et al*., 1999a) that DPs lead to an increase in the frequency of miniature GABA$_A$-PSCs between P3 and P6, with no significant change in their mean amplitude, ruling out an increase in the number or efficacy of postsynaptic GABA$_A$ receptors at functional synapses as possible mechanisms underlying the DP-induced GABAergic plasticity in neonates. The results presented in the present study provide further evidences supporting this hypothesis. Whilst a uniform postsynaptic change would have led to a

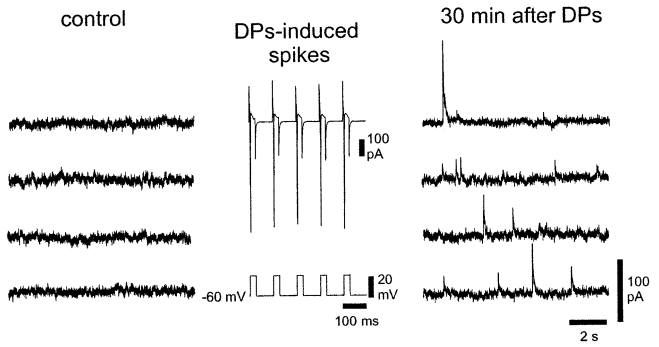

FIG. 6. sGABA$_A$-PSCs appeared in a P1 'silent cell' after the DPs. Traces show electrophysiological recordings performed at P1 (holding potential +10 mV), (left) before and (right) 30 min after the DPs bursts (5 DPs, 30 ms each, at 7 Hz) repeated 20 times at 0.1 Hz at a holding potential of –60 mV (each DP evoked a spike current. A sample is given in the centre.) Recordings were performed in the absence of both CNQX and DL-APV, with a K-gluconate-filled electrode. Before the application of DPs no sGABA$_A$-PSCs were recorded. After DPs, sGABA$_A$-PSCs were recorded.

parallel shift of the cumulative amplitude histogram of sGABA$_A$-PSCs, we found that DPs always resulted in an increase or in the appearance of large-amplitude events. An increase in the probability of GABA release following DPs can explain both the increase in the frequency of sGABA$_A$-PSCs and, by temporal summation, the increase in the number of large-amplitude events. Alternatively, DPs may transform previously silent GABAergic synapses into functional ones and these new functional synapses may be responsible for the large amplitude events. The functional appearance of new synapses could have occurred for example by means of the activation of previously inactive synaptic terminals (Charpier *et al*., 1995; Tong *et al*., 1996) and/or postsynaptic receptors clustering (Isaac *et al*., 1995; Durand *et al*., 1996; Tong *et al*., 1996). Alternatively, the DP conditioning protocol may have changed the excitability of GABAergic cells, leading to the recruitment of previously inactive GABAergic cells. In support of the functional appearance of new synapses, we found that DPs were able to induce the appearance of sGABA$_A$-PSCs in synaptically silent cells at P0–1. How can DPs lead to a concomitant decrease in sGABA$_A$-PSC frequency and increase in the number of large amplitude events is more complicated to envisage. The elucidation of the mechanisms underlying the DP-induced differential effects on frequency and amplitude of sGABA$_A$-PSCs, and the origin of large-amplitude sGABA$_A$-PSCs await further studies.

Physiological relevance of DP-induced GABAergic synaptic plasticity

The functional maturation of the GABAergic synaptic transmission onto hippocampal pyramidal neurons takes place during the early postnatal period (Ben-Ari *et al*., 1997; Dupuy & Houser, 1996; Tyzio *et al*., 1999). During this time, there is also an important maturation of the excitatory glutamatergic synaptic transmission (reviewed in Ben-Ari *et al*., 1997). Therefore, the strength and/or the number of GABAergic synaptic contacts also increases in order to balance and adjust the increase in the glutamatergic drive. This is demonstrated by the progressive increase in the frequency of sGABA$_A$-PSCs during postnatal development. We found that DP-induced increases in GABAergic synaptic activity are restricted to the early postnatal period, as reported for the cortex (Komatsu, 1994) and the lateral superior olive (Kotak & Sanes, 2000). By the end of the second postnatal week of life, when the frequency of sGABA$_A$-PSCs has reached a plateau value, the DP conditioning protocol failed to induce any significant long-lasting change in their frequency or amplitude. Thus, the critical period for functional maturation of GABAergic synaptic transmission and the susceptibility to express DP-induced GABAergic synaptic plasticity seem to correlate. This observation emphasizes a possible role of this form of plasticity in the functional maturation of the GABAergic synaptic transmission.

In an earlier study of our group (McLean *et al*., 1996), we reported that neonatal GABAergic synapses express a bi-directional plasticity after tetanic stimuli that is also restricted to the first postnatal week of life. However, this type of stimulation never occurs *in vivo*. Therefore, in addition to the temporal correlation, the relevance of the conditioning protocol has to be taken into account to establish a causal link between synaptic modifications and functional maturation. The discovery that back-propagating action potentials could serve as an associative signal to induce LTP or LTD at glutamatergic synapses (Magee & Johnston, 1997; Markram *et al*., 1997) has provided evidence that physiological stimuli could lead to synaptic modifications. In the present study, we have shown that postsynaptic spike bursting was sufficient to increase the GABAergic synaptic drive onto hippocampal cells. We also have shown that, to be efficient, the DPs must be applied at a frequency of 0.1 Hz. In spinal neurons, the expression of specific phenotypes is also keyed to the frequency at which Ca^{2+} transients were evoked (Gu & Spitzer, 1995). Thus, Ca^{2+} spikes are effective in promoting the maturation of I_{kv} when applied at a frequency of 2 per hour, but not at a frequency of 1 per hour. The induction of LTP at glycinergic connections onto Mauthner cells is also tightly linked to the frequency at which the auditory stimulation was delivered, being maximal at 200–800 Hz and not effective at 80–110 Hz or 1–3 kHz (Oda *et al*., 1998). It is important to stress that, in these studies, the frequencies of stimulation effective in achieving maturation or synaptic plasticity are similar to those of endogenous Ca^{2+} spikes or sensory stimuli occurring naturally. The DP conditioning protocol used in the present study is also related to the sort of activity that neonatal CA3 pyramidal neurons may experience *in vivo*. Thus, clusters of action potentials can occur as a result of endogenous bursting behaviour of CA3 pyramidal cells (Hablitz & Johnston, 1981) or from the presence of network driven activity present at early stage of postnatal development (Ben-Ari *et al*., 1989). We have further shown that the DP conditioning protocol can lead to the appearance of spontaneous GABAergic synaptic activity in synaptically silent cells. In other words, the conditioning protocol mimics or accelerates the functional maturation of the GABAergic synaptic transmission that occurs *in vivo* (Tyzio *et al*., 1999).

Several lines of evidence have pointed to the link between long-term changes in synaptic efficacy and the refinement and functional maturation of excitatory connections (Crair & Malenka, 1995; Kirkwood *et al*., 1995). We propose to further extend this link to GABAergic synaptic transmission in the neonatal hippocampus, according to the observation that long-lasting changes in GABAergic synaptic activity are induced during a restricted period of development, one that closely matches the period of functional maturation, and by a conditioning protocol relevant to the physiological conditions.

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Abbreviations

ACSF, artificial cerebrospinal fluid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; DL-APV, DL-2-amino-5-phosphovaleric acid; DPs, depolarizing pulses; GABA, γ-aminobutyric acid; IEI, inter-event interval; LTD, long-term depression; LTP, long-term potentiation; P, postnatal day; R_m, membrane resistance; R_s, series resistance; $sGABA_A$-PSCs, spontaneous $GABA_A$ receptor-mediated postsynaptic currents; TS, tetanic stimulation.

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