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Developmental patterns and plasticities: the hippocampal model

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Abstract

Because developmental activity-dependent synaptic plasticity has been hypothesized to participate in network refinement, leading to the precise mapping of synaptic contacts constituting a functional brain, it is important to investigate the spatio-temporal structure of immature network activities. This article is briefly reviewing 15 years of studies on the immature rat hippocampus which, together with recent results obtained from awake rat pups, represent an important step toward the understanding of spontaneous patterns of activity and their potential implication in network maturation. Due to synergistic excitatory actions of GABA and glutamate receptor mediated signals during early postnatal life, spontaneous patterns of hippocampal activity that have been characterized both in vitro and in vivo are likely to provide hebbian modulation of developing glutamatergic and GABAergic synapses. Together with studies on trophic actions of these transmitters, study of the immature hippocampal network patterns and plasticities allows for multiple technical and conceptual approaches and represents an interesting experimental model for development studies.

Keywords: Hippocampus; Immature; Network; Oscillations; Plasticity

1. Introduction

The hippocampus is a popular brain structure among neurobiologists, and is probably the most studied structure for neuronal plasticity. Indeed, it has a number of major advantages: (i) the cellular organisation with an identified tri-synaptic pathway is relatively simple; (ii) the efficacy of several identified synaptic pathways can be modified by hebbian stimulation, associating pre- and postsynaptic discharge; (iii) it is potentially involved in memory formation and in identified pathologies such as epilepsies. In the adult rat hippocampus, as well as in other brain structures and species including humans, a whole range of synchronous oscillations is expressed in association with specific behaviours (for review: [10,51]). Theta (5–10 Hz) and superimposed gamma (30–80 Hz) oscillations can be expressed continuously for tens of seconds during motion and REM sleep. Faster (150–200 Hz) "ripple" oscillations lasting 100–200 ms have an irregular but frequent occurrence during immobility and slow wave sleep. A striking effect of such background synchronous oscillations is to organize neuronal discharge and lock it to a definite window of time (phase-locking). Neurons that will tend to discharge collectively within a few milliseconds can efficiently cooperate to discharge their target cells and thereby reach hebbian conditions of synchronous pre- and postsynaptic firing, which has been described as a favourable condition for induction of synaptic plasticity. Therefore, even though the exact roles of these collective patterns remain to be clarified, one major hypothesis is that the sculpting of neuronal discharge, which may be involved in information coding ("binding" problem, spatial coding), may also play a decisive role in information storage via synaptic plasticity [10,51].

More recently, it has been suggested that the rules governing adult synaptic plasticity also apply for immature synaptic connexions and represent an important step in the maturation of neuronal networks [23,28,53]. Spontaneous patterns of synchronized activities would help determine precise mapping of synaptic contacts, following the rule that the connexions between cells that fire together would be reinforced, consolidated, while the contacts between cells that would be out of synchrony would be depressed or even eliminated [40]. In fact, synchronized patterns of activity were proposed to be a fundamental feature of developing neuronal networks, providing hebbian conditions for the modulation

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of developing synapses and building of functional networks. However it remains to be clarified for most brain structures what are the spontaneous patterns expressed at the early stages of development, in order to know whether hebbian conditions occur spontaneously in developing networks and what are the cells that actually fire together. This article is going to briefly review 15 years of studies on the immature rat hippocampus which, together with recent results obtained from awake rat pups, represent an important step toward the understanding of spontaneous patterns of activity and their potential implication in network maturation, and represent an interesting experimental model for development studies.

2. Specific properties of immature synapses

The properties of immature synapses can be very different from those of adult ones (cf. Fig. 1). In this respect, a major finding was that GABA, the main mediator of inhibition in the adult brain, operates as an excitatory neurotransmitter at the embryonic stage and early postnatal life (for review: [5,6,12]). Observed 14 years ago in the hippocampal formation [4], excitatory effects of GABA have since been shown in developing neurons from various brain structures [11,41,52,56,57, 63,66]. $GABA_A$ receptors activation in immature neurons produces depolarization instead of hyperpolarization, due to an elevated intracellular concentration of

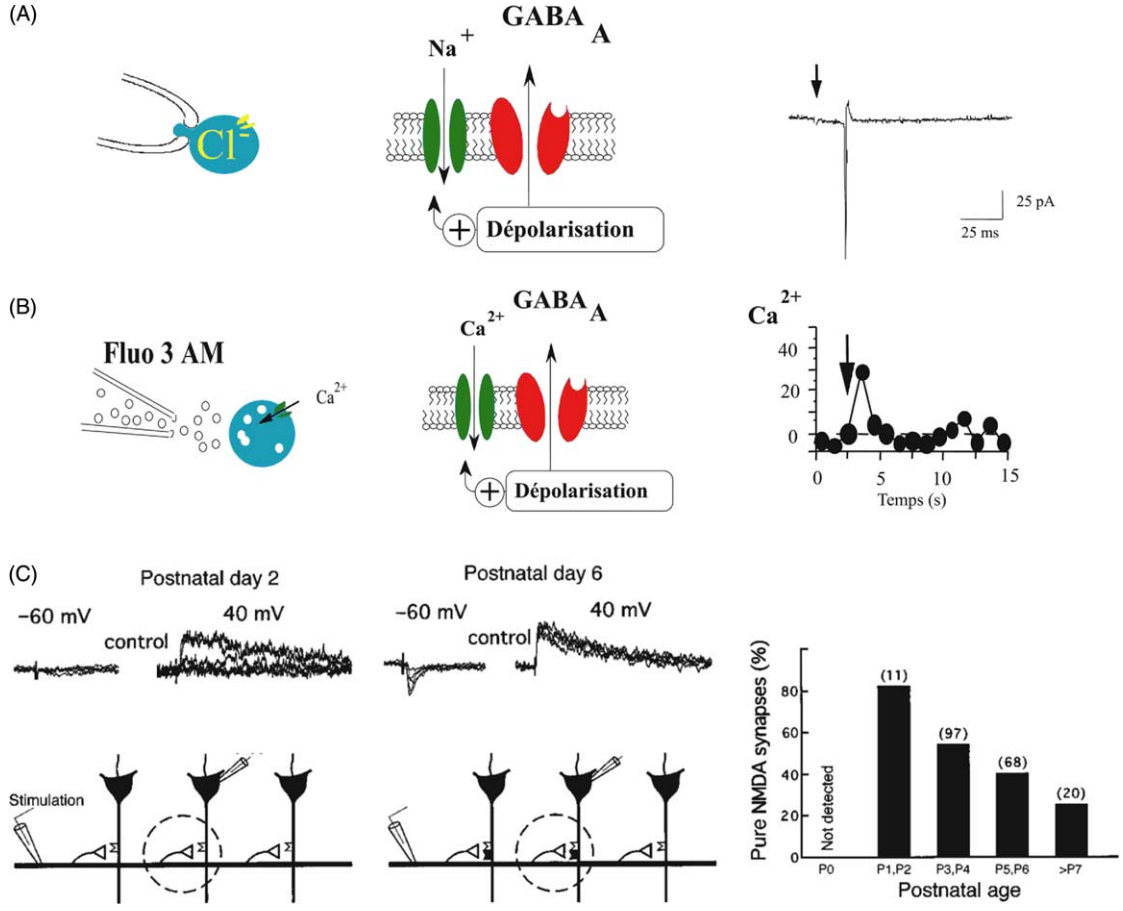

Fig. 1. Specific properties of immature GABAergic and glutamatergic synapses. In the neonatal rat hippocampus (age P0–P5), immature GABAergic synapses exert a depolarizing effect due to reversed Cl gradient. (A) In patch-clamp cell-attach recordings from CA3 pyramidal cells in hippocampal slices, a condition that preserves the Cl gradient, electrical stimulation of GABAergic afferents (arrow) in presence of glutamatergic antagonists (APV + CNQX) activates voltage dependant sodium channels, triggering an action potential in the postsynaptic cell (reproduced with permission from Ref. [35]). (B) Using confocal microscopy on hippocampal slices with the calcium sensitive die Fluo 3 AM, intracellular Ca^{2+} changes were monitored from cells loaded extracellularly, thus preserving Cl gradient. Synaptic stimulation of $GABA_A$ receptors (arrow), in presence of the glutamatergic antagonists APV and CNQX, activates voltage dependant Ca^{2+} channels, triggering an increase in intracellular Ca^{2+} (reproduced with permission from Ref. [33]). (C) Measuring patch clamp whole-cell responses to minimal afferent stimulation in presence of GABAergic antagonists allows to estimate the relative contributions of AMPA and NMDA receptors to glutamatergic synaptic transmission. At P0-2, the high failure rates of synaptic responses at hyperpolarized potentials (−60 mV) but not at depolarized potentials (40 mV) suggests the presence of glutamatergic synapses transmitting via NMDA but not AMPA receptors. The proportion of such pure NMDA synapses (plot) strongly decreases during the first postnatal week (reproduced with permission from Ref. [15]).

chloride that keeps a depolarized reversal potential for $GABA_A$ receptor mediated responses [42,56,57,67]. Originally observed in acute hippocampal slices using electrophysiological recordings [4], developmental (young > adult) and spatial (dendrites > soma) gradients of chloride concentration have since been observed directly using chloride-sensitive fluorescent dyes in dendrites and soma of hippocampal cultured cells [25,26]. The reason for the elevated intracellular chloride concentration in immature neurons is likely the delayed maturation of chloride homeostasis systems [25,26,67]. Emphasis has lately been put on delayed expression of the Cl extruders KCC2 [20,55] and ClC2 [61], and early over-expression of the Cl accumulating NKCC, a Na–K–Cl cotransporter [45].

Depolarizing effects of GABA in neonatal neurons determine very different interactions, as compared to adult, between $GABA_A$ and other voltage-gated and receptor operated ionic channels. Importantly, this depolarization is above the threshold of the sodium action potential generation, the reversal potential for postsynaptic $GABA_A$ signals shifting from −50 mV in most neurons at P0-3 to −65 mV in most neurons at P12 [4]. Consequently, synaptic $GABA_A$ receptor mediated currents can trigger action potentials in neonatal neurons. This observation was originally made using intracellular microelectrode recordings [4]. Disadvantage of this approach is that any intracellular recording is invasive and therefore can modify $[Cl^-]_i$ because of cell dialysis. Also, these results have since been confirmed by studies that utilized approaches that do not modify $[Cl^-]_i$, such as use of voltage sensitive dyes [17], patch-clamp technique in the cell-attached mode [31,35,57] (cf. Fig. 1A) or perforated patch clamp with the chloride-impermeable ionophore gramicidin [11,16,20,41,52,56]. Voltage-gated calcium channels is another type of voltage-gated channels activated by GABA in the immature neurons. Using calcium imaging techniques, it has been demonstrated that $GABA_A$ receptor agonists induce Ca^{2+} influx through voltage-gated calcium channels (including high threshold type) in a number of immature preparations [11,14,20,33,39,41,50,52,66]. Importantly, synaptically released GABA can also increase $[Ca^{2+}]_i$ in neonatal hippocampal neurons via activation of voltage-gated Ca^{2+} channels [33] (cf. Fig. 1B).

$GABA_B$ receptor mediated inhibition also undergoes significant developmental changes. Postsynaptic $GABA_B$ receptor mediated synaptic responses are not expressed in neonatal granular cells and CA3 pyramidal neurons and were shown to appear at about the end of the first postnatal week [18,19]. Lack of postsynaptic $GABA_B$ inhibition is due to the absence of functional postsynaptic $GABA_B$ receptors since application of the agonist of $GABA_B$ receptors baclofen fails to produce postsynatic response in neonatal neurons. Interestingly, other G-protein mediated postsynaptic responses have also delayed maturation in the neonatal hippocampus [19]. In contrast, $GABA_B$ receptors localized on the presynaptic GABAergic and glutamatergic terminals are already functional at birth [19] and can provide an important inhibitory control of the neonatal hippocampal network [47].

Importantly, glutamatergic synaptic transmission also undergoes significant changes at its earliest stages of development (cf. Fig. 1C). In the adult, glutamatergic synaptic transmission typically involves both AMPA and NMDA types of ionotropic receptors. In the immature brain, glutamatergic synaptic responses seem to be devoid of AMPA receptor mediated component [15,37,64]. Since NMDA receptors are largely blocked at resting potential due to voltage dependent Mg^{2+} block, which is as efficient in immature as in adult neurons [30,62], these immature glutamatergic synapses are silent at resting potential. However, the glutamatergic response mediated by NMDA receptors can be revealed at depolarized potentials [15,64]. Therefore, glutamatergic synaptic signals are first purely NMDA receptor mediated, due to either absence of AMPA receptors from the synapse [38,58] or insufficient transmitter release at immature glutamatergic synapses [13,22,54]. Transformation of these synapses into the classical AMPA/NMDA receptors functional synapses occurs during the first week after birth.

Since network activity is largely determined by synapses properties, the patterns of activity expressed at these early stages of development may be quite different from those expressed in the adult brain.

3. Hippocampal in vivo patterns

In order to know what kind of activity was expressed in the brains of immature rats, we have recently performed electrophysiological recordings from 4 to 6 day-old animals in vivo (cf. Fig. 2, [36]). In order to identify collective field patterns as well as unitary activities, we used bundles of 8 extracellular electrodes with tips positioned at various depths, above and below the CA1 pyramidal layer (1800–2000 μm below brain surface), allowing to record electrophysiological activity from the CA1 region in freely moving rat pups. We observed that at P4-6, neuronal firing was mostly distributed among multi-unit bursts of 0.5–3 s duration (cf. Fig. 2A), which recurred at preferred frequencies of 0.3 and 0.1 Hz and were often accompanied by large field deflections (cf. Fig. 2B). Between these collective events, hippocampal EEG was largely flat and neuronal firing almost absent, in strike contrast with the situation in adults. In fact, adult patterns such as ripples and theta oscillations appear progressively during the second postnatal week, while the long multi-unit bursts disappear around P10.

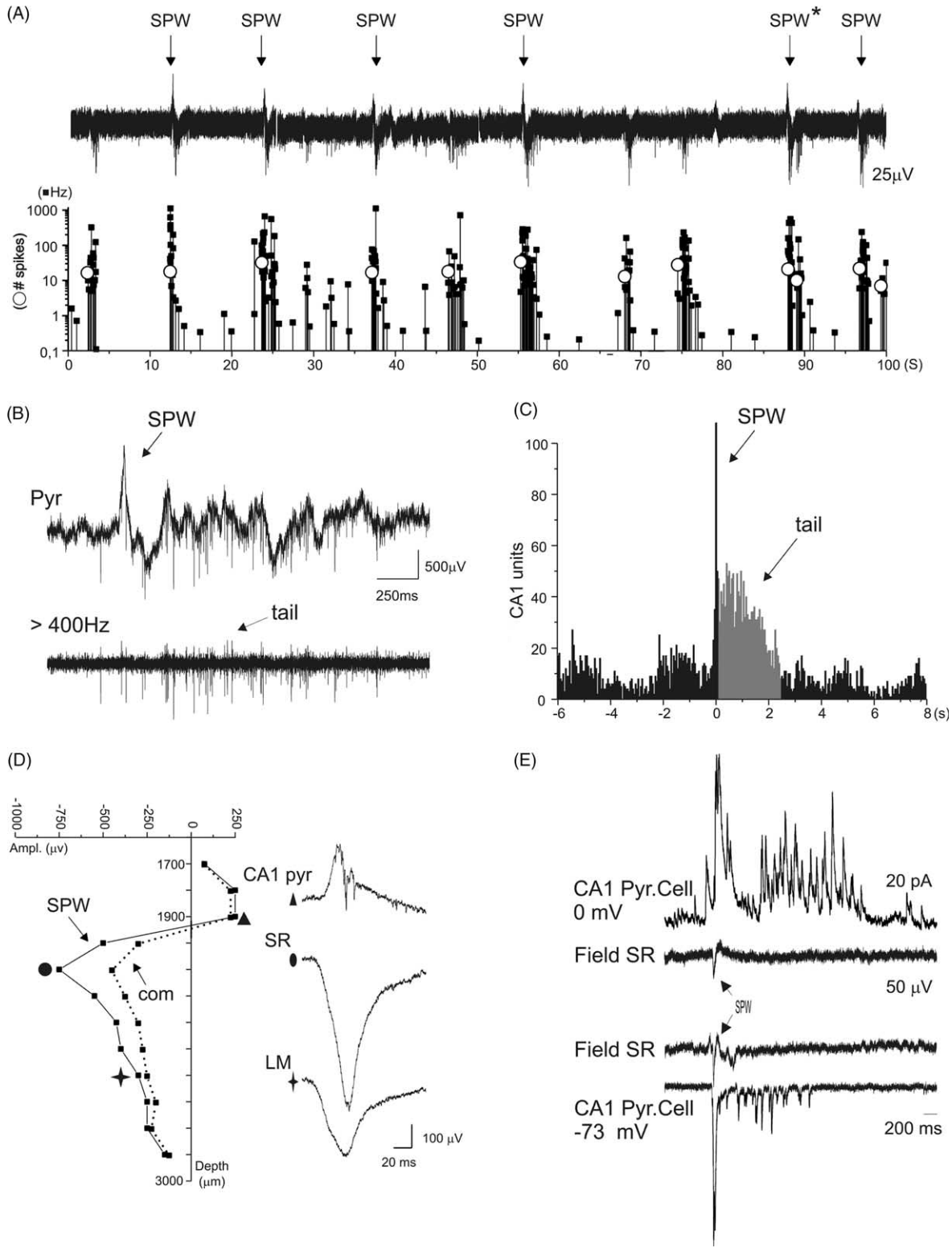

Fig. 2. Correlated bursts of activity in the developing hippocampus in vivo. In the neonatal rat hippocampus (age P4-6), most of neuronal activity concentrates within recurrent multi-unit bursts often associated to population field sharp waves (SPWs) and driven by GABAergic and glutamatergic synapses (reproduced with permission from Ref. [36]). (A) Filtered trace (>200 Hz) of multi-unit activity in the CA1 pyramidal layer of a non anesthetized rat pup. Below, corresponding multiple unit activity over time. Each vertical line terminating by a black square represents the firing of 1 spike (height proportional to the instantaneous frequency of spikes). Vertical lines terminating by a white circle correspond to multi-unit bursts (heights proportional to the number of spikes in the burst). (B) SPW-burst under urethane anesthesia. (C) Cross correlogram of CA1 multi-unit activity and simultaneously recorded field SPWs (reference). (D) Amplitude versus depth profile of SPWs using 16 sites silicon probe. (E) GABAergic (0 mV) and glutamatergic (−73 mV) synaptic correlates of hippocampal SPW-bursts (field SR, arrows).

The next interesting observation was that a similar pattern of activity was observed under urethane anesthesia, although the bursts were a bit less frequent. This allowed us to perform more sophisticated recordings and further characterize the synaptic events underlying hippocampal bursts. Multi-site extracellular recordings using a silicon probe with 16 recording sites (100 μm vertical separation), inserted vertically into the CA1—dentate gyrus axis to record from various layers of the hippocampus, revealed that the amplitude versus depth distribution of the large field deflections associated to multi-unit bursts had a negative pic in stratum radiatum and reversed polarity in the CA1 pyramidal layer (cf. Fig. 2D). This profile is similar to that of adult sharp wave bursts (SPW), that occur spontaneously in the rat hippocampus during immobility and slow wave sleep [65]. It is also similar to the CA1 field responses evoked by electrical stimulation of the Schaffer collaterals, suggesting that the large deflections that are often associated to CA1 immature bursts originate in CA3 and are conveyed to CA1 through the activation of Schaffer collaterals.

Taking advantage of the stability offered by the anesthetized preparation, we also performed patch clamp whole-cell recordings from CA1 neurons. Using a low chloride intracellular solution ($E_{Cl} = -70$ mV), $GABA_A$ receptor mediated postsynaptic currents (PSCs) had a reversal potential around −70 mV while glutamatergic PSCs reversed around 0 mV. Therefore, in the voltage clamp mode, we could identify $GABA_A$ PSCs at glutamate reversal potential (0 mV), and glutamatergic PSCs at $GABA_A$ reversal potential (−70 mV), and observe that the large synaptic currents underlying neonatal hippocampal SPW-bursts included both glutamatergic and GABAergic components (cf. Fig. 2E).

Therefore, we suggest that hippocampal SPW-bursts that occur in awake and sleeping rat pups as well as in urethane anesthetized animals are the main hippocampal field pattern during the first postnatal days. These events are driven by synaptically activated $GABA_A$ and glutamate receptors. Because CA3 neurons provide the main afferent pathway to the CA1 region, the high correlation between CA1 multi-unit activity and SPWs in neonates suggests that SPW-bursts provide synchronized pre- and postsynaptic firing, a condition that may favour hebbian modification of developing Schaffer collateral synapses. Since neurons rarely discharged between bursts at this age, these synchronous events represent the major source of correlated neuronal activity for both glutamatergic and GABAergic synapses in the neonatal hippocampus.

4. In vitro giant depolarizing potentials and GABA–NMDA synergy

The activity we have observed in vivo displays striking similarities with previously described immature hippocampal pattern in vitro. Therefore, a number of observations made during the last 15 years in in vitro preparations are likely to apply to the in vivo situation.

During the first postnatal week, hippocampal spontaneous activity in vitro is characterized by periodical neuronal discharges, so-called giant depolarizing potentials (GDPs), which expression coincides with the temporal window when GABA exerts an excitatory action via $GABA_A$ receptors [4–6,21,31,35,49] while postsynaptic $GABA_B$ receptor mediated inhibition is hardly functional [19]. Network discharge during GDPs is however limited by presynaptic inhibition via $GABA_B$ receptors, because blockade of $GABA_B$ receptors changes GDPs into ictal-like events [47].

Simultaneous electrophysiological recordings and Ca^{2+} imaging using confocal microscopy in the hippocampal slices of neonatal rats revealed that spontaneous GDPs were driving synchronous augmentations of intracellular Ca^{2+} [21,35], which were mainly due to Ca^{2+} influx via voltage-dependent Ca^{2+} channels on the level of soma and dendrites (cf. Fig. 3A). Ca^{2+} increases at discrete dendritic spots in the conditions preventing activation of voltage-gated calcium channels were also observed during GDPs [34], suggesting that Ca^{2+} can also enter into the cell through NMDA channels during GDPs, at the sites of glutamatergic synapses.

In previous studies, GDPs recorded from pyramidal cells were thought to be entirely $GABA_A$ receptor mediated [4]. Since GDPs were blocked by glutamate receptors antagonists, the hypothesis had been made that they resulted from the synchronous discharge of GABAergic interneurons, following their recurrent excitation by pyramidal cells. Indeed, dual recordings showed that stratum radiatum CA3 interneurons fire bursts of action potentials during GDPs simultaneously recorded from pyramidal cells [31]. However, the use of novel approaches including $[Ca^{2+}]_i$ monitoring by confocal microscopy and patch clamp allowed new insights into the mechanisms of GDPs generation, and an important role in the excitation of pyramidal cells and interneurons has been attributed to synergistic excitatory actions of $GABA_A$ and glutamate (preferentially NMDA) receptors (cf. Fig. 3).

In addition to voltage-gated conductances, depolarizing GABA has been demonstrated to potentiate the activity of NMDA receptors. Since the voltage dependent Mg^{2+} block of NMDA channels operates not only in adult but also in neonatal neurons [30,62], their activation during synaptic activity requires external sources of depolarization. In adult neurons, this depolarization is largely provided by glutamate acting on AMPA receptors. In contrast, GABA, which hyperpolarizes adult neurons, prevents the activation of NMDA receptors [27]. An opposite situation prevails in neonatal neurons in which GABA has depolarizing action. In cell-attached recordings, activation of $GABA_A$ receptors

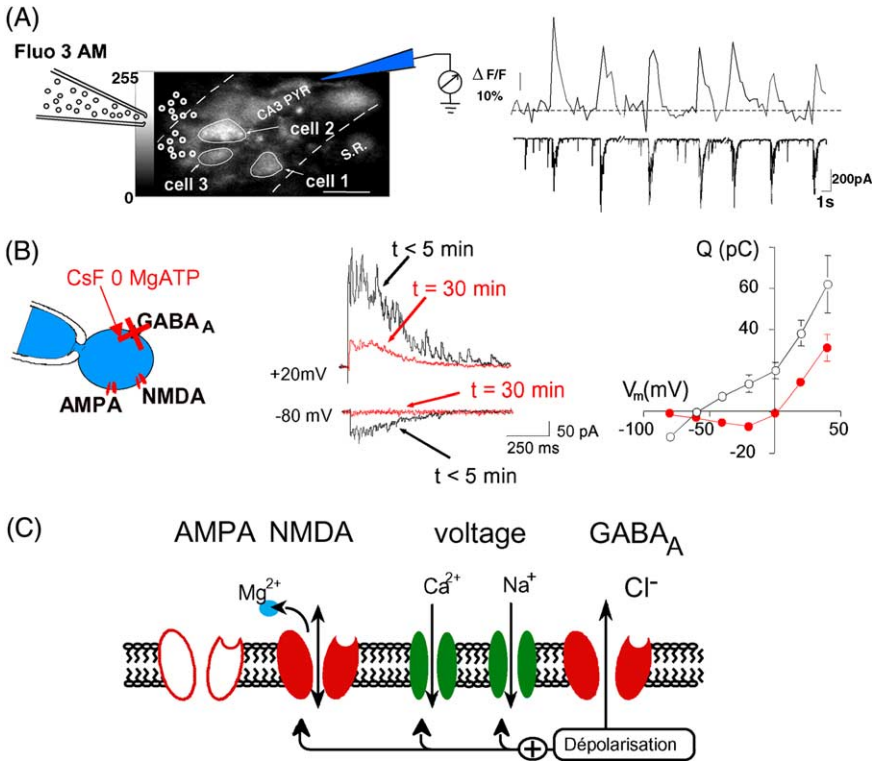

Fig. 3. Hebbian conditions during the neonatal hippocampal pattern. In neonatal hippocampal slices, spontaneously expressed giant depolarizing potentials (GDPs) provide increases in Ca^{2+} by the synergistic excitation mediated by $GABA_A$ and NMDA receptors. (A) Simultaneous patch clamp (whole-cell) recording and Ca^{2+}-dependant fluorescence measurements using confocal microscopy from extracellularly loaded neurons in the CA3 region. Spontaneously occurring recurrent GDPs are associated to postsynaptic Ca^{2+} increase. (B) Whole-cell recordings with an intracellular solution containing Cs–F and no Mg-ATP causes run-down of $GABA_A$-mediated responses, thus revealing the NMDA component of GDPs ($t < 5$ min: empty circles; $t = 30$ min: filled circles). (C) In the neonatal hippocampus, at a time when AMPA receptors hardly contribute to glutamatergic synaptic transmission, $GABA_A$ and NMDA receptors act in synergy. Their co-activation during GDPs provides spontaneous hebbian stimulation of developing synapses (reproduced with permission from Ref. [35]).

in neonatal hippocampal neurons potentiated the activity of single NMDA channels via depolarization and a resulting reduction of voltage-dependent Mg^{2+}-block. Activation of $GABA_A$ receptors also potentiated the influx of Ca^{2+} via NMDA channels from cells loaded by extracellular application of the Ca^{2+} sensitive dye Fluo3-AM and recorded using confocal microscopy [35]. Thus, in contrast to adult, where hyperpolarizing $GABA_A$ responses inhibit the activity of NMDA receptors by strengthening their voltage-dependent Mg^{2+}-block, $GABA_A$ receptor mediated depolarization in neonatal neurons removes the Mg^{2+}-block of NMDA channels and potentiates their activities.

A major issue concerning the expression of $GABA_A$–NMDA synergy however was to show that $GABA_A$ and NMDA receptors are co-activated during spontaneous hippocampal activity. Because the large increase in conductance produced by the GABAergic component of GDPs may have masked an NMDA component in early intracellular recordings [4] due to shunting mechanisms [60], intracellular blockade of $GABA_A$ receptors by whole-cell dialysis with an internal solution containing Fluoride and no Mg-ATP [9,29,31,35] was used to investigate for activation of glutamatergic receptors during GDPs. This approach revealed that in both pyramidal cells and interneurons, GDPs are generated by both $GABA_A$ and glutamate receptors (cf. Fig. 3B). Neuronal excitation during GDPs may thus result from the cooperation between two types of synaptic connexions: (i) excitatory GABAergic synapses from interneurons and (ii) glutamatergic inputs that presumably originate from pyramidal cells. Therefore at a time when synaptic transmission via AMPA receptors is relatively quiescent, GABAergic innervation provides an important contribution to the excitation of the immature neurons and to the activation of NMDA receptors (cf. Fig. 3C).

Although the field SPWs that often preceded hippocampal bursts in vivo are not expressed in the in vitro preparation, and the glutamatergic component of in vivo bursts is more pronounced than during in vitro GDPs, the duration, the relatively rhythmic recurrence of the burst events and the associated $GABA_A$ receptor mediated synaptic currents suggest that these events are the in vivo counterparts of GDPs described in vitro. Because GDPs and SPW-bursts have been shown to pro-

vide spontaneous hebbian stimulation of developing synapses, these spontaneous patterns of activity may be involved in the activity-dependant maturation of the hippocampal network.

5. Activity-dependent maturation of the hippocampal network: developmental synaptic plasticities and trophic effects of neurotransmitters

Long term potentiation (LTP) and long term depression (LTD) are the classical models to study activity-dependent synaptic plasticity at excitatory glutamatergic synapses. Electrical stimulation, or other paradigms that remove the voltage dependent Mg^{2+} block from NMDA channels and increase intracellular calcium concentration ($[Ca^{2+}]_i$), generate long term changes in synaptic efficacy that are expressed by AMPA receptors [7]. As mentioned above, glutamatergic synapses switch during maturation from AMPA-silent (pure NMDA) synapses into functional AMPA + NMDA synapses. Interestingly, this process has been shown to be triggered by presynaptic firing together with postsynaptic depolarization, in an NMDA dependent manner ([15] cf. Fig. 4). Therefore, GDPs or neonatal SPW-bursts, that spontaneously provide such hebbian conditions with localized Ca^{2+} influx through NMDA receptors due to GABA–NMDA synergistic excitation, are likely to play a crucial role in the control of the maturation of glutamatergic synapses by synaptic plasticity.

It has moreover appeared that not only glutamatergic synapses but also GABAergic synapses are subject to long term changes in which activation of pre- and/or postsynaptic neurons and subsequent rise in $[Ca^{2+}]_i$ are required. In fact, both NMDA-dependent LTD and NMDA-independent LTP of $GABA_A$ receptor mediated synaptic transmission ($LTD_{GABA\text{-}A}$ and $LTP_{GABA\text{-}A}$) have been reported in the neonatal hippocampus following a high frequency train [46]. Activation of

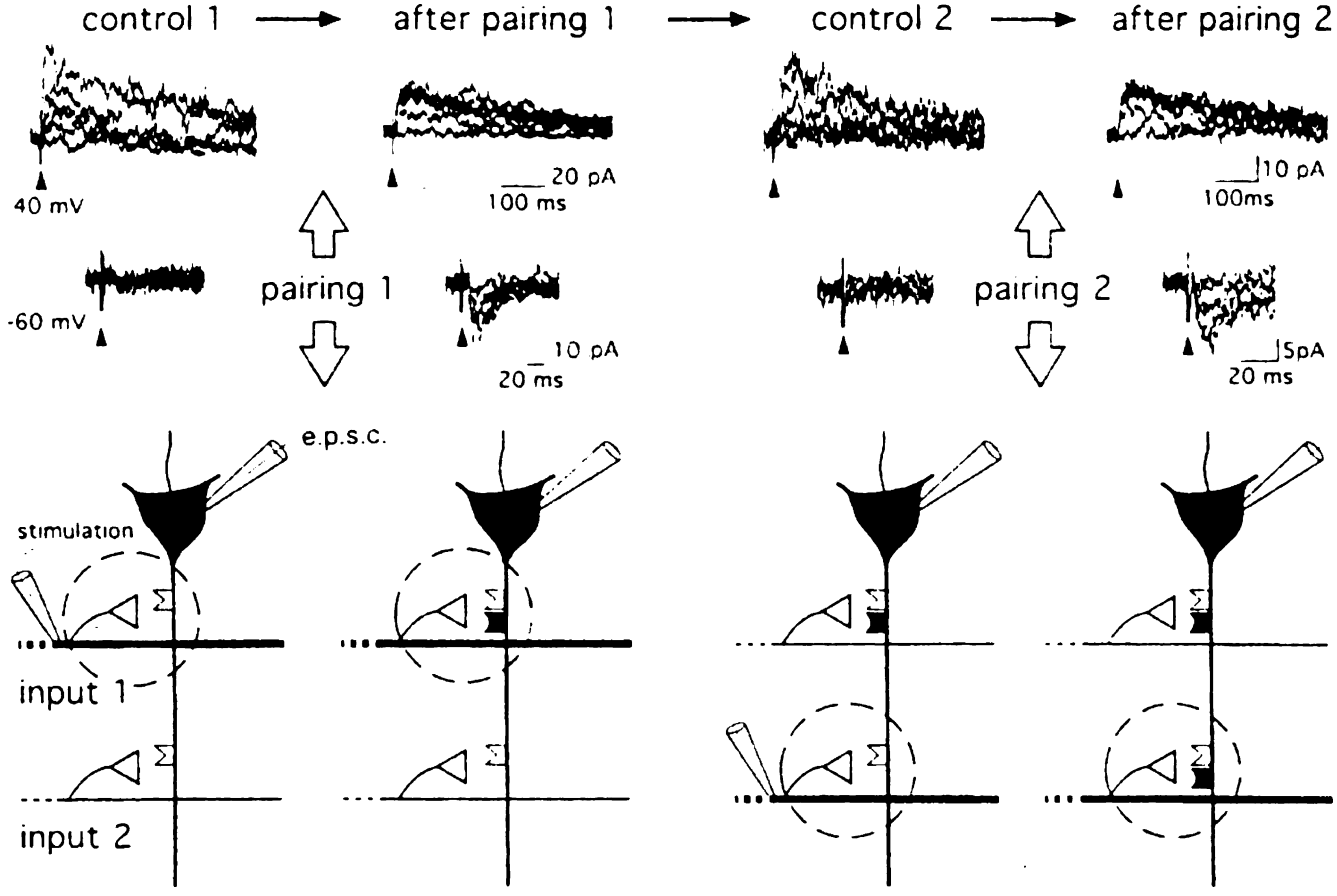

Fig. 4. Glutamatergic plasticity in the immature hippocampus. The conversion of AMPA silent (pure NMDA) synapses into mixed AMPA + NMDA functional synapses can be triggered by the coincident activity of pre- and postsynaptic sites, activation of NMDA receptors during hebbian stimulation, in analogy to adult LTP paradigms. A pure NMDA synapse from a CA1 pyramidal cell at P3 (control 1, input 1) was converted to mixed AMPA + NMDA synapse by pairing 1. Another AMPA-silent synapse (input 2) on the same postsynaptic neuron (control 2) was not converted by heterosynaptic stimulation of input 1 but was converted to mixed AMPA + NMDA synapse by hebbian stimulation (pairing 2) of input 2, suggesting that "functional glutamatergic synapses induction" is input specific (reproduced with permission from Ref. [15]).

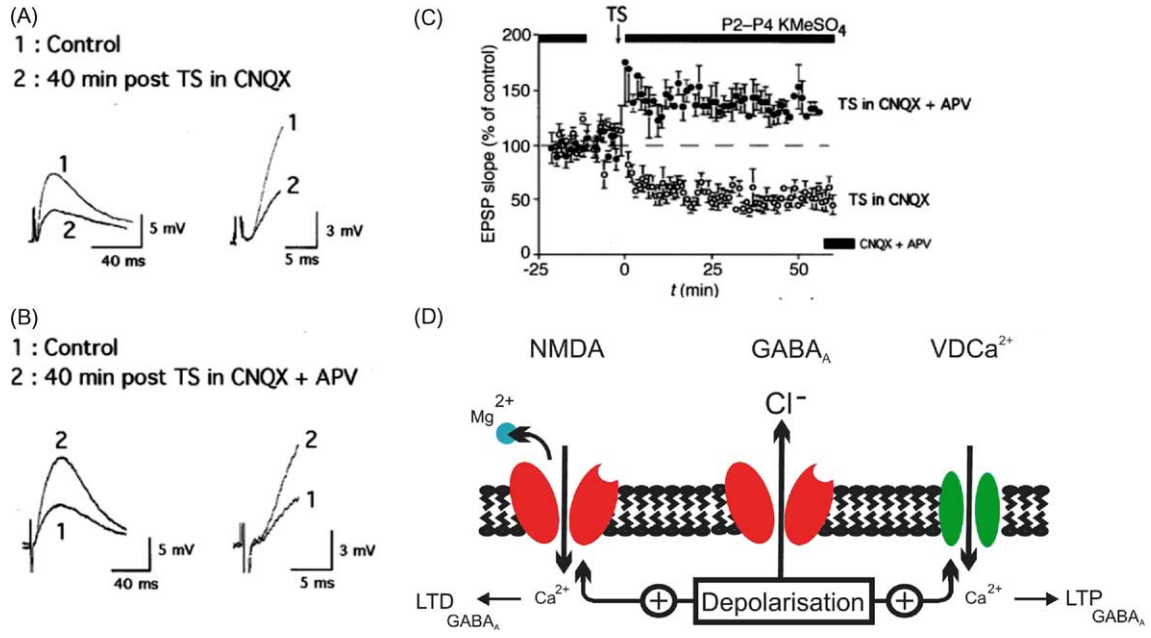

Fig. 5. GABAergic plasticity in the immature hippocampus. In analogy with the adult forms of glutamatergic LTD–LTP that are induced and expressed by AMPA receptors, GABAergic LTD–LTP induced and expressed by $GABA_A$ receptors ($LTD_{GABA\text{-}A}$ and $LTP_{GABA\text{-}A}$) have been reported in the neonatal hippocampus. During a high frequency train (TS), $GABA_A$ receptors activation provides the depolarizing source that releases NMDA channels from Mg^{2+} block to induce NMDA-dependent LTD_I or to activate voltage-gated calcium channels and trigger NMDA-independent LTP_I (reproduced with permission from Ref. [46]).

$GABA_A$ receptors is required for the induction of both $LTD_{GABA\text{-}A}$ and $LTP_{GABA\text{-}A}$. In the case of $LTD_{GABA\text{-}A}$, it provides the depolarization that releases NMDA channels from Mg^{2+} block. When NMDA channels are not activated though, the activation of voltage-gated calcium channels and associated Ca^{2+} increase triggers $LTP_{GABA\text{-}A}$ (cf. Fig. 5). It is interesting to note here that in the neonatal hippocampus, at a time when GABA exerts an excitatory role similar to that later devoted to glutamatergic AMPA receptors, GABAergic synaptic transmission can be modulated by activity-dependant LTD and LTP, in analogy with the adult forms of glutamatergic LTD–LTP that are induced and expressed by AMPA receptors in the adult hippocampus.

In addition to synaptic plasticity, trophic effects of the neurotransmitters GABA and glutamate, released during the spontaneous patterns of activity of the neonatal hippocampus, may also participate in the maturation of the network. During the last 15 years, a large body of evidence accumulated indicating that neurotransmitters may behave as morphogenic agents during development (for review see [23,32,43,48]). Like glutamate, GABA has been shown to play a variety of morphogenic roles at embryonic and early postnatal stages. Trophic effects of GABA are mimicked by $GABA_A$ receptor agonists and are blocked by $GABA_A$ receptor antagonists, suggesting that these effects are mediated via $GABA_A$ receptor that are massively activated during hippocampal GDPs or SPW-bursts. LoTurco et al. [41] have shown that exogenously applied GABA decreases the rate of cellular proliferation in the ventricular zone of the rat embryonic neocortex. GABA also stimulates directed migration as well as random motility of postmitotic cortical neuroblasts [2]. Once neurons have reached their final locations, GABA acts as a morphogenic agent promoting neurite outgrowth and differentiation [1,41,48,59], expression of receptors [3,24], synaptogenesis [59] and an increase in the density of organelles involved in protein synthesis [24,59]. Interestingly, experimental work on developing hippocampal neurons maintained in culture has recently suggested that GABA itself can promote the switch in $GABA_A$ receptor mediated signals from excitation to inhibition [20]. This switch would involve $[Ca^{2+}]_i$ increases mediated by $GABA_A$ dependent neuronal depolarization, leading to increased expression of the Cl^- extruder KCC2 [20]. In fact, several observations support the idea that the depolarization mediated by GABA and the associated rise in $[Ca^{2+}]_i$ underlie the different morphogenic actions of GABA. First, the window during which GABA acts as a morphogenic agent and depolarizes the cells coincide [8,20]. In addition, some morphogenic actions of GABA cease when cells are treated with the permeant calcium chelator BAPTA-AM [2] or VDCC blockers [8,20] and these morphogenic actions are mimicked by agents increasing $[Ca^{2+}]_i$ through the activation of VDCC [20,41]. The steps following the increase in $[Ca^{2+}]_i$ are presently

unknown but may involve activation of various genes, including immediate early genes and growth factor genes. For instance, it has been demonstrated that GABAergic stimulation also switches from enhancing to repressing BDNF mRNA synthesis during the maturation of hippocampal neurons in vitro, linked to a loss in its ability to activate voltage-dependent calcium channels [8]. Furthermore, the $GABA_A$ receptor agonist muscimol and BDNF increase both the size and neuropeptide Y (NPY) immunoreactivity of hippocampal interneurons [44]. However, GABAergic stimulation fails to increase NPY immunoreactivity in cultures from BDNF-knockout embryos. At later developmental stages, when GABA hyperpolarizes neurons and represses BDNF synthesis, stimulation of $GABA_A$ receptors reduces cell size and NPY immunoreactivity of interneurons. GABAergic interneurons might thus control their phenotype through the regulation of BDNF synthesis [44].

6. Conclusion

Because developmental activity-dependent synaptic plasticity has been hypothesized to participate in network refinement, leading to the precise mapping of synaptic contacts constituting a functional brain, further studies are awaited to get more details about the spatio-temporal structure of immature network activities. From what has already been described concerning the neonatal hippocampus, in which it seems that a large proportion of cells are firing together, it may not be obvious to understand how such massive synchronisation may drive the establishment of precise mapping of synaptic contacts via activity-dependent synaptic plasticity. Alternatively, developmental plasticities expressed by GDPs or SPW-bursts may be implicated more globally in the maturation of the hippocampal network, setting for example quantitative constraints on the dynamic development of inhibitory and excitatory signalling in order to keep the balance between inhibition and excitation within definite physiological limits, favouring coordinated neuronal growth and differentiation.

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