Review TRENDS in Neurosciences Vol.27 No.7 July 2004

Interneurons set the tune of developing networks

Yehezkel Ben-Ari, Ilgam Khalilov, Alfonso Represa and Henri Gozlan

Institut de Neurobiologie de la Méditerranée (INMED), Parc Scientifique de Luminy, BP13, 13009 Marseille, France

Despite a rather long migratory journey, interneurons are functional before vertically migrating pyramidal neurons and they constitute the source and target of the first functional synapses in the developing hippocampus. Interneuron-driven network patterns are already present *in utero* while principal cells are mostly quiescent. At that early stage, GABAergic synapses – which are formed before glutamatergic ones – are excitatory, suggesting that GABA is a pioneer, much like the neurons from which it is released. This review discusses this sequence of events, its functional significance and the role that interneurons might play in the construction of cortical networks.

Much like the construction of a building, the formation and operation of a cortical network depends on the interactions between vertical and horizontal building blocks constituted by glutamatergic principal cells and GABAergic interneurons, respectively. The principal cells provide most of the long interconnections, the interneurons primarily a significant amount of the intrinsic inputs. There is now extensive evidence to suggest that interneurons play a central role in the generation of oscillations and network-driven patterns as well as the functions associated with these patterns, including integrative perception, sleep and memory processes (reviewed notably in the *TINS Interneuron Diversity series* [1–3]). This review will concentrate on the developmental aspects of the crosstalk between these two building blocks. GABA receptors and synapses are functional before glutamate ones in a wide range of brain structures and species [4]. Recent studies suggest that interneurons that release GABA are also functional before the principal cells and thus could control network-driven patterns at an early developmental stage. One tentative proposal is that, much as an engine, interneurons are programmed to ignite the circuit and generate primitive patterns that are instrumental in shifting from a silent ensemble of immature neurons to an adult network that communicates via millions of synapses.

Different source and different journeys

GABAergic interneurons in rodents originate essentially in the ganglionic eminences and enter the developing cortex via a tangential migration [5] that appears not to require a glial scaffold. In humans [6] and non-human primates (Z. Petanjek *et al.*, unpublished), interneurons also originate in the subventricular zone, suggesting interesting evolutionary changes in the development of the cortical mantle. By contrast, pyramidal neurons are generated near the surface of the cerebral ventricle [7] and migrate towards the marginal zone, forming the characteristic layered structure of the cerebral cortex [8–11]. A similar arrangement takes place in the hippocampal formation, with pyramidal and granule cell layers having a similar chronological arrangement but at different time points (antenatal and perinatal, respectively).

Pyramidal cell are generated in rodents primarily during the last gestational week, whereas in primates and humans this takes place during mid-gestation (between the 6th and the 17th weeks of gestation) [7]. GABAergic interneurons are clearly generated before glutamatergic cells [12] but they have a longer journey before they reach their final destination. Nevertheless, the developmental profile of markers of GABA receptors and GABA synapses takes place very early and provides a rationale for the predominant role of GABA in maturing tissue [13–15]. In the hippocampus, the formation of the hippocampal primordium is concomitant with the arrival of migrating GABA cells, so that the genesis and migration of pyramidal cells occur more or less simultaneously with the arrival of interneurons.

GABAergic synapses are established before glutamatergic ones on interneurons and principal cells

Studies using selective glutamate and GABA receptor antagonists suggest that GABA receptors and synapses are operative before glutamatergic synapses in all brain structures and species studied to date [16–18] (see also references in Ref. [4]). This sequence was demonstrated more directly in studies in which hippocampal neurons were patch-clamp recorded from the CA1 region of hippocampal slices obtained *in utero* and at birth [19,20]. The synaptic activity of both interneurons and pyramidal cells was determined and the neurons were reconstructed after intracellular injections of dyes. Three morphologically different types of cells were identified in each case: small silent cells with no functional synapses, cells with GABA but not glutamate synapses and cells with both types of synapses that were also more developed. $GABA_A$ synapses appeared before glutamate ones in both pyramidal cells [19,20] and interneurons [20] (Figure 1a,b) and cells with only glutamate synapses have not been found over a population of several hundred of neurons. Thus,

Corresponding author: Yehezkel Ben-Ari (ben-ari@inmed.univ-mrs.fr).
Available online 19 May 2004

 0166-2236/$ - see front matter doi:10.1016/j.tins.2004.05.002

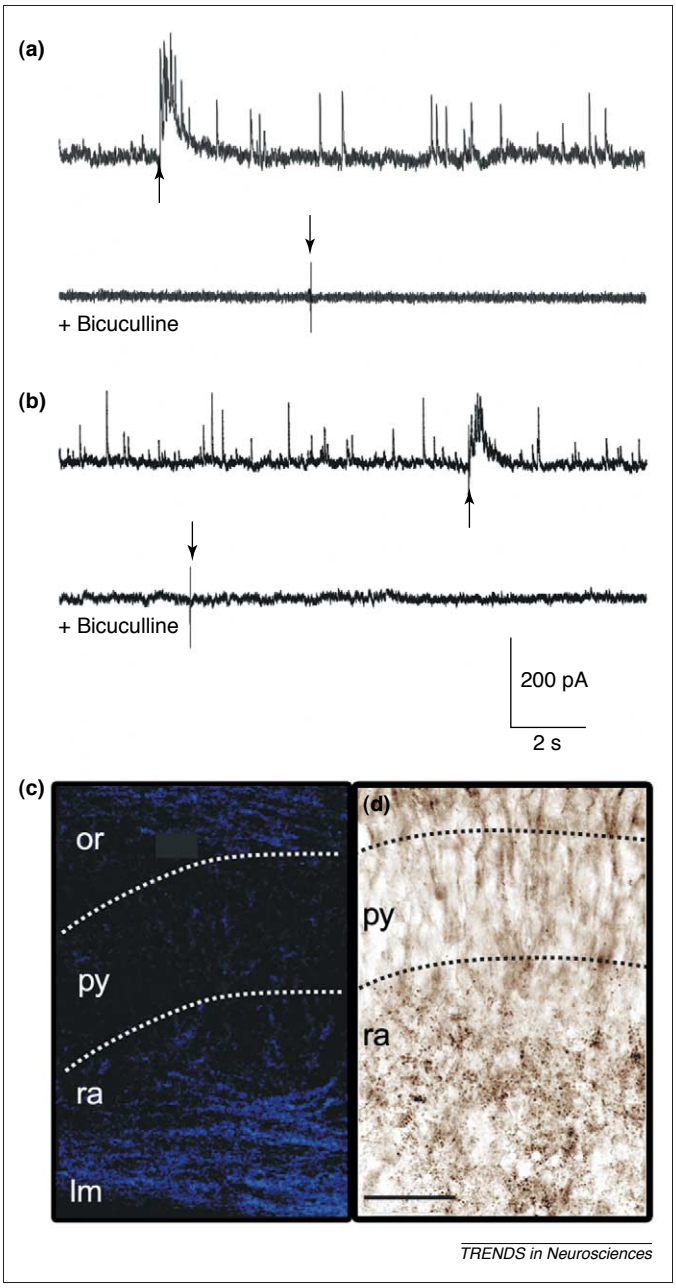

Figure 1. Morpho-functional characteristics of CA1 neurons at birth. (a,b) $GABA_A$ synapses appeared before glutamate synapses: patch pipettes were filled with a K^+ gluconate solution to visualize simultaneously $GABA_A$ (outward) and AMPA (inward) receptor-mediated responses, the reversal potentials for $GABA_A$ currents being around −60 mV. Spontaneous and evoked postsynaptic currents were recorded in postnatal day (P)0 of rat hippocampal slices at −45 mV. Traces recorded in a CA1 pyramidal cell **(a)** and in an interneuron from stratum radiatum **(b)** display only GABA-mediated currents. No NMDA response could be evoked at +40 mV in these conditions (not shown). Application of bicuculline (10 μM, 10 min) completely suppressed spontaneous and evoked (arrow) $GABA_A$ responses in the two cell types. Note the absence of interictal seizures. Reproduced, with permission, from Ref. [23] © (2003) Oxford University Press. (c,d) Immunocytochemistry in P0 hippocampal slices: glutamate decarboxylase 67 (GAD 67) **(c)** and synaptophysin **(d)** labeling was not detected in the CA1 pyramidal layer at birth but was clearly observed in the other CA1 layers. Panel (d) is reproduced, with permission, from Ref. [19] © (1999) the Society for Neuroscience. Scale bar, 40 μm. Abbreviations: lm, stratum lacunosum moleculare; or, stratum oriens; py, pyramidal cell layer; ra, stratum radiatum.

there is sequential expression of GABA and glutamate synapses during maturation, the formation of glutamate synapses taking place only in more developed neurons (Figure 2). As glutamatergic axons are present at an earlier stage, this sequence is not due to a late arrival of the inputs. Clearly, different conditions are required for the formation of GABA and glutamate synapses. The same sequence of synapse formation was also observed in pyramidal cells of the macaque hippocampus but the principal difference was its earlier occurrence – that is, *in utero* [21]. Thus, GABA synapses are formed first in the entire population of neurons, providing a crucial role to interneurons.

GABAergic synapses are formed first on apical dendrites

Studies using synapsin and glutamate decarboxylase (GAD) immunoreactivity suggest that the first synapses on pyramidal neurons are probably made on the apical dendrites and not on the soma [19] (Figure 1c,d) and are GABAergic [14]. In fact, 'silent' pyramidal cells – those with no synaptic activity – have no apical dendrites, suggesting that synapses are formed only once pyramidal neurons have extended apical dendrites [19,20]. In keeping with this, dendritic projecting interneurons such as basket cells develop before somatic projecting interneurons [20,22]. Interestingly, although cholecystokinin-containing interneurons (a subpopulation of basket cells) are present at birth in rats [23,24], they form initially synapses on the dendrites of pyramidal cells and shift after postnatal day (P)4 to their adult pattern of somatic innervation [24]. In a more general perspective, the developing network appears to control sequentially first its dendritic glutamatergic inputs and only then the action potentials and the outputs generated in the cell body of principal neurons.

Interneurons supply initially most of the activity

A comparative analysis of the morphological–functional maturation of interneurons and pyramidal cells reveals that the former mature before the latter, despite a similar GABA–glutamate sequence [20]. Thus, at rat embryonic day (E)18–E20, 12% of pyramidal neurons and 65% of interneurons had functional synapses [20]. A similar ratio is found at birth [19,20]. If the first functional synapses are between interneurons, the initial patterns recorded will be generated primarily, if not solely, by interneurons. This is indeed the case, at least in the hippocampus, because at E18, when most of pyramidal cells are silent and interneurons express mainly GABA synapses, 4-aminopyridine (4-AP) triggers oscillations (Figure 3a). These oscillations are suppressed by bicuculline. Furthermore, synchronized $GABA_A$-receptor-mediated oscillations were recorded in pairs of CA1 interneurons (Figure 3b), indicating that the first functional network of activity is probably constituted by interconnected interneurons that use excitatory GABA as a neurotransmitter (H. Gozlan *et al.*, unpublished). The morphological substrate for these observations is probably provided by an early maturation of calretinin-positive interneurons that exclusively innervate other interneurons [25,26]. These neurons are among the first interneurons present [23,27]. Therefore, interneurons first innervate other interneurons before innervating the dendrites and then the cell bodies of the principal cells.

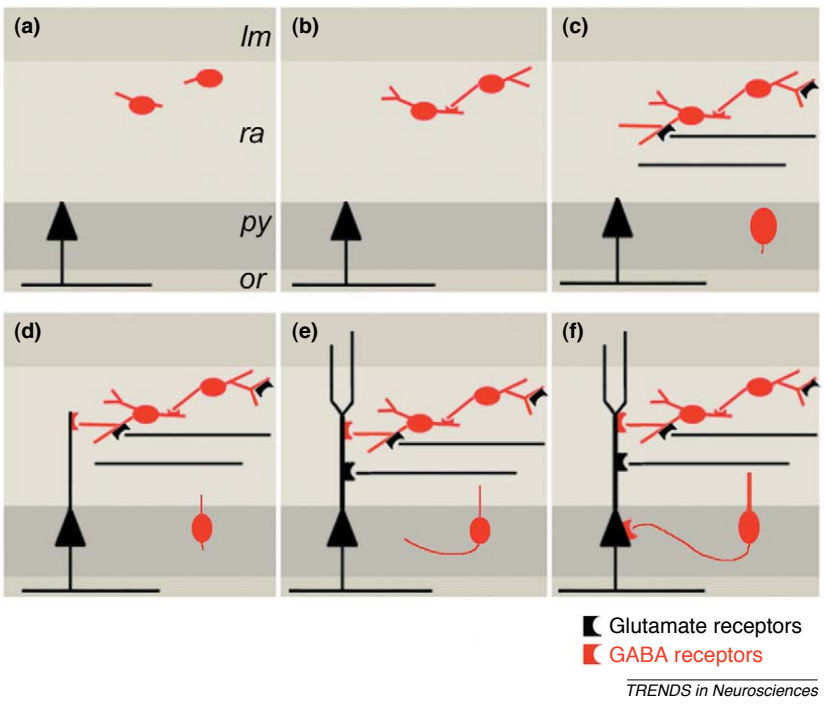

Figure 2. Sequential formation of synapses in interneurons and pyramidal cells during development of the rat. Interneurons are represented by red ovals, pyramidal cells by black triangles. This six-step diagram schematizes the sequential formation of synapses but does not take into account its heterogeneity. The ages mentioned are likely to represent the ages at which a significant number of new synapses are found, bearing in mind that their acquisition by a whole population of neurons probably extends over three or four days. **(a)** Neither type of neuron is innervated. (b,c) Interneurons, but not pyramidal cells, express $GABA_A$ **(b)** then glutamate **(c)** synapses. Note that excitatory fibers (black) selectively synapse with interneurons and not with the poorly developed apical dendrites of pyramidal cells. These three developmental steps take place mainly between embryonic day (E)17 and E19. (d,e) Pyramidal neurons express sequentially $GABA_A$ **(d)** and glutamate **(e)** synapses on apical dendrites. Note that the apical dendrites of pyramidal cell progressively extended in stratum radiatum (d) and stratum lacunosum moleculare (e). These two steps are likely to start at around E19 and continue after birth. Synapses between interneurons and the soma of pyramidal cells are formed later, at around postnatal-day 4 **(f)**. Abbreviations: lm, stratum lacunosum moleculare; or, stratum oriens; py, pyramidal cell layer; ra, stratum radiatum.

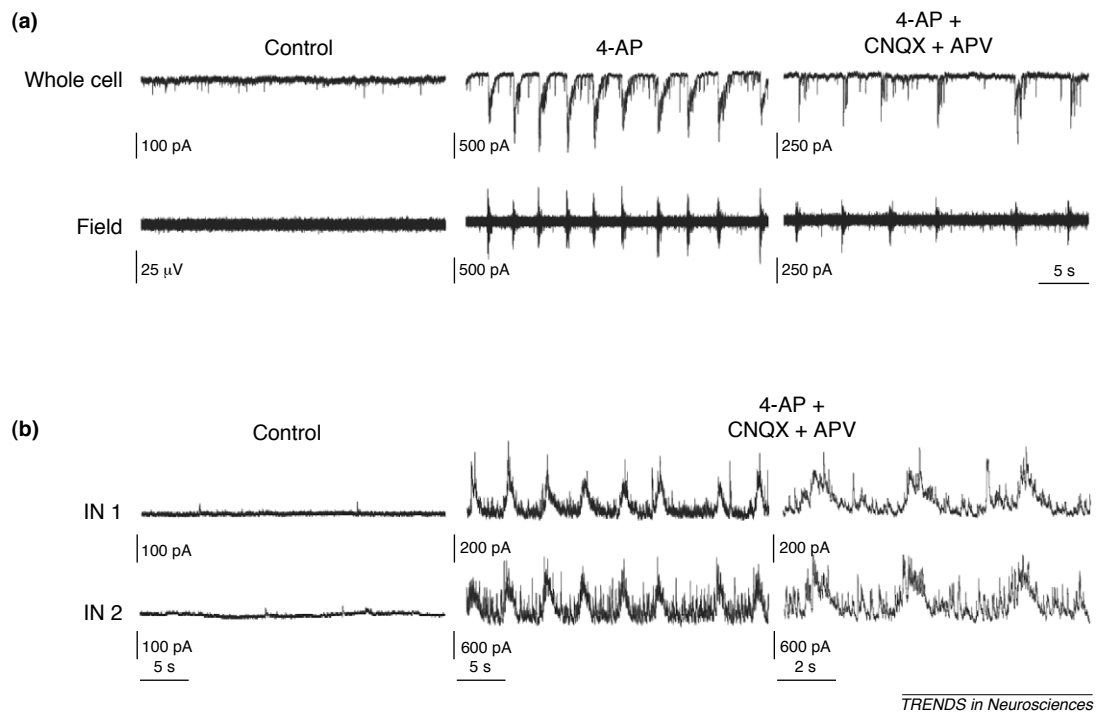

Figure 3. An early functional network of interneurons in the CA1 area of the hippocampus at rat embryonic-day 20. **(a)** 4-Aminopyridine (4-AP) induces oscillations. A whole-cell recording (−40 mV) from an interneuron from stratum oriens and a simultaneous extracellular recording in the stratum radiatum are shown. Application of 4-AP (100 μM) induces oscillations in interneurons that are synchronized with field potentials. These oscillations persist in the presence of ionotropic glutamate antagonists 6-cyano-7-nitroquinoxaline (CNQX; 10 μM) and D-2-amino-5-phosphonovaleric acid (APV; 20 μM). **(b)** 4-AP-induced oscillations are synchronized in pairs of interneurons. Double whole-cell recordings (+40 mV) of two interneurons (IN 1 and IN 2) from stratum radiatum are shown. These interneurons display only $GABA_A$ synapses. Addition of 4-AP in the presence of ionotropic glutamate antagonists induces synchronous oscillations in the two cells.

GABA excites at early developmental stages

Studies performed in the hippocampus suggest that immature neurons have a higher intracellular Cl^- concentration, leading to depolarization by GABA of immature neurons [28], generation of action potentials [23,29,30], activation of NMDA receptors [29] and a rise of intracellular Ca^{2+} concentration [31]. These properties are not limited to hippocampal neurons, because they are found in a wide range of brain structures and species [4].

The K^+–Cl^- cotransporter 2 (KCC2), which usually extrudes Cl^- below its electrochemical equilibrium potential, plays a central role in the excitation of GABA in immature tissues [32,33]. KCC2 expression progressively increases from embryonic stages to P15 [34] and parallels the shift of GABA properties from excitatory to inhibitory. Furthermore, antisense oligonucleotide inhibition of KCC2 expression produces a marked positive shift in the reversal potential of $GABA_A$ responses in functionally mature hippocampal pyramidal neurons [33]. Poo and co-workers have suggested that GABA itself controls the shift from excitation to inhibition because blockade of GABA receptors in cultures prevents the shift from taking place [35]. However, this has not been confirmed by other groups [36,37]. Nevertheless, KCC2 plays a central role in the developmental switch of $GABA_A$-mediated responses from depolarizing to hyperpolarizing [38]. By contrast, other studies suggest that the Na^+–K^+–Cl^- cotransporter 1, which accumulates Cl^- in neurons, might also contribute to this sequence by its early maturation, which would contribute to the depolarizing actions of GABA [39,40].

Discussion

In conclusion, these observations suggest that the operation of GABAergic synapses is a key mechanism in the development of cortical networks. The heterogeneity of interneurons, with its endowed capacity to control a wide range of selective actions, is highly suitable to begin the sequence of events required for the shift from an ensemble of immature neurons that communicate via primitive paracrine systems (Box 1) to one that operates via synapses.

The first synapses are GABAergic; they occur between GABAergic neurons and the first network pattern they generate is also characteristic of GABA. GABA can be considered as a pioneer transmitter that might have been selected early during evolution. Why are GABA receptors and synapses suitable for this early task? Probably because,

Box 1. Paracrine communication between neurons before synapse formation

In all systems studied, functional receptors are expressed before synapses and the crosstalk between presynaptic and postsynaptic elements is of paramount importance in the formation of synaptic complexes [49]. This is also the case for GABA and glutamate that are present in very immature 'silent' neurons with no synaptic currents [19,49]. Thus in the neocortex, blocking GABA receptors generates currents in neurons [49]. This has been more directly shown in the hippocampus, where clearly GABA and glutamate released to the extracellular space from axonal growth cones or glia diffuse and activate in a paracrine manner synaptically silent neurons [50]:

- A single stimulation of the stratum radiatum evoked in CA1 pyramidal cells a slow current that developed and decayed within several seconds (Figure Ia). This response was mediated mainly by $GABA_A$ receptors and to a lesser extent by NMDA receptors, but not by AMPA/kainate receptors. Spontaneous events (Figure Ib) with similar properties were also recorded.
- Application of $GABA_A$ receptor antagonists in CA1 pyramidal cells generated, in resting conditions and in the absence of any electrical stimulation, a current revealing tonic stimulation of pyramidal cells by GABA (Figure Ic). By contrast, glutamate receptor antagonists failed to evoke any current.
- Glutamate but not GABA transporters are operational perinatally (late embryonic stages to postnatal-day 0), suggesting that the removal of GABA at that stage is not efficient, thus allowing diffusion of GABA and distal paracrine actions.
- GABA and glutamate could be released thorough Ca^{2+}-independent processes that do not require soluble *N*-ethylmaleimide-sensitive fusion-attachment-protein receptor (SNARE). In fact, slow and tonic currents are generated in maturing pyramidal neurons in slices incubated with botulinium toxin (to cleave SNARE) and in sections from murine uncoordinated 18-1 (Munc 18-1) knockout mice (this mutation abolishes any vesicular release). The mechanisms involved remains to be determined. These observations raise the possibility that nonsynaptic release of transmitters might precede vesicular release.

In conclusion, transmitters released through a non-conventional mechanism diffuse within the extracellular space and activate cells not yet synaptically contacted. Such activity constitutes the first mode of intercellular communication via transmitters in maturing tissues. GABA (probably released from maturing interneurons) is the main actor of this paracrine form of activity, owing to the poor function of GABA transporters. A paracrine mode of action of glutamate could occur after activation or through modulation of glutamate transporter activity.

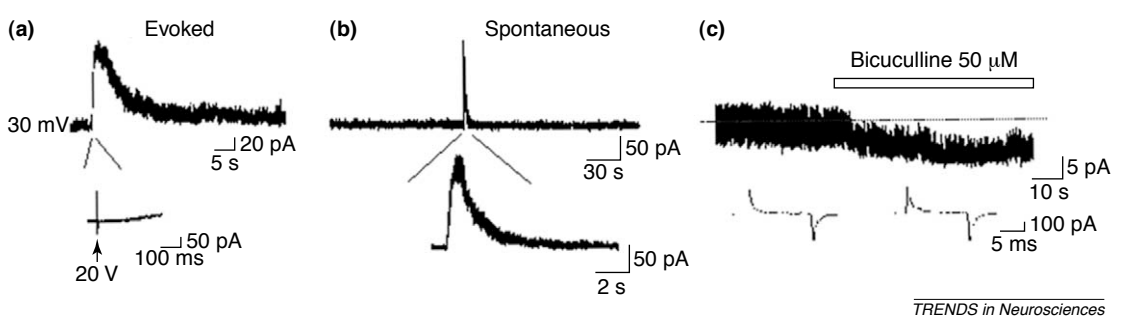

Figure I. Paracrine activation of maturing pyramidal cells. Electrophysiological recordings from murine uncoordinated 18-1 (Munc 18-1)-deficient mice. **(a)** Electrical stimulation (20V, 40 μs duration) evokes a slow current but not a postsynaptic current. **(b)** Spontaneous slow current in a 5 min recording. **(c)** Tonic current produced by bicuculline. This is associated with a decrease in the basal noise. Resistance does not change (18 MΩ) before and during the application of bicuculline. Reproduced, with permission, from Ref. [50].

in contrast to glutamatergic synapses, in both depolarizing and hyperpolarizing conditions, the reversal potential of GABA currents is close to rest and is thus not toxic. When depolarizing, GABA synapses are thus ideally suited to provide the excitatory drive required to generate Ca^{2+} currents and to remove the Mg^{2+} block from NMDA receptors without having the potentially toxic effects of glutamate synapses [4,31]. Fully operative inhibition with powerful hyperpolarization is required only once a sufficient density of glutamatergic synapses and a powerful excitation is established. Further studies are required to determine whether the expression of KCC2 is indeed modulated [35] or not [36,37] by activity. Additional questions concern the part played in the developmental role of GABA by the high Cl^- concentration and the endowed rapid shift of GABA action according to maturation stage or level of activity.

What are the advantages of this sequence? The longer duration of GABA postsynaptic currents will facilitate the likelihood that synaptic currents generated in neurons endowed with a few synapses summate and generate network driven events. In addition, if the percentage of neurons endowed with burst-generation capacity is particularly high during early postnatal life [41,42], these neurons will act as cellular pacemakers and resonators. Pacemaker cells coupled by electrotonic junctions could also contribute to the generation of the first signature of a network mediated initially by giant depolarizing potentials (GDPs) of GABA synapses in the immature hippocampus [43]. The excitation of other interneurons in a primitive ensemble enhances this action and promotes the occurrence of GDPs as a universal pattern present in immature neurons [28]. With the maturation of the network and the formation of large number of synapses, the central role of the primitive pattern is replaced by a larger repertoire of behaviourally relevant patterns.

There are several important implications of the proposed scenario. If GABA receptors are expressed first, their trafficking and sorting mechanisms might differ from those that control glutamate receptors. Perhaps the expression of these receptors and the formation of GABA synapses are 'automatic', requiring only the actual interaction of the axon and its future target. By contrast, the formation of glutamate synapses might require additional conditions, notably mature targets (i.e. mature dendrites in the hippocampus). Pyramidal cells establish synapses first on GABAergic neurons that are more mature than other pyramidal cells. Soriano and colleagues have also suggested that inter-hemispheric glutamatergic axons also innervate first GABAergic interneurons that are destined to die before shifting their attention to other glutamatergic neurons [44]. Whatever the underlying mechanism, the consequence is that synapses between interneurons provide a primitive pattern in a network composed primarily of silent pyramidal neurons. This initial pattern of GABA-dominated activity might modulate maturation of the arbor of the principal cells. In keeping with this, in neuronal cultures, slices cultures and intact preparations, blockade of NMDA and GABA receptors produce respectively a stimulation and reduction of neuronal arborization [45,46]. The negative results obtained with GAD 65 and GAD 67 double knockout mice (in which brain histogenesis does not seem to be affected) [47] are not compatible with this scenario unless alternative sources of modulation are present. However, these mutants die at birth, when neurons are poorly developed, and synaptic and network activities in these animals have not yet been analyzed. Interestingly, it has been shown that juvenile GAD 65 knockout mice [48] have a defect of visual-experience-dependent plasticity, thus reinforcing the suggestion that GABA is involved on developmental processes.

At least in the developing hippocampal network, interneurons exert a control over the network at a very early stage, when the principal cells are largely immature with few or no synapses. In addition, GABAergic synapses are functional very early in all brain structures and species studied, raising the possibility that this principle might be valid in other brain structures. Therefore, the interneuronal network that plays a central role in generating behaviorally relevant network-driven patterns in the adult brain might be programmed to control the developing network even when the mode of communications between neurons is of more a paracrine type, with few or no synapses. Despite of the long journey of these neurons, they might be tuned to control and modulate essential functions in the construction of the cortical network. It will be important to determine the role of the heterogeneity of interneurons at an early stage and how the scaffold of the vertically oriented principal cells is modulated by the horizontally oriented interneurons.

References

1 Lawrence, J.J. *et al.* (2003) *Interneuron Diversity series*: Containing the detonation – feedforward inhibition in the CA3 hippocampus. *Trends Neurosci.* 26, 631–640
2 Freund, T.F. (2003) *Interneuron Diversity series*: Rhythm and mood in perisomatic inhibition. *Trends Neurosci.* 26, 489–495
3 Maccaferri, G. *et al.* (2003) *Interneuron Diversity series*: Hippocampal interneuron classifications – making things as simple as possible, not simpler. *Trends Neurosci.* 26, 564–571
4 Ben-Ari, Y. (2002) Excitatory actions of GABA during development: the nature of the nurture. *Nat. Rev. Neurosci.* 3, 728–739
5 Marin, O. *et al.* (2001) A long, remarkable journey: tangential migration in the telencephalon. *Nat. Rev. Neurosci.* 2, 780–790
6 Letinic, K. *et al.* (2002) Origin of GABAergic neurons in the human neocortex. *Nature* 417, 645–649
7 Rakic, P. (1995) A small step for the cell, a giant leap for mankind: a hypothesis of neocortical expansion during evolution. *Trends Neurosci.* 18, 383–388
8 Marin-Padilla, M. (1998) Cajal-Retzius cells and the development of the neocortex. *Trends Neurosci.* 21, 64–71
9 Angevine, J.B. *et al.* (1961) Autoradiographic study of cell migration during histogenesis of cerebral cortex of the mouse. *Nature* 192, 766–776
10 Rakic, P. (1971) Neuron-glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electronmicroscopic study in *Macacus Rhesus*. *J. Comp. Neurol.* 141, 283–312
11 Sidman, R.L. *et al.* (1973) Neuronal migration, with special reference to developing human brain: a review. *Brain Res.* 62, 1–35
12 Soriano, E. *et al.* (1986) Asynchronism in the neurogenesis of GABAergic and non-GABAergic neurons in the mouse hippocampus. *Brain Res.* 395, 88–92
13 Rozenberg, F. *et al.* (1989) Distribution of GABAergic neurons in late fetal and early postnatal rat hippocampus. *Brain Res. Dev. Brain Res.* 50, 177–187
14 Dupuy, S.T. *et al.* (1996) Prominent expression of two forms of glutamate decarboxylase in the embryonic and early postnatal rat hippocampal formation. *J. Neurosci.* 16, 6919–6932
15 Tanaka, D. *et al.* (2003) Multimodal tangential migration of neocortical GABAergic neurons independent of GPI-anchored proteins. *Development* 130, 5803–5813

16 Walton, M.K. *et al.* (1993) Sodium channels, $GABA_A$ receptors, and glutamate receptors develop sequentially on embryonic rat spinal cord cells. *J. Neurosci.* 13, 2068–2084
17 Chen, G. *et al.* (1995) GABA receptors precede glutamate receptors in hypothalamic development; differential regulation by astrocytes. *J. Neurophysiol.* 74, 1473–1484
18 Koller, H. *et al.* (1990) GABA and glutamate receptor development of cultured neurons from rat hippocampus, septal region, and neocortex. *Synapse* 5, 59–64
19 Tyzio, R. *et al.* (1999) The establishment of GABAergic and glutamatergic synapses on CA1 pyramidal neurons is sequential and correlates with the development of the apical dendrite. *J. Neurosci.* 19, 10372–10382
20 Hennou, S. *et al.* (2002) Early sequential formation of functional $GABA_A$ and glutamatergic synapses on CA1 interneurones of the rat foetal hippocampus. *Eur. J. Neurosci.* 16, 197–208
21 Khazipov, R. *et al.* (2001) Early development of neuronal activity in the primate hippocampus *in utero*. *J. Neurosci.* 21, 9770–9781
22 Nitsch, R. *et al.* (1990) Late appearance of parvalbumin-immunoreactivity in the development of GABAergic neurons in the rat hippocampus. *Neurosci. Lett.* 118, 147–150
23 Gozlan, H. *et al.* (2003) Interneurons are the source and the targets of the first synapses formed in the rat developing hippocampal circuit. *Cereb. Cortex* 13, 684–692
24 Morozov, Y.M. *et al.* (2003) Postnatal development and migration of cholecystokinin-immunoreactive interneurons in rat hippocampus. *Neuroscience* 120, 923–939
25 Gulyas, A.I. *et al.* (1996) Interneurons containing calretinin are specialized to control other interneurons in the rat hippocampus. *J. Neurosci.* 16, 3397–3411
26 Freund, T.F. *et al.* (1996) Interneurons of the hippocampus. *Hippocampus* 6, 347–470
27 Jiang, M. *et al.* (1997) Expression of calretinin in diverse neuronal populations during development of rat hippocampus. *Neuroscience* 81, 1137–1154
28 Ben-Ari, Y. *et al.* (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol.* 416, 303–325
29 Leinekugel, X. *et al.* (1997) Ca^{2+} oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus. *Neuron* 18, 243–255
30 Tyzio, R. *et al.* (2003) Membrane potential of CA3 hippocampal pyramidal cells during postnatal development. *J. Neurophysiol.* 90, 2964–2972
31 Leinekugel, X. *et al.* (1995) Synaptic $GABA_A$ activation induces Ca^{2+} rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *J. Physiol.* 487, 319–329
32 Delpire, E. (2000) Cation–chloride cotransporters in neuronal communication. *News Physiol. Sci.* 15, 309–312
33 Rivera, C. *et al.* (1999) The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397, 251–255
34 Stein, V. *et al.* (2004) Expression of the KCl cotransporter KCC2 parallels neuronal maturation and the emergence of low intracellular chloride. *J. Comp. Neurol.* 468, 57–64
35 Ganguly, K. *et al.* (2001) GABA itself promotes the developmental switch of neuronal GABAergic responses from excitation to inhibition. *Cell* 105, 521–532
36 Ludwig, A. *et al.* (2003) Developmental up-regulation of KCC2 in the absence of GABAergic and glutamatergic transmission. *Eur. J. Neurosci.* 18, 3199–3206
37 Titz, S. *et al.* (2003) Hyperpolarizing inhibition develops without trophic support by GABA in cultured rat midbrain neurons. *J. Physiol.* 550, 719–730
38 Payne, J.A. *et al.* (2003) Cation–chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 26, 199–206
39 Delpire, E. *et al.* (2002) Human and murine phenotypes associated with defects in cation–chloride cotransport. *Annu. Rev. Physiol.* 64, 803–843
40 Russell, J.M. (2000) Sodium–potassium–chloride cotransport. *Physiol. Rev.* 80, 211–276
41 Wellmer, J. *et al.* (2002) Long-lasting modification of intrinsic discharge properties in subicular neurons following status epilepticus. *Eur. J. Neurosci.* 16, 259–266
42 Yaari, Y. Plasticity of intrinsic neuronal excitability after status epilepticus: time course and ionic mechanisms. *Epilepsia* (in press)
43 Strata, F. *et al.* (1997) A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. *J. Neurosci.* 17, 1435–1446
44 Super, H. *et al.* (1998) Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. *J. Neurosci.* 18, 4616–4626
45 Savic, N. *et al.* (2003) *N*-methyl-D-aspartate receptor blockade during development lowers long-term potentiation threshold without affecting dynamic range of CA3-CA1 synapses. *Proc. Natl. Acad. Sci. U.S.A.* 100, 5503–5508
46 Barbin, G. *et al.* (1993) Involvement of $GABA_A$ receptors in the outgrowth of cultured hippocampal neurons. *Neurosci. Lett.* 152, 150–154
47 Ji, F. *et al.* (1999) GABA and histogenesis in fetal and neonatal mouse brain lacking both the isoforms of glutamic acid decarboxylase. *Neurosci. Res.* 33, 187–194
48 Hensch, T.K. *et al.* (1998) Local GABA circuit control of experience-dependent plasticity in developing visual cortex. *Science* 282, 1504–1508
49 Misgeld, T. *et al.* (2002) Roles of neurotransmitter in synapse formation: development of neuromuscular junctions lacking choline acetyltransferase. *Neuron* 36, 635–648
50 Demarque, M. *et al.* (2002) Paracrine intercellular communication by a Ca^{2+}- and SNARE-independent release of GABA and glutamate prior to synapse formation. *Neuron* 36, 1051–1061