Developing networks play a similar melody

Yehezkel Ben-Ari

During development, when synapses start to be established, a primitive form of network-driven activity provides most of the synaptic activity. This pattern enables a high degree of synchrony in immature neurons in spite of the small number of functional synapses and could participate in activity-dependent growth and synapse formation. Relying on the giant depolarizing potentials that provide most of the synaptic activity in the developing hippocampus, this article reviews the common properties and generating mechanisms of these patterns, and particularly the role of the early depolarizing action of $GABA_A$ and glycine receptors and the sequential expression of GABA and glutamate synapses. Patterns similar to giant depolarizing potentials have been observed in a wide range of structures and species suggesting that there is a temporal template throughout evolution that constitutes an essential step in the formation of functional networks.

A fundamental question in developmental neurobiology is that of the role of activity in synapse and network formation. There are several indications that the construction of the brain involves 'nature and nurture' with both a predetermined set of genes that controls the general organization of the developing brain and activity-dependent mechanisms that modulate several essential developmental processes including migration and synapse formation or elimination[1–3]. The contribution of activity-dependent mechanisms is also reflected by the long-lasting deleterious consequences of seizures and other conditions that perturb neuronal activity during development in both humans and experimental models[4]. Thus, it is important to determine the properties of neuronal activity at initial developmental stages.

An intriguing feature of neuronal activity of developing neurons and circuits is the presence, during a limited period of time, of primitive patterns of synchronized activity. These patterns are observed first, before the formation of synapses. Thus, in embryonic *Xenopus* spinal cord neurons[5], spontaneous transient elevations of Ca^{2+} occur before synapse formation and modulate developmental processes. Several excellent reviews of these patterns have been published[5,6]. Recurrent patterns are also observed after the formation of the first synapses and in many structures these provide most of the activity for a transient period that extends from days to weeks in different species. The present review analyses these patterns, their mechanisms of generation and propagation, and possible functional significance. Relying on the giant depolarizing potentials (GDPs) that provide most of the synaptic activity of the developing hippocampus, the mechanisms underlying the generation and propagation of synchronized patterns in immature structures are reviewed. Further, this article suggests that synchronized activity plays an important role in the strategy followed by the developing brain to shift from a silent structure with no electrical activity and no synapses to an active one that possesses a highly diversified range of electrical signals and billions of selective synapses. Because

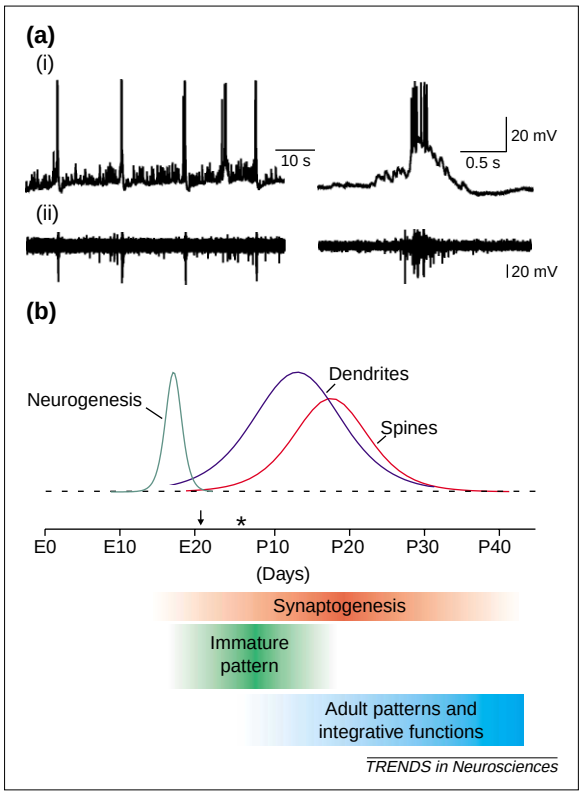

Fig. 1. Patterned spontaneous activity in the developing hippocampus. (a) In the neonatal rat hippocampus, neuronal activity is synchronized in spontaneous network discharges (GDPs). Simultaneous recordings of CA3 pyramidal cell (i) and extracellular field in CA3 pyramidal cells layer (ii) in postnatal day (P) 6 hippocampal slice. Single GDP is shown on the expanded time scale on the right. (b) Principal events in the development of the rat hippocampal network. Principal pyramidal cells are generated before birth and differentiate during the first postnatal month. Hippocampal neurons start to establish synaptic connections around birth [birth occurs at embryonic day (E) 21, indicated by arrow] and during the two postnatal weeks, most synaptic activity is synchronized in immature patterns – GDPs. Adult hippocampal patterns and hippocampal-dependent integrative functions emerge later, during the second to third postnatal weeks. Asterisk indicates P6. Curves for neurogenesis, dendritic growth and spine generation represent the first derivatives of the Boltzman fits of data from Refs 22,23,58.

Yehezkel Ben-Ari
INMED, INSERM U29, Avenue de Luminy, B.P. 13, 13273 Marseille Cedex 09, France.
e-mail: ben-ari@inmed.univ-mrs.fr

  PII: S0166-2236(00)01813-0

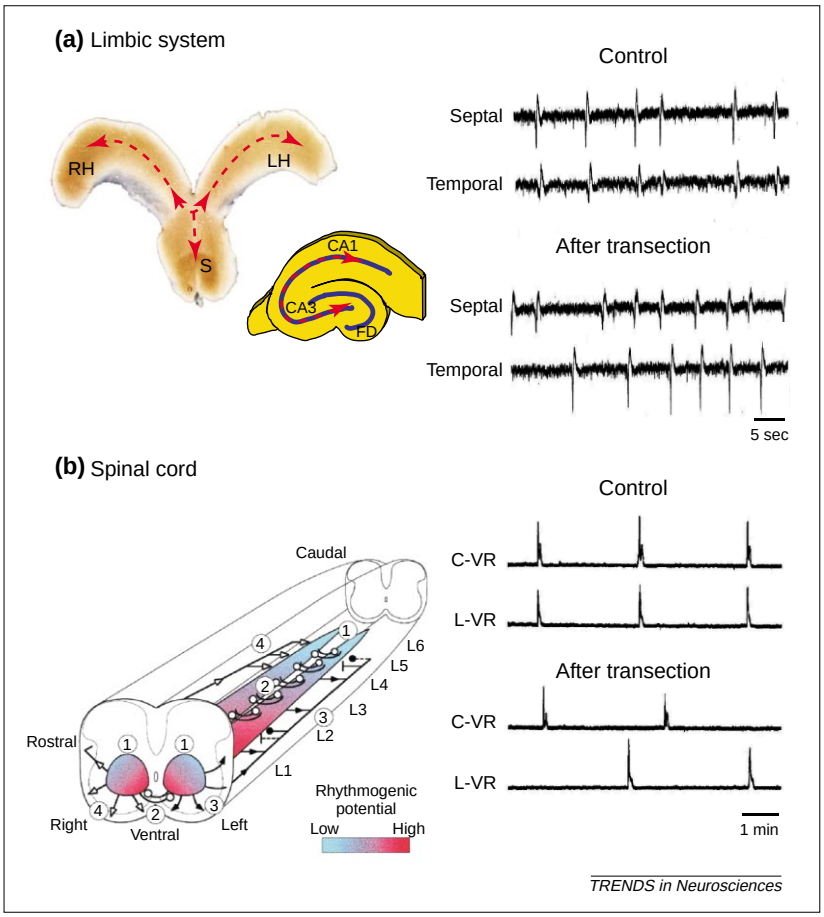

Fig. 2. Propagating waves of activity in the developing limbic system and spinal cord. (a) In the intact rat limbic system preparation *in vitro* (left), giant depolarizing potentials (GDPs) originate in the septal poles of hippocampi and propagate towards the temporal poles (field recordings, right) and septum (S). GDPs are synchronized in the left and right hippocampi (LH and RH) via commissural connections. After transection, both septal and temporal halves of hippocampus can generate GDPs, but the frequency is higher in the natural pacemaker – septal pole. Adapted, with permission, from Ref. 9. (b) In the intact neonatal rat spinal cord *in vitro* (left), spontaneous network discharges originate in the cervical part and propagate caudally (field recordings, right). After transection, both rostral and caudal parts are capable of generating spontaneous discharges (lower traces). Scheme on the left also illustrates the rhythmogenic potential in the spinal cord (based on the phase relations of the 5-HT and NMDA induced rhythmic activity). Scheme is adapted from Ref. 59 and recordings are from Ref. 51.

similar patterns are present in a wide range of structures and species this suggests that there is a temporal developmental program that underlies the formation of neuronal circuits and that has been conserved throughout evolution.

Characteristic features of hippocampal GDPs

GDPs are recurrent network-driven large synaptic events that are recorded in the rat hippocampal slices between postnatal day (P) 0 to P10 (Fig. 1a)[7]. GDPs are also observed in slice cultures (M. Demarque and H. Becq, personal observations) in the intact hippocampus *in vitro*[8,9] and *in vivo*[10]. In addition they are observed in rabbit hippocampal slices[11] and the rhesus macaque *in utero*[12] suggesting that they are conserved throughout mammalian evolution. Although, the term GDPs was used in most further studies[11,13–16], other terms have recently been used to describe the same phenomenon, including: 'giant GABAergic potentials'[17], 'early network oscillations'[18] and 'population bursts'[19,20]. This review uses the terminology GDP. The major features of GDPs are described below.

GDPs constitute the first synaptic pattern observed in the developing hippocampus

In the rat hippocampus, neurogenesis of pyramidal neurons and interneurons proceeds during the last days of embryonic life[21]; synaptic connections start to be established around the time of birth with an extensive growth of axons and dendrites that reach adult-like features at the end of the second postnatal week[22–25]. During this period of intensive growth, GDPs provide most of the on-going activity in pyramidal neurons and interneurons[7,15]. GDPs constitute the first synchronized activity; other 'adult' hippocampal patterns, including theta activity, emerge later[10,26,27]. Interestingly, in the rat, hippocampal-dependent integrative functions – learning and memories – also emerge later at the end of the first postnatal month[28–30] suggesting that GDPs occur at an early period during which the hippocampus does not generate the 'adult' patterns of activity and does not perform the integrative functions associated with these patterns (Fig. 1b).

GDPs are slowly propagating waves of activity within the limbic system

GDPs are population discharges synchronized locally within a temporal frame of several hundreds of milliseconds and recurring at a frequency of about 0.1 per s. In the transverse slice of rabbit hippocampus, GDPs mainly originate in the CA3 subfield and propagate to CA1 at a speed of 25 mm/s, however, ~20% of GDPs originate in CA1 and propagate backwards to CA3 (Ref. 11). GDPs recorded in the granular cells were less frequent than in the hippocampus proper but correlated with GDPs in CA3 (Ref. 11). In both rats and rabbits, GDPs are also observed in mini-slices of CA3 and CA1 subfields and hilus and fascia dentata[11,15,18]. This is in contrast with the observation that GDPs cease in CA3 and CA1 after removal of hilus[17]. Multisite recordings did not reveal significant delays of GDPs inside CA3 (Ref. 15). GDPs are associated with intracellular Ca^{2+} oscillations in neuronal ensembles in CA3 (Ref. 31) and CA1 (Ref. 18). Therefore, although the network elements required for the generation of GDPs are present in all hippocampal subfields, the CA3 region has the highest pacemaker activity and triggers most GDPs that propagate to the CA1 region and to fascia dentata (Fig. 2a).

Longitudinal propagation of GDPs has been addressed in the intact hippocampus *in vitro* preparation that preserves the entire hippocampal network[8,9]. Using multisite extracellular and whole-cell recordings from the CA3 hippocampal region it was shown that spontaneous GDPs can be initiated

Box 1. Dual role of GABA in the developing hippocampus

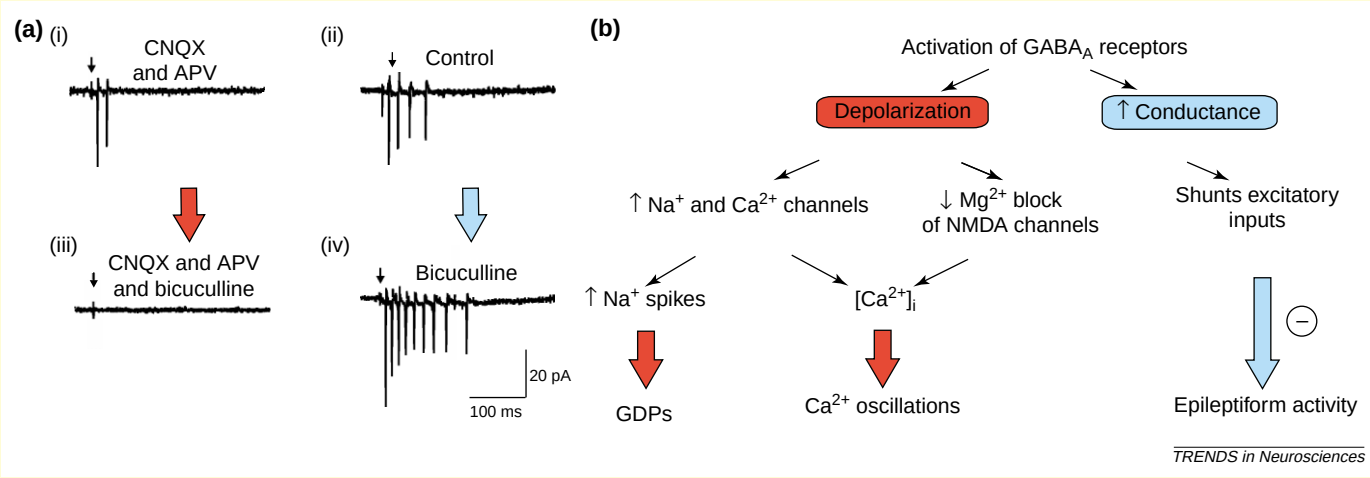

Fig. I. Activation of the $GABA_A$ receptors exerts dual – excitatory and inhibitory – effects on the immature hippocampal neurons. (a) GDPs evoked by electrical stimulation (arrow) evoke a burst of action potentials in the P4 CA3 pyramidal cell recorded in cell-attached mode. (i) Blockade of glutamate ionotropic AMPA and NMDA receptors by CNQX and APV reduces the response to two action potentials and this response is blocked by further addition of the $GABA_A$ receptor antagonist bicuculline. (ii) By contrast, application of bicuculline directly induces epileptiform discharge. This suggests that GABA excites this immature neuron but prevents over excitation produced by glutamate. Adapted from Ref. g. (b) Dual actions of GABA via activation of $GABA_A$ receptors on the immature hippocampal neurons. The dual effects of GABA will both depolarize and facilitate the generation of Na^+ and Ca^{2+} currents and remove the voltage-dependent Mg^{2+} block from NMDA receptors and at the same time shunt the glutamatergic currents when these are present, thus preventing the generation of epileptiform activities.

Although there is considerable evidence for the excitatory actions of GABA on immature neurons[a], recent studies support the idea that in the immature networks, GABA exerts dual – both excitatory and inhibitory – actions. Dual effects of GABA are caused by the reversal potential of the $GABA_A$ receptor-activated chloride conductance (E-GABA) reverses at ~35 mV (Ref. b). On the one hand, $GABA_A$ receptor-mediated depolarization excites the immature neurons. Figure Ia shows cell-attached recordings (that do not modify the intracellular Cl^-) from postnatal day (P) 4 rat CA3 pyramidal neuron. In the presence of the glutamate receptor antagonists CNQX and APV, electrical stimulation evokes two action potentials that are blocked by the $GABA_A$ receptor antagonist bicuculline (red arrow), providing evidence for the excitatory action of GABA. In addition, $GABA_A$ receptor-mediated depolarization activates voltage-gated Ca^{2+} channels and potentiates the activity of the NMDA receptors via attenuation of their voltage-dependent Mg^{2+} block[c,d] (Fig. Ib). On the other hand, E-GABA is more negative than the reversal potential of the glutamate receptor-mediated currents (near 0 mV). Therefore, when $GABA_A$ and glutamate receptors are co-activated during GDPs, $GABA_A$ receptor-activated conductance shunts the glutamate receptor-mediated currents and prevents the epileptiform activity (Fig. Ia, blue arrow)[g–k] (See Fig. 3 in main text). Interactions between $GABA_A$ and glutamate receptors in the immature neurons also depends on the temporal relationship of their activation. The glutamate-induced depolarization can be shunted during the peak and potentiated during the decay phase of the GABA-induced responses[e,f]. Thus, the net effect of GABA in the immature neurons will depend on various factors, including the values of E-GABA, the ratio of the GABA:NMDA:AMPA receptor-activated conductances and their spatial distribution and timing of activation (Fig. Ib).

References

a Ben-Ari, Y. *et al.* (1997) GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage a trois'. *Trends Neurosci.* 20, 523–529
b Ben-Ari, Y. *et al.* (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol.* 416, 303–325
c 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
d Leinekugel, X. *et al.* (1995) Synaptic GABAA activation induces Ca^{2+} rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *J. Physiol.* 487, 319–329
e Chen, G. *et al.* (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. *J. Physiol.* 494, 451–464
f Gao, X.B. *et al.* (1998) GABA-dependent firing of glutamate-evoked action potentials at AMPA/kainate receptors in developing hypothalamic neurons. *J. Neurophysiol.* 79, 716–726
g Khalilov, I. *et al.* (1999) Dual role of GABA in the neonatal rat hippocampus. *Dev. Neurosci.* 21, 310–319
h Palva, J.M. *et al.* (2000) Fast network oscillations in the newborn rat hippocampus *in vitro*. *J. Neurosci.* 20, 1170–1178
i Autere, A.M. *et al.* (1999) Synaptic activation of GABAA receptors induces neuronal uptake of Ca^{2+} in adult rat hippocampal slices. *J. Neurophysiol.* 81, 811–816
j Bolea, S. *et al.* (1999) Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. *J. Neurophysiol.* 81, 2095–2102
k Wells, J.E. *et al.* (2000) GABAergic inhibition suppresses paroxysmal network activity in the neonatal rodent hippocampus and neocortex. *J. Neurosci.* 20, 8822–8830

anywhere along the longitudinal septo-temporal axis (Fig. 2a). However, GDPs are most often initiated in the septal pole of the hippocampus and propagate towards the temporal pole at a speed of 7.5 ± 2 mm/s. Also, there is a higher probability that a GDP propagates from front to backward than in the other direction. This rostro-caudal gradient of propagation is associated with a parallel gradient of

Box 2. Sequential maturation of GABA and glutamate

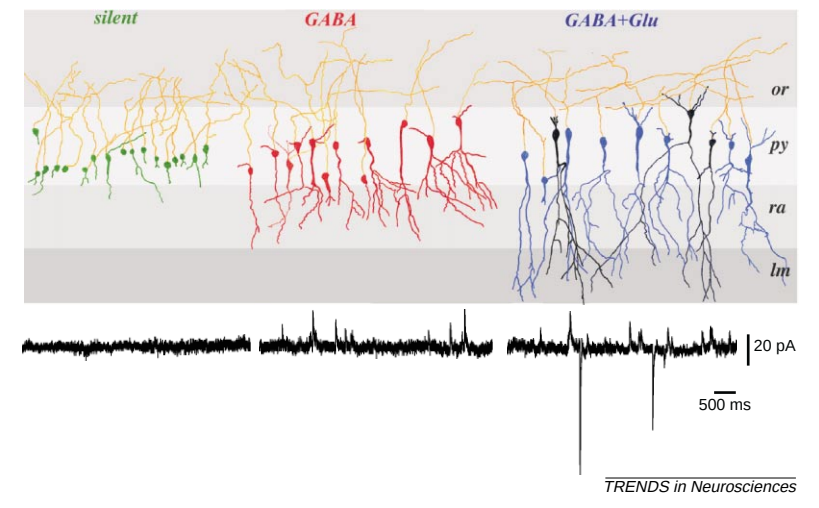

Fig. I. Sequential maturation of the GABAergic and glutamatergic synaptic transmission in the neonatal rat hippocampus. Patch-clamp whole-cell recordings of CA1 pyramidal neurons at birth. The neurons were recorded at a holding potential of 40 mV (upper traces) and –70 mV (lower traces). Three different type of CA1 pyramidal cells are encountered at birth: cells that are synaptically silent (A1), cells with only GABAergic synaptic activity (A2) and cells with both GABAergic and glutamatergic synaptic activity.

In the rat, at birth, CA1 pyramidal neurons can be differentiated into three groups: (1) neurons that have an axon but no dendrites (80% of the neurons) and have no functional synapses (no spontaneous or evoked PSCs); (2) neurons that have an axon, a small apical dendrite (10% of the neurons), these neurons have GABAergic synapses only; (3) neurons that have extended apical dendrites and basal dendrites (10% of the neurons), and also have GABA and glutamate synapses[a]. Therefore, GABAergic synapses are established before glutamatergic ones and the formation of glutamatergic synapses requires that the target neuron has an apical dendrite that reaches the distal superficial regions. The capacitance of the neurons – an estimation of its arbor – allows the neuron group to which it belongs to be determined. Furthermore, synaptophysin and GAD-immunolabeling are enriched in the apical dendrites at an early stage – they are virtually absent from the pyramidal layer suggesting that the first GABAergic synapses are established on the apical dendrites of the principal neurons. Interestingly, GABAergic interneurons become postmitotic before pyramidal neurons[b] and have the same sequence but at an earlier stage (H. Gozlan and Y. Ben-Ari, unpublished). Tracing studies show that the principal glutamatergic fibers are present before birth[c] suggesting that the afferent fibers are present but glutamate synapses will not be established as long as the target neuron has not reached a certain degree of maturity. Consistent with this, in rat fetal hippocampi, the first glutamatergic synapses are established with GABAergic interneurons[c] suggesting that interneurons are the source and the target of the first synapses established in the hippocampus. GABAergic synapses also have an earlier development than glutamatergic ones in other structures and in cultures[d,e]. Within the various families of glutamatergic receptors, NMDA receptor-mediated PSCs appear to be functional before AMPA receptor-mediated PSCs. Durand and collaborators[f] have also suggested that pairing stimuli or GDP-like current pulses generate the functional expression of AMPA receptor PSCs. Although other interpretations are possible[g,h], these observations are compatible with an earlier expression of NMDA receptors, followed by an activity-dependent progressive expression of AMPA receptor-mediated PSCs. Because GABA provides the sole source of excitatory synaptic activity at an early stage and acts in synergy with NMDA receptors, it is suggested that the repetitive activation of GABA and NMDA receptors will control the expression of AMPA receptor-mediated synapses. The long duration of GABA and NMDA receptor-mediated currents, will facilitate the summation of synaptic currents. Interestingly, there is also a sequential maturation of pre- and postsynaptic G-protein-mediated K^+ currents, with presynaptic but not postsynaptic $GABA_B$, adenosine and 5-HT receptors being functional at an early stage[i]. Therefore, the only transmitter-gated type of inhibition that operates at an early stage is the presynaptic control of transmitter release by G-protein-coupled receptors (see Fig. 3 in main text).

References

a 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

b Soriano, E *et al.* (1989) Neurogenesis of glutamic acid decarboxylase immunoreactive cells in the hippocampus of the mouse. I: regio superior and regio inferior. *J. Comp. Neurol.* 281, 586–602

c Diabira, D. *et al.* (1999) Late embryonic expression of AMPA receptor function in the CA1 region of the intact hippocampus *in vitro*. *Eur. J. Neurosci.* 11, 4015–4023

d Chen, G. *et al.* (1995) GABA receptors precede glutamate receptors in hypothalamic development; differential regulation by astrocytes. *J. Neurophysiol.* 74, 1473–1484

e Gao, X.B. and van den Pol, A.N. (2001) Long-term potentiation and functional synapse induction in developing hippocampus. *J. Neurophysiol.* 85, 425–434

f Durand, G.M. *et al.* (1996) Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* 381, 71–75

g Asztely, F. *et al.* (1997) Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. *Neuron* 18, 281–293

h Gasparini, S. *et al.* (2000) Silent synapses inthe developing hippocampus: lack of functional AMPA receptors or low probability of glutamate release? *Proc. Natl. Acad. Sci. U. S. A.* 97, 9741–9746

i Gaïarsa, J.-L. *et al.* (1995) Postnatal development of pre- and postsynaptic $GABA_B$-mediated inhibitions in the CA3 hippocampal region of the rat. *J.Neurophysiol.* 73, 246–255

GDP frequency in isolated portions of hippocampus. In the two interconnected hippocampi preparations[9] GDPs were highly synchronized in the septal poles of both hippocampi and propagated temporally to the caudal poles of both hippocampi and to the medial septum (Fig. 2a). The early inter-hemispheric propagation of GDPs suggest that commissural connections are functional at an early stage. These observations suggest that although local circuits from any region of the neonatal

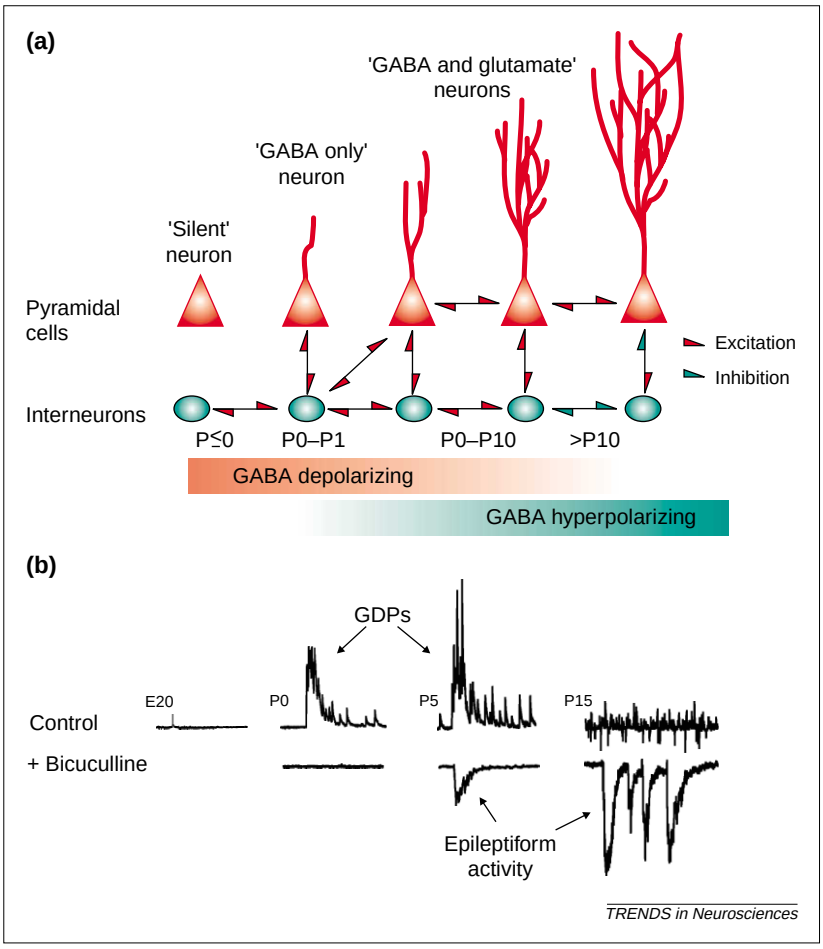

Fig. 3. Development of the hippocampal network and coordinated activity. (a) Scheme of development of the hippocampal circuitry in the rat. Hippocampal neurons start to receive first GABAergic and later glutamatergic synaptic inputs around birth. During the temporal window, when GABA depolarizes the immature neurons, all synaptic loops are excitatory and network generates GDPs. GDPs cease when GABA becomes hyperpolarizing. (b) Developmental changes in the synaptic activity associated with maturation of the hippocampal network. At E20, all pyramidal neurons are silent with no functional synapses (trace shows the evoked response). At P0, a small percentage of pyramidal neurons have GABAergic inputs, whereas almost all interneurons express both GABA and glutamate synapses (see Box 2). Consequently, in 'GABA only' neurons, GDPs are mediated exclusively by $GABA_A$ receptors and blocked by the $GABA_A$ receptor antagonist bicuculline. At P2–P10 (P5 is shown as an example), the percentage of GABA-only neurons is rapidly reduced and the expression of glutamate synapses increases in parallel, thus GDPs are generated by GABA and glutamate receptors. At that stage, blockade of $GABA_A$ receptors induces epileptiform activity synchronized by the recurrent glutamatergic collateral synapses (see also Box 1). At P15, GABA is hyperpolarizing in all neurons and GDPs are not present in the slice. The age-dependence of the scheme differs in various structures and probably depends on various biological parameters that might modify the speed at which the developmental program is executed (i.e. nutrition conditions, number of offspring per litter, the level of hormones that modulate the depolarizing effects of GABA, etc.).

hippocampus can generate GDPs, in the intact network the septal pole of CA3 paces most GDPs in the transverse and longitudinal hippocampal planes, respectively. The immature hippocampus is reminiscent of the heart in which there are several pacemakers but only one normally paces the activity because of its higher frequency.

Mechanisms of generation of GDPs: GABA sets the tune but is not the only player

In the initial description of GDPs (Ref. 7), using intracellular recordings, several observations were made suggesting that, in immature neurons, GABA has a depolarizing action that plays a central role in the generation of GDPs. The depolarizing effects of GABAergic synapses during development are considered a milestone of developing networks because they have been confirmed in every species and structure studied[32,33]. This is as a result of higher concentrations of intracellular concentrations of chloride $[Cl^-]_i$ in immature neurons following late development of chloride transporters[34]. However, glutamate receptor antagonists can also reduce the frequency of GDPs (Ref. 7) suggesting a participation of these receptors for the generation of GDPs. We proposed a model based on a mutual excitation of interneurones by glutamatergic synapses that originate in pyramidal neurons and by the recurrent excitation of pyramidal neurons by excitatory GABAergic synapses.

More recent studies suggest that the depolarization produced by GABAergic synapses can excite neurons if the depolarization exceeds the threshold of Na^+ action and leads to the activation of voltage-gated Ca^{2+} channels thus increasing the concentration of intracellular Ca^{2+} $[Ca^{2+}]_i$ (Ref. 35). GABA also acts in synergy with NMDA channels by reducing the voltage-dependent Mg^{2+} block, thus leading to activation of NMDA receptors and a further rise of $[Ca^{2+}]_i$ (Ref. 31). These events operate in physiological conditions to generate LTP and LTD of developing GABAergic synapses[36,37]. However, more recent studies also suggest that GABA produces an inhibition of glutamatergic synapses, an effect mediated by shunting mechanisms (Box 1). In addition, using an intracellular dialysis method that enables the blockade of $GABA_A$ receptors in the recorded pyramidal neuron[15], GDPs were found to include AMPA and NMDA receptor-mediated currents (see also Refs 14,19). Therefore, although GABAergic synapses play a central role in GDPs, there is also a substantial contribution of glutamate PSCs. One factor to consider is the high degree of heterogeneity of pyramidal neurons and interneurons that prevail at this early developmental stage. Indeed, in a recent study, we reported a sequential expression of GABA and glutamate, the latter becoming functional in more-developed neurons (Box 2). Therefore, within the same slice, some neurons will have only GABAergic synapses – and GABAergic GDPs – whereas other neurons will have GABA and glutamate synapses and therefore a variable degree of participation of glutamatergic PSCs in the GDPs. Thus, maturational gradients might be determinant in the relative contribution of GABA and glutamate in GDPs.

The mechanism by which GDPs terminate has been addressed in rat hippocampal slices. It was shown that bath application of the $GABA_B$ receptor antagonist lengthened spontaneous GDPs (Ref. 38), an effect that is probably mediated by the blockade of presynaptic $GABA_B$ receptors that, similar to

adenosine and serotonine receptors, are functional early in development, in contrast to postsynaptic receptors[39,40]. Other mechanisms explaining the termination of GDP-like patterns in the spinal cord also exist[41].

GDP-like patterns: a hallmark of developing networks

The presence of patterned, spontaneous activity is a remarkably well preserved feature of the developing neuronal network. In vertebrates, patterned spontaneous activities have been described in an impressive number of structures, including the spinal cord[41], the retina[42], the auditory system[43], the trigeminal nucleus[44] and neocortex[45]. In the retina, following the first description by Galli and Maffei[42] in rat fetuses *in vivo* of spontaneous bursting activity, *in vitro* studies have shown that amacrine and ganglion cells are recruited in waves that sweep across a restricted portion of the retina well before vision develops in species as different as chick, ferret, rabbit, and turtle (for review see Ref. 46). A co-ordinated, spontaneous rise in $[Ca^{2+}]_i$ has also been reported in groups of neighboring neocortical ventricular zone neurons[47] and neocortical networks[45]. GDPs similar to those observed in the rat are also present in hippocampal slices of rhesus macaques *in utero*[12].

In spite of some differences in mechanisms of generation, GDP-like patterns have, in all structures and species studied, the following common features.

- They are present during a restricted window of development corresponding to a period of intense synaptogenesis and neuronal growth. They are also the first pattern of synaptic activity observed in immature circuits.
- They are globally similar in different structures, characterized by relatively slowly propagating recurrent waves of activity and random firing of units in a temporal frame of several hundreds of milliseconds.
- They propagate both within structures and between connected structures that will be part of functional ensembles. Thus, in *in vitro* slices that include the retina and lateral geniculate nucleus (LGN) the bursting activity of ganglion cells is transmitted to LGN. In addition, retinal waves drive LGN neurons to fire action potentials, suggesting that the activity is further conveyed to the visual cortex[48]. In the spinal cord, synchronous GDPs propagate according to various gradients (dorso-ventral, rostro-caudal, medio-lateral, Fig. 2b).
- They are always associated with $[Ca^{2+}]_i$ oscillations. These can be generated by a Ca^{2+} influx through NMDA channels and voltage-gated Ca^{2+} channels initiated by the activation of $GABA_A$ receptors in the neonatal hippocampus[31,35] or by the formation of inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3] that diffuses through gap junctions into neighboring cortical cells and causes Ca^{2+} release[49,50].
- They coincide with the depolarizing action of GABA and glycine even when other transmitters are responsible for their generation (see below). In the retina, spinal cord, hypothalamus and neocortex, application of GABA or glycine receptors antagonists initially abolishes or reduces the frequency of spontaneous activity[18,45,51–54].
- The mechanisms of generation of patterns are modified during development. Thus, in the retina, excitation is initially provided by ACh and GABA (depolarization) and later switches to glutamate[52,55,56]. Similarly, in the spinal cord, glycine[53] or ACh and GABA (Ref. 57) trigger spontaneous network activities early in development, and subsequently glutamate plays an important role (for review see Ref. 56). However, in most structures, GABA (or glycine) contributes initially to their generation[54].

Therefore, extremely different neuronal networks generate patterned, spontaneous activity governed by similar fundamental rules. I suggest that the shift during development of the chloride permeable channels – GABA and glycine – is instrumental in the generation of GDPs. From the available published data an initially depolarizing action of GABA and a delayed shift to hyperpolarizing effects have been observed in every structure and species studied, including the primate hippocampus[15], suggesting that similar to GDPs, this constitutes a fundamental feature of developing circuits. Currently, however, it is not clear whether in addition to the hippocampus, GABAergic synapses are also formed before glutamatergic synapses in other structures (Box 2). Although speculative, it is important to stress that depolarizing GABAergic synapses are endowed with potentially important features for a developing circuit: they simultaneously provide an excitatory drive – necessary to increase $[Ca^{2+}]_i$ and stimulate growth – and a shunting action that is inherent to GABA synapses. This will prevent epileptogenesis and excitotoxic effects that would have been caused by an early expression of glutamate synapses. In this scenario, the shift from depolarizing to hyperpolarizing actions of GABA will take place once the target neuron has reached a certain degree of maturation and has a significant number of glutamatergic synapses.

Conclusion

The immature brain is not a small adult brain. Several processes take place primarily or exclusively in the developing brain including cell division, differentiation and migration, synapse formation and elimination, programmed cell death and the formation of brain structures. To perform these functions, several receptors, ionic channels, second messengers, growth factors and signaling molecules have unique features during development. The pace at which these changes take place is highly variable depending on the species, the brain structure and the

multiple developmental gradients that operate. Consequently, the immature nervous system exhibits a high degree of heterogeneity: two neurons from the same region that will be difficult to distinguish at the adult stage, can be extremely different at birth: one having a well developed dendrite and functional synaptic currents, the other having no dendrites and no functional synapses. In spite of this heterogeneity, both the shift from depolarizing to hyperpolarizing actions of GABA and glycine, and the presence of a universal primitive pattern of synchronized activities appear to be a hallmark of developing networks.

The functional significance of GDP-like patterns remains to be determined. In general these patterns are not suited for fast information integrative function and processing because of their time window of operation (~100 ms –1 s) and slow speed of propagation between structures (~1 mm/s). Also, they predominate at a time when sensory information and higher functions are not present. Indeed, oscillations, observed at an early stage before the formation of synapses, control extension and motility of neurites in addition to synthesis and expression of the GABAergic phenotype[5,6]. With the expression of the first synapses, patterns change their mode of generation progressively (from pacemaker intrinsic to synaptic driven) with initially GABAergic synapses and a progressive increase of the contribution of glutamate synapses. I suggest that the functional significance of GDP-like patterns also depends on maturational gradients: initially, a 'nonselective metabolic' role providing, via the large rise of $[Ca^{2+}]_i$, a signal for growth and maturation. Progressively, with the maturation of the dendrites and the formation of a large number of glutamate synapses, GDPs exert a more selective control of synapse formation and the hyperpolarizing actions of GABA prevail (Fig. 3). Future studies will hopefully determine the role of activity in the various synchronized developmental alterations.

Acknowledgements
I am extremely indebted to Jean-Luc Gaiarsa and Rustem Khazipov for their help in putting together this review, their suggestions and their enthusiasm.

References

1 Goodman, C.S. (1996) Mechanisms and molecules that control growth cone guidance. *Annu. Rev. Neurosci.* 19, 341–377
2 Katz, L.C. and Shatz, C.J. (1996) Synaptic activity and the construction of cortical circuits. *Science* 274, 1133–1138
3 Feller, M.B. (1999) Spontaneous correlated activity in developing neural circuits. *Neuron* 22, 653–656
4 Holmes, G.L. and Ben-Ari, Y. (1998) Seizures in the developing brain: perhaps not so benign after all. *Neuron* 21, 1231–1234
5 Spitzer N.C. (1995) Spontaneous activity: functions of calcium transients in neuronal differentiation. *Perspect. Dev. Neurobiol.* 2, 379–386.
6 Spitzer, N.C. (1994) Spontaneous Ca^{2+} spikes and waves in embryonic neurons: signaling systems for differentiation. *Trends Neurosci.* 17, 115–118
7 Ben-Ari, Y. *et al.* (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J. Physiol.* 416, 303–325
8 Khalilov, I. *et al.* (1997) A novel in vitro preparation: the intact hippocampal formation. *Neuron* 19, 743–749
9 Leinekugel, X. *et al.* (1998) Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septohippocampal complex *in vitro*. *J. Neurosci.* 18, 6349–6357
10 Leinekugel, X. *et al.* (1999) Network activity in the hippocampus of neonatal rats *in vivo*. *Soc. Neurosci. Abstr.* 25, 2266
11 Menendez de la Prida, L. *et al.* (1998) Origin of the synchronized network activity in the rabbit developing hippocampus. *Eur. J. Neurosci.* 10, 899–906
12 Khazipov, R. *et al.* (1999) Early maturation of hippocampal network in the fetal cynomolgus monkey. *Soc. Neurosci. Abstr.* 25, 2266
13 Xie, X. and Smart, T.G. (1991) A physiological role for endogenous zinc in rat hippocampal synaptic neurotransmission. *Nature* 349, 521–524
14 Bolea, S. *et al.* (1999) Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. *J. Neurophysiol.* 81, 2095–2102
15 Khazipov, R. *et al.* (1997) Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. *J. Physiol.* 498, 763–772
16 Strata, F. *et al.* (1995) Cyclic AMP-dependent modulation of giant depolarizing potentials by metabotropic glutamate receptors in the rat hippocampus. *J. Physiol.* 489, 115–125
17 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
18 Garaschuk, O. *et al.* (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. *J. Physiol.* 507, 219–236
19 Lamsa, K. *et al.* (2000) Synaptic $GABA_A$ activation inhibits AMPA-kainate receptor-mediated bursting in the newborn (P0-P2) rat hippocampus. *J. Neurophysiol.* 83, 359–366
20 Palva, J.M. *et al.* (2000) Fast network oscillations in the newborn rat hippocampus *in vitro*. *J. Neurosci.* 20, 1170–1178
21 Bayer, S.A. (1980) Development of the hippocampal region in the rat II. Morphogenesis during embryonic and early postnatal life. *J. Comp. Neurol.* 190, 115–134
22 Minkwitz, H.G. (1976) Zur Entwicklung der neuronenstruktur des hippocampus während des prä- unf postnatalen ontogenese des albinoratte *J. Hirnforsch.* 17, 233–253
23 Englisch, H.J. *et al.* (1974) Distribution of spines on the pyramidal neurons in the CA-1 region of the hippocampus in the rat. *Z. Mikrosk. Anat. Forsch.* 88, 85–102
24 Gaïarsa, J-L. *et al.* (1992) Effect of neonatal degranulation on the morphological development of rat CA3 pyramidal neurons : Inductive role of mossy fibers on the formation of thorny excrescences. *J. Comp. Neurol.* 321, 612–625
25 Gomez-Di Cesare, C.M. *et al.* (1997) Axonal remodeling during postnatal maturation of CA3 hippocampal pyramidal neurons. *J. Comp. Neurol.* 384, 165–180
26 Leblanc, M.O. and Bland, B.H. (1979) Developmental aspects of hippocampal electrical activity and motor behavior in the rat. *Exp. Neurol.* 66, 220–237
27 Creery, B.L. and Bland, B.H. (1980) Ontogeny of fascia dentata electrical activity and motor behavior in the Dutch belted rabbit (*Oryctolagus cuniculus*). *Exp. Neurol.* 67, 554–572
28 Rudy, J.W. *et al.* (1987) Ontogeny of spatial navigation behaviors in the rat: dissociation of 'proximal'- and 'distal'-cue-based behaviors. *Behav. Neurosci.* 101, 62–73
29 Freeman, J.H. and Stanton, M.E. (1991) Fimbria-fornix transections disrupt the ontogeny of delayed alternation but not position discrimination in the rat. *Behav. Neurosci.* 105, 386–395
30 Altman, J. *et al.* (1973) The hippocampus and behavioral maturation. *Behav. Biol.* 8, 557–596
31 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
32 Cherubini, E. *et al.* (1991) GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci.* 14, 515–519
33 Leinekugel, X. *et al.* (1999) GABA is the principal fast-acting excitatory transmitter in the neonatal brain *Adv. Neurol.* 79, 189–201
34 Rivera, C. *et al.* (1999). The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* 397, 251–255
35 Leinekugel, X. *et al.* (1995) Synaptic GABAA activation induces Ca++ rise in pyramidal cells

and interneurons from rat neonatal hippocampal slices. *J. Physiol.* 487, 319–329
36 Caillard, O. *et al.* (1999) Mechanisms of induction and expression of long-term depression at GABAergic synapses in neonatal rat hippocampuc. *J. Neurosci* 19,
37 McLean, H.A. *et al.* (1996) Bidirectional plasticity expressed by GABAergic synapses in the neonatal rat. *J. Physiol.* 496, 471–477
38 McLean, H.A. *et al.* (1996) Spontaneous release of GABA activates $GABA_B$ receptors and controls network activity in the neonatal rat hippocampus. *J. Neurophysiol.* 76, 1036–1046
39 Caillard, O. *et al.* (1998) Ontogenesis of presynaptic GABAB receptor-mediated inhibition in the CA3 region of the rat hippocampus. *J. Neurophysiol.* 79, 1341–1348
40 Gaiarsa, J.L. *et al.* (1995) Postnatal maturation of gamma-aminobutyric acidA and B-mediated inhibition in the CA3 hippocampal region of the rat. *J. Neurobiol.* 26, 339–349
41 O'Donovan, M.J. (1989) Motor activity in the isolated spinal cord of the chick embryo: synaptic drive and firing pattern of single motoneurons. *J. Neurosci* 9, 943–958
42 Galli, L. and Maffei, L. (1988) Spontaneous impulse activity of rat retinal ganglion cells in prenatal life. *Science* 242, 90–91
43 Lippe, W.R. (1994) Rythmic spontaneous activity in the avian auditory system. *J. Neurosci.* 14, 1486–1495
44 Ho, S.M. and Waite, P.M. (1999) Spontaneous activity in the perinatal trigeminal nucleus of the rat. *NeuroReport* 10, 659–664
45 Garaschuk, O. *et al.* (2000) Large-scale oscillatory calcium waves in the immature cortex. *Nat. Neurosci.* 3, 452–459
46 Wong, R.O. (1999) Retinal waves and visual system development. *Annu. Rev. Neurosci.* 22, 29–47
47 Owens, D.F. and Kriegstein, A.R. (1998) Patterns of intracellular calcium fluctuation in precursor cells of the neocortical ventricular zone. *J. Neurosci.* 18, 5374–5388
48 Mooney, R. *et al.* (1996) Thalamic relay of spontaneous retinal activity prior to vision. *Neuron* 17, 863–874
49 Owens, D.F. and Kriegstein, A.R. (1998) Patterns of intracellular calcium fluctuation in precursor cells of th eneocortical ventricular zone. *J. Neurosci.* 18, 5374–5388
50 Kandler, K. and Katz, L.C. (1998) Coordination of neuronal activity in developing visual cortex by gap junction-mediated biochemical communication. *J. Neurosci.* 18, 1419–1427
51 Nakayama, K. *et al.* (1999) Rostrocaudal progression in the development of periodic spontaneous activity in fetal rat spinal motor circuits *in vitro*. *J. Neurophysiol.* 81, 2592–2595
52 Fischer, K.F. *et al.* (1998) Age-dependent and cell class-specific modulation of retinal ganglion cell bursting activity by GABA. *J. Neurosci.* 18, 3767–3778
53 Fellipa-Marques, S. *et al.* (2000) Spontaneous and locomotor-related GABAergic input onto primary afferents in the neonatal rat. *Eur. J. Neurosci.* 12, 155–164
54 Gao, B-X. and van den Pol, A.N. (2001) GABA, not glutamate, a primary transmitter driving action potentials in developing hypothalamic neurons. *J. Neurophysiol.* 85, 434
55 Feller, M.B. *et al.* (1996) Requirement for cholinergic synaptic transmission in the propagation of spontaneous retinal waves. *Science* 272, 1182–1187
56 O'Donovan, M.J. (1999) The origin of spontaneous activity in developing networks of the vertebrate nervous system. *Curr. Opin. Neurobiol.* 9, 94–104
57 Milner, L.D. and Landmesser, L.T. (1999) Cholinergic and GABAergic inputs drive patterned spontaneous motoneuron activity before target contact. *J. Neurosci.* 19, 3007–3022
58 Bayer, S.A. (1980) Development of the hippocampal region in the rat II. Morphogenesis during embryonic and early postnatal life. *J. Comp. Neurol.* 190, 115–134
59 Kjaerulff, O. and Kiehn, O. (1996) Distribution of networks generating and coordinating locomotor activity in the neonatal rat spinal cord in vitro: a lesion study. *J. Neurosci.* 16, 5777–5794

Editorial policy

Most articles published in *TINS* are commissioned by the Editor. However, authors who wish to contribute to any section of the journal should contact the Editor with the names of all authors and a point-by-point outline of the proposed article including 10–12 key references. Outlines may also be sent to members of the Advisory Editorial Board. Completed manuscripts submitted without liaison with the *TINS* Editorial Office cannot be considered. *TINS* readers who wish to suggest that a particular topic be covered are also invited to send their suggestions to the Editor.

Correspondence and comment on any aspect of *TINS* or the field of neuroscience are welcomed, and should be sent directly to the Editor.

Are you interested in writing short Headline articles?

We are planning to extend our In Brief section and are looking to recruit another news writer.

We are looking for an enthusiastic scientist who would act both as section editors and neuroscience journalists to compile these reports. The role would be particularly suitable for research scientists with an interest in writing who have a broad interest in neuroscience and could act to filter and distil highlights of news for each issue.

Source material can include online news and information, printed news sources and primary literature. If you are interested, then please contact The Editor, *Trends in Neurosciences*, Elsevier Science London, 84, Theobald's Road, London WC1X 8RR, United Kingdom (Tel +44 [0]20 7611 4130; Fax +44 [0]20 7611 4485), or e-mail The Editor at tins@current-trends.com for further details.