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GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations

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Ben-Ari Y, Gaiarsa JL, Tyzio R, Khazipov R. GABA: A Pioneer Transmitter That Excites Immature Neurons and Generates Primitive Oscillations. *Physiol Rev* 87: 1215–1284, 2007; doi:10.1152/physrev.00017.2006.—Developing networks follow common rules to shift from silent cells to coactive networks that operate via thousands of synapses. This review deals with some of these rules and in particular those concerning the crucial role of the neurotransmitter γ-aminobuytric acid (GABA), which operates primarily via chloride-permeable $GABA_A$ receptor channels. In all developing animal species and brain structures investigated, neurons have a higher intracellular chloride concentration at an early stage leading to an efflux of chloride and excitatory actions of GABA in immature neurons. This triggers sodium spikes, activates voltage-gated calcium channels, and acts in synergy with NMDA channels by removing the voltage-dependent magnesium block. GABA signaling is also established before glutamatergic transmission, suggesting that GABA is the principal excitatory transmitter during early development. In fact, even before synapse formation, GABA signaling can modulate the cell cycle and migration. The consequence of these rules is that developing networks generate primitive patterns of network activity, notably the giant depolarizing potentials (GDPs), largely through the excitatory actions of GABA and its synergistic interactions with glutamate signaling. These early types of network activity are likely required for neurons to fire together and thus to "wire together" so that functional units within cortical networks are formed. In addition, depolarizing GABA has a strong impact on synaptic plasticity and pathological insults, notably seizures of the immature brain. In conclusion, it is suggested that an evolutionary preserved role for excitatory GABA in immature cells provides an important mechanism in the formation of synapses and activity in neuronal networks.

I. INTRODUCTION AND HISTORICAL PERSPECTIVES

γ-Aminobutyric acid (GABA) is an inhibitory transmitter, acting on a receptor channel complex permeable mainly to chloride anions that act to reduce neuronal excitability. As such, GABAergic signaling plays a major role in brain physiology, and dysfunction of GABAergic signaling can result in pathological conditions such as epilepsies that are generated when the balance between excitation and inhibition is impaired (16, 178, 179, 181, 284, 525–527). Recent studies suggest a more complex scope of functions for GABAergic signaling than just global inhibition. For example, the heterogeneity in types of GABAergic synapses and interneurons unraveled in the last decade suggests that an array of GABAergic signaling functions may exist (139, 165, 201, 497, 497). GABAergic neurons also control the generation of behaviorally relevant patterns and oscillations that may turn out to be far more important than inhibition per se. In addition, GABA depolarizes neurons because of a "reversed" chloride gradient in a wide range of neuron types and animal species, notably invertebrates (82, 163, 177, 178, 182, 229, 285, 355, 356, 674). Even in adult mammalian cortical neurons, dendritic GABAergic action is depolarizing because of a locally reversed Cl^- gradient and not a different ionic mechanism, as was thought for some years (14; also see Refs. 111, 244, 418, 587; for reviews, see Refs. 420, 589). However, the vast majority of central actions of GABA are inhibitory.

In this review, we discuss these issues from the standpoint of brain maturation. Studies on brain development have greatly increased our understanding of how the brain operates and how cortical networks integrate neuronal activity. The observation that has renewed interest in studying GABA in development is the discovery of a higher $[Cl^-]_i$ in immature neurons that leads to excitatory actions of GABA in immature neurons (50). The progressive reduction of $[Cl^-]_i$ has now been confirmed in every animal species and brain structure investigated, suggesting that the depolarizing to hyperpolarizing (D-H) switch associated with an excitatory to inhibitory (E-I) shift has been preserved during evolution and provides a solution to a major developmental problem. Hence, the central issues are as follows: Why is the chloride gradient reduced during brain maturation? What are the underlying mechanisms and functional significance? What are the implications of these rules in the construction of cortical networks? It is has been suggested (49) that this sequence enables developing neurons and networks to equilibrate glutamatergic and GABAergic drives and avoid transient overexcitation or overinhibition if the former or the latter predominate.

Here, we first review the main features of GABA receptors and GABAergic synapses. Bearing in mind that GABA exerts a multitude of actions on developmental processes well before synapses are functional, we shall then review the early actions of GABA on migration, cell growth, and synapse formation. The earlier formation of GABAergic synapses, the initial excitatory actions of GABA, and the generation of primitive activity patterns are then analyzed. The $GABA_B$ metabotropic receptor G protein-activated channels during development will be briefly reviewed. We then review the mechanisms of GABAergic synapse plasticity. Finally, we discuss the role of depolarizing GABA in relation to the high prevalence of seizures during early development and the pathological plasticity of GABA signaling in epileptogenesis. Since studies using the hippocampus have provided many of the initial observations and the concepts derived from them, we shall review these first before discussing other brain structures. We shall review only in brief the organization of GABA receptor subunits as this has been extensively reviewed recently (490, 492).

II. BASIC PROPERTIES OF GABA SIGNALING

The amino acid GABA prevails in the adult central nervous system (CNS) as an inhibitory neurotransmitter that mediates most of its effects through two classes of receptors: $GABA_A$ and $GABA_B$ receptors.

A. $GABA_A$ Receptors

$GABA_A$ receptors consist of pentameric assembly of distinct subunits that forms a central ion channel permeable to chloride, and to a lesser extent, bicarbonate anions. To date, 19 $GABA_A$ receptors subunits have been cloned in the mammalian CNS. This diversity offers a great potential heterogeneity of $GABA_A$ receptor subunit composition, which is further increased by alternative splicing. The molecular composition of the $GABA_A$ receptors has important functional consequences as it determines the properties, pharmacological modulation, and targeting of the native receptors.

$GABA_A$ receptors are ligand-gated ion channels permeable to chloride and bicarbonate with a net effect that depends on the electrochemical gradient of these anions (297). Under physiological conditions, $GABA_A$ receptor activation generates a membrane hyperpolarization and a reduction of action potential firing. However, this classical view has been challenged by recent studies showing that $GABA_A$ receptor-mediated responses reversal potential (E_{GABA}) is close to, or even at a more depolarized potential than, the resting membrane potential (E_m), thus leading to a membrane depolarization (244, 418). Shunting inhibition is an alternative mechanism of inhibition, in which hyperpolarizing and depolarizing $GABA_A$ receptor-mediated responses reduce dendritic excitatory glutamatergic responses via a local increase in conductance across the plasma membrane. $GABA_A$ receptor-mediated shunting occurs in a narrow window near the peak of $GABA_A$ receptor-induced synaptic responses and requires a close temporal overlap between glutamatergic and GABAergic synaptic responses (244, 586). Hyperpolarizing and depolarizing $GABA_A$ receptor-mediated synaptic responses can enhance cell excitability; thus hyperpolarizing responses trigger rebound spikes that can pace population activity (202). Dendritic GABAergic depolarizing responses combined with subthreshold membrane depolarization can elicit action potentials in adult cortical pyramidal neurons (244). In some cerebellar interneurons, $GABA_A$ receptor-mediated responses reversed at -58 mV, and activation of presynaptic GABAergic afferents leads to postsynaptic firing (111). The polarity of $GABA_A$ receptor-mediated responses can also change during physiological cycles or pathological conditions. In the suprachiasmatic nucleus, GABA triggers excitation during the day and inhibition during the night (645). Following repeated activation, $GABA_A$ receptor-mediated responses can switch from a hyperpolarizing to depolarizing direction and can enhance cell firing (499). This activity-dependent switch also occurs during epileptiform activity where it may contribute to generate epileptiform activity (206, 325, 339).

The activation of $GABA_A$ receptors by the release of GABA leads to both phasic inhibitory postsynaptic currents (IPSCs) and tonic currents as revealed by the outward holding current and decrease in background noise induced by $GABA_A$ receptors antagonists (303, 477, 554). Tonic $GABA_A$ receptor-mediated currents were observed early in pre- and postnatal life (158) but not in adult pyramidal cells, unless the concentration of GABA was increased (96, 554, 665). The tonic current results from GABA spillover acting on extrasynaptic receptors with different subunit composition and pharmacological profile compared with the synaptic receptors (250, 477, 554, 590). The functional role of the tonic current remains to be determined. The net effect of the tonic current is an increase in input conductance, thus decreasing the input-output relationship of the neurons (107). Moreover, the total charge carried by the tonic current in granule cells and interneurons is larger than the averaged charge carried by the spontaneous phasic current, thus pointing to an important role in regulating the network excitability.

B. $GABA_B$ Receptors

GABA also acts on $GABA_B$ receptors that operate through G_i and G_o proteins (68, 140, 449) localized on both pre- and postsynaptic membranes. Activation of

postsynaptic receptors generally causes activation of inwardly rectifying potassium channels (GIRK or Kir3) that underlie the late phase of inhibitory postsynaptic potentials (170, 407). Activation of presynaptic $GABA_B$ receptors decreases neurotransmitter release by inhibiting voltage-activated Ca^{2+} channels of the N or P/Q types (13, 435, 448, 508, 545), although mechanisms independent of changes in membrane conductance have also been proposed (449). Activation of $GABA_B$ receptors also modulates cAMP production (256, 564), leading to a wide range of actions on ion channels and proteins that are targets of the cAMP-dependent kinase (protein kinase A or PKA), and thus modulate neuronal and synaptic functions (228, 538).

To date, genes encoding two different subunits, $GABA_{B1}$ and $GABA_{B2}$, have been identified (81, 283, 310, 503, 551). Fully functional $GABA_B$ receptors require the coassembly of the two different subunits, since neither the $GABA_{B1}$ nor the $GABA_{B2}$ is active when expressed independently (194, 294, 311, 361, 524, 662). However, when coexpressed, recombinant $GABA_{B1,2}$ receptors mediate all predominant effects of native receptors, i.e., modulation of cAMP production, activation of GIRK channels, and inhibition of P/Q- and N-type Ca^{2+} channels (175, 194, 416). Moreover, in $GABA_{B1}$ or $GABA_{B2}$ knockout mice, all $GABA_B$ receptor-mediated functions were absent (226, 511, 547). However, the general assumption that heterodimerization of $GABA_{B1}$ and $GABA_{B2}$ subunits is required has been recently challenged by the observation of responses with receptor subunit expressed in isolation (226, 417).

III. DEPOLARIZING/EXCITATORY ACTIONS OF GABA DURING DEVELOPMENT

A. Early Studies on Actions of GABA on Immature Neurons

Contradictory observations were made in early studies concerning the maturation of GABAergic inhibition (174, 254, 512, 549). Thus in vivo studies of kitten hippocampus suggested that inhibition is the predominant form of early synaptic activity (512). In contrast, studies in hippocampal slices suggested that excitatory synaptic events are more common in young animals and that inhibitory synaptic activity appears fairly late in the kitten (549), rabbit (548), and rat (166, 254) hippocampus. Probably the first suggestion of a developmentally regulated shift of GABA actions was made by Obata et al. (481) in spinal cord neurons. Applications of GABA or glycine depolarized 6-day-old chick spinal neurons in culture and hyperpolarized 10-day-old embryos (481). Using intracellular recordings, Schwartzkroin and colleagues found depolarizing responses to somatic GABA application and a depolarizing GABAergic component of synaptic responses in neonatal (P6–10) rabbit hippocampal CA1 pyramidal neurons. The E_m was of −53 mV, and the reversal potentials of the somatic responses to GABA and GABAergic postsynaptic potentials were of −36 and −46 mV, respectively (467), although a more negative value of −54 mV of the somatic E_{GABA} was reported in a previous study (466). The authors suggested that depolarizing GABA inhibits via shunting mechanisms. In mature pyramidal cells, the E_m was of −59 mV, and the reversal potentials of the somatic responses to GABA and GABAergic IPSPs were of −71 and −67 mV, respectively. The authors suggested that these developmental changes are due to two types of GABA receptors/channels: a hyperpolarizing type permeable to chloride and a depolarizing type permeable to sodium and/or calcium in addition to chloride. Although subsequent studies suggested different actions of GABA in dendrites and somata of adult neurons (9, 10, 14, 612), the developmental changes in GABAergic signaling are clearly due to alterations of $[Cl^-]_i$.

In a study performed in 1989, the developmental changes of GABAergic signaling in neonatal hippocampal slices were investigated using intracellular recordings from CA3 pyramidal cells (56). The principal findings of this study can be summarized as follows: *1*) GABA acting via $GABA_A$ receptors depolarizes and excites the immature neurons, due to an elevated concentration of $[Cl^-]_i$ in immature cells that is reduced progressively with development; *2*) neuronal activity at an early developmental stage is provided by a network primarily driven by synchronized $GABA_A$-mediated giant depolarizing potentials (GDPs); *3*) GABAergic activity is expressed first and precedes glutamatergic (AMPA receptor-mediated) synaptic transmission during development; and *4*) early glutamatergic synapses are predominantly based on postsynaptic NMDA receptors. The developmental excitatory to inhibitory (E-I) switch in the action of GABA and reversal potential of GDPs and GABAergic responses occurred at postnatal day P5–P7. Although various details of these observations have been recently revised (see below), the principal conclusions of this study have been confirmed in a wide range of preparations suggesting that the progressive reduction of $[Cl^-]_i$ is a general developmental rule that has been conserved throughout evolution.

B. Multiple Facets of Depolarizing and Excitatory GABA During Development

1. GABA depolarizes immature neurons

a) INTRACELLULAR RECORDINGS. Early demonstrations of depolarizing actions of GABA on immature neurons were obtained mainly using intracellular recordings (56, 405, 467, 481). However, intracellular recordings introduce several sources of errors including alterations in the intracellular ionic composition which affects E_{GABA} and

neuronal depolarization, both errors being particularly important in immature neurons. Indeed, sharp electrodes used for intracellular recordings are filled with electrolyte in the molar range that exceeds severalfold the ionic composition in intact cells. Dialysis of the cell during recordings with such electrodes will alter intracellular ionic composition. For example, using intracellular recordings, E_{GABA} in the neonatal CA3 pyramidal cells at P2–5 was estimated at −25 mV with KCl-filled electrodes and at −51 mV with potassium methylsulfate-filled electrodes (56). In addition to direct dialysis of anions, $[Cl^-]_i$ may change as a result of the alteration in the activity of the cation-chloride cotransporters. For example, potassium-filled sharp electrodes could elevate $[K^+]_i$ and increase KCC2 driving force, whereas cesium-filled electrodes could block KCC2. Besides the dialysis problem, intracellular recordings using sharp electrodes introduce leak conductance in the range of 500 MΩ that could also affect $[Cl^-]_i$ because of exchange of Cl^- between the cell and external solution via leak conductance. Leak conductance also introduces an important error in the estimation of E_m causing neuronal depolarization, the artifact being maximal in small neurons with high membrane resistance (37, 626). Nevertheless, the depolarizing effects of GABA have now been confirmed with less invasive recording techniques.

B) GRAMICIDIN PERFORATED PATCH RECORDINGS. To overcome the problem of intracellular dialysis, Marty and colleagues have developed a technique of perforated patch recordings. This is based on ionophores (polyene antibiotics) that are inserted in plasma membrane in cell-attached configuration to obtain electrical access to cell (278, 419, 514). Polypeptide antibiotic gramicidin forms channels in membranes that are selectively permeable to small cations but not anions (469) and therefore are suitable for noninvasive recordings of $GABA_A$ and glycine responses (1, 176, 364, 517).

With the use of gramicidin perforated patch, GABA- and glycine-evoked depolarization were found in cultured rat dorsal horn neurons (515, 653), cortical neurons (244, 399, 418, 488, 632), hippocampal pyramidal neurons (31, 215, 367, 625) and interneurons (31, 205), cerebellar interneurons and Purkinje cells (111), hypothalamic neurons (113), and chick cochlear neurons (401) (Table 1). Examples of the depolarizing and excitatory responses evoked by the $GABA_A$ agonist isoguvacine and synaptic activation of the $GABA_A$ receptors in a P2 CA3 pyramidal cell recorded using gramicidin perforated patch are shown in Figure 1. In CA3 pyramidal cells, E_{GABA} measured using gramicidin perforated patch was of −55 mV during the early neonatal period and progressively shifted to −74 mV by the end of the second postnatal week (625) (see also Ref. 31). However, while gramicidin perforated patch recordings eliminate the problem of intracellular dialysis and thus provide accurate estimate of E_{GABA}, it does not solve the problem of leak conductance and associated neuronal depolarization that particularly affects immature cells with a gigaohms range membrane resistance. For example, gramicidin perforated patch measurements gave an estimate of E_m in P0–2 CA3 pyramidal cells at around −40 to −50 mV, whereas noninvasive measurements of E_m using cell-attached recordings of NMDA channels gave value of −77 mV (626).

C) CELL-ATTACHED RECORDINGS OF $GABA_A$ CHANNELS. Depolarizing actions of GABA on immature neurons have been reported with cell-attached recordings of single $GABA_A$ channels. This noninvasive technique has substantial advantages over other electrophysiological approaches to measure $GABA_A$ driving force (DF_{GABA}) because it affects neither E_{GABA} nor E_m. In addition, this methodology is not compromised by space-clamp problems. In cell-attached recordings, the driving force on ions across $GABA_A$ channels is $-DF_{GABA} - V_p$, where V_p is the pipette potential. Therefore, DF_{GABA} is $-V_p$ at the reversal potential of the currents through $GABA_A$ channels. Using cell-attached recordings of single $GABA_A$ channels in embryonic rat spinal and olfactory bulbar cells, Serafini et al. (557) have shown that GABA-activated chloride channels reverse polarity when the on-cell patch pipette potential was approximately −30 mV, indicating that E_{GABA} is more depolarized than the resting membrane potential by 30 mV (557). A similar approach revealed strongly depolarizing values of DF_{GABA} in CA3 pyramidal cells during fetal (40 mV) and postnatal (20 mV) periods and a transient depolarizing-to-hyperpolarizing switch in the action of GABA at term (624). While cell-attached recordings of $GABA_A$ channels give an accurate estimate of DF_{GABA}, to deduce E_{GABA} it is necessary to know E_m, which can be achieved by cell-attached recordings of NMDA (377, 626) or K^+ channels (31, 204, 638). These types of recordings confirmed that the depolarizing action of GABA is due to the positive values of E_{GABA} in immature neurons.

D) CELL-ATTACHED RECORDINGS OF K^+ AND NMDA CHANNELS. Cell-attached recordings of K^+ (204, 401, 573, 638, 638, 679) and NMDA channels (377, 624, 626) used to monitor membrane potential have also revealed depolarizing actions of GABA in immature neurons. This technique was first used in adult neurons by Zhang and Jackson (679), who used changes in the amplitude of currents through single K^+ channels to monitor changes in the membrane potential in response to GABA in the membranes of peptidergic nerve terminals of the posterior pituitary (679). With K^+ concentration in the recording pipette equal to intracellular K^+ concentration, K^+ channels reversed at pipette potential equivalent to the membrane potential. A similar approach was used to determine the depolarizing actions of GABA on adult granular cells and hilar neurons (573). Miles and colleagues (638) studied the developmental profile of GABA actions using K^+ channels to monitor membrane potential in hippocampal interneurons. Application of $GABA_A$ agonists depolarized CA1 interneurons

TABLE 1. *Depolarizing and excitatory effects of GABA and glycine in developing brain structures*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Retina	Chick E3	Retina	Ca^{2+} imaging, intracellular recordings	GABA and muscimol depolarize and increase intracellular Ca^{2+} via activation of VGCCs.	Yamashita and Fukuda, 1993
Retina	Ferret P0–25	Intact retina, retinal slices	Ca^{2+} imaging, whole cell	Both GABA and glycine increase Ca^{2+} in retinal ganglion cells at P0–10 and decrease Ca^{2+} after P15; $GABA_A$ antagonists suppress spontaneous bursting activity of ganglion cells at P0–10; diverse effects at P15 and increase in burst frequency at >P21. ON and OFF cells bursting difference emerges as GABA becomes inhibitory.	Fischer et al., 1998
Retina	Turtle S22-PH3	Retina	Ca^{2+} imaging	GABA is excitatory at S25 and switches to inhibitory around hatching, coinciding with a transformation of retinal waves to stationary patches.	Sernagor et al., 2003
Retina	Rabbit E29-P26	Retina starburst cells	Ca^{2+} imaging	Muscimol increases Ca^{2+} in starburst cells at E29 and has no detectable effect at P5, concomitant with an emergence of strong inhibitory GABA action on waves.	Zheng et al., 2004
Spinal cord	Chick E6–10	Spinal cord explants	Intracellular recordings	Deplarizing and excitatory at E6–8 and hyperpolarizing at E10.	Obata et al., 1978
Spinal cord	Chick E11–16	Isolated spinal cord	Intracellular recording	Hyperpolarization and inhibition at E11–16.	Velumian 1984
Spinal cord	Chick E10–11	Isolated spinal cord	MEQ intracellular Cl^- measurements	Depolarizing; Cl^- reduces during network burst and recovers partly via NKCC1.	Chub et al., 2006
Spinal cord	Rat E14–18	Dissociated cells	Voltage-sensitive dye	No detectable response before E14; depolarizing at E14–18.	Mandler et al., 1990
Spinal cord	Rat E16–P2	Hemisected spinal cord preparation, motoneurons	Intracellular recordings	Depolarization at E16–21 and almost isoelectric at P1–2; glycine and $GABA_A$ receptor antagonists block response to dorsal root stimulation at E16–18 and increases it at E19-P2.	Wu et al., 1992
Spinal cord	Rat E15–16+ 3–28 div	Neuronal culture, dorsal horn	Gramicidin perforated patch, whole cell, Ca^{2+} imaging	GABA and glycine increase intracellular Ca^{2+} via activation of VGCCs and cause neuronal depolarization during the first week in culture; number of responding cells decreases with age, and none of cells responds at >30 div.	Wang et al., 1994; Reichling et al., 1994
Spinal cord and olfactory bulb	Rat E15	Dissociated cells	Cell-attached rec. of $GABA_A$ channels	Depolarization by 30 mV; $GABA_A$ channel openings occasionally trigger action potentials.	Serafini et al., 1995
Spinal cord	*Xenopus laevis* larvae 3–8 days old	Spinal cord in vivo; primary sensory Rohon-Beard neurons and dorsolateral interneurons	Intracellular, whole cell, amphotericin B and gramicidin perforated patch recordings	Depolarizing, action of $GABA_A$ via $GABA_A$ receptors on Rohon-Beard neurons and hyperpolarizing effects in dorsolateral interneurons; bumetanide negatively shifts E_{GABA} in Rohon-Beard neurons.	Rohrbough and Spitzer, 1996
Spinal cord	Rat E13–18	Spinal cord preparation	Ventral root potentials	Depolarizing and excitatory at E13–15; GABA and glycine antagonists suppress network bursts at E14–5.	Nishimaru et al., 1996
Spinal cord	Rat E15–19	Slices, lumbar motoneurons	Perforated patch clamp; Ca^{2+} imaging	Depolarization; entry of Ca^{2+} via Ca^{2+} channels.	Kulik et al., 2000
Spinal cord	Mouse KCC2 knockout E18.5	Slice, ventral part, motoneurons	Perforated patch clamp, field potentials	$KCC2^{-/-}$ mice die after birth due to motor deficits that also abolish respiration. GABA and glycine are more depolarizing in $KCC2^{-/-}$ mice than in wild type.	Hubner et al., 2001
Spinal cord	Rat P0–5	Brain stem-spinal cord preparation	Intracellular recording from L2–5 motoneurons; extracellular root recordings	Isoelectric inhibitory in motoneurons; depolarizing and excitatory in primary afferents.	Vinay and Clarac 1999; Fellippa-Marques et al., 2000
Spinal cord	Rat P0–7	Slices, dorsal horn (mainly L11 neurons)	Gramicidin perforated patch	Depolarizing in 40% of neurons at P0–2; hyperpolarizing in all cells by P6–7.	Baccei & Fitzgerald, 2004

TABLE 1—*Continued*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Spinal cord	Rat P0–60	Slices, dorsal horn L1 neurons	Gramicidin perforated patch, whole cell, Ca^{2+} imaging	Depolarizing during the first week, D-H switch is complete by P7; GABA causes Ca^{2+} increase until P21 coincides with biphasic H-D response to GABA; chloride extrusion does not reach maturity by P10–11.	Cordero-Erausquin et al., 2005
Brain stem	Chick E17-P10	Slices, cochlear mucleus	Cell-attached potassium channels, gramicidin perforated patch	Depolarizing, dual excitatory and inhibitory depending on context of their activation.	Lu and Trussel, 2001, Monsivais and Rubel, 2001
Brain stem	Rat E18-P17	Slices, LSO neurons	Intracellular recording	Depolarizing at E18-P4 and hyperpolarizing after P8.	Kandler and Friauf, 1995
Brain stem	Rat P2–13	Slices, LSO neurons	Gramicidin perforated patch	Depolarizing in 59%, hyperpolarizing in 5%, and biphasic in 34% of cells; $E_{Glycine}$ shifts positively during the response to glycine, independently of HCO_3.	Backus et al., 1998
Brain stem	Rat P2–11	Slices, LSO neurons	Gramicidin perforated patch	Depolarizing and excitatory (30% of cells) at P1–4 and hyperpolarizing at P9–11; D-H switch around P5–8.	Ehrlich et al., 1999
Brain stem	Rat, mice P3–12	Slices, LSO neurons	Gramicidin perforated patch, molecular biology	Depolarizing during the first week, D-H switch occurs at P8 in wild-type but not in KCC2 KO; KCC2 is present early but is not active; NKCC1 mRNA not detected during the early D phase.	Balakrishnan et al., 2003
Brain stem	Rat, mice P1–15	Slices, LSO neurons	Ca^{2+} imaging	Both GABA and glycine (exogenous and evoked by MNTB stimulation) increase Ca^{2+} during the first postnatal week; slightly decrease Ca^{2+} in 2-wk-old animals.	Kullman et al., 2002
Brain stem	Rat, E18-P10	Slices, LSO, MSO, MSN, MTNB	Voltage-sensitive fluorescent dye, gramicidin perforated patch	D-H switch was determined in four superior olivary complex nuclei: MSO P5–9, SPN E18-P1, no D-H switch in MTBN neurons, LSO P4–5 but delayed in low-frequency regions; mere expression of KCC2 did not correlate with depolarizing GABA.	Lohrke et al., 2005
Brain stem	Rat P0–18	Slices, hypoglossal motoneurons	Gramicidin perforated patch	Depolarizing in P0–3 and hyperpolarizing in P10–18 motorneurons.	Singer et al., 1998
Brain stem	Mouse P0–15	Slices, Pre-Botzinger complex neurons	Gramicidin perforated patch from PBC neurons, field potentials from XII rootlets	Depolarizing at P0–2, hyperpolarizing at P4 (D-H switch at around P3), depolarizing action persists in bicarbonate-free saline. Bicuculline does not affect the frequency of rhythmic XII motor output at P0–3 and increases it after P3.	Ritter and Zhang, 2000
Basal ganglia	Rat P4–26	Slices, substantia nigra pars reticulata	Gramicidin perforated patch	Depolarizing in neonatal cells; D-H switch occurred in males at around P17 and in females at around P10.	Kyrosis et al., 2006
Hypothalamus	Rat	Neuronal culture E15–18 + 2–25 div	Ca^{2+} imaging	GABA elevates, and $GABA_A$ antagonist depresses intracellular Ca^{2+} via VGCCs in the immature neurons; both effects on intracellular Ca^{2+} switch between 8–13 div.	Obrietan and van den Pol, 1995
Hypothalamus	Rat	Neuronal culture E15 +1–7 div	Ca^{2+} imaging	GABA increases and bicuculline reduces Ca^{2+} levels in the growth cones.	Obrietan and van den Pol, 1996
Hypothalamus	Rat	Neuronal culture	Gramicidin perforated patch clamp	GABA depolarizes and often excites neurons in young cultures (1–7 div) and hyperpolarizes and inhibits at 20–33 div. E_{GABA} shifts from −40 to −70 mV. E_{GABA} is more negative in neurons dissociated from P5 compared the E15. Endogenous GABA excites as bicuculline reduces ongoing firing. GABA-evoked depolarization could facilitate or shunt other depolarizing input depending on temporal relationship between GABA-evoked depolarization and other excitatory events.	Chen et al., 1996

TABLE 1—*Continued*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Hypothalamus	Rat	Neuronal culture E15–18+2–5 div	Gramicidin perforated patch, whole cell	Depolarizing; shunts glutamate-mediated depolarization at the peak and facilitates excitatory action of glutamate during the decay phase of GABA response.	Chen et al., 1998
Hypothalamus (and spinal cord)	Mice	Slice, P1–10 neuronal cultures E15–18+4–>20 div	Gramicidin perforated patch, extracellular	Depolarizing and excitatory in the immature neurons (slices: P1–4; cultures: 4–7 div) and inhibitory in mature (slices: P8–10; cultures: >20 div). $GABA_A$ antagonist decreases firing in immature neurons and increases in mature neurons. $GABA_A$ excitatory drive is more important than glutamatergic in the immature neurons.	Gao et al., 2001
Hypothalamus	Mice	Slice and neuronal culture	Gramicidin perforated patch, whole cell	Depolarizing and excitatory at P2–9 (exogenous and synaptic GABA) and 2–23 div cultures; patterns of GABA-dependent action potentials (single or multiple spikes) depend on intrinsic membrane properties of hypothalamic neurons.	Wang et al., 2001
Inferior colliculus	Gerbil	Slices	Ca^{2+} imaging, intracellular	Ca^{2+} increase, biphasic hyperpolarizing-depolarizing response.	Lo et al., 1998
Cerebellum	Rat P3−5+4–30 div	Explant culture, granule cells	Ca^{2+} imaging	Ca^{2+} increase that outlasts the exposure to GABA by several minutes.	Connor et al., 1987
Cerebellum	Rat P2–22	Slice, Purkinje neurons	Ca^{2+} imaging, gramicidin perforated patch	Depolarizes and increases Ca^{2+} during the first postnatal week; D-H switch and loss of Ca^{2+} increase to GABA occurs at the end of the 1st postnatal week.	Eilers et al., 2001
Cerebellum	Rat P4–28	Slice, granule cells	Gramicidin perforated patch, whole cell	Depolarizing and occasionally excitatory at P7; isoelectric and shunting at P18–21.	Brickley et al., 1996
Hippocampus	Kitten P1–18	In vivo, responses to fornix stimulation	Intracellular recordings	Inhibitory (hyperpolarizing) IPSPs are evoked in hippocampal neurons by fornix stimulation at P1–3; spontaneous IPSPs are similar in duration, EPSPs are not prominent in young kittens.	Purpura et al., 1968
Hippocampus	Kitten P2–28	Slice, CA1 neurons	Intracellular recordings	IPSPs and EPSPs are seen at all ages. IPSPs block cell discharge even in the youngest preparations.	Schwartzkroin and Altschuler, 1977
Hippocampus	Rabbit P6-1 mo	Slice, CA1 pyr. cells	Intracellular recordings	Depolarizing at P6–10 in soma and dendrites; depolarizing in dendrites and hyperpolarizing in soma in adult.	Mueller et al., 1993, 1984; Janigro and Schwartzkroin, 1988
Hippocampus	Rat P1–64	Slice, CA1	Field Potential	Paired-pulse simulation reveals facilitation at P1–5 and inhibition at P6–64; "spontaneous unison firing" pattern (GDPs?) blossoms at P4–5 and disappears at P6.	Harris and Teyler, 1983
Hippocampus	Rat P0–P18	Slice, CA3 pyr. cells	Intracellular recordings	Depolarizing and excitatory at P2–5; hyperpolarizing and inhibitory from P6 onwards. Giant depolarizing potentials (GDPs) are generated by excitatory GABA. Blockade of $GABA_A$ receptors suppresses GDPs until P5 and induces epileptoform activity from P6 onwards. Glycine action displays similar D-H developmental profile.	Ben-Ari et al., 1989; Cherubini et al., 1990; Ito and Cherubini 1991; Chesnut and Swann 1989
Hippocampus	Rat P10–P15	Slice	Field Potential, intracellular recordings	Muscimol produces epileptiform activity and disinhibition at P10–15.	Chesnut and Swann, 1989
Hippocampus	Rat P3-adult	Slice, CA3 and CA1	Intracellular and field potential recording	Hyperpolarizing IPSPs are first observed by P5–6 in CA3 and by P9 in CA1 neurons. In CA3, bicuculline causes epileptiform events in slices from immature but not month-old rats. Equilibrium potential of IPSPs in CA3 neurons was similar when made during the first postnatal week and at 1 mo of age.	Swann et al., 1989
Hippocampus	Rat E17–21 and P5–7	Dissociated cells	Fluorescence-activated cell sorter	Depolarizing at E17–21; depolarizing and hyperpolarizing in cell subpopulations at P5–7.	Fiszman et al., 1990

TABLE 1—*Continued*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Hippocampus	Rat P2–20	Slice, CA1 pyramidal cells	Whole cell recordings	Isoelectric response at P2–5; E_{GABA} at P2–5 is predicted by GHK, hyperpolarizing and more negative than GHK value at P8–20.	Zhang et al., 1991
Hippocampus	Rat P2–5	Slice, CA3 pyr. cells	Cell-attached action potentials; cell-attached NMDA channels; Ca^{2+} imaging	Depolarizing and excitatory; decrease in Mg^{2+} blockade of NMDA receptors via depolarization; influx of Ca^{2+} via VGCCs and NMDA channels. Synergistic action of $GABA_A$ and NMDA receptors is involved in Ca^{2+} oscillations associated with the GDPs.	Leinekuge et al., 1995; 1997
Hippocampus	Rat E17+5–8 div	Neuronal culture	MQAE Cl^- measurements in dendrites and soma	Pericarionic $[Cl^-]_i$ is lower than dendritic $[Cl^-]_i$; furosemide and bumetanide stronger decrease in $[Cl^-]_i$ in dendrites than in soma.	Hara et al., 1992
Hippocampus	Rat P0–16	Slice, CA1	Ca^{2+} imaging, whole cell	Early network oscillations (ENOs = equivalent of GDPs) in CA1 are blocked by bicuculline; muscimol increases intracellular Ca^{2+} in all cells at P1–12 involved in ENOs. Ca^{2+} increase gradually decreases over the first 2 postnatal weeks.	Garaschuk et al., 1998
Hippocampus	Rat P0–2	Slice, CA3 and CA1 pyramidal cells	Field potentials, gramicidin perforated patch, whole cell	Depolarizing and excitatory at cellular level but inhibitory at network level; Muscimol blocks GDPs; bicuculline induces interictal activity.	Lamsa et al., 2000
Hippocampus	Rat P2–5	Slice, CA3 interneurons	Cell-attached action potentials	Depolarizing and excitatory; synergistic action of GABA and glutamate (AMPA and NMDA) receptors in interneuron excitation during GDPs; blockade of $GABA_A$ receptors induces epileptiform activity from P2.	Khazipov et al., 1997
Hippocampus	Rat (Wistar) P0–5	Slice, intact hippocampus in vitro, CA3 pyramidal cells	Cell-attached; whole cell	Excitatory at P2–5 and inhibitory at P10–15 (synaptic-GABA on single unit); $GABA_A$ agonist and diazepam transiently increase GDPs frequency; $GABA_A$ antagonist blocks GDPs and induces interictal events from P0 and ictal events from P2.	Khalilov et al., 1999
Hippocampus	Rat P1–34 (Sprague-Dawley)	Slice, CA3 pyramidal cells	Field potentials, whole cell, cell-attached	Excitatory from P1 to P10, excitation-to-inhibition switch from P10 to P14; inhibitory after P15 ($GABA_A$ agonist test on MUA); dual role in seizure control.	Khazipov et al., 2004
Hippocampus	Rat P1–30	Slice, CA3 pyramidal cells	Field potential	Bicuculline action switches from depressing to increasing CA3 MUA at P12 (3.5 mM external K^+) and at P23 (8.5 mM external K^+). Ictal events caused by 8.5 mM K^+ are blocked by gabazine and bicuculline and are enhanced by isoguvacine and muscimol.	Dzhala et al., 2003
Hippocampus	Rat	Slice, CA3 pyramidal cells	Field potential, whole cell	Depolarizing and excitatory, NKCC1 blockade shifts E_{GABA} negatively and alleviates high-potassium seizures in vitro and kainate seizures in vivo.	Dzhala et al., 2005
Hippocampus (and neocortex)	Mouse P0–7, rat P1–5	Slice	Field potentials	Anticonvulsive; starting from P2–3, $GABA_A$ blockade induces paroxysmal activity both in the hippocampus and neocortex.	Wells et al., 2000
Hippocampus	Rat P1–21	Slice, CA1 interneurons	Cell-attached potassium channels	Depolarizing response to $GABA_A$ agonist at P1–4 and at P7–21 to similar level (−55 mV).	Verhuegen et al., 1999
Hippocampus	Mice P3–32	Neuronal culture; slice, CA1 pyr. cells	Whole-cell, local GABA photolysis	Chloride extrusion progressively develops during the two first postnatal weeks.	Khirug et al.,
Hippocampus	Rat E18+ div4–18	Neuronal culture	Ca^{2+} imaging, gramicidin perforated patch	Induces influx of Ca^{2+} via voltage-gated Ca^{2+} channels and depolarizes during 2 wk after plating; D-H switch correlates with KCC2 expression and is promoted by GABA.	Ganguly er et al., 2001
Hippocampus	Rat P1–9	In vivo P7–9 and slices P1–8	Field potentials, gramicidin perforated patch	Depolarizing, blockade of NKCC1 renders GABA hyperpolarizing and blocks sharp waves in vivo and GDPs in vitro.	Sipila et al., 2006

TABLE 1—*Continued*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Hippocampus	Rat P0–2	Slices, CA3 and CA1 pyramidal cells	Field potentials, gramicidin perforated patch, whole cell	Depolarizing and excitatory but inhibits AMPA-kainate receptor-mediated excitatory inputs to pyramidal cells and limits the size of activated population during GDPs.	Lamsa et al., 2000
Hippocampus	Rat P0–30, guinea pig P0–40	Slices (acute and short-term slice cultures)	Intracellular recordings, molecular biology	Rat: depolarizing at P0–4 and hyperpolarizing at P12–30; D-H switch is coupled to expression of KCC2; guniea pig: hyperpolarizing at P0–40.	Rivera et al., 1999
Hippocampus	Rat P4 + up to 6 wk in culture	Organotypic slice; CA3 and CA1	Gramicidin perforated patch, whole cell	Depolarizing but inhibitory in a majority of cells and excitatory in a minority of cells; GDPs are present from 12 div; GDPs are transiently blocked by bicuculline and increased in frequency by isoguvacine.	Mohajerani and Cherubini, 2005
Hippocampus	Rat P0–6	Slices, CA3 pyramidal cells	Whole cell, cell-attached, perforated patch, field potentials	Tonic and phasic depolarizing GABAergic input is crucial to promote intrinsic bursting of CA3 pyramidal cells and generation of GDPs.	Sipila et al., 2005
Hippocampus	Rat P0–15	Slices, CA3 pyramidal cells	Gramicidin perforated patch, field potential	Gradual developmental shift in E_{GABA} over P0–15 with a midpoint of disappearance of the excitatory effects of GABA at P8. The effect of isoguvacine and synaptic GABA on MUA switches from an increase to a decrease at P10 and P8, respectively.	Tyzio et al., 2006
Dentate	Mice adult	Newly generated granular cells	Gramicidin perforated patch, whole cell	Strongly depolarizing in newborn granular cells; GDP-like GABAergic events in a subpopulation of newly generated granular cells.	Ge et al., 2006; Overstreet-Wadiche et al., 2006
Neocortex	Rat P4–41	Slices, layer II/III pyramidal and nonpyramidal cells	Intracellular recordings	Depolarizing at P4–10 and hyperpolarizing at P11–41.	Luhmann and Prince, 1991
Neocortex	Rat, cat	Slices	Ca^{2+} imaging	GABA increases Ca^{2+} in the neonatal neocortical neurons via VGCCs.	Yuste and Katz, 1991
Neocortex, visual	Rat E18-P30	Slices	Ca^{2+} imaging	Ca^{2+} increase at E18-P5, reduction in Ca^{2+} increase after P5 and no detectable response after P20.	Lin et al., 1994
Neocortex	Rat E16–18	Slices, ventricular zone	Gramicidin perforated patch; Ca^{2+} imaging	Strongly depolarizing in VZ cells (E_{GABA} = −5 mV); induces influx of Ca^{2+} via voltage-gated Ca^{2+} channels.	LoTurco et al., 1995
Neocortex	Rat E16-P16	Slice	Gramicidin perforated patch, Ca^{2+} imaging	Strongly depolarizing in fetal and neonatal neurons; progressive negative shift in E_{GABA} over development; induces influx of Ca^{2+} via voltage-gated Ca^{2+} channels in embryonic and early postnatal cells.	Owens et al., 1996
Neocortex	Rat E19-P5	Slices	Whole cell	GABA application increases frequency of GABA-PSCs, suggesting excitation of interneurons at P3–4.	Owens et al., 1999
Neocortex	Rat P0–11	Slices, Layer 2–6, stimulation of layer 1	Whole cell, gramicidin perforated patch	Depolarizing and excitatory during the first postnatal week.	Dammer man et al., 2000
Neocortex	Rat P0–12	Slices	Ca^{2+} imaging	GABA induces Ca^{2+} transients in the neonatal neurons; number of responsive cells reduces from nearly 100% during the first postnatal week to ~20% at P12; Ca^{2+} ENOs are not blocked by bicuculline.	Garaschuk et al., 2000
Neocortex	Rat 2nd half of gestation (mainly E19)	Single-cell suspensions from CP/SP and VZ/SVZ zones	Flow cytometry and microscopy of Ca^{2+} and potentiometric signals, whole cell	GABA and muscimol depolarize and increase Ca^{2+} mainly in cells undergoing neuronal differentiation and only in a fraction of precursor and progenitor cells; autocrine GABAergic signaling tonically depolarizes and increases Ca^{2+} in CP/Sp neurons and is critical to neurite outgrowth.	Marie et al., 2001

TABLE 1—*Continued*

Structure	Species/Age	Preparation	Methods	GABA (and/or Glycine) Action	References
Neocortex	Rat P1–21	Slices, Cortical plate, layer II/III and layer V/VI pyramids	Gramicidin perforated patch, Ca^{2+} imaging, single-cell RT-PCR	Depolarizing at P1–3, hyperpolarizing at P11–20, E_{GABA} correlates with high NKCC1 and low KCC2 and is more positive in CP than in layers II/III and V/VI; NKCC1 blockade negatively shifts E_{GABA} and blocks Ca^{2+} increase.	Yamada et al., 2004
Neocortex	Rat E19-P4	Slices, CP, pyramids	Whole cell, Ca^{2+} imaging	Depolarizing and excitatory, glycine (and taurine) increases frequency of GABA-PSCs, $[Ca^{2+}]_i$.	Flint et al., 1998
Neocortex, hippocampus	P1–17	Slices, CA1 pyramidal cells	Cl^- imaging, gramicidin perforated patch	Transient decrease in intracellular Cl^- at P1–4 and increase at P16–17.	Marandi et al., 2002
Neocortex	Rat	Neuronal culture E17+11 div	Intracellular ^{36}Cl measurement	Transient decrease in intracellular ^{36}Cl that stimulates NKCC1.	Schomberg et al., 2003
Neocortex (and amygdala)	Rat, guinea pig P21–28	Slice	Gramicidin perforated patch, cell-attached, whole cell	Depolarizing both in soma and dendrites and can facilitate action potential generation when combined with proximal excitatory input.	Martina et al., 2001; Gulledge and Stuart, 2003, DeFazio et al., 2000
Neocortex	Rat P3–28	Slice	Whole cell	Manipulation with extracellular K^+ affects E_{GABA} in P18–28 but not at P3–6 as predicted from alteration in KCC2 expression.	DeFazio et al., 2000
Neocortex	Rat	Neuronal culture E17+11 div	Bumetanide-sensitive K^+ influx	NKCC1 activity measured as bumetanide-sensitive K^+ influx using ^{86}Rb as a tracer for K^+ is found at 4–8 div, peaking at 12–14 div.	Sun et al., 1999
Neocortex	Rat E18-P13	Slice, Cajal-Retzius cells	Whole cell, cell-attached K^+ channels, gramicidin perforated patch	Depolarizing and excitatory at all ages.	Mienville, 1998
Neocortex	Rat P0–3	Slice, (tangential) Cajal-Retzius cells	Whole cell, gramicidin perforated patch	Depolarizing and excitatory, shunting inhibition of concurrent excitatory input.	Kild et al., 2002

E, embryonic; P, postnatal; D-H switch, depolarizing to hyperpolarizing switch; E_{GABA} and $E_{Glycine}$, the reversal potentials of the $GABA_A$ and glycine receptor-mediated responses; VGCC, voltage-gated calcium channels; div, days in vitro; LSO, lateral superior olive. Table is reproduced with permission from *The Inmed Journal;* visit the website *http://inmednet.com/Table R.xls* for the latest updates, and send your suggestions to khazipov@inmed.univ-mrs.fr.

but seldom excited neurons from P1-P21 rats. However, Chavas and Marty (111) raised some concern on this technique and suggested that K^+ channel reversal method gives a value that may be too hyperpolarized.

NMDA channels recorded in cell-attached configuration were also used to monitor membrane potential and to study the depolarizing effects of GABA (377, 626). Since currents through NMDA channels reverse near 0 mV (475), NMDA currents should reverse their polarity at a holding potential on the pipette $V_p = E_m$. With the use of this approach, it was shown that bath application of the $GABA_A$ agonist isoguvacine causes depolarization of P2–5 CA3 pyramidal cells from −82 to −59 mV (in the presence of tetrodotoxin to block network-driven activity) (377).

GABA-mediated depolarization is due to the efflux of chloride ions via $GABA_A$ channels, and because the chloride ions are negatively charged, efflux of negative ions results in inward electric current producing depolarization $\cdot V = I_{GABA} \times R_m$. Efflux of chloride ions results in a reduction of intracellular negative charge ($\cdot V = \cdot Q/C$, where Q is the charge and C is the cell capacitance). Therefore, a similar current will produce a larger depolarization in small neurons having high R_m and small C. Theoretical maximal level of GABA-mediated depolarization is equal to E_{GABA}. However, in reality, GABA-mediated depolarization affects the activity of voltage-dependent membrane conductance, and the integral response to GABA is a result of complex interactions between the $GABA_A$ and voltage-dependent sodium, calcium, and potassium channels. In physiological conditions, GABA responses also interact with other conductance (including glutamate-activated AMPA/kainate and NMDA receptors), and modeling of such interactions in simplified systems reveals that GABA receptors can produce different types of responses depending on the temporal and spatial context of their activation. Prolonged activation of $GABA_A$ receptors can also result in a redistribution of chloride ions ("collapse of chloride gradient") and increase the

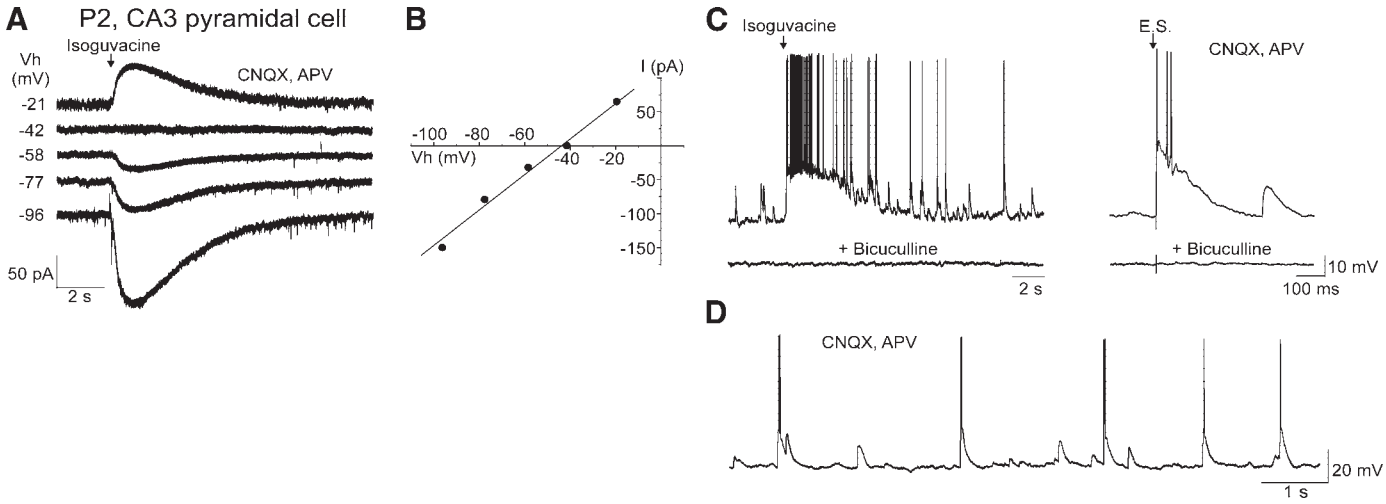

FIG. 1. $GABA_A$-mediated postsynaptic responses in a P2 CA3 pyramidal cell with gramicidin perforated patch. *A*: responses evoked by puff of the $GABA_A$ agonist isoguvacine in voltage-clamp mode at different holding potentials. *B*: dependence of the peak of the isoguvacine-induced responses on the membrane potential. Note that the responses reverse at −44 mV. *C*: current-clamp recordings of the same neuron. Brief application of isoguvacine (*left*) and electrical stimulation (E.S.) of the slice in the presence of the ionotropic glutamate receptors antagonists CNQX and D-APV (*right*) evoke depolarization and action potentials. The responses are blocked by bicuculline (20 μM, traces below). *D*: in the presence of the glutamate receptors antagonists CNQX and D-APV, spontaneous $GABA_A$-mediated postsynaptic potentials often result in action potentials. In *C* and *D*, the membrane potential is held at −80 mV. [From Tyzio et al. (625).]

relative contribution of bicarbonate permeability of $GABA_A$ channels producing a delayed depolarization (587). This mechanism does not, however, operate in immature cells because of delayed expression of intracellular carbonic anhydrase (520, 533).

2. GABA triggers sodium action potentials

Depolarizing GABA is also often excitatory in immature neurons, i.e., neurons generate action potentials in response to GABA. This occurs when $GABA_A$-mediated depolarization reaches directly or via voltage-gated conductance the threshold for the generation of action potentials. For example, synaptic activation of $GABA_A$ receptors by electrical stimulation in the presence of the glutamate receptors antagonists (CNQX and APV) evoked one or two action potentials in P2–5 CA3 pyramidal cells and interneurons recorded in the cell-attached configuration, and the response was blocked by the $GABA_A$ antagonist bicuculline (Fig. 2) (325, 326, 377). Similar excitatory responses can be recorded with gramicidin perforated patch recordings (625). Excitatory responses are also evoked by application of GABA or $GABA_A$ agonists (325, 625). Multiple unit activity recorded with extracellular electrodes is also enhanced by application of the $GABA_A$ agonists (325, 625). Synaptic activation of $GABA_A$ receptors augmented and reduced multiple unit activity (MUA), respectively, in neonatal and adult hippocampal neurons; both effects were sensitive to the $GABA_A$ antagonist bicuculline (325, 625). The positive allosteric modulator of $GABA_A$ receptors diazepam, which prolongs openings of $GABA_A$ channels and slows down the decay of the GABAergic responses, also increased GDPs frequency, synaptic activity, and MUA in neonatal rats (316). Blockade of $GABA_A$ receptors reduces and increases MUA in neonatal and adult rat CA3 hippocampus neurons, respectively (126, 172), probably via suppression of background GABAergic tone (567). Thus GABA clearly excites immature pyramidal cells and interneurons (see Table 1 for excitatory actions of GABA in other brain structures).

Interestingly, hyperpolarizing GABA can also act as an excitatory transmitter in adult neurons where it activates excitatory conductance and rebound firing. For example, GABAergic inhibitory postsynaptic potentials (IPSPs) are often followed by a rebound firing of the cells due to activation of the low-threshold calcium and hyperpolarization-activated cationic I_h currents in cerebellar nuclear neurons (6, 390). In thalamic relay cells of ferret dorsal lateral geniculate nucleus in vitro, spindle waves are associated with barrages of inhibitory postsynaptic potentials generated by burst firing of interneurons in the perigeniculate nucleus. These IPSPs result in rebound burst firing in thalamic relay cells triggered by activation of low-threshold calcium spikes (28). Rebound excitation is suppressed by blockade of $GABA_A$ receptors. Whereas excitatory action of depolarizing GABA occurs during GABAergic excitatory postsynaptic potentials (EPSPs), rebound firing occurs at the decay of GABAergic IPSPs.

3. GABA increases $[Ca^{2+}]_i$

Activation of $GABA_A$ receptors increases $[Ca^{2+}]_i$ in virtually all immature types of neurons. This effect is mainly due to the activation of voltage-gated calcium

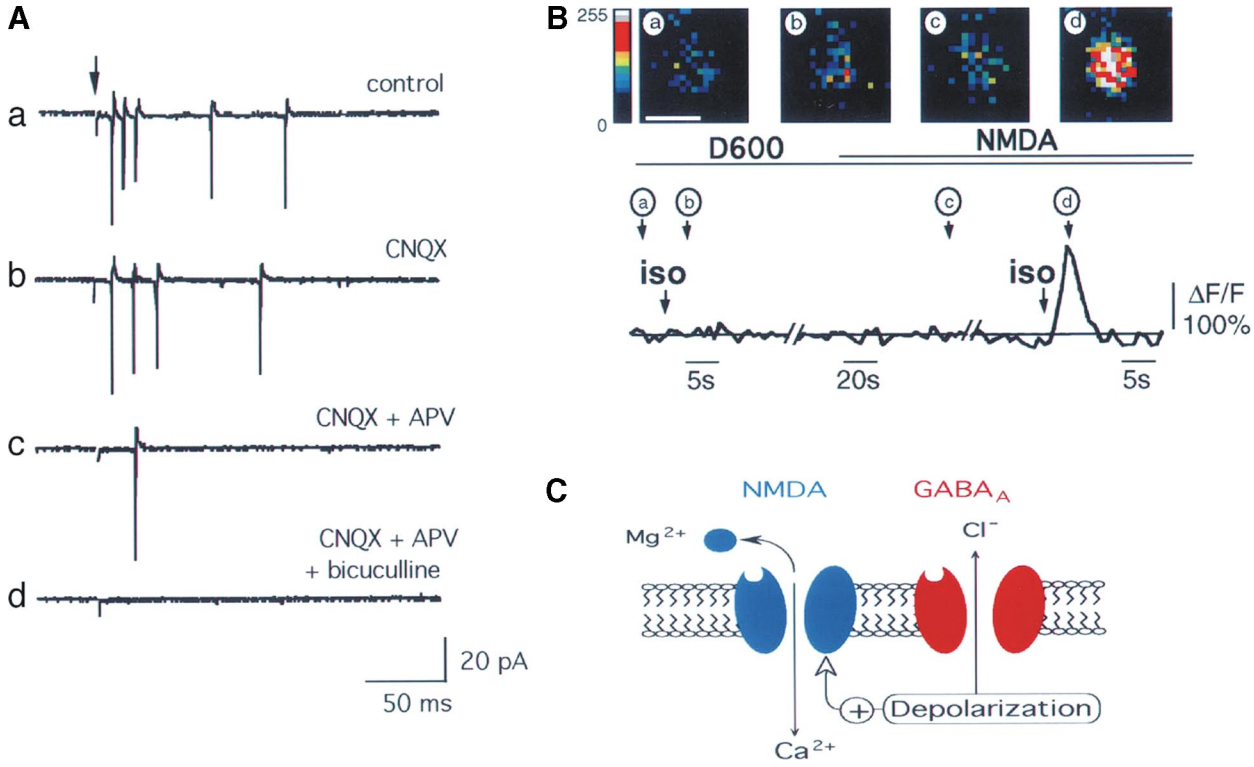

FIG. 2. GABA potentiates the activity of NMDA receptors in the neonatal hippocampus. *A*: synaptically elicited responses in neonatal CA3 pyramidal neurons (P5) recorded in cell-attached configuration. *a*: In control conditions, electrical stimulation elicited a burst of five action potentials. The number of action potentials was slightly affected by AMPA receptor antagonist CNQX (10 μM) (*b*) and strongly reduced by further addition of the NMDA receptor antagonist APV (50 μM) (*c*). *d*: The remaining response was blocked by the $GABA_A$ receptor antagonist bicuculline (10 μM). *B*: a CA3 pyramidal neuron (P5) was loaded extracellularly with the Ca^{2+}-sensitive dye fluo-3 AM, and the slice was continuously superfused with the voltage-gated Ca^{2+} channel blocker D600 (50 μM). Focal pressure ejection of a $GABA_A$-receptor agonist, isoguvacine (100 μM), or bath application NMDA (10 μM) had no effect on $[Ca^{2+}]_i$ fluorescence. However, a combined activation of $GABA_A$ and NMDA receptors resulted in a significant increase of $[Ca^{2+}]_i$ fluorescence. *C*: scheme of the interactions between $GABA_A$ and NMDA receptors in immature neurons. [From Leinekugel et al. (377).]

channels as it persists when sodium channels are blocked and is suppressed by calcium channel blockers. Interestingly, blockade of $GABA_A$ receptors induces a significant decrease in $[Ca^{2+}]_i$, indicating that tonic release of GABA is sufficient to increase Ca^{2+} in immature neurons (484).

Connor et al. (132) were among the first to use digital imaging of the Ca^{2+} to study the depolarizing responses of developing granule cells in culture to GABA. Application of GABA induced a transient increase in membrane conductance and caused $[Ca^{2+}]_i$ increase that outlasted the exposure to GABA by several minutes. Glutamate or kainate also elevated $[Ca^{2+}]_i$, but unlike GABA, this $[Ca^{2+}]_i$ response reversed rapidly upon removal of the transmitter. In keeping with these results, GABA and glycine increased $[Ca^{2+}]_i$ in a number of embryonic and neonatal neurons including neocortex (223, 385, 399, 675), hippocampus (222, 377, 378), spinal cord (359), dorsal horn neurons (515, 653), hypothalamus, olfactory bulb, cortex, medulla, striatum, thalamus, hippocampus, and colliculus (484) (see Table 1).

Synaptically released GABA also increases intracellular calcium in immature neurons. Obrietan and van den Pol (484) have shown that addition of bicuculline to monosynaptically connected hypothalamic neurons decreased $[Ca^{2+}]_i$, indicating that hypothalamic neurons were secreting GABA at an early age of development, and that sufficient GABA was released to elicit an increase in $[Ca^{2+}]_i$. This effect was seen even after blocking all glutamatergic activity with glutamate receptor antagonists (484). Leinekugel et al. (378) have demonstrated that electrical stimulation of afferent fibers induces a transient increase in $[Ca^{2+}]_i$ in neonatal pyramidal cells and interneurons (P5). This elevation of $[Ca^{2+}]_i$ was reversibly blocked by bicuculline but not by glutamate receptor antagonists. During simultaneous electrophysiological recording in current-clamp mode and $[Ca^{2+}]_i$ monitoring from P5 pyramidal cells, electrical stimulation of afferent fibers, in the presence the glutamate receptors antagonists, caused synaptic depolarization accompanied by a few action potentials and a transient increase in $[Ca^{2+}]_i$. In voltage-clamp mode, however, there was no increase in $[Ca^{2+}]_i$ following synaptic stimulation, showing that it is depolarization dependent (378).

An additional factor to consider is that voltage dependence of calcium conductance is developmentally regulated. Ganguly et al. (215) have shown that mild depolarization produced by application of 6 mM extracellular potassium evokes robust increase in $[Ca^{2+}]_i$ in P7 neurons but no response at P13 in organotypic slices (215). Only 8–10 mM $[K^+]_o$ produced calcium signals in P13 neurons. Whole cell study of the voltage dependence of calcium currents revealed a developmental shift in the activation profile of calcium currents toward more hyperpolarized potentials (215). Calcium fluorescence measurements can be also affected by the developmental changes in the Ca^{2+}-buffering properties of neurons. Several developmental studies indicate that calcium-binding proteins are progressively expressed during development in various types of neurons including calretinin (543), parvalbumin (130), and calbindin (79). Recently, Chavas and Marty (111) raised concern on using $[Ca^{2+}]_i$ to monitor depolarizing actions of GABA showing that, in cerebellar interneurons, $GABA_A$ agonists induce a somatodendritic $[Ca^{2+}]_i$ rise that persists at least until postnatal day 20 and is not mediated by depolarization-induced Ca^{2+} entry. A local $[Ca^{2+}]_i$ elevation could likewise be elicited by repetitive stimulation of presynaptic GABAergic afferent fibers. Following $GABA_A$ receptor activation, bicarbonate-induced Cl^- entry led to cell depolarization, Cl^- accumulation, and osmotic tension. The authors proposed that this tension induces the $[Ca^{2+}]_i$ rise as part of a regulatory volume decrease reaction (110).

4. *GABA reduces the voltage-dependent magnesium block of NMDA channels*

The depolarization produced by GABA also attenuates the voltage-dependent magnesium block of NMDA channels (Fig. 2). Using cell-attached recordings of single NMDA channels from P2–5 CA3 pyramidal cells, Leinekugel et al. (377) have shown that activation of $GABA_A$ receptors strongly reduces the magnesium block of NMDA channels by reducing the affinity of magnesium ions to NMDA channels from 16 to 118 μM. This effect was entirely due to neuronal depolarization from −82 to −59 mV. Confocal microscopy with the permanent dye fluo 3-AM revealed that in the presence of the calcium channels blocker D600, applications of isoguvacine and NMDA increase $[Ca^{2+}]_i$ when applied together but not separately. In the presence of an AMPA receptor antagonist (CNQX), electrical stimulation evoked on average 3.6 action potentials in immature pyramidal cells and interneurons (326); adding an NMDA receptor antagonist (APV) further reduced the response to 1.4 action potentials, and the remaining spikes were fully blocked by bicuculline (Fig. 2). Therefore, synaptic activation of $GABA_A$ receptors attenuates the magnesium voltage-dependent block of NMDA receptors. This "synergistic" interaction between $GABA_A$ and NMDA receptors contributes to the generation of the physiological pattern of GDPs (59, 326, 377) (and see below).

5. *GABA interferes with ionotropic glutamatergic transmission*

In developing hypothalamic neurons in culture, GABA acting via $GABA_A$ receptors exerts depolarizing actions that will exert different effects on AMPA-mediated responses depending on the delay between the activation of $GABA_A$ and AMPA receptors (219). GABAergic depolarization reduced and augmented glutamatergic postsynaptic responses at short and longer latencies, respectively. The reduction is due to the shunting effect of $GABA_A$-mediated conductance. In contrast, subthreshold glutamatergic responses summated with $GABA_A$-mediated depolarization generated spikes if they occurred at the end of $GABA_A$ depolarization, when the shunting $GABA_A$ conductance ceased. These observations suggest that under certain temporal conditions $GABA_A$ and AMPA/kainate receptors may work in synergy to excite the immature neurons. Similar synergistic excitatory actions of GABA-mediated depolarization and glutamatergic EPSPs may also occur in mature cortical neurons (244).

C. Developmental Changes in Chloride Homeostasis

Developmental changes in GABA signaling are determined by the progressive negative shift in E_{GABA} that in turn reflects the developmental reduction of $[Cl^-]_i$. In addition to electrophysiological and calcium-imaging experiments (see above), elevated $[Cl^-]_i$ in the immature neurons has been demonstrated directly with Cl^- indicator dyes. With the use of membrane-permeable MQAE and two-photon microscopy, activation of $GABA_A$ receptors induced a chloride influx and efflux in second and first postnatal week, respectively, in CA1 pyramidal neurons (412). Immature dissociated neurons also possess a somatodendritic chloride gradient (253). Kuner and Augustine (362) constructed an optical indicator for chloride ions by fusing the chloride-sensitive yellow fluorescent protein with the chloride-insensitive cyan fluorescent protein (clomeleon) and showed that $[Cl^-]_i$ decreases during development of hippocampal neurons in culture (362).

Neuronal chloride homeostasis is controlled by the activity of several chloride cotransporters, exchangers, and channels (reviewed in Refs. 157, 436, 499, 506). Developmental changes in two cation-chloride cotransporters, accumulating chloride NKCC1 and chloride extruder KCC2, play a pivotal role in the developmental changes in $[Cl^-]_i$ (Fig. 3).

NKCC is a membrane transport protein that mediates chloride uptake across the plasma membrane internaliz-

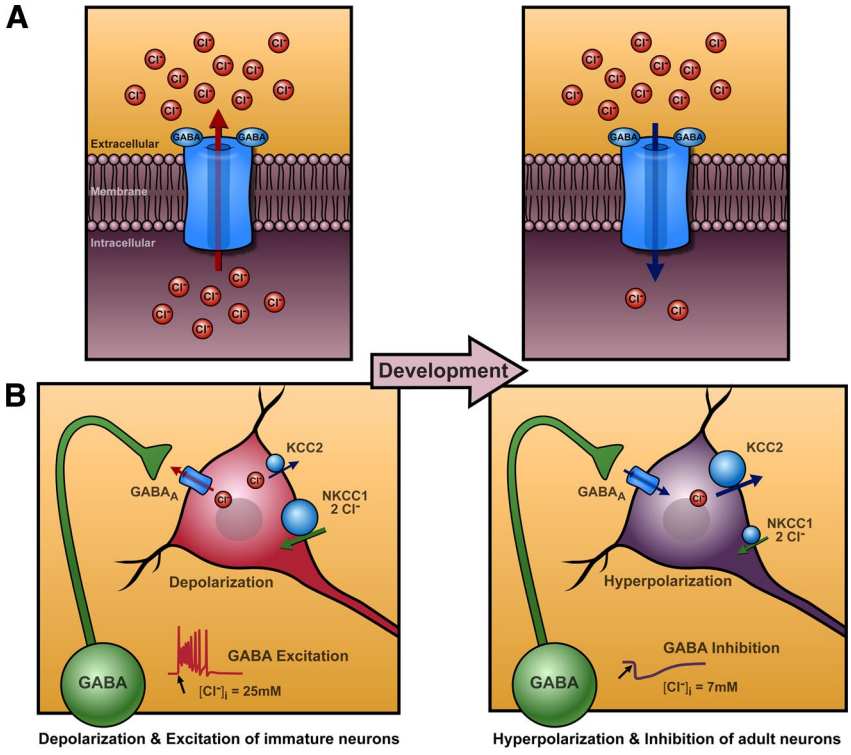

FIG. 3. Developmental changes in chloride homeostasis during development. *A*: during development, the intracellular chloride concentration decreases. In the immature neurons, efflux of the negatively charged chloride ions produces inward electric current and depolarization. In the mature neurons, chloride enters the cell and produces outward electric current and hyperpolarization. *B*: developmental change in the intracellular chloride is due to the changes in the expression of the two major chloride cotransporters, KCC2 and NKCC1. Chloride extruder KCC2 is expressed late in development, whereas NKCC1, which accumulates chloride in the cell, is more expressed in the immature neurons.

ing one Na^+, one K^+, and two Cl^- in electroneutral coupled fashion (499). Cloning and functional expression studies have identified two major isoforms of NKCC cotransporters that are products of distinct genes. The NKCC1 isoform is the so-called secretory isoform best characterized in secretory epithelial cells. NKCC1 is expressed in virtually all mammalian cells and is thought to play a housekeeping role in cell volume homeostasis and the common control of cytosolic ion content. NKCC1 does not use ATP but operates using electrochemical gradient for Na^+ and K^+ produced by Na^+-K^+-ATPase. There is considerable evidence that uptake of Cl^- in immature neurons is mediated by NKCC1. High expression of NKCC1 in immature neurons plays an important role in maintaining high intracellular Cl^- (157, 173, 207, 383, 440, 499, 506, 528, 596, 651, 671).

KCC2 is the principal transporter for Cl^- extrusion from neurons. KCC2 extrudes K^+ and Cl^- using the electrochemical gradient for K^+. Cl^- extrusion is weak in immature neurons and increases with neuronal maturation (330, 405, 678). The KCC2 isoform of KCC cotransporters is expressed in mature neurons, thus underlying the developmental changes in Cl^- extrusion (400, 521, 562, 651, 671). K^+-Cl^- cotransport also contributes to the low $[Cl^-]_i$ in mature neurons (156, 286, 450, 613–615). Additionally, the KCC1, KCC3, and KCC4 isoforms have been also found in the central nervous system but with a limited expression in neurons (499).

Using ribonuclease protection analysis and in situ hybridization, Clayton et al. (121) determined the developmental expression of the members of the cation-Cl^- cotransporter gene family in rat brain (121). Of the inwardly directed cotransporters, NKCC-1, NKCC-2, and NCC-1, only NKCC-1 was detected at significant levels in brain. NKCC-1 was expressed in neurons, appearing first in the cortical plate but not in the ventricular or subventricular zone. Expression levels peaked by the third postnatal week and were maintained in adults. Outwardly directed cotransporters demonstrated a different time course of expression: KCC-1 was expressed prenatally at low levels that increased slightly over the course of development; KCC-2 expression appeared around birth and increased dramatically after the first week of postnatal life.

Using single-cell PCR, Rivera et al. (521) showed that at birth, KCC2 mRNA was barely detectable; a steep increase in the expression was evident at P5, reaching adult level by P15. Interestingly, mature dorsal root ganglion neurons that have depolarizing GABA (160) did not express KCC2. In contrast, KCC2 mRNA was present in abundant amounts at embryonic day E42 in the hippocampus of guinea pig, a species with early maturation. Spatiotemporal expression pattern of KCC2 mRNA expression follows a caudorostral gradient reflecting functional maturation of various brain areas. Electrophysiological recordings using Cl^--free sharp electrodes revealed strong correlation between the hyperpolarizing $GABA_A$-mediated responses and KCC2 mRNA expression in the pyramidal hippocampal neurons of the rat, guinea pig, and dorsal root ganglion

neurons. Antisense oligodeoxynucleotides against KCC2 mRNA produced a fivefold reduction in KCC2 protein in P11–13 rat hippocampal slices associated with a strong reduction of DF_{GABA} (from −10.9 to −2.8 mV). Similarly, early overexpression of KCC2 in immature cortical neurons, before the upregulation of KCC2, produced a negative shift in GABA reversal potential and reduced GABA-elicited calcium responses in cultured neurons (118, 374, 588). Taken together, these results indicated that developmental expression of KCC2 is pivotal for development of hyperpolarizing $GABA_A$-mediated inhibition.

Using perforated patch-clamp method and single-cell multiplex RT-PCR to measure cation-Cl^- cotransporter mRNAs in postnatal rat neocortical neurons, Yamada et al. (671) reported that the mRNA expression levels of NKCC1 and KCC2 were positively and negatively correlated, respectively, with E_{GABA} (671). $[Cl^-]_i$ and NKCC1 mRNA were higher in cortical plate (CP) neurons than in the presumably older layer V/VI pyramidal neurons in a given slice. The pharmacological effects of selective NKCC1 blocker bumetanide on E_{GABA} were consistent with the different expression levels of NKCC1 mRNA. The expression of NKCC1 and KCC2 was also analyzed by Western blot and immunofluorescence and double labeling in the rat and human cortex (173). In the rat cortex, NKCC1 expression reached 14-fold higher levels between P3–14 than at P21 and adulthood. In contrast, KCC2 levels were significantly lower during the first two postnatal weeks than at P21 and adulthood. These ontogenic findings were confirmed by immunofluorescence double labeling using the neuronal marker NeuN and either NKCC1 or KCC2 specific antibodies. In human cortex, NKCC1 expression was significantly higher at 31–41 postconceptional weeks than at 1 year and older. During the first year of life, NKCC1 expression rapidly decreased to levels of the adult. Between 31 and 41 postconceptional weeks, when NKCC1 levels were peaking, KCC2 expression was 2–25% of adult levels, rising over the first year of life. Immunofluorescence double labeling with neuronal marker NeuN and antibodies against NKCC1 and KCC2 was consistent with Western blot results. Whole cell recordings from P4–6 rat CA1 pyramidal cells demonstrated that blockade of NKCC1 activity with bumetanide shifted E_{GABA} from -37 ± 2.7 to -40.4 ± 2.7 mV. Modest effect of bumetanide on E_{GABA} in these experiments could be due to the error of E_{GABA} measurements introduced by whole cell recordings. Taken together, these observations suggest that neocortical neurons, like hippocampal neurons, express a D-H change mediated by a developmental shift from NKCC1 to KCC2.

D. Bicarbonate-Mediated $GABA_A$ Excitation

Strong activation of GABA receptors produces depolarizing shift in E_{GABA} and excitatory action of GABA in mature neurons (9, 10, 14, 298, 533, 570, 587, 612). This effect is maximal in small cell compartments with high receptor-to-volume ratio such as dendrites (587). The activity-dependent $GABA_A$-mediated depolarization/excitation is contingent on HCO_3^-, which is permeable via $GABA_A$ receptor channels (74, 297). HCO_3^- equilibrium potential is set at approximately −10 mV, and therefore, bicarbonate currents via $GABA_A$ receptors are depolarizing. Efflux of HCO_3^- is compensated by rapid synthesis of HCO_3^- from CO_2 by carbonic anhydrase (498). Depolarization caused by the HCO_3^- current leads to accumulation of $[Cl^-]_i$, a positive shift in E_{GABA} (299, 300, 644), and an increase of extracellular K^+ that depolarizes neurons to more positive levels than E_{GABA} (300, 570). Furthermore, inhibitors of carbonic anhydrase suppress GABAergic excitation (533).

However, HCO_3^--dependent excitation is expressed relatively late in development and does not contribute to depolarizing and excitatory actions of GABA in immature neurons. Also, GABA-mediated excitation of immature neurons persists after removal of bicarbonate/CO_2 from the external medium and its substitution for HEPES-based buffer (378). Also, carbonic anhydrase VII, a key molecule in the generation of HCO_3^--dependent GABAergic excitation, is not expressed at an early stage, and high-frequency stimulation of GABAergic inputs generates excitatory actions of GABA at P10–12 but not before (520, 533). HCO_3^- efflux however contributes to the depolarizations mediated by GABA and glycine in fetal spinal cord motoneurons (359).

E. Voltage-Gated Chloride Channels

Several members of the voltage-gated chloride channel family are expressed in the CNS including ClC-2, ClC-3, ClC-4, ClC-5, ClC-6, and ClC-7 (121, 122). ClC-2 channels are inwardly rectifying, with significant conductance only at membrane potentials more negative than Cl^- equilibrium potential. They play a role in regulation of cell volume regulation and the maintenance of a balance between electrolytes and intracellular ions (241; see also Ref. 534). Acute hyposmotic challenges increase cell volume and activate ion fluxes (K^+ and Cl^-), and conversely, hyperosmotic challenge causes shrinkage consequently to the activation of K^+ and Cl^- conductances. These channels are intracellular, membrane bound, or both (119). In mature hippocampal neurons, hyperpolarization activated Cl^- conductance mediated by ClC-2 channels is of sufficient magnitude and duration to stabilize the relationship between E_{Cl} and resting membrane potential indepen-

dently of electroneutral Cl^- transport (that is KCC2) (571, 584). Transgenic expression of ClC-2 in neurons that accumulate Cl^- is sufficient to reduce intracellular Cl^- levels to concentrations that approach the passive Cl^- equilibrium potential (585). ClC-2 is expressed early in development and may play a role in the developmental decline in $[Cl^-]_i$ (122). The relatively low expression of ClC-2 at an early stage may contribute to the depolarizing actions of GABA and glycine in the hippocampus (451). More recent studies suggest that CLC-3 may play an important role in maturation. Thus Strobawa et al. (592) have reported an almost complete degeneration of the hippocampus and retina in CLC-3 knockouts and have shown that this effect is mediated by an intracellular action of the protein since the volume changes evoked identical responses in controls and CLC-3 knockouts. More recently however, Nelson and co-workers (655) showed a chloride conductance activated by calmodulin kinase II that in hippocampal cultures increases the duration and amplitude of exogenous and synaptic currents generated by NMDA receptors in immature neurons at a time when $[Cl^-]_i$ is elevated. This effect is reduced with maturation, when $[Cl^-]_i$ is lower, and thus acts to augment excitability and NMDA receptor-mediated events in immature neurons. The channels are postsynaptically located close to NMDA receptors and may play an important role in parallel to the synergistic actions of GABA with glutamate to augment the contribution of NMDA receptor-mediated events. This important observation points to an alternative mechanism for increasing excitability when $[Cl^-]_i$ is elevated.

F. Time Course of the Excitatory to Inhibitory Developmental Switch

The general conclusion derived from studies on the time course of the developmental E-I switch in GABAergic actions is that it parallels the negative shift in E_{GABA}. The timing of the shift also depends on the species, sex, brain structures, and neuronal type. Studies using viral infections to label proliferating neurons in adult networks strongly suggest that the shift of $GABA_A$ signaling reflects the "age" of the recorded neuron (227, 487; and see below). Timing of the E-I switch in the GABA actions has been most thoroughly investigated in the rat hippocampus. Originally it was reported in rat CA3 pyramidal cells that E-I switch occurs at around P5 (56). In agreement with these results, intracellularly recorded inhibitory GABAergic postsynaptic potentials were observed in the majority of CA3 pyramidal cell by P5–6 and by P9 in CA1 pyramidal cells (600). Different estimates were made with whole cell recordings from CA1 pyramidal cells (678). However, these techniques are invasive and will affect E_{GABA}, reflecting the importance of combining noninvasive imaging with electrophysiological techniques.

With measurement of $[Ca^{2+}]_i$ using fluorescent calcium-sensitive dyes, the activation of GABA receptors induced an influx of calcium ions via voltage-gated calcium channels at P2–5 CA3 pyramidal neurons and interneurons but not at P12–13, suggesting that the switch occurs during the second postnatal week (378). With the use of a similar approach, the switch in CA1 pyramidal cells was estimated to be around P5–6; at P7–10, ~20% of cells were still excited by GABA, and none from the beginning of the third postnatal week onwards (222). In cultures of hippocampal neurons, the midpoint of disappearance of the $GABA_A$-mediated increase in $[Ca^{2+}]_i$ occurred at ~11 days in vitro (215).

Noninvasive extracellular and cell-attached recordings revealed a developmental switch in the effect of $GABA_A$ agonist isoguvacine on MUA in CA3 Sprague-Dawley rat hippocampus at around P13 (325) (Fig. 4). Similar experiments performed in Wistar rat hippocampus revealed that the effect of isoguvacine switched from an increase to a decrease MUA at around P10 (625), that is 3 days earlier than in Sprague-Dawley rats, suggesting a strain difference in the timing of the E-I switch. The effect of synaptic $GABA_A$-mediated responses evoked by electrical stimulation in the presence of the glutamate ionotropic and $GABA_B$ receptors antagonists on MUA in the Wistar rat CA3 pyramidal cell layer switched from an increase to a decrease at around P8 (625). With gramicidin perforated patch recordings, the E-I switch was also centered at P8 (625). The $GABA_A$ antagonist bicuculline switched from decreasing to increasing MUA in Wistar CA3 pyramidal cell layer at around P12 (172). The time course of the E-I switch of GABA also depends on the sex of the animal: with the use of the gramicidin perforated patch-clamp recordings from the rat substantia nigra pars reticularis, it has been shown that the switch occurs in males around P17 and in females around P10 (363). Since the postnatal development is associated with dramatic changes in physiological activity patterns and seizure susceptibility, these minor differences may have an important physiological and clinical relevance (see sects. VI and IX).

G. Intrinsic and Extrinsic Factors Modulate the Developmental Switch

What are the genetic and environmental mechanisms underlying the developmental switch? So far, our knowledge on this topic is fragmentary. Information is now available on the developmental expression of molecules that control chloride concentrations (see sect. III), but the control of the expression of these molecules remains to be fully determined.

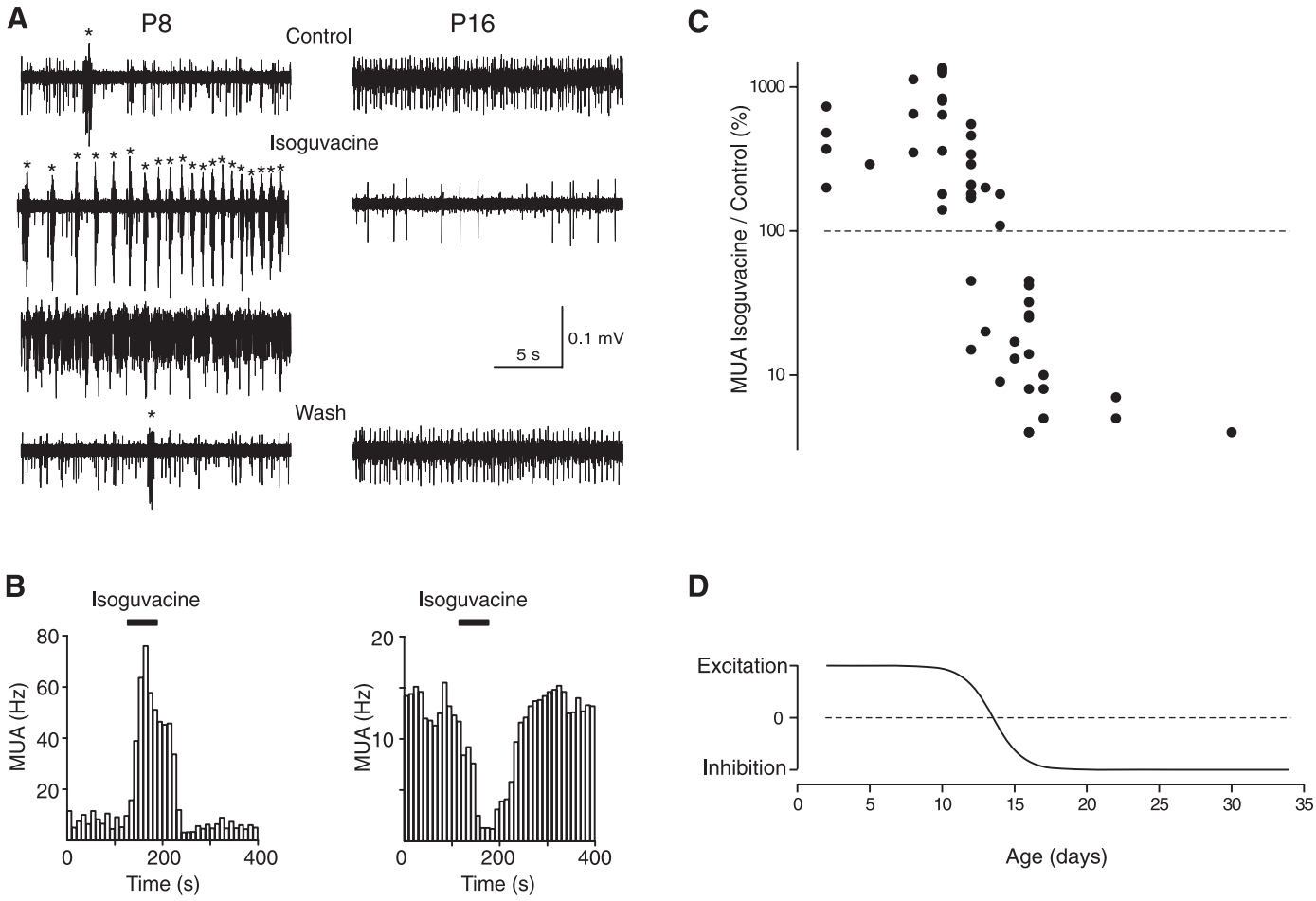

FIG. 4. Developmental switch in the $GABA_A$ signaling in the Sprague-Dawley rat CA3 hippocampus. *A*: bath application of the $GABA_A$ receptor agonist isoguvacine (10 μM for 1 min) increased the frequency of extracellularly recorded action potentials in CA3 pyramidal cell layer at P8 but decreased the frequency at P16. Field giant depolarizing potentials (GDPs) are marked with asterisks. Note that isoguvacine induced an increase in the field GDP frequency (*top trace*) followed by increased asynchronous firing (*bottom trace*). Upon wash of isoguvacine, neuronal activity returned to the control level. *B*: the time course of the effect of isoguvacine on multiple unit activity (MUA) in the experiments shown above. Note that activation of $GABA_A$ receptors transiently increased MUA frequency (excitatory effect) at P8 and reduced MUA frequency (inhibitory effect) at P16. *C*: plot of the age dependence in the effect of isoguvacine on neuronal firing. Each point represents the ratio (as a percentage) of the MUA frequency at the peak of the isoguvacine effect to the MUA frequency in control. Pooled data from 49 slices are shown. *D*: Boltzmann fit of the age dependence of the effect of isoguvacine on neuronal firing; note that the switch in $GABA_A$ signaling from excitation to inhibition occurs at P13.5 ± 0.4. [From Khazipov et al. (325).]

1. *GABA itself regulates the developmental switch in the action of GABA*

Ganguly et al. (215) determined the regulation of the developmental switch in rat hippocampal cells culture. Using gramicidin perforated patch recordings and intracellular calcium imaging, the authors showed that GABA increased $[Ca^{2+}]_i$ in neurons cultured for 4–9 days but not after day 13. The response was blocked by nimodipine, an antagonist of L-type calcium channels, but not by depleting intracellular calcium stores with thapsigargin, suggesting the GABA-activated rise in $[Ca^{2+}]_i$ is mediated by L-type calcium channels. Developmental decrease of the GABA-evoked calcium response was paralleled with a negative shift of E_{GABA} from −44 mV at days 6–7 to −61 mV at days 13–14. Chronic blockade of $GABA_A$, but not glutamate, receptors prevented the loss of calcium responses and the negative shift of E_{GABA} while chronic depolarization with 10 mM KCl accelerated the switch. Blockade of $GABA_A$ receptors also decreased by 68% the developmental increase in the expression of mRNA encoding for KCC2. Interestingly, blockade of sodium spikes with tetrodotoxin (TTX) did not affect the switch, and $GABA_A$ receptor antagonists prevented the switch in presence of TTX. These observations suggest that the developmental switch is triggered by miniature $GABA_A$ postsynaptic potentials generated in the absence of action potentials. These findings suggested that $GABA_A$-activated Ca^{2+} influx regulates the expression of chloride extruder KCC2, raising the issue of the intracellular cascades underlying this phenomenon. According to this

work, GABA may play a self-regulatory role in the kinetics of the developmental switch in the GABA actions. Poo and co-workers (196) have recently also reported that prolonged postsynaptic spiking of hippocampal neurons led to a shift in the reversal potential of GABA-induced Cl^- currents toward positive levels in a duration- and frequency-dependent manner. This shift requires an elevation of $[Ca^{2+}]_i$ and is occluded by inhibition of KCC2. Interestingly, these changes are larger in mature neurons that express the cotransporter than in immature neurons. These observations further reinforce the link between activity and the actions of GABA (196).

Using a turtle model and calcium imaging, Sernagor and colleagues (379) investigated the effects of bicuculline on the GABAergic polarity switch (from 1 wk before hatching until 4 wk after hatching) and the presence of patterns of spontaneously generated retinal waves in the retinal ganglion cell layer. During that period, spontaneous activity normally switches from propagating waves to stationary patches of coactive cells, until correlated activity completely disappears. The authors reported that in the presence of bicuculline, $GABA_A$ responses remain excitatory and spontaneous waves were generated and propagated across the retinal ganglionic layer. This action was associated with a reduction of the developmentally regulated expression of the KCC2 transporter, thus in keeping with the observations of Ganguly et al. (215).

However, other studies suggest that GABAergic activity is not required for the switch to take place. In hippocampal cell cultures, the developmental increase in the KCC2 expression and hyperpolarizing shift of E_{GABA} can take place in spite of a chronic blockade of $GABA_A$ receptors (404, 616). A similar conclusion was reached in a recent study in which the vesicular amino acid transporter was mutated leading to a cleft palate, but did not impair the development of inhibitory synapses or of the expression of KCC2 (666).

An additional mechanism has been proposed by Ouardouz and Sastry (486). The authors showed that in developing deep cerebellar nuclei, a train of high-frequency stimulations triggered the D-H shift, an effect mediated by an apparent upregulation of the KCC cotransporter. This action was prevented by protein synthesis inhibitors and by protein phosphatase inhibitors, suggesting that a phosphorylation of the protein and the synthesis of new molecules may be involved in the transformation.

2. Brain-derived neurotrophic factor and tyrosine kinase receptors regulate the developmental switch

Other factors have been implicated in the D-H developmental switch of GABA. Thus cultured hippocampal neurons initially contain an inactive form of the KCC2 protein, which becomes activated with neuronal maturation (314). This process is accelerated by transient stimulation of insulin-like growth factor I (IGF-I) receptors. Because the transporter could be rapidly activated by coapplication of IGF-I and Src kinase and deactivated by membrane-permeable protein tyrosine kinase inhibitors, it was suggested that activation of K^+/Cl cotransporter function by endogenous protein tyrosine kinases mediates the developmental D-H switch of GABAergic responses (314). Brain-derived neurotrophic factor (BDNF) acting via tyrosine kinase receptor also increased expression of KCC2 during the early development (5). This upregulation of KCC2 expression may be mediated by Shc pathway (522). Interestingly, at older developmental stages, BDNF affected E_{GABA} in the hippocampal interneurons in an opposite direction (656). BDNF induced a shift in E_{GABA} to more positive levels for GABAergic but not glutamatergic postsynaptic neurons. It was concluded that BDNF may decrease the efficacy of inhibitory transmission by acute postsynaptic downregulation of Cl^- transport, in addition to its well-known presynaptic effect. TrkB-mediated downregulation of KCC2 in adult neurons requires activation of the two major cascades: phospholipase C (PLC)-γ and Shc (444). Thus the polarity of the effect of BDNF on KCC2 expression is age dependent.

Khirug et al. (330) compared the developmental expression and function of KCC2 in acute slices and cultures. Imposing a fixed Cl^- load via patch pipette, they measured the resultant somatodendritic gradients in reversal potential of GABAergic currents to determine the time course of functional maturation of KCC2-mediated Cl^- extrusion. In both preparations, the gradient was initially small or not detectable in immature neurons. An abrupt increase occurs at around days 13–14 in culture, while a more gradual increase occurs between postnatal days 5–14 in slices. Consistent with the presence of a nonfunctional form of KCC2 in immature hippocampal neurons grown in culture, application of the broad-spectrum kinase inhibitor staurosporine produced a rapid and potent upregulation of KCC2 function in these cultured neurons, but not in neonatal slices. These results indicated that the functional activity of KCC2 in vivo parallels the developmental expression of the protein, whereas cultured neurons require an additional activation step (mimicked by staurosporine) for KCC2 to become functional.

H. A Dramatic Shift of E_{GABA} During Delivery

A dramatic illustration of the importance of the shift of the actions of GABA was shown recently in a study in which using single $GABA_A$ channel recordings in slices, Tyzio et al. (624) investigated the properties of GABA around delivery time (Fig. 5). GABA was expected to depolarize neurons in utero and a few days after delivery,

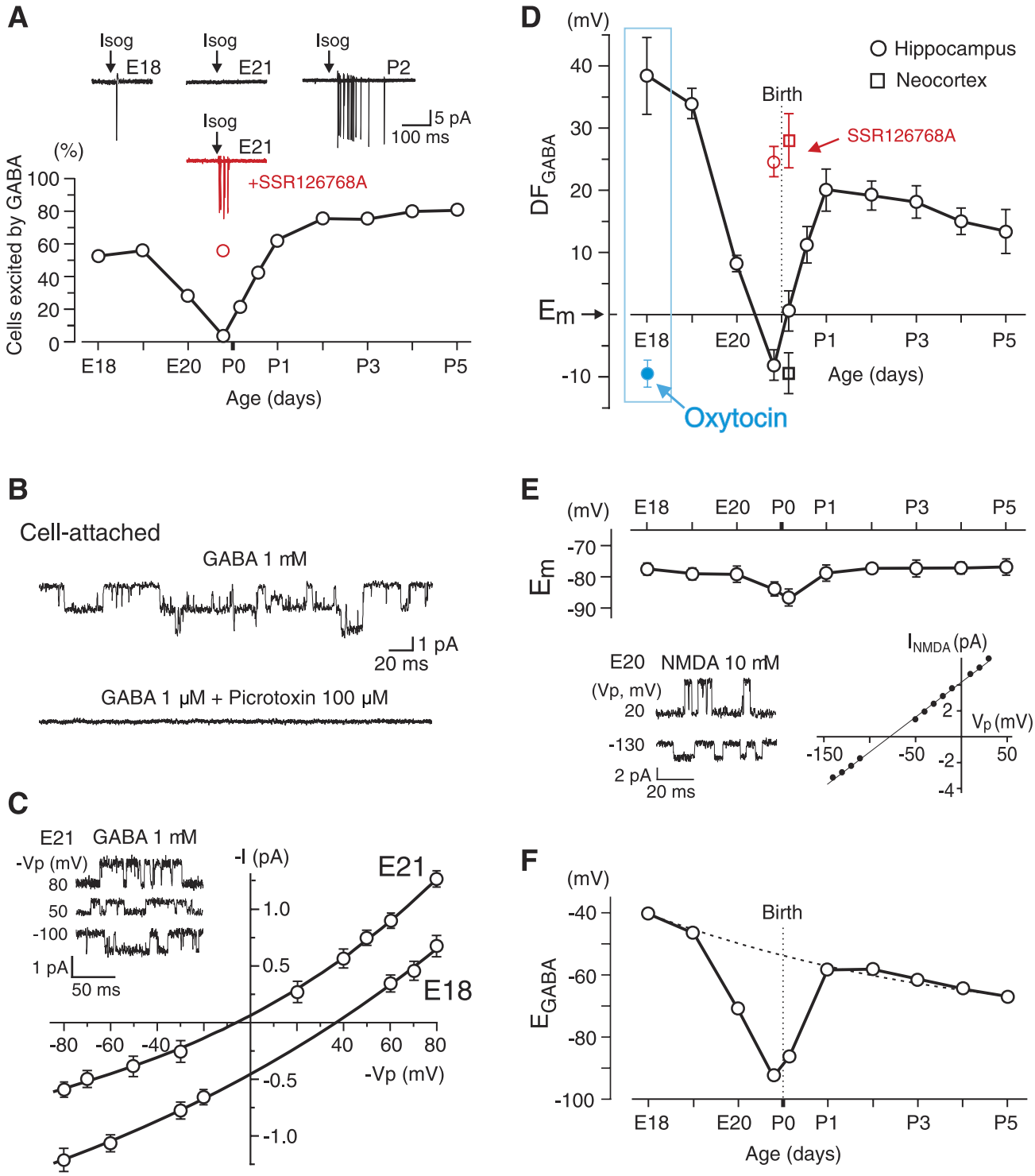

FIG. 5. Oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery. *A*: responses of CA3 pyramidal cells recorded in cell-attached mode to the $GABA_A$ agonist isoguvacine. *Bottom*: summary plot of the proportion of cells excited by isoguvacine during the perinatal period. Note transient loss of the excitatory effect of isoguvacine near term. Red code corresponds to the fetuses whose mothers received the oxytocin receptor antagonist SSR126768A. [E, embryonic; E21 corresponds to the early phase of delivery (1–2 h before birth); P, postnatal; P0 is the day of birth; pooled data from 146 neurons]. *B*: cell-attached recordings of single $GABA_A$ channels with 1 μM GABA in patch pipette (*top trace*); the channels were not observed in the presence of the $GABA_A$ antagonist picrotoxin (100 μM; *bottom trace*). *C*: *I-V* relationships of the currents through $GABA_A$ channels in two cells, at E21 and E18; their reversal potential corresponds to the $GABA_A$ driving force (DF_{GABA}). *D*: summary plot of the age dependence of DF_{GABA} inferred from single $GABA_A$ channels recordings [means ± SE; 209 CA3 pyramidal cells (○) and 17 neocortical pyramidal cells (□); 6–24 patches for each point]. Red code refers to pretreatment with SSR126768A (n = 25 hippocampal and 9 neocortical patches). Bath application of oxytocin (1 μM) induced hyperpolarizing switch in DF_{GABA} at E18 (blue code). *E*: age dependence of the resting membrane potential (E_m) of CA3 pyramidal cells inferred from the reversal of single NMDA channels recorded in cell-attached mode (n = 84 cells; 4–12 patches for each point). *F*: age dependence of the $GABA_A$ reversal potential ($E_{GABA} = E_m + DF_{GABA}$). Note a transient hyperpolarizing shift of E_{GABA} near birth. [From Tyzio et al. (624).]

but shortly before, during, and after delivery, there was a dramatic transient fall of the concentration of $[Cl^-]_i$ (from >25 to 4 mM) and a D-H shift in DF_{GABA}. Single NMDA channel recordings made in parallel showed that E_m was slightly hyperpolarized by 7 mV during delivery, indicating a genuine shift of E_{GABA}. Cell-attached recordings confirmed that GABA transiently lost its excitatory action during this narrow window. The mechanisms underlying the perinatal D-H shift were due to the maternal hormone oxytocin that is released by the mother to trigger labor as oxytocin and antagonists of oxytocin receptors, respectively, produced and blocked this shift. Most significantly, administration of the antagonist to the mother prevented perinatal DF_{GABA} shift in neocortical and hippocampal fetal neurons. Administration of bumetanide alleviated the actions of oxytocin receptor antagonists, suggesting that the actions of the hormone are mediated by a rapid reduction of NKCC1 activity leading to a reduction of $[Cl^-]_i$. The authors suggested that the fetus is informed of the imminence of delivery by maternal release of a hormone that both triggers labor and exerts a massive inhibitory action on fetal neurons. These effects are neuroprotective. Indeed, blocking oxytocin receptors in pregnant rats, using oxytocin receptor antagonists also used to avoid preterm labor, aggravated the effects of hypoxic episodes in fetal neurons. This study illustrates the physiological relevance of the developmental shift of GABA actions (624).

I. Depolarizing Actions of GABA in Other Brain Structures and Animal Species

Studies performed in a wide range of developing brain structures confirmed a developmental switch in the actions of GABA from depolarizing (and often excitatory) to hyperpolarizing (and often inhibitory). These include the neocortex, the cerebellum, the spinal cord, the olfactory bulb, sensory structures, and several subcortical and peripheral structures. Table 1 lists available information with references.

IV. EARLY OPERATION OF $GABA_A$ SIGNALING PRIOR TO SYNAPSE FORMATION

The starting point is that like at all central or peripheral synapses, postsynaptic receptors are functional well before synapses are formed and prior to the arrival of presynaptic axons. The earlier formation of receptors suggests that the postsynaptic neuron or muscle receives some information from the extracellular environment and can communicate with arriving terminals to engage the sequence of events that will ultimately lead to the formation of a synapse. This has been extensively investigated in the neuromuscular junction that has provided most of the concepts concerning the role of presynaptic axons on the construction of the postsynaptic complex (360, 465).

GABA and GABAergic markers are expressed well before synapses are formed, and GABA is released at an early developmental stage and acts as a trophic factor to modulate several essential developmental processes including neuronal proliferation, migration, differentiation, synapse formation, neuronal growth, or network construction. This early intercellular communication is based on diffusion and distal paracrinic actions that contrasts with the local fast communication provided by synaptic currents. These early effects are briefly summarized here as they provide an excellent illustration of the multiple facets of the actions of GABA and the unique role of GABA as an early communicating signal. Excellent reviews of these effects have been published (36, 352, 492, 579).

GABA, like other neurotransmitters, is released by growth cones (220) and can act on distal sites to generate receptor-mediated currents. Neurotransmitters have now been shown to influence directly or indirectly several aspects of neuronal maturation in various experimental systems (36, 94, 183, 368, 370, 492). The depolarizing action of GABA on developing systems is particularly suited and constitutes a common denominator to its actions on a wide range of processes.

A. GABA Signaling Is Present at a Very Early Stage

In the neocortex, all the components of GABA signaling are present at an early stage. GABA immunoreactive neurons are found in all fetal zones of the cerebral anlage starting from embryonic day 15 (123, 633), and a second population of GABAergic cells is observed in the intermediate zone. Beginning on day 16 of gestation and continuing throughout gestation, GABAergic neurons are observed in the marginal zone, the zone, the cortical plate, and the ventricular and subventricular zones (30). As with NMDA-generated currents, GABA-mediated currents are present in proliferative zones well before synapses have been established (398, 399, 493). Other physiological studies that failed to detect synaptic currents at an early stage reported tonic outward currents in response to applications of GABA receptor antagonists (492, 493). Therefore, a tonic release of GABA acts can generate long-lasting currents in neurons that bear no functional synapse (also see below).

In the rodent hypothalamus, immunogold and peroxidase studies showed that GABA immunoreactivity is present in fibers and weakly stained perikarya at the beginning of hypothalamic neurogenesis (E15) (631). Studies of neurons in culture suggest an earlier presence of $GABA_A$ receptors and currents than other transmitters

including glutamate and glycine. Thus, in cultured hypothalamic embryonic neurons, GABA evoked currents already a few hours after plating (E15), and all neurons recorded expressed $GABA_A$ receptors, whereas only some expressed glutamate or glycine receptors (112). The same group (483) showed also that applications of GABA elevate calcium in growth cones, suggesting that GABA receptors may play a significant role in modulating the formation of early connections in the hypothalamus.

Similar conclusions were reached in studies of the maturation of the cerebellum. GABA immunoreactivity is present throughout the dendrites, cell bodies, and growth cones (607). In contrast, the vesicular GABA transporter (VGAT) was confined to the growth cones and axon varicosities where GABA receptors are not present, suggesting that GABA may be extrasynaptically released from axon varicosities and growth cones by vesicular secretion "exocytosis" at a time when synapses have not formed. In addition, GABA release can occur from all part of the developing neuron by nonvesicular secretion that the authors suggest may be due to a paracrine type of release through a reversal of the transporters (also see Ref. 673 and below). GABA is present in forebrain neurons at birth and can be released from growth cones without being associated with synaptic vesicles (610). This is in keeping with the Ca^{2+}-independent release of endogenous and ^{3}H-labeled GABA from growth cones (see below).

In rat embryonic dorsal spinal cord, GABAergic markers are present already at embryonic day 13 (E13), while neurons are still proliferating. GABA-evoked responses were inhibited by bicuculline and picrotoxin and potentiated by benzodiazepines (97, 556). Similarly, embryonic rat striatal neurons respond selectively to the $GABA_A$ agonist muscimol (195).

The developmental regulation of neurotransmitter synthesis has been extensively studied in the central nervous system of the *Xenopus* embryo (579). GABA immunoreactivity appeared at an early stage, also suggesting that GABA may play additional roles early in development (580, 657). This system is an attractive model for investigation, since action potentials are elicited from spinal neurons at the time of closure of the neural tube and can be used to study the role of activity in the phenotypic expression of transmitters. Spitzer and co-workers (52, 75, 576, 577) have shown that the GABA phenotype is regulated by calcium signaling providing a direct link between activity and network operation.

B. A Nonvesicular Release of GABA in Developing Cortical Networks

Barker and co-workers (629, 630) also reported a series of observations that suggest a tonic release of GABA on cultured hippocampal neurons. Washing the surface of neurons with a steam of saline rapidly and reversibly reduced the baseline current and fluctuations, both of which were completely eliminated by bicuculline (629, 630). Using hippocampal embryonic and early postnatal slices, Demarque et al. (158) confirmed that bicuculline generates tonic currents during maturation. A brief electrical pulse applied to the vicinity of the recorded neuron generated a GABAergic "nonsynaptic" current in silent neurons that have no spontaneous or evoked postsynaptic currents. Procedures that release GABA also failed to generate a response indicating that vesicular release mechanisms are not operant at that stage. This early slow current (ESC) is much longer than synaptic currents, several seconds duration, and the electric charges are several orders of magnitude larger than that of conventional synaptic responses. ESCs are observed shortly after birth but disappear subsequently, around 2 wk postnatal. Thus, during a transient postnatal period, neurons can have a slow GABAergic ESC generated with or without the faster more conventional synapse-driven GABAergic currents. In contrast to EPSCs, ESCs are not blocked by a wide range of agents that block calcium channels and vesicular release, including cobalt, TTX, zero calcium media, or botulinum toxin, and are observed in knockout mice in which the snare family proteins have been knocked out and vesicular release completely obliterated (637). Therefore, a nonvesicular release of GABA precedes the conventional vesicular mechanisms and operates to flood in postsynaptic target neurons prior to synapse formation. The source of this release has not been identified. However, growth cones do release GABA both in a calcium-dependent (220) or -independent manner (611). The presence of this "noncanonical release" (158) of transmitter suggests that procedures that block vesicular release may be inadequate to infer on the role of transmission on brain construction. Blocking sodium/calcium spikes or even vesicular release by knockout strategies does not prevent the action of nonvesicular release of transmitter. The nonvesicular release of GABA may be sufficient to modulate functions that will develop normally in the absence of ongoing network dependent activity (516).

C. GABA Influences DNA Synthesis in Precursor Neocortical Cells

Activation of GABA in the neocortical proliferative zone leads to significant decrease in DNA synthesis and a reduction in the number of bromodeoxyuridine (BrdU)-labeled cells (399). Depolarization by high K^+ produced the same effect (148, 399), and blocking GABA receptors prevented the actions of GABA and increased DNA synthesis, suggesting that ongoing GABA release may reduce protein synthesis (399). More recent experiments re-

vealed, however, a more complex situation with the actions of GABA involving basic fibroblast factor (bFGF) (21). Furthermore, the polarity of GABA actions strongly depends on the experimental conditions and the target cells. Thus GABA increased the number of BrdU-labeled cells in the ventricular zone but decreased it in the subventricular zone (261). Neither the mechanisms of release nor the intracellular signals have been determined.

Patch-clamp recordings made from embryonic and early postnatal cortical neurons show that they express functional GABA receptors that change properties significantly with development. $GABA_A$ receptors expressed in proliferating precursors have higher apparent affinity, long channel kinetics (491), and long channel open times in neuroepithelial cells (414). The different properties of $GABA_A$ receptors are most likely due to different subunit composition, although other factors play an important role including intracellular calcium concentration, degree of receptor phosphorylation, association with anchoring proteins, and degree of clustering of the receptors. These issues have been discussed recently in excellent reviews and need no further comments here (491, 492). The important message that stems from these studies is that receptors expressed in immature cells tend to have slow kinetics both prior to synapse formation and once immature synapses are formed. The slow desensitization will facilitate the generation of long-lasting currents and increase intracellular calcium.

D. GABA Modulates Neuronal Migration

Whole cell patch-clamp recordings from fetal rat neuroblasts revealed that they are coupled by gap junctions into clusters of 15–90 cells and generate large responses when GABA is applied (392). Other observations also suggest that migrating neurons express at an early developmental stage GABA and glutamate receptors. Thus calbindin-positive neurons in the intermediate zone express a glutamate transporter (438) and inwardly rectifying kainate receptors but not NMDA or GABA receptors (437). This suggests that AMPA/kainate receptors present on migrating interneurons at an early stage are activated by glutamate released from corticofugal axons. In contrast to a calcium imaging technique, NMDA, AMPA, or GABA agonists induced a rise of calcium in tangentially migrating cells (574).

Somewhat different observations were made for the migration of the principal cells. In their study, Represa and co-workers (410) used 5-bromo-2′-deoxyuridine labeling and a mixed coculture of hippocampal slices composed of one slice from naive mice and one from green fluorescent protein (GFP) mice (Fig. 6). This coculture enables one to follow the migration of visually identified GFP-positive pyramidal neurons on naive slices. The authors showed that migrating neuroblasts express NR1 and GABA immunolabeling. When patch-clamp recorded, agonists of GABA or NMDA but not AMPA receptors generated currents. Interestingly, while many neurons expressed GABA but not NMDA receptors, only 1 of 10 neurons expressed NMDA but not GABA receptors, suggesting that GABA receptors are established before

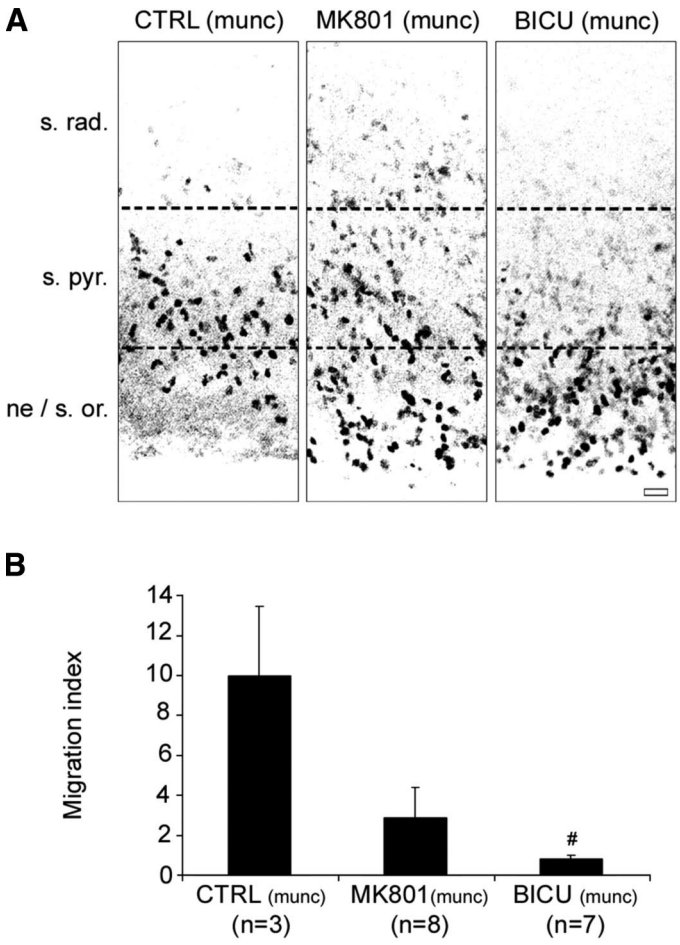

FIG. 6. $GABA_A$ and NMDA receptor antagonists impaired the migration of BrdU-labeled cells in hippocampal organotypic slice culture from munc18–1 mutant mice. *A*: immunostaining for BrdU on hippocampal organotypic slices from munc18-1 mutant mice cultured for 1 DIV without any treatment [control condition (munc); *left*], in the presence of 10 μM MK801 (*middle*), and in the presence of 50 μM bicuculline (*right*). In the absence of any treatment in munc18-1 hippocampal slices, the BrdU+ cells after 1 DIV are distributed partly into the migration area [i.e., neuroepithelium (ne)/stratum oriens (s.or.)] and partly into the stratum pyramidale (s.pyr.). After 1 DIV in the presence of MK801 and to a greater extent in the presence of bicuculline, BrdU+ cells that have failed to migrate are distributed into the migration area. Scale bar, 20 μm. *B*: histogram showing the migration indices obtained after treatment for 1 DIV with 10 μM MK801 or 50 μM bicuculline compared with the untreated control (munc) condition in hippocampal organotypic slice cultures from munc18-1 mutant mice. The migration indices are expressed ($\pm$ SE) as the ratio between the percentage of cells that reached the stratum pyramidale after 1 DIV and the percentage of cells that were still in the migration area (i.e., from the neuroepithelium to the stratum oriens), with the average of the control values being set to 10. #$P < 0.01$. The number of experiments is given in parentheses. CTRL, Control; BICU, bicuculline. [From Manent et al. (410).]

NMDA receptors. This sequential expression of receptors follows the general rules shown in acute slices for the formation of synapses (see sect. VI). Also, GABA but not glutamate receptor antagonists generated tonic currents. The generation of currents in migrating neurons could control neuronal excitability and motility (555). The presence of functional AMPA currents in neocortical neurons (438) but not hippocampal (410) principal neurons could reflect genuine differences between different neuronal populations. In keeping with this, Represa and co-workers (411) confirmed that tangentially migrating interneurons express AMPA but not GABA or NMDA receptors. Therefore, migrating principal cells and interneurons may be modulated by different receptors during their journey.

Are these receptors functional and involved in modulating neuronal migration? Using mouse cerebellar slice preparations and a laser scanning confocal microscope, Komuro and Rakic (345) showed that selective blockade of N-type functional calcium channels in granule cells retarded cell movement. In contrast, blocking T- or L-type calcium channels or sodium and potassium channels had no effect on granule cell migration. In another study, the same group (346) showed that blockade or activation of NMDA receptors, respectively, retarded and increased cell migration. Blocking uptake of glutamate accelerated the rate of cell migration reflecting a contribution of endogenous extracellular glutamate to the process. The amplitude and frequency components of Ca^{2+} fluctuations correlated positively with the rate of granule cell movement, suggesting a modulation of migration by spikes and waves of calcium oscillations (347, see also Ref. 348). These observations collectively suggest that migration speed and directions are modulated by extrinsic calcium signals to which transmitters participate prior to synapse formation. Molnar and co-workers (396) studied the role of $GABA_B$ receptors by combining preembedding immunocytochemistry with cell tracking in embryonic brain slice cultures. The authors found that a $GABA_B$ receptor antagonist produced a dose-dependent accumulation of tangentially migrating neurons in the ventricular/subventricular zones of the cortex as well as a shorter leading process. The effects of the antagonist are most likely presynaptic as agonists did not generate currents in these neurons in keeping with the observations that $GABA_B$ agonists mature first presynaptically and are expressed only subsequently in the postsynaptic target neurons (see sect. IX). Other studies suggest developmentally regulated expression of different types of glutamate and GABA receptor subunits; some have prominent expression in the embryonic and/or postnatal brain, whereas others are mainly present in the adult brain (406). Changes of subunit composition may constitute essential factors in the development of particular brain regions and specific neuronal populations within these regions.

The role of $GABA_A$ receptors in the neuronal migration has also been investigated in the newborn rat parietal cortex in vivo and in vitro. Local in vivo application of bicuculline methiodide via cortical surface Elvax implants revealed heterotopic cell clusters in the upper layers (264). In organotypic neocortical slices from embryonic day 18–19 embryos, bicuculline increased the migration speed, suggesting an inhibitory action of GABA on migration (264). Similarly, in embryonic rat cortical slices, bicuculline enhanced migration of neurons at E18 (44).

Bolteus and Bordey (71) studied the role of GABA in proliferation and migration of neuronal precursor migration in the anterior subventricular zone and the rostral migratory stream. In acute brain slices, application of low concentrations of GABA reduced the rate of cell migration, whereas bicuculline enhanced the migration rate significantly, suggesting that endogenous GABA tonically reduces the speed of cell migration via $GABA_A$ receptor activation. Interestingly, inhibition of the uptake of GABA or enhancement of the release of GABA by high K^+ from neuronal precursors reduced migration, the later effect being blocked by bicuculline. The authors suggested that astrocytes generate a microenvironment that controls the degree of $GABA_A$ receptor activation and the migration of neuronal precursors. In an earlier study, the same group showed that GABA is depolarizing at resting membrane potential, suggesting that the depolarization by means of a rise of calcium constitutes the basis for a paracrine signal to dynamically regulate their proliferation and/or migration (43, 652).

Different results were obtained in the hippocampus. Using two-slice cocultures obtained from GFP and naive mice (see above), Represa and co-workers (410) measured the effects of various agents on the migration of the GFP neurons that can be readily visualized when they migrate on the naive slice. The authors showed that both bicuculline and a NMDA receptor antagonist retard considerably the migration of GFP neurons. Most interestingly, when the GFP slice was apposed to a slice obtained from a Munc18 knockout mouse, in which there is no vesicular release, the effects of bicuculline and NMDA receptor antagonist were preserved, suggesting that an endogenous nonvesicular release of GABA and glutamate modulates the migration of neurons. Therefore, the stimulation of electrically silent neurons induces the release of GABA and glutamate in a "noncanonical" mechanism (410) that modulates the migration of adjacent neurons by an action on receptors in the absence of functional synapses (see Ref. 516 for review). The reasons for the different actions of bicuculline in the hippocampus and neocortex are not known; one possibility is the generation by bicuculline of seizures in adjacent more developed neocortical neurons or a different involvement of astrocytes as suggested by Bolteus and Bordey (see below).

Collectively, these observations suggest that receptors are expressed well before synapses and are activated during migration, providing a possibility for extrinsic factors to modulate migration. This is in keeping with the need for intracellular calcium alterations for cytoskeletal changes that are indispensable for motility and migration (348). Other major functions are also regulated by calcium oscillations including expression of the GABA phenotype (see below). These observations have important clinical implications considering the importance of migration disorders and the effects of widely used transmitter acting drugs during pregnancy (see sect. IX).

E. Early Trophic Actions of GABA

Early studies showed that applications of GABA to cultures of mouse neuroblastoma cells triggered a series of changes including proliferation of coated vesicles and the appearance of electron-dense material aggregated at the inner aspect of the plasma membrane (136, 186). The same group showed that GABA treatments affect the growth and development of embryonic chick cortical and retinal neurons in culture, including the length and branching of the neurites (582). GABA treatment also increased the density of neurotubules, rough endoplasmic reticulum (RER), and Golgi apparatus. Similar observations were made by Schousboe and colleagues (46, 252, 428). Treatment of cultures with the GABA agonist 7-tetrahydroxyazolo[5,4-c]pyridin-3-ol (THIP) of 7-day-old cultures but not older ones increased the cytoplasmic density of RER and Golgi apparatus.

GABAergic embryonic hippocampal neurons express a high potential for growth and sprouting in vitro and in vivo. Thus Robain et al. (523) compared the maturation of neurons taken from E18-E19 embryos, dissociated in suspension, and either grafted to an adult hippocampus or cultivated. The authors reported a substantial proportion of GABAergic cells in vivo and in vitro that exceeds the percentage of GABA positive neurons in situ. The same group then tested directly the effects of GABA or GABA receptor antagonists on the maturation and neuronal growth in vitro (35). Whereas neuritic outgrowth of cultured hippocampal neurons was not affected by muscimol, a $GABA_A$ agonist, addition of bicuculline to the culture medium severely reduced the number of primary neurites and branching points (35). The lack of effects of the $GABA_A$ agonist may be due to a degradation of the agonist or a desensitization of receptors (or the fact that endogenous GABA saturates the receptors, thus adding more GABA will have no effect). The powerful effects of $GABA_A$ antagonists suggest that an endogenous release of GABA stimulates growth of hippocampal neurons probably via the excitatory actions of GABA at an early stage.

Groc et al. (239, 240) investigated in vivo the early roles for GABA- and glutamate-driven hippocampal neuronal network activity in the morphological differentiation of CA1 pyramidal cells. Injection of tetanus toxin into hippocampus of P1 rats induced strong reduction in the frequency of spontaneous GABA and glutamatergic synaptic currents and led to a complete blockade of GDPs during the first postnatal week. Morphology of neurobiotin-filled CA1 pyramidal cells was analyzed at the end of the first postnatal week (P6–10). In activity-reduced neurons, the total length of basal dendritic tree was three times less than control. The number, but not the length, of basal dendritic branches was affected. The growth impairment was restricted to the basal dendrites. The apical dendrite, the axons, or the soma grew normally during activity deprivation. Thus the in vivo neural activity in the neonate hippocampus seems to promote neuronal growth by initiating novel branches (239). However, quantitative analysis of dendritic parameters and spine density from reconstructed CA1 pyramidal cells showed that young adult CA1 pyramidal (postnatal day 31–34) cells that were exposed to activity deprivation in the neonatal period were almost indistinguishable from control cells of the same age. These results suggest that the early hippocampal activity driven by depolarizing GABA and glutamate controls the growth rate but is not necessary for the generation of an adult normal basal dendritic tree (240).

F. Conclusion: GABA Is an Ancillary Communicating Signal With Multiple Actions at Early Developmental Stages

GABA is not a novel signaling molecule. GABA and its receptors appeared very early during evolution (531). Glutamic acid decarboxylase (GAD) is ubiquitous in prokaryotes and eukaryotes. In hydra, neurosteroids and GABA antagonists like picrotoxin act on $GABA_A$ receptors with a modulation of the feeding response (131, 153). GABA exerts important roles in plants, and these actions are not only metabolic (77, 78). The synthesizing enzyme of GABA, GAD, has been identified in several plants including arabidopsis where it is involved in several important functions. Severe morphological abnormalities, such as short stems, are induced in transgenic tobacco plants expressing a mutant GAD lacking the calmodulin-binding domain (38). In plants, disruption of enzymes for GABA degradation results in abnormal development (76), and a GABA gradient is involved in guidance of the pollen tube (494). Interestingly, stress leads to extremely high GABA and low glutamate. Thus the synthesis of GABA is rapidly stimulated in wounded plant tissues consequent to the activation of GAD, and GABA is activated by infection of plants (117).

Fibers showing GABA-like immunoreactivity are present in abundance in the longitudinal nerve cords, lateral nerves, and fibers forming commissures in the brain of a flatworm *Dugesia tigrina* (188). GABA medi-

ates enteric muscle contraction in the nematode *Caenorhabditis elegans* via a receptor coupled to a cation-selective ligand-gated ion channel (EXP-1) (42). The EXP-1 protein resembles ionotropic GABA receptor subunits, but the pore-forming domain has residues that confer cation selectivity. Hence, GABA exerts a cation-mediated excitatory action. GABAergic neurons that are also GAD positive are present in the first brain nerves and cerebral and caudal ganglia of the urochordate *Oikopleura dioica* (575). Therefore, although GABA has a major metabolic role at a very early stage of evolution, its multiple facets have served many functions including early signaling actions notably as a transmitter.

These observations raise the possibility that at least some of the fundamental properties associated with the actions of GABA have been exploited early on in evolution. The anionic permeability of its channels and the chloride fluxes associated with its operation appear to be useful at an early developmental stage well before synapses are established. The most parsimonious hypothesis is that GABA as an informative molecule is a pioneer signal that contributes to organize a heterogeneous population of immature neurons. The paracrine actions of GABA at that stage and the use of a nonvesicular release of GABA to flood the environment and the delayed development of GABA transporters (see below) will facilitate distal actions of GABA. The stimulation of neuronal growth appears to be mediated by a positive loop whereby the release of GABA excites postsynaptic neurons that release BDNF leading to an enhancement of the actions of GABA (see sect. VIII). Although important shifts in the action of other transmitters during development have been reported, those involving GABA are of particular importance as GABA receptors are present in every neuron and GABAergic inhibition in every circuit. In addition, the reversal of GABA actions has no parallel situation in glutamatergic synapses.

Since glutamatergic signaling operates somewhat later that GABAergic synapses (see sect. V), GABA will be de facto the principal excitatory transmitter early on. An additional factor to consider is the earlier maturation of functional NMDA receptors than AMPA receptor-mediated EPSCs, the so-called "silent" synapses (169; but see also Refs. 225, 238). There are some indications that the voltage-dependent Mg^{2+} blockade is less efficient in neonatal neurons than adults (57, 80, 279, 338, 606; but see Ref. 328) consequent to important changes in subunit composition that occur during maturation (458, 519). Interestingly, GABA is an important source to remove the blockade and enable the activation of NMDA receptor channels (59). This "synergistic" action of GABA with NMDA receptors (377) contrasts with adult neurons and constitutes a major aspect of early GABA signaling in addition to the generation by GABA of action potentials (see sect. III). Although glutamate is likely also released at an early stage, in physiological conditions, circulating concentrations of GABA are more elevated than those of glutamate because of the earlier maturation of glutamate transporters than GABA transporters (see sect. VII). This explains why GABA but not glutamate tonic currents have been recorded at an early stage. Therefore, the system is tuned to favor the actions of GABA early on.

V. GABAERGIC SYNAPSES ARE ESTABLISHED BEFORE GLUTAMATE SYNAPSES

A. GABA Receptor Antagonists Block Early Ongoing Activity in the Hippocampus

Since the actions of GABA and glutamate receptor antagonists are selective on immature neurons (56, 467), they can be used to determine the contribution of GABA and glutamate PSCs to ongoing activity. In their initial observations, Ben-Ari and colleagues (56, 115) reported that during the first postnatal week, bicuculline blocked ongoing activity and often fails to generate seizures as in adults. At an early stage, ongoing activity is dominated by the so-called GDP, a transient network-driven pattern mediated primarily by GABA that disappears progressively with the D-H shift (see sect. VI). GDPs reversed at a potential close to that of currents generated by exogenous applications of GABA, suggesting that immature PSCs are mediated primarily by $GABA_A$ receptors. Both occurred at more depolarized potentials in immature neurons than adult ones, confirming the early depolarizing actions of GABA (see sect. III). GDPs were, however, also blocked by glutamate receptor antagonists, notably by the NMDA receptor antagonist APV (135, 210, 212), suggesting a synergistic contribution of depolarizing GABA and NMDA receptors (see also below).

B. GABAergic Synapses Are the First Functional Synapses on Principal Cells of the Hippocampal Formation

The use of GABA receptor antagonists to determine the sequential maturation of GABA and glutamate synapses has however several limitations in immature networks as reflected by the observation that in P0-P1 slices: bicuculline generates seizures in some studies but not in others (see sects. VI and IX). Antagonists can affect differently various neuronal populations reducing the excitatory drive and disinhibiting principal neurons (see, for instance, Refs. 600, 602). Bicuculline also blocks potassium channels (155). GABA receptor antagonists generate seizures more readily in intact hippocampal preparations than in slices, most likely because of the loss of a significant number of axonal inputs and synapses that at that

developmental stage are needed to generate network drive synchronized patterns (316). In addition, the heterogeneous intrinsic properties of developing networks constitute major handicaps. Adjacent neurons that belong to the same neuronal population can have different GABAergic signaling at different developmental stages. Clearly, a more direct demonstration that GABAergic synapses are formed and operative before glutamatergic ones must rely on more direct morphofunctional observations.

This was first performed in the rodent CA1 pyramidal neurons in hippocampal slices at birth (P0) (627) (Fig. 7). Neurons were patch-clamp recorded, their synaptic activity determined, and neurons reconstructed post hoc for morphological studies (627). Three populations of neurons were observed in P0 slices: *1*) The majority of the neurons (80%) were "silent" with no spontaneous or evoked PSCs including when strongly stimulated by various agents; neurons have small somata and either no dendrites or an anlage of apical dendrite; *2*) 10% of the neurons are "GABA only," i.e., they have GABAergic but no glutamatergic PSCs. These neurons have a small apical dendrite restricted to the stratum radiatum and PSCs mediated only by $GABA_A$ receptors; and *3*) 10% of the neurons are "GABA and glutamate," i.e., express PSCs mediated by $GABA_A$ and glutamate receptors. These have an apical dendrite that reaches the stratum lacunosum moleculare and basal dendrites, indicating that the former develop first.

None of the recorded neurons had glutamate without GABAergic PSCs, confirming the earlier maturation of GABAergic synapses. Interestingly, only pyramidal neurons that had a small apical dendrite expressed functional GABAergic synapses, suggesting that GABAergic synapses are formed first on the apical dendrites, not on cell bodies. Also, only neurons that had an extended apical dendrite that reached the distal lacunosum moleculare expressed functional glutamate synapses. This may suggest that glutamate synapses are formed first on the distal dendrites of lacunosum moleculare. Alternatively, glutamatergic synapses are only formed on principal cells when the target neuron has reached a more developed stage. Clearly, the conditions required for the formation of GABA and glutamate synapses differ (see below). The three neuronal populations differed significantly in terms of their arbor and extension as reflected also by their different capacitance that was predictive of the functional synapses present on the recorded neurons. Although a few neurons, <4% of pyramidal neurons, expressed NMDA but not AMPA receptors, there was no morphological difference between these neurons, indicating that neurons with "silent" glutamate synapses do not constitute a unique population. Basal dendrites were formed last, suggesting that the first synapses established in the developing hippocampus are located on the apical dendrites of principal cells (see below). Therefore, at least in pyramidal neurons, GABAergic systems mature before glutamatergic ones, and GABA provides all the early initial excitatory drive.

The conclusions derived from that study were that neurons that have a soma but not apical dendrites will have receptors but no functional synapses. Then the apical dendrites start growing and GABA synapses are first formed. Only when the apical dendrites reach the distal lacunosum moleculare are glutamatergic synapses established. This reinforces the need for detailed morphological investigations when immature neurons are investigated to take into account their extreme heterogeneity. Another factor to consider is the presence of pioneer pyramidal neurons or interneurons with arborized den-

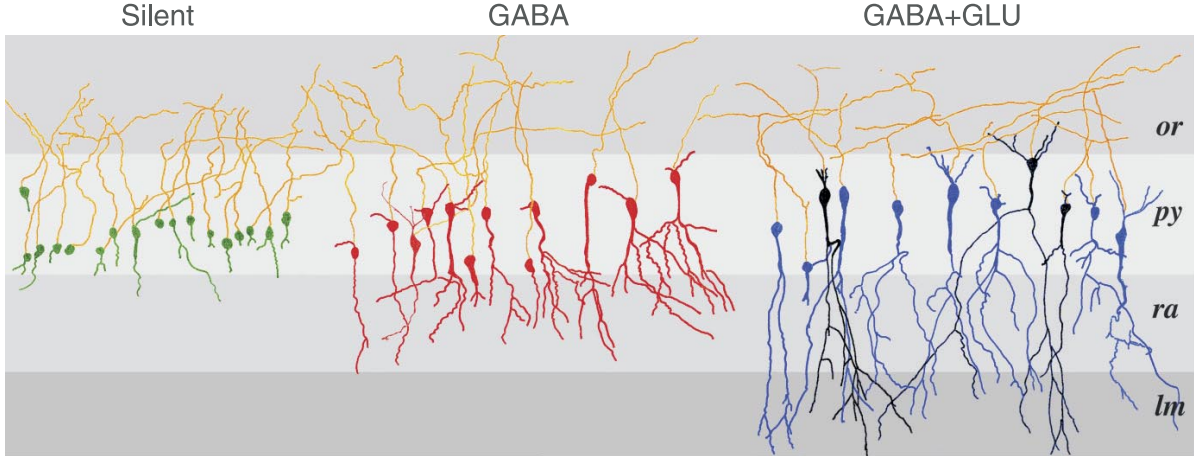

FIG. 7. Camera lucida reconstruction of injected P0 pyramidal cells grouped accordingly to their synaptic properties. The neurons not represented here are shown in Figure 9*A*. Silent cells are shown in green, GABA-only in red, and neurons with GABA and NMDA or GABA plus NMDA plus AMPA receptor-mediated PSCs are in black and blue, respectively. Note that each group of cells has a relative homogeneous degree of maturation. GABA-active and GABA plus glutamate-active neurons differ essentially by the presence of an apical dendrite in the stratum lacunosum moleculare. Note also that there are no morphological differences between GABA plus NMDA and GABA plus NMDA plus AMPA-active cells. [From Tyzio et al. (627).]

drites at an early stage when most neurons are quiescent with little or no functional synapses; this may explain the observations of AMPA and NMDA receptor-mediated population field EPSPs already at birth in CA1 (161).

One important implication of this study (627) is that the formation of synapses is strongly dependent on information provided by the target neuron. Indeed, as glutamatergic axonal afferents are present at an early developmental stage in the appropriate layer (see below), the GABA-glutamate sequence is not due to a later arrival of the inputs. Therefore, glutamatergic axons that meet immature apical dendrites will not establish synapses, whereas GABAergic axons will: different clues are required for the formation of glutamatergic and GABAergic synapses. Studies in culture offer several convenient tools to examine these issues. GABAergic and glutamatergic neurons develop in culture and express similar morphological and biological features as in vivo (62). With the use of this preparation, a detailed description of the formation of GABA and glutamate has been reported and the role of neuronal activity analyzed (87, 141, 224, 456, 684). Thus the axons of cultured hippocampal neurons form presynaptic specializations soon after they emerge and do not require conditions other than encountering appropriate targets (197). However, cell bodies and dendrites cannot induce the formation of presynaptic specializations until they have reached a critical stage of "maturation" (197). These and other observations are in keeping with the notion that axons develop early and reach their targets and then wait for appropriate cues from the targets to establish functional synapses. One possibility is a retrograde messenger that could be targeted to glutamate but not GABA synapses and would be required for the formation of synapses. In several systems, specific cues required for synapse formation between pre- and postsynaptic neurons have been investigated (151).

Poo and colleagues (544) have shown recently that a trophic factor, BDNF, induced rapid and persistent potentiation of evoked glutamate release when the postsynaptic neuron was glutamatergic but not when it was GABAergic. In a single presynaptic neuron innervating glutamatergic and GABAergic neurons, only the former target was modulated by BDNF, suggesting a target-specific action of BDNF on individual terminals. Cortical cocultures containing neurons that were obtained early during development (E16) readily evoked GABA PSCs, whereas glutamate PSCs were mainly evoked when the cocultures were obtained from more adult neurons (E19) (454). This was due to a reduction of the number of release sites, the quantal amplitude, but not the mean release probability. This suggests that the formation of glutamatergic but not GABAergic synapses is influenced by a postsynaptic factor. In another study (641), the role of axonal guidance in the choice of the correct target neuron was investigated using an in vitro system in which neuritic outgrowth of rat cortical neurons is accurately guided along the narrow pathways of a surface micropattern. The authors found that precise axonal guidance is insufficient for a target-specific synapse formation; additional cues are required to enable recognition between individual cells (641). Molecular matches between pre- and postsynapse play a central role in the establishment of the correct connections. The role of extrinsic and intrinsic cues in brain development and the establishment of neuronal phenotypes has been extensively documented at earlier developmental stages (183, 368). However, more studies are required to examine in parallel the maturation of GABA and glutamate synapses and compare their development and cross-relations. This information will in turn facilitate the definition of cues that govern the formation of an equilibrated network with the appropriate density of GABA and glutamate synapses. We shall not review here homeostatic mechanisms in the formation of synapses as this has been almost exclusively concerned with glutamatergic synapses and reviewed extensively recently (623).

The earlier maturation of GABAergic synapses has also been validated in another major component of the hippocampal formation: the granule cells of the fascia dentate. As developing granule cells have a developmental period that is primarily postnatal, they are useful candidates to examine this rule in postnatal slices. In this region, inhibitory interneurons are generated in utero in rodents, and their circuitry is well developed even as their target excitatory connections are still being formed. GABAergic neurotransmission was found to prevail in immature cells and the contribution of glutamatergic neurotransmission increased with neuronal maturation (387). In another study (12), two GABA currents evoked in immature granule cells by medial perforant path stimulation showed only depolarizing GABAergic synaptic input at first and then also glutamatergic synaptic inputs. Neurons were identified on the basis of their decreasing input resistance and resting membrane potential getting more hyperpolarized with maturation. Miniature GABA PSCs have a slower kinetics in immature granule cells (P0-P15) resulting in a larger decrease in synaptic charge transfer than in adults (270). Other unique features of immature GABAergic currents have been reported (125, 387, 388). Studies by Soltez and co-workers (269, 270) using pair recordings from neonatal granule cells show that the spontaneous activation of $GABA_A$ receptors displays unique properties that are distinct from the temporal patterns and biophysical features observed following $GABA_A$ receptor activation in the adult dentate gyrus. Therefore, the earlier formation of GABA-operated synapses is a general rule that applies to the various subpopulations of glutamatergic neurons in the hippocampus.

Parallel studies have suggested that GABA neurons play a pioneer role in the formation of glutamatergic synapses. Thus Soriano and colleagues (598) have shown that GABA interneurons are the first targets of commissural projections; over 60% of the targets of commissural axons according to quantitative electron microscopic (EM) studies. The authors suggested that early GABAergic neurons may constitute pioneer cells that guide the formation of commissural axons and die subsequently (291). The targets of commissural connections were identified in this study as being on interneurons, and signs of cell death were observed with EM techniques. In this model, early maturing neurons will help guiding glutamatergic axons to their targets. In contrast, another study suggests that when genes required for the tangential migration of interneurons are deleted and interneurons fail to reach the hippocampus, the organization of hippocampal connections remains quite normal (505; see also below).

C. GABAergic Interneurons Mature Before Principal Neurons and Follow the Same Sequence

If GABAergic synapses are operative before glutamate synapses, then GABAergic interneurons and their axons must mature before glutamatergic pyramidal neurons. Morphological studies suggest an earlier maturation of GABAergic neuronal elements. GABAergic neurons in the rat hippocampus appeared at the 18th day of gestation deeply in the intermediate zone near the ventricular zone, and superficially in the marginal zone near the hippocampal fissure (39, 529). GABA immunolabeling is enriched in the neuropil initially rather than in the principal layers in keeping with the early formation of dendrite targeted synapses. At E19, most pyramidal neurons have just finished their formation (11) and have not developed the apical dendrites (445, 446) required for the formation of synaptic contacts in the marginal zone (197, 627). The early formation of GABAergic neurons is also reflected by a variety of markers including GAD (168), GABA (529), and calbindin (598). These and other synaptic markers including synapsin-1 and synaptophysin labeling are present in dendritic layers but not in the stratum pyramidale, suggesting that the first synapses formed in the hippocampus are GABAergic peridendritic synapses that precede perisomatic GABAergic synapses (see below). The presence of GAD containing terminals in utero and at birth in the neuropil of stratum radiatum (529) also suggests that afferent fibers are present prior to synapse formation. Golgi impregnation was used to study the dendritic differentiation of granule cells in the rat fascia dentate (403). This was followed by gold-toned analysis to identify the fine structural study of the same identified neurons and of the input synapses onto their cell bodies and dendrites. Authors reported that the plexus of GABAergic axons is already well developed at an early stage when granule cells are still forming.

A direct validation of the proposed model came from morphofunctional studies in which a large number of interneurons recorded in the CA1 region of slices of newly born rat pups (P0) and reconstructed after physiological characterization (51, 266; also see Refs. 211, 213). At birth, three types of interneurons were found: *1*) noninnervated "silent" interneurons (5%) with no spontaneous or evoked synaptic currents, *2*) GABA only interneurons (17%) with $GABA_A$ but no glutamate synapses, and *3*) GABA and glutamate interneurons with GABA and glutamatergic synapses (78%).

Silent neurons remained silent when ongoing activity was artificially augmented and latrotoxin applied to deplete vesicular pools confirming the lack of functional synapses. Also, high-frequency electrical stimuli in the vicinity of visually patched clamped neurons did not evoke PSCs or action potentials/currents when recorded with cell-attached recordings. In contrast, functional GABA and glutamate receptors were present on silent interneurons since applications of agonists evoked the expected currents. Reconstruction of these neurons revealed that the three types of interneurons correspond to three developmental stages: noninnervated neurons that have little or no dendrites, interneurons with only $GABA_A$ synapses having a more extended dendritic arbor, and interneurons with both GABA and glutamate synapses that are extensively developed. In fact, the total surface of the dendrites, axons, and somata were significantly lower in silent neurons than in GABA only, and the latter were also significantly smaller than neurons endowed with operative GABA and glutamate synapses. Neuronal capacitance enabled the prediction of the recorded electrical response: the curve relating surface and capacitance revealed three groups of neurons that corresponded to the three neuronal populations.

These studies confirmed the early maturation of interneurons at a time when pyramidal neurons are primarily silent. Thus, at birth, 80% of CA1 pyramidal neurons but only 5% of interneurons are silent, although $<10\%$ of pyramidal neurons and $>60\%$ of interneurons have both GABA and glutamate synapses at that stage. In fetuses (E18–20), the majority of interneurons (65%) had operative synapses, whereas nearly 90% of principal neurons were silent. Similarly, the ratio of GABA only versus GABA and glutamate interneurons was more than sixfold higher at E18–20 than at birth, confirming the late formation of glutamate synapses on interneurons that are only established once target neurons have functional GABAergic synapses. Putative pioneer interneurons were also reconstructed; these had an extensive dendritic and axonal arbor that innervated the CA3 region, the distal

lacunosum moleculare, or the subiculum. These neurons are similar to the back-projecting interneurons described in adults (201). Therefore, interneurons follow the same sequence as principal cells but earlier: at all developmental stages, there is a GABAergic source of synaptic activity to stimulate neuronal growth.

D. GABAergic Interneurons Innervate First the Dendrites of Principal Neurons

As stressed above, GABAergic interneurons constitute an extremely heterogeneous population of neurons, and there has been enormous difficulties in classifying the various neuronal types relying on morphological and electrophysiological or biochemical markers (201, 497). This is even more difficult in developing networks because immature neurons lack many of the chemical markers and the extended dendritic or axonal arbor required to identify them (266, 369). Determining the sequence of maturation of various interneurons is important to determine whether indeed GABAergic synapses are formed on the dendrites of principal neurons before their somata. This is also important since the first patterns recorded in the developing hippocampus are generated by interneurons (51, 236).

In spite of these difficulties, morphofunctional studies suggest that interneurons that innervate other interneurons and/or the dendrites of principal cells mature before those that innervate the somata of principal cells. Thus, in the adult hippocampus, calretinin immunoreactive interneurons innervate exclusively GABAergic dendrites and other calretinin or calbindin D28k or vasoactive intestinal polypeptide (VIP)-containing basket cells, whereas parvalbumin-containing basket and axo-axonic cells are selectively avoided (245). The unique connectivity of these neurons confers important contribution to control the generation of synchronous, rhythmic hippocampal activity. Its target specificity is also an important asset to determine the sequential maturation of GABAergic and glutamatergic terminals. Calretinin neurons are detected as early as embryonic day 15 in the primordial hippocampus in the primitive plexiform layer (292, 397).

In a more recent study (670), neurons from the proliferative zone from GFP mouse embryos were transplanted onto neonatal cortical feeder cells and assessed for their ability to generate specific interneuron subtypes. Parvalbumin- and somatostatin-expressing interneurons originate primarily within the medial ganglionic eminence, whereas the calretinin-expressing interneurons derive mainly from the caudal ganglionic eminence (see also Ref. 88). Therefore, GABAergic interneurons that innervate dendrites and somata of principal cells may have a different origin and migration trajectory. Since the fate of these neurons and their discharge properties appear to be determined by their origin (88, 353), it will be of interest to examine which factors control their phenotype during migration and their integration within the networks. One prediction is that interneurons that innervate other interneurons or the dendrites of principal cells are functional before neurons that innervate cell bodies.

An interesting example is also provided by the maturation of cholecystokinin (CCK)-immunoreactive interneurons. These neurons innervate the dendrites of principal cells in all hippocampal subfields at birth, and the axon arbors begin to concentrate around pyramidal cell bodies only at P8 (463). Synapses formed on CCK-containing interneurons are primarily symmetric initially and subsequently shift to asymmetric synapses, suggesting an earlier formation of GABAergic terminals (463). These observations also suggest that the interconnections between interneurons mature first. The authors suggested that the shift from dendritic to somatic targets occurs when the D-H shift occurs. In sum, these observations are in accord with the suggestion that the earliest functional synapses are between interneurons, then on dendrites of principal neurons, and only lastly on the somata of principal neurons.

The maturation of endocannabinoid systems has also been investigated since type 1 cannabinoid receptors that are selectively located on axon terminals of GABAergic interneurons mediate a retrograde synaptic signaling that controls the release of GABA (308, 663). Type 1 cannabinoid receptors are expressed at an early stage, and its cellular and subcellular patterns of expression during early postnatal life are similar to that of CCK-containing axons (462). This distribution suggests that endocannabinoid receptors can modulate at an early stage, well before the D-H shift has occurred, the dominant ongoing GABAergic activity (461, 462). This hypothesis was tested recently in vitro and in vivo (66). The authors reported that ongoing activity during early maturation is modulated by endocannabinoid receptors and GDPs, and other polysynaptic events activate these presynaptic receptors leading to a retrograde negative-feedback loop acting on the terminals of CCK interneurons to reduce the release of GABA (see sects. VI and VII). In summary, interneurons innervate other interneurons before innervating principal cells, with functional GABAergic synapses operating at a time when glutamatergic activity is sparse or nonexistent. Furthermore, dendritic targets are favored by axons and by connections established in neurons that have little or no operative synapses.

E. Use of Knockout Strategies to Determine the Role of GABA in Development

Obata and co-workers (22) have generated GAD 67 and/or GAD 65 knockouts to investigate the conse-

quences on brain development. Deleting the 65 isoform of GAD did not produce gross behavioral alterations, and the brain was histologically quite normal, although with a lower threshold for seizure generation (22). In contrast, mice lacking the 67 isoform of GAD died of severe cleft palate during the first morning after birth. As expected, these mice have much reduced GAD activities and GABA contents in the cerebral cortex (to 20% for the 65 and 7% for the 67, respectively). Their brain, however, did not show any discernible defects in its construction (23). Mice with both knockouts (290) also do not survive after birth (cleft palate) and have quite normal brains on gross histological examination in spite of an almost complete absence of GABA. However, the compensatory development of other transmitters cannot be discarded as GABA and glycine are released from the same terminals (see below), and reorganizations during brain development could explain some of these relatively minor consequences of knocking out GAD 65 or 67. A detailed microscopic investigation of these connections and distribution of various cell types in the knockouts remains to be performed. A detailed study of this phenotype is also needed as studies in this laboratory (R. Tyzio, A. Represa, and Y. Ben-Ari, unpublished data) have revealed a high density of GABAergic terminals and a quite normal frequency of GABAergic synaptic currents in GAD 65 knockouts.

Other studies have performed more selective deletions of GABA signaling. Thus the deletion of genes required for the migration of GABAergic interneurons to the hippocampus and cortex has been investigated by Rubenstein and colleagues (124). Mice lacking two homeodomain proteins needed for migration, DLX-1 and DLX-2, show no detectable cell migration from the subcortical telencephalon to the neocortex and a massive loss of GABA-expressing cells in the neocortex (17, 415). Loss of Dlx1/2 homeobox function also prevents migration of most GABAergic interneurons to the hippocampus (505). It is possible to differentially disrupt the arrival to various populations of neurons to the hippocampus. Thus deleting Nkx2.1 homeobox function depletes the hippocampus of a distinct subset of hippocampal interneurons. In contrast to the conclusions of Soriano et al. (598), loss of hippocampal interneurons had neither major effects on the early development of hippocampal projection neurons nor on the path-finding of afferent tracts: the commissural, entorhinal, and Schaeffer collaterals were intact. These mutations led to a reduction of GABAergic IPSCs in neocortex and hippocampus and cortical dysrhythmia in vivo. Dlx1 mutant mice show generalized electrographic seizures and histological evidence of seizure-induced reorganization. Whether this mutation leads to abnormal migration of principal cells and organization in the pyramidal layer of the hippocampus has not been determined.

F. GABA Transporters are Functional After Glutamate Transporters

In adult networks, the extracellular concentration of glutamate in the synaptic cleft is controlled by high-affinity Na^+-dependent transporters required both for physiological processes and for prevention of receptor overactivation and possible excitotoxic actions (152). Glutamate transporters also contribute to the synthesis of GABA via the supply of glutamate to GABAergic synaptic terminals (423). A different situation prevails in the developing brain where glutamate transporters are functional well before synapses have been established (410, 490), suggesting that the tight control of glutamate is essential to prevent possible toxic actions of glutamate. In keeping with this, blockade of glutamate transport in immature but not adult neurons by DL-threo-β-benzyloxyaspartate (TBOA) that blocks the three main neuronal transporters (561) generates in hippocampal slices, a pattern of recurrent synchronous oscillations that is readily blocked by a NMDA receptor antagonist (159). Intracerebroventricular injections of the blocker to pups generate NMDA receptor-dependent seizures. These oscillations are network driven and associated with an activity in the beta frequency range (15–20 Hz). Applications of NMDA also generated similar oscillations that are blocked by glutamate but not GABA receptor antagonists, reflecting the important role of NMDA receptors in their generation. Similar oscillations are readily generated in the intact hippocampus in vitro where their propagation can be investigated. In more recent studies, the same group has shown that the electroencephalogram (EEG) signature of these oscillations in pups is reminiscent of the seizures with suppression bursts recorded in various encephalopathies in children (442). Interestingly, a familial form of Otahara encephalopathy is associated with a mutation of a mitochondrial glutamate transporter (455). Therefore, deficiencies of glutamate transport can be deleterious during development in keeping with extensive data (431).

At birth, glutamate but not GABA transporters are functional. Thus the current generated by local applications of GABA were not modified by bath applications of SKF899756A, a selective powerful and irreversible blockade of GAT1 (72, 158). A similar experiment in P14 neurons revealed a consistent increase in currents generated by exogenous GABA (158). In contrast, TBOA augmented the currents produced by pressure ejections of glutamate and generated a large and persistent tonic current, suggesting that glutamate transporters operate in physiological conditions to prevent an accumulation of glutamate in the extracellular space. These effects do not require vesicular release of transmitters as they are observed also in the presence of botulinum toxin (158). The retarded functional expression of a GABA transporter is also reflected by the observation that in adult but not neonatal neurons,

an inhibitor of GABA transporters unravels a $GABA_B$ receptor-dependent paired pulse depression (92). Kaila and co-workers (566) showed that blockers of GABA transporters increase the frequency of GDPs at the ages studied (P4-P5), suggesting, in agreement with observations made by the authors of this review (92, 158), that blockers of GABA transport are functional when tested on polysynaptic PSCs, like GDPs, but not on single PSCs or currents evoked by local applications of GABA. In contrast, the efficient glutamate transport mechanisms lead to a rapid removal of glutamate. The generation at birth of tonic currents by glutamate but not GABA receptor antagonists is also in keeping with an early maturation of glutamate transporters. The lack of efficient GABA transporters will facilitate the diffusion of GABA to distal sites well before synapses are functional.

G. The GABA-Glutamate Sequence in Primate Hippocampal Neurons in Utero

Are these trends restricted to rodents and rabbits, or are they general to other species including human and subhuman primates? GABA receptor sites are present in cortical samples in autopsies of normal infants during the third trimester of gestation. In monkeys, GABA markers are present in utero, and labeling is increased during postnatal life (672). The activity of GAD increases twofold during postnatal development (672).

The basket cell subclass of GABAergic interneurons reaches their cellular target cells several weeks before birth (64). Huntley et al. (280) reported that in the monkey sensory motor cortex, GABA receptor immunoreactivity is enriched in the neuropil, often outlining the nonlabeled pyramidal cells and interneurons. The laminar distribution of GABA immunolabeling and GABA receptors suggests that GABAergic circuits may contribute to the establishment of afferent connections (559). In a systematic study of the emergence and distribution of GABA-containing cells in both the association (prefrontal) and primary sensory (visual) cortices using light and electron microscopic immunohistochemistry (430), the authors reported that GABA-immunolabeled neurons were present throughout the full thickness of the cerebral wall, as early as E41. In addition, migrating bipolar neurons expressed the markers, suggesting that a subset of cortical neurons may be committed to a given phenotype prior to reaching their adult position. In the occipital lobe of the embryonic monkey (429), subplate neurons contain the GABA machinery. The establishment of GABAergic neuronal circuits may be involved in the development of long tract connections that are present in the region prior to their transfer to the overlying cortical plate. This putative role of subplate neurons has received strong support recently from various studies in rodents (167, 306, 460). Therefore, in spite of their long journey in monkeys, GABAergic interneurons are present at an early stage and may modulate major functions.

The maturation of GABA and glutamatergic PSCs was investigated in a study in which primate neurons were recorded during the fetal stage of development (323) (Fig. 8). The authors made patch-clamp recordings of CA1 hippocampal pyramidal neurons in slices of macaque cynomolgus fetuses delivered by cesarean sections from midgestation to a few weeks before delivery. After functional identification of GABA and glutamate PSCs, neurons were biocytin-filled and reconstructed, and the information was used to provide a quantitative estimate of the time frame of maturation. Several major factors were quantified including GABA and glutamate PSCs, axonal and dendritic arbors, and spine density as a measure of synapse formation. As in the rodent, the reconstructed neurons consisted of three neuronal populations that correlated with the level of morphological differentiation of pyramidal cells: *1*) silent neurons with no apical (or basal) dendrites and no synaptic currents (these constitute the majority of CA1 pyramidal neurons around mid-gestation); *2*) "GABA only neurons" with no operative glutamate synapses appeared somewhat later, at a time when neurons expressed an apical dendrite that penetrated into stratum radiatum; and *3*) "GABA and glutamate" neurons that were first observed around E100 and that expressed functional GABAergic and glutamatergic PSCs. These pyramidal neurons had a well-developed axodendritic tree and dendritic spines.

The estimation made on the basis of this analysis was that hundreds of spines and functional glutamatergic synapses are established every day during the last trimester of gestation, leading to a shift from almost no synapses at midgestation in pyramidal neurons of CA1 to over 7,000 synapses before delivery. Also, axons are formed before dendrites and grow at a fast speed with a peak reached at mid gestation. The Boltzmann equation of these functions (Fig. 9) provides a good template of maturation of cortical networks in primates and is in excellent parallelism with postnatal rodent data. Results suggest that this template has been conserved across the mammalian evolution but shifted toward fetal life in primates. These observations have also important clinical implications that are discussed in section IX.

H. Glutamatergic Mossy Fiber Synapses Have an Early Mixed GABA/Glutamate Phenotype in the Developing Hippocampus

In adults, the granule cells mossy fiber synapses (MFs) form excitatory glutamatergic synapses with CA3 principal cells and on inhibitory interneurons. Gutierrez and co-workers (246–248) showed that in contrast devel-

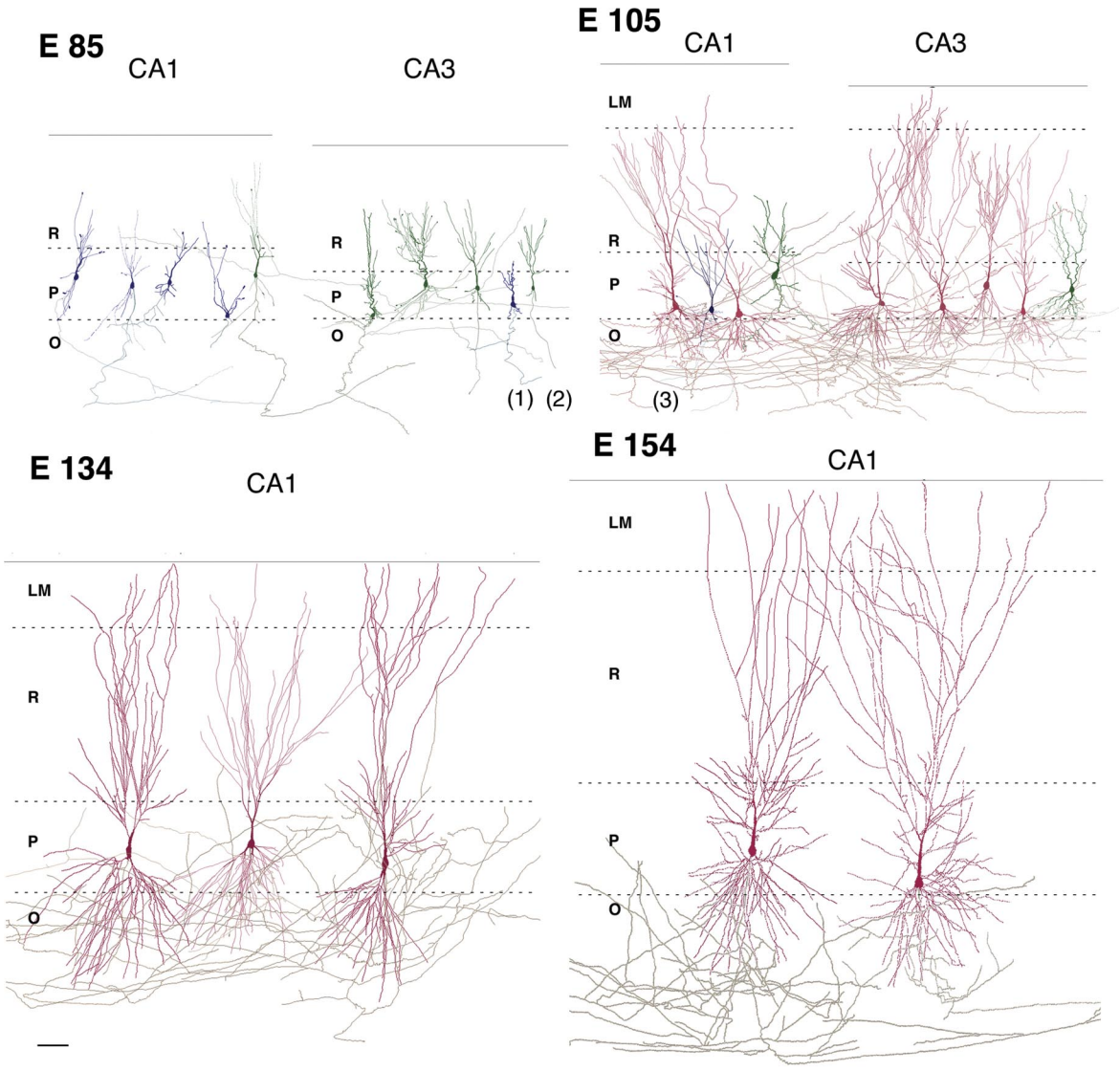

FIG. 8. Morphological differentiation of pyramidal cells in the cynomolgus monkey hippocampus during the second half of gestation. Reconstruction of biocytin-filled pyramidal cells in CA1 and CA3 hippocampal subfields at various ages in utero (E85–E154). Note an intensive growth of the pyramidal cells that reach a high level of morphological differentiation at E134, 1 mo before birth. O, stratum oriens; P, pyramidal cell layer; R, stratum radiatum; LM, stratum lacunosum moleculare; the dashed lines indicate the limits between layers, and the solid line indicates the hippocampal fissure. Axons (gray), color of the dendritic arborization indicate the expression of synaptic currents (blue, silent; green, GABA only; red, GABA + glutamate neurons). Electrophysiological recordings from neurons 1–3 are shown on Figure 3*A*. Scale bar, 100 μm. [From Khazipov et al. (323).]

oping hippocampal granule cells express markers of both glutamatergic and GABAergic phenotypes. Activation of these neurons produces glutamate receptor- and GABA receptor-mediated PSCs in their postsynaptic targets (246–248). Thus, in developing rats until day 22–23 of age, in the presence of glutamate receptor antagonist, stimulation of granule cells evoked PSCs in pyramidal cells with GABAergic properties (248). The adult purely glutamatergic phenotype was observed in more adult preparations. In guinea pig slices, Kullmann and co-workers (646) reported similar observations with a mixed phenotype that in contrast persisted in adult animals. Thus, in hippocampal slices from 3- to 5-wk-old guinea pigs, electrical stimuli that recruit dentate granule evoked in CA3 pyramidal cells monosynaptic $GABA_A$ receptor-mediated synaptic signals (646). In a more recent study, Cherubini and co-workers (535) reported that in rats, mossy fibers release GABA in addition to glutamate during the first postnatal week but not thereafter. Thus the stimulation of granule cells in the dentate gyrus gave rise to monosynaptic $GABA_A$-mediated responses in principal cells and interneurons, and chemical stimulation of granule cell dendrites by focal applications of glutamate generated barrages of $GABA_A$-mediated PSCs. Therefore, in MFs,

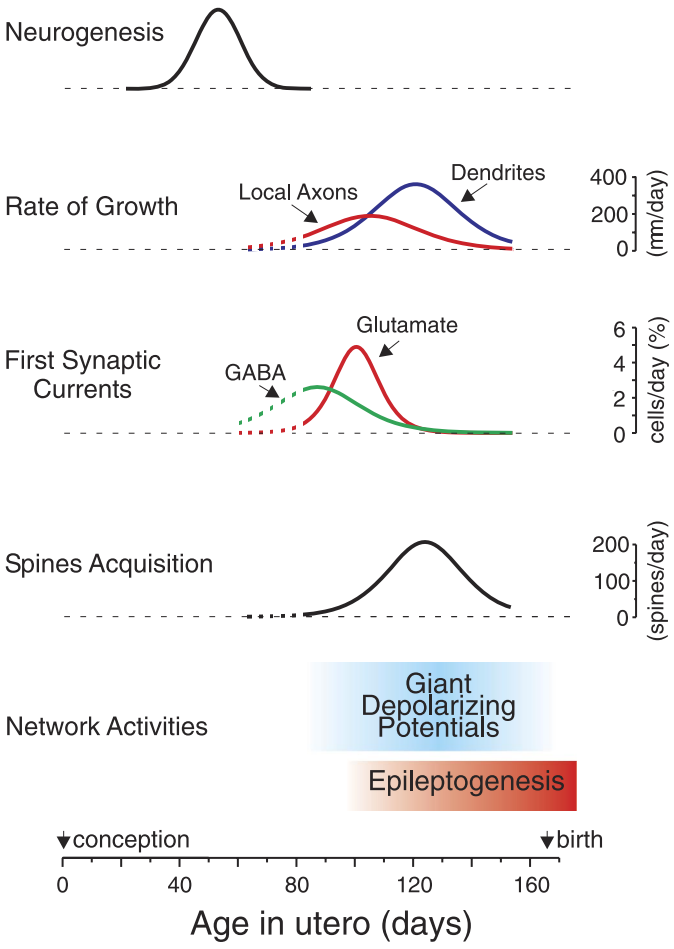

FIG. 9. Developmental template of the fetal macaque hippocampus. Hippocampal neurons are generated during the first half of gestation, with a peak at E55. During the second half of gestation, pyramidal cells grow intensively with the maximal rate of axonal and dendritic growth attained at ~E105 and E120 (200 and 400 μm/day), respectively. Already at midgestation (E85), half of the pyramidal cells receive GABAergic synaptic inputs. Glutamatergic synaptic currents appear later, and their expression coincides with the appearance of the first dendritic spines. The acquisition of glutamatergic synapses (as deduced from the number of dendritic spines) proceeds with a maximal rate of ~200 synapses formed every day on a pyramidal cell at ~E125. During the phase of intensive neuronal growth and synaptogenesis, most of the neuronal activity is synchronized in a particular pattern of spontaneous network activity, the GDPs. By the last third of gestation, the hippocampal network also becomes capable of generating epileptiform activities. [From Khazipov et al. (323).]

GABA can operate as a fast transmitter transiently during development before synapses adopt its adult glutamatergic phenotype. This suggests that the GABA phenotype predominates, including in some classical glutamatergic synapses, at an early stage. This is also observed in glycinergic synapses (see below).

Depolarizing axonal $GABA_A$ receptors on mossy fibers are involved in the activity-dependent facilitation during development. In immature mouse hippocampal slices, short-train stimulation (five pulses at 25 Hz) caused frequency-dependent facilitation of not only postsynaptic responses but also presynaptic fiber volleys that represent presynaptic activities. This fiber volley facilitation was inhibited by selective $GABA_A$ receptor antagonists, or by enkephalin that selectively suppresses excitability of interneurons. This facilitation resulted from depolarization of mossy fibers in imaging experiments using a voltage-sensitive dye. This increased mossy fiber excitability caused by depolarizing action of GABA gradually decreased with development and eventually disappeared at around postnatal day 30. These results suggested that GABA released from interneurons acted on axonal GABA receptors on mossy fibers and contributed at least partially to the activity- and age-dependent facilitation in the hippocampus (472).

Gutierrez and co-workers also reported that after severe temporal seizures, in which mossy fibers play a unique role (48), there is a reexpression of the GABA phenotype transiently. Thus, after kindling, the activation of mossy fibers generated $GABA_A$ receptor-mediated synaptic field responses in CA3 and GABA receptor-mediated collateral inhibition (247, 621). This observation is keeping with the extensive expression of early genes and markers after severe seizures (48), including some that are only expressed during development, keeping with the suggestion that epileptogenesis recapitulates ontogenesis (507). Therefore, the machinery required for the release of GABA and its postsynaptic actions is present on immature mossy fibers and may be reexpressed after severe insults, reflecting the important plasticity associated with development and after seizures.

I. The GABA-Glutamate Sequence in Proliferating Neurons in Adult Networks

Two brain regions generate new neurons in adulthood: the olfactory bulb and the granule layer of the fascia dentata. This raises the question of whether the GABA/glutamate sequence is also respected in proliferating neurons in an adult environment: is there a replay of early programs in adults? Studies using retroviral tools have shown that indeed GABA cells are formed first and establish excitatory synapses initially.

Using transgenic mice that express GFP in newly born neurons, Westbrook and colleagues (487) showed that dentate granule cells have only GABAergic PSCs initially. In a more recent paper, this group (487a) showed that the sequential expression of GABA and glutamate PSCs follows neuronal maturation in that neurons with small dendrites are silent, neurons that are more developed have GABA but no glutamate PSCs, and neurons with an extensive dendritic arbor have both GABA and glutamate PSCs. Interestingly, immature neurons also generate long-lasting currents that are reminiscent of GDPs (see above), suggesting that all developmental sequences are respected in pro-

liferating neurons. Similar conclusions were reached by Ge et al. (227) using a retroviral strategy to express GFP specifically in proliferating cells and their progeny. They reported that newly born granule cells of the dentate gyrus experience first a GABA tonic current, followed by GABAergic synapse formation, and then glutamate synapses. Perforated patch recordings showed that the concentration of $[Cl^-]_i$ is reduced progressively with an initial depolarization produced by GABA. However, E_m also changed during maturation, the effect most likely due to the inherent problems related to the use of patch-clamp recording in immature neurons (37, 626). Interestingly, the authors also showed that the polarity of the actions of GABA is dependent on the expression of the NKCC1 and KCC2 transporters as in neonates, suggesting that the D-H shift is respected during the formation of neurons in adults. The density of glutamatergic PSCs is reduced when these transporters are knocked out and/or when GABA synapses are blocked at an early stage, suggesting that the formation of glutamatergic synapses requires an earlier D-H shift and the formation of GABAergic synapses (227). This also confirms the important actions of GABA as a trophic agent as the growth of dendrites in the principal cells is retarded when GABA signaling is blocked. The early expression and importance of GABA signaling has also been demonstrated in the maturation of olfactory bulb interneurons where GABAergic responses develop before glutamatergic responses in adult-born granule and periglomerular neurons (47, 249, 389). Bordey and colleagues (71, 386, 652) have shown that neuronal progenitors of the subventricular zone contain GABA and are depolarized by GABA release, suggesting a paracrine signal among neuronal progenitors that regulates proliferation and/or migration.

Therefore, the integration of newly formed neurons follows the same rules as during brain maturation. This implies that even in an adult environment, the activation of GABA receptors and synapses exerts excitatory actions on neurons, and only once they are fully integrated do they progressively acquire the adult properties. This is in keeping with a developmental program endowed in these neurons that includes the sequential establishment of receptors, transporters, chloride regulation, and synapse formation.

J. The GABA-Glutamate Sequence in Other Brain Regions

Morphofunctional studies comparing both the physiological and morphological maturation of identified neurons have, to the best of our knowledge, not been performed in structures other than the hippocampus. However, there are several indications in many brain structures that GABAergic signaling matures before other transmitters signals including glutamate but also glycine in the spinal cord. We shall briefly review these studies before discussing the transient GABA phenotype and the development of mixed synapses that use GABA and glycine or GABA and glutamate.

1. The GABA-Glutamate sequence in the neocortex

The migration of GABAergic interneuron of the neocortex has been described in detail recently. In contrast to principal cells, interneurons are generated in the medial ganglionic eminence and migrate tangentially to their final cortical destinations (352, 415). However, there are also species-specific programs for the generation of neocortical local circuit neurons, since many primate cortical interneurons are generated in the ventricular and subventricular zone (381). Neurons that are just born and migrate to the rodent cortical plate (493) have a spontaneous synaptic activity, the majority of which is GABAergic. First detected as early as embryonic day 18 (E18), spontaneous GABAergic PSCs are present at birth in ~50% of recordings. Kriegstein and collaborators (150) have also shown that electrical stimulation of layer I generates a GABAergic excitatory PSC in pyramidal neurons, whereas activation of intrinsic layer I neurons with a glutamate agonist fails to produce PSCs in pyramidal cells. This pathway provides spatially restricted excitatory GABAergic innervations of the distal apical dendrites of pyramidal neurons during the peak period of cortical synaptogenesis. This group has also reported major changes in GABA receptor subunits that result in important alterations of GABAergic currents during the transition from precursor to fully active neurons. GABA receptors expressed in proliferating cells have a higher apparent affinity to GABA, long channel open times, and a poorly desensitizing response to activation of GABA receptors (reviewed in Ref. 492). Kriegstein and Prince (354) reported longer and slower synaptic currents in immature neurons than in adult ones as well as a lack of inhibitory IPSPs associated with less prominent actions of GABA receptor antagonists (354). In contrast, GABAergic responses were readily evoked at all developmental stages in pyramidal and nonpyramidal neurons from layers II and III of the neocortex (P4 to P10) (405). A differential role of somatic and axon hillock targeted GABAergic axons in the critical periods has been reported recently, pointing to the important differences of various interneuronal populations on the formation of cortical circuits (267). In cortical neurons in culture, operative GABA receptors are present as early as 1 day after plating, whereas the sensitivity to glutamate was retarded (340). Therefore, a parsimonious conclusion of these observations is that the neocortex follows a similar sequence during development even as far as interneurons are concerned. This is also reinforced by the observations that

initial actions of GABA are excitatory in the neocortex (Table 1). Platel et al. (504) showed recently that application of glycine to embryonic E12 slices generated a depolarization and an increase of $[Ca^{2+}]_i$ via activation of Na^+ channels and Na^+-Ca^{2+} exchanger. This in turn triggers the release of glutamate that will activate AMPA and NMDA receptors, suggesting that tonic release of glycine may activate chloride-permeable glycine channels providing in the neocortex the excitatory drive needed to activate glutamate ionotropic signaling.

2. The GABA-glutamate sequence in the spinal cord

Early formation of GABA and/or glycine synapses has been reported in spinal cord neurons in cultures and in situ in several species. In the frog *Xenopus laevis*, GABA-immunoreactive spinal cord neurons (Kolmer-Agduhr cells) began to appear by 1.2 days shortly after neural tube closure (69). The pattern of GABA-immunoreactive cells emerged during embryogenesis, as the density of GABAergic neurons increased. Early maturation of GABA signaling and the early depolarizing actions of GABA in Rohon-Beard spinal neurons of *Xenopus* larvae have also been extensively studied by Spitzer and coworkers (528, 657). Interestingly, this early maturation of GABA signaling is also found in cultures with a similar time course as in vivo, and the process requires calcium influx mediated by spontaneous voltage-dependent channels as well as transcriptional mechanisms (579, 580). Specific GABA immunoreactivity developed in spinal neurons in dissociated cell culture with the same time course previously defined in vivo (579, 580).

In the isolated spinal cord preparation from rat fetuses (E13.5-E18.5), spontaneous bursts are mediated by glycine receptors at E14.5 and GABA receptors at E15.5; blockers of glutamate synapses have no effects at that stage (473). Serial sagittal sections of embryos processed for GAD-65 showed positive axons that extended rostrally out of the spinal cord into the midbrain at an early developmental stage (E12–15) (618). Clarac et al. (120) developed an isolated brain stem/spinal cord from newborn rats. An early maturation of GABAergic control at various levels of the spinal locomotor network was reported including a locomotor-related GABAergic input onto primary afferent terminals (120, 192). O'Donnovan and coworkers (20, 65) extensively investigated the maturation of GABA signaling in various preparations including the embryonic and posthatched chick lumbosacral spinal cord. GABA immunoreactivity was expressed very early (E4), well before glycine labeling that appeared relatively late (E8) in embryonic development (20, 65). The authors concluded that glycine and GABA provide the earliest functional synapses and function transiently as excitatory synapses at a time when glutamate synapses have not been established.

An early expression of GABAergic markers was also extensively studies by Barker and co-workers. Thus, in embryonic and postnatal rat spinal cord, GABA is present as early as embryonic day 13 (E13) (97, 542). Using a fluorescence technique to unravel the maturation of various receptors in spinal cord embryos, Barker and coworkers (649) observed, starting from E13, a sequential expression of sodium channels followed by GABA and then only kainate activated glutamate channels.

3. The GABA-glutamate sequence in the auditory system

Peripheral auditory development proceeds rapidly in kittens between the 7th and 20th postnatal days, and input/output functions achieve maturity only at that stage (647). Yet, inhibition evaluated by the effects of ionophoresis of GABA and other amino acids operates at an early stage (647). In a subsequent study (648), the same group showed that capacity of GABA to reduce spontaneous or acoustically evoked discharge rates was directly related to control discharge rates in both immature and mature animals. The authors suggested that the GABA signaling operates prior to the arrival of sensory information. Kandler and collaborators (231) have shown recently that in the lateral superior olive (LSO), a nucleus in the mammalian sound localization system that receives inhibitory input from the medial nucleus of the trapezoid body (MNTB), there is a specific elimination/strengthening of GABAergic and glycinergic synapses. This step is essential for the formation of a precise tonotopic map. The immature GABA/glycinergic synapses in the rat LSO also release glutamate, which activates postsynaptic NMDA receptors. Vesicular glutamate and GABA transporter are colocalized in single MNTB terminals. At an early stage, glutamatergic transmission relying on synapse-specific activation of NMDARs may play an important role in synapse elimination and in activity-dependent refinement of inhibitory circuits.

4. Other structures

An electronic microscopy analysis of the development of the inferior olivary complex of the rat also revealed a delayed maturation of GAD-containing terminals with the classical morphological organization being reached only after P10-P15 (234). Before that stage, GABAergic projecting elements appear to remain in a waiting compartment. In cultures of septum and hippocampus, Koller et al. (340) determined the maturation of functional receptors by applications of selective agonists. The authors reported that the $GABA_A$ receptor developed prior to the glutamate receptors in both regions and appeared as early as a few days after plating, whereas over 9 days were required for the appearance of glutamate NMDA and non-NMDA receptor subtypes. Interestingly,

the receptors appeared earlier in the hippocampus than in the septum (340).

In a pioneer study on hypothalamic neurons in cultures and slices, Van Den Pol and co-workers (221) showed that the synaptic release of GABA is not only excitatory but plays a more robust role than glutamate in generating spike activity initially. The authors suggested "GABA not glutamate provides the initial excitatory drive." Applications of the $GABA_A$ receptor antagonist bicuculline induced a dramatic decrease in spike frequency (83% decrease) in developing neurons, three times greater than that generated by glutamate receptor antagonists APV and CNQX that block NMDA and AMPA subtype of receptors, respectively. The membrane properties of immature neurons are compatible with robust excitation, since GABA generates bursts of action potentials (221, 482).

In the developing retina (365), GABAergic neurons are immature at birth and become functionally mature by ~9 days after birth. Receptive-field properties of many neurons are determined by GABAergic inhibitory processes that operate around P11–P28 in the visual cortex of kitten (667).

K. A Developmental Switch From GABA to Glycine

Since GABA and glycine receptors are permeable to chloride and other anions, they should follow a similar D-H shift during maturation. This indeed has been shown to be the case in several systems (see sect. III). An additional interest in studying the maturation of glycine receptors during development stems from the fact that in adult neurons, single terminals can release GABA and glycine (293). Thus, in the spinal cord, unitary IPSCs composed of a strychnine-sensitive, glycine receptor-mediated component and a bicuculline-sensitive, GABA receptor-mediated component have been recorded (293).

In spinal cord slices of 17- to 18-day-old embryos (E17–18) and 1- to 3-day-old postnatal rats (P1–3), Ziskind-Conhaim and colleagues (217) have shown an earlier maturation of GABAergic signaling in motoneurons. Relying on the different time courses and the effects of selective antagonists, the authors suggested developmental switch from predominantly long-duration GABAergic synaptic currents to short-duration glycinergic. During embryonic development, the conductance increase generated by GABA was sevenfold larger than that generated by glycine in utero but not after birth (216–218). At birth, whole cell patch-clamp recordings from rat superficial dorsal horn neurons suggest that GABAergic but not glycinergic PSCs are present (26). At all ages, between P0 and P13–P14, GABAergic mechanisms are initially dominant over glycinergic events (26; also see Refs. 313, 515). Large, long-lasting spontaneous IPSPs with mini IPSCs were also recorded in cultures of rat spinal cord neurons (382). These appear to reflect an increased intracellular calcium concentration in the presynaptic terminals.

In the auditory system, GABA and glycine are present in several neuronal types. Thus, in the cochlear nucleus of the guinea pig, the vast majority of putative inhibitory endings contain glycine, GABA, or both, suggesting that in at least a proportion of terminals GABA may be released with glycine (296). In the medial nucleus of the trapezoid body, Trussell and co-workers (25) showed that in young animals (P5–P7), 80% of the responses evoked by electrical stimuli are blocked by GABA receptor antagonists and 20% by glycine receptor antagonists. This contrasts with the situation at the older age group (P13–P15) where glycine but not $GABA_A$ receptor antagonists blocked the responses. Since GABAergic PSCs have slower kinetics than glycinergic PSCs, this progressive maturation of glycine PSCs has important functional consequences concerning the development of hearing. Thus, at P25, IPSCs are 10 times briefer than at birth (25; see also Ref. 622). A similar switch from GABA to glycine is also observed in the lateral superior olive during the first two postnatal weeks, with GABAergic IPSCs declining from almost 80% at postnatal days 3–5 to close to 10% at P12–P16. There was an equal and opposite increase in the glycinergic component during this same period (349). In "mixed" mIPSCs, resulting from corelease of glycine and GABA from the same vesicles, there is a similar developmental switch in this structure (470). In the gerbil lateral superior olive (LSO), there is a transition from GABAergic to glycinergic transmission during the first two postnatal weeks (349). Several other studies have examined this transition and the role of activity in refinement and maturation of chloride-dependent glycine and GABA channels (41, 98, 231, 237, 281, 333, 560). Collectively, these studies possibly reflect the pioneer role of GABA that is expressed at an early stage prior to synapse refinement and expression of the mature phenotype, as if GABA signaling is "automatically" expressed first in developing systems.

L. Conclusion: A Model That Integrates These Findings

The observations summarized above lead to a hypothetical model that can be tested for its functional significance. The main features of the models are *1*) a sequential formation of GABA then glutamate synapses; *2*) axons develop before dendrites; *3*) dendrites are innervated before somata and interneurons that innervate dendrites of principal cells mature before those that are targeted to innervate the soma; and *4*) interneurons follow a similar GABA/glutamate sequence of synapse formation.

At an early stage, GABAergic interneurons and preferentially interneurons that innervate the dendrites of

principal cells establish synapses with other interneurons, i.e., the first synapses that operate in utero in the hippocampus are synapses between GABAergic interneurons. Pyramidal cells must have a small apical dendrite to have functional synapses. Immunocytochemical observations suggest that GABAergic axons do not invade the somatic layer at an early stage and wait until apical dendrite grow and penetrate the stratum radiatum. Then, glutamatergic synapses are formed on GABAergic interneurons before innervating other principal cells. The extensive early arborization of the axons of principal neurons in stratum oriens, from which originate many of the dendrites targeted interneurons, raises the possibility that glutamatergic synapses are formed first on interneurons of stratum oriens. The principal cell then elaborates apical dendrites that are innervated by GABAergic axons first, presumably by axons of dendrites targeted interneurons that develop and extend an axonal arbor earlier than other interneurons. Finally, once pyramidal neurons have extended apical dendrites, glutamatergic axons from other principal cells establish functional connections. This scheme, however, does not exclude an important role to various types of transiently expressed neurons such as the cholinergic interneurons present in the developing but not the adult hippocampal network (301). These may play a hitherto unsuspected role in the generation of early patterns.

In more general terms, axons are known to develop at a very early stage. Thus neocortical neurons have already long axons that reach the other hemisphere while they are still migrating on radial glia and have neither dendrites nor synapses (563). The earlier maturation of axons suggests that developing neurons communicate first with their targets before having operative inputs. Interestingly, recent studies in adult hippocampal (139) and neocortical neurons (165) suggest a strong correlation between the kinetics of GABA and glutamate PSCs and their axonal targets. Neurons that project to the same target have a similar PSC kinetics even when the somata of the parent cell is located in a different layer (139). Two neurons having their somata in different layers but their axons in the same one will have similar PSCs. This observation raises the possibility that neurons are programmed to innervate certain targets and to express accordingly the subunit composition of receptors. This is difficult to test at present; the use of viral labeling of interneurons (88) may help overcome this difficulty.

Other aspects of this model require further investigation. Thus, although the GABA/glutamate sequence is not due to a sequential arrival of GABAergic and glutamatergic inputs (161, 597), the mechanisms underlying this sequence and corresponding intracellular cascades are not known. This applies also to the preference of dendrites for the formation of glutamatergic synapses and to the contribution of GABAergic and chloride signaling in the construction of cortical networks. The functional significance of early GABAergic interneuronal patterns in utero and the pathogenic consequences of blocking that early pattern also remain to be clarified (see sect. IX).

VI. PRIMITIVE PATTERNS IN THE DEVELOPING BRAIN ARE LARGELY BASED ON EXCITATORY GABA

A. GDPs in the Hippocampus: A Prototype of Early Network Patterns

Recurrent GDPs were discovered in a study during which intracellular recordings were made from several hundred CA3 and CA1 pyramidal neurons in slices of neonatal rats (P0 to P18) (56). GDPs are highly reminiscent of the "spontaneous unison firing" pattern described using extracellular field potential recordings (254). Spontaneous GDPs are present in the vast majority of neurons and readily evoked by electrical stimuli in an all-or-none manner. GDPs are slow network-driven polysynaptic events (several hundreds of milliseconds usually) that are restricted to an early developmental window in the rat. An example of GDPs recorded using gramicidin perforated patch and extracellular field potential recordings is shown on Figure 10.

Considerable evidence indicates that depolarizing GABA plays a pivotal role in the generation of GDPs because *1*) the developmental profile of GDPs coincides with the excitatory action of GABA, and GDPs disappear when E-I switch occurs (56, 222, 269, 325, 326, 377, 432–434, 567, 593); *2*) GDP's reversal potential is close to E_{GABA} and depends on $[Cl^-]_i$ (56, 326, 377); *3*) intracellular blockade of $GABA_A$ receptors reveals glutamatergic component of GDPs, but the magnitude of GABAergic conductance significantly exceeds glutamatergic component (326, 377); *4*) depending on age and cell type, $GABA_A$ receptor antagonists either completely block GDPs or transform them to paroxysmal activity (56, 222, 265, 269, 316, 320, 321, 323, 325, 326, 377, 432–434, 567, 593); *5*) GABA agonists and positive allosteric modulators (diazepam) increase GDPs frequency (316, 325); and *6*) blockade of NKCC1 by bumetanide, which renders GABA inhibitory, suppresses GDPs (173, 569).

GDPs are not restricted to slices; they have been reported in intact hippocampi in vitro (318, 375), neurons in cultures (642), and organotypic slices (453). Extensive investigations have also confirmed that GDPs are present in all the regions of the hippocampal formation. In agreement with earlier studies (56), GDPs were observed in the CA3 and CA1 neonatal pyramidal neurons of the rabbit (432–434), with the CA3 region being most frequently the pacemaker region of the pattern. Menendez de la prida et al. (434) also showed that GDPs are present in granule

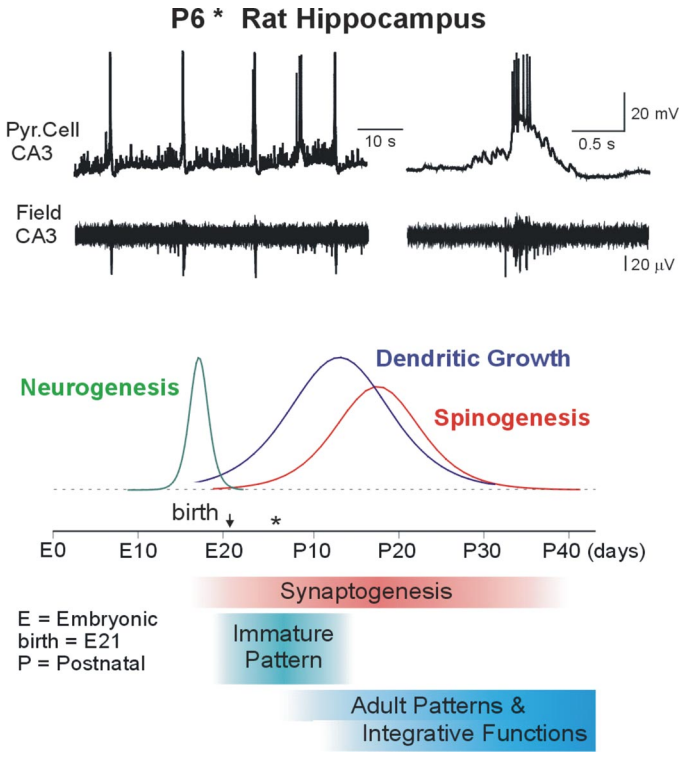

FIG. 10. Giant depolarizing potentials in the neonatal rat hippocampus. *A*: gramicidin perforated patch recordings from a CA3 pyramidal cell in P6 rat hippocampus. Concomitant field potential recordings are shown on trace below. Note that GDPs in CA3 pyramidal cell coincide with the field potential population burst. One GDP is shown on expanded time scale in the *right panel*. *B*: the developmental template of the rat hippocampus. Note that GDPs are present during the phase of the intense growth of the hippocampal neurons and synaptogenesis.

cells of the fascia dentate (see also Ref. 269) where they follow the GDPs generated in CA3. These groups and others also showed that although CA3 is the most frequent pacemaker, all hippocampal regions can generate GDPs when separated from the other components, a small region containing a couple of thousand neurons will suffice (432). Therefore, the entire hippocampal network possesses the capacity to generate GDPs, but in situ some regions are more apt to impose their frequency on other regions, and the best candidate for a pacemaker region is the CA3 region that is particularly equipped to generate oscillations. CA3 pyramidal neurons mature before CA1 pyramidal neurons, and granule cells can generate synchronized patterns somewhat later (see above).

As other polysynaptic events, GDPs are more sensitive than monosynaptic currents to many agents and procedures that alter neuronal excitability, including high Mg^{2+} solutions (513), anoxoischemic episodes (142), and manipulations that affect pre- or postsynaptic efficacy including adenosine receptors (536), acetylcholine receptors that modulate the release of GABA (409), endogenous acetylcholine that enhances the release of GABA (70), glutamate metabotropic receptors that enhance the synchronous release of GABA as in adults (509) through cAMP-dependent protein kinase (594), ATP operating via distinct P2X and P2Y receptors (537), and cannabinoid signaling that mediates depolarization induced suppression of GABA release (66). Lauri and co-workers (371, 372) suggested that kainate receptors that are highly expressed throughout the neonatal brain shift function during maturation and modulate the occurrence of GDPs. The activation of these receptors by ambient glutamate tonically reduces glutamate release. In contrast, the activation of these receptors at an early stage strongly upregulates GABAergic transmission and thus may regulate the occurrence of GDPs. Activation level of kainate receptors is finely tuned to permit synchronized network activity in the neonatal hippocampus, because both increased activation and inhibition of GluR5 kainate receptors inhibits GDPs. Apparently, these effects are dependent on distinct cellular mechanisms: activation of kainate receptors vastly increases asynchronous GABA release and thus shunts the network, whereas inhibition of the kainate receptors reduces interneuronal synchronization, therefore mitigating the build-up of network bursts (371, 372).

GDPs are also modulated by presynaptic NMDA receptors. Thus Gaiarsa et al. (212) have shown that glycine acting on NMDA receptors augments the release of GABA and the frequency of GDPs. This action is mimicked by D-serine that also acts on that site and fully prevented by NMDA receptor antagonists. Interestingly, Poo and colleagues (384) have shown recently that in the developing tadpole retinotectal system, visual stimuli generate a long-term depression of GABAergic synapses mediated by presynaptic NMDA receptors that at that developmental stages modulate the release of GABA.

Similar to GDPs, patterns of sharp waves and population bursts have also been observed in the hippocampus of neonatal rats in vivo (376). Using multisite extracellular and patch-clamp recordings in the hippocampus of freely moving and anesthetized rat pups, respectively, the authors observed spontaneous recurrent bursts of synchronized neuronal activity that lasted 0.5–3 s like GDPs and in which GABA and glutamate PSCs contribute. Firing of CA1 pyramidal neurons occurred mostly within sharp waves and population bursts that were separated by long periods of silence. This electrographic pattern was recorded during sleep, immobility, or feeding behavior. Sharp waves are generated by synchronous discharge of CA3 pyramidal cells and are conveyed to CA1 via Schaffer collaterals; thus CA3 like in vitro appears as the most likely pacemaker. A major difference between immature sharp waves and adult ones is the occurrence of fast ripples that the neonatal network cannot generate and that appear from the end of the second postnatal week (85, 376; see also below). The developmental time course of the ripple parallels the switch in the $GABA_A$ receptor-

mediated signaling from excitation to inhibition. Population bursts were characterized by increase of unit activity without prominent field potential typically followed by sharp waves but could also occur independently. Whole cell recordings in situ revealed again in agreement with in vitro recordings a contribution of both GABA and glutamate receptor-mediated synaptic signals in their generation (376). Importantly, the NKCC1 blocker bumetanide that renders GABA inhibitory efficiently suppressed both sharp waves in vivo and GDPs in vitro during the postnatal period, suggesting that depolarizing GABA is instrumental in the generation of the early hippocampal patterns both in vivo and in vitro (173, 569).

Although depolarizing and excitatory GABA plays a pivotal role in the generation of GDPs in the neonatal hippocampus, GABA also exerts inhibitory action in immature networks. Indeed, blockade of GABA receptors induces interictal-like epileptiform activity in the neonatal hippocampal slices (316, 325, 326, 367, 659) and ictal-like tonic-clonic seizures in the intact hippocampus in vitro (320, 321) and in vivo (282). Although $GABA_A$ agonists and positive allosteric modulators transiently increase GDPs frequency, the effect is followed by depression due to shunting inhibition (316, 325). Also, activation of GluR5 kainate receptors increases asynchronous GABA release and inhibits GDPs (372). Thus GABA exerts dual excitatory and inhibitory actions in the neonatal hippocampal network. Depolarizing GABA inhibits via a shunting mechanism by increasing membrane conductance and limiting depolarization above E_{GABA}. In addition, depolarizing GABA can also activate voltage-gated K^+ channels (401, 457) and attenuate the amplitude of the presynaptic spike through a combination of shunting and Na^+ channel inactivation, a phenomenon observed in invertebrate sensory fibers, dorsal root ganglion cell terminals, and secretory nerve endings (100, 180, 184, 185, 315, 530, 679).

B. A Problem of Terminology

GDPs have been identified initially with intracellular current-clamp recordings, hence the name. The terminology therefore does not correspond to events recorded with other techniques. Relying on field recordings and imaging techniques, bursting activity, unison firing pattern, and early networks oscillations (ENOs) have, respectively, been proposed to term the same phenomenon (222, 254, 496). Clearly none of the names proposed fully represents all the features of the events, and we shall use the original terminology and restrict it to define patterns present transiently in immature networks consequent to the presence of a large number of excitatory GABAergic currents.

C. How Are GDPs Generated? How do They Propagate?

1. The GABA D-H population model

In this model (56, 325, 326, 377), hippocampal local networks composed of a few hundred GABAergic interneurons and glutamatergic pyramidal neurons are endowed with autorhytmicity largely based on synergistic interactions between recurrent excitatory GABAergic and glutamatergic PSCs. In this model, both glutamatergic and GABAergic neurons and synapses are the key generators. GDPs are considered as a signature of the state of maturation of the network at a given developmental stage: GDPs are present as long as a substantial percent of GABAergic synapses are excitatory. In addition to the E-I shift in the actions of GABA, the late establishment of functional perisomatic interneurons-basket cells and axoaxonic neurons may provide an additional important signal for the cessation of GDPs. A central aspect of the model proposed is that in spite of the requirement of glutamatergic synapses, the level of neuronal depolarization and excitation during GDPs is mainly determined by GABAergic synapses and interneurons. This model is compatible with the main features of GDPs, its developmental occurrence that parallels excitatory actions of GABA, and its modulation by various conditions and agents that control network excitability.

2. The gap junction pacemaker hilar interneurons model

Strata et al. (593) have suggested that GDPs are generated by an endogenous pacemaker current present in hilar interneurons "paced" by a hyperpolarization-activated current, with properties of I_h and synchronized via gap junctions. The excitatory actions of GABA are not required for that model to operate. The I_h current mediated by HCN1 channels has also been suggested to contribute to GDPs recently (61). The selective I_h blocker ZD7288 disrupted GDP generation and abolished spontaneous bursting of the CA3 pyramidal cells at frequencies typical of GDPs without major influence on interneuronal firing (61). In addition, the "hilar interneurons" model cannot explain the generation by all components of the hippocampal circuit of GDPs including subslices of various hippocampal regions that do not contain the hilar zone, and they are blocked by the glutamate receptors antagonists (56, 60, 432, 434). It is also difficult to reconcile with all other studies that point to crucial roles of the GABA E-I shift in the generation of GDPs by local networks.

3. A combined intrinsic current and recurrent glutamatergic excitation model

Another model (567, 568) incorporates the depolarizing actions of GABA and intrinsic bursting activity of CA3 pyramidal neurons generated by voltage-dependent currents. In this model, pacemaker intrinsic pyramidal bursting neurons are the generators and GABA only acts as a tonic source of depolarization that enables these neurons to reach the threshold for burst firing. Phasic excitation by GABA plays little role in the generation of GDPs as GDPs in interneurons do not precede GDPs recorded in field recordings, suggesting that the drive is endogenous to pyramidal cells. The triggering and termination of intrinsic bursts and of GDPs are determined, respectively, by a sodium persistent slow current for the regenerative depolarization that triggers intrinsic bursts and a slow Ca^{2+}-activated K^+ current for the postburst afterhyperpolarization. The frequency of GDPs is determined by the kinetics of these currents and not by synapse-driven events. These intrinsic events are translated to network-driven GDPs by means of the recurrent glutamatergic excitatory collaterals present in CA3 pyramidal neurons (567). In that respect, GDPs are similar to interictal patterns generated when inhibition is blocked in CA3 neurons (567) except that they require tonic GABA for their generation. This model is of obvious interest as it incorporates basic properties of the CA3 pacemaker region in developing networks (see above and Ref. 70).

However, other observations cannot be reconciled with this model. First, in an earlier intracellular recording study, systematic bursting in CA3 (or CA1) pyramidal neurons was seldom observed (56), in keeping with Yaari and co-workers (374) who reported spontaneous bursts in CA3 after but not before P11 reaching a peak around P18. Also, GDPs are recorded in granule cells where they are clearly not mediated by intrinsic bursting properties but by GABAergic interneurons (269, 434). GDPs can be generated in the isolated CA1 region where neurons are not endowed with intrinsic burst patterns and poorly developed glutamatergic collaterals (222; L. Anikstejn, unpublished data). Interneurons clearly generate GDPs, even at an embryonic stage when most pyramidal neurons are quiescent, suggesting that interneurons set the pace of hippocampal patterns (265). Also, antagonists of $GABA_B$ receptors augment significantly the duration (by several-fold) of GDPs, suggesting that the release of GABA and other presynaptic mechanisms are responsible for terminating the event (427). Patterns equivalent to GDPs are present in a wide range of structures (see below) that do not seem to possess a pacemaker region equivalent to CA3 pyramidal neurons. Also worth stressing, the membrane potential threshold required for the generation of a persistent sodium channel is closer to the threshold of sodium spikes than to the resting membrane potential (200). However, clearly several aspects of this model are important, and more detailed investigations are needed to clarify this issue.

The propagation of GDPs was studied in detail in the intact hippocampal formation in vitro (320, 375), in which it is possible to investigate with more detail the propagation of GDPs across the entire hippocampal formation, including the septum and entorhinal cortex. Patch-clamp recordings from a large sample of neurons revealed GDPs (375) that are identical to those recorded in slices. With the use of multiple field recordings and dual patch recordings, GDPs were found to propagate along the septotemporal axis of the hippocampus with short delays between adjacent neurons and long ones between distal sites (375). The spontaneous activity of the septal pole was higher than that of the temporal pole, and separating the septal and temporal poles did not affect the frequency of GDPs in the former (11/min) but reduced by half that of the temporal pole (to 5/min). Also, GDPs generated in the septal pole had a much higher probability for propagation to the temporal pole than the converse, suggesting that the hippocampus operates like a syncytium in which all components can generate rhythms but some are more developed, have a higher GDP frequency, and impose their patterns. The septal pole paces the rest of the hippocampus (375), as it has a higher density of active glutamatergic synapses and a higher spontaneous activity. The differential maturation of interneurons in the septal and temporal poles most likely also plays a role in this gradient (89). Recordings from the septum indicate that GDPs originate in the hippocampus as cutting the connections between the septum and hippocampus blocked the GDPs in the former but not the latter. GDPs are also synchronized between both hippocampi by the commissural axons, and both septal poles generate synchronized GDPs, suggesting that commissural axons are operative at birth already (see also Ref. 319). GDPs propagate to other limbic structures in vitro, including the entorhinal cortex (39).

D. GDPs in Subhuman Primates

In primates, the prenatal differentiation of pyramidal neurons, granular neurons (164), and interneurons (63, 64) and the early establishment of synaptic connections provide a basis for the in utero emergence of synchronized activities. Patch-clamp recordings of hippocampal neurons were performed in slices of macaque cynomolgus fetuses delivered by cesarean section during the second half of gestation (323) (see Figs. 8 and 9). No spontaneous network-driven activity was observed at midgestation (E85) and seldom close to term (E154; term is 165 days). In contrast, between E105 and E134, the majority of pyramidal neurons displayed periodic network-driven

events occurring at a frequency of 0.13 ± 0.04 Hz. This activity was highly reminiscent of GDPs. Fetal monkey GDPs displayed the typical features of polysynaptic network-driven events: *1*) they were synchronized in simultaneously recorded neurons; *2*) they could be evoked in an all-or-none manner by electrical stimulation; *3*) their frequency was independent of the membrane potential; and *4*) they were completely blocked by TTX. GDPs were synchronously generated in paired recordings from pyramidal cells and interneurons and were mediated by synaptically activated $GABA_A$ and glutamate receptors. As in the rat hippocampus, the GDPs were blocked by glutamate receptor antagonists CNQX and APV and were transformed to paroxysmal discharges by the $GABA_A$ receptor antagonist bicuculline. The total count of synaptic events revealed that nearly half of GABAergic and glutamatergic synaptic activity is synchronized in GDPs. Thus similar to the neonatal rat hippocampus, GDPs in the fetal macaque hippocampal slices are generated by GABAergic inputs from interneurons and glutamatergic recurrent collateral synapses, and the synaptic activity is highly synchronized during the phase of intensive neuronal growth and synaptogenesis.

E. Other Early Patterns in the Developing Hippocampus

1. Periodic inward currents

Periodic inward currents (PICs) (19) are readily generated in neonatal slices by several agents in the presence of TTX, TEA, and CsCl to block most sodium and potassium channels. Thus recurrent applications of the metabotropic agonist ACPD generated PICs that persist for several hours. PICs are generated by nonspecific cationic currents and mediated by a pulsatile release of glutamate that activates ionotropic glutamate receptors. PICs are blocked by removing calcium from the external medium as well as the addition of caffeine and thapsigargin but not by ryanodine. PICs are triggered by elevation of $[Ca^{2+}]_i$, after mobilization of Ca^{2+} from inositol 1,4,5-trisphosphate ($InsP_3$)-sensitive stores that in turn will lead to a pulsatile release of glutamate from presynaptic nerve terminals. The relation of PICs to other unique features of the developing network remains to be investigated; GABAergic receptors do not appear to play a role in PICs contrary to GDPs, yet their developmental curves are quite similar, suggesting that features other than the E-I shift are involved in the generation of early patterns.

2. Gap junction-mediated oscillations

Gap junction-mediated oscillations were recently identified in immature hippocampal networks using fast multibeam two-photon calcium microscopy to record hundreds of neurons simultaneously in immature hippocampal slices from P0 to P8 (141a). With this technique, it is possible to record the activity of a large number of neurons simultaneously and identify the pattern present in every neuron (138). Using this tool, the authors showed that before birth, most immature neurons in the CA1 region do not produce a coherent pattern but intrinsic recurrent calcium spikes. The first coherent pattern appears around delivery and is characterized by a plateau potential that synchronizes a small population of gap junction-coupled neurons. At a subsequent stage, GDPs appear. They are present simultaneously in hundreds of neurons and propagate to the entire network. Therefore, activity shifts from a voltage-gated localized pattern involving selected neuronal assemblies to a synaptic one that reaches most neurons via excitatory GABA and glutamtergic synapses. These observations suggest that in addition to a sequential maturation of individual ionic channels (490) there is a sequential maturation of network events with gap junction-mediated signals preceding synapse-mediated synchronized events (see Ref. 459 for review). Interestingly, in the neocortex, gap junction-mediated oscillations are generated by carbachol primarily through the action of subplate cortical neurons (167).

F. Oscillations in Other Developing Structures

1. The neocortex

A) IN VITRO PATTERNS. Oscillations and network patterns are frequent in various parts of the developing neocortex, consistent with the intrinsic capacity of developing networks to generate oscillations (302). These patterns are thought to play important roles in wiring essential connections notably cortical maps and in other important functions including migration and proliferation (346, 347, 489, 658). Calcium oscillations were described by Kriegstein and co-workers in the neocortical ventricular zone (489); calcium waves also propagate spontaneously through radial glial cells in the proliferative cortical ventricular zone. They require connexin hemichannels, P2Y1 ATP receptors, and intracellular $InsP_3$-mediated calcium release. Disrupting these waves decreases proliferation during of embryonic neurogenesis (658). The same group also showed that metabotropic glutamate receptors also generate oscillations in the developing neocortex (198).

Using imaging techniques and visual patch recordings, Yuste and co-workers (676, 677) showed that neonatal neocortical neurons oscillate when strongly stimulated by 0 Mg^{2+} and 5 mM K^+, or high K^+ containing ACSF (500, 501). These correlated activities were mediated by the depolarizing actions of GABA at that age that also increases intracellular calcium. However, TTX or blocking glycine receptors did not affect the activity,

whereas cholinergic and glutamatergic antagonists modulated it, suggesting a complex interaction of several signals. The authors favored the hypothesis that a release of calcium from intracellular stores plays an important role in keeping with other lines of evidence. As in other brain structures, gap junctions between developing neocortical neurons are frequent and may play an important role in oscillations (302, 500). Interestingly, the authors showed that these patterns might presage adult functional architecture as the cortex could be partitioned in domains of spontaneously coactive neuronal populations coupled by gap junctions (302, 677).

Using two photon dynamic imaging, Konnerth and co-workers (2, 222) showed the presence of cortical waves to which they referred to as cortical ENOs (C-ENOs) in the rat neonatal neocortical slices and in vivo (P0 to P12) (2, 222). ENOs that are expressed only during the first postnatal week much like hippocampal GDPs (see above) occur at a very low rate (1–12/min) and propagate across the entire cortical mantle at a speed of 2 mm/min, a rate that is much slower than GDPs and other patterns and that may suggest a nonsynaptic propagation mechanism. The duration of single events was tens of seconds. C-ENOs are completely blocked by AMPA or NMDA receptor antagonists but not by GABA receptor antagonists at least until the end of the first postnatal week. In more adult slices, bicuculline generated oscillations most likely of an epileptogenic type in keeping with the parallel to the E-I shift of the actions of GABA during the transition from immature to more adult pattern. C-ENOs are quite different from GDPs by the early contribution of glutamate signaling and limited contribution of GABA. In contrast to these observations, Luhmann and co-workers (264) showed that at birth bicuculline but also muscimol block calcium oscillations in P0 neocortical slices. Clearly, a detailed morphofunctional description of the sequential maturation of GABA and glutamatergic synapses is required to gain a better picture for the development of neocortical patterns.

Luhmann and co-workers (167) have described a pattern of carbachol-induced propagating network beta oscillations in the neonatal mouse neocortex. In the newborn mouse, this activity required an intact subplate and was strongly synchronized within a cortical column by gap junctions. With the developmental disappearance of the subplate at the end of the first postnatal week, activation of NMDA receptors in the immature cortical network was needed to generate this columnar activity pattern. It was suggested that during a brief developmental period the cortical network switches from a subplate-driven, gap junction-coupled syncytium to a synaptic network acting through NMDA receptors to generate synchronized oscillatory activity, which may function as an early functional template for the development of the cortical columnar architecture (167). These oscillations were not affected by blockade of $GABA_A$ receptors, suggesting limited participation of GABA interneurons in their generation (167).

Voigt et al. (642) have performed a detailed study of the oscillations present in neocortical cultures. In an initial study, these authors (642) reported the presence of network oscillations driven by GABA and glutamate PSCs in neuronal cultures that are identical to GDPs. Giant GABAergic neurons are key elements in their generation as they are only present when at least two of these neurons per square millimeter were present in the culture, with lower density GDPs not present. Changes in the density of other neurons did not influence the presence of oscillations. These neurons form an interconnected network with extended axonal arborization enabling them to "collect" information from large regions of the cortex; they were also identified as corresponding to large GABAergic neurons derived from the primordium plexiform layer that reside in the subplate at the time of birth. Therefore, a subpopulation of giant GABAergic neurons that the authors consider as being a type of basket cells extends its axonal arbor and triggers network oscillations. Using this preparation, the authors suggested in more recent studies that the survival of a subpopulation of GABAergic interneurons that the authors consider may correspond to the calretinin/vasoactive intestinal polypeptide interneurons, critically depends on the presence of functional NMDA receptors, since chronic blockade of these receptors led to a dramatic loss of these neurons (154). This observation reinforces the important links between GABA and NMDA signaling. Since GABA and NMDA receptors act in synergy to generate GDPs, this feedback control will provide a mechanism for cross control of the maturation of GABA and NMDA signaling. In keeping with this, chronic blockade of NMDA receptors exerts a more severe action than blocking AMPA receptors. In another study, the same group (642) showed that GABA receptor antagonists fully block GDPs during the first 3–4 days after their emergence but not later, suggesting that GABA acts as an excitatory neurotransmitter in these young networks. This observation is in contradiction with the observations of Konnerth and co-workers (222) who reported that in intact cortices, GABA receptor antagonists do not block GDPs (or ENOs) at an early stage. In older cultures, oscillations persist in the presence of bicuculline but with a smaller burst frequency and a more sustained depolarization and burst-associated Ca^{2+} transients (485). In a more recent study, the same group used an interesting preparation to investigate the relation between network oscillations and transformation of silent synapses (with NMDA receptors alone) to active AMPA receptor containing synapses within a few minutes (643). Since oscillatory activity appears a short delay after cultures, the authors used a preparation that allows neighboring networks and their axons to arrive to the culture

either before or after the presence of oscillations. The network interconnected in the former but not in the latter case. The authors stress the importance of taking into account the developmental stage, as the actions of antagonists will differ to an important extent.

Generation and propagation of oscillations were studied in detail using calcium-imaging techniques in mice organotypic neocortical slices (134). At birth, cortical neurons generated widespread, highly synchronous $[Ca^{2+}]_i$ transients that propagate to large areas and involve the majority of cells. These oscillations are both TTX and nifedipine sensitive and disappear a few days later, suggesting that voltage-gated events precede synapse-driven oscillations like in other brain regions. When slices were exposed to TTX to block ongoing synaptic activity, the pattern was present until P13, suggesting that the development of synaptic activity mediates the developmentally regulated loss of activity (424; see also Ref. 459 for review).

Therefore, these observations suggest that in the neocortex like in the hippocampus and other brain regions, neuronal populations are entrained initially in widespread patterns that are restricted to the developing network before being replaced by a multitude of patterns. The generation of this pattern relies on gap junctions at least in an initial developmental stage, whereas in contrast to the hippocampus, GABAergic signaling is not a key player, although the loss of these early patterns occurs when the immature action of GABA has subsided. However, a full picture of the operation of these oscillations will only be achieved once the link between the occurrence of these patterns and the morphofunctional maturation of individual neurons has been achieved. Also intriguing is the need for external excitatory drives, since most of these patterns are only observed in vitro in the presence of high K^+ or other conditions increasing excitability.

B) IN VIVO PATTERNS. The dominant pattern of electrical activity in the neonatal rat neocortex in vivo is a spindle-burst (251, 329, 447). Spindle-bursts are spatially confined spindle-shaped oscillations at alpha-beta frequency which are associated with phase-locked neuronal firing and activation of the glutamatergic and GABAergic synapses. Spindle-bursts are a self-organizing pattern that persists after deafferentation. In the intact animal, compartmentalized spindle-bursts in somatosensory cortex are triggered by sensory feedback resulting from spontaneous movements in a somatotopic manner (329). In visual cortex, spindle-bursts are triggered by spontaneous retinal waves in an eye-specific manner (251). Spindle-bursts in the neonatal rats are homologous to the pattern of delta-brushes expressed in human neocortex during the fetal period of development (327, 366, 443), also known as slow activity transients (SATs) (634, 635).

Network mechanisms of spindle-bursts were studied in neonatal rat barrel cortex using a superfused cortex preparation (324, 447). Pharmacological analysis revealed that spindle-bursts are driven by glutamatergic synapses with a major contribution of AMPA/kainate receptors, but slight participation of NMDA receptors and gap junctions. Whole cell recordings revealed GABAergic synaptic currents phase-locked with the local field potential oscillations, suggesting that interneurons are activated during spindle-bursts. However, blockade of $GABA_A$ receptors did not significantly affect the frequency of the rapid oscillations associated with spindle-bursts. NKCC1 antagonist bumetanide, which shifts the reversal potential of the $GABA_A$-mediated responses towards negative values (671), did not affect spindle-bursts. This is in contrast to the inhibitory effects of bumetanide on hippocampal GDPs in vitro and sharp waves in vivo (173, 569). Therefore, it appears that early hippocampal patterns of sharp waves/GDPs are more dependent on the depolarizing/excitatory GABA than the neocortical pattern of spindle-burst, which is consistent with the observations made in vitro (223).

Although GABAergic interneurons are not directly involved in setting rhythm of spindle-burst oscillations, they play important role in their horizontal compartmentalization. Blockade of $GABA_A$ receptors significantly increased the area of activation during spindle-bursts evidenced by increase in the amplitude and power of oscillations, duration of spindle-bursts, and their horizontal spread. Thus compartmentalization of spindle-bursts is determined not only by the vertical segregation of the sensory feedback-driven essentially AMPA/kainate receptor-mediated somatotopic excitation (3, 86, 193, 268, 329, 331, 502) but also by surround GABAergic inhibition which prevents horizontal spread of the activity via long-range glutamatergic cortical connections, a pattern observed in the adult neocortex (108, 199, 595). The inhibitory action of GABA at the network level is probably due to the shunting mechanisms amplified by the activation of the voltage-gated potassium channels and inactivation of sodium channels (73, 219, 244, 401). These results are in general agreement with the findings that administration of $GABA_A$ antagonists induces hypersynchronous seizure-like activity in the neocortex in vivo by P3 (34) and in vitro by P2 (659).

Thus, in contrast to hippocampal GDPs, depolarizing GABA exerts mainly an inhibitory role in the neonatal neocortical network in vivo, providing surround inhibition and compartmentalization of spindle-bursts.

2. The spinal cord

The presence of spontaneous synchronized patterns in the developing spinal cord has long been known and related to the ongoing motor activity in embryos of vari-

ous species. In humans, the presence of these patterns is clinically relevant as their absence is thought to reflect a bad prognosis (510). In a series of investigations, O'Donovan and collaborators (190, 478, 480) reported that the embryonic chick spinal cord has spontaneous activity generated by somewhat hyperexcitable networks that can be recorded already in ovo. They exhibit rhythmic discharge both with slow episodes (lasting minutes) and fast intraepisode cycling (~1 Hz frequency). The mechanisms that initiate these patterns and control their durations were investigated. The duration of an episode of activity was found to depend on the network excitability at the beginning of the episode (604) and a model proposed whereby a bistable network endowed with a slow activity-dependent depression that switches periodically between the active and inactive states (605). Interestingly, a network of interneurons appears to play a central role in the generation of this pattern as electrical stimulations in conditions that activate these interneurons triggered episodes prematurely, and this was prevented by pharmacological blockade of cholinergic inputs to interneurons or GABAergic outputs from other interneurons (660). In keeping with an early important role for GABA/glycine synapses, the authors also reported that early rhythmically active interneurons project to motoneurons and are most likely interconnected by recurrent excitatory synaptic connections (20, 518 and review in Ref. 479). A comparison of the effects of GABA and glutamate antagonists revealed more persistent and generalized actions of the former, suggesting that although spontaneous rhythmic activity is a general property of developing spinal networks, GABA and glycinergic networks are plastic and act rapidly to compensate for the blockade of excitatory transmission (190). The isolated lumbosacral spinal cord of the neonatal mouse (P0–7) also generated rhythmic motor activity that included several patterns including spontaneous, alternating discharge that occurs two to three times over a 10-min interval (661). The contribution of GABA signaling to these spontaneous and locomotor-related patterns and the role of the D-H shift was shown by Clarac and coworkers (192). A contribution of presynaptic $GABA_B$ receptors was also reported (102).

3. Retinal waves

Retinal waves are among the most investigated early patterns of activity and will not be reviewed here in detail (see Refs. 144, 191, 617). They are thought to play a central role in the construction of cortical maps and other essential features of the visual cortex operation (see Refs. 95, 144–146, 309). Probably the first indications that retinal ganglion cells discharge in utero well before being subject to sensory stimulation came from the elegant studies by Maffei and Gali-Resta (214, 408). The spontaneous discharges of neighboring retinal ganglion cells were recorded simultaneously in situ in embryonic days 18 and 21 while still connected to the anesthetized mother rat. They found correlated activity between adjacent neurons, and the patterns reported in fact are quite similar to the GDPs as recorded with extracellular recordings in the hippocampus. Subsequent work studied the mechanisms that underlie the generation of these patterns and the role of various transmitter systems and lead to quite controversial conclusions (e.g., Ref. 617 for discussion). A central role for starburst neurons and chlolinergic systems has been suggested, although $GABA_A$ and $GABA_B$, glycine, and other receptors appear to play important roles in addition to endogenous voltage-gated calcium currents.

Studies using the embryonic chick retina showed the presence of highly patterned large-amplitude, rhythmic waves of excitation that propagate across the retina. These patterns are most prominent between embryonic days 13 and 18, coinciding with the developmental period during which retinal axons refine their connections in their targets (668). Antagonists of glutamatergic and glycinergic transmission and of gap junctional communication suppress spontaneous activity, whereas antagonists to GABAergic transmission potentiate it. In another study of embryonic chick retinas, $GABA_B$ receptors were reported to play a central role as shown by pharmacological studies and immunocytochemistry (99).

An important role of GABA in the generation and maintenance of retinal waves was suggested by Sernagor and co-workers in the turtle retina and recording from ganglionic cells (379). The authors reported that the D-H shift of GABA actions coincides with the shift of spontaneous activity from propagating waves to stationary patches of coactive cells. Also, blocking $GABA_A$ receptors chronically leads to both maintenance of the D phenotype of GABA actions, and spontaneous waves keep propagating across the RGC layer. This is mediated by a blockade of the upregulation of the chloride cotransporter KCC2. Therefore, the activity, largely mediated by GABAergic signals initially is required for the chloride extrusion mechanism to occur as well as for the changes of the ongoing pattern (379).

Studies by Feller and co-workers (565) reported a central role of L-type Ca^{2+} channels in newborn mice. Applications of agonists that selectively activate these channels induce large, frequent, rapidly propagating waves. These waves propagate independently of fast synaptic transmission, and nicotinic acetylcholine, AMPA, NMDA, glycine, and $GABA_A$ receptor antagonists did not affect their frequency. More recent studies by Zhou and co-workers (682, 683) have clarified these issues using the perinatal rabbit retina. Thus, in a flat-mount perinatal rabbit retinal preparation, a cholinergic and a strychnine-sensitive system in the inner retina plays an obligatory and developmentally regulated role in the initiation and propagation of spontaneous retinal waves. In that study,

glycine not GABA appeared essential and provided an excitatory action initially before the D-H shift that occurred immediately after birth. Using a dual patch-clamp recording and Ca^{2+} uncaging method, the same group reported a Ca^{2+}-dependent corelease of ACh and GABA from starburst cells that is developmentally regulated with a dramatic reduction in the action of nicotinic receptors that parallels the D-H shift of GABAergic synapses (681). In a subsequent study, the same authors insisted on the multiple developmental stages that rely on very different transmitters and voltage-dependent calcium signals (603). A central role of endogenous voltage-dependent calcium channels was suggested more recently in pharmacologically isolated starburst amacrine cells (SACs) that generate spontaneous semiperiodic calcium spikes and long-lasting afterhyperpolarizations (AHPs) (680). In the presence of a cocktail of blockers that include GABA receptor antagonists, SACs generate spontaneously calcium spikes and long-lasting AHPs that provide the initial stimulation for retinal waves at an early developmental stage. The model proposed suggests that retinal waves are generated by an interaction between spontaneously active SAC cells and recurrent synaptic connections mediated in part by excitatory GABA. This mechanism is somewhat similar to that suggested by Kaila and co-workers to explain the GDPs (567) with intrinsic neuronal clocks that rely on voltage-gated signals and synaptic connections to enable their propagation. Conceptually, these models rely on the hypothesis that some neurons are endowed with unique features to play the role of conductors of early oscillations. This is an obvious attractive hypothesis that however will have to be reconciled with the developmental sequences that appear to be intrinsic to all neurons and not restricted to one neuronal population. How do the early patterns conducted by a unique population of neurons shift to the plethora of patterns generated by various neuronal populations? The use of selective knockouts combined with dynamic imaging techniques will help clarify several issues (95, 230), in particular if the genetic ablation is developmental stage and neuron dependent.

G. Conclusions

In conclusion, this summary reflects the similarities and differences between the hippocampus model and other structures. All these structures tend to oscillate more readily at an early developmental stage and to express the D-H shift even when GABAergic signals are not instrumental in their generation. Therefore, the D-H shift represents an important check-point after which the pattern changes signature and possibly function. It remains to be determined if these reflect different developmental stages or genuine different structure-dependent developmental programs. It is also important to stress the major differences between oscillations (GDPs or the like) and seizures generated by blocking GABA receptors: these are recurrent network-driven patterns but have little in common otherwise (also see sect. IX). Quantitative imaging studies will be essential to compare in detail the generating neurons and signaling cascades.

We suggest that one common feature appears to be universal: the occurrence of a transitional stage during which a single pattern provides all the activity of emerging networks. This may differ in various structures in the role of GABA, voltage-gated channels, or glutamatergic inputs, but the common aspect is that the transition from silent neurons to networks that generate a multitude of behaviorally relevant patterns is not direct, it includes a transition dominated by the presence of a "primitive" pattern that in many brain structures shares many common mechanisms with hippocampal GDPs. This primitive pattern is poor in information content, not necessarily associated with specific information, presence in the retina before eye opening, but is indispensable to turn on the machine and ignite the network. It remains to be investigated whether a single neuronal population coordinates this emerging pattern or whether it is organized with a developmental organized contribution, according to their developmental stage, various neurons are endowed with the properties required to conduct the oscillation. Stated differently, it will be important to determine the coincidence between the sequential maturation of neuronal and network signaling.

VII. PLASTICITY OF DEVELOPING GABAergic AND GLYCINERGIC SYNAPTIC TRANSMISSION

Persistent activity-dependent changes of synaptic efficacy such as those associated with long-term potentiation (LTP) or long-term depression (LTD) contribute to synapse formation during development. These have been extensively investigated in adult glutamatergic synapses. However, in the past decade, long-term changes in the strength of GABAergic and glycinergic synapses have been reported in different adult and developing brain regions (209). Here we will mainly focus on the plasticity of GABAergic/glycinergic transmission in the developing brain.

A. Induction of Long-Term Alterations of Synaptic Efficacy in Developing GABAergic and Glycinergic Synapses

Both homosynaptic and heterosynaptic long-term plasticity have been reported in the developing lateral superior olive (350), cortex (343), and hippocampus

(307, 426). Long-term plasticity was observed on pharmacologically isolated IPSPs or IPSCs or on unitary IPSCs evoked by direct stimulation of the interneurons.

Not surprisingly, an increase in $[Ca^{2+}]_i$ is required to shape GABAergic/glycinergic synapses, although the source of the Ca^{2+} rise may differ from one structure to another (208). In the neonatal rat hippocampus, the induction of LTP_{GABA-A} requires a membrane depolarization, provided by the activation of $GABA_A$ receptors during tetanic stimulation (90, 243, 427) that activates voltage-dependent calcium channels (VDCCs), which trigger a cascade of intracellular signals leading to LTP_{GABA-A}. In the developing rat visual cortex, LTP_{GABA-A} induction requires the activation of postsynaptic $GABA_B$ receptors, which facilitate the release of Ca^{2+} from $InsP_3$-sensitive stores triggered by monoamines (342). In the neonatal rat hippocampus (91, 426) and cortex (343), tetanic stimulation triggers heterosynaptic LTD_{GABA-A}. In both cases, LTD_{GABA-A} is triggered postsynaptically via the activation by glutamate of NMDA receptors on neonatal CA3 pyramidal cells (90, 91, 426, 427) and cortex (343). LTD_{GABA-A} requires that $GABA_A$ and NMDA receptors work in synergy in the former, whereas in the visual cortex, LTD_{GABA-A} was only observed when $GABA_A$ receptors were blocked by bicuculline. This difference is likely due to the depolarizing and hyperpolarizing actions of GABA in the neonatal rat hippocampus and the visual cortex. In the developing *Xenopus* retinotectal system, visual stimuli induced LTD_{GABA-A} (384) mediated by the activation of presynaptic NMDA receptors and a coincident high level of GABAergic activity.

Interestingly, in the developing rat hippocampus (426) and visual cortex (343), the same tetanic stimulation can lead to both LTP_{GABA-A} and LTD_{GABA-A} depending on whether or not NMDA receptors are activated during the conditioning protocol. Because of their specific distribution and restricted axonal terminals on target cells (203), GABAergic interneurons can selectively influence the efficacy of afferent inputs and the emergence and maintenance of network oscillations. Thus, depending on the type of interneurons, local changes in the efficacy of GABA/glycinergic synapses will have different consequences on the input-output relationship of the target neurons. For instance, heterosynaptic depression that results from activation of postsynaptic NMDA receptors will be only expressed by dendritic synapses where glutamatergic inputs impinge on target cells. The NMDA-dependent LTD_{GABA-A} may locally decrease the shunting effect of glutamatergic inputs mediated by $GABA_A$ receptor activation, thereby increasing the glutamatergic drive received by the target developing neurons. The LTP_{GABA-A} may increase the shunting effect of $GABA_A$ receptor activation and decrease the glutamatergic drive, but also increase the excitatory actions of GABA thereby increasing the contribution of $GABA_A$ receptors to the ongoing synaptic activity.

B. Long-Term Changes in the Efficacy of GABAergic and Glycinergic Synapses are Mediated by Presynaptic Mechanisms

In the developing rat hippocampus, GABAergic synaptic plasticity is associated with modifications in the frequency, but not amplitude, of quantal IPSCs (90, 91), with changes in the coefficient of variation of evoked IPSCs amplitude (91, 307) and change in failure rate of minimal stimulations (307, 384). Long-term plasticity of GABAergic synapses is therefore expressed as modifications in the number of functional synapses or changes in the probability of transmitter release. In the neonatal rat hippocampus, application of a conditioning protocol leading to LTP_{GABA-A} also leads to the appearance of functional GABAergic synapses in previously "silent" CA3 pyramidal neurons (243), supporting the idea that a modification in the number of functional synapses accounts for the expression of LTP_{GABA-A}.

If the locus of expression is presynaptic and the induction postsynaptic, a retrograde messenger should modulate the efficacy of presynaptic GABAergic terminals. Such retrograde or *trans*-synaptic feedback contributes to both short- and long-term modulation of inhibitory synapses in the adult cerebellum (640) and hippocampus (116, 304, 664). In the developing rat hippocampus, the identification of messengers involved in LTP_{GABA-A} induction awaits further studies. However, a recent study has demonstrated the requirement of BDNF in the induction of Ca^{2+}-LTP_{GABA-A} (242). Thus activation of postsynaptic voltage-dependent Ca^{2+} channel leading to LTP_{GABA-A} in control conditions had no effect on the strength of GABAergic synaptic transmission in the developing rat hippocampus in the presence of TrkB immunoglobulin, a "scavenger" on endogenous BDNF, or in the presence of k252a, an inhibitor of TrkB receptors coupled-tyrosine kinase LTP_{GABA-A}. Interestingly, this protein is primarily produced by excitatory neurons such as pyramidal and granule cells in the hippocampus (189) and is targeted to the axonal as well as the somato-dendritic compartment of the cells (84, 257). Moreover, using time lapse video microscopy of BDNF-GFP fluorescence, a postsynaptic Ca^{2+}-dependent release of BDNF from the dendrites of hippocampal and cortical neurons in culture has been demonstrated (84, 255). Exogenously applied BDNF modulates GABAergic synaptic efficacy through both pre- (29, 242, 425) and postsynaptic (114, 187, 295) modifications in different brain structures. Altogether, these data suggest that BDNF in the developing postsynaptic target cell may function as an autocrine or retrograde messenger that acts locally to strengthen GABAergic inputs. However, it

remains to be determined whether BDNF has an instructive or a permissive role in the induction of GABAergic synaptic plasticity.

The stability of $GABA_A$ receptors is another important factor for regulating the efficacy of GABAergic synaptic transmission (reviewed in Ref. 337). A direct relationship between the number of $GABA_A$ receptors and the strength of the synapses has been reported in adult tissue (476). Moreover, blocking clathrin-dependent endocytosis of $GABA_A$ receptors induced a large increase in quantal size in cultured hippocampal cells (336), and insulin evoked a translocation of $GABA_A$ receptors to the surface, thereby increasing the amplitude of miniature IPSCs in cultured neurons (650). Therefore, insertion or removal of $GABA_A$ receptors can result in changes in the GABAergic strength.

Activation of protein kinases or phosphatases is another mechanism through which the postsynaptic Ca^{2+} rise can be translated into long-term changes of synaptic efficacy (reviewed in Ref. 337). Thus in the adult hippocampus, the expression of $LTD_{GABA\text{-}A}$ involves a downregulation of $GABA_A$ receptors by the calcium-sensitive phosphatase calcineurin (402, 654). In the cerebellum, the expression of $LTP_{GABA\text{-}A}$ required the activation of postsynaptic calcium/calmodulin-dependent kinase II (CaMKII) (305, 312). In the developing auditory brain stem, $LTD_{GABA\text{-}A}$ is expressed postsynaptically, as the amplitude but not frequency of miniature synaptic events was reduced after LTD induction (109). In this structure, the induction of LTD required the activation of postsynaptic kinases (351).

Changes in the strength of inhibitory inputs can also result from modification in the reversal potential of GABAergic synaptic responses as documented in the hippocampus (196, 669). In both culture and acute hippocampal, coincident pre- and postsynaptic spiking or postsynaptic firing of hippocampal neurons led to a persistent decrease in GABAergic synaptic strength, associated with a depolarizing shift of the reversal potential of $GABA_A$ receptor-mediated synaptic potentials (E_{GABA}). Similarly, pairing exogenously applied GABA with postsynaptic depolarization leads to a long-lasting transformation of hyperpolarizing GABAergic responses into depolarizing responses (129). $GABA_A$ receptors are permeable to chloride, and the intracellular concentration is regulated by different chloride cotransporters (499). In their study, Woodin et al. (669) have shown that coincident pre- and postsynaptic spiking decreases the cation/chloride cotransporter KCC2 activity, resulting in the shift of E_{GABA} to more positive values. Long-lasting change in E_{GABA} has been also reported in epileptic tissue (127, 321), supporting the contribution of such phenomenon in the emergence and maintenance of pathological network activity.

C. Long-Term Plasticity: Contribution to the Establishment of GABAergic and Glycinergic Synapses in the Developing Brain

Extensive investigations primarily centered on excitatory glutamatergic synaptic transmission (335, 393, 394) suggest that activity plays a role in shaping the principal steps of brain maturation including cell migration and differentiation, axonal path finding, and the establishment of functional synaptic connections. The mechanisms underlying these effects are not well understood but include long-term changes in synaptic efficacy (133, 558). More recent studies suggest that long-term changes in synaptic efficacy may also contribute to the establishment of functional inhibitory synapses in the developing brain.

Thus, in all developing structures where GABAergic and glycinergic synaptic plasticity has been described, the induction is restricted to a limited critical period of development. This period closely matches the period of functional maturation of networks in the visual cortex (341), hippocampus (243, 426), and auditory system (350). In the auditory system, the tonotopic organization of glycinergic projections is achieved through synapse elimination (539), a process involving activity-dependent mechanisms (540). The structural refinement of axonal arbors emerges gradually and is preceded by a functional elimination and strengthening of GABA/glycine connections (333). The period during which LTD is induced in this structure coincides with the period of functional elimination of inhibitory synapses (350). In the cortex, $LTP_{GABA\text{-}A}$ and $LTD_{GABA\text{-}A}$ occurs in young rats in which development of selective visual responsiveness is ongoing. Interestingly, the maintenance of $LTP_{GABA\text{-}A}$ requires firing of presynaptic inhibitory terminals and presynaptic calcium influx through VDCCs in the developing rat cortex (344).When stimulation of the test pathway was stopped after $LTP_{GABA\text{-}A}$ induction, potentiated responses returned to baseline level. This observation suggests that, if GABAergic synaptic plasticity contributes to experience-dependent refinement of visual inputs early in life, this refinement may not persist unless strengthened synapses are activated by visual stimulation.

The second evidence came from the observation that the spontaneous synaptic activity involved in the functional maturation of inhibitory synapses also induces long-term plasticity at theses synapses. Thus the spontaneous patterned synaptic activity that is a universal hallmark of developing networks plays a crucial role in the refinement of neuronal circuits (55). In the developing rat hippocampus, pyramidal cells and interneurons fire action potentials during GDPs (56, 326) associated with an increase in intracellular calcium concentration (377). Experimentally induced postsynaptic firing of CA3 pyramidal neurons triggers a Ca^{2+}-dependent $LTP_{GABA\text{-}A}$ during a narrow postnatal time window and leads to the appear-

ance of functional GABAergic synapses on previously silent cells (243), thus mimicking the functional maturation of GABAergic synapses that occurs in vivo (627). Moreover, pharmacological blockades of the GDPs prevent the functional maturation of GABAergic synapses (128), and pairing evoked GABAergic synaptic responses with spontaneous GDPs leads to LTP_{GABA-A} in the neonatal rat hippocampus (307). Therefore, early in postnatal life, GDPs may represent the physiological pattern of activity leading to the functional maturation of GABAergic synapses through LTP/LTD-like mechanisms. More recently, Poo and collaborators (384) have shown that repeated light stimuli can trigger LTD of GABAergic synaptic transmission in the developing *Xenopus* retinotectal system, thus showing that sensory experience can modify the efficacy of developing GABAergic synapses.

The third evidence linking activity-dependent maturation of inhibitory synapses and long-term plasticity came from the observation that both phenomena share a similar mechanism. Thus BDNF also participates in the induction of LTP_{GABA-A} in the developing rat hippocampus via trk receptor-coupled protein tyrosine kinase (242). BDNF also exerts long-term effects on the formation and activation of GABAergic synapses in vivo and in vitro. In cerebellar and hippocampal cultures, activity deprivation decreases the number of GABAergic synapses, an effect reversed by the application of BDNF or neurotrophin-4 (422, 553). Overexpression of BDNF (5) or chronic treatment with different neurotrophins (421, 552, 639) accelerates the functional maturation of GABAergic synapses. Several studies have also shown that neurotrophins can modulate the efficacy of inhibitory synaptic transmission (29, 114, 187, 242, 295, 425, 608).

Interestingly, $GABA_A$ receptor activation switches from enhancing to repressing BDNF mRNA synthesis during hippocampal maturation (67). Similarly, GABAergic stimulation enhances the expression of BDNF through the activation of the mitogen-activated protein kinase signaling cascade during early development, but not in mature hypothalamic neurons (482). These observations therefore suggest a feedback loop between GABA and BDNF early in development, where GABA facilitates the expression, and likely the Ca^{2+}-dependent release, of BDNF, and BDNF facilitates the functional maturation and plasticity of developing GABAergic synapses. In agreement with this hypothesis, GABAergic stimulation regulates the phenotype of GABAergic interneurons (421) and the developmental switch from depolarizing to hyperpolarizing GABAergic response (656) through a BDNF-dependent mechanism. It remains now to determine whether spontaneous synaptic activity and excitatory GABA early in life are able to trigger a BDNF release from the target cells, and to determine the biological effect of the released BDNF on the efficacy of GABAergic synaptic efficacy.

D. Conclusion

Developing GABAergic and glycinergic synapses clearly undergo calcium-dependent long-term changes in synaptic efficacy notably following conditioning protocols that are relevant to physiological conditions. The association between wiring of neurons and their firing during maturation provides a rational approach to determine just how activity during development enables refinement of the construction of functional units and networks and what role GABA and its developmentally regulated shifts plays in this process.

VIII. PATHOGENIC ASPECTS OF DEPOLARIZING GABA

A. GABA and the High Incidence of Seizures of the Immature Brain

The immature brain is prone to seizure, and there are important age-dependent differences in the efficiency of $GABA_A$ acting anticonvulsive drugs. The incidence of seizures is highest early in life, particularly during the first few postnatal months (258, 259). A large number of pathological processes could lead to seizures including inborn errors of metabolism, genetic factors, hypoxic-ischemic insults or congenital brain anomalies. Fever which rarely causes seizures in adults is a frequent cause of seizures during development (260, 271, 274, 553).

Critical periods of seizure susceptibility have also been documented in animal models. Thus, in the postnatal rat hippocampus, there is a bell-shaped age dependence of susceptibility to various epileptogenic agents and conditions including kainic acid (8, 317, 620), electrical stimulation (464), hypoxia (288), $GABA_A$ antagonists (233, 323, 599), fever (32, 277, 546), $GABA_B$ receptor antagonists (427), adenosine A1 antagonists (171), and high potassium (172, 282, 325). The developmental changes in GABAergic function (50, 53) could explain the higher incidence of seizures of immature neurons in addition to other candidate mechanisms (for reviews, see Refs. 33, 272, 274, 601).

In the adult brain, hyperpolarizing and inhibitory GABA prevents generation and propagation of paroxysmal activity (203, 441), and many antiepileptic drugs act by enhancing GABAergic transmission. Depolarizing and excitatory action of GABA can be a potential factor for enhanced excitability in the immature brain. Thus GABA-enhancing drugs have a limited anticonvulsive efficiency or even a proconvulsive action, and $GABA_A$ receptor antagonists exert anticonvulsive effects in several models of ictogenesis (172, 173, 319, 321, 325). Anticonvulsive effects of GABA in immature neurons have also been re-

ported (316, 326, 439, 659), possibly because of the shunting actions of depolarizing GABA (113, 316, 367, 401, 659).

Studies in hippocampal slices using the high-potassium model of ictogenesis that is relevant as elevation of $[K^+]_o$ occurs during seizures (287, 289, 380, 532, 619, 685) showed that a rise in $[K^+]_o$ from physiological level (3.5 mM) to 8.5 mM induced seizure-like events (SLEs) between P7 and P16 peaking at postnatal day 11 (325). SLEs included an initial bursting discharge as well as a tonic and clonic-like after discharge period ending with postictal depression. The developmental curve of these events correlates well with hypoxia (288) and fever (32)-induced seizures in vivo.

Studies performed in the hippocampus in vivo (282) or in vitro (172, 325) showed that blockade of $GABA_A$ receptors caused different effects depending on the background epileptiform activity. Thus $GABA_A$ receptor antagonists either replaced SLEs by recurrent interictal-like events (325) or reduced the frequency of SLEs while increasing the amplitude of population spikes. In the high-K^+/low-Mg^{2+} model, ictal-like activity was reduced by the $GABA_A$ agonist isoguvacine and exacerbated by bicuculline (282). In another study, $GABA_A$-enhancing drugs potentiated SLEs, whereas NKCC1 antagonist bumetanide negatively shifted E_{GABA} and suppressed SLE during the second postnatal week, emphasizing the proconvulsive role of depolarizing GABA (172, 173). Intrahippocampal injection of epileptogenic agents (high K^+ and low Mg^{2+}) in vivo induced in P8–P12 rat hippocampal generated electrographic seizures that were facilitated by coinfusions of the $GABA_A$ receptor antagonist and blocked by coinfusions of isoguvacine or diazepam, suggesting anticonvulsants effects of GABA (282). Although barbiturates and benzodiazepines suppressed seizures in neonatal rats in various models of ictogenesis (357, 358, 572, 636), hippocampal seizures induced by systemic injection of kainate were suppressed by bumetanide, phenobarbital, and bicuculline, suggesting that depolarizing GABA facilitates seizures in the developing brain (173).

Interestingly, the effects of $GABA_A$ receptor antagonists are strongly age dependent as they generate interictal-like activity in slices at birth (316, 367, 659), ictal-like SLEs starting from P2 in the intact hippocampus in vitro (316, 320), and SLEs in slices only around the end of the first postnatal week (233, 282, 599). Interictal- and ictal-like activity were also induced by bicuculline in the macaque hippocampal slices during the last third of gestation (323). In the mirror-focus model of secondary epileptogenesis, there is a strong depolarizing shift in E_{GABA} and powerful excitatory action of GABA and bicuculline suppressed interictal-like events in slices prepared from epileptic hippocampi, suggesting proconvulsive action of GABA (319).

Taken together, these observations suggest a complex contribution of GABA to the initiation and generation of paroxysmal activity in the immature hippocampus. On one hand, GABA excites immature neurons, but on the other hand, depolarizing GABA also inhibits because of shunting mechanisms due to increase in membrane conductance as a result of opening $GABA_A$ channels and voltage-gated K^+ channels and Na^+ channel inactivation (401, 586). These dual effects of GABA probably explain the complexity of the phenomena observed during development.

When comparing the sequence of events that occur during brain maturation (325), it is manifest that the peak susceptibility to seizures triggering events or procedures corresponds to the intersection of three curves: the fall of the depolarizing drive of GABA signaling that at P10 or so GABA is clearly less excitatory than at earlier stages but has not reached the inhibitory efficacy that will be attained a couple of days later at a time when the density of glutamatergic synapses has increased. The peak susceptibility therefore does not correspond to the maximal excitatory drive of GABAergic signaling, this occurs in utero, but to the convergence of a reduced GABAergic excitation and a significant density of glutamatergic inputs that are not fully compensated by the shunting actions of GABA.

Depolarizing and excitatory actions of GABA during seizure have also been reported in adults. Indeed, there is now considerable evidence that the dynamic switch in the action of GABA from inhibitory to excitatory as a result of massive release of GABA occurs during seizures in adults (24, 395). Moreover, $GABA_A$-mediated excitation contributes substantially to neuronal synchronization during SLEs generated in a low-magnesium model (339), long-term exposure to the $GABA_B$ receptor antagonists (628), the posttetanic afterdischarges (206), and interictal activity in slices of subiculum from TLE patients (127). A fundamental age difference in the $GABA_A$-depolarizing responses is that in adult neurons, GABA is hyperpolarizing at resting state in a majority of neurons (but see Refs. 40, 127, 244, 401, 418, 586, 645) and becomes depolarizing/excitatory only as a result of intense activation of $GABA_A$ receptors. In contrast, in the immature neurons, because of the delayed expression of chloride extruder KCC2 (286, 450, 499, 521) and early expression of chloride loader NKCC1 (157, 173, 499, 506, 528, 596, 671), $GABA_A$-mediated responses are depolarizing/excitatory at rest. This is likely to be one of the major factors contributing to enhanced seizure susceptibility.

B. Seizures Beget Seizures: GABA and High-Frequency Oscillations

The suggestion that "seizures beget seizures" (235) has long been considered as a basic mechanism in epilepsies, but experimental compelling evidence has been dif-

ficult to obtain. Extensive investigation in adults, notably using the kindling paradigm, suggests that recurrent seizures lead to a reduced threshold for further seizures and the transformation of a naive hippocampus or amygdala to one that generate seizures permanently (232, 541). Studies confirm the high degree of susceptibility and the relative resistance of limbic and neocortical structures, respectively, as well as the difficulties in kindling brain structures in primates. In general, these studies are in keeping with similar observations made using kainate or pilocarpine as models of temporal lobe epilepsies that illustrate how recurrent seizures generate a chronic situation with networks generating spontaneous and evoked epileptiform activities (48, 101). However, the conditions required for the transformation to take place cannot be investigated in vivo because of difficulties in establishing a causal relation between the propagation of seizures and the outcome.

Recent studies performed on developing networks in vitro have provided direct evidence that seizures beget seizures. This is of particular importance because of the so-called "paradox of the developing brain." Indeed, in humans and animal models, seizures cause long-lasting deleterious sequels (272, 273, 276) but lead less frequently to neuronal loss and brain damage because of the relative higher resistance of immature neurons to insults (8, 48, 274, 275, 474, 620). Therefore, it is assumed that their deleterious sequels are mediated by long-lasting effects of the seizures on developmental programs. It is therefore essential to determine the effects of seizures on developing networks. A preparation that enables us to do that in good conditions has been developed recently. It is based on an intact neonatal preparation that includes the entire hippocampus and its directly connected structures including the septal complex and the entorhinal cortex (320). Because of a better preservation of intrinsic connections, this generates readily network-driven oscillations including ictal high-frequency oscillations and other patterns that are seldom recorded in slices. This preparation can be placed in a three-independent compartment chamber that enables the perfusion of the two hippocampi and the commissures with different liquids (322). It is thus possible to apply a convulsive agent to one hippocampus and determine the consequences of the propagation of recurrent seizures in the naive hippocampus relying on field and patch-clamp recordings in both hippocampi. It is also possible to interrupt the interhemispheric propagation of seizures reversibly by perfusing the commissural connections with TTX to block action potentials. With the use of this preparation, it was shown (319) that the propagation of an ictal event from a kainate-treated hippocampus to a naive hippocampus was not sufficient to transform the latter to a chronically epileptic one. In contrast, when several epileptiform episodes are allowed to propagate to the contralateral side, the latter becomes epileptic; it generates spontaneous ictal episodes once disconnected from the kainate-treated side.

With the use of this preparation, the conditions required for the epileptogenic transformation were determined (319). Applications of kainate to the treated side and an NMDA receptor antagonist to the naive chamber blocked the long-term effect in the latter, indicating that active NMDA receptors are required for seizures to beget seizures. This observation reinforces the links between LTP and long-term actions of seizures. In adult slices, seizures lead to a long-term enhancement of glutamatergic PSCs when NMDA receptors are operative (58, 91, 452, 591).

The mechanisms involved in the generation of a mirror focus were investigated in a subsequent study (321). It was found that seizures beget seizures only when they include high-frequency oscillations (HFOs, over 40–80 Hz). A wide range of conditions that did not generate HFOs also failed to generate a mirror focus including blockade of NMDA receptors but also unexpected applications of a GABA receptor antagonist to the naive hippocampus. This suggests that operative GABA signaling is required for seizures to beget seizures. This is perhaps best illustrated by the observation that coapplications of kainate and a GABA receptor antagonist to the same hippocampus blocked both HFOs and the long-term effects of kainite. In contrast, the contralateral hemisphere, in which both GABA and NMDA receptors are functional, became a mirror focus. Therefore, the synergistic actions of GABA and NMDA receptors are required for seizures to beget seizures, suggesting that GABA receptor antagonists are proconvulsive but antiepileptic. This unexpected conclusion illustrates the duality of the actions of GABAergic signals in immature networks: GABA excites but also inhibits; blocking GABA generates seizures but also prevents the long-term consequences of seizures by blocking the generation of HFOs (Fig. 11) (53, 321, 373). Although the exact mechanisms that underlie the link between HFOs and formation of a mirror focus have not been determined, the synergistic actions of GABA and NMDA acting to generate HFOs most likely play a central role. It is suggested that they enable the generation of gamma (and higher) band frequency oscillations that will trigger a cascade of signals that lead to long-term consequences including a depolarizing shift of the actions of GABA that further facilitates the activation of NMDA receptors leading to the long-term sequels.

Interestingly, the developing brain is not capable of generating physiologically relevant HFOs at an early developmental stage. Thus physiological ripple oscillations >140 Hz are observed in vivo in the hippocampus after the end of the second postnatal week (85). Also, studies in slices indicate that several procedures that generate HFOs including bath application of carbachol to the intact cerebral cortex of newborn rats (332) do not generate HFOs at an early stage. In humans, the first

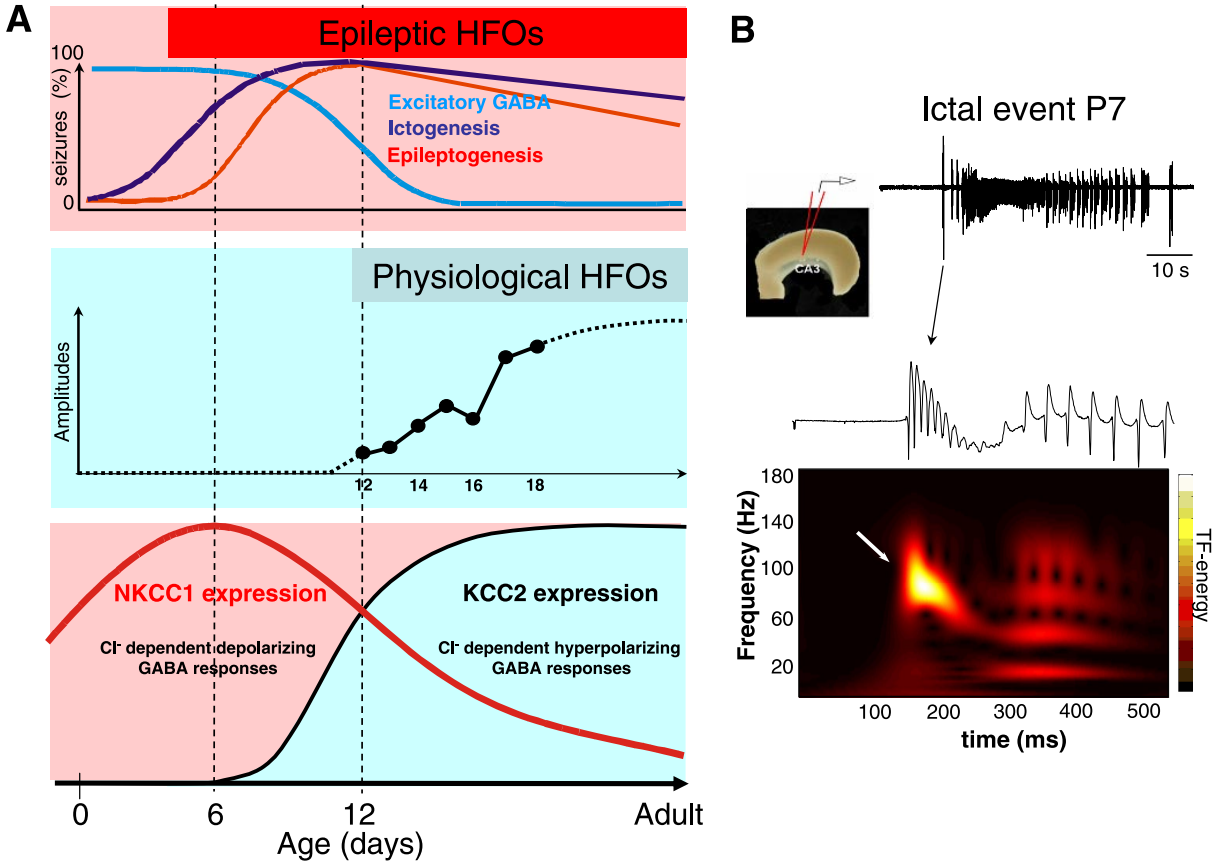

FIG. 11. Developmental switches and postnatal development of physiological and epileptic high-frequency oscillations (HFOs). *A*: *i*) Age dependence of ictogenesis (the generation of seizures) and epileptogenesis (the permanent transformation by seizures of a naive network into an epileptic one) in rat hippocampus. Epileptogenesis can be observed only from P6 onwards, suggesting that sufficient maturation of GABAergic and glutamatergic synapses is required to trigger long-term epileptic alterations. *ii*) Energy of hippocampal physiological HFOs (ripples, 140–200 Hz) expressed in vivo in rat pups from P12–P20. Note that the HFOs do not begin until the second week of life and that the developmental time course of these HFOs parallels the switch of GABA from excitation to inhibition at P13–P15. This developmental switch also coincides with the end of the physiological pattern of the giant depolarizing potentials (GDPs). *iii*) In neonatal animals, neurons accumulate Cl^- through the Na^+-K^+-$2Cl^-$ cotransporter NKCC1, resulting in a depolarized E_{Cl} and the excitatory action of GABA. Later in development, increased expression of the neuron-specific K^+-Cl^- cotransporter KCC2 results in Cl^- extrusion and a negative shift in the Cl^- equilibrium across the cell membrane, leading to the inhibitory action of GABA. *B*: a spontaneous ictal-like event recorded extracellularly in the CA3 area of a P6 rat intact immature hippocampus (that had been rendered chronically epileptic after several applications of kainate) started with HFOs in the gamma range (60–100 Hz), as shown by a time-frequency representation that provides a representation of the dominant frequency during an ictal episode. [From Le Van Quyen et al. (373).]

cognitive HFOs can be detected after 8 mo as viewed with EEG recordings consistent with the development of higher information processing (147). At that stage, as shown above, seizures do generate HFOs that can form an epileptogenic mirror focus most likely because the density of glutamatergic synapses is not sufficient to generate HFOs. In these conditions, very immature networks lack the critical density of functional glutamatergic synapses required for these oscillatory activities. These observations suggest that seizures may not lead to long-term consequences at an early developmental stage at least until the network can generate HFOs.

Are these conclusions valid for the adult brain? Applications of kainate also lead to long-term consequences, since after an episode of hyperactivity, an electrical stimulation that generated an EPSC in control conditions induced an all-or-none epileptiform activity (58). Blocking NMDA receptors also prevented this transformation. However, applications of a GABA receptor antagonist failed to prevent the HFOs and the long-term consequences, indicating that other mechanisms in adults provide a source for HFOs including gap junctions as HFOs are blocked by gap junctions blockers (162).

C. Excitatory GABA in Migration Disorders

Developmental epilepsies are due to a variety of disorders including migration, proliferation, and other insults that often have a genetic cause, although epigenetic causes are also important (413, 495, 550, 609). Migration disorders are among the most investigated causes of ep-

ilepsies, and recent studies performed in human and animal preparations have investigated those roles of GABAergic signaling in seizure generation.

Mathern and co-workers (103, 105, 106; for review, see Ref. 104) have extensively investigated the properties of neurons in slices obtained after surgical interventions in migration disorders. In a variety of cortical dysplasia (CD) types, various neuronal populations were identified on the basis of post hoc reconstruction including balloon cells, large cytomegalic neurons, and atypical and immature neurons (106). Cytomegalic neurons generated readily recurrent calcium currents (103, 105, 106) and exhibited a lower frequency of glutamatergic currents and a higher frequency of GABAergic PSCs (103). The authors suggest that oscillations produced by GABAergic PSCs may generate synchronized patterns that would propagate to normal neurons located within the focus and generate epileptiform activities. Other observations suggest a deficit in GABAergic mechanisms. Thus a reduction in IPSC frequency and a potentially compensatory decrease in transporter-mediated GABA reuptake function have been reported (93). Interestingly, GABAergic signaling is involved in the relationship between ictal activity onset and the occurrence of slow interictal-like events (149). In focal dysplastic slices, decreasing or increasing $GABA_A$ receptor function abolished or potentiated ictal discharges, respectively. The initiation of ictal events is due to the occurrence of synchronized GABAergic PSCs leading to $[K^+]_o$ elevations that promote the generation of ictal events (149; also see Refs. 143, 583). The decrease in NR2B subunit expression in cytomegalic neurons from CD tissue also can lead to a reduction of the voltage-dependent Mg^{2+} blockade of NMDA receptors and the generation of synchronized patterns (18, 471). Further studies are clearly required to determine the role of depolarizing GABAergic signals in seizure generation in developmental malformations.

IX. GENERAL CONCLUSIONS: A SEQUENCE THAT EQUILIBRATES GABA AND GLUTAMATE DURING DEVELOPMENT

The construction of brain networks faces several issues. First, the process must take into account the extreme heterogeneity of immature neurons that will at any point in time be in different developmental stages. This will complicate the generation of network-driven patterns that are common to neurons endowed with many active synapses and to others that have few if any operative synapses. If "neurons that fire together wire together," then slow kinetics and widespread propagation of primitive patterns are mandatory to overcome this heterogeneity and set together neurons in different degrees of maturation. Second, neurons have to balance their glutamatergic and GABAergic inputs both to generate the environmental support for activity-dependent mechanisms that regulate many essential developmental processes. This is also needed to prevent deleterious consequences of a disruption of the balance between GABA and glutamate: a glutamatergic excitation that is not compensated by GABAergic signaling will lead to excitotoxicity, and a dominating GABAergic inhibitory drive will slow down developmental processes. Third, neurons and networks must shift from a silence ensemble of newly differentiating neurons to an ensemble of coactive neurons that generate a plethora of patterns and oscillations. Observations discussed in this review may provide a solution to these issues (also see Ref. 49). The fact that these sequences described for the hippocampus have been generally confirmed for many other brain structures and animal species suggest that they have been kept throughout evolution and reinforce their relevance for development.

At an early stage, GABA receptors are already functional when most synapses are not operative. In fact, there is most likely a sequential formation of GABA receptors followed by NMDA and AMPA receptors at least in principal cells of the hippocampus. In the neocortex, there are some indications that GABA receptors are present early (399; also see Refs. 504 and 459 for review), but GABAergic inhibition is delayed probably because of a delayed synaptic drive to GABAergic interneurons (4, 334, 391, 398, 399, 405, 490). In the hippocampus, the lack of an efficient GABA transport system at that early stage will facilitate the diffusion of GABA and its distal actions on receptors. The long-lasting spontaneous and evoked tonic currents generated by a noncanonical release of GABA will exert actions on distal neurons. To prevent excitotoxicity, two mechanisms operate: *1*) an early maturation of functional glutamate transporters that prevent excessive overactivation of NMDA receptors in addition to the voltage-dependent Mg^{2+} blockade and *2*) the excitatory actions of GABA that can generate sodium and calcium spikes but with a driving force that is much lower than that of glutamate (30–40 mV at best compared with 80 mV for glutamate). The shunting actions of GABA will also reduce the glutamatergic drive. In addition, several voltage-gated channels are operative to prevent seizures and excitotoxicity. The early expression of presynaptic $GABA_B$ and other G protein-mediated signaling will control transmitter release. Collectively, despite excitatory GABA, the balance between excitation and inhibition is preserved thanks to other mechanisms including a presynaptic control of transmitter release.

There are only a few exceptions to the rule that during maturation there is a reduction of $[Cl^-]_i$ (401). According to the age of the species, the brain structure, and the neuron, this will take place in utero or after birth. This is by no means incompatible with excitatory actions of GABA on certain types of neurons or dendrites of

neurons. The point here is that the concentrations of $[Cl^-]_i$ present at an early stage are not reached in adults except in very special conditions (seizures and damage). Perhaps even more importantly, the developmental curve (of a reduction of $[Cl^-]_i$) has not been challenged and is in fact valid for peripheral neurons and for other organs, indicating that it has served other purposes during development than converting excitation to inhibition of GABA/glycine. Although speculative, it is possible that the removal of chloride from the intracellular milieu was a more recent selective adaptation for central neurons.

Within this general scheme, there are several points to consider. Studies using single-channel cell-attached recording show that E_m is not modified during maturation (626). Therefore, the driving force that mediates the actions of GABA is strictly dependent on $[Cl^-]_i$ and not on a shift of E_m. However, this developmental gradient includes at least two checkpoints to the excitatory actions of GABA, the generation of sodium and calcium action potentials, and the activation of NMDA receptors by removing the voltage-dependent Mg^{2+} blockade. At an early stage, when $[Cl^-]_i$ is particularly high, the estimated difference between resting membrane potential and E_{Cl} is ~40 mV, the activation of GABA will generate action potentials. The high impedance of small cells will compensate the smaller density of sodium channels. The developmental time course of the GABA/NMDA synergy is an additional important criterion that has not been studied in detail. This leads to long-term changes in synaptic efficacy of GABA, i.e., a long-term potentiation or depression when calcium channels or NMDA receptors are activated, respectively. This synergistic action of GABA and NMDA is instrumental in the developmental actions of GABA as it intervenes in neuronal growth, synapse formation, and network construction.

The GABA-glutamate sequence is another major component of the model proposed. The earlier formation of GABAergic synapses provides excitatory drive early on that will entrain very immature neurons in primitive network-driven patterns. In keeping with this, in utero, exclusively GABAergic patterns can be recorded in interneurons that have matured before principal cells. Therefore, interneurons that modulate many behaviorally relevant patterns and oscillations in the adult circuit play a central role in the generation of synchronized patterns well before a significant proportion of principal cells have established a dense network of excitatory synapses. By developing first and extending an elaborated pattern of connections initially with other interneurons, GABAergic neurons generate the first GDPs, setting the pace for the modulation by activity of the formation of glutamatergic synapses. The longer kinetics of GABAergic PSCs and the synergistic actions of GABA and NMDA receptors will facilitate the generation of primitive patterns. Combined together, GABA-NMDA PSCs will have an ~300 ms duration that will readily facilitate the synchronization of long-lasting polysynaptic PSCs or GDPs. Once the excitatory drive and the density of glutamatergic synapses are significant and an efficient inhibitory signaling is required, then the mechanisms that shift the chloride gradient are activated, and neurons and networks resume their adult operation. This scenario offers a less stringent and programmed way to shift from silent to active neurons. Indeed, the alternative solution, an equilibrated parallel maturation of excitatory and inhibitory synapses, would have entrained a very strict series of control of the synthesis and operation of all the proteins and mechanisms needed to equilibrate GABA and glutamate PSCs during development including transporters, uptake mechanisms, release proteins, vesicular transport and trafficking, potentiation, and depression, etc. The earlier formation of slightly excitatory GABAergic synapses with their inborn inhibitory device eliminates the risk for overexcitation yet supplies the excitatory drive and the calcium signaling needed for maturation.

The GABA/glutamate sequence is paralleled by a sequential maturation of interneurons before principal cells. At this early stage, pyramidal neurons are in essence silent except a few pioneer neurons. Therefore, GABAergic interneurons are organized to operate as the main pacemaker generators of early pattern. They are endowed with the properties to trigger oscillations and to ignite the sequences that will run up the engine before it operates properly. Extensive investigations by Spitzer and co-workers (577, 581) have shown that calcium waves and spikes enable the establishment of the GABA phenotype by modulating the synthesis of GAD, procedures that block these oscillations also block the expression of this phenotype.

Several elements of this model remain to be confirmed. First, do other brain structures indeed follow the same rules? If in a given network glutamatergic synapses are formed first, i.e., the neocortex, it will be interesting to determine how the issue of toxicity is avoided. Second, the role of voltage-gated channels and notably K^+ channels known to play a crucial role in inhibitory signaling and present early on, has not been discussed at length. Third, the role of GABAergic signaling in the formation of glutamate synapses and connections remains to be clarified. Studies using antagonists or knockout strategies are less than adequate because of the multitude of actions and heterogeneity of immature neurons. More efficient will be the use of conditional time- and neuron-specific deletions of siRNA that have already shown recently to be extremely useful and informative (27). A recent study shows that GABAergic transmission controls the balance of excitation and inhibition in the developing retinotectal circuit, since a premature reduction of $[Cl^-]_i$ in by the expression of the Cl^- transporter KCC2 blocked the developmental increase in AMPA receptor-mediated trans-

mission (7). Fourth, present understanding of the control of $[Cl^-]_i$ suggest that both intrinsic and extrinsic signals are involved in the developmental reduction of its concentration. However, this conclusion is based on a static and often qualitative estimation of $[Cl^-]_i$. The regulation of $[Cl^-]_i$ is a dynamic process, and very few studies measuring in physiological conditions alterations of chloride have been performed. To determine the alteration of $[Cl^-]_i$ during GDPs or synaptic currents may require dynamic analysis to fully take into account the speed with which $[Cl^-]_i$ resumes its control concentration. If physiological activity indeed regulates rapidly and persistently $[Cl^-]_i$, it will be essential to determine the cascade of signals that underlies these actions using physiologically relevant preparations. An additional major concern is the identification of the postmitotic age of the recorded neuron. With present available information, it is clearly difficult to rely on data grouped on the basis of the age of the animal without neuronal reconstruction or neuronal birth dating. The use of lentivirus tranfection techniques to record neurons after identifying their age will be essential. Transfection studies of the KCC2 cotransporter are also very useful as they enable the testing of the consequences of overexpression at an early stage of the transporter. This is particularly useful if an early expression of KCC2 augments selectively the density of GABAergic synapses (7, 118), raising the possibility that $[Cl^-]_i$ and the actions of GABA influence the formation of synapses on the postsynaptic targets.

The pathological implications of these observations and sequences should not be underestimated. With the advent of imaging tools in humans, it was determined that many neurological disorders originate early in life because of the adverse effects of mutations or environmental hazards. There is a concern on the possible deleterious actions of the widely used GABA-acting drugs during gestation. Thus GABA-acting antiepileptic agents can have deleterious actions on neuronal migration and cortical construction. The long-lasting actions of seizures on $[Cl^-]_i$ and the excitatory actions of GABA in human and animal epileptic material suggest that epileptogenesis recapitulates ontogenesis. This general scheme appears to be common to many neurological disorders, suggesting that $[Cl^-]_i$ is a nodal point in the posttraumatic response of neurons and networks. One hypothesis is that the accumulation of chloride during insults overcomes the capacity of the neuron to extrude chloride by affecting persistently cotransporters. This in turn leads to a cascade of pathogenic consequences.

In conclusion, the study of how GABAergic signals are established during brain maturation has illustrated how studies on development lead to a major breakthrough in our understanding of how networks operate. This topic is also central to perhaps better understanding the complex interactions between nature and nurture and the roles of genetic programs and activity-dependent mechanisms. Perhaps the most exciting is to see how the apparently simple question "what comes first inhibition of excitation" leads to the discovery of fundamental developmental rules that appear to have been kept throughout evolution and that may represent a favorable solution to these issues.

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