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GABA_A Receptors, Anesthetics and Anticonvulsants in Brain Development

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Abstract

GABA, acting *via* GABA_A receptors, is well-accepted as the main inhibitory neurotransmitter of the mature brain, where it dampens neuronal excitability. The receptor's properties have been studied extensively, yielding important information about its structure, pharmacology, and regulation that are summarized in this review. Several GABAergic drugs have been commonly used as anesthetics, sedatives, and anticonvulsants for decades. However, findings that GABA has critical functions in brain development, in particular during the late embryonic and neonatal period, raise worthwhile questions regarding the side effects of GABAergic drugs that may lead to long-term cognitive deficits. Here, we will review some of these drugs in parallel with the control of CNS development that GABA exerts *via* activation of GABA_A receptors. This review aims to provide a basic science and clinical perspective on the function of GABA and related pharmaceuticals acting at GABA_A receptors.

Keywords

GABA; anesthesia; epilepsy; neurogenesis; glutamate; proliferation; apoptosis; synaptic

INTRODUCTION

Although γ -aminobutyric acid (GABA) was detected in bacteria in the early 19th century, about 40 more years passed before it was detected in the mammalian brain [1]. In 1967, an elegant study confirmed previous suggestions that GABA could be the main inhibitory transmitter in the cerebral cortex. In addition, this study showed that Cl⁻ ions were responsible for GABA's inhibitory effect [2]. Today, GABA is well-known to be the most abundant inhibitory neurotransmitter in the mammalian central nervous system (CNS) where about 20% of all neurons are GABAergic [3]. GABA acts by binding to two different types of receptors, ionotropic receptors called type A and C GABA receptors and metabotropic receptors called type B GABA receptors. Being ligand-gated ion channels, GABA_A receptors facilitate rapid responses, while GABA_B receptor activation causes slower and more complex responses involving G proteins and second messengers. Here, we focus on GABA_A receptors, briefly describing their structure and pharmacology, and referring to recent reviews on these topics when appropriate. In particular, we encourage readers to refer to more extensive reviews for details on the gene expression, structure, expression and drugs [4,5].

In addition to acting as a neurotransmitter, GABA strongly influences the different stages of cell development from proliferation to synaptic integration. GABA's action is mediated through

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paracrine/autocrine signaling prior to synapse formation and then through classic synaptic signaling later on. The budding field of neonatal and adult neurogenesis has recently seen a wave of articles describing GABA's function on the development of newly born neurons in the postnatal brain. Findings from these articles will be discussed in this review following some remarks on the electrophysiological properties of GABA_A receptors.

Our concise review of GABA_A receptor properties and GABA's function in CNS development sets the stage for a discussion of GABAergic drugs to which the developing brain may be exposed. Such drugs include the common drug of abuse ethanol, as well as antiepileptics and anesthetics that must sometimes be used during pregnancy or during the first few years of life. We will review evidence that each of these drug classes can trigger premature apoptosis in animal models and subsequent cognitive deficits in animals or humans. Finally, we will comment on the relevance of the present data for clinical practice and pharmaceutical development, and on the need for further studies.

I. GABA_A RECEPTOR PROPERTIES

Subunit Composition

GABA_A receptors belong to the large family of ligand-gated ion channels that are heteromeric pentamers like nicotinic receptors (Fig. 1A). These receptors are formed by subunits of different subclasses. Nineteen of these subunits grouped into eight classes have been identified (α 1-6, β 1-3, γ 1-3, δ , ϵ , θ , π , ρ 1-3) although more variations exist through alternate splicing. For example, γ 2 exists as a long (γ 2L) and short (γ 2S) variant increasing the diversity of GABA_A receptors [6,7]. Many of the genes encoding these subunits are located in clusters in the order of $\beta\alpha\gamma$ and $\beta\alpha\gamma$ on different chromosomes. Phylogenetic tree analysis showed that the different clusters derived from an original ancestor cluster by multiple duplication steps. So far, four such clusters have been mapped in humans. The cluster with the genes for the subunits β 1 α 4 γ 1 is located on the chromosome 4p14-q12 where the β 1 and α 4 genes face head-to-head and are separated by 60 kbp [8]. A second gene cluster for the subunits β 2 α 6 γ 2 can be found on chromosome 5q31.2-q35 with less than 60 kbp distance between the β 2 and α 6 genes facing head-to-head. The β 3 α 5 γ 3 gene cluster was mapped on chromosome 15q11-q13 where the β 3 and α 5 genes also show head-to-head orientation and transcription of the γ 3 gene points away from the cluster [9]. A gene cluster for $\theta\alpha$ 3 ϵ was mapped on chromosome Xq28 where the θ and α 3 genes show head-to-head orientation. Like the γ gene in the other clusters, the ϵ gene shows the opposite direction of transcription compared to the α gene.

The subunit composition of a particular receptor determines its kinetics and the specific effects of allosteric modulators of GABA_A receptors. It is thus important to determine the subunit composition of receptors in brain regions and individual cells (see next section). Although the multiple subunits could allow a broad variation of receptors, findings suggest that the majority of GABA_A receptors is composed of α , β , and γ or δ subunits. To reveal the subunit composition in native tissue, different methods have been used, including immunohistochemistry to localize the different subunits and electrophysiology to compare receptor properties in native tissue and heterologous expression systems. Data suggest that receptors composed of α 1 β 2 γ 2 subunits are the most abundant, followed by receptors composed of α 2 β 3 γ 2 and α 3 β 3 γ 2 subunits. Other combinations of these three subunits were less prominent. Interestingly, expression studies in heterologous expression systems showed that each of the three subunits (α , β , γ) is retained in the endoplasmic reticulum and gets degraded when it is expressed alone [10-12]. On the other hand, co-expression of α and β leads to functional GABA_A receptors, but with a single-channel conductance (12 pS) that is half of normal [13]. In some GABA_A receptors, the γ subunit could be replaced by the δ , ϵ , or π subunit, whereas the θ subunit could substitute for the β subunit [12,14,15]. The ρ 1-3 subunits are mostly expressed in the retina where they form mono- or

hetero-oligomers known as GABA_A receptors. These GABA_A receptors are sensitive to picrotoxin like GABA_A receptors but not bicuculline and baclofen like GABA_A and GABA_B receptors, respectively. Because their structure, function and genetics were similar to those of GABA_A receptors, they were classified as another class of ionotropic GABA receptors called GABA_C receptors [16].

Expression in Adult Tissue

Some subunits, such as $\alpha 1$, $\beta 1$ and 2, and $\gamma 2$, are detected throughout the brain although differences in their distribution have been observed (for review see [17]). Other subunits such as $\alpha 2-6$, $\gamma 1$, and δ are restricted to specific areas, like the cerebellar granular cell layer and the cochlear nucleus for the $\alpha 6$ subunit [18-21](for review see [17]). In addition to this regional diversity, there are different subunit compositions in the same cell leading to different subcellular localizations of the receptors. For example, the γ subunit is important for postsynaptic targeting [22], while receptors containing $\alpha 4$, $\alpha 5$, $\alpha 6$ or δ are located extrasynaptically [23,24].

The clustering of genes encoding the subunits is very attractive to serve as an explanation for the different expression patterns. For example, the $\alpha 4$, $\beta 1$, and $\gamma 1$ subunits, whose genes are in one cluster, are co-expressed in the undifferentiated neuroepithelium in the embryonic rat [17]. On the other hand, $\alpha 6$ is expressed in cerebellar granular cells, while no other subunit of the same cluster can be detected [17]. Thus, the clustering of subunits is not solely responsible for the different pattern of subunit expression, although neighboring genes could have an impact on subunit expression. One example was the targeted disruption of the gene for the $\alpha 6$ subunit with neomycin gene insertions that caused a decrease of mRNA transcription and protein levels of the neighboring cluster genes for the $\alpha 1$ and $\beta 2$ subunits [25]. Interestingly, although the $\alpha 6$ subunit is mainly expressed in the cerebellum, a decrease in the $\alpha 1$ and $\beta 2$ subunits occurred in the forebrain but not in the cerebellum. One possible explanation for this finding is that the binding of fore-brain-specific transcription factors was prevented and the distance between some regulatory DNA sequences was changed. Further evidence for the different regulatory mechanisms of GABA_A receptor subunit expression came from other studies of gene knockouts. A targeted deletion of δ results in a decrease in the $\alpha 4$ subunit expression and an upregulation of the $\gamma 2$ subunit expression in the brain regions where the δ subunit is normally expressed [26,27]. Another example for such compensatory mechanisms was found in mice lacking the $\alpha 1$ subunit, where an increase of $\alpha 2$ - and $\alpha 3$ -containing receptors in the cerebellar cortex was detected [28]. Together these findings suggest that the expression of subunits depends on tightly controlled transcription and on shared regulatory elements within DNA sequences. Recent computational analysis of the GABA_A receptor subunit genes revealed putative promoters and regions for long range transcriptional regulatory components in the intergenic sequences [29]. Nevertheless, it remains to be experimentally verified whether the coordinate control of subunit expression and assembly might be responsible for the observed compensatory mechanisms between subunits.

Structure

GABA_A receptors have the same pentameric structure as some other ligand-gated ion channels, for example the nicotinic acetylcholine receptor (nAChR), the glycine receptor, and the 5-hydroxytryptamine type 3 receptor (Fig. 1A). Structural information for these receptors came from the X-ray crystallography structure of the *Lymanaea stagnalis* acetylcholine binding protein, a homologue of the N-terminal domain of the nAChR, and from the cryoelectron microscopy structure of the nAChR of *Torpedo marmorata* [30,31]. These data were used for modeling the structure of GABA_A receptors, which was assumed to be ideally composed of two α and β subunits together with one γ subunit [32,33]. Both α and β subunits contribute to the formation of the GABA binding pocket. Every subunit has an extracellular short C-terminus

and a long N-terminal domain lining four α -helical transmembrane domains (TM1-4) with a large cytoplasmic loop between TM3 and TM4 (Fig. 1B). TM2 faces the lumen of the pore and TM4 is anchored in the lipid membrane. TM1 and TM3 interact with the neighboring subunit, respectively. This modeled arrangement suggests that some grooves or cavities between the subunits would allow conformational flexibility of the receptor and provide space for putative binding sites. The functional state of the receptor could change these cavities so that drugs could bind to stabilize or switch to the activation state. Because the model relies on comparable sequence data, the proposed binding sites could not always be confirmed experimentally. For example, Leu232 was experimentally shown to lie in the alcohol and anesthetic binding pocket, whereas the model could not confirm this finding [34]. Nevertheless, the model allows proposing new drug binding sites and gives insight into the conformational mechanisms following the binding of GABA and antagonists. The intracellular loop contains multiple protein-protein interaction sites as well as different phosphorylation sites to modulate trafficking and membrane clustering (see section on trafficking).

Ligands

An increasing number of drugs, like steroids, anesthetics, benzodiazepines, barbiturates and others, can modulate the activity of GABA_A receptors. Three different binding sites were identified: GABA/muscimol, t-butylbicyclophosphorothionate (TBPS)/picrotoxin, and benzodiazepine [5]. TBPS and picrotoxin are pro-convulsants that noncompetitively block GABA-gated chloride flux by binding to one or more sites located within or close to the chloride channel (for review see [4]). Picrotoxin can inhibit TBPS binding [35]. Electrophysiological and radioligand binding studies have been helpful for determining these different drug binding sites at GABA_A receptors. Unfortunately, these methods could not clearly rule out possible allosteric effects through binding to different sites on the diverse GABA_A receptors. Therefore, the development of specific agonists or antagonists has been limited [36-38].

Benzodiazepines—Many compounds are known to bind to the benzodiazepine site, for example the drug diazepam. In general, benzodiazepines are muscle relaxant, anticonvulsive, sedative and anxiolytic drugs [39]. They act by increasing the frequency of channel opening, thus requiring GABA to be present and classifying them as allosteric agonists [40]. The presence of a γ subunit is essential and most of the receptors in the brain contain a $\gamma 2$ subunit that confers the highest degree of sensitivity to most benzodiazepines and related compounds in clinical use. The less abundant $\gamma 1$ and $\gamma 3$ subunits confer decreased sensitivity, but do allow a degree of modulation by a subset of benzodiazepine-like ligands (for review see [41]). Because the benzodiazepine binding site is thought to be located in a binding pocket between the α and γ subunits of the GABA_A receptor [42], the composition of the receptor determines its sensitivity to benzodiazepine. The binding pocket for benzodiazepines is affected by the identity of the $\alpha 1$ subunit, in particular a histidine residue located in the $\alpha 1$ subunit is critical for the high sensitivity to benzodiazepines [43]. This residue is a histidine in $\alpha 1$, $\alpha 2$, $\alpha 3$ and $\alpha 5$ but arginine in $\alpha 4$ and $\alpha 6$, which have a modified binding site less sensitive to ‘classical’ benzodiazepines such as diazepam, flunitrazepam, or clonazepam but still sensitive to other benzodiazepine-site ligands such as Ro15-45 and flumazenil [44]. Although some drugs, such as zolpidem, show selectivity for the $\alpha 1$ -containing receptor, the clinical spectrum of action of benzodiazepines is quite similar since most of the benzodiazepine-site ligands modulate $\alpha 1\beta\gamma 2$, $\alpha 2\beta\gamma 2$, $\alpha 3\beta\gamma 2$, or $\alpha 5\beta\gamma 2$ receptors. As a result, research programs aimed at discovering subtype-selective benzodiazepines were initiated during the past few years with a major focus on developing compounds interacting with either $\alpha 2$, $\alpha 3$ or $\alpha 5$ -containing receptors (for review and additional references see [45]). One of these compounds is SL651498, which has muscle relaxant and anxiolytic-like effects, and has fewer side-effects than classical benzodiazepines [46]. It behaves as a full agonist at recombinant GABA_A receptors containing the $\alpha 2$ and $\alpha 3$ subunit, and as a partial agonist at recombinant receptors expressing $\alpha 1$ and $\alpha 5$ subunits [47].

Another action of benzodiazepines is to inhibit the binding of TBPS to the TBPS/picrotoxin-binding site, but only in the presence of micromolar GABA concentrations. This is consistent with the finding that benzodiazepines affect GABA_A receptor conductance only in the presence of GABA (for review see [45]). Interestingly, the level of binding of TBPS reflects the functional state of GABA_A receptors [45].

Barbiturates—Barbiturates are another class of compounds affecting the activity of GABA_A receptors. They increase the channel open duration, but have no effect on opening frequency or channel conductance [40]. Several sites of interactions of barbiturates with GABA_A receptors have been proposed (for review and references see [4,45,48]). At concentrations above 50 μ M barbiturates directly open GABA_A receptor-associated chloride channels in the absence of GABA, and at higher concentrations change the desensitization of the receptor. Interestingly, action of the sedative-hypnotic barbiturates like pentobarbital or phenobarbital can be detected in homo-oligomeric GABA_A receptors composed of either α , β , γ or δ subunits, suggesting a highly conserved binding sites among the subunits (for review see [5]).

Steroids—Steroids are synthesized both in the periphery and in the brain, where they are called neurosteroids. They are well known to have slowly developing effects on mood and behavior following an action at the genomic level. They also directly (nongenomic) act at GABA_A receptors resulting in fast modulating of synaptic transmission (for review see [49, 50]). Like for the barbiturates, steroids are thought to have at least two different sites of interaction at GABA_A receptors. The evidence is indicated by the dose-dependent action of some steroids [51]; at concentrations in the nano-molar ranges, the anesthetic alphaxalone and some metabolites of progesterone enhance the GABA-stimulated chloride conductance. At higher doses (≥ 100 nM), they directly activate GABA_A receptors in the absence of GABA. The neuro-active steroids, such as allopregnanolone and allotetrahydrodeoxycorticosterone (THDOC), are potent positive allosteric modulators of GABA_A receptors with sedative, anxiolytic, and anticonvulsant properties. However, the sulfated neuro-active steroids pregnenolone sulfate and dehydroepiandrosterone sulfate are negative GABA_A receptor modulators and induce anxiogenic and proconvulsant effects (for review see [52]). There is evidence that the δ subunit is involved in modulating the effect of neurosteroids, since their action is reduced in mice lacking the δ subunit [53].

Anesthetics—Although compounds used as anesthetics belong to different classes, many have direct effects on the activity of GABA_A receptors. Like barbiturates and steroids, low concentrations of isoflurane, enflurane, halothane, and propofol enhance GABA induced-chloride currents and thus act as allosteric modulators. At higher doses, they directly open GABA_A receptors (for review see [54]). Studies demonstrated that the β subunit plays a role in the binding of these anesthetics to GABA_A receptors, but the exact binding site has not been identified. A point mutation in the $\beta 3$ subunit strongly attenuates the effects of two intravenous anesthetics, etomidate and propofol, but only slightly decreases the action of the volatile anesthetics enflurane and halothane [55,56]. As a result, $\beta 3$ subunit-containing GABA_A receptors are responsible for the respiratory depressant effect of etomidate and propofol in the CNS, while their cardiac action (i.e. negative chronotropy) depends on different molecular targets [57].

Trafficking

Like all other cell surface proteins, GABA_A receptors are mobile at the cell surface and undergo removal coincident with insertion of new receptors. The number of receptors is critical, as increased receptor endocytosis (also called internalization) suppresses efficacy of inhibitory synaptic transmission [58]. Although ubiquitin-dependent or caveolin/lipid-raft-dependent

mechanisms may be involved in GABA_A receptor internalization, the clathrin pathway has been shown to be critical for GABA_A receptor endocytosis in cultured hippocampal neurons [59] (for review on ubiquitin see [60,61], and for review on clathrin and non-clathrin-mediated endocytosis see [62]). Using fluorescent GABA_A receptors, Bogdanov *et al.* showed that GABA_A receptor endocytosis and insertion occurred at extrasynaptic sites in cultured hippocampal neurons [63]. In addition, pulse-chase analysis revealed that newly inserted GABA_A receptors at extrasynaptic sites were recruited to synaptic sites in ~25 minutes. The process of GABA_A receptor endocytosis begins with binding of an adaptor protein (e.g. the adaptin complex AP2) to the cytoplasmic domain of the receptor. This interaction of the receptors with the AP2 adaptin is critical for the recruitment of receptors into clathrin-coated pits. After invagination and dynamin-dependent fission of the vesicles, the latter are moved inside the cell to be uncoated and fused with endosomes. Dynamin is a GTPase involved in membrane constriction and fission during endocytosis [64]. The binding site of GABA_A receptors to the adaptin complex AP2 is provided by the intracellular loops of the β and γ subunits. A specific leucine motif in the β subunit (LL 343/344) is important for receptor internalization [65]. The $\gamma 2$ subunit binds to several proteins, including gephyrin, which was first found as an anchor protein for glycine receptors [66]. Gephyrin is a tubulin-binding protein linking the receptor to the cytoskeleton. It was shown that gephyrin and the $\gamma 2$ subunit are necessary for trafficking and clustering of GABA_A receptors at postsynaptic sites [22]. Interestingly it was also found that clustered GABA_A receptors at postsynaptic sites exhibit significantly lower rates of mobility at the cell surface compared with their extrasynaptic counterparts. Gephyrin was found to reduce the lateral diffusion of GABA_A receptors, facilitating their accumulation at inhibitory synapses [67]. Another protein, which stabilizes the receptors on the cell surface, is the ubiquitin-like protein Plic-1. Plic-1 facilitates GABA_A receptor cell surface expression without affecting the rate of receptor internalization. It does so by binding to the intracellular loops of the α and β subunits through its carboxy-terminal ubiquitin-associated domain [68]. Internalized receptors are either rapidly recycled back to the cell surface, or on a slower time scale, targeted for lysosomal degradation. This sorting decision is regulated by a direct interaction of GABA_A receptors with huntingtin-associated protein 1 (HAP1), which inhibits receptor degradation thus facilitating receptor recycling [69].

Plic-1 also enhances the stability of intracellular GABA_A receptors, thus increasing the number of receptors available for insertion into the plasma membrane. Thus, GABA_A receptor trafficking at different sites in the cell membrane, transport to the cell surface, and internalization are dependent on a number of critical adaptor proteins, some of which are shared with other receptors.

Electrophysiological Properties in Adult Tissue

Extracellular binding of two GABA molecules between the α and β subunits is necessary for efficient gating of GABA_A receptors [70]. When GABA is removed from the extracellular space and thus unbinds from GABA_A receptors, the receptor-channel switches from the open-state back to the closed state. Following a desensitization period, the channel can be re-opened upon subsequent GABA binding. The timing and rate of these transitions is dependent on the subunit composition of GABA_A receptors and the availability of extracellular GABA. Following GABA binding, a conformational switch occurs resulting in an increase in chloride (Cl^-) and, to a lesser extent, bicarbonate (HCO_3^-) permeability. The relative $\text{HCO}_3^-/\text{Cl}^-$ permeability according to the Goldman-Hodgkin-Katz equation ranges from 0.18 in cultured mouse spinal neurons [71] to 0.6 in hippocampal pyramidal neurons [72]. Neurons contain low amounts of intracellular Cl^- (~8 mM) due to the presence of a powerful Cl^- extrusion mechanism, the K^+ - Cl^- co-transporter 2 (KCC2) [73] (for review see [74]), resulting in a Cl^- equilibrium potential lower than the resting membrane potential (V_m around -70 mV in

neurons). Thus, GABA_A-channel opening predominantly results in Cl⁻ influx and thus hyperpolarization of the neuronal membrane potential (for review see [75]). GABA is thus considered to be an inhibitory neurotransmitter in neuronal networks. However, there are exceptions to this rule. As mentioned above, HCO₃⁻ permeates GABA_A-channels and its equilibrium potential is much more positive than the resting membrane potential due to active regulation of intra-cellular pH. As a result in some neuronal populations (e.g. pyramidal neurons in the neocortex), the HCO₃⁻ component of the GABA_A current (i.e. HCO₃⁻ efflux) is strong enough to produce a substantial depolarization despite an opposing current mediated by Cl⁻. GABA_A-depolarization happens specifically in small neuronal compartments (i.e. dendrites) following intense GABA_A receptor activation. It results from the activity-dependent collapse of the opposing concentration gradients of Cl⁻ and HCO₃⁻, and accumulation of intra-cellular Cl⁻ [76]. The depolarizing action of GABA in mature networks has been the subject of a recent review [74].

As mentioned in a previous section, GABA_A receptors are located at both postsynaptic and extrasynaptic sites, and their numbers are dynamically regulated. Synaptic GABA_A receptors open transiently upon vesicular synaptic release of GABA briefly reaching up to one millimolar concentration in the synaptic cleft (for review see [77]). Extrasynaptic GABA_A receptors see much lower GABA concentrations that may be maintained for longer time periods, resulting in tonic receptor activation (for review see [78]). Clearly, transient synaptic and tonic extrasynaptic receptor activation will have different effects on synaptic input integration and resulting neuronal excitation (for review see [79]). Tonic activation is thought to lower neuronal excitability upon a depolarizing input [80,81]. Both synaptic and extrasynaptic receptor gating, the number of receptors, and the concentration time course of GABA (release kinetics and uptake mechanisms) will shape GABA_A-currents and thus their effects on cellular function.

II. GABA_A RECEPTOR EXPRESSION AND FUNCTION IN DEVELOPING TISSUE

The important role of GABA in development is reflected by its production in many brain regions, as well as the differential distribution of GABA_A receptors in the brain. In the developing brain including embryonic, early postnatal and adult germinative zones, GABA acts as a signaling molecule, influencing proliferation, migration and synaptogenesis as previously extensively reviewed [82-94]. The next paragraphs describe the pattern of receptor expression and the receptor electrophysiology in developing tissue, and discuss studies related to the action of GABA or GABAergic blockers on cell development *in vitro* and in slices from rodents. Studies related to the apoptotic action of GABAergic mimetics *in vivo* will be further described in the section on clinical use and implications.

Expression in Developing Tissue

Several regions in the developing cortex have been identified where cells contain GABA [95]. GABA is present very early compared to other neurotransmitters [96]. As early as embryonic day (E) 13 in rodents, GABAergic fibers are detected in the brainstem, mesencephalon and diencephalon. At E14, GABAergic cell bodies are found in the lateral cortical anlage and by E16 they are in the forebrain and all cortical regions. Similarly in adults, a zone sandwiched between the lateral ventricle and the striatum, called the subventricular zone (SVZ), retains neurogenic potential throughout life. In the adult SVZ, neuroblasts release GABA even in adulthood in their surrounding environment, and neuroblasts and surrounding cells express functional GABA_A receptors [97-99]. GABA_A receptors are detected as early as E13 [100-102]. Each subunit exhibits a unique regional and temporal developmental expression pattern suggesting that the composition and properties of embryonic GABA_A receptors differ markedly from those expressed in the adult brain with a few exceptions like

receptors in cerebellar Purkinje cells [103,104]. The subunits $\alpha 5$ and $\gamma 2$ are present in most brain regions throughout embryonic development whereas other subunits (e.g. $\alpha 6$) are present only after birth. Other subunits (e.g. $\alpha 2$ and $\alpha 3$) show various expression profiles in different brain regions [103,104]. After birth, receptors containing a $\alpha 2$ subunit are gradually replaced by $\alpha 1$ -containing receptors [105]. Whereas the $\gamma 2$ subunit shows a constant level of expression, the levels of $\gamma 1$ and $\gamma 3$ subunits decrease during development. Although the $\gamma 2$ subunit level was constant during development, this subunit is critical for normal development [106]. Indeed, mice were generated containing GABA_A receptors with a targeted disruption of the $\gamma 2$ subunit gene, which is necessary for the benzodiazepine sensitivity. As a result the single channel conductance was reduced to values consistent with recombinant GABA_A receptors composed of α/β subunits. No alteration of embryonic development (i.e. normal body weight and histology of newborn mice) was observed. However, postnatally the reduced GABA_A receptor function was associated with retarded growth, sensorimotor dysfunction, and drastically reduced life-span.

Electrophysiology in Developing Tissue

In neuroblasts (i.e. still proliferative cells) and immature neurons (i.e. postmitotic), the activity of an inwardly directed Na⁺–K⁺–Cl[–] co-transporter (NKCC1) acts to maintain a relatively high internal Cl[–] concentration, resulting in a Cl[–] equilibrium potential and thus a GABA_A reversal potential, that is more positive than the resting membrane potential (for review see [83,107]). Thus, GABA_A receptor activation in immature neurons results in a net efflux of anion and cell depolarization [108,109]. During development, neurons progressively acquire KCC2, which decreases the internal chloride concentration. GABA is thought to affect cell development (see below) *via* depolarization-induced changes in intracellular Ca²⁺ dynamics (for review see [83,84]). GABA-induced depolarization is thought to lead to voltage-gated calcium channel opening and calcium entry. However, as part of a recent review, we argued that this mechanism may not be valid for every cell type such as radial glia or subventricular astrocytes [88], but an alternative mechanism leading to calcium entry remains to be proposed. In addition, in embryonic or adult germinative zones, GABAergic signaling occurs in the absence of synapses leading to tonic as opposed to transient (i.e. phasic) receptor activation [99,110]. Transient receptor activation may nevertheless be possible as previously discussed [88].

GABA_A Receptor Function in Cell Development

Simplified description of the developing zones (Fig. 2)—The primary germinative zone is a layer of proliferating neuroepithelial cells all along the neural tube in early phases of neurogenesis. These cells divide symmetrically and generate identical progeny, thus expanding the germinative zone. Later, neuroepithelial cells progressively generate the well-known radial glia that still behave as neural progenitors and divide in the ventricular zone along the ventricles [111] (Fig. 2A). They can divide asymmetrically resulting in equal distribution of genomic DNA, but unequal distribution of cytoplasmic regulatory elements. As a result radial glia generate neuroblasts (still able to proliferate in the embryonic subventricular zone) and neuronal precursors (i.e. postmitotic, committed to become neurons) that migrate along their long radial processes to reach their final destination in the cortex. Different zones from the VZ and pre-plate are formed during development such as the subventricular zone (SVZ) above the VZ, the intermediate zone and the cortical plate on top. The radial migration is well described in the developing cerebral and cerebellar cortex (for review see [112]). During neonatal development, neurogenesis slows while gliogenesis predominates for two to three weeks after birth in mice, and one to three years in humans. These two postnatal weeks are characterized by intense neuronal maturation and synaptic integration that require dendritic development, synapse formation, and pruning along with intense gliogenesis. It is important to mention that it remains unclear whether the birth of cortical neurons persists during the neonatal period. In the cerebellum strong neurogenesis (both proliferation and migration) persists for 2-3 weeks

after birth in rodents. Similarly in humans, cerebellar granule cell precursors are located in an external layer all around the cerebellum, and continue migrating inside the cerebellum on radial processes of the future Bergmann glial cells during 1-2 years after birth [113]. As part of gliogenesis, radial glia are also well-known to transform into astrocytes, including cerebellar Bergmann glia, during the neonatal period (for review see [112]). At the ventricle wall of the future striatum, the proliferative zone forms the primordia of the medial and lateral ganglionic eminences where precursors of the GABAergic cortical neurons are born and tangentially migrate to the cortex (for review see [114-116]). In addition, along this ventricle wall, the radial glia also progressively transform into astrocytes, which retain the capacity of behaving as neural progenitors [117]. The cell production capacity of this zone, called the subventricular or subependymal zone sandwiched between the lateral ventricle and the striatum, persists until adulthood in mammals, including humans [89,118] (Fig. 2B). SVZ astrocytes generate transit amplifying cells and then neuroblasts that migrate throughout the SVZ and along the rostral migratory stream to reach the olfactory bulb or the piriform cortex [200] where they differentiate into GABAergic and dopaminergic interneurons. SVZ neuroblasts also migrate and integrate into the piriform cortex. A layer of proliferative cells also persists beneath the granule cell layer of the dentate gyrus, is called the subgranular zone, and generates granule cells throughout adult life.

Function during embryonic development (Table 1)—During embryonic development, the influence of GABA on cell proliferation was originally shown on VZ cells, which are presumably radial glia [119]. Inhibition of GABA_A receptors with bicuculline was shown to increase DNA synthesis, resulting in an increased number of proliferating cells in the ventricular zone in slices from E16-E19 rats. Table 1 summarizes some of these studies. Another study showed that GABA increased the proliferation rate in the ventricular zone in slices from E13-E14 mice by shortening the cell cycle, whereas proliferation in the SVZ was decreased [95]. These studies suggest that GABA has differential effects on cell proliferation depending on the location and developmental stage. GABA *via* GABA_A receptor activation increased the proliferation of cultured cerebellar granule cell suspensions from 6-8-day-old rats [120], a time period when neurogenesis is active in the external granule cell layer of rodents. GABA also affects cell migration by either modulating the speed of migration or playing a role as a chemoattractant. Interestingly, it was shown in a pioneer study that femtomolar GABA stimulated chemotaxis (migration along a chemical gradient), whereas micromolar GABA initiated chemokinesis (increased random movement) of cultured embryonic rat cortical neurons [121]. The same group showed that GABA promoted the migration of VZ/SVZ cells into the intermediate zone of E18 cortical slices *via* picrotoxin-sensitive GABA_A receptors, but that bicuculline-sensitive receptors blocked migration into the cortical plate [122]. This again suggests differential effects of GABA on cell migration depending on the cell location. More recently, inhibition of GABA_A receptors by bicuculline increased the speed of migration of neuronal precursors in E18-E19 neocortical slices [123]. In addition, local *in vivo* application of bicuculline or the agonist muscimol *via* cortical surface Elvax implants induced prominent alterations in the cortical architecture. GABA promotes embryonic neuron survival *in vitro* [124], and affects cell differentiation [125] and maturation such as dendrite growth [126,127] that is necessary for synaptic integration. Low levels of GABA alone or with diazepam were without effect on dendrite outgrowth of cultured embryonic hippocampal pyramidal neurons while higher levels caused moderate reductions in outgrowth [127]. Neither GABA nor the anticonvulsants affected cell survival. However, GABA plus diazepam significantly reduced the dendritic regression and cell death normally caused by glutamate. This illustrates that GABA works in cooperation with other neurotransmitters like glutamate, and this will be further detailed in the clinical section of this review. In addition, erroneous conclusions could be drawn from *in vitro* work where the cell micro-environment is not preserved compared to the slice or *in vivo* environment. Finally, an elegant demonstration of the dependence of cell

development on GABA synthesis was demonstrated in a mouse model, where a conditional knock-out of the GABA synthesizing enzyme GAD67 led to deficits in axon branching and limited connectivity in the cortex [128].

Function on adult and neonatal neurogenesis—GABA strongly affects the different stages of cell development in the adult neurogenic zones, i.e. the SVZ and the subgranular zone (see Table 1). Briefly, a system of GABAergic signaling has been described in the SVZ and along the rostral migratory stream where neuroblasts release GABA, which activates GABA_A receptors on both neuroblasts and neural progenitors, which display characteristics of astrocytes (for review see [88,129]). GABA_A receptor activation was shown to limit the proliferation of both SVZ neuroblasts and neural progenitors [99,130]. In addition, GABA acting at GABA_A receptors reduces the speed of neuroblast migration and this effect is tightly regulated by GABA uptake in surrounding astrocytes [97]. GABA also promotes dendrite growth in newly born olfactory bulb neurons and hippocampal granule cells [131,132]. The latter results in promoting synaptic integration of newly born granule cells into a mature network [132].

The function of GABA on cell development has not received as much attention as its role in synaptic transmission and the properties of GABA_A receptors. Nevertheless, GABA has clearly a fundamental role in the different stages of cell development during embryonic, neonatal and adult life.

III. CLINICAL USE AND IMPLICATIONS OF ANESTHETICS AND ANTICONVULSANTS

The importance of ligand-gated ion channels, particularly GABA_A receptors, in both embryonic and postnatal development, described herein, leads to questions about the effects of common pharmaceuticals in fetuses, infants, and small children, and opens new challenges for pharmaceutical development. Commonly used GABAergic agents include ethanol and other drugs of abuse, anticonvulsant medications, and both intravenous and inhaled anesthetics. These drugs are now known to accelerate apoptotic cell death in the CNS of some mammals during a period of synaptogenesis and physiologic apoptosis (reviewed by [133]), frequently called the brain growth spurt, that spans from the sixth month of gestation through the third postnatal year in humans and from birth through the second postnatal week in rodents [134]. Longstanding evidence supports the effects of drugs of abuse on developing fetuses, including the Fetal Alcohol Syndrome that affects many children. More recent evidence describes detrimental effects of anticonvulsant medications on CNS development in fetuses and infants. To date, there is a lack of data regarding outcomes following exposure to anesthetics for procedures or sedation during the brain growth spurt. In this section, the evidence supporting the effects of ethanol, anticonvulsants, and anesthetics on the developing CNS and their mechanisms of action (Table 2) will be discussed, with comments concerning implications for clinical practice and pharmaceutical development.

Anticonvulsants

Seizures affect 1-2% of people worldwide, and occur most commonly during the first postnatal year [135]. Anticonvulsant medications are also used to treat a variety of other neurological disorders [136], and are frequently used during pregnancy or in children. The impact of antiepileptic medications (AEDs) on neural development and later cognitive functioning has been recently reviewed by Kaindl *et al.* (2006) [137]. Anticonvulsant medications work to decrease neuronal excitation through multiple mechanisms, including GABA_A receptor potentiation, NMDA- and AMPA/kainate-type glutamate receptor inhibition, sodium channel inhibition, or inhibition of voltage-gated calcium channels. These medications, like ethanol

and general anesthetics, have been shown to induce widespread neural apoptosis when applied during the brain growth spurt period in rodents [138]. There is also extensive evidence of learning and cognitive deficits in rodents and humans following early exposure to antiepileptic medications. These defects in humans include teratogenicity in some instances, but also more subtle learning deficits and decrements in I.Q. that are sometimes not measurable until later in childhood. Specifically, among those AEDs that act primarily by enhancing GABA_A receptor activity, several negative effects on cognition were observed. For example, phenobarbital use during pregnancy, particularly during the third trimester, has been associated with cognitive deficits and lower verbal intelligence scores later in childhood and into adulthood [139-141]. Detrimental effects on cognition and intelligence were also observed following barbiturate exposure during the first three postnatal years [140,142-144]. In contrast, two AEDs, topiramate and levetiracetam, were found not to induce neuronal apoptosis in neonatal rat brain when used at therapeutic doses [145,146]. Topiramate is thought to act by inhibiting AMPA/kainate-type glutamate receptors [147,148], with possible effects at GABA_A receptors [149, 150]. Levetiracetam is thought to act by inhibiting high-voltage-activated calcium channels [151,152]. The considerable evidence pointing to negative developmental effects of AEDs, along with the preliminary evidence of improved toxicity profiles of AEDs that act by mechanisms other than GABA_A receptor potentiation or NMDA receptor inhibition, suggests that the search for AEDs with alternate molecular targets holds promise for effective seizure control with fewer developmental side effects. Clinical studies are needed to evaluate the cognitive effects of topiramate and levetiracetam during pregnancy and the first postnatal years in human subjects.

Fetal Alcohol Syndrome

Several detrimental effects seen in the children of women who used ethanol while pregnant began to be recognized in the late 1970's [153-155]. These effects, now known as fetal alcohol syndrome (FAS) or the less-severe fetal alcohol effects (FAE) include craniofacial abnormalities, microcephaly, mental retardation, learning impairment and attention-deficit/hyperactivity disorder [156], other behavioral problems, and psychiatric disorders of childhood and adulthood [157,158].

Ethanol's effects in the CNS include potentiation of currents through GABA_A receptors [159] and inhibition of currents through NMDA receptors [160-163]. Exposure of infant rat brains to ethanol yields widespread apoptotic cell death, likely accounting for the neurologic phenotype in FAS. The apoptosis appears to be triggered through actions on GABA and NMDA receptors, as the distribution of cell death with ethanol is roughly equal to the sum of those distributions caused by other GABA mimetics and NMDA receptor antagonists combined [158]. It is believed that the neurologic phenotype of FAS results mainly from ethanol exposure during the third trimester of pregnancy, as this coincides with the period of synaptogenesis and physiologic apoptosis. Although children with FAS were often exposed repeatedly to ethanol in utero, research in infant rats has demonstrated increased apoptosis following brief exposures. A minimum exposure of 200 mg/dl for four consecutive hours was initially required to see increased apoptosis (at least 15-fold over baseline) using silver staining, TUNEL, and electron microscopy 24 hours after the treatment in postnatal day 7 (P7) rats [158]. Lasting spatial learning deficits, as measured in the Morris water maze, were observed following similar neonatal ethanol treatments in later studies [164-167]. A recent study has observed increased apoptosis in P7 mouse brain using only 50 mg/dl ethanol (a serum concentration that may be achieved easily during social drinking) for 30-45 minutes when they detect apoptosis earlier using staining for caspase-3 activation 4 hours after the treatment [168]. Recent evidence also suggests that neurogenesis in the adolescent rat brain is inhibited by ethanol intake [169], in agreement with the role of GABAergic signaling in the postnatal neurogenic centers of the brain. The hastened apoptosis observed in the developing rodent brain upon ethanol exposure

likely explains the reduced brain mass and extensive neurologic phenotype in children with FAS. Ethanol shares its mechanism of action with many common intravenous and inhaled anesthetic agents, whose potential impact on the developing human CNS is discussed next.

Anesthetics

Worldwide, many fetuses and infants are exposed to anesthetic agents each year. While these agents are unquestionably necessary and beneficial to children in need of pain control or sedation for procedures or intensive care (reviewed by [170-172]), research over the past several years has raised the possibility of neurological sequelae, particularly in cases of prolonged anesthesia or sedation. Commonly used anesthetic drugs include benzodiazepines (e.g. midazolam), barbiturates (e.g. thiopental), propofol, ketamine, and inhaled anesthetics (e.g. isoflurane, desflurane, sevoflurane, nitrous oxide (N₂O, also known as laughing gas)). Each of these agents acts to inhibit neuronal excitation by potentiating GABA_A receptor activity, blocking NMDA receptors, or both, among other mechanisms (reviewed by [54, 173,174]). Furthermore, these agents are routinely used in combination with one another in balanced anesthesia, a technique by which anesthesiologists achieve adequate anesthesia while seeking to avoid potential toxicity from any one agent. Animal studies suggest that this combination of agents is most likely to generate an unwanted apoptotic response in the CNS of young patients [158,175]. Here, we will review literature describing the ability of NMDA receptor antagonists, GABA mimetics, and anesthetic combinations to trigger CNS apoptotic responses in young rodents and primates, and the possibility that these effects lead to cognitive deficits.

NMDA receptors are thought to be the main targets for two commonly used pediatric anesthetic agents, ketamine and N₂O [176-180]. During the brain growth spurt period of synaptogenesis and physiologic apoptosis, developing neurons are susceptible to death by excitotoxicity through over-activation of glutamate receptors, including NMDA receptors [181,182]. However, they have a concurrent susceptibility to NMDA receptor blockade. Application of ketamine or N₂O, both NMDA receptor antagonists, in infant rats has been shown to trigger a large increase in apoptosis in certain regions of the CNS [158,183,184]. The brain regions affected may change over time within the brain growth spurt period [183]. These apoptotic events, in some cases, led to subsequent learning deficits [175]. Additionally, application of ketamine (5 µg/ml for 4 hours or with 0.01 µg/ml for longer periods) to cultured neural progenitors from the rodent SVZ has an inhibitory effect on the formation of dendritic arbors [185]. Similar ketamine treatments also led to retractions of established dendritic arbors in culture [186], raising the possibility that NMDA receptor antagonists may pose threats to development other than accelerated apoptosis. An increase in neuroapoptosis following 24 hours of surgical ketamine anesthesia has now been observed in developing rhesus monkeys [187].

GABA receptor potentiation is thought to be the principal mechanism of action by which many general anesthetics function, including intravenous agents like propofol, etomidate, thiopental, and midazolam, as well as inhaled anesthetics like isoflurane, desflurane, and sevoflurane. The prevalence of their use in the induction and maintenance of general anesthesia is nearly universal. Like for NMDA receptor antagonists, studies have demonstrated accelerated apoptosis in the developing rodent brain after application of the GABA mimetics, specifically benzodiazepines (including diazepam [158]), barbiturates (including pentobarbital and phenobarbital [158]), and isoflurane [188]. Many such studies have been criticized for using doses and durations of application that exceed those commonly used in clinical practice, but a few studies have also confirmed effects with reasonable clinical doses. In particular, one study has demonstrated apoptosis, inhibition of hippocampal long-term synaptic potentiation, and learning deficits following treatment with a clinically-relevant combination of isoflurane,

N₂O, and ketamine [188]. Studies have also shown that the co-administration of NMDA receptor antagonists and GABA mimetics have greater detrimental effects with respect to apoptosis and learning deficits than either drug class alone [158,175,188,189]. As described in section II, GABA_A receptors in the developing brain regulate different phases of cell development, including proliferation and dendritic arborization, which allows neurons to receive and integrate thousands of synaptic inputs. Anesthetic agents commonly used in children for anesthesia, sedation in the intensive care unit, and/or acute and chronic pain control have also been shown to affect this process in cell cultures of developing interneurons derived from the subventricular zone progenitors [190]. In this model system, concentrations as low as 1 µg/ml of the GABA potentiator propofol for as little as 4 hours reduced the growth of developing dendritic arbors, measured as a decrease in dendritic length and branch points compared to untreated controls. These authors also found that high concentrations (>25 µg/ml) of the benzodiazepine midazolam did not have obvious effects on dendritic development [190]. They seek to explain this difference by pointing out that propofol acts *via* a different molecular mechanism on a different subunit of GABA_A receptors, and that propofol has known concurrent effects on the NMDA receptor, nicotinic acetylcholine receptors, calcium channels, and actin phosphorylation [190,191]. It has been difficult to determine the relevance of these rodent studies to human anesthesia, and confirmatory studies in non-human primates and in clinical trials are presently lacking.

The exposure of young patients to anesthesia or sedation is frequently of shorter duration than the exposures to ethanol or anticonvulsants that lead to demonstrable cognitive deficits. Furthermore, many of the neurons present in the developing brain are likely to undergo apoptosis in the course of normal development. It seems likely, therefore, that the potential insult from a brief anesthetic exposure may be tolerated by the developing human brain without functional deficit. There is evidence for poorer neuropsychological outcomes in children who have undergone surgery early in life, but these studies do not directly address the role of anesthesia in these outcomes [192]. A randomized multinational trial regarding infants undergoing hernia surgery with general anesthesia versus regional anesthesia is in progress and may begin to address this issue more clearly. Meanwhile, in the absence of conclusive data it may be wise for the anesthesia community to consider options for achieving adequate anesthesia safely while minimizing the concurrent use of multiple anesthetic agents in children less than three years of age.

CONCLUDING REMARKS

Since 1967 when GABA was found to be the main inhibitory transmitter in the cerebral cortex, we have uncovered the structure and subunit distribution of GABA_A receptors in the developing and mature CNS. Progress has been made in understanding GABA receptor trafficking both at synaptic and extrasynaptic sites. However, only more recently did we realize the importance of GABA on brain development, from proliferation of radial neuroepithelial cells to synaptic integration of adult-born interneurons. These recently uncovered functions of GABA through GABA_A receptors remain puzzling and require additional studies to determine the subunit composition of the GABA_A receptors involved and the downstream intracellular pathway activated for regulating cell development. Nevertheless, the important role of GABA on cell development raises questions regarding the side-effects of anesthetics and anticonvulsants in pregnant women and infants. The wealth of data concerning the fetal alcohol syndrome, along with the studies of infants exposed to antiepileptic drugs (AEDs) in utero, provide strong evidence that the developing human brain is sensitive to insults from GABA mimetics and NMDA receptor antagonists, and that these insults can be of clinical importance. Studying the neuropsychiatric consequences of anesthetics or AEDs in young children has frequently been hindered by an inability to separate the effects of medical treatment from the effects of the underlying disease process that necessitated the surgery, sedation, or

anticonvulsant treatment. The study currently underway involving outcomes from general versus regional anesthesia for a relatively benign surgical indication (hernia repair) promises to provide needed information to the field.

Due to species differences, it remains unclear what degree or duration of GABA_A receptor potentiation, NMDA receptor inhibition, or both might be required to produce neuroapoptosis in humans like that seen in rodents and rhesus monkeys. Furthermore, against the backdrop of physiologic apoptosis that characterizes the brain growth spurt, it is unknown what amount of aberrant apoptosis the human brain can tolerate before suffering a cognitive deficit. One could think the human brain has evolved to tolerate apoptotic loss during stressful, early life events such as birth, and the poor and variable nutritional environment that may have existed frequently in human history.

An important point raised by studies in rodents is the greater effect, both on neuroapoptosis and learning, of GABA mimetics and NMDA receptor antagonists used concurrently than either drug class alone. This brings to mind the widespread practice of balanced anesthesia, where agents with different mechanisms of action are used concurrently to achieve adequate anesthesia while minimizing potential toxicity from any one drug. Acknowledging the lack of data about the neuropsychological outcomes of balanced anesthesia in this age group, anesthesiologists may wish to avoid concurrent administration of agents with multiple actions when practical in their youngest patients. Due to the longer duration of exposure during sedation for intensive care or during antiepileptic treatment, the pharmacologic strategies chosen in these instances for young patients are even more likely to impact their neuropsychiatric outcomes.

Looking forward, the use of anesthetics and AEDs in young children and the neuropsychiatric outcomes in these patients are likely to be influenced by three factors. (1) New clinical data detailing the cognitive effects of different drugs and drug combinations used in young children are needed to inform medical decisions. In the absence of clinical data, studies in nonhuman primates may prove useful. (2) Pharmaceutical development of drugs to different or more specific molecular targets may offer improved toxicity profiles. One may think that anesthetics and AEDs are destined to alter CNS development when used during the brain growth spurt simply because they quiet neuronal activity as part of their clinical efficacy, regardless of their specific molecular targets. However, the apparently favorable toxicity profiles of the AEDs topiramate and levetiracetam relative to other AEDs, as described above, provides hope that further drug development may be fruitful. The development of novel anesthetics may be complicated by the requirements for very specific pharmacokinetics and lipid solubility in general anesthesia, and the relatively brief duration of anesthetic exposures during surgery may reduce the effect of these agents on long-term outcome. By contrast, the longer treatment times involved in sedation and anticonvulsive treatment, along with the more relaxed requirements for physiochemical drug attributes for these indications, may make this more fruitful territory for continued drug development. (3) Last, adjuvant treatments designed to minimize the impact of anesthetics and AEDs on the developing CNS may hold some promise. 17Beta-estradiol [193], erythropoietin [194], and minocycline [195], each of which is thought to affect the AKT serine/threonine kinase pathway, plus the antioxidant melatonin [196] are being investigated in this regard.

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Fig. (1). GABA_A receptor structure and location of drug binding sites

(A) Diagram illustrating the pentameric structure of GABA_A receptors containing 2α, 2β and 1γ subunits. (B) Schematic illustration of the α6 subunit with its three domains (extracellular, transmembrane and intracellular) and binding sites for GABA and other drugs. (C) Diagram illustrating the binding sites of different GABAergic drugs.

Fig. (2). Diagram of the developing zones where GABA regulates cell development

(A) Schematic representation of an embryonic (E) 15 coronal section of the forebrain. Radial glia (RG, green) proliferate in the ventricular zone (VZ) and extend their processes to the pial surface across the ganglionic eminence and the neocortex. Neuronal precursors (blue) migrate along radial glial processes and some proliferate in the SVZ (not shown on the diagram). Both proliferation and migration are regulated by GABA (#1-3). The migration of the precursors of cortical GABAergic interneurons (orange) born in the VZ of the ganglionic eminence is also affected by GABA (#4). S: primordia of septum, H: primordia of hippocampus. **(B)** Schematic representation of an adult sagittal section of the forebrain illustrating the neurogenic zones, the SVZ along the lateral ventricle and the subgranular zone (SGZ). GABA decreases the proliferation of neural progenitors and neuroblasts in the SVZ (#5), and the speed of migration of neuroblasts (#6) on their way to the olfactory bulb. **(C)** Simplified lineage summary.

Table 1Summary of the Roles of GABA Through GABA_A Receptors on the Different Stages of Neurogenesis

	Region/Age	GABA Action via GABA_A Receptors	Ref.
Proliferation ----- <i>Embryonic</i> ----- <i>Postnatal</i>	<ul style="list-style-type: none"> SVZ cells, i.e. radial glia in E15-E19 rat neocortical slices SVZ and VZ cells in E13-14 mouse neocortical slices cultured rat embryonic cortical cells 	<ul style="list-style-type: none"> Decreases DNA synthesis -1- Increases and decreases proliferation rate in SVZ and VZ, respectively -2- Inhibits the bFGF proliferative effect 	<ul style="list-style-type: none"> [120] [95] [198]
	<ul style="list-style-type: none"> adult SVZ neuroblasts in slices postnatal SVZ astrocytes (i.e. neural progenitors) cultured cerebellar granule cells from P6-8 rats 	<ul style="list-style-type: none"> inhibits proliferation -5- inhibits proliferation -5- Increases proliferation 	<ul style="list-style-type: none"> [131] [99] [121]
Migration ----- <i>Embryonic</i> ----- <i>Postnatal</i>	<ul style="list-style-type: none"> cultured embryonic cortical neurons embryonic cortical and ganglionic eminence neurons in slices 	<ul style="list-style-type: none"> chemokinesis and chemotaxis effects depending on concentrations various effects on neuronal migration depending on location -3 and 4- 	<ul style="list-style-type: none"> [122] [123,124,199]
	<ul style="list-style-type: none"> postnatal SVZ neuroblasts in acute mouse slices 	<ul style="list-style-type: none"> decreases speed of migration -6- 	<ul style="list-style-type: none"> [97]
Survival	<ul style="list-style-type: none"> cultured striatal neurons from E18 rats 	<ul style="list-style-type: none"> increases survival 	<ul style="list-style-type: none"> [125]
Differentiation ----- <i>Embryonic</i> ----- <i>Postnatal</i>	<ul style="list-style-type: none"> mesencephalic precursors into dopaminergic neurons <i>in vitro</i> 	<ul style="list-style-type: none"> prevents differentiation to DA neurons 	<ul style="list-style-type: none"> [126]
	<ul style="list-style-type: none"> cultured hippocampal slices 	<ul style="list-style-type: none"> promotes acquisition of neuronal transcription factors 	<ul style="list-style-type: none"> [200]
Maturation ----- <i>Embryonic</i> ----- <i>Postnatal/adult</i>	<ul style="list-style-type: none"> cultured embryonic pyramidal neurons 	<ul style="list-style-type: none"> promotes increases in dendritic growth 	<ul style="list-style-type: none"> [128]
	<ul style="list-style-type: none"> olfactory bulb and subgranular newly born neurons 	<ul style="list-style-type: none"> promotes increases in dendritic growth and synaptic integration -7- 	<ul style="list-style-type: none"> [132,133]

The numbers refer to the numbers in Fig. 2.

Table 2

Summary of the Sites of Action of Anesthetics and Anticonvulsants*

Drugs	GABA _A Receptors	AMPA/Kainate Receptors	NMDA Receptors	Use
Barbiturates	Potentialiation	Inhibition	-	AED, intravenous anesthetics
Topiramate	Potentialiation ($\alpha 6\beta 2\gamma 2$) and inhibition of $\alpha 1$ or $\alpha 2\beta 2\gamma 2$ [150]	Inhibition [148,149]	-	AED
Benzodiazepines, e.g. midazolam	Potentialiation	-	-	intravenous anesthetics
Propofol	Potentialiation [191,192]	-	Slight inhibition [191,192]	Intravenous anesthetics
Ketamine	Slight potentialiation	-	Inhibition [177-179]	intravenous anesthetics
Isoflurane	Potentialiation	Mixed	Slight inhibition	Inhaled anesthetics
Nitrous oxide	Slight potentialiation	Mixed	Inhibition [180,181]	Inhaled anesthetics

* Table 2 is compiled from [54,174].