Early in development, γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the mature brain, depolarizes and excites targeted neurons by an outwardly directed flux of chloride, resulting from the peculiar balance between the cation-chloride co-transporter NKCC1 and the extruder KCC2. The low expression of KCC2 at birth leads to accumulation of chloride inside the cell and to the equilibrium potential for chloride positive respect to the resting membrane potential. GABA exerts its action via synaptic and extrasynaptic GABA<sub>A</sub> receptors mediating phasic and tonic inhibition, respectively. Here, recent data on the contribution of "ambient" GABA to the refinement of neuronal circuits in the immature brain have been reviewed. In particular, we focus on the hippocampus, where, prior to the formation of conventional synapses, GABA released from growth cones and astrocytes in a calcium- and SNARE (soluble N-ethylmaleimide-sensitive-factor attachment protein receptor)-independent way, diffuses away to activate in a paracrine fashion extrasynaptic GABA receptors localized on distal neurons. The transient increase in intracellular calcium following the depolarizing action of GABA leads to inhibition of DNA synthesis and cell proliferation. Tonic GABA exerts also a chemotropic action on cell migration. Later on, when synapses are formed, GABA spilled out from neighboring synapses, acting mainly on extrasynaptic β<sub>2</sub>, β<sub>3</sub>, and γ-containing GABA<sub>A</sub> receptor subunits, provides the membrane depolarization necessary for principal cells to reach the window where intrinsic bursts are generated. These are instrumental in triggering calcium transients associated with network-driven giant depolarizing potentials which act as coincident detector signals to enhance synaptic efficacy at emerging GABAergic and glutamatergic synapses.

Keywords: development, hippocampus, tonic GABA<sub>A</sub> conductance, network activity, extrasynaptic GABA<sub>A</sub> receptor

y-aminobutyric acidergic (GABAergic) signaling plays a crucial role for processing and storage of information in the brain. By releasing γ-aminobutyric acid (GABA) into distinct targeted subcellular compartments, GABAergic interneurons regulate cells excitability and dictate the temporal dynamics of principal cells firing giving rise to networks oscillations thought to support distinct brain states and high cognitive functions (Klausberger and Somogyi, 2008). The action of GABA relies on the temporally and spatially regulated expression of GABA<sub>A</sub> receptors which mediate two distinct forms of inhibition: phasic and tonic (Semyanov et al., 2004; Caviedes et al., 2005; Farrant and Nusser, 2005). Phasic inhibition is mediated by local release of GABA from presynaptic vesicles (Cherubini, 2012). Once released, GABA binds to synaptic GABA<sub>A</sub> receptors facing presynaptic release sites and trigger fast inhibitory postsynaptic potentials (IPSPs), regulating point-to-point communication between neurons. In this case, synaptic GABA<sub>A</sub> receptors are exposed for a very short period of time to high concentrations of GABA. GABA diffuses throughout the neuropil before being taken up by selective plasma membrane transporters, which contribute to the clearance of the neurotransmitter and to shape synaptic currents (Cherubini and Conti, 2001). This transient inhibitory action is important for timing-based signaling, setting the temporal window for synaptic integration (Pouille and Scanziani, 2001) and synchronization of neuronal networks (Cobb et al., 1995).

Tonic inhibition is mediated by "ambient" GABA originated from spillover of the neurotransmitter escaping the synaptic cleft (Kaneda et al., 1995; Bricklely et al., 1996; Wall and Usowicz, 1997), from astrocytes via a non-vesicular calcium-independent process (Liu et al., 2000; Schousboe, 2003) or from the reversed transport (Artwell et al., 1993; Wu et al., 2001). In all these cases extrasynaptic GABA<sub>A</sub> receptors are persistently exposed to submicromolar concentrations of GABA present in the extracellular space. This requires extrasynaptic GABA<sub>A</sub> receptors with high affinity for GABA and relatively insensitive to desensitization. Selective plasma membrane transporters contribute to the clearance of GABA thus regulating its concentration in the extracellular space, in particular during massive release (Bragina et al., 2008). The resulting GABA-mediated tonic conductance is involved in regulating network excitability, cell firing and oscillatory behavior. In addition, the persistent increase in tonic conductance may affect the magnitude and duration of voltage responses to injected currents and increase the decrement of voltage with distance (Farrant and Nusser, 2003). Synaptic and extrasynaptic GABA<sub>A</sub> receptors are thought to belong to separate entities since they appear to be composed of...
ambient" GABA in sculpting neuronal circuits at early developmental stages. GABA depolarizes and excites targeted cells via an outflux of chloride. GABAergic signaling is unique in that the polarity of its action largely depends on the intracellular chloride concentration ([Cl\(^{-}\)]\(_{i}\)), leading in certain conditions to depolarization and even excitatory effects. Neuronal [Cl\(^{-}\)]\(_{i}\) is under the control of cation-chloride co-transporters (CCCs), intrinsic membrane proteins that transport Cl\(^{-}\) ions, together with Na\(^{+}\)and/or K\(^{+}\) ions, in an electro-neutral manner due to the stoichiometric coupling and directionality of translocated ions. The two main CCCs which control chloride concentration inside the cell are the Na–K–2Cl⁻ importer NKCC1 and the K–Cl exchanger KCC2. The low expression of KCC2 at birth leads to accumulation of chloride inside the cell and to the equilibrium potential for chloride (E\(_{Cl}^{\text{eq}}\)) positive with respect to the resting membrane potential (V\(_{m}\)). The progressive reduction in [Cl\(^{-}\)]\(_{i}\) with age, due to the developmentally up-regulated expression of KCC2 and the concomitant down-regulated expression of NKCC1 (Yamada et al., 2004; Dzhala et al., 2005), leads to relatively low [Cl\(^{-}\)]\(_{i}\) (E\(_{Cl}^{\text{eq}}\) close to V\(_{m}\)).

Interestingly, GABAergic signals can be shifted in polarity by activity. This unique form of plasticity involves changes in the expression of CCCs able to convert GABA responses from hyperpolarizing to depolarizing and vice versa (Fiuzzi and Woodin, 2007). While in adult hippocampal neurons, coincident detection of pre and postsynaptic signals alters the activity of KCC2 leading to changes in E\(_{Cl}^{\text{eq}}\) and in the strength of inhibition (Woodin et al., 2003), in immature cells modifies the expression of NKCC1 causing E\(_{Cl}^{\text{eq}}\) to shift toward more negative values (Balena and Woodin, 2008). Therefore, the dynamic regulation of intracellular chloride strictly depends on the ongoing activity generated by neurons and this may affect not only synaptic but also extrasynaptic GABA receptors.

Synapses start developing from birth following a well-defined sequence of events: GABAergic signals develop before glutamatergic ones whose operation correlates with the level of dendritic arborization (Tyzio et al., 1999; Ben-Ari et al., 2007). This occurs first in interneurons, and then in principal cells, indicating that GABAergic interneurons provide the early source of activity in otherwise silent networks (Gozlan and Ben-Ari, 2003).

Interestingly, prior to the formation of conventional synapses, GABA released from growth cones and astrocytes in a calcium- and soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE)-independent way, diffuses away to activate in a paracrine fashion extrasynaptic receptors (Figure 1; Demasque et al., 2002). These are expressed at very early stages of development by neuronal precursors and by neurons in several brain areas giving rise to a tonic conductance (Nguyen et al., 2001). The existence of such conductance can be estimated by the shift in the baseline current obtained by blocking GABA\(_{A}\) receptors with selective antagonists. The tonic current persists after treatment with calcium channel blockers or botulin toxin which draws SNAP-25 (synaptosomal-associated protein 25), a SNARE protein that prevents vesicular release. In addition, a tonic current is present in Munc18-1 deficient mice lacking vesicular release (Demasque et al., 2002). It has been proposed that a relatively poor clearance system (but see Sipilä et al., 2004; Safalina et al., 2006) enables GABA to accumulate in the extracellular space and reach a concentration sufficient to exert its depolarizing and excitatory effects on distal neurons making this form of intercellular communication very effective. In addition, since the main neuronal GABA transporter GAT-1 carries chloride along with GABA and sodium, the possibility that a high intracellular chloride may decrease the efficacy of GABA uptake cannot be excluded.
Ambient GABA mediates tonic conductance in development

**Cellot and Cherubini**

In the immature hippocampus, ambient GABA depolarizes targeted cells and contributes to generate network-driven GDPs. (A) Late in embryonic, early in postnatal life, prior to synapses formation, GABA released from growth cones diffuses in the extracellular space (light blue), binds to GABAA receptors located on the membrane of a neighboring cell (gray) and depolarizes the membrane through an outwardly directed flux of chloride. This results from the peculiar balance between the cation-chloride importer NKCC1 (large circle in green) and the poorly expressed cation-chloride extruder KCC2 (small circle in green), leading to accumulation of chloride inside the cell \([\text{Cl}^-]\). Tonic GABA current can be unveiled by applying picrotoxin (PTX, 100 μM; bar) as illustrated in the inset on the right. (B) After birth, during the first postnatal week, chemical GABAergic and glutamatergic synapses start appearing. GABA released by exocytosis from presynaptic vesicles (dark blue) acts on postsynaptic GABAA receptors located in face to presynaptic release sites and generate synaptic currents (inset on left). Once released GABA spills out (light blue) to activate extrasynaptic GABAA receptors. At this stage, glutamatergic synapses are also formed (yellow). The synergistic action of GABA and glutamate, both depolarizing and excitatory is crucial for GDPs generation. At this developmental stage, both phasic and tonic GABA are depolarizing since the cation-chloride extruder KCC2 expression is still poorly expressed on the membrane surface (small circle in green). (C) In adulthood, GABA acting on both synaptic and extrasynaptic GABAA receptors hyperpolarizes the membrane. This occurs because, due to the developmental up-regulation of the cation-chloride extruder KCC2 expression (large circle in green), \([\text{Cl}^-]\) is maintained at very low levels and when GABA opens GABAA receptor channels, causes a net flux of chloride inside the cell leading to membrane hyperpolarization and inhibition of cell firing. In addition, the concomitant down-regulation of the cation-chloride importer NKCC1 with age (small circle in green), contributes to maintain a very low \([\text{Cl}^-]\). (D) During the first postnatal week, depolarizing the membrane in the presence of AMPA/kainate/NMDA and GABAA receptor antagonists (DNQX, APV, and PTX), induces the appearance of intrinsic voltage-dependent bursts which are instrumental in triggering GDPs (see text). On the right a single burst recorded on an expanded time scale. The shadow (light blue) represents the membrane depolarization induced by tonic GABA. (E) Individual whole cell (neonatal CA3 pyramidal neuron; upper trace) and concomitant extracellular field recordings of spontaneous network-driven GDPs (bottom trace). On the right a single GDP and a concomitant field potential are represented on an expanded time scale. (F) Intrinsic bursts induced in adulthood by depolarizing the membrane in the presence of DNQX, APV, and PTX. Note the difference in burst duration between adults and neonates. (G) Whole cell (upper trace) and concomitant extracellular recordings (bottom trace) obtained from an adult CA3 pyramidal cell. On the right a single spike is shown on an expanded time scale. Note the absence of network-driven correlated activity such as GDPs (D and F modified from Safiulina et al., 2008; E modified from Ben-Ari et al., 2007).

**Ambient GABA regulates cell migration and synapses formation**

The construction of the cerebral cortex from a single sheet of neuroepithelium relies on a sequence of well-orchestrated developmental processes. Neurons must be generated in the correct number, migrate to the proper position, and form connections with neighboring cells. Of the many cell-intrinsic and -extrinsic signals involved in neocortical development, ambient GABA plays a central role in these processes (Wang and Kriegstein, 2009).
During corticogenesis, endogenous GABA present in the extracellular space, binds with high affinity (higher than in post-migratory neurons) to extrasynaptic GABAA receptors (relatively insensitive to desensitization), expressed on migrating neurons as well as on radial glia and causes a membrane depolarization and a transient increase in calcium via voltage-dependent calcium channels. This leads to inhibition of DNA synthesis and cell proliferation as assessed by the reduced number of progenitors incorporating BrDU (Los turco et al., 1995; Owens and Kriegstein, 2002). Furthermore, tonic GABA exerts a chemotactic action on cell migration as demonstrated by the observation that blocking GABAA receptors with bicuculline in organotypic hippocampal slices reduces the migration of neuroblasts (Mann et al., 2005). Ambient GABA may also regulate the speed of migration of young neurons in the rostral migratory stream (Roberts and Bordey, 2004) and provide a stop signal for ending migration (Behar et al., 2000). The effect of GABA on cell migration has been recently questioned. Using in vitro electroporation, Cancareda et al. (2007) have clearly demonstrated that the premature expression of the cation-chloride exchanger KCC2, which eliminates the depolarizing action of endogenous GABA in a subpopulation of newly born cortical neurons, does not alter their migration while at P4-P6 severely affects their morphological structure (neurons exhibit few short dendrites). However, as highlighted in a recent study (Inoue et al., 2012), the ectopic expression of KCC2 is functional only in the postnatal, but not in the embryonic brain. This is probably related to endogenous taurine, particularly abundant in the fetal brain that, via the with-no-lysine protein kinase 1 (WNK1) signaling pathway, exerts an inhibitory action on KCC2 activation.

Increasing evidence suggests that a tonic GABAA-mediated membrane depolarization provides the first excitatory drive necessary for promoting neurite outgrowth and synapse formation. Unlike glutamate in fact, GABA depolarizes and at the same time, by clamping the membrane potential close to ECl (in immature neurons about ~40 mV) exerts a shunting effect that would prevent an excessive calcium entry through voltage-dependent calcium channels with consequent excitotoxicity (Safinulina et al., 2010). GABA-mediated membrane depolarization has been shown to regulate the formation of glutamatergic synapses in the developing cortex in vitro, an effect that needs the contribution of NMDA receptors. This will allow a proper balance between excitation and inhibition, essential for the correct functioning of neuronal circuits (Wang and Kriegstein, 2008). Cortical neurons begin to express NMDA receptors during migration to the cortical plate. However, these receptors do not conduct at rest because they are blocked by magnesium. By facilitating the relief of the voltage-dependent magnesium block, GABA via its depolarizing action renders these receptors conductive. The systemic blockade of early GABA-mediated depolarization during a critical period between E17-P7 in mice with bumetanide, a selective NKCC1 inhibitor, leads to lasting disruption of AMPA receptors-mediated glutamatergic transmission in the adult cortex and to an excitatory/inhibitory imbalance (Wang and Kriegstein, 2011). Morphological analysis of bumetanide-treated mice revealed reduced spines density and dendritic arborization in cortical neurons (Wang and Kriegstein, 2011). This is in contrast with the data obtained by Pfeffer et al. (2009) from Nkcc1−/− mice. Compensatory mechanisms responsible for the slightly depolarizing action of GABA in the genetic model may account for this discrepancy (Sipila et al., 2009).

Interestingly, in the adult brain, GABA-mediated tonic excitation drives synaptic integration of newly generated neurons in pre-existing functional circuits suggesting that adult neurogenesis recapitulates the sequence of events occurring in immature cells at embryonic and early stages of postnatal development (Ge et al., 2006). As in postnatal development, conversion of GABA-induced depolarization into hyperpolarization in newborn dentate gyrus cells leads to significant defects in the formation of GABAergic and glutamatergic synapses as well as in dendritic arborization (Ge et al., 2006).

IMMEDIATELY AFTER BIRTH, A TONIC GABA-MEDIATED CONDUCTANCE CONTRIBUTES TO THE TRIGGERING OF NETWORK-DRIVEN GDPs IN THE HIPPOCAMPUS

In the neonatal hippocampus a GABAa receptor-mediated tonic conductance has been well-characterized in both CA1 (Demarque et al., 2002; Marchi onni et al., 2007) and CA3 regions (Marchionni et al., 2007; Sipila et al., 2007). In these areas, principal cells exhibit a sustained tonic conductance (larger in the CA3 region; Marchionni et al., 2007) which plays a crucial role in enhancing cell excitability and neuronal firing, thus contributing to GDPs generation (Sipila et al., 2005, 2009).

Tonic GABAa-mediated currents are more pronounced in neonates than in adults (Figure 1; Stell and Mody, 2002; Semyanov et al., 2003; Stell et al., 2003; Caraiscos et al., 2004). This has been attributed to the relatively poor GABA clearance caused by the low expression of GABA transporters. However, at least GAT1 has been found to be present and functional from birth as shown by the ability of NO-711, a selective GAT-1 inhibitor, to enhance the decay kinetics of GABAa-mediated synaptic currents (Safinulina et al., 2006) and the duration of GDPs (Sipila et al., 2004), indicating that GABA uptake controls GABAergic transmission and limits the action of GABA on GPDs. It is worth mentioning that while NO-711 affects the shape of synaptic currents (Safinulina et al., 2006), it only slightly alters tonic currents (Marchionni et al., 2007; Sipila et al., 2007), suggesting that GAT1, due to its preferential localization on axon terminals (Minelli et al., 1995), is more efficient at removing GABA from synapses than from the extracellular space.

In both CA1 and CA3 principal cells, “ambient” GABA may originate at least in part from spillover of the neurotransmitter from neighboring synapses during activity as shown by the reduction in the tonic current by tetrodotoxin (TTX) that blocks sodium currents and propagated action potentials (Marchionni et al., 2007; but see Sipila et al., 2007 for the CA3 region). Interestingly, in contrast with adult guinea pigs (Semyanov et al., 2003) or rats (Marchionni et al., 2007), in neonatal animals stratum radiatum GABAergic interneurons fail to exhibit any sustained background conductance. This may be due either to a more efficient uptake system, able to maintain extracellular GABA at very low levels, or to a low expression of extrasynaptic receptors able to sense GABA. While the first possibility is unlikely since NO-711 did not modify the holding current, the second one remains
to be demonstrated. Whether other transporters, different from GAT-1 are actively involved in removing GABA from GABAAergic interneurons cannot be excluded.

In immature CA1 and CA3 principal cells, the selective use of GABA$_A$ receptor agonists and antagonists has unveiled the presence of various extrasynaptic receptor subunits. These include the a1, a3 (Sipilä et al., 2007), a5, f2, f3, and y2 (Marchionni et al., 2007). While a5 GABA$_A$ receptor subunits are highly expressed in the neonatal hippocampus (Laurie et al., 1992; Dideleon et al., 2000), b-subunits are relatively scarce (Dideleon et al., 2000) and this may explain why allosteric (d)reducencies of extrasynaptic GABAA receptors leading to an increased firing in pyramidal cells but not in interneurons. This may enhance glutamate release from principal cells.

Giant depolarizing potentials, which represent a primordial form of synchonym between neurons, are generated by the synergistic action of glutamate and GABA, both of which are depolarizing and excitatory (Figure 1; Cherubini et al., 1991; Ben-Ari, 2002). In analogy with the synchronized activity generated in the disinhibited hippocampus (De la Prida et al., 2006), GDPs emerge when a sufficient number of cells fire and the excitability of the network attains a certain threshold within a restricted period of time.

Although the entire hippocampal network possesses the capacity to generate GDPs, the CA3 area is centrally involved because:

(i) in this area GABAAergic interneurons with large azonal arborizations operate as functional hubs to synchronize large ensembles of cells (Bonifazi et al., 2009; Picardo et al., 2011; Allene et al., 2012);
(ii) principal cells are connected by extensive glutamatergic recurrent collaterals; (iii) principal cells give rise to intrinsic bursts that drive other neurons to fire (Sipilä et al., 2003; Safinina et al., 2008).

In this context, GABA$_A$-mediated tonic inhibition provides the membrane depolarization needed for principal cells to reach the window where intrinsic bursts are generated (Figure 1; Sipilä et al., 2007; Ben-Ari et al., 2012).

Since GDPs are instrumental for enhancing synaptic efficacy at emerging glutamatergic and GABAAergic synapses and for converting silent synapses into conductive ones (Kasyanov et al., 2004; Mohajerani et al., 2007), we can speculate that, immediately after birth, ambient GABA exerts a crucial role in synaptic wiring.

Whether changes in synaptic efficacy are associated with structural modifications necessary to rewire local neuronal circuits remain to be demonstrated.

Early in Postnatal Development, Tonic GABAAergic Currents Differently Regulate Cell Excitability in Various Brain Regions

Dentate Gyrus Granule Cells

A powerful tonic GABAergic signaling has been described in granule cells of the dentate gyrus already at P3 (Holter et al., 2010). Using subunit-specific pharmacological modulators, it has been demonstrated that, as in adults (Glykys et al., 2008), this conductance is mediated by a5 and d subunits containing extrasynaptic GABA$_A$ receptors. The relative contribution of receptors containing a5-subunits decreases with age, while that of receptors containing b-subunits increases, indicating that as principal cells the expression of d subunits is developmentally regulated. The increased expression of d subunits parallels that of tonic conductance that reaches its maximum during the second postnatal week and then declines (Holter et al., 2010). Surprisingly, unlike immature CA1 and CA3 principal cells (Marchionni et al., 2007), this conductance exerts mainly an inhibitory effect via a shunting inhibition. The inhibitory effect would prevent the occurrence of neonatal seizures (Holter et al., 2007).

Neocortical Neurons

A GABA$_A$-mediated tonic conductance has been detected in layer V pyramidal cells of the somatosensory cortex of newborn animals (Sebe et al., 2010). This conductance, which is mainly mediated by a5 and d subunits containing extrasynaptic GABA$_A$ receptors, is very large immediately after birth and then decreases dramatically during the second postnatal week. The developmental change appears to be related to the enhanced clearance of GABA from the extracellular space (due to the increased activity of GABA transporters) and to the reduced expression of b subunits (the a5 subunits are expressed also in adults; Yamada et al., 2007). In cell-attach recordings GABA has been found to exert opposite effects on immature cell excitability, decreasing or increasing cell firing in accord with the value of $E_C$ relative to resting membrane potential (Sebe et al., 2010).

Unlike the somatosensory cortex, in the visual cortex tonic GABAAergic currents increase with age (Jang et al., 2010). This may be related to the role of GABA in shaping neuronal circuits during a well-defined period of early postnatal development called critical period (Sale et al., 2010). In this period, the experience-dependent maturation of the visual acuity closely relies on the development of the intracortical GABAergic inhibition. Dark rearing, which causes a permanent loss of visual acuity reduces also GABAergic signaling mainly from parvalbumin positive interneurons (Hensch, 2005).

Cerebellar Granule Cells

The cerebellum is the first brain structure where a persistent background GABA$_A$-mediated conductance has been characterized (Kaneda et al., 1995). This clearly exhibits a developmental profile, increasing progressively after P7. This may reflect the increasing number of GABAergic terminals within the glomerulus, a structure that limiting GABA diffusion may render the action of ambient GABA more efficient (Birchley et al., 1996; Wall and Usowicz, 1997). In addition, while at early developmental stages tonic GABA exerts a depolarizing and excitatory action, later it...
Table 1 | Tonic GABAergic currents in various brain regions during early development.

| Structure                        | Age                        | GABA<sub>A</sub> R subunits | Direction of GABA action | Reference                   |
|----------------------------------|----------------------------|------------------------------|--------------------------|-----------------------------|
| CA1 region of hippocampus        | Late embryonic, early postnatal life (1st week) | α5, γ2                     | Depolarizing             | Demarque et al. (2002)      |
| CA3 region of hippocampus        | 1st postnatal week         | α1, α3, α5, β3, γ2          | Depolarizing             | Marchionni et al. (2007)    |
| Dentate gyrus                    | Newborn cells in adulthood | Unknown                     | Depolarizing             | Gop et al. (2006)           |
| Somatosensory cortex             | 1st postnatal week         | α5, k                       | Depolarizing             | Sebe et al. (2010)          |
| Cerebellum                       | 1st postnatal week         | Unknown                     | Depolarizing             | Brickley et al. (1996)      |
| Thalamus (ventrobasal)           | 1st postnatal week         | α4, k                       | Hyperpolarizing          | Wall and Usowicz (1997)     |
| Striatum                         | 2nd postnatal week         | α5, k                       | Unknown                  | Ade et al. (2008)           |
|                                  | 4th postnatal week         | α5, k, β3                  | Unknown                  | Janssen et al. (2011)       |

The inhibitory GABA<sub>A</sub>-mediated conductance is larger in MSNs expressing dopamine D2 receptors, which project to the globus pallidus respect to those expressing dopamine D1 receptors which project to the substantia nigra pars reticulata (Ade et al., 2008; Santhakumar et al., 2010). While the first are supposed to inhibit movements, the latter to facilitate them (Gerfen et al., 1990). The greater expression of α5 subunit containing GABA<sub>A</sub> receptors in D2- respect to D1-positive MSNs may account for the observed effects (Ade et al., 2008). In addition, using conditional b3 subunit knock-out mice Janssen et al. (2011) have demonstrated that receptors containing the b3 subunit also contribute to tonic inhibition in D2-positive neurons. These data are in agreement with previous mRNA expression studies showing that young but not adult striatal tissue primarily expresses α2, α5, β3, and γ GABA<sub>A</sub> receptor subunits (Laurie et al., 1992). Differences in tonic currents between D1- and D2-positive cells observed in juvenile animals may be crucial for regulating MSNs excitability and motor output.

CONCLUSION

From the data reviewed here it is clear that a tonic GABA<sub>A</sub>-mediated conductance plays an important role in brain development. This conductance is developmentally regulated and differs in various brain regions (see Table 1). While in some areas (i.e., hippocampus and somatosensory cortex) it diminishes with maturation, in others (i.e., thalamus, visual cortex, striatum, and cerebellum) it increases. This depends on several factors including stage of cell maturity, geometry of the synapses, interneuronal firing, neurotransmitter diffusion, expression and distribution of extrasynaptic GABA<sub>A</sub> receptors, and/or GABA transporters. In addition, according to the direction of GABA signaling, accumulation of the neurotransmitter in the extracellular space may differently affect network excitability.

In spite progress in the field much remains to be known on how early activity or experience regulates receptors trafficking and

exerts an inhibitory effect via shunting inhibition (E<sub>G</sub> is close to the resting membrane potential; Brickley et al., 1996).

THALAMIC RELAY NEURONS

A similar sequence of events takes place in thalamic ventrobasal relay neurons, where GABA<sub>A</sub>-mediated tonic current is developmentally regulated. This conductance can be detected from the first week of postnatal life (Bolldini et al., 2005). It progressively increases with age. This effect is not related to changes in the expression of GABA transporters, since bicuculline-induced shift in baseline current does not change by blocking GABA uptake with selective inhibitors (Pedem et al., 2008). The ontogenetic up-regulation of the tonic conductance depends on marked changes in subunit composition of extrasynaptic GABA<sub>A</sub> receptors with increased expression of α4 and β3 subunits, as assessed with electrophysiology and immunohistochemistry. While in adult neurons, the persistent activation of extrasynaptic receptors by ambient GABA leads to a membrane hyperpolarization needed to promote low threshold burst firing (Cope et al., 2005), in neonates its function is not clear since it depends on the hyperpolarizing or depolarizing action of GABA.

STRIATAL MEDIUM SPINY NEURONS

In GABAergic medium spiny striatal output neurons (MSNs), a tonic GABA<sub>A</sub>-mediated conductance starts appearing toward the end of the second postnatal week (Ade et al., 2008; Kirmse et al., 2008). Before P14, it can be detected only in the presence of GSK-3 inhibitors suggesting that this transporter operates in the extracellular space. An increase in synaptic GABA release, due to an up-regulation of the glutamatergic drive to MSNs may account for the persistent activation of extrasynaptic GABA<sub>A</sub> receptors with age. Ambient GABA originates mainly from action potential-dependent synaptic release, since TTX reduces the tonic GABA<sub>A</sub>-mediated conductance in development. In GABAergic medium spiny striatal output neurons (MSNs), the depolarizing action of GABA leads to a membrane hyperpolarization needed to promote low threshold burst firing (Cope et al., 2005), in neonates its function is not clear since it depends on the hyperpolarizing or depolarizing action of GABA.
exchanges between synaptic and extrasynaptic pools. While the interaction of scaffold proteins with synaptic receptors has been at least in part elucidated that related to extrasynaptic receptors is still poorly understood. Furthermore, it would be of interest to know how selectively silencing in embryonic or early postnatal life a particular extrasynaptic GABA receptor subunit may alter the computational properties of neuronal circuits and synaptic gene expression. This would help to better understand the role of GABA as a developmental signal and its implications in neurodevelopmental disorders.

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**CONTRIBUTION**

Giada Cellot and Enrico Cherubini wrote the paper.

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Cellot and Cherubini

GABA-mediated tonic conductance in development
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August 2013 | Volume 7 | Article 126 | 8

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Cellot and Cherubini

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