Neuroscience and Behavioral Physiology, Vol. 43, No. 5, June, 2013

Excitatory Effects of GABA during Ontogeny

G. R. Valeeva,[1,2,3] R. N. Khazipov,[1,3] and E. E. Nikolsky[2]

Translated from Rossiiskii Fiziologicheskii Zhurnal imeni I. M. Sechenova, Vol. 97, No. 11, pp. 1179–1186, November, 2011. Original article submitted March 11, 2011. Revised version received June 29, 2011.

Gamma-aminobutyric acid (GABA) is the main inhibitory transmitter in the central nervous system. However, at the early stages of development, GABA has excitatory influences on immature neurons. This review presents contemporary views on the mechanisms of GABAergic excitation and the physiological role of excitatory GABA in generating patterns of network activity in the developing brain.

Keywords: GABA, interneurons, cerebral cortex, inhibition, excitation, synapse, development.

Gamma-aminobutyric acid (GABA) is the main inhibitory transmitter in the central nervous system of animals. GABA is released by a special class of neuron – interneurons – which account for about 10–20% of all neurons. Release of GABA in inhibitory synapses leads to activation of chloride ion-permeable $GABA_A$ receptors on the postsynaptic membrane. This induces a transient increase in cell membrane permeability (which underlies shunting inhibition) and hyperpolarization (which leads to inhibition due to membrane potential being moved away from the action potential (AP) threshold). A similar mechanism underlies glycine-mediated inhibition. As each interneuron forms connections with thousands of target cells, synchronous inhibition is an important mechanism for synchronizing neuron populations and generating physiological patterns of network activity, such as, for example, oscillations in the gamma frequency range or sleep spindles [1, 13, 27]. Impairments to GABAergic inhibition induce imbalance between excitation and inhibition, resulting in changes to the activity of neural networks. Changes in this balance in the cerebral cortex are often accompanied by epileptic discharges. Thus, a significant number of congenital and acquired epileptic syndromes results from impairment of the operation of the GABAergic inhibitory system. It is interesting that in a number of epileptic syndromes, as well as in post-trauma situations or hypoxia, there are changes in the polarity of GABAergic responses, from hyperpolarizing to depolarizing [7, 18, 44]. This occurs as a result of an increase in the intracellular chloride ion concentration and a positive shift in the reversion potential of GABA-mediated responses (E-GABA). Thus, an important component of GABAergic inhibition, associated with hyperpolarization, is lost. In addition, in the presence of significant increases in intracellular chloride ion concentrations, the depolarizing action of GABA can lead to cell excitation, i.e., there is a qualitative change in the action of GABA from inhibitory to excitatory.

While the excitatory action of GABA in the CNS in adult mammals occurs mainly in pathological states, excitation resulting from activation of $GABA_A$ receptors is the rule at the early stages of ontogeny [2, 4, 25]. As in pathological states, the excitatory action of GABA in ontogeny is explained in terms of an increase in chloride ion content in immature neurons, such that E-GABA is more positive than the resting potential (RP). Depolarization induced by activation of $GABA_A$ receptors rarely reaches the action potential (AP) generation threshold. However, GABA-mediated depolarization leads to activation of voltage-dependent calcium and sodium channels and reverses magnesium blockade of NMDA glutamate receptors, which brings the membrane potential of the postsynaptic cell towards the threshold and induces AP. The excitatory action of GABA is therefore probably the result of integration of GABA, glutamate, and various voltage-dependent conductivities.

[1] Mediterranean Neurobiology Institute, Marseilles, France; e-mail: roustem.khazipov@inserm.fr.
[2] Kazan Institute of Biochemistry and Biophysics, Russian Academy of Sciences, Kazan, Russia.
[3] Laboratory of Neurobiology, Kazan Federal University, Kazan, Russia.

0097-0549/13/4305-0656

Evidence for the depolarizing and excitatory actions of GABA on immature neurons has been obtained using very diverse experimental approaches, including electrophysiological and optical methods. The first experiments suggesting an excitatory action for GABA in neonatal rats used intracellular recording methods [3]. It should, however, be noted that although these data were subsequently confirmed using less invasive methods, the initial results obtained using intracellular recordings and patch clamping in the whole-cell configuration included a significant number of artifacts. These were primarily associated with cell dialysis leading to changes in the intracellular chloride ion concentration, as well as shunting of the cell membrane via electrical conductivity formed at the site of electrode insertion (or close contact (gigaseal) of the pipette with the membrane in conditions of patch clamp recording), which leads to depolarization of the RP [42]. Contemporary electrophysiological methods which have demonstrated the depolarizing and excitatory actions of GABA in immature rodents are:

1) *perforated patch*, where electrical access to the cell is obtained by artificial insertion of cation channels, for example, gramicidin, into the cell membrane which does not produce any change in the intracellular chloride ion concentration [12]; a variant of this method consists of cell-attached recording with electrical access to the cell via potassium channels [21];

2) the "*cell-attached*" patch-clamp configuration, in which the current-voltage characteristics and reversion potential of currents via single GABA-activated channels provides direct evaluation of the driving force acting on chloride ions [37, 41], while the reversion potential of currents via NMDA or potassium channels can be used to assess the membrane potential [14, 41, 42, 45]. As with extracellular recording of AP (a quite old and simple non-invasive recording method), this method of measuring AP frequency can demonstrate that GABA has excitatory or inhibition actions;

3) *optical methods*, including measurements of intracellular calcium ion concentrations (increased on cell depolarization due to activation of voltage-dependent calcium channels) using calcium-sensitive fluorescent probes [8]; measurement of intracellular chloride ion concentrations using ion-selective electrodes, the fluorescent chloride indicator MQAE [26], and chloride ion-sensitive fluorescent proteins such as clomeleon [10, 23] and their analogs [47]; measurement of membrane potential using fluorescent voltage-sensitive probes inserted into the cell membrane.

The excitatory action of GABA on neurons during development has been demonstrated in virtually all nervous system structures in various species of animals. This suggest that the excitatory action of GABA is a universal property of the developing brain. This raises the question of what mechanism underlies the depolarizing and excitatory actions of GABA on the immature neurons, what the role of the excitatory action of GABA in the developing nervous system is, and how this effect of GABA affects the generation of synchronous activity in neural networks. Below, we will try to answer these questions and consider a number of questions which will in all probability be the subject of future studies.

Increased Chloride Ion Concentrations as a Mechanism of the Depolarizing Actions of GABA in Immature Neurons. As noted above, the main mechanism of the depolarizing (and, thus, excitatory) effect of GABA is an increase in the chloride ion concentration in immature neurons, which is associated with the expression of specific transporters carrying chloride ions across the cell membrane, the set of which changes with age [17, 36, 48] (for reviews of intracellular chloride ion contents see [9, 29, 31, 33]. In situ hybridization and PCR studies of individual cells have shown that the cotransporter NKCC-1, which accumulates intracellular chloride, starts to be expressed in rat brain neurons during the embryonic period and reaches an expression peak during the second week after birth [11]. This is followed by a gradual decrease in NKCC-1 expression and, starting from the fourth week after birth, the expression level becomes comparable with that in adult animals. These changes also occur in humans, where the NKCC-1 expression peak occurs between weeks 30 and 40 of fetal development. At the same time, expression of the KCC2 transporter, which expels chloride from cells, starts from the second week after birth and reaches the adult level by the beginning of the fourth week of postnatal development [36]. Thus, increases in the expression of the NKCC-1, which "pumps" chloride ions into cells, and the virtual absence of the chloride ion-eliminating KCC2 facilitates the accumulation of intracellular chloride, leading to a significant depolarizing electromotive force driving the chloride ion efflux current on opening of GABA-activated channels. The fundamental roles of NKCC-1 in maintaining high intracellular chloride levels and the depolarizing activity of GABA are also supported by the observation that bumetanide, a selective (at concentrations of up to 10 μM) NKCC-1 blocker (which underlies its therapeutic use as a diuretic substance inhibiting NKCC-1 expressed in the kidneys), suppresses the depolarizing and excitatory actions of GABA on immature neurons [41, 43, 48].

Role of the Depolarizing Effect of GABA in Generating Early Patterns of Activity in Developing Neural Networks. The depolarizing and excitatory actions of GABA have important consequences determining the nature of activity in developing neural networks. As demonstrated in studies on hippocampal slices from neonatal rat pups, the age period during which an excitatory action of GABA is typical (the first two weeks after birth) coincides with the period during which a specific pattern of network activity exists, i.e., so-called giant depolarizing potentials (GDP) [3, 19]. GDP are network discharges reminiscent of interictal epileptiform discharges during which activation

of both pyramidal cells and interneurons occurs in a time window constituting hundreds of milliseconds; cell activation during GDP results from the synergistic excitatory actions of glutamate and GABA [5, 20, 25]. A typical feature of GDP is that excitation does not arise simultaneously in all areas in hippocampal slices but, rather, is reminiscent of a wave originating in field CA3 and then propagating across the whole slice [28]. GDP is most wavelike in nature in the intact hippocampus in vitro: in these preparations, GDP usually originate in the septal part of the hippocampus and appear with delays of up to 0.5 sec in the temporal pole of the intact hippocampal preparation [24]. The reason for such slow conduction of GDP is the long-lasting and variable delay in conduction of excitation in GABAergic synapses [43].

Recent studies have shown that the chloride ion concentration in postsynaptic cells determines the time characteristics of excitation in the developing GABAergic synapse: the greater the intracellular chloride concentration, the shorter the delay in AP origination in the postsynaptic neuron [43]. At physiological intracellular chloride concentrations, the transmission of excitation in GABAergic synapses is quite slow – AP arise with long (up to hundreds of milliseconds) and very variable delays. This is associated with the fact that depolarization evoked by activation of $GABA_A$ receptors rarely reaches the threshold level, AP generation requiring additional activation of subthreshold non-inactivating sodium conductivity [38, 39, 43]. It is interesting to note that decreases in intracellular chloride contents due to partial blockade of NKCC-1 using low bumetanide concentrations induce significant lengthening of delays in excitatory GABA synapses, which are accompanied by powerful slowing of the rate of GDP conduction. This blockade of NKCC-1 completely suppresses the excitatory action of GABA and leads to the disappearance of GDP. GDP-like patterns of activity whose generation also involves excitatory GABA have been described in various structures of the developing nervous system.

Role of the Depolarizing Effect of GABA in Neuron Development and the Formation of Synaptic Connections. While the role of the excitatory effect of GABA in generating GDP has been well studied, significantly less investigated is the question of the physiological role of GDP and the excitatory influence of GABA itself. It should be noted that at different stages of ontogeny, synapses between neurons are only just forming and their numbers are very small. In conditions of small numbers of contacts between cells, synchronization of neuronal ensembles can occur as a result of the excitatory action of GABA. Thus, the role of the excitatory effect of GABA may consist of providing the minimal level of excitation required for synchronization of neuronal ensembles. As GDP support the synchronous activation of pre- and postsynaptic neurons, as well as NMDA receptors, which is one of the Hebbian mechanisms of synaptic plasticity, it has been suggested that this may lead to long-term changes in the efficiency of synaptic transmission and thus take part in forming neural networks [30]. Another consequence of GDP consists of synchronous oscillations of intracellular calcium – a factor of importance for development [15, 16, 25] – and the secretion of growth factors such as BDNF [22]. A further result of increased intracellular chloride concentrations may be an increase in intracellular osmotic pressure, which should create favorable conditions for increasing cell membrane area and neuron growth, by analogy with the mechanisms acting on neuron regeneration after axotomy (where NKCC-1 activation and acquisition of the GABA-depolarized phenotype are also seen [32]. A number of recent studies using chronic exposure to bumetanide during embryonic and neonatal development in rat pups have shown that blockade of NKCC-1 does in fact lead to significant retardation of cell growth and differentiation, as well as slowing of synaptogenesis [46]. Similar observations were made in conditions of artificial expression of KCC2 in embryonic tissues [34].

Regulation of the Excitatory Effect of GABA. Apart from the developmental change in the action of GABA from excitatory to inhibitory, which is associated with changes in the expression of chloride transporters, there are humoral mechanisms regulating the excitatory action of GABA on immature neurons. The clearest example of this modulation is the temporary switching in the action of GABA from excitatory to inhibitory in the rat fetus brain induced by oxytocin during birth [41]. Oxytocin is a neurohypophyseal hormone which induces uterine contraction during birth. However, it also crosses the placental and blood-brain barriers of the fetus and induces decreases in intracellular chloride ion concentrations, which are accompanied by a shift in E_{GABA} towards more negative values and loss of the excitatory action of GABA on fetal cortical neurons. The effects of oxytocin on GABAergic transmission develop very quickly (over a number of minutes) and disappear just as quickly when oxytocin is removed. In terms of its effects, the actions of oxytocin are reminiscent of those of bumetanide, so it has been suggested that NKCC-1 is the target for oxytocin. What might the physiological role of elimination of the excitatory effect of GABA during birth be? Deprivation of the excitatory effect of GABA was found to lead to a significant increase in the resistance of neurons to ischemia, which is always a serious threat to the fetal brain during birth. In addition, by modulating GABAergic transmission in nociceptive neurons, oxytocin has an analgesic action, which appears to prevent pain induced by compression of the fetus in the birth canal during parturition.

Another example of humoral modulation is provided by modulation of the excitatory action of glycine (which activates chloride channels similar to GABA-activated channels) by serotonin in developing *Danio rerio* fish [6]. During the formation of locomotor neural networks, endogenous serotonin increases motor activity, decreasing

the duration of inactive periods but not affecting movement periods. Serotonin receptor blockers increased the duration of inactivity periods, which was associated with decreases in the depolarizing effects of glycine on spinal neurons, and these effects, both at the level of motor behavior and at the level of the depolarizing action of glycine, were reproduced by bumetanide.

Oxytocin and serotonin are evidently only some of the several thus far identified modulators of the excitatory effects of GABA and glycine; it is probable that the range of these modulators is very extensive. It will be very interesting if future studies establish a complete list of these modulators and their relationships with behavioral functions and with various biological cycles in the developing organism. Completion of this task will inevitably involve in vivo recording of responses induced by GABA in the intact animal. It should be noted that all data on the depolarizing and excitatory actions of GABA on immature neurons have been obtained in brain slices or isolated cells and that how GABA acts on immature neurons in vivo remains unknown. It would seem that it is impossible to produce a complete in vitro reproduction of the in vivo situation. This applies both to the composition of the extracellular fluid, including electrolytes, metabolites, and humoral factors, and to the delivery of oxygen and removal of carbon dioxide, as well as problems associated with the trauma imposed on neurons as slices are cut or cells are isolated for cultures, etc. Thus, it has recently been suggested that the excitatory action of GABA seen in brain slices is an artifact resulting from incorrect composition of the artificial cerebrospinal fluid used for perfusion of slices, i.e., the absence of ketone bodies [35]. Ketone bodies (hydroxybutyrate, acetoacetate, and acetone), as well as lactate and pyruvate, along with glucose, are important metabolites, though the only energy substrate in the traditionally used extracellular solution is glucose. Addition of hydroxybutyrate to the traditional solution led to complete elimination of the depolarizing and excitatory actions of GABA on immature neurons in hippocampal slices from neonatal rats, and also blocked GDP. Unfortunately, attempts to repeat these results in a number of laboratories were unsuccessful [21, 40]. In addition, pyruvate at millimolar concentrations was found to suppress GDP, though these concentrations were an order of magnitude greater than the physiological, while physiological concentrations of pyruvate had no significant influence on E-GABA or GDP. Although humoral regulation of E-GABA is of some interest, the question of how GABA acts in the developing brain in vivo remains key, and this question is currently one of the most relevant in this field. There is also clear interest in the question of how data on the excitatory effects of GABA obtained mainly in experiments on laboratory animals – rats and mice – relate to humans. Although the expression profile of chloride transporters suggests a depolarizing action of GABA in the human fetus at the intrauterine stages of development [11], there is as yet no experimental evidence for the existence of this phenomenon in humans. The answer to this question is important for understanding how the fetal brain functions in utero and for developing methods for treating nervous system diseases involving developmental changes in the actions of GABA.

REFERENCES

1. M. Bartos, I. Vida, and P. S. Jonas, "Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks," *Nat. Rev. Neurosci.*, **8**, No. 1, 45–56 (2007).
2. Y. Ben Ari, J. L. Gaiarsa, R. Tyzio, and R. Khazipov, "A pioneer transmitter that excites immature neurons and generates primitive oscillations," *Physiol. Rev.*, **87**, 1215–1284 (2007).
3. Y. Ben-Ari, E. Cherubini, R. Corradetti, and J.-L. Gaiarsa, "Giant synaptic potentials in immature rat CA3 hippocampal neurones," *J. Physiol. (London)*, **416**, 303–325 (1978).
4. Y. Ben-Ari, R. Khazipov, X. Leinekugel, et al., "$GABA_A$, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois,'" *Trends Neurosci.*, **20**, No. 11, 523–529 (1997).
5. S. Bolea, E. Avignone, N. Berretta, et al., "Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices," *J. Neurophysiol.*, **81**, No. 5, 2095–2102 (1999).
6. R. Brustein and P. Drapeau, "Serotoninergic modulation of chloride homeostasis during maturation of the locomotor network in zebrafish," *J. Neurosci.*, **25**, 10,606–10,616 (2005).
7. I. Cohen, V. Navarro, S. Clemenceau, et al., "On the origin of interictal activity in human temporal lobe epilepsy in vitro," *Science*, **298**, No. 5597, 1418–1421 (2002).
8. J. A. Connor, H. Y. Tseng, and P. E. Hockberger, "Depolarization- and transmitter-induced changes in intracellular Ca^{2+} of rat cerebellar granular cells in explant cultures," *J. Neurosci.*, **7**, 1384–1400 (1987).
9. E. Delpire, "Cation-chloride cotransporters in neuronal communication," *News Physiol. Sci.*, **15**, No. 6, 309–312 (2000).
10. J. Duebel, S. Haverkamp, W. Schleich, et al., "Two-photon imaging reveals somatodendritic chloride gradient in retinal On-type bipolar cells expressing the biosensor clomeleon," *Neuron*, **49**, No. 1, 81–94 (2006).
11. V. I. Dzhala, D. M. Talos, D. A. Sdrulla, et al., "NKCC1 transporter facilitates seizures in the developing brain," *Nat. Med.*, **11**, No. 11, 1205–1213 (2005).
12. S. Ebihara, K. Shirato, N. Harata, and N. Akaike, "Gramicidin-perforated patch recording: GABA response in mammalian neurones with intact intracellular chloride," *J. Physiol. (London)*, **484**, No. 1, 77–86 (1995).
13. T. Freund and G. Buzsaki, "Interneurones of the hippocampus," *Hippocampus*, **6**, 345–470 (1996).
14. D. Fricker, J. A. Verheugen, and R. Miles, "Cell-attached measurements of the firing threshold of rat hippocampal neurones," *J. Physiol.*, **517**, No. 3, 791–804 (1999).
15. O. Garaschuk, E. Hanse, and A. Konnerth, "Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampal," *J. Physiol.*, **507**, No. 1, 219–236 (1998).
16. O. Garaschuk, J. Linn, J. Eilers, and J. Konnerth, "Large-scale oscillatory calcium waves in the immature cortex," *Nature Neurosci.*, **3**, No. 5, 452–459 (2000).
17. Y. Kazaku, N. Akaike, S. Komiyama, and J. Nabekura, "Regulation of intracellular chloride by cotransporters in developing lateral superior olive neurons," *J. Neurosci.*, **19**, No. 8, 2843–2851 (1999).

18. I. Khalilov, G. L. Holmes, and Y. Ben Ari, "In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures," *Nat. Neurosci.*, **6**, No. 10, 1079–1085 (2003).
19. R. Khazipov, I. Khalilov, R. Tyzio, et al., "Developmental changes in GABAergic actions and seizure susceptibility in the rat hippocampus," *Eur. J. Neurosci.*, **19**, No. 3, 590–600 (2004).
20. R. Khazipov, X. Leinekugel, I. Khalilov, et al., "Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices," *J. Physiol. (London)*, **498**, No. 3, 763–772 (1997).
21. K. Kirmse, O. W. Witte, and K. Holthoff, "GABA depolarizes immature neocortical neurons in the presence of the ketone body ss-hydroxybutyrate," *J. Neurosci.*, **30**, No. 47, 16,002–16,007 (2010).
22. N. Kuczewski, C. Porcher, N. Ferrand, et al., "Backpropagating action potentials trigger dendritic release of BDNF during spontaneous network activity," *J. Neurosci.*, **28**, 7013–7023 (2008).
23. T. Kuner and G. J. Augustine, "A genetically encoded ratiometric indicator for chloride: capturing chloride transients in cultured hippocampal neurons," *Neuron*, **27**, No. 3, 447–459 (2000).
24. X. Leinekugel, I. Khalilov, Y. Ben Ari, and R. Khazipov, "Giant depolarizing potentials: the septal pole of the hippocampus paces the activity of the developing intact septohippocampal complex in vitro," *J. Neurosci.*, **18**, No. 16, 6349–6357 (1998).
25. X. Leinekugel, I. Medina, K. Khalilov, et al., "Ca^{2+} oscillations mediated by the synergistic excitatory actions of $GABA_A$ and NMDA receptors in the neonatal hippocampus," *Neuron*, **18**, No. 2, 243–255 (1997).
26. N. Marandi, A. Konnerth, and O. Garaschuk, "Two-photon imaging in neurons of brain slices," *Pflügers Arch.*, **445**, 357–365 (2002).
27. D. A. McCormick and T. Bal, "Sleep and arousal: thalamocortical mechanisms," *Annu. Rev. Neurosci.*, **20**, 185–215 (1997).
28. P. Menendez, S. Bolea, and J. V. Sanchez-Andres, "Origin of the synchronized network activity in the rabbit developing hippocampus," *Eur. J. Neurosci.*, **10**, No. 3, 899–906 (1998).
29. A. Mercado, D. B. Mount, and G. Gamba, "Electroneutral cation-chloride cotransporters in the central nervous system," *Neurochem. Res.*, **29**, No. 1, 17–25 (2004).
30. M. H. Mohajerani and E. Cherubini, "Role of giant depolarizing potentials shaping synaptic currents in the developing hippocampus," *Crit. Rev. Neurobiol.*, **18**, 13–23 (2006).
31. J. A. Payne, C. Rivera, J. Voipio, and K. Kaila, "Cation-chloride co-transporters in neuronal communication, development and trauma," *Trends Neurosci.*, **26**, No. 4, 199–206 (2003).
32. S. Pieraut, V. Laurent-Matha, C. Sar, et al., "NKCC1 phosphorylation stimulates neurite growth of injured adult sensory neurons," *J. Neurosci.*, **27**, No. 25, 6751–6759 (2007).
33. M. D. Plotkin, E. Y. Snyder, S. C. Hebert, and E. Delpire, "Expression of the Na-K-2Cl cotransporter is developmentally regulated in postnatal rat brains: a possible mechanism underlying GABA's excitatory role in immature brain," *J. Neurobiol.*, **33**, 781–795 (1997).
34. A. Reynolds, E. Brustein, M. Liao, et al., "Neurogenic role of the depolarizing chloride gradient revealed by global overexpression of KCC2 from the onset of development," *J. Neurosci.*, **28**, 1588–1597 (2008).
35. S. Rheims, C. D. Holmgren, G. Chazal, et al., "GABA action in immature neocortical neurons directly depends on the availability of ketone bodies," *J. Neurochem.*, **110**, No. 4, 1330–1338 (2009).
36. C. Rivera, J. Voipio, J. A. Payne, et al., "The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation," *Nature*, **397**, No. 6716, 251–255 (1999).
37. R. Serafini, A. Y. Valeyev, J. L. Barker, and M. O. Poulter, "Depolarizing GABA-activated Cl^- channels in embryonic rat spinal and olfactory bulb cells," *J. Physiol. (London)*, **488**, 371–386 (1995).
38. S. T. Sipila, K. Huttu, I. Soltesz, et al., "Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus," *J. Neurosci.*, **25**, No. 22, 5280–5289 (2005).
39. S. T. Sipila, K. Huttu, J. Voipio, and K. Kaila, "Intrinsic bursting of immature CA3 pyramidal neurons and consequence giant depolarizing potentials are driven by a persistent Na current and terminated by a slow Ca-activated K current," *Eur. J. Neurosci.*, **23**, 2330–2338 (2006).
40. R. Tyzio, C. Allene, R. Nardou, et al., "Depolarizing actions of GABA in immature neurons depend neither on ketone bodies nor on pyruvate," *J. Neurosci.*, **31**, No. 1, 34–45 (2011).
41. R. Tyzio, R. Cossart, I. Khalilov, et al., "Maternal oxytocin triggers a transient inhibitory switch in GABA signaling in the fetal brain during delivery," *Science*, **314**, 1788–1792 (2006).
42. R. Tyzio, A. Ivanov, C. Bernard, et al., "Membrane potentials of CA3 hippocampal pyramidal cells during postnatal development," *J. Neurophysiol.*, **90**, No. 5, 2964–2972 (2003).
43. G. Valeeva, A. Abdullin, R. Tyzio, et al., "Temporal coding at the immature depolarizing GABAergic synapse," *Front. Cell. Neurosci.*, **4**, (2010).
44. A. van den Pol, K. Obrietan, and G. Chen, "Excitatory actions of GABA after neuronal trauma," *J. Neurosci.*, **16**, No. 13, 4283–4292 (1996).
45. J. A. Verheugen, D. Fricker, and R. Miles, "Noninvasive measurements of the membrane potential and GABAergic action in hippocampal interneurons," *J. Neurosci.*, **9**, No. 7, 2546–2555 (1999).
46. D. D. Wang and A. R. Kriegstein, "Blocking early GABA depolarization with bumetanide results in permanent alterations in cortical circuits and sensorimotor gating deficits," *Cereb. Cortex*, **21**, 574–587 (2011).
47. T. Waseem, M. Mukhtarov, S. Buldakova, et al., "Genetically encoded Cl-sensor as a tool for monitoring of Cl-dependent processes in small neuronal compartments," *J. Neurosci. Meth.*, **193**, No. 1, 14–23 (2010).
48. J. Yamada, A. Okabe, H. Toyoda, et al., "Cl-uptake promoting depolarizing GABA actions in immature rat neocortical neurones is mediated by NKCC1," *J. Physiol.*, **557**, No. 3, 829–834 (2004).