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The Depolarizing Action of GABA Controls Early Network Activity in the Developing Hippocampus

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Abstract Early in postnatal life γ-aminobutyric acid (GABA), the primary inhibitory transmitter in adults, excites targeted neurons by an outwardly directed flux of chloride which results from the unbalance between the cation–chloride cotransporters NKCC1 and KCC2, involved in chloride uptake and extrusion, respectively. This effect contributes to generate synchronized network activity or giant depolarizing potentials (GDPs) in the developing hippocampus. Here, we review some recent data concerning the mechanisms by which GDPs are generated and their functional role in enhancing synaptic efficacy at poorly developed GABAergic and glutamatergic synapses. In adulthood, reshaping neuronal circuits due to changes in chloride homeostasis and to the shift of GABA from hyperpolarizing to depolarizing, has been implicated in several neurological disorders, including epilepsy. Evidence has been recently provided that in chronically nerve growth factor-deprived mice expressing a progressive age-dependent neurodegenerative pathology resembling that observed in patients with Alzheimer's disease, the reduced expression of mRNA encoding for the *Kcc2* gene and the depolarizing action of GABA lead to the reorganization of the neuronal hippocampal network. This may represent a novel mechanism by which GABAergic signaling counterbalances the loss of synaptic activity in neurodegenerative diseases.

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Introduction

The construction of the brain relies on genetic and environmental determinants "nature and nurture." While nature provides a set of genes that control the general organization of the brain, nurture ensures that genetically built neuronal circuits adapt to the environment. Learning constitutes an adaptive process involving experience- or activity-dependent mechanisms regulating several developmental steps, including synapses formation and elimination [1, 2].

Spontaneous correlated neuronal activity occurring at late embryonic, early postnatal stages of development constitutes a hallmark of developmental networks, well preserved during evolution, that have been observed in almost every brain structure examined, including the retina [3], the neocortex [4–7], the hippocampus [8], the hypothalamus [9], the cerebellum [10], and the spinal cord [11, 12]. This activity is crucial for the proper wiring and the construction of the adult brain according to the Hebbian rule "neurons that fire together wire together."

This review will focus on a particular form of synchronized neuronal activity, observed in the immature hippocampus and characterized by giant depolarizing potentials or GDPs [8]. GDPs, which have been proposed as the in vitro counterpart of "sharp waves" recorded in vivo in rat pups during immobility periods, sleep, and feeding [13] can be considered a primordial form of synchrony between neurons, which precedes more organized forms of activity such as theta and gamma rhythms [14].

GDPs Are Generated by the Synergistic Action of Glutamate and GABA

GDPs are generated synchronously in a neuronal population. They consist in recurrent long lasting depolarizing potentials, with amplitudes up to 50 mV and lasting several hundreds of milliseconds separated by long lasting intervals [8]. They depend on the synergistic action of glutamate and γ-aminobutyric acid (GABA) that at this developmental stage is depolarizing and excitatory. As expected from a mixed glutamatergic and GABAergic potential, blocking the $GABA_A$ receptor-mediated component with an intracellular solution containing potassium fluoride reveals a glutamatergic response which reverses polarity close to the equilibrium potential for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-mediated responses (E_{AMPA}; [15]); however, the magnitude of the GABAergic conductance exceeds that of the glutamatergic one since GDPs' reversal is close to E_{GABA} [8, 15]. Electrophysiological recordings associated with fura-2-based fluorometric calcium imaging have demonstrated that within a particular hippocampal area, GDPs involve the synchronous discharge of principal cells and interneurons [16]. Local GABAergic interneurons usually drive principal cells, as demonstrated by the temporal relationship of glutamatergic and GABAergic inputs during GDPs. By clamping two neighboring pyramidal cells at the relevant reversal potentials, it is possible to measure the GABAergic and glutamatergic components in isolation. Thus, when two neighboring pyramidal cells were voltage-clamped at −70 mV (close to E_{GABA}), the glutamatergic components occurred synchronously in both cells; however, when one cell was held at 0 mV (the assumed reversal potential for glutamate) and the other at −70 mV, the GABAergic component always preceded the glutamatergic one by several milliseconds [17]. On average, in four out of five cells during GDPs, the GABAergic components preceded the glutamatergic ones by 29.1±6.4 ms (Fig. 1).

Using network dynamics imaging, online reconstruction of functional connectivity and targeted whole-cell recordings from immature hippocampal slices, it was recently demonstrated that functional hubs composed of subpopulations of GABAergic interneurons with large axonal arborizations are able to synchronize large ensembles of cells [18]. In addition, evidence has been provided that in the CA3 region, GDPs can still be generated in small islands, isolated from the rest of the hippocampus, containing a relatively small proportion of principal cells and interneurons [15, 16, 19]. The depolarizing action of GABA during GDPs leads to calcium influx through the activation of voltage-dependent calcium channels and *N*-methyl-D-aspartate (NMDA) receptors [16, 20]. The transient elevation in intracellular calcium level activates signaling pathways

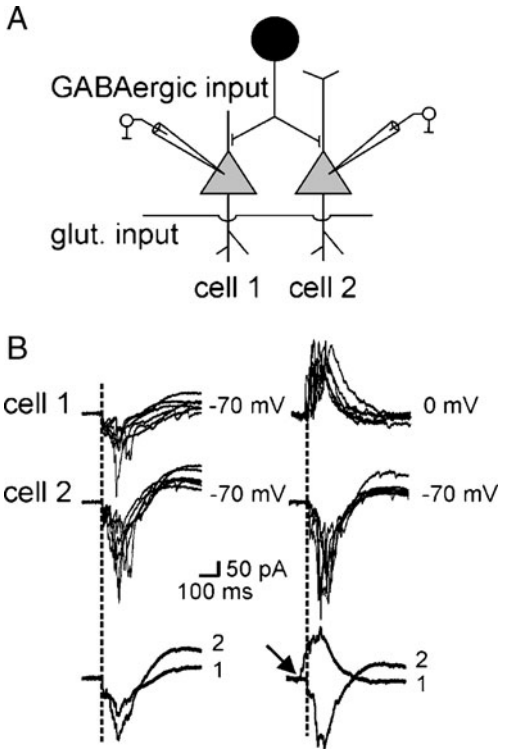

Fig. 1 GABA released from interneurons drives principal cells. **a** Schematic diagram showing two pyramidal cells receiving GABAergic innervation from an interneuron (*black*). The glutamatergic input is not represented. **b**. Double voltage clamp recordings from neighboring CA3 pyramidal cells (cell 1 and cell 2) held both close to the reversal potential for GABA (at −70 mV, *left*) or one to the reversal potential for glutamate (0 mV, *upper right*) and the other close to the reversal potential for GABA (−70 mV, *middle right*). Six individual sweeps are superimposed in each condition. The averages of individual traces are shown below. Notice that the GABAergic component of GDPs precedes the glutamatergic one (*arrow*) indicating that GABA released from a GABAergic interneuron drives the pyramidal cell. Modified from ref. 17

known to control several developmental processes, including DNA synthesis, neuronal migration, differentiation, and synaptogenesis [21]. GDPs disappear spontaneously towards the end of the second postnatal week in concomitance with the shift of GABA from the depolarizing to the hyperpolarizing direction.

GABA, which is the main inhibitory neurotransmitter in the adult CNS, early in postnatal life depolarizes and excites target cells through an outwardly directed flux of chloride [17, 21–24]. $GABA_A$ receptors are mainly permeable to chloride and to HCO_3^-; however, in immature neurons, the influence of HCO_3^- on E_{GABA} is negligible because of the relatively high intracellular chloride level $[Cl^-]_i$ [25]. Chloride homeostasis is controlled by the Na-K-2Cl cotransporter, NKCC1, and by the K-Cl cotransporter, KCC2, that enhance and lower $[Cl^-]_i$, respectively [26]. Due to the low expression of the KCC2 extruder at birth, chloride accumulates inside the neuron via NKCC1. The progressive increase in the expression of KCC2 is respon-

sible for the developmental shift of GABA from the depolarizing to the hyperpolarizing direction [27]. KCC2 extrudes K^+ and Cl^- using the electrochemical gradient for K^+. Cl^- extrusion is weak in immature neurons and increases with neuronal maturation [28, 29].

Transfection of the *Kcc2* gene into immature hippocampal neurons in cultures converts the action of GABA from excitatory to inhibitory [30]. Furthermore, the premature expression of KCC2 by in utero electroporation eliminates the excitatory action of endogenous GABA in a subpopulation of newly born cortical neurons and severely impairs the morphological maturation of cortical cells without altering their radial migration [31]. This effect can be mimicked by overexpressing the inwardly rectifying K^+ channel which lowered the membrane potential and reduced cell excitability, strongly suggesting that membrane depolarization caused by the early GABA excitation is essential for the functional maturation of cortical circuits in vivo.

It is worth noting that KCC2, independent of its chloride transport function, interacts with the cytoskeleton to regulate spine morphology at excitatory synapses, thus acting as a possible synchronizing factor in the development of inhibitory and excitatory neurotransmission [32]. The direct structural interaction of KCC2 with the cytoskeleton-associated protein 4.1 N and with the actin cytoskeleton seems to regulate neuronal differentiation and migration early in embryonic development [33].

In similar experiments, using in utero electroporation of a short hairpin RNA sequence against *Nkcc1* to knock down in vivo the expression of NKCC1 and to abolish $GABA_A$-mediated excitation leads to a significant reduction in AMPA receptor-mediated synaptic transmission associated with a disruption of dendritic arborization and spine density further indicating that the depolarizing and excitatory action of GABA plays a permissive role in the formation of excitatory synapses [34]. A similar alteration of excitatory synapses which persists in adulthood has also been observed when NKCC1 is blocked with the diuretic bumetanide during a critical period of postnatal development. Interestingly, these effects could be rescued by overexpressing mutant voltage-independent NMDA receptors, indicating that GABA depolarization cooperates with NMDA receptor activation to regulate the formation of excitatory synapses [34]. GABAergic depolarization would facilitate the relief of the voltage-dependent magnesium block from NMDA receptors, thus allowing calcium entry to initiate intracellular signaling cascades. Cooperation with NMDA receptor activation represents an early form of coincidence detection between GABAergic and glutamatergic inputs.

Interestingly, deletion of the gene encoding for glutamic acid decarboxylase (GAD) 65, the enzyme responsible for producing vesicular GABA, leads to a decrease in GABA content associated with a reduced network activity (GDPs) and a delayed neuronal maturation in the hippocampus [35]. In contrast, mice lacking GAD67 or both isoforms of GAD (GAD65 and GAD67), which die immediately after birth because of a cleft palate and inability to suckle, exhibit a normal brain cytoarchitecture [36], suggesting possible compensatory mechanisms maybe mediated via some other neurotransmitter.

Most of the studies so far reported on the depolarizing action of GABA refer to in vitro experiments. Does GABA exert a depolarizing action also in vivo? As already mentioned, GDPs are thought to be the in vitro counterpart of the in vivo hippocampal early sharp waves, and several lines of evidence suggest that similar mechanisms govern these phenomena. Hence, in experiments from the hippocampus of freely moving rat pups, Sipila et al. [37] have clearly shown that intraperitoneal application of the specific NKCC1 inhibitor bumetanide blocks sharp waves, strengthening the view that sharp waves and GDPs are largely homologous network events, both controlled by the depolarizing action of GABA.

It is worth noting that GABA-mediated excitation has been demonstrated to drive synaptic integration of newly generated granule cells in the adult dentate gyrus [38].

GDPs Emerge when Synaptic Trafficking and Cell Firing Exceed a Certain Threshold

How GDPs are generated is still a matter of debate. In particular, it is unclear how neuronal ensembles synchronize to produce a population response. Although the entire hippocampal network possesses the capacity to generate GDPs for its anatomical characteristics, including extensive glutamatergic connections via recurrent collaterals, the CA3 area is particularly well equipped to generate oscillations and can be considered the pacemaker region. Functionally, excitatory synaptic interactions would facilitate neuronal synchronization and the initiation of population bursts [39–41]. In addition, CA3 principal cells exhibit, in the absence of synaptic transmission, intrinsic bursts upon membrane depolarization which, by virtue of their spontaneous discharges and large spike output, can drive other neurons to fire in synchrony [42, 43]. The regenerative depolarization due to persistent slow sodium current would trigger intrinsic bursts [44]. To reach the threshold for activating the persistent sodium current, neurons should depolarize from rest and this can be achieved through the activation of a tonic $GABA_A$-mediated conductance (by ambient GABA) which further enhances cell excitability and the glutamatergic drive to principal cells [42, 45]. Interestingly, prior to synapses formation, immature neurons communicate via the paracrine action of GABA. According to Demarque et al. [46], transmitter receptors are operative before synapses formation as indicated by the observation that cells synaptically silent

respond to application of GABA or glutamate. Therefore, GABA released by non-conventional release sites such as growth cones and astrocytes in a calcium- and SNARE-independent manner, would diffuse away to activate extrasynaptic $GABA_A$ receptors [46]. The absence of an efficient uptake system will enable GABA to accumulate in the extracellular space and to reach a concentration sufficient to exert its distal action. By activating extrasynaptic $GABA_A$ receptors, GABA would produce a slow tonic current which would depolarize principal cells to the voltage range necessary to activate the persistent sodium current and intrinsic bursts [42]. During a burst, calcium entering into the cell through voltage-activated calcium channels opens calcium-dependent potassium conductances responsible for burst termination. Early in postnatal development, intrinsic bursting activity would be facilitated by the low expression of Kv7.2 and Kv7.3 channels responsible for the non-inactivating, low-threshold M current (I_m), which in adulthood controls spike after-depolarization and burst generation [47]. In a recent study, we observed that the density of I_m is very low at birth and markedly increases after the first postnatal week [43]. This leads to intrinsic bursts which, in comparison to adults, are longer and more robust and recur more regularly (Fig. 2; [43]).

Neuronal synchronization would occur when a sufficient number of cells fire within a restricted time window and the excitability of the network attains a certain threshold. This process has been well described in the disinhibited hippocampus, where population synchrony has been proposed to depend on an active process consisting in a buildup period during which synaptic traffic and cell firing exceeds a certain threshold [48]. A similar process may be involved in the generation of GDPs early in postnatal life [49, 50] when synaptic interactions are facilitated by the excitatory action of GABA [22, 24]. Simultaneous recordings from pairs of proximal CA3 pyramidal neurons showed a concurrent increment in the instantaneous firing frequency previous to GDPs' onset which correlated with an increase in the number of synaptic potentials [49]. Along this line, we found that the number of spontaneously occurring synaptic events preceding GDPs in the time interval between 0 and 0.5 s was significantly higher from that occurring between 0.5 and 1 s, indicating that the frequency of synaptic events is related to the occurrence of GDPs (Fig. 3).

In thalamocortical neurons, a striking contribution to rhythmogenesis is given by the slowly activating inwardly rectifying cationic current I_h, carried by hyperpolarization-activated cyclid neucleotide-gated (HCN) channels. This current which has the unique property to be activated upon hyperpolarization beyond resting potential provides the pacemaker depolarization during rhythmic oscillations [51]. Although the inward current carried by I_h is generally not sufficient to bring the membrane back to the original

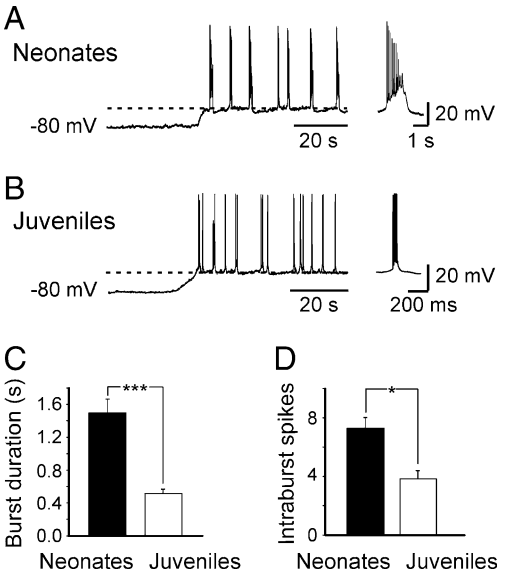

Fig. 2 Intrinsic bursting activity in neonatal and juvenile neurons. **a** Depolarizing the membrane from −80 mV to −56 mV (*dashed line*) in a P3 neuron (synaptic activity and GDPs were blocked with DNQX, 20 μM; APV, 50 μM; and picrotoxin, 100 μM) induced the appearance of intrinsic voltage-dependent bursts (shown on the *right* in an expanded time scale). **b** Representative tracing from a P21 neuron (no GDPs were present). Depolarizing the membrane to −65 mV (*dashed line*) in the presence of DNQX, APV, and picrotoxin revealed intrinsic bursts which differed in frequency and shape from those recorded in neonatal neurons (see individual bursts on the *right*). **c**, **d** Mean burst duration and number of intraburst spikes in neonatal (n=26) and in juvenile neurons (n=20). Note that in juvenile neurons, burst duration was significantly shorter and the number of intraburst spikes was significantly smaller than in neonatal neurons. *Single asterisk*, $p<0.05$; *triple asterisk*, $p<0.001$. Modified from ref. 43

level, it can reach threshold for activation of the persistent sodium current. In a previous study from the neonatal hippocampus, we have suggested that, at least at the level of hilar interneurons, GDPs could be triggered by a pacemaker current with biophysical properties similar to I_h [52]. Later on, Bender et al. (2006; [53]) have clearly demonstrated that the selective I_h blocker ZD 7288 disrupts GDPs generation in CA3 pyramidal cells and interneurons known to be endowed since birth with the HCN1 isoform of HCN channels. It should be stressed, however, that although I_h may favor neuronal synchronization it is not essential for GDPs' generation since these may occur also in neurons lacking HCN channels.

Another factor which facilitates network synchronization is electrical coupling between neurons via gap junctions [54]. This way of signaling is particularly well developed in immature neurons [52]. Using fast multibeam two-photon calcium microscopy (to record simultaneously hundreds of neurons) small cell assemblies coupled by gap junctions have been detected in the hippocampus at birth [55]. Thus, small groups of intrinsically electrically coupled active neurons generate synchronous calcium plateaus nesting

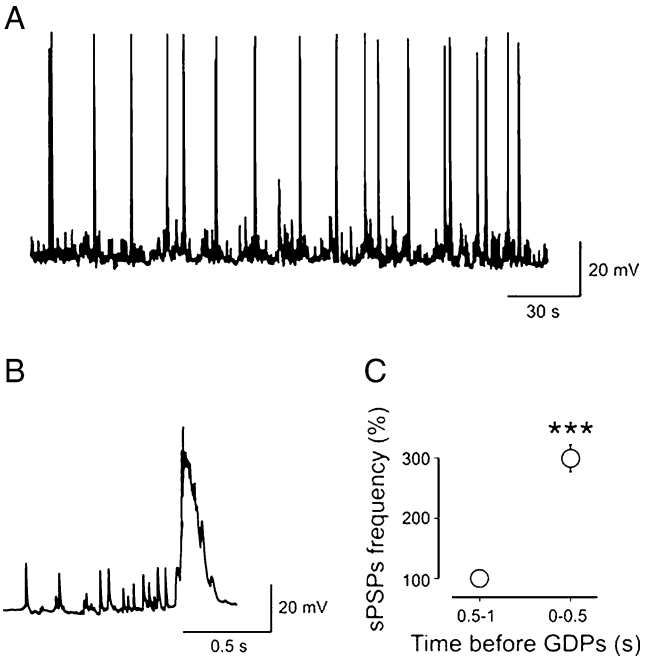

Fig. 3 Early in postnatal life, spontaneous synaptic activity contributes to hippocampal network synchronization. **a** GDPs recorded from a CA3 pyramidal neuron at P3. GDPs are typically preceded by a number of spontaneously occurring synaptic events. **b** A GDP preceded by spontaneous synaptic activity shown in an expanded time scale. **c** Frequency of spontaneous synaptic activity recorded within 0–0.5 s and 0.5–1 s intervals preceding GDPs' onset and normalized to that of 0.5–1 s interval. *Triple asterisk*, $p<0.001$. Safiulina et al. unpublished observations

non-synaptic recurrent membrane potential oscillations. These voltage-gated localized patterns involving selected neuronal assemblies would precede synaptic-driven activity such as GDPs which progressively emerge during the first week of postnatal life [55]. Interestingly, this primitive pattern of activity was found to be potentiated by oxytocin, a maternal hormone essential for labor induction, suggesting an endogenous regulation by signaling molecules involved in delivery.

GDPs Act as Coincidence Detector Signals to Enhance Synaptic Efficacy at Both GABAergic and Glutamatergic Synapses

One essential question is the functional role of correlated network activity in the immature hippocampus. We tested the hypothesis that GDPs may act as coincidence detectors to enhance synaptic efficacy in an associative and activity-dependent manner. Activity-dependent modifications in synaptic strength such as long-term potentiation (LTP) or long-term depression are critical for information storage in the brain and for the development of neuronal circuits. These processes are particularly important in the developing brain, characterized by an elevated number of "silent" synapses [56]. These are synapses that do not conduct at rest because the neurotransmitter is not released when the presynaptic terminal is invaded by an action potential (presynaptically silent) or because they are unable to detect the release of the neurotransmitter due to the lack of the respective receptors on the subsynaptic membrane (postsynaptically silent). Silent synapses can be converted into active ones by activity-dependent processes and this represents the most common mechanism for LTP induction, not only during development but also in the mature brain [57].

We addressed this issue at poorly developed MF-CA3 synapses, which early in development are GABAergic [58]. A pairing procedure was developed, consisting in triggering MF stimulation with the rising phase of GDPs in such a way that calcium transients associated with GDPs occurred simultaneously with MF-evoked GABAergic currents (MF-GPSCs). After a control period of 5–10 min in which GPSCs were evoked by stimulation of granule cells in the dentate gyrus at 0.05 Hz, the patch was switched from voltage-clamp to current-clamp mode and MF responses were evoked by spontaneously occurring GDPs (pairing typically lasted 5 min). After this period, the patch was switched back to voltage clamp mode and synaptic currents were recorded as in control. As illustrated in Fig. 4, in the case of presynaptically silent synapses, the pairing protocol caused the appearance of responses to the first stimulus and increased the number of successes to the second one [59]. In the case of non-silent low-probability synapses, the pairing procedure produced a strong and persistent potentiation of MF responses associated with a significant increase in the number of successes and, in double pulse experiments, with a significant reduction in the paired-pulse ratio and a significant increase in the inverse squared value of the coefficient of variation. This suggests that an increased probability of transmitter release accounts for the persistent increase in synaptic efficacy.

In the absence of pairing, no significant changes in synaptic efficacy occurred. Moreover, when the interval between GDPs and MF stimulation was progressively increased, the potentiation declined and reached the control level when presynaptic signals were activated 2–3 s after GDPs [59]. In addition, pairing induced long lasting increase in synaptic efficacy was prevented when cells were loaded with the calcium chelator 1,2-Bis(2-aminophenoxy)ethane-N,N,N′,N′-tetra acetic acid or when nifedipine was added to the extracellular medium. In contrast, the NMDA receptor antagonist D-(-)-2-amino-5-phosphonopentanoic acid failed to prevent pairing-induced potentiation, indicating that calcium rise through NMDA receptors is not involved in this form of plasticity. These data clearly demonstrate that during development, coincidence detection signals provided by GDPs are crucial for enhancing GABAergic transmission at emerging MF-CA3 connections.

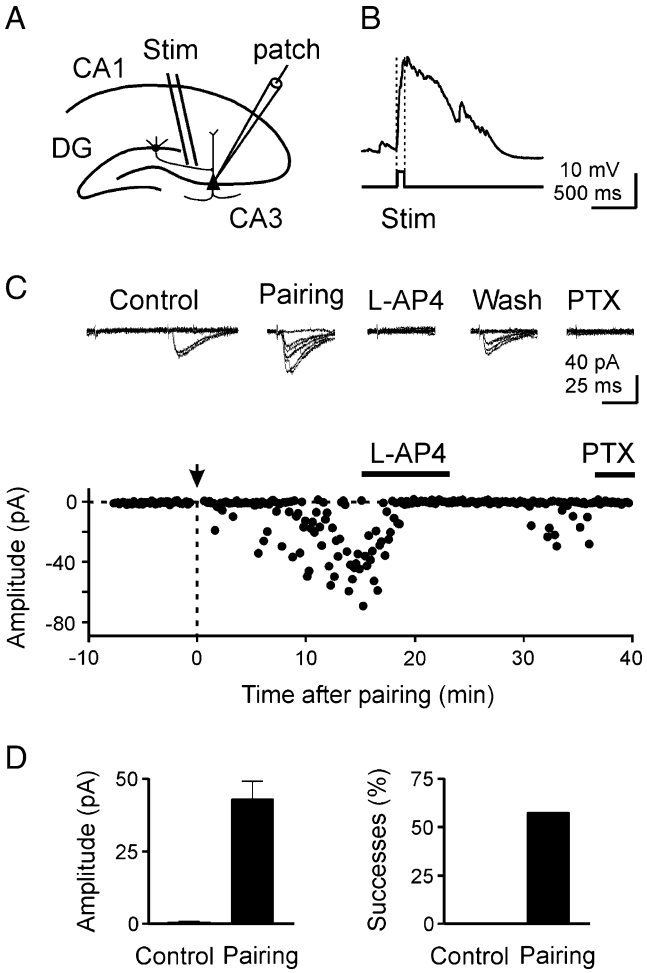

Fig. 4 Pairing induced the appearance of synaptic responses in presynaptically silent neurons. **a** Schematic diagram showing the arrangement of stimulating electrode and patch pipette. **b** A single GDP recorded from the hippocampus of a P2-old rat with a patch pipette containing QX 314 to block action potentials. The rising phase of GDPs (between the *dotted lines*) was used to trigger synaptic stimulation (*Stim*). **c** Amplitudes of synaptic responses (*dots*) evoked by minimal stimulation of MF before and after pairing (*arrow* at time 0) are plotted against time. The traces above the graph represent individual responses evoked before and after pairing in different experimental conditions as indicated. This synapse was considered "presynaptically" silent because it did not exhibit any response to the first stimulus over 48 trials (at 0.1 Hz) but two responses to the second one. For clarity after "pairing," only synaptic responses evoked by the first stimulus are shown. **d** Mean excitatory postsynaptic current amplitude (*left*) and mean percentage of successes (*right*) before and after pairing (n=5). Modified from ref. 59

Likewise, GDPs have been shown to be instrumental in increasing synaptic strength at glutamatergic connections [60]. A similar pairing protocol used to trigger Schaffer collateral stimulation caused a persistent increase in spontaneous and evoked AMPA-mediated glutamatergic currents (sEPSCs), an effect that required calcium influx through postsynaptic L-type calcium channels. As for MF-CA3 synapses, the induction of LTP was clearly postsynaptic (dependent on the postsynaptic rise of calcium through GDPs) while its expression was presynaptic, as suggested by the decrease in PPR and the increase in the frequency, but not in the amplitude of sEPSCs. Therefore, the postsynaptic cell should provide a retrograde signal to the presynaptic neuron. One attractive candidate is brain-derived neurotrophic factor (BDNF), which can be released in a calcium-dependent way by depolarization of the postsynaptic cell [61–63] and plays a crucial role in synaptic plasticity [64]. BDNF acts on tropomyosin-related kinase receptor B (TrkB), and this interaction activates different signaling pathways. Indeed, this neurotrophin mimicked the effects of pairing on synaptic transmission and BDNF scavengers or tropomyosin-related kinase receptor B (TrkB) antagonists blocked pairing-induced synaptic potentiation. Blocking TrkB receptors in the postsynaptic cell did not prevent the effects of pairing, suggesting that BDNF, possibly secreted from the postsynaptic cell during GDPs, acts on TrkB receptors localized on presynaptic neurons. Pairing-induced synaptic potentiation was blocked by ERK inhibitors, suggesting that BDNF activates the MAPK/ERK cascade, which may lead to transcriptional regulation and new protein synthesis in the postsynaptic neuron. Therefore, BDNF can act presynaptically to alter the probability of glutamate release but also postsynaptically to produce the morphological modifications necessary for the formation of new synapses and the refinement of the adult neuronal circuit.

It has been recently demonstrated that the release of BDNF is regulated by metabotropic $GABA_B$ receptors [65]. Early network activity such as GDPs would provide the critical amount of GABA needed to activate extrasynaptic $GABA_B$ receptors which would play an instructive role in shaping neuronal circuits. Thus, mice lacking functional $GABA_B$ receptors exhibit a deficit in GABAergic synaptic transmission associated with a reduced perisomatic GABAergic innervation in the hippocampus [65].

Overall, these results support the hypothesis that during a critical period of postnatal development GDPs act as coincidence detectors to establish the appropriate GABAergic and glutamatergic connections leading to the development of the adult neuronal circuit.

In Some Pathological Conditions the Switch of GABA from the Hyperpolarizing to the Depolarizing Direction May Affect the Operation of Neuronal Networks

As emphasized earlier, GDPs are generated by GABA which early in development is depolarizing and excitatory and to a lesser extent by glutamate. GDPs disappear spontaneously after birth when GABA becomes hyperpolarizing; however, in some pathological conditions, including epilepsy [66, 67], axonal injury [68], trauma [69], neuropathic pain [70], inflammatory hyperalgesia,

allodynia [71], and neurodegeneration [72] GABA may become depolarizing and excitatory also in adulthood.

In temporal lobe epilepsy, for instance, depolarizing GABA responses in neurons localized downstream to the sclerotic area may contribute to the generation of interictal discharges [66]. In slices prepared from human tissue from patients affected by mesial temporal lobe epilepsy, interictal activity seems to be generated by a minority of subicular neurons, including interneurons and a subset of pyramidal cells. The reciprocal interaction between these neurons would be facilitated by the depolarizing action of GABA [66]. Deafferentation may resume a genetic program which would switch GABA polarity from the hyperpolarizing to the depolarizing direction. Combined intracellular recordings with biocytin-filled electrodes and immunochemistry for KCC2 have revealed that the majority of cells depolarized by GABA are immunonegative for KCC2, indicating that changes in Cl^- homeostasis contribute to human epileptiform activity [66]. Interestingly, the diuretic bumetanide, which suppresses NKCC1-mediated accumulation of intracellular Cl^- [73], is able to restore hyperpolarizing GABA responses and to block the epileptiform activity, thus opening new therapeutic avenues for the treatment of this severe form of epilepsy which typically drug-resistant.

While the relative contribution of NKCC1 and KCC2 to chloride homeostasis is well understood, the molecular mechanisms regulating their expression are still unclear. Evidence has been provided that nicotine cholinergic signaling contributes to the developmental changes in the expression of chloride transporters. During postnatal development, calcium influx through nicotine acetylcholine receptors would promote the maturation of chloride gradient by upregulating the KCC2 extruder via the transcription factor CREB, thus shifting the equilibrium potential for Cl^- towards more negative values [74]. Since cholinergic signaling is under the control of nerve growth factor (NGF), the possibility that this neurotrophin contributes to regulate chloride homeostasis cannot be excluded.

Using adult (6 months old) transgenic mice deprived of NGF (AD11; [75, 76]), which exhibits a severe deficit of the cholinergic function associated with an age-dependent progressive neurodegenerative pathology resembling that observed in Alzheimer's disease (AD) patients, we have recently found that the depolarizing and excitatory action of GABA leads to a modification of the neuronal hippocampal circuit [72, 77] The neurodegenerative phenotype of AD11 mice is characterized by impairment in retention and transfer of spatial memory tasks associated with cholinergic atrophy, neuronal loss, tau hyperphosphorylation and insolubility, abnormalities of the neuronal cytoskeleton reminiscent of tangles [75], β-amyloid (Aβ) plaques [78] and deficit in cortical synaptic plasticity [79]. Perforated-patch and cell-attached recordings have allowed estimating the reversal of GPSCs and GABA-evoked single-channel currents, respectively. In the hippocampus of AD11 mice, E_{GABA} was found to be positive with respect to the resting membrane potential. Real-time qRT-PCR and immunocytochemical experiments revealed a reduced expression of mRNA encoding for the *Kcc2* gene and of the respective protein. In addition, in line with a depolarizing action of GABA, the $GABA_A$ agonist isoguvacine significantly

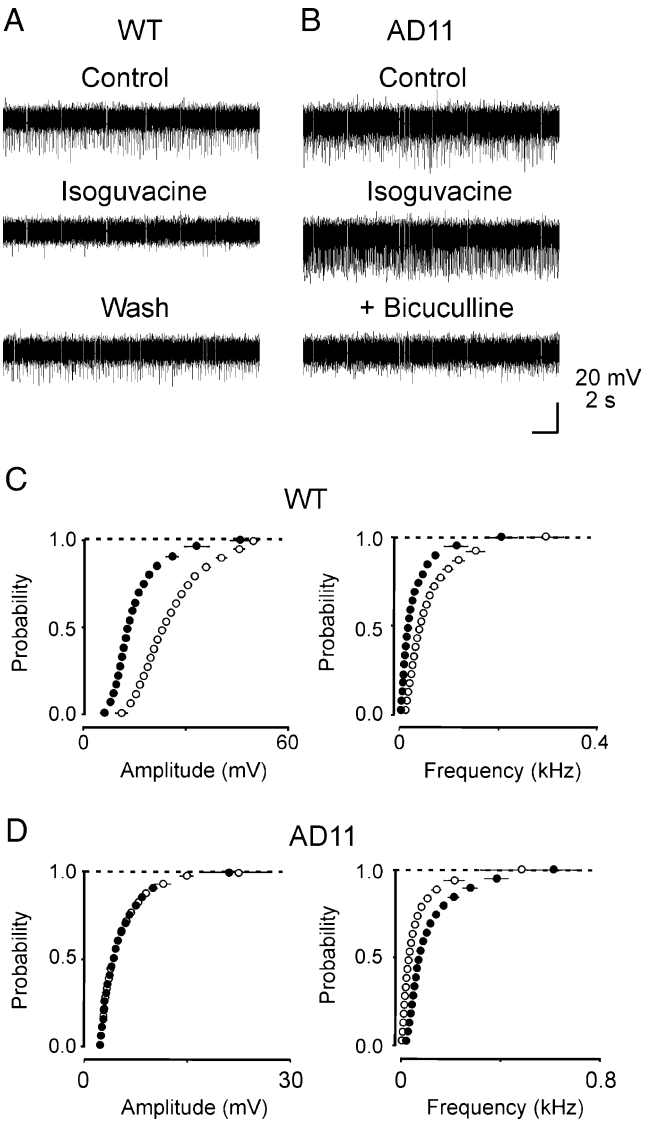

Fig. 5 Changes in the effects of isoguvacine on multiunit activity recorded from WT and AD11 mice. **a** A brief application of isoguvacine in the bath to hippocampal slices from WT animals reduced the frequency of MUA and eliminated higher amplitude events in a reversible manner. **b** In AD11 mice, isoguvacine increased the frequency of MUA, an effect that was blocked by bicuculline (10 μM). **c**–**d** Amplitude and frequency probability distribution plots obtained from WT (**c**; $n=7$) and AD11 (**d**; $n=6$) mice in the absence (*open circles*) or in the presence (*closed circles*) of isoguvacine. Differences in amplitude (*WT*) and in frequency (*AD11*) obtained in the presence of isoguvacine were significantly different from controls $P<0.001$; Wilcoxon signed rank test. Modified from ref. 72

increased the firing of CA1 principal cells and at the network level, the frequency of multiunit activities (MUA; Fig. 5), indicating that indeed the shift of GABA from hyperpolarizing to depolarizing contributes to reshaping the neuronal network [77]. If this process is common to other animal models of AD, it remains to be established.

Conclusions

The data reviewed here clearly indicate that GABA, through its depolarizing action, not only controls early network oscillations in the hippocampus but is instrumental in reshaping neuronal circuits in some pathological conditions occurring in adulthood. Much remains to be known about the genetic program and the molecular mechanisms regulating these processes. Among the molecules which may contribute to control the direction of GABAergic signaling, an attractive candidate is brain-derived neurotrophic factor, BDNF. This neurotrophin, through the activation of TrkB receptors, has been implicated in the developmental regulation of chloride homeostasis. In hippocampal slices, the elevated neuronal activity would enhance BDNF expression leading to a down regulation of KCC2 [80]. A similar mechanism may account for KCC2 reduction found in AD11 mice where the enhanced network excitability may increase BDNF levels and TrkB activation; however, although BDNF levels have not been measured, this hypothesis seems unlikely since BDNF has been shown to be down regulated in AD patients or in the animal model of AD [81].

Interestingly, in a recent study the possibility that the microRNA-92 regulates the *Kcc2* gene expression has been highlighted [82]. MicroRNAs are evolutionary conserved, non-coding RNA molecules, 18–25 nucleotides in length that by interacting with specific targeted mRNAs posttranscriptionally regulate gene expression. Whether similar mechanisms control the direction of GABA signaling also in adulthood in pathological conditions such as epilepsy and AD, remains to be established.

References

1. Ben-Ari Y (2001) Developing networks play a similar melody. Trends Neurosci 24:353–360
2. Spitzer NC (2006) Electrical activity in early neuronal development. Nature 444:707–712
3. Feller MB, Butts DA, Aaron HL, Rokhsar DS, Shatz CJ (1997) Dynamic processes shape spatiotemporal properties of retinal waves. Neuron 19:293–306
4. Yuste R, Katz LC (1991) Control of postsynaptic Ca2+ influx in developing neocortex by excitatory and inhibitory neurotransmitters. Neuron 6:333–344
5. Owens DF, Boyce LH, Davis MB, Kriegstein AR (1996) Excitatory GABA responses in embryonic and neonatal cortical slices demonstrated by gramicidin perforated-patch recordings and calcium imaging. J Neurosci 16:6414–6423
6. Dammerman RS, Flint AC, Noctor S, Kriegstein AR (2000) An excitatory GABAergic plexus in developing neocortical layer 1. J Neurophysiol 84:428–434
7. Maric D, Liu QY, Maric I, Chaudry S, Chang YH, Smith SV, Sieghart W, Fritschy JM, Barker JL (2001) GABA expression dominates neuronal lineage progression in the embryonic rat neocortex and facilitates neurite outgrowth via GABA(A) autoreceptor/Cl- channels. J Neurosci 21:2343–2360
8. Ben-Ari Y, Cherubini E, Corradetti R, Gaiarsa JL (1989) Giant synaptic potentials in immature rat CA3 hippocampal neurones. J Physiol 416:303–325
9. Chen G, Trombley PQ, van den Pol AN (1996) Excitatory actions of GABA in developing rat hypothalamic neurones. J Physiol 494:451–464
10. Eilers J, Plant TD, Marandi N, Konnerth A (2001) GABA-mediated Ca2+ signalling in developing rat cerebellar Purkinje neurones. J Physiol 536:429–437
11. O'Donovan MJ (1999) The origin of spontaneous activity in developing networks of the vertebrate nervous system. Curr Opin Neurobiol 9:94–104
12. Wang J, Reichling DB, Kyrozis A, MacDermott AB (1994) Developmental loss of GABA- and glycine-induced depolarization and Ca2+ transients in embryonic rat dorsal horn neurons in culture. Eur J Neurosci 6:1275–1280
13. Leinekugel X, Khazipov R, Cannon R, Hirase H, Ben-Ari Y, Buzsáki G (2002) Correlated bursts of activity in the neonatal hippocampus in vivo. Science 296:2049–2052
14. Buzsáki G, Draguhn A (2004) Neuronal oscillations in cortical networks. Science 304:1926–1929
15. Bolea S, Avignone E, Berretta N, Sanchez-Andres JV, Cherubini E (1999) Glutamate controls the induction of GABA-mediated giant depolarizing potentials through AMPA receptors in neonatal rat hippocampal slices. J Neurophysiol 81:2095–2102
16. Garaschuk O, Hanse E, Konnerth A (1998) Developmental profile and synaptic origin of early network oscillations in the CA1 region of rat neonatal hippocampus. J Physiol 507:219–236
17. Mohajerani MH, Cherubini E (2005) Spontaneous recurrent network activity in organotypic rat hippocampal slices. Eur J Neurosci 22:107–118
18. Bonifazi P, Goldin M, Picardo MA, Jorquera I, Cattani A, Bianconi G, Represa A, Ben-Ari Y, Cossart R (2009) GABAergic hub neurons orchestrate synchrony in developing hippocampal networks. Science 326:1419–1424
19. Khazipov R, Leinekugel X, Khalilov I, Gaiarsa JL, Ben-Ari Y (1997) Synchronization of GABAergic interneuronal network in CA3 subfield of neonatal rat hippocampal slices. J Physiol 498:763–772
20. Leinekugel X, Medina I, Khalilov I, Ben-Ari Y, Khazipov R (1997) Ca2+ oscillations mediated by the synergistic excitatory actions of GABA(A) and NMDA receptors in the neonatal hippocampus. Neuron 18:243–255
21. Owens DF, Kriegstein AR (2002) Is there more to GABA than synaptic inhibition? Nat Rev Neurosci 3:715–727
22. Cherubini E, Gaiarsa JL, Ben-Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. Trends Neurosci 14:515–519
23. Ben-Ari Y, Khazipov R, Leinekugel X, Caillard O, Gaiarsa JL (1997) GABAA, NMDA and AMPA receptors: a developmentally regulated 'ménage à trois'. Trends Neurosci 20:523–529
24. Ben-Ari Y (2002) Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728–739
25. Kaila K (1994) Ionic basis of GABAA receptor channel function in the nervous system. Prog Neurobiol 42:489–537

26. Blaesse P, Airaksinen MS, Rivera C, Kaila K (2009) Cation–chloride cotransporters and neuronal function. Neuron 61:820–838
27. Rivera C, Voipio J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K (1999) The K+/Cl- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251–255
28. Khirug S, Huttu K, Ludwig A, Smirnov S, Voipio J, Rivera C, Kaila K, Khiroug L (2005) Distinct properties of functional KCC2 expression in immature mouse hippocampal neurons in culture and in acute slices. Eur J Neurosci 21:899–904
29. Luhmann HJ, Prince DA (1991) Postnatal maturation of the GABAergic system in rat neocortex. J Neurophysiol 65:247–263
30. Chudotvorova I, Ivanov A, Rama S, Hubner CA, Pellegrino C, Ben AY, Medina I (2005) Early expression of KCC2 in rat hippocampal cultures augments expression of functional GABA synapses. J Physiol 566:671–679
31. Cancedda L, Fiumelli H, Chen K, Poo MM (2007) Excitatory GABA action is essential for morphological maturation of cortical neurons in vivo. J Neurosci 27:5224–5235
32. Li H, Khirug S, Cai C, Ludwig A, Blaesse P, Kolikova J, Afzalov R, Coleman SK, Lauri S, Airaksinen MS, Keinänen K, Khiroug L, Saarma M, Kaila K, Rivera C (2007) KCC2 interacts with the dendritic cytoskeleton to promote spine development. Neuron 56:1019–1033
33. Horn Z, Ringstedt T, Blaesse P, Kaila K, Herlenius E (2010) Premature expression of KCC2 in embryonic mice perturbs neural development by an ion transport-independent mechanism. Eur J Neurosci 31:2142–2155
34. Wang DD, Kriegstein AR (2008) GABA regulates excitatory synapse formation in the neocortex via NMDA receptor activation. J Neurosci 28:5547–5558
35. Overstreet-Wadiche LS, Bensen AL, Westbrook GL (2006) Delayed development of adult-generated granule cells in dentate gyrus. J Neurosci 26:2326–2334
36. Asada H, Kawamura Y, Maruyama K, Kume H, Ding RG, Kanbara N, Kuzume H, Sanbo M, Yagi T, Obata K (1997) Cleft palate and decreased brain gamma-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. Proc Natl Acad Sci USA 94:6496–6499
37. Sipilä ST, Schuchmann S, Voipio J, Yamada J, Kaila K (2006) The cation–chloride cotransporter NKCC1 promotes sharp waves in the neonatal rat hippocampus. J Physiol 573:765–773
38. Ge S, Goh EL, Sailor KA, Kitabatake Y, Ming GL, Song H (2006) GABA regulates synaptic integration of newly generated neurons in the adult brain. Nature 439:589–593
39. Traub RD, Wong RK (1982) Cellular mechanism of neuronal synchronization in epilepsy. Science 216:745–747
40. Miles R, Wong RK (1987) Latent synaptic pathways revealed after tetanic stimulation in the hippocampus. Nature 329:724–726
41. Traub RD, Miles R (1991) Multiple modes of neuronal population activity emerge after modifying specific synapses in a model of the CA3 region of the hippocampus. Ann NY Acad Sci 627:277–290
42. Sipilä ST, Huttu K, Soltesz I, Voipio J, Kaila K (2005) Depolarizing GABA acts on intrinsically bursting pyramidal neurons to drive giant depolarizing potentials in the immature hippocampus. J Neurosci 25:5280–5289
43. Safiulina VF, Zacchi P, Taglialatela M, Yaari Y, Cherubini E (2008) Low expression of Kv7/M channels facilitates intrinsic and network bursting in the developing rat hippocampus. J Physiol 586:5437–5453
44. Sipilä ST, Huttu K, Voipio J, Kaila K (2006) Intrinsic bursting of immature CA3 pyramidal neurons and consequent giant depolarizing potentials are driven by a persistent Na+ current and terminated by a slow Ca2+-activated K+ current. Eur J Neurosci 23:2330–2338
45. Marchionni I, Omrani A, Cherubini E (2007) In the developing rat hippocampus a tonic GABAA-mediated conductance selectively enhances the glutamatergic drive of principal cells. J Physiol 581:515–528
46. Demarque M, Represa A, Becq H, Khalilov I, Ben-Ari Y, Aniksztejn L (2002) Paracrine intercellular communication by a Ca2+- and SNARE-independent release of GABA and glutamate prior to synapse formation. Neuron 36:1051–1061
47. Yue C, Yaari Y (2004) KCNQ/M channels control spike after-depolarization and burst generation in hippocampal neurons. J Neurosci 24:4614–4624
48. Menendez de la Prida LM, Huberfeld G, Cohen I, Miles R (2006) Threshold behavior in the initiation of hippocampal population bursts. Neuron 49:131–142
49. Menendez de la Prida LM, Sanchez-Andres JV (1999) Nonlinear frequency-dependent synchronization in the developing hippocampus. J Neurophysiol 82:202–208
50. Menendez de la Prida L, Sanchez-Andres JV (2000) Heterogeneous populations of cells mediate spontaneous synchronous bursting in the developing hippocampus through a frequency-dependent mechanism. Neuroscience 97:227–241
51. Pape HC (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. Annu Rev Physiol 58:299–327
52. Strata F, Atzori M, Molnar M, Ugolini G, Tempia F, Cherubini E (1997) A pacemaker current in dye-coupled hilar interneurons contributes to the generation of giant GABAergic potentials in developing hippocampus. J Neurosci 17:1435–1446
53. Bender RA, Galindo R, Mameli M, Gonzalez-Vega R, Valenzuela CF, Baram TZ (2005) Synchronized network activity in developing rat hippocampus involves regional hyperpolarization-activated cyclic nucleotide-gated (HCN) channel function. Eur J Neurosci 22:2669–2674
54. Connors BW, Long MA (2004) Electrical synapses in the mammalian brain. Annu Rev Neurosci 27:393–418
55. Crépel V, Aronov D, Jorquera I, Represa A, Ben-Ari Y, Cossart R (2007) A parturition-associated nonsynaptic coherent activity pattern in the developing hippocampus. Neuron 54:105–120
56. Durand GM, Kovalchuk Y, Konnerth A (1996) Long-term potentiation and functional synapse induction in developing hippocampus. Nature 381:71–75
57. Voronin LL, Cherubini E (2004) 'Deaf, mute and whispering' silent synapses: their role in synaptic plasticity. J Physiol 557:3–12
58. Safiulina VF, Fattorini G, Conti F, Cherubini E (2006) GABAergic signaling at mossy fiber synapses in neonatal rat hippocampus. J Neurosci 26:597–608
59. Kasyanov AM, Safiulina VF, Voronin LL, Cherubini E (2004) GABA-mediated giant depolarizing potentials as coincidence detectors for enhancing synaptic efficacy in the developing hippocampus. Proc Natl Acad Sci USA 101:3967–3972
60. Mohajerani MH, Sivakumaran S, Zacchi P, Aguilera P, Cherubini E (2007) Correlated network activity enhances synaptic efficacy *via* BDNF and the ERK pathway at immature CA3-CA1 connections in the hippocampus. Proc Natl Acad Sci USA 104: 13176–13181
61. Goodman LJ, Valverde J, Lim F, Geschwind MD, Federoff HJ, Geller AI, Hefti F (1996) Regulated release and polarized localization of brain-derived neurotrophic factor in hippocampal neurons. Mol Cell Neurosci 7:222–238
62. Lessmann V, Gottmann K, Malcangio M (2003) Neurotrophin secretion: current facts and future prospects. Prog Neurobiol 69:341–374
63. Magby JP, Bi C, Chen ZY, Lee FS, Plummer MR (2006) Single-cell characterization of retrograde signaling by brain-derived neurotrophic factor. J Neurosci 26:13531–13536
64. Poo MM (2001) Neurotrophins as synaptic modulators. Nat Rev Neurosci 2:24–32
65. Fiorentino H, Kuczewski N, Diabira D, Ferrand N, Pangalos MN, Porcher C, Gaiarsa JL (2009) GABA(B) receptor activation triggers BDNF release and promotes the maturation of GABAergic synapses. J Neurosci 29:11650–11661

66. Cohen I, Navarro V, Clemenceau S, Baulac M, Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. Science 298:1418–1421
67. Huberfeld G, Wittner L, Clemenceau S, Baulac M, Kaila K, Miles R, Rivera C (2007) Perturbed chloride homeostasis and GABAergic signaling in human temporal lobe epilepsy. J Neurosci 27: 9866–9873
68. Nabekura J, Ueno T, Okabe A, Furuta A, Iwaki T, Shimizu-Okabe C, Fukuda A, Akaike N (2002) Reduction of KCC2 expression and GABAA receptor-mediated excitation after in vivo axonal injury. J Neurosci 22:4412–4417
69. van den Pol AN, Obrietan K, Chen G (1996) Excitatory actions of GABA after neuronal trauma. J Neurosci 16:4283–4292
70. Coull JA, Beggs S, Boudreau D, Boivin D, Tsuda M, Inoue K, Gravel C, Salter MW, De Koninck Y (2005) BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. Nature 438:1017–1021
71. Funk K, Woitecki A, Franjic-Würtz C, Gensch T, Möhrlen F, Frings S (2008) Modulation of chloride homeostasis by inflammatory mediators in dorsal root ganglion neurons. Mol Pain 4:32–44
72. Lagostena L, Rosato-Siri M, D'Onofrio M, Brandi R, Arisi I, Capsoni S, Franzot J, Cattaneo A, Cherubini E (2010) In the adult hippocampus, chronic nerve growth factor deprivation shifts GABAergic signaling from the hyperpolarizing to the depolarizing direction. J Neurosci 30:885–893
73. Payne JA (1997) Functional characterization of the neuronal-specific K-Cl cotransporter: implications for [K+]o regulation. Am J Physiol 273:C1516–C1525
74. Liu Z, Neff RA, Berg DK (2006) Sequential interplay of nicotinic and GABAergic signaling guides neuronal development. Science 314:1610–1613
75. Capsoni S, Ugolini G, Comparini A, Ruberti F, Berardi N, Cattaneo A (2000) Alzheimer-like neurodegeneration in aged antinerve growth factor transgenic mice. Proc Natl Acad Sci USA 97:6826–6831
76. Ruberti F, Capsoni S, Comparini A, Di Daniel E, Franzot J, Gonfloni S, Rossi G, Berardi N, Cattaneo A (2000) Phenotypic knockout of nerve growth factor in adult transgenic mice reveals severe deficits in basal forebrain cholinergic neurons, cell death in the spleen, and skeletal muscle dystrophy. J Neurosci 20:2589–2601
77. Rosato-Siri M, Cattaneo A, Cherubini E (2006) Nicotine-induced enhancement of synaptic plasticity at CA3-CA1 synapses requires GABAergic interneurons in adult anti-NGF mice. J Physiol 576:361–377
78. Capsoni S, Giannotta S, Cattaneo A (2002) Beta-amyloid plaques in a model for sporadic Alzheimer's disease based on transgenic anti-nerve growth factor antibodies. Mol Cell Neurosci 21:15–28
79. Pesavento E, Capsoni S, Domenici L, Cattaneo A (2002) Acute cholinergic rescue of synaptic plasticity in the neurodegenerating cortex of anti-nerve-growth-factor mice. Eur J Neurosci 15:1030–1036
80. Rivera C, Voipio J, Thomas-Crusells J, Li H, Emri Z, Sipilä S, Payne JA, Minichiello L, Saarma M, Kaila K (2004) Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. J Neurosci 24:4683–4691
81. Peng S, Garzon DJ, Marchese M, Klein W, Ginsberg SD, Francis BM, Mount HT, Mufson EJ, Salehi A, Fahnestock M (2009) Decreased brain-derived neurotrophic factor depends on amyloid aggregation state in transgenic mouse models of Alzheimer's disease. J Neurosci 29:9321–9329
82. Barbato C, Ruberti F, Pieri M, Vilardo E, Costanzo M, Ciotti MT, Zona C, Cogoni C (2010) MicroRNA-92 modulates K(+) Cl(−) co-transporter KCC2 expression in cerebellar granule neurons. J Neurochem 113:591–600