Functional hallmarks of GABAergic synapse maturation and the diverse roles of neurotrophins

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INTRODUCTION

What does the construction of the very first synapses during embryonic development and the generation of new synapses after a stroke in the aged brain have in common? What enables or constrains the formation of new synapses after transplantation of exogenous neurons into an adult brain? How could we possibly stimulate inhibitory synapse formation in an epileptic cortex – or repair the sad consequences of alcohol consumption of a pregnant mother when too many inhibitory neurons are lost in her baby’s brain? – We are still far from an all-embracing answer to these questions, and more work is needed to understand the impact of synaptogenesis on the performance of each given functional system during a particular stage of development. Nonetheless we will try to delineate some basic principles of synapse development that can be regarded as common, at least in the sense that they are applicable to synaptic connections using the inhibitory γ-amino-butyric acid (GABA) as a neurotransmitter of both the hippocampus and the superior colliculus, i.e., two brain structures requiring intact GABAergic inhibition while fulfilling clearly different functions.

The hippocampus is involved in learning and memory formation, but apart from its functions, it has served as a classical model system for very many aspects of synaptic transmission and synapse development (see Ben Ari et al., 2007; McBain and Kauer, 2009). Likewise, the superior colliculus of mammals, or the optic tectum of avians, amphibians, and fish, is a structure that has become quite popular for its retina-, head-, and body-related sensory and motor maps and its role as novelty detectors (see Boehnke and...
Munoz, 2008; Stein et al., 2009 for recent review). As a nearly two-dimensional projection area of retinal and visual cortical afferents it served to identify a number of molecules relevant for the formation of orderly connections (Feldheim and O’Leary, 2010). It also appears to be well suited to study inhibitory synaptogenesis since the superior colliculus is reputed to contain the largest amounts of GABA and the highest density of GABAergic synaptic terminals in the brain (see Grantyn et al., 2004 for references).

In the following we shall highlight eight principles that appear to determine the performance of GABAergic synapses during embryonic and early postnatal development. Findings from the rodent superior colliculus will be discussed and, if available, compared to results from hippocampal preparations under the common assumption that fundamental principles of synapse development and function are shared among many brain regions despite regional tailoring of synapse parameters to the specific developmental and functional requirements. The focus of the discussion will be on functional parameters of synaptic transmission at the expense of molecular determinants of developmental changes. Building on this, we explore to what extent these principles are governed by neurotrophin signaling during development and how they predict characteristics of GABAergic synapses in lesioned brain tissue or during adult neurogenesis.

**FUNCTIONAL MATURATION OF GABAergic SYNAPSES**

**SYNAPTIC TRANSMISSION STARTS WITH GABA**

The role of GABA as a “pioneer transmitter” (Ben Ari et al., 2007) implies a variety of paracrine actions of this neurotransmitter along with a lead in synaptogenesis. The rule of GABAergic lead during the formation of network activity is based on patch clamp recordings from hippocampal slices of newborn rats where AMPAR-mediated synaptic events were rare or entirely missing while GABAergic inhibitory postsynaptic currents (IPSCs) could either be induced by electrical stimulation or detected as spontaneously occurring events (Hollrigel and Soltesz, 1997; Tyzio et al., 1999).

In the superior colliculus, action potential-mediated spontaneous and unitary evoked synaptic currents did indeed occur in the absence of glutamatergic synaptic activity at E17 (Grantyn et al., 2004), i.e., at an age when inhibitory synaptic currents were first seen in the retina (Unsoeld et al., 2008). Furthermore, evaluation of double immunostaining using antibodies against synaptophysin and the vesicular GABA transporter vGAT showed that at E17 all presynaptic puncta were GABAergic (Figure 1A). Even at postnatal day (P) 0 the fraction of GABAergic terminals was as high as 87% (J. Walter and R. Grantyn, unpublished result).

The GABAergic lead during development has been attributed to earlier differentiation and functionality of GABAergic interneurons as opposed to glutamatergic principal cells (Gozlan and Ben Ari, 2003). As for the function of GABAergic pioneer synapses, one has to consider their depolarizing polarity (see below, part 6) and the associated capacity to generate local Ca\(^{2+}\) transients which make them a good substitute for the lack of glutamatergic inputs. Synaptic GABA release could also contribute to some initial steps of Ca\(^{2+}\)-dependent neuron differentiation, such as dendrite and axon outgrowth (see Sernagor et al., 2010), and it might assist the formation of glutamatergic afferents by producing giant depolarizing potentials. The latter concept of GABAergic excitation driving maturation of the glutamatergic synaptic phenotype, also termed “ménage à trois,” has attracted particular attention (Ben-Ari et al., 1997). Interestingly, GABAergic synaptic transmission could not only precede glutamatergic transmission within distinct sets of synapses, but one and the same synapse type was shown to shift from an initial GABAergic phenotype to a mixed glutamatergic/GABAergic and, later on, to a predominantly glutamatergic phenotype.
A distinct feature of GABAergic synaptic transmission in the embryonic mouse brain is the large amplitude of action potential-mediated spontaneous or evoked IPSCs (sIPSCs, eIPSCs, respectively; Cohen et al., 2000; Kirischuk et al., 2005) and their pronounced fatigue under condition of rapid and repetitive activation. This is illustrated in Figure 1B. In an E17 superior colliculus slice, the well-defined eIPSCs induced by the first stimulus preceded a complete failure after the second. When applying a stimulus train larger eIPSCs were only generated after preceding failures, and there was a tendency for asynchronous release after the stimulus trains (Figure 1C), suggesting a protracted elevation of presynaptic Ca\(^{2+}\) concentration (Kirischuk and Grantyn, 2003).

Were these large responses induced by one or several synaptic terminals? – According to the “one site-one vesicle”-hypothesis of synaptic transmission (Korn and Faber, 1987) one might be tempted to suggest that the connections in the embryonic colliculus must be composed of multiple contacts and/or multiple active zones per ending. However, the electron microscopy (EM) images from P1 colliculi and evaluation of immunostained synaptic terminals in E17 tecta indicate that this is rather unlikely (Lund and Lund, 1972; Juettner et al., 2005). Taking into account that under the given experimental conditions miniature IPSC (mIPSC) amplitudes amounted to 25–30 pA we concluded that 10–15 vesicles may simultaneously be released from just one site.

The possibility of multivesicular release has also been considered for inhibitory synapses in hippocampal cultures (Fedulova and Veselovsky, 2002) and hippocampal slices (Biro et al., 2006) while data from small glutamatergic synapses appears contradictory (see Oertner et al., 2002; Huang et al., 2010 vs. Chen et al., 2004). Kirischuk et al. (1999) characterized the release of GABAergic from single synaptic terminals in dissociated cultures from the embryonic rat tectum. Selective stimulation of axon terminals after loading a Ca\(^{2+}\) indicator allowed us to record single-bouton-evoked stimulus-locked IPSCs (sIPSCs) along with the respective asynchronous delayed IPSCs (dIPSCs; Figures 2A,B) and presynaptic Ca\(^{2+}\) transients. An estimate of quantal size (Q) can be extracted from amplitude distributions of sbIPSCs and dIPSCs (Figure 2C). Dividing maximal or mean sbIPSC amplitudes by the experimentally derived values of Q (Figures 2D,E) one directly obtains the maximal or mean quantal content (m) (Kirischuk and Grantyn, 2002). Even under physiological conditions \(\frac{\left[Ca^{2+}\right]}{[Mg^{2+}] = 2}\), the mean m value was larger than 1 in 30 out of 40 tested boutons (range: 0.4–6), and the maximal m reached 8–12. At any given synapse, the mean sIPSC amplitudes changed with the third power of the presynaptic bulk Ca\(^{2+}\) concentration (Figure 2F). With few exceptions, these boutons had one active zone per terminal only, which lead us to suggest that these immature GABAergic terminals had the capacity to release several vesicles from just one docking site (Kirischuk and Grantyn, 2002).

It should be mentioned that simultaneous release of several transmitter quanta can even occur spontaneously, due to random elevations of presynaptic \([Ca^{2+}]\), as observed in basket cell terminals in slices of the postnatal rat cerebellum (Llano et al., 2000b). Lowering extracellular \([Ca^{2+}]\) would decompose “giant” mIPSCs and eventually limit spontaneous synaptic activity to monoquantal events.

### IMMATURE SYNAPTIC TERMINALS RELEASE VESICLES WITH HIGHER PROBABILITY THAN MATURE SYNAPSES

Presynaptically, synapse maturation is characterized by the formation of multiple release sites and the differentiation of the release machinery which encompasses a complex set of changes affecting docking, molecular and positional priming, fusion, site clearing, and several pathways for replenishment of the vesicle pool (Neher and Sakaba, 2008; Pang and Sudhof, 2010). Most commonly, changes in the release are characterized by invoking the statistical parameter “average probability of release” (Pr), often defined as the likelihood that any contact of a synaptic connection would liberate one quantum of the transmitter in response to a presynaptic action potential. In most cases Pr is not directly accessible for measurement, but determined by binomial fitting (for instance, Stricker et al., 1996), variance–mean analysis (Clements and Silver, 2000) or covariance analysis (Scheuss and Neher, 2001). In the frame of the binomial model of synaptic transmission, the unitary postsynaptic response (i.e., the response obtained by activation of just one presynaptic neuron) would reflect the product of N, Pr, and Q. N being the total number of synapses/active zones/docking sites formed by the presynaptic cell.

In synapses with multivesicular release, presynaptic differentiation can also be characterized on the basis of changes of m, the mean quantal content (Taschenberger et al., 2005). In view of the difficulties to accurately determine the number of active zones participating in release (or the structure-based “histological N”) it has become acceptable to disregard the site-dependent heterogeneity of individual release sites and to conceptually merge all the vesicles to one common pool, an approach first introduced for the calyx of Held (Sakaba et al., 2002) and later extended to general models of quantal synaptic transmission (Neher and Sakaba, 2008; Pan and Zucker, 2009), where N becomes the number of vesicles in the readily releasable pool (RRP). The average probability that a given vesicle is released from that pool is \(p_{\text{ex}}\). (Heterogeneous vesicle pools and different modes of vesicle fusion are complexities to
FIGURE 2 | Multivesicular release from single GABAergic boutons in cultures from the E20 rat superior colliculus. (A) Types of synapses selected for direct application of depolarizing stimuli to single presynaptic terminals in the presence of action potential block with tetrodotoxin. Left panel: Phase contrast images; middle panels: fluorescent images showing same view fields after up-take of FM1-43; right panels: magnified synaptic sites with phase contrast optics. (B) Specimen records of single-bouton-activated IPSCs (sbIPSCs, lower trace) and respective stimulating current (upper trace). (C) Amplitude distribution and binomial fitting of sbIPSCs (bars and solid line) and dIPSCs (dashed line) for the solitary bouton illustrated in the upper row images of (A). dIPSCs were sampled during a period of 250–500 ms after the pulse. (D,E) Quantification of the results for maximal and mean sbIPSCs suggesting a quantal content >1. (F) Relationship between the mean sbIPSC amplitude and the maximal amplitude of the presynaptic bulk Ca2+ transient [Ca2+]pre recorded from a presynaptic area delineated on the basis of vesicular staining. (Modified from Kirischuk et al., 1999).
be considered in more elaborate reflections on presynaptic vesicle release).

As a first approximation, $p_{ves}$ can be determined using experimental protocols that deplete the RRP, for example a high-frequency stimulation (Schneggenburger et al., 1999; Kirischuk and Grantyn, 2000; Hanse and Gustafsson, 2001). The GABAergic synapses of the postnatal mouse superior colliculus frequency stimulation (Schneggenburger et al., 1999; Kirischuk et al., 2005). The GABAergic synapses have been studied at some detail in our lab using single-bouton activation in the presence of TTX or Ca$^{2+}$ channel blockers to suppress co-activation of other boutons in contact with the postsynaptic cell (Kirischuk et al., 2002). This approach has the advantage that one and the same synaptic terminal is reliably stimulated with each trial, presynaptic depolarizations can be graded and presynaptic Ca$^{2+}$ transients could serve as indicators of presynaptic activation (Kirischuk et al., 1999). The experiments showed that at short intervals (<100 ms) different depressant mechanisms occur simultaneously but differ in their recovery kinetics.

The rapidly recovering paired pulse depression (PPD$_{fast}$), as seen at interstimulus intervals of 25–50 ms, is release-dependent (the amplitude of the second eIPSC being inversely proportional to the amplitude of the first one) and strongly affected by the extracellular Ca$^{2+}$ concentration (Jensen et al., 1999; Chen et al., 2004). Developmental changes of PPD$_{fast}$ can be expected due to the maturation of presynaptic Ca$^{2+}$ buffering (for instance, Llano et al., 2000a) or changes in the patterns of presynaptic G-protein coupled receptor expression. Both mechanisms account for neuron-specific differences of paired pulse plasticity in the adult brain (see, for instance, Senn et al., 1998; Foncer et al., 2000) while at the onset of GABAergic synaptogenesis modulatory diversity is low. Even a GABA(B)R-mediated contribution to paired pulse plasticity is missing in immature GABAergic synapses (Wilcox and Dichter, 1994; Jensen et al., 1999; Kirischuk et al., 2002), and the predominant type of paired pulse behavior is depression.

With further synapse maturation the amount of PPD decreases (Juettner et al., 2001), and this change can be ascribed to a decrease in $p_{ves}$ (Kirischuk et al., 2005). Raising the presynaptic Ca$^{2+}$ influx by increasing extracellular Ca$^{2+}$ levels (Senn et al., 1998), prolonging presynaptic depolarization (Kirischuk et al., 2002) or blocking G-protein mediated depression by N-ethylmaleimide (NEM) (Kirmse and Kirischuk, 2006) can enhance $p_{ves}$ and turn an already established paired pulse facilitation (PPF) into PPD.

A much more slowly recovering form of PPD can be isolated at intervals of 1 s (PPD$_{slow}$). In contrast to PPD$_{fast}$, PPD$_{slow}$ found was to be calcium- and release-independent. This type of depression has first been described for neuromuscular junctions (Betz, 1970), the squid giant synapse (Hsu et al., 1996), the calyx of Held (Bellingham and Walmsley, 1999; Borst and Saksman, 1999), and a synapse formed by the Mauthner cell axon in the goldfish (Waldeck et al., 2000). PPD$_{slow}$ has been incorporated in the contemporary models of transmitter release under the term “transient refractoriness” (Zucker and Regehr, 2002; Pan and Zucker, 2009) or “site clearing” (Neher and Sakaba, 2008). Despite its prominence, at least in immature synapses (see Kirischuk et al., 2002), it still awaits detailed characterization at a molecular level along with a more systematic testing for developmental changes.

It should be noted that the probability of obtaining a postsynaptic response would depend both on $p_{ves}$ and the probability that a given site (active zone) is available for release. Therefore,
to obtain a full description of the developmental changes in the performance of a given type of synaptic connections binomial analysis or variance–mean analysis (Silver, 2003) need to be performed to determine Pr in addition to \( \rho_{\text{pres}} \).

Apart from estimating the quantal parameters of synaptic transmission, the reliability or fatigability of a synaptic connection could be tested using the average eIPSC amplitude reached during the last 10 trials of a series of high-frequency pulses (50–100 Hz). This reveals the so-called tetanic depression of synaptic transmission. If normalized to Q and RRP, this parameter is best suited to reflect the age-dependent changes in the release performance (Kirischuk et al., 2005). Tetanic eIPSC depression was strong at P3–6, but decreased at P11–P15. Although developmental acceleration of the RRP replenishment rate cannot be excluded, the observed decrease of release probability accompanied with the increase of RRP size may underlie these changes. Interestingly, the decrease of tetanic depression coincides with the time of in-growth of cortical afferents and the massive up-regulation of glutamatergic synaptic transmission prior to eye opening at day P14–15 (see Aamodt and Constantine-Paton, 1999; Grantyn et al., 2004 for more). Experimentally, presynaptic GABA release can be modified by allowing cortical afferents to grow into tectal tissue (Henneberger et al., 2007) indicating that indeed changes in glutamatergic innervation shape the function of pre-existing GABAergic synapses.

**SYNAPSE MATURATION IS CHARACTERIZED BY A DOMINANCE OF SYNCHRONOUS OVER ASYNCHRONOUS RELEASE**

A developmental study in the calyx of Held has reported a changing relationship between synchronous and asynchronous release (Chuhma et al., 2001; Yang and Xu-Friedman, 2010). Delayed release might even be present when evoked release is missing, and the two modes of release display a differential dependency on the presynaptic Ca\(^{2+}\) concentration in the vesicle area ([\(\text{Ca}^{2+}\)]\(_{\text{ves}}\)) (Kirischuk and Grantyn, 2003; Yang and Xu-Friedman, 2010). Asynchronous IPSCs (aIPSCs) were sampled during a train of high-frequency stimulation and a synchrony index of release was defined for the period of the last 10 intervals of the high-frequency train by dividing the charge transfer of stimulus-locked eIPSCs by the charge transfer of unlocked aIPSCs. It was found that this index increased with age, showing that the relative number of vesicles released in a stimulus-locked manner increases when neurons mature. As the delayed component of asynchronous release (charge of dIPSCs) displayed a developmental decrease as well, one can conclude that asynchronous release is a characteristic feature of immature synapses, being replaced by action potential–locked transmission at older age. Again, the changes were biggest around the time of eye opening (i.e., shortly after the time of massive in-growth of glutamatergic synapses).

While asynchronous release may be more pronounced in immature GABAergic connections, its persistence at more mature stages will depend on the type of interneuron activated and the Ca\(^{2+}\)-binding proteins expressed (Daw et al., 2010).

**IN IMMATURE NEURONS GABA ACTS AS DEPOLARIZING TRANSMITTER**

The pioneer role of GABA at initial stages of circuit formation in the brain has much to do with its depolarizing action (Cherubini et al., 1991; Ben Ari et al., 2007). The latter has long ago been discovered in the immature rat striatum (Mitsudome et al., 1987) and the immature rabbit and rat hippocampus (Mueller et al., 1984; Cherubini et al., 1990). In the superior colliculus the depolarizing/excitatory action of GABA is prominent at P0–P1 (Juettner et al., 2001), but it already disappears by P3 (Grantyn et al., 2004). In the hippocampus, the change in the polarity of GABA action occurs around P5 (Ben Ari et al., 1989) and has been attributed to the developmental up-regulation of the expression and membrane targeting of KCC2, a Cl\(^{−}\) exporter (Rivera et al., 1999; Ganguly et al., 2001; Hubner et al., 2001). High activity of KCC2 in relation to the activity of NKCC1, a Cl\(^{−}\) importer, would ensure low intracellular Cl\(^{−}\) concentrations and, consequently, a hyperpolarizing GABA action (Blaesse et al., 2009).

However, a developmental switch from depolarizing to hyperpolarizing GABA is not observed in all developing neurons (Banke and McBain, 2006) and, where present, even parts of the somatodendritic plasma membrane can differ with regard to their local chloride gradients (Gulledge and Stuart, 2003). Whether or not at the end shunt inhibition or excitatory GABA actions will dominate the overall effect of GABA on the neuronal output will, first of all, depend on the spatio-temporal relationships of the respective chloride channels with the glutamatergic inputs (see Bracci and Panzeri, 2006).

**SYNAPSE MATURATION IMPLIES IPSC SHORTENING DUE TO AN INCREASE IN ALPHA1 SUBUNIT EXPRESSION**

The developmental shortening of postsynaptic currents is a widely observed phenomenon in many brain areas, including the rodent hippocampus (Cohen et al., 2000; Hutcheon et al., 2004) and superior colliculus (Juettner et al., 2001; Henneberger et al., 2005a; Kirischuk et al., 2005). The slow decay kinetics of IPSCs shortly after birth has been associated with the high expression level of the alpha3 subunit of the GABA(A)R, while the subsequent shortening of IPSCs was attributed to an up-regulation of the alpha1/alpha3 ratio.

Again, the most interesting questions concern the cause and the function of this phenomenon. We have tried to determine the timing of the developmental switch from slow to fast IPSCs in the superior colliculus and found that most of the change occurs during days P6 and 15, i.e., prior to eye opening (around P14–15 in mice, the day of birth being P0). It also coincided with the developmental peak of the NMDAR-mediated charge transfer in the glutamatergic synaptic currents and might be an activity-dependent phenomenon.

We therefore considered the possibility that glutamatergic/NMDAR-mediated activity provided the drive for the switch in the GABA(A)R subunit composition. Respective culture experiments with chronic exposure to MK-801 confirmed this suggestion, while block of mGluR receptor activity with S-MCPG had no effect on IPSC decay kinetics (Henneberger et al., 2005a).

It is particularly telling that the decrease of IPSC duration also coincided with the increase of the synchrony index and the reduction of tetanic depression of the phasic stimulus-locked release. One may speculate that by the time of onset of patterned vision GABAergic synapses assume...
As this effect could be reversed by transfecting hippocampal neurons with the chloride exporter KCC2, it was suggested that the chloride conductance, RRP or p_{res} of evoked release, but it augmented asynchronous release (Figures 3E,G) – the characteristic feature of immature collicular synapses (see above, part 5). This could be a consequence of increased levels of the GABA-synthesizing enzyme GAD after BDNF treatment. Indeed, like hippocampal neurons (Aguado et al., 2003) collicular neurons reacted to BDNF with an increase in presynaptic GAD65 levels (Henneberger et al., 2005b), and the latter is known to facilitate asynchronous release (Tian et al., 1999). In some preparations, including hippocampal slice cultures, addition of exogenous BDNF was reported to produce a higher yield of GAD-labeled presynaptic terminals (Marty et al., 2000).

On the postsynaptic side (Figure 3H), BDNF treatment of cultured collicular neurons resulted in a suppression of GABAergic synaptic transmission (Tanaka et al., 1997; Brüning et al., 2001) which could be attributed to a reduction in the number of open channels contributing to the IPSCs without affecting their single channel conductance, RRP or p_{res} (Henneberger et al., 2005b). In principle, the depressant effect of BDNF on the postsynaptic response to GABA could reflect reduced neurotransmitter loading into vesicles and thus a smaller number of open postsynaptic receptors/channels during transmission. However, we are not aware of any direct evidence supporting this interesting alternative hypothesis and would tentatively conclude that a reduction in postsynaptic receptor number accounts for most of the BDNF-mediated inhibition of GABAergic transmission elicited by single action potentials (Brüning et al., 2001).

Similar or related results were obtained in the intact superior colliculus of bdnf−/− mice, however only after P13/14, i.e., at a more mature stage of development.
FIGURE 3 | Measurement of RRP, $p_{var}$, and Q in collicular neurons, and the effects of BDNF. (A) Specimen trace of eIPSCs induced by high-frequency (HF) stimulation (20 pulses at 50 Hz) of a single GABAergic axon. For clarity stimulus artifacts are replaced by triangles. (B) Cumulative plot of eIPSC amplitudes vs. stimulus number. The eIPSC amplitudes were normalized to the median dIPSC amplitude of the same unitary connection (inset). Back-extrapolation to the y intercept indicates RRP. (C,D) Tests for applicability of the estimates of $p_{var}$. (E) Sample records to illustrate BDNF effects on IPSCs induced by HF stimulation. To obtain the amplitude of the steady state current, the current integral was normalized to the total time of integration for the last five stimulus intervals. Arrow heads denote the peak level of the eIPSC after the first pulse in the train. Note that BDNF does not affect the steady state current but significantly reduces the ratio between steady state current and first eIPSC (inset). (F) BDNF increases the time constant of decay of the “synaptic tail current,” i.e., the current produced by dIPSCs after the stimulus train, as estimated by single exponential fit. (G) Sample record of the postsynaptic response to HF stimulation of a single GABAergic axon. Note the presence of dIPSCs (inset: enlarged) after the end of stimulation. (H) Reduction of dIPSC amplitudes as evidence for a depressant postsynaptic effect of BDNF, in contrast to the absence of significant changes in the range of coefficient of variation (C), paired pulse ratio, and $p_{var}$. (Modified from Henneberger et al., 2005b).
Among the stimuli inducing inhibitory synaptogenesis are factors liberated from damaged tissue. Several labs have described the near future. Still very little is known on the possible role of NGF during in situ development of GABAergic synapses.

We have explored some acute effects of added NGF in hippocampal cultures and found that NGF promoted GABAergic synaptogenesis (Salama-Cohen et al., 2006). The effects of NGF included:

1. TrkA-mediated up-regulation of VGAT expression
2. An increase in the number of VGAT-immunopositive synaptic terminals in contact with hippocampal neurons
3. A prominent reduction in the E/I ratio of contacting boutons.

The final outcome of exogenous NGF depended on its depressant action on the proneural gene neurogenin 3 (Ngn3), a nuclear transcription factor that is also under the control of Hes1/5.

Our results suggest that with regard to the glutamate/GABA or E/I balance of synaptic transmission and development BDNF and NGF might assume antagonistic roles. Therefore, to further explore the significance of NGF for the development and function of GABA synapses might be one of the most rewarding tasks in the near future.

**ACUTE GABAergic SYNAPTGENESIS AFTER LESION**

Among the stimuli inducing inhibitory synaptogenesis are factors liberated from damaged tissue. Several labs have described “inhibitory sprouting” in the immediate environment (Mittmann and Eysel, 2001) or the terminal area (Deller et al., 1995) of lesioned cells.

Consistent with the pioneer role of GABA at initial stages of synaptogenesis (see above, part 6), our investigation of synapse formation after slicing of late embryonic or neonatal rodent superior colliculi revealed that at this age lesion preferentially facilitates the formation of new GABAergic synapses (Meier et al., 2003). The up-regulation of GABAergic synapses was inferred from the increase of vGAT- or GAD65-positive terminals, mIPSC frequency, and postsynaptic GABA(A)R immunofluorescence. GABAergic synaptogenesis could be prevented/facilitated by blocking/enhancing, respectively, PKC activation, suggesting the involvement of phosphorylation-dependent mechanisms. Manipulations assisting the return of globally elevated intracellular Ca2+ levels to resting Ca2+ concentrations promoted this form of reactive synaptogenesis in the neonatal superior colliculus (J. Walter, C. Henneberger, J. C. Meier and R. Grantyn, unpublished observation).

**GABAergic SYNAPTGENESIS DURING INTEGRATION OF NEWLY GENERATED NEURONS IN THE ADULT BRAIN**

Adult neurogenesis represents an important response of the damaged as well as the learning brain (see Ma et al., 2010). Notably in the dentate gyrus newly formed neurons integrate into pre-existing networks, which requires the formation of new inhibitory synapses (Tozuka et al., 2005; Wang et al., 2005; Toni and Sultan, 2011). The functional properties of nascent GABAergic synapses and their subsequent development were shown to reproduce some essential features of immature synapses in the embryonic and neonatal brain: there was a GABA lead in the innervation of newborn granule cells, the initial action of GABA was depolarizing, the decay kinetic was slow, and synaptic currents were relatively insensitive to zolpidem (Overstreet-Wadiche et al., 2005; Ge et al., 2006; Karten et al., 2006). Preventing a depolarizing GABA action by knock-out of NKCC1 reduced and delayed GABAergic synaptogenesis (Ge et al., 2006).

These results validate major milestones, as defined above for the ontogeny of inhibitory synaptic connections in the brain, and suggest that the outlined developmental mechanisms may apply irrespective of the given local conditions of a neuronal network.
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