J Physiol 587.9 (2009) pp 1881–1888

The Journal of Physiology

SYMPOSIUM REPORT

Activity-dependent development of inhibitory synapses and innervation pattern: role of GABA signalling and beyond

Z. Josh Huang

Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA

GABA-mediated synaptic inhibition is crucial in neural circuit operations. The development of GABAergic inhibitory synapses and innervation pattern in mammalian neocortex is a prolonged process, extending well into the postnatal period, and is regulated by neural activity and experience. Accumulating evidence supports the hypothesis that GABA signalling acts beyond synaptic transmission and regulates inhibitory synapse development; in other words, similar to glutamate signalling at developing excitatory synapses, GABA may coordinate pre- and post-synaptic maturation at inhibitory synapses. These findings raise numerous questions regarding the underlying mechanisms, including the role of GABA receptors and their link to synaptic adhesion molecules. Since synapse formation is a crucial component of axon growth, GABA signalling may also shape the axon arbor and innervation pattern of inhibitory neurons. A mechanism unique to GABAergic neurons is activity-dependent GABA synthesis, largely mediated through activity-regulated transcription of the rate-limiting enzyme GAD67. Such cell-wide as well as synaptic regulation of GABA signalling may constitute a mechanism by which input levels and patterns onto GABAergic neurons shape their innervation pattern during circuit development.

(Received 19 December 2008; accepted 19 January 2009; first published online 2 February 2009)
Corresponding author Z. J. Huang: Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA.
Email: huangj@cshl.edu

In many areas of the mammalian brain, such as the neocortex, neural circuits rely on inhibition mediated by γ-aminobutyric acid (GABA) from diverse cell types to control the spatiotemporal patterns of electrical signalling (Markram *et al.* 2004). The inhibitory output of GABAergic neurons is distributed in the network through their axons and synapses, which constitute elaborate and cell-type-specific innervation patterns (Huang *et al.* 2007). A prominent feature of GABAergic axon arbors in neocortex is their local exuberance: a single interneuron often produces extensive local arbors that innervate hundreds of neurons in its vicinity and form multiple clustered synapses onto each target neuron (Tamas *et al.* 1997; Wang *et al.* 2002). Such an innervation pattern probably contributes to their efficient control over the activity patterns in local cell populations. For example, a single parvalbumin-containing (PV) basket interneuron innervates hundreds of pyramidal neurons at the soma and proximal dendrites, and controls the output and synchrony of pyramidal neurons (Fig. 1; Cobb *et al.* 1995; Miles *et al.* 1996; Tamas *et al.* 1997). Furthermore, PV basket cells form extensive mutual innervation (Tamas *et al.* 2000) and, together with their unique physiological properties, contribute to the generation of coherent network oscillations that might organize functional neural ensembles (Bartos *et al.* 2007).

The development of a mature GABAergic innervation pattern is often a prolonged process, extending well into the postnatal period. In the dentate gyrus of hippocampus, basket cell axon arbors undergo marked maturation between the first and fourth week, and increased connectivity among basket cells contributes to the enhanced coherence of gamma oscillation in local networks (Doischer *et al.* 2008). In primary visual cortex, the maturation of perisomatic inhibition by basket interneurons proceeds into the fifth postnatal week and

This report was presented at *The Journal of Physiology* Symposium on *Mechanisms of neocortical development*, which took place on 14 November 2008 at the annual meeting of the Society for Neuroscience in Washington, DC. It was commissioned by the Editorial Board and reflects the views of the authors.

DOI: 10.1113/jphysiol.2008.168211

may contribute to the regulation of the critical period of plasticity (Huang *et al.* 1999; Morales *et al.* 2002). Importantly, the maturation of inhibitory innervation in visual and somatosensory cortex is regulated by sensory experience (Morales *et al.* 2002; Chattopadhyaya *et al.* 2004; Jiao *et al.* 2006). Such activity-dependent development of inhibitory synapses and innervation pattern is a major component of neural circuit assembly, yet the underlying cellular and molecular mechanisms are poorly understood.

GABA signalling regulates inhibitory synapse development

As key mediators of neural activity, neurotransmitters are particularly well suited to couple synaptic signalling with synaptic wiring (Zhang & Poo, 2001; Hua & Smith, 2004). Glutamate, the major excitatory transmitter in vertebrate brain, has been implicated in regulating many aspects of synapse formation, maturation and plasticity (Zheng *et al.* 1994; Shi *et al.* 1999; Carroll *et al.* 1999; Wong & Wong, 2001; Bonhoeffer & Yuste, 2002; Malinow & Malenka, 2002; Tashiro *et al.* 2003). In addition, through regulating synaptogenesis, glutamate receptor signalling contributes to activity-dependent development of axonal and dendritic arbors (Ruthazer *et al.* 2003; Hua & Smith, 2004; Hua *et al.* 2005; Cline & Haas, 2008).

Initially discovered as an inhibitory transmitter, GABA has since been implicated in multiple processes of neural development, from cell proliferation to circuit formation (Owens & Kriegstein, 2002). The trophic effects of GABA on neuronal migration and neurite growth during the embryonic and perinatal period are largely explained by

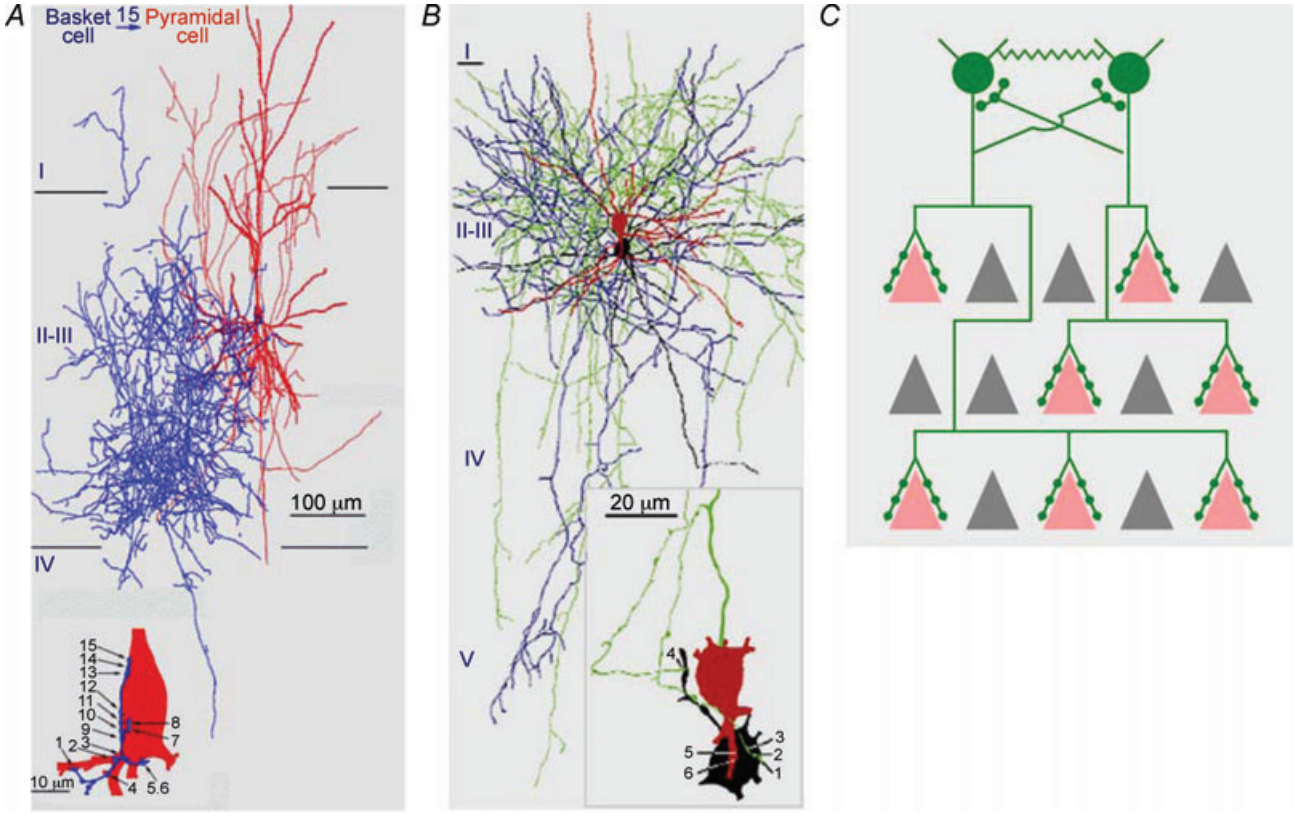

Figure 1. Perisomatic innervation pattern of the neocortical basket interneurons
A, highly exuberant axonal arborization of a neocortical basket interneuron (blue) and one of its many postsynaptic pyramidal cells (red). Although the basket axon overlaps with a large part of the pyramidal basal dendritic tree, all 15 electron microscopically verified synaptic junctions (bottom panel, right) are clustered around the soma or the most proximal dendrites (bottom panel, left) (adapted from Tamas *et al.* 1997). *B*, reconstructions of the two PV basket cells connected by both chemical and electrical synapses (presynaptic cell: soma and dendrites, red; axon, green; postsynaptic cell: soma, dendrites, black; axon blue). Cortical layers (I–V) are indicated on the left. The electron microscopically identified synaptic junctions (1 – 4) and gap junctions (5, 6) mediating the interaction between the coupled cells were found nearby on the soma and a proximal dendrite (inset) (adapted from Tamas *et al.* 2000). *C*, a schematic showing prominent features of the innervation pattern of cortical basket interneurons. A single basket cell axon (green) innervating the many pyramidal neurons (pink) in its vicinity with clusters of perisomatic synapses (green dots). Basket cells also innervate other basket cells via chemical and electrical (zigzaged lines) synapses. Grey triangles represent pyramidal neurons that are not innervated by these basket cells.

its depolarizing action in immature neurons, resulting from chloride ion efflux through the $GABA_A$ receptor, which triggers calcium influx and signalling (Ben-Ari *et al.* 1989; Leinekugel *et al.* 1995). During the post-natal period, the up-regulation of the chloride transporter KCC2 in neurons results in increased extrusion of intracellular chloride (Rivera *et al.* 1999), and GABA assumes its classic role as an inhibitory transmitter (Ben-Ari *et al.* 2007).

Recently, several studies converge and suggest that, in addition to mediating synaptic inhibition in more mature circuits, GABA signalling promotes and coordinates pre- and post- synaptic maturation during activity-dependent development of inhibitory synapses and innervation (Fig. 2). A main line of evidence came from studying the effects of altering GABA synthesis on the development of perisomatic synapses from PV basket interneurons in the visual cortex. The maturation of many features of basket cell axon arbors and perisomatic synapses can be recapitulated in cortical organotypic cultures (Di Cristo *et al.* 2004) and is strongly regulated by neuronal activity (Klostermann & Wahle, 1999; Chattopadhyaya *et al.* 2004). Genetic knockdown of GABA synthesis implicates GABA signalling itself in the development of perisomatic synapses (Chattopadhyaya *et al.* 2007). GABA is synthesized by two glutamate decarboxylases, GAD67 and GAD65 (Soghomonian & Martin, 1998). Of these two enzymes, GAD67 is the rate-limiting enzyme and influences cellular GABA contents in a dosage-dependent manner (Asada *et al.* 1997; Ji *et al.* 1999). Knockdown of GAD67 in single GABAergic interneurons, which should have minimum impact on circuit activity levels, results in profound cell autonomous deficits in synapse formation, axon branching and innervation field in cortical organotypic cultures; such deficits were partially rescued by blocking GABA re-uptake or enhancing $GABA_A$ or $GABA_B$ receptor function (Chattopadhyaya *et al.* 2007). Similar deficits were found in visual cortex of Gad67 germline heterozygotes, which show ~40% reduction of GABA levels (Chattopadhyaya *et al.* 2007). Conversely, overexpression of Gad67 in single basket interneurons promotes the maturation of perisomatic synapses (Chattopadhyaya *et al.* 2007). These results demonstrate that GABA acts beyond inhibitory

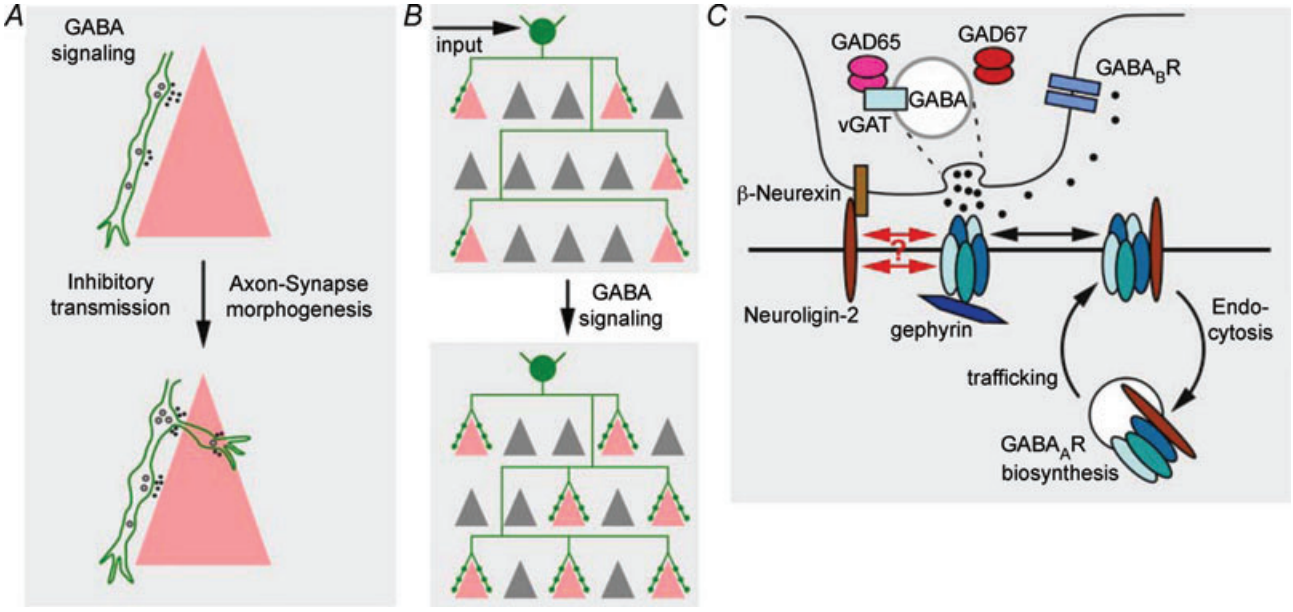

Figure 2. GAD67 and GABA act beyond inhibitory transmission and regulate inhibitory synapse development and innervation patterns
A, GABA signalling may regulate the morphogenesis of inhibitory synapses. *B*, since synapse formation is an integral part of axon growth and branching, activity-dependent GABA signalling may further influence the development of GABAergic axon arbor and innervation pattern. *C*, a hypothetical model depicting how GABA–GABA receptor signalling and neuroligin–neurexin adhesion may interact and co-operate to regulate the development of inhibitory synapses. Pentameric $GABA_A$Rs are assembled in the endoplasmic reticulum. Most $GABA_A$Rs are first delivered to extrasynaptic locations, they then either diffuse to and become trapped at postsynaptic sites or undergo endocytosis. NL2 and synaptic $GABA_A$Rs stabilize each other, either through intracellular reciprocal interactions aided by scaffolding proteins such as gephyrin or through extracellular *cis* interaction. In addition, GABA activation of $GABA_A$Rs might further stabilize synaptic $GABA_A$Rs through structural changes or signalling mechanisms. Such activity- and GABA-mediated stabilization of $GABA_A$R might further increase the levels of NL2 at cell–cell contacts and, in turn, stabilize presynaptic terminals through trans-synaptic interactions with neurexins.

transmission in juvenile and adolescent brain, and regulates the maturation of inhibitory synapses and innervation patterns (Fig. 2), thus revealing a new facet of GABA function distinct from its early tropic action in neonatal brain.

Structural role of $GABA_A$ receptors: coupling transmission to synapse maturation and stability

Another line of evidence supporting a role of GABA on the development of inhibitory synapses came from studying the effects of manipulating $GABA_A$ receptor ($GABA_AR$) subunits. $GABA_ARs$ are heteropentameric chloride channels composed of several classes of subunits (Michels & Moss, 2007). Although over 19 subunits have been identified, giving rise to a large number of possible subunit combinations, the vast majority of $GABA_ARs$ consist of α, β and $\gamma 2$ subunits in a 2 : 2 : 1 stoichiometry. In the mature brain, $GABA_ARs$ are primarily localized at postsynaptic and extrasynaptic membranes where they mediate phasic and tonic inhibition, respectively.

The $\gamma 2$ subunit is essential for accumulation of cell surface $GABA_ARs$ at postsynaptic sites (Essrich *et al.* 1998; Schweizer *et al.* 2003). Acute suppression of $\gamma 2$ expression in cultured hippocampal neurons not only disrupts $GABA_AR$ clustering but also results in a profound reduction of GABAergic innervation of $\gamma 2$-deficient neurons (Li *et al.* 2005; Fang *et al.* 2006). Moreover, when palmitoylation of the $\gamma 2$ subunit was suppressed by knockdown of the Asp-His-His-Cys (DHHC) family palmitoyltransferase GODZ, trafficking of GABARs to postsynaptic sites was perturbed and GABAergic innervation was reduced (Fang *et al.* 2006). As both presynaptic GABA and postsynaptic $GABA_A$ receptors influence GABAergic synapse development, a simple hypothesis is that activity-dependent GABA signalling promotes the differentiation of pre- and post-synaptic sites, and coordinates the maturation and stabilization of inhibitory synapses.

Further evidence regarding the role of $GABA_ARs$ in synapse formation came from studies of Purkinje neurons in the cerebellum. Purkinje cells are themselves GABAergic neurons but also receive two types of GABAergic inputs: the axo-somatic synapses from basket interneurons and the axo-dendritic synapses from stellate interneurons, both with $GABA_ARs$ containing the $\alpha 1$ subunit. Deletion of the $\alpha 1$ subunit gene results in a complete loss of functional $GABA_ARs$ in Purkinje cells by postnatal day 18 (Fritschy *et al.* 2006). In these $\alpha 1^{-/-}$ mice, GABAergic terminals from stellate axons are initially formed normally onto the Purkinje dendritic shaft. However, starting from postnatal day 11, synaptogenesis is significantly reduced and perturbed (Fritschy *et al.* 2006; Patrizi *et al.* 2008). Instead, stellate cell terminals form aberrant and mismatched contacts with post-synaptic specialization on the spines of Purkinje dendrites. These results suggest that initial steps of GABAergic synapse formation can proceed in the absence of $\alpha 1$, but $GABA_A$ receptors appear crucial for activity-dependent regulation of synapse density, possibly through promoting the stabilization of transient axodendritic contact into mature synapses. The mechanism linking GABA signalling to synapse maturation are still unclear. Activation of $GABA_ARs$ may result in the local release of trophic factors which promote inhibitory synapse maturation, and/or act as protective signals that prevent synapses elimination. The failure to stabilize presynaptic terminals after post-synaptic loss of $GABA_ARs$ suggests the presence of a retrograde signal that is regulated by synaptic activity or by association with postsynaptic $GABA_ARs$. Amongst the molecular mechanisms that may contribute to such an activity-regulated trans-synaptic signal, the neuroligin and neurexin complex represents one of the plausible candidates.

From $GABA_A$ receptors to synaptic adhesion and activity-dependent retrograde signalling

Neuroligins and neurexins are heterophilic synaptic adhesion molecules broadly expressed in the central nervous system (Brose, 1999; Sudhof, 2008). Cell biological studies have revealed potent 'synaptogenic' or synapse-organizing activities for these proteins (recently reviewed in (Levinson & El-Husseini, 2005; Craig & Kang, 2007). Postsynaptic neuroligins promote assembly of functional presynaptic specializations in axons, while presynaptic neurexins – through interaction with neuroligins – recruit postsynaptic scaffolding proteins and transmitter receptors in dendrites.

While neuroligin–neurexin complexes are common building blocks of glutamatergic and GABAergic synapses, analysis of mutant mice so far support their particularly critical roles in the organization of GABAergic synapses. Triple knockout mice lacking the three alpha-neurexin transcripts, although they die at birth, show a 50% reduction in the density of GABAergic synapses in the brainstem (Missler *et al.* 2003). In double knockout mice, some of which reach adulthood, GABAergic synapse density is reduced by 30% whereas glutamatergic synapse density is apparently unchanged (Dudanova *et al.* 2007). As for neuroligins, mice lacking the three major isoforms (NL1, 2 and 3), also perinatal lethal, show only a relatively small (15–20%) reduction in the number of synapses in the brainstem, but a severe loss of $GABA_ARs$ and the scaffolding protein gephyrin from postsynaptic

sites (Varoqueaux *et al.* 2006). Among the different isoforms, NL2 is exclusively localized to GABAergic synapses. NL2$^{-/-}$ mice display a selective decrease in the number of inhibitory synapses in the postnatal neocortex (Chubykin *et al.* 2007). In addition, layer 2/3 neurons in acute cortical slices from NL2$^{-/-}$ mice show a selective impairment of GABAergic transmission whereas glutamatergic transmission is normal. Overexpression of NL2 in cultured neurons increases the density GABAergic terminals (Chih *et al.* 2005) and the amplitude of inhibitory postsynaptic currents (Chubykin *et al.* 2007). Notably, this overexpression-induced increase in GABAergic transmission is blocked by pharmacologically reducing network activity in the culture. Therefore, neuronal and synaptic activity might either regulate the presynaptic response to NL2 or postsynaptic stabilization induced by NL2.

As both neuroligins and $GABA_A$ receptors play important roles in the maturation of postsynaptic specializations and the differentiation and stabilization of presynaptic terminals at inhibitory synapses, an obvious question is: how do GABA/$GABA_A$R-mediated synaptic signalling and neuroligin/neurexin-mediated synaptic adhesion interact and cooperate to regulate activity-dependent development of inhibitory synapse? It is currently unknown at what stage of their biosynthetic pathway $GABA_A$Rs first interact with NLs, and how such interactions might be regulated. One possibility is that NL2 and synaptic $GABA_A$Rs would stabilize each other, either through intracellular reciprocal interactions aided by scaffolding proteins such as gephyrin or through extracellular *cis* interactions (Fig. 2). In addition, GABA activation of $GABA_A$Rs might further stabilize $GABA_A$Rs at synapses through as yet unknown structural or signalling mechanisms. Such activity- and GABA-mediated stabilization of $GABA_A$Rs might further increase the levels of NL2 at postsynaptic sites; this, in turn, would stabilize the presynaptic terminals through trans-synaptic interactions with neurexins. Evidence consistent with this model include: (1) *in vitro* studies demonstrated a co-aggregation of NL2 and the $GABA_A$Rα2 subunit in heterologous cells (Dong *et al.* 2007); (2) the residence time of $GABA_A$Rs on the plasma membrane and their targeting to synapses is regulated by synaptic activity (Saliba *et al.* 2007); (3) pharmacological blockade of neuronal activity in cultured neurons diminishes the synaptogenic activity of NL2 (Chubykin *et al.* 2007); (4) reduced GABA synthesis and release result in a reduction of inhibitory synapses (Chattopadhyaya *et al.* 2007). Moreover, there is precedent for such mechanisms in activity-dependent recruitment of glutamate receptor and trans-synaptic signalling at glutamatergic synapses. Local spontaneous activity and glutamate release reduce diffusion exchange of GluR1 between synaptic and extrasynaptic domains, resulting in postsynaptic accumulation of GluR1 (Ehlers *et al.* 2007). In addition, PSD-95 and NL1 retrogradely modulate presynaptic release probability and may coordinate post- and pre-synaptic morphological changes (Ehrlich *et al.* 2007; Futai *et al.* 2007). It remains to be seen whether analogous mechanisms for GABA and NL2 signalling exist at inhibitory synapses.

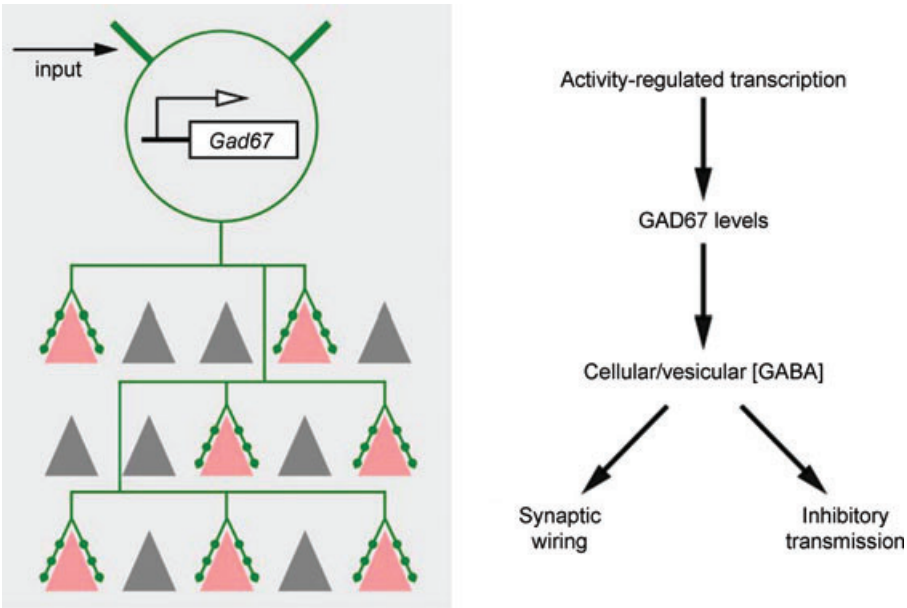

Figure 3.
A scheme showing that the level and pattern of neuronal activity may regulate inhibitory synaptic morphogenesis and innervation patterns through GAD67-mediated GABA synthesis and signalling.

In an alternative model, the expression and/or localization of NL2 might be regulated by GABA signalling, either through regulating NL2 protein levels or NL2-interacting proteins involved in its synaptic localization. It is also possible that GABA binding to $GABA_A$Rs might modulate their coupling to NL2, thereby increasing the potency and affinity of NL2 towards neurexin in the presynaptic terminals.

Activity-regulated Gad67 transcription as a cell-wide mechanism for modulating GABA signalling and innervation pattern

A mechanism unique to GABAergic neurons is activity-dependent GABA synthesis. Unlike glutamate, which is both the precursor and product of many essential metabolic and signalling processes in the cell, GABA can only be synthesized by two glutamate decarboxylases, and the main function of GABA is intercellular signalling (Soghomonian & Martin, 1998). In most brain regions, GAD67 activity is rate-limiting for GABA synthesis (Asada *et al.* 1996; Kash *et al.* 1997). Since GAD67 is produced at a limiting level in the brain (Asada *et al.* 1997), alterations in GAD67 levels influence cellular and vesicular GABA content (Murphy *et al.* 1998; Engel *et al.* 2001). Unlike GAD65, which is relatively stable, GAD67 protein has a rather quick turn-over rate, with a half-life of several hours (Christgau *et al.* 1991; Pinal & Tobin, 1998). The major step in the physiological regulation of GAD67 activity is *Gad1* transcription, which is dynamically regulated during development (Kiser *et al.* 1998), by neural activity (Patz *et al.* 2003; Kinney *et al.* 2006) and experience (Benson *et al.* 1989; Benevento *et al.* 1995; Liang *et al.* 1996; Gierdalski *et al.* 2001; Kobori & Dash, 2006). Therefore, activity-dependent transcription may result in adjustment of GAD67 levels and the intracellular GABA pool for release. As alterations in GAD67 and GABA levels profoundly influence interneuron axon growth and synapse formation during the development of inhibitory circuits, neuronal activity might shape the pattern of inhibitory synaptic innervation through GAD67-mediated GABA synthesis (Fig. 3). Such activity-dependent and cell-wide regulation of a 'transmitter resource' implies a novel logic for the maturation of inhibitory synapses and innervation pattern. This hypothesis needs to be tested by disrupting the activity regulation of GAD67 transcription in GABAergic neurons and examining the impact on inhibitory synapse development.

More questions than answers

The converging findings that GABA and GABA receptor signalling regulate inhibitory synapse development raise numerous questions regarding the underlying mechanisms and their functional implications. The many steps from GABA signalling to receptor trafficking/stability and neuroligin–neurexin function remain to be defined. In addition, it is unknown whether and how postsynaptic activity in pyramidal neuron might influence the action of GABA signalling on inhibitory synapse development. Furthermore, because cortical GABAergic neurons not only innervate pyramidal neurons but also other GABAergic neurons, an obvious question is whether and how GABA signalling might regulate the development of inhibitory synapses onto inhibitory neurons. Addressing such questions will require methods to visualize inhibitory synapses onto inhibitory neurons. Finally, although activity regulation of GAD67 transcription has been well demonstrated in numerous developmental and plasticity paradigms, its impact on GABA signalling and inhibitory synapse development and plasticity remains to be established *in vivo*. Compared with our understanding of the role of glutamate in excitatory synapse development, we are only beginning to scratch the surface of the role of GABA in the development of inhibitory synapses. Progress in this area will not only enhance our understanding of activity-dependent development of inhibitory synapses, axon arbors and innervation patterns, but also might have implications in the construction of cortical subnetworks, such as reciprocally connected groups of excitatory and inhibitory neurons (Yoshimura & Callaway, 2005).

References

Asada H, Kawamura Y, Maruyama K, Kume H, Ding R, Ji FY, Kanbara N, Kuzume H, Sanbo M, Yagi T & Obata K (1996). Mice lacking the 65 kDa isoform of glutamic acid decarboxylase (GAD65) maintain normal levels of GAD67 and GABA in their brains but are susceptible to seizures. *Biochem Biophys Res Commun* **229**, 891–895.

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 γ-aminobutyric acid in mice lacking the 67-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A* **94**, 6496–6499.

Bartos M, Vida I & Jonas P (2007). Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. *Nat Rev Neurosci* **8**, 45–56.

Ben-Ari Y, Cherubini E, Corradetti R & Gaiarsa JL (1989). Giant synaptic potentials in immature rat CA3 hippocampal neurones. *J Physiol* **416**, 303–325.

Ben-Ari Y, Gaiarsa JL, Tyzio R & Khazipov R (2007). GABA: A pioneer transmitter that excites immature neurons and generates primitive oscillations. *Physiol Rev* **87**, 1215–1284.

Benevento LA, Bakkum BW, Cohen RS & Port JD (1995). γ-Aminobutyric acid and somatostatin immunoreactivity in the visual cortex of normal and dark-reared rats. *Brain Res* **689**, 172–182.

Benson DL, Isackson PJ, Hendry SH & Jones EG (1989). Expression of glutamic acid decarboxylase mRNA in normal and monocularly deprived cat visual cortex. *Brain Res Mol Brain Res* **5**, 279–287.

Bonhoeffer T. & Yuste R (2002). Spine motility. Phenomenology, mechanisms, and function. *Neuron* **35**, 1019–1027.

Brose N (1999). Synaptic cell adhesion proteins and synaptogenesis in the mammalian central nervous system. *Naturwissenschaften* **86**, 516–524.

Carroll RC, Lissin DV, von Zastrow M, Nicoll RA & Malenka RC (1999). Rapid redistribution of glutamate receptors contributes to long-term depression in hippocampal cultures. *Nat Neurosci* **2**, 454–460.

Chattopadhyaya B, Di Cristo G, Higashiyama H, Knott GW, Kuhlman SJ, Welker E & Huang ZJ (2004). Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. *J Neurosci* **24**, 9598–9611.

Chattopadhyaya B, Di Cristo G, Wu CZ, Knott G, Kuhlman S, Fu Y, Palmiter RD & Huang ZJ (2007). GAD67-mediated GABA synthesis and signaling regulate inhibitory synaptic innervation in the visual cortex. *Neuron* **54**, 889–903.

Chih B, Engelman H & Scheiffele P (2005). Control of excitatory and inhibitory synapse formation by neuroligins. *Science* **307**, 1324–1328.

Christgau S, Schierbeck H, Aanstoot HJ, Aagaard L, Begley K, Kofod H, Hejnaes K & Baekkeskov S (1991). Pancreatic beta cells express two autoantigenic forms of glutamic acid decarboxylase, a 65-kDa hydrophilic form and a 64-kDa amphiphilic form which can be both membrane-bound and soluble. *J Biol Chem* **266**, 21257–21264.

Chubykin AA, Atasoy D, Etherton MR, Brose N, Kavalali ET, A & Sudhof TC (2007). Activity-dependent validation of excitatory versus inhibitory synapses by neuroligin-1 versus neuroligin-2. *Neuron* **54**, 919–931.

Cline H & Haas K (2008). The regulation of dendritic arbor development and plasticity by glutamatergic synaptic input: a review of the synaptotrophic hypothesis. *J Physiol* **586**, 1509–1517.

Cobb SR, Buhl EH, Halasy K, Paulsen O & Somogyi P (1995). Synchronization of neuronal activity in hippocampus by individual GABAergic interneurons. *Nature* **378**, 75–78.

Craig AM & Kang Y (2007). Neurexin-neuroligin signaling in synapse development. *Curr Opin Neurobiol* **17**, 43–52.

Di Cristo G, Wu C, Chattopadhyaya B, Ango F, Knott G, Welker E, Svoboda K & Huang ZJ (2004). Subcellular domain-restricted GABAergic innervation in primary visual cortex in the absence of sensory and thalamic inputs. *Nat Neurosci* **7**, 1184–1186.

Doischer D, Hosp JA, Yanagawa Y, Obata K, Jonas P, Vida I & Bartos M (2008). Postnatal differentiation of basket cells from slow to fast signaling devices. *J Neurosci* **28**, 12956–12968.

Dong N, Qi J & Chen G (2007). Molecular reconstitution of functional GABAergic synapses with expression of neuroligin-2 and GABAA receptors. *Mol Cell Neurosci* **35**, 14–23.

Dudanova I, Tabuchi K, Rohlmann A, Sudhof TC & Missler M (2007). Deletion of alpha-neurexins does not cause a major impairment of axonal pathfinding or synapse formation. *J Comp Neurol* **502**, 261–274.

Ehlers MD, Heine M, Groc L, Lee MC & Choquet D (2007). Diffusional trapping of GluR1 AMPA receptors by input-specific synaptic activity. *Neuron* **54**, 447–460.

Ehrlich I, Klein M, Rumpel S & Malinow R (2007). PSD-95 is required for activity-driven synapse stabilization. *Proc Natl Acad Sci U S A* **104**, 4176–4181.

Engel D, Pahner I, Schulze K, Frahm C, Jarry H, Ahnert-Hilger G & Draguhn A (2001). Plasticity of rat central inhibitory synapses through GABA metabolism. *J Physiol* **535**, 473–482.

Essrich C, Lorez M, Benson JA, Fritschy JM & Luscher B (1998). Postsynaptic clustering of major $GABA_A$ receptor subtypes requires the $\gamma 2$ subunit and gephyrin. *Nat Neurosci* **1**, 563–571.

Fang C, Deng L, Keller CA, Fukata M, Fukata Y, Chen G & Luscher B (2006). GODZ-mediated palmitoylation of $GABA_A$ receptors is required for normal assembly and function of GABAergic inhibitory synapses. *J Neurosci* **26**, 12758–12768.

Fritschy JM, Panzanelli P, Kralic JE, Vogt KE & Sassoe-Pognetto M (2006). Differential dependence of axo-dendritic and axo-somatic GABAergic synapses on $GABA_A$ receptors containing the $\alpha 1$ subunit in Purkinje cells. *J Neurosci* **26**, 3245–3255.

Futai K, Kim MJ, Hashikawa T, Scheiffele P, Sheng M & Hayashi Y (2007). Retrograde modulation of presynaptic release probability through signaling mediated by PSD-95-neuroligin. *Nat Neurosci* **10**, 186–195.

Gierdalski M, Jablonska B, Siucinska E, Lech M, Skibinska A & Kossut M (2001). Rapid regulation of GAD67 mRNA and protein level in cortical neurons after sensory learning. *Cereb Cortex* **11**, 806–815.

Hua JY, Smear MC, Baier H & Smith SJ (2005). Regulation of axon growth *in vivo* by activity-based competition. *Nature* **434**, 1022–1026.

Hua JY & Smith SJ (2004). Neural activity and the dynamics of central nervous system development. *Nat Neurosci* **7**, 327–332.

Huang ZJ, Di Cristo G & Ango F (2007). Development of GABA innervation in the cerebral and cerebellar cortices. *Nat Rev Neurosci* **8**, 673–686.

Huang ZJ, Kirkwood A, Pizzorusso T, Porciatti V, Morales B, Bear MF, Maffei L & Tonegawa S (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* **98**, 739–755.

Ji F, Kanbara N & Obata K (1999). GABA and histogenesis in fetal and neonatal mouse brain lacking both the isoforms of glutamic acid decarboxylase. *Neurosci Res* **33**, 187–194.

Jiao Y, Zhang C, Yanagawa Y & Sun QQ (2006). Major effects of sensory experiences on the neocortical inhibitory circuits. *J Neurosci* **26**, 8691–8701.

Kash SF, Johnson RS, Tecott LH, Noebels JL, Mayfield RD, Hanahan D & Baekkeskov S (1997). Epilepsy in mice deficient in the 65-kDa isoform of glutamic acid decarboxylase. *Proc Natl Acad Sci U S A* **94**, 14060–14065.

Kinney JW, Davis CN, Tabarean I, Conti B, Bartfai T & Behrens MM (2006). A specific role for NR2A-containing NMDA receptors in the maintenance of parvalbumin and GAD67 immunoreactivity in cultured interneurons. *J Neurosci* **26**, 1604–1615.

Kiser PJ, Cooper NG & Mower GD (1998). Expression of two forms of glutamic acid decarboxylase (GAD67 and GAD65) during postnatal development of rat somatosensory barrel cortex. *J Comp Neurol* **402**, 62–74.

Klostermann O & Wahle P (1999). Patterns of spontaneous activity and morphology of interneuron types in organotypic cortex and thalamus-cortex cultures. *Neuroscience* **92**, 1243–1259.

Kobori N & Dash PK (2006). Reversal of brain injury-induced prefrontal glutamic acid decarboxylase expression and working memory deficits by D1 receptor antagonism. *J Neurosci* **26**, 4236–4246.

Leinekugel X, Tseeb V, Ben-Ari Y & Bregestovski P (1995). Synaptic $GABA_A$ activation induces Ca^{2+} rise in pyramidal cells and interneurons from rat neonatal hippocampal slices. *J Physiol* **487**, 319–329.

Levinson JN & El-Husseini A (2005). Building excitatory and inhibitory synapses: balancing neuroligin partnerships. *Neuron* **48**, 171–174.

Li RW, Yu W, Christie S, Miralles CP, Bai J, Loturco JJ & De Blas AL (2005). Disruption of postsynaptic GABA receptor clusters leads to decreased GABAergic innervation of pyramidal neurons. *J Neurochem* **95**, 756–770.

Liang F, Isackson PJ & Jones EG (1996). Stimulus-dependent, reciprocal up- and downregulation of glutamic acid decarboxylase and Ca^{2+}/calmodulin-dependent protein kinase II gene expression in rat cerebral cortex. *Exp Brain Res* **110**, 163–174.

Malinow R & Malenka RC (2002). AMPA receptor trafficking and synaptic plasticity. *Annu Rev Neurosci* **25**, 103–126.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G & Wu C (2004). Interneurons of the neocortical inhibitory system. *Nat Rev Neurosci* **5**, 793–807.

Michels G & Moss SJ (2007). $GABA_A$ receptors: properties and trafficking. *Crit Rev Biochem Mol Biol* **42**, 3–14.

Miles R, Toth K, Gulyas AI, Hajos N & Freund TF (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* **16**, 815–823.

Missler M, Zhang W, Rohlmann A, Kattenstroth G, Hammer RE, Gottmann K & Sudhof TC (2003). Alpha-neurexins couple Ca^{2+} channels to synaptic vesicle exocytosis. *Nature* **423**, 939–948.

Morales B, Choi SY & Kirkwood A (2002). Dark rearing alters the development of GABAergic transmission in visual cortex. *J Neurosci* **22**, 8084–8090.

Murphy DD, Cole NB, Greenberger V & Segal M (1998). Estradiol increases dendritic spine density by reducing GABA neurotransmission in hippocampal neurons. *J Neurosci* **18**, 2550–2559.

Owens DF & Kriegstein AR (2002). Is there more to GABA than synaptic inhibition? *Nat Rev Neurosci* **3**, 715–727.

Patrizi A, Scelfo B, Viltono L, Briatore F, Fukaya M, Watanabe M, Strata P, Varoqueaux F, Brose N, Fritschy JM & Sassoe-Pognetto M (2008). Synapse formation and clustering of neuroligin-2 in the absence of $GABA_A$ receptors. *Proc Natl Acad Sci U S A* **105**, 13151–13156.

Patz S, Wirth MJ, Gorba T, Klostermann O & Wahle P (2003). Neuronal activity and neurotrophic factors regulate GAD-65/67 mRNA and protein expression in organotypic cultures of rat visual cortex. *Eur J Neurosci* **18**, 1–12.

Pinal CS & Tobin AJ (1998). Uniqueness and redundancy in GABA production. *Perspect Dev Neurobiol* **5**, 109–118.

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.

Ruthazer ES, Akerman CJ & Cline HT (2003). Control of axon branch dynamics by correlated activity *in vivo*. *Science* **301**, 66–70.

Saliba RS, Michels G, Jacob TC, Pangalos MN & Moss SJ (2007). Activity-dependent ubiquitination of $GABA_A$ receptors regulates their accumulation at synaptic sites. *J Neurosci* **27**, 13341–13351.

Schweizer C, Balsiger S, Bluethmann H, Mansuy IM, Fritschy JM, Mohler H & Luscher B (2003). The $\gamma 2$ subunit of $GABA_A$ receptors is required for maintenance of receptors at mature synapses. *Mol Cell Neurosci* **24**, 442–450.

Shi SH, Hayashi Y, Petralia RS, Zaman SH, Wenthold RJ, Svoboda K & Malinow R (1999). Rapid spine delivery and redistribution of AMPA receptors after synaptic NMDA receptor activation. *Science* **284**, 1811–1816.

Soghomonian JJ & Martin DL (1998). Two isoforms of glutamate decarboxylase: why? *Trends Pharmacol Sci* **19**, 500–505.

Sudhof TC (2008). Neuroligins and neurexins link synaptic function to cognitive disease. *Nature* **455**, 903–911.

Tamas G, Buhl EH, Lorincz A & Somogyi P (2000). Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat Neurosci* **3**, 366–371.

Tamas G, Buhl EH & Somogyi P (1997). Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neurone in the cat visual cortex. *J Physiol* **500**, 715–738.

Tashiro A, Dunaevsky A, Blazeski R, Mason CA & Yuste R (2003). Bidirectional regulation of hippocampal mossy fiber filopodial motility by kainate receptors: a two-step model of synaptogenesis. *Neuron* **38**, 773–784.

Varoqueaux F, Aramuni G, Rawson RL, Mohrmann R, Missler M, Gottmann K, Zhang W, Sudhof TC & Brose N (2006). Neuroligins determine synapse maturation and function. *Neuron* **51**, 741–754.

Wang Y, Gupta A, Toledo-Rodriguez M, Wu CZ & Markram H (2002). Anatomical, physiological, molecular and circuit properties of nest basket cells in the developing somatosensory cortex. *Cereb Cortex* **12**, 395–410.

Wong WT & Wong RO (2001). Changing specificity of neurotransmitter regulation of rapid dendritic remodeling during synaptogenesis. *Nat Neurosci* **4**, 351–352.

Yoshimura Y & Callaway EM (2005). Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. *Nat Neurosci* **8**, 1552–1559.

Zhang LI & Poo MM (2001). Electrical activity and development of neural circuits. *Nat Neurosci* **4**, 1207–1214.

Zheng JQ, Felder M, Connor JA & Poo MM (1994). Turning of nerve growth cones induced by neurotransmitters. *Nature* **368**, 140–144.