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REVIEW

The Neuroplastin adhesion molecules: key regulators of neuronal plasticity and synaptic function

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Abstract

The Neuroplastins Np65 and Np55 are neuronal and synapse-enriched immunoglobulin superfamily molecules that play important roles in a number of key neuronal and synaptic functions including, for Np65, cell adhesion. In this review we focus on the physiological roles of the Neuroplastins in promoting neurite outgrowth, regulating the structure and function of both inhibitory and excitatory synapses in brain, and in neuronal and synaptic plasticity. We discuss the underlying molecular and cellular mechanisms by which the Neuroplastins exert their physiological effects and how these are dependent upon the structural features of Np65 and Np55, which enable them to bind to a diverse range of protein partners. In turn this enables the Neuroplastins to interact with a number of key neuronal signalling cascades. These include: binding to and activation of the fibroblast growth factor receptor; Np65 *trans*-homophilic binding leading to activation of p38 MAPK and internalization of glutamate (GluR1) receptor subunits; acting as accessory proteins for monocarboxylate transporters, thus affecting neuronal energy supply, and binding to $GABA_A$ α1, 2 and 5 subunits, thus regulating the composition and localization of $GABA_A$ receptors. An emerging theme is the role of the Neuroplastins in regulating the trafficking and subcellular localization of specific binding partners. We also discuss the involvement of Neuroplastins in a number of pathophysiological conditions, including ischaemia, schizophrenia and breast cancer and the role of a single nucleotide polymorphism in the human Neuroplastin (*NPTN*) gene locus in impairment of cortical development and cognitive functions.

Keywords: GABA receptors, immunoglobulin superfamily, long-term potentiation, monocarboxylate transporter, neurite outgrowth, postsynaptic density.

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Neuroplastin structure and isoforms

The Neuroplastins Np65 and Np55 are neuronal and synapse-enriched immunoglobulin superfamily (IgSF) members that contain three and two Ig domains respectively. Np65, but not Np55, mediates cell adhesion by *trans*-homophilic binding. At present, an adhesive function for Np55 has not been established. The Neuroplastins were originally identified as glycoprotein components of rat brain synaptic membrane (SM) and postsynaptic density (PSD) fractions (Hill *et al.* 1988). Sequence comparisons showed that the peptide structures of Np65 and Np55 are identical except for the N-terminal Np65-specific Ig1 domain (Fig. 1), and they arise from a single gene by alternative splicing. The Ig domains are

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Abbreviations used: aa, amino acid; CAM, cell adhesion molecule; Fc, fragment crystallizable region of immunoglobulin; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; Ig, immunoglobulin; IgSF, immunoglobulin superfamily; LTD, long-term depression; LTP, long-term potentiation; MCT, monocarboxylate transporter; mEPSC, miniature excitatory postsynaptic current; mIPSC, miniature inhibitory postsynaptic current; np, neuroplastin; *NPTN*, neuroplastin gene; P, postnatal day; PSD, postsynaptic density; SM, synaptic membrane; SNP, single nucleotide polymorphism.

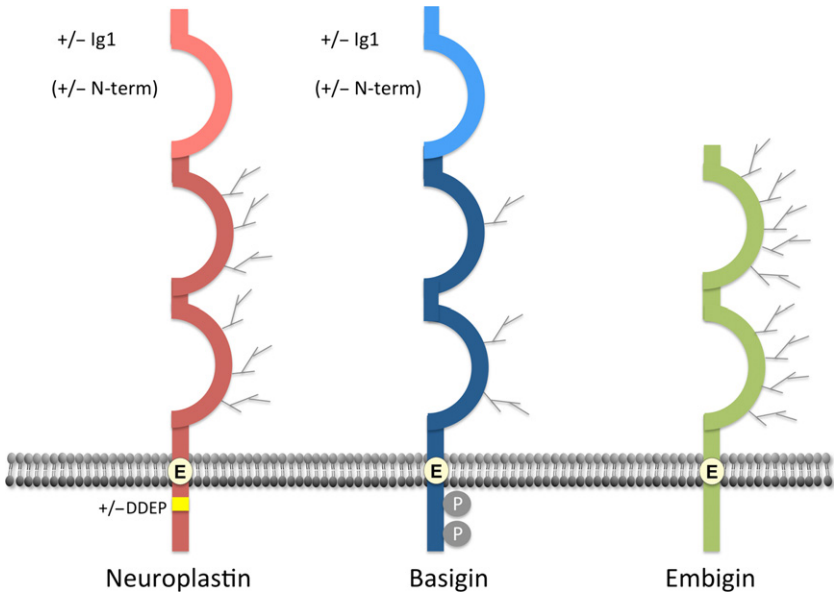

Fig. 1 Structural features of the Basigin group of the IgSF, Neuroplastin, Basigin and Embigin. All family members have two Ig domains that project into the extracellular space. Neuroplastin and Basigin are also present as isoforms containing an additional N-terminal Ig domain (± Ig1). All members contain a glutamate (E) at exactly the same position in the transmembrane domain. This residue may be important for molecular interactions within the membrane region. Neuroplastins have additional alternative splice isoforms that contain a four-amino acid (aa) mini exon (i.e. four isoforms; Np65 and Np55 ± DDEP) within the cytoplasmic domain. The number and position of predicted *N*-glycosylation sites (grey symbols) varies not only between the family members but also between different species. Here, *N*-glycosylation sites of the human and rat proteins are indicated. Furthermore, for Basigin, two phosphorylation sites in the cytoplasmic domain have been identified, but for Neuroplastin and Embigin, analogous data are not available.

followed by a single 21 amino acid (aa) membrane-spanning sequence and a short (34 aa) intracellular domain (Langnaese *et al.* 1997). The intracellular domain comprises mainly charged or neutral, but also several hydrophobic residues. A 28 aa signal peptide which is cleaved in the mature proteins is present at the N-terminus. In humans, the Neuroplastin (*NPTN*) gene is located on chromosome 15q22 and comprises nine exons (NCBI; Gene ID 27020). A total of four splice variants of Np65 and Np55 either containing a 4 aa acidic 340-Asp-Asp-Glu-Pro-(DDEP)-343 insert in the intracellular domain or not (Np + DDEP and Np − DDEP; aa numbering is for Np65) have been identified (Langnaese *et al.* 1997; Kreutz *et al.* 2001).

Genome-wide sequence searches showed that the Neuroplastins are most closely related to Basigin/CD147 (also variously termed HT7 antigen, 5A11 antigen or Neurothelin in the chick, M6 antigen or EMPPRIN, extracellular matrix metalloproteinase inducer, in the human and MRC OX47 antigen in the rat) and that they are members of the Basigin/CD147 group (Fig. 1) of the IgSF (Langnaese *et al.* 1997). The other family member besides Basigin and the Neuroplastins is Embigin (gp70) (Muramatsu and Miyauchi 2003; Iacono *et al.* 2007). Basigin, like Neuroplastin, is expressed as 2- or 3-Ig domain isoforms (Fig. 1) termed Basigin 1 and 2 respectively (Miyauchi *et al.* 1990; Kanekura *et al.* 1991; Ochrietor *et al.* 2003). Sequence identity between the Neuroplastins and Basigin ranges from 40% to 45%, although it is much higher in specific regions. The highest degree of identity is within and around the transmembrane sequence (including the charged glutamate residue) and intracellular domains. The transmembrane glutamate plays a major role in trafficking Basigin to the plasma membrane (Manoharan *et al.* 2006).

Basigin is also termed EMMPRIN as it induces several extracellular matrix metalloproteinases (for reviews, see Muramatsu and Miyauchi 2003; Gabison *et al.*2005; Yan *et al.* 2005; Nabeshima *et al.* 2006; Iacono *et al.* 2007) via interaction with the Ig1 domain of Basigin 1. Sequence identity between the Neuroplastins and Basigin is only 20% for this domain. The Basigin–extracellular matrix metalloproteinase interaction is responsible for the prominent roles of Basigin in cancer metastasis, wound healing and tissue remodelling. Basigin also interacts with a range of other proteins, including α3, α6 and β1 Integrins, Caveolin, Annexin II, Cyp60 and monocarboxylate transporters (MCTs). The initial data suggested that the transmembrane glutamate residue was important in the interaction of all three members of the Basigin group with MCTs. However, subsequent studies have shown that although these interactions are mediated via the transmembrane domain of the Basigin group, they are primarily hydrophobic (Wilson *et al.* 2002; Manoharan *et al.* 2006; Finch *et al.* 2009).

The Neuroplastins contain six potential sites for *N*-glycosylation in the rat and human brain (nine in the mouse), all located on the common Ig2 and 3 domains (Fig. 1). One *O*-glycosylation site is predicted at serine 95 in the Ig1 domain. Most, if not all, sites are glycosylated since the apparent molecular weights of Np65 and 55 are reduced from 65 and 55 kDa to 40 and 28 kDa respectively following complete deglycosylation (Willmott *et al.* 1992). X-ray crystallographic analysis of recombinant protein encoding the Ig2 and Ig3 domains of Np55 (ecto Np55) shows that *N*-acetylglucosamine is present at four of the six potential *N*-glycosylation sites in the rat (Owczarek *et al.* 2010). There is considerable heterogeneity of Np55 glycoforms between tissues (Langnaese *et al.* 1998), thus supporting a tissue-specific role for the carbohydrate moieties in Neuroplastin function.

In contrast to earlier reviews (Owczarek and Berezin 2012; Beesley *et al.* 2013) which focus primarily on the adhesive and signalling properties of Neuroplastins and on their involvement in behaviour and disease, here we focus on their physiological functions and the associated underlying molecular and cellular mechanisms. Thus, having outlined the structural features of Neuroplastins and their relationship to the Basigin group of the IgSF in subsequent sections, we discuss their expression and localization, and developmental profiles, key information for understanding Neuroplastin function. The major focus of the review is on the physiological functions of the Neuroplastins including: interaction with the fibroblast growth factor receptor (FGFR) and induction of neurite outgrowth; their role in regulation of synaptic structure and function; the importance of Neuroplastins in long-term activity-dependent synaptic plasticity, including long-term potentiation (LTP); their role in neuronal energy supply by monocarboxylates, principally lactate; their role in trafficking and localization of specific binding partners, and a putative role for Np55 in cerebellar long-term depression (LTD). In later sections we review the behavioural effects of Neuroplastins and their roles in a number of pathophysiological conditions, finally discussing perspectives for future research.

Expression and localization of the Neuroplastins

Despite the close structural identity between Np65 and Np55, early studies showed important differences in their localization and functions. Thus, Np65 and Np55 show different patterns of tissue, cellular and subcellular expression and localization; different developmental profiles in brain, and important differences in some binding interactions and physiological functions.

Expression of Np65 is brain- and neuron-specific, whereas Np55 is expressed in a wide range of tissues (Hill *et al.* 1988; Langnaese *et al.* 1997; Smalla *et al.* 2000). Strikingly and similar to the Neuroplastins the 3 Ig domain containing Basigin 2 isoform exhibits a much more restricted expression than the 2 Ig domain Basigin 1 isoform (Ochrietor *et al.* 2003). It is solely expressed by photoreceptors in the retina where it plays a key role in lactate transport. This argues for a strictly tissue-specific splicing mechanism in the biosynthesis of both cell adhesion molecules (CAMs).

Immunocytochemical, biochemical fractionation and gene expression studies show that Np65 levels exhibit an anterior–posterior axis of expression within the brain (Hill *et al.* 1988; Smalla *et al.* 2000; Marzban *et al.* 2003). Thus, Np65 is predominantly expressed by subsets of forebrain neurones in the cortex, hippocampus and striatum. It is present in lower amounts in midbrain regions such as the thalamus and hypothalamus, but is barely detectable in brainstem regions. It is absent from the spinal cord and peripheral nerve. In contrast to Np65, there is no anterior–posterior axis of expression for Np55, and it is readily detectable in all brain regions, though there are variations in its level. Immunocytochemical studies show that expression of Np65 is laminar in both the cerebral cortex and in the hippocampus, and occurs as punctate deposits concentrated in neuropil regions, i.e. layers II, III and Vb/VI in the cerebral cortex and in the stratum radiatum and stratum oriens in the hippocampus, (Hill *et al.* 1988; Smalla *et al.* 2000). Strikingly, the level of hippocampal Np65 immunoreactivity in rodent brain is much lower in the CA3 compared to the CA1 region, with the dentate gyrus (DG) showing an intermediate level of expression.

The punctate nature and localization of Np65 immunoreactivity suggested that it is located, at least in part, at the synapse. Indeed, initial biochemical fractionation studies showed that Np65, but not Np55, is concentrated in forebrain PSDs. Co-localization studies with PSD markers such as PSD95 confirm that a fraction of Np65 is localized to the postsynaptic membrane and PSD, but it is also detected presynaptically. Thus, Neuroplastins have been detected in a fraction containing many presynaptic membrane proteins, but from which almost all of the postsynaptic proteins have been removed (Boyken *et al.* 2013).

The localization of Np55 has been studied in detail in the mouse cerebellum (Marzban *et al.* 2003). Here 95% of the total Neuroplastin is Np55 and furthermore no cerebellar immunoreactivity is detected with an Np65-specific antibody. These data suggest that the immunocytochemical staining in cerebellum is specific to Np55. The cerebellar Np55 immunoreactivity is primarily synaptic, and is concentrated at parallel fibre synapses on Purkinje cells in the molecular layer and on synaptic glomeruli in the granule cell layer. This pattern of localization suggests that Np55 also plays important functional roles at the synapse. Strikingly, Np55 is expressed in parasagittal (zebrin) stripes (Marzban *et al.* 2003; Beesley *et al.* 2013). These parasagittal bands have been described for numerous molecules (Ozol *et al.* 1999; Armstrong and Hawkes 2000) and most thoroughly

investigated for Zebrin II/aldolase C (Brochu *et al.* 1990; Ahn *et al.* 1994). Np55 is enriched in Zebrin II-negative stripes (also see the sections on Developmental profile of the Neuroplastins and Does Np65 play a role in cerebellar long-term depression?).

While many features of Neuroplastin expression are common to rodent and human brain, some striking differences have been reported (Bernstein *et al.* 2007). In marked contrast to the rodent, in the human brain, Np65 is expressed at comparable levels in cerebral and cerebellar cortices, and no parasagittal bands of Neuroplastin immunoreactivity are detected in the human cerebellum. Furthermore, the sharp demarcation in Neuroplastin immunoreactivity observed between CA1 and CA3 regions of the rodent hippocampus is not observed in the human brain.

Both Neuroplastins are expressed in retina (Kreutz *et al.* 2001). Immunocytochemical studies show that Neuroplastin immunoreactivity is confined to the two synaptic layers of the retina – the inner and outer plexiform layers. Np65 immunoreactivity is associated with the ribbon synapses between the photoreceptors and the bipolar cells in the inner plexiform layer.

Developmental profile of the Neuroplastins in brain

Expression of the *NPTN* gene in the mouse is ontogenetically controlled with highest mRNA levels being detected in the first postnatal month and in differentiating human progenitor cells, *NPTN* gene expression increases 1.5-fold during the first 7 days of differentiation *in vitro* (Desrivières *et al.* 2014). Developmental studies show that in the rodent brain, Np55 expression precedes Np65, and that it is expressed in low levels in the embryonic brain. Its level increases rapidly during the first two postnatal weeks, thereafter increasing gradually to reach the stable adult level between postnatal weeks 4 and 5 (Hill *et al.* 1989; Langnaese *et al.* 1997; Marzban *et al.* 2003). In contrast, Np65 is not detected in embryonic brain, and its levels increase rapidly only during the second and third postnatal weeks, thereafter reaching the stable adult level.

In mouse cerebellum, transient expression of punctate deposits of Np55 immunoreactivity is observed outlining the Purkinje cell somata at postnatal day (P)7, but this is not detected by P10 (Marzban *et al.* 2003). These may correspond to the transient synapses made between the developing climbing fibres and the Purkinje cell somata at this stage and which are eliminated later in development (Mason 1987). The parasagittal (zebrin) stripes of Neuroplastin immunoreactivity are detected by P5. However, as cerebellar compartmentation and afferent topography is already established by P5, it is unlikely that the Neuroplastins are involved in these processes, although it is likely they are important in later events leading to refinement of connectivity.

Interestingly, Neuroplastin expression is linked to the ganglioside composition of neural membranes (Mlinac *et al.* 2012). In mice deficient for the enzyme B4galnt1 and thus lacking complex gangliosides, Neuroplastin mRNA and particularly Np55 protein expression is increased in the hippocampus. Strikingly, not only the amount but also the subcellular distribution of Neuroplastin immunoreactivity is affected by the lack of complex gangliosides, with drastically reduced staining of CA1 and CA3 pyramidal neurones. These findings point to interactions between Neuroplastins and complex gangliosides because brain regions with higher levels of complex gangliosides in wild-type mice are particularly affected by the alterations of Neuroplastin expression in B4galnt1 knockout mice. In particular, an association of Neuroplastins with lipid rafts might be changed in a way similar to that described for neural cell adhesion molecule (NCAM) in the mono (M) and disialic (D) ganglioside (G) GM2/GD2 synthase knockout, GD3 synthase and the GM2/GD2/GD3 synthase double knockout mice (Ohmi *et al.* 2011). However, the increase in Neuroplastin mRNA expression in B4galnt1 knockout mice might also be caused by indirect effects of complex gangliosides on *NPTN* gene regulation.

Physiological functions of the Neuroplastins

Np65 and Np55 support neurite outgrowth via *trans*-homophilic binding and activation of the FGF receptor

Many CAMs, including NCAM, L1 and the cadherins, signal by binding to and activating the FGFR tyrosine kinase (Walsh and Doherty 1997; Hansen *et al.* 2008). Fibroblast growth factor (FGF) receptors are composed of up to three Ig domains, D1–D3. The D2 (Ig2) and D3 (Ig3) domains are sufficient for FGF binding. On the basis of a high homology between a 13 aa sequence in the Ig2 domain of Np55 (121RIVTSEEVIIRDS134 of Np65) and a motif in the Ig1 (D1) domain of the FGFR1 structure (RI-TGEEVEVRDS), Owczarek *et al.* (2010) predicted that Np55 might bind to and signal through activation of the FGFR. However, the putative FGFR-Neuroplastin binding site is distinct from that described for NCAM, L1 and the cadherins, which primarily comprises a cluster of acidic aa (the acid box) located on the linker region between the D1 and D2 domains. Furthermore, neither of the other two members of the Basigin group has the Neuroplastin-FGFR sequence similarity, thus suggesting that this interaction is specific to the Neuroplastins (Owczarek *et al.* 2010).

Surface plasmon resonance experiments using ecto Np55, a recombinant protein encoding the two Np55 Ig domains, showed that it bound to recombinant protein comprising the D2 and D3 domains of the FGFR1 receptor (Owczarek *et al.* 2010) with a K_D of 11 μM. These FGF receptor domains have been shown to be sufficient for the receptor dimerization, which leads to activation of the tyrosine kinase domain

(Mohammadi *et al.* 2005). Although the K_D value indicates relatively weak binding, it is of the same order as the K_Ds for NCAM (10 μm) and L1 (3.25 μM) binding to the FGFR (Kiselyov *et al.* 2003; Kulahin *et al.* 2008).

The observation that Np55 supports neurite outgrowth in cultured cerebellar granule cells and hippocampal neurones (Owczarek *et al.* 2010; Owczarek and Berezin 2012), but does not exhibit homophilic or heterophilic binding to other CAMs suggested that the underlying mechanism is by activation of the FGFR. A synthetic peptide, narpin (Fig. 2), comprising a tetramer of the Np55 FGFR homology sequence, was used to show that this is indeed the case. Treatment of Trex 93 cells expressing the FGFR1 IIIc splice variant with either ecto Np55 or narpin induced FGFR autophosphorylation indicating receptor activation. Thus, activation of the FGFR by Np55 is at least in part responsible for mediating Np55-induced neurite outgrowth since both ecto Np55 and narpin-induced neurite outgrowth are inhibited by the FGFR inhibitor SU5402. Furthermore, transfection of hippocampal neurones with a dominant negative kinase deleted FGFR1 blocked ecto Np55- and narpin-induced neurite outgrowth.

As MAPK signalling pathways are implicated in the signal transduction cascades activated by other IgSF family members (Hansen *et al.* 2008), they are prime candidates for mediating Np55-induced neurite outgrowth. Interestingly, increased phosphorylation, and hence activation of p38MAPK and extracellular signal-regulated kinase (ERK) 1/2, was observed in neurones treated with ecto Np55 (Owczarek *et al.* 2010). Strikingly, FGF2, a cognate ligand for the FGFR, and which has much higher receptor activation potency than Np55, does not induce neurite outgrowth from primary hippocampal neurones. However, FGF2 does inhibit Np55-induced neurite outgrowth in a dose-dependent manner. These results led Owczarek *et al.* (2010) to suggest that Np55 and FGF2 may have overlapping, but not identical, binding sites on the FGFR. Indeed, Np55 and FGF2 differentially activate MAPK signalling pathways. Thus, while both ecto Np55 and FGF2 increase phosphorylation of ERK 1/2, the FGF2-elicited response is stronger than that observed for ecto Np55. Strikingly, while ecto Np55 also increases phosphorylation and thus activation of p38MAPK, FGF2 does not. Evidence for the involvement of p38MAPK in the neurite outgrowth effect of Np55 is provided by the observation that SB202190, a specific p38MAPK inhibitor, blocked this response. Significantly, ecto Np55 also induced a ca. 40% increase in synaptic calcium concentration which was reduced by the FGFR1 receptor inhibitor SU5402 and by lavendustin A, a general protein tyrosine kinase antagonist. These data implicate calcium in the Np55-induced signalling cascade(s) leading to increased neurite outgrowth.

Although the later developmental expression of Np65 compared to Np55 initially suggested that it did not play a role in neurite outgrowth, its early expression in hippocampal neurones in culture provided evidence that this might not be the case (Owczarek *et al.* 2011). Indeed, recombinant protein containing all three Ig domains of Np65 (ecto Np65) induces neurite outgrowth both in cultures of hippocampal neurones

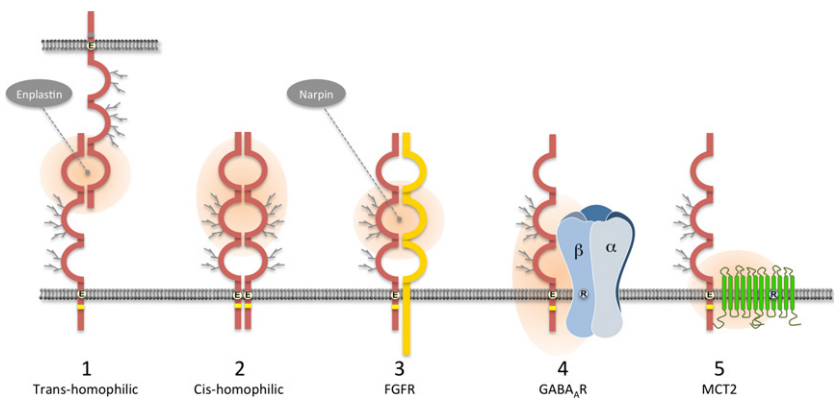

Fig. 2 Neuroplastin binding interactions. *cis* and *trans*-Neuroplastin homophilic interactions and several Neuroplastin-binding proteins have been identified to date. Potential regions for interaction between Neuroplastin and these binding partners are highlighted in ochre. (1) *Trans*-homophilic binding: the peptide enplastin inhibits this interaction. (2) *cis*-homophilic binding: analogous to Basigin (Yoshida *et al.* 2000), the interaction is likely to be between adjacent Ig2 domains. (3) *cis* interaction of Neuroplastins with fibroblast growth factor (FGF) receptors: the interaction is between the two middle Ig domains; the peptide narpin interferes with this heterophilic binding. (4) Heterophilic binding between Neuroplastin and $GABA_A$ receptors: Np65 Interacts with α1, α2 and β2 $GABA_A$ receptor subunits. Although the interaction surface has not been identified, both β2 and β3 subunits have an arginine (R) at the same position in their C-terminal transmembrane domain which plausibly binds to the glutamate (E) in the Neuroplastin transmembrane region. (5) Direct heterophilic interaction between Neuroplastin. Although the interaction sites have not been identified, analogy with the Basigin-monocarboxylate transporter (MCT)1 interaction suggests that the interaction is primarily hydrophobic.

and cerebellar granule cells. The mechanism involves Np65 *trans*-homophilic adhesion (see the section on Np65 *trans*-homophilic binding) since it is blocked by enplastin, a tetrameric peptide whose design is such that each monomeric sequence contains the Np65 *trans*-homophilic binding sequence. Thus, enplastin can act as a peptide mimetic of Np65. Although enplastin specifically blocks the *trans*-homophilic Np65–Np65 interaction (Owczarek *et al.* 2011), it can also act as a partial agonist, supporting neurite outgrowth by hippocampal neurones grown on HEK cells that do not express Np65. As for Np55-mediated neurite outgrowth, the Np65 *trans*-homophilic interaction appears to activate several signalling cascades, including the FGFR1 receptor, ERK 1/2 and p38MAPK.

Ca^{2+} influx and subsequent activation of CaM kinase II is important in mediating Np65-induced neurite outgrowth as has been shown for many other CAMs which exhibit *trans*-homophilic binding (Hansen *et al.* 2008). Thus, KN-93 (a compound which inhibits binding of calmodulin to CaM kinase II) blocked Np65-mediated induction of neurite outgrowth. Furthermore, application of either ecto Np65 or enplastin to hippocampal neurones induced an increase in synaptic calcium levels. The observations that both Np65 and enplastin-induced neurite outgrowth are abrogated by SU 5402 implicate the FGFR in mediating this response.

Np65 regulates synaptic structure and function in the hippocampus

Recent studies using hippocampal neurones derived from mice in which expression of the *NPTN* gene is disrupted show that both the structure and function of glutamatergic and the function of GABAergic synapses in hippocampal CA1 and DG regions are regulated by Np65 (Herrera-Molina *et al.* 2014). Western blotting and immunocytochemistry confirm that there is no detectable Neuroplastin in brain in Neuroplastin-deficient mice. Analysis of the number of excitatory synapses both *in situ* and in hippocampal neuronal cultures shows that these are significantly reduced for CA1 and DG neurones in the Neuroplastin-deficient compared to wild-type neurones. Detailed comparison of the localization of pre- (Synapsin) and postsynaptic (Homer) markers showed an increased synaptic mismatch and a reduced number of postsynapses between them in mature hippocampal cultures prepared from the Neuroplastin-deficient compared to wild-type mice (localization of Np65 at excitatory synapses is shown in Fig. 3). These results were confirmed by perturbation of Np65 function in cultures prepared from wild-type mice by the addition of Np65Fc constructs. Np65Fc and Np55Fc are recombinant proteins comprising either all three or the two common (Ig 2 and 3) Neuroplastin Ig domains respectively fused to the human Ig Fc domain (Smalla *et al.* 2000). Addition of Np65Fc reduced the match of pre- and postsynaptic elements of excitatory synapses in cultures from wild-type mice. As Np55Fc or Fc alone had no effect, this perturbation of synaptic function is Np65 specific. Immunocytochemical studies of Np65Fc binding confirmed that it binds to Np65-positive CA1 and DG neurones, suggesting that Np65 regulation of synapse number and coupling between pre- and postsynaptic elements is caused by Np65 *trans*-homophilic binding. Electrophysiological studies showed that both the amplitude and frequency of miniature excitatory postsynaptic currents, mEPSCs, are reduced by some 33% as a result of Neuroplastin deficiency (Herrera-Molina *et al.* 2014). In contrast, GABAergic synapses showed no effect of Np65 deficiency on either number or matching of pre-and postsynaptic elements. Surprisingly, analysis of miniature inhibitory postsynaptic currents (mIPSCs) showed that their amplitude was increased in Neuroplastin-deficient compared to wild-type neurones, though mIPSC frequency remained unchanged. However, it is possible that the increased amplitude of the mIPSCs is a consequence of reduced glutamatergic transmission. It will be interesting to study this early in neuronal network formation when GABAergic synapses are still excitatory (Cherubini *et al.* 1991; Ben-Ari *et al.* 1994) to learn about the potential impact of Np65, which starts to be expressed at

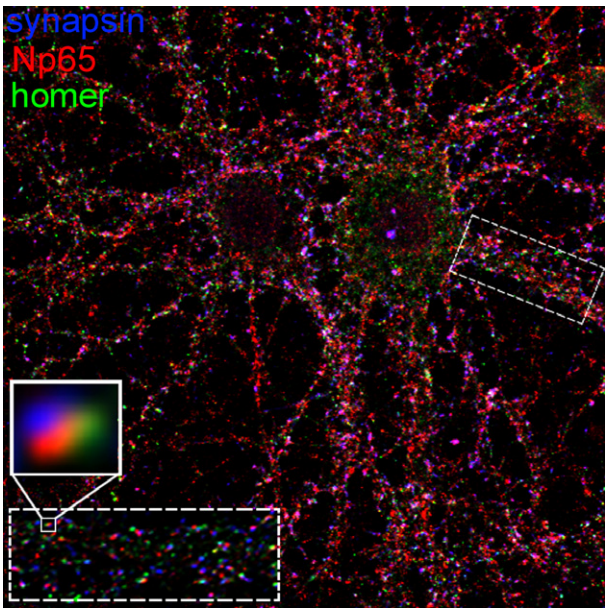

Fig. 3 Np65 is a component of the pre- and postsynaptic elements of hippocampal excitatory synapses. Rat hippocampal neurones were cultured for 28 DIV (Kaech and Banker 2006) and live stained for Np65 (red) using an anti-Np65 isoform-specific antibody according to published protocols (Herrera-Molina *et al.* 2014). Cells were then fixed, permeabilized and stained with antibodies against pre-(Synapsin I, blue) and postsynaptic (Homer, green) markers followed by secondary antibodies. The image was acquired using high resolution confocal microscopy. The digital magnification shows a dendrite segment after image deconvolution using Huygens software (Scientific Volume Imaging B.V., Hilversum, the Netherlands). Note that Np65 is clearly detected between excitatory pre- and postsynaptic elements.

a significant level around this time, on the initial establishment of a network's setpoint.

Analysis of $GABA_A$ receptor subunit composition revealed a reduced co-localization of α2 subunits with Gephyrin-positive GABAergic postsynaptic structures in Neuroplastin-deficient mice confirming the earlier data of Sarto-Jackson *et al.* (2012), showing that treatment of hippocampal cultures with Np65 shRNA down-regulates the level of α2 subunits (also see the section Np65 is important in localization of $GABA_A$ receptors). The α1-to-α2 subunit ratio at synapses is decreased in Neuroplastin-deficient neurones at 12 days in vitro (DIV), but increased at DIV 21. Np65 protein levels are strongly up-regulated at the same time that α1 replaces α2 subunits in hippocampal neurones (Panzanelli *et al.* 2011). This strengthens the hypothesis that Neuroplastin plays a role in the developmental switch of $GABA_R$ composition. This increased α1-to-α2 ratio at 21 DIV may account for the increased amplitude and faster decay time of the mIPSCs because of the increased conductance of α1 compared to α2 subunits (Brussaard *et al.* 1997; Ortinski *et al.* 2004). These data support a role for Np65 as an important regulator of the ratio and function of excitatory and inhibitory synapses in the hippocampus and suggest that Np65 plays an important role in regulating the network properties of hippocampal neuronal circuits.

Np65 *trans*-homophilic binding: its role in regulation of hippocampal long-term potentiation by activation of p38MAPK leading to internalization of GluR1 receptors

Binding experiments using covaspheres coated with Np65Fc or Np55Fc showed that Np65, but not Np55, exhibits *trans*-homophilic binding (Smalla *et al.* 2000). Crystallographic analysis of the structure of the Ig1 domain of ecto Np65 has identified the *trans*-homophilic binding site as a sequence on the Ig1 F–G loop (Owczarek *et al.* 2011). This loop is oriented perpendicular to the surface and therefore is able to bind to the corresponding F–G loop of the Ig1 domain on an opposing Np65 molecule. A recent study comparing the binding of Np65Fc to hippocampal neurones from wild-type and Neuroplastin-deficient mice confirms that the recombinant protein does indeed bind to endogenous Np65 molecules (Herrera-Molina *et al.* 2014).

In common with other CAMs including L1, NCAM and Cadherins (Luthi *et al.* 1994; Cremer *et al.* 1998; Tang *et al.* 1998), the Neuroplastins play key roles in long-term activity-dependent synaptic plasticity. Thus, several Neuroplastin antibodies including one that is specific to the Np65 N-terminal Ig1 domain almost completely suppress the maintenance, but not the induction of LTP at CA1 synapses in hippocampal slices (Smalla *et al.* 2000). This effect is also observed with Np65Fc, but not Np55Fc. These observations suggest that the effect is Np65-specific and is therefore likely to be mediated by *trans*-homophilic binding between pre- and postsynaptic Np65 molecules.

Since molecular modelling studies predict that Np65 molecules extend some 13–16 nm into the synaptic cleft, and since the synaptic cleft is some 20–30 nm (Gray 1987), Np65 can link the pre- and postsynaptic membranes by *trans*-homophilic binding. Therefore, presumably the anti-Np65 antibodies or recombinant peptides disrupt existing synaptic Np65 *trans*-homophilic interactions in addition to perturbing the formation of new trans-synaptic Np65 interactions.

The mechanism by which Np65 inhibits the maintenance phase of LTP involves activation of p38MAPK, which in turn down-regulates surface GluR1 containing α-amino-3-hydroxy-5-methylisoxazole-4-propionate-type AMPA glutamate receptors by internalization (Empson *et al.* 2006). Treatment of organotypic hippocampal slice cultures with Np65Fc resulted in an increase in phosphorylation of p38MAPK concomitant with the inhibition of LTP. P38MAPK is activated by dual phosphorylation on threonine 180 and tyrosine 182 residues (Paul *et al.* 1997; Mielke and Herdegen 2000; Ono and Han 2000). The specific p38 MAPK inhibitor SB202190 blocks the Np65Fc-induced inhibition of LTP, thus confirming the role of the kinase in mediating this response.

The availability of surface glutamate receptors is critical to the induction and maintenance of hippocampal LTP (Malinow and Malenka 2002), and multiple signal transduction pathways have been shown to regulate α-amino-3-hydroxy-5-methylisoxazole-4-propionate receptor incorporation into synapses during LTP. These include CaM kinase II (Lisman *et al.* 2002), MAPK (Zhu *et al.* 2002), protein kinase C (Boehm *et al.* 2006) and PI 3 kinase (Arendt *et al.* 2010). Strikingly, the number of GluR1 receptors at the cell surface, but not the total GluR1 receptor number, was reduced following treatment of hippocampal slices with Np65Fc. This down-regulation of surface GluR1 receptors is mediated by p38MAPK since the response was blocked by SB202190. However, the mechanism by which Np65Fc activates p38MAPK remains unclear, but is unlikely to involve direct binding of Np65 to the enzyme since there is no known kinase binding motif on the Neuroplastin intracellular domain. Since application of ecto Np65 to hippocampal neurones in culture induces an increase in intracellular synaptic calcium Owczarek *et al.* (2011), it is plausible that this triggers a signalling cascade resulting in p38MAPK activation.

The synaptic level of Np65 is regulated by synaptic activity

A number of paradigms which result in sustained increases in synaptic activity alter the level of Np65 present in the PSD. Thus, kainate-induced seizures result in a significant increase in the amount of Np65 present in PSDs prepared from seizured compared to kainate-treated non-seizured and control animals (Smalla *et al.* 2000). Similarly, the level of Np65 in a PSD-enriched fraction prepared from hippocampal

slices showed a marked increase following induction of LTP. Transient global ischaemia also results in a large increase in the level of PSD-associated Np65 (Beesley *et al.* 2013). A dynamic model for Np65 translocation into the PSD is supported by recent data which suggest that Np65 is a loosely bound rather than an integral PSD protein. PSDs can be prepared from SMs either using phase partitioning with *n*-octyl glucoside and polyethylene glycol (Gurd *et al.* 1982), or a double Triton X-100 procedure (Cho *et al.* 1992). The latter is more rigorous and solubilizes a number of loosely bound PSD proteins. The level of Np65 in PSDs prepared using the more rigorous Triton X-100 procedure is much lower than is observed using the milder phase partitioning protocol. In contrast, the level of PSD95, an integral protein component of PSDs at glutamatergic synapses, is comparable in PSDs prepared using either method. Taken together, these data suggest that Np65 is a loosely bound PSD protein, which can be translocated into the PSD in response to sustained changes in synaptic activity. However, it is also possible that the increased level of PSD-located Np65 is caused by increased stabilization, possibly associated with altered Np65 binding interactions.

Np65 is important in localization of $GABA_A$ receptors

Sarto-Jackson *et al.* (2012) have shown that Np65 binds to specific $GABA_A$ receptor subtypes and affects their localization. $GABA_A$ receptors comprise five subunits, most usually two α, two β and one γ subunit (Olsen and Sieghart 2008). Depending on subunit composition, the various receptor subtypes exhibit different pharmacological properties and different localization at synaptic and extrasynaptic sites. $GABA_A$ receptors containing α1, 2 or 3 subunits are localized mainly at synaptic sites and interact with the scaffolding protein Gephyrin, which anchors the receptor to the underlying postsynaptic complex and prevents their lateral diffusion (Kneussel and Loebrich 2007; Tretter *et al.* 2012). Receptors containing the α5 subunit are mainly extrasynaptic and link to the actin cytoskeleton via Radixin (Loebrich *et al.* 2006). Co-purification experiments using affinity columns of antibodies specific to the intracellular domain of Neuroplastin or for the $GABA_A$ receptor β2 subunit demonstrate a specific interaction between Neuroplastin and $GABA_A$ receptors. Co-immunoprecipitation experiments using antibodies directed against Neuroplastin or the extracellular domain of $GABA_A$ receptor α-subunits confirm both the interaction and that the Np65–$GABA_A$ complex is present on the cell surface. Fluorescence resonance energy transfer confirms a direct interaction between Np65 and $GABA_A$ receptors. Immunocytochemical studies in hippocampal neuronal cultures and sections show that Np65 co-localizes with α1 and α2, but not α3 subunits at GABAergic synapses and α5 subunits at extrasynaptic sites in cultures. Strikingly, the co-localization is confined to several synaptic sites along the same dendrite, but is absent from others. Significantly, only a small proportion of Gephyrin co-localized with Np65 and synaptic clusters of Np65 that co-localized with synaptic $GABA_A$ receptors, but not with Gephyrin were detected. A functional role for Np65 in the subcellular localization of specific $GABA_A$ receptor subtypes is suggested by the Np65 shRNA-induced down-regulation of α2-containing $GABA_A$ receptors at GABAergic synapses. In the shRNA-treated neurones, α2 subunit staining was more diffuse and did not co-localize with vesicular inhibitory aa transporter, a presynaptic marker of GABAergic synapses. These data together with the involvement of Neuroplastins in: (i) regulating the structure and function of hippocampal excitatory synapses, (ii) regulating the function of hippocampal inhibitory synapses and (iii) in recycling of GluR1 receptors suggest multiple roles for these CAMs in modulating synaptic signalling through receptor localization and recycling.

The role of the Neuroplastins in regulating neuronal energy supply

Monocarboxylates such as lactate, pyruvate and ketone bodies are important respiratory fuels for the developing and adult nervous system, lactate being the major monocarboxylate in the adult brain (Bergersen 2007). Specific 12-pass membrane proteins, the MCTs mediate the rapid uptake of these fuels across the cell membrane. Currently, 14 members of the MCT family have been identified, but only MCT1–MCT4, MCT8 and MCT10 have been functionally characterized. MCT1–MCT4 are proton-linked transporters which catalyse transport of monocarboxylate energy substrates across the plasma membrane (for reviews, see Halestrap and Wilson 2012; Halestrap 2013). Of these, MCT1, MCT2 and MCT4 are expressed in the brain. MCT1 is predominantly localized in glia, but also in some neurones, MCT4 in glia and cerebral vasculature while MCT2 is the major neuronal MCT in rodents, but not humans (Koehler-Stec *et al.* 1998; Karin *et al.* 2002; Debernardi *et al.* 2003; Rafiki *et al.* 2003; Halestrap and Wilson 2012). Lactate, principally released by glial cells, is an important energy substrate for neurones and sustains neuronal activity. MCT2 is suggested to be the major MCT isoform responsible for lactate uptake by neurones (Pierre and Pellerin 2005). Furthermore, immunocytochemical studies suggest that MCT2 is concentrated in the PSD (Bergersen *et al.* 2001). MCTs 1–4 all require ancillary proteins to be expressed in an active form at the plasma membrane, and the interaction between the two proteins is essential for their monocarboxylate transport function (for reviews, see Halestrap and Wilson 2012; Halestrap 2013). Basigin is the preferred ancillary protein for MCT1 (Kirk *et al.* 2000; Wilson *et al.* 2002) and Embigin the preferred ancillary protein for MCT2 in some tissues, for example kidney, though this has not been directly demonstrated for the brain (Wilson *et al.* 2005; Ovens *et al.* 2010).

Several observations provide strong evidence that the Neuroplastins can act as the preferred ancillary protein for MCT2 (Wilson *et al.* 2013). First, co-transfection of COS cells (a fibroblast-like cell line) with enhanced yellow (EYFP)fluorescent protein-tagged Neuroplastin and cyan fluorescent protein (CFP)-tagged MCT2 results in translocation of MCT2 to the plasma membrane, whereas in cells transfected with CFP-MCT2 alone, no MCT2 is detected at the cell surface and it remains in the perinuclear compartment. Fluorescence resonance energy transfer confirms a direct binding interaction between the Neuroplastins and MCT2 in double transfected COS cells.

Use of antisense technology in *Xenopus laevis* oocytes confirms that the Neuroplastins can act as accessory proteins for MCT2. These oocytes do not express endogenous MCT2 (Broer *et al.* 1999), but do express Basigin and Neuroplastin. Immunofluorescence studies show, as expected, that MCT2 is concentrated at the plasma membrane of control oocytes injected with MCT2 cRNA. However, in oocytes treated with Neuroplastin antisense RNA, there is no significant MCT2 at the plasma membrane (Wilson *et al.* 2013). In contrast, in oocytes treated with Basigin antisense RNA, there is only a partial reduction in the plasma membrane level of MCT2.

The functional consequences of the reduced transport and localization of MCT2 at the oocyte plasma membrane are shown by the fact that lactate transport is significantly increased in oocytes injected with MCT2 cRNA. However, if these oocytes are also treated with Neuroplastin antisense RNA, lactate transport is reduced to the level observed in control uninjected oocytes. However, the key question is: do Neuroplastins support MCT2 function in neurones *in vivo*? Immunocytochemical studies of cerebellum give strong indications that this proposition is correct. MCT2 and Neuroplastin show parallel patterns of localization on Purkinje cell somata and dendrites and most strikingly both are concentrated in the same parasagittal Zebrin II-negative stripes in the cerebellar vermis. These data suggest that Neuroplastins are the preferred accessory proteins for MCT2 in at least some neuronal populations (Wilson *et al.* 2013).

Previous electron microscope immunogold studies of MCT2 localization in the cerebellum showed a concentration of MCT2 immunoreactivity at the PSDs of parallel fibre–Purkinje cell synapses (Bergersen *et al.* 2001, 2002). Interestingly, the distribution of MCT2 immunoreactivity paralleled that of δ2-glutamate receptors. As the intracellular domains of both these transmembrane proteins have an SXI PDZ binding motif, it was suggested that both proteins could be anchored to the PSD by interaction with one of the PDZ domains of PSD95. Various reports show that lactate, mainly released by astrocytes, is used as an important energy substrate by neurones and sustains neuronal activity (Pierre and Pellerin 2005). Furthermore, Suzuki *et al.* (2011) have demonstrated the importance of astrocyte–neurone lactate transport in long-term memory formation. They show that disrupting the expression of MCT1 and MCT4, both of which are expressed in astrocytes, leads to amnesia. This can be rescued by lactate, but not glucose. However, while disruption of the neuronally expressed MCT2 also causes amnesia, significantly this can be rescued by glucose, but not lactate. Overall, these data support MCT2 as the key player in ensuring an adequate supply of lactate to energize neurones and synapses after stimulation.

The role of Basigin in monocarboxylate transport in sensory systems

The role of Basigin in sensory system function has been most extensively studied in the retina (for a review, see Ochrietor and Linser 2004), but also in the olfactory system. In the retina, photoreceptors can function when provided with lactate, but not glucose (Winkler 1981). The retinal Muller glial cells have high glycolytic activity and release lactate in response to light (Poitry-Yamate *et al.* 1995). Thus, there is a cycle of glial lactate release followed by photoreceptor uptake and utilization for energy production. In the retina, Basigin 1 is expressed by the Muller glial cells and Basigin 2 by the photoreceptors. Strikingly homozygous Basigin null mice are blind from the time of eye-opening, exhibiting retinal dysfunction followed by degeneration of the photoreceptors (Hori *et al.* 2000; Philp *et al.* 2003; Ochrietor and Linser 2004). The expression of MCT1 and Basigin overlaps in the retina and the level of the MCT1 protein is markedly reduced in retinal membrane preparations from Basigin null mice, although the level of MCT1 transcript is not affected (Philp *et al.* 2003). The photoreceptor degeneration is suggested to result from the observed failure of MCT1 to integrate into the plasma membranes of the Muller glial cells and photoreceptors, disrupting the lactate cycle from Muller glial cells to photoreceptors, thus leading to photoreceptor degeneration. Ochrietor has investigated whether a similar lactate shuttle operates in the olfactory system. Although Basigin and MCT1 are both expressed in the nasal epithelium and in the olfactory bulb their patterns of expression do not overlap (J.D. Ochrietor, unpublished results). Interestingly, MCT2 is expressed by olfactory neurones. As detailed in the section 'The role of the Neuroplastins in regulating neuronal energy supply', there is clear evidence that Neuroplastins can act as the accessory protein for MCT2, although as yet there is only circumstantial evidence that Neuroplastins are the preferred accessory proteins for MCT2 in neurones. As the nasal sensory neurones do not express Embigin and Basigin is not the preferred partner (Wilson *et al.* 2005, 2013; Ovens *et al.* 2010), this provides an opportunity to demonstrate unequivocally that Neuroplastin is the accessory protein for MCT2 in at least some neuronal populations *in vivo*.

Naruhashi *et al.* (1997) reported abnormalities of sensory and memory functions in homozygous Basigin knockout

mice, including impaired performance in a Y-maze task which assesses short-term memory and in a water finding task which examines latent learning without any motor dysfunction. Subsequently, it has been proposed that these learning and memory deficiencies are caused by disruption of a lactate shuttle similar to that in the retina, but between neurones and astrocytes in specific brain regions (Howard *et al.* 2010).

Does Np55 play a role in cerebellar long-term depression?

The preferential localization of Neuroplastins and MCT2 to subsets of Purkinje cells in parasagittal Zebrin II-negative stripes may be related to synaptic plasticity and LTD. This proposition is supported by reports that different parallel fibre synapses have different postsynaptic constituents in different Purkinje cell compartments. Furthermore, several proteins associated with cerebellar glutamatergic transmission, cellular signalling pathways and with LTD show differential expression between Purkinje cells localized in the parasagittal Zebrin II-positive and negative stripes (reviewed in Armstrong and Hawkes 2000), i.e. paralleling the localization of the Neuroplastins and MCT2. Key examples of such molecules include the metabotropic glutamate receptor mGluR1b (Mateos *et al.* 2001), excitatory aa transporter 4 (Dehnes *et al.* 1998), an inositol 1,4,5-trisphosphate receptor (Furutama *et al.* 2010), Phospholipase Cβ3/4 (Sarna *et al.* 2006), and protein kinase C (Barmack *et al.* 2000). The concentration of Np55 at cerebellar synapses suggests that, like Np65 in the hippocampus, it may play a role in synaptic plasticity, and in the cerebellum this suggests a role in LTD. Taken together, the data on the concomitant differences in localization of Np55 and MCT2 between subsets of Purkinje cells may, at least in part, be related to a differential requirement for lactate as an energy source, which in turn may relate to differences in LTD between the subsets of Purkinje cells.

Behavioural effects of Neuroplastins

The potential of peptide mimetics that target the binding interactions and physiological responses of the Neuroplastins to modulate behavioural responses is of considerable interest. Np55-mediated activation of FGFR1 coupled with the observation that its cognate ligand FGF2 decreases depressive behaviour in rats (Evans *et al.* 2004) led Owczarek *et al.* (2011) to investigate whether narpin, the synthetic peptide which encompasses the FGFR1 binding motif of Np55, has antidepressant activity using the forced swim test as a model paradigm. Antidepressant treatment of rodents in this test results in decreased immobility coupled with increases in swimming and climbing behaviour (Porsolt *et al.* 1978). Narpin treatment resulted in antidepressant-like changes in behaviour, i.e. decreased floating behaviour and increased climbing behaviour. These effects are not caused by enhanced locomotor activity as evidenced by determining the effects of narpin in the open field test. At present, the mechanism for the antidepressant effect of narpin is not clear. However, the NCAM-derived FGL, FG-loop, synthetic peptide, and the growth factor FGF2, both of which exert an antidepressant effect, increase ERK phosphorylation (Garcia-Maya *et al.* 2006; Aonurm-Helm *et al.* 2008). These observations lead to the proposal that ligands for the FGFR modulate behaviour via the ERK pathway (Owczarek *et al.* 2011). Overall, the data suggest that narpin may be a useful therapeutic agent for the treatment of mental disorders.

Further evidence for a role of Np65 in human depressive behaviour is provided by studies on the effects of chronic stress on synaptic Np65 levels. Exposure to chronic stress induces behavioural changes associated with depressive behaviour (anhedonia, learned helplessness and anxiety) and induces changes to synaptic structure and function that parallel those observed in animal models of depressive behaviour, namely dendritic spine retraction together with dysregulation of synaptic proteins (Dagnino-Subiabre *et al.* 2009). In a preliminary study in rats, it has been shown that after chronic stress, the levels of Np65 in forebrain PSDs and SMs are significantly reduced. The changes in synaptic Np65 level are reversed by treatment with the selective serotonin reuptake inhibitor Fluoxetine (P. Farias, E. Ampuero, R. Herrera-Molina, K-H. Smalla and U. Wyneken, unpublished results). These results suggest an involvement of Np65 in the concomitant synaptic reorganization processes after chronic stress, and also a functional importance of serotonergic transmission for Np65 localization.

As Np65 plays a key role in activity-dependent synaptic plasticity, Owczarek *et al.* (2011) tested the effect of enplastin, the Np65-specific peptide which targets the *trans*-homophilic binding site, on spatial learning behaviour using the Morris water maize. Although enplastin treatment resulted in increased times for animals to find the hidden platform on day 1, no effects were observed on subsequent days. Thus, the peptide has an inhibitory effect on the initial, but not later, phases of the learning process. In this context, the behaviour of Neuroplastin-deficient mice will be a valuable tool for establishing the functional significance of Neuroplastin-mediated cell adhesion *in vivo*.

A role for Neuroplastin in pathophysiology and human disease

So far, no direct role for the Neuroplastins in human disease and neurological disorders has yet been clearly defined. However, a single nucleotide polymorphism (SNP) in the human Neuroplastin locus is associated with reduced cortical thickness and impaired intellectual ability in adolescents (Desrivières *et al.* 2014). In this paper, SNPs in genes showing altered expression during growth and differentiation of a human neuronal stem cell line were correlated with changes in cortical thickness. One variant, rs7171755 that is located < 2 kb downstream of the *NPTN* gene, was

associated with a thinner left hemisphere cortex, particularly in the frontal and temporal lobes, resulting in impairment of verbal and non-verbal intellectual abilities. Strikingly cortical thickness and *NPTN* expression exhibit a hemispheric asymmetry, both being higher in the right than in the left cortex. Changes in cortical thickness in the adolescent are consistent with known cellular maturational parameters such as synaptic density. Given the role of Neuroplastin 65 in regulating synapse structure and function in the mouse hippocampus, it seems likely that Neuroplastins also play key roles in regulating synapse function and neuronal network activity in the human brain.

Linkage to schizophrenia

Neuroplastin has been identified as a risk factor for schizophrenia (Ouchi *et al.* 2005; Saito *et al.* 2007). These studies used two animal models for schizophrenia, namely treatment of rodents with the psychotomimetics methamphetamine or phencyclidine. Chronic methamphetamine use induces a psychotic state closely resembling schizophrenia in humans (Sato *et al.* 1983), and administration of phencyclidine mimics both the positive and negative symptoms of schizophrenia (Javitt and Zukin 1991). Gene expression profiling of either phencyclidine- or methamphetamine-treated rats revealed that both Neuroplastin and Basigin are up-regulated in both groups. Only 41 genes were either up-or down-regulated by these treatments (Ouchi *et al.* 2005). Subsequent studies of schizophrenic patients identified four SNPs in the 5′ upstream putative promoter and 5′ untranslated region (Saito *et al.* 2007) of the *NPTN* gene. Of these, one (del-G-G) exhibited increased frequency in schizophrenia. A second (T-G-T) exhibited decreased frequency and significantly lowered Neuroplastin transcription as determined from experiments with reporter constructs. These data led Saito *et al.* (2007) to propose that the T-G-T SNP, through its inhibitory role in transcription of the *NPTN* gene, could lower the onset risk for schizophrenia.

Role in ischaemia

A significant role for Np65 in recovery from ischaemic insult is suggested by the threefold increase in Np65 in forebrain PSDs following transient ischaemia (Beesley *et al.* 2013). It is plausible that the increased Np65 level in PSDs is at least in part related to its function as an accessory protein and chaperone for MCT2, and possibly MCT1. The glucose and oxygen deprivation, which results from the ischaemia, disrupts cerebral energy metabolism. Lactate is suggested to be an obligatory substrate for recovery following hypoxia (Schurr *et al.* 1997). Furthermore, blockade of lactate transport exacerbates delayed neuronal damage in rat brain following ischaemia (Schurr *et al.* 2001). These results are consistent with an increased postsynaptic level of MCT2 as a neuroprotective mechanism in the rodent brain. This also raises the question as to whether the increased PSD level of Np65 observed in late-phase LTP or following kainate-induced seizures is related to the requirement for increased energy supply under these conditions.

Neuroplastin as a biomarker for breast cancer

In a screen to select potential tumour antigens in B-cell actively proliferating regions of tumour-draining lymph nodes from human breast cancer patients, Rodriguez-Pinto *et al.* (2009) identified Neuroplastin as a potential candidate. Subsequent analysis of human breast carcinoma tissue revealed the presence of both 55 and 45 kDa Np55 glycoforms. Significant Neuroplastin immunoreactivity was detected in some 20% of breast tissue showing invasive carcinoma compared with only 2.5% of control breast tissue. Interestingly a larger percentage of Neuroplastin-positive tumours was found in tissue showing distant (50% Neuroplastin-positive) rather than lymph node (20% Neuroplastin-positive) metastasis, leading to the suggestion that Neuroplastin expression may promote tumour invasion. Strikingly Neuroplastin over-expression in a breast cancer cell line transplanted into nude mice resulted in a significant increase in tumour growth and angiogenesis. The Neuroplastin-induced increase in angiogenesis observed *in vivo* is mediated by an increase in production of vascular endothelial growth factor. These data led to the suggestion that aberrantly expressed Neuroplastin might promote breast tumour growth and metastasis. Thus, Np55 is a potential biomarker for breast tumour screening and for development of therapeutic approaches. Importantly, a range of FGFR abnormalities associated with breast cancer has been identified, including FGFR1 amplification which is found in 8–15% of all breast cancers, and amplification of the chromosomal region harbouring FGFR1 has been detected in 10–15% of breast cancers (reviewed in Fearon *et al.* 2013). Thus, it is plausible that activation of FGFR by Np55 may play a role in the pathophysiology of some types of breast cancer.

In the context of Neuroplastin links to cancer, it has been reported that *NPTN* is 1 of 166 genes that were differentially expressed in adenomatous and normal intestinal mucosas, i.e. colorectal carcinoma tissue (Gaspar *et al.* 2008), exhibiting a down-regulation.

Neuroplastin localization in a model of Niemann–Pick disease

Niemann–Pick disease refers to a group of related genetic disorders in which sphingomyelin accumulates in lysosomes. It is fatal in early childhood for severe forms of the disease and early adulthood for less severe forms. In the classical Niemann–Pick type A disease, there is a complete loss of acid sphingomyelinase resulting in early death. An animal model for this disease has been generated by targeted disruption of the acid sphingmyelinase gene to produce a knockout (ASKMO) mouse (Horinouchi *et al.* 1995).

Disruption of the acid sphingomyelinase gene in turn disrupts cholesterol metabolism, resulting in widespread axonal and dendritic abnormalities and ultimately Purkinje cell death (Otterbach and Stoffel 1995; Sarna *et al.* 2001). The pattern of Neuroplastin immunoreactivity in the cerebellum has been studied in the ASKMO mouse. Neuroplastin immunoreactivity in Purkinje cells in homozygous wild-type mice is confined to the dendrites whereas in homozygous ASKMO mice high levels of Neuroplastin immunoreactivity are often detected in the surviving Purkinje cell somata (Marzban *et al.* 2003). This altered cellular localization is thought to be associated with a generalized defect in protein trafficking. Interestingly, Purkinje cell Neuroplastin expression and localization is not dependent either upon the normal histotypic organization of the cerebellum or formation of normal parallel fibre–Purkinje cell synaptic connections since Neuroplastin is readily detected in the Purkinje cell dendrites of both the *disabled (dab)* and *cerebellar folia deficient (cdf)* mice (Marzban *et al.* 2003). In the *dab* mouse, there is a targeted disruption of the Reelin signalling pathway, resulting in a profound Purkinje cell ectopia as these neurones remain as ectopic clusters among the deep cerebellar nuclei rather than migrating to their normal destination in the Purkinje cell layer. Furthermore, these ectopic cells receive little or no parallel fibre input (Howell *et al.* 1997; Gallagher *et al.* 1998). Similarly, *cdf* mice also exhibit ectopic Purkinje cells which express abundant dendritic Neuroplastin immunoreactivity (Marzban *et al.* 2003).

Conclusions and future perspectives

It is clear that considerable progress has been made in understanding the diverse physiological functions of Neuroplastins ranging from cell to cell adhesion, synapse stabilization and modification both in development and in activity-dependent synaptic plasticity, to regulation of neuronal energy supply provided by monocarboxylates such as lactate. The availability of Neuroplastin-deficient mice has facilitated the characterization of Neuroplastin functions *in vivo*. Although a diverse range of Neuroplastin binding partners have been identified, and interaction with a number of key cellular signalling pathways established as discussed below, further Neuroplastin-interacting proteins and signalling pathways await identification and characterization. The identification of an SNP in the human *NPTN* gene locus which impairs cortical development and intellectual ability, and the evidence linking Neuroplastin to a number of pathophysiological conditions as discussed in the section on A role for Neuroplastin in pathophysiology and human disease above, highlights the importance of Neuroplastin function in the human brain. The current state of the art as reviewed here suggests a number of priority areas for further investigation as follows.

Comprehensive characterization of Neuroplastin functions *in vivo*

To date, most of the Neuroplastin functional studies are *in vitro*. Analysis of mice in which expression of the *NPTN* gene is disrupted will provide a valuable tool for investigating which of these functions are physiologically important *in vivo* and provide insight into the underlying cellular and molecular mechanisms which give rise to the phenotype.

In particular, the development of inducible and region-specific Neuroplastin-deficient mice and mice which specifically lack Np65 will be of key importance for establishing the specific functions of Neuroplastin isoforms.

Identification of novel Neuroplastin binding partners

Although some Neuroplastin binding partners have already been identified, blue native gel separations of detergent-solubilized synaptic fractions show several high-molecular-weight Neuroplastin complexes indicating that further binding partners remain to be identified (T. Kaehne, P. Klemmer and K.-H. Smalla, unpublished results). Therefore, establishing the complete Neuroplastin interactome(s) and its dynamics in different neuronal activity states is a key goal.

Linking physiological function to cellular and molecular mechanisms

Despite the identification of several Neuroplastin binding partners and signalling pathways, the critical link between functional effect and mechanism is for the most part not clear and a major focus of future research must be to establish these links.

Neuroplastin function in receptor complex assembly

An emerging theme is the role of the Neuroplastins in the chaperoning and trafficking of several molecules including MCT2, glutamate receptor GluR1 and $GABA_A$ receptor subunits. However, the molecular mechanisms involved and the role of Np65 in the assembly of the intact receptors are areas for future investigation.

Functional overlap between the members of the Basigin group of the IgSF

While the differences in structure and binding partners suggest that some functions of each of the three members of the Basigin group of the IgSF are specific to one family member, e.g. induction of extracellular matrix metalloproteinases by Basigin, there is undoubtedly some redundancy of function between family members. It is already clear that there is promiscuity between the three family members with respect to their function as accessory proteins for MCTs. Strikingly the level of Basigin is up-regulated in the forebrain of Neuroplastin-deficient mice (D. Montag, T. Kaehne and K.-H. Smalla, unpublished results), suggesting that although it has lower affinity for MCT2 than Neuroplastin, it may compensate for some Neuroplastin functions. A complete

analysis of overlap between functions for the three group members is required. Furthermore, it is not clear whether heterodimers between the three group members occur *in vivo*.

Can Neuroplastin act as the accessory protein to MCT1 in human brain?

Although MCT2 is the major neuronal MCT in rodents, it is much less abundant in the human brain. This raises the question of whether other MCTs, primarily MCT1, fulfil this role in humans. Interestingly, YFPN (yellow fluorescent protein)-Neuroplastin is able to efficiently chaperone CFPN-MCT1 to the cell surface when both are co-transfected into COS cells (M.C. Wilson and A.P. Halestrap, unpublished observations).

The roles of Neuroplastin in pathophysiological conditions

The association of the human *NPTN* gene locus with cortical thickness and intellectual abilities (Desrivières *et al.* 2014) together with data from the studies on the behavioural effects of Neuroplastin-related peptides suggests that these CAMs may be clinically relevant to a number of genetically complex neurological disorders. They therefore offer a starting point for novel approaches to the development of diagnosis and/or therapy.

Undoubtedly our understanding of the importance of the Neuroplastins in neuronal and synaptic function and in specific pathophysiologies will be significantly increased by studies addressing these priority areas.

Acknowledgments and conflict of interest disclosure

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