Adhesion G protein-coupled receptors in nervous system development and disease

Tobias Langenhan[1], Xianhua Piao[2] and Kelly R. Monk[3]

Abstract | Members of the adhesion G protein-coupled receptor (aGPCR) class have emerged as crucial regulators of nervous system development, with important implications for human health and disease. In this Review, we discuss the current understanding of aGPCR functions during key steps in neural development, including cortical patterning, dendrite and synapse formation, and myelination. We focus on aGPCR modulation of cell–cell and cell–matrix interactions and signalling to control these varied aspects of neural development, and we discuss how impaired aGPCR function leads to neurological disease. We further highlight the emerging hypothesis that aGPCRs can be mechanically activated and the implications of this property in the nervous system.

Extracellular matrix
(ECM). A network of secreted molecules, including glycoproteins and polysaccharides. ECM molecules secreted by a cell remain closely associated to provide adhesive, signalling and structural functions.

[1]*Department of Neurophysiology, Institute of Physiology, University of Würzburg, Röntgenring 9, 97070 Würzburg, Germany.*
[2]*Division of Newborn Medicine, Department of Medicine, Children's Hospital and Harvard Medical School, 300 Longwood Avenue, Boston, Massachusetts 02115, USA.*
[3]*Department of Developmental Biology and Hope Center for Neurological Disorders, Washington University School of Medicine, 660 South Euclid Avenue, Saint Louis, Missouri 63110, USA.*
tobias.langenhan@uni-wuerzburg.de; xianhua.piao@childrens.harvard.edu; monkk@wustl.edu

doi:10.1038/nrn.2016.86
Published online 28 Jul 2016

Diverse steps are required for proper nervous system development, assembly and connectivity. Precursors must proliferate, commit to a fate and differentiate into specialized cells. Neural cells (neurons, glia and their precursors) must migrate to distinct destinations from their origins, guided by cell and planar polarity cues. Neurons and glia then form essential cellular specializations, including dendrites, synapses and the myelin sheath. These processes rely on the ability of neural cells to sense and communicate with their environment, including neighbouring cells and the extracellular matrix (ECM), and to relay key extracellular cues into intracellular signalling events. Remarkably, one class of proteins — the adhesion G protein-coupled receptors (aGPCRs) — can carry out all of these varied functions.

The aGPCRs represent the second largest GPCR class in the human genome[1], but they are considerably understudied compared with other GPCR classes. Like all GPCRs, aGPCRs have a seven-transmembrane (7TM) heptahelical domain and an intracellular domain (ICD) (FIG. 1) through which the receptor can couple to heterotrimeric G proteins[2]. Uniquely, aGPCRs are also defined by two additional features: first, a long amino-terminal extracellular domain (ECD) (FIG. 1) that for many family members contains motifs that are classically involved in cell–cell adhesion (hence the name 'adhesion GPCR'); and second, the presence of a GPCR autoproteolysis-inducing (GAIN) domain within the ECD, which encompasses the highly conserved GPCR proteolysis site (GPS)[3,4] (FIG. 1a,b). Many aGPCRs undergo autoproteolysis at the GPS, which results in a protein that is separated into an N-terminal fragment (NTF) and a carboxy-terminal fragment (CTF)[5] (FIG. 1). It is now generally accepted that aGPCRs can function as adhesion molecules due to the NTF and can function as classical GPCRs through the CTF[2]. Recently, receptor autoproteolysis was shown to expose a cryptic tethered agonist ligand, known as the *Stachel* sequence (stalk), which is a potent receptor activator[6–10] (FIG. 1a,b). Receptor autoproteolysis and *Stachel*-mediated activation are discussed in more detail below. Importantly, the unique structural features of aGPCRs allow this receptor class to fulfil diverse functions in nervous system development, and the importance of these roles is clear given the fact that mutations in aGPCRs cause numerous nervous system diseases and pathologies.

aGPCRs in neurodevelopment and disease

aGPCRs in early nervous system development. One of the earliest stages of nervous system development in vertebrates is neural tube closure. The exact sequence of morphogenetic movements varies depending on the species but neural folds ultimately merge at the midline of the embryo, generating the neural tube that will give rise to the brain, spinal cord and neural crest[11]. At this stage, aGPCRs are already key components of neural development. Mice with mutations in the gene encoding the aGPCR CELSR1 (also known as ADGRC1) exhibit craniorachischisis, a severe neural tube defect in which the brain and spinal cord remain open owing to the failure of neural tube closure[12]. A mechanism for this phenotype was suggested by work in chicks, in which CELSR1 is normally localized at a restricted population

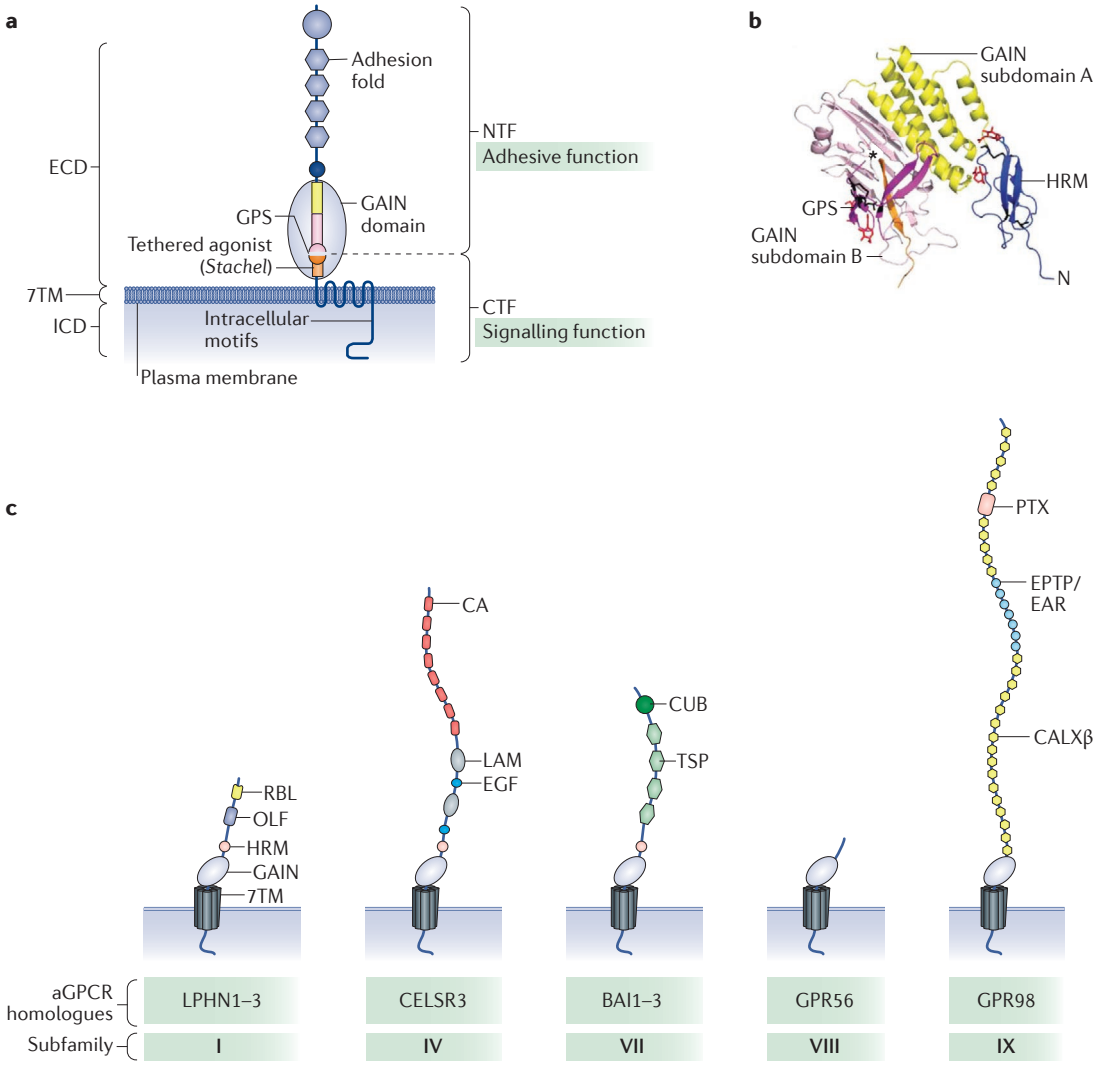

Figure 1 | **Molecular architecture of aGPCRs. a** | Adhesion G protein-coupled receptors (aGPCRs) possess structural elements of adhesion molecules and GPCRs. Their extended extracellular domain (ECD) usually contains a collection of adhesion motifs that can engage with cellular and matricellular interaction partners, and a juxtamembrane GPCR auto-proteolysis-inducing (GAIN) domain, which is present in all aGPCRs. GAIN subdomain A (yellow rectangle), GAIN subdomain B (pink rectangle) and the GPCR proteolysis site (GPS) motif (pink and orange semicircles) are shown. The GAIN domain is directly connected to the seven-transmembrane (7TM) unit through a linker sequence of approximately 20 amino acids, known as the *Stachel* (stalk). Recently, this structural component of aGPCRs was identified as a tethered agonist, which stimulates metabotropic activity of several aGPCR homologues. Similar to the ECD, the intracellular domains (ICDs) of aGPCRs can be unusually large. It is estimated that more than one-half of all known aGPCRs undergo auto-proteolytic cleavage that is catalysed through the GAIN domain, which is present on the cell surface as a non-covalent heterodimer between an amino-terminal fragment (NTF) and a carboxy-terminal fragment (CTF). The cleavage occurs at the evolutionarily highly conserved GPS. **b** | The crystal structure of the GAIN domain of rat latrophilin 1 (LPHN1; PDB accession code: 4DLQ) shows that it is composed of two subdomains A (yellow) and B (pink), with subdomain B containing the GPS motif (purple), where self-cleavage occurs at the GPS (asterisk); colours correspond to the aGPCR diagram in part **a**. This cleavage event separates the contiguous receptor protein into the NTF and the CTF. After proteolysis, the last β-strand of subdomain B of the GAIN domain (orange) belongs to the CTF and corresponds to the *Stachel* sequence. The structure demonstrates that both cleavage fragments of the GAIN domain remain firmly attached to each other through non-covalent interactions. The GAIN domain of LPHN1 was crystallized together with its neighbouring hormone receptor motif (HRM) domain (blue). **c** | Molecular layout of representative certain aGPCRs. Subfamily I contains LPHN1–3 (also known as ADGRL1–3). CELSR3 (also known as ADGRC3) is a member of subfamily IV. BAI1–3 (also known as ADGRB1–3) are members of subfamily VII. GPR56 (also known as ADGRG1) is a member of subfamily VIII. GPR98 (also known as ADGRV1) is the only member of subfamily IX. CA, cadherin domain; CALXβ, calnexin-β domain; CUB, C1r/C1s, Uegf, Bmp1 domain; EGF, epidermal growth factor domain; EPTP/EAR, epitempin/epilepsy-associated repeat; LAM, laminin domain; OLF, olfactomedin domain; PTX, pentraxin domain; RBL, rhamnose-binding lectin domain; TSP, thrombospondin domain. Part **a** is adapted with permission from REF. 95, AAAS. Part **b** is reproduced with permission from REF. 4, European Molecular Biology Organization.

Adhesion G protein-coupled receptors
(aGPCRs). A family of receptors within the GPCR superfamily structurally characterized by a large extracellular domain (ECD), a seven-transmembrane domain (7TM) and an intracellular domain (ICD). aGPCR ECDs often contain motifs that are involved in cell–cell and cell–matrix interactions.

GPCR autoproteolysis-inducing (GAIN) domain
An evolutionarily conserved domain (~320 amino acids) shared by adhesion G protein-coupled receptors and polycystic kidney disease proteins. The GAIN domain is both necessary and sufficient for receptor autocleavage.

***Stachel* sequence**
(Stalk). From the German word for 'stinger', the *Stachel* sequence is generated by adhesion G protein-coupled receptor (aGPCR) cleavage, is connected to the seven-transmembrane domain and functions as an intramolecular tethered agonist for aGPCRs.

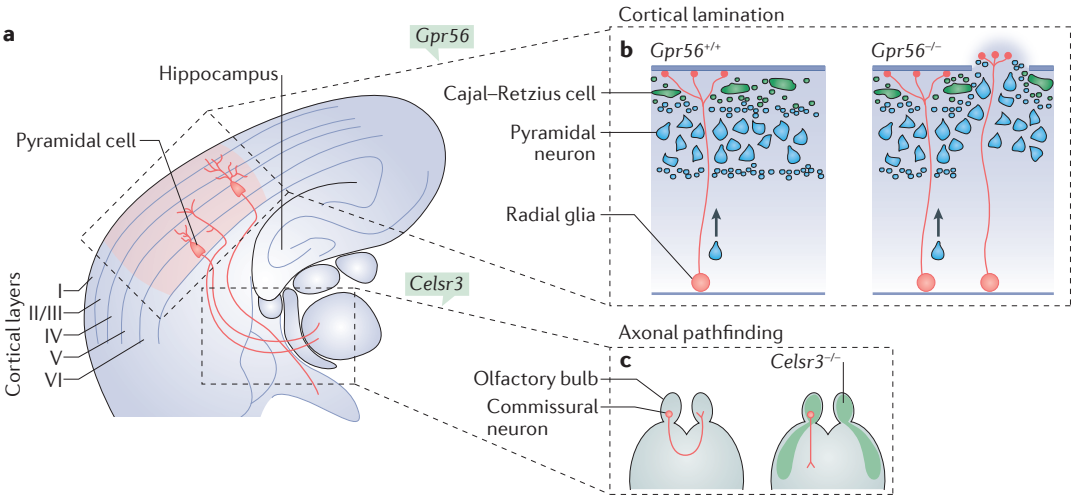

Figure 2 | **Organ-scale functions of aGPCRs. a** | Adhesion G protein-coupled receptors (aGPCRs) affect the overall architecture and wiring of cortical and subcortical brain areas. Left, schematic of the forebrain shown in sagittal section. The red shaded area marks the parietal cortex where cortical defects occur in the absence of GPR56. **b** | The protein encoded by *Gpr56* regulates pial basement membrane integrity and cortical lamination. Wild type (*Gpr56*$^{+/+}$) is shown on the left. Deleting *Gpr56* results in the overmigration of preplate neurons (blue) to regions normally occupied by Cajal–Retzius cells (green), misplacement of radial glial endfeet (red) and a breached pial basement membrane (purple). **c** | The protein encoded by *Celsr3* (also known as *Adgrc3*) exerts potent control over the migration of many fibre tracts in the brain, including commissural axons connecting olfactory nuclei (red), corticoefferent and thalamocortical fibres (not shown). Removal of *Celsr3* results in axonal pathfinding defects and concomitant miswiring of affected brain regions[30,39]. Part **a** is adapted from REF. 114, Nature Publishing Group. Part **b** is adapted with permission from REF. 115, Landes Bioscience and Springer Science + Business Media. Part **c** is adapted from REF. 18, Nature Publishing Group.

of adherens junctions that are oriented towards the mediolateral axis of the neural plate[13]. *In vivo* knockdown studies indicated that *CELSR1* cooperates with planar cell polarity (PCP) proteins to upregulate RHO kinase at these adherens junctions. Without localized RHO activation, the contraction and apical constriction of the neural plate cells is impaired, leading to defects in neural tube closure[13]. In humans, *CELSR1* mutations are associated with craniorachischisis, as well as other neural tube defects, suggesting that the functions of this aGPCR in neural tube closure are conserved[14–16]. CELSR proteins that function as PCP components are also required for proper cilia positioning in ependymal cells[17], as discussed in a recent review on this topic[18].

aGPCRs shape the nervous system. As in neural tube closure and ependymal organization, CELSR proteins also regulate neuronal migration, dendritogenesis and axon guidance[18]. *celsr2*-knockdown zebrafish and *Celsr2*-mutant mice display impaired caudal migration of facial branchiomotor neurons in the brainstem, and, in mice, these defects are enhanced by the loss of *Celsr3* (also known as *Adgrc3*)[19,20]. These phenotypes are also observed in other PCP pathway mutants[18], although it is unclear whether canonical PCP signalling is required. Interestingly, in *Celsr1*- mutant mice, a large subset of facial branchiomotor neurons aberrantly migrate rostrally rather than caudally[20]. This unique phenotype has not been described in any other mutant, PCP pathway or otherwise, suggesting that CELSR1 controls the directionality of neuron migration in a PCP-independent pathway.

Beyond the brainstem, aGPCRs are also involved in neuronal migration in the developing cerebral cortex. This role was first suggested by the discovery that *GPR56* (also known as *ADGRG1*) mutations in humans cause a form of polymicrogyria, bilateral frontoparietal polymicrogyria (BFPP)[21,22]. Further studies in *Gpr56*-mutant mice, and in one post-mortem brain, revealed that BFPP is a cobblestone-like lissencephaly (that is, a pathologically smooth brain that lacks normal folding), which is characterized by overmigrating neurons and the formation of neuronal ectopias on the surface of the brain[23,24]. In mutant mice, the pial basement membranes develop normally at first, but the membranes are subsequently breached by overmigrating preplate neurons[23] (FIG. 2). At the pial surface, binding of the ECM protein collagen III to GPR56 activates RHOA by coupling to $G\alpha_{12/13}$, which in turn inhibits neuronal migration[25].

Following neuronal migration in development, dendritic elaboration occurs that enables appropriate functional domains to be established, axons must undergo pathfinding, and these two neuronal protrusions must correctly wire together to produce functional circuits. Once again, aGPCRs represent key mediators of these developmental events, with functions conserved in invertebrates and vertebrates. *Drosophila melanogaster* and *Caenorhabditis elegans* possess one CELSR orthologue: Flamingo (Fmi (also known as Starry night and Stan) in *D. melanogaster* and FMI-1 in *C. elegans*). In *D. melanogaster*, *fmi* mutants display dendritic overgrowth phenotypes at the midline (in the peripheral nervous system (PNS) and in the

Planar cell polarity
(PCP). The global polarized organization of cells within the plane of a tissue.

Polymicrogyria
A developmental brain malformation characterized by many small folds (gyri) in the cortex. Bilateral frontoparietal polymicrogyria (BFPP) and bilateral perisylvian polymicrogyria (BPPR) are subtypes of polymicrogyria caused by mutations in the adhesion G protein-coupled receptor gene *GPR56*.

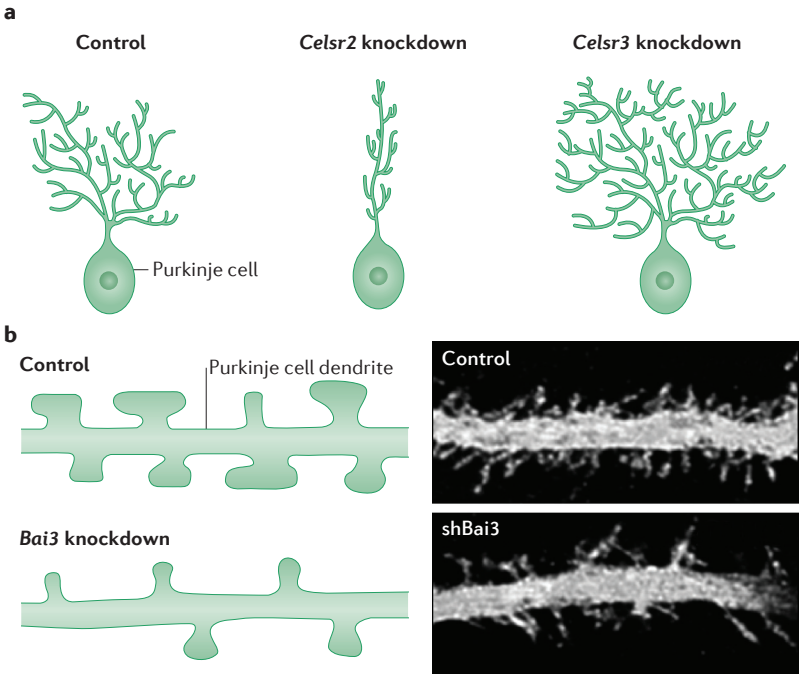

Figure 3 | **Cellular-scale functions of aGPCRs. a** | The architecture of neurons is governed by adhesion G protein-coupled receptors (aGPCRs). The extensive dendritic arbours of Purkinje cells are controlled by receptors of the CELSR group of aGPCRs. Shown are the dendritic arbours of a generic mouse Purkinje neuron upon knock down of *Celsr2* (also known as *Adgrc2*; middle), which leads to a reduction in arbour complexity compared with the control neuron (left). Knock down of *Celsr3* (also known as *Adgrc3*) causes the opposite effect, resulting in a more intricate dendritic structure (right)[31]. **b** | The function of BAI3 (also known as ADGRB3) is involved in the establishment of synaptic connections between Purkinje cells and the excitatory inputs coming from climbing and parallel fibre afferents. Shown is the schematic morphology of Purkinje cells (left) and a confocal image of these cells (right) after knock down of *Bai3*, which causes the depletion of synapse and spine density on its dendrites[44,45]. shBai3, short hairpin ribonucleic acid to silence *Bai3* expression. Part **a** is adapted from REF. 116, Nature Publishing Group. Part **b** is reproduced with permission from REF. 44, Elsevier.

mushroom body neurons (CNS)[26–29], supporting the idea that Fmi inhibits dendrite extension. In mammals, CELSR proteins may have more nuanced roles in dendritogenesis. No phenotype was observed in dendrite development of *Celsr3*-mutant cortical neurons at perinatal stages *in vivo*, but, similar to the dendritic overgrowth observed in *fmi*-mutant flies, knock down of *Celsr3* enhances the growth of dendritic arbours in rat cerebral slice cultures[30,31]. By contrast, knock down of *Ceslr2* in slice culture reduces both dendrite length and complexity[31,32] (FIG. 3a). Although future work is required to recapitulate these findings in a genetic model, it is intriguing to note that this opposing effect in neurite growth (CELSR2 enhances neurite growth, whereas CELSR3 suppresses it) can be attributed to a single amino acid change in the first intracellular loop of both CELSR proteins: I2413 in Fmi and in CELSR3 compared with R2573 in CELSR2 (REF. 31). This amino acid change has consequences for receptor signalling, as homophilic interactions of CELRS2 or CELSR3 augment intracellular concentrations of calcium ions ($[Ca^{2+}]_i$) differently. CELSR2 homodimers evoke higher $[Ca^{2+}]_i$ than CELSR3 homodimers[31], which is likely to further contribute to the differential modulation of dendrite growth by these closely related aGPCRs.

In addition to regulating proper dendrite morphology, CELSR aGPCRs also function in axon guidance, with roles conserved from *C. elegans* and *D. melanogaster* to mammals. This topic has been discussed in depth in several recent reviews[18,33,34]; thus, we only highlight a few key studies here and we direct the reader to these reviews for further information. In *C. elegans*, *fmi-1* mutants exhibit defective axon pathfinding of pioneer and follower axons in the ventral nerve cord, as well as of GABAergic ventral D-type neurons[35,36]. In the case of the ventral nerve cord axons, pioneer axons require the CTF but not the NTF for pathfinding, and follower axons require the NTF but not the CTF[35]. This suggests a bimodal function for this aGPCR in axon guidance, whereby FMI-1 drives intracellular signalling in the case of pioneer axons but promotes cell–cell adhesion and/or trans-signalling in the case of follower axons. In *D. melanogaster*, Fmi is required for photoreceptor neuron axon–axon and axon–target interactions such that in *fmi* mutants, photoreceptor axons misroute to inappropriate targets[37,38]. Finally, in mammals, *Celsr3*-mutant mice show marked defects in axon guidance in several major CNS tracts, including the anterior commissure, internal capsule and corticospinal tract[18,30] (FIG. 2c). Analysis of cell type-specific mutants demonstrated that *Celsr3* is required in intermediate targets in order to guide axons from the cortex to subcortical structures[39]. Given the crucial roles of CELSR proteins in dendrite and axon development, it is not surprising that aGPCRs are also crucial for axon–dendrite connections, synapses.

aGPCRs in synapse formation and function. Members of two aGPCR subfamilies, brain-specific angiogenesis inhibitor (BAI; also known as ADGRB) and latrophilin (LPHN; also known as ADGRL, CIRL1, CL1 and lectomedin 2), are required for synapse development and biology. In mammals, there are three BAI orthologues, of which BAI3 (also known as ADGRB3) is the best studied in this context for its role in mediating synapse development. BAI3 is mostly a brain-specific molecule with the levels of BAI3 peaking during neonatal development and persisting at lower levels throughout adult life[40]. BAI3 is present in biochemical preparations of synapses in the forebrain and cerebellum[41,42], supporting a role for this aGPCR in synapse development and maintenance. Indeed, work from several laboratories has elegantly demonstrated that BAI3 functions together with its ligand C1QL1 to strengthen winning climbing fibre (CF)–Purkinje synapses and to promote pruning of the remaining CFs[43–45] (FIG. 3b). Given the current model for the development of schizophrenia as a consequence of aberrant brain wiring[46], it is perhaps not surprising that two single nucleotide polymorphisms (SNPs) in human *BAI3* have been linked to schizophrenia in genome-wide association studies[47].

The NTF of BAI3 contains a complement C1r/C1s, Uegf, Bmp1 (CUB) domain and four thrombospondin type 1 repeats (TSRs) (FIG. 1c). Endogenous BAI3 in the

brain is cleaved via GAIN domain-mediated autoproteolysis, although exogenously expressed BAI3 in HEK293 cells remains uncleaved[4]. The NTF of BAI3 binds to C1QL proteins with 10 nM and 2 μM affinity for C1QL3 and C1QL1, respectively[43,45]. The C-terminal globular domain of C1QL proteins is responsible for their binding to BAI3 (REF. 48), although the exact binding domain for C1QL proteins has been reported to vary depending on the binding partner. C1QL3 binds BAI3 via the TSR domain, whereas C1QL1 binds via the CUB domain[43,45].

In mammals, there are four C1q-like proteins, C1ql1–C1ql4. Of these, mRNAs for *C1ql1*, *C1ql2* and *C1ql3* are expressed almost exclusively in the CNS[49]. Neuronal C1q regulates the postnatal elimination of inactive synapses between retinal ganglion cells and the lateral geniculate nucleus[50]. C1q-like proteins are postulated to be involved in synaptic development and maintenance, and compelling evidence suggests a role for BAI3–C1QL interactions in synaptic formation *in vitro*. The addition of recombinant C1QL3 globular domain protein to cultured hippocampal neurons significantly decreased the number of excitatory synapses. This effect can be rescued by adding an equimolar concentration of the recombinant BAI3 fragment that binds to C1QL3 (REF. 43). Moreover, in cultured cerebellar slices, knock down of *Bai3*, *C1ql1* or both, resulted in reduced spine density[44].

Consistent with these reports *in vitro*, recent studies have also supported a function for BAI3 in synapse development *in vivo*. In the mouse cerebellum between postnatal days (P) 3 and 7, axons of multiple inferior olivary (IO) neurons make initial innervations on a single Purkinje cell (PC) soma. These projections mature into CFs and PCs develop their dendritic arbours. Beginning at P9, a single winning CF translocates its synapse from the soma to the thorny spines of PC proximal dendrites[51,52], while the rest of the CFs remaining on the soma are eliminated by P21. *BAI3* is expressed in the PC postsynaptic terminal, whereas C1QL1 is produced by IO neurons[44,45]. Although C1QL proteins are widely considered to be secreted, in IO neurons, C1QL1 was found to be restricted to the presynaptic terminal by immunohistochemistry and immunoelectron microscopy[45]. The interaction between BAI3 and C1QL1 strengthens the winning CF–PC synapses and promotes the pruning of the remaining CFs, as both *C1ql1*-null and PC-specific *Bai3*-null cerebella manifest fewer CF synapses and reduced constant CF-evoked excitatory postsynaptic currents[45].

LPHN proteins and excitatory synapse formation. There are three LPHN receptors in mammals, LPHN1–3 (also known as ADGRL1–3). LPHN1 was originally cloned as a presynaptic receptor for α-latrotoxin, which is a toxin in the venom of black widow spiders that stimulates strong neuronal exocytosis[3,53–55]. Similar to CELSR proteins, LPHNs are conserved in invertebrates, supporting critical and evolutionarily conserved functions for these aGPCRs. Several endogenous binding partners have so far been identified for LPHN aGPCRs: teneurins, fibronectin leucine-rich transmembrane (FLRT) family proteins and neurexins.

Teneurin 2 (also known as Lasso) binds to LPHN1 more strongly than to LPHN2. Although teneurin 2 does not bind to LPHN3, this aGPCR does interact with teneurin 3 (REFS 56–58). Of these interactions, teneurin 2 and LPHN1 has been the most extensively studied. Teneurin 2 is restricted to postsynaptic terminals, and its globular domain binds to the NTF of LPHN1. Interestingly, the addition of the teneurin 2 globular domain induces Ca^{2+} signals in the presynaptic boutons of cultured hippocampal neurons, suggesting that teneurin 2 and LPHN1 function in *trans* to regulate synaptic function[56].

All three mammalian LPHN proteins also interact with members of the FLRT family: FLTR1 interacts with LPHN1 and LPHN3; FLTR2 interacts with LPHN3; and FLRT3 interacts with all LPHN aGPCRs[57,59–61]. LPHN3 and FLRT3 interactions may regulate glutamatergic synapse density and function[57], and the crystal structures of LPHN3 and FLRT complexes demonstrate that the olfactomedin domain of LPHN3 binds to the leucine-rich region (LRR) of FLTR2 and FLTR3 (REFS 59–61).

Finally, LPHN1 has been shown to bind neurexin 1α, neurexin 1β, neurexin 2β and neurexin 3β, which are presynaptic cell adhesion molecules that also function as α-latrotoxin receptors[62]. It is interesting to note that all LPHN binding partners that have been identified to date are single-pass transmembrane molecules with key roles in axon guidance, neural connectivity and synaptogenesis[2,57,63–65]. Thus, LPHN aGPCRs may have a general function in binding neuronal transmembrane molecules to shape nervous system development and function. Accordingly, LPHN receptor variants have been associated with attention deficit hyperactivity disorder (ADHD)-like phenotypes in animal models and genetic linkage studies in humans[66–69].

As aGPCRs are key regulators of early nervous system development, as well as axon, dendrite and synapse biology, it might be expected that they would also be involved in neurological disease, and this has indeed been shown to be the case in humans for several aGPCR family members (TABLE 1). In addition to the examples noted above of CELSR1 in neural tube defects, GPR56 in BFPP, BAI3 in schizophrenia and LPHN proteins in ADHD, other aGPCR variants are also associated with or causative for disorders of the nervous system (TABLE 1). *GPR56* mutations also cause autosomal recessive bilateral perisylvian polymicrogyria (BPPR), which is a polymicrogyria limited to the cortex surrounding the Sylvian fissure[70]. Similar to patients with BFPP, individuals with BPPR have intellectual and language difficulties, seizures and, to a lesser degree, motor disability[70]. Additionally, mutations in *GPR98* (also known as *ADGRV1*, *VLGR1* and *MASS1*) are linked familial febrile seizures and are causative for Usher syndrome type IIC[71–74]. With further study into the physiology and pathophysiology of aGPCRs in the nervous system, additional disease linkages are likely to be revealed in the future.

aGPCRs in cell–environment interactions

The diverse functions of aGPCRs in neural tube closure, cortical development, axon guidance, and dendritogenesis and synaptogenesis are all mechanistically linked in

Table 1 | **aGPCRs in human neurological disease**

Gene symbol (also known as)	Disease	Refs
BAI3 (ADGRB3)	SNPs associated with schizophrenia	47
CELSR1 (ADGRC1)	Neural tube defects (OMIM 182940)	14–16
GPR56 (ADGRG1)	Bilateral frontoparietal polymicrogyria (BFPP; OMIM 606854) and bilateral perisylvian polymicrogyria (BPPR; OMIM 615752)	21,70
GPR126 (ADGRG6)	Lethal congenital contracture syndrome 9 (OMIM 616503)	81
LPHN3 (ADGRL3)	SNPs associated with attention deficit hyperactivity disorder (ADHD)	66,67
GPR98 (ADGRV1)	Usher syndrome, type IIC (OMIM 605472)	72

OMIM, Online Mendelian Inheritance in Man; SNP, single nucleotide polymorphism.

that cell–cell and cell–matrix interactions underlie most, if not all, of these roles (as detailed above). Moreover, recent work has firmly established that aGPCRs also control one of the best examples of a specialized cell–cell interaction in the nervous system: the interaction between myelinating glia and axons.

aGPCRs in CNS myelination. In addition to its key functions in cortical development, GPR56 has also recently been implicated in the development of oligodendrocytes, which are the myelinating glia of the CNS. Work in zebrafish and mice supports a model in which GPR56 functions autonomously in oligodendrocyte precursor cells (OPCs) to control proliferation. In both species, the loss of GPR56 function leads to reduced myelin in the CNS, which is caused by reduced OPC proliferation and the premature differentiation of these cells before the full cohort of oligodendrocyte lineage cells can be established[75,76]. These functions are mediated by $G\alpha_{12/13}$ signalling and RHOA activation, although the ligand that might stimulate GPR56 in OPCs is currently unknown. Notably, patients with BFPP present with reduced white matter volume by MRI[21,22,24,77], suggesting that the functions of GPR56 in OPC development may be conserved in humans. Furthermore, GPR98 is enriched in oligodendrocytes *in vivo*; *in vitro*, *Gpr98* knockdown decreases myelin-associated glycoprotein (MAG; a key myelin protein) levels, but overexpression of *Gpr98* increases MAG levels. Signalling studies revealed that GPR98 activates protein kinase A (PKA) and PKC via $G\alpha_s$ and $G\alpha_q$ in response to extracellular calcium[78]. Given that GPR98 is enriched in myelinated regions of the superior and inferior colliculi, which are crucial for the initiation and propagation of audiogenic seizures, it will be interesting in the future to determine how this pathway might affect seizures that are linked to mutations in this aGPCR.

GPR126 and ECM proteins in PNS myelination. In the PNS, Schwann cells (SCs) are specialized glial cells that generate the multilamellar myelin sheath that surrounds axons. The aGPCR GPR126 (also known as ADGRG6) is an evolutionarily conserved regulator of SC myelination; without this aGPCR, SCs in both zebrafish and mouse mutants can ensheathe axons but fail to spiral their membrane to generate the myelin sheath[79,80] (FIG. 4). In humans, mutations in *GPR126* cause lethal congenital contracture syndrome 9, which is a severe form of arthrogryposis multiplex congenita, and peripheral nerves from these patients show reduced expression of myelin genes, suggesting that the function of GPR126 is conserved in humans[81]. Work *in vivo* and *in vitro* supports a model whereby GPR126 functions autonomously in SCs to initiate myelination by coupling to $G\alpha_s$ proteins, increasing levels of cyclic AMP and activating PKA, which ultimately leads to the upregulation of the genes required for SC terminal differentiation and myelination[6,79,82,83]. During development, SCs synthesize ECM components that are incorporated into the basal lamina of the SCs. The basal lamina is crucial for many stages of SC development, from axon selection to myelination[84,85]. Two binding partners that reside in the SC basal lamina have so far been described for GPR126: collagen IV and laminin 211, which interact with the NTF of GPR126 via different domains[86,87].

GPR126 can undergo two proteolytic processes: intracellularly via GAIN domain-mediated autoproteolysis to generate the NTF and the CTF; and extracellularly via furin-mediated cleavage at an S2 site within the NTF that produces CUB and PTX-containing and GAIN domain-containing fragments[88], although it is unclear to what extent S2 site cleavage occurs in different cellular contexts *in vivo*. Collagen IV binds to the CUB and PTX fragment of GPR126 with a high affinity, and exogenous application of this ECM molecule can increase cAMP levels in heterologous cells that express GPR126 (REFS 86,87). By contrast, laminin 211 binds to the GAIN domain-containing fragment of GPR126; however, in this case, exogenous application of laminin 211 in heterologous systems suppresses cAMP accumulation under static conditions and increases cAMP under dynamic conditions[87]. These results raise the interesting question of whether aGPCRs 'sense' binding partners in the traditional chemoreceptive mode of metabotropic receptor–ligand pairs, or whether the interaction with binding partners is a prerequisite for transmitting mechanical forces on the receptors, which then elicits downstream signals.

Mechanisms of aGPCR signal perception

Mechanical activation of aGPCRs. Mounting evidence suggests that aGPCRs bestow a cell with a sense of its mechanical environment through the adhesive interactions described above, as well as a novel, mechanical force-based mechanism of aGPCR activation. Mechanical properties that are sensed by aGPCRs may encompass features of low dynamic range, such as the rigidity of the ECM of the tissue. A recent example to support this scenario was described for the GPR126 and laminin 211 interaction. During development, laminin 211 is secreted by SCs in a monomeric form and polymerizes during the development of the basal lamina. This process 'stiffens' the ECM surrounding the SCs, which could subject the anchored NTF of GPR126 to

Arthrogryposis multiplex congenita
A congenital disorder defined by the presence of at least two major joint contractures caused by reduced or absent fetal movement.

Basal lamina
A component of basement membranes that is linked to the basal side of cell membranes and comprises organized extracellular matrix molecules.

Metabotropic receptor
A membrane receptor that acts through second messengers.

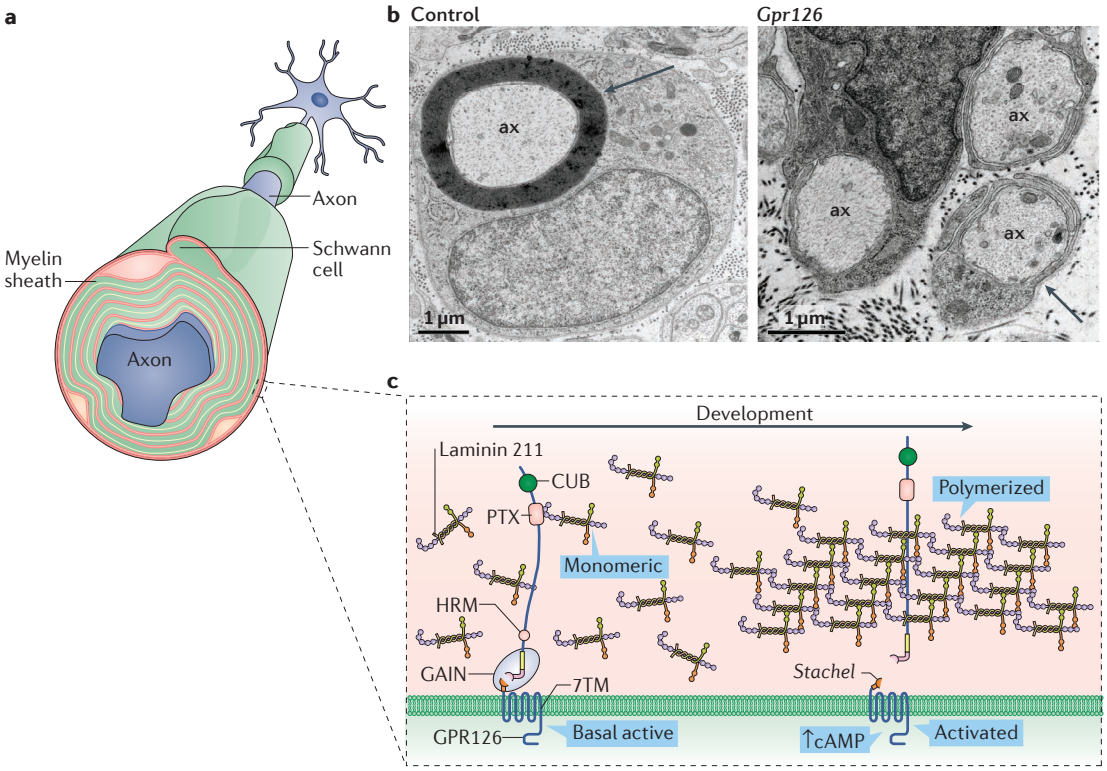

Figure 4 | **Laminin 211 may facilitate mechanical activation of GPR126 in Schwann cell development. a** | Diagram depicting Schwann cell myelination of an axon in the peripheral nervous system. **b** | GPR126 (also known as ADGRG6) is essential for Schwann cell myelination. In wild-type mice (control; left), Schwann cells generate the myelin sheath (arrow) that surrounds axons (labelled 'ax'; as shown in part **a**). In *Gpr126*-mutant mice (right), Schwann cells ensheath axons (part **a**) but fail to generate myelin. The basal lamina is noted in *Gpr126* mutants (arrow). **c** | A model for GPR126 activation in Schwann cells. At early stages, monomeric laminin 211 and GPR126 interactions are not sufficient to increase cyclic AMP. As development progresses, the basal lamina matures and as laminin 211 polymerizes, enough force may be exerted on GPR126 to facilitate *Stachel* (stalk)-mediated signalling and myelin initiation. 7TM, seven-transmembrane domain; CUB, C1r/C1s, Uegf, Bmp1 domain; GAIN, GPCR autoproteolysis-inducing domain; HRM, hormone receptor motif; PTX, pentraxin domain. Part **a** is adapted from REF. 117, Nature Publishing Group. Part **b** is republished with permission of The Company of Biologists Ltd, from Gpr126 is essential for peripheral nerve development and myelination in mammals, Monk, K. R., Oshima, K., Jörs, S., Heller, S. & Talbot, W. S., **138**, 2011; permission conveyed through Copyright Clearance Center, Inc. Part **c** is adapted from REF. 118, Nature Publishing Group.

mechanical force. This hypothesis was supported by genetic and pharmacological assays, and mechanical stimulation of GPR126 by laminin 211 was shown to require signalling via the *Stachel* sequence, which is predicted to be buried within the GAIN domain[4,87]. This suggests that mechanical forces on aGPCRs may be required to expose the *Stachel* sequence for receptor activation. However, specific details regarding the force generation by laminin 211 on GPR126, as well as how other ECM proteins such as collagen IV interact to control the signalling of this aGPCR in SCs *in vivo*, remain to be resolved.

aGPCRs may also sense mechanical changes in the higher dynamic range such as shear, load, bend, vibration and stretch. Indeed, investigations into the role of latrophilins have uncovered physiological stimulus perception in these categories. In *D. melanogaster*, the latrophilin homologue *Cirl* is produced in mechanosensory neurons, which are arrayed in dedicated chordotonal organs. Neuronal responses from these mechanosensory neurons can be directly recorded and matched to stimulus properties, as well as to structural modifications of the receptor. In a recent study, it was shown that the ability of *Cirl*–knockout mutants to correctly register mechanical stimuli — sound, stretch and touch — was severely impaired[89]. Electrophysiological recordings demonstrated that the mechanoceptive function of latrophilin is stimulus frequency-dependent, suggesting that this aGPCR is narrowly tuned to a specific force range (FIG. 5). Further, latrophilin activation through mechanical signals probably crosstalks with transient receptor potential (TRP) channels, which are classical ionotropic mechanosensors that reside in the cilia of mechanosensory neurons and which gate receptor currents upon mechanical challenge[89,90]. Interestingly, two latrophilin binding partners that are conserved in invertebrates, neurexin and teneurin, are also present in mechanosensory organs[91]. Further, latrophilin homologues in *C. elegans* and mammals signal via $G\alpha_s$ and $G\alpha_q$, and can be activated by a *Stachel*-tethered agonist[55,92]. Taken together, this suggests that stimulus perception and

Chordotonal organs
Peripheral compound mechanosensory organs in insects and other arthropods that perceive mechanical signals such as sound, touch and muscle stretch.

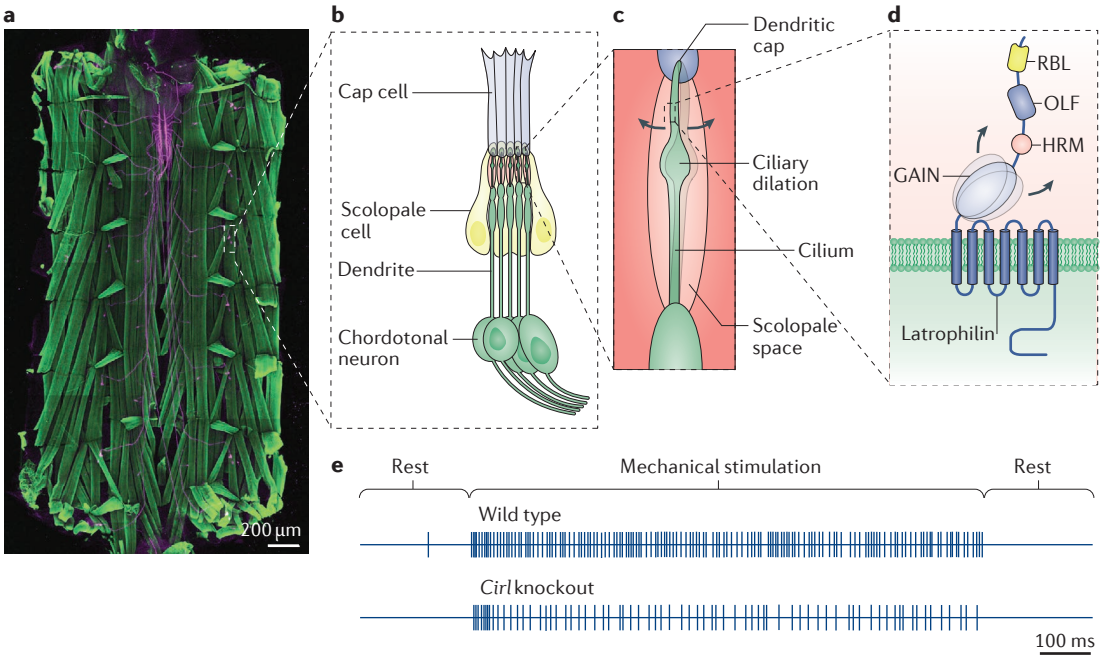

Figure 5 | **The aGPCR latrophilin sensitizes mechanosensory neurons in *Drosophila melanogaster*. a** | *Drosophila melanogaster* larvae possess eight mechanosensory neurons per hemisegment, known as chordotonal neurons (purple). Within each hemisegment, there are also three singular neurons and a pentascolopidial chordotonal organ (lch5), in which five mechanosensory neurons (green) are bundled together. The image is a collage of high-resolution confocal images. **b** | Schematic representation of the lch5 organ. Each unit contains a mechanosensory neuron (green), a scolopale cell (yellow) and a cap cell (grey). **c** | The scolopale and cap cells form a cavity around the apical dendrite of the neuron, known as the scolopale space (pink), into which a single cilium extends (green). Inside the scolopale space, the cilium is fixed within an extracellular matrix, the dendritic cap (blue), which is secreted by the cap cell. The cilium is thereby mechanically coupled to the relative motion between the cap cell and the neuron exerted through sounds, touch and stretch. Latrophilin (also known as CIRL) is expressed in chordotonal neuron dendrites and cilia. **d** | Upon mechanical stimulation, the amino-terminal fragment (NTF) and carboxy-terminal fragment (CTF) of latrophilin may move relative to each other or even disengage at the G protein-coupled receptor (GPCR) proteolysis site (GPS), thereby modulating the signalling state of the receptor. **e** | Representative electrophysiological recordings from lch5 axons of control and *Cirl*-knockout *D. melanogaster* larvae upon mechanical stimulation with a piezo probe at 900 Hz. The traces show that removal of the adhesion GPCR (aGPCR) causes a drop in the firing frequency of action currents, demonstrating that latrophilin sensitizes the neurons for mechanical stimulation. GAIN, GPCR autoproteolysis-inducing domain; HRM, hormone receptor motif; OLF, olfactomedin domain; RBL, rhamnose-binding lectin domain. Image in part **a** courtesy of M. Nieberler, Univeristy of Würzburg, Germany. Parts **b**, **c** and **e** are adapted with permission from REF. 89, Elsevier.

signal transduction by CIRL in mechanosensory neurons may function similarly to GPR126 through exposure of its *Stachel* sequence at the NTF–CTF junction.

That aGPCRs may generally function as mechanoreceptors is also supported by findings outside the nervous system. In skeletal muscles, GPR56 resides on myocytes[93], where it interacts with its extracellular ligand collagen III[25,94]. Like GPR126 and latrophilin, GPR56 also contains a *Stachel* sequence, the exposure of which results in $G\alpha_{13}$ recruitment[10], which confirms previous findings of this second messenger target[25]. Recent findings have demonstrated that the GPR56–$G\alpha_{12/13}$–PKA pathway controls anabolic responses and stimulates muscle hypertrophy, which is triggered through exercise[94]. In this process, GPR56 senses the mechanical overload of muscle that is inflicted by physical activity, possibly through tension changes that are applied to the receptor, which is suspended between muscle cell membrane and its anchoring matrix.

aGPCR proteolysis and agonism. Taken together, the ligand profile and cellular responses of aGPCRs support a model in which at least some signalling is mediated by mechanical activation. Accordingly, several biochemical properties of aGPCRs are consistent with members of this class functioning as mechanosensitive metabotropic receptors, including aGPCR proteolysis and *Stachel* sequence-mediated agonism. The GAIN domain resides in close proximity to the 7TM domain of every aGPCR homologue[4,95], which can act as an autoprotease, processing the pro-receptors into the NTF and the CTF at the GPS[4,5].

Protein folding, expression levels and cell surface delivery were originally suggested to rely on aGPCR autoproteolysis, but several aGPCRs may not undergo self-cleavage but may instead remain as single polypeptide chains after biosynthesis[96]. Further, investigations of receptor mutants with disabled auto-proteolytic activity have produced ambiguous results regarding the biological and pharmacological activities of these

receptors. Some receptors exhibit GAIN domain cleavage-dependent properties[97], whereas others are unaffected by the loss of GAIN domain auto-proteolysis[6,8,96,98]. Self-cleavage and subsequent fragment association through a dedicated structural fold is also found in other cell surface molecules with potential mechanical tasks, such as mucins, which belong to a class of proteins that line the surface of mucous epithelia. Their adhesive ectodomains can measure more than 20,000 amino acids in length and contain a SEA (sea urchin sperm protein, enterokinase, agrin) domain, which auto-proteolyses itself similarly to GAIN domains[99]. This property was suggested to confer epithelial cells with a system to evade mechanical overload by NTF shedding, or to register mechanical activity by the loss of the NTF and its potential interaction partners[100].

In a non-mutually exclusive model, aGPCR auto-proteolysis may also provide a means to separate the functions of the NTF and the CTF, and to partition their biological effects in response to mechanical changes. In this context, the pre-formation of an NTF–CTF heterodimer could be a prerequisite for defining the physical threshold for the separation of biological functions, as the CTF remains anchored inside the cleaved GAIN domain through hydrophobic interactions[4]. It is currently unclear how much force would be required to separate the two fragments and, consequently, it is not currently feasible to estimate where GAIN domain dissociation energies rank among the forces that are required to split known adhesion partners[101].

The recent identification of the tethered agonist activation mode for aGPCRs lends support to the model that these receptors are mechanosensors. It is known that the NTF of aGPCRs suppresses their metabotropic activities[102–105], suggesting that this extracellular domain either functions as an inverse agonist of the 7TM domain, repressing its function, or that it restricts the availability of a tethered agonist that stimulates 7TM domain activity[4,95,106]. Structure–function studies using intermolecular complementation experiments with the nematode latrophilin homologue LAT-1 first suggested that the tethered agonist model may be pertinent to aGPCR function. Co-expression of two signalling defective receptor variants — one without a 7TM domain, one with a foreign GPS motif— displayed full biological activity in rescuing assays, indicating that the extracellular portion of the receptor agonistically interacts with the 7TM signalling unit[96].

Further, the crystal structure of the auto-proteolytically cleaved GAIN domain of LPHN1 shows that its last β-strand (the most N-terminal secondary structural element of the CTF in the case of auto-proteolysed aGPCRs) is enveloped by the NTF portion of the domain. A short linker connects the β-strand with the first transmembrane segment of the 7TM domain. This β-strand linker region represents the *Stachel* sequence, which was first shown to function as a tethered agonist for the aGPCRs GPR126 and GPR133 (REF. 6).

Removal or mutation of the *Stachel* sequence precludes metabotropic signalling, whereas addition of a synthetic *Stachel* peptide rescues this defect *in vitro* and *in vivo*[6]. *Stachel*-mediated agonism has also been demonstrated for several other aGPCRs: GPR56, GPR110 (also known as ADGRF1)[10], GPR64 (also known as ADGRG2)[7], GPR114 (also known as ADGRG5)[8], LAT-1 and LPHN1 (REF. 92), suggesting that the tethered agonist model may be generally applicable to the activation of many, if not all, aGPCRs. Notably, this aGPCR activation mechanism has many features of protease-activated receptor (PAR) activation, which was discovered more than 20 years ago. PARs belong to a class of Rhodopsin-like GPCRs and have a cryptic tethered agonist at a similar position and size as the *Stachel* sequence of aGPCRs. The PAR agonist is exposed through receptor cleavage by an exogenous protease, thrombin, which then potently stimulates the metabotropic activity of its receptor, and cleavage-deficient PAR mutants are signalling defective, underscoring the importance of proteolysis in PAR activation[107].

By contrast, *Stachel*-mediated activation can also be observed in aGPCRs without an auto-proteolytically active GAIN domain, or in GPS cleavage-disabled point mutants[6,8]. Further, cleavage-competent aGPCRs arrive at the membrane as pre-cleaved but associated heterodimers. Mechanical strain, which is enacted through stretch, vibration, matrix stiffening and other means, and which is relayed through the adhesive interactions between the receptor extracellular domain and its associated binding partners, thus seems to be a plausible factor that allows for *Stachel* sequence signalling. This may occur through the complete disruption of the NTF–CTF dimer in some aGPCRs, but cannot account for cleavage-deficient receptors. Alternatively, under mechanical tension and interactions with the appropriate binding partners, the GAIN domain conformation may be altered in such a way that allows the *Stachel* to contact the 7TM interface.

Taken together, the current model of aGPCR stimulation suggests that mechanical forces expose a tethered agonist, which is incorporated in the receptor molecule. This agonist is nudged into the extracellular groves of the 7TM domain, stabilizing one activity state of the receptor. The remarkable biochemical properties of aGPCRs allow this receptor class to function as adhesion, signalling and mechanosensitive molecules, which facilitates their myriad and diverse functions in nervous system development and disease.

Conclusions and future directions

aGPCRs represent an extensive group of receptor molecules that have previously been generally neglected by cell biological and pharmacological research. Their molecular composition is uniquely suited to interact with a wide variety of extracellular partners that define the structural and mechanical environment of a cell. The effect of aGPCRs on the nervous system was first uncovered by pioneering genetic studies that showed the indispensability of these receptors during the generation of cortical cytoarchitectures in the case of BFPP. Since then, several more neurobiological contexts have been associated with aGPCR dysfunction. These include psychiatric disorders[108], Usher syndrome[72] and ADHD[66].

GPS motif
(GPCR proteolysis site motif). A small motif (~50 amino acids) contained within the larger G protein-coupled receptor (GPCR) autoproteolysis-inducing (GAIN) domain of adhesion GPCRs and polycystic kidney disease proteins where receptor autocleavage occurs. Many adhesion GPCRs are autocleaved at the GPCR proteolysis site inside the motif. The GPS motif alone is necessary, but not sufficient, for receptor autocleavage.

In most cases, the exact pathogenetic course of these diseases is unclear, partly owing to a lack of knowledge about how these peculiar receptors work.

Recent years, however, have witnessed a concerted effort to define the main properties of aGPCR biology, including the discovery of a conserved activation mechanism for the receptor class via the tethered agonist, as well as the emerging concept that aGPCRs serve as metabotropic mechanoreceptors in the nervous system and beyond. These important discoveries now provide a firm conceptual framework to dissect and combat the effects of aGPCR dysfunction. An obvious direction of this venture is the development of drugs that mimic or interfere with the *Stachel* sequence, though much work is required to define the pharmacological, pharmacochemical and pharmaceutical properties of such compounds.

These efforts will need to be paralleled by clinical investigations to identify genetic lesions of aGPCR loci that are associated with human illness, especially for diseases of the nervous system. Although aGPCRs are also recognized as possessing exceptional vulnerability for the occurrence of somatic mutations in the context of cancer[109,110], it is unclear whether such alterations may also precede the outbreak of neuropathologies or psychiatric conditions. It is also unknown whether disease-causing mutations affect the properties of aGPCR signalling cascades, and understanding their interrelationship will shed light on both directions: on the molecular events that underlie aGPCR functions and on the cellular effects that are controlled through them.

A fascinating aspect of aGPCRs in neurobiology is the recent discovery that the developmental functions of aGPCRs with their multiple roles in neuronal and glial ontogenesis extend to the evolution of brain structures. GPR56 was shown to drive the increase in neocortical complexity along a phylogenetic axis, conceivably through the constant expansion of its cortical expression domain within the neocortex[70]. Analysis of transcriptional profiles of the aGPCR class suggests that many homologues are highly abundant in the CNS and the PNS[111–113]. Once these expression data are matched with neurobiological functions for individual aGPCR homologues, it will be an intriguing task to study them in an evolutionary context. It would not be surprising to learn that aGPCRs are also eminent players on this field.

1. Bjarnadóttir, T. K. *et al.* The human and mouse repertoire of the adhesion family of G-protein-coupled receptors. *Genomics* **84**, 23–33 (2004).
2. Hamann, J. *et al.* International Union of Basic and Clinical Pharmacology. XCIV. Adhesion G protein-coupled receptors. *Pharmacol. Rev.* **67**, 338–367 (2015).
3. Krasnoperov, V. G. *et al.* α-Latrotoxin stimulates exocytosis by the interaction with a neuronal G-protein-coupled receptor. *Neuron* **18**, 925–937 (1997).
Biochemical screens were used to identify latrophilin 1 as the first known aGPCR in the nervous system.
4. Araç, D. *et al.* A novel evolutionarily conserved domain of cell-adhesion GPCRs mediates autoproteolysis. *EMBO J.* **31**, 1364–1378 (2012).
This paper describes the structural and functional identification of the GAIN domain and demonstrates its general presence in aGPCRs and PKD1 proteins.
5. Lin, H. H. *et al.* Autocatalytic cleavage of the EMR2 receptor occurs at a conserved G protein-coupled receptor proteolytic site motif. *J. Biol. Chem.* **279**, 31823–31832 (2004).
Together with reference 4, this paper defined the mechanism underlying the autocatalytic cleavage at the GPS of aGPCRs.
6. Liebscher, I. *et al.* A tethered agonist within the ectodomain activates the adhesion G protein-coupled receptors GPR126 and GPR133. *Cell Rep.* **9**, 2018–2026 (2014).
Using pharmacological and *in vivo* approaches, this study identified the *Stachel* sequence tethered agonist for the aGPCRs GPR126 and GPR133. This study further provided evidence that *Stachel*-mediated GPR126 signalling is required for Schwann cell myelination *in vivo*.
7. Demberg, L. M., Rothemund, S., Schöneberg, T. & Liebscher, I. Identification of the tethered peptide agonist of the adhesion G protein-coupled receptor GPR64/ADGRG2. *Biochem. Biophys. Res. Commun.* **464**, 743–747 (2015).
8. Wilde, C. *et al.* The constitutive activity of the adhesion GPCR GPR114/ADGRG5 is mediated by its tethered agonist. *FASEB J.* **30**, 666–673 (2015).
9. Kishore, A., Purcell, R. H., Nassiri-Toosi, Z. & Hall, R. A. Stalk-dependent and stalk-independent signaling by the adhesion G protein-coupled receptors GPR56 (ADGRG1) and BAI1 (ADGRB1). *J. Biol. Chem.* **291**, 3385–3394 (2016).
10. Stoveken, H. M., Hajduczok, A. G., Xu, L. & Tall, G. G. Adhesion G protein-coupled receptors are activated by exposure of a cryptic tethered agonist. *Proc. Natl Acad. Sci. USA* **112**, 6194–6199 (2015).
Using pharmacological approaches, this study identified the *Stachel* sequence tethered agonist for the aGPCRs GPR56 and GPR110. Together with reference 6, this study described a new activation mechanism for the aGPCR class.
11. Gilbert, S. F. *Developmental Biology* (Sinauer Associates, 2000).
12. Curtin, J. A. *et al.* Mutation of *Celsr1* disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr. Biol.* **13**, 1129–1133 (2003).
13. Nishimura, T., Honda, H. & Takeichi, M. Planar cell polarity links axes of spatial dynamics in neural-tube closure. *Cell* **149**, 1084–1097 (2012).
14. Robinson, A. *et al.* Mutations in the planar cell polarity genes *CELSR1* and *SCRIB* are associated with the severe neural tube defect craniorachischisis. *Hum. Mutat.* **33**, 440–447 (2012).
15. Allache, R., De Marco, P., Merello, E., Capra, V. & Kibar, Z. Role of the planar cell polarity gene *CELSR1* in neural tube defects and caudal agenesis. *Birth Defects Res. Part A Clin. Mol. Teratol.* **94**, 176–181 (2012).
16. Lei, Y. *et al.* Identification of novel CELSR1 mutations in spina bifida. *PLoS ONE* **9**, e92207 (2014).
17. Boutin, C. *et al.* A dual role for planar cell polarity genes in ciliated cells. *Proc. Natl Acad. Sci. USA* **111**, E3129–E3138 (2014).
18. Tissir, F. & Goffinet, A. M. Shaping the nervous system: role of the core planar cell polarity genes. *Nat. Rev. Neurosci.* **14**, 525–535 (2013).
19. Wada, H., Tanaka, H., Nakayama, S., Iwasaki, M. & Okamoto, H. Frizzled3a and Celsr2 function in the neuroepithelium to regulate migration of facial motor neurons in the developing zebrafish hindbrain. *Development* **133**, 4749–4759 (2006).
20. Qu, Y. *et al.* Atypical cadherins Celsr1-3 differentially regulate migration of facial branchiomotor neurons in mice. *J. Neurosci.* **30**, 9392–9401 (2010).
21. Piao, X. *et al.* G protein-coupled receptor-dependent development of human frontal cortex. *Science* **303**, 2033–2036 (2004).
In this paper mutations of *GPR56* are identified as the underlying cause for cortical development defects in patients with BFPP. Together with reference 72, this study links the dysfunction of aGPCRs with a neurological ailment for the first time.
22. Piao, X. *et al.* Genotype-phenotype analysis of human frontoparietal polymicrogyria syndromes. *Ann. Neurol.* **58**, 680–687 (2005).
23. Li, S. *et al.* GPR56 regulates pial basement membrane integrity and cortical lamination. *J. Neurosci.* **28**, 5817–5826 (2008).
24. Bahi-Buisson, N. *et al. GPR56*-related bilateral frontoparietal polymicrogyria: further evidence for an overlap with the cobblestone complex. *Brain* **133**, 3194–3209 (2010).
25. Luo, R. *et al.* G protein-coupled receptor 56 and collagen III, a receptor-ligand pair, regulates cortical development and lamination. *Proc. Natl Acad. Sci. USA* **108**, 12925–12930 (2011).
26. Gao, F. B., Brenman, J. E., Jan, L. Y. & Jan, Y. N. Genes regulating dendritic outgrowth, branching, and routing in *Drosophila*. *Genes Dev.* **13**, 2549–2561 (1999).
27. Sweeney, N. T., Li, W. & Gao, F.-B. Genetic manipulation of single neurons *in vivo* reveals specific roles of flamingo in neuronal morphogenesis. *Dev. Biol.* **247**, 76–88 (2002).
28. Kimura, H., Usui, T., Tsubouchi, A. & Uemura, T. Potential dual molecular interaction of the *Drosophila* 7-pass transmembrane cadherin Flamingo in dendritic morphogenesis. *J. Cell Sci.* **119**, 1118–1129 (2006).
29. Reuter, J. E. *et al.* A mosaic genetic screen for genes necessary for *Drosophila* mushroom body neuronal morphogenesis. *Development* **130**, 1203–1213 (2003).
30. Tissir, F., Bar, I., Jossin, Y., de Backer, O. & Goffinet, A. M. Protocadherin Celsr3 is crucial in axonal tract development. *Nat. Neurosci.* **8**, 451–457 (2005).
In this study, the pivotal role of the aGPCR CELSR3 in neuronal migration and cortical connectivity is described.
31. Shima, Y. *et al.* Opposing roles in neurite growth control by two seven-pass transmembrane cadherins. *Nat. Neurosci.* **10**, 963–969 (2007).
32. Shima, Y., Kengaku, M., Hirano, T., Takeichi, M. & Uemura, T. Regulation of dendritic maintenance and growth by a mammalian 7-pass transmembrane cadherin. *Dev. Cell* **7**, 205–216 (2004).
33. Berger-Müller, S. & Suzuki, T. Seven-pass transmembrane cadherins: roles and emerging mechanisms in axonal and dendritic patterning. *Mol. Neurobiol.* **44**, 313–320 (2011).
34. Chai, G., Goffinet, A. M. & Tissir, F. Celsr3 and Fzd3 in axon guidance. *Int. J. Biochem. Cell Biol.* **64**, 11–14 (2015).

35. Steimel, A. *et al.* The Flamingo ortholog FMI-1 controls pioneer-dependent navigation of follower axons in *C. elegans*. *Development* **137**, 3663–3673 (2010).
36. Najarro, E. H. *et al. Caenorhabditis elegans* flamingo cadherin *fmi-1* regulates GABAergic neuronal development. *J. Neurosci.* **32**, 4196–4211 (2012).
37. Senti, K. A. *et al.* Flamingo regulates R8 axon-axon and axon-target interactions in the *Drosophila* visual system. *Curr. Biol.* **13**, 828–832 (2003).
38. Lee, R. C. *et al.* The protocadherin Flamingo is required for axon target selection in the *Drosophila* visual system. *Nat. Neurosci.* **6**, 557–563 (2003).
39. Zhou, L. *et al.* Early forebrain wiring: genetic dissection using conditional *Celsr3* mutant mice. *Science* **320**, 946–949 (2008).
40. Kee, H. J. *et al.* Expression of brain-specific angiogenesis inhibitor 3 (BAI3) in normal brain and implications for BAI3 in ischemia-induced brain angiogenesis and malignant glioma. *FEBS Lett.* **569**, 307–316 (2004).
41. Collins, M. O. *et al.* Molecular characterization and comparison of the components and multiprotein complexes in the postsynaptic proteome. *J. Neurochem.* **97**, 16–23 (2006).
42. Selimi, F., Cristea, I. M., Heller, E., Chait, B. T. & Heintz, N. Proteomic studies of a single CNS synapse type: the parallel fiber/purkinje cell synapse. *PLoS Biol.* **7**, e83 (2009).
43. Bolliger, M. F., Martinelli, D. C. & Südhof, T. C. The cell-adhesion G protein-coupled receptor BAI3 is a high-affinity receptor for C1q-like proteins. *Proc. Natl Acad. Sci. USA* **108**, 2534–2539 (2011).
44. Sigoillot, S. M. *et al.* The secreted protein C1QL1 and its receptor BAI3 control the synaptic connectivity of excitatory inputs converging on cerebellar Purkinje cells. *Cell Rep.* **10**, 820–832 (2015).
This manuscript demonstrated that loss of BAI3 function decreases presynaptic bouton number.
45. Kakegawa, W. *et al.* Anterograde c1ql1 signaling is required in order to determine and maintain a single-winner climbing fiber in the mouse cerebellum. *Neuron* **85**, 316–329 (2015).
This study showed that C1ql1 from CFs interacts with the aGPCR BAI3 at Purkinje cell synapses. Together with reference 44, a role for C1ql1 and BAI3 in synaptogenesis was defined.
46. Rapoport, J. L., Addington, A. M., Frangou, S. & Psych, M. R. C. The neurodevelopmental model of schizophrenia: update 2005. *Mol. Psychiatry* **10**, 434–449 (2005).
47. DeRosse, P. *et al.* The genetics of symptom-based phenotypes: toward a molecular classification of schizophrenia. *Schizophr. Bull.* **34**, 1047–1053 (2008).
48. Ressl, S. *et al.* Structures of C1q-like proteins reveal unique features among the C1q/TNF superfamily. *Structure* **23**, 688–699 (2015).
49. Iijima, T., Miura, E., Watanabe, M. & Yuzaki, M. Distinct expression of C1q-like family mRNAs in mouse brain and biochemical characterization of their encoded proteins. *Eur. J. Neurosci.* **31**, 1606–1615 (2010).
50. Stevens, B. *et al.* The classical complement cascade mediates CNS synapse elimination. *Cell* **131**, 1164–1178 (2007).
51. Hashimoto, K., Ichikawa, R., Kitamura, K., Watanabe, M. & Kano, M. Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum. *Neuron* **63**, 106–118 (2009).
52. Kano, M. & Hashimoto, K. Synapse elimination in the central nervous system. *Curr. Opin. Neurobiol.* **19**, 154–161 (2009).
53. Davletov, B. A., Shamotienko, O. G., Lelianova, V. G., Grishin, E. V. & Ushkaryov, Y. A. Isolation and biochemical characterization of a Ca^{2+}-independent α-latrotoxin-binding protein. *J. Biol. Chem.* **271**, 23239–23245 (1996).
54. Krasnoperov, V. G. *et al.* The calcium-independent receptor of α-latrotoxin is not a neurexin. *Biochem. Biophys. Res. Commun.* **227**, 868–875 (1996).
55. Lelianova, V. G. *et al.* α-Latrotoxin receptor, latrophilin, is a novel member of the secretin family of G protein-coupled receptors. *J. Biol. Chem.* **272**, 21504–21508 (1997).
Using biochemical approaches, this study together with reference 3, defines latrophilin 1 as the first known aGPCR in the nervous system.
56. Silva, J.-P. *et al.* Latrophilin 1 and its endogenous ligand Lasso/teneurin-2 form a high-affinity transsynaptic receptor pair with signaling capabilities. *Proc. Natl Acad. Sci. USA* **108**, 12113–12118 (2011).
57. O'Sullivan, M. L. *et al.* FLRT proteins are endogenous latrophilin ligands and regulate excitatory synapse development. *Neuron* **73**, 903–910 (2012).
58. Boucard, A. A., Maxeiner, S. & Südhof, T. C. Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. *J. Biol. Chem.* **289**, 387–402 (2014).
59. Jackson, V. A. *et al.* Structural basis of latrophilin-FLRT interaction. *Structure* **23**, 774–781 (2015).
60. Lu, Y. C. *et al.* Structural basis of latrophilin-FLRT-UNC5 interaction in cell adhesion. *Structure* **23**, 1678–1691 (2015).
61. Ranaivoson, F. M. *et al.* Structural and mechanistic insights into the Latrophilin3-FLRT3 complex that mediates glutamatergic synapse development. *Structure* **23**, 1665–1677 (2015).
62. Boucard, A. A., Ko, J. & Südhof, T. C. High affinity neurexin binding to cell adhesion G-protein-coupled receptor CIRL1/latrophilin-1 produces an intercellular adhesion complex. *J. Biol. Chem.* **287**, 9399–9413 (2012).
63. Lisé, M.-F. & El-Husseini, A. The neuroligin and neurexin families: from structure to function at the synapse. *Cell. Mol. Life Sci.* **63**, 1833–1849 (2006).
64. Kenzelmann, D., Chiquet-Ehrismann, R., Leachman, N. T. & Tucker, R. P. Teneurin-1 is expressed in interconnected regions of the developing brain and is processed *in vivo*. *BMC Dev. Biol.* **8**, 30 (2008).
65. Bottos, A., Rissone, A., Bussolino, F. & Arese, M. Neurexins and neuroligins: synapses look out of the nervous system. *Cell. Mol. Life Sci.* **68**, 2655–2666 (2011).
66. Arcos-Burgos, M. *et al.* A common variant of the latrophilin 3 gene, *LPHN3*, confers susceptibility to ADHD and predicts effectiveness of stimulant medication. *Mol. Psychiatry* **15**, 1053–1066 (2010).
67. Ribases, M. *et al.* Contribution of *LPHN3* to the genetic susceptibility to ADHD in adulthood: a replication study. *Genes Brain Behav.* **10**, 149–157 (2011).
68. Lange, M. *et al.* The ADHD-susceptibility gene lphn3.1 modulates dopaminergic neuron formation and locomotor activity during zebrafish development. *Mol. Psychiatry* **17**, 946–954 (2012).
69. van der Voet, M., Harich, B., Franke, B. & Schenck, A. ADHD-associated dopamine transporter, latrophilin and neurofibromin share a dopamine-related locomotor signature in *Drosophila*. *Mol. Psychiatry* **21**, 565–573 (2015).
70. Bae, B. I. *et al.* Evolutionarily dynamic alternative splicing of *GPR56* regulates regional cerebral cortical patterning. *Science* **343**, 764–768 (2014).
This investigation uses GWAS data, mRNA–Seq and mouse genetics to directly implicate the regional expression of GPR56 in the forebrain as a phylogenetic driving force of neural stem cell proliferation, gyral patterning and neocortex evolution in higher mammals.
71. Nakayama, J. *et al.* A nonsense mutation of the *MASS1* gene in a family with febrile and afebrile seizures. *Ann. Neurol.* **52**, 654–657 (2002).
72. Weston, M. D., Luijendijk, M. W. J., Humphrey, K. D., Möller, C. & Kimberling, W. J. Mutations in the *VLGR1* gene implicate G-protein signaling in the pathogenesis of Usher syndrome type II. *Am. J. Hum. Genet.* **74**, 357–366 (2004).
This study reports that mutations of *GPR98* (also known as *VLGR1* and *ADGRV1*) are responsible for the sensory defects associated with Usher syndrome type IIC.
73. Besnard, T. *et al.* Non-*USH2A* mutations in USH2 patients. *Hum. Mutat.* **33**, 504–510 (2012).
74. Ebermann, I. *et al. PDZD7* is a modifier of retinal disease and a contributor to digenic Usher syndrome. *J. Clin. Invest.* **120**, 1812–1823 (2010).
75. Giera, S. *et al.* The adhesion G protein-coupled receptor GPR56 is a cell-autonomous regulator of oligodendrocyte development. *Nat. Commun.* **6**, 6121 (2015).
76. Ackerman, S. D., Garcia, C., Piao, X., Gutmann, D. H. & Monk, K. R. The adhesion GPCR Gpr56 regulates oligodendrocyte development via interactions with $Gα_{12/13}$ and RhoA. *Nat. Commun.* **6**, 6122 (2015).
77. Piao, X. *et al.* An autosomal recessive form of bilateral frontoparietal polymicrogyria maps to chromosome 16q12.2-21. *Am. J. Hum. Genet.* **70**, 1028–1033 (2002).
78. Shin, D., Lin, S. T., Fu, Y. H. & Ptácek, L. J. Very large G protein-coupled receptor 1 regulates myelin-associated glycoprotein via $G_{αs}/G_{αq}$-mediated protein kinases A/C. *Proc. Natl Acad. Sci. USA* **110**, 19101–19106 (2013).
79. Monk, K. R. *et al.* A G protein-coupled receptor is essential for Schwann cells to initiate myelination. *Science* **325**, 1402–1405 (2009).
This study defined the essential function of GPR126 in Schwann cell myelination. This study was also one of the first to provide *in vivo* evidence that aGPCRs probably couple to heterotrimeric G proteins.
80. Monk, K. R., Oshima, K., Jörs, S., Heller, S. & Talbot, W. S. Gpr126 is essential for peripheral nerve development and myelination in mammals. *Development* **138**, 2673–2680 (2011).
81. Ravenscroft, G. *et al.* Mutations of *GPR126* are responsible for severe arthrogryposis multiplex congenita. *Am. J. Hum. Genet.* **96**, 955–961 (2015).
82. Glenn, T. D. & Talbot, W. S. Analysis of Gpr126 function defines distinct mechanisms controlling the initiation and maturation of myelin. *Development* **140**, 3167–3175 (2013).
83. Mogha, A. *et al.* Gpr126 functions in Schwann cells to control differentiation and myelination via G-Protein activation. *J. Neurosci.* **33**, 17976–17985 (2013).
84. Monk, K. R., Feltri, M. L. & Taveggia, C. New insights on Schwann cell development. *Glia* **63**, 1376–1393 (2015).
85. Feltri, M. L., Poitelon, Y. & Previtali, S. C. How Schwann cells sort axons: new concepts. *Neuroscientist* **22**, 252–265 (2015).
86. Paavola, K. J., Sidik, H., Zuchero, J. B., Eckart, M. & Talbot, W. S. Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. *Sci. Signal.* **7**, ra76 (2014).
87. Petersen, S. C. *et al.* The adhesion GPCR GPR126 has distinct, domain-dependent functions in Schwann cell development mediated by interaction with laminin-211. *Neuron* **85**, 755–769 (2015).
This study describes how GPR126 and laminin 211 interactions control different stages of Schwann cell development and myelination. Together with reference 89, it contains the first description of a mechanosensory role of aGPCRs in the nervous system.
88. Moriguchi, T. *et al.* DREG, a developmentally regulated G protein-coupled receptor containing two conserved proteolytic cleavage sites. *Genes Cells* **9**, 549–560 (2004).
89. Scholz, N. *et al.* The adhesion GPCR Latrophilin/CIRL shapes mechanosensation. *Cell Rep.* **11**, 866–874 (2015).
This study reports the aGPCR latrophilin as a modulator of mechanosensory neuron sensitivity in *D. melanogaster*. Together with reference 87, it contains the first description of a mechanosensory role of aGPCRs in the nervous system.
90. Yan, Z. *et al. Drosophila* NOMPC is a mechanotransduction channel subunit for gentle-touch sensation. *Nature* **493**, 221–225 (2013).
91. Baumgartner, S. *et al.* A *Drosophila* neurexin is required for septate junction and blood-nerve barrier formation and function. *Cell* **87**, 1059–1068 (1996).
92. Müller, A. *et al.* Oriented cell division in the *C. elegans* embryo is coordinated by G-Protein signaling dependent on the adhesion GPCR LAT-1. *PLoS Genet.* **11**, e1005624 (2015).
93. Wu, M. P. *et al.* G-protein coupled receptor 56 promotes myoblast fusion through serum response factor- and nuclear factor of activated T-cell-mediated signalling but is not essential for muscle development *in vivo*. *FEBS J.* **280**, 6097–6113 (2013).
94. White, J. P. *et al.* G protein-coupled receptor 56 regulates mechanical overload-induced muscle hypertrophy. *Proc. Natl Acad. Sci. USA* **111**, 15756–15761 (2014).
95. Langenhan, T., Aust, G. & Hamann, J. Sticky signaling–adhesion class G protein-coupled receptors take the stage. *Sci. Signal.* **6**, re3 (2013).
96. Prömel, S. *et al.* The GPS motif is a molecular switch for bimodal activities of adhesion class G protein-coupled receptors. *Cell Rep.* **2**, 321–331 (2012).
97. Qian, F. *et al.* Cleavage of polycystin-1 requires the receptor for egg jelly domain and is disrupted by human autosomal-dominant polycystic kidney disease 1-associated mutations. *Proc. Natl Acad. Sci. USA* **99**, 16981–16986 (2002).

98. Bohnekamp, J. & Schöneberg, T. Cell adhesion receptor GPR133 couples to G_s protein. *J. Biol. Chem.* **286**, 41912–41916 (2011).
99. Ligtenberg, M. J. *et al.* Cell-associated episialin is a complex containing two proteins derived from a common precursor. *J. Biol. Chem.* **267**, 6171–6177 (1992).
100. Macao, B., Johansson, D. G. A., Hansson, G. C. & Härd, T. Autoproteolysis coupled to protein folding in the SEA domain of the membrane-bound MUC1 mucin. *Nat. Struct. Mol. Biol.* **13**, 71–76 (2006).
101. Benoit, M. Cell adhesion measured by force spectroscopy on living cells. *Methods Cell Biol.* **68**, 91–114 (2002).
102. Okajima, D., Kudo, G. & Yokota, H. Brain-specific angiogenesis inhibitor 2 (BAI2) may be activated by proteolytic processing. *J. Recept. Signal Transduct. Res.* **30**, 143–153 (2010).
103. Paavola, K. J., Stephenson, J. R., Ritter, S. L., Alter, S. P. & Hall, R. A. The N terminus of the adhesion G protein-coupled receptor GPR56 controls receptor signaling activity. *J. Biol. Chem.* **286**, 28914–28921 (2011).
104. Yang, L. *et al.* GPR56 regulates VEGF production and angiogenesis during melanoma progression. *Cancer Res.* **71**, 5558–5568 (2011).
105. Ward, Y. *et al.* CD97 amplifies LPA receptor signaling and promotes thyroid cancer progression in a mouse model. *Oncogene* **32**, 2726–2738 (2013).
106. Liebscher, I., Schöneberg, T. & Prömel, S. Progress in demystification of adhesion G protein-coupled receptors. *Biol. Chem.* **394**, 937–950 (2013).
107. Vu, T. K., Hung, D. T., Wheaton, V. I. & Coughlin, S. R. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* **64**, 1057–1068 (1991).
108. Lanoue, V. *et al.* The adhesion-GPCR *BAI3*, a gene linked to psychiatric disorders, regulates dendrite morphogenesis in neurons. *Mol. Psychiatry* **18**, 943–950 (2013).
109. Kan, Z. *et al.* Diverse somatic mutation patterns and pathway alterations in human cancers. *Nature* **466**, 869–873 (2010).
110. O'Hayre, M. *et al.* The emerging mutational landscape of G proteins and G-protein-coupled receptors in cancer. *Nat. Rev. Cancer* **13**, 412–424 (2013).
111. Haitina, T. *et al.* Expression profile of the entire family of *Adhesion*G protein-coupled receptors in mouse and rat. *BMC Neurosci.* **9**, 43 (2008).
112. Zhang, Y. *et al.* An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J. Neurosci.* **34**, 11929–11947 (2014).
113. Harty, B. L., Krishnan, A., Sanchez, N. E., Schiöth, H. B. & Monk, K. R. Defining the gene repertoire and spatiotemporal expression profiles of adhesion G protein-coupled receptors in zebrafish. *BMC Genomics* **16**, 62 (2015).
114. Lőpez-Bendito, G. & Molnár, Z. Thalamocortical development: how are we going to get there? *Nat. Rev. Neurosci.* **4**, 276–289 (2003).
115. Strokes, N. & Piao, X. in *Adhesion-GPCRs: Structure to Function* Ch. 7 (eds Yona, S. & Stacey, M.) (Springer, 2010).
116. Cerminara, N. L., Lang, E. J., Sillitoe, R. V. & Apps, R. Redefining the cerebellar cortex as an assembly of non-uniform Purkinje cell microcircuits. *Nat. Rev. Neurosci.* **16**, 79–93 (2015).
117. Sherman, D. L. & Brophy, P. J. Mechanisms of axon ensheathment and myelin growth. *Nat. Rev. Neurosci.* **6**, 683–690 (2005).
118. Mouw, J. K., Ou, G. & Weaver, V. M. Extracellular matrix assembly: a multiscale deconstruction. *Nat. Rev. Mol. Cell Biol.* **15**, 771–785 (2014).

Acknowledgements
The authors thank their colleagues in the Adhesion GPCR Consortium (www.adhesiongpcr.org) for their continuous feedback on results and scientific concepts that are discussed in this Review. The authors apologize to all colleagues whose work could not be covered owing to scope and space restrictions. This work was supported by grants of the Deutsche Forschungsgemeinschaft (FOR 2149/P1 + P3, SFB 1047/A5, SFB-TR 166/C3 and LA2861/7-1 to T.L.), the US National Institutes of Health (NS085201 and NS094164 to X.P. and NS079445 and HD080601 to K.R.M.), and the National Multiple Sclerosis Society (RG-1501-02577 to X.P. and Harry Weaver Neuroscience Fellowship to K.R.M.).

Competing interests statement
The authors declare no competing interests.

DATABASES
BFPP: http://www.omim.org/entry/606854
BPPR: http://www.omim.org/entry/615752
Lethal congenital contracture syndrome-9: http://www.omim.org/entry/616503
Neural tube defects: http://www.omim.org/entry/182940
Online Mendelian Inheritance in Man (OMIN): http://www.omim.org/
Usher syndrome, type IIC: http://www.omim.org/entry/605472

ALL LINKS ARE ACTIVE IN THE ONLINE PDF