Transient cell–cell interactions in neural circuit formation

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Abstract | The wiring of the nervous system requires a complex orchestration of developmental events. Emerging evidence suggests that transient cell–cell interactions often serve as positional cues for axon guidance and synaptogenesis during the assembly of neural circuits. In contrast to the relatively stable cellular interactions between synaptic partners in mature circuits, these transient interactions involve cells that are not destined to be pre- or postsynaptic cells. Here we review the roles of these transient cell–cell interactions in a variety of developmental contexts and describe the mechanisms through which they organize neural connections.

En passant synapses
Synapses formed along the shaft of the axon, in contrast to terminal synapses, which are formed only at the end of the axon.

Pioneer axon
An axon that develops early in a fascicle and acts as a scaffold for later-developing neurons to grow on.

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How the nervous system assembles into the neuronal circuits that lead to complex behaviours is a question that has fascinated neuroscientists for many years. The assembly of neural circuits requires the intricate coordination of multiple developmental events. First, cells differentiate to their proper fate and migrate to the appropriate location. This is followed by the outgrowth of axons and dendrites, which are guided by various axon guidance cues to the appropriate target field. Once axons reach their target field, they then must identify their appropriate postsynaptic targets and initiate synapse formation.

How is the formation of neural circuits achieved? Traditionally, neuronal connectivity has been thought to arise from direct interactions between pre- and postsynaptic neurons, as the final product of neural development is the establishment of functional synaptic connections between appropriate synaptic partners. However, many studies have demonstrated that specific cell populations that are not part of the eventual neural circuit have important roles in the assembly of these circuits. Since the initial discovery of 'guidepost cells' that guide grasshopper axons, transient cell–cell interactions that are important for axon guidance and synaptogenesis have been identified in many different organisms and in various developmental contexts. In contrast to classically described environmental axon guidance cues, which act through gradients to attract or repel axons over long distances, these cell–cell interactions transiently mark a specific spatial coordinate in a particular developmental stage and delineate choice points for axon growth or synaptogenesis. Conceptually, these cells have been shown to have diverse roles in neural circuit formation, including acting as intermediate targets in axon guidance (FIG. 1a), serving as a scaffold for axon growth and guidance (FIG. 1b), acting as 'placeholders' by forming a transient circuit with presynaptic targets (FIG. 1c), providing positive or negative signals to direct spatial localization of *en passant* synapses (FIG. 1d), and coordinating neural circuit formation by providing cues for both synapse formation and axon guidance (FIG. 1e). In this Review, we compare and contrast the roles and mechanisms through which transient cell–cell interactions enable neural circuit formation in different organisms and developmental contexts.

One of the first demonstrations that cells extrinsic to the neural circuit act as intermediate targets in axon guidance was that of the role of guidepost cells in pioneer sensory axon guidance in the embryonic grasshopper hindlimb. In the grasshopper, sensory neuron cell bodies are located near the tip of the hindlimb and extend their axons from the appendage along a stereotyped route to reach the CNS[1] (FIG. 2). This route is not straight, but quite circuitous and includes dramatic turns in the trajectory of the axons at certain points[2] (FIG. 2). At these points of change, morphologically distinct cells described as 'guideposts' have been observed. These cells may contain cues that could reorient the trajectory of the axon at strategic points and this could be involved in a 'connect-the-dots' strategy for axon guidance. A key aspect of this idea is that these cells are not continuously present along the axon path; instead they are present at discrete points, providing non-continuous positional information that sequentially guides axonal growth.

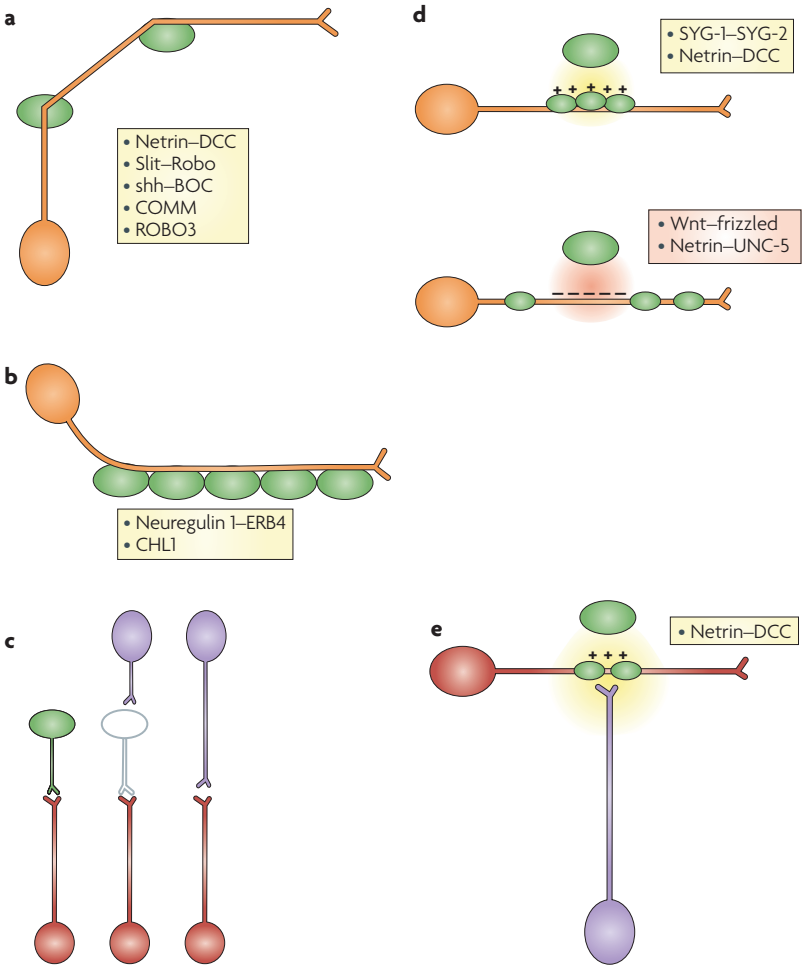

Figure 1 | **Conceptual roles for transient cell–cell interactions in neural circuit formation. a** | Guidepost cells as intermediate targets in axon guidance. A neuron (shown in orange) extends its axon and contacts a non-continuous set of guidepost cells (shown in green) in a sequential fashion. Each guidepost cell attracts the growth cone, allowing stepwise guidance of the axon. Examples of intermediate targets in axon guidance include the floor plate in the spinal cord and guidepost cells in the grasshopper limb. Molecules involved in intermediate-target axon guidance include the netrins, the Slits and sonic hedgehog (shh), and their respective receptors DCC, the Robos and BOC, as well as COMM and ROBO3. **b** | Cells as a scaffold for axonal tract formation. A neuron uses cells present along the path of the axonal trajectory as a substrate for axonal growth. Examples include 'axon runway cells' in leeches, LOT1a cells in the telencephalon, 'corridor cells' in the internal capsule and Bergmann glia in the cerebellum. Molecular factors implicated in this process include neuregulin 1 and its receptor ERBB4, and close homologue of L1 (CHL1). **c** | Cells as placeholders in neural circuit assembly. A presynaptic neuron (shown in red) extends its axonal process into the target field before the final postsynaptic targets are present and forms transient synapses onto placeholder cells (shown in green) in the target field (left-hand image). At a later developmental stage (middle image) the placeholder cell undergoes regulated cell death and the postsynaptic cell (shown in purple) is born and begins to extend its dendrite to the target field. Later in development (right-hand image) the postsynaptic dendrite grows into the target field and synapses are formed between pre- and postsynaptic cells. Examples of such placeholder cells include Cajal–Retzius cells in the hippocampus and subplate cells. **d** | Guidepost cells regulate the subcellular localization of synapses. Guidepost cells express membrane molecules (such as SYG-2) or secrete signals (such as Wnts or netrins) that promote or inhibit synaptogenesis in a defined subcellular region of the axon by binding their receptor (SYG-1, frizzled or DCC, respectively). Axon guidance is not affected by the guidepost cells themselves in this case. **e** | Guidepost cells as organizers of neural circuit formation. Guidepost cells can secrete signals (such as netrins) that both promote the local assembly of presynaptic specializations in the presynaptic axon and guide postsynaptic dendrites to their proper location.

Further characterization of these cells identified some characteristics that were used as initial criteria for defining guidepost cells[3]: guideposts are present along the path of growing axons, they are contacted by axonal growth cones, they are separated from one another but within filopodial reach of one another, they can be distinguished from adjacent cells using antibodies and they can form cellular junctions with axons (dye can pass between an axon and a guidepost cell). Although these criteria have been useful as a guideline for what constitutes a guidepost cell, subsequently discovered guidepost cells have not been definitively shown to form dye-passing cellular junctions with axons.

A causal role for guidepost cells in axon guidance was discovered in cell ablation experiments: specific ablation of these cells resulted in pioneer axons wandering off their correct trajectory and forming ectopic branches[4]. This suggested that cells present along the path of an axon in discrete locations could serve as positional cues in a stepwise fashion to guide axons to their appropriate targets.

Guidepost cells in axon guidance

The floor plate as an intermediate target in commissural axon guidance. Since the discovery of intermediate targets in grasshopper axon guidance, other cells in many organisms have also been shown to function as guideposts in axon guidance. The role of guidepost cells as intermediate targets in axon guidance is well characterized at the vertebrate midline (FIG. 3a). In bilaterally symmetric animals, commissural neurons cross from one side of the spinal cord to the other, connecting the two halves of the CNS. Commissural axons first travel ventrally, and then cross the midline to travel rostrally on the other side of the spinal cord. What are the cellular mechanisms that guide these commissural axons? Cells at the midline serve as intermediate targets for the crossing axons. In *Drosophila melanogaster*, midline glial cells serve this function, as mutations that affect the development of these cells disrupt midline crossing of commissural axons[5]. In the vertebrate spinal cord, the floor plate serves this function. In Danforth's short tail mouse mutants, a further mutation in the zinc finger transcription factor GLI2 results in the loss of the floor plate, and consequently many commissural axons have aberrant trajectories that do not cross the midline[6]. Additionally, in zebrafish *cyclops* (also known as *ndr2*) mutants, which lack a floor plate, a similar axon guidance defect was observed whereby many commissural axons were no longer able to cross the midline[7]. However, the floor plate does not seem to be absolutely required for commissural axon guidance, as additional analyses of mouse and zebrafish mutants lacking a floor plate have demonstrated that a substantial number of commissural axons have wild-type trajectories[7–10]. This suggests that, in addition to the floor plate, there are other sources of guidance cues that influence commissural axon growth.

As an intermediate target, the floor plate must first attract the commissural axons and then repel them so

Floor plate
Specialized neural epithelial cells found at the ventral midline of the spinal cord that secrete various factors involved in organizing the nervous system.

Netrins
Secreted proteins similar to laminin that were first discovered through their involvement in axon guidance.

Slits
Axon guidance factors that were first discovered through their roles as chemorepellents.

Optic chiasm
The anatomical landmark where retinal ganglion cell axons from both eyes converge and cross.

Radial glia
Cells with astrocytic characteristics and processes that span the region from the lumen of the ventricle to the pial surface. They have multiple roles during neural development.

that they reach their next target. Study of the floor plate has been quite fruitful for identifying molecular cues associated with guidepost cells.

First, how does the floor plate attract commissural neurons to the midline? Netrins, secreted extracellular proteins, were identified as long-range chemoattractants of commissural axons to the midline[11] (FIG. 3a). Netrins are secreted from the floor plate and bind their receptor, UNC-40 (also known as DCC), which is present on commissural axons. Genetic analyses in vertebrates, *D. melanogaster* and *Caenorhabditis elegans* have demonstrated that the netrin–DCC interaction is crucial for midline guidance (reviewed in REF. 12). A subset of these commissural axons is guided to the midline by the morphogen sonic hedgehog, which is also secreted from the floor plate and binds to its receptor, BOC, which is present on commissural axons[13,14]. In addition to long-range chemoattraction, adhesion molecules such as axonin I (also known as CNTN2) and neuronal cell-adhesion molecule (NRCAM), which are expressed by the floor plate, may be involved in attractive guidance[15,16]. Thus, the floor plate secretes multiple factors, which allow long-range attraction of commissural axons towards the midline.

Second, once commissural axons reach the floor plate, how do they grow away from the midline after crossing and proceed to their target? Floor plate cells secrete another set of extracellular molecules, the Slits, which repel commissural axons by binding to their receptors, the Robos, which are expressed on commissural axons. Mutations in *D. melanogaster slit* as well as in both isoforms of *D. melanogaster robo* cause dramatic midline-crossing phenotypes[17–19]. A similar role for Slits and Robos is found at the vertebrate midline, as deletions of all three Slit proteins results in the stalling of many commissural axons at the midline[20].

Attractive and repulsive molecules are secreted from the floor plate at the same time. So how is commissural axon receptivity to attractive and repulsive cues coordinated? In *D. melanogaster*, this process seems to be mediated through an intracellular protein, COMM, which is involved in the trafficking of Robos and prevents them from being expressed on the growth cone membrane before midline crossing[21–24]. On midline crossing, COMM is downregulated, allowing surface expression of Robos on commissural axons and resulting in repulsion. Although vertebrates have no structural homologue of COMM, its role is fulfilled by a Robo-like receptor, ROBO3 (also known as RIG1)[25]. Alternative splicing of *Robo3* generates two molecular species, one of which has a role similar to that of the *Drosophila* COMM. The other splice isoform acts similarly to ROBO1 and ROBO2 (also known as LEA) to repel axons after crossing[26]. Additionally, interaction between the attractive and the repulsive pathways is further required to coordinate the midline crossing. It has been demonstrated that Robos can bind to the cytoplasmic tail of DCC and silence the netrin-induced attractive response, providing an elegant solution to the potential 'tug of war' between the attractive and the repulsive response[27] (FIG. 3b,c). Thus, floor plate cells secrete various attractive and repulsive factors that are intricately regulated to allow the floor plate to function as an intermediate target in axon guidance. These cells may also provide a substrate for the axons to grow on and pass through the midline, although the mechanism by which this occurs is not yet understood.

Interestingly, floor plate cells also secrete an as yet unidentified neurotrophic factor that promotes commissural cell survival provided that the commissural axon grows near the floor plate[28]. This *en passant* neurotrophic action may represent a mechanism to ensure the fidelity of axonal wiring by allowing only neurons that grow towards the intended target to survive.

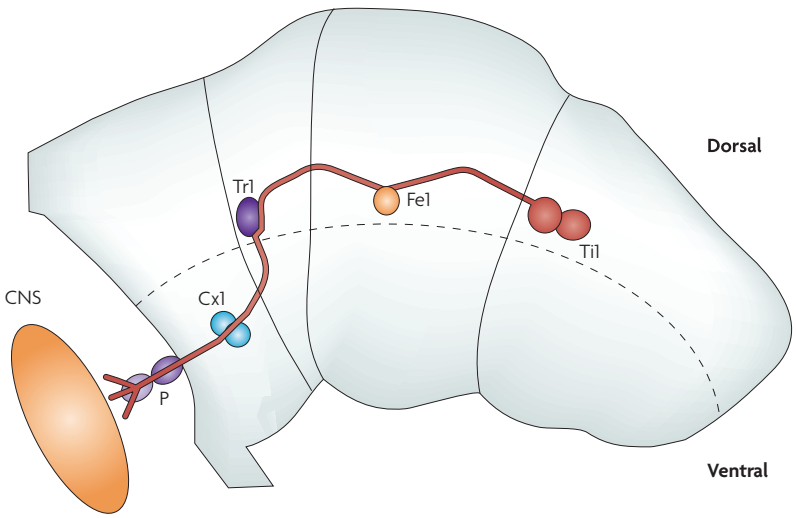

Figure 2 | **Guidepost cells in grasshopper pioneer neuron axon guidance.** The stereotyped axonal trajectory of Ti1 neurons. Ti1 axons reorient their trajectory at points of contact with specific cell types (Fe1, Tr1, Cx1 and P). Ablation of Cx1 cells causes Ti1 axons to wander and form ectopic branches. Figure is modified, with permission, from REF. 2 © (1986) Society for Neuroscience.

CD44+ neurons and radial glia in retinal axon guidance. An additional example of guidepost cells directing midline axon guidance occurs in the mammalian retina. During the development of retinal axon projections, separate populations of retinal ganglion cells (RGCs) extend axons to contralateral and ipsilateral targets (FIG. 4). This pattern of axonal projection is necessary for binocular vision in vertebrates. What are the factors that determine whether a neuron connects with contralateral or ipsilateral targets? There is a population of early-born neurons, which express the cell surface protein CD44, present at the site of the future optic chiasm before retinal axons reach this landmark[29] (FIG. 4). Interestingly, these cells are homologous to those at the tract of the post-optic commissure in the fish optic chiasm[30]. Ablation of these CD44+ cells causes retinal axons to stall at the chiasm, preventing the formation of contralateral projections at early stages of development[31]. This suggests that CD44+ cells are necessary for RGC axons to cross the midline and form the contralateral projection. In addition, radial glia are also found at the site of the optic chiasm. Radial glia are contacted by RGC axons while the axons cross the midline[32], and are important in forming the

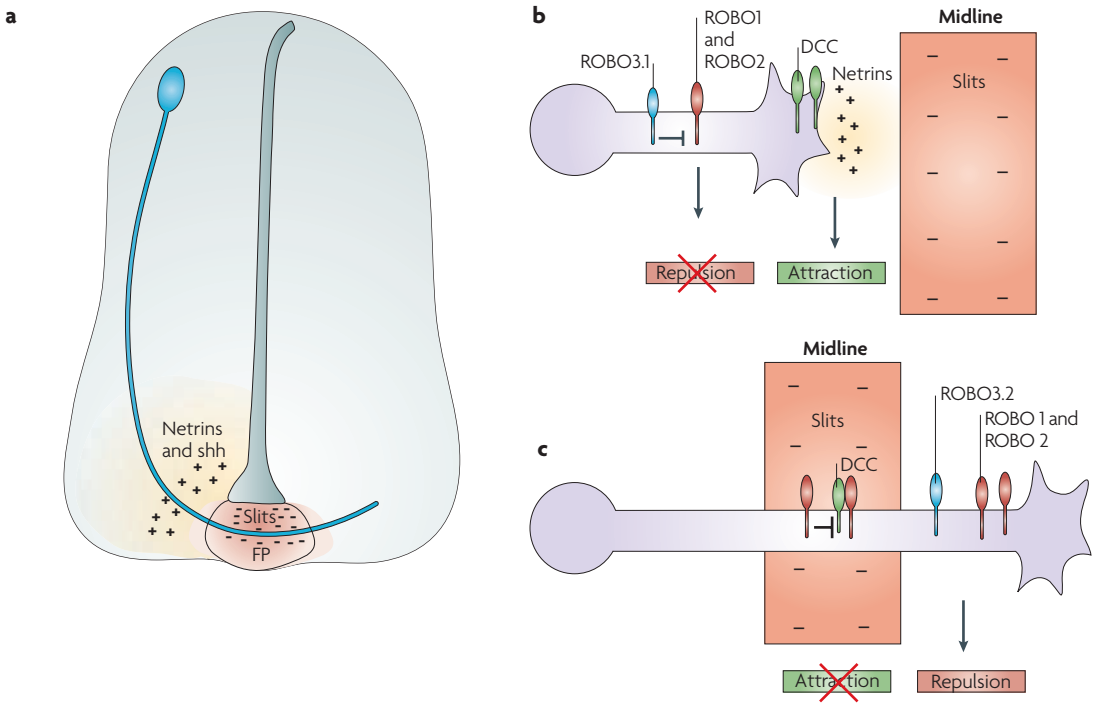

Figure 3 | **The floor plate as an intermediate target in axon guidance. a** | A cross section of the developing vertebrate spinal cord. Commissural axons from only one side of the spinal cord are shown for simplicity. Commissural axons (shown in blue) are attracted to the floor plate (FP) by secretion of the chemoattractants netrins and sonic hedgehog (shh) (yellow shading). On crossing the midline, chemoattraction is silenced and commissural axons become receptive to the repulsive Slit cue (red shading), which is also secreted by the floor plate. This allows commissural axons to cross the midline only once. **b** | Molecular events that underlie commissural axon attraction to the floor plate. Netrins secreted from the floor plate bind their commissural axon-expressed receptor, DCC, allowing axon attraction towards the floor plate. The activities of the chemorepellent receptors ROBO1 and ROBO2 are inhibited by ROBO3.1, preventing repulsion of the axons away from the floor plate by Slits. **c** | Molecular events that coordinate the switch from attraction to repulsion in commissural axons. As commissural axons cross the floor plate, the activity of the netrin receptor DCC is inhibited by the Slit receptor ROBO1 through interactions between their cytoplasmic tails. Upregulation of ROBO3.2, ROBO1 and ROBO2 allows repulsion away from the midline through the chemorepellents the Slits. Data from REF. 26.

ipsilateral projection as they repel a subset of RGC axons away from the chiasm. Radial glia express ephrin B2, and a subset of RGC axons express EPHB1, its receptor[33] (FIG. 4). In EPHB1-deficient mice, fewer axons project ipsilaterally (more RGC axons cross the midline), suggesting that the ephrin B2–EPHB1 interaction plays a part in repelling RGC axons. These experiments suggest that radial glia are important for repelling a subpopulation of axons from the optic chiasm to form the ipsilateral projection. Thus, radial glia and CD44+ neurons act together to regulate RGC axon guidance.

An additional midline glial cell population, the 'glial wedge', has been shown to be involved in callosal axon guidance[34]. Cortical axons cross from one hemisphere to the other medially through an axon tract called the corpus callosum. The glial wedge is a collection of glia with cell bodies located in the ventricular zone on either side of the midline below the corpus callosum. Reorientation of the glial wedge in organotypic slice cultures causes callosal axons to inappropriately turn away from the midline, and replacement of the glial wedge with a piece of cortex causes callosal axons to grow in aberrant trajectories, suggesting that the glial wedge provides positional cues for callosal axon guidance. This positional cue is thought to be SLIT2, expressed in the glial cells, which binds the SLIT2 receptors expressed on the callosal axons[34], ROBO1 and ROBO2, to repel axons across the midline.

Mitral cell
The main efferent output of the olfactory bulb.

Cells as scaffolds for axon guidance

In some situations, cells may serve as substrates for axon growth and tract formation. These cells do not fit the classical definition of a guidepost cell, as some of them are present along the entire axonal trajectory of the neuron and thus provide continuous guidance. These cells are present along the future axonal path before the axon reaches the target, and some have been found to be required for proper axonal growth and targeting. In the leech, an 'axonal runway' cell is present along the entire axonal path of a peripheral motor axon and is required for its proper targeting[35]. In moths, motor neurons grow precisely on a scaffold of 'strap' and 'bridge' cells to reach their final target[36,37]. In the vertebrate olfactory system, mitral cells project axons into the lateral telencephalon through a fascicle called the lateral olfactory tract (LOT). A specific population of cells, labelled by a LOT1 antibody, is present in the future LOT before mitral cells extend their axons into the telencephalon[38].

Bergmann glial cell
An astrocyte that extends long radial fibres throughout the entire cerebellum.

In a co-culture system, ablation of these LOT1-labelled cells prevented elongation of mitral cell axons into the telencephalon, suggesting that these cells were required for mitral cell outgrowth in the telencephalon[38].

'Corridor' cells in thalamocortical axon guidance. Another example of cells acting as a scaffold for axon guidance is the role of 'corridor' cells in thalamocortical axon guidance. Dorsal thalamic axons have a stereotyped trajectory and form an axon tract through the medial ganglionic eminence (MGE) of the internal capsule to reach their specific cortical targets. Elegant work from Lopez-Bendito *et al.* demonstrated that tangentially migrating neuregulin 1-expressing cells from the lateral ganglionic eminence (LGE) form a corridor for dorsal thalamic axons to grow through the internal capsule[39]. In *Mash1* (also known as *Ascl1*)-null mice, LGE-derived corridor cells are not present and thalamocortical axons fail to extend into the MGE. Transplantation of wild-type LGE progenitors into *Mash1*-null mice was able to re-form the corridor and restored the ability of dorsal thalamic axons to grow into the internal capsule. Ectopic transplantation of corridor cells into the caudal ganglionic eminence (CGE) was also sufficient to recruit thalamic axons to the CGE. Taken together, these data suggest that these corridor cells are both necessary and sufficient for dorsal thalamic axon guidance through the internal capsule. At the molecular level, various isoforms of neuregulin 1 expressed on corridor cells, and the neuregulin 1 receptor, ERBB4, expressed on thalamic axons mediate the short-range attraction to allow proper thalamic axon guidance[39].

Glia as scaffolds for stellate axon arborization. A striking example of subcellular specificity in wiring can be found in the cerebellum, where stellate interneurons form synapses only on the distal portions of the Purkinje cell dendrites (FIG. 5). How is this specificity achieved? It was observed that Bergmann glial fibres serve as a scaffold for stellate axons as they contact Purkinje cell dendrites[40]. Bergmann glia form an elaborate scaffold of radial fibres throughout the cerebellum, which has been suggested to play a part in organizing the cerebellar circuitry. Stellate axons were found to grow along these Bergmann glial fibres, and synapses were observed at the intersection of Bergmann glial fibres and Purkinje cell dendrites, suggesting that Bergmann glia serve as an intermediate scaffold for stellate–Purkinje cell innervation (FIG. 5). This adhesion between stellate axons and Bergmann glial fibres is mediated by an adhesion molecule, close homologue of L1 (CHL1). In CHL1-deficient animals, stellate axons no longer closely adhere to Bergmann glial fibres; instead they grow in aberrant trajectories and form a reduced number of synapses onto Purkinje cells. Thus, Bergmann glia act as an intermediate template to direct stellate axon arborization and, consequently, synapse formation onto Purkinje cell dendrites.

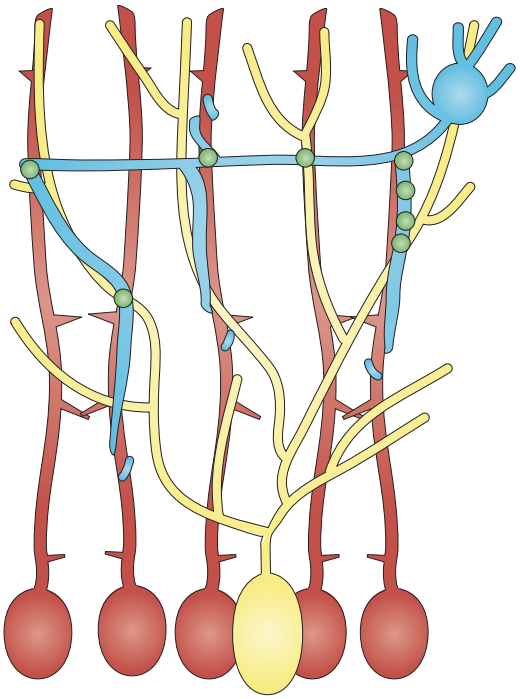

Figure 5 | **Bergmann glia direct stellate interneuron arborization onto Purkinje cell dendrites.** Purkinje cells (shown in yellow) receive synaptic inputs (shown in green) from stellate interneurons (shown in blue) at only the distal portions of their dendrites. Bergmann glia (shown in red) cell processes act as a scaffold for stellate axon guidance and synaptogenesis onto Purkinje cell dendrites. Figure is modified from REF. 40.

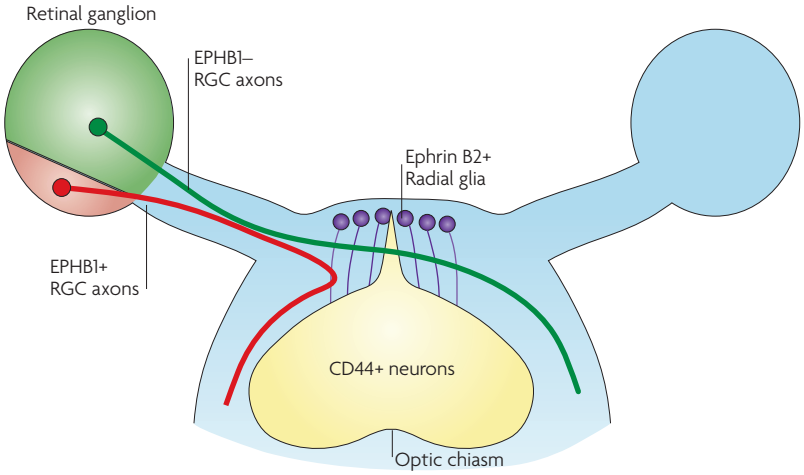

Figure 4 | **Radial glia and CD44+ cells in retinal axon guidance.** Retinal ganglion cell (RGC) axons from one eye only are shown for simplicity. Ventral temporal (VT) RGC axons (shown in red) contact radial glia and are repelled from the midline to form the uncrossed projection. This repulsion is in part due to interactions between ephrin B2, expressed on radial glia, and its receptor, EPHB1, expressed on VT RGC axons. Non-VT RGC axons (shown in green) do not express EPHB1 and so cross the midline to form the contralateral projection. This crossing is dependent on CD44+ neurons located at the optic chiasm[78].

Synaptic placeholders in neural circuit formation

In certain instances during neural development, presynaptic neurons will extend their axonal processes in the appropriate target field before the postsynaptic neurons are present. How are these axons guided in the absence of their postsynaptic target? Two examples suggest that cells can act as placeholders to provide cues for target field selection.

Cajal–Retzius cells and GABAergic interneurons in the hippocampus. A striking example of the precision in neural circuit formation is found in the establishment of layer specificity in the vertebrate hippocampus (reviewed in REF. 41). Afferent axons from the entorhinal cortex form synapses on the distal dendrites of pyramidal neurons in the stratum lacunosum-moleculare (SLM) layer, whereas commissural or associational fibres form synapses on the more proximal part of these same pyramidal dendrites in the stratum radiatum (SR) (FIG. 6a). Entorhinal axons enter the SLM layer before most pyramidal dendrites have arrived in this area. How is this laminar specificity achieved?

When entorhinal and commissural axons enter the target field, calretinin-positive Cajal–Retzius cells and GABA (γ-aminobutyric acid)- and calbindin-positive interneurons (both early-born neurons) are present in the SLM and SR, respectively[42–44]. Cajal–Retzius cells and GABAergic interneurons form transient synaptic contacts with entorhinal axons and commissural axons, respectively, and both populations of cells undergo cell death later during development[43]. Thus, these two classes of cells may be part of a transient neural circuit that is necessary for layer-specific targeting.

A functional role for Cajal–Retzius cells in layer-specific innervation was tested in an entorhinal

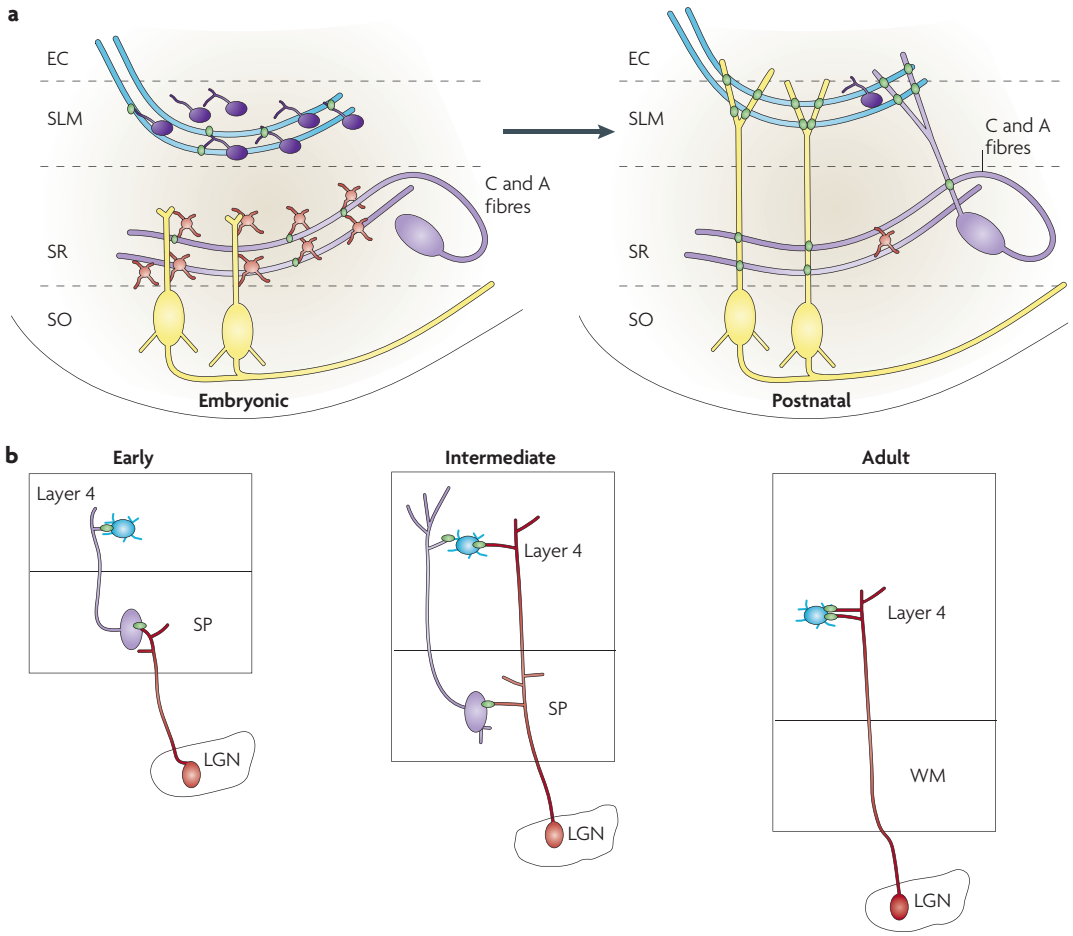

Figure 6 | **The role of Cajal–Retzius cells and subplate cells in neural circuit formation. a** | Cajal–Retzius cells and GABA (γ-aminobutyric acid)-ergic interneurons have roles in establishing layer specificity in the hippocampus. In the vertebrate embryo, pyramidal dendrites (shown in yellow) are found only in the stratum radiatum (SR) layer. Commissural and associational (C and A) axon fibres (shown in light purple) preferentially form synaptic contacts (shown in green) onto GABAergic interneurons (shown in red), which are also found in the SR layer. Axons from the entorhinal cortex (EC) (shown in light blue) form synapses onto Cajal–Retzius cells (shown in dark purple), found in the stratum lacunosum-moleculare (SLM) layer, before the pyramidal dendrites reach the SLM. Dashed lines delineate the SLM and SR layers. In the postnatal vertebrate many Cajal–Retzius cells and GABAergic interneurons are removed through regulated cell death, and pyramidal dendrites extend into the SLM to form synapses on both entorhinal and C and A afferents on distinct dendritic domains. **b** | Subplate neurons act as transient synaptic targets in the formation of the thalamocortical circuit. Early in development, lateral geniculate nucleus (LGN) axons (shown in red) accumulate in the subplate (SP) and form synaptic connections (shown in green) with subplate neurons (shown in purple). Subplate neurons send axons into the cortical plate and form synaptic connections with layer 4 cortical neurons (shown in blue). Later in development, LGN neurons grow into cortical layer 4 and form synapses onto both subplate and layer 4 neurons. In the adult, subplate neurons are eliminated by regulated cell death and LGN axons form mature synaptic connections on cortical layer 4 neurons[47]. WM, white matter. Data from REF. 41 and REF. 47.

Entorhinal cortex
The brain region that provides the main input to the hippocampus.

Reelin
A secreted glycoprotein that is implicated in neuronal migration and other signalling pathways.

Cajal–Retzius cell
One of the earliest-born classes of neurons. They are located along the surface of the entire cortex during most of cortical development.

Lateral geniculate nucleus
The area of the thalamus that receives projections from the retina and sends projections to the visual cortex.

Subplate
A transient cell layer located below the cortical plate early in development that is important for establishing thalamocortical projections.

Box 1 | Subplate and Cajal–Retzius cells and disease

It has been observed that subplate neurons are selectively vulnerable to injury in a rodent model of neonatal hypoxia–ischaemia[59]. Early hypoxic events in humans can cause periventricular white matter injury, resulting in many cognitive and sensory problems, including cerebral palsy, cortical visual impairments and learning difficulties[60–62]. Subplate neurons are located in areas of diffuse subcortical signal abnormalities observed by MRI in premature infants with periventricular white matter injury[63]. Thus, loss of subplate cells is a potential mechanism for the pathology observed in neonatal hypoxia–ischaemia. It is unknown why subplate cells are particularly vulnerable to early hypoxic events, although some possible reasons are their early maturation and their intrinsic ability to undergo cell death later in development[46,47,64].

What are the consequences if guidepost cells persist throughout development? In patients with temporal lobe epilepsy it was shown that there is an abnormally high number of Cajal–Retzius cells in the hippocampus, and that this larger number of cells correlates with a higher incidence of early-onset febrile seizures[65]. This suggests that the persistence of Cajal–Retzius cells in the hippocampus may be involved in the pathology of temporal lobe epilepsy. In addition, perturbations in the protein reelin, which is secreted by Cajal–Retzius cells early in development, have also been associated with various neurological disorders, including schizophrenia and autism[66–69], although it is unclear whether this is due to the role of reelin in cell migration or to a guidepost role of Cajal–Retzius cells. Future studies are required to identify the precise roles of these cells in the pathogenesis of disease.

hippocampus slice co-culture system[45]. Ablation of the Cajal–Retzius cells prevented layer-specific innervation by entorhinal axons, suggesting that Cajal–Retzius cells are required for layer-specific targeting of these axons[45]. Thus, these cells seem to function as placeholders that enable layer-specific innervation in the hippocampus. Interestingly, Cajal–Retzius cells have also been shown to form transient synaptic contacts with pyramidal cells in the cortex[33], suggesting that this place-holding function might be involved in cortical maturation.

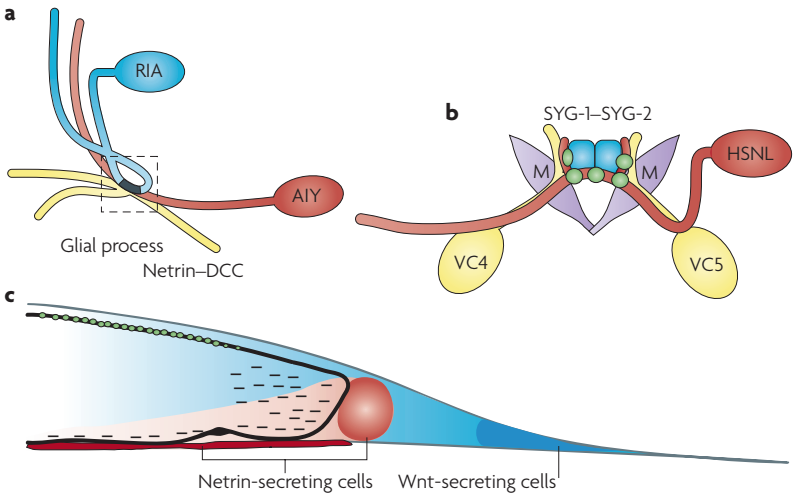

Figure 7 | **Non-neuronal cells as guideposts in synaptic specificity.** **a** | AIY–RIA connectivity is determined by ventral cephalic sheath glial cells. In the *Caenorhabditis elegans* nerve ring neuropil, the interneuron AIY forms synapses onto the interneuron RIA at a specific spatial coordinate (indicated by the box and the black shading). This coordination of synapse formation is mediated by netrins expressed by cephalic sheath glia (shown in yellow), which ensheath the contact area between AIY and RIA. **b** | The presynaptic location of the neuron HSNL is determined by vulval epithelial guidepost cells. In the *C. elegans* egg-laying circuit, HSNL forms synapses (shown in green) on VC4 and VC5 neurons and vulval muscles (M). This synaptic pattern is determined by vulval epithelial cells (shown in blue) that express the cell adhesion molecule SYG-2, which localizes its HSNL-expressed receptor, SYG-1. SYG-1 is necessary for synapse formation[54]. **c** | A diagram of a *C. elegans* tail with the DA9 neuron (shown in black) and its synaptic pattern (shown as green circles). Wnt-secreting cells and netrin-secreting cells cooperate to prevent synapse formation in the commissural and dendritic region of the DA9 neuron. Wnts act through their receptor, frizzled, and netrin acts through its receptor, UNC-5, to inhibit synapse formation. Data from REF. 76 and REF. 58.

Subplate cells in thalamic development. In the mature mammalian visual system, neurons from the lateral geniculate nucleus (LGN) innervate the visual cortex neurons in layer 4. However, during development, LGN axons arrive in the cortical target field long before layer 4 neurons have migrated into the region. What happens during this waiting period? It seems that subplate neurons serve as placeholders for thalamic axons. Thalamic axons from the LGN grow into the subplate and form transient synapses with the subplate cells, and subplate cells form transient connections with layer 4 neurons[46,47]. Thus, subplate cells serve as a relay between LGN neurons and the cortex. As layer 4 cortical neurons begin to mature, subplate cells begin to be removed by regulated cell death, and LGN axons extend into the cortex and form synapses with their final targets — layer 4 cortical neurons (FIG. 6b). Ablation studies have shown that subplate neurons are necessary for the proper guidance of LGN axons into layer 4 of the cortex[48], as well as for the formation of ocular-dominance columns[49]. Additional work suggests that the subplate may be important for functional maturation of the thalamocortical circuit. Ablation of subplate cells causes weak synaptic transmission and the absence of orientation columns[50]. In addition, ablation of the subplate prevents upregulation of the genes responsible for GABAergic gene expression, and may prevent the formation of inhibitory circuits in layer 4 neurons[51]. Thus, subplate cells not only play a part in guiding LGN projections, they also have roles in the synaptic maturation of the circuit.

Subplate cells and Cajal–Retzius cells serve as two examples of transient synaptic targets in the vertebrate nervous system. Interestingly, subplate cells and Cajal–Retzius cells have been associated with various neurological diseases, although a causal link between disturbances of these cells and disease has not yet been established (BOX 1).

Synaptic guidepost cells

In addition to roles in axon guidance, transient cell–cell interactions have been shown to provide positional cues for synapse target selection and synaptogenesis.

Box 2 | **Multifunctional roles of guidepost cells**

Many of the guidepost cells are born very early during development, and play multiple parts in the development of the nervous system. For example, in addition to attracting commissural axons, floor plate cells specify neuronal and glial cell fates through the secretion of sonic hedgehog (reviewed in REFS 70–72). Similarly, Cajal–Retzius cells are some of the earliest-born neurons in the cerebral cortex and have multiple functions in neocortical development, including neuronal migration[73,74]. *Caenorhabditis elegans* vulval epithelial cells provide positional cues for the migration of muscle precursors, the axonal arborization of the VC neurons, and the initiation of HSNL neuron synaptogenesis[54–56,75]. Ventral cephalic sheath cells not only specify the synaptic locations of certain sets of neurons, they also affect the guidance of multiple axons in the *C. elegans* nervous system[52,53,76]. One strategy that may have evolved to streamline the development of the nervous system is to reuse key cellular organizers for many different functions.

Another interesting feature of certain guidepost cells, such as subplate cells and Cajal–Retzius cells, is that they are present only transiently during development. How might their death be regulated? A study has shown that the presence of entorhinal axons is necessary for the death of Cajal–Retzius cells in co-culture experiments[77]. This is in contrast to the situation for Cajal–Retzius cells in the neocortex, where neuronal activity seems to be important for their survival. Subplate cells also undergo regulated cell death postnatally[46], but the mechanism for this death is unknown.

Glial cells. Glia-like cells have been observed to play a part in orchestrating the formation of the thermotaxis circuit in *C. elegans*[52]. The interneuron AIY forms synapses on the interneuron RIA at a stereotyped spatial coordinate in the *C. elegans* nerve ring neuropil (FIG. 7a). The glia-like ventral cephalic sheath cells (VCSCs) extend thin processes and contact the RIA in a manner that closely resembles the synaptic contact between AIY and RIA. Genetic manipulation showed that the VCSCs have an instructive role in specifying the localization of synapses between AIY and RIA. This process is mediated by the expression of netrin (also known as UNC-6) by VCSCs and of the netrin receptor, DCC, by both AIY and RIA. Interestingly, DCC has different roles in AIY and RIA. In AIY it specifies the localization of presynaptic specializations, whereas in RIA it has a canonical axon guidance role in bringing the RIA axon in contact with the AIY process. Thus, this is an example of a guidepost cell secreting a positional cue with diverse effects on neural circuit assembly depending on the cellular context. Interestingly, these glia-like cells are likely to have many roles in organizing the *C. elegans* nervous system (BOX 2): ablation of VCSCs affects sensory dendrite extension, axon guidance and nerve ring assembly[53].

Vulval epithelial cells. Another example of non-neuronal cells specifying synapse location is found in the *C. elegans* egg-laying circuit. The HSNL neuron forms synapses clustered around the vulva on two postsynaptic targets: the VC4 and VC5 neurons and vulval muscles[54] (FIG. 7b). Ablation of neighbouring vulval epithelial cells, but not ablation of the postsynaptic targets, causes defects in presynapse assembly[54]. Vulval epithelial cells transiently express the immunoglobulin cell adhesion molecule SYG-2, which clusters its HSNL-expressed receptor, SYG-1, and this interaction is responsible for specifying synapses in HSNL[54,55] (FIG. 7b). Interestingly, these same epithelial cells have been shown to be responsible for axonal branching of the VC neurons[56], suggesting that guidepost cells may release multiple signals that globally organize neural circuitry through diverse mechanisms.

Non-neuronal cells in long-range synaptic patterning. In addition to non-neuronal cells having a guidepost role through contact and cell adhesion, non-neuronal cells have also been shown to secrete long-range cues that can influence synapse formation. In the tail of *C. elegans*, the bipolar DA9 motor neuron forms synapses in a discrete portion of its axon (FIG. 7c). Secretion of Wnts by tail hypodermal cells localizes the Wnt receptor frizzled to the asynaptic domain in the posterior part of the DA9 axon, which acts to locally inhibit synapse formation[57] (FIG. 7c). In a similar manner, secretion of netrin has been shown to locally inhibit synapse formation in the dendrite and the proximal axon of DA9 through interactions with the netrin receptor UNC-5 (REF. 58) (FIG. 7c). Thus, non-neuronal cells can provide long-range extrinsic cues to pattern the subcellular distribution of synaptic connections.

Conclusions and future directions

Transient cell–cell interactions have been shown to have functions in many diverse contexts in patterning neuronal connectivity, from axon guidance to synapse target selection. However, many mysteries remain about how exactly these cell populations function in wiring the nervous system.

Many unresolved questions surround the transient nature of cell–cell interactions in axon guidance. A key question is how axons switch from being attracted to an intermediate target to moving away from the cell and continuing along their trajectory. Although the work on COMM and ROBO3 in the *D. melanogaster* and vertebrate midline provides one model of how this occurs, presumably other mechanisms are acting in other developmental contexts. Another question is whether factors are transmitted between intermediate targets and the axonal growth cone during axon guidance. As hinted by the original guidepost studies in grasshoppers, certain guidepost cells can form dye-passing junctions with axons. Are molecules transmitted between guidepost cells and growth cones? If so, what are they and what might their roles be?

Another area that is poorly understood is the molecular mechanisms of transient circuit assembly. What are the cues that allow for transient synapses to be formed on Cajal–Retzius cells and subplate cells, and what are the signals that regulate the disassembly of these synapses?

Wnts
A family of well-conserved secreted molecules that have multiple roles in cell–cell interactions.

How is cell death regulated in transient cell populations such as the cells of the subplate and Cajal–Retzius cells? What are the consequences if cell death does not occur?

The molecular mechanisms that underlie the 'transfer' of synapse formation from guidepost cells to proper postsynaptic targets is also poorly understood. For instance, in the *C. elegans* HSNL system, the SYG-1–SYG-2 adhesion between HSNL and vulval epithelial cells somehow leads to proper synapse formation between HSNL and VC neurons and vulval muscles. Presumably, there must be some kind of transfer between guidepost cells and postsynaptic targets. How does this occur?

A final question is determining how widespread is the strategy of using guidepost cells during neural circuit formation. With newer tools and approaches enabling us to visualize neural circuit formation at single-cell resolution, it is plausible that many additional transient cell–cell interactions will be implicated in neural circuit assembly.

The discovery of transient cell–cell interactions with cells extrinsic to the final neural circuit has increased our understanding of the wiring of the nervous system. These cells have multifunctional roles in the assembly of the nervous system, which are mediated by various secreted proteins and cell adhesion molecules. Many mysteries remain, and future studies on these cell populations will lead to better insight into the diversity of strategies used to assemble the nervous system.

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DATABASES
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
axonin 1 | BOC | CD44 | CHL1 | COMM | *cyclops* | EPHB1 | ephrin B2 | ERBB4 | frizzled | GLI2 | *Mash1* | neuregulin 1 | NRCAM | ROBO1 | ROBO2 | ROBO3 | SLIT2 | sonic hedgehog | SYG-1 | SYG-2 | UNC-5 | UNC-6 | UNC-40

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