

# Sonic hedgehog in CNS development: one signal, multiple outputs

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Sonic hedgehog (SHH) is a member of the hedgehog family of signalling molecules. SHH was initially described as a protein secreted from two signalling centres, the notochord and the floor plate. Subsequently, it was identified as a morphogen that is directly responsible for dorso-ventral patterning of the CNS. More recently, additional sites of SHH expression have been identified and multiple actions of SHH during CNS development discovered, including the specification of oligodendrocytes, proliferation of neural precursors and control of axon growth. Despite these various activities, it appears that the SHH signalling pathway is well conserved and that the same mechanisms are utilized to achieve a variety of cellular responses. Therefore, a more precise understanding of the molecular mechanisms that underlie the different responses to SHH signalling is the next step in the study of this molecule and its role in the regulation of neural development.

The vertebrate CNS is an organized structure consisting of thousands of cell types, all of which require multiple cell-cell interactions to generate a functional circuit. Each of the different cell types, from neurons to glia, arise from a unique population of progenitors, the neuroepithelial cells of the embryonic neural plate. During development, the neural plate undergoes multiple processes of growth, patterning and morphogenetic movements to generate the highly complex three-dimensional structure of the mature, vertebrate CNS. Concomitantly, the developmental programme must ensure that appropriate cell types are generated at the correct time in specific positions and in the correct numbers. It is the proper orchestration of these events that generates a functional CNS.

The molecular mechanisms responsible for the completion of this developmental programme involve the action of inductive signals, secreted either from tissues other than the CNS or from signalling centres within the CNS itself. Interpretation of these signals leads to the activation of transcriptional events that then mediate the acquisition of different cell fates. The first major insight into the understanding of these processes came with the discovery of Sonic hedgehog (SHH) [1–4]. SHH is produced by two ventral midline signalling centres: the notochord, the axial mesoderm that underlies the ventral neural plate; and the floor plate, a specialized population of cells at the ventral midline of the CNS [5]. It is now clear that SHH is both necessary and sufficient for the induction of the floor plate [6–8] and for the differentiation of ventral neurons along the axis [6–10]. However, the specification of ventral-cell fates is not the only function of SHH during development of the CNS, as recent research has revealed additional sites of SHH

production and identified multiple roles for this molecule in the coordination of neuronal development.

## What is SHH and how does it signal?

SHH is a member of the hedgehog family of signalling molecules identified by homology to the *Drosophila* hedgehog (HH; reviewed in Ref. [11]). SHH is proteolytically cleaved to produce two secreted proteins [12,13], a 19 kDa N-terminal protein (N-SHH) that mediates all signalling activities in vertebrates and invertebrates (reviewed in Ref. [14]) and a 25 kDa C-terminal protein (C-SHH) that possesses protease activity [13,15]. Although N-SHH has inductive properties *in vitro*, this form does not exist as such *in vivo*, as it is further modified by addition of a cholesterol moiety to the C-terminal amino acid and a palmitoyl group to the N-terminal of the processed N-SHH (Fig. 1a) [16–18]. The cholesterol modification, which is auto-introduced by C-SHH [16], plays a critical role in SHH signalling by facilitating the regulated secretion and long-range activity of the SHH protein to which it is covalently coupled [19,20]. Furthermore, the N-terminal palmitate modification, introduced in *Drosophila* by a different enzyme [17], increases the inductive potency of SHH, both *in vitro* [18] and *in vivo* [21].

N-SHH was described initially as a morphogen responsible for a number of early patterning processes; it is involved in the control of left-right asymmetry, dorso-ventral patterning of the CNS and somites, patterning of the limb, as well as in some aspects of organogenesis (reviewed in Ref. [14]). Despite these various functions, the HH-signalling pathway is well conserved from invertebrates to mammals, and progress in defining the HH pathway has been reviewed extensively [22–26]. At the cell surface, SHH binds with high affinity to patched (Ptc), a 12-transmembrane protein (Fig. 1b). In mammals, two isoforms of Ptc are encoded by *Ptc1* and *Ptc2*, although *Ptc1* appears to be active in the CNS [27–34]. Binding of SHH to Ptc prevents the normal inhibition of smoothed (Smo), a seven-transmembrane protein with a topology reminiscent of G-protein-coupled receptors, which is the signalling component of the SHH-receptor complex [35–39]. During development of the vertebrate CNS, either inhibition of G<sub>i</sub> proteins [38] or expression of a constitutively active form of Smo [39] is sufficient to trigger some actions of SHH. Further regulators of the pathway, which act at the surface of cells responding to SHH have been identified in the

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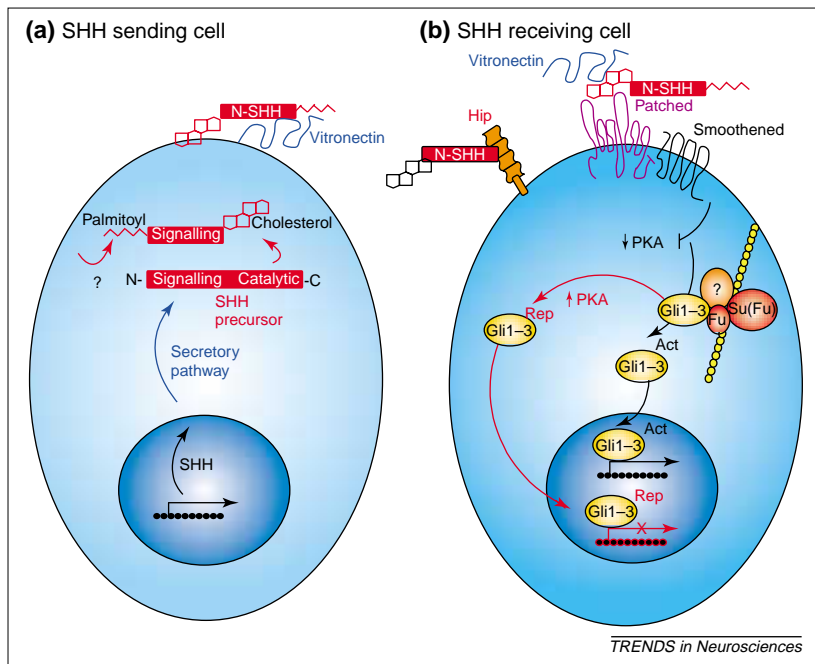


Fig. 1. Schematic representation of the sonic hedgehog (SHH) signalling pathway. Only components of the signalling pathway identified in vertebrates are represented. (a) The SHH precursor is processed to generate the N-SHH morphogen, to which two modifications are introduced: a cholesterol group at the C-terminal and a palmitoyl moiety at the N-terminal. Cholesterol is added by C-SHH, whereas palmitoyl is added by an unknown component (?) in vertebrates. (b) Several signalling components have been identified in vertebrates because of their homology to *Drosophila* proteins: the two components of the receptor Patched and Smoothened, and the intracellular complex formed by Fused (Fu), Suppressor of Fused [Su(Fu)] and possibly some other components (?), which might be associated with microtubules (as in *Drosophila*). Zinc-finger transcription factors of the Ci/Gli (Gli 1–3) family, which might be associated with the intracellular complex and translocated to the nucleus by activation of the pathway, act at the last step of SHH signalling. Abbreviations: Act, activation; Hip, hedgehog-interacting protein; PKA, protein kinase A; Rep, repression.

vertebrate CNS. Hedgehog-interacting protein (Hip) is a type I transmembrane protein that attenuates SHH signalling by binding N-SHH with an affinity similar to that of Ptc1 [40], whereas vitronectin, an extracellular matrix glycoprotein, enhances SHH activity during motor-neuron differentiation, also by binding SHH directly (Fig. 1b) [41].

Within the nucleus of the responding cell, zinc-finger transcription factors of the Ci/GLI family (GLI1–3) act at the last known step of the SHH-signal-transduction pathway [42,43], although it is still unclear whether GLI proteins mediate all aspects of SHH signalling during vertebrate CNS development [44–46]. GLI proteins are processed *in vitro*, to yield truncated forms with different, tightly regulated transcriptional activities and subcellular locations [47,48]. GLI-mediated transcription of SHH target genes is regulated, at least in part, by protein kinase A (Fig. 1b) [49,50], an inhibitor of SHH activity [51,52]. However, cytoplasmic–nuclear shuttling of GLI proteins also depends on the complex formed by the kinesin-like cytoplasmic protein costal-2 (which has no vertebrate homologue, to date), the serine–threonine kinase Fused [53] and Suppressor of Fused [53–55], although this mechanism is not yet fully understood. Further research is also required to link Smo activation to the release of transcriptionally active forms of the GLI proteins.

Despite the conservation of this signalling pathway, SHH acts at many different steps of CNS development. These various activities, which include determination of ventral neural phenotypes, induction of oligodendrocyte precursors, proliferation of specific neuron progenitor populations, eye patterning and modulation of growth cone movements, will all be reviewed in this article.

#### Neuronal specification in the ventral CNS: the initial and best characterized functions of SHH

The first detailed analysis of the distribution of SHH during early development of the CNS revealed the presence of SHH in what were considered the two main signalling centres responsible for ventralizing the neural tube (the notochord and the floor plate), precisely at the times when these centres were known to have inducing capacities [5]. Subsequently, gain- and loss-of-function experiments demonstrated that SHH is both necessary and sufficient to induce ventral-neural-cell types [6–8,56] and much effort has been applied to elucidate how this is achieved. SHH was postulated to function in a concentration-dependent way as a gradient morphogen, acting both directly and at long-range to pattern the ventral neural tube. However, attempts to detect SHH outside SHH-producing cells were largely unsuccessful [5] until a recent study visualized SHH in SHH-target tissues under conditions that preserved proteoglycan/glycosaminoglycans [57]. Further, *in vivo* expression of either a constitutively active form of Smo [39] or a mutant form of Ptc1 that does not bind SHH but retains the ability to inhibit Smo [58] produced switches in progenitor-cell identity and neuronal fate, thus demonstrating that SHH does indeed function both directly and at long range to direct ventral-cell identity.

Signalling by a SHH gradient establishes distinct progenitor domains by regulating the expression of a set of homeodomain proteins that comprises members of the Pax, Nkx, Dbx and Irx families [59–61]. Homeodomain proteins have been subdivided into class I and class II proteins, based on their differential regulation by SHH signalling. Class I proteins are synthesized by neural progenitor cells in the absence of SHH, whereas production of class II proteins depends upon exposure to SHH [8,59–63]. By the induction or the repression of the levels of these transcription factors, SHH defines five progenitor domains in the ventral neural tube (Fig. 2a). In turn, the pairs of homeodomain proteins that abut a common progenitor-domain boundary repress each other's expression, contributing to the definition of the neural progenitor domains and the positions at which postmitotic neurons are generated (Fig. 2) (reviewed in Refs [64,65]). It is possible that similar mechanisms are responsible for determining ventral cell fate in more anterior areas of the CNS because SHH also mediates the induction of dopamine-releasing neurons in the midbrain [66] and serotonergic neurons in the ventral forebrain

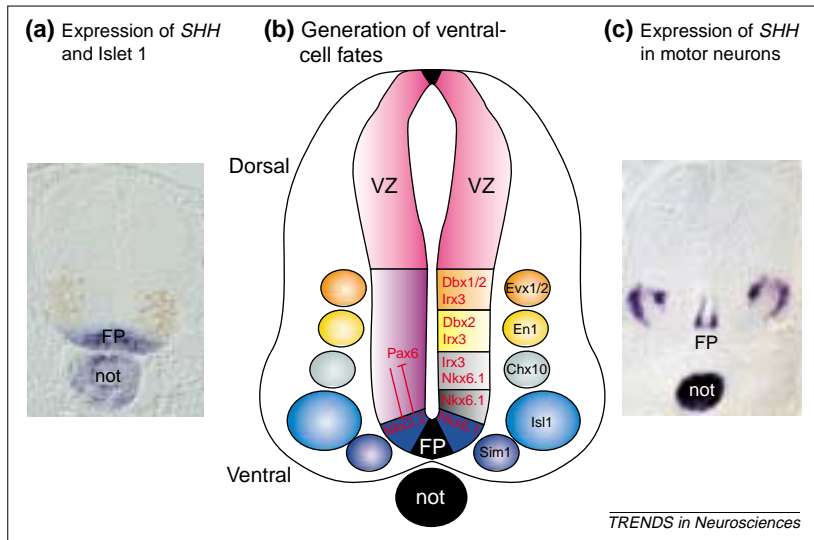


Fig. 2. Generation of ventral neural fates by sonic hedgehog (SHH) signalling. (a) Double detection of mRNA encoding SHH (purple) and islet 1 protein (orange) in a section from a stage-16 chick. SHH is expressed in the notochord (not) and the floor plate (FP) of the spinal cord and is responsible for the generation of islet-1-positive motor neurons in the ventral spinal cord. (b) Graded SHH signalling establishes the expression domains of class I (Pax6, Irx3 and Dbx1/2; repressed by SHH) and class II (Nkx2.2 and Nkx6.1; induced by SHH) homeodomain proteins in progenitor cells located in the ventricular zone of the spinal cord. The expression domains of Pax6 and Nkx2.2, which are mutually regulated, are represented on the left-hand side of the ventricular zone. The small ovals represent five classes of neurons that arise in the ventral spinal cord, and indicate gene expression specific for each neuronal subtype. Adapted from Ref. [121]. (c) Expression of mRNA (purple) encoding SHH in the notochord, floor plate and differentiated motor neurons in an E5 chick spinal-cord section.

[21,67,68]. However, further investigation is required to precisely define these rostral activities, as well as the role of SHH in fully differentiated spinal motor neurons (Fig. 2c) [69,70]. Interestingly, the definition of ventral neural fates along the anterior–posterior axis is not the only role played by SHH in the ventral CNS and increasing evidence indicates a role for SHH in the specification of the oligodendrocyte fate, as discussed in the next section.

#### Oligodendrocyte specification: a later role for SHH in ventral CNS development

Oligodendrocytes, the myelin-forming glial cells in the CNS, arise from multiple restricted foci of the neuroepithelium and then disperse throughout the

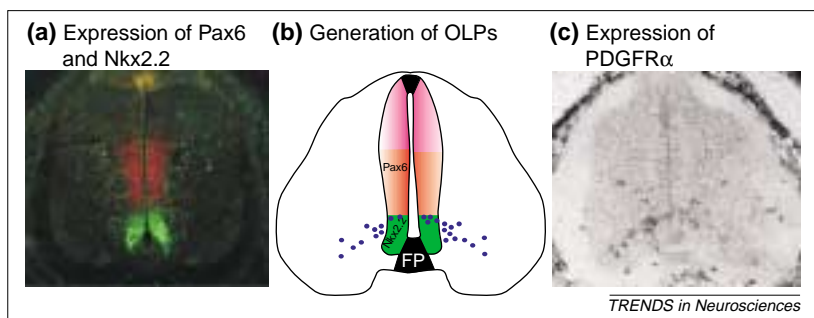


Fig. 3. Generation of oligodendrocyte precursors in the ventral spinal cord by SHH-mediated signalling. (a) Double detection of Nkx2.2 (green) and Pax6 (red), showing mutually exclusive spatial domains, in an E5 chick spinal-cord section. (b) OLPs (blue dots) originate from the Nkx2.2 domain of the ventral ventricular zone close to the floor plate. (c) Expression of PDGFR $\alpha$  receptor in migrating OLPs in an E6 chick spinal cord section. (a) and (c) reproduced with permission from Ref. [88]. Abbreviations: FP, floor plate; OLPs, oligodendrocyte precursors; PDGFR $\alpha$ , platelet-derived growth factor receptor  $\alpha$ ; SHH, sonic hedgehog.

developing grey matter. All along the spinal cord, hindbrain, midbrain and caudal forebrain, oligodendrocyte precursors (OLPs) originate from two narrow ventral columns in the neuroepithelium at either side of the floor plate (reviewed in Ref. [71]). However, in the anterior forebrain, OLPs emerge from the anterior entopeduncular area and probably from the zona limitans intrathalamica [72,73]. OLPs are identified and defined by the presence of early markers including the basic helix–loop–helix transcription factors Olig1 and Olig2 [74–76], plp and dm-20 (the major protein components of mature CNS myelin) [77], and/or the expression of the receptor for platelet-derived growth factor  $\alpha$  [78]. Whether these markers define a single or multiple lineages of OLPs is controversial [79]. However, all OLPs originate in areas of the CNS that either express SHH themselves or are in close proximity to SHH-expressing cells [5,80], raising the question of whether SHH is involved in the acquisition of oligodendrocyte cell fate.

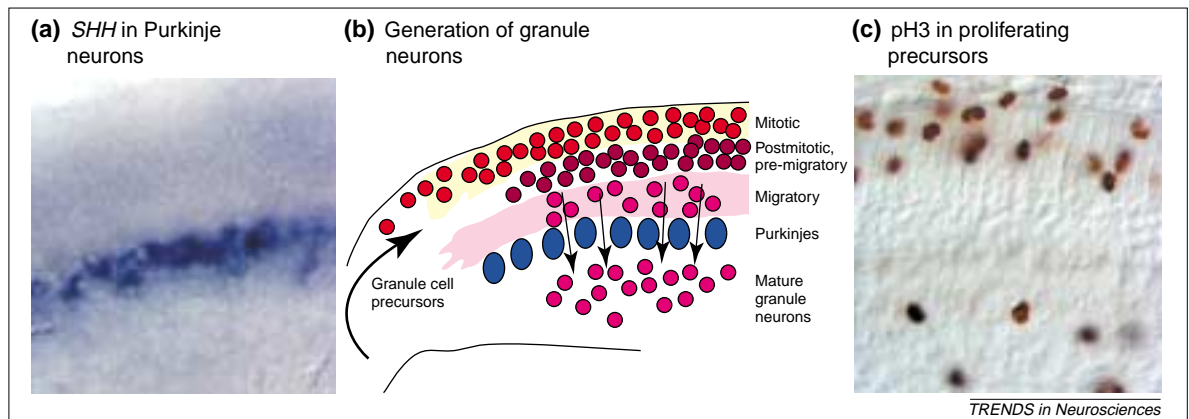
Notochord- and floor plate-grafting experiments, as well as neural explant studies, demonstrated initially that SHH can induce oligodendrocyte differentiation [80–84] and subsequently that SHH signalling is required for specification of OLPs in chick spinal cord [85]. Gain- and loss-of-function experiments in mouse embryos further confirmed that SHH is both necessary and sufficient for the specification of OLPs in the spinal cord [85] and forebrain [86,87]. OLP specification occurs late, at a time when ventral neuroepithelial cells have ceased to produce somatic motor neurons [88]. However, generation of motor neurons is not required for this activity as OLP specification occurs in islet 1<sup>-/-</sup> mice that lack motor neurons [89,90].

As discussed above, ventral neuroepithelial cells can read and interpret different concentrations of SHH and so acquire different neuronal fates. That similar concentrations of SHH are required for the induction of both ventral neurons and OLPs, suggests that a common precursor may give rise to both ventral neurons and oligodendrocytes [84,85,91]. A recent report nicely demonstrates that, in the chick, SHH directly induces OLPs and that these cells are produced from the domain within the ventral neuroepithelium that expresses Nkx2.2. At the time of OLP production, this region no longer synthesizes Pax6 and the capacity to produce somatic motor neurons has also been lost (Fig. 3) [88]. Therefore, the key question remains. How do neuroepithelial cells switch in time, from generating ventral neurons to the production of OLPs? A timely burst of SHH activity that expands the domain of Nkx2.2 expression, together with promiscuous interactions between different transcriptional regulators, such as Olig2, are mechanisms delineated in a series of new and exciting reports (reviewed in Ref. [92]).

#### The role of SHH in dorsal CNS development

Early in the development of the CNS the synthesis of SHH is ventrally restricted. However, as development

Fig. 4. Proliferation of cerebellar granule cell precursors is mediated by sonic hedgehog (SHH) signalling. (a) Expression of mRNA encoding SHH (blue) in Purkinje neurons in the cerebellar cortex of an E14 chick embryo. (b) SHH signalling induces proliferation of granule cell precursors in the outer EGL (red) in a laminin-rich environment (yellow). Differentiation of granule neurons (purple) commences in a vitronectin-rich environment (pink) in the inner EGL. Adapted from Ref. [96]. (c) Immunostaining of a section of an E14 chick-embryo cerebellum with the mitosis marker phospho-histone 3 (pH3) shows that proliferation of granule cell precursors takes place in the outer EGL. Abbreviation: EGL, external germinal layer.



proceeds, areas of SHH synthesis appear in dorsal domains, the first of which is a narrow transverse boundary, the zona limitans intrathalamica that separates two prosomeres, P2 and P3, in the prosencephalon. SHH is found in the zona limitans intrathalamica [5], and much has been postulated about this location and its putative role as an organizer centre [93]. However, although this is an attractive hypothesis, much work is required to prove it.

SHH and components of the SHH-signalling pathway are also found in dorsal CNS cortical structures (cerebral cortex, optic tectum and cerebellar cortex) during late development and adulthood [94]. The cellular mechanisms involved in the development of these layered structures are well described. In particular, the cerebellar cortex is formed from two distinct proliferative zones, a typical ventricular zone and a displaced germinal zone, termed the external germinal layer. The external germinal layer exists transiently on the surface of the cerebellar anlage and contains many granule cell precursors that are mitotically active [95]. After clonal expansion in the superficial external germinal layer, granule cells exit the cell cycle, extend axons and migrate through the field of Purkinje neurons to their final destination, the inner layer of granule cells.

Recent studies provide insights into the molecular nature of the signals directing proliferation of granule cell precursors. SHH is expressed in migrating and settled Purkinje neurons (Fig. 4a) and acts as a potent mitogenic signal to expand the granule cell progenitor population [96–98]. This active proliferation takes place in the outer part of the external germinal layer in a laminin-rich environment (Fig. 4b) [99]. Subsequent steps of granule cell differentiation require exit from the cell cycle, induction of differentiation and migration through the Purkinje cell layer, events that occur in the presence of SHH. Therefore, termination of granule cell proliferation is not due to a reduced exposure to SHH; rather it probably results from the conversion of a proliferative response to one that directs the differentiation of these cells. The extracellular matrix glycoprotein vitronectin appears responsible for this conversion. Thus vitronectin downregulates the response of

granule cell precursors to SHH, an effect that is independent of the mitogen-activated protein (MAP) kinase pathway. However, this response involves cAMP response element binding protein (CREB) phosphorylation, which terminates SHH-mediated proliferation and promotes differentiation of granule neurons [99]. Additional mechanisms underlying SHH-mediated proliferation of granule cell precursors are possible, as Ptc1 interacts directly with cyclin B1 to regulate cell-cycle progression in embryonic kidney cells [100]. Whether this is also the case in the vertebrate CNS remains to be elucidated.

**The role of SHH in eye patterning, retinal-cell specification and axon guidance of retinal ganglion cells**  
Control of morphogenetic as well as differentiation events are functions of SHH that also contribute to the development of a sensory structure: the eye. The eye is a bilateral organ that originates from a single field positioned in the anterior portion of the neural plate. Anterior migration of diencephalic precursor cells along the midline separates the primordial eye field into two regions [101], proliferation and evagination of which gives rise to two visible optic primordia, the optic vesicles. The infolding of the vesicle into optic cups and their progressive determination further originates functionally specialized eye tissues: the optic stalk, the neural retina and the retinal pigment epithelium. Targeted disruption of the gene encoding SHH in mice and mutations in the human gene cause severe defects in the anterior neural tube, including formation of a cyclopic eye (Fig. 5a) [8,102]. Although it is possible that SHH contributes to the downregulation of eye-specific genes in the eye anlage midline as proposed for the bilateralization of the eye field in the chick [103,104], the cyclopic-eye phenotype is easily explained by the observation that SHH activity regulates the spatial expression of genes encoding Pax6 and Pax2, which normally demarcate the distal (retina and lens) and proximal (optic disk and optic stalk) optic primordium, respectively. Thus, widespread overexpression of SHH (or the related *tiggywinkle* hedgehog) causes an expansion of the Pax2 domain at the expense of the Pax6-positive

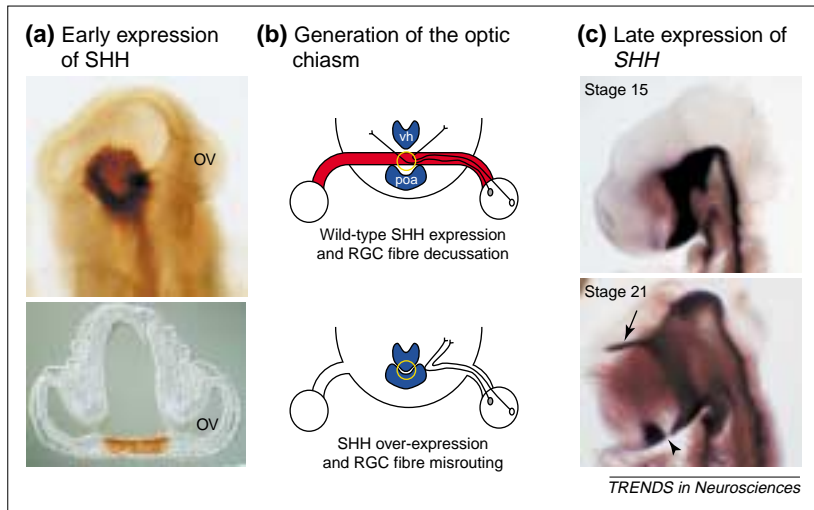


Fig. 5. Sonic hedgehog (SHH) is involved early in morphogenesis of the eye vesicle and late in the control of axon growth of retinal-ganglion cells. (a) A stage-11 chick embryo immunostained with anti-SHH antibody showing expression in the prechordal plate (top panel) and the ventral diencephalon between the optic vesicles (bottom panel). (b) Downregulation of SHH at the optic recess is a prerequisite for decussation of RGC fibres and formation of the optic chiasm. (c) *In situ* hybridization of stage-15 and stage-21 chick embryos showing late expression of the gene encoding SHH. The arrowhead points to the optic recess and the arrow points to the zonal limitans intrathalamica. Abbreviations: OV, optic vesicle; poa, pre-optic area; RGC, retinal ganglion cell; vh, ventral hypothalamus.

retinal field. By contrast, absence of SHH and *tiggywinkle* hedgehog in cyclops zebrafish mutants correlates with a severe reduction in Pax2 concentration and a corresponding increase in Pax6 across the midline, which leads to the fusion of the two retinal fields [105,106]. Absence of SHH in BF1-deficient mice or disruption of SHH activity in chicks using blocking antibodies causes alterations in the ventral ocular tissue and an expansion in the expression domains of dorsal-specific genes, indicating that SHH activity is also required to establish dorso-ventral polarity of the optic cup [107,108].

Production of SHH in the ventral diencephalic midline accounts for the early patterning activities of SHH in the eye whereas endogenously expressed members of the SHH-signalling pathway participate in the control of proliferation and differentiation of retinal precursor cells [109–111]. SHH, and the related *tiggywinkle* and *desert hedgehog*s, as well as the SHH receptors Ptc1 and Ptc2, have been detected in differentiating eye tissues [109,110,112]. SHH secreted by differentiated retinal ganglion cells (RGC) drives the wave of neurogenesis from the centre to the periphery of the retina by a mechanism that involves the induction of its own expression as well as the activation of the Ras-MAP kinase pathway [111], which is strikingly similar to HH activity observed during the differentiation of *Drosophila* retina [113]. Furthermore, as interfering with SHH activity in the retina modifies the overall number of RGC, it appears that SHH secreted by layered RGC behind the differentiation wavefront regulates the generation of additional RGC [114].

If *SHH* expression at the midline of neurulating embryos is required for proximo-distal and dorso-ventral patterning of the eye, subsequent downregulation of *SHH* expression at the optic recess may represent a prerequisite for correct pathfinding behaviour of RGC axons across the midline (Fig. 5b,c). In fact, N-SHH directly suppresses the growth of RGC axons by a mechanism that involves a decrease in cAMP concentration in the growth cone itself [115],

consistent with the idea that propagation of HH signalling involves the activity of protein kinase A [22–26]. This hypothesis is further supported by the fact that sustained expression of SHH in Pax2/*noi*-deficient mice/zebrafish mutants produces abnormal ipsilateral projection of the visual axons [115–117]. Whether SHH modulates the behaviour of growth cones other than those of retinal ganglion cells is unknown. However, SHH inhibits the migration of neural crest cells [118], reinforcing the idea that the already widely accepted range of SHH activities must be updated to incorporate control of cell movement, including the progression of the axonal growth cone.

### Conclusions

SHH is a potent morphogen capable of patterning the vertebrate CNS. However, it is clear that SHH contributes also to proliferation, differentiation and axon growth. This wide variety of functions seems to be transduced essentially by a single pathway and the challenge for future research is to understand how this occurs. Once again, the molecular regulation of the graded response to SHH in the ventral neural tube seems to be leading the way in solving at least part of the puzzle. In a recent publication, Muhr *et al.* [119] demonstrate that the acquisition of neuronal fates in the ventral neural tube is tightly and spatially controlled through the repression of homeodomain proteins (transcriptional repressors that themselves are targets of SHH signalling), thus describing a novel derepression strategy for neuronal-fate specification. Further, the capacity of transcriptional repressors such as Olig2 to interact equally well with genes that are repressed or activated by SHH seems to be a key step that regulates the switching of progenitors to form either neuronal or glial cells in the ventral neural tube [92].

Thus it seems that in the ventral neural tube, where neuroepithelial cells need to read and interpret graded concentrations of SHH, interactions between transcriptional targets that are themselves transcriptional regulators ensure a tight control over the spatially and temporally restricted production of different neural cell types. As these interactions are unravelled, it will be interesting to analyse how SHH signalling is diversified to orchestrate ventral cell differentiation along the antero-posterior axis of the CNS. This can be achieved by interaction with other signalling pathways, as seen with the specification of midbrain dopaminergic neurons [68]. This may also be the case for the proliferative activity of SHH in the cerebellum, where granule cell precursors have to switch off SHH-mediated proliferation, even in the presence of a constant, high supply of SHH, to complete their developmental programme. Whether SHH is utilizing the consensus-signalling pathway to induce proliferation remains to be demonstrated. However, if this is the case, interactions with other signalling cascades, either extracellular or intracellular, could be crucial for inactivation of the SHH pathway. In this regard, interactions between SHH and other

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# Neural encoding of behaviourally relevant visual-motion information in the fly

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Information processing in visual systems is constrained by the spatial and temporal characteristics of the sensory input and by the biophysical properties of the neuronal circuits. Hence, to understand how visual systems encode behaviourally relevant information, we need to know about both the computational capabilities of the nervous system and the natural conditions under which animals normally operate. By combining behavioural, neurophysiological and computational approaches, it is now possible in the fly to assess adaptations that process visual-motion information under the constraints of its natural input. It is concluded that neuronal operating ranges and coding strategies appear to be closely matched to the inputs the animal encounters under behaviourally relevant conditions.

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The goal of neuroethology is to explain behaviour in terms of the activity of nerve cells and their interactions. This can only be achieved if the experimental animal can be analysed at different levels ranging from behaviour to individual neurons. Cellular mechanisms underlying processing of neuronal information are frequently analysed using *in vitro* preparations where artificial stimulation replaces the natural sensory input. Although such studies provide fascinating insights into the complex computational abilities of neurons [1], the results may not be extrapolated easily to *in vivo* conditions, where the range of response amplitudes of neurons and their temporal activity patterns may differ considerably from artificially induced activity. In systems such as the retina of the horseshoe crab

*Limulus* [2], and various brain areas of pigeons [3], cats [4] and monkeys [5,6], it is now feasible to analyse the neuronal representation of visual input as it is experienced during behaviour (reviewed in Refs [7,8]). Until now, however, in most systems the underlying neuronal mechanisms have been difficult to unravel.

In the fly it is possible to employ both quantitative behavioural approaches as well as *in vivo* electrophysiological and imaging methods to analyse how behaviourally relevant visual input is processed [9–20]. Although the latter techniques are mainly employed in the blowfly, which is relatively big, they are complemented by studies of the smaller fruitfly, *Drosophila*, where a broad range of genetic approaches can be applied to dissect the visual system in an increasingly specific way [21,22].

We review recent progress on the encoding of optic-flow information in the blowfly. Optic flow is an important source of information about self-motion and the three-dimensional layout of the environment, not only for flies but for most moving animals including humans (Box 1, [4,23–25]). Flies exploit optic flow to guide their locomotion [13] and to control compensatory head movements [26], and understanding the computational principles underlying optic-flow processing in flies could provide insights into visual-motion analysis in general.