OPINION

From nerve net to nerve ring, nerve cord and brain — evolution of the nervous system

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Abstract | The puzzle of how complex nervous systems emerged remains unsolved. Comparative studies of neurodevelopment in cnidarians and bilaterians suggest that this process began with distinct integration centres that evolved on opposite ends of an initial nerve net. The 'apical nervous system' controlled general body physiology, and the 'blastoporal nervous system' coordinated feeding movements and locomotion. We propose that expansion, integration and fusion of these centres gave rise to the bilaterian nerve cord and brain.

How animals progressed from a simple nerve net to a complex centralized nervous system remains one of the most exciting and unsolved questions of animal evolution[1–11]. Nerve nets are loose networks of interconnected neurons that cover the bodies of animals in groups that branched off early from the animal evolutionary tree (FIG. 1) — most prominently, in the cnidarians, a group comprising jellyfish, corals, sea anemones and other soft-bodied polyps. The complex nervous systems found in vertebrates and other bilaterians are thought to have emerged from an ancient nerve net; however, it is not clear when, how and how many times this transition occurred in animal evolution.

As nervous systems rarely fossilize (with a few spectacular exceptions[12]), one approach to reconstruct and understand their evolution is to compare the nervous systems of animals that live today and to identify traits that, by specific similarity, probably existed in the last common ancestor of the compared groups (a state referred to as homology). Such conserved traits can be proteins (such as ion channels, receptors or enzymes) that, owing to sequence similarity, probably belonged to the genetic repertoire of the common ancestor[13], or specific aspects of the phenotype that are unlikely to have evolved twice[13] — be it at the subcellular, cellular or tissue level. In this Opinion article, we focus on comparison of neurodevelopment across species, which is especially informative[14–16]: shared developmental events often 'recapitulate' evolutionary processes common to the compared groups[17], so this approach is expected to reveal conserved and basic organizational features that will help in understanding the early steps in nervous system evolution.

A neurodevelopmental comparison. FIGURE 2 illustrates developmental stages of a vertebrate (*Xenopus laevis*; FIG. 2a), an annelid (*Platynereis dumerilii*; FIG. 2b) and a cnidarian (*Nematostella vectensis*; FIG. 2c). At gastrula stages, each of these animals is covered by ectoderm, and blastoporal ectodermal tissue (around the closing blastopore) and apical pole ectodermal tissue can be distinguished molecularly. At subsequent stages (called neurula in vertebrates, metatrochophora in annelids and planula in cnidarians), the ectoderm gives rise to neurogenic tissue, and recent molecular studies in sea anemones show that, just as in bilaterians, this neurogenic ectodermal tissue is subdivided into developmental regions[18]. Remarkably, our comparison of these regions, as detailed below, reveals that they are similar in molecular identity and overall spatial arrangement to the corresponding regions in annelids and vertebrates, as illustrated by matching colours in FIG. 2. In this Opinion, we accordingly propose that these regions already existed in the cnidarian–bilaterian ancestor and, based on our comparison of their neural progeny in cnidarians and bilaterians, hypothesize what cell types they might have ancestrally produced. One of these conserved neurogenic tissues is the apical region, which gives rise to a specialized part of the nervous system that we call the apical nervous system (ANS), referring to its developmental origin. Likewise, conserved molecular identities are apparent in developmental regions centred on the blastopore (FIG. 2). We refer to the neural tissue emerging from these regions as the blastoporal nervous system (BNS).

A new scenario of nervous system evolution. Building on these comparisons of developmental regions (including the neural cell types that they produce) in phylogenetically very distant groups, we put forward an evolutionary scenario of the early evoluton of the nervous system in metazoans, as summarized in FIG. 3. We posit that nervous system evolution started with the emergence of a homogenous, diffuse nerve net that covered most of the ectoderm in a gastrula-shaped ancestor (FIG. 3a), which was probably benthic (living at the sea floor) and used mucus and motile cilia to trap microparticles for feeding[151]. The ANS (FIG. 3) evolved around the apical tip of the gastrula-shaped ancestors; it served as a sensory-integration centre for the global, modulatory control of body physiology and motor activity. We also propose that the BNS (FIG. 3) emerged at the opposite end of the body, as a specialized part of the nerve net around the digestive opening that directly controlled feeding movements. We further hypothesize that, in the common cnidarian–bilaterian ancestor, the BNS coordinated the contraction of two bilateral rows of contractile pouches in the adjacent inner layer, which subsequently gave rise to tentacle pouches and mesenteries (folds of the inner layer) in the cnidarians and to somites (blocks of mesodermal tissue) in the bilaterians (FIG. 3b). Finally, we propose that the urbilaterian nervous systems evolved by the transformation of the BNS into a pair

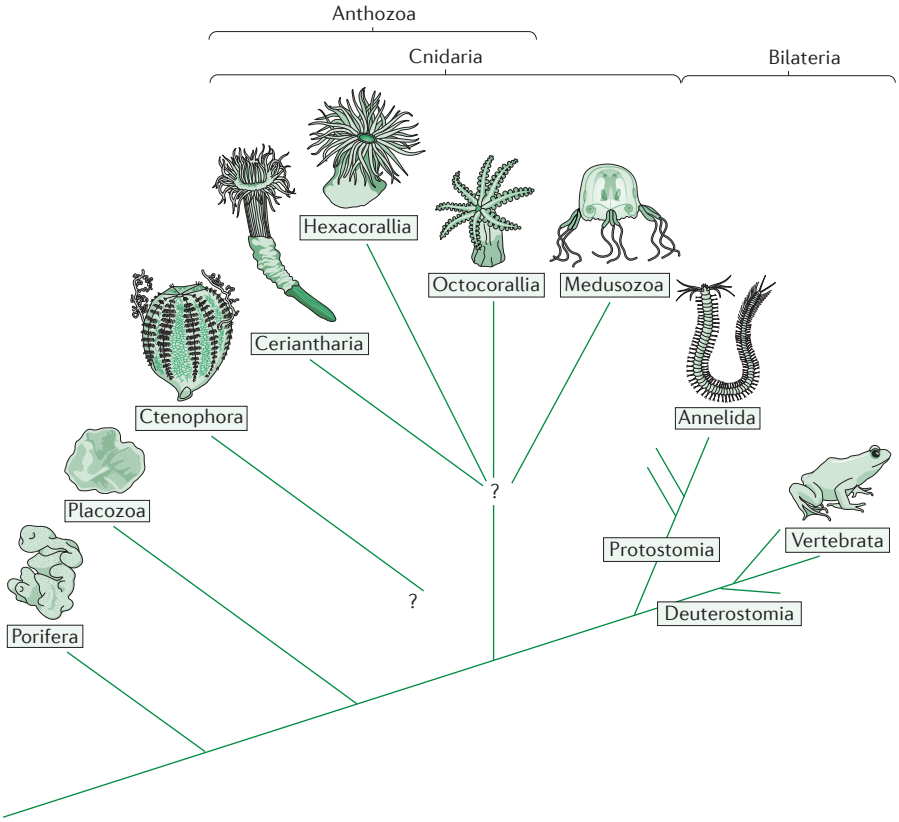

Figure 1 | **Animal phylogeny.** A simplified animal phylogenetic tree (showing the evolutionary history of animals), in which lines represent evolutionary diversification. The lengths of the lines are arbitrary, as they do not indicate evolutionary distance. For a brief characterization of the Anthozoa, Bilateria, Ceriantharia, Cnidaria, Ctenophora, Medusozoa and Neuralia, see the glossary. The phylogenetic position of the Ctenophora is not settled, as indicated by a question mark. The Ctenophora image is adapted with permission from REF. 43, Wiley. The Porifera and Placozoa images are reprinted with permission from REF. 139 (Nielsen, C. *Animal Evolution: Interrelationships of the Living Phyla* p31 and p39 (2012)) by the permission of Oxford University Press. The Annelida image is adapted with permission from REF. 140, Schweizerbart Science Publishers (www.schweizerbart.de).

of bilateral nerve cords, enabling directed and coordinated locomotion, and by the fusion of the anterior end of the BNS with the ANS into the new bilaterian brain — the 'chimeric brain hypothesis' (REF. 19) (FIG. 3c).

Although our comparison mostly centres on cnidarians and bilaterians, we also refer to available ctenophore data. The ctenophores, or comb jellies, are another important group of non-bilaterian marine invertebrates with a nerve net. Recent genome sequencing indicates that the ctenophores branched off the evolutionary lineage leading to the bilaterians earlier than the cnidarians[20,21]; however, their placement in the phylogenetic tree remains contentious (FIG. 1). Overall, ctenophore neurodevelopment seems to be more divergent, which might be explained by the more-basal position of the ctenophores in the animal evolutionary tree, by their derived nature[22], or simply by lack of data, a problem that might be overcome in future studies.

Starting point: a diffuse nerve net

A nerve net is a network of dispersed epithelial sensory neurons and so-called ganglion cells[9,23,24], which are neurons that send out neurites, forming an intricate mesh of fibres on the basal side of the epithelium[23,25]. In agreement with many other authors (for example, REFS 26–28), we put forward the notion that the first manifestation of a nervous system in animals was a rather homogenous (that is, diffuse) nerve net that innervated the bodies of ancestors of the Neuralia (FIG. 1). We propose that this nerve net evolved in the ectoderm of gastrula-shaped ancestors and directly innervated strands of longitudinal muscle fibres.

An ectodermal nerve net in gastrula-shaped ancestors. Gastrula-like developmental stages are common to almost all phyla[17] and also occur in the most basal metazoans, the sponges[29]. Such stages are cup shaped, with an outer tissue layer (the ectoderm) and an inner tissue layer (the gastroderm), which are connected around the opening of the 'cup', called the blastopore (see the upper panels in FIG. 4). In cnidarians (FIG. 4a) and ctenophores (FIG. 4b), a gastrula-like basic organization persists through development and into the adult body, modified only by multiple folding events in the outer and inner layers. Nerve net-like arrangements of neurons develop in the outer ectoderm in cnidarians and ctenophores (FIG. 4c,d). If we presume that neuralian ancestors were gastrula-like in organization, it follows that a similar nerve net may have existed in the ectoderm of these organisms and mediated simple behaviour (FIG. 3a). In cnidarians, a nerve net also forms from the inner layer[3,11]; however, in the basal cerianthids, the nerve net seems to be purely ectodermal[30], which suggests that inner layer neurons may have evolved independently in the cnidarian and bilaterian lineages[11].

Homology of ectodermal nerve net neurons is well supported by recent molecular investigations of ectodermal neurogenesis in *N. vectensis*[18,31–36], which shows extensive similarities to bilaterian neurogenesis. For example, WNT–β-catenin signalling acts upstream of neurogenesis[18,36], activating basic helix–loop–helix (bHLH) proneural genes of the achaete–scute and atonal families, as well as *soxB2* (REF. 36), a high-mobility group (HMG) box transcription factor with a conserved role in promoting neuronal differentiation[34,35]. Conservation of SOXB2 is especially interesting, because the regulatory elements that control ectodermal *soxB2* expression in cnidarians also drive expression in bilaterian neuroectoderm and sensory placodes[34]. This makes a strong case that *SOXB2*-expressing ectoderm was present in cnidarian–bilaterian ancestors or, in other words, that *SOXB2*-expressing cnidarian and bilaterian neurogenic ectoderm is homologous (corresponding to the red, yellow and blue neurogenic developmental regions in the middle panels of FIG. 2). Notch signalling has likewise been implicated in *N. vectensis* neurogenesis, as disruption of Notch signalling results in an upregulation of bHLH genes and an increase in the number of ectodermal neurons[31]. Overexpression of bHLH family members increases the number of ectodermal neurons, whereas their knockdown reduces them[32,36]. Embryonic lethal abnormal vision 1 (*elav1*; a highly conserved RNA-binding protein implicated in neuronal differentiation across Bilateria) is likewise required for ectodermal neuronal differentiation in *N. vectensis*[33].

The neuromuscular orthogon. If early neuralians (that is, the last common ancestors of ctenophores, cnidarians and bilaterians; see FIG. 1) indeed possessed a nerve net, what did it innervate? In all cnidarian groups and in ctenophores, the presence of a well-developed nerve net correlates with the presence of basiepithelial muscle fibres directly innervated by nerve net neurons[1,3,8,9,11,24,26,30,37–40]. Hence, the evolutionary origin of the nerve net seems to be closely linked to that of the musculature, as a system to control contractions through simple sensory–effector circuits. Remarkably, as a rule, the cnidarian ectoderm produces longitudinal muscle fibres, whereas the inner layer, or gastroderm, forms circular fibres[9,41]. This matches the situation in the cerianthids[9] and is also commonly observed in the medusozoans[41]. Furthermore, longitudinal muscle fibres are the first to develop underneath the epidermis in the ctenophore *Mnemiopsis leidyi*[42], followed later by circular fibres[43]. We thus hypothesize that a cup-shaped, gastraea-like ancestor possessed an ectodermal nerve net that directly innervated outer longitudinal fibres and, via paracrine secretion across germ layers, also modulated the activity of inner circular fibres. We refer to this arrangement as the neuromuscular orthogon.

Nerve net subfunctionalization: the first integrative units. When a nerve net was in place, subsequent evolution was about partitioning the nerve net and optimizing these parts towards specific functions[26] in ctenophores, cnidarians and bilaterians. For example, both the cnidarian and the ctenophore nervous systems show strong tendencies towards nerve net specialization (and even centralization). This is evident from the complex behaviours exhibited by cnidarians, such as tentacle movements in anthozoans for feeding and swimming[44–46], or swimming in medusozoans[23,39,47]. These movements are coordinated by specialized sensory–motor integrative units that show higher densities of neurons and neuropil than the rest of the nerve net, indicating considerable degrees of centralization[26,38,43,48]. The same is true for the ctenophore 'apical sense organ', which controls a very different mode of swimming via ciliated comb plates[43,49].

However, it is unclear when these initial steps of nerve net subfunctionalization first occurred in evolution. Did they take place entirely independently or are they, at least in part, shared among the cnidarians, bilaterians and/or even ctenophore lineages? To approach this question, we reason that any such step required diversification of an initially homogenous population of neurons by division of labour (the evolutionary transition from a multifunctional cell type to more-specialized sister cell types with distributed functions)[50–52]. Developmentally, this process should be mediated by the spatially diverging activity of transcription factors[50–52], thus defining new molecular neurogenic subdivisions within the developing nerve net. In bilaterians, neural regions are established by the restricted expression of transcription factors of the NK cluster, paired box (PAX), homeotic (HOX) and Sine oculis (SIX) homeodomain families[15,53,54]. Below, we ask whether and to what extent neurodevelopmental regions in cnidarians and bilaterians express similar sets of transcription factors and thus qualify as possible evolutionary counterparts. We thus identify and discuss two possible ancient 'hot spots' of regionalization and specialization in animal nervous systems: the ANS and the BNS.

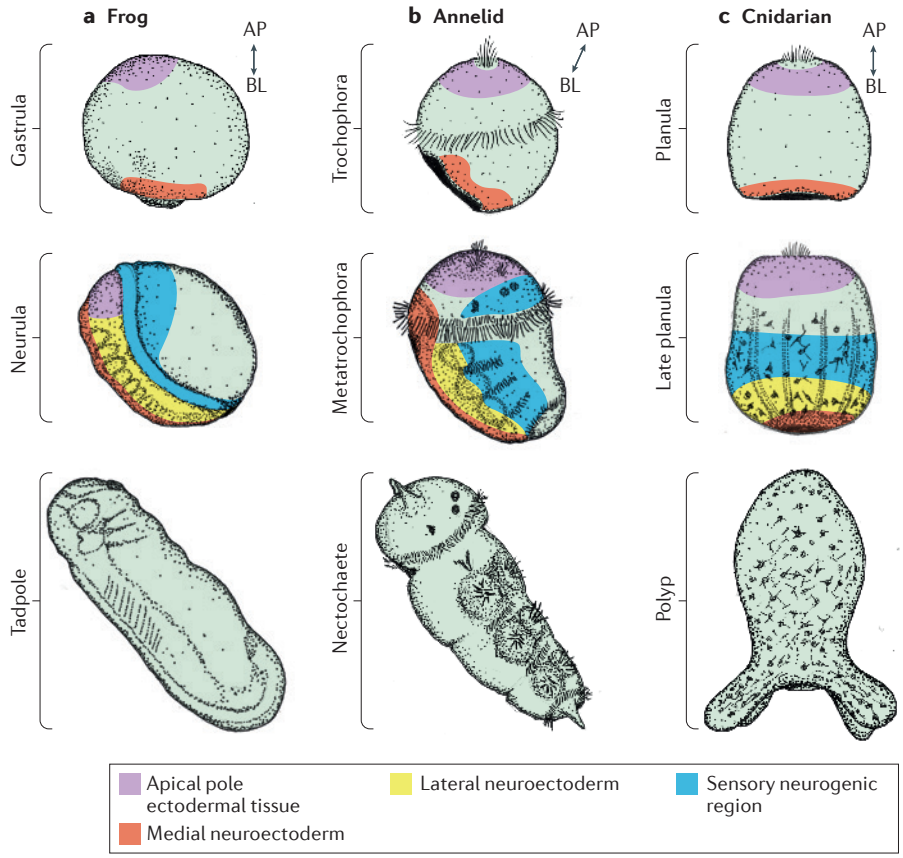

Figure 2 | **Comparison of neurodevelopment in the frog, annelid and sea anemone.** The frog *Xenopus laevis* (part **a**), the annelid *Platynereis dumerilii* (part **b**) and the cnidarian *Nematostella vectensis* (part **c**) are depicted in their gastrula-like stages (gastrula, trochophora and planula, respectively; upper panels), intermediate developmental (neurula, metatrochophora and late planula, respectively; middle panels) and juvenile stages (tadpole, nectochaete and polyp, respectively; lower panels). Colours demarcate developmental neurogenic regions, and double-headed arrows show the apical (AP)–blastoporal (BL) axis. All views in parts **a**–**c** are lateral. At gastrula stages (upper panels), blastoporal ectodermal tissue (around the closing blastopore; red) and apical pole ectodermal tissue (violet) can be distinguished. At subsequent stages, a large part of the ectoderm — incluing the former apical and blastoporal regions — gives rise to neurogenic tissue. The neurogenic tissue comprises regions of distinct molecular identity (indicated by different colours), which will give rise to different parts of the nervous system. In the frog (part **a**), the neural plate (violet, red and yellow) comprises future forebrain tissue, as well as medial and lateral neural tube tissue; it is laterally bounded by developing peripheral nervous system components (blue). Similar regions are apparent in the annelid (part **b**), and these give rise to the brain, medial and lateral nerve cord and peripheral nervous system. As reasoned in this article, these regions also exist in the cnidarian (part **c**). In the frog and annelid worm, these regions are further subdivided into specific subregions by the activity of molecular organizing signals[15].

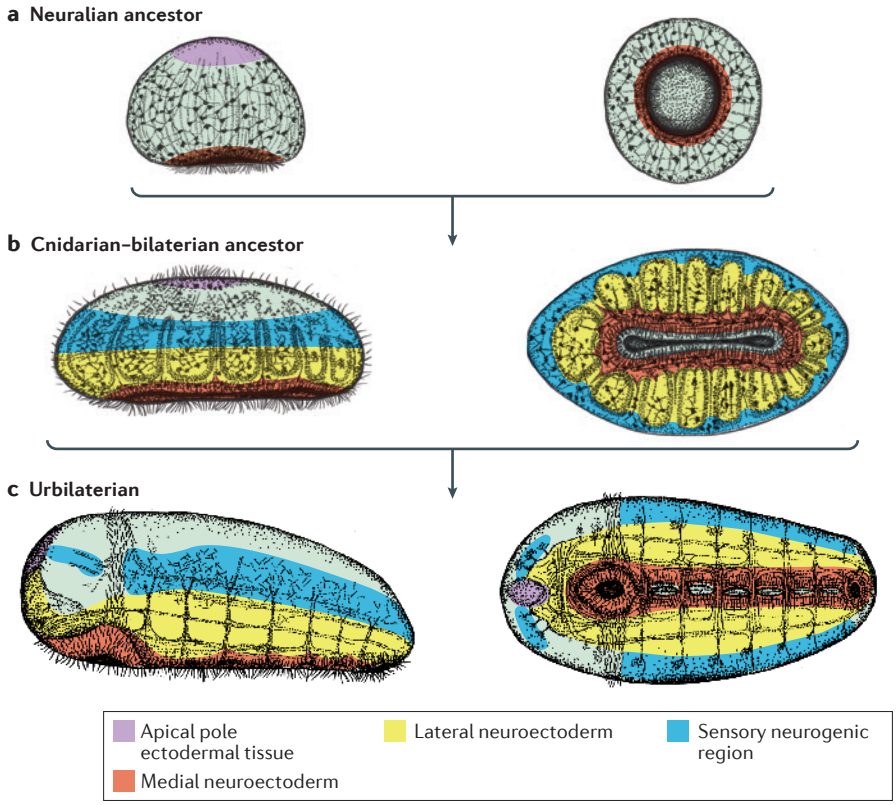

Figure 3 | **A hypothetical scenario of nervous system evolution. a** | Neuralian ancestor. A nerve net covers the gastrula-shaped animal. Nerve-net neurons situated around the digestive opening (red) control mucociliary feeding. Specialized sensory cells detecting environmental stimuli are located around the apical pole (violet). This organism evolved into the cnidarian–bilaterian ancestor. **b** | Cnidarian–bilaterian ancestor. Specialized parts of the nerve net are centred on the slit-like digestive opening: the 'blastoporal nervous system' (BNS; red and yellow) controls the contraction of a bilateral series of contractile gastric pouches. More laterally, the nerve net has evolved into a sensory plexus, which is shown in blue. The 'apical nervous system' (ANS; violet) controls body physiology. This animal evolved into the bilaterian ancestor, the urbilaterian. **c** | Urbilaterian. This organism has a semi-centralized nervous system with longitudinal axon tracts and commissures. The BNS, comprising two regions (the medial motor (red) and lateral sensory-integrative (yellow) regions), forms the ventral nerve cord. The bilaterian brain represents a fusion of the ANS (violet) and the anterior BNS (yellow). The peripheral nervous system is shown in blue. The views in the left panels are lateral. The views in the right panels are blastoporal (parts **a** and **b**) and ventral (part **c**).

The ANS

In the ciliated swimming larvae of many cnidarians and bilaterians (FIG. 5), the first manifestation of the ANS is the sensory–neurosecretory apical organ: an assembly of paracrine sensory cells with long motile cilia that develops around the apical pole (FIG. 5a), forming the apical tuft. The apical organ is involved in the regulation of larval settlement and metamorphosis. It is positioned on top of a prominent apical neuropil formed by the neurites of surrounding sensory neurons[33,55–60]. In cnidarians, the apical organ cells seem not to synapse on the neurites of the neuropil[33,55] but to basally secrete peptides and neuromodulators that potentially modulate activity of these neurites[55]. The apical region gives rise to the foot of the adult polyp, which also shows a concentration of neurons and neuropil in anthozoans and medusozoans (FIG. 5b); however, not much is known about ANS neuroanatomy at these stages. A prominent ANS also exists in ctenophores in the form of an apical sense organ[43] specialized in the coordination and control of ciliary swimming. This organ comprises mechanosensory ciliated cells that specialize in sensing gravity and that are in continuity with the rows of ciliated cells of the comb plates[43,49]. Underlying the apical sense organ is a nerve net neuropil and neurosecretory endings of paracrine cells. The organ also contains photo- and chemosensory cells and interneurons.

Homology of apical organs. Intriguingly, in cnidarians and bilaterians, a conserved set of transcription factors — SIX3, Forkhead box Q2 (FOXQ2) and retinal homeobox (RX; also known as RAX)[18,54,61–63] — specifies the apical region[18,62], which gives rise to the ANS. Based on the common origin of neurodevelopmental regions that express SIX3, FOXQ2 and RX and the similarity of some of the characteristic cell types that constitute these areas at larval stages, we[63] and others[60,64] have recently proposed that the ANS is a specialized part of the nervous system that is homologous across cnidarians and bilaterians (FIG. 5c). For example, in cnidarians[55,65,66] and bilaterians[64], apical organ cells bear long, sensory-motile cilia. These cilia form a conspicuous apical tuft, which has been directly implicated in the perception and integration of environmental signals for the control of larval swimming and for the selection of an appropriate substrate for settlement and metamorphosis. Furthermore, in both groups the apical organ comprises sensory-neurosecretory cells[55,64,67], many of which are chemosensory[63] and express non-visual opsins for ambient light detection[18,63,68]. Supporting homology, screening for changes in gene expression in *N. vectensis* larvae with suppressed apical organ development revealed more than 80 downregulated genes, many of which also have a role in apical organ development in sea urchins[69]. These genes represent excellent candidates for conserved elements of the gene regulatory network controlling ANS specification and differentiation[69].

In cnidarians, the sensory neurons innervating the apical neuropil release the neuropeptides RFamide or LWamide, which have been implicated in the inhibition and induction of metamorphosis, respectively[11,58]. Likewise, in bilaterian larvae such as those of annelids[67,70], molluscs[71], flatworms[72], echinoderms[73] and hemichordates[73], sensory-peptidergic neurons innervate the apical neuropil, releasing neuropeptides and hormones that belong to evolutionarily conserved families and, most importantly, include RFamide and LWamide[74,75]. For example, myoinhibitory peptide (MIP), which shows sequence similarity to cnidarian LWamides, has recently been implicated in the change of locomotion in the annelid *P. dumerilii* when the larvae settle on the sea floor[64]. Other neuropeptides exert accelerating or inhibitory effects on global ciliary beating[76], indicating that, at least during larval stages, major conserved output of the ANS is the global control of ciliary locomotion.

The adult ANS: an ancient control centre for body physiology? In adult polyps of the cnidarian *Hydra* spp., the ANS persists as a concentration of neurons in the peduncle, and its ascribed activity (FIG. 5b) is the neuropeptidergic control of muscular pumping movements involved in internal fluid transport[77]. These data indicate that, in cnidarians, the adult ANS may have a role in the overall control of body homeostasis via the systemic release of neuropeptides or hormones — an interesting perspective given the similar and conserved role of the bilaterian adult ANS[19,67,68]. Further comparisons at the cell-type level will be especially rewarding. Based on these (limited) cnidarian–bilaterian comparisons, we propose that the ANS evolved as a sensory-integration centre for the control of whole-body physiology and motor activity (mostly ciliary in larvae; mostly muscular in adults). Its main conserved effector organ may have been a neurosecretory release site for hormones and neuropeptides, representing a specialized aggregation of paracrine nerve net activities. If so, when did the ANS first come into place?

Interestingly, the apical sense organ of adult ctenophores resembles the apical organ of larval cnidarians and bilaterians, at least functionally, combining light sensation, chemosensation and mechanosensation for the control of ciliary locomotion. The ctenophore apical sense organ might thus represent a highly derived ANS variant specialized for ciliary comb plate locomotion. However, nothing is known about developmental *six3* or *foxq2* expression in ctenophores, and the expression of several Lin11, Isl-1 and Mec-3 (LIM) homeodomain proteins in the developing apical sense organ in the ctenophore *M. leidyi*[78] is not shared with the cnidarians[79]. Small peptides are present in ctenophores[49], and the presence of cells immunopositive for FMRF (H-Phe-Met-Arg-Phe-NH_2) and vasopressin neuropeptides in the floor of the apical sense organ may suggest conservation of a restricted set of ANS sensory-neurosecretory cell types among ctenophores, cnidarians and bilaterians[43]. In any case, the structural complexity of the ctenophore apical sense organ neuroanatomy far exceeds that of the cnidarian ANS and represents an impressive case of convergent cerebralization[43] (that is, the transformation of part of the nervous system into a superordinate control centre; the brain). The question of whether a simple ANS precursor existed in ancestral ctenophores remains open.

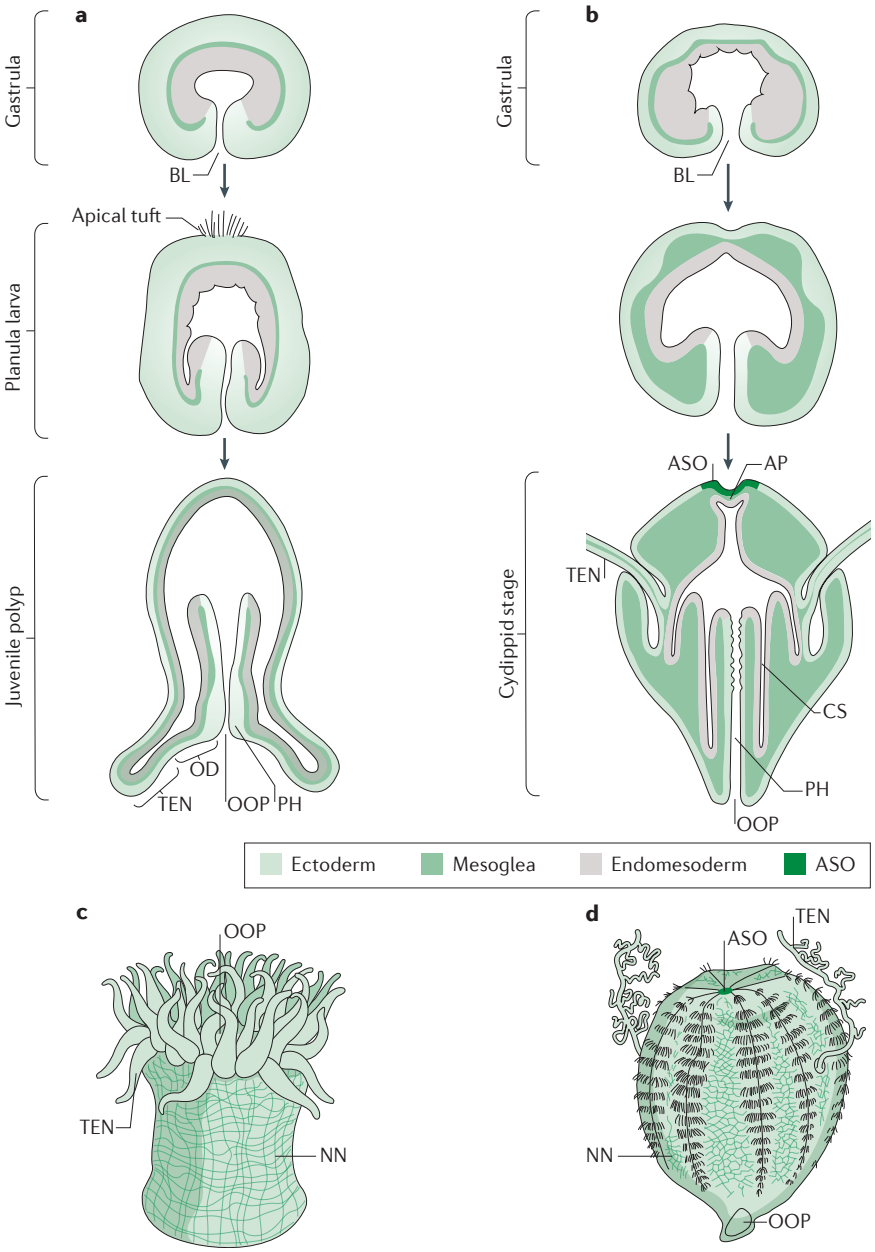

Figure 4 | **Development of gastrula-like body forms with an ectodermal nerve net in cnidarians and ctenophores. a**, **b** | The simple cup-shaped morphology of a developing cnidarian (sea anemone) and ctenophore (comb jelly), from the gastrula stages (upper panels) to the larval or intermediate (middle panels) and juvenile stages (lower panels). The views in parts **a** and **b** are sections along the primary axis. During sea anemone development, the gastrula stage (part **a**, upper panel) develops into a swimming larva, the planula (part **a**, middle panel), with an apical tuft. In the developing polyp (part **a**, lower panel), the oral opening (OOP) is bent inwards, bounded by an oral disk (OD) and pharynx (PH) and, in the periphery of the oral disk, inner and outer layers are pulled out into tentacles (TENs). On the inside, the lumen of the TEN is continuous with pouches that arise by inward folding of the inner layer into so-called mesenteries. In the adult polyp, the OOP is used for both food intake and defecation and thus represents both mouth and anus. A thin layer of gelatinous tissue, called the mesoglea, separates the ectoderm from the endomesoderm. Ctenophore development also starts with a gastrula stage (part **b**, upper panel), which develops directly, via repeated folding events (part **b**, middle panel) into a young ctenophore (part **b**, lower panel), retaining the overall gastrula-like morphology. The PH and a complex canal system (CS) develop from multiple invaginations of the inner gastroderm. Anal pores (APs) form alongside the 'apical sense organ' (ASO). The TENs result from the ectoderm folding inwards and outwards. **c** | The nerve net (NN) in an adult sea anemone. **d** | The NN in an adult ctenophore (*Pleurobrachia pileus*). The views in parts **c** and **d** are lateral. BL, blastopore. Part **c** is adapted with permission from REF. 141, from Sinauer Associates. Part **d** is adapted with permission from REF. 43, Wiley.

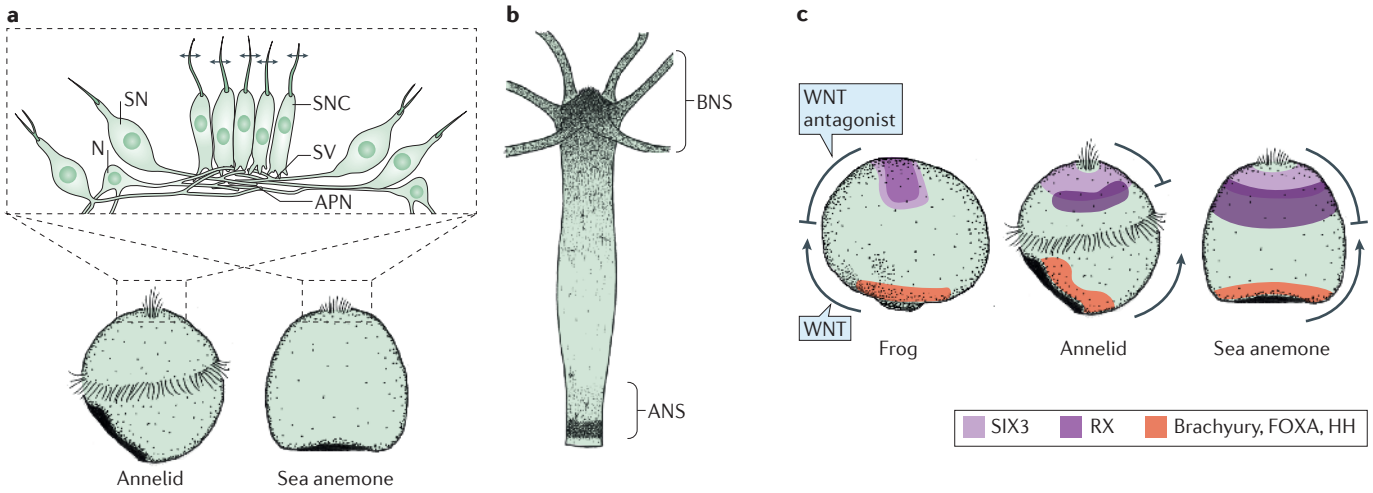

Figure 5 | **The ANS in cnidarians and bilaterians. a** | A prototypical 'apical organ' with bipolar peripheral sensory neurons (SNs) and neurons (Ns) arranged around central, flask-shaped sensory-neurosecretory cells (SNCs) with locomotor cilia, releasing secretory vesicles (SVs) into the underlying apical neuropil (APN). A similar arrangement of cells is found in both annelid and sea anemone swimming larvae (boxes). **b** | Drawing of the RFamide-immunopositive nervous system (black staining) in the medusozoan *Hydra* spp., showing agglomerations of neurons in the peduncle (denoting the 'apical nervous system' (ANS)) and around the mouth (denoting the 'blastoporal nervous system' (BNS)). **c** | Apical view of a late frog gastrula (left panel), an early annelid trochophora larva (middle panel) and a sea anemone planula larva. Expression of Sine oculis homeobox homologue 3 (*SIX3*) and retinal homeobox (*RX*) genes (light and dark violet) in frog[142], annelid[54,63] and sea anemone[18,62] apical regions are shown alongside the blastoporal expression of brachyury, Forkhead box A (FOXA) and Hedgehog (HH) (red). Black arrows refer to WNT signalling, and black inhibitory arrows refer to inhibition of WNT signalling by WNT antagonists. Figure part **b** is adapted with permission from REF. 143, Springer Science+Business Media: *Cell & Tissue Research*, Antisera to the sequence Arg-Phe-amide visualize neuronal centralization in hydroid polyps, 241, 1985, 171–182, Grimmelikhuijzen, C. J., figure 9a.

The BNS

On investigation of six species of sea anemone, Hertwig and Hertwig[9] noticed that the ectoderm of the tentacles and the oral disc (the region between the tentacle rosette and the oral opening) had a much thicker neuropil and was richer in ganglion cells than other body regions. In the oral disk, they observed radial aggregations of densely packed neurons in line with the neuron populations of the individual tentacles[9], which were assumed to coordinate movements between tentacles, oral disk and mesenteries[80]. They referred to these as 'some kind of nervous central organ' (REF. 9). Specialized (but not necessarily more condensed) neuronal populations have been described more recently in *N. vectensis*[56], and concentrations of neurons and neuropil in and around the mouth and tentacles are also found in medusozoan polyps[7,11,23,26,38,81,82] (FIG. 5b) and in the rhopalia (sensory organs with concentrations of neurons) of medusae[23,39]. These are often accompanied by circumferential neuropil, so-called nerve rings[9,23,33,39,56]; however, the nerve rings in anthozoan polyps and in medusae are topologically distinct and have probably evolved independently. The cnidarian BNS contains sensory neurons, interneurons and motor neurons; for example, anthozoan[83] and hydrozoan[84] tentacles are covered both with mechanosensory neurons and with ganglion cells, some of which innervate the muscle fibres. Therefore, the major function of the BNS is to coordinate feeding movements in polyps[45,85] and swimming in medusae[39].

Homology of the BNS across the Neuralia?

Homology between cnidarian nerve rings and (parts of) the bilaterian centralized nervous system has been proposed repeatedly[26,82], following the suggestion by Balfour[86] that if a circumoral nerve ring became longitudinally extended it might result in two nerve cords joined at the front and back (also supported by Sedgwick[6]), as depicted in FIG. 3. Here, we extend and refine this proposal, based on comparison of molecular regions of cnidarians and bilaterians as outlined in the following sections. In essence, we propose that a specialized set of sensory neurons, interneurons and motor neurons developed from ectodermal regions around the blastopore in the cnidarian–bilaterian ancestor. Through multiple (and independent) diversification events, these BNS precursor cell types gave rise to the sensory neurons, interneurons and motor neurons of today's cnidarian BNS and bilaterian nerve cords. The bilaterian nerve cords evolved by fusion of the two halves of the BNS along the new ventral midline. Consistent with this hypothesis, the annelid neuroectodermal midline represents the line of fusion of a slit-like blastopore[60,87] (FIG. 2b), and the vertebrate neuroectodermal midline (known as the floor plate) is derived from blastoporal tissue[88] (FIG. 2a).

Importantly, this hypothesis does not extend to ctenophores. Although a higher density of the oral nerve net has been reported[43], the regionally expressed transcription factors, as well as most WNT ligands and cell type markers shared between cnidarians and bilaterians, seem to be absent in ctenophores (see below).

Conserved molecular regions in the BNS

Similar combinations of transcription factors seem to specify the neural regions developing from ectodermal blastoporal tissue in cnidarians and bilaterians (FIG. 6). Numerous factors show differential concentric expression in the oral ectoderm of sea anemone planula larvae and early polyps[18,56,89–91] (FIG. 6a), anticipating the later developing anatomical and physiological BNS subdivisions (the pharynx, oral disk and tentacles)[91].

Importantly, many of these factors are well-established players in the mediolateral patterning of the bilaterian

neuroectoderm[15,53], expressed in a similar sequence of domains on both sides of the neural midline in annelids[15,92] (FIG. 6b) and also in insects, vertebrates[53,93–95] (FIG. 6c), cephalochordates[96] and hemichordates[97]. Thus, in bilaterians as in cnidarians, FOXA, FOXB, NK2.2 and LIM homeobox (LMX) demarcate the most-medial region; PAX family members are expressed more laterally, where they overlap with NK6 (REFS 15,53); and Msh homeobox (MSX), Iroquois homeobox (IRX) and Distal-less homeobox (DLX) demarcate the most-peripheral or lateral region. We consider this to be strong evidence that the same transcription factors already specified developmental regions around the blastopore in the common cnidarian–bilaterian ancestor. Given that, in both cnidarians and bilaterians, these regions topologically correspond to subregions of the developing BNS, it is tempting to speculate that the evolutionary origin of these regions is related to the emergence of the first specialized neural cell types at the onset of nervous system centralization. But what were these cell types?

WNT signalling and neural identities. Conserved WNT signals seem to control similar transcription factor networks in cnidarian and bilaterian blastoporal tissues (FIG. 7). Of the 12 WNT ligands differentially expressed along the *N. vectensis* apical–blastoporal body axis, 4 (WntA, Wnt1, Wnt7 and Wnt4) demarcate staggered domains in the BNS ectoderm[98], and Wnt2 demarcates the adjacent body column ectoderm (FIG. 7a). Except for WntA, which is lost in vertebrates, orthologous WNT ligands are reported in similar positions in the vertebrate neural tube (including WNT1 (REF. 99), WNT7 (REFS 100,101),WNT4 (REF. 102) and WNT2 (REF. 103); FIG. 7b). Intriguingly, evidence is accumulating that these WNT ligands may have conserved roles in the specification and differentiation of ancient neural cell types[98,47].

Monoaminergic cells. In vertebrates, canonical WNT1 in conjunction with Sonic hedgehog (SHH) signalling triggers the specification of monoaminergic neurons (dopaminergic and serotonergic) along the neural midline, via FOXA, Orthodenticle homologue (OTX) and LMX activation[99,104–109]; SHH-dependent NKX2.2 favours serotonergic over dopaminergic fate[99,110]. In *N. vectensis,* ectopic activation of canonical WNT signalling upregulates FoxA, FoxB and Lmx and Nk2 genes, orthologous to vertebrate NKX2.2 (REF. 90); furthermore, expression of three *N. vectensis otx* genes in the oral ectoderm[111] and of the Hedgehog ligand in the pharynx[112] is fully consistent with an overall conservation of this network (FIG. 7a,b). Serotonin is specifically detected in the *N. vectensis* pharynx (D.A., H.M. and J. Renno, unpublished observations), in line with locally restricted activity of monoamine-synthesizing enzymes. Besides the modulation of contractile activities around the gastric opening, another function of these monoaminergic cells might have been the control of ciliary beating for microparticle trapping with the mucociliary sole[151].

Motor neurons. Motor neurons might represent another set of ancient BNS neuron types. In the vertebrate neuroectoderm, non-canonical WNT4 in conjunction with SHH signalling specifies medial motor column (MMC) neurons[102]. One key factor regulating MMC neuron identity is the transcription factor motor neuron and pancreas homeobox (MNX; also known as HLXB9 and HB9). Importantly, *N. vectensis hlxb9* demarcates small sets of ectodermal cells of unknown identity between tentacles in the developing *N. vectensis* polyp[113]. Further to a (possibly) conserved function in specification, vertebrate WNT4s also seem to have an ancient role in establishing neuromuscular junctions: together with WNT11 ligands, they initiate the formation of neuromuscular junctions[114,115], and this role is conserved in *Drosophila*[116]. Given that *WNT4* and *WNT11* (with shared neuromuscular functions) are ancient duplicates[98,117], it is likely that the WNT4 and WNT11 precursor — which predated the cnidarians — already had the same function. Notably, *N. vectensis wnt11* is expressed in scattered ectodermal cells[98]. Likewise, vertebrate WNT7 has been found to exert multiple roles in differentiating neurons, including dendrite outgrowth,

Glossary

Anthozoans
A group of cnidarians with bilateral symmetry including the sea anemones, corals and sea pens. Anthozoans have a biphasic life cycle involving swimming planula larvae and adult polyps with tentacles; they do not have a medusa stage.

Apical nervous system
(ANS). An integrative nervous centre developing from the apical plate and composed of sensory-neurosecretory cells and of sensory neurons and interneurons projecting into the apical neuropil.

Apical neuropil
A neuropil located at the apical pole of cnidarian larvae and bilaterian primary larvae, underneath the 'apical organ'. The apical neuropil is formed by neurons that belong to the 'apical nervous system' and receives paracrine input from the apical organ.

Bilaterians
A group of animals with bilateral symmetry, including the vertebrates, cephalochordates, sea urchins, insects, annelids and molluscs. Characteristic features relevant for nervous system evolution include a through-gut with mouth and anus, commissural interneurons, a chimeric brain and paired sensory organs.

Blastoporal nervous system
(BNS). An integrative nervous centre developing from ectoderm surrounding the blastopore.

Cerianthids
A group of tube-dwelling cnidarian polyps that branched off early in the cnidarian tree and are thus especially informative about early cnidarian conditions

Cnidarians
A group of animals characterized by their name-giving stinging cells (cnidocytes) and simple gut with a single opening; the cnidarians include jellyfishes, corals and sea anemones. Adults in this group are polyps and/or medusae.

Ctenophore
One of a group of jelly-like animals with bilateral symmetry that spend their entire life swimming in the water, propelled by cilia in a comb-like arrangement; they have a nerve net and a primitive gut. The phylogenetic position of this group is not settled.

Homology
Traits present in distinct groups that are thought to be inherited from a similar trait in their last common ancestor. Homologous traits often share specific internal structures, similar positions within the body and continuity of phylogenetic distribution.

Medusozoans
A group of cnidarians that form medusae as part of their adult life cycle. Medusozoans include the stinging jellyfish, box jellyfish and the developmental model systems *Clytia hemisphaerica* and *Hydra* Spp.

Nerve cords
The most-prominent longitudinal part of the central nervous system in bilaterian animals, extending along the animal's anterior–posterior axis. In vertebrates, the nerve cords correspond to the hindbrain and spinal cord.

Nerve rings
In Cnidaria, concentrations of neurons and axons around the pharynx or interconnecting the rhopalia.

Neuralia
A group of animals that includes the Cnidaria, Ctenophora and Bilateria but excludes more basal metazoans, such as the sponges (the Porifera). The defining feature of this group is the presence of neurons.

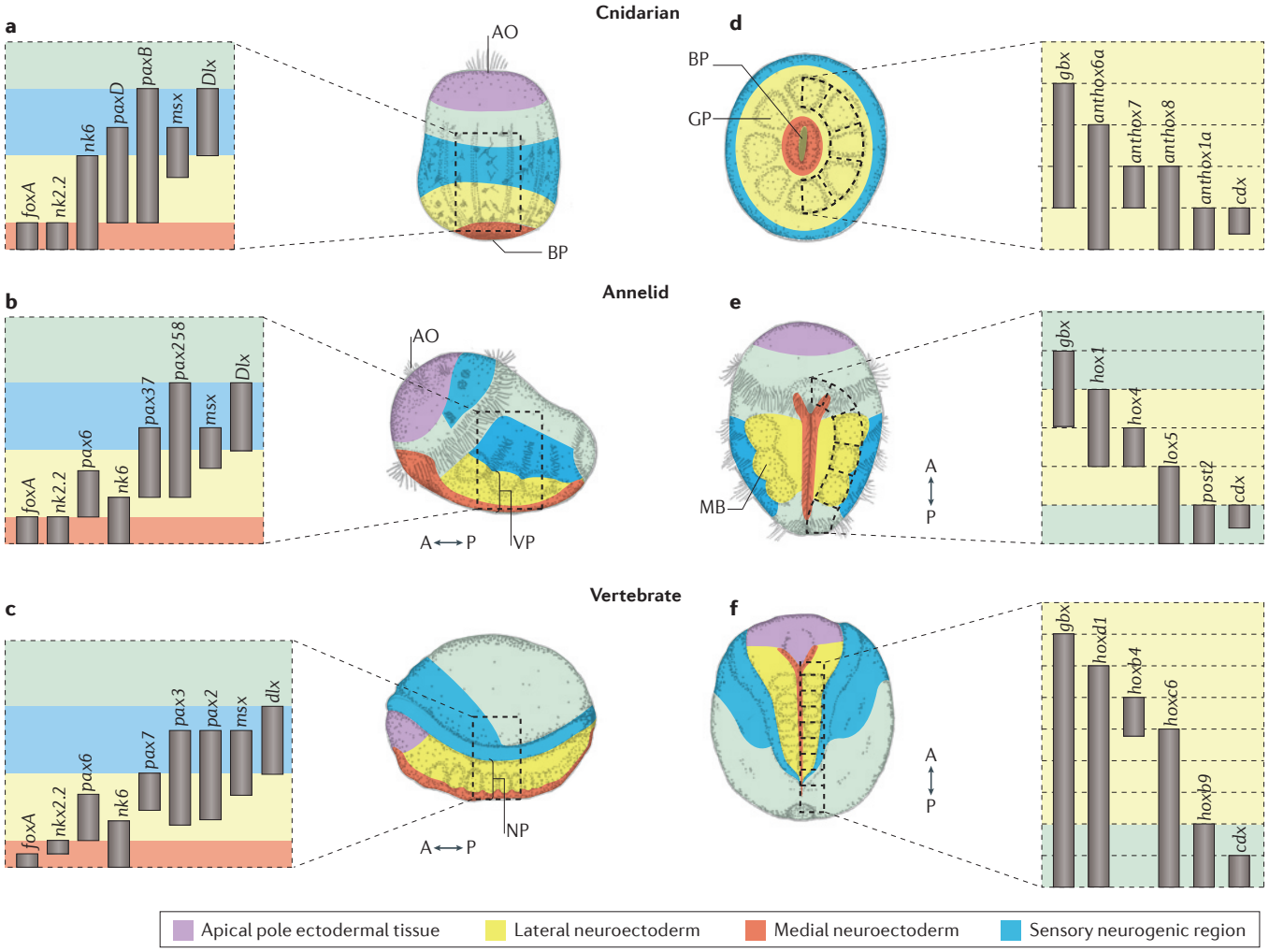

Figure 6 | **Molecular regions in the BNS.** Mediolateral patterning in a developing cnidarian (the sea anemone *Nematostella vectensis*; late planula larva), an annelid (*Platynereis dumerilii;* late trochophora larva) and a vertebrate (the frog *Xenopus laevis*; stage 14). For each animal, corresponding developmental neurogenic regions are indicated in similar colours. For the areas indicated by dashed lines, spatially restricted gene expression is illustrated by bars. **a** | In the sea anemone, Forkhead box A (*foxA*) (and *foxB*)[56,90,144] and *nk2.2*-related homeodomain[79,90,91,145] transcription factors have proven specific for the most-medial (pharyngeal) portion of the oral or blastoporal ectoderm (red), which gives rise to the pharyngeal nerve ring[56]. By contrast, paired box D (*paxD*; orthologous to *pax37*), *paxB* (*pax258*) and *paxA* family members are excluded from the most-medial region but expressed more peripherally[123] (blue and yellow); and an *nk6*-related homeodomain factor is expressed in the tentacle ectoderm. Finally, Msh homeobox (*msx*), Iroquois homeobox (*irx*, not shown) and Distal-less homeobox (*dlx*) homeodomain factors are expressed in a peripheral concentric domain at a distance from the blastopore that represents the future upper body column (capitulum) of the polyp in *N. vectensis*[18] (blue and yellow) and *Acropora millepora*[91]. **b**,**c** | Expression of orthologous genes in annelids[15,92] and vertebrates[53,93–95]. **d**–**f** | Spatially staggered expression of gastrulation and brain-specific homeobox (*GBX*) and homeobox (*HOX*) genes in a bilateral series of pouches in *N. vectensis*[113], *P. dumerilii*[146] and *X. laevis*[147,148]. In parts **d**–**f** the animals are oriented as seen from below in parts **a**–**c** (with the anterior (A) and posterior (P) shown using double-headed arrows). The overlying ectoderm is shown transparent, so that the pouches and somites of the inner layer are visible. In the sea anemone *N. vectensis*, expression of *anthox6a* overlaps with expression of *anthox7*, *anthox8a* and *anthox8b*, and is followed by *anthox1a*, the limit of which coinciding with expression of the presumed caudal-type homeobox (*cdx*) gene (**d**). Notably, as revealed by phylogenetic analyses[113,149,150], *anthox6a* is most-closely related to annelid *hox1*, and *anthox1a* is most-closely related to annelid *hox3 to hox9*, whereas *anthox7*, *anthox8a* and *anthox8b* are cnidarian-specific duplicates of a *HOX* gene of unclear affinity but located in between the anterior *HOX* and posterior *HOX* genes in the hypothetical eumetazoan HOX cluster[113,149,150], which we refer to as the 'middle *HOX*' (not shown). Note that the identity of the presumed *cdx* gene is not firmly established[113,1549,150]. This implies that the expression limits of the cnidarian *hox* genes show spatial colinearity with their chromosomal order, as is also observed in bilaterians. Note also that *gbx* is expressed in somites and overlying neuroectoderm in *X. laevis* and *P. dumerilii*. AO, 'apical organ'; BP, blastopore; *lox5*, leech homeobox gene 5; GP, gastric pouch; MB, mesodermal band; NP, neural plate; *post2*, posterior 2.

presynaptic active zone formation and synaptic vesicle recycling[118]. Possibly, non-canonical WNT4, WNT11 and WNT7 signalling shared an ancestral neural function inside the BNS.

Rohon–Beard-like mechanosensory cells. Finally, mechanosensory Rohon–Beard-like cells[119] may have belonged to the ancient BNS. In vertebrates, formation of specialized mechanosensory Rohon–Beard cells requires MSX[120] and, non-cell autonomously, DLX function in the adjacent non-neural ectoderm[121]. Also, PAX3 and zinc-finger protein of the cerebellum 1 (ZIC1) co-specify Rohon–Beard cells via the activation

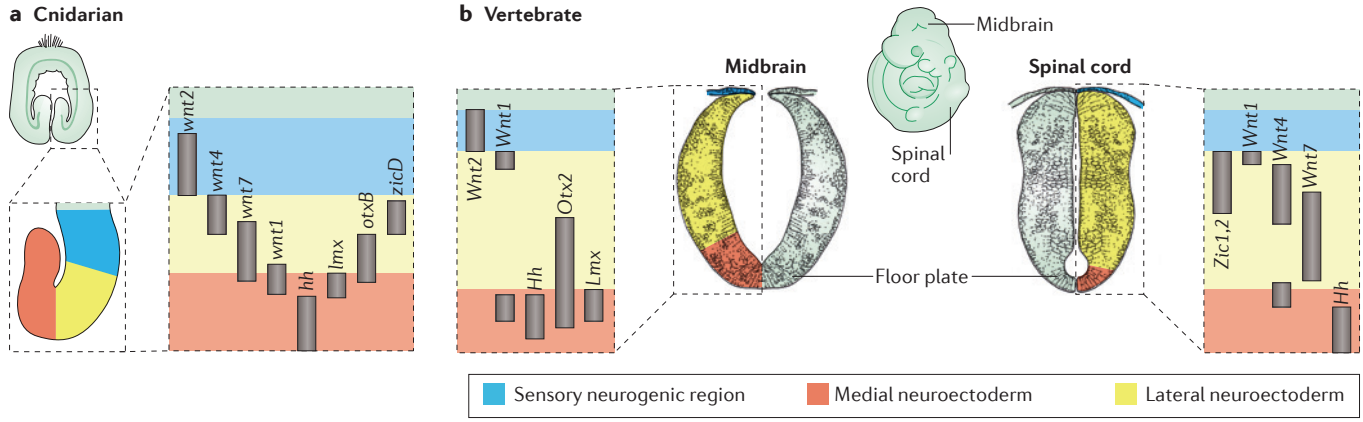

Figure 7 | **WNT and HH signalling in cnidarians and vertebrates.** **a**, **b** | Expression of Hedgehog (HH), WNT1, WNT2, WNT4 and WNT7 in the developing pharyngeal, oral and columnar ectoderm of the *Nematostella vectensis* planula larva (**a**) and in the mouse embryonic day 9.5 neural tube (**b**) at the level of the mesencephalon (midbrain; left) and spinal cord (right). The dashed box in part **a** demarcates the lateral limit of the 'blastoporal nervous system' (BNS). Expression of orthologous *WNT* genes occurs at comparable mediolateral positions. A limited set of downstream transcription factors (zinc-finger protein of the cerebellum (ZIC), Orthodenticle homologue (OTX) and LIM homeobox (LMX)) also shows similar mediolateral coordinates.

of Runt-related transcription factor 1 (*RUNX1*)[122]. *RUNX1* expression in turn requires canonical WNT8 signalling from the underlying paraxial meseoderm[122]. Strikingly, all of these network players seem to be specifically co-expressed in the lateral *N. vectensis* BNS ectoderm (FIGS 6a,7a): besides *msx* and *dlx*, *wnt8* is expressed in gastrodermis, underlying the body column[98]; *paxD3* (REF. 123) expression matches that of *zic* paralogues in the tentacle ectoderm[124]; and the single *N. vectensis runx* gene is also expressed in the tentacle ectoderm, in scattered sensory neurons or ganglion cells[125]. It will be helpful to determine the identity of these cells and whether and how their formation depends on *msx*, *dlx*, *paxD*, *zic*, *runx* and/or canonical WNT signalling. The ancestral nature of Rohon–Beard-like sensory neurons has been postulated before[126] and could well extend to cnidarian–bilaterian ancestors. Rohon–Beard cells express mechanosensory TRP channels and are glutamatergic and GABAergic[127]; intriguingly, transient receptor potential vanilloid (TRPV) and glutamic acid decarboxylase (GAD), the GABA-synthesizing enzyme, are strongly expressed in polyp tentacles and along the body column[56] (H.M. and D.A., unpublished observations).

Importantly, except for *wntA*, which is expressed around the mouth margin in adult ctenophores[128], most of the genes encoding the WNT ligands discussed above (*wnt2*, *wnt4*, *wnt11*, *wnt7* and *wnt8*) seem to be missing in the ctenophore *M. leidyi*[129], and the orthology relationships of *nk* and *pax* genes are unclear[20,130]. By contrast, *nk2*, *nk6*, *msx* and *pax258* genes are present in the genome of the ctenophore *Pleurobrachia bachei*[21]. The future study of these genes in *Pleurobrachia* Spp. might be informative with regard to whether a BNS was secondarily lost in the ctenophore lineage or never existed.

BNS control of gastric pouches

In both cnidarians and bilaterians, the neurogenic ectoderm of the BNS and trunk neuroectoderm develops in close association with contractile tissue developing from the inner layer; and in both groups this contractile tissue is subdivided into metameric units — somites in bilaterians and gastric pouches in cnidarians. We hypothesize that the evolution of these metameric contractile units paralleled and triggered the emergence of the BNS (see also REF. 151). Homology between gastric pouches and bilaterian somites (which similarly develop from gastric pouches in many bilaterians) had initially been proposed by Sedgwick[6] and others[131] and finds new support in the observation that, in sea anemones as in bilaterians, the bilateral sets of pouches and somites express the EGH homeobox gene gastrulation and brain-specific homeobox (*GBX*) gene and the *HOX* genes in a colinear fashion (FIG. 6d–f). Note that ctenophores also show bilateral symmetry manifest in outpocketings from the primitive gut that give rise to an internal canal system. However, any relatedness to the cnidarian pouches remains highly speculative as no molecular data are available for the specification of these canals.

We propose that the evolving BNS gained control over the contraction of muscles differentiating from an ancient set of bilateral pouches. Initially, this control must have been paracrine, as it necessarily occurred across germ layers (as explained above for the neuromuscular orthogon). It is tempting to speculate that these bilateral pouches probably corresponded to the presumed digestive diverticula present in fossils from the Precambrian, such as *Dickinsonia* spp.[132]; these animals presumably grazed on algal mats with their blastoporal side facing downwards[133]. Homology between cnidarian gastric pouches and bilaterian somites can be tested further by the study, in *N. vectensis*, of conserved transcription factors that spatially subdivide the bilaterian somites (such as the bHLH factors *paraxis* and *hand*).

Outlook: the bilaterian nervous system

The molecular evidence compiled in the previous sections supports a far-reaching hypothesis; namely that the ANS and BNS can be regarded 'nucleation centres' for the emergence of the bilaterian centralized nervous system, which merged at the level of the bilaterian forebrain (FIG. 3c), as recently proposed[19]. This Opinion article elucidates the origin of these two integrative centres in early animal evolution. Notably, a similar dual origin of the centralized nervous system has been proposed for protostomes[60,134] and, more recently, the chordate neural tube has been postulated to be derived from an initial circumblastoporal nerve ring[60].

If so, a number of important changes (synapomorphies) must have occurred on the bilaterian evolutionary lineage

(FIG. 3c). A first truly bilaterian innovation would have been the commissures interconnecting the fused halves of the BNS. The comparative study of the homeodomain transcription factor DBX, which has a conserved role in the specification of commissural interneurons in vertebrates and insects, may elucidate this process. With the emergence of commissural interconnections, the stage would have been set for new left–right coordinated movements (such as simple alternate bending of the body), which require coordination between the two body halves.

A concomitant trend would have been the vast expansion of the BNS by duplicating motor and interneuron types. An ipsilateral and contralateral elaboration of sensory-motor circuits, in conjunction with the acquisition of commissural interneurons via Roundabout homologue (ROBO) and Slit repulsion would have resulted in the expanded mediolateral sequence of neurogenic columns and neuron types proposed for urbilaterians and, ultimately, in the complex pattern present in the vertebrate neural tube[53]. Developmentally, the elaboration of mediolateral patterning would have involved graded Hedgehog signalling[112] and an elaboration of blastoporal WNT signalling in the neuroectoderm[102], integrating with bone morphogenetic protein (BMP) signalling from adjacent non-neural ectoderm.

The medial closure of the former single opening into a separate mouth and anus[88] would have imposed a directional flow on food movement with the mucociliary sole[151] and triggered additional nervous specializations for forward locomotion. Chemosensory input into the new mouth region might have fed into a new 'primordial locomotor control centre' (FIG. 3c), which might have evolved at the level of the first (GBX-negative) pair of somites. Composed of specialized interneurons and motor neurons, it would have started to act as an upstream command centre for coordinated contraction of left and right muscle fibres. The evolution of this centre might have enabled simple forward locomotion in early bilaterians.

Finally, the most-striking innovations of the bilaterian nervous system are the brain and associated sensory organs. The multifaceted evolution of the brain goes far beyond the scope of this article; here, we only briefly mention two fundamental steps that, following the evolutionary scenario developed herein, would have shaped the bilaterian brain. First, the former ANS (FIG. 3) and the primordial locomotor centre would have fused to form the new bilaterian brain. We refer to this idea as the chimeric brain hypothesis (elaborated elsewhere[19]). Second, the paired anterior sensory organs (FIG. 3c) would have evolved as an elaboration and specialization of the peripheral sensory plexus, feeding into the new brain. In vertebrates[135], insects[136] and annelids[137], these are specified by SIX1 and/or SIX2 and include both the chemosensory organs and the eyes. The evolution of anterior sense organs might have been accompanied by the emergence of the first sensory-associative brain centres (giving rise to the pallium in vertebrates and the mushroom bodies in annelids and insects)[138].

Concluding remarks

Building on previous concepts and on rich new neurodevelopmental data sets available for cnidarians, we present a comprehensive view of how the cnidarian and bilaterian nervous systems may be related in animal evolution. Most importantly, we provide a comparative developmental framework for nervous system regionalization that will allow the identification of homologous neuron types in animals as remote as humans and sea anemones in the future. Starting from a homogenous nerve net that covered the entire gastrula-shaped body, the evolution of the ANS and BNS as integration centres on opposite sides of the body appears to have predated the cnidarian–bilaterian ancestor. The ANS enabled more-complex control of general body physiology, whereas the BNS is most likely to have co-evolved with a mucociliary sole and contractile gastric pouches. We speculate that both systems merged in bilaterians at the level of the anterior brain. Our scenario of nervous system evolution is consistent with available comparable data and can be tested by further study and comparison at the gene-regulatory and cellular level.

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