

Convergent evolution of bilaterian nerve cords

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It has been hypothesized that a condensed nervous system with a medial ventral nerve cord is an ancestral character of Bilateria. The presence of similar dorsoventral molecular patterns along the nerve cords of vertebrates, flies, and an annelid has been interpreted as support for this scenario. Whether these similarities are generally found across the diversity of bilaterian neuroanatomies is unclear, and thus the evolutionary history of the nervous system is still contentious. Here we study representatives of Xenacoelomorpha, Rotifera, Nemertea, Brachiopoda, and Annelida to assess the conservation of the dorsoventral nerve cord patterning. None of the studied species show a conserved dorsoventral molecular regionalization of their nerve cords, not even the annelid *Owenia fusiformis*, whose trunk neuroanatomy parallels that of vertebrates and flies. Our findings restrict the use of molecular patterns to explain nervous system evolution, and suggest that the similarities in dorsoventral patterning and trunk neuroanatomies evolved independently in Bilateria.

The nervous systems of Bilateria, in particular their trunk neuroanatomies, are morphologically diverse¹ (Fig. 1a). Groups such as arthropods, annelids, and chordates exhibit a medially condensed nerve cord, which is ventral in arthropods and annelids, and dorsal in chordates. By contrast, other lineages have multiple paired longitudinal nerve cords distributed at different dorsoventral levels. There are even bilaterians with only weakly condensed basiepidermal nerve nets, similar to those in cnidarians (Fig. 1a), which supports the idea that this net-like neural arrangement predates the Cnidaria–Bilateria split^{2,3} (Fig. 1a). However, the earliest configuration of the bilaterian central nervous system (CNS) is still debated^{2,4–7} (Fig. 1a), and thus it is unclear when and how often nerve cords evolved in Bilateria.

The conserved deployment of signalling molecules and transcription factors along the bilaterian anteroposterior and dorsoventral axes grounds most scenarios for the evolution of the CNS^{2,4,7–12}. In particular, the similar expression of the transcription factors *nkx2.1/nkx2.2*, *nkx6*, *pax6*, *pax3/7*, and *msx* in the ventral neuroectoderm of the fly *Drosophila melanogaster* and the annelid *Platynereis dumerilii*, and the dorsal neural plate of vertebrates (Fig. 1b), is a core argument for proposing an ancestral CNS comprising a medial ventral nerve cord (VNC) in Bilateria^{2,4,7,12,13}. In *P. dumerilii* and vertebrates, and to some extent in *Drosophila*, the staggered expression of these genes correlates with the spatial location of neuronal cell types along their trunks^{4,9,12}. Serotonergic neurons form in the ventromedial *nkx2.2*⁺/*nkx6*⁺ region, cholinergic motor neurons develop in the *nkx6*⁺/*pax6*⁺ area, and *dbx*⁺ interneurons and lateral sensory trunk neurons differentiate in the more dorsolateral *pax6*⁺/*pax3/7*⁺ and *pax3/7*⁺/*msx*⁺ domains, respectively (Fig. 1b). The dorsoventral arrangement of these transcription factors and neuronal cell types is absent in hemichordates^{10,11,14}, nematodes^{15,16}, and planarians¹⁷, consistent with the idea that the most recent ancestor of Bilateria had a dorsoventrally patterned, medially condensed VNC that has been repeatedly lost in these and perhaps other groups¹². However, there is an alternative explanation: that a CNS with a single nerve cord and the similar dorsoventral patterning is the

trait that repeatedly evolved, and thus was absent in the most recent common bilaterian ancestor^{5,8,10,11}.

Neuroectodermal patterning in Xenacoelomorpha

To explore the conservation of neuroectodermal patterning systems in Bilateria, we first studied Xenacoelomorpha (Extended Data Fig. 1), which is the sister group to all remaining bilaterian lineages^{18,19} (that is, Nephrozoa). We focused our analyses on *Xenoturbella bocki*, the nemertodermatids *Meara stichopi* and *Nemertoderma westbladi*, and the acoele *Isodiametra pulchra*. As in the acoele *Hofstenia miamia*²⁰ and most other bilaterians^{7,10}, these xenacoelomorphs differentially express anteroposterior marker genes along their primary body axis^{21,22} (Extended Data Figs 2a, c and 3). The bone morphogenetic protein (BMP) pathway, which has an ancestral dorsoventral patterning role^{20,23} and an anti-neural role in *Drosophila* and vertebrates⁹, is also similarly deployed in all studied xenacoelomorphs²⁰, with *bmp* ligands expressed dorsally and antagonists located more ventrolaterally (Fig. 2a, d and Extended Data Figs 2d and 4). However, the dorsoventral transcription factors that we found in our genomic resources (Supplementary Table 1) did not show a clear staggered expression (Fig. 2b, e). Therefore, Xenacoelomorpha only exhibits the anteroposterior and BMP ectodermal patterning systems, which is reminiscent of the cnidarian condition²⁴.

Importantly, ectodermal patterning systems are deployed independently of the trunk neuroanatomy in Xenacoelomorpha. Similar to cnidarians, xenacoelomorphs have a uniformly distributed, diffuse basiepidermal nerve net^{3,25–27}. *Xenoturbella* species only have this network²⁶. However, nemertodermatids have additional longitudinal basiepidermal nerve cords²⁵, located dorsally in *M. stichopi*²⁸ (Fig. 2c), and ventrally in *N. westbladi* (Extended Data Fig. 2e). The acoele *I. pulchra* also has four pairs of subepidermal nerve cords distributed along the dorsoventral axis²⁷ (Fig. 2f). Genes commonly involved in neurogenesis (Extended Data Fig. 5a, d) and neural transmission (Extended Data Figs 2b, f and 5b, c, e) are consistently expressed in the

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