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The evolution of nervous system centralization

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It is yet unknown when and in what form the central nervous system in Bilateria first came into place and how it further evolved in the different bilaterian phyla. To find out, a series of recent molecular studies have compared neurodevelopment in slow-evolving deuterostome and protostome invertebrates, such as the enteropneust hemichordate *Saccoglossus* and the polychaete annelid *Platynereis*. These studies focus on the spatially different activation and, when accessible, function of genes that set up the molecular anatomy of the neuroectoderm and specify neuron types that emerge from distinct molecular coordinates. Complex similarities are detected, which reveal aspects of neurodevelopment that most likely occurred already in a similar manner in the last common ancestor of the bilaterians, Urbilateria. This way, different aspects of the molecular architecture of the urbilaterian nervous system are reconstructed and yield insight into the degree of centralization that was in place in the bilaterian ancestors.

Keywords: evo–devo; Bmp signalling; dorsoventral axis inversion; Urbilateria; nervous system centralization

1. INTRODUCTION

Surprisingly, little is known about the evolutionary origin of central nervous systems (CNS). It is not known when they first appeared in animal evolution and what their initial structure and function was. It is also unclear whether the CNS of vertebrates and invertebrates trace back to a common CNS precursor (Arendt & Nübler-Jung 1999) or whether they are of independent evolutionary origin (Holland 2003; Lowe *et al.* 2003). This review addresses the questions of when and in what form the CNS first came into place and how it further evolved in different animal phyla. To track the evolutionary transition from 'diffuse' to 'centralized' in bilaterian nervous system evolution (figure 1), we first define these terms. We then explain what the study of bilaterian neurodevelopment can reveal about this transition. Specifically, we focus on the role of *Decapentaplegic* (*Dpp*) signalling in triggering neurogenesis in a polarized manner along the dorsoventral body axis. We then outline the conserved mediolateral molecular anatomy of the bilaterian neuroectoderm (figure 2) and pinpoint a set of conserved neuron types that develop from corresponding regions (figure 3). We finally discuss the significance of these data for reconstructing the urbilaterian nervous system.

(a) *What is a CNS?*

In physiological terms, a CNS integrates and processes sensory information coming from the periphery, and initiates body-wide responses via neurosecretion into the body fluid or direct stimulation of the body musculature. Anatomically, a CNS is a delimited nervous tissue that comprises distinct agglomerations of functionally specialized neurons (nuclei) interconnected by axon tracts (neuropil). The CNS may be subdivided into separate parts (ganglia). It connects to the periphery via nerves. A CNS thus defined is found in various shapes and degrees of complexity in different animal phyla, including vertebrates and many invertebrates, such as echinoderms, arthropods, nematodes, molluscs and annelids (figure 1*a*).

In contrast, a diffuse nervous system receives sensory input and processes locomotor or neurosecretory output only locally, without central integration. This is achieved by the direct interconnection of sensory neurons and effector neurons (Westfall *et al.* 2002). For example, a diffuse nervous system is present in the body wall epithelium of adult cnidarians (figure 1*b*).

Even though these definitions are straightforward, the categorization of some animal nervous systems remains ambiguous (Miljkovic-Licina *et al.* 2004). For example, some cnidarian medusae possess an elaborate nerve ring around their central opening (manubrium) in addition to their diffuse nerve net (Mackie 2004). This nerve ring reflects a considerable degree of centralization. Also, the nervous system of deuterostome enteropneusts exhibits aspects of both central and diffuse organization (reviewed and discussed in Holland 2003). On one hand, enteropneusts have axon tracts that run along the longitudinal body axis and show a strong concentration of neurons in the anterior part of the body, reflecting nervous integration. On the other hand, enteropneusts have a 'nerve net' interconnecting the cell bodies, dendrites and axons of sensory neurons, interneurons and motor neurons, and neurons are embedded in the epidermis, as an indicative of a diffuse system, rather than forming an anatomically distinct structure (Lowe *et al.* 2003).

Given the vast differences in nervous system organization in Bilateria, what can we learn from comparative studies about the urbilaterian nervous system? So far,

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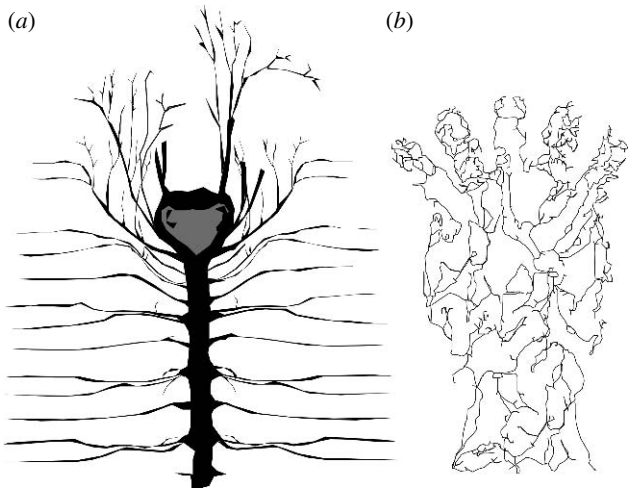

Figure 1. Different degrees of centralization in metazoan brains. (*a*) Centralized nervous system of an oligochaete worm. (*b*) Nerve net of a cnidarian polyp representing a typical non-centralized nervous system. Schematized drawings modified with permission from Bullock & Horridge (1965).

insight has been very limited and proposals about complexity and shape of the urbilaterian nervous system ranged from diffuse (Mineta *et al.* 2003; Lowe *et al.* 2006) to centralized (Denes *et al.* 2007). Assuming a diffuse urbilaterian nervous system would imply independent centralization events at least in protostomes and deuterostomes (Holland 2003; Lowe *et al.* 2006). Assuming a centralized urbilaterian nervous system, on the other hand, would imply secondary simplification of the nervous system of enteropneusts and many other invertebrate groups (Denes *et al.* 2007). These two conflicting hypotheses can now be tested. If centralization occurred independently in protostomes and deuterostomes, we would expect the neurodevelopment and molecular architecture of their CNS to be generally divergent. Instead, if centralization predated Bilateria, this should be reflected by similarities in neurodevelopment and CNS molecular architecture between the bilaterian superphyla.

2. NERVOUS SYSTEM CENTRALIZATION: THE EVO–DEVO APPROACH

A key strategy to unravel the degree of centralization that was in place in the urbilaterian nervous system is the comparison of CNS development between protostome and deuterostome groups. However, depending on the amount of evolutionary change these groups have accumulated, their neurodevelopment will be more or less informative about ancestral characteristics of nervous system centralization in Bilateria. Ancestral features will be most apparent in the neurodevelopment of species that have changed relatively little during evolution and will be modified to a larger extent in faster evolving species (Raible *et al.* 2005). Distinct aspects of neurodevelopment are currently under study in a broad range of protostome and deuterostome model species.

(i) *Polarized distribution of neuronal precursors with respect to the main body axes*. One important aspect of nervous system centralization is the early developmental segregation of the ectoderm into a 'non-neural' and a 'neural' portion, the neuroectoderm. In bilaterians, the neuroectoderm is located anterior where the brain and associated sensory organs develop, and on the neural trunk side which is ventral in most invertebrates and dorsal in vertebrates due to dorsoventral axis inversion (Arendt & Nübler-Jung 1994; De Robertis & Sasai 1996; Lowe *et al.* 2006). What are the signals that polarize the bilaterian ectoderm and to what extent are they comparable between phyla?

(ii) *Subdivision of the neural anlage into regions* ('*molecular anatomy*'). Another aspect of nervous system centralization amenable to comparative studies is how the developing nervous system relates to the molecular anatomy of the body. Bilaterians have in common an early subdivision of the developing embryo (or larva) into regions of distinct molecular identities (St Johnston & Nüsslein-Volhard 1992; Arendt & Nübler-Jung 1996; Lowe *et al.* 2003; Schlosser & Ahrens 2004; Yu *et al.* 2007). These are referred to as molecular anatomy and can be used as a molecular map. A similar molecular anatomy of the CNS anlage at early developmental stages has been considered as a good indication of CNS homology (Arendt & Nübler-Jung 1996; Lichtneckert & Reichert 2005). Note however that the structures that develop from corresponding regions in two species are not necessarily homologous (Lowe *et al.* 2003). How similar is the molecular anatomy between species, of the whole body and of the developing CNS in particular, and what is the significance of conserved expression regions for our understanding of CNS evolution?

(iii) *Spatial segregation of neuron types in the CNS*. Nervous system centralization not only implies local concentration of neurons but also their functional and spatial segregation and interrelation ('operational centralization'). This is exemplified by Herrick's longitudinal neuron columns in the vertebrate spinal cord, which comprise distinct sets of motor- and interneuron types. With the recent progress in the identification of conserved neuron types by molecular fingerprint comparisons (Arendt & Nübler-Jung 1999; Thor & Thomas 2002; Arendt *et al.* 2004), and using the conserved molecular anatomies as universal molecular maps, the localization and spatial segregation of neuron types can now be compared between remote bilaterians (Denes *et al.* 2007; Sprecher *et al.* 2007; Tessmar-Raible *et al.* 2007). To what extent had neuron types already been spatially arranged in Urbilateria and what does this tell about the ancestral state of nervous system centralization?

(a) *Central nervous systems develop from the non-*Dpp *body side*

In all bilaterian animals investigated (with the exception of the nematodes), the Bmp signalling system sets up tissue polarity along the dorsoventral axis (Mizutani *et al.* 2005; Lowe *et al.* 2006; Levine & Brivanlou 2007;

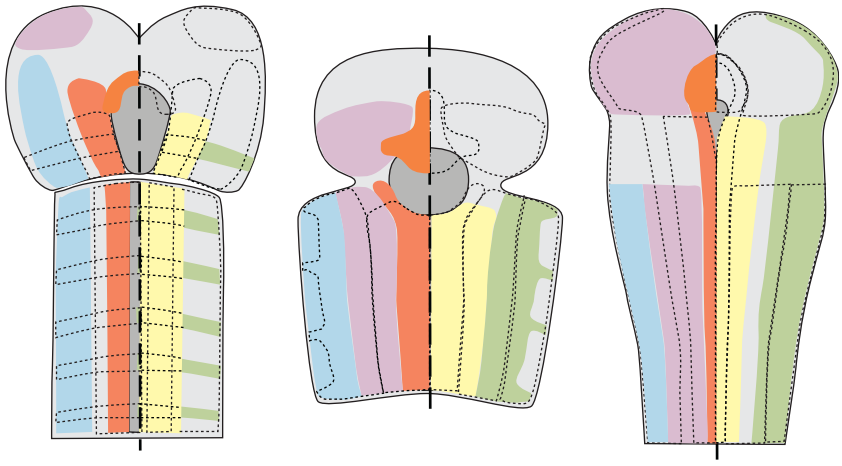

Figure 2. Comparison of mediolateral neurogenic columns across Bilateria. Expression of *nk2.2*/*nk2.1* (orange; Shimamura *et al.* 1995), *Nk6* (yellow; Rubenstein *et al.* 1998), *Pax6* (violet; Mastick *et al.* 1997; Urbach & Technau 2003*a*,*b*), *gooseberry*/*Pax3*/*7* (green; Matsunaga *et al.* 2001; Puelles *et al.* 2003) and *msh*/*Msx* (blue; Shimeld *et al.* 1996) orthologues in the neuroectoderm of *Drosophila*, *Platynereis* and mouse at pre-differentiation stages. The *Drosophila* and *Platynereis* schematics represent ventral views, and the mouse one is a dorsal view with the neural tube unfolded into a neural plate for better comparison. Neurogenic columns are demarcated by expression boundaries and represent cells with a unique combination of transcription factors. All expression patterns are symmetrical but are shown on only one side for clarity.

Yu *et al.* 2007). The Bmp system predates the emergence of the bilaterian CNS (Matus *et al.* 2006; Rentzsch *et al.* 2006) and was thus in place to be adapted for nervous system centralization, i.e. for the differential distribution of neuronal precursors along this axis. How similar is the role of Bmp signalling with respect to nervous system centralization in various bilaterians?

Whenever a CNS is present, it develops from the non-Bmp body side, in insects (Mizutani *et al.* 2005, 2006), vertebrates (Sasai *et al.* 1995; Levine & Brivanlou 2007), amphioxus (Yu *et al.* 2007) and also annelids (Denes *et al.* 2007). Also, in early vertebrate (Harland & Gerhart 1997) and fly development (Mizutani *et al.* 2006), the antineurogenic activity of Bmps sets the limit of the neuroectoderm. These findings first suggested that Bmp signalling had an ancient role in the overall restriction of neurogenesis to the neural body side (e.g. Padgett *et al.* 1993). Yet, this simple notion was not supported by recent additional comparative data: in enteropneusts (Lowe *et al.* 2006) and in polychaetes (Denes *et al.* 2007), the pan-neural marker *elav* is *not* downregulated by exogenously applied BMP4. How can we reconcile these findings?

The available data are consistent with a refined evolutionary scenario, which assumes that in early bilaterians the antineurogenic effect of Bmp signalling was only on specific sets of motor neurons (and interneurons), restricting them to the neural body side, while there was a positive effect on the formation of sensory neurons that do not form part of the CNS proper (Rusten *et al.* 2002). In line with this, Bmp signalling has been shown to trigger the formation of the peripheral sensory neurons at later developmental stages, at the neural plate border and adjacent lateral placodes in the vertebrates (Schlosser & Ahrens 2004) and in the lateral 'epidermal' ectoderm in *Drosophila* (Rusten *et al.* 2002). In annelids, the types of sensory neurons characterized so far arise from the lateral and dorsal sides as opposed to motor- and interneurons that form from the ventral body side (Denes *et al.* 2007); indeed, exogenous BMP4 strongly upregulates the

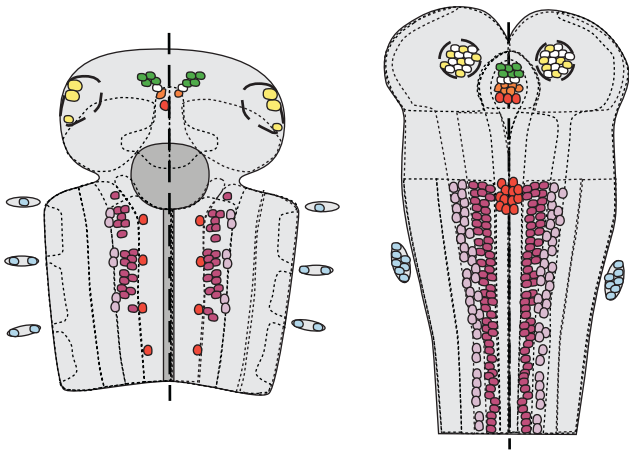

Figure 3. Conserved neural cell types in annelid and vertebrate. The neuron types emerging from homologous regions in the molecular coordinate systems in annelid and vertebrate and expressing orthologous effector genes are marked with the same colour. Homologous cell types include the molecular clock cells positive for *bmal* (dark green), ciliary photoreceptors positive for *c-opsin* and *rx* (white), rhabdomeric photoreceptors positive for *r-opsin*, *atonal* and *pax6* (yellow), vasotocinergic cells positive for *nk2.1*, *rx* and *otp* (orange), serotonergic cells positive for *nk2.1*/*nk2.2* (red), cholinergic motor neurons positive for *pax6*, *nk6* and *hb9* (violet), interneurons positive for *dbx* (pink), as well as trunk sensory cells positive for *atonal* and *msh* (light blue).

sensory marker *atonal*, consistent with a conserved role of *Dpp*/BMP in the specification of peripheral sensory neurons (Denes *et al.* 2007). Also, in enteropneusts where postmitotic neurons are spread all around the circumference of the trunk (Lowe *et al.* 2003), the distribution of motor-, inter- and sensory neuron precursors may not be uniform (Lowe *et al.* 2006): for example, there is a small population of putative motor neurons in the ventral ectoderm (expressing conserved motor neuron markers) and motor neurons are reported to be enriched in the ventral axon tract. A more in-depth analysis of the role of Bmp signalling and of other signalling systems active along the DV axis will elucidate

a possible conservation of neuron type segregation in annelid and enteropneust neuro-development.

Our revised scenario, that the ancestral role of Bmp signalling was to promote sensory over motor neuron fates, rather than a general antineurogenic effect, fits well with the actual distribution of motor and sensory neurons in many invertebrates, where it appears to be the rule rather than the exception that sensory neurons emerge outside of the neuroectoderm on the non-neural='*Dpp*/Bmp' body side. If this were indeed an ancestral bilaterian trait, this would imply that a certain degree of centralization was present in Urbilateria (i.e. the sorting out of motor versus sensory neurons along the secondary body axis).

(b) *A conserved pattern of mediolateral regions extending from head to trunk*

To estimate the complexity of the urbilaterian CNS, we need to know the complexity of the underlying molecular anatomy that was in place in Urbilateria. Although comparative studies have addressed this for both the anterior–posterior (Slack *et al.* 1993; Schilling & Knight 2001) as well as for the mediolateral (dorsoventral=neural/non-neural) axes (Cornell & Ohlen 2000), our focus here is on mediolateral patterning. Previous comparisons of the molecular anatomy of the insect and vertebrate neuroectoderm had revealed a similar mediolateral sequence of *nk2.2*+, *gsx*+ and *msx*+ neurogenic domains (reviewed in Arendt & Nübler-Jung 1999; Cheesman *et al.* 2004) that also extend into the brain anlage (Urbach & Technau 2003*a*,*b*; Sprecher *et al.* 2007). Notably, in the developing forebrain, medial *nk2.2* expression is complemented by the medial expression of its sister gene, *nk2.1* (Zaffran *et al.* 2000). *Nk6* genes also play a conserved role in mediolateral patterning because the neuroectodermal expression of the *Drosophila* orthologue shows medial restriction as observed in the vertebrates (Cheesman *et al.* 2004).

Our recent work on the mediolateral anatomy of the developing annelid nerve cord has revealed an even higher degree of conservation in mediolateral patterning (figure 2). In addition to the previously detected protostome–deuterostome similarities, we find that annelids and vertebrates share a *pax6*+ column at similar mediolateral level that likewise extends up to the forebrain (violet in figure 2; Denes *et al.* 2007). In both groups, the medial portion of the *pax6*+ column overlaps the *nk6*+ column (yellow in figure 2). Adding to this, annelids and vertebrates share a lateral *pax3/7*+ column (green in figure 2; note that this gene is expressed strictly segmentally in the *Drosophila* neuroectoderm; Davis *et al.* 2005). Our data also revealed that the positioning of the *gsx*+ column is more variable than initially assumed and the vertebrate *dbx*+ interneuron columns are probably vertebrate-specific evolutionary acquisitions (Denes *et al.* 2007).

The conservation of mediolateral columns between vertebrates, annelids and (to a lesser extent) insects is in stark contrast to the situation in enteropneusts, where similar columns have not been observed with the exception of the dorsal *dll*+ column and the ventral midline column (Lowe *et al.* 2006).

Two conclusions can be drawn. First, if the complex molecular mediolateral anatomy shared between annelids and vertebrates is indeed due to evolutionary conservation—and this notion seems inescapable given the overall complexity of this pattern (figure 2)—it must have been present in Urbilateria. Then, the immediate question arises: what was the difference in developmental fate between these regions in Urbilateria? One plausible scenario is that these regions gave rise to distinct and segregated ancestral neuron types, as will be discussed in the next section. Second, these findings would suggest that the mediolateral molecular anatomy in enteropneusts is secondarily simplified (Denes *et al.* 2007), consistent with the notion of evolutionary loss in a slow-evolving species (see discussions in Lowe *et al.* 2006; Denes *et al.* 2007).

(c) *Conserved neuron types develop from similar mediolateral progenitor domains*

In insects and vertebrates, neuron types emerging from the medial *nk2.2*+ column have to pioneer the medial longitudinal fascicles as well as peripheral nerves (Arendt & Nübler-Jung 1999 and references therein). Among these, neuron populations that send out ascending and descending projections in the vertebrate hindbrain are serotonergic and modulate spontaneous locomotor activity (Briscoe *et al.* 1999; Schmidt & Jordan 2000; Pattyn *et al.* 2003). In *Platynereis*, serotonergic neurons likewise emerge from the medial *nk2.2* columns and pioneer the longitudinal tracts and segmental nerves (red in figure 3; Denes *et al.* 2007). One type of serotonergic neurons also emerges from the *nk2.1*+ brain regions, as evidenced for *Platynereis* and fishes (Tessmar-Raible *et al.* 2007) as well as sea urchin (Takacs *et al.* 2004).

The *nk2.1*+ region in the developing forebrain of vertebrate and annelid gives rise to another conserved neuron type, early differentiating neurosecretory cells that synthesize the highly conserved neuropeptide arg-vasotocin/neurophysin (orange in figure 3). These cells form in the vicinity of ciliated photoreceptor cells in the brain that share the expression of *rx* and of *c-opsin* orthologues in vertebrate and annelid (white in figure 3) and of molecular clock cells positive for *bmal/cycle* (green in figure 3; Arendt *et al.* 2004).

Somatic motor neurons exhibit the same transcription factor signature (*hb9*+, *lim3*+, *islet-1/2*+) in insects, nematodes and vertebrates (Thor & Thomas 2002). In the vertebrates, these neurons are cholinergic and emerge from the *pax*+, *nk6*+ progenitor domain (violet in figure 3; Ericson *et al.* 1997). We found that the same is true for *Platynereis*, where the first cholinergic motor neurons that innervate the longitudinal musculature have the same transcription factor signature and emerge from the *pax6*+, *nk6*+ column (Denes *et al.* 2007; A. S. Denes *et al.* 2007, unpublished data).

Taken together, these data identify a considerable number of conserved neuron types that emerge from similar molecular coordinates in annelid and vertebrate. Obviously, this comparison is far from complete and awaits further characterization and localization of neuron types in both taxa.

As to the peripheral nervous system, we have so far identified and compared rhabdomeric photoreceptor cells in annelids and retinal ganglion cells in vertebrates (yellow in figure 3) that form from the eye anlage in both species (dashed circles in figure 3). In the trunk, we found some conserved sensory neuron types that emerge from similar lateral molecular coordinates in annelid and vertebrate (blue in figure 3; *ath*+ or *trpv*+; Denes *et al.* 2007); this comparison is ongoing.

3. RECONSTRUCTING THE URBILATERIAN NERVOUS SYSTEM

In conclusion, the comparison of neurodevelopment between protostome and deuterostome animal models reveals a conserved molecular architecture of considerable complexity that was inherited from the Urbilateria. Departing from a diffuse nerve net with homogeneously distributed neuron types, a first segregation of motor and sensory neurons occurred along the D–V axis in the line of evolution leading to the bilaterians. This involved Bmp signalling and possibly other signalling cascades. These signals established a refined mediolateral molecular anatomy, involving at least four longitudinal neurogenic regions with distinct molecular identities (*nk2.2*+/*nk6*+, *pax6*+/*nk6*+, *pax6*+/*pax3/7*+, *msx*+/*pax3/7*+; figure 2) that gave rise to spatially segregated neurons. Among these were medial serotonergic neurons, intermediate cholinergic motor neurons, some sort of interneurons and lateral sensory neurons (figure 3; Denes *et al.* 2007). These neuron types presumably controlled ancestral locomotor patterns such as undulatory swimming and/or peristalsis. In the head region, specialized light-sensitive cell types evolved, integrating different kinds of photic input to set the molecular clock and to control neurosecretory and motor output (Tessmar-Raible *et al.* 2007). While this already reflects a considerable degree of nervous system centralization that was presumably in place in Urbilateria, a renewed push in research combining developmental genetics with classical neuroethology in slow-evolving protostomes and deuterostomes will be needed to refine and complete this picture.

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