

The origin and evolution of the nervous system

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ABSTRACT The nervous systems of animals as diverse as flies and mice share many conserved features, suggesting that such features were already present in their last common ancestor. As our knowledge of neural development increases, so does the list of conserved features, pointing to the existence of a highly sophisticated, single species as the origin of most extant nervous systems. Possible reasons for this unexpected monophyly are discussed, leading to the conclusion that the appearance of very different life forms, lifestyles and habitats requires the previous attainment of a neural circuitry that is sufficiently robust to cope with large changes without losing its overall coherence.

KEY WORDS: *Urbilateria, conservation, tolerance, adaptation*

*Rien n'est mort que ce qui n'existe pas encore
Près du passé luisant demain est incolore
Il est informé aussi près de ce qui parfait
Présente tout ensemble et l'effort et l'effet*

From "Cortège" by Guillaume Apollinaire¹

Prologue

Twelve years ago, *The International Journal of Developmental Biology* published a special issue on "Developmental Biology in Belgium". On that occasion I was asked to write a review on the development of the nervous system (Ghysen, 1992). At the time, the nervous systems of epineurians (chordates) and hyponeurians (annelids/arthropods) were still considered highly dissimilar, and were supposed to have diverged independently from the diffuse nerve net present in diploblasts (cnidarians and ctenarians). My review developed the idea that, on the contrary, the nervous systems of all triploblasts share many essential features, and must have derived from a common ancestor that already enjoyed a fairly sophisticated nervous system. This idea has now gained large support, and the ancestor has gained a name: *Urbilateria* (de Robertis and Sasai, 1996).

Many discoveries have been made over the past decade that brought substantial changes in our view of phylogeny. On one hand, the classical distinction between diploblasts and triploblasts is questioned by recent discoveries demonstrating not only that typical mesoderm derivatives such as smooth and striated muscles

do exist in so-called diploblasts, but also that their formation depends on a conserved set of mesodermal determinants, including the gene *twist* (Spring *et al.*, 2000, 2002; Muller *et al.*, 2003). Furthermore, patterns of gene expression during ctenarian embryogenesis reveal a clear plane of bilateral symmetry (Yamada and Martindale, 2002), and ablation experiments demonstrate that the radial symmetry supposedly typical of diploblasts is a derived character superimposed on a basically bilateral body plan (Houliston *et al.*, 1993). This re-appraisal of the «lowly diploblasts» is only just beginning, however, and too few data are yet available to draw meaningful conclusions about their neural development. Cnidarians and ctenarians will therefore not be considered in this paper.

The classical views about triploblast phyletic relations have also changed dramatically during the past decade, with the conventional separation between acoelomates, pseudocoelomates and coelomates being replaced by a subdivision that separates ecdysozoans (including nematodes and arthropods) and lophotrochozoans (including annelids, molluscs and platyhelminths) (Aguinaldo *et al.*, 1997; rev. in Adoutte *et al.*, 2000). Thus model organisms that were once thought to be very distantly related, e.g. *Caenorhabditis* and *Drosophila*, are now considered to belong to the same superphylum.

Due in part to these changes in perspective, and also to the accumulated knowledge, the list of features that are common to most triploblast nervous systems has been considerably extended during the past decade. As such features were presumably also present in their last common ancestor, we can now form a more complete view of the urbilaterian nervous system and of its devel-

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Note 1: "Nothing is dead but that which lives not yet / Next to the shining past, tomorrow is colorless / It is shapeless too next to the accomplished / Which reveals at the same time both effort and effect". [Translation by A. Ghysen].

opment than was possible ten years ago. In this paper I will review some of these features, to illustrate the unexpected sophistication of *Urbilateria's* neural equipment.

As the special issues of the *Int. J. Dev. Biol.* are a privileged place to venture into somewhat speculative grounds, I will also explore possible explanations for the puzzling conclusion that a single, highly evolved species is the ancestor of all living triploblasts, while one would have thought that the earliest metazoans had many evolutionary avenues open to them.

1. The nervous system of *Urbilateria*

Sensory nervous system: mechanosensory organs

The sensory systems of all triploblasts comprise various types of sense organs, underlying distinct sensory modalities. Obvious similarities have been noted between sense organs of arthropods and of vertebrates, highly suggestive of a common origin. Here I will concentrate on the mechanoreceptors, as I know this system best. The case of olfaction and gustation is equally compelling, however, and the case of the photoreceptors will be discussed in another chapter of this issue.

The mechanosensory bristles of flies (Fig. 1A) are composed of a bipolar sensory neuron with a specialized microtubular body at the tip of its dendrite. The dendrite is surrounded by different types of support cells, one of which produces the stiff shaft that amplifies the touch sensitivity of the neuron. The axon is accompanied by a glial cell. The entire set of cells is generated from a precursor cell by a fixed lineage. Precursor cells arise within an epithelial field of cells made competent through the expression of one or more control genes of the bHLH type (proneural genes). The choice of the precursor cell involves a process of lateral inhibition mediated by the Notch-Delta interaction.

The most primitive mechanosensory organs of vertebrates are the lateral line organs (neuromasts) that are found on the body of fish and amphibians (Fig. 1B). The lateral line is a wave-detecting system closely related to the inner ear. The neuromasts are composed of sensory hair cells, which form an elongated microtubular process called the kinocilium. The hair cells are surrounded by two types of support cells, which secrete the stiff cupula that ensheathes the kinocilia and modulates their sensitivity. They are innervated by bipolar neurons which are accompanied by glial cells. The different types of cells are not generated by a fixed lineage, but they all arise from an epithelial field, or placode. The formation of this field, and the choice of cell fates within the placode, involves the expression of one or more proneural genes and the action of the Notch system.

The remarkable similarities between the fly bristles and the vertebrate octavolateral system (inner ear + lateral line) have already been noted, and leave little doubt that both are derived from a common ancestor (Adam *et al.*, 1998, Eddison *et al.*, 2000, Fritsch *et al.*, 2001). Differences like the fixed lineage that generates the bristle organs in arthropods, or the specialized hair cells that mediate wave detection in vertebrates, are presumably late additions to the original system. The latter may have consisted of superficial sensory neurons endowed with a mechanosensory dendrite, of support cells that would both protect and modulate the sensitivity of the dendrite, and of glial cells. All three cell types probably originated from epithelial cell patches rendered competent by the local expression of proneural genes. The allocation of

cell fates within these patches may have involved cell interactions mediated by the Notch system.

It is still not clear whether the ancestral sensory neuron evolved into the specialized hair cell, or whether the hair cell arose *de novo* (as did the ciliary photoreceptor cells in the vertebrate retina, whose ganglion cells seem to be homologous to the rhabdomeric photoreceptors of invertebrates, Arendt *et al.*, 2002). One possibility is that the ancestral sensory neuron duplicated, with one copy becoming specialized in the sensory function (the hair cell), and the other retaining the signal transmitting function (the neuron). Indeed, the hair cell retains many properties of a true neuron, including its presynaptic specialization. Not only could this separation of functions have facilitated cell specialization, but it would also open the possibility of coupling a single neuron to a larger number of sensory cells, thereby increasing the sensitivity of the system. Interestingly the same situation occurs in the visual system, where single ganglion cells are also coupled to a large number of ciliated photoreceptors.

Central nervous system: orthogonality

The central nervous system of all triploblasts is essentially a three-dimensional structure derived from a two-dimensional epithelium. It is built along three axes, antero-posterior, dorso-ventral, and apico-basal. Development along each axis relies on its own developmental mechanisms, and generates its own range of cell specificities. The central nervous system is, therefore, basically an orthogonal structure.

The vertebrate and arthropod nervous systems have long been considered to be extremely different due to their position in the body plan (dorsal in vertebrates and ventral in arthropods). Back in the XIXth century, however, it had already been suggested that the difference was more superficial than profound (for a scholarly review of this long-standing dispute, see Nübler-Jung and Arendt, 1994). The idea that the nervous systems of protostomians and deuterostomians are homologous in spite of their different positions has been revived more recently (Arendt and Nübler-Jung, 1994, Ghysen, 1992). This view is completely supported by the discovery that the genetic system underlying the D/V axis is actually conserved between the two phyla, with the neural (dorsal) region of deuterostomians being truly homologous to the neural (ventral) region of protostomians (De Robertis and Sasai, 1996).

Thus, what has long been considered as an inversion of the body plan may turn out to be a trivial consequence of the formation of a new mouth in the deuterostomian lineage (Nübler-Jung and Arendt, 1996). In this context it is interesting to note that when the chordate *Amphioxus* is swimming it has its neural side up, as decent chordates do, but when it lies buried in the sand it has its neural side down, much as a legitimate protostomian would, whereby its mouth opens in free water (Nübler-Jung, pers. comm.).

Central nervous system: connectivity

Axon guidance along the longitudinal and transversal axes has now been largely clarified in protostomians (mostly in the fly and the nematode). Analyses in deuterostomians (chicken, toad and mouse) have demonstrated the implication of conserved sets of molecules in conserved aspects of axonal guidance (rev. in Arendt and Nübler-Jung, 1999). For example, the role of the *robd/slit* system in the control of midline crossing has been well documented, and is strongly conserved across phyla (Hivert *et al.*, 2002). It has been

shown recently that the same system is also involved in the setting up of longitudinal fiber tracts at various distances from the midline (Rajagopalan *et al.*, 2000, Zlatic *et al.*, 2003). Whether this function is conserved in vertebrates has not yet been established. Whatever the case, the universal implication of the *robo/slit* system in everything that concerns the relation between neurons and midline indicates that this system must have been firmly rooted in the development of the urbilaterian nerve chord.

Likewise, the roles of the netrin/DCC system have been convincingly demonstrated in protostomes (fly, nematode) as well as in deuterostomes (chick, mouse, zebrafish). The molecular and functional conservation of axon guidance mechanisms suggests that a robust orthogonal organization already characterized the urbilaterian nerve chord. Whether elementary functional circuits were already genetically designed within this orthogonal net, and could have been inherited by all derived animals, has not been demonstrated but seems plausible enough.

Central nervous system: cell diversity

The previous sections strongly suggest that the urbilaterians already possessed the molecular mechanisms that underly axon guidance relative to the body axes. Their axons were able therefore to build the orthogonal scaffold of fibers that is so typical of all triploblastic nervous systems. Such design, however, requires that different neuronal cell types be specified during development. Is there any indication that such a diversity of neuronal identities already existed in the urbilaterians?

An important difference between arthropods and vertebrates is the mode of formation of their nerve chord. While in arthropods neuroblasts delaminate from the epithelium, in vertebrates the entire neuroepithelium invaginates to form the neural tube. This difference may be more impressive than profound, however (rev. in Arendt and Nübler-Jung, 1999). Indeed, in both cases neurons are produced by unequally dividing cells, where one of the two progenies continues dividing in a stem-cell mode, while the other becomes neuronal (or undergoes a very limited number of additional divisions).

Given the major importance of the capability to generate defined types of neurons in a defined succession, it may be that no complex nervous system could evolve without the ability to program and control asymmetric divisions. In flies, where the mechanism of this asymmetry has been best analysed, one of its major protagonists is the product of the gene *numb*. The Numb protein is localized at one pole of the dividing cell as a cortical crescent, and determines the fate of the daughter cell by which it is inherited (Rhyu *et al.*, 1994). There is now good evidence that this process has been conserved in vertebrates (Shen *et al.*, 2002, Cayouette and Raff, 2002), indicating that mechanisms to program cell diversity already existed in the urbilaterian central nervous system.

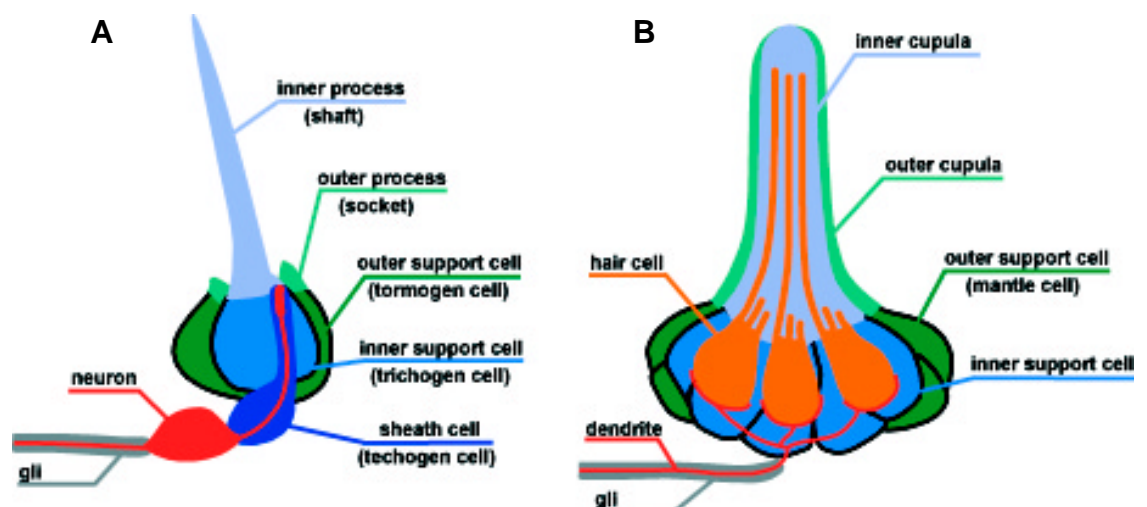
Neuronal cell diversity depends on the differential expression of a large number of transcriptional regulators which are common to all triploblasts. The corresponding genes have been grouped in families according to their type of DNA-binding domain. Most families already comprised many members in the *Urbilaterian* ancestor, suggesting a high level of genetic complexity in neuronal specification. To take but one example, it has been estimated that the urbilaterian genome already comprised 35 distinct bHLH genes, each of which is still recognizable in the genomes of flies, nematodes and vertebrates (Ledent and Vervoort, 2001). Most of these bHLH genes are involved in the determination of cell diversity within the nervous system.

Antero-posterior organization of the central nervous system

A prominent feature of the central nervous system is its longitudinal subdivision into distinct domains. Based on morphological and embryological grounds, the vertebrate CNS is traditionally subdivided in two major parts, the brain and the spinal cord, with the brain itself being subdivided in an anterior (forebrain), an intermediate (midbrain) and a posterior (hindbrain) region (Fig. 2A). The arthropodian CNS is subdivided in an anterior part, the cerebral ganglion (also called brain), an intermediate part extending from the oesophagus to the neck (suboesophageal ganglion) and a caudal part, the ventral nerve cord made of a succession of segmental ganglia (Fig. 2B).

Fig. 1. Schematic diagrams of mechanosensory organs in an insect and in a fish. (A) depicts a mechanosensory bristle in *Drosophila*, while (B) depicts a lateral line neuromast in *Danio rerio*.

In both cases the basic structure of the organ is one or more mechanosensory cell(s) surrounded by several support cells which generate the external structure of the organ, and accompanied by glial cells. In the fly bristle, mechanoreception and signal transduction are done by the same cell, the sensory neuron. In the fish organ (and in the inner ear sensory epithelium, which is evolutionarily related), mechanoreception takes place in specialized hair cells, while signal transduction occurs in the peripheral and central axons of the sensory neuron. This difference may have allowed for an increased sensitivity in the vertebrate system, since the information from several hair cells converges on a single sensory neuron.



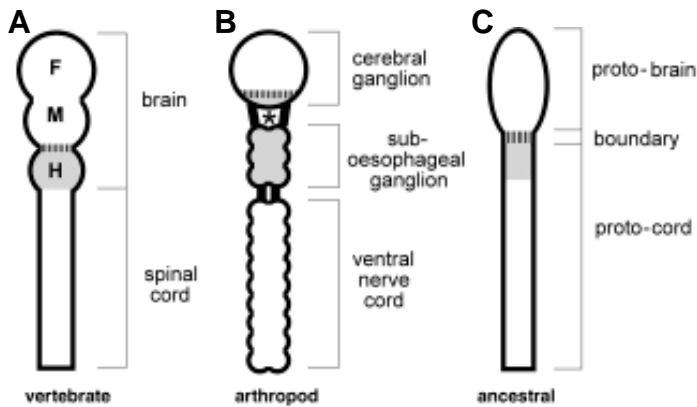


Fig. 2. Basic structure of the mammalian (A) and arthropodian (B) central nervous systems. At the right of each system are indicated its traditional subdivisions. The common (ancestral) structure is deduced in (C). The vertical hatching identifies the midbrain-hindbrain boundary in vertebrates and the homologous structure in insects. The light gray identifies the cephalized regions of the nerve cord: hindbrain in vertebrates, tritocerebrum and suboesophageal ganglion in *Drosophila*. Abbreviations: F, forebrain; M, midbrain; H, hindbrain; *, oesophagus.

The search for homologies between these two systems, and with other type of CNS such as the molluscan or the echinodermal, has long seemed doubtful at best, meaningless at worst. The situation has now changed with the recognition of a special region called midbrain-hindbrain boundary (MHB; vertical stripes in Fig. 2A), which plays a major organizing role in the development of the vertebrate brain (rev. in Wurst and Bally-Cuif, 2001). Not only is this «boundary» essential for the patterning of the CNS on either side, but it also defines the transition between two regions with very different genetic requirements.

The region anterior to the MHB expresses the *Otx* genes, which are homologous to the *orthodenticle* (*otd*) gene of the fly. *Otx* genes are necessary for the development of this region (rev. in Simeone, 1998), much as the *otd* gene is required for the development of the anteriormost part of the fly CNS, and indeed the mammalian *Otx* can functionally substitute for the fly gene in *otd* mutants (Leuzinger *et al.*, 1998, Nagao *et al.*, 1998). Homeotic genes, on the other hand, are expressed in the region of the nervous system posterior to the MHB, but are excluded from the anterior region both in mammals and in flies (rev in Hirth and Reichert, 1999).

The MHB region itself (striped section in Fig. 2A) is characterized by the expression of the *Pax2/5/8* genes (rev. in Wada and Satoh, 2001). Hirth *et al.* (2003) have recently shown that the fly counterparts of the *Pax 2/5/8* genes, *pax2* and *poxn*, do not have adjacent expression domains except at one place: the boundary between the second and third subdivision of the fly brain (striped section in Fig. 2B). As the anteriormost homeotic gene *labial* is expressed in the third subdivision of the fly brain, the *pax2/poxn* stripe separates an anterior domain expressing *otd* from a posterior domain expressing the homeotic genes. There is little doubt, therefore, that the *Otx-Pax-Hox* subdivision of the mammalian brain is strictly homologous to the similar subdivision now reported in flies, and therefore was in all likelihood present in urbilaterians (Hirth *et al.*, 2003).

In order to facilitate the discussion, and since neither the mammalian nor the fly traditional subdivisions of the CNS take

into account this major organisational feature, we will adopt the nomenclature proposed in Fig. 2C, where the region of the CNS anterior to the MHB will be called protobrain, and the posterior region will be called protocord. The case of the anterior, cephalized part of the protocord (the mammalian hindbrain; light gray in Fig. 2) will be discussed separately.

Protobrain

The overall design of brain connectivity is surprisingly similar in arthropod and vertebrate representatives (Fig. 3). The details of the processes by which such early tracts are established, and the genes that are responsible for this establishment, have not yet been elucidated. It is therefore difficult to decide whether or not the apparent similarity of wiring diagrams reflects a shared ancestry or not. Nevertheless what has been learned about axonal pathfinding in general, and about the formation of commissural tracts in particular, suggests that similarities in the basic connectivity may well represent homology rather than analogy, and therefore that a pattern not too dissimilar from what is shown in the figure may have existed in the urbilaterian and have been inherited by arthropods and chordates alike.

An important component of the brain are the olfactory centers: the antennal lobes in flies, and the olfactory bulbs in vertebrates. In both cases, the basic organization of the olfactory projection relies on the convergence of sensory axons that share the same odorant receptor onto a single projection center - a glomerulus (Hildebrand and Shepherd, 1997). This type of projection is relatively unusual in that most other sensory systems establish a somatotopic organization where the position of each axon terminal reflects the position of the sense organ, rather than its specificity. The similarity in glomerular organization between flies and mice suggests that the major circuitry of the olfactory system, based on the convergence of similar inputs to specific glomeruli, already existed in the urbilateria.

Another important component of the protobrain is the visual system, which is associated to this region both in vertebrates and in arthropods (Fig. 3). There seems to be an important difference between the two types of visual systems, as the vertebrate retina arises as a part of the CNS, while the arthropod retina develops peripherally. Yet, structures as dissimilar as the vertebrate and the many types of invertebrate eyes are now recognized to have derived from a simple, prototypic eye already present in urbilaterians. The apparent differences in the visual systems of arthropods and vertebrates may therefore have to be reexamined when we know better the genetic networks involved in setting up the neuronal circuitry that underlies vision.

Hindbrain genes

Just posterior to the MHB boundary region lies a region which is called hindbrain in chordates, and sub-oesophageal ganglion in insects. As mentioned previously, this region differs from the brain in that it is clearly segmented, and its segmental diversity is patterned by the *Hox* genes (which are excluded from the protobrain). This region also differs from the more caudal part of the nerve chord in that it is massively involved in the control of head-related and vegetative functions. It can therefore be considered as a cephalized part of the protocord (light gray in Fig. 2).

In arthropods this region comprises three segments, each subdivided in two compartments. The recent discovery that the

most posterior part of the fly brain, the tritocerebrum, is posterior to the boundary and expresses a homeotic gene, suggests that the cephalized cord actually comprises four segments. In chordates there is some doubt as to whether the intermediate region comprises 8 segments, as usually considered, or 4 segments each subdivided in two compartments, as many features suggest.

While it may be that this specialized region appeared independently in arthropods and in chordates as a cephalization of the anteriormost part of the segmented nerve cord, it would seem more economical to imagine that urbilaterians already possessed a four-tiered CNS with a brain proper (protobrain), a boundary (MHB) region, a segmented hindbrain, and a nerve cord.

Hindbrain function

In vertebrates the hindbrain is largely devoted to the afferent and efferent innervation of the branchial arches. As the mouthparts are derived from the branchial arches, gustation is well represented in the hindbrain, as is facial touch perception. The chordate hindbrain is also involved in the control of many essential vegetative functions such as breathing (that is, forced aeration of the lungs), blood circulation (heart action and blood pressure), nutrition, and other reflex mechanisms such as coughing and vomiting (Kuhlenbeck, 1975).

In spite of major changes in habitat and physiology, major features of the hindbrain organization have remained constant throughout vertebrates. For example, the afferent projection of the lateral line of fish is very similar to the projection of one of its mammalian derivatives, the cochlea (Alexandre and Ghysen, 1999), even though the former is involved in a sense of «distant touch» (Dijkgraaf, 1989) and has disappeared in terrestrial tetrapods, while the latter is involved in sound perception. Likewise, the control center maintaining the respiratory rhythm in man still occupies its primitive position in the medulla, even though the respiratory mechanism of air breathing vertebrates is completely different from that of gilled fishes (Herrick, 1926). Thus, many features of the hindbrain are highly conserved in all vertebrates, in spite of large functional changes in the peripheral organization.

Since branchial arches are considered to be a typical chordate structure, one could imagine that the hindbrain is a chordate invention without counterpart in insects. This may be untrue, however. In insects, the corresponding region of the central nervous system comprises the three gnathal segments, which together form the sub-oesophageal ganglion. These segments are associated with the mouthparts, and are correspondingly specialized. They are largely concerned with gustation and facial touch perception (Mitchell and Itagaki, 1992), and they receive the afferent projection of the taste-sensitive neurons on the proboscis.

As for the control of vegetative functions such as breathing and circulation, there would seem again to be little common ground between chordates and insects, as in the latter breathing mostly involves passive diffusion through the tracheal system. It has recently become clear that breathing is more sophisticated in arthropods than previously assumed, however (e.g. Westneat *et al.*, 2003), and subject to a tight CNS control which however remains to be elucidated. It will be interesting to see if some of the centers involved in the control of arthropod vegetative functions are located in the suboesophageal ganglion.

In summary, then, it seems at least possible that the anteriormost section of the protochord was already specialized in Urbilateria,

dealing with gustation and «facial» sensitivity, as well as with the control of rhythmic vegetative functions.

2. Conservation and change in evolution

In this second, more speculative section, I will explore the idea that all extant triploblasts arose from a single, highly sophisticated ancestral species. The proposal I will argue for is that this unexpected monophyly derives at least in part from two linked factors. First, a major constraint of metazoan evolution is that, because of the complexity of the developmental programme of any organism, changes are not allowed (rule of conservative changes). Second, only those developmental processes that have become extremely stable can withstand substantial variation without collapsing.

The rule of conservative changes

The rule of conservative changes states that only those changes can be tolerated, that change essentially nothing. This rule applies to any set of interacting elements, where changes in any one component will alter all the interactions in which this component is involved, and adversely affect the function of the entire set. The stringency of this rule will obviously increase with the number of interactions, as it becomes more and more unlikely that a single change in one element can improve, or at least not harm, the result of the total sum of all interactions.

Biological organisms all undergo a developmental process that leads from the zygote to the reproductive stage. The rule of conservative changes implies, therefore, that any mutational change is first and foremost screened for its compatibility with every step of the developmental program. This is because the only absolute requirement is that the entire program remains functional, i.e. retains its coherence.

Given the complexity of the nervous system, the requirement for maintaining coherence must have been most stringent during the evolution of neural development, and the rule of conservative changes must have had particular relevance for this process. It is no surprise, therefore, that this rule seems to pervade all aspects of developmental neurobiology, from molecules to mind. This I will illustrate by two examples, one at either end of the spectrum of application.

At the level of molecules, to take but one example, the majority (8/14) of the most conserved amino-acids of the homeodomain

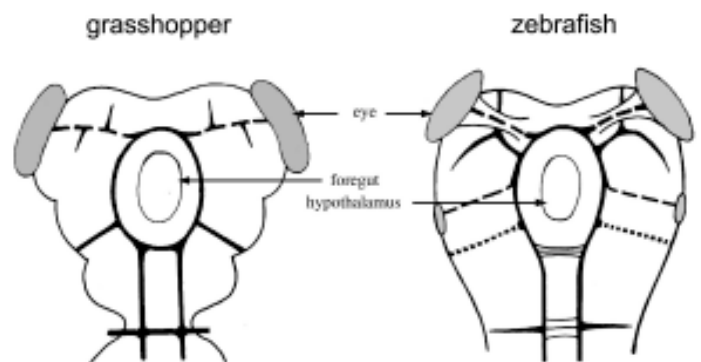


Fig. 3. Early tracts in the anterior central nervous system of an insect (locust, left) and of a vertebrate (zebrafish, right). Adapted from Arendt and Nübler-Jung, 1996.

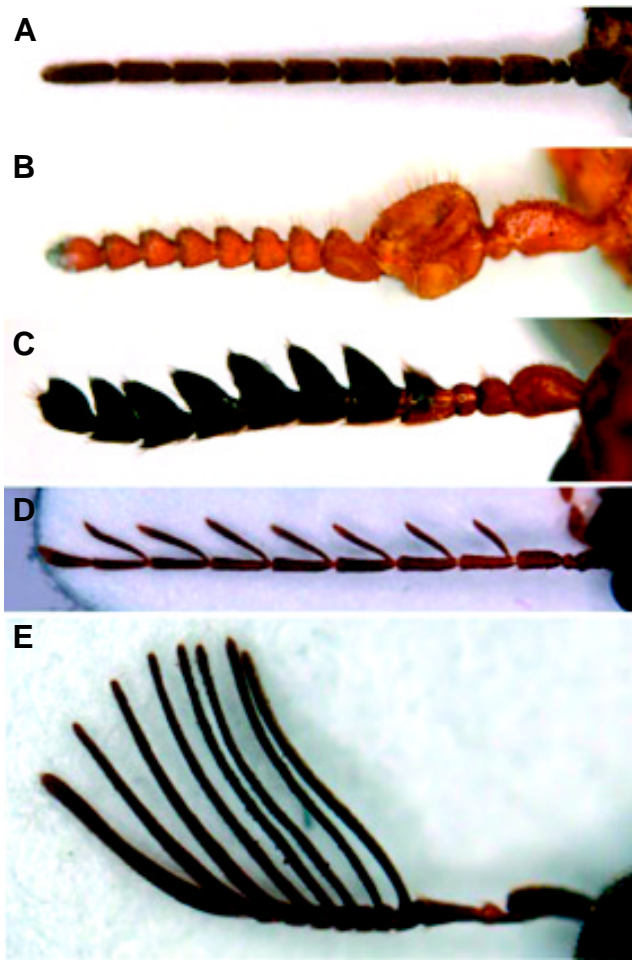


Fig. 4. Eleven-segmented antennae in various coleopteran species. (A) *Cupedidae*: *Tenomerga cinereus*; (B) *Melyridae*: *Malachiinae*; (C) *Chrysomelidae*: *Anomoea* sp.; (D) *Ptilodactylidae*: *Ptilodactyla* sp.; (E) *Eucnemidae*: *Isorhipis obliqua*. The *Ptylodactylidae* (D) are unique among all insects in that the antennal segments 4 to 10 have a basal articulated segment, rather than a simple expansion as in e.g. the *Eucnemidae* (E). Anterior is up in all panels. All micrographs and identifications kindly provided by Dr. N. Gompel.

are not involved in the biological function of the domain - binding to DNA. Of the 14 most conserved residues of a set of 346 homeodomain sequences, only 3 bind to bases, and 3 bind to the sugar-phosphate backbone. The 8 remaining residues ensure the structural stability of the domain by contributing to the hydrophobic internal core (Duboule, 1994). Thus many changes can be (and have been) accepted and eventually fixed, as long as they do not affect the internal coherence of the homeodomain.

At the other extreme, that of mental processes, any new information appears to be instantly screened by the human brain for its coherence with previous information, a pre-attentive process which in the auditory system is associated to an electrical brain response called mismatch negativity (Näätänen, 1992, Koelsch *et al.*, 2002). Interestingly, this process seems to be accentuated in autistic patients, and the acute awareness of discordances may be at the root of their inability to adjust to changing conditions (Seri *et al.*, 1999, Gomot *et al.*, 2002).

Conservative development and tolerance

Coming back to the issue of the developmental programme, one is led to the idea that once a coherent organization has been produced, it is further stabilized by the progressive accumulation of additional mechanisms. Because all changes are screened for their consistency with the existing organization, such additional mechanisms will necessarily reinforce and ensure developmental stability in a wide range of adverse situations. The result of this evolution would be an ever increasing robustness of the system, and an increased capability to follow, or revert to, a relatively normal course in the presence of perturbations - what Waddington called «canalization».

As pointed out recently, the progressive reinforcement and stabilization of developmental processes will buffer the individual from the effect of mutation, and the population as a whole may therefore carry more genetic variation (Kirschner and Gerhart, 1998). Populations with a wider polymorphism may diversify faster under selective conditions, thus improving the «evolvability» of the species, and the success of its genome. This potential for rapid diversification would of course be particularly advantageous during the extensive radiations that followed massive extinctions.

The rule of conservative changes puts the emphasis on negative selection - screening for the adaptation of each new element to all other elements with which it interacts, and removing any change that alters the pattern of interactions (and thereby the developmental programme). This view is complementary to the more usual view of evolution as based on positive selection - selection for the fittest. While the latter focuses on the interaction of the individual with the external world, the former focuses on the adaptation of individual gene products to the internal world. Given that all internal interactions are optimized in any given species (for, if they were not, positive selection would quickly ensure that they are), it follows that any non-conservative change will be detrimental and can only be, at best, tolerated (Garcia-Bellido, 2000).

Thus the concept of tolerance to genetic changes that reduce the fitness of the individual may be an important factor to understand evolution, in particular in the case of the extensive radiations that follow massive extinctions. It may be urgent to incorporate the massive knowledge gained over the past 20 or so years in our understanding of the genetic bases of development, into our current views of evolution, selection and adaptation.

Odd numbers

The accumulation of largely (but not entirely) redundant mechanisms will lead, as a corollary, to the puzzling observation that seemingly arbitrary patterns appear inordinately resistant to change. This conclusion is somewhat different from the classical comment «if it works, why fix it»? According to the latter, once the number of fingers settled on five (for whatever reason, and more likely than not without any reason), it has remained so ever since because there was no pressing reason for it to change. My proposal differs from this simple inertia in one important feature: the progressive accumulation of developmental additions that are consistent with the original pattern will make it ever more resistant to change, much like «plugins» may add to the performance of a system but can never change its basic structure, and indeed generally contribute to its stability.

Pushing the argument one step further, one can speculate that it is only those processes that are highly robust, which can then be modified to extraordinary extents without losing their develop-

mental stability. Indeed the five fingers of tetrapods are famed for the incredible range of structures they can give rise to, from wings to fins through toes and our agile hands. Likewise the nonsensical fixation of most coleopters for 11-segmented antennae is accompanied by large variations in the identities of the various segments (shape, length, color, sense organs present). Roughly speaking one can apparently get the most insane-looking antenna, as long as it counts 11 segments (Fig. 4). It is tempting to invert the argument, and to imagine that it is because the 11-odd developmental system had reached an extreme robustness in some ancient coleopteran that it could accept large variations in many of its components without collapsing. From that stage on, this developmental system could then generate antennae with many different morphologies - yet necessarily obeying the rule of eleven.

Robustness and adaptation in the nervous system

Let us now consider how this general idea could be applied to the nervous system. In a penetrating short paper published in *IJDB*, Michael Bate made the important point that «neurons are born and differentiate in ways that are not conditioned by their future functions as elements of neural circuits» (Bate, 1998). He went on to say that «To understand how functions ... can emerge from these beginnings, it is worth remembering that fundamental attributes of the nervous system such as the circuitry underlying locomotion or escape behavior are probably also present as a rather stereotyped and evolutionarily conserved set of cells and connections. It is at least possible to envisage that there is a fundamental framework of circuitry just as there is a scaffolding of initial pathways».

One would imagine that there has been a strong selective pressure to make such «fundamental frameworks of circuitry» as stable as possible from a developmental point of view. This involves not only stabilizing the formation of the individual circuits, but also providing for general means to adapt them to unforeseeable perturbations, i.e. general mechanisms of functional plasticity (e.g., learning) and of developmental plasticity (e.g., elimination). Both types of plasticity are indeed found in all triploblasts, and seem to rely on similar mechanisms, suggesting again that they were already present in our urbilaterian ancestors (e.g. Goda, 1995, Friedrich, 2000). It has been argued that there must also be a mechanism to assess and adjust the functional connectivity of the circuit to optimize its performance (Bate, 1992). The nature of this mechanism is still not understood, however.

The central nervous system would then be largely composed of a combination of proven, well-tried, trustworthy elementary circuits, many of which were already developed in our urbilaterian ancestors. Such circuits may have become integrated in various physiological functions as diversification proceeded, a diversification that presumably took place both in space and in time. For example, mutations in the *HoxA1* gene, which in mice is expressed only transiently in rhombomeres r3 and r4 at a very early stage of hindbrain development, result in the formation of a supernumerary respiratory center (del Toro *et al.*, 2001). This result reveals the presence of groups of interconnected cells in r3, that can form a fully active respiratory center provided it is not modified into some other function by *HoxA1*.

In the previous section I developed the argument that it is only after a process has become developmentally stabilized that it can undergo large changes without compromising its function. Applying this argument to the nervous system, one is led to the conclu-

sion that once the «fundamental frameworks of circuitry» have become stable enough, changes and adjustments can be introduced in individual circuits without perturbing the functioning of the entire system. This, of course, requires that there are genetic means to single out one circuit among all others (Brunet and Ghysen, 1999). The patterned expression of transcriptional regulators with homeotic, bHLH, paired-box and other conserved motifs in the central nervous system may be a simple way to single out neural subsets. Once genetically identified, each subset can evolve in its own way: neural subsets become «uncoupled» from each other, and freed to explore new directions without impairing the development and function of the entire system - provided, again, that such changes are compatible with the basic structure common to all subsets.

The extreme variability of behaviors and survival strategies among triploblasts would then be subordinate on the previous attainment by the urbilaterians of a high level of developmental stability in the building of elementary functional circuits. According to this view, the initial triploblast radiation may have been contingent upon reaching this highly evolved stage of neural development. Conversely, once this stage of neural development had been reached, and once the radiation had become possible, it did not leave much room for a reiteration of the entire process. It follows that the major radiation is bound to have occurred only once, and only from one ancestor: the one whose neural development had reached a level of complexity, and of developmental stability, robust enough to withstand large changes without losing sense.

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