Larval nervous systems: true larval and precocious adult

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ABSTRACT

The apical organ of ciliated larvae of cnidarians and bilaterians is a true larval organ that disappears before or at metamorphosis. It appears to be sensory, probably involved in metamorphosis, but knowledge is scant. The ciliated protostome larvae show ganglia/nerve cords that are retained as the adult central nervous system (CNS). Two structures can be recognized, viz. a pair of cerebral ganglia, which form the major part of the adult brain, and a blastoporal (circumblastoporal) nerve cord, which becomes differentiated into a perioral loop, paired or secondarily fused ventral nerve cords and a small perianal loop. The anterior loop becomes part of the brain. This has been well documented through cell-lineage studies in a number of spiralian lineages, and homologies with similar structures in the ecdysozoans are strongly indicated. The deuterostomes are generally difficult to interpret, and the nervous systems of echinoderms and enteropneusts appear completely enigmatic. The ontogeny of the chordate CNS can perhaps be interpreted as a variation of the ontogeny of the blastoporal nerve cord of the protostomes, and this is strongly supported by patterns of gene expression. The presence of ‘deuterostomian’ blastopore fates both in an annelid and in a mollusk, which are both placed in families with the ‘normal’ spiralian gastrulation type, and in the chaetognaths demonstrates that the chordate type of gastrulation could easily have evolved from the spiralian type. This indicates that the latest common ancestor of the deuterostomes was very similar to the latest common pelago-benthic ancestor of the protostomes as described by the trechaean theory, and that the neural tube of the chordates is morphologically ventral.

KEY WORDS: Deuterostomy, Metamorphosis, Nervous systems, Dorsal–ventral orientation

Introduction

Studies on ontogeny are very important for our understanding of nervous system evolution and diversification, and new methods have made important contributions to knowledge of the structure and development of larval nervous systems and their relationships to adult nervous systems. The nervous system has for a long time been considered one of the most conservative animal organ systems and has therefore been given high importance in studies of animal evolution. More than a century ago, Hatschek (Hatschek, 1888) introduced the names Zygoneura (now Protostomia), Ambulacraria and Chordonia (now Chordata) for three major groups of the Bilateria, based on the structure and position of the central nervous system (CNS). The Zygoneura were characterized by a paired longitudinal ventral nerve cord [a cluster of nervous cells including their cell bodies, as opposed to a nerve, which is a bundle of axons (Richter et al., 2010); the ventral nerve cord may be specialized into rows of ganglia connected by connectives] and the Chordonia by an unpaired dorsal neural tube. The division of the Bilateria into Protostomia and Deuterostomia (=Cordonia + Ambulacraria) (Grob, 1908) is still universally accepted and is now supported by numerous phylogenomic studies (Hejnol et al., 2009; Wheeler et al., 2009; Edgecombe et al., 2011). However, the interpretation of the dorsal/ventral orientation of the two groups has been challenged, and it now appears that the two longitudinal nerve cords are homologous (see below).

The topology of the bilaterian part of the animal tree of life is relatively well established, but there is not agreement about the inter-relationships of the basal metazoan groups. In particular, the position of the Ctenophora has recently come into focus. The classical view has been that the Ctenophora together with Cnidaria form the clade Coelenterata, but a number of recent phylogenomic studies (Dunn et al., 2008; Hejnol et al., 2009; Maxwell et al., 2012; Nesnidal et al., 2013) have placed the Ctenophora as the sister group of all the remaining metazoans, and the recent studies of the whole genomes of Mnemiopsis leidyi (Ryan et al., 2013) and Pleurobrachia belcheri (Mroz et al., 2014) support to this position. This implies either that the nervous systems (and muscles) have been lost in sponges and placozoans or that the nervous systems and muscles of Ctenophora and Neuralia (Cnidaria + Bilateria) have evolved convergently. However, loss of the nervous system appears highly unlikely, and the phylogenomic analyses may suffer from long branch attraction, because the latest common ancestor of the living ctenophores could have been as recent as around the K–T (Cretaceous–Tertiary) boundary (Podar et al., 2001; Jékely et al., 2015). The cydippid stage characteristic of the ctenophore life cycles must be interpreted as a juvenile rather than a larva (Ryan et al., 2013), both because it lacks special larval organs and because it can already carry out sexual reproduction (Martindale, 1987). The ‘apical organ’ of the mainly holoplagic ctenophores is situated at the apical pole, but its structure is completely different from that of cnidarians and bilaterians (Hernandez-Nicaise, 1991) and it is not considered homologous of the neuralian apical organ (Jager et al., 2011). The discussion of larval nervous systems can therefore be restricted to the nervous systems of Cnidaria and Bilateria, i.e. Neuralia.

Ciliated primary larvae are found in numerous lineages of Porifera, Cnidaria, Spiralia (Lophotrochozoa) and Ambulacraria (Echinodermata + Hemichordata), but are absent in Ecdysozoa and Chordata (except for the non-feeding amphioxus larva). This larval type has been called primary larvae (Jagersten, 1972).

Within the Neuralia, the position of the Acoelomorpha (Acoela, Nemertodermatida and Xenoturbellida) is still debated, with the more traditional view that they are the sister group of the remaining bilaterian groups (Hejnol et al., 2009; Nielsen, 2010; Ryan et al., 2013), or the alternative view that they are the sister group of the Ambulacraria (Philippe et al., 2011). A discussion of their phylogenetic position falls outside the scope of the present paper, and the uncertainty dictates that they will not contribute to the present discussion.

Most recent authors agree that the eumetazoan ancestor was a gastraea, although this is rarely discussed directly (e.g. Knoll and Carroll, 1999; Brusca and Brusca, 2003). Most ciliated larvae have a ciliated sensory organ at the apical pole, and I have called the corresponding ancestor neurogastraea (Nielsen, 2008). Its organization was retained in the larvae when the pelago-benthic life cycle(s) evolved through addition of adult benthic stages; this and alternative theories for the evolution of the pelago-benthic life cycles have been discussed elsewhere (Nielsen, 2013). New nervous centers developed in the benthic adults and gradually became established in the larval stages, through the evolutionary process called adulthood (Jägersten, 1972), so that two types of nervous centers can be recognized in the larvae, viz. the exclusively larval apical organ and the larval–adult nervous system.

The following discussions are based on the framework of the trochaen theory (Nielsen, 2012), which appears to be the only theory that explains both the origin of the ciliary bands of the trochophora larva and the morphology of the protostomian CNS in a continuous series of adaptational modifications of existing structures.

**The apical organ: the ancestral neuralutive brain**

The apical organ is a characteristic, ciliated, putative sensory structure in most of the ciliated neuralian larvae. It develops from the most apical blastomeres, as shown in numerous cell-lineage studies (Nielsen, 2004; Nielsen, 2005). The apical group of long cilia works together as a compound cilium (cirrus) in many species (Fig. 1), but other species show a more generally ciliated area. The apical organ consists of a group of columnar or flask-shaped cells usually with one cilium, but in some species with several; it does not fit the narrow definition of a ganglion, because it apparently comprises only sensory cells (Richter et al., 2010). Many spiralians develop lateral (cerebral) ganglia in close apposition to the apical organ, and this compound structure has been called the apical organ in most of the older morphological literature, and this is also seen in some studies on gene expression (Tosches and Arendt, 2013). However, most recent papers use the term apical organ in the restricted sense in accordance with the terminology of Richter et al. (Richter et al., 2010). The homology of apical organs of cnidarians and bilaterians has been questioned because the animal–vegetal axis has the same orientation as the apical–blastoporal axis in the bilaterians, whereas the two axes have opposite directions in the ‘coelenterates’ (Dunn et al., 2007). However, new observations of gene expression clearly demonstrate that the apical pole of cnidarians and the apical pole of bilaterians are homologous (Sinigaglia et al., 2013; Marlow et al., 2014; Fritzenwanker et al., 2014).

**Cnidaria**

The apical organ of cnidarian larvae is well studied, especially that of the sea anemone *Nematostella*, where it consists of a group of monociliated nerve cells (Marlow et al., 2014). The organization of the organ seems rather similar in all groups, but the prominent ciliary cirrus seen in anthozoans (Fig. 1A) is lacking in the medusozoans (Nakanishi et al., 2008).

The apical organ is necessary for settling (Rentzsch et al., 2008). It is lost when the larva settles with cells around the apical pole (Yuan et al., 2008). The nervous system becomes reorganized with degeneration of the larval nerve net and development of a net of new neurons; a brain is lacking both in the polyps and in the medusae (Martina, 2000; Nakanishi et al., 2008).

**Protostomia**

A slightly twisted cirrus of long apical cilia is seen in many ciliated larvae of annelids, mollusks, nemerteans and phoronids (Nielsen, 1987; McDougall et al., 2006; Maslakova, 2010; Temereva and Wanninger, 2012) (Fig. 1B). There is usually only one cilium per cell, but multiciliate cells are known both from mollusks and from nemerteans (Cantell et al., 1982; Page, 2002). There is much variation in the number and structure of the cells in the organ. A characteristic pattern of eight flask-shaped serotoninergic cells, each with one cilium, is found in many species, but the number may increase during development, for example in the phoronid *Phoronopsis* (Temereva and Wanninger, 2012; Temereva and Tistrin, 2014). Other types of serotoninergic cells may also be present (Page, 2002).

Several annelids, mollusks, nemerteans and platyhelminths have been the subject of classical cell-lineage studies (reviewed in Nielsen, 2004; Nielsen, 2005) and the few modern studies have confirmed the earlier results in almost all details (Ackermann et al., 2005; Hejnol et al., 2007; Meyer et al., 2010). The apical organ differentiates from the most apical cells, named 1a1–1d1 in the spiral-cleavage terminology. The organ degenerates before or at the time of settling (Dickinson and Croll, 2003), and the cells may undergo apoptosis (Gifondorwa and Leise, 2006). In the gastropod *Crepidula*, the four apical cells degenerate at an early developmental stage, long before the larva hatches from the egg mass (Conklin, 1897). In the pildium larvae, the apical organ is shed at metamorphosis together with the whole larval body, which in some cases becomes ingested by the juvenile (Maslakova, 2010).

All entoproct larvae have a large apical organ with several types of cells; it disappears at metamorphosis (Nielsen, 1971).

Bryozoan larvae show wide variation in the structure of the apical region. Phylactolaemate ‘larvae’ show a concentration of...
serotonergic sensory cells at the apical pole, but an apical tuft is not present (Gruhl, 2010); the whole ciliated epithelium including the apical pole is invaginated at metamorphosis and degenerates (Brien, 1953). Nerve cells have not been observed in cyclostome larvae. Eurystome larvae show considerable variation. The planktotrophic cyphonautes larvae have a well-defined apical organ with a central area of neurons connected to a basal nerve plexus surrounded by ring-shaped areas of mononucleated and non-ciliated cells and one epitheliosmuscular cell. The function of this organ is unknown, but it is connected to the pyriform sensory organ, which is used in testing the substrate for settling (Stricker et al., 1988). All the larval organs degenerate after settling (Atkins, 1955). The lecithotrophic coronate larvae have a much more complicated apical structure, with a radial array of wedge-shaped multiciliated cells (Woollacott and Zimmer, 1971; Reed and Cloney, 1982; Reed et al., 1988). These cells are underlain by a nervous plexus, which sends a prominent nerve to the pyriform organ. A ring-shaped blastema below the ciliated cells surrounds the nerve. At settling, the neural plate is pulled down and disintegrates, and the ciliated cells and the lower blastema develop into the polypide of the first zoid (Reed and Woollacott, 1983; Fuchs et al., 2011). There is no connection between the apical organ and the nervous system of the polypides.

The function of the protostomian apical organ is poorly known, but certain cells in the organ of the gastropod Phestilla are necessary for recognition of the right substrate for settling (Hadfield et al., 2000).

Deuterostomia

In the ambulacarians (hemichordates + echinodermates), ciliated apical organs are found in enteropneusts and in species of all classes of echinoderms, although only some species show the typical, slightly twisted tuft and only in some stages, for example in some enteropneusts (Stiasny, 1914) (Fig. 1C). The various types of larvae show some variation in the structure of the nervous system at the apical pole, with more scattered groups of flask-shaped cells located at the most apical parts of the circumoral ciliary band (neotroch) (Byrne et al., 2007). So the morphology is not very reminiscent of the apical organ in cnidarians and protostomes, but gene expression supports the homology (Yaguchi et al., 2010), and the apical organs always degenerate before or at metamorphosis, both in echinoderms (Byrne et al., 2007) and in enteropneusts (Miyamoto et al., 2010).

The chordates do not have ciliated larvae and an apical organ is not present. The theory for the origin of the chordate CNS proposed by Garstang (Garstang, 1928) and presented in many textbooks shows the apical organ being internalized by the fusion of lateral ciliary bands and becoming situated in the brain. This idea has found some support from ultrastructure and gene expression studies (Lacalli et al., 1994; Tagawa et al., 2000), but it is incompatible with the theory for the evolution of the chordate CNS presented below.

The larval–adult CNS

The adult CNS begins to develop already in an early larval (or embryonic) stage in all bilaterians. In protostomes, it consists of a pair of cerebral ganglia and a blastoporal (circumblastoporal) nerve ring, where the anterior part of the ring and the cerebral ganglia together become the adult brain. The deuterostomes are more difficult to interpret, but both embryology and gene expression patterns of the chordate CNS indicate homology with the blastoporal nerve ring of the protostomes.

The recent papers by Tosches and Arendt (Tosches and Arendt, 2013) and Marlow et al. (Marlow et al., 2014) recognize the dual nature of the nervous system of the vertebrates; the cerebral ganglia are called the apical legacy or apical plate and the blastoporal nerve cord is called the blastoporal legacy or medio-lateral patterning. The ‘forebrain’ is described as a chimera. This is in good accordance with the descriptions and interpretations below.

Cnidaria

A concentration of nerve cells is seen around the mouth, but they cannot be characterized as a nerve cord (Koizumi, 2007; Marlow et al., 2009).

Protostomia

Both development and morphology of the adult central nervous system is quite well known in many spiralians (reviews in Nielsen, 2004; Nielsen, 2005), and its evolution has been explained by the trochaea theory (Nielsen, 2012). Typically, it consists of a pair of cerebral ganglia and a blastoporal nerve cord, which differentiates into the paired or fused ventral nerve cord with a perioral and a perianal loop (Fig. 2). This is best seen in annelids (Meyer et al., 2010), but the pattern can clearly be recognized, for example in mollusks (Dickinson and Croll, 2003).

The cerebral ganglia differentiate from two cells of the first micromere quartet in the spiral cleavage, from the cells 1c and 1d in the polychaete Platynereis (Ackermann et al., 2005), and from 1a and 1c in the gastropod Crepidula (Hejnol et al., 2007). The eyes found in many annelid and mollusk larvae develop from cells of the cerebral ganglia (Conklin, 1897; Ackermann et al., 2005).

![Diagram of the central nervous system of a protostome larva](Image)

Fig. 2. Central nervous systems (CNSs) in protostomes, exemplified by annelids. The upper two illustrations show the CNS of a trochophora larva and a juvenile, as interpreted by the trochaea theory [modified from Nielsen (Nielsen, 2012)], and the lower illustration shows the CNS of a late lecithotrophic larva of Capitella as demonstrated through a cell-lineage study (see Meyer et al., 2010). The shape of the circumblastoporal nerve ring in Capitella is exactly as predicted, except that the anal loop is missing; it may have been overlooked, and it is present in many other annelids.
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The embryological origin of the cerebral ganglia and blastoporal nervous system is difficult to deduce in groups without spiral cleavage. Phoronids and brachiopods seem to lack cerebral ganglia and are usually believed to lack the blastoporal nervous system. However, the large larva of Phoronopsis harmeri (Temereva, 2012) and the larva of the brachiopod Novocrania anomala (Altenburger and Wanninger, 2010) show paired longitudinal blastoporal nerves with a number of commissures, and this is very similar to the blastoporal nervous system of the annelids.

Entoproct larvae have a paired or unpaired frontal organ/ganglion, which may be modified cerebral ganglia. The organ degenerates after metamorphosis (Nielsen, 1971). Some observations on Loxosomella larvae indicate the presence of longitudinal nerves in the foot (Wanninger et al., 2007). Their fate is unknown. Bryozoan larvae lack cerebral ganglia and ventral nerve cords.

Both morphology and gene expression indicate that the cerebral ganglia of the annelids are homologous with the protocerebrum of the arthropods (Harzsch, 2004; Scholtz and Edgecombe, 2006) respectively (Meyer and Seaver, 2010). Observations of gene expression (Tosches and Arendt, 2013) are in full agreement with this interpretation (see below).

The neural tube in the tail is resorbed at settling. In the ascidians, ascidian larvae have a neural tube without nerve cells in the tail and are never seen in chordates (Kaul and Stach, 2010). Neither morphology nor gene expression give unambiguous information about the dorsal–ventral organization of this structure involves a number of genes also active in neurulation, and the radial nerve cords have been interpreted as homologs of the chordate neural tube (Haag, 2005). However, this must be interpreted as an example of homoplasy (Nielsen and Martinez, 2003; Nielsen, 2006). The tornaria larva has a posterior ring of large, compound cilia, which has often been called a telotroch, but its homology is uncertain (Nielsen and Hay-Schmidt, 2007). There is a nerve along this ciliary band (Lacalli and Gilmour, 2001), and both the ciliary band and the nerve begin to degenerate at metamorphosis. The nervous system of juvenile and adult enteropneusts comprises a dorsal collar cord in the region just behind the mouth, a longitudinal nerve cord extending from the collar cord posteriorly along the length of the body, and nerves around the pharynx to a longitudinal nerve cord along the ventral side. The collar cord develops through a chordate-like neurulation, and a number of the cell types and their distribution resemble those seen in chordates (Kaul and Stach, 2010). Also, gene expression shows strong similarities (Pani et al., 2012), but the gene expression patterns in the dorsal and ventral nerve cords of the body are very similar (Nomaksteinsky et al., 2009). Neither morphology nor gene expression give unambiguous information about the dorsal–ventral orientation (Holland et al., 2013).

The CNS of the chordates develops from the characteristic neural tube, which is specialized in different ways in the three phyla Cephalochordata, Urochordata and Vertebrata. The dorsal–ventral orientation of the cephalochordates can only be inferred through comparisons with the other chordates, because their ontogeny clearly indicates that their mouth is the modified first gill opening (Ruppert, 1997; Benito-Gutiérrez and Arendt, 2009). The anterior part of the neural tube differentiates into a brain vesicle, and various types of photoreceptors and other sensory organs are found along its length (Wicht and Lacalli, 2005).

The orientation of most of the adult urochordates is difficult to make out because of their metamorphoses with the rotation of the gut, but the development of certain ascidians, such as Ciona and Clavelina, shows that the stomodeaum/mouth is situated at the same side as the neural tube, which then ought to be described as ventral (Seeliger and Hartmeyer, 1893-1911; Veeman et al., 2010). The ascidian larvae have a neural tube without nerve cells in the tail and an anterior brain vesicle, in some species with an eye, a statocyte and various other sensory structures (Burighel and Cloney, 1997). The neural tube in the tail is resorbed at settling. In the ascidians,
A fresh view on the classical description of the embryology of the frog *Xenopus* (Hausen and Riebesell, 1991) with the apical pole kept in the same position during development clearly shows how the anterior end of the neural tube extends around the anterior end of the embryo (and the blastoporal/anal area around the posterior end) so that both the mouth and anus become situated on the side called dorsal in the protostomes (Fig. 5).

The character used for distinguishing Protostomia and Deuterostomia has always been the fate of the blastopore. The protostomian nerve cords typically differentiate along the fusing lateral blastopore lips, or from homologous areas in direct developing species, with the blastopore developing into the mouth, or in more modern interpretation into the mouth + anus (amphistomy). In the deuterostomes, the blastopore becomes the anus (Grobben, 1908). Amphistomy has never been observed in any deuterostome, but it is remarkable that an embryology with the blastopore becoming only the anus has been observed both in the urochordates and vertebrates as a modified anterior opening of an amphistome blastopore closure.

The question then arises whether a homolog of the protostomian cerebral ganglia could be present in vertebrates. The ‘apical legacy’ area of the vertebrate brain (Tosches and Arendt, 2013) is situated in the area corresponding to the episphere of the spiralian larva, so it could represent the cerebral ganglia with the eyes of the

Fig. 6. ‘Deuterostomy’ in protostomes. Median sections of embryos. In Viviparus, the blastopore directly becomes the anus and the whole archenteron becomes the stomach; the stomodeum develops from an area just behind the prototroch. The ventral foot area is the general area of the blastoporal nervous system, although there are no nerves in the midline. Modified from Otto and Tönninger (Otto and Tönninger, 1906). In Eunice, the archenteron becomes solid, but the anus breaks through in the area of the blastopore at a later stage; the stomodeum develops from an area just behind the prototroch. The ventral nerves develop from the ventral area but there are no nerves in the midline. Modified from Åkesson (Åkesson, 1967).

protostomes, but the vertebrate eye develops from the apical plate, i.e. in the blastoporal area (Eagleson and Harris, 1990), so this is very questionable.

Conclusions

The CNs of the neuralsian (cnidarians + bilaterians) comprise three main components. (1) The apical organ, which is present in almost all ciliated larvae. It degenerates before or at metamorphosis. It is the ancestral neuralsian brain and the only cnidian ‘brain’. (2) A pair of cerebral ganglia that develop in bilaterian larvae/embryos (from the first micromere quartet in the spiralians). They form the main part of the protostomian brain, but it is difficult to identify homologous structures in the vertebrate brain. (3) A blastoporal (circumblastoporal) nerve cord, which is represented by the posterior part of the brain and the ventral nerve cords in the protostomes (developing from the second micromere quartet in the spiralians) and by the neural tube in the chordates (brain plus spinal cord in the vertebrates).

A further conclusion is that the latest common ancestor of the bilaterians resembled the pelago-benthic ancestor of the protostomes as envisaged by the trochaea theory (Fig. 2). The blastopore fate of the deuterostomes could be a specialization of the protostomian lateral blastopore closure (amphistomy), as illustrated by the occurrence of ‘deuterostomy’ in the annelid Eunice, the gastropod Viviparus and the chaetognaths. This indicates that the deuterostomes could be an early offshoot from the stem-lineage of the protostomes.

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