What arthropod brains say about arthropod phylogeny

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What can we learn from the brain of a single member of a species? If the brain is that of a remipede, the focus of a report by Fanenbruck *et al.* (1) in this issue of PNAS, the answer is something new about arthropod phylogeny. The Remipedia, discovered in 1979, are crustaceans found deep in the waters of coastal caves (2, 3). To date, twelve species of remipedes are known: one from a single cave in Western Australia and the others from the Caribbean. The remipede body is divided into a head and an elongated trunk with up to 32 segments. The trunk segments feature paddle-like appendages that give the taxon its name ("oar feet"). Remipedes show adaptations to life in darkness, including the absence of pigmentation and eyes, but like other crustaceans they have two pairs of antennae.

The evolutionary history of these rare arthropods is uncertain. In their study of the brain of *Godzilliognomus frondosus*, Fanenbruck *et al.* (1) provide a fresh look at remipede phylogeny. Their neuroanatomical studies suggest that the Remipedia are most likely part of a larger clade that includes the Malacostraca and the Hexapoda, two arthropod groups well known for their complex brains.

This result is surprising because, at first inspection, the simplicity of the remipede body plan suggests that this group provides a glimpse of untagmatized ancestors with numerous unspecialized appendages and should be placed in a basal position in the crustacean lineage (4). This view, based on gross morphology, is contradicted by molecular analyses suggesting that the remipedes are a sister group to the hexapods (six-legged arthropods, including insects) (5, 6). These studies were based on comparisons of sequences for RNA polymerase II and elongation factors 1 and 2. The results not only elevated the Remipedia from a basal status, but also provided support for the much-discussed idea that hexapods are more closely related to crustaceans than to the other tracheated arthropods, the centipedes and millipedes (Chilopoda and Diplopoda; collectively, Myriapoda). This hypothesis unites the hexapods and crustaceans in a larger clade designated Pancrustacea (7, 8). Recent phylogenetic reconstructions based on complete mitochondrial genomes go a step further by

joining crustaceans and insects but placing a non-insect hexapod group, the Collembola, outside of Pancrustacea (9).

These molecular phylogenies are at odds with striking morphological synapomorphies that unite the Myriapoda and Hexapoda, such as tracheae, Malpighian tubules, and the absence of second antennae. A reexamination of these morphological characters from a detailed cellular, molecular, and developmental perspective therefore is warranted.

Phylogenetic Reconstruction and Neuroanatomy

As Strausfeld (10) has indicated, inference of phylogeny from shared neural characters is by no means a new approach, but it was not until the 1990s that modern researchers sought inspiration from the early 20th century investigations of Holmgren and Hanström on the brains of arthropods. Recent endeavors in this field have naturally been stimulated by the opportunity to apply advanced cell-labeling techniques to arthropod brains, but techniques alone do not drive the renascence of comparative neuroanatomy. Some of the most exciting new studies rely on venerable methods such as Bodian's reduced silver stain and the Golgi technique. Instead, it is controversy produced by discrepancies between morphological and molecular phylogenies of the arthropods that has shown neuroanatomists "where to look."

Three arthropod brain regions have received much attention in recent studies: the optic lobes, mushroom bodies, and central complex. Other major brain regions have been less extensively analyzed. Comparisons of the aforementioned brain regions generally provide support for the pancrustacean hypothesis. Brain-based conclusions regarding arthropod phylogeny, however, typically remain tentative because certain groups, such as the Chilopoda and Diplopoda, are understudied, and others, such as the Remipedia, have until now not been studied at all.

Optic Lobes

The optic lobes of insects comprise a set of three nested, retinotopic neuropils. The outermost of these neuropils, the lamina, receives direct inputs from photoreceptors in the compound eye. The lamina and the medulla, and in turn the medulla and the lobula complex, the

innermost neuropil, are linked by chiasmata. Similar optic neuropils can be readily discerned in crustaceans, which, like insects, also have compound eyes. The lamina in both of these groups is organized into columnar visual sampling units called optic cartridges of similar cellular composition. The Malacostraca (decapods such as crabs and lobsters, and the isopods) also have three distinct optic neuropils joined by two chiasmata. By contrast, other crustaceans such as the shrimps (Branchiopoda) and copepods (Maxillopoda) have no lobula complex and lack a chiasma between the lamina and the medulla. Analysis of a large library of arthropod brains led Strausfeld to suggest that all optic lobe features proximal to the medulla are products of convergence (homoplasies), with similarities reflecting constraints imposed initially by the organization of the photoreceptors and secondarily by the organization of the lamina and medulla (10, 11). Strausfeld's groundbreaking analysis, based not just on the optic lobes but on 100 neural features scored as present or absent in 28 taxa, yielded a tree identifying hexapods and crustaceans as sister groups.

This topic was readdressed by Harzsch (12) in a study of insect, malacostracan, and branchiopod optic neuropils by antihistamine immunocytochemistry. Harzsch interpreted his data as evidence against convergence, arguing that the third optic neuropil and the two optic chiasmata are synapomorphies for Malacostraca and Insecta (13). Strausfeld and colleagues (14) revisited the issue of optic lobe architecture in a study with antisera to the inhibitory neurotransmitter γ-aminobutyric acid (GABA). One striking finding was the absence of evidence that the crustacean medulla is divided into inner and outer regions, leading (along with other details of optic neuropil structure and development) to the conclusion that the lamina and outer medulla of insects are homologous to the lamina and the entire medulla of the malacostracans. These considerations highlight a characteristic tension in this field. It can be argued that differences in the morphology of GABAergic strata in insect and crustacean medullas

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are simply details; it can be equally argued that they change everything. Note, however, that both data sets are consistent with a malcostracan/insect clade, although the proposed neuroanatomical synapomorphies are different.

Deutocerebrum and Mushroom Bodies

The mushroom bodies are the insect brain structure best known to non-entomologists because of their functional association with learning and memory (15) and their structural plasticity in adult insects (16). A set of lobed neuropils originating from dorsal clusters of numerous small neurons (globuli or Kenyon cells), they are typically the most prominent features of the insect protocerebrum. One of their functions is to serve as a second order olfactory neuropil, and they receive massive ipsilateral projections from the deutocerebral olfactory interneurons, but mushroom bodies are present even in primarily and secondarily anosmic insects (17). The presence of recognizable mushroom body-like structures in other invertebrates such as annelids and onychophorans diminishes the usefulness of the mushroom bodies for phylogenetic reconstruction. Either mushroom bodies were present deep in the ancestry of groups that we recognize today as being significantly diverged, or they have evolved independently many times.

Surprisingly, there are no mushroom bodies in crustaceans. Instead, the protocerebral projections of olfactory interneurons project bilaterally to dense neuropils called hemiellipsoid bodies (18), which are associated with numerous globuli cells but do not have either the lobed structure or parallel fibers of the insect mushroom body. Although early 20th century neuroanatomists assumed that the hemiellipsoid bodies were highly modified mushroom bodies, this assertion of homology remains unproved.

Central Complex

The third set of characters that has attracted the attention of comparative neuroanatomists is the central complex, a set of protocerebral midline neuropils. In insects, these complex neuropils include a distinctive ellipsoid body, a fan-shaped body, a pair of lateral neuropils connected by a protocerebral bridge, and a pair of noduli immediately ventral to the ellipsoid body. Unpaired midline neuropils are present in crustaceans, but they are simpler in structure and contain fewer subunits than the insect central complex (19). In a study with antisera directed against tachykinin-related peptide (TRP) and allatostatins, Loesel *et al.* (20) reported that all groups examined but the Diplopoda have the same protocerebral midline structures. Although the key finding in this report is refutation of the monophyly of the Myriapoda, new information is presented on the midline structures of malocostracans, who match the insects in many details of central complex structure, including the presence of distinct allatostatin- and TRP-positive layers.

The Remipede Brain

It is against this background that the rich implications of the Fanenbruck *et al.* study (1) of *Godzilliognomus frondosus* can be appreciated. Because this remipede is eyeless, there are no optic neuropils to consider. In the protocerebrum, this leaves us with the hemiellipsoid bodies and the midline neuropils. The hemiellipsoid bodies strongly resemble those of malacostracans. The major components of the malacostracan midline neuropils are also recognizable. Fanenbruck *et al.* also estimate that the number of neurons in the remipede protocerebrum is larger than in branchiopods, comparable to the greater numbers found in malacostracans and insects.

The absence of optic neuropils in Remipedia has led to a focus on the less-studied deutocerebrum and tritocerebrum. In the deutocerebrum, the presence of glomeruli comparable to the olfactory glomeruli of malacostracans (and insects) is convincingly argued. These glomeruli appear to receive inputs from the aesthetascs, rows of chemosensory neurons located on the basal segments of the first pair of antennae. The tritocerebrum, which receives inputs from the nonchemosensory second pair of antennae and innervates the cephalic shield, is similar in organization to the tritocerebrum of malacostracans. These observations, particularly the organization of the deutocerebrum, leave us with a brain like that of an eyeless malacostracan: distinct olfactory glomeruli, bipartite antenna 1 neuropils, and a prominent olfactory globular tract that links the olfactory glomeruli with the hemiellipsoid bodies. The results of Fanenbruck *et al*. (1) therefore provide support for the hypothesis of a Remipedia–Malacostraca–Hexapoda clade.

Adult Brains Are Useful, Too

Comparative studies of gene expression during early development have contributed significantly to our understanding of arthropod relationships and the evolution of the arthropod body plan (21). The present study joins a growing number of articles demonstrating the contributions comparative neuroanatomy of adult brains can make to phylogenetic reconstruction (10, 12, 14, 17), even when, as in the case of the Remipedia, developmental studies are impossible. This study further draws attention to the relative lack of detailed comparative studies of regions of the arthropod brain beyond the protocerebrum and its associated optic lobes. The comparative neuroanatomy of the deutocerebrum and tritocerebrum need a level of scrutiny equal to that afforded by recent studies to the optic lobes, the mushroom bodies, and the central complex. Elucidating details of cell cytoarchitectonics and patterns of immunoreactivity are the first steps, but, following the lead of the developmental biologists, comparative studies of gene expression must follow when possible so that we do not overlook homologies concealed by the complexity of the adult arthropod brain. A synthesis of evo–devo approaches to nervous system development with comparative studies of adult brains could be the key that unlocks the phylogeny of the arthropods.

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