Review Article

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The Bivalve Nervous System and its Relevance for the Physiology of Reproduction

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Abstract

Bivalves are widespread invertebrates that are mostly marine and benthic, with great impacts in the aquatic systems food chains. Their soft body is laterally compressed and covered with a hard shell, often having bilateral symmetry. Strong adductor muscles help in the shell movement. Various species are used as bioindicators of environmental quality. Many, such as mussels, clams, scallops, or oysters, are heavily harvested/reared for human consumption. Bivalves availability, adaptability and simple anatomy make them attractive for both fundamental and applied research. One particular target for such studies is the nervous system. It is typically made of a central nervous system holding three types of ganglia (cerebral, pedal, visceral), organized into an outer neuron- and glia-rich cortex and an inner axon-rich medulla. Nerves interconnect the ganglia as well as these and peripheral nervous system components, made of sensorial structures such as eyes (mantle, tentacles), and osphradia (gills) and statocysts (foot); They are involved in photoreception or are mechano or chemoreceptors. Among other roles, the nervous system governs reproduction, via influences in the sexual development, gametogenesis, fertilization and spawning. Such modelling is via neurotransmitters and neurohormones, interplaying with direct/ indirect impacts of biotic (*eg*, food abundance) and abiotic (*eg*, temperature, pH, salinity) factors. We know now that many pollutants can disrupt the nervous system and gonads and their poorly known interaction. Knowing the nervous system functional morphology is critical to understand such disruptions and foreseen reproductive consequences. Accordingly, this work offers a systematic overview about the bivalve nervous system and related reproductive events.

Keywords: Anatomy; Histology; Bivalves; Nervous system; Ganglia; Neurons; Glial cells; Neurocytology; Neurophysiology; Reproduction.

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Introduction

In almost all metazoans, the coordination is accomplished by two main mechanisms, hormones and nervous system signals. These two central systems interact with each other to maintain the homeostasis of animals and to respond appropriate information to the environmental stimulus [1-2]. In addition to these basic vital functions, the nervous systems of higher organisms are able to perceive and react to a greater range of environmental stimuli in intricate and varied way including responsible for feeling, thinking, and learning [3]. In vertebrates there are more complicated components of the nervous system. Anatomically, there are two systems: the central nervous system (CNS) and the peripheral nervous system (PNS). CNS consists of brain and spinal cord. The PNS comprises the somatic and autonomic nervous systems. Somatic afferents carry sensory information from the skin, muscle, and joints to the CNS, while motor efferent nerves innervate skeletal muscle to cause the movement contraction. [4] The autonomic nervous system can be thought of as a motor system for visceral organs, because it projects to these organs to innervate and control the function of smooth muscle, cardiac muscle, endocrine, and exocrine glands. The autonomic nervous system is typically further divided anatomically and functionally into the sympathetic and parasympathetic subdivisions. [2, 4, 5]

In lower invertebrates of the animal kingdom, like Coelenterates or Cnidarians, the nervous system consist of specialized nerve cells of ectoderm called nerve net that consists of sensory and muscle cells diffusely distributed. [6, 7] The most highly evolved groups, like flatworms, show the first real CNS because their sensory cells are grouped into special anatomical collection forming a nerve ring or ganglia organized in the bilaterally symmetrical longitudinal body axis as nerve cords. Their ganglia can assume a segment-like structure as a result of the more or less regular array of cross-connections innervating the whole body. [8] In the head region, there are specialized structures, such as primitive "eye or ocelli". These structures are also be found in annelids, in which in the anterior end there is a distinct brain and segmented body plan, with ganglia organized into a ladder-like chain in each segment. [9] The dorsal brain is connected to the ventral chain of segmental ganglia via circumesophageal connectives. Each segmental ganglion, which typically is said to consist of about 1000 neurons, is organized in a bilaterally symmetrical way. Both halves are linked to one another by commissures and to neighboring ganglia by connectives. Peripheral nerves, typically three pairs, projects from each ganglion and innervate the segmental body wall. [10] All ganglia have a structure which is characteristic for higher invertebrates; neurons within cortex and the neuronal processes (dendrites and axons) lie in a neuropil in the core of the ganglion. In some annelids, distinctive giant neurons occur, and these play an important role in fast escape responses. [11] In some species the structure of optic ganglia is formed. [12, 13]

In arthropods, however, the body organization is different from that of annelids with articulated appendages and the fusion of originally unitary, metameric segments into the functional entities comprising the head, thorax, and abdomen. For such insects and crustaceans, their head region tends to form a complex brain consisting of extensively fused cerebral ganglia. These are often associated with the processing of information from specialized sensory organs, for example, a protocerebrum of insects, which receives visual sensory input from both compound eyes and from the simple ocelli, a deutocerebrum which receives sensory input from the antennae, and a tritocerebrum which receives input from the head surface. [14] These brain structures together contain about 90% of the neurons in the central nervous system, which in the larger crustaceans sum about one million nerve cells. In the higher arthropods, there are brain regions which consist of associative neuropil centres, cell body regions, and aggregates of neurosecretory cells. [15, 16] The requirement for accurate motor control of the articulated body appendages, especially the thoracic legs, has led to an increasing specialization of the ventral segmental ganglia. [17] There are the thoracic ganglia typically containing more interneurons, efferent projecting motor neurons and afferent sensory fibers than the abdominal ganglion. The latter, often associated with specialized structures, are located in the posterior end of the animal. In addition, there is a tendency toward fusion of the segmental ganglia into fewer (in some cases single) ganglia. [18] One such ganglion, the subesophageal, it is formed by several ganglia and controls the mouthparts – this is generally found enclosed in the head capsule. [19] The segmental specializations of the arthropod nervous system allow complex motor activity to be generated. This includes flying, running, jumping, manipulation, and sound production. [12, 18]

In molluscs, there are variations in the organization of the nervous system. In order to get sensations they have a collection of neurons in the ventral cord which are called ganglia. The basic organization of their CNS comprises about five pairs of ganglia which are arranged around the gut, normally near the head, and are linked to one another by connectives and commissures. It is possible to distinguish cerebral, buccal, pleural, pedal, and abdominal ganglia. [13, 20] The basic organizational plan can vary significantly among individual molluscan species, to the extent that the various ganglia can change their position and even fuse with one another. [12, 13, 20] In Gastropoda, Scaphopoda, Polyplacophora, Monoplacophora (slow-moving animals) and Cephalopoda (active predatory lifestyle), there is cephalisation. [7, 11, 21] There are numerous studies on the nervous system of the gastropod mollusc *Aphysia californica*, which is an animal model for the neurobiologists' study of behaviours, namely learning and memory. [22, 23] In bivalves, such as, clams, mussels, and scallops, there is bilateral symmetry and soft body. They have an interesting simple model of CNS, recognized as very useful for studies ranging from neurobiochemistry to neurophysiology. [7, 24, 25]

However, little seems to be known about the detailed anatomy of components in the bivalve nervous system. This Chapter reviews two major parts in bivalves. The first concerns the structure and function of nervous system. The second describes the neural control of reproduction.

General morphology and functions of nervous system in bivalves

Knowledge about the morphology and functioning of central nervous system in bivalves is still somewhat scarce and needing further study. The reasons for this limitation are varied. The histology, though studied to a certain extent, is different from that of vertebrates; most of the available forms are small, and the few experimental work has been performed using methods fruitful in vertebrates but, possibly, inadequate or insufficient for bivalves. A great feature of the bivalve nervous system is the small number of neuronal elements within ganglia and that contribute to the peripheral tissue. [20, 26] This makes possible a type of analysis that is difficult to achieve in vertebrates. Also, interesting direct correlations between the size of the ganglia and their function can be disclosed in bivalves.

The central nervous system

Anatomy of the ganglia

The basic plan of organization of all bivalves nervous system is bilaterally symmetric which each half body segment possessing a ganglion. In typical bivalves, they consist of three pairs of ganglia: cerebropleural (commonly called as cerebral), visceral and pedal; along with two pairs of long nerve cords. Both cerebral ganglia are interconnected to visceral and pedal ganglia by bilaterally running nerve cords. Each ganglion gives rise to nerve fibers that supply the organs and tissues in close proximity. [24, 26, 28] For instance, the cerebral ganglia innervate labial palps, anterior adductor muscle, anterior part of the mantle, and sensory organs, including statocysts (equilibrium organs) and osphradia (a chemo-mechanical sense organ). [29] The visceral ganglion innervates the gills, heart, posterior adductor muscle, posterior part of the mantle, siphons, and sensory organs in the mantle. [30] As in other bivalve species, the visceral ganglion of *Venus verrucosa* comes from the fusion of two original ganglia, thus showing bilateral symmetry; pairs of symmetrical nerves emerge from each pole and diverge. Lastly, the pedal ganglion, as the name indicates, innervates the foot. [28, 31, 32]

Cerebral ganglia

In most bivalves, the paired cerebral ganglia are well separated from each other (left and right) and they are usually triangular in shape, with the color varying from milky white to bright red. These ganglia are situated between the base of the labial palps and the first esophageal subdivision of the digestive tract, being shortly cross-connected by a commissure arching over the esophagus, as well as, longitudinal linked between pedal and visceral by connectives. In reality, they are formed by fusion of the cerebral and pleural ganglia around the anterior part, and that is why they are commonly referred as cerebropleural ganglia or cerebral ganglia in the literature [20, 32]; and herein we shall use the latter term henceforth for consistency. From each cerebral ganglion not only the principal two pair of nerves cords lead toward the posterior of the animal: one, cerebro-pedal connectives that extend posterior and ventrally to the pedal ganglia in the foot; another, cerebro-visceral connectives, running directly back from the cerebral ganglia to the visceral ganglion, which is located on the surface of the posterior adductor muscle. But, there are also the pallial nerves innervating the labial palp, anterior adductor muscle, gill [33], and part of mantle margin, as well as the statocysts and osphradia. In the absence of cephalic sense organs the cerebral ganglia are weakly developed and small. [33] In snails, the central ganglia are more concentrated and the visceral loop is so short that all of the principal ganglia are in the anterior nerve ring above the esophagus. [20, 21, 34]

Pedal ganglia

In general bivalves, the pedal ganglia is positioned below the esophagus and is anterior to the base of the foot. They have the same type of coloration but are larger than the cerebral ganglia and more rounded in appearance. The pedal commissures are rare; in most forms the right and left ganglia have met together in the middle line. Each ganglia extend the following nerves: 1) the pedal nerve, which innervates the foot, originates from the ventral posterior surface; 2) in genus *Mytilus*, the ventral byssus retractor nerve, innervating the byssus organ and muscle and arising from the posterior ventral side of the ganglion; 3) the dorsal byssus retractor nerve, which also innervates the upper byssus muscles arise from the posterior dorsal of the ganglion. In *Crassostrea virginica*, there are as well no pedal ganglia in line with the lack of a foot for moving. [24, 32, 35]

Visceral ganglia

In typical bivalves the visceral ganglion is the largest ganglia, being derived from the fusion of two original ganglia. Visceral ganglia either appear as "rounded triangles" or else having multiple lobules, with milky white to bright red in colour at the ventral end of the visceral mass, on the anteroventral border next to adductor muscle. The visceral ganglia are much larger than the cerebral and nerves emanating from it innervate the mantle, gills, intestine, anus, skin, posterior part of the genital apparatus, kidney, the main digestive gland and posterior adductor muscle. [32-36] In addition to their usual autonomic functions, the visceral ganglia also receive sensory inputs from the sensory tentacles of the mantle. The tentacles are photoreceptive, mechanoreceptive, and even chemoreceptive organs. [24, 32] It is of interest to note that the distribution of the nerves which originate from the visceral ganglia is not always identical for each ganglion. Processes could be seen to extend from nerve cell bodies. Fibres could be seen in the cerebro-visceral connective and in the origin of the branchial nerve. [36] The large white visceral ganglion can be revealed by opening the exhalant chamber and cloaca and looking between the pyloric process and the posterior adductor muscle. [30, 37, 38]

Histology of the ganglia

Irrespective of the ganglia types, they typically consist of three layers, an outermost perineurium, the outer cortex and the inner medulla, which can be called neuropil. [20, 39] Accordingly, the typical structural organization of the ganglia , bivalves like those of most invertebrates, consist of a multilayered rind of neuronal cell bodies which send their processes to a central core, are sheathed by a connective tissue perineurium and contain two types of cells: nerve cells (neurons) and glial cells.[13, 20]

Perineurium

Ultrastructural analysis of the *V. verrucosa* ganglia

shows — from the ganglion periphery the perineurium — a limiting envelope formed by a sheath of connective tissue that consists of collagen fibers and fibroblasts; they are arranged in a loose three-dimensional network, alternating with sheaths of dense microfilamentous material with the appearance of a basal lamina surrounding the ganglia. [36] As for its function, the perineurium is likely to provide not only a protective envelope, but also a permeability barrier, which may be particularly important in bivalve ganglia which probably lack a glial blood-brain barrier. [13, 20] But the perineurium in vertebrates is different from that in invertebrates, because it is a concentric layer of bundled nerves that it is a protective layer of connective tissue located around nerves in the body and the internal organs. Indeed, it is composed of concentric layers of connective tissue that form a protective sheath around bundles of nerve fibers. This structure is a transparent tube-shaped layer that is easily pulled away from the bundled nerves. Perineurium nerve coverings are a part of the peripheral nervous system (PNS), which is responsible for transmitting messages from the central nervous system (CNS) in the brain to the effectors, like arms, legs, and internal organs. [2]

The cortical part of ganglia (cortex)

The cortex, a multilayered area of neuron and satellite glial cells in *V. verrucosa* [36], is to be the complex network centre of neuronal cell bodies and glial cells. The cortex is not only involved in the control of many internal, homeostatic regulatory processes, but also in the production of complex behaviours. Many of nerve cell bodies located in the cortex were radially oriented and closely associated with the connective tissue sheath. Many of the neurons send their axons into the neuropil ganglion (inner) zone.[40]

Medulla or neuropil region

As previous mention, in the most invertebrate ganglia such as arthropods and annelids, the cell bodies of neurons occur in a thin rind on the periphery of the ganglion, and the core that contains axons and dendrites is called the neuropil, a ganglionic core containing the axonal processes of the cortical neurons in *V. verrucosa*. [36] These nerve cell bodies appeared to be extensively innervated, as indicated by the specific staining of endings on their surface their process of the nerve cell body tapered as it extended from the body. The neuropil region has a fibre organization of axons in the nerve tracts that form clustered areas of complex synapses, i.e., glomeruli. [20, 37]

Ganglionic structure follows a common pattern in virtually all invertebrates, with an outer rind of neuronal somata surrounding an inner core of axons and dendrites. The somata are clustered in groups. The axonal processes of motor neurons leave the ganglion through the lateral nerves to innervate their targets in the periphery (often muscles). Most motor neurons have just one axon leaving the ganglion, but a few have axons in several nerves that innervate different targets. In this way a single motor neuron can exert coordinated control over sets of muscles that need to act together. Individual muscles are generally innervated by just one or a few excitatory motor neurons. [13, 20]

Neurons and glial cells (ganglionic cells)

There is no doubt that in all bivalves the number of central neurons is smaller if compared to more complex animals. Notwithstanding, each neuron has a specific and often complicated task to perform which involves receiving and making many synaptic connections.[41, 42] In certain instances, differences between the pair of neurons in each half of the central nervous system are slight, so that one can replace the other to a considerable extent. But in many other cases the loss of one fibre must involve considerable loss of function, which may be mitigated to a certain extent by the overlapping fields of different neurons. The nervous system also contains cells that surround, nourish, and support the neurons and their process, and these are called glial cells. [40]

Nerve cells or neurons

As in most invertebrates, unipolar neurons predominate, even though a few bipolar and even multipolar nerve cells have been described. [13, 20, 43] Neuronal cell bodies have overall ultrastructural features similar to those of most vertebrate and invertebrate neuron. They contain a pale round or oval nucleus with one or more prominent nucleoli. The cytoplasm is rich in granular and agranular endoplasmic reticulum, free ribosomes, mitochondria and glycogen deposits. Some mitochondria have a paracrystalline structure, similar to that found in the neurons of *Spisula solidissima* [32, 44], which may be related to the accumulation of proteins and lipid; as it is known to occur in a variety of vertebrate and invertebrate cells. Microtubules and microfilaments are bare. Golgi complexes are numerous and developed, being formed by long cured cisternae filled with finely granular electron-opaque material and by vesicular profiles of variable size and electron density. In most cell bodies, dense core vesicles are an important component and can be found in large amounts dispersed in the cytoplasm. They display a great variability of size, shape and electron-opacity and represent the only distinctive feature of the neuron, which are comparable in other ultrastructural respects. [37, 45]

Most neuronal bodies are in the cortex and close to the perineurium sheath of the ganglia. There are also the beginnings of the nerves fibres that are made of axons (i.e., neurites in unipolar neurons) and eventual dendrites. [46] Pigments can also be found within neuron, namely as granules designated by cytosomes or lipochondria, exactly alike described in gastropods. [20, 37, 47] The cytoplasmic membranes of neuronal cell bodies, which are in extensive reciprocal contact, do not show particular specializations, except for the presence of subsurface cisternae in peripheral neurons of *S. solidissima*. [44]

The neuronal cell process originates from a large, cone-shaped extension of the soma which gradually taper. The cytoplasm contains microtubules, neurofilaments, mitochondria and vesicles displaying the same ultrastructural heterogeneity as those in the cell bodies. The ganglionic core is formed by a complex network of processes of different diameters. Nerve processes containing cytoskeletal elements are intermingled with others filled with vesicles. Tracts are formed by wider axons of passage, while non-glomerular neuropil contains finer processes which arborise and establish synaptic contacts. [20] Different types of neurons can be identified from their branched process pattern and in terms of function, and so they can be grouped into three basic categories: a) neurons with specialized endings that respond to energy from the environment are called sensory neurons; b) neurons that have axons terminating on muscle fibers are called motor neurons c) all other neurons, that are interneurons. [1, 21, 48]

The majority of synaptic contacts occur in the neuropil between nerve processes, even if rare, axomatic synapses have also been recognized within the cortex. The presynaptic sides can be identified both the presence of neurotransmitter vesicles and of electron dense areas collate to the membrane. In these synaptic areas, organelles such as mitochondria and cytoskeleton elements are sparse. Post synaptic sites are simpler, being the most significant feature the unevenness of the membrane. The synaptic space (cleft) typically does not vary in width (H” 20 nm) across the synapse. Despite this key features, more than one type of synaptic characteristics may occur. For instance, in the genus *Mytilus* there are synapses with vesicles that only have a lucid content while other have vesicles having either dense or clear cores. In addition to the vesicle discharges at synapses, it is accepted that neuromediators are released at non-synaptic sites; a process that is not exclusive of bivalves. More details on the above can be read elsewhere. [46, 49]

Glial cells

As in vertebrates, the glial cells of invertebrates have a vast array of structural and functional specializations. [50] They can be feature of the higher invertebrate groups like, the Arthropods, Annelids and Molluscs. Their location is around the neurons, especially at the nervous tissue interface. Glial cells have an oval nucleus with chromatin clumped in the periphery. Generally, two types of electron-dense, membrane-bound inclusions can be discerned: cytosome-like bodies and oval granules called gliosomes (450-650 nm in length and 250-350 nm in width). These later are a distinctive feature of glial cells in several bivalve species (and also gastropods). Their role in nervous activity appears to be necessary when the neurons become aggregated into ganglia. [13, 50, 52] In the *Mytilus edulis*, glial cells have an oval or indented nucleus with chromatin clumped in the periphery. Their cytoplasm is usually scanty but nevertheless contains microfilaments, mitochondria, cisternae of rough endoplasmic reticulum, free ribosomes, and small Golgi complexes.[53] Neuronal cell bodies in the cortex of the pedal ganglion are subdivided in clusters by septa formed by glial cell bodies and their processes, among which there is a system of intercellular channels, mainly evident in the subperineurial zone. In this region, even in well-fixed tissues, there are clusters of empty vesicular profiles of variable size, which seem to bud off from glial processes: the nature of dark glial cells characterized by a dense cytoplasm, which are present in the deepest regions of the cortex and in the neuropil. Glial cells appear less frequently in the ganglion central fiber core, being completely absent from wide neuropil regions. [20, 51]

The peripheral nervous system

The peripheral nervous system of bivalve is made up of sensory structures regulated through the lateral nerves. The organs are usually tentacles and most are typically mechanoreceptors and chemoreceptors. The sensory organs of bivalves are not well developed, and are largely a function of the posterior mantle margins. In scallops have complex eyes with a lens and retina, but most other bivalves have much simple eye or ocelli. In Septibranchs, the inhalant siphon is surrounded by vibration-sensitive tentacles for detecting prey.[7, 54]

Primary ciliary receptors

In bivalves, three types of ciliated sensory receptors were described. [55, 57] The most common consists of 35-40 nonmotile cilia on a cluster of four to six sensory neurons, apparently mechanoreceptors associated with a pair of glandular cells. The second type, a monociliary receptor, has a long, stiff kinocilium surrounded at the base by a corolla of nine short, club-shaped microvilli. The third type consists of 17-20 nonmotile cilia in a circle on a single sensory neuron that distally envelops a gland cell. These structures work as mechanoreceptors and can be seen in the tentacles of the scallop *Placopecten magellanicus* [58], mantle edge of *Donax serra* and *Donax sordidus* and on the siphon of *Macoma balthica*. [56]

Ocelli (eye spots)

Bivalves have two types of eyes: paired cerebral eyes, as well illustrated in the veliger (the planktonic larval stage) and adults of *M. edulis*, and pallial eyes. [32] The latter eyes are found on the siphons of *Cardium edule* and on the middle mantle fold of the *Pecten maximus*. [59] This organ is the light receptor, containing pigmented cells. In *M. edulis*, cerebral eyes appear as dark spots located at the bases of the first ctenidial filaments of the left and right inner demibranchs. Each ocellus is an open cup, and the retina is composed of sensory and pigment cells. Eyes in *P. magellanicus* are on the middle of the mantle skirt. [32] The photoreceptoral organelles are directed toward the incoming light. The sensory cell has a bulbous nuclear region, a slender cell process, and, apically, rhabdomeres, and, compared to the eye of genus *Pecten*, there are very few receptor cells. [59] In *Pecten maximus*, more than 60 eyes are located in the sensory fold of the mantle. [58] Each consists of a cornea, a large cellular lens, a distal and proximal retina, a reflecting argentea, and a layer of pigment cell around the eye. The lens cells contain few organelles and rest on a thick basal lamina. Beneath the lamina there are nerve fibres of the distal retinal cells that bear few microvilli among numerous cilia at their distal surface. The axon leaves the distal retinal cell from the side, passes up to the basal membrane, and joins other distal nerve fibers to form the optic nerve. There are glial supporting cells between the distal and the proximal retina, the cells of which face in the opposite direction from the distal cells. [58, 59]

Statocyst

In bivalves, paired statocysts are located in both dorsolateral sides of the pedal ganglia, and there are nerve connecting them to the cerebral ganglia. In the genus *Pecten*, each statocyst consists of a sac of hair cells and supporting cells. Inside the sac is a statolith composed of crystals, and a static nerve extends from the sac and eventually connects to the cerebral ganglion. [60] Hair cells have kinocilia, microvilli at their distal ends, and one or more striated roots that pass deeply into the cell cytoplasm. They function to allow animal to maintain orientation. [32, 61]

Osphradium

The osphradium can detect incoming water as a chemo- or mechanoreceptor around the ctenidial axis, exhalent, and suprabranchial section of mantle cavity. In a number of bivalve species, osphradia have sensory processes, sensory cells, supporting cells, and innervation of the ridge by nerves from ventral ganglion. [29] The osphradium is an ancient sensory structure in Mollusca, and it is better developed in Gastropoda, where it is a strategically located chemo-mechanical organ in the pallial cavity. [32, 62]

Abdominal sense organs

Abdominal sense organs are situated on the ventral surface of the posterior adductor muscles in bivalves. [32] The sensory epithelium is tall and consists of two predominant cell types, electron-dense supporting cells with microvilli only, pigment granules and oval distal nuclei, and sensory cells with round proximal nuclei and electron-lucent cytoplasm. The narrow sensory processes always are bunched and reach the surface bearing long stiff cilia. Surrounding the cilium is nine 'stereomicrovilli' forming a basal plate in connection with the basal body. In the prosobranch *Nucula sulcata* there is the so-called Stempell's organ, a tube-like sense organ, situated immediately dorsal to the anterior adductor muscle. Collar receptors in the sensory portion of the organ indicated a mechanoreceptive function. [32]

The cellular components of an invertebrate nervous system include: sensory neurons, which convert physical variables (e.g., light level or muscle force) into electrical signals; motor neurons, which make synapses with muscles or other effector organs (e.g., light-producing organs, glands); interneurons, which transmit information between other neurons; and glial cells, which are electrically excitable, that influence the ionic environment surrounding neurons and the transmission of signals between them. [13] The transport of signalling of neurotransmitters is considered to be a major function of ganglia in most bivalves division of the ganglia. The central nervous system of bivalves have neurons that contain the biogenic amines dopamine (DA), norephinephrine (NE) and serotonin (5-HT), each type might inhibit the synthesis of the other transmitters.

Neuroactive substances

There are various techniques to study in nervous tissue of bivalves, and one of the important technique is immunocytochemistry, which for instance characterized the neurons containing neuroactive substances in *M. edulis*.[46]

Serotonin or 5-hydroxytrytamine (5-HT)

5-HT is found in the central nervous system of vertebrates and invertebrates. [63] It is thought as the key neurotransmitter that control reproductive process of many invertebrates, such as the crustacean *Macrobrachium rosenbergii*. [64] In *M. edulis*, serotonin immunoreactive neurons were seen in light microscopic immmunocytochemical studies. Most often, those neurons are unipolar (8.5-25 μm) and very numerous both in the pedal and the cerebral ganglia. [65] Moreover, a great number of labelled nerve processes were shown in the ganglionic cores, in the connectives and in the nerves. In the bivalves *Anodonta cygnea* and *Mactra stultorum*, auto radiographic studies indicated that there is a selective uptake of 3H5-HT by ganglionic nerve processes containing dense core vesicles. The neuropil of the pedal ganglia has small dopamine-containing neurons closely associated with it. Situated ventrally in the pedal ganglia is a large group of 5HT-containing neurons. Both dopamine and 5-HT are present in the cells at the junction of the visceral and right parietal ganglia, and that dopamine and 5-HT varicosities are present in the neuropil of the pedal ganglia in molluscs. [63]

Neuropeptides

Neurons immunoreactive for gamma-aminobutyric acid (GABA) have been verified in all the ganglia using an antibody directed against the amino acid itself. [66] GABA immunoreactive neurons are represented more in the pedal and cerebral ganglia than in the visceral ganglia, but are less numerous than neurons displaying 5-HT-positivity. For the majority, GABA-positive neurons are small, unipolar (10 μm in diameter), the exceptions being represented by a few small bipolar and multipolar cells present almost exclusively in the pedal ganglia. [48] In these latter there are also two pairs of bilaterally symmetric, large (30 μm in diameter) multipolar neurons with long processes projecting widely throughout the neuropil. Immunoreactive processes form networks in the ganglionic cores and run in all the connectives and nerves; even so, GABAergic fibers are very rare in the foot. [48] Whether peptide releases occur at synaptic contacts remains to be fully elucidated, as synaptic terminals positive to neuropeptides have not yet been recognized. In addition to the substances above-mentioned, there is physiological and pharmacological evidence for the presence of other peptides, both in the central and peripheral nervous system, such as the case of FMRFamide (Phe-Met-Arg-Phe-NH2). [43]

Acetylcholine (Ach)

Acetylcholine has long been recognized as a neurotransmitter. In most bivalves Ach acts as an inhibitory neurotransmitter whereas in some it may have an excitatory role. Ach actions can be even inconsistent within a species. Ach has, therefore, a wide variety of effects, e.g., on the heart where it is a cardioinhibitory neurotransmitter. [67]

Dopamine

Dopamine is widely distributed in the invertebrate nervous system and has a diverse effect of reproduction in bivalves. [68] Dopamine was shown to inhibit spawning activity in serotonin-treated *Dreissena polymorpha* mussels, indicating that spawning activity is stimulated by serotonin but negatively controlled by dopamine (i.e. dopamine is linked to gametogenesis rather than spawning and fertilization) [69]. In the gonads of *Mizuhopecten yessoensis*, dopamine acts both as a neurotransmitter and neurohormone to rise the levels of cAMP, that seem to play a regulatory role in the reproduction. [70] This does not mean that dopamin have actions restricted to reproduction, exemplified by its role in the contro of ciliary beating as elegantely demonstrated in *C. virginica*. [42]

Mechanisms of neuronal transmission

Knowing that nerve impulses were mediated by chemical neurotransmitters, it became possible to isolate the inhibitory and excitatory effects of nerve stimulation and to identify the probable neurotransmitter substances.

The action potential

Just as a quick reminder, a basic function of most neurons is ability to produce nerve impulses or action potentials along the cell membrane. Potential differences cross the membrane known as the membrane potential. In the resting potential membrane, it is approximately -65 mV. When the membrane potential is raised enough to reach the threshold result in voltage-gated, sodium channels open up and allowing Na+ to flow into the cell and depolarizing the membrane. This is an action potential (AP), the rapid depolarization is soon opposed by the closing of Na+ channels (stopping its influx from the exterior) and opening of K+ channels (allowing the efflux of K+, during both the repolarisation and hyperpolarization phases for restoring the resting potential). Finally, both Na+ and K+ channels close and the membrane potential return to resting stage and along the membrane is passively extended and excited adjacent areas to do the same step. The presynaptic terminal contains synaptic vesicles-packets containing a chemical neurotransmitter. The type of neurotransmitter varies depending on the neuron. [1-5]

Neurotransmitter activity

We know that there are different neuropeptides and that small-molecule transmitters exist in the neuron bivalves, including acetylcholine, monoamines, and amino acids. [71] For the events underpinning impulse conduction, the synapse plays a critical role in integrating activities of the nervous system. This synapse is one in which transmission is chemically mediated, i.e., a substance liberated from the nerve ending of one cell brings about excitation in the plasma membrane of the next. In many cases acetylcholine fulfils this function just as it does in the classical myoneural junction. In other instances norepinephrine plays a similar role, although in these cases some structural differences in the synapse appear. Indeed, specialized low-resistance connections exist, coupling the pre and postsynaptic neurons and resulting in extremely rapid transmission. Finally, in all cases in which electrical transmission has been seen a particular structural type of intercellular junction has also been present. [1, 20]

Neural modulation of the physiology of the reproduction in bivalves

Many substances have been candidates as neurotransmitters in bivalves. Acetylcholine, 5-hydroxytryptamine, dopamine, and FMRF amide, they might be physiologically significant in a few species. Acetylcholine and 5-hydroxytryptamine are almost certainly neurotransmitter substances in the gonad whether or not any other neuroactive endocrine substances are release at sites remote from the gonad. [1, 65] Bivalves possess large identifiable nerve cells in their ganglia, and some of these have been shown to be reproductive-regulatory. [72]

For example, in green lipped mussel, *Perna canaliculus*, neurons in the visceral ganglia of both male and female were characterized by immunohistochemical techniques, and found that there are immunoreactivity of anti-5HT and anti-DA in large type and anti-APGWamide in small type of neurons.[38] In the gastropod *Haliotis asinina*, which has a predictable spawning cycle, there are various neuropeptides secreted from anterior ganglia that play a regulatory role in reproduction, like APGWamide, myomodulin, and FMRamide. [73]

Morphological and physiological aspects of gonads and breeding cycles

Sexual differentiation

Gonochorism is the condition of most bivalves, with no external morphological differences between the sexes. [7, 74, 75] However, the presence of some hermaphrodites in wild populations was reported, e.g. in the form of oocytes within the normal testicular tissue (ovotestis), namely in individuals of *Scrobicularia plana*.[76, 77] Some species are naturally predominantly hermaphrodites, with distinct male and female portions of the gonad, like seen in scallop *Pecten maximus*; the mature gonad is divided into two areas: dorsal testis with white colour and ventral ovary with orange-red colour. [7, 78] In *Anadara broughtoni* (48.3-52.5 mm in size), gonads are present at sexual maturity and the sexes were reported as being separated. In *Anadara senilis* from Nigerian coast, studies on the sexuality concluded that it is a protandrous hermaphrodite (monoecious), with animals developing as males first and then changing to be females. [79]

Gametogenesis

Gametogenesis involves the production of gametes in the gonad that occupy a major portion of the visceral mass as in bivalves. Spermatogenesis and oogenesis is related to a period of reproductive cycle that is influenced by external environmental factors. Spermatogenesis occurrence located along the inner periphery of acinus. Spermatogonia are the first cells to become primary spermatocytes by mitotic divisions, later these cells undergo into meiosis to become secondary spermatocytes and spermatids, respectively, then following the differentiation of mature spermatids into spermatozoa without further cell divisions. [28, 32] As to oogenesis, the primary oogonia have potential to do repeated mitosis and in the process differentiate to secondary oogonia, which ingress in the meiotic process until stopping at the prophase stage of meiosis I — the completion of meiosis occurs at fertilisation. During oogenesis, the oocytes greatly increase in size by a process named vitellogenesis, which basically consists in the assemblage of lipids and some glycogen in the ooplasm. [7, 78]

Spawning

In most bivalves, there are various stimuli suggested as being importance in control the breeding cycle, like water temperature, pH range, tide, latitude, and food abundance. [75-80] Whilst extreme temperatures may inhibit spawning, these seem to be less limiting in warmer climates than in temperate waters. It is a widely suggested that in each species may occur only over a critical spawning period and also depending on the physiological condition of the animals and/or their geographical distribution. [81, 82] Generally, gametes are discharged into the mantle cavity and then into the environment by valve movements, relaxation of adductor muscles, enlargement of ostia, and increased ciliary action of the ctenidia [32-79] and are fertilized externally. Internal fertilization in some bivalves females collect sperm in the mantle cavity or gill chamber and then the developing larvae are brooded. The zygote continues develop in various larval forms (trochophore and veliger) up to reaching the juvenile stage. [32] Differences exist even in species of the same genera. For example, the major period of spawning of *Anadara granosa* in southern Europe is from July to October with a peak in August, and larvae can be found for over a two month period. [83] This is different from *A. senilis,* as it appeared that the major spawning period is in October, and some spawning of *A. gmnosa* probably takes place throughout the year. [79] But there are evidences of a peak period in between June and September. *In A. broughtoni* from Japan has spawning time in beginning of August to the end of September. [79]

Evidence for neurosecretory (neuroendocrine) substances involved in reproduction

Bivalve reproduction consists of many critical steps, beginning in nerve centres and ending in the gonads. The steps include sexual development, gametogenesis, fertilization and spawning. On the whole, sexual differentiation processes of bivalves are still in doubt but some aspects are gaining a better understanding. Serotonin, dopamine and sex steroids are some agents that are involved in the sexual differentiation process. [84]

Monoamine oxidase (MAO) regulated by serotonin level is the main elimination pathway for monoamines such as dopamine, serotonin, octopamine and noradrenaline. The MAO activity could be induced by a variety of secondary amines in the environment and could likely modulate serotonin levels in nerve tissues and perhaps sex differentiation. For example, MAO activity in the nerve ganglia and gonad was shown to be induced with a concomitant decrease in serotonin and dopamine in mussels exposed for 90 day, 10 km downstream from a primary-treated municipal effluent plume. [85] Indolamines (serotonin and tryptomine) and catecholamines (i.e., dopamine and noradrenaline) are particular neurotransmitters involved in the integrated actions of neuronal populations that implicate at the sexual differentiation in bivalves.[86-87] The level of dopamine increases after injections of E2 in the sea scallop, but it dropped during active spawning period.[88] Moreover, dopamine was shown to inhibit spawning activity in serotonin-treated *D. polymorpha* mussels. [69] There has been a quest to locate the involved neurons. For instance, an immunohistochemical study was made in the green-lipped mussel, *Perna canaliculus*, using anti-sera raised against neuropeptides and neurotransmitters known to control reproduction and spawning. The authors concluded that there are neurons positive for serotonin (5-HT), dopamine (DA), APGWamide, and egg-laying hormone (ELH) within the visceral ganglia, despite not being able to prove the physiological functions in the control of the reproduction of the studied species. [38]

Many of the hormones in invertebrates are neurohormones, so they are produced by nerve cells. [89] As with conventional neurons, neurosecretory cells are able to receive signals from other neurons. However, unlike ordinary neurons that have cell-to-cell communication over short distances at synapses, neurosecretory cells ultimately release their product into an extracellular space that may be at some distance from the target cells. [89] In an organism with a circulatory system, the neurohormones are typically sent by the vascular route to their target. In contrast, in lower invertebrates that lack an organized circulatory system, the neurohormones apparently simply diffuse from the release site to the target. In molluscs, the neurosecretory cells and nerve cells in ganglia are described as endocrine cells producing neurohormones (dopamine, noradrenaline and serotonin). [89] In *V. verrucosa*, 5-HT was studied by immunohistochemistry, and it was found in serotonergic neurons that were located at a region of the cortex of the visceral ganglion, in serotonergic fibers at the root of branchial nerve, and along the walls of the ovarian follicles and also running between the seminiferous acini. [36] In *Lamelliden scorrianus*, two types of neurosecretory cells were observed on the dorsal surface of cerebropleural ganglia, which accumulate the neurosecretory material at low temperature. [90]

By all the above, it is logically possible to hypothesize that there is a large potential for xenobiotic endocrine disruption effects on the nervous system controlled reproduction.

Effects of endocrine-disrupting chemicals on bivalves

Endocrine-disrupting chemicals (EDCs) are substances that can interfere with the endocrine system of animals, being this simplistic definition subject to refinements.[91] EDCs are known for a wide range of chemical compounds, including, natural estrogen and synthetic hormones (ethynylestradiol), industrial chemicals (such as alkylphenols, bisphenol A, ethoxylates and tributyltin) and pesticides (*eg*, chlormephos and atrazine). [92-94] Evidence of the effects of these compounds has been presented in the majority of studies with fish, crustaceans, annelids and molluscs. [95, 97] Certain alarming concerns have been increased in human health of EDCs, such as decline in sperm quality, increase in the frequency of development abnormalities of the male reproductive tract, precocious puberty, and altered neuronal development. [98, 99]

Aquatic organisms are being subjected to contact with these substances because they are discharged into the water, and thus appear in rivers, estuaries and sea. [100] This lead to numerous studies on wildlife and consequently the interest on endocrine disruption of invertebrates is obtaining more attention. Nowadays there are facts pointing that bivalves seem to be affected by EDCs, as revealed by the appearance of oocytes in the testes (ovotestis-intersex) of the peppery furrow shell, *S. plana*, from the Avon Estuary, United Kingdom, where there was a likely source of estrogenic chemical from agriculture, and also in the Guadiana Estuary, in Portugal, where the presence of EDCs was thought to mainly derived from urban, industrial and agricultural discharges.[76, 77] In the freshwater mussel, *Elliptio complanata*, waterborne exposure to estrogenic compounds present in municipal effluents (and also direct exposures by injection), were able to alter the metabolism of serotonin and dopamine (both players in the sexual differentiation), likely via E2 receptor-mediate pathway and serotonin receptors.[85] All these examples do show the current pertinence to address EDCs impacts over the nervous system of bivalves, and looking for the impacts of the gonadal maturation events.

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