Chapter 3

Structure-Function of Serotonin in Bivalve Molluscs

Sayyed Mohammad Hadi Alavi, Kazue Nagasawa,

Keisuke G. Takahashi and Makoto Osada

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.69165

Abstract

It has been observed that 5-HT excites the heart nerves in hard clam and regulates contraction and relaxation of the anterior byssus retractor muscle in the blue mussel. It is now known that 5-HT regulates several neurobehavioral systems such as mood, appetite, sleep, learning, and memory. It also plays critical roles in the physiological functions of peripheral organs involved in stress, growth, and reproduction in the animal kingdom. The present study reviews conserved 5-HT biosynthesis and its localization in the nervous system, and its physiological contribution to regulate reproduction in bivalves. In the cytosol of neurons, tryptophan hydroxylase catalyzes hydroxylation of L-tryptophan to 5-hydroxytryptophan, which is converted to 5-HT by aromatic L-amino acid decarboxylase. A 5-HT transporter and a monoamine oxidase reuptakes and metabolizes 5-HT to control the amount of released 5-HT in the nervous system and peripheral organs. Perikarya and fibers of 5-HT neurons are mostly located in the cortices and neuropil of ganglia, respectively, and innervate the gonad. However, distribution and 5-HT content differ among species and sexes and undergo seasonal variations associated with gonadal development. The present review pays a special attention to future research perspectives to better understand 5-HT regulation of reproduction in bivalves.

Keywords: gonad, nervous system, oocyte, serotonin biosynthesis, serotonin metabolism, reuptake, sperm

1. Introduction

5-Hydroxytryptamine called serotonin (5-HT) is a transmitter substance of the nervous system in animal kingdom. From its first discovery in the 1940s, many laboratories have been directing their studies toward understanding the biology of 5-HT and its physiological functions on various biological systems especially on mammals as model organism [1–15]. However, 5-HT has been also identified in bivalves from the period of its first discovery and earlier studies on these animals have led to convince the neurobiologist that it acts as a neurotransmitter.

A brief bibliography of 5-HT discovery and its physiological functions is provided in **Table 1**. Rapport et al. [16] was the first who isolated a vasoconstrictor substance from the blood serum in a crystalline form and tentatively identified it as 5-HT in a creatinine sulfate complex [17].

Year	Scientists	Contribution to discovery of identification, localization, and characterization of 5-HT	References
1947	Rapport et al.	Isolation of a substance from the blood serum that constricts blood vessels and contracts isolated intestinal strips	[16]
1948	Rapport et al.	The substance contains an indole ring	[42]
1949	Rapport	Identification of chemical structure of 5-HT as a creatinine sulfate complex	[17]
1951	Hamlin and Fisher	Synthesis of 5-HT	[18]
1953	Twarog and Page	Identification of 5-HT in the extract of the brain of mammals (dog, rat, and rabbit)	[19]
1953	Gaddum	Assigning a role for 5-HT in normal cerebral function in mammals	[43]
1953	Welsh	5-HT, in contrast to acetylcholine, excites the heart nerves in hard clam (Bivalvia, Mollusca) originating from visceral ganglion	[21]
1954	Amin et al.	Localization of 5-HT in the central nervous system (brain) of mammals (dog)	[20]
1954	Wooley and Shaw	Human schizophrenia might be due to 5-HT deficiency	[44]
1954	Twarog	5-HT-mediated relaxation of byssus retractor muscle in the blue mussel (Bivalvia, Mollusca) is antagonist of acetylcholine contracting the muscle	[22]
1956	Hoyle and Lowy	5-HT is a putative neurotransmitter controlling contraction and relaxation of the anterior byssus retractor muscle in the blue mussel	[23]
1957	Brodie and Shore	Assigning 5-HT function as a neurotransmitter	[24]
1957	Welsh	Identification of 5-HT in the extract of nervous system of various bivalve mollusks	[25]
1962	Falck et al.	Development of Falck-Hillarp method to visualize monoamine-containing cells as intense yellow-green fluorescence	[45]
1964	Dahlström and Fuxe	Identification of 5-HT cell bodies in the pons and midbrain, from where they project with their axons to the forebrain, medulla, and spinal cord	[46]
1968	Sweeney	Identification and localization of 5-HT in whole body extract and in the nervous system of blue mussel using Falck-Hillarp's method	[47]

Year	Scientists	Contribution to discovery of identification, localization, and characterization of 5-HT	References
1982	Matsutani and Nomura	Serotonin stimulates spawning in Yesso scallop (Bivalvia, Mollusca)	[33]
1984	Hirai and Koide	5-HT stimulates oocyte maturation in surf clam	[48]
1985	Osanai	5-HT regulation of the oocyte signaling required to undergo germinal vesicle breakdown	[49]

Species: Blue mussel, *Mytilus edulis*; hard clam, *Mercenaria mercenaria*; surf clam, *Spisula solidissima*; Yesso scallop, *Patinopecten yessoensis*.

Table 1. Bibliography of 5-hydroxytryptamine (serotonin, 5-HT): from discovery to physiological characterization.

Within next 5 years, 5-HT has been synthesized [18], identified in the extract of mammalian brain [19], and localized in the brain of mammals [20]. Along with these studies on mammals, Welsh [21], Twarog [22], and Hoyle and Lowy [23] demonstrated that 5-HT excites the heart nerves in hard clam (*Mercenaria mercenaria*), and regulates contraction and relaxation of the anterior byssus retractor muscle in the blue mussel (*Mytilus edulis*) that both belong to Bivalvia, Mollusca. These observations resulted in identification of 5-HT as a neurotransmitter in the nervous system of mammals [24]. In the same year, Welsh [25] identified 5-HT in the nervous system of bivalves and demonstrated that 5-HT content in these animals is higher than other invertebrates and vertebrates [26]. Moreover, bivalves have served some advantages to be used as experimental model: (A) they are small which is a great opportunity to conduct serial examinations on the whole organism, (B) they have a simple nervous system, (C) the nervous system is relatively large in size and easy to dissect ganglia and connectives, and (D) the nervous system contains high amount of 5-HT.

Serotonin regulates various neurobehavioral systems (such as mood, appetite, sleep, learning, and memory). However, studies have revealed that it also plays critical roles in physiological functions of peripheral organs such as stress and growth [3–5]. One of the major systems that 5-HT contributes to is the regulation of reproduction. In both mammals and bivalves, it has been observed that 5-HT regulates reproductive endocrine system, oocyte maturation, and sperm motility [27–38].

Although 5-HT biosynthesis and its receptor structure have been reviewed in bivalves [39–41], there is a gap of review on physiological signaling of 5-HT in these animals. The present study reviews the biology of 5-HT in bivalves; particularly its contribution to reproduction. Biosynthesis pathway of 5-HT in the nervous system and cellular localization of 5-HT neurons in the nervous system are studied. Particular attention has then paid to 5-HT content and distribution of 5-HT neurons in the gonad. This study provides future perspectives that await investigation to better understand 5-HT network and signaling in bivalve reproduction.

2. Biosynthesis, metabolism, and reuptake of 5-HT in the nervous system

Hamlin and Fisher [18] were the first who synthesized 5-HT from tryptophan. A year later, Blaschko [50] suggested that 5-hydroxytryptophan (5-HTP) is the substrate for 5-HT. This

suggestion led to the discovery of an enzyme in mammalian kidney [51], later called aromatic L-amino acid decarboxylase (AADC) [52] that mainly decarboxylates 5-HTP to 5-HT [53]. In parallel, studies have shown that the extract of mammalian brain contains 5-HT [19], and administration of exogenous 5-HTP or tryptophan increases 5-HT level in the brain and peripheral organs [54, 55]. A year later, Welsh and Moorhead [56] observed that homogenates of ganglia of hard clam are capable of synthesizing 5-HT from 5-HTP, *in vitro*. Further studies using the blue mussel (*Mytilus edulis*) demonstrated presence of precursors of 5-HT (either tryptophan or 5-HTP) [57–59], and decarboxylation of 5-HTP to 5-HT [60, 61]. Thus, 5-HT biosynthesis in bivalves is similar to those of higher vertebrates. Although aforementioned studies have shown biosynthesis pathway of 5-HT and demonstrated that both nervous system and peripheral organs contain 5-HT; however, it was still unknown where the 5-HT biosynthesis takes place and how it gets transferred to other organs.

In 1960s, Bertaccini [62] and Gal et al. [63] demonstrated that the brain contains 5-HT even after partial or complete removal of 5-HT in the gastro-intestinal tissues and the brain produces 5-HT after intracerebral injection of radioactive labeled tryptophan. It is worth noting that it has previously been shown that the intestine contains large amount of 5-HT [64]. These studies provided the scientists with very important information that the brain independently synthesizes 5-HT from L-tryptophan, and suggested that exogenous 5-HT administration incorporates to 5-HT contents in the nervous system. Next studies resulted in molecular identity of two major enzymes in 5-HT biosynthesis pathway: tryptophan hydroxylase (TPH) and AADC [6, 14, 65, 66] (**Figure 1**). In the cytosol of the nerve cells, TPH catalyzes hydroxylation of L-tryptophan to produce 5-HTP by incorporation of an atom of atmospheric oxygen into L-tryptophan and the other is reduced to water, in the presence of the cofactor agent, tetrahydrobiopterin. The pathway is rate-limiting step meaning that suppression of TPH activity results in stopping 5-HT biosynthesis. The AADC catalyzes conversion of 5-HTP to 5-HT which is not rate-limiting step. It has also been shown that the rate at which 5-HT is produced in the central nervous system highly depends on availability of tryptophan, tryptophan uptake into the brain, and dietary contents of tryptophan and other amino acids (such as tyrosine and phenylalanine) that compete with tryptophan uptake or transport carrier into the brain [8, 14, 67].

In the snail, it has been observed that certain nerves are capable of accumulating radioactive labeled 5-HT [68]. Using bivalves, Stefano and Aiello [69] observed that fluorescence intensity of 5-HT-immunoreactive (5-HT-IR) neurons increases in the blue mussel after administration of exogenous 5-HT. Thus, as in mammals, 5-HT biosynthesis in bivalve mollusks also takes place in the nervous system.

Further studies have shown that there are biological systems through which external amounts of the released 5-HT is regulated, as its rise may cause abnormal physiological functions or might be lethal for cells. Reuptake and metabolism of 5-HT are key determinants to remove and/or inactivate significant amount of released 5-HT, respectively. Metabolism of 5-HT is mediated by monoamine oxidase (MOA) located in the outer membrane of mitochondria, and catalyzes the oxidative deaminative of 5-HT to 5-hydroxy-3-indolacetaldehyde (5-HIAL), which is further metabolized into 5-hydroxy-3-indolacetic acid (5-HIAA) by an

NAD^+-dependent aldehyde dehydrogenase. In addition, an NADH-dependent aldehyde reductase or an NADPH-dependent alcohol-dehydrogenase converts 5-HIAL to 5-hydroxytryptophol (5-HTOL) [6, 70] (**Figure 1**). In mollusks, small amount of MOA has been reported [71]. Boutet et al. [72] cloned MOA molecular structure in the Pacific oyster. Administration of MAO inhibitor leads to increase in the number and intensity of 5-HT-IR neurons in the blue mussel [69]. Thus, metabolism of 5-HT is active in bivalve mollusks. However, studies have demonstrated that 5-HT action at the synapse is mostly terminated by its reuptake across the presynaptic membrane [73–77].

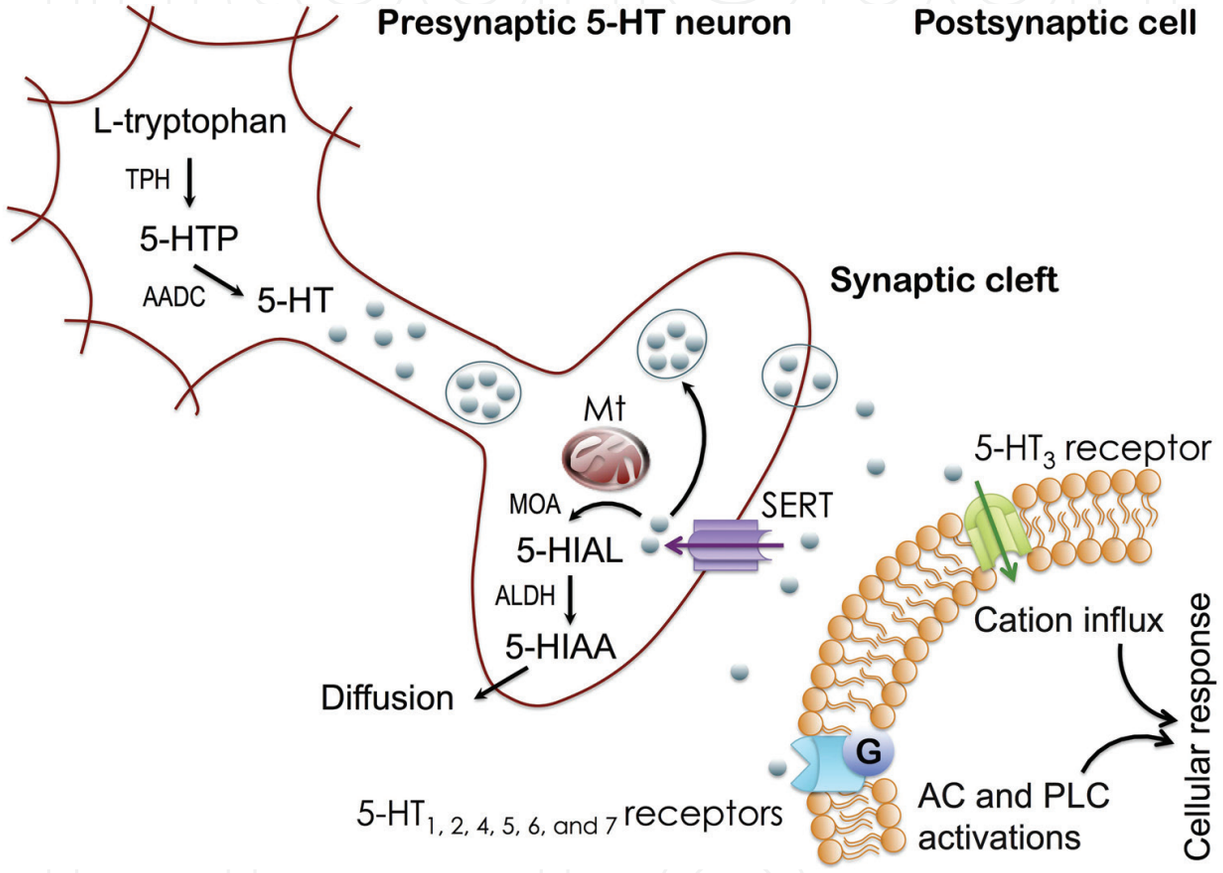

Figure 1. Biosynthesis, metabolism and reuptake of 5-hydroxytryptamine (serotonin, 5-HT) in bivalves. In the cytosol of the 5-HT neurons, tryptophan hydroxylase (TPH) catalyzes hydroxylation of L-tryptophan to produce 5-hydroxytryptophan (5-HTP) that becomes converted to 5-HT by aromatic L-amino acid decarboxylase (AADC). Conversion of L-tryptophan to 5-HTP is rate-limiting step meaning that suppression of TPH activity results in stopping 5-HT biosynthesis, however AADC-catalyzed conversion of 5-HTP to 5-HT is not rate-limiting pathway. The 5-HT vesicles are transferred to axon terminal and released to synaptic cleft. Reuptake and metabolism of 5-HT are key determinants to inactivate significant amount of the released 5-HT. In mollusks including bivalves, 5-HT reuptake from synaptic cleft is more than the enzymatic destruction. It is an ionic-coupled system and mediated by a serotonin transporter (SERT) that transports 5-HT from synaptic cleft to the presynaptic 5-HT neuron. However, enzymatic destruction of 5-HT also exists which is mediated by monoamine oxidase (MOA) located in the outer membrane of mitochondria (Mt). The MOA catalyzes the oxidative deaminative of 5-HT to 5-hydroxy-3-indolacetaldehyde (5-HIAL) that is metabolized into 5-hydroxy-3-indolacetic acid (5-HIAA) by aldehyde dehydrogenase (ALDH). Released 5-HT binds to its receptor(s) on the surface of a postsynaptic cell or postsynaptic neuron (not shown in the figure) to trigger intracellular signaling required for a cellular response, e.g., stimulation of oocyte and sperm maturation. The 5-HT receptors are mainly G-protein coupled receptor ($5\text{-HT}_{1, 2, 4, 6, 5, \text{and } 7}$ receptors), which induce adenylate cyclase (AC) or phospholipase C signaling (PLC). However, the 5-HT_3 receptor is a ligand-gated ion channel and regulates ionic influx.

The 5-HT reuptake is also similar between mollusks and mammals. It is an ionic-coupled pathway mediated by a serotonin transporter (SERT) that transport 5-HT from synaptic cleft to the presynaptic neuron [9, 12, 78]. SERT first binds a Na^+ ion, followed by 5-HT, and then a Cl^- ion in the synaptic cleft and transport to presynaptic neuron. After releasing 5-HT, K^+ efflux is involved in the translocation mechanism of SERT. This is an energy dependent process and a Na^+/K^+ ATPase maintains the extracellular Na^+ concentration as well as the intracellular K^+ concentration [79]. This mechanism results in the inactivation of 5-HT by removing it from the synaptic cleft. Studies have also shown that a 5-HT reuptake inhibitor (SRI) interferes with SERT function to inhibit or suppress 5-HT reuptake [80, 81].

3. Anatomy of the nervous system in bivalves

3.1. Nervous system

In bivalves, the nervous system is bilaterally symmetrical, decentralized, and consists of cerebral ganglia (CG), pedal ganglia (PG), and visceral ganglia (VG). The ganglia are joined by a cerebral commissure, a visceral commissure, and cerebral-pedal, cerebral-visceral, and cerebral-visceral-pedal connectives [82–86] (**Figure 2**). Each ganglion is surrounded by a perineurium.

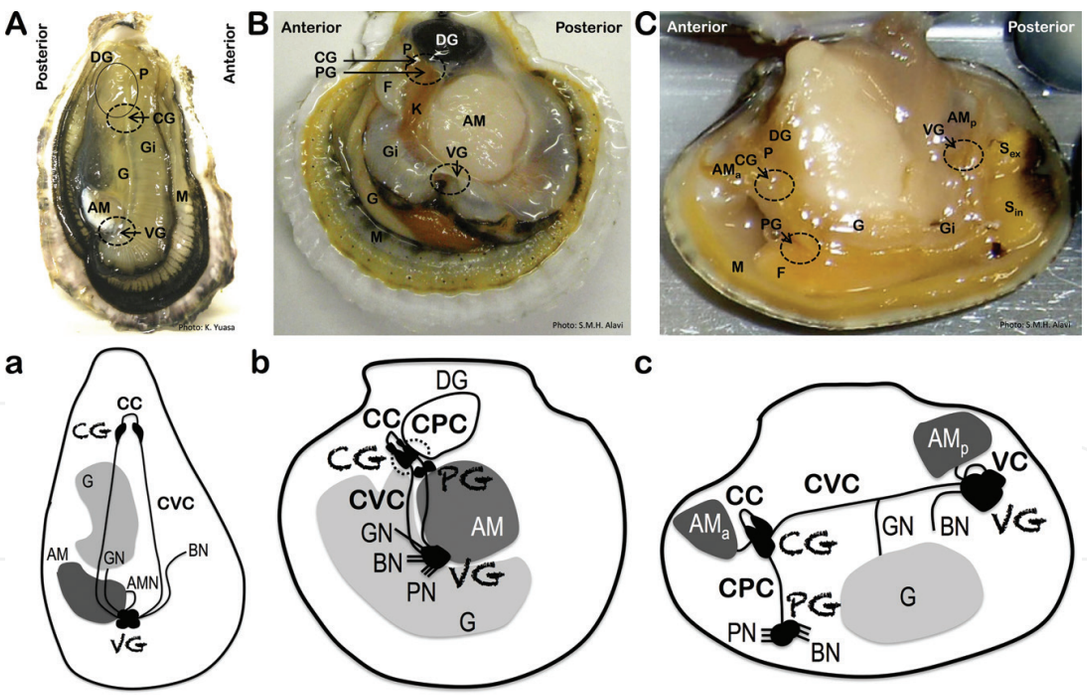

Figure 2. Anatomy of the nervous system in bivalves. It is decentralized and consists of bilaterally symmetrical cerebral ganglia (CG), pedal ganglia (PG), and visceral ganglia (VG). The locations of ganglia highly differ among species; however, they are connected by nerve connectives. The PG are absent in oysters (e.g., Pacific oyster, *Crassostrea gigas*, A). All parts of the nervous system exist in scallops (e.g., Yesso scallop, *Patinopecten yessoensis*, B) and clam species (e.g., Manila clam, *Ruditapes philippinarum*, C). Panels a, b, and c are representative schematics of intercommunicating ganglia in Pacific oyster [83], Yesso scallop (the authors), and soft-shell clam, *Mya arenaria* [89], respectively. In most bivalves, VG innervates the gonad. $AM_{a\ and\ p}$, anterior and posterior adductor muscle; AMN, adductor muscle nerves; BN, bronchial nerves; CC, cerebral commissure; CPC, cerebral-pedal connective; CVC, cerebral-visceral connective; DG, digestive gland; F, foot; G, gonad; GN, gonad nerves; Gi, gills; K, kidney; M, mantle; P, labial palp; PN, pallial nerve; S_{in}, incurrent siphon; S_{ex}, excurrent siphon.

The neuronal cell bodies "perikarya" are located at the cortices and the axonal processes lie at central core called "neuropil".

The pairs of CG lie on the sides of esophagus and are connected by a cerebral commissure in bivalves. In oyster species, CG are less developed and positioned at the sharp angle anterior to the labial palp, gills, and digestive gland [83]. In mussel and clam species, CG are located anterior to the digestive gland, and beneath the anterior adductor muscle [82, 84]. In freshwater pearl mussel (*Hyriopsis bialata*), CG are fused [87]. In scallop species, the foot is positioned anterior to CG, and adductor muscle and digestive gland are located posterior to CG [82, 86]. Each CG consists of an anterior lobe and a posterior lobe [88]. The CG innervate the palps, anterior adductor muscle, and parts of mantle [83, 84, 86].

In most bivalves, the pairs of PG lie on the foot and are connected by a pedal commissure [84–86]. However, PG are absent in oyster species [83]. In soft-shell clam (*Mya arenaria*), the PG are fused [89]. In freshwater pearl mussel, PG are positioned in the visceral mass [87]. The PG innervate the foot [84, 86].

The paired VG are located on the ventral side of the adductor muscle, usually posterior to foot. In most bivalves, ganglia of VG are fused into a single organ [83, 89–91]. In scallop species, VG consist of five lobes; two anterior lobes, a posterior lobe, and two lateral lobes [88, 90]. There is an accessory ganglion that locates at the point of the lateral lobes. The CG and VG are joined by a pair of cerebral-visceral connective that pass through the digestive gland or gonad. The VG innervate various organs, including gonads, gills, hearts, sensory organs, posterior adductor muscle, and parts of mantle [83, 84, 86].

3.2. Anatomy and annual cycle of neurosecretory cells in bivalves

Rawitz [92] seems to be first who isolated pear- or club-shaped neurons from the European flat oyster (*Ostrea edulis*). The neurons are classified into unipolar, bipolar, and multipolar neurons (**Figure 3**) [93]. Illanes-Bucher [94] classified the neurosecretory cells into A1, A2, A3, and A4 in the blue mussel. The A1-type neurons are small (6–15 μm), unipolar, and nucleus is located opposite to the axonal cone. The A2-type nerve cells are large (20–30 μm), multipolar, and nucleus is eccentric. The A3-type nerve cells are large (20–25 μm), unipolar, and nucleus is eccentric. The A4-type nerve cells are medium in size (12–15 μm), apparently unipolar, and contain numerous vacuoles surrounded by neurosecretory granules. Blake [95] observed that the neurosecretory cycle of neurons in the CG of the Bay scallop (*Argopecten irradians*) appeared identical to that of the VG. The neurosecretory cells also undergo distinct annual cycle [96–99]. Seasonal changes in the activity of neurosecretory cells are also associated with gonadal development, and the cells release their products at maturity stage [96]. Moreover, number of active neurosecretory cells positively correlates with progress of the gonad development in the Bay scallop [95], clam (*Katelysia opima*) [100], blue mussel [101], and green-lipped mussel (*Perna canaliculus*) [102].

3.3. Identification and cellular localization of 5-HT

Cellular localization of 5-HT neurons and its quantitative bioassay in the nervous system and gonads provide us with highly satisfactory knowledge to elucidate ontogeny and developmental

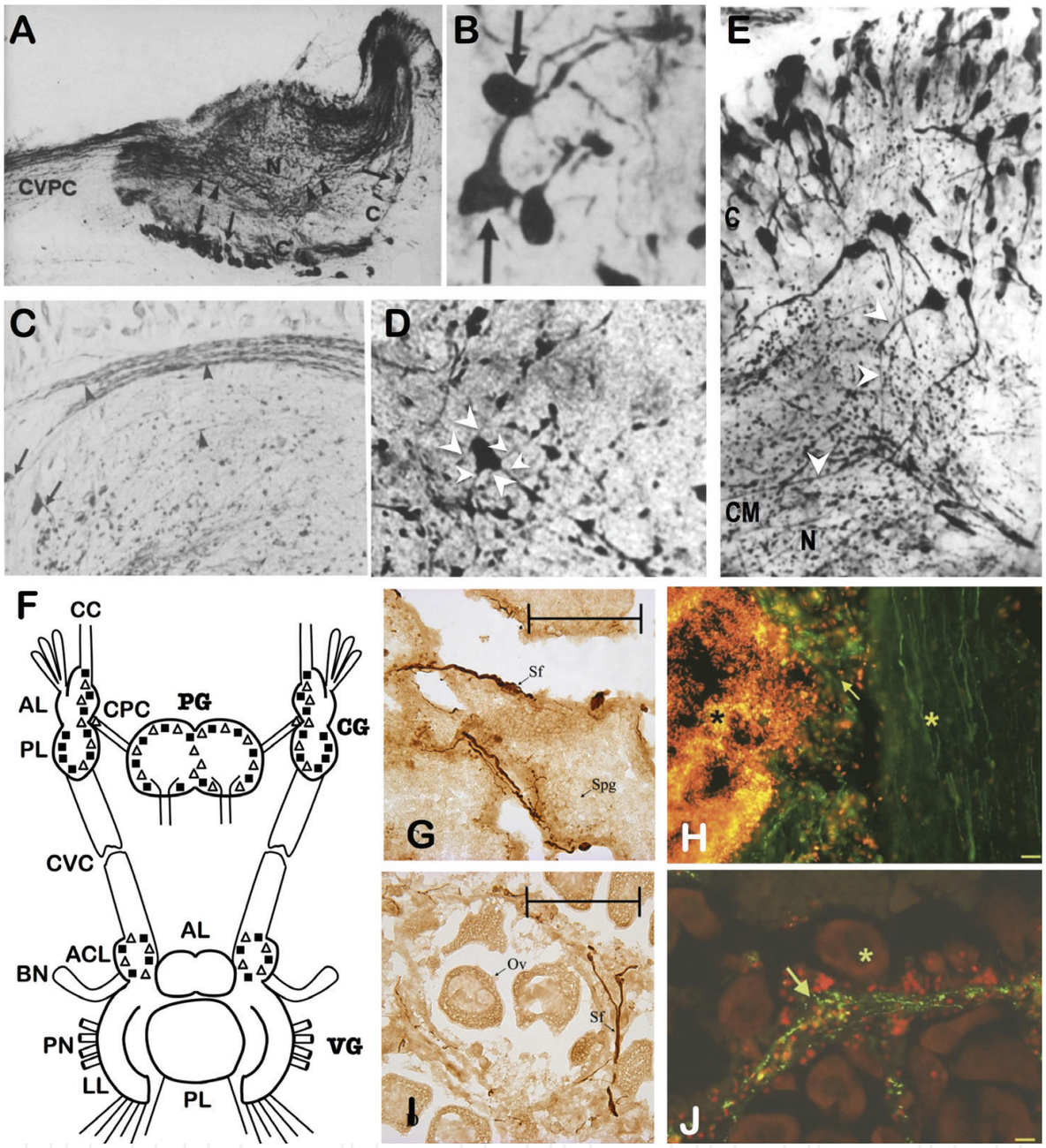

Figure 3. Cellular localization of 5-hydroxytryptamine (serotonin, 5-HT) in the nervous system (A–F) and gonad (G–J) of bivalves. (A) The 5-HT immunoreactive (5-HT-IR) cell bodies (arrows) and fibers (arrowheads) in the cortex (C) and neuropil (N) of cerebral ganglia (CG) (135×). (B) A few 5-HT-IR unipolar neurons with cell bodies (arrows) and their process in the CG (360×). (C) 5-HT-IR neurons (arrows) and fibers (arrowheads) in the visceral ganglion (380×). (D) a 5-HT-IR multipolar neuron with its processes (arrows) in pedal ganglion (PG) (800×). (E) Pear-shaped unipolar 5-HT-IR neurons and fibers in cortex (C) and neuropil (N) of PG. The arrowheads show long process of (the axon) of a 5-HT-IR neuron that runs toward commissure (CM) (315×). CVPC is cerebral-visceral-pedal connective. (A)–(C) [103], (D) and (E) [104] show localization of the 5-HT neurons in *Mytilus galloprovincialis*. (F) A schematic of localization of the 5-HT neurons in Yesso scallop, *Patinopecten yessoensis* (■) [105] and great scallop, *Pecten maximus* (Δ) [90]. (G) and (H) the 5-HT-IR fibers in the testis of *Mya arenaria* and *Venus verrucosa*, respectively. The 5-HT-IR fibers (Sf) are seen in the testis containing spermatogonia (Spg) at early stage II of the development (I). The 5-HT-IR fibers (arrows) originated from cerebral-visceral connective (yellow asterisk) surround acini full of sperm (black asterisk) (H). (I) and (J) The 5-HT-IR fibers in the ovary of *M. arenaria* and *V. verrucosa*, respectively. The 5-HT-IR fibers (Sf) surround the ovary containing post-vitellogenic oocytes (Ov) (I). The 5-HT-IR fibers (arrow) surround the wall of the follicles filled with mature oocytes (asterisk) (J). Scale bar G and I = 100 μm [106] and H and J = 20 μm [91].

biology of 5-HT biosynthesis, release, and reuptake, and to understand 5-HT regulation of reproduction in bivalves.

3.3.1. 5-HT in the nervous system of bivalves

Welsh [25] was the first who identified 5-HT in the nervous system of the hard clam using a paper chromatography method. Then, Welsh and Moorhead [26, 56, 107] used a spectrofluorometric method to measure 5-HT in over 60 species from 11 different phyla that includes 7 bivalve species (**Table 2**) [108]. They reported that (A) the nervous system of bivalves contains much higher 5-HT than that of other invertebrates. In the phylum Annelida, 5-HT is measured 0.1–10.4 μg/g wet in the nerve cords. In the phylum of Arthropoda, 5-HT is measured from <1.0 μg/g wet in the nerve cords, ventral ganglia, and green ganglia. In vertebrates, 5-HT is measured 0.3–2.6 μg/g wet in different parts of cat brain [109]. (B) Content of 5-HT is higher in the nervous system than the peripheral organs. (C) Content of 5-HT differs among various parts of the nervous system. It is higher in the ganglia than the connective nerves. In addition, they observed that 5-HT content is slightly lower in VG than those of CG and PG (10 vs. 15 μg/g wet) in the blue mussel. (D) The blood does not contain 5-HT. The authors suggested that 5-HT is produced in the nervous system: in cell bodies or synaptic region of neurons.

Species	Notes	Nervous system	Gonad	Reference
Brown mussel *Perna perna*	M: HPLC-ED V: pg/mg wet (mean ± SEM) Jul.: Resting stage Sep.: Developmental stage I–II Mar.: Maturation stage IIIA Apr.: Egg-laying stage	5-HT 74 ± 16 (PG), 51 ± 7 (CG) (Jul.) 115 ± 20 (PG), 61 ± 6 (CG) (Sep.) 293 ± 54 (PG), 63 ± 7 (CG) (Mar.) 302 ± 47 (PG), 150 ± 9 (CG) (Apr.) 5-HIAA 79 ± 22 (PG), 56 ± 30 (CG) (Jul.) 122 ± 30 (PG), 11 ± 1 (CG) (Sep.) 166 ± 46 (PG), 46 ± 12 (CG) (Mar.) 56 ± 16 (PG), 83 ± 40 (CG) (Apr.)	5-HT 8.7 ± 0.6 (Jul.) 31 ± 5.7 (Sep.) 142 ± 49.6 (Mar.) 142 ± 14.3 (Apr.) 5-HIAA 188 ± 36 (Jul.) 443 ± 70 (Sep.) 29 ± 6 (Mar.) 51 ± 5 (Apr.)	[110]
Pacific lion's paw scallop *Nodipecten subnodosus*	M: HPLC V: ng/mg dry (mean ± SD) I: Resting stage II: Initial development stage III: Maturing stage IV: Mature stage V: Partially spent stage VI: Fully spent stage		5-HT I: ND (O), 0.35 ± 0.63 (T) II: ND (O), 0.87 ± 0.94 (T) III: 0.04 ± 0.07 (O), 0.65 ± 0.72 (T) IV: 0.12 ± 0.19 (O), 2.04 ± 2.18 (T) V: ND (O), 0.42 ± 0.56 (T) VI: ND (O), ND (T)	[111]

Species	Notes	Nervous system	Gonad	Reference
Surf clam *Spisula solidissima*	M: HPLC V: ng/g wet (mean ± SEM) I. Active stage II. Ripe stage III. Spawning stage IV: Spent stage * shows $P < 0.05$ compared to stages I and IV		5-HT I: 625 ± 100 (O), 550 ± 100 (T) II: 175 ± 50* (O), 225 ± 65 (T) III: 350 ± 95* (O), 500 ± 150 (T) IV: 1050 ± 250 (O), 575 ± 400 (T)	[112]
Peruvian scallop *Argopecten purpuratus*	M: Spectrofluorometer V: ng/mg wet (mean ± SEM) It is a hermaphroditic species VG innervates mainly the female portion of the gonad CG and PG innervate mainly the male portion of the gonad * shows $P < 0.05$ compared to before spawning	5-HT CG + PG + VG 29.4 ± 4.3 (before spawning) 17.9* ± 0.6 (after sperm release) 22.5 ± 0.5 (after oocyte release) 21.3* ± 2.3 (24 h after spawning) CG + PG 107.3 ± 12.9 (**b**efore spawning) 63.6 ± 2.1* (spawned) 100.0 ± 16.3 (unspawned) VG 50.7 ± 4.3 (before spawning) 51.8 ± 5.1 (spawned) 53.3 ± 12.4 (unspawned)	5-HT Ovary portion of gonad 1.0 ± 0.03 (before spawning) 0.6* ± 0.02 (after sperm release) 0.5* ± 0.05 (after oocyte release) 0.7 ± 0.15 (24 h after spawning) Testis portion of gonad 1.7 ± 0.15 (before spawning) 0.8* ± 0.05 (after sperm release) 0.7* ± 0.09 (after oocyte release) 1.2 ± 0.05 (24 h after spawning)	[113]
Atlantic deep-sea scallop *Placopecten magellanicus*	M: HPLC-ED V: pg/mg wet (mean ± N.D.) Samples of March	CG + PG + VG 5-HTP: 1650 ± 715 5-HT: 1150 ± 525 5-HIAA: 180 ± 90	5-HTP: 2035 ± 520 5-HT: 1000 ± 180 5-HIAA: 90 ± 15	[114]
Atlantic deep-sea scallop *Placopecten magellanicus*	M: HPLC-ED V: pg/mg wet (mean ± N.D.) Samples of March–May	CG + PG + VG 5-HT: 1483 ± 828	5-HT: 791 ± 408	[115]
Peruvian scallop *Argopecten purpuratus*	M: Spectrofluorometer V: ng/mg wet (mean ± SEM)	5-HT CG + PG + VG 48.3 ± 7.2 (0 d) 46.2 ± 9.7 (0.5 d) 40.0 ± 5.6 (1 d) 37.9 ± 3.5 (7 d) 44.5 ± 5.7 (14 d) 39.0 ± 6.0 (21 d) 47.2 ± 6.2 (28 d) 63.3 ± 12.6 (35 d)	5-HT Gonad ovary (O) or testis (T) portion 1.3 ± 0.02 O, 6.8 ± 0.5 T (0 d) 0.7 ± 0.03 O, 2.2 ± 0.7 T (0.5 d) 0.7 ± 0.02 O, 2.5 ± 0.5 T (1 d) 1.5 ± 0.34 O, 3.0 ± 0.5 T (7 d) 1.6 ± 0.02 O, 4.8 ± 0.4 T (14 d) 1.4 ± 0.03 O, 4.6 ± 1.3 T (21 d) 1.0 ± 0.04 O, 4.4 ± 0.4 T (28 d) 1.1 ± 0.01 O, 4.9 ± 0.9 T (35 d)	[116]

Species	Notes	Nervous system	Gonad	Reference
Great scallop *Pecten maximus*	M: HPLC-ED V: ng/g.p. (mean ± SEM) Samples of mature individuals (3-year old)	CG + PG 330 (Jul., 1991) 405 (Aug., 1991) 510 (Nov., 1991) 510 (Dec., 1991) 180 (Jan., 1992) 270 (Feb. 1992) 240 (beginning of Mar., 1992) 210 (middle of Mar., 1992) 180 (end of Mar., 1992) 225 (Apr., 1992) 300 (May, 1992) 300 (June, 1992) VG 350 (Jul., 1991) 410 (Aug., 1991) 550 (Nov., 1991) 405 (Dec., 1991) 290 (Jan., 1992) 350 (Feb. 1992) 290 (beginning of Mar., 1992) 200 (middle of Mar., 1992) 315 (end of Mar., 1992) 350 (beginning of Apr., 1992) 450 (middle of Apr., 1992) 350 (beginning of May, 1992) 425 (end of May, 1992) 425 (June, 1992)		[90]
California mussel *Mytilus californianus*	M: HPLC-ED V: nM/ganglia pair (mean ± SEM) Samples of mature individuals in March–May	0.09 ± 0.02 (CG) 0.22 ± 0.05 (PG) 0.41 ± 0.07 (VG)		[117]
Blue mussel *Mytilus edulis*	M: HPLC-ED V: nM/g.p. (mean ± SEM) Samples of mature individuals in March–May	0.04 ± 0.01 (CG) 0.06 ± 0.003 (PG)		[117]
Gaper clam *Tresus capax*	M: HPLC-ED V: nM/g.p. (mean ± SEM) Samples of mature individuals in March–May	0.70 ± 0.11 (CG) 0.39 ± 0.06 (PG) 0.48 ± 0.06 (VG)		[117]
Cockle clam *Clinocardium nuttallii*	M: HPLC-ED V: nM/g.p. (mean ± SEM) Samples of mature individuals in March–May	0.22 ± 0.01 (PG) 0.24 ± 0.04 (VG)		[117]

Species	Notes	Nervous system	Gonad	Reference
Bent-nose clam *Macoma nasuta*	M: HPLC-ED V: nM/g.p. (mean ± SEM) Samples of mature individuals in March–May	0.20 ± 0.06 (CG) 0.15 ± 0.004 (VG)		[117]
Blue mussel *Mytilus edulis*	M: Spectrofluorometer V: μg/g wet (mean ± SEM) *, **, and *** show $P < 0.005$, $P < 0.001$, and $P < 0.05$ compared to Jan, respectively	2 CG + 2 PG + 2 VG 25.10 ± 2.71 (Jan.) 26.96 ± 2.11 (Feb.) 32.17 ± 3.85 (Mar.) 41.98 ± 1.22* (Apr.) 48.15 ± 1.02** (May) 53.13 ± 1.71** (Jun.) 51.74 ± 3.14** (Jul.) 57.28 ± 2.49** (Aug.) 48.90 ± 1.13* (Sep.) 44.80 ± 1.51* (Oct.) 35.71 ± 2.70*** (Nov.) 28.97 ± 2.64 (Dec.)		[118]
Blue mussel *Mytilus edulis*	M: Spectrofluorometer V: ng/ganglion pair (mean ± SD)	5-HT 123 ± 12 – 252 ± 34 (PG)		[119]
Blue mussel *Mytilus edulis*	M: Spectrofluorometer V: μg/g wet (mean ± N.D.)	5-HT 5.4–8.6 (PG, Mar.) 26.2-42 (PG, Apr.)		[120]
Fingernail clam *Sphaerium sulcatum*	M: Spectrofluorometer V: ng/individual (mean ± N.D.)	13.4 ± 2.5 (whole body extracts)		[47]
Ocean quahog *Arctica islandica*	M: Spectrofluorometer V: μg/g wet	5-HT CG + PG + VG 20		[26]
Atlantic jackknife clam *Ensis directus*	M: Spectrofluorometer V: μg/g wet	5-HT CG + PG + VG 21-39		[26]
Soft-shell clam *Mya arenaria*	M: Spectrofluorometer V: μg/g wet	5-HT CG + PG + VG 22		[26]
Hard clam *Venus mercenaria*	M: Spectrofluorometer V: μg/g wet 26 assays during 16 months	5-HT CG + PG + VG 30–40		[26, 107]
Atlantic surf clam *Spisula solidissima*	M: Spectrofluorometer V: μg/g wet	5-HT CG + PG + VG 8.0–14.3 Ganglia connectives 2.2		[26]
Atlantic deep-sea scallop *Placopecten magellanicus*	M: Spectrofluorometer V: μg/g wet	5-HT 36 (VG)		[26]

Species	Notes	Nervous system	Gonad	Reference
Blue mussel *Mytilus edulis*	M: Spectrofluorometer V: μg/g wet	5-HT 15 (CG) 15 (PG) 10 (VG)		[26]

Abbreviation: CG, cerebral, cerebral-pleural or cerebroid ganglion; d, day; g.p., ganglia pair; HPLC-ED, high-performance liquid chromatography coupled with electrochemical detection; M, methods; N.D., not determined; O, ovary; PG, pedal ganglion; T, testis; SD, standard deviation; SEM, standard error of mean; V, values; VG, visceral ganglion.

Table 2. Identification of 5-hydroxytryptophan, (5-HTP), serotonin (5-hydroxytryptamine, 5-HT), and 5-hydroxyindoleacetic acid (5-HIAA) in the nervous system and gonad of bivalve mollusks.

Following development of cellular and molecular methods, 5-HT has been localized in the nervous system and gonad of several bivalve species (**Table 3**). Firstly, Falck-Hillarp's method has been used to localize 5-HT in fingernail clam (*Sphaerium sulcatum*) [47], blue mussel [69], and Yesso scallop [88]. In this method, histological sections are exposed to gaseous formaldehyde or glyoxylic acid to visualize monoamine containing neurons [45, 121, 122]. In all examined bivalve species, 5-HT-IR neurons are observed in CG, PG, and VG (**Table 3**). However, the Falck-Hillarp's method is not always useful as 5-HT fluorescence tends to faint rapidly. In addition, catecholamines neurons show similar intensity to that of 5-HT neurons at high concentrations [123]. In 1978, Steinbusch et al. [124] developed a rat monoclonal antibody against a 5-HT-bovine serum albumin conjugate to localize 5-HT in nervous system. Further studies have used monoclonal or polyclonal antibody against 5-HT to localize 5-HT-IR neurons in the nervous system and peripheral organs of bivalves (**Table 3**). The advantage of immunohistochemistry method using antibodies against 5-HT is to describe morphology of 5-HT neurons, and to localize 5-HT distribution within different parts of nervous system, precisely. The 5-HT-containing neurons are mostly unipolar, although their sizes may differ among species (**Table 3**). Using an electron microscopy, it has been observed that 5-HT-IR neurons are often in close connection with each other, but without indication of gap junctions or other specialized junctions. The neurons possess numbers of granular vesicles (100–180 nm in Mediterranean mussel) containing 5-HT that concentrated at the cell periphery [104, 125]. It has confirmed that 5-HT-IR fibers are the axon or axon terminals of 5-HT containing neurons that transport 5-HT to peripheral organs. Within the nervous system, 5-HT-IR fibers seem to be synaptic region, an area where release and reuptake of 5-HT occur.

In general, studies on bivalves show that 5-HT-IR neurons are mostly located in the cortices, and 5-HT-IR fibers are located in the neuropil of CG, PG, and VG (**Table 3**). In Yesso scallop, 5-HT-IR neurons are located in the cortices of the right side of the left lobe and in the left side of the right lobe in anterior lobe (AL) of CG, while they are located throughout their cortices in PG and the posterior lobe (PL) of CG [105] (**Figure 3**). In the great scallop [90], distribution of 5-HT-IR neurons in the posterior lobe of CG slightly differs compared to Yesso scallop. In VG, 5-HT-IR neurons are restrictively scattered in the accessory lobe of scallop species [90, 105, 115] or at the roots of branchial nerves in clams [89]. Large numbers of 5-HT-IR fibers have also been observed in the cerebral-pedal, and cerebral-visceral-pedal connectives [90, 103], suggesting that 5-HT transports from CG to VG [69, 89, 90, 105]. Comprehensive overview of

Species	Methods	Cerebral ganglia	Visceral ganglia	Pedal ganglia	Gonad	Reference
Fingernail clam *Sphaerium sulcatum*	Histochemistry using a paraformaldehyde-induced fluorescence method	5-HT-IR unipolar cells (μm length) are located in the cortices at the dorsal and anteriomedial surfaces of the ganglion. 5-HT-IR fibers are located in the anterior pallial nerve, the CVC, CC, and CPC	No traces of 5-HT-IR neurons are observed in the VG. 5-HT-IR fibers are observed	5-HT-IR fluorescences are uniformly distributed in the cytoplasm of unipolar neurons (10–25 μm length). Green-yellow fibers extend throughout neuropil and across the PC		[47]
Blue mussel *Mytilus edulis*	Histochemistry using a paraformaldehyde-induced fluorescence method	5-HT-IR neurons (9–14 μm d.) are only located in the cortex. Fluorescence is observed in the perikarya	A few 5-HT-IR neurons (11–14 μm d.) are located in the cortex and neuropil. 5-HT-IR fibers are observed in the CVC			[69]
Yesso scallop *Patinopecten yessoensis*	Histochemistry using a glyoxylic acid-induced fluorescence method	Fluorohistochemical reaction is detected in the neuropil, and its tendency is higher than PG and VG	Fluorohistochemical tendency is high in the accessory ganglia	Fluorohistochemical reaction is detected in the neuropil close to CPC	Muscles of the gonoduct stretched under the epithelium in the gonad	[88]
Yesso scallop *Patinopecten yessoensis*	Immunohistochemistry using a rat monoclonal 5-HT antibody against a 5-HT-bovine serum albumin conjugate (coded YC5/45 HL, Sera-Lab, UK)	5-HT-IR neurons are distributed in the AL (right side of the left lobe and left side of the right lobe), and throughout the cortex in PL	ND	5-HT-IR neurons are distributed throughout the cortex		[105]
Mediterranean mussel *Mytilus galloprovincialis*	Immunogold labeling of nerve cells using an anti-5-HT raised in rabbits against formaldehyde cross-linked 5-HT-bovine serum albumin (Immunonuclear, Incstar Co, Stillwater, MN)	5-HT-IR unipolar neurons are mostly located in the cortex with a few numbers in the neuropil. 5-HT-IR fibers are seen in the CC and CVPC	5-HT-IR neurons are unipolar and located in the cortex. Number of 5-HT-IR neurons is lower than CG.5-HT-IR fibers are seen in the visceral commissure and CVC	Large numbers of 5-HT-IR unipolar neurons and a few bipolar or multipolar are clustered in the cortex. 5-HT-IR fibers are observed in neuropil		[103, 104, 125, 126]

Species	Methods	Cerebral ganglia	Visceral ganglia	Pedal ganglia	Gonad	Reference
Great scallop *Pecten maximus*	Immunohistochemistry using an anti-5-HT polyclonal antibody (coded, PS10, TEBU)	5-HT-IR neurons are mostly located in the cortex: 10 or 20–25 μm d. 5-HT-IR fibers are seen in the CVC	A small number of 5-HT-IR neurons are seen in VG, restricted to ACL at the base of CVC	5-HT-IR neurons are mostly located in the cortex with size of 10 μm d. (small cells) or 20–25 μm d. (large cells)	5-HT-IR fibers surround periphery of gonadal lobules (acini) and in the subepithelial layer of the gonoducts	[90]
Atlantic deep-sea scallop *Placopecten magellanicus*	Immunohistochemistry using a rabbit anti-5-HT antibody (Incstar Co., Stillwater, MN)	5-HT-IR neurons are widely distributed over the anterior surface and only sparsely over the posterior surface. 5-HT-IR fibers are located in neuropil	5-HT-IR neurons are mainly distributed in the accessory ganglia. 5-HT-IR neurons and fibers are far fewer than CG and PG	5-HT-IR neurons are unipolar (5–15 μm d.) and located along the medial, dorsal, and ventral margins, of the anterior surface of each PG. 5-HT-IR fibers are located in neuropil	5-HT-IR fibers occasionally surround periphery of acini at early gametogenesis. After spawning, 5-HT-IR fibers abundantly surround the empty germinal acini	[115]
Surf clam *Spisula solidissima*	Immunohistochemistry using a rabbit anti-5-HT antibody (Incstar Co., Stillwater, MN)				5-HT-IR fibers surrounds periphery of gonadal lobules (acini) in males and females throughout reproductive cycle. The 5-HT-IR fibers are interrupted or expelled from each acinus after spawning	[112]
Warty venus *Venus verrucosa*	Immunohistochemistry using a rabbit anti-5-HT antibody (Biogenesis, UK)	5-HT-IR oval perikarya are clustered at the roots of the branchial nerves in the cortex. They are unipolar (15–25 μm d.). 5-HT-IR fibers are located in the neuropil			5-HT-IR fibers are observed at the periphery of the follicle and seminiferous acini filled with mature oocytes and sperm, respectively	[91]
Soft-shell clam *Mya arenaria*	Immunohistochemistry using a rabbit polyclonal anti-5-HT antibody (Sigma-Aldrich Co. LLC.)	Largest number of 5-HT-IR cells scattered throughout the cortex	5-HT-IR cells are symmetrically restricted to clustered population called "glomeruli"	5-HT-IR cells are symmetrically distributed in the cortex	Early spermatogenesis stage in males and post-vitellogenic stage in females	[89, 106]

Species	Methods	Cerebral ganglia	Visceral ganglia	Pedal ganglia	Gonad	Reference
Freshwater pearl mussel *Hyriopsis bialata*	Immunohistochemistry using a rabbit polyclonal anti-5-HT IgG (Zymed Laboratories, San Francisco, CA or Sigma-Aldrich Co. LLC.)	5-HT-IR neurons are large (10 × 30 μm d.) and located at the periphery of CG. 5-HT-IR fibers are occasionally detected	5-HT-IR perikarya are large (10 × 30 μm d.) and located in the cortex of VG. 5-HT-IR fibers are mostly observed in the neuropil. Expression of 5-HT-IR fibers or neurons is higher in females than males	5-HT-IR neurons are large (10 × 30 μm d.) and located at the periphery of PG		[87, 127]

Abbreviations: 5-HT-IR, serotonin-immunoreacted; ACL, accessory lobe; AL, anterior lobe; CC, cerebral commissure; CPC, cerebral-pedal connective; CVC, cerebral-visceral connective; CVPC, cerebral-visceral-pedal connective; PC, pedal commissure; d., diameter; ND, no 5-HT-IR neurons or fibers are detected; PL, posterior lobe.

Table 3. Cellular localization of 5-hydroxytryptamine (serotonin 5-HT) in the nervous system and gonad of bivalve mollusks.

cellular localization of 5-HT indicates that localization and distribution of 5-HT-IR neurons may differ among subclasses of bivalve, for instance between Heterodonta (genus *Mya*, *Ruditapes*, and *Venus*) and Pteriomorphia (genus *Pecten, Patinopecten*, and *Mytilus*) (**Table 3**). It might be due to differences in location of various parts of nervous system in the body to innervate peripheral organs.

Using histochemistry or immunohistochemistry methods, studies have shown that a few 5-HT-IR neurons are located in the cortex and neuropil of VG compared to those of the CG or PG, for instances in the blue mussel [47, 69, 128], Mediterranean mussel (*Mytilus galloprovincialis*) [103], great scallop [90], Atlantic deep-sea scallop (*Placopecten magellanicus*) [115], and soft-shell clam [89]. Matsutani and Nomura [105] reported no 5-HT-IR neurons in the VG of the Yesso scallop. Although VG contain a few 5-HT-IR neurons, they are usually rich in 5-HT-IR fibers. These studies confirm the Welsh and Moorhead's observation that 5-HT content differs among various parts of the nervous system.

Studies used spectrofluorometric method [26, 47, 56, 118–120] or electrochemical detection coupled with a high-performance liquid chromatography (HPLC-EC) to study 5-HT content in the nervous system of bivalves [90, 110, 114, 115, 117] (**Table 2**). Results confirm aforementioned differences in 5-HT content among various parts of the nervous system, for instance it is higher in the CG than the VG of gaper clam (*Tresus capax*) and bent-nose clam (*Macoma nasuta*) [117]. In addition, the metabolite of 5-HT (5-HIAA) is detected in the nervous system of the brown mussel (*Perna perna*) [110] and Atlantic deep-sea scallop [114], suggesting that metabolism of 5-HT takes place in the nervous system.

Welsh and Moorhead [56] observed that *in vitro* 5-HT synthesis by the nerve tissues undergoes a seasonal variation and suggested seasonal variation of amine oxidase. Further studies have shown that 5-HT content in the nervous system undergoes seasonal variation along with gonadal development in bivalves (**Table 2**). Content of 5-HT increases in the nervous system from early gonadal development to maturity stage in the brown mussel [110] and decreases following spawning in Peruvian scallop (*Argopecten purpuratus*) [113]. York and Twarog [120] reported that 5-HT in the PG of blue mussel is higher in April than March. It has also observed that 5-HT content in the whole nervous system of the blue mussel increases from April to October [118]. As the blue mussel spawns from late spring to late summer [129, 130], these data suggest that 5-HT content increases during spawning. 5-HT content also correlates with the content of its metabolite (5-HIAA), suggesting that metabolism of 5-HT is in parallel to its biosynthesis in the nervous system [110].

3.3.2. 5-HT in the gonad of bivalves

Localization of 5-HT in the gonad has studied in a few species of bivalves (**Table 3**). Using method of Falck-Hillarp, Sweeney [47] and Matsutani and Nomura [88, 105] observed the 5-HT-IR fibers in the gonoduct and epithelium around gonad in the Fingernail clam and Yesso scallop, respectively, and suggested that the 5-HT-IR fibers originate from CVC to innervate the gonad. Further studies using antibodies against 5-HT confirmed existence of 5-HT-IR fibers in the gonad of Yesso scallop [105], great scallop [90], Atlantic deep-sea scallop [115], surf clam [112], warty venus [91], and soft-shell clam [106]. These studies clearly indicated

that the nervous system innervation of the gonads is mostly emerged from VG or derived from CVC. The 5-HT-IR fibers surround periphery of collecting tubes and of gonadal lobules (acini) in males and females filled with sperm and oocytes, respectively (**Figure 3**).

As seasonal-dependent 5-HT content in the nervous system, distribution of 5-HT fibers also changes in the gonad throughout reproductive cycle [91, 106, 112, 115] (**Figure 3**; **Tables 2** and **3**). Generally, the 5-HT-IR fibers are occasionally observed around the germinal acini, and extensively distributed around the collective tubes at early developmental stage. However, the 5-HT-IR fibers around the acini are more frequent at maturity stage [112]. After spawning, the 5-HT-IR fibers still exist around collecting tubes, and are abundant around gamete empty acini.

Using spectrofluorometric or HPLC-EC method, 5-HT content has been measured in the gonad of the Atlantic deep-sea scallop [114, 115], surf clam [112], Pacific lion's paw scallop (*Nodipecten subnodosus*) [111], and brown mussel [110]. Matsutani [131] reported a tendency toward an increase and a decrease of 5-HT content in the testis and ovary of Japanese scallop (*Chlamys farreri nipponensis*) during spawning, respectively. It has shown that 5-HT content increases from early developmental stage of the gonad to maturity stage in males and females [110, 111]. In surf clam, Masseau et al. [112] reported that changes in 5-HT content are uncertain in males during testicular development and after spawning. However, in females, 5-HT is high at early development stage, decreases at maturity stage and spawning, and then increases after spawning. They also reported that 5-HT content does not differ between males and females when they are compared at similar gonadal development stage. Klouche et al. [110] pooled the data of males and females in brown mussel, as there are no differences between sexes, and observed that 5-HT content increases toward maturation of gonad. In Peruvian scallop, 5-HT content decreases in the male and female portions of gonad following spawning [113, 116]. Observed differences in 5-HT content among studies may represent inter-species differences associated with 5-HT regulation of reproduction that might also be different between sexes. Klouche et al. [110] reported that the gonadal content of 5-HT metabolite (5-HIAA) in brown mussel is high at early development and become decreased at maturity stage. As 5-HT content is high at maturity, these suggest that 5-HT-dependent reproduction associates with decreasing 5-HT inactivation mediated by its metabolism.

A few studies show 5-HT content in both nervous system and gonad, for instance in the Peruvian scallop [113, 116] and brown mussel [110]. Results show higher 5-HT content in the nervous system than gonadal tissue as 5-HT content is lower in connective nerves than 5-HT neurons [26, 56].

Croll et al. [115] observed that distribution of 5-HT-IR neurons and fibers is similar between juvenile and adult in the Atlantic deep-sea scallop or between sexes in the surf clam [112]. However, abundance or distribution of 5-HT neurons and 5-HT content may differ between sexes. Martínez and Rivera [116] observed that 5-HT content is higher in the male portion than female portion of the gonad of the Peruvian scallop. Expression of 5-HT-IR fibers or neurons has been seen to be higher in the VG of females than that of males [127]. These studies may suggest inter-sex difference in 5-HT biosynthesis or inter-sex difference in 5-HT regulatory function of reproduction.

4. Conclusion and future research perspectives

The essential components of 5-HT biosynthetic pathway are highly conserved in the animal kingdom. The 5-HT biosynthesis from the essential amino acid L-tryptophan is catalyzed by TPH, which convert L-tryptophan to 5-HTP, and by AADC, which convert 5-HTP to 5-HT. All precursors of 5-HT are identified in the nervous system of bivalves. In mammals, there are two isoforms of TPH (TPH1 and TPH2), which are predominantly expressed in the peripheral organs and in the nervous system, respectively. However, TPH1 is the primary form and expresses earlier in neural development [132, 133]. Molecular sequence of the gene encoding AADC has also been identified and localized in mammals [134, 135]. It has a non-specific tissue distribution and is expressed in wide range of cell types [66]. In bivalves, molecular identity, localization, and characterization of TPH and AADC are unknown. These studies will provide us with satisfactory information to better understand ontogeny of 5-HT neurons in the nervous system and to elucidate developmental biology of 5-HT regulation of reproduction.

It has been seen that the first 5-HT-IR neurons appearing within the nervous system correspond to the location of the CG and apical ganglion (AG) during the late trochophore stage: 30–32 h postfertilization in blue mussel [136], 24 h postfertilization in surf clam [137], and 27 h postfertilization in the Bay mussel (*Mytilus trossulus*) [138]. Kreiling et al. [137] reported that the 5-HT-IR neurons appear in VG of surf clam at 48 h postfertilization. Following 72 h postfertilization, the 5-HT-IR neurons emerging from the CG and AG extend their processes to the VG, through which connections of the 5-HT-IR neurons between CG/AG and VG are formed at 96 h postfertilization. During the embryonic development, the size of the 5-HT area in the CG/AG and VG increases from 24 h to 96 h postfertilization, which is associated with an increase in 5-HT content. Cann-Moisan et al. [139] reported that 5-HT content undergoes variation throughout the larval and postlarval stages. It rises from 2 d to 27 d postfertilization (15–50 pg/μg of protein, respectively); however, it decreases to less than 1 pg/μg of protein after 55 d postfertilization. These indicate that 5-HT neurons form at the embryonic stage, and 5-HT content increases from embryonic development to metamorphosis, and decreases after metamorphosis. Voronezhskaya et al. [138] observed that 5-HT-IR neurons innervate the peripheral organs in the postmetamorphic stage, suggesting that 5-HT biosynthesis undergoes developmental variation. This might be related to the availability of the 5-HT precursors or inactivation mechanisms of 5-HT. However, further studies are required to investigate development of 5-HT fibers in the gonad through developmental stage.

As animals lost the ability to synthesize tryptophan, there possess developed biological mechanisms through which animals obtain tryptophan from their diets. Thus, 5-HT biosynthesis highly depends on dietary factors including availability of tryptophan and competitive uptake or transport of tryptophan with other amino acids (such as tyrosine and phenylalanine) into the 5-HT neurons. Studying nutritional effects on 5-HT biosynthesis will lead to better understanding of physiological relationships between seasonal variation in 5-HT content and gonadal development. In addition, it can help us to investigate the impacts of parental nutrition on gamete maturation and fertility in bivalves. These studies can provide us with

knowledge to better understand 5-HT controls of feeding behaviors such as appetite and satiety, which have been demonstrated in mammals [140].

Mechanisms of 5-HT inactivation in the nervous system and peripheral organs of bivalves are poorly understood. It requires molecular identity, localization, and characterization of SERT and MOA. In this regard, several types of SERT and MOA inhibitors are available [80, 114, 141] that provide us with useful tools to elucidate molecular signaling that control 5-HT reuptake and metabolism. A few studies show that selective 5-HT reuptake inhibitors modulate 5-HT-induced spawning in bivalves. Fong [142] and Fong et al. [143, 144] reported spawning of Zebra mussel treated with selective 5-HT reuptake inhibitors (fluvoxamine, fluoxetine, zimelidine, and paroxetine). Both males and females are capable of releasing their gametes after treatment with fluvoxamine at 10^{-7} and 10^{-6} M, respectively. Following treatment with fluoxetine, 100% of males have spawned at 10^{-4} to 10^{-5} M, however spawning has induced in 50–60% of females at 10^{-5} M. Zimelidine induces spawning in 100 and 60–70% of males and females at 10^{-4} M. Paroxetine induces spawning in 50 and 20% of males and females at 10^{-6} and 10^{-5} M, respectively. Considering spawning of males and females at 10^{-3} M 5-HT, these results indicate that selective 5-HT reuptake inhibitors stimulate spawning in Zebra mussel at concentrations lower than that of 5-HT. Further examinations have revealed that mianserin and cyproheptadine interfere with fluvoxamine-, fluoxetine-, and zimelidine-induced spawning [144] suggesting that antagonists of 5-HT_2 receptor block stimulatory function of selective 5-HT reuptake inhibitors in spawning. Inhibition of 5-HT reuptake may increase the synaptic 5-HT concentrations, which in turn activate postsynaptic 5-HT receptor to induce spawning. It is also possible that selective 5-HT reuptake inhibitors act as ligands at postsynaptic receptor rather than inhibition of SERT. Overall, these studies suggest that 5-HT transport plays a key role in reproduction; however, the mechanisms of action are largely unknown.

So far, histochemistry and immunohistochemistry methods have been employed to localize the 5-HT neurons and fibers, and spectrofluorometric and HPLC-EC methods have been used to identify 5-HT content in the nervous system and gonad of various bivalve species. Successful implication of various mammalian monoclonal or polyclonal antibodies indicates that 5-HT structure is highly conserved through evolution across the animal kingdom. However, mechanisms through which 5-HT acts on a biological system may differ. The present review shows that 5-HT content highly differs in the nervous system and gonad of bivalve species. The inter-species differences in 5-HT content might be related to capability of nervous system to synthesize 5-HT, differences in 5-HT inactivation or 5-HT transport from nervous system to the gonad. In the latter case, 5-HT content in the gonad may correspond to 5-HT concentration that requires to stimulate spawning. The present review shows that 5-HT concentration to induce spawning highly differs between sexes, and among species. It is worth to note that tissue sampling, extraction procedure, and analytical method affect the results of 5-HT content. In addition, 5-HT content undergoes seasonal variation and change following spawning.

Conflict of interest

The authors declare no conflicts of interest, financial or otherwise.

Acknowledgements

This study was supported by Tohoku Ecosystem-Associated Marine Sciences (TEAMS) grants from the Ministry of Education, Culture, Sports, Science and Technology (MEXT)-Japan, JSPS KAKENHI (16H04978), JSPS postdoctoral fellow (23-01404), and JAMBIO (23-02) to M.O.

Author details

Sayyed Mohammad Hadi Alavi, Kazue Nagasawa, Keisuke G. Takahashi and Makoto Osada*

*Address all correspondence to: makoto.osada.a8@tohoku.ac.jp

Laboratory of Aquacultural Biology, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai, Japan

References

[1] Costa E, Gessa GL, Sandler M, editors. Serotonin—New Vistas, Biochemistry and Behavioral and Clinical Studies, Advances in Biochemical Psychopharmacology. Vol. 11. New York: Raven Press; 1974

[2] Sanders-Bush E, editor. The Serotonin Receptors. New Jersey: Humana Press Inc; 1988

[3] Baumgarten HG, Göthert M, editors. Serotoninergic Neurons and 5-HT Receptors in the CNS, Handbook of Experimental Pharmacology. Vol. 129. Berlin: Springer; 2000

[4] Roth BL, editor. The Serotonin Receptors: From Molecular Pharmacology to Human Therapeutics. New Jersey: Humana Press Inc; 2006

[5] Müller CP, Jacobs BL, editors. Handbook of the Behavioral Neurobiology of Serotonin. Handbook of nehavioral neuroscience. Vol. 21. Amsterdam: Elsevier; 2010

[6] Maximino C. Serotonin in the nervous system of vertebrates. In: Maximino C, editor. Serotonin and Anxiety: Neuroanatomical, Pharmacological, and Functional Aspects, Springer Briefs in Neuroscience. New York: Springer-Verlag; 2012. pp. 15-36

[7] Blenau W, Baumann A. Serotonin Receptor Technologies. Series of Neuromethods. Vol. 95. New Jersey: Humana Press Inc; 2015

[8] Fernstrom JD. Dietary effects on brain serotonin synthesis: Relationship to appetite regulation. The American Journal of Clinical Nutrition. 1985;**42**:1072-1082

[9] Kanner BI, Schuldiner S. Mechanism of transport and storage of neurotransmitter. Critical Reviews in Biochemistry 1987;**22**:1-38

[10] Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. International union of pharmacological classification of receptors for 5-hydroxytryptamine (serotonin). Pharmacological Reviews. 1994;**46**:157-193

[11] Barnes NM, Sharp T. A review of central 5-HT receptors and their function. Neuropharmacology. 1999;**38**:1083-1152

[12] Torres GE, Gainetdinov RR, Caron MG. Plasma membrane monoamine transporters: Structure, regulation and function. Nature Reviews Neuroscience. 2003;**4**:13-25

[13] Millan MJ, Marin P, Bockaert J, Mannoury la Cour C. Signaling at G-protein-coupled serotonin receptors: Recent advances and future research directions. Trends in Pharmacological Sciences. 2008;**29**:454-464

[14] Gershon MD. Biochemistry and physiology of serotonergic transmission. Comprehensive Physiology. 2011. Supplement 1: Handbook of Physiology, The Nervous System, Cellular Biology of Neurons;573-623

[15] Pytliak M, Vargova V, Mechirova V, Felsoci M. Serotonin receptors—from molecular biology to clinical applications. Physiological Research. 2011;**60**:15-25

[16] Rapport MM, Green AA, Page IH. Purification of the substance which is responsible for vasoconstrictor activity of serum. Federation Proceedings. 1947;**6**:184

[17] Rapport MM. Serum vasoconstrictor (serotonin). V. Presence of creatinine in the complex. A proposed structure of the vasoconstrictor principle. The Journal of Biological Chemistry. 1949;**180**:961-969

[18] Hamlin KE, Fisher FE. Synthesis of 5-hydroxytryptamine. Journal of the American Chemical Society. 1951;**73**:5007-5008

[19] Twarog BM, Page IH. Serotonin content of some mammalian tissues and urine and a method for its determination. American Journal of Physiology. 1953;**175**:157-161

[20] Amin AH, Crawford BB, Gaddum JH. Distribution of substance P and 5-hydroxytryptamine in the central nervous system of the dog. The Journal of Physiology. 1954; **126**:596-618

[21] Welsh JH. Excitation of the heart of *Venus mercenaria*. Archiv for Experimentelle Pathologie und Pharmakologie. 1953;**219**:23-29

[22] Twarog BM. Responses of a molluscan smooth muscle to acetylcholine and 5-hydroxytryptamine. Journal of Cellular Physiology. 1954;**44**:141-163

[23] Hoyle G, Lowy J. The paradox of *Mytilus* muscle: A new interpretation. The Journal of Experimental Biology. 1956;**33**:295-310

[24] Brodie BB, Shore PA. A concept for a role of serotonin and norepinephrine as chemical mediators in the brain. Annals of the New York Academy of Sciences. 1957;**66**:631-642

[25] Welsh JH. Serotonin as a possible neurohumoral agent: Evidence obtained in lower animals. Annals of the New York Academy of Sciences. 1957;**66**:618-630

[26] Welsh JH, Moorhead M. The quantitative distribution of 5-hydroxytryptamine in the invertebrates, especially in their nervous systems. Journal of Neurochemistry. 1960;**6**: 146-169

[27] Dufau ML, Tinajero JC, Fabbri A. Corticotropin-releasing factor: An antireproductive hormone of the testis. The FASEB Journal. 1993;**7**:299-307

[28] Sirotkin AV, Schaeffer HJ. Direct regulation of mammalian reproductive organs by serotonin and melatonin. Journal of Endocrinology. 1997;**154**:1-5

[29] Hull EM, Muschamp JW, Sato S. Dopamine and serotonin: Influences on male sexual behavior. Physiology & Behavior. 2004;**83**:291-307

[30] Dubé F, Amireault P. Local serotonergic signaling in mammalian follicles, oocytes and early embryos. Life Sciences. 2007;**81**:1627-1637

[31] Fujinoki M. Serotonin-enhanced hyperactivation of hamster sperm. Reproduction 2011; **142**:255-266

[32] Jiménez-Trejo F, Tapia-Rodriguez M, Cerbon M, Kuhn DM, Manjarrez-Gutiérrez G, Mendoza-Rodriguez CA, Picazo O. Evidence of 5-HT components in human sperm: Implications for protein tyrosine phosphorylation and the physiology of motility. Reproduction. 2012;**144**:677-685

[33] Matsutani T, Nomura T. Induction of spawning by serotonin in the scallop *Patinopecten yessoensis*. Marine Biology Letters. 1982;**3**:353-358

[34] Hirai S, Kishimoto T, Kadam AL, Kanatani H, Koide SS. Induction of spawning and oocyte maturation by 5-hydroxytryptamine in the surf clam. Journal of Experimental Zoology. 1988;**254**:318-321

[35] Deguchi R, Osanai K. Serotonin-induced meiosis reinitiation from the first prophase and from the first metaphase in oocytes of the marine bivalve *Hiatella flaccida:* Respective changes in intracellular Ca^{2+} and pH. Developmental Biology. 1995;**171**:483-496

[36] Guerrier P, Durocher Y, Gobet I, Leclerc C, Moreau M. Reception and transduction of the serotonin signal responsible for oocyte meiotic reinitiation in bivalves. Invertebrate Reproduction and Development. 1996;**30**:39-45

[37] Krantic S, Rivailler P. Meiosis reinitiation in molluscan oocytes: A model to study the transduction of extracellular signals. Invertebrate Reproduction and Development. 1996;**30**:55-69

[38] Alavi SMH, Matsumura N, Shiba K, Itoh N, Takahashi KG, Inaba K, Osada M. Roles of extracellular ions and pH in 5-HT-induced sperm motility in marine bivalve. Reproduction. 2014;**147**:331-345

[39] Rózsa KS. The pharmacology of molluscan neurons. Progress in Neurobiology. 1984;**23**: 79-150

[40] Walker RJ. Transmitters and modulators. In: Willows AOD, editor. The Mollusca. Vol. 6. Neurobiology and Behavior Part 2. Academic Press Inc. Orland; 1986. pp. 279-485

[41] Tierney AJ. Structure and function of invertebrate 5-HT receptors: A review. Comparative Biochemistry and Physiology Part A. 2001;**128**:791-804

[42] Rapport MM, Green AA, Page IH. Serum vasoconstrictor (serotonin). IV. Isolation and characterization. The Journal of Biological Chemistry. 1948;**176**:1243-1251

[43] Gaddum JH. Antagonism between lysergic acid diethylamide and 5-hydroxytryptamine. The Journal of Physiology London. 1953;**121**:15

[44] Woolley DW, Shaw E. A biochemical and pharmacological suggestion about certain mental disorders. Science. 1954;**119**:587-588

[45] Falck B, Hillarp NA, Thieme G, Torp A. Fluorescence of catechol amines and related compounds condensed with formaldehyde. Journal of Histochemistry and Cytochemistry. 1962;**10**:348-354

[46] Dahlström A, Fuxe K. Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. Acta Physiologica Scandinavica Supplementum. 1964;**232**:1-55

[47] Sweeney D. The anatomical distribution of monoamines in a fresh water bivalve mollusca, *Sphaerium sulcatum*. Comparative Biochemistry and Physiology. 1968;**25**:601-614

[48] Hirai S, Kishimoto T, Koide SS, Kanatani H. Serotonin induction of spawning and oocyte maturation in *Spisula*. The Biological Bulletin. 1984;**167**:518

[49] Osanai K. In vitro induction of germinal vesicle breakdown in oyster oocyte. The Bulletin of the Marine Biological Station of Asamushi Tôhoku University. 1985;**18**:1-9

[50] Blaschko H. Amine oxidase and amine metabolism. Pharmacological Reviews. 1952;**4**: 415-458

[51] Udenfriend S, Clark CT, Titus E. 5-Hydroxytryptophan decarboxylase: A new route of metabolism of tryptophan. Journal of the American Chemical Society. 1953;**75**:501-502

[52] Lovenberg WH, Weissbach H, Udenfriend S. Aromatic L-amino acid decarboxylase. The Journal of Biological Chemistry. 1962;**237**:89-93

[53] Clark CT, Weissbach H, Udenfriend S. 5-Hydroxytryptophan decarboxylase: Preparation and properties. The Journal of Biological Chemistry. 1954;**210**:139-148

[54] Udenfriend S, Weissbach H, Bogdanski DF. Increase of tissue serotonin following administration of its precursor 5-hydroxytryptophan. The Journal of Biological Chemistry. 1957;**224**:803-810

[55] Udenfriend S, Weissbach H. Turnover of 5-hydroxytryptamine (serotonin) in tissues. Proceedings of the Society for Experimental Biology and Medicine. 1958;**97**:748-775

[56] Welsh JH, Moorhead M. *In vitro* synthesis of 5-hydroxytryptamine from 5-hydroxytryptophan by nervous tissues of two species of mollusks. The Gunma Journal of Medical Sciences. 1959;**8**:211-218

[57] Aiello E. Factors affecting ciliary activity on the gill of the mussel *Mytilus edulis*. Physiological Zoology. 1960;**33**:120-135

[58] Aiello E. Identification of the cilioexcitatory substance present in the gill of the mussel *Mytilus edulis*. Journal of Cellular Physiology. 1962;**60**:17-21

[59] Gosselin RE, Moore KE, Milton AS. Physiological control of molluscan gill cilia by 5-hydroxytryptarnine. The Journal of General Physiology. 1962;**46**:277-296

[60] Blaschko H, Milton A. Oxidation of 5-hydroxytryptamine and related compounds by *Mvtilus* gill plates. British Journal of Pharmacology and Chemotherapy. 1960;**15**:42-46

[61] Aiello E. The fate of serotonin in the cell of the mussel *Mytilus edulis*. Comparative Biochemistry and Physiology. 1965;**14**:71-82

[62] Bertaccini G. Tissue 5-hydroxytryptamine and urinary 5-hydroxyindole acetic acid after partial or total removal of the gastro-intestinal tract in the rat. The Journal of Physiology. 1960;**153**:239-249

[63] Gal EM, Poczik M, Marshal Jr FD. Hydroxylation of tryptophan to 5-hydroxytryptophan by brain tissue *in vivo*. Biochemical and Biophysical Research Communications. 1963;**12**:39-43

[64] Toh CC. Release of 5-hydroxytryptamine (serotonin) from the dog's gastrointestinal tract. The Journal of Physiology London. 1954;**126**:248-254

[65] Joh TH. Tryptophan hydroxylase: Molecular biology and regulation. In: Baumgarten HG, Göthert M, editors. Serotoninergic neurons and 5-HT receptors in the CNS, Handbook of experimental pharmacology. Vol. 129. Berlin Heidelberg: Springer; 2000. pp. 117-129

[66] Hasegawa H, Nakamura K. Tryptophan hydroxylase and serotonin synthesis regulation. In: Müller CP, Jacobs BL, editors. Handbook of the behavioral neurobiology of serotonin, Handbook of behavioral neuroscience. Vol. 21. Amsterdam: Elsevier; 2010. pp. 183-202

[67] Gessa GL, Tagliamonte A. Possible role of free serum tryptophan in the control of brain tryptophan level and serotonin synthesis. In: Costa E, Gessa GL, Sandler M, editors. Serotonin – New vistas, biochemistry and behavioral and clinical studies, Advances in biochemical psychopharmacology. Vol. 11. New York: Raven Press; 1974. pp. 119-131

[68] Pentreath VW, Cottrell GA. Selective uptake of 5-hydroxytryptamine by axonal processes in *Helix pomatia*. Nature. 1972;**239**:213-214

[69] Stefano GB, Aiello E. Histofluorescent localization of serotonin and dopamine in the nervous system and gill of *Mytilus edulis* (Bivalvia). The Biological Bulletin. 1975;**148**:141-156

[70] Bortolato M, Chen K, Shih JC. The degradation of serotonin: Role of MAO. In: Müller CP, Jacobs BL, editors. Handbook of the behavioral neurobiology of serotonin, Handbook of behavioral neuroscience. Vol. 21. Amsterdam: Elsevier; 2010. pp. 203-218

[71] Guthrie PB, Neuhoff V, Osborne NN. Dopamine, noradrenaline, octopamine and tyrosine hydroxylase in the gastropod *Helix pomatia*. Comparative Biochemistry and Physiology Part C. 1975;**52**:109-111

[72] Boutet I, Tanguy A, Moraga D. Molecular identification and expression of two non-P450 enzymes, monoamine oxidase A and flavin-containing monooxygenase 2, involved in phase I of xenobiotic biotransformation in the Pacific oyster, *Crassostrea gigas*. Biochimica et Biophysica Acta. 2004;**1679**:29-36

[73] Cardot J. La monoamine oxidaze chez le mollusque *Helix pomatia*: activité sur quatre substrates. Comptes rendus des séances de la Société de biologie (Paris). 1966;**160**:1264-1268

[74] Gerschenfeld HM, Stefani E. 5-Hydroxytryptamine receptors and synaptic transmission in molluscan neurones. Nature. 1965;**205**:1216-1218

[75] Gerschenfeld HM, Stefani E. Evidence for an excitatory transmitter of serotonin in molluscan central synapses. Advances in Pharmacology. 1968;**6A**:369-392

[76] Hiripi L, Rakonczay Z, Nemcsok J. The uptake kinetics of serotonin, dopamine and noradrenaline in the pedal ganglia of the fresh water mussel (*Anodonta cygnea* L., Pelecypoda). Annals of Biology (Tihany). 1975;**42**:21-28

[77] Osborne NN, Hiripi L, Neuhoff V. The in vitro uptake of biogenic amines by snail (*Helix pomatia*) nervous tissue. Biochemical Pharmacology. 1975;**24**:2141-2148

[78] Rudnick G, Clark J. From synapse to vesicle: The reuptake and storage of biogenic amine neurotransmitters. Biochimica et Biophysica Acta. 1993;**1144**:249-263

[79] Rudnick G. Bioenergetics of neurotransmitter transport. Journal of Bioenergetics and Biomembranes. 1998;**30**:173-178

[80] Fuller W. Uptake inhibitors increase extracellular serotonin concentration measured by brain microdialysis. Life Sciences. 1994;**55**:163-167

[81] Daws LC, Gould GG. Ontogeny and regulation of the serotonin transporter: Providing insights into human disorders. Pharmacology & Therapeutics. 2011;**131**:61-79

[82] Bullough WS. Practical Invertebrate Anatomy. London: Macmillan & Co, Limited; 1950

[83] Galtsoff PS. The American oyster *Crassostrea virginica* Gmelin. Fish Bull US Fish Wildlife Service. Vol. 64. Washington: US Government Printing Office; 1964

[84] Gosling E. Morphology of Bivalves. In: Bivalve molluscs: Biology, Ecology and Culture. Ed.by Gosling E. Oxford: Blackwell Publishing; 2003. pp. 7-43

[85] Grizel H, Auffret M, Barille L, Besnard-Cochennec N, Blanc F, Boucaud-Camou E, Chollet B, Henry M, Jabbour-Zahab R, Le Pennec M, Lubet P, Mathieu M, Thielley M. An atlas of histology and cytology of marine bivalve molluscs. Plouzané, France: Ifremer Publication; 2003. pp. 159-168

[86] Beninger PG, Le Pennec M. Structure and function in scallops. In: Shumway SE, Parsons GJ, editors. Scallops: Biology, Ecology and Aquaculture. Amsterdam: Elsevier; 2006. pp. 123-227

[87] Meechonkit P, Kovitvadhi U, Chatchavalvanich K, Sretarugsa P, Weerachatyanukul W. Localization of serotonin in neuronal ganglia of the freshwater pearl mussel, *Hyriopsis* (*hyriopsis*) *bialata*. Journal of Molluscan Studies. 2010;**76**:267-274

[88] Matsutani T, Nomura T. Localization of monoamines in the central nervous system and gonad of the the scallop, *Patinopecten yessoensis*. Bulletin of the Japanese Society for the Science of Fish. 1984;**50**:425-430

[89] Garnerot F. Contribution à l'amélioration des connaissances sur la physiologie de *Mya arenaria* (mollusque bivalve): description du système nerveux, des structures fonctionnelles de la gonade et de leurs interactions. [Thesis]. Rimouski, Québec, Canada: Université du Québec à Rimouski; 2007. p. 244

[90] Paulet YM, Donval A, Bekhadra F. Monoamines and reproduction in *Pecten maximus*, a preliminary approach. Invertebrate Reproduction and Development. 1993;**23**:89-94

[91] Siniscalchi A, Cavallini S, Sonetti D, Sbrenna G, Capuano S, Barbin L, Turolla E, Rossi R. Serotonergic neurotransmission in the bivalve *Venus verrucosa* (Veneridae): A neurochemical and immunohistochemical study of the visceral ganglion and gonads. Marine Biology. 2004;**144**:1205-1212

[92] Rawitz B. Das zentrale nervensystem der Acephalen. Jenaische Zeitschrift fur Naturwissenschaft, Neue Folge, Band. 1887;**13**:384-460

[93] Sastry AN. Pelecypoda (excluding Ostreidae). In: Giese AC, Pearse JS, editors. Reproduction of Marine Invertebrates. Vol. V, Molluscs: Pelecypods and lesser classes. New York: Academic Press; 1979. pp. 113-292

[94] Illanes–Bucher J. Recherches cytologiqueset expérimentales sur la neurosécré- tion de la moule *Mytilus edulis* L. (*Mollusque, Lamellibranche*). France: Thèse de 3eme cycle, Université de Caen; 1979

[95] Blake NJ. Environmental regulation of neurosecretion and reproductive activity in the bay scallop, *Acquipecten irradians* (Lamarck). [Dessertation]. Kingston: University of Rhode Island; 1972

[96] Lubet P. Recherches sur le cycle sexual et I'emission des gamètes chez les Mytilidés et les Pectinidés. Revue des travaux de l'Institut des pêches maritimes. 1959;**23**:387-548

[97] Antheunisse LJ. Neurosecretory phenomena in the zebra mussel *Dreissena polymorpha* Pallas. Archives Néerlandaises de Zoologie. 1963;**15**:237-314

[98] Nagabhushanam R. Neurosecretory changes in the nervous system of the oyster *Crasssostrea virginica* induced by various experimental conditions. Indian Journal of Experimental Biology. 1964;**2**:1-14

[99] Lubet P, Pujol JP. Incidence de la neurosécrétion sur l'euryhalinité de Mytilus galloprovincialis, Lmk. Variation de la tenure en eau. Rapports et Proces-Verbaux des Reunions - communication International Pour L'Exploration de la Mer Mediterranee. 1965;**18**:148-154

[100] Nagabhushanam R, Mane UH. Seasonal variation in the biochemical composition of the clam, *Ketelysia opima*. Rivista di Biologia. 1975;**67**:279-301

[101] Lubet P, Aloui N, Karnaukova N. Etude comparee de l'action de la temperature sur le cycle de reproduction de *Mytilus galloprovincialis* Lmk. Comparasion avec *Mytilus edulis* L. Comptes Rendus de l'Académie des Sciences (Paris). 1986;**303**:507-512

[102] Mahmud S, Mladenov PV, Chakraborty SC, Faruk MAR. Relationship between gonad condition and neurosecretory cell activity in the green-lipped mussel, *Perna canaliculus*. Progressive Agriculture. 2007;**18**:135-148

[103] Vitellaro-Zuccarello L, De Biasi S, Bernardi P, Oggioni A. Distribution of serotonin-, gamma-aminobutyric acid- and substance P-like immunoreactivity in the central and peripheral nervous system of *Mytilus galloprovincialis*. Tissue and Cell. 1991;**23**:261-270

[104] De Biasi S, Vitellaro-Zuccarello L. Distribution of 5HT-immunoreactivity in the pedal ganglion of *Mytilus gallopro vincialis:* A light- and electron-microscopic study. Cell and Tissue Research. 1987;**249**:111-116

[105] Matsutani T, Nomura T. Serotonin-like immunoreactivity in the central nervous system and gonad of the scallop, *Patinopecten yessoensis.* Cell and Tissue Research. 1986;**244**:515-517

[106] Garnerot F, Pellerin J, Blaise C, Mathieu M. Immunohistochemical localization of serotonin (5-hydroxytryptamine) in the gonad and digestive gland of *Mya arenaria* (Mollusca: Bivalvia). General and Comparative Endocrinology. 2006;**149**:278-284

[107] Welsh JH, Moorhead M. Identification and assay of 5-hydroxytryptamine in molluscan tissues by fluorescence method. Science 1959;**129**:1491-1492

[108] Bogdanski DF, Pletscher A, Brodie BB, Udenfriend S. Identification and assay of serotonin in brain. The Journal of Pharmacology and Experimental Therapeutics. 1956;**117**:82-88

[109] Kuntzman R, Shore PA, Bogdanski D, Brodie BB. Microanalytical procedures for fluorometric assay of brain dopa-5-HTP decarboxylase activity in brain. Journal of Neurochemistry. 1961;**6**:226-232

[110] Klouche MS, De Deurwaerdère P, Dellu-Hagedorn F, Lakhdar-Ghazal N, Benomar S. Monoamine content during the reproductivte cycle of *Perna perna* depends on site of origin on the Atlantic Coast of Morocco. Scientific Reports. 2015;**5**:13715

[111] López-Sánchez JA, Maeda-Martínez AN, Croll RP, Acosta-Salmón H. Monoamine fluctuations during the reproductive cycle of the Pacific lion's paw scallop *Nodipecten subnodosus*. Comparative Biochemistry and Physiology Part A. 2009;**154**:425-428

[112] Masseau I, Bannon P, Anctil M, Dubé F. Localization and quantification of gonad serotonin during gametogenesis of the Surf clam, *Spisula solidissima*. The Biological Bulletin. 2002;**202**:23-33

[113] Martínez G, Saleh F, Mettifogo L, Campos E, Inestrosa N. Monoamines and the release of gametes by the scallop *Argopecten purpuratus*. Journal of Experimental Zoology. 1996;**274**:365-372

[114] Pani AK, Croll RP. Distribution of catecholamines, indoleamines, and their precursors and metabolites in the scallop, *Placopecten magellanicus* (Bivalvia, Pectinidae). Cellular and Molecular Neurobiology. 1995;**15**:371-386

[115] Croll RP, Too CKL, Pani AK, Nason J. Distribution of serotonin in the sea scallop *Placopecten magellanicus*. Invertebrate Reproduction and Development. 1995; **28**:125-135

[116] Martínez G, Rivera A. Role of monoamines in the reproductive process of *Argopecten purpuratus*. Invertebrate Reproduction and Development. 1994;**25**:167-174

[117] Smith JR. A survey of endogenous dopamine and serotonin in ciliated and nervous tissues of five species of marine bivalves, with evidence for specific, high-affinity dopamine receptors in ciliated tissue of *Mytilus californianus*. Comparative Biochemistry and Physiology Part C. 1982;**71**:57-61

[118] Stefano GB, Catapane EJ. Seasonal monoamine changes in the central nervous system of *Mytilus edulis* (Bivalvia). Experientia. 1977;**33**:1341-1342

[119] Stefano GB, Catapane J, Aiello E. Dopaminergic agents: influence on serotonin in the molluscan nervous system. Science. 1976;**194**:539-541

[120] York B, Twarog BM. Evidence for the release of serotonin by relaxing nerves in molluscan muscle. Comparative Biochemistry and Physiology Part A. 1973;**44**:423-430

[121] Axelsson S, Bjorkland A, Falck B, Lindvall O, Svensson LA. Glyoxylic acid condensation: A new fluorescence method for the histochemical demonstration of biogenic monoamines. Acta Physiologica Scandinavica. 1973;**87**:57-62

[122] Lindvall O, Bjorklund A, Falck A, Svensson LA. Combined formaldehyde and glyoxylic acid reactions. I. New possibilities for microspectrofluorometric differentiation between phenylethylamines, indolylethylamines and their precursor amino acids. Hisrochemistry. 1975;**46**:27-52

[123] Bjorkland A, Falck B, Lindvall O, Svensson LA. New aspects on reaction mechanisms in the formaldehyde histofluorescence method for monoamines. Journal of Histochemistry and Cytochemistry. 1973;**21**:17-75

[124] Steinbusch HWM, Verhofstad AAJ, Joosten HWJ. Localization of serotonin in the central nervous system by immunohistochemistry: Description of a specific and sensitive technique and some applications. Neuroscience. 1978;**3**:811-819

[125] Vitellaro-Zuccarello L, De Biasi S, Bairati A. Subcellular localization of serotonin-immunoreactivity in the pedal ganglion of *Mytilus galloprovincialis* (Mollusca, Bivalvia). Journal of Submicroscopic Cytology and Pathology. 1988;**20**:109-113

[126] Vitellaro-Zuccarello L, De Biasi S, Amadeo A. Immunocytochemical demonstration of neurotransmitters in the nerve plexuses of the foot and the anterior byssus retractor muscle of the mussel, *Mytilus galloprovincialis*. Cell and Tissue Research. 1990;**261**:467-476

[127] Meechonkit P, Asuvapongpatana S, Jumromn W, Kovitvadhi U, Weerachatyanukul W. Sexual differences in serotonin distribution and induction of synchronous larval release by serotonin in the freshwater mussel *Hyriopsis bialatus*. Journal of Molluscan Studies. 2012;**78**:297-303

[128] Dahl E, Falck B, von Mecklenburg C, Myhrberg H, Rosengren E. Neuronal localization of dopamine and 5-hydroxytryptamine in some mollusca. Zeitschrift Fur Zellforschung Und Mikroskopische Anatomie. 1966;**71**:489-498

[129] Pieters H, Kluytmans JH, Zandee DI, Cadee GC. Tissue composition and reproduction of *Mytilus edulis* in relation to food availability. Netherlands Journal of Sea Research. 1980;**311**:101-115

[130] Pronker AE, Nevejan NM, Peene F, Geijsen P, Sorgeloos P. Hatchery broodstock conditioning of the blue mussel *Mytilus edulis* (Linnaeus 1758). Part I. Impact of different micro-algae mixtures on broodstock performance. Aquaculture International. 2008;**16**:297-307

[131] Matsutani T. Endogenous factors controlling spawning in marine bivalves. In: Hoshi M, Yamashita O, editors. Advances in Invertebrate Reproduction. Vol. 5. Amsterdam: Elsevier; 1990. pp. 231-237

[132] Nakamura K, Hasegawa H. Developmental role of tryptophan hydroxylase in the nervous system. Molecular Neurobiology. 2007;**35**:45-54

[133] Nakamura K, Sato T, Ohashi A, Tsurui H, Hasegawa H. Role of a serotonin precursor in development of gut microvilli. The American Journal of Pathology. 2008;**172**:333-344

[134] Ichinose H, Kurosawa Y, Titani K, Fujita K, Nagatsu T. Isolation and characterization of a cDNA clone encoding human aromatic L-amino acid decarboxylase. Biochemical and Biophysical Research Communications. 1989;**164**:1024-1030

[135] Scherer LJ, McPherson JD, Wasmuth JJ, Marsh JL. Human dopa decarboxylase: localization to human chromosome 7p11 and characterization of hepatic cDNAs. Genomics. 1992;**13**:469-471

[136] Flyachinskaya LP. Localization of serotonin and FMRFamide in the bivalve mollusc *Mytilis edulis* at early stages of its development. Journal of Evolutionary Biochemistry and Physiology. 2000;**36**:66-70

[137] Kreiling JA, Jessen-Eller K, Miller J, Seegal RF, Reinisch CL. Early development of the serotonergic and dopaminergic nervous system in *Spisula solidissima* (surf clam) larvae. Comparative Biochemistry and Physiology Part A. 2001;**130**:341-351

[138] Voronezhskaya EE, Nezlin LP, Odintsova NA, Plummer JT, Croll RP. Neuronal development in larval mussel *Mytilus trossulus* (Mollusca: Bivalvia). Zoomorphology. 2008;**127**:97-110

[139] Cann-Moisan C, Nicolas L, Robert R. Ontogenic changes in the contents of dopamine, norepinephrine and serotonin in larvae and postlarvae of the bivalve *Pecten maximus*. Aquatic Living Resources. 2002;**15**:313-318

[140] Lee MD, Clifton PG. Role of the serotonergic system in appetite and ingestion control. In: Müller CP, Jacobs BL, editors. Handbook of Behavioral Neurobiology of Serotonin, Handbook of Behavioral Neuroscience. Vol. 21. Amsterdam: Elsevier; 2010. pp. 331-345

[141] Robinson DS. Monoamine oxidase inhibitors: A new generation. Psychopharmacology Bulletin. 2002;**36**:124-138

[142] Fong PP. Zebra mussel spawning is induced in low concentrations of putative serotonin reuptake inhibitors. The Biological Bulletin. 1998;**194**:143-149

[143] Fong PP, Huminski PT, D'-Urso LM. Induction and potentiation of parturition in fingernail clams (*Sphaerium striatinum*) by selective serotonin re-uptake inhibitors (SSRIs). Journal of Experimental Zoology. 1998;**280**:260-264

[144] Fong PP, Philbert CM, Robert BJ. Putative serotonin reuptake inhibitor-induced spawning and parturition in freshwater bivalves is inhibited by mammalian 5-HT2 receptor antagonists. Journal of Experimental Zoology Part A. 2003;**298**:67-72

IntechOpen