Serotonin and Synaptic Transmission at Invertebrate Neuromuscular Junctions

Wen-Hui Wu
*University of Kentucky*

Robin L. Cooper
*University of Kentucky*, rlcoop1@uky.edu

Follow this and additional works at: [https://uknowledge.uky.edu/biology_facpub](https://uknowledge.uky.edu/biology_facpub)

Part of the Biology Commons

Right click to open a feedback form in a new tab to let us know how this document benefits you.

Repository Citation

Wu, Wen-Hui and Cooper, Robin L., "Serotonin and Synaptic Transmission at Invertebrate Neuromuscular Junctions" (2012). *Biology Faculty Publications*. 60.
[https://uknowledge.uky.edu/biology_facpub/60](https://uknowledge.uky.edu/biology_facpub/60)

This Article is brought to you for free and open access by the Biology at UKnowledge. It has been accepted for inclusion in Biology Faculty Publications by an authorized administrator of UKnowledge. For more information, please contact UKnowledge@lsv.uky.edu.
Serotonin and Synaptic Transmission at Invertebrate Neuromuscular Junctions

Digital Object Identifier (DOI)
http://dx.doi.org/10.5607/en.2012.21.3.101

Notes/Citation Information
Published in Experimental Neurobiology, v. 21, no. 3, p. 101-112.

Copyright © Experimental Neurobiology 2012.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (http://creativecommons.org/licenses/by-nc/3.0) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.
GENERAL BACKGROUND OF 5-HYDROXYTRYPTAMINE AND RECEPTORS

5-Hydroxytryptamine (5-HT, serotonin) is a common biogenic amine found in both vertebrates and invertebrates as well as in plants [1, 2]. The precursor to 5-HT, tryptophan, is likely important in the early evolution of life and perhaps the early presence of tryptophan is a reason for 5-HT to be potentially the first neurotransmitter noted with the development of a nervous system [2]. 5-HT acts as both a neurotransmitter and neurohormone and as a potent modulator of neurons and various tissues in many animal species [3]. Generally 5-HT actions are elicited by transmembrane G protein coupled receptors (GPCRs), which then activate or inhibit different intracellular second messenger cascades. 5-HT receptors from some organisms have been classified based on sequence or pharmacology [4, 5]; for example in the vertebrates, 7 families (5-HT1-7), 14 subtypes have been identified, whereas in Drosophila four 5-HT receptors named 5-HT1Adro, 5-HT1Bdro, 5-HT2dro, 5-HT7dro [5-10] have been classified. 5-HT receptors appear to be present on invertebrate presynaptic nerve terminals and on muscle membranes; receptors of a cricket (Gryllus domesticus) mandibular muscle have a similar pharmacological profile as a 5-HT2-like receptor subtype [11]. Profiling the 5-HT receptor subtypes directly on skeletal muscle within invertebrates is an area of research that is lacking. The 5-HT4 and 5-HT7 receptors are shown to have alternate splice variants which increase the number of receptor subtypes and may...
alter the selectivity to pharmacological agents [12]. In addition, 
5-HT2 receptors can have different RNA-edited isoforms [13, 14].

With the use of the genetically modifiable model D. melanogaster, a number of studies have examined over-expression and under-expression of receptors subtypes on the effects of development, behavior and physiology as well as the general actions of 5-HT in D. melanogaster [7, 15-19]. Based on physiological and pharmacological studies in crustaceans there may be a larger number of 5-HT receptors present than in D. melanogaster [20-26]. Two receptors types have been cloned and characterized in crustaceans [9, 27, 28] and in a pond snail [29]. A 5-HT receptor 5-HT1(apAC1) has been cloned, sequenced and characterized in Aplysia sensory neurons [30]. 5-HT receptors are being cloned in a variety of invertebrates and surely more will be forth coming with the rapid development in genomic sequencing abilities. There are a plethora of reports on the effects of 5-HT for sensory and central neurons as well as on behaviors in invertebrates which are worthy of multiple reviews. However, for this brief review we focus on the physiological effects of 5-HT at the skeletal neuromuscular junctions in some of the key model invertebrates. The invertebrate neuromuscular junctions (NMJs) are very diverse across species and within species in structure and function [31-37]. The recent majority of reports on structure and function of NMJs are of D. melanogaster due to the genetic approaches and manipulations being utilized [38-41].

WHY FOCUS ON NMJS?

The synaptic communication between neurons and target cells depends on the specialized anatomy and physiology of the synapses [42]. The regulation and modulation of neurotransmitter release is the basis of chemical synaptic transmission. For nervous systems to function properly, the efficacy of synapses are finely regulated and adjustable to respond to changing circumstance and requirement. Too high or too low synaptic input both result in inappropriate communication of target cells. Both pre- and postsynaptic factors can influence the synaptic strength. The amount of neurotransmitter released and the sensitivity of the postsynaptic membrane both are important for measuring synaptic strength. Each step in the process of synaptic transmission can be the target of many factors that lead to alteration of synaptic strength. For example, the phosphorylation state of SNARE proteins that are involved in vesicle docking, or the density of active zones where transmitter is released, can influence the number of quantal units released per impulse (presynaptic mechanism). Postsynaptically, the number of active receptors, the postsynaptic input resistance, the area and the ultrastructure of subsynaptic reticulum, all can alter the effectiveness of quantum release and thus influence synaptic strength.

The ease in accessibility to the synaptic sites at NMJs allows one to record intracellular or very close to synapses by extracellular recordings (focal macropatch over a varicosity) in order to minimize cable properties in signal decrement [43-45]. Such signal loss occurs with recordings in a neuron cell body to measure synaptic function in the dendritic trees. The localized recording over a NMJ allows one the ability to measure properties of single and multiple vesicular quanta for very precise quantal analysis (occurrences, size and shape) to index synaptic function [46-49]. In addition, invertebrate NMJs are relatively stable for hours in a minimal saline at room temperature as compared to mammalian NMJs. Since most muscles in invertebrates are innervated by relatively few motor neurons, for the most part, they are identifiable anatomically and physiologically from preparation to preparation [50, 51]. Since the fine structure and detailed quantal analysis is feasible for many invertebrate NMJs, the acute and chronic actions of modulators on structure and function can be examined for their mechanic actions [52-54].

INSECTS

Given such a diverse group of animals within the class Insecta, it would not be surprising to find a wide range of anatomic and physiologic profiles in the innervation of skeletal NMJs. For example, the innervation of the genital chamber of the female cricket, Acheta domestica, shows 5-HT-immunoreactive nerve terminals that contact the muscle fibers which likely releases 5-HT in a type of volume transmission over the muscle as there are no defined synapses [55]. Such 5-HT containing nerve endings are also present in earthworm skeletal muscles [56]. However, no serotonin is associated with the oviducts or the innervation to the oviducts in the locust [57]. Earlier studies did not elucidate if the effect of 5-HT was directly on the presynaptic terminal or on the muscle but reported overall changes in force of muscle contraction. In a locust leg muscle, 5-HT produces an overall decrease in force development [58] but the mechanism of action still needs to be determined. It is suggested that in some of the earlier studies with insects, the high concentrations of 5-HT used may indeed block synaptic transmission by impeding the postsynaptic receptors [36, 58].

Despite the intense investigations in synaptic structure and plasticity in D. melanogaster related to genetic and mutational manipulations, there are few reports on the modulation of synaptic efficacy by peptides or modulators at the skeletal NMJ [54, 59-64]. As for the influence of 5-HT at the NMJ, the scantiness
of studies is likely due to the mild effects observed by using 5-HT itself as well as pharmacological agonists/antagonists of 5-HT receptors. However, application of 5-HT to the intact larval CNS does enhance the drive of motor neurons (MN) [17]. The most commonly studied Drosophila neuromuscular junctions are those in the most prominent ventral longitudinal abdominal muscle fiber muscles 6 and 7 [65], which have the simplest innervation pattern among the Drosophila body wall muscles. Both electrophysiological and morphological studies imply that each of these two muscles is innervated by only 2 axons [66, 67]. Application of 5-HT to these NMJs appears to slightly depress synaptic strength [68,69]. We are not aware of any attempt to investigate actions of 5-HT on adult skeletal NMJs. However, with the recent advent of designer receptors exclusively activated by designer drugs (DREAD) in motor neurons allows one to examine mechanisms of activating second messenger cascades as if receptors for modulators existed on presynaptic nerve terminals or on the muscles themselves [70, 71].

CRUSTACEANS

The NMJs in crustaceans offer many advantages for addressing mechanism of action in modulation of synaptic efficacy at NMJs, but crustaceans do fall short in being able to genetically modify the properties for investigations. Potentially approaches with RNAi might be practical to address more species-specific manipulations in synaptic function in a variety of crustaceans [72-75]. The same physiological and anatomical advantages of the Drosophila NMJs apply for the crustaceans, but in addition, the wide range in known diversity in synapses within crustaceans makes them attractive for comparative studies in commonalities of mechanisms in low- and high-output synapses or ones that facilitate or depress rapidly [31, 32, 76]. The parallels to vertebrate central synaptic physiology of phenomenon described at crustacean NMJs are likely one reason of continual interest to a wide variety of researchers investigating synaptic transmission. In addition, the historical contribution of crustaceans in synaptic physiology is unsurpassed [77-80]. The ability to combine direct structure and function in defined labeled synapses offers the ability to unravel synaptic structural complexity with function [31, 32, 43-45, 81].

It was demonstrated as early as 1954 that 5-HT enhances synaptic transmission at the crustacean NMJs [82, 83] and that the effect was likely a presynaptic enhancement of mean quantal content came afterwards [84]. The 5-HT that modifies most crustacean skeletal NMJs does so through the exposure of hemolymph. 5-HT is released from nerve endings in thoracic roots and from the pericardial organs into the hemolymph [85]. Thus, 5-HT is accessible to all the exposed NMJs. The excitatory as well as inhibitory NMJs are enhanced in transmission by 5-HT [86, 87]. The quantal effects are explained by increased probability of vesicular fusion during evoked transmission likely caused by an increase in the number of vesicular vesicles being docked and possibility their sensitivity of fusing due to enhanced Ca\(^{2+}\) sensitivity or presence of free Ca\(^{2+}\) within the terminals [88]. However, several studies have shown that a presynaptic rise in free Ca\(^{2+}\) is not substantial enough to account as a primary mechanism of 5-HT’s action [86, 89-91]. Since there is a steep rise in sensitivity to Ca\(^{2+}\) for enhancing synaptic efficacy at crustacean NMJs [92] a low release from internal stores may account well enough for part of the effect [20]. This notion of an internal release of Ca\(^{2+}\) is also supported by experiments conducted by Glusman and Kravitz [91] in which they showed that a calcium-free bath, along with EGTA and high MgCl\(_2\), 5-HT could still cause spontaneous release of transmitter for lobster NMJs. The enhanced spontaneous and evoked fusion events relates to an increase in ‘n’ (number of sites) and ‘p’ (probability of release) to explain the enhanced ‘m’ (mean quantal content; m=np) after exposure to 5-HT [69, 93, 94]. An interesting observation, but not yet explained mechanistically, is that 5-HT produced an effect with low or zero extracellular calcium at a crayfish NMJ but 5-HT’s effect depended on extracellular sodium concentration [89].

Low- and high-output NMJs in crayfish and crab show differential responses to 5-HT [95-97]. This could be due to the larger reserve pool of vesicles in tonic (low-output) terminals than the phasic (high-output) terminals and the fact that higher-output synapses in crustaceans have more complex synapses containing more active zones in close apposition on synapses than lower output synapses [45, 98-100].

NMJs investigated in lobster and crab revealed similar findings to those of the crayfish. 5-HT also enhances both excitatory and inhibitor NMJs that have been examined in Homarus americanus (lobster) [101, 102]. 5-HT also promotes the force of nerve-evoked contractions of the gastric mill muscle of the crab, Cancer borealis [103].

The differential responses and cellular mechanism of 5-HT’s action at crustacean NMJs is likely accounted for by the density and receptor subtypes on the presynaptic terminals. Vertebrate 5-HT\(_2\)-like receptors were physiologically identified for Procambarus clarkii at NMJs [21-24, 69]. Since this subtype of receptor has been sequenced in a crab and crayfish [28] these may be the subtypes present at the NMJs; however the blockers for the vertebrate 5-HT\(_2\)-like receptors could not block the entire 5-HT enhancement of synaptic enhancement [24]. Also 5-HT\(_2\) agonists did not mimic the responses fully at the crayfish NMJ [24], so
potential affinity in binding 5-HT and pharmacological agents differ in crustaceans to vertebrate subtype receptor analogs. The pharmacology of monoamines in the cardiac ganglion of lobsters also does not mimic vertebrate classifications [104]. Care needs to be taken in assuming the pharmacology of mammalian 5-HT receptors applies to invertebrates [22].

Given there is at least some pharmacological and sequence similarity to vertebrate 5-HT1 receptor subtype present in crayfish and that injection of an IP3 analog (adenophostin-A) in the presynaptic motor nerve terminals enhances release [20], a potential mechanism is that 5-HT receptors on the presynaptic membrane mediate activation of G coupled receptors which leads to activation of phospholipase C (PLC) which in turn produces 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) [105]. The production of IP3 can directly result in Ca2+ release from internal stores (i.e., ER) through IP3 receptors on the ER [106].

Since vertebrate 5-HT1 receptor family activates phospholipase C (PLC) [9] a similar receptor activated cascade is possible at the crayfish NMJs. Such mechanisms are established in other systems [105, 107, 108] and given that caffeine and ryanodine actions are in concurrence with IP3 receptors potentially on the ER in crayfish presynaptic motor nerve terminals [20] we have to consider this mechanism as a likely possibility. The rise is Ca2+, even a slight rise, could activate calmodulin and in turn activate CaM-Kinase (CaM-K), which can lead to phosphorylation of proteins such as synapsin. The possibility is that vesicles would then be able to leave the tethers to the cytoskeleton and dock to the presynaptic membrane, which is also a phosphorylation step [109-111]. The increased docked vesicles could be subjected to the calcium influx and release from internal stores [112]. This would account for the increase in the occurrence of spontaneous quantal events and enhanced evoked responses with 5-HT exposure. In the invertebrate Aplysia, it was shown that exposure of neurons to 5-HT results in phosphorylation of synapsins [113]. cAMP was also suggested to be involved in 5-HT action [30, 114-116]. cAMP has been shown to activate Protein Kinase A (PKA) which then can lead to phosphorylation of transcriptional factors such as CREB. Such action can regulate synthesis of proteins used in synaptic transmission [117-119]. It has also been suggested that the cAMP and calmodulin pathways may work together and promote transcription [120]. When phosphatases are inhibited at the crayfish NMJ the effect of 5-HT is enhanced, thus demonstrating the significance of phosphorylation [121] which is known to occur with exposure to 5-HT at crustacean NMJs [122].

In a recent study addressing the potential mechanisms of 5-HT, as well as stimulation of the motor nerve terminal, in recruiting vesicles from a reserve pool (RP) to a readily releasable pool (RRP) within the presynaptic nerve terminals of crayfish NMJs, we developed a model to account for the observations and previous reports. In a current study, we inhibited the packaging of glutamate by blocking the vesicular glutamate transporter (VGlut) with the drug bafilomycin A1 (BA) [123-125]. In this way, the rapidly recycling vesicles within the RRP will be empty with repetitive stimulation. However, if the RP is spared from being recruited by low frequency stimulation and if they are already packaged with transmitter, prior to exposure to BA, then 5-HT should be able to recruit these RP vesicles to the RRP and synaptic transmission restored temporarily. This is exactly what was observed indicating that the RP and RRP can be physiologically differentiated into distinct functional groups and that 5-HT recruits the RP into action [126, 127]. To deplete or use up the packaged RRP vesicles, continuous stimulation was provided since the opener NMJ preparation is low-output and fatigue resistant. A high frequency of 40 Hz was used for comparative purposes to 20 Hz continuous stimulation. As expected, preparations stimulated at 40 Hz depressed faster than the ones stimulated at 20 Hz and there was a reduced effect for the 40 Hz stimulated preparations to exposure of 5-HT. This suggests that a higher stimulation frequency is able to recruit some of the RRP. This is illustrated in a model (Fig. 1). To address if PLC is an intermediate step within the cascade of events activated by 5-HT mediated responses, we used a PLC inhibitor (U73122) and an inactive analog (U73343) to serve as a negative control [128]. We found that the treatment of U73122 caused a significant decrease of 5-HT effect on synaptic transmission. This result confirmed the involvement of PLC signaling cascade in inducing the enhancement of synaptic transmission by 5-HT at a different physiological condition.

There are observations in other preparations that indicate the presence of two distinct vesicle pools: RRP and RP. In the cat superior sympathetic ganglion, Prado et al. [129] separated the two pools by electrically stimulating the nerve to deplete the RRP of acetylcholine, and then recruit RP vesicles by tityustoxin. Using FM 1-43 dye, the two pools have been identified in a temperature-sensitive mutant Drosophila line, shibire, and later in WT [130, 131]. However in our study with the crayfish NMJ, a novel approach with bafilomycin A1 was used together with continuous stimulation to deplete the RRP, and then 5-HT was applied to recruit RP vesicles and the recruitment involves a PLC signaling cascade. A mechanistic illustration is detailed in Fig. 1.

There does not appear to be a substantial direct effect on crustacean skeletal muscle to account for an increase in EPSP or IPSP amplitude due to an increase in input resistance of the fibers [82, 114, 132, 133]. A small increase in input resistance, by exposure to 5-HT, accounts for a slight increase in the EPSP...
amplitude for superficial flexor muscle fibers of crayfish [94]. More substantial alteration in input resistance can occur in crustacean neurons due to 5-HT exposure [134] so there could be some effect on the presynaptic motor nerve terminals.

In comparison to the smooth muscle in the intestine of vertebrates, the muscles of the crayfish hindgut are striated with gap junctions and generate intrinsic pacemaker activity [135, 136]. Application of 5-HT [137] and octopamine [138] to GI tract increases the frequency and strength of contractions. 5-HT and dopamine are highly concentrated in CNS and GI tract and they are directly responsible for the peristalsis and muscle contraction [137, 139].

**ANNELIDS**

The leech has served as a model organism in neurobiology for many years [140] but few studies have directly focused attention at NMJs in the leech and even fewer on the effects of 5-HT in synaptic transmission at NMJs. However, studies have examined the effect of 5-HT on the drive of motor neurons and innervation patterns [141-147]. 5-HT exposure has a relaxing effect on skeletal muscle in the leech [148] and enhances muscle force and work production during locomotion and feeding [149]. This is physiological relevant since Retzius neurons do directly innervate skeletal muscle in the leech and these cells do release 5-HT [144, 150-152]. In the earthworm and polychaete (*Sabellastarte magnifica*) muscle contraction is reduced by 5-HT [153, 154] which lead to the idea that 5-HT might be acting as inhibitory transmitter in these preparations [155].

**GASTROPODS**

A few studies with gastropods have been approached for the direct effect of 5-HT at the NMJ. 5-HT produces facilitation for an evoked response in buccal muscle within *Aplysia* [156]. The presynaptic actions of 5-HT is to enhance transmitter release [157]. Like for some of the actions in annelids, 5-HT can also produce muscle relaxation and reduce force in *Aplysia* [158]. Such effects on muscle contraction and force maybe dependent on 5-HT concentration and the species studied, since in *Aplysia brasiliana* 5-HT increases a Ca$^{2+}$ influx that promotes muscle contraction used for swimming [159].

**OTHER INVERTEBRATES**

In a sea urchin (*Parechinus*), 5-HT apparently had no effect at the NMJ [160]. However in a sea cucumber (*Apostichopus*...
5-HT inhibited evoked contractions induced by acetylcholine and there appears to be 5-HT innervation directly to muscles of the body wall [161].

**SUMMARY**

Although headway has been made in describing the various actions of 5-HT at NMJs in invertebrates, the cellular mechanisms of these actions are still lacking. Additional pharmacological and molecular profiling in a variety of invertebrate preparations will increase our knowledge of both the uniqueness and similarities among the invertebrates. As history has taught us in physiology, and in particular neurobiology, what is learned in invertebrate preparations paves the way to new views and mechanistic cellular understanding of complex processes within the vertebrates.

**ACKNOWLEDGEMENTS**

We thank Mr. Josh Titlow, Ms. Rachel Holsinger and Mr. Zana Majeed for reviewing this manuscript and their constructive suggestions. Supported by personal funds of authors.

**REFERENCES**

1. Azmitia EC (2001) Modern views on an ancient chemical: serotonin effects on cell proliferation, maturation, and apoptosis. Brain Res Bull 56:413-424.
2. Azmitia EC (2007) Serotonin and brain: evolution, neuroplasticity, and homeostasis. Int Rev Neurobiol 77:31-56.
3. Bunin MA, Wightman RM (1999) Paracrine neurotransmission in the CNS: involvement of 5-HT. Trends Neurosci 22:377-382.
4. Monastirioti M (1999) Biogenic amine systems in the fruit fly Drosophila melanogaster. Microsc Res Tech 45:106-121.
5. Tierney AJ (2001) Structure and function of invertebrate 5-HT receptors: a review. Comp Biochem Physiol A Mol Integr Physiol 128:791-804.
6. Blenau W, Baumann A (2001) Molecular and pharmacological properties of insect biogenic amine receptors: lessons from Drosophila melanogaster and Apis mellifera. Arch Insect Biochem Physiol 48:13-38.
7. Colas JF, Launay JM, Maroteaux L (1999) Maternal and zygotic control of serotonin biosynthesis are both necessary for Drosophila germband extension. Mech Dev 87:67-76.
8. Saudou F, Boschert U, Amlaiky N, Plassat JL, Hen R (1992) A family of Drosophila serotonin receptors with distinct intracellular signalling properties and expression patterns. EMBO J 11:7-17.
9. Saudou F, Hen R (1994) 5-Hydroxytryptamine receptor subtypes in vertebrates and invertebrates. Neurochem Int 25:503-532.
10. Witz P, Amlaiky N, Plassat JL, Maroteaux L, Borrelli E, Hen R (1990) Cloning and characterization of a Drosophila serotonin receptor that activates adenylate cyclase. Proc Natl Acad Sci U S A 87:8940-8944.
11. Baines RA, Downer RG (1991) Pharmacological characterization of a 5-hydroxytryptamine-sensitive receptor/adenylate cyclase complex in the mandibular closer muscles of the cricket, Gryllus domesticus. Arch Insect Biochem Physiol 16:153-163.
12. Hoyer D, Hannon JP, Martin GR (2002) Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533-554.
13. Burns CM, Chu H, Rueter SM, Hutchinson I.K, Canton H, Sanders-Bush E, Emeson RB (1997) Regulation of serotonin-2C receptor G-protein coupling by RNA editing. Nature 387:303-308.
14. Niswender CM, Sanders-Bush E, Emeson RB (1998) Identification and characterization of RNA editing events within the 5-HT2C receptor. Ann N Y Acad Sci 861:38-48.
15. Colas JF, Launay JM, Kellermann O, Rosay P, Maroteaux L (1995) Drosophila 5-HT2 serotonin receptor: coexpression with fushi-tarazu during segmentation. Proc Natl Acad Sci U S A 92:5441-5445.
16. Colas JF, Launay JM, Vonesch JL, Hickel P, Maroteaux L (1999) Serotonin synchronises convergent extension of ectoderm with morphogenetic gastrulation movements in Drosophila. Mech Dev 87:77-91.
17. Dasari S, Cooper RL (2004) Modulation of sensory-CNS-motor circuits by serotonin, octopamine, and dopamine in semi-intact Drosophila larva. Neurosci Res 48:221-227.
18. Dasari S, Cooper RL (2006) Direct influence of serotonin on the larval heart of Drosophila melanogaster. J Comp Physiol B 176:349-357.
19. Dasari S, Wang L, Harrison DA, Cooper RL (2009) Reduced and misexpression of 5-HT2 receptors alters development, behavior and CNS activity in Drosophila melanogaster. Int J Zool Res 5:101-114.
20. Dropic AJ, Brailoiu E, Cooper RL (2005) Presynaptic mechanism of action induced by 5-HT in nerve terminals: possible involvement of ryanodine and IP3 sensitive Ca^2+ stores. Comp Biochem Physiol A Mol Integr Physiol 142:355-361.
21. Sosa MA, Spitzer N, Edwards DH, Baro DJ (2004) A
crustacean serotonin receptor: cloning and distribution in the thoracic ganglia of crayfish and freshwater prawn. J Comp Neurol 473:526-537.

22. Sparks GM, Brailoiu E, Brailoiu GC, Dun NJ, Tabor J, Cooper RL (2003) Effects of m-CPP in altering neuronal function: blocking depolarization in invertebrate motor and sensory neurons but exciting rat dorsal horn neurons. Brain Res 969:14-26.

23. Sparks GM, Dasari S, Cooper RL (2004) Actions of MDMA at glutamatergic neuromuscular junctions. Neurosci Res 48:431-438.

24. Tabor JN, Cooper RL (2002) Physiologically identified 5-HT2-like receptors at the crayfish neuromuscular junction. Brain Res 932:91-98.

25. Tierney AJ, Mangiamele LA (2001) Effects of serotonin and serotonin analogs on posture and agonistic behavior in crayfish. J Comp Physiol A 187:757-767.

26. Tierney AJ, Greenlaw MA, Dams-O’Connor K, Aig SD, Perna AM (2004) Behavioral effects of serotonin and serotonin agonists in two crayfish species, Procambarus clarkii and Orconectes rusticus. Comp Biochem Physiol A Mol Integr Physiol 139:495-502.

27. Clark MC, Dever TE, Dever JJ, Xu P, Rehder V, Sosa MA, Baro DJ (2004) Arthropod 5-HT2 receptors: a neurohormonal receptor in decapod crustaceans that displays agonist independent activity resulting from an evolutionary alteration to the DRY motif. J Neurosci 24:3421-3435.

28. Spitzer N, Edwards DH, Baro DJ (2008) Conservation of structure, signaling and pharmacology between two serotonin receptor subtypes from decapod crustaceans, Panulirus interruptus and Procambarus clarkii. J Exp Biol 211:92-105.

29. Mapara S, Parries S, Quarrington C, Ahn KC, Gallin WI, Goldberg JI (2008) Identification, molecular structure and expression of two cloned serotonin receptors from the pond snail, Helisoma trivolvis. J Exp Biol 211:900-910.

30. Lee YS, Choi SL, Lee SH, Kim H, Park H, Lee N, Lee SH, Chae YS, Jang DJ, Kandel ER, Kaang BK (2009) Identification of a serotonin receptor coupled to adenyllyl cyclase involved in learning-related heterosynaptic facilitation in Aplysia. Proc Natl Acad Sci U S A 106:14634-14639.

31. Atwood HL, Cooper RL (1995) Functional and structural parallels in crustacean and Drosophila neuromuscular systems. Am Zool 35:556-565.

32. Atwood HL, Cooper RL (1996) Synaptic diversity and differentiation: crustacean neuromuscular junctions. Invert Neurosci 1:291-307.

33. Collins CA, DiAntonio A (2007) Synaptic development: insights from Drosophila. Curr Opin Neurobiol 17:35-42.

34. Govind CK, Walrond JP (1989) Structural plasticity at crustacean neuromuscular synapses. J Neurobiol 20:409-421.

35. Westfall IA (1996) Ultrastructure of synapses in the first-evolved nervous systems. J Neurocytol 25:735-746.

36. Gerschenfeld HM (1973) Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. Physiol Rev 53:1-119.

37. Whim MD, Church PJ, Lloyd PE (1993) Functional roles of peptide cotransmitters at neuromuscular synapses in Aplysia. Mol Neurobiol 7:335-347.

38. Lee JY, Bhatt D, Bhatt D, Chung WY, Cooper RL (2009) Furthering pharmacological and physiological assessment of the glutamatergic receptors at the Drosophila neuromuscular junction. Comp Biochem Physiol C Toxicol Pharmacol 150:546-557.

39. Li H, Cooper RL (2001) Effects of the ecdysoneless mutant on synaptic efficacy and structure at the neuromuscular junction in Drosophila larvae during normal and prolonged development. Neuroscience 106:193-200.

40. Li H, Harrison D, Jones G, Jones D, Cooper RL (2001) Alterations in development, behavior, and physiology in Drosophila larva that have reduced ecdysone production. J Neurophysiol 85:98-104.

41. Li H, Peng X, Cooper RL (2002) Development of Drosophila larval neuromuscular junctions: maintaining synaptic strength. Neuroscience 115:505-513.

42. Sherrington CS (1906) The integrative action of the nervous system. Scribner, New York.

43. Cooper RL, Marin L, Atwood HL (1995) Synaptic differentiation of a single motor neuron: conjoint definition of transmitter release, presynaptic calcium signals, and ultrastructure. J Neurosci 15:4209-4222.

44. Cooper RL, Harrington CC, Marin L, Atwood HL (1996) Quantal release at visualized terminals of a crayfish motor axon: intraterminal and regional differences. J Comp Neurol 375:583-600.

45. Cooper RL, Winslow JL, Govind CK, Atwood HL (1996) Synaptic structural complexity as a factor enhancing probability of calcium-mediated transmitter release. J Neurophysiol 75:2451-2466.

46. Cooper RL, Stewart BA, Wojtowicz JM, Wang S, Atwood HL (1995) Quantal measurement and analysis methods compared for crayfish and Drosophila neuromuscular junctions, and rat hippocampus. J Neurosci Methods 61:67-78.
Wen-Hui Wu and Robin L. Cooper

47. Viele K, Stromberg AJ, Cooper RL (2003) Estimating the number of release sites and probability of firing within the nerve terminal by statistical analysis of synaptic charge. Synapse 47:15-25.

48. Viele K, Lancaster M, Cooper RL (2006) Self-modeling structure of evoked postsynaptic potentials. Synapse 60:32-44.

49. Lancaster M, Viele K, Johnstone AF, Cooper RL (2007) Automated classification of evoked quantal events. J Neurosci Methods 159:325-336.

50. Atwood HL, Cooper RL (1996) Assessing ultrastructure of crustacean and insect neuromuscular junctions. J Neurosci Methods 69:51-58.

51. Worden MK (1998) Modulation of vertebrate and invertebrate neuromuscular junctions. Curr Opin Neurobiol 8:740-745.

52. Cooper RL, Ruffner ME (1998) Depression of synaptic efficacy at intermolt in crayfish neuromuscular junctions by 20-Hydroxyecdysone, a molting hormone. J Neurophysiol 79:1931-1941.

53. Logsdon S, Johnstone AF, Viele K, Cooper RL (2006) Regulation of synaptic vesicles pools within motor nerve terminals during short-term facilitation and neuromodulation. J Appl Physiol 100:662-671.

54. Ruffner ME, Cromarty SI, Cooper RL (1999) Depression of synaptic efficacy in high- and low-output Drosophila neuromuscular junctions by the molting hormone (20-HE). J Neurophysiol 81:788-794.

55. Elekes K, Hustert R (1988) The efferent innervation of the genital chamber by an identified serotonergic neuron in the female cricket Acheta domestica. Cell Tissue Res 252:449-457.

56. Nishihara H (1967) The fine structure of the earthworm body wall muscle. Acta Anat Nippon 42:38-39.

57. Lange AB (2004) A neurohormonal role for serotonin in the control of locust oviducts. Arch Insect Biochem Physiol 56:179-190.

58. Hill RB, Usherwood PN (1961) The action of 5-hydroxytryptamine and related compounds on neuromuscular transmission in the locust Schistocerca gregaria. J Physiol 157:393-401.

59. Badre NH, Martin ME, Cooper RL (2005) The physiological and behavioral effects of carbon dioxide on Drosophila melanogaster larvae. Comp Biochem Physiol A Mol Integr Physiol 140:363-376.

60. Chen X, Ganetzky B (2012) A neuropeptide signaling pathway regulates synaptic growth in Drosophila. J Cell Biol 196:529-543.

61. Cooper RL, Neckameyer WS (1999) Dopaminergic modulation of motor neuron activity and neuromuscular function in Drosophila melanogaster. Comp Biochem Physiol B Biochem Mol Biol 122:199-210.

62. Dunn TW, Mercier AJ (2005) Synaptic modulation by a Drosophila neuropeptide is motor neuron-specific and requires CaMKII activity. Peptides 26:269-276.

63. Middleton CA, Nongthomba U, Parry K, Sweeney ST, Sparrow JC, Elliott CJ (2006) Neuromuscular organization and aminergic modulation of contractions in the Drosophila ovary. BMC Biol 4:17.

64. Nagaya Y, Kutsukake M, Chigusa SI, Komatsu A (2002) A trace amine, tyramine, functions as a neuromodulator in Drosophila melanogaster. Neurosci Lett 329:324-328.

65. Crossley CA (1978) The morphology and development of the Drosophila muscular system. In: The genetics and biology of drosophila (Ashburner M, Novitski E, eds), pp 499-599. Academic Press, New York.

66. Jan LY, Jan YN (1976) Properties of the larval neuromuscular junction in Drosophila melanogaster. J Physiol 262:189-214.

67. Sink H, Whitington PM (1991) Location and connectivity of abdominal motoneurons in the embryo and larva of Drosophila melanogaster. J Neurophysiol 22:298-311.

68. Dasari S, Viele K, Turner AC, Cooper RL (2007) Influence of PCPA and MDMA (ecstasy) on physiology, development and behavior in Drosophila melanogaster. Eur J Neurosci 26:424-438.

69. Sparks GM, Cooper RL (2004) 5-HT offsets homeostasis of synaptic transmission during short-term facilitation. J Appl Physiol 96:1681-1690.

70. Nichols CD, Becnel J, Johnson O, Majeed ZR, Tran V, Yu B, Roth BL, Cooper RL (2012) DREADD receptor control of behavior, signalling, and physiology in the model organism Drosophila melanogaster. Thorlabs (Photonics) meeting abstract. Optogenetics and Pharmacogenetics in Neuronal Function and Dysfunction. New Orleans, LA, USA. 11-12 October 2012.

71. Majeed ZR, Cooper RL, Nichols CD (2012) The influence of DREAD receptors activation in the CNS of Drosophila melanogaster. Annual Meeting of Society for Neuroscience, 2012 Oct 13-17. New Orleans, LA, USA.

72. Estrada B, Gisselbrecht SS, Michelson AM (2007) The transmembrane protein Perdido interacts with Grip and integrins to mediate myotube projection and attachment in the Drosophila embryo. Development 134:4469-4478.

73. Kato Y, Shiga Y, Kobayashi K, Tokishita S, Yamagata H, Iguchi
T. Watanabe H (2011) Development of an RNA interference method in the cladoceran crustacean Daphnia magna. Dev Genes Evol 220:337-345.
74. Estrada MP, Lugo JM, Acosta J, Carpio Y, Borroto I, Morera Y, González O, Rodriguez T, Ramos L, Huberman A (2007) Effects of RNA interference on gene functions of aquatic organisms. Biotechnol Apl 24:178-182.
75. Pékhletsik R, Cooper RL, Atwood HL, Hampson DR (1996) Expression profiling of mRNA obtained from single identified crustacean motor neurons: determination of specificity of hybridization. Invert Neurosci 1:341-349.
76. Wu WH, Cooper RL (2010) Physiological recordings of high and low output NMJs on the Crayfish leg extensor muscle. J Vis Exp (45):e2319. Available from: http://www.jove.com/index/details.stp?id=2319.
77. Atwood HL (1976) Organization and synaptic physiology of crustacean neuromuscular systems. Prog Neurobiol 7:291-391.
78. Atwood HL (1967) Variation in physiological properties of crustacean motor synapses. Nature 215:57-58.
79. Cooper AS, Cooper RL (2009) Historical view and physiology demonstration at the NMJ of the crayfish opener muscle. J Vis Exp (33):e1595. Available from: http://www.jove.com/index/details.stp?id=1595.
80. Wiese K (2002) The crustacean nervous system. Springer, Berlin.
81. Johnstone AF, Viele K, Cooper RL (2011) Structure/function assessment of synapses at motor nerve terminals. Synapse 65:287-299.
82. Florey E, Florey E (1954) Uber die mogliche Bedeutung von Enteramin (5-oxytryptamin) als nervoser Aktimssubstanz bei cephalopoden und dekapoden Crustacean. Z Naturforsch 9B:58-68.
83. Fischer L, Florey E (1983) Modulation of synaptic transmission and excitation-contraction coupling in the opener muscle of the crayfish. Astacus leptodactylus, by 5-hydroxytryptamine and octopamine. J Exp Biol 102:187-198.
84. Dudel J (1965) Facilitatory effects of 5-hydroxy-tryptamine on the crayfish neuromuscular junction. Naunyn Schmiedebergs Arch Pharmacol 249:515-528.
85. Beltz BS, Kravitz EA (1983) Mapping of serotonin-like immunoreactivity in the lobster nervous system. J Neurosci 3:585-602.
86. Vyshedskiy A, Delaney KR, Lin JW (1998) Neuromodulators enhance transmitter release by two separate mechanisms at the inhibitor of crayfish opener muscle. J Neurosci 18:5160-5169.
87. Wang C, Zucker RS (1998) Regulation of synaptic vesicle recycling by calcium and serotonin. Neuron 21:153-167.
88. Dudel J (1988) Modulation of quantal synaptic release by serotonin and forskolin in crayfish motor nerve terminals. In: Modulation of synaptic transmission and plasticity in nervous systems, vol. 19 (Hertting G, Spatz HC, eds), pp 259-270. Springer, Berlin.
89. Dixon D, Atwood HL (1985) Crayfish motor nerve terminal's response to serotonin examined by intracellular microelectrode. J Neurobiol 16:409-424.
90. Delaney K, Tank DW, Zucker RS (1991) Presynaptic calcium and serotonin-mediated enhancement of transmitter release at crayfish neuromuscular junction. J Neurosci 11:2631-2643.
91. Glusman S, Kravitz EA (1982) The action of serotonin on excitatory nerve terminals in lobster nerve-muscle preparations. J Physiol 325:223-241.
92. Dudel J (1981) The effect of reduced calcium on quantal unit current and release at the crayfish neuromuscular junction. Pflugers Arch 391:35-40.
93. Southard RC, Haggard J, Crider ME, Whiteheart SW, Cooper RL (2000) Influence of serotonin on the kinetics of vesicular release. Brain Res 871:16-28.
94. Strawn JR, Neckameyer WS, Cooper RL (2000) The effects of 5-HT on sensory, central and motor neurons driving the abdominal superficial flexor muscles in the crayfish. Comp Biochem Physiol B Biochem Mol Biol 127:533-550. (See Erratum 128:377-378, 2001).
95. Cooper RL, Dönmez A, Shearer J (2003) Intrinsic differences in sensitivity to 5-HT between high- and low-output terminals innervating the same target. Neurosci Res 45:163-172.
96. Djokaj S, Cooper RL, Rathmayer W (2001) Presynaptic effects of octopamine, serotonin, and cocktails of the two modulators on neuromuscular transmission in crustaceans. J Comp Physiol A 187:145-154.
97. Johnstone AF, Kellie SS, Cooper RL (2008) Presynaptic depression in phasic motor nerve terminals and influence of 5-HT on vesicle dynamics. Open Neurosci J 2:16-23.
98. Bradacs H, Cooper R, Msghina M, Atwood H (1997) Differential physiology and morphology of phasic and tonic motor axons in a crayfish limb extensor muscle. J Exp Biol 200:677-691.
99. King MJ, Atwood HL, Govind CK (1996) Structural features of crayfish phasic and tonic neuromuscular terminals. J Comp Neurol 372:618-626.
100. Millar AG, Zucker RS, Ellis-Davies GC, Charlton MP,
Atwood HL (2005) Calcium sensitivity of neurotransmitter release differs at phasic and tonic synapses. J Neurosci 25:3113-3125.

101. Hamilton JL, Edwards CR, Holt SR, Worden MK (2007) Temperature dependent modulation of lobster neuromuscular properties by serotonin. J Exp Biol 210:1025-1035.

102. Harris-Warrick RM, Kravitz EA (1984) Cellular mechanisms for modulation of posture by octopamine and serotonin in the lobster. J Neurosci 4:1976-1993.

103. Jorge-Rivera JC, Sen K, Birmingham JT, Abbott LF, Marder E (1998) Temporal dynamics of convergent modulation at a crustacean neuromuscular junction. J Neurophysiol 80:2559-2570.

104. Berlind A (2001) Monoamine pharmacology of the lobster cardiac ganglion. Comp Biochem Physiol C Toxicol Pharmacol 128:377-390.

105. Berridge MJ, Irvine RF (1989) Inositol phosphates and cell signalling. Nature 341:197-205.

106. Hisatsune C, Nakamura K, Kuroda Y, Nakamura T, Mikoshiba K (2005) Amplification of Ca$^{2+}$ signaling by diacylglycerol-mediated inositol 1,4,5-trisphosphate production. J Biol Chem 280:11723-11730.

107. Mattson MP, LaFerla FM, Chan SL, Leissring MA, Shepel PN, Geiger JD (2000) Calcium signaling in the ER: its role in neuronal plasticity and neurodegenerative disorders. Trends Neurosci 23:222-229.

108. Petersen OH, Cancela JM (1999) New Ca$^{2+}$-releasing messengers: are they important in the nervous system? Trends Neurosci 22:488-495.

109. Chi P, Greengard P, Ryan TA (2003) Synaptic vesicle mobilization is regulated by distinct synapsin I phosphorylation pathways at different frequencies. Neuron 38:69-78.

110. He P, Southard RC, Chen D, Whiteheart SW, Cooper RL (1999) Role of alpha-SNAP in promoting efficient neurotransmission at the crayfish neuromuscular junction. J Neurophysiol 82:3406-3416.

111. Tolar LA, Pallanck L (1998) NSF function in neurotransmitter release involves rearrangement of the SNARE complex downstream of synaptic vesicle docking. J Neurosci 18:10250-10256.

112. Yang SN, Tang YG, Zucker RS (1999) Selective induction of LTP and LTD by postsynaptic [Ca$^{2+}$], elevation. J Neurophysiol 81:781-787.

113. Fiumara F, Giovedi S, Menegon A, Milanese C, Merlo D, Montarolo PG, Valtorta F, Benfenati F, Ghirardi M (2004) Phosphorylation by cAMP-dependent protein kinase is essential for synapsin-induced enhancement of neurotransmitter release in invertebrate neurons. J Cell Sci 117:5145-5154.

114. Battelle BA, Kravitz EA (1978) Targets of octopamine action in the lobster: cyclic nucleotide changes and physiological effects in hemolymph, heart and exoskeletal muscle. J Pharmacol Exp Ther 205:438-448.

115. Dixon D, Atwood HL (1989) Conjoint action of phosphatidylinositol and adenylate cyclase systems in serotonin-induced facilitation at the crayfish neuromuscular junction. J Neurophysiol 62:1251-1259.

116. Goy MF, Kravitz EA (1989) Cyclic AMP only partially mediates the actions of serotonin at lobster neuromuscular junctions. J Neurosci 9:369-379.

117. Geppert M, Goya D, Stevens CF, Sudhof TC (1997) The small GTP-binding protein Rab3A regulates a late step in synaptic vesicle fusion. Nature 387:810-814.

118. Bolshakov VY, Golan H, Kandel ER, Siegelbaum SA (1997) Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. Neuron 19:635-651.

119. Yao J, Qi J, Chen G (2006) Actin-dependent activation of presynaptic silent synapses contributes to long-term synaptic plasticity in developing hippocampal neurons. J Neurosci 26:8137-8147.

120. Dash PK, Karl KA, Colicos MA, Prywes R, Kandel ER (1991) cAMP response element-binding protein is activated by Ca$^{2+}$/calmodulin- as well as cAMP-dependent protein kinase. Proc Natl Acad Sci U S A 88:5061-5065.

121. Swain JE, Robitaille R, Dass GR, Charlton MP (1991) Phosphatases modulate transmission and serotonin facilitation at synapses: studies with the inhibitor okadaic acid. J Neurobiol 22:855-864.

122. Goy MF, Schwarz TL, Kravitz EA (1984) Serotonin-induced protein phosphorylation in a lobster neuromuscular preparation. J Neurosci 4:611-626.

123. Bowman EJ, Graham LA, Stevens TH, Bowman BJ (2004) The bafilomycin/concanamycin binding site in subunit c of the V-ATPases from Neurospora crassa and Saccharomyces cerevisiae. J Biol Chem 279:33313-33318.

124. Cavelier P, Attwell D (2007) Neurotransmitter depletion by bafilomycin is promoted by vesicle turnover. Neurosci Lett 412:95-100.

125. Juge N, Gray JA, Omote H, Miyaji T, Inoue T, Hara C, Uneyama H, Edwards RH, Nicoll RA, Moriyama Y (2010) Metabolic control of vesicular glutamate transport and release. Neuron 68:99-112.
5-HT Modulation of Invertebrate NMJs

126. Wu WH, Cooper RL (2012) The regulation and packaging of synaptic vesicles as related to recruitment within glutamatergic synapses (in review, doi: http://dx.doi.org/10.1016/j.neuroscience.2012.08.037).

127. Wu WH, Cooper RL (2012) The regulation and packaging of synaptic vesicles related to recruitment within glutamatergic synapses. Annual Meeting of Society for Neuroscience, 2012 Oct 13-17. New Orleans, LA, USA.

128. Bleasdale JE, Thakur NR, Grehan RS, Bundy GL, Fitzpatrick FA, Smith RJ, Bunting S (1990) Selective inhibition of receptor-coupled phospholipase C-dependent processes in human platelets and polymorphonuclear neutrophils. J Pharmacol Exp Ther 255:756-768.

129. Prado MA, Gomez MV, Collier B (1992) Mobilization of the readily releasable pool of acetylcholine from a sympathetic ganglion by tityustoxin in the presence of vesamicol. J Neurochem 59:544-552.

130. Betz WJ, Mao F, Bewick GS (1992) Activity-dependent fluorescent staining and destaining of living vertebrate motor nerve terminals. J Neurosci 12:363-375.

131. Kuromi H, Kidokoro Y (2000) Tetanic stimulation recruits vesicles from reserve pool via a cAMP-mediated process in Drosophila synapses. Neuron 27:133-143.

132. Grundfest H, Reuben JP (1961) Neuromuscular synaptic activity in lobster. In: Nervous inhibition (Florey E, ed), pp 92-104. Pergamon, New York.

133. Kravitz EA, Glusman S, Harris-Warrick RM, Livingstone MS, Schwarz T, Goy MF (1980) Amines and a peptide as neurotransmitters in lobsters: actions on neuromuscular preparations and preliminary behavioural studies. J Exp Biol 89:159-175.

134. Cooper RL, Ward E, Braxton R, Li H, Warren WM (2003) The effects of serotonin and edysone on primary sensory neurons in crayfish. Microsc Res Tech 60:336-345.

135. Brenner TL (1999) The physiology of crayfish intestinal striated muscle: histology, histochemistry, and excitation-contraction coupling. MSc thesis. University of Calgary, Calgary, AB, Canada.

136. To TH, Brenner TL, Cavey MJ, Wilkens JL (2004) Histological organization of the intestine in the crayfish Procambarus clarkii. Acta Zool 85:119-130.

137. Musolf BE (2007) Serotonergic modulation of the Crayfish hindgut: effects on hindgut contractility and regulation of serotonin on hindgut. Biology Dissertations. Paper 32. Available from: http://digitalarchive.gsu.edu/biology_diss/32.

138. Orchard I, Lange AB (1985) Evidence for octopaminergic modulation of an insect visceral muscle. J Neurobiol 16:171-181.

139. Cooper AS, Lekrisawat B, Gilberts AB, Mercier AJ, Cooper RL (2011) Physiological experimentation with the crayfish hindgut: a student laboratory exercise. J Vis Exp (47):e2324. Available from: http://www.jove.com/details.php?id=2324.

140. Nicholls JG, Stent GS, Muller KJ (1981) Neurobiology of the leech. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

141. Brodfuehrer PD, Debski EA, O'Gara BA, Friesen WO (1995) Neuronal control of leech swimming. J Neurobiol 27:403-418.

142. Stuart AE (1970) Physiological and morphological properties of motoneurones in the central nervous system of the leech. J Physiol 209:627-646.

143. Ort CA, Kristan WB, Stent GS (1974) Neuronal control of swimming in the medicinal leech. J Comp Physiol 94:121-154.

144. Kuffler DP (1978) Neuromuscular transmission in longitudinal muscle of the leech Hirudo medicinalis. J Comp Physiol 124:333-338.

145. Cline HT (1983) 3H-GABA uptake selectively labels identifiable neurons in the leech central nervous system. J Comp Neurol 215:351-358.

146. O'Gara BA, Illuzzi FA, Chung M, Portnoy AD, Fraga K, Frieman VB (1999) Serotonin induces four pharmacologically separable contractile responses in the pharynx of the leech Hirudo medicinalis. Gen Pharmacol 32:669-681.

147. Sawada M, Coggeshall RE (1976) A central inhibitory action of 5-hydroxytryptamine in the leech. J Neurobiol 7:477-482.

148. Schain RJ (1961) Effects of 5-hydroxytryptamine on the dorsal muscle of the leech (Hirudo medicinalis). Br J Pharmacol Chemother 16:257-261.

149. Gerry SP, Ellerby DJ (2011) Serotonin modulates muscle function in the medicinal leech Hirudo verbana. Biol Lett 7:885-888.

150. Mason A, Kristan WB (1982) Neuronal excitation, inhibition and modulation of leech longitudinal muscle. J Comp Physiol 146:327-356.

151. Mason A, Sunderland AJ, Leake LD (1979) Effects of leech Retzius cells on body wall muscles. Comp Biochem Physiol C 63C:359-361.

152. Yaksta-Sauerland BA, Coggeshall RE (1973) Neuromuscular junctions in the leech. J Comp Neurol 151:85-99.

153. Hidaka T, Kuriyama H, Yamamoto T (1969) The mechanical properties of the longitudinal muscle in the earthworm. J Exp Biol 50:431-443.
154. Del Carmen Alvarez M, Del Castillo J, Sánchez V (1969) Pharmacological responses of the dorsal longitudinal muscle of Sabellastarte magnifica. Comp Biochem Physiol 29:931-942.

155. Díaz-Miranda L, de Motta GE, García-Arrarás JE (1992) Monoamines and neuropeptides as transmitters in the sedentary polychaete Sabellastarte magnifica: actions on the longitudinal muscle of the body wall. J Exp Zool 263:54-67.

156. Fox LE, Lloyd PE (2002) Mechanisms involved in persistent facilitation of neuromuscular synapses in aplysia. J Neurophysiol 87:2018-2030.

157. Lotshaw DP, Lloyd PE (1990) Peptidergic and serotonergic facilitation of a neuromuscular synapse in Aplysia. Brain Res 526:81-94.

158. Evans CG, Vilim FS, Harish O, Kupfermann I, Weiss KR, Cropper EC (1999) Modulation of radula opener muscles in Aplysia. J Neurophysiol 82:1339-1351.

159. Laurienti PJ, Blankenship JE (1997) Serotonergic modulation of a voltage-gated calcium current in parapodial swim muscle from Aplysia brasiliana. J Neurophysiol 77:1496-1502.

160. Boltt RE, Ewer DW (1963) Studies on the myoneural physiology of Echinodermata. V. the lantern retractor muscle of Parechinus: responses to drugs. J Exp Biol 40:727-733.

161. Inoue M, Tamori M, Motokawa T (2002) Innervation of holothurian body wall muscle: inhibitory effects and localization of 5-HT. Zoolog Sci 19:1217-1222.