The Gill and Siphon Withdrawal Reflex in *Aplysia californica*

Eman F. Khwaja

Aplysia californica is a principal model organism in the field of neurobiology due to its famously known gill and siphon withdrawal reflex. It was first used in the 1960s by neurobiology researcher Eric Kandel and has since gained popularity due to its easily distinguishable neurons and simple nervous system. Since then, *Aplysia californica* has been the model organism for research studies across the globe and has contributed to increasing knowledge of ion channels, neurotransmitters, mutations, and learning and memory. The gill and siphon reflex that made this organism popular is a defensive mechanism that results in the gill and siphon of the organism to retract upon exposure to disruptive and threatening stimuli. The unique aspect of this reflex is its synaptic plasticity and learning abilities. The organism can display several types of learning in association with this specific reflex, which include habituations, sensitization, classical conditioning, and operant conditioning. This reflex of *Aplysia californica* utilizes a number of aspects of its nervous system and has provided to be extremely influential on studies associated with memory and learning, molecular biology, behavior, genetics, synaptic plasticity, and even aging.

Aplysia californica, more commonly known as the California Sea Hare, is a species of sea slugs and is part of the phylum Mollusca. Since the 1960s, this *Aplysia californica* has become increasingly popular as a lab model due to the structure of its central nervous system and presence of its gill and siphon withdrawal reflex (Rankin, et al., 2008). It was first studied by Eric Kandel, the father of modern neuroscience, and his colleagues when they set out to determine the biochemical and neurological basis of learning (Walter, et al., 1983). In the 1970s, they discovered that cyclic AMP and serotonin were both necessary for short-term memory formation and that cAMP-dependent protein kinase and potassium channels were integral to long-term memory (Walter, et al., 1983). Since then, Aplysia California has been significantly studied across the world and across a variety of fields. Studies on this organism have led to a variety of scientific advancements and even to two Nobel prizes.

Within the nervous system of *Aplysia californica* are nine separate ganglia and about 10,000 neurons (Rankin, et al., 2009). Each of the neurons are accessible from the surface of the ganglia and are distinctive enough from each other that researchers are able to be identify each one individually based on their synaptic connections, function within the nervous system, and physiological structure (Rankin, et al., 2008). Despite the simple structure of this organism's nervous system, countless complex behaviors are highly associated with it, such as the gill and siphon withdrawal reflex. This gill and siphon reflex behavior is known as a defensive reflex mechanism that causes the siphon and gill of the organism to be retracted upon the presence of disrupting stimuli (Moroz, et al., 2006). There is a two-step process that occurs when the reflex is triggered through a stimulus being applied to either the siphon or the mantle shelf of the organisms (Moroz, et al., 2006). First, there is a siphon-withdrawal and following the siphon-withdrawal, there is the gill-withdrawal. These two components will occur extremely close in time, producing a single reflex termed the gill and siphon withdrawal reflex.

Due to the limited number of neurons, roughly 10,000 neurons, within the nervous system of this organism, it is possible for researchers to observe experimental questions on this organism. There are even less neurons associated with the gill and siphon withdrawal reflex, and even then only a few are integral to producing this behavior and its plasticity during various forms of learning. Some of the integral neurons necessary to producing this reflex in *Aplsyia californica* include mechanosensory neurons, interneurons, and motor neurons (Carew, et al., 1983). Mechanosensory neurons are neurons that innervate the siphon and are activated by mechanical stimuli to collect information for processing (Frost, et al., 1985). Interneurons are located between neurons that receive input and neurons that supply an output, and these neurons receive input from sensory neurons (Frost, et al., 1985). Finally, the motor neurons innervate the muscles in the gill of the organism and receive processed information to elicit a response (Frost, et al., 1985). In spite of the seemingly simple structure of the nervous system of *Aplysia californica*, it has become a common lab model and integral organism to neuroscience research due to the complex processes associated with its nervous system

Current Advancements

Aplysia californica first became a lab model in the 1960s and has since contributed to significant neuroscience advancements through the work of Eric Kendal, Ph.D., and others who studied the gill and siphon withdrawal reflex. Since then, research conducted on this organism has included a wide variety of topics within neuroscience, including genomics, brain function, toxicology, and behavior. Morez, et al., (2006) found that *Aplysia californica* has 104 counterpart genes that has aided in the study of neurodegenerative diseases and treatments, such as Alzheimer's, Parkinson's, and dementia. Greer, et al., (2018) completed a study on gene expression which suggested a mechanism for which aging in sensory and motor neurons affected the development of mutations that impact learning in *Aplysia californica*. These researchers have continued to develop their research by assessing the ideal time to develop long-term memories during human development based on expression of certain genes, neurotransmitters, and neuronal development. Akhmedov, et al., (2013) completed research on the topic of aging in *Aplysia californica* which also suggested that mutations in genetic information that influence action potentials have led to a change in the responsiveness to stimuli and neurotransmitters, such as acetylcholine.

One of the behavioral biological topics *Aplysia californica* is most known for is the study of learning and the gill and siphon withdrawal reflex. Research completed on this reflex found that it is not only a behavioral reflex, but relies heavily on neurotransmitters, ion channels, action potentials, among other aspects of the nervous system of *Aplysia californica* (Agranoff, et al., 1999). Changes in these components of the central nervous system can seriously impact the gill and siphon withdrawal reflex. Mutations in aspects of the central nervous system can also impede on the organism's ability to complete this behavior properly, such as mutations to the ion channels or to the neurotransmitter receptors on the postsynaptic neuron. The gill and siphon withdrawal reflex is also capable of being adapted through a number of learning variations, including habituation, sensitization, operant conditioning, and classical condition (Bailet, et al., 2008).

Habituation occurs when a repeated stimulus is presented to an organism, resulting in a decrease reaction or response to the particular stimulus (Houwer, et al., 2018). In *Aplysia californica*, certain stimuli that are repeatedly presented to the organism to trigger the gill and siphon withdrawal response can lead to a decreased responsiveness to that particular stimuli. Sensitization occurs when there is an increase in responsiveness to a stimulus because it is a novel stimulus (Houwer, et al., 2018). In studies with *Aplysia californica* as a lab model, operant conditioning is used to allow the model organism to learn the association between the gill and siphon withdrawal reflex and receiving food. Operant conditioning occurs when the organism learns the association between a behavior and a consequence which increases or decreases a specific behavior, such as a reward (Houwer, et al., 2018). In the case of this organism, operant conditioning can occur when the organism receives a positive consequence when it completes its reflex and protects itself from a predator. Classical conditioning is another type of learning that *Aplysia californica* is capable of, which is when organisms learn the association between two stimuli and can anticipate events, therefore eliciting a response (Houwer, et al., 2018). *Aplysia californica* uses this type of learning to change their rate of responsiveness to normalized stimuli with their gill and siphon withdrawal reflex. The ability of *Aplysia californica* to learn and adapt a reflex signifies heightened cognitive control and also provides a mechanism for energy reduction by reducing the organism's focus on the production of an unnecessary behavior.

Genetics

According to Cotman, et al., (1999) *Aplysia californica* has 10,000 active genes, many of which encode behaviors, such as the gill and siphon withdrawal reflex. These behaviors can be influenced by learned responses and repetitive stimuli, thus allowing the organism to adapt its own gill and siphon withdrawal reflex when presented with repeated stimuli. This research has also suggested that the development of these learned adaptations to encoded behaviors are a results of protein synthesis and methylation involvement, as well as storage of the memories in the nucleus of the organism. Research completed by Tamvakis, et al., (2018) has also suggested that RNA that is transferred from one organism to another can induce long-term sensitization and epigenetic nonsynaptic changes which result in a adapted behavioral effects implicated in the gill and siphon withdrawal reflex. These behavioral implications can include a decreased response without going through the same situations to induce habituations as other organisms might have to.

Esdin, et al., (2010) found that long-term habituation of the gill and siphon withdrawal reflex required gene transcription, as well as adaptations of certain types of voltage-gated calcium channels. These researchers found that phosphatases 1 and 2A were necessary for the protein synthesis required for long-term habituation. They also found that the gill and siphon withdrawal reflex depended highly on RNA synthesis and the blocking of certain transcription factors, such as actinoymcun-D and DRB (6,6-dichlorobenzimidazole riboside). Most of these changes in the genetics required presynaptic and postsynaptic mechanisms (Esdin, el al., 2010). The results of these studies suggest that the gill and siphon withdrawal reflex relies heavily on genes, as well as various other physiological and neurobiological systems and structures.

Subcellular Organelles

Despite the gill and siphon withdrawal reflex being a genetically-encoded behavior found in *Aplysia californica* and requiring little cognitive processes to perform, this behavior employs a number of subcellular organelles in order to properly function and defend the organism. According to Lee, et al., (2015) the nucleus and cytoplasm are all implicated in the process of developing the reflex (Lee, et al., 2015). In addition, the mitochondria and rough endoplasmic reticulum, which are both located within the nucleus of the cell, were also implicated in playing a functional role of the reflex (Lee, et al., 2015). According to Miao, et al., (1977), proteins are one of the main factors in encoding long-term memories and contributing to habituation. These proteins are a product of the organelles ribosomes, endoplasmic reticulum, and rough endoplasmic reticulum. The cytoplasm is also implicated in the process of making and folding these proteins which provide necessary functions to the subcellular organelles involved in the gill and siphon withdrawal reflex. The golgi apparatus is also an essential organelle within the cells to ensure that the proteins are being packaged and transported adequately. All these organelles function collaboratively to ensure that the proteins necessary for aiding in the process of encoding memory and learning are functional and available. According to research conducted Dash, et al., (1990), protein synthesis is the basis of encoding long-term memories for this organism (Dash, et al., 1990). For short-term memory, the synthesis of CREB was necessary for influencing the synaptic connections (Dash, et al., 1990). Long-term memory is more associated with changes in the density of synaptic connections (Dash, et al., 1990). These subcellular organelles are involved in key processes to produce a functional siphon and withdrawal reflex and are therefore essential to the protection and anti-predator behaviors of *Aplysia californica*.

Mutations

Mutations in any of the genes that impact subcellular organelles that are necessary to memory encoding, learning, and the gill and siphon withdrawal reflex can lead to detrimental effects on the entire organism. These mutations would prevent the organism from eliciting the proper response to stimuli, or even eliciting a response that was not necessary, therefore harming itself. Mutations in the genes that encode the golgi apparatus, endoplasmic reticulum, or ribosomes can result in an inability for the organism to produce and transport proteins that have the proper structure necessary to be functional (Miao, et al., 2005). These mutations might result in a failure of the gill and siphon withdrawal reflex.

Chung, et al., (2014) completed a study on *Aplysia californica* which implies that aging can lead to stress, thereby resulting in neuronal mutations. These aging mutations can cause mutations in the DNA that encode for the motor neurons associated with the gill and siphon withdrawal reflex. The DNA mutations that occur can alter the structure and function of the macromolecules, as well as change the functional outcomes of voltage-gated ion channels for sodium, potassium, and calcium (Chung, et al., 2014). This combined damage prevents neuronal cell survival and mutates structures of proteins, ultimately preventing the gill and siphon withdrawal reflex from functioning properly. Kempsell, et al., (2015) suggested that using heat shock as a treatment for these aging mutations can allow the organism to refold proteins, thus preventing some of the damage that occurs as a result of this aging stress (Kempsell, et al., 2015). This study has been specifically targeted towards humans and looking at neurodegenerative diseases and aging. Researchers have identified that mutations can lead to changes in memory, learning, and functionality of the organism. It has also been hypothesized that similar changes may be occurring in humans that have disorders such as Alzheimer's and Parkinson's (Greer, et al., 2018).

Neurotransmitters

The neurotransmitters that are highly associated

with learning in *Aplysia californica* are serotonin, glutamate, GABA, and acetylcholine (Tritt, et al., 1982). Serotonin (5-HT) is a neurotransmitter that is involved in sleep, memory, digestion, and social behavior (Hochner, et al., 1986). In some cases, such as with invertebrates, it is associated with stress signals. Acetylcholine is the main neurotransmitter at neuromuscular junctions and is also highly involved in muscle and motor actions, heart rate, pain response, and endocrine functions (Hochner, et al., 1986). Glutamate is an excitatory neurotransmitter and is vital to processes involving learning, brain development, memory. Glutamate is released onto the interneurons and motor neurons when a stimulus disrupts the mechanosensory neurons within the siphon of the organism, thus promoting an action potential to complete the gill and siphon withdrawal reflex (Drake, et al., 2005). Glutamate release is also increased during habituation of the gill and siphon withdrawal reflex (Drake, et al., 2005). GABA is a neurotransmitter associated with inhibition in the brain (Drake, et al., 2005). Depending on the type of learning and synaptic plasticity, the concentrations of these chemical messengers are changed within the nervous system to implicate a variation in the response of the organism. During habituation, the release of glutamate between the sensory and motor neurons is depressed, which is potentially the cause of the decreased activity in the reflex (Drake, et al., 2005). The cause of glutamate depression is a result of limited synaptic vesicles available to release neurotransmitters to the synaptic cleft during an action potential (Drake, et al., 2005). During sensitization, there is an increase in the amount of serotonin that is released which excites the interneurons and sensory neurons. Serotonin enhances the transmitter release from the sensory neurons which produces a greater response in the motor neurons (Lodish, et al., 2000). The neurotransmitters that are involved in the production of the gill and siphon withdrawal reflex are also integral aspects to the various forms of learning that manipulate the organism's reflex.

Action Potentials

The changes in neurotransmitters released is influenced by both the action potentials and the ion channels in the central nervous system of *Aplysia californica*. Action potentials occur when either a chemical or electrical stimulus is applied to the dendrites of the neuron cell, which can result in changes in the charge of the membrane of the neuron (Hochner, el al., 1986). If the charge reaches a certain threshold, an action potential will occur and send a message down the axon of the neuron and into the cleft to message the next neuron.

In sensitization, the gill and siphon withdrawal reflex is highly associated with an increase in the duration of a presynaptic action potential. This duration increase is measured at roughly 10-30% (Klein, el al., 1978). Habituation of *Aplysia californica*'s gill and siphon withdrawal reflex occurs at the synapse between sensory and motor neurons, and it is also highly associated with a reduction in action potentials. Action potentials are a result of sodium channels and inhibition in sodium channels can reduce the likelihood that action potentials will be produced due to the inability for the membrane to change its charge to the threshold. Some neurons, however, are still able to produce action potentials despite the influenced sodium channels (Geduldig, et al., 1968).

Cleary, et al., (1995) completed research which found that sensitization is mediated by interneurons called facilitator neurons. These neurons have been found to increase the release of GABA, thereby increasing the inhibitory signals in the central nervous system. Short-term sensitization and conditioning was also found to occur with increase inhibitor signals, or increase GABA levels (Cleary, et al., 1995). Long-term sensitization of the gill and siphon withdrawal reflex required an increase in serotonin to be released by facilitator neurons, as well as an increased amount of GABA (Cleary, et al., 1995).

Ion Channels

As a result of learned behavior associated with the gill and siphon withdrawal reflex, modifications to the ion channels occur to encourage changes in action potentials and neurotransmitters release. Loechner, et al., (1989) has suggested that as short-term memories are formed, there is modifications in the activity of neuronal synapses to increase or decrease the amount of activity (Loechner, et al., 1989). By changing the amount of activity in the synapses, there is a change in the chemical and electrical signals that are needed for completion of the gill and siphon withdrawal reflex. In sensitization, there is an enhancement of glutamatergic transmission during which serotonin releases and binds to the interneurons involved in the gill and siphon withdrawal reflex (Chesnoy-Marchais, et al., 1986). This results in a series of actions which lead to g-protein-coupled receptors to stimulate the

production of cyclic AMP, which binds to protein kinase A. These actions cause a reduction in the opening of the voltage-gated K+ channels and prolong the opening of the presynaptic Ca^{2+} channels, which increase the amount of neurotransmitters the synaptic vesicles are able to release (Castellucci, et al., 1985). These changes in neurotransmitter release and concentration have integral impacts on the learning associated with the gill and siphon withdrawal reflex.

Neurotransmitter Release

The modifications to voltage-gated calcium ion channels can lead to changes in the release of neurotransmitters. These modifications in neurotransmitter release impact the chemical signals that sent and perceived by the neurons, ultimately reflecting changing in the behavioral displays shown by the organism. During habituation of the gill and siphon withdrawal reflex, the action potentials encoding the information of the stimuli on the gill or siphon will open calcium channels in the presynaptic cells to release the neurotransmitter (Hawkins, et al., 1985). During the opening of these voltage-gated channels, there is a decrease in the amount of calcium released; this results in a decrease in the amount of chemical signals that are transported into the synaptic cleft and picked up by receptors in the postsynaptic cell (Hawkins, et al., 1985). These forms of plasticity and learning that are associated with decreased responsiveness to stimuli in the gill and siphon withdrawal reflex have also been found to be associated with an increase amount of glutamate receptors in motor neuron postsynaptic cells. Skehel, et al, (1995) found that a type of protein called VAP-33, or Vesicle-Associated Membrane Protein, is involved in the release of a significant number of neurotransmitters, including serotonin, glutamate, and acetylcholine. (Skehel, et al., 1995). During neurotransmitter release in the presynaptic cell, endocytosis occurs and during the process of recycling the vesicles or exocytosis, various types of proteins must coat the membrane of the cell and pick off a piece of the membrane to form the vesicle (Skehel, et al., 1995). VAP-33 is necessary for the exocytosis of synaptic vesicles in the presynaptic cell because they provide necessary aspects of coating the membrane of the neuron during the first stages of exocytosis (Skehel, et al., 1995). Antonov, et al., (2001) also suggested that *Aplysia californica* can consciously change their behavior by inhibiting activity in the neurons in the central nervous system by producing and releasing an excess amount of nitric oxide, which is known to act as an inhibitor in the nervous system (Antonov, et al., 2001). This research suggests that *Aplysia californica* are capable of recognizing stimuli that they should be less reactive to.

Discussion

The marine mollusk *Aplysia californica* continues to push the boundaries of our understanding of neuroscience, behavior, and learning in the fields of science through the study of the gill and siphon withdrawal reflex. Beginning in the 1960s with Eric Kandel and his colleagues groundbreaking studies on the neuobiological basis of learning (Rankin, et al., 2008), this organism has continued to provide necessary advancements to the field of neuroscience. The unique ability of *Aplysia californica* to develop and adapt a reflex through various forms of learning, such as sensitization and habituation, signifies heightened cognitive control. This ability also allows the organism to have a mechanism for energy reduction by reducing the organism's focus on the production of an unnecessary behavior. Information about the mutations associated with aging (Greer, et al., 2018), as well as the subcellular organelles involved in learning (Miao, et al., 2005) have greatly changed society's understanding of encoding processes and memory. The neurotransmitters highly involved in the gill and siphon withdrawal reflex include serotonin, GABA, glutmate, and acetylcholine. For learning to occur in *Aplysia californica,* the release of these neurotransmitters are altered to either inhibit or enable the reflex (Cleary, et al., 1995). Completed studies on these organisms have been applied to and tested on other organisms to increase our understanding of these processes beyond the systems in *Aplysia californica*, and these results continue to provide context to future studies on similar themes and diseases in other organisms. Despite having a simple nervous system, the nervous system of *Aplysia californica* has provided and is continuing to provide a greater understanding for complex behaviors associated with simple nervous systems, as well as a greater understanding of influences on the functions of neurons, ion channels, and action potentials. Future studies on this organism include increasing our understanding of the way in which aging changes and adapts the central nervous system, particularly in the form of neurodegenerative mutations and disorders. Other studies are also continuing to understand processes

associated with learning and memory and determining the prime ages to develop long-term memories.♦

References

1. Cash D, Carew TJ. A quantitative analysis of the development of the central nervous system in juvenile *Aplysia californica*. J Neurobiol. 1989;20:25–47.
2. Agranoff BW, Cotman CW, Uhler MD. Invertebrate Learning and Memory. Lippincott-Raven; 1999.
3. Single neuron serotonin receptor subtype gene expression correlates with behavior within and across three molluscan species *Proc R Soc B* August 29, 2018 285: 20180791
4. Abrams TW. 1985. Activity-dependent presynaptic facilitation: an associative mechanism in *Aplysia*. Cell Mol Neurobiol 5: 123–145
5. Choi SL, Lee YS, Rim YS, Kim TH, Moroz LL, Kandel ER, Bhak J, Kaang BK. 2010. Differential evolutionary rates of neuronal transcriptome in *Aplysia kurodai* and*Aplysia californica* as a tool for gene mining. J Neurogenet 24: 75–82.
6. Lee, C. H., Flint, J. J., Hansen, B., & Blackband, S. J. (2015). Investigation of the subcellular architecture of L7 neurons of Aplysia californica using magnetic resonance microscopy (MRM) at 7.8 microns. *Scientific reports*, *5*
7. Subcellular Analysis of d-Aspartate. Hai Miao,Stanislav S. Rubakhin, and, and Jonathan V. Sweedler *Analytical Chemistry* (2005)
8. Baur P. S. Lipochondria and the light response of Aplysia giant neurons,. J Neurobiol 8, 19–42, (1977).
9. Kandel E. R. & Kupfermann I. The functional organization of invertebrate ganglia. Annu Rev Physiol 32, (1970)
10. Lewis E. R., Everhart T. E. & Zeevi Y. Y. Studying neural organization in Aplysia with the scanning electron microscope. *Science 165.* (1969)
11. Edstrom JP, Lukowiak KD. Frequency-dependent action potential prolongation in Aplysia pleural sensory neurones. *Neuroscience*. (1985)
12. Geduldig, D., & Junge, Sodium and calcium components of action potentials in the Aplysia giant neurone. *The Journal of physiology*, (1968).
13. Hochner, B., Klein, M., Schacher, S., & Kandel, E. R. Action-potential duration and the modulation of transmitter release from the sensory neurons of Aplysia in presynaptic facilitation and behavioral sensitization. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(21), (1986)
14. Klein M, Kandel ER. Presynaptic modulation of voltage-dependent Ca2+ current: mechanism for behavioral sensitization in Aplysia californica. *Proc Natl Acad Sci U S A.* (1978)
15. Ye X., Xie F., Romanova E. V., Rubakhin S. S. & Sweedler J. V. Production of nitric oxide within the Aplysia californica nervous system. *ACS Chem Neurosci* 1, (2010).
16. Lodish H, Berk A, Zipursky SL, et al. *Molecular Cell Biology*. 4th edition. New York: W. H. Freeman; (2000)
17. Tritt SH, Byrne JH. Neurotransmitters producing and modulating opaline gland contraction in Aplysia californica. *J Neurophysiol*. (1982)
18. Akhmedov K, Rizzo V, Kadakkuzha BM, Carter CJ, Magoski NS, Capo TR, Puthanveettil SV. Decreased response to acetylcholine during aging of aplysia neuron R15. PLoS One. (2013).
19. Drake TJ, Jezzini S, Lovell P, Moroz LL, Tan W. Single cell glutamate analysis in *Aplysia* sensory neurons. *J Neurosci Methods*. (2005)
20. Walters ET, Byrne J, Carew T, Kandel E. Mechanoafferent neurons innervating tail of Aplysia. I. Response properties and synaptic connections. J *Neurophysiol*. (1983)
21. Hochner B, Klein M, Schacher S, Kandel ER. Additional component in the cellular mechanism of presynaptic facilitation contributes to behavioral dishabituation in Aplysia. *Proc Natl Acad Sci U S A.* (1986)
22. Hawkins RD, Abrams TW, Carew TJ, Kandel ER. A cellular mechanism of classical conditioning in Aplysia: activity-dependent amplification of presynaptic facilitation. *Science. (*1983)
23. Antonov I, Antonova I, Kandel ER, Hawkins RD. The contribution of activity-dependent synaptic plasticity to classical conditioning in *Aplysia*. *J Neurosci* (2001)
24. Castellucci VF, Nairn A, Greengard P, Schwartz JH, Kandel ER. Inhibitor of adenosine 3':5'-monophosphate-dependent protein kinase blocks presynaptic facilitation in Aplysia. *J Neurosci.* (1982)
25. Loechner KJ, Kaczmarek LK. Regulation of ion channels in Aplysia neurons by autoactive peptides and second messengers. *Soc Gen Physiol*

Ser. 1989;44:69-82. Review. PubMed
26. Chesnoy-Marchais D, Evans MG. Nonselective ionic channels in Aplysia neurones. *J Membr Biol*. (1986)
27. Chung SG, van Rey EM, Bai Z, Rogers MW, Roth EJ, Zhang L-Q. Aging-related neuromuscular changes characterized by tendon reflex system properties. *Arch Phys Med Rehabil*. (2005)
28. Kempsell AT, Fieber LA. Behavioral aging is associated with reduced sensory neuron excitability in Aplysia californica. *Front Aging Neurosci*. (2014)
29. Kempsell AT, Fieber LA. Aging in sensory and motor neurons results in learning failure in Aplysia californica. *PLoS One* (2015)
30. Greer, J. B., Schmale, M. C., & Fieber, L. A. Whole-transcriptome changes ingene expression accompany aging of sensory neurons in Aplysia californica. *BMC genomics*, *(2015)*
31. H., Shaw, R., Kalachikov, S., Panchin, Y. V., Farmerie, W., Russo, J. J., Ju, J., … Kandel, E. R. Neuronal transcriptome of Aplysia: neuronal compartments and circuitry. *Cell*, *127 (2006)*
32. Rankin, C. H., Abrams, T., Barry, R. J., Bhatnagar, S., Clayton, D. F., Colombo, J., Coppola, G., Geyer, M. A., Glanzman, D. L., Marsland, S., McSweeney, F. K., Wilson, D. A., Wu, C. F., … Thompson, R. F. Habituation revisited: an updated and revised description of the behavioral characteristics of habituation. *Neurobiology of learning and memory*, *92*(2), 135-8. (2008).
33. Hernandez, P. J., & Abel, T. . The role of protein synthesis in memory consolidation: progress amid decades of debate. *Neurobiology of learning and memory*, *89*(3), 293-311. (2007)
34. Skehel PA, Martin KC, Kandel ER, Bartsch D. A VAMP-binding protein from Aplysia required for neurotransmitter release. *Science*. (1995)
35. Aplysia. *Curr Biol*. 2011;21(2):R60-1. Moroz, Leonid L. "Aplysia." *Current Biology : CB*, U.S. National Library of Medicine, (2011)
36. Walters, E. T., & Moroz, L. L. Molluscan memory of injury: evolutionary insights into chronic pain and neurological disorders. *Brain, behavior and evolution*, *74*(3), (2009).
37. Bailey CH, Kandel ER. Synaptic remodeling, synaptic growth and the storage of long-term memory in *Aplysia*. *Prog Brain Res*. (2008)
38. Houwer, J. D., Barnes-Holmes, D., & Moors, A. (n.d.). What is Learning? On the nature and merits of a functional definition of learning. *Psychonomic Society*.
39. Cleary, L. J., Byrne, J. H., & Frost, W. N.. Role of Interneurons in Defensive Withdrawal Reflexes in Aplysia. *Neurobiology and Anatomy*. (1995)
40. Frost, W. N., Castellucci, R. D., Hawkins, R. D., Kandel, E. R.. Monosynaptic connections made by the sensory neuropns of the gill – and siphon-withdrawal reflex in *Aplysia* participate in storage of long-term memory for sensitization (1985)