Journal Pre-proof

Lymnaea stagnalis as model for translational neuroscience research: from pond to bench

V. Rivi, C. Benatti, C. Colliva, G. Radighieri, N. Brunello, F. Tascedda, J.M.C. Blom

PII: S0149-7634(19)30654-2

DOI: https://doi.org/10.1016/j.neubiorev.2019.11.020

Reference: NBR 3608

To appear in: *Neuroscience and Biobehavioral Reviews*

Received Date: 26 July 2019

Revised Date: 24 September 2019

Accepted Date: 25 November 2019

Please cite this article as: Rivi V, Benatti C, Colliva C, Radighieri G, Brunello N, Tascedda F, Blom JMC, *Lymnaea stagnalis* as model for translational neuroscience research: from pond to bench, *Neuroscience and Biobehavioral Reviews* (2019), doi: https://doi.org/10.1016/j.neubiorev.2019.11.020

Lymnaea stagnalis as model for translational neuroscience research: from pond to bench

Rivi V.[1], Benatti C.[2,4], Colliva C[1,4]., Radighieri G[1]., Brunello N[2]., Tascedda F.[2,4§], Blom JMC.[3,4§]

[1] Dept. of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy

[2] Dept. of Life Sciences, University of Modena and Reggio Emilia, Modena, Italy

[3] Dept. of Education and Human Sciences, University of Modena and Reggio Emilia, Modena, Italy

[4] Centre of Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, Modena, Italy

§ these authors contributed equally to the manuscript

*Corresponding author:

Johanna MC Blom

Professor Psychobiology

Dept. of Education and Human Sciences, Pediatric Neuroscience Lab

Center for Neuroscience and Neurotechnology

University of Modena and Reggio Emilia

Via Campi 287

41125 Modena, Italy

Email: joan.blom@unimore.it

Tel: 0039-0592055162

Key-words: memory, snails, behavioural test, translational medicine, stress, aging

Index

Highlights

1) *Lymnaea stagnalis*, a reductionistic, yet sophisticated model to address fundamental questions in learning and memory
2) Learning and memory in snails have been highly conserved
3) The "molecular actors" memory are similar both across phylogenetic groups and learning paradigms
4) *Lymnaea stagnalis* teach us the importance of context,
5) *Lymnaea stagnalis* to understand in what conditions we memorize, we eat, we memorize, we age
6) *Lymnaea* is a valid and reliable model to move research from pond to bench to bedside

Abstract

The purpose of this review is to illustrate how a reductionistic, but sophisticated, approach based on the use of a simple model system such as the pond snail *Lymnaea stagnalis (L. stagnalis)*, might be useful to address fundamental questions in learning and memory. *L. stagnalis,* as a model, provides an interesting platform to investigate the dialog between the synapse and the nucleus and vice versa during memory and learning. More importantly, the "molecular actors" of the memory dialogue are well-conserved both across phylogenetic groups and learning paradigms, involving single- or multi-trials, aversion or reward, operant or classical conditioning. At the same time, this model could help to study how, where and when the memory dialog is impaired in stressful conditions and during aging and neurodegeneration in humans and thus offers new insights and targets in order to develop innovative therapies and technology for the treatment of a range of neurological and neurodegenerative disorders.

1. Background

Understanding the molecular and physiological mechanisms involved in brain disorders is one of the most important challenges in neuroscience today. In the last decades, the animal models of choice used in neuroscience research have been mostly small mammals (i.e. rats and mice). However, this approach may not be always the most appropriate, and has fuelled many protests and triggered many scientific, economic, ethical and social discussions (Alberts, 2010).

During the evolutionary process that prompted the diversity among species, numerous molecular pathways have been almost entirely conserved ~~across species~~ (Ottaviani et al., 2007), which has led to the recognition of invertebrate models as a more flexible tool to study the basic and conserved mechanisms of central nervous system (CNS) physiology and pathology (Corning, Dyal and Willows, 1973; Kaang et al., 1993; Ottaviani et al., 2013; Tascedda et al., 2015), exceeding the practical and conceptual limitations of experimentation on mammals and cell cultures. Moreover, this approach, reduces the time and the costs of experimentation. On balance, using an interdisciplinary approach, that combines different methods and fields including evolution, genetics, molecular biology and behaviour, a model such as *Lymnaea stagnalis* will allow us to open new frontiers towards translational neuroscience research, starting from simple model systems, passing through more complex organisms, until arriving at *Homo sapiens sapiens*.

2. Attributes of *L. stagnalis* as model for studies in the field of Neuroscience

It is well-known that molluscan gastropods are good models to study the molecular and cellular mechanisms of neuronal function and dysfunction (Nestler et al., 2010; Burne et al., 2011; Tascedda et al., 2015; Stefano et al., 2015). Among them, attention should be paid to the pond snail *Lymnaea stagnalis* (*L. stagnalis*, Linnaeus, 1758), a pulmonate gastropod, widely used as model system in basic and applied neuroscience research (Murakami et al., 2013a; Takigami et al., 2013). The CNS of *L. stagnalis* consists of approximatively 20,000 readily identifiable neurons, organized in a ring of 11 interconnected ganglia, whose functional roles within specific networks can be defined and directly attributed to observable behaviours, such as reproduction (van Minnen et al., 1989; Ter Maat et al., 1992), respiration (Syed et al., 1990; Winlow et al., 1992), feeding (Yeoman et al., 1994; Straub et al., 2002) and locomotion (Syed et al., 1991). Not only, many neurons are large in size (diameter up to ~100 μm), offering a large amount of biological material for molecular, morphological and functional analyses, which have led to the validation of the function of specific genes and the study of the molecules and metabolic pathways involved in neuronal regeneration (Hermann et al., 2000; Koert et al., 2001), synapse formation (Syed et al., 1992; Feng et al., 1997; Gardzinki et al., 2007), synaptic plasticity (Smit et al., 2001), neurodevelopment (Croll et al., 2000), aging (Wildering et al., 1991; Klaassen et al., 1998; Patel et al., 2006), adaptive responses to stress (Hermann et al., 1998; Fei et al., 2007) and, last, but not least, learning and memory formation (Benjamin et al., 2000; Lukowiak et al., 2003).

We centred this review around two key-questions:

1) What changes in the brain during learning?
2) Once something is learned, how is that information stored ~~memorized~~ in the brain?

To answer these questions, we think it is necessary to start from the simplest examples of memory storage (Kandel, 2001) and the most experimentally manageable animal models.

We recognize that most of the molecular studies in *L. stagnalis* are the result of studies of homology and we also realise that the use of a reductionist approach in the 21th-century is an arduous undertaking, but if elementary forms of learning are common to all animals with an evolved nervous system, conserved processes must exist in the molecular mechanisms of learning that can be studied more effectively in simple invertebrate animals (Kandel, 2001). Thus, our purpose is to illustrate how this reductionistic, but not simplistic, approach based on the use of a simple model system such as *L. stagnalis*, might be useful to address fundamental questions in learning and memory. The reconstruction of the extensive dialog between the synapse and the nucleus, and the nucleus and the synapse during memory and learning, could also help to study how, where and when this dialog is impaired in stressful conditions and during aging and neurodegeneration in humans.

3. *L. stagnalis* as model for the integrative molecule-to behaviour study of memory and learning

Learning about the predictive association between events and the consequences of specific behaviours is indispensable for animals to adapt and survive in complex and ever-changing environments. Remembering these associations, animals alter their behaviour appropriately and this alteration can be defined as learning (Dalesman and Lukowiak, 2012; Lapiedra et al., 2017). While non-associative learning (i.e. habituation and sensitization) is the simplest and most primitive form of learning, associative learning is more complex and requires that stimuli occur in close temporal contiguity and in a fixed sequence (Byrne and Hawkins, 2015). Among the various categories of associative learning, classical and operant conditioning are the best known and well-studied. In particular, in classical conditioning a neutral conditioned stimulus (CS) paired with a forceful unconditioned stimulus (US) is hypothesized to evoke the unconditional response. On the other hand, operant conditioning is response-contingent and involves the presentation of a reinforcing stimulus when the animal performs a specific behaviour. If the reinforcing stimulus is negative, animals learn to avoid engaging in the behaviour, if the stimulus is positive, animals spontaneously perform the behaviour more often (Mackintosh, 1974). In this complex scenario, *L. stagnalis* has proven to be a useful model for the study of the molecular, cellular, and neuronal networks related to memory, as well as the behavioural aspects of learning and its consolidation in long-term memory (LTM) (Ito et al., 1999; Lukowiak et al., 2000; Murakami et al, 2013b).

Indeed, the knowledge of the architecture of the essential neural circuits of behaviours that can be conditioned (i.e. respiration and feeding), allowed the identification and the study of neurons exhibiting plasticity. Using intact vertebrate biological systems, these goals are more difficult to attain. Many studies in the last decades demonstrated that *L. stagnalis* can be both classically and operantly conditioned (Kojima et al., 1996; Sakakibara et al., 1998; Kobayashi et al., 1998; Spencer et al., 1999; Lukowiak et al., 2000). In particular, in food reward classical conditioning (Kemenes and Benjamin, 1989; Kemenes, et al., 2006), a neutral conditioned stimulus (CS – i.e. amyl acetate, gentle taps to the lips or visual cues) was paired with a strong unconditioned feeding stimulus (US – usually sucrose) and the temporal-contingent repeated presentation of these stimuli resulted in a sequence of rhythmic and stereotyped feeding movements when snails were exposed to the CS alone in the post-training phase.

This behavioural-conditioned response suggested that snails learned that the CS "means" food (Kemenes and Benjamin, 2009). Interestingly, when *Lymnaea* is moderately food-deprived, it is capable to acquisition and extensive retention (for at least 19 days) of an appetitively reinforced feeding response after only a single training trial, offering the possibility to perform detailed analyses of the neural mechanisms underlying plasticity (Alexander Jr. et al.1982, 1984; Fulton et al., 2005; Ribeiro et al., 2005).

L. stagnalis have been also trained using conditioned taste aversion (CTA) learned and subsequently formed memory to suppress the feeding response to an appetitive CS (usually sucrose) when paired with an aversive US (usually KCl or tactile stimulus) that ultimately inhibited feeding. Consequently, snails learned that the presentation of a CS signal was associated with the imminent arrival of the aversive stimulus (Kojima et al., 1997). Moreover, the aerial respiratory behaviour of snails, which is characterized by the spontaneous opening and closing of the pneumostome (the respiratory orifice) at the water surface (Boycott, 1936; Jones et al., 1961), can be operantly conditioned. When the animal attempted to open its pneumostome as a reaction to hypoxic water, it received a gentle tactile stimulus to the pneumostome area, evoking, as escape-withdrawal reflex, its closure. Prolonged tactile stimulation of the pneumostome every time the animal attempted to breathe resulted in significantly fewer attempts to open the pneumostome as training proceeded (Lukowiak et al., 1996). In a recent study, Lukowiak and co-workers demonstrated the capacity of snails to perform configural learning, that resulted in the ability to treat stimuli experienced together as different from the simple sum of their elements (Swinton et al., 2019). These results are consistent with the hypothesis that stimulus–stimulus learning is an important adaptive learning mechanism that helps animals, from invertebrates to mammals, to decipher the meaning of important stimuli in their environment. The ability of snails to undergo configurational learning is an ulterior confirmation of its potentiality in neuroscience and behavioural research (Swinton et al., 2019). Depending on the training procedure used, either intermediate term-memory (ITM; persisting up to 3 h) or LTM (persisting for at least 24 h) occurred in *L. stagnalis* (Kojima et al., 1996; Benjamin et al., 2000; Sangha et al., 2003c; Ito et al., 2013; Otsuka et al., 2013; Takahashi et al., 2013; Lukowiak et al., 2014; Sunada et al., 2014). Because the inhibition of transcription or translation blocked the formation of LTM in a variety of model systems, but did not affect short-term memory (STM), it has been concluded that this phase, lasting only minutes, does not require neither *de novo* protein nor RNA synthesis (De Zazzo and Tully, 1995). In addition to the differences in the length of time that memory persisted, a difference between LTM and ITM in requiring altered gene activity was observed. In fact, while ITM required the translation of new proteins from pre-existing RNA and only depended on new protein synthesis, LTM depended on altered gene activity and required both the transcription of new RNA, and their translation into new proteins (Lukowiak et al., 1996; McGaugh, 2000; Inda et al., 2005; Sangha et al., 2005). Thus, LTM is represented at the cellular level by activity-dependent modulation of both the function and the structure of specific synaptic connections that, in turn, depend on the activation of specific patterns of gene expression. In recent years much effort has gone into identifying the signalling cascades that ultimately lead to the production of new proteins for the process of memory formation, such as, proteins required for the maturation of particular synapses that store the remembered information. A variety of different molecular and biochemical tools have been used to measure changes in the expression or activation levels of specific molecules during LTM and ITM in *L. stagnalis* and enhanced expression or activation of these key factors has been observed in specific stages of memory formation and consolidation.

As would be expected with such vital processes, learning and memory are observable across a vast array of species. It is thus reasonable to hypothesize that such a fundamental conserved mechanism, may occur as the result of a well conserved set of underlying molecular mechanisms. On balance, in this complex and dynamic scenario, *Lymnaea* could give an enormous contribution to understand the molecular mechanism by which organisms acquire, store, and eventually use their experiences.

4. Toward a molecular pathway for memory and learning in *L. stagnalis*

4.1 Lym-CREB in the synaptic enhancement of memory consolidation

One transcription factor that plays a major role in LTM formation in *Lymnaea* is the homologous of cAMP response element-binding protein, LymCREB (Silva et al., 1998). The cDNA sequences for the activator type of CREB, LymCREB1, and the repressor type, LymCREB2, in *L. stagnalis* have successfully been cloned and analysed (Sadamoto et al, 2004). In particular, LymCREB homodimers were demonstrated at both the mRNA and protein level in cerebral giant cells (CGCs) (Ribeiro et al., 2003; Sadamoto et al., 2004) and in the right pedal dorsal 1 interneuron (RPeD1), which are necessary for CTA (Kojima et al. 1997; Nakamura et al. 1999; Scheibenstock et al., 2002) and operant conditioning of respiration (Taylor and Lukowiak, 1992; Scheibenstock et al., 2002; Sangha et al. 2003b), respectively. In particular, memory training in these cells enhanced both the levels of phosphorylated LymCREB1 (Ribeiro et al., 2003) as well as LymCREB1 gene expression (Sadamoto et al. 2010), indicating that memory training increased both the gene expression of LymCREB transcriptional activator and the level of its activation by phosphorylation. After phosphorylation, LymCREB1, in turn, initiated a cascade of altered gene activity and new protein synthesis, necessary for synaptic enhancement in memory consolidation (Nakamura et al., 1999; Ribeiro et al., 2003; Sadamoto et al., 2004). In contrast, LymCREB2 inhibited the function of LymCREB1 (Nakamura et al., 1999) and the ratio of activator/repressor LymCREBs has been proposed to act as a "molecular switch" in determining whether LTM is formed (Sadamoto et al, 2004). Similar findings have been obtained in invertebrates, including *D. melanogaster* (Perazzona et al., 2004) and *A. californica* (Bartsch et al., 1995), and in mammals (Karpinski et al., 1992; Yin et al., 1994; Josselyn et al., 2001). Most of the upstream signalling cascade leading to the activation of CREB appears to be conserved through evolution, and many aspects of the role of CREB in synaptic plasticity described in invertebrates have also been observed in the mammalian CNS (Barco et al., 2003).

Evidence from numerous model systems indicate that CREB-driven transcription results downstream of the activation of Cyclic Adenosine Monophosphate (cAMP), which mediates almost all of its actions through protein kinase A (PKA). Furthermore, in various model systems ranging from invertebrates to mammals, CREB1 works as a transcriptional activator only after its phosphorylation by either PKA, mitogen-associated protein kinase (MAPK) or calcium calmodulin-dependent protein kinase II (CaMKII) (Montminy, 1997). Similar to mammals and *Aplysia*, LymCREB1 contains a kinase inducible domain which presents consensus sequences of several kinases (Pinna and Ruzzene, 1996), such as LymPKA, protein kinase C (LymPKC), LymCaMKII and protein kinase G (PKG) (Sadamoto et al., 2004). On the other hand, LymCREB2 has two consensus sequences for the LymMAPK phosphorylation site and one PKC recognition site (Sadamoto et al., 2004), suggesting that these kinases act as memory promoter genes that up-regulate the expression of LymCREB or down-regulate the suppressor activity of LymCREB2.

The contribution to synaptic plasticity and memory of these kinases, together with highly conserved molecular targets, such as N-methyl-D-aspartate (NMDA) glutamate receptors (GRINs) and nitric oxide (NO), have been investigated in *Lymnaea*.

4.2 Involvement of LymGRIN in associative plasticity processes

NMDA receptors are required for memory formation across several types of memory and numerous species (Szapiro et al., 2003; Xia et al., 2005; Zhang et al., 2005; Glanzman et al., 2008; Kano et al., 2008). The main characteristics of NMDA receptors, such as the permeability to calcium, the voltage dependent magnesium block, the slow kinetics, together with the numerous binding sites for cofactors, make them well suited for associative plasticity processes, that are specifically mediated by the entry of calcium, which, in turn, activates a variety of cell signalling cascades, involving PKC and CaMKII pathways and NO synthase (NOs), which all contribute to memory formation (Ha et al., 2006; Wan et al., 2010; Rosengeer et al., 2010). Data obtained from *L. stagnalis* using agents that block the receptors suggest that the activation of the homologous of NMDA receptors, LymGRINs, are required in order to allow LTM formation following conditioning (Rosenegger and Lukowiak, 2010; Wan et al., 2010). Evidence of this, is the effect of ketamine, a NMDA blocker, which compromised the consolidation phase of memory, by acting on transcriptional events that are exclusive for early LTM but not for ITM (Browning and Lukowiak, 2008), or late LTM (Wan et al., 2010).These findings are consistent with the vertebrate and invertebrate literature on learning (Shimizu et al., 2000; Silva et al., 2003; Irvine et al., 2005; Bevilaqua et al., 2005), where the entry of calcium through NMDARs during robust synaptic stimulation triggered synapse-to-nucleus signalling cascades that resulted in the activation of CREB through PKA and MAPK-mediated phosphorylation (Montminy, 1997).

4.3 Lym-PKC in memory acquisition and maintenance

The entrance of Ca^{2+} through NMDARs activates directly or indirectly numerous protein kinases, including PKC (Malinow et al., 1988). The role of the PKC family has been investigated in many learning paradigms and animal models, including numerous invertebrate models (Choi et al., 1991; Muzzio et al., 1997). For more than two decades, PKC activation has been implicated in the formation of associative memory in a variety of species, including the mollusc, *Hermissenda crassicornis*, rodents and rabbits (Bank et al., 1988, Olds et al., 1989; Nelson et al., 1990), providing a valid support for a mechanism that has been conserved across the evolution of species ranging from invertebrate molluscs to higher mammals (Takigami et al., 2014a). Interestingly, data from *Lymnaea* indicated that the administration of bryostatin, a PKC activator, before the conditioning training procedure enhanced both ITM and the length of LTM (Rosenegger et al., 2008; Takigami et al., 2014), whereas injection of a PKC inhibitor (GF109203X) blocked both ITM and LTM formation (Rosenegger et al., 2008). In this regard, Rosenegger, Parvez, and Lukowiak (2008) demonstrated that pre-treatment of *Lymnaea* with bryostatin before operant conditioning of aerial respiration not only makes it easier to produce LTM, but also makes the memory persist much longer (Rosenegger et al., 2008).

Moreover, Tagikami and colleagues (2014) demonstrated that the mechanism by which STM becomes consolidated in LTM involved the activation of PKC-mediated phosphorylation, following CTA (Tagikami et al., 2014a). In fact, when bryostatin was injected within the early time period following CTA trials, memory consolidation was progressively enhanced, suggesting that PKC initiated the synthesis of new proteins necessary for LTM formation and enhanced mRNA translation following DNA transcription (Takigami et al., 2014a). In turn, protein synthesis, seems to be critical for providing essential biochemical and structural components to the synaptic apparatus required for the implementation of the memory storage process. This hypothesis is fully supported by similar findings indicating that bryostatin-induced PKC phosphorylation extended memory duration in both *Hermissenda* and mammals following Pavlovian-conditioning (Alkon et al., 2005; Kuzirian et al., 2006; Hongpaisan and Alkon, 2007; Sun and Alkon, 2008).

Because PKC dysfunctions are involved in several types of memory impairments in both humans and rodents (Pascale et al., 1998) and considering the highly-conserved involvement of this pathway in memory, *L. stagnalis* represents an attractive tool to elucidate the potentiality of PKC as pharmacological target for the treatment of memory decline and dementias.

4.4 LymCaMKII in late consolidation of associative memory

The involvement of CaMKII in memory acquisition has been well documented in several organisms (Cammarota et al., 2002; Silva, 2003; Elgersma et al., 2004). CaMKII, like other CaM-kinases, is activated by the transient influx of Ca^{2+} (e.g., through NMDA receptors) and plays a role in subsequent transcriptional and translational processes that involved CREB (Silva et al., 1998; Abel and Lattal, 2001; Hudmon and Schulman, 2002; Wang et al., 2006). Similarly, CaMKII is an highly suitable molecular substrate for LTM storage due to its unique ability to maintain an active auto-phosphorylated state even after the decay of external stimuli (i.e., when Ca^{2+} influx stops) (Hook and Means, 2001). The homologous of CaMKII has been cloned in *Lymnaea* (LymCaMKII) and shared important functional roles in learning and memory (Wan et al., 2010) with its mammalian counterpart. Previous studies identified a critical time window (occurring approximatively at 24 hours after training) during which the activation of LymCaMKII was required for the late consolidation of associative memory. In contrast, no evidence was found for a role of the activation of LymCaMKII (or other LymCaM-kinases) in early or intermediate consolidation, for up to 20 hours after training (Wan et al., 2010). Because LymGRINs are only involved in the acquisition of LTM, whereas LymCaMKII participates in the late consolidation phase, there is a dissociation of NMDA receptor function and CaMKII activation between these two different phases of memory formation. This is different from what has been described in NMDA receptor and CaMKII knock-out mice, where the activation of CaMKII resulted from the upstream activation of NMDA receptors (Wang et al., 2003). In this regard, it was hypothesised that the intrinsic activation of LymCaMKII leads to sustained high levels of auto-phosphorylated CaMKII, which the ensuing learning-induced delayed rise in Ca^{2+} from glutamatergic receptors.

Additionally, using CaMKII and NMDA inhibitors it was found that, while memory consolidation depended on both NMDA receptors and CaMKII activation, CaMKII-dependent late memory consolidation did not require the activation of NMDA receptors (Wan et al., 2010). This suggested that the rise of Ca^{2+} was mediated by non-NMDA type voltage-gated calcium channels or intracellular calcium stores in the CGC axon terminals (Kemenes et al., 2006). Actually, it has yet to be

established whether this function has been conserved in other more complex organisms. While LymCaMKII was not involved in late consolidation, LymNO and LymPKA were required for early memory formation (Kemenes et al., 2002, 2006). The complementary roles played by LymCaMKII, LymNO, and LymPKA suggest that the dynamic consolidation phase involves both parallel and sequential activation of different signalling cascades in the different phases of the consolidation of long-term memory.

4.5 Lym-PKA activity induced distinct temporal patterns are correlated with different memory phases

Studies in *Aplysia* first revealed the participation of the cAMP/PKA-signalling pathway in synaptic facilitation and sensitization (Brunelli et al., 1976). Moreover, in both invertebrates and vertebrates the dynamic network of molecular signalling cascades activated by learning, involved highly conserved PKA-mediated mechanisms (Selcher et al. 2002; Roberts and Glanzman 2003; Schwärzel et al. 2007). In *L. stagnalis*, an increase in PKA during the first 10 minutes after training was essential for an early phase of LTM (6 hours). On the other hand, prolonged activation of LymPKA in the late phase of LTM (24 hours) was involved in memory reconsolidation (Michel et al., 2008). Thus, very early PKA-mediated events that are necessary for 6-hour memory formation, are not sufficient for a 24 hours memory trace to form, which depends on more prolonged PKA activity. This finding implies a distinct temporal pattern induced by PKA activity that is related to the formation of different phases of memory, which has been observed in rodents as well (Tronson et al., 2006). Various protein phosphatases regulate the local activity of PKA acting as inhibitory constraints on memory formation. In this regard, it has been suggested that in snails an equilibrium between both kinase and phosphatase activities exists and regulates both memory storage as well as retrieval (Sharma et al., 2003).

4.6 Consequences of LymPACAP learning-induced activation of adenylate cyclase

Studies concerning the role of PKA in the consolidation of memory in *Lymnaea* indicate that, similar to other systems, activation of adenylate cyclase (AC) forms a key step in LTM formation. Interesting, in the *L. stagnalis* nervous system, the protein homologous to the vertebrate pituitary adenylate cyclase-activating polypeptide, LymPACAP, and its receptors were involved in learning-induced activation of AC (Pirger et al., 2010). In particular, activation of AC by PACAP was necessary for LTM to occur in a food-reward conditioning paradigm. Moreover, the application of PACAP at the beginning of multi-trial conditioning accelerated the formation of transcription-dependent memory. This memory-boosting effect of exogenously applied PACAP was blocked by the PACAP receptor antagonist PACAP6-38 in both single-trial chemical and multi-trial tactile conditioning, suggesting that LymPACAP is released in response to chemical and tactile conditioning (Pirger et al., 2010).

4.7 LymNO-dependent cascade and memory and learning

The nitric oxide (NO)-cGMP signalling system, together with the cAMP system, plays a critical role in the protein synthesis-dependent formation of LTM in many vertebrates and invertebrates (Moroz et al., 1994; Lu et al. 1999; Roberson et al. 1999). In *Lymnaea,* mRNA transcripts from the two related nNOS genes, Lym-nNOS1 and Lym-nNOS2, are expressed in CGCs (Korneev et al., 2005) and there is ample evidence that the consolidation of a memory trace following one-trial chemical conditioning depends on the LymNO-GMP signalling pathway. In fact, 6 hours after chemical conditioning, Lym-nNOS1 was up-regulated and a critical period of sensitivity up to 5 hours after conditioning was observed when blocking this pathway thus preventing the formation of LTM (Kemenes et al., 2002). This is in line with studies in bees and mice, where the NO-cGMP-pathway, together with the PKA-cascade, is necessary in the early post-training phase of memory formation (Müller, 2000). As previously shown in numerous model systems, it was assumed that in *Lymnaea* the LymNO-cGMP cascade (Park et al., 1998; Sadamoto et al., 1998) activates different signalling pathways via LymCREB1 phosphorylation (Lu et al., 1999). In fact, the LymNO-cGMP cascade involves the phosphokinase G (PKG), that, in turn, binds PKG kinase consensus sequences in the kinase inducible domain of LymCREB1, thereby activating LymCREB1 by phosphorylation (Sadamoto et al., 2004). Moreover, in *Lymnaea* NO modulated the strength of serotonergic neurotransmission between CGCs and motoneurons in the feeding system (Straub et al., 2007) which could be involved in conditioning.

4.8 LymMAPK and its role in intermediate and long-term memory

The complex molecular signalling cascades activated by behavioural training in *L. stagnalis* also results in the activation of the orthologous of mitogen-activated protein kinase (LymMAPK), as shown in numerous model system and training paradigms (Morris et al., 1986; Tsien et al., 1996; Silva et al. 1998; Müller 2000; Valjent et al., 2001; Sharma and Carew 2004). In *Aplysia*, for example, the translocation of MAPK in the nucleus after stimuli presentation results in long-term facilitation at the sensory motor neuron synapse (Martin et al., 1999). In *Lymnaea*, classical food-reward conditioning training resulted in elevated levels of activated LymMAPK in protein extracts from the cerebral and buccal ganglia and lip tissue surrounding the mouth (Ribeiro et al. 2005), indicating that both the CNS and the peripheral nervous system are involved in memory formation. In addition, in rats, the inhibition of MAPK activity blocked the formation of both ITM and LTM (Rosengger et al., 2010), where fear conditioning was observed as a result of the activation of this cascade (Atkins et al., 1997). Based on these observations, it was hypothesized that in *Lymnaea* LymMAPK was active both during ITM, which only required translation, and during the formation of LTM, providing correlative evidence that for LTM to form, ITM must occur first (Parvez et al., 2006). In light of this, in *Lymnaea*, as in other model systems, LymMAPK and LymPKC-mediated intracellular cascades and play a fundamental role in either signalling, initiating and maintaining processes that alter gene activity and induce new protein synthesis necessary for the formation of memories that persist longer than a few minutes. Remarkably, unlike other factors, after single-trial reward conditioning, LymMAPK was activated not only in response to contingent CS-US application, but also, when the stimuli were applied alone, suggesting that this kinase is necessary but not sufficient for the consolidation of associative LTM (Wan et al., 2010).

Not only, food-reward conditioning selectively increased LymCREB phosphorylation in the same ganglia that expressed LymMAPK to regulate feeding behaviours (Ribeiro et al. 2003), suggesting that phosphorylation of LymCREB in neurons could result in downstream activation of LymMAPK, as is observed in mammals (Thomas and Huganir 2004).

L. stagnalis studies based on single-trial food-reward classical conditioning have yielded information on a variety of general and specific, molecular and cellular, mechanisms necessary for the consolidation of memory, that involve the regulation of gene expression by transcription factors, such as LymCREB and the activation of LymPKA, LymPKC, and MAPK signalling pathways, as well as LymGRIN receptors and LymCaMKII (Fig. 1). Mounting evidence suggests that these molecular pathways have been highly conserved in learning, both across phylogenetic groups and learning paradigms, involving single- or multi-trials, aversion or reward, operant or classical conditioning. Even if LymPKA, LymNMDA receptors, LymCaMKII, LymCREB, and LymNOS/NO are selectively activated or upregulated, it seems likely that these and other signalling molecules are part of a synergistic effort and together contribute to the memory consolidation process, with none of them alone being sufficient for LTM (Kemenes, 2013) (Fig. 2).

4.9 LymC/EPB synaptic plasticity and memory consolidation

In line with data from *Aplysia, Helix* and mammals, LymCREB seems to regulate the expression of the homologous of CCAAT/enhancer binding protein, LymC/EBP, (Alberini et al., 1994; Niehof et al., 1997), which is an immediate-early gene involved in synaptic plasticity necessary for memory consolidation after CTA (Hatakeyama et al., 2006).

In particular, early consolidation of memory after CTA learning in *L. stagnalis* involved not only the rapid synthesis and phosphorylation of LymC/EBP, but also required the rapid breakdown of its mRNA, suggesting that a pool of LymC/EBP mRNA is rapidly translated and degraded after CTA learning. This fast turnover of newly transcribed mRNA was necessary for the prolonged *de novo* synthesis of LymC/EBP, fundamental for the consolidation phase of memory formation (Hatakeyama et al., 2004). These results led to the formation of a general rule stating that effective gene activation by a transcription factor involved in LTM consolidation requires an increase in the amount of the transcription factor itself (Hatakeyama et al., 2006). Although is not yet known which genes act downstream of LymC/EBP activation, based on data from *Aplysia*, *Helix*, and mammals (Alberini, 1994; Niehof et al., 1997; Hatakeyama et al., 2006), it is hypothesized that the expression of LymC/EBP is likely regulated by LymCREB. The potential downstream targets of C/EBP likely are the LymnNOS genes. These targets are, in fact, co-localized in B2 motoneurons, and LymnNOS genes have three putative Lym-C/EBP binding sites, which would provide the necessary structural conditions for the interaction of C/EBP with NOs genes in the *Lymnaea* feeding network. In addition, a link has been hypothesized between LymC/EBP and insulin-like growth factor 2 (IGF2), as previously demonstrated in mammals (Alberini et al., 2012). If confirmed, these data would contribute to a better understanding of the role of IGF genes in memory enhancements.

4.10 LymMIP involvement in long-term synaptic enhancement

Another intriguing factor involved in memory formation is the molluscan insulin-related peptide II (MIP-II), that belongs to the insulin superfamily. LymMIP-II was first cloned in *Lymnaea* (Smit et al., 1991; Li et al., 1992) and is expressed in the growth-controlling neuroendocrine light green cells, which are located in the cerebral ganglia (Meester et al., 1992; Smit et al., 1992). Because insulin receptors, including MIP receptors (Roovers et al., 1995), are homologous across *phyla* (Jonas et al., 1996), and ligand-binding sites are well-conserved, the use of an antibody against the extracellular domain of the mammal insulin receptor was assumed to act as an antagonist for MIP receptor (Murakami et al., 2013b). Indeed, injection of the insulin receptor antibody into the snail abdomen before CTA training blocked the memory consolidation process (Murakami et al., 2013a). On the contrary, when partially purified MIPs or bovine insulin were applied to the isolated nervous system of snails, long-term synaptic enhancement was observed at sites thought to play key roles in CTA learning and LTM formation (i.e. CGCs) (Hatakeyama et al., 2013; Murakami et al., 2013a).

Thus, up-regulation of LymMIP-II stimulates neurite formation (Smit et al., 1988; Kits et al., 1990), confirming that one of the physical manifestations of LTM formation is change in the morphology of the synapse during memory formation (Geraerts, 1992), similar to what was observed in *Aplysia* (Bailey and Kandel, 1993), *C. elegans* (Kodama et al., 2002) and rodents (Dou et al., 2005; Ramsey et al., 2005). Because the expression levels of LymMIP-II do not change when LymCREB1 is inhibited (Azami et al., 2006), the upstream transcription factors that regulate the expression of MIP-II do not directly involve LymCREB interaction. Insulin plays an important role in cognitive function across species and numerous human clinical studies suggest a link between type 2 diabetes mellitus, insulin resistance, and cognitive dysfunction (Biessels and Reagan, 2015; Heni et al., 2015; Kim and Feldman, 2015; Mainardi et al., 2015; Tramutola et al., 2018). Consequently, *Lymnaea* as a model system could help to elucidate the involvement of insulin dysregulation and memory impairment.

4.11 The monoaminergic system of *L. stagnalis* and its role in memory-related changes

Accumulating evidence suggest that when memory scores in CTA are better, the monoamine contents in the nervous system of *Lymnaea* are lower and when the insulin content in the CNS decreases, so do the monoamine contents correlated with higher memory scores. Thus, the ratio of synaptic monoamine concentration is important for memory formation and consolidation while at the same time memory-related changes may occur when the total monoamine contents in the CNS are low (Totani et al., 2019). In this regard, serotonin (5-hydroxytryptamine: 5-HT) plays a key role in the mediation of learning and memory in molluscs (Dyakonova and Sakharov, 2001). The best known example of this is dishabituation and sensitization of the gill withdrawal reflex in *Aplysia* that is mediated by the release of 5-HT from interneurons (Kandel, 2001).

Moreover, 5-HT drives both feeding behaviour and food satiety in *L. stagnalis* (Kemenes et al., 1990; Croll et al., 1999; Yamanaka et al., 2000; Kawai et al., 2011; Dyakonova et al., 2015). CGCs, that are serotoninergic, are sensitive to the concentration of glucose in the hemolymph (Dyakonova et al., 2015). As to 5-HT and memory in *Lymnaea*, an injection with a 5-HT receptor antagonist after CTA induced reversible amnesia (Nikitin and Solntseva, 2013).

Also, 5-HT levels are required to be low for learning and LTM to occur. In fact, while a decrease in the ability to learn and remember was observed in snails immersed in 5-HT, an injection with insulin rescued the ability of snails to learn CTA and form LTM (Mita et al., 2014). Finally, the amount of 5-HT released is controlled by a cAMP-PKA-CREB cascade in the CGC (Nakamura et al., 1999; Sadamoto et al., 2011). Together these data, underscore the suitability of *L. stagnalis* as a model to unravel the complexity of the serotonin signalling pathway (Benatti et al., 2017). Furthermore, dopamine (DA) pathways play an essential role in reward systems in both vertebrates and invertebrates. In *L. stagnalis,* DA is involved in LTM consolidation of reward classical conditioning (Kemenes et al., 2011) and consolidation of appetitive conditioning (i.e. sucrose as the US) (Eliott et al., 2011). Similar to dopamine, octopamine, a neurotransmitter first discovered by Erspamer in octopus (Erspamer, 1948), is also thought to be a reward related neurotransmitter and acts in a similar manner as dopamine, mediating feeding behaviour (Elliott and Vehovszky, 2000) and participating in the formation of LTM after aversive food conditioning (i.e. KCl as the US) (Kemenes et al., 2011). Thus, appetitive and aversive food conditioning in *Lymnaea* are mediated by the dopaminergic and octopaminergic system (Kemenes et al., 2011).

5. Memory extinction: when the memory for conditioning is masked by another form of learning

Memory persistence depends in part on the training procedure used, as proven by numerous studies performed in both mammals (human and rodent) and invertebrates (i.e. *Drosophila, Aplysia, Lymnaea, Apis*) that demonstrated that while 'massed-training' and 'spaced training' result in similar behavioural phenotypes, the latter results in a longer-lasting memory (Hovland, 1940; Carew et al., 1972; Hintzman, 1974; Bitterman et al., 1983; Frost et al., 1985; Lukowiak et al., 1998; Sakakibara et al., 1998; Hermitte, 1999; Lukowiak et al., 2000; Commins et al., 2003; Takahashi et al., 2013; Takigami et al., 2014b). In particular, studies using *Lymnaea* indicated that spaced training is more effective than massed training in both classical conditioning (Sakakibara, 2006; Sakakibara, 2008; Takahashi et al., 2013) and operant conditioning (Kobayashi et al., 1998). Moreover, the different behavioural outcome of these training produces reflects the dynamic molecular modifications underlying STM, ITM and LTM formation, respectively (Takigami et al., 2014b).

On the contrary, because memory transience depends from events that interfere after the formation of memory (McGeoch, 1932; Minami and Dallenbach, 1946), manipulation of snails' post-training environment in order to prevent the occurrence of 'interfering events', extends the persistence of memory (Shanga et al., 2003).

The consolidation phase, in fact, requires time, and under some circumstances consolidation related processes appear to be susceptible to a variety of influences, both facilitating and impairing the stabilization of the memory trace (McGaugh, 1966). Interference-based forgetting occurs when new information acquired either before or after a learning event attenuates memory expression (proactive and retroactive interference, respectively) (Dudai, 2004).

Multiple learning events, in fact, often occur in rapid succession, leading to competition between consolidating memories. In this contest, *Lymnaea* offers the opportunity to study the effect of proactive or retroactive interference when the consolidating memory is either in a stable or labile stage. Recently Crossley and colleagues (2019) demonstrated that when new learning takes place during a stable stage, proactive interference only occurs if the two consolidating memories engage the same circuit mechanisms. On the other hand, if different circuits are used, both memories survive.

They also demonstrated that, even if there is some interaction between the memory systems during the acquisition phase of the new memory, the original memory is only vulnerable to interference when it is in a labile state (Crossley et al., 2019). Despite forgetting (Sangha et al., 2005), that is the loss of the learned behaviour (Schacter, 2001), extinction is the gradual loss of a learned behaviour when a reinforcing stimulus was no longer applied (Pavlov, 1927). Previous studies demonstrated that extinction does not result in the destruction of the earlier formed memory, but is thought to be an active process, where the original memory is temporarily occluded by a new memory (Lattal et al., 2006). This process occurs across paradigms and species, passing from *C. elegans* to humans (Myers and Davis, 2002). Extinction in *Lymnaea*, as in other model systems, is not the unlearning of the 'old' memory, on the contrary, it requires new protein synthesis, suggesting that during this process new learning occurs which suppresses, but does not abolish, the memory for previous conditioning (Sangha et al., 2003a). In particular, in *L. stagnalis,* extinction training enhanced the activity of LymGRIN receptors and LymMAPK, both involved in memory formation, implying that changes in the same molecular machinery serves a number of purposes (Rosenegger and Lukowiak, 2010).

These results are consistent with previous studies from rodents, where treatments with NMDA agonists prior to extinction training severely inhibited its acquisition (Cammarota et al., 2005), whereas MAPK was involved in the extinction of conditioned fear (Szapiro et al., 2003) and inhibitory avoidance (Rossato et al., 2006). In this context, *L. stagnalis* represents a good model to determine the differences between the types of memory (extinction and 'original') and more fully understand their mechanisms.

6. Key-molecular factors involved in stress-induced memory block: beyond the Yerkes–Dodson/Hebb law

A large body of evidence from humans and rodents affirms that stress has complex influences on memory performance, with both negative and positive consequences depending on the nature and the "intensity" of the stressor (Baldi and Bucherelli, 2005; Lupien et al., 2007; Sandi and Pinelo-Nava, 2007). According to the 'Yerkes–Dodson Law', too much or too little stress obstructs LTM formation, while 'just the right amount' enhances LTM (Yerkes and Dodson, 1908). Moreover, single-acute versus repetitive-chronic stressors influence memory in distinct ways (Sandi and Loscertales, 1999; Byrne et al., 2014). Many studies provide evidence that opposing effects are induced by stress during the phases of consolidation (generally facilitating) and retrieval (generally impairing) of information (Roozendaal, 2002). That is, stress can be defined as a state that requires dynamic physiological, psychological or behavioral readjustment or modification in order to maintain allostatic load of the organism low, which would help the organism to sustain a 'neuronal cost' (in terms of gene activity and new protein synthesis) to form LTM to 'relevant' events (Lukowiak et al., 2014). It is also important to consider that stress only facilitates learning and memory when experienced in the same context and around the time of the event that needs to be remembered (Joëls et al., 2006). The effects of stress on learning and memory could produce contradictory results: the same stimulus may be perceived as a stressor by one organism but not by another, or may be perceived as a stressor only at certain times in the same organism.

From the literature, disagreement emerges regarding the different effects of stress on memory function (including facilitating, impairing, or the lack of effects). In this regard, *L. stagnalis* represents a compelling integrative model to understand how stress affects memory formation (Benjamin et al., 2000; Otsuka et al., 2013; Takahashi et al., 2013; Lukowiak et al., 2014; Sunada et al., 2014).

Using ecologically relevant stressors that snails are likely to encounter in their natural environment, it is possible to study how learning and memory formation are modified by stressors (Lukowiak et al., 2010). *Lymnaea*, in order to live long and prosper, require adequate quantity of food and a balanced source of calcium, necessary to grow the shell and to detect predators (Dalesman and Lukowiak, 2010). Consequently, restriction of food and/or calcium are considered to be environmental stressors. At the same time, because crowding increases competition for resources, it is considered a social stressor. There is ample evidence that some stressors (e.g. predator detection) lead to enhanced memory formation (Orr and Lukowiak, 2008), whereas other stressors (e.g. crowding) lead to suppression of memory formation (de Caigny and Lukowiak, 2008). Not only, when stressors are experienced in combination, the outcome results in unpredictable consequences on snails' ability to learn and form memory and cannot be predicted based on the impact of the stressors on memory formation when the stressors are presented individually (Huges et al. 2017).

6.1 Enhancement of LTM formation and the initiation of HSPs production in *L. stagnalis*

The exposition of *Lymnaea* for a brief period to heat (1 h at 30°C) before operant conditioning training not only enhances the formation of LTM (Teskey et al., 2012), but also increases the synthesis of two heat shock proteins (HSPs): HSP40 and HSP70 (Foster et al., 2015). Studies from rodents indicated that the flavonoid quercetin blocks the effects on memory formation of those stressors that act via HSPs (Mohammadi et al., 2014) by altering the expression levels of CREB (Costa-Mattioli et al., 2009). Future studies using *Lymnaea* could help to better comprehend the link between the enhancement of LTM formation and the start of HSPs production (Sunada et al., 2016).

6.2 The cooling-induced modification on ITM and LTM

Even if cooling can be considered a more naturally event, in particular circumstance, it can be used as stressful stimulus. Numerous studies established that brief periods of hypothermia after conditioning training interfere with memory consolidation in both *Lymnaea* and in other species because of a reduction in protein synthesis (Sekiguchi et al., 1994; Xia et al., 1998; Sangha et al., 2003d). In fact, it seems that cooling interferes with the metabolic processes necessary for memory formation (Fulton et al., 2008; Takahashi et al., 2014). In this regard, Sangha and colleagues (2003) demonstrated that cooling the snails for 1 hour immediately after training blocked ITM or LTM, whereas delaying the same cold-block by 10 or 15 minutes allowed for the formation of ITM and LTM, respectively (Sangha et al., 2003d). These data imply that cooling-sensitive processes required during the establishment of ITM and LTM operate through a brief time-window immediately following learning. Furthermore, it seems that the processes leading to stabilization of the learned behaviour, once started, are not broken down by cooling (Sangha et al., 2003d). Moreover, Takahashi and colleagues (2013) showed that prolonging the cold-block up to 180 minutes following training inhibited memory formation as well, suggesting that there are two critical periods for LTM formation. In fact, the application of the cold-block immediately after training

interferes with the macromolecular protein synthesis required for memory consolidation, whereas the cooling effect observed 180 minutes after training alters a second round of protein synthesis occurring following memory formation (Takahashi et al., 2013). Finally, exposure of snails to 4°C for 8 days once LTM is consolidated, resulted in disruption of the events downstream memory formation that are responsible for forgetting. In this way, LTM that normally persisted for 2 days was extended for at least 8 days (Sangha et al., 2003d). These data are consistent with the hypothesis that forgetting is an active process due to the learning and remembering of interfering events. If confirmed, it should be possible to disrupt the process of forgetting (i.e., block the new consolidation process for 'interfering events') by using cooling. In this contest, *L. stagnalis* represents a good model to study the time-window required for ITM and LTM using reversibly cold-block induced amnesia which, in turn, is a non-toxic and easily reversible procedure, that can be applied and removed for discrete amounts of time (Takahashi et al., 2013). Moreover, cooling can be utilized to study how loss of memory may be prevented.

6.3 DNA methylation in memory persistence in relation to longer-term stressors or environmental changes

Considering that the interval between stress experienced by snails and the enhancing effect of this stressor on the formation of LTM is in the order of days, epigenetic mechanisms have been hypothesized to play a key role. In this regard, epigenetic changes, such as DNA methylation, have emerged as common mechanisms involved in memory formation across species (Zovkic et al., 2013). In particular, DNA methylation was involved in olfactory LTM in honeybees (Biergans et al.,2012) as well as in long-term potentiation at sensory-motor synapses in Aplysia (Rajasethupathy et al., 2012). Studies from rodents suggest that DNA methylation represents a dynamic state, that can be strongly influenced by various environmental manipulations, including exposure to stress (Chertkow-Deutsher et al., 2010). Stressful stimuli, in fact, alter DNA methylation state which, in turn, is the result of upstream events, including increased glutamate and neuropeptide transmission and enhanced activation of transcription factors (Stankiewicz et al., 2013). In this context, the enhancement of LTM or the length of its persistence in stressed snails required DNA methylation (Lukowiak et al., 2014). In fact, treatment with a DNA methylation blocker (5-Aza-2'-deoxycytidine (5-AZA)) 1 hour before exposing snails to a memory-promoting stressor, altered the persistence of LTM (Lukowiak et al.,2014; Sunada et al., 2016). Interestingly, in the absence of stress-enhanced memory, 5-AZA was not active (Lukowiak et al.,2014). Because drugs of abuse, such as cocaine and methamphetamines, activate stress pathways (Moldow and Fischman, 1987), it has been hypothesised that intense memories characteristic of post-traumatic stress disorder (PTSD) and drug addiction, may be resistant to forgetting because memories appear to be stabilized by epigenetic changes (Carter et al., 2006; Kennedy et al., 2010; Debiec, 2012; Nestler, 2014; Schmidt et al., 2013). Therefore, *Lymnaea* represents a good model to study the involvement of DNA methylation in memory persistence related to longer-term stressors or environmental changes, while at the same time contributes to elucidate the role of epigenetic changes in memory impairments (Carter et al., 2006).

6.4 Neuro-modulatory role of the endocannabinoid system in how stress modifies LTM formation

The mechanisms by which various stressors affect memory formation are not entirely clear. In this contest, the endocannabinoid system and, in particular cannabinoid receptors (CBs), seems to play a key neuro-modulatory role in how stress modifies LTM formation (Campolongo et al., 2009; Atsak et al., 2012; Tan et al., 2014). In mammals, in fact, the activation of the endocannabinoid system not only enhanced the effects of a stressor on adaptive behaviours (Morena and Campolongo, 2014; Goodman and Packard, 2015), but it also suppressed the formation of working memory and LTM. On the contrary, both forms of memory were enhanced when the endocannabinoid system was inhibited. Because the endocannabinoid system is phylogenetically ancient (McPartland, 2004) and well conserved among species, Sunada and colleagues (2017) hypothesized that in *Lymnaea* putative cannabinoid receptors (LymCBrs) are involved in learning and memory formation in stressful conditions (Sunada et al., 2017). Indeed, *L. stagnalis*, expressed two G-protein-coupled neuronal receptor genes, which encoded proteins closely related to well characterized vertebrate CBrs (Sunada et al., 2017). Injecting a mammalian CBr agonist (i.e. WIN 55) in snails, mimicked the traumatic event of exposure to severe stressor and rendered snails unable to learn and form memories for up to one week after the traumatic event. On the contrary, injection of a mammalian CBr antagonist (i.e. AM 251) before snails received the traumatic stimulus reduced the effect on learning and memory. Injection of the same antagonist into untrained and not traumatized snails enhanced their ability to form LTM (Sunada et al., 2017). Evidence from the literature also supports these data. Previous research in rodents, in fact, indicated that blocking of CBrs enhanced spatial and associative memory (Terranova et al., 1996; Robinson et al., 2008). While the effects of WIN 55 was prolonged and maintained for at least 4 days, the effect of other stressors on LTM formation persisted for only a few hours (Orr and Lukowiak, 2008). Consequently, it was hypothesized that WIN 55 causes a state of extreme fear that is incompatible with learning and memory, as demonstrated in mammals, where the endocannabinoid system plays a key role in the neuronal regulation of anxiety and responses to fear (Ruehle et al., 2012). Future research could benefit from our simple model system and use it to better understand how the endocannabinoid system is involved in the acquisition of learning and memory. At the same time, gaining better comprehension as to how behaviorally relevant stressors may alter LTM formation and/or its persistence focusing on simple systems may lead us to understand how to treat 'memory disorders' such as phobias, PTSD and substance abuse more effectively (Dębiec,2012; Agren,2014).

7. Necessity knows no law: when the conditioned stimulus sucrose becomes a source of energy

It is well-established that different stress states resulting from different durations of food deprivation alter the ability of snails to express LTM. According to the Yerkes–Dodson/Hebb law, while some degree of a food-deprivation-induced stressed state must exist for the CTA to successfully occur, the length of food deprivation alters learning and LTM formation (Ito et al., 2015). In particular, food deprivation for 1 day resulted in optimal learning and memory, whereas food deprivation for 5 days before training resulted in little or no learning and memory (Sugai et al., 2007; Mita et al., 2014). Because memory formation is energetically expensive (Barnard et al., 2006; Burns et al., 2011), if energy intake is too much restricted, LTM formation should be impaired in order to conserve energy. Thus, snails are hypothesized to learn and form LTM, but in an overly stressed state associated with prolonged food deprivation, their ability to express the LTM phenotype should be suppressed, suggesting that hunger triumphs over the memory not to respond to the CS. That is, starvation generates a conflict between memory formation versus the desire or necessity to eat (Ito et al., 2015). Moreover, the context-specificity of memory expression (Haney and Lukowiak, 2001) played an important role in the lack of LTM observed in 5-day severely food-deprived snails. In fact, LTM is only observed in 5-day deprived snails when tested for LTM following *ad libitum* access to food for additional 7 days while in a 1-day food-deprived state (day 13 snails). In contrast, snails did not express the memory phenotype if they had recovered from 5-days of food deprivation. These data support the fact that 1 day of starvation after refeeding reinstalls the optimal context in which snails memorize (Ito et al., 2015). Based on the observation that during CTA training there was an up-regulation of LymMIP-II (Azami et al., 2006), it has been hypothesized that sucrose on the lips, which represents the CS, induced an insulin spike, that, in turn, modulated the neural circuit underlying CTA-LTM (Murakami et al., 2013b). In addition, considering that an injection of insulin to 1-day food-deprived snails improved learning and CTA memory (Mita et al., 2014), it is likely that the occurrence of an insulin spike correlates with the acquisition and retention of associative learning. Finally, previous studies indicated that 5 days food-deprived snails trained in the presence of a food smell, no longer learned nor formed memory (Lukowiak et al., 2014), which stresses the importance of the 'state' of the organism and how and when it perceives a stimulus as a stressor. Thus, the expression of memory is both context and state-dependent and may only be expressed following the resolution of the conflict between the homeostatic drive to eat versus having a memory of learning under what conditions not to eat (Mita et al., 2014).

8. *L. stagnalis* as a model for age-associated memory decline

Evidence is accumulating affirming that aging affects memory. However, the role of molecular dysregulation in age-associated memory deficits is not well understood. Learning and memory impairments are a common and evolutionarily conserved feature of the normal aging brain (Burke and Barnes, 2006; Luck et al., 2010).

8.1 Age-associated memory decline and oxidative stress

The (neuro)biological foundations of the natural decline in plasticity are not completely understood but appear to involve a progressive alteration of neuronal excitability resulting from an impaired activity in glutamate receptor subunits, such as glutamate ionotropic receptor AMPA type subunit 2 and NMDA type subunit 2B (Disterhoft and Oh, 2006, Hermann et al., 2007; Kashiyae et al 2009), together with a shift in the mechanisms that regulate synaptic plasticity , including Ca^{2+} channel function and Ca^{2+}-dependent processes (Fukaya et al., 2007). Moreover, there is extensive evidence for a role of oxidative stress, and particularly lipidic peroxidation, as a key factor in aging and age-associated neural impairment (Harman, 1956; Dröge and Schipper, 2007; Sultana and Butterfield, 2010). Lipid peroxidation results in loss of membrane polyunsaturated fatty acids and oxidized phospholipids as polar species contributing to increased membrane rigidity (Farooqui and Horrocks, 1998). Alterations in the neural membrane phospholipid components, in turn, not only influence crucial intra- and inter-cellular signalling, but also alter many physical properties of the membrane, such as fluidity, phase permeability, bilayer thickness and lateral domains (Horrocks and Farooqui, 2004). Polyunsaturated fatty acids (PUFAs) are released from membrane phospholipids by a number of enzymic mechanisms involving the receptor-mediated stimulation of phospholipase A2 (PLA_2) and phospholipase C/diacylglycerol lipase pathways (Farooqui and Horrocks, 1998; Niki, 1990). Thus, PLA_2 represents a key factor in age-associated decline in neuronal excitability and the related impairment in activity-dependent forms of learning and memory formation (Niki, 1990). Similar results, obtained in different model systems, suggest that a decline of electrical activity/excitability and synaptic functions, associated with lipid peroxidation, are conserved characteristic of neuronal aging (Arundell et al., 2006; Disterhoft and Oh, 2007; Hermann et al., 2007; Spiteller, 2010; Watson et al., 2012). In this regards, Watson and colleagues (2012) demonstrated that PLA_2-dependent free fatty acids (FFAs) release was significantly enhanced in CNS of old snails, and that experimental oxidative stress raised PLA_2-dependent FFAs release in CNS of younger snails to the level observed in older brains. Moreover, both experimentally induced and naturally senescent phenotypes were fully reversed by blocking the activity of PLA2. This suggests a central role of lipid metabolism, particularly oxidative stress-induced activation of PLA2, in the process of neuronal aging and age-associated learning and memory impairment in *L. stagnalis* (Watson et al., 2012). Similarly, evidence regarding PLA2-mediated excision of fatty acids and fatty acid metabolism in the genesis of age-related cognitive impairment has been observed in mammals including humans as well (Adibhatla and Hatcher, 2008; Darios et al., 2007; Sanchez-Mejia and Mucke, 2010). In this regard, numerous studies from mammals showed that PUFAs deficiency markedly affects neurotransmission, ion channel activities and synaptic plasticity (Yehuda et al., 2002).

On the other hand, prolonged PUFAs supplementation in the diet restored membrane fluidity and calcium homeostasis in the brain, improved electrophysiological parameters (i.e. hippocampal long-term potentiation) and learning ability in aged rats and, not least, enhanced cognitive function in humans with memory deficits (Kotani et al., 2003; Kotani et al., 2006; Fukaya et al., 2007; Kashiyae et al., 2009). Moreover, studies from senescent rats demonstrated that the dysregulation of calcium-dependent neuronal processes could be restored to the state observed in young rats with prolonged PUFA-supplementation (Fukaya et al., 2007). These parallelisms between species that are phylogenetically distant lead to converging evidence which postulates that lipid peroxidation-dependent PLA2 activity is a fundamental, evolutionary conserved aspect of neuronal aging and a cause of age-associated changes in neuronal signalling and cognitive decline in the normal aging process of brain.

8.2 The role of PKA/CREB1 and PACAP38 in age-associated memory decline

As illustrated before, the conserved activation of adenylate cyclase by LymPACAP and the consequent activation of the LymPKA-LymCREB1 molecular cascade are fundamental during the formation of LTM (Sadamoto et al., 2004).

Previous studies indicated that the exogenous application of PACAP before training rescued memory impairments in old snails, suggesting that the inactivation of this target represents an useful tool for the study of age-associated memory impairment, that, in turn, seems to be only suspended but not irreversibly extinguished (Watson et al., 2010). Along a similar vein, treatment with insulin-like growth factor-1 (IGF-1), which in vertebrates activates PACAP type I receptors (Delcourt et al., 2007), increased memory formation in aged snails (Pirger et al., 2014), suggesting that IGF-1 may exert a comparable memory-boosting effect on aged snails as PACAP. Based on these observations, the PKA-CREB1 pathway, together with PA2 and IGF-1, have been proposed as targets for therapeutic interventions for age-related memory loss (Pirger et al., 2014).In this contest, due to the evolutionarily conserved nature of these polypeptides and their established role in memory and synaptic plasticity in snails (Kemenes et al., 2013), *Lymnaea* should be a considered as an excellent model in which to conduct drug discovery studies.

9. Limitations of molecular analysis of the nervous system of *L. stagnalis*

The purpose of this review was to reassume the data and illustrate that *L. stagnalis* relies on a set of core molecules required for learning and memory in a similar way as observed in a number of other species, ranging from invertebrates to mammals. Thanks to the simplicity and accessibility of neuronal circuits, *Lymnaea* has provided important insights into the fundamental cellular and synaptic elements required for establishing conserved cognitive functions, such as memory and learning. However, a serious drawback in the molecular analysis of the nervous system of *L. stagnalis* is the lack of large-scale genomic or neuronal transcriptome information. In fact, although its genome has been sequenced, gene characterization has not yet been performed. This turns out to be an important obstacle for the use of this model in comparative molecular studies (Feng et al., 2009). The molecular information available has been obtained by cloning of partial cDNA sequences, together with *in situ* hybridization and immunohistochemistry. Thus, an important scientific puzzle to solve in the near future is the characterization of the gene networks that play central roles in the functioning of the CNS in *L. stagnalis*. A pre-requisite for such exploration is the knowledge of gene sequences, which can be used to monitor when, where, and how particular genes are expressed. Investment in such a research effort, will not only add a new experimental model to the limited number of invertebrate models already used in translational neuroscience research.

10. New directions of neuroscience translational research using *L. stagnalis*

Understanding how biological aging processes affect the brain and how they contribute to the onset and progress of age-associated neurodegenerative diseases is a central research goal in neuroscience. In this context, *Lymnaea* provides a powerful model system to learn more about the cellular and molecular details of memory processes. Unlike *D. melanogaster* and *C. elegans,* the most common and best characterized invertebrate models (Yamaguchi and Yoshida, 2018; Möller et al., 2018), *Lymnaea* has a relative long life span (lasting approximately 9–12 months) which offers a powerful new tool to study age-related modifications involving genetic, molecular, and cellular mechanisms, which usually take time to manifest their full effects (Nestler et al., 2010; Tascedda et al., 2015). This last factor is of particularly interest in studies on neuro-aging, chronic human pathologies, especially neurodegenerative diseases such as Alzheimer's, Parkinson's, and chronic psychiatric diseases such as major depression, schizophrenia or bipolar disorder.

As previously illustrated, *L. stagnalis* embodies a useful tool for translation-oriented research aimed at developing new therapeutic approaches to age-associated memory dysfunction. In particular, PA2, PACAP38, and IGF-1 and their related biochemical cascades, represent fascinating “memory rejuvenating” agents and their (genetic) characterization could help to elucidate some of the mechanisms underlying cognitive decline in the aging human brain.

At the same time, *Lymnaea* represents an excellent model for the comprehension of the causal mechanisms of memory extinction. Identifying the molecular substrates of extinction could promote the development of pharmacological treatments for psychiatric disorders, such as fear disorders, and substance addiction in humans.

However, because similar molecular processes contribute to the development and persistence of both memory consolidation and extinction, pharmacological interventions designed to facilitate extinction should be explored carefully.

In addition, *Lymnaea* as model system provides an outstanding platform to investigate the crosstalk between neuronal metabolism (energy) and the formation of memory and how such mechanisms are altered during aging and neurodegenerative disorders. As explained, insulin and IGF-1 modulate aspects of plasticity in the CNS of *Lymnaea* and enhance learning abilities in older learning-impaired snails (Murakami et al., 2013; Pirger et al., 2014).

These findings echo very well with the growing evidence suggesting a the role of ILPs and insulin resistance in aging (Alcedo et al., 2013).

The *Lymnaea* research platform we portrayed in this review will also be a great tool to investigate fundamental mechanisms of stress-mediated memory impairments.

In this regard, stressors or bioactive agents that cause enhancement of LTM formation in *Lymnaea* have been hypothesized to act via putative CBrs. If confirmed, these studies will help to elucidate the role of the endocannabinoid system in learning and in memory in mammals including humans.

Moreover, because epigenetic changes, such as DNA methylation, are emerging as a common mechanism in synaptic plasticity and memory formation across species (Zovkic et al., 2013) and are involved in the maintenance of memories in OTSD and drug addiction, research using *Lymnaea* will add to this growing area of research and will further highlight the common mechanisms of memory formation in vertebrates and invertebrates species.

Beside investigating molecular and cellular aspects of numerous human diseases, *Lymneaea* could also open interesting perspectives concerning both the validation of the mechanism of action of existing drugs and the preclinical studies of drugs discovery (Tascedda et al., 2015). Given the high costs and the long time needed to identify and develop new drugs, a faster and less expensive system of drug discovery is both necessary and urgent. Therefore, *Lymnaea*, represents a versatile platform for the screening of new compound, the identification of innovative drug targets and for the deciphering of mechanisms underlying drug action. Snails, in fact, are aquatic invertebrates with an open circulatory system, allowing the use of membrane-permeant drugs that can be easily absorbed, to unravel the complexity of various signalling pathways and to provide new insights on how drugs and molecules can modulate different neuronal functions and behaviours (Benatti et al., 2017). At present, very few pharmacological studies using *Lymnaea* as model system are available up today. Benatti and colleagues (2017), for example, evaluated the transcriptional effects of a serotonergic stimulation on selected targets involved in 5-HT signalling and neurotransmission in the CNS of *Lymnaea*. They treated chronically (48 hours) or acutely (6 hours) snails with the immediate precursor of serotonin (5-hydroxy trypthophan - 5HTP), with fluoxetine (FLX) or with a combination of the two compounds. They demonstrated that transcription of Lym-CREB1 was strongly induced following chronic, but not acute, exposure to 5-HTP. This pivotal study suggested that *Lymnaea* could significantly contribute to finding novel functions of known drugs or molecular targets and for the identification of new drugs and their validation.

Not least, *Lymnaea* represents a valid model for neuro-engineering research. Counting on the fact that many neurons in the CNS of *Lymnaea* have large somata, the formation of high-quality neuron/semiconductor interfaces is facilitated (Birmingham et al., 2004). Thus, in the last years, considerable efforts have been made to develop techniques through which individual neurons can be noninvasively coupled to electronic microstructures of a semiconductor substrate, such as capacitors for stimulation and transistors for recording (Fromherz et al., 1991, 1995; Zeck and Fromherz, 2001; Bonifazi and Fromherz, 2002). Using this approach, Kaul and co-workers (2004) interfaced the pre- and postsynaptic neuron of an excitatory chemical synapse of *Lymnaea* by a silicon chip (Kaul et al., 2004). In this way, stimulating the presynaptic cell with a chip capacitor and recording the activity of the postsynaptic cell with a transistor, they enhanced the strength of the soma-soma synapse by repetitive capacitor stimulation (Kaul et al., 2004).

Moreover, Zeck and Fromherz (2001) demonstrated that isolated *Lymnaea* neurons can be anchored to a semiconductor chip and neurite growth cones can be guided along specified pathways (Zeck and Fromherz, 2001).

Using this approach, it would be possible to reconstruct chemical synapses between specific neurons to form neuroelectronic circuits that exhibit various forms of synaptic plasticity and specific neuro-modulatory properties, adding different degree of flexibility to an already dynamic circuit (Birmingham et al., 2004).

Thus, studies of *Lymnaea* neural networks on silicon chips not only are promoting our understanding of the long-term dynamics and plasticity of relatively simple neural circuits, but are also providing the basis for reliable interfaces for new neuro-devices for humans (Birmingham et al., 2004).

On the basis of these considerations, although animal models can never summarize the full phenotype of a human clinical disorder, in particular neurological ones, *L. stagnalis* offers a new important and innovative tool for neuroscientists (Tascedda et al., 2015), representing a remarkable model system in which to study the genetic and molecular basis of human CNS physiology and pathology (Benatti et al., 2017). We think that this model allows for a better vantage point from which innovative therapies and technology can be developed in order to treat a range of neurological and neurodegenerative disorders, and that the ultimate success of neuroscientists engaged in translational research will depend on collecting and improving the quality and quantity of knowledge to “translate” (Alberts, 2010) obtained in invertebrates and in more complex organisms, until arriving at human beings. Because research on invertebrate models represents the shortest and most efficient tool to study and treat human diseases, *L. stagnalis* offers a model to bridge the gap between traditional *in vitro* and preclinical animal assays, and to move from pond to the bench.

11. Conclusions

In conclusion, what have we learn from *L. stagnalis* to date?

7) A variety of general and specific, molecular and cellular, mechanisms necessary for the consolidation of memory in snails have been highly conserved in learning, both across phylogenetic groups and learning paradigms, involving single- or multi-trials, aversion or reward, operant or classical conditioning;
8) similarly, the model contributes to define more clearly in what conditions we form memory in the most optimal way, "teaching" us the importance of context in which we eat, age, form memories and consolidate them;
9) *Lymnaea* allows us to move beyond simple homology, representing a valid and reliable model in which to study the genetic and molecular basis of human CNS physiology and pathology

Acknowledgement

In memory of Prof. Enzo Ottaviani for the precious suggestions and for the encouragement to work on this model.

References

1. Abel T, Lattal KM. Molecular mechanisms of memory acquisition, consolidation and retrieval. Current Opinion in Neurobiology. 2001 Apr;11(2):180–7.
2. Adibhatla RM, Hatcher JF. Phospholipase A $_2$, reactive oxygen species, and lipid peroxidation in CNS pathologies. BMB Reports. 2008 Aug 31;41(8):560–7.
3. Agren T. Human reconsolidation: A reactivation and update. Brain Research Bulletin. 2014 Jun;105:70–82.
4. Alberini CM, Chen DY. Memory enhancement: consolidation, reconsolidation and insulin-like growth factor 2. Trends in Neurosciences. 2012 May;35(5):274–83.
5. Alberini CM, Ghirardl M, Metz R, Kandel ER. C/EBP is an immediate-early gene required for the consolidation of long-term facilitation in Aplysia. Cell. 1994 Mar;76(6):1099–114.
6. Alberini CM. Genes to remember. Journal of Experimental Biology. 1999 Nov 1;202(21):2887–91.
7. Alberts B. Model Organisms and Human Health. Science. 2010 Dec 24;330(6012):1724–1724.
8. Alcedo J, Flatt T, Pasyukova EG. Neuronal Inputs and Outputs of Aging and Longevity. Front Genet [Internet]. 2013 [citato 23 settembre 2019];4. Available at: http://journal.frontiersin.org/article/10.3389/fgene.2013.00071/abstract
9. Alexander J, Audesirk TE, Audesirk GJ. One-trial reward learning in the snailLymnea stagnalis. J Neurobiol. 1984 Jan;15(1):67–72.
10. Alexander JE, Audesirk TE, Audesirk GJ. Rapid, nonaversive conditioning in a freshwater gastropod. Behavioral and Neural Biology. 1982 Dec;36(4):391–402.
11. Alkon DL, Epstein H, Kuzirian A, Bennett MC, Nelson TJ. Protein synthesis required for long-term memory is induced by PKC activation on days before associative learning. Proceedings of the National Academy of Sciences. 2005 Nov 8;102(45):16432–7.
12. Alkon DL, Sun M-K, Nelson TJ. PKC signaling deficits: a mechanistic hypothesis for the origins of Alzheimer's disease. Trends in Pharmacological Sciences. 2007 Feb;28(2):51–60.
13. Arundell M, Patel BA, Straub V, Allen MC, Janse C, O'Hare D, et al. Effects of age on feeding behavior and chemosensory processing in the pond snail, Lymnaea stagnalis. Neurobiology of Aging. 2006 Dec;27(12):1880–91.
14. Atkins CM, Selcher JC, Petraitis JJ, Trzaskos JM, Sweatt JD. The MAPK cascade is required for mammalian associative learning. Nat Neurosci. 1998 Nov;1(7):602–9.
15. Atsak P, Roozendaal B, Campolongo P. Role of the endocannabinoid system in regulating glucocorticoid effects on memory for emotional experiences. Neuroscience. 2012 Mar;204:104–16.
16. Azami S, Wagatsuma A, Sadamoto H, Hatakeyama D, Usami T, Fujie M, et al. Altered gene activity correlated with long-term memory formation of conditioned taste aversion inLymnaea. J Neurosci Res. 2006 Nov 15;84(7):1610–20.
17. Bailey CH, Kandel ER. Structural Changes Accompanying Memory Storage. Annu Rev Physiol. 1993 Oct;55(1):397–426.
18. Baldi E, Bucherelli C. The Inverted "U-Shaped" Dose-Effect Relationships in Learning and Memory: Modulation of Arousal and Consolidation. Nonlinearity in Biology, Toxicology, Medicine. 2005 Jan;3(1):nonlin.003.01.0.
19. Bank B, LoTurco JJ, Alkon DL. Learning-induced activation of protein kinase C: A molecular memory trace. Mol Neurobiol. 1989 Mar;3(1–2):55–70.
20. Barco A, Pittenger C, Kandel ER. CREB, memory enhancement and the treatment of memory disorders: promises, pitfalls and prospects. Expert Opinion on Therapeutic Targets. 2003 Feb;7(1):101–14.
21. Barnard CJ, Collins SA, Daisley JN, Behnke JM. Odour learning and immunity costs in mice. Behavioural Processes. 2006 Mar;72(1):74–83.
22. Bartsch D, Ghirardi M, Skehel PA, Karl KA, Herder SP, Chen M, et al. Aplysia CREB2 represses long-term facilitation: Relief of repression converts transient facilitation into long-term functional and structural change. Cell. 1995 Dec;83(6):979–92.
23. Benatti C, Colliva C, Blom JMC, Ottaviani E, Tascedda F. Transcriptional effect of serotonin in the ganglia of Lymnaea stagnalis. Invertebrate Survival Journal. 2017;14(1):251–258.
24. Benjamin PR, Staras K, Kemenes G. A systems approach to the cellular analysis of associative learning in the pond snail Lymnaea. Learn Mem. 2000 Jun;7(3):124–31.
25. Bevilaqua LR, Medina JH, Izquierdo I, Cammarota M. Memory consolidation induces N-methyl-d-aspartic acid-receptor- and Ca2+/calmodulin-dependent protein kinase II-dependent modifications in α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor properties. Neuroscience. 2005 Jan;136(2):397–403.
26. Biergans SD, Jones JC, Treiber N, Galizia CG, Szyszka P. DNA Methylation Mediates the Discriminatory Power of Associative Long-Term Memory in Honeybees. Zars T, editor. PLoS ONE. 2012 Jun 18;7(6):e39349.
27. Biessels GJ, Reagan LP. Hippocampal insulin resistance and cognitive dysfunction. Nat Rev Neurosci. 2015 Nov;16(11):660–71.
28. Birmingham JT, Graham DM, Tauck DL. Lymnaea stagnalis and the development of neuroelectronic technologies. J Neurosci Res. 2004 May 1;76(3):277–81.
29. Bitterman ME, Menzel R, Fietz A, Schäfer S. Classical conditioning of proboscis extension in honeybees (Apis mellifera). Journal of Comparative Psychology. 1983;97(2):107–19.
30. Bonifazi P, Fromherz P. Silicon Chip for Electronic Communication Between Nerve Cells by Non-invasive Interfacing and Analog–Digital Processing. Advanced Materials. 2002;14(17):1190–3.
31. Boycott AE. The Habitats of Fresh-Water Mollusca in Britain. The Journal of Animal Ecology. 1936 May;5(1):116.
32. Browning K, Lukowiak K. Ketamine inhibits long-term, but not intermediate-term memory formation in Lymnaea stagnalis. Neuroscience. 2008 Aug;155(3):613–25.
33. Brunelli M, Castellucci V, Kandel E. Synaptic facilitation and behavioral sensitization in Aplysia: possible role of serotonin and cyclic AMP. Science. 1976 Dec 10;194(4270):1178–81.
34. Burke SN, Barnes CA. Neural plasticity in the ageing brain. Nat Rev Neurosci. 2006 Jan;7(1):30–40.
35. Burne T, Scott E, van Swinderen B, Hilliard M, Reinhard J, Claudianos C, et al. Big ideas for small brains: what can psychiatry learn from worms, flies, bees and fish? Mol Psychiatry. 2011 Jan;16(1):7–16.
36. Burns JG, Foucaud J, Mery F. Costs of memory: lessons from 'mini' brains. Proc R Soc B. 2011 Mar 22;278(1707):923–9.
37. Byrne JH, Hawkins RD. Nonassociative Learning in Invertebrates. Cold Spring Harb Perspect Biol. 2015 May;7(5):a021675.
38. Byrne K, Sunada H, Teskey M, Lukowiak K, Dalesman S. Environmentally relevant stressors alter memory formation in the pond snail Lymnaea. J Exp Biol. 2014 Jan 1;217(Pt 1):76–83.
39. Cammarota M, Bevilaqua LRM, Barros DM, Vianna MRM, Izquierdo LA, Medina JH, et al. Retrieval and the Extinction of Memory. Cell Mol Neurobiol. 2005 Jun;25(3–4):465–74.
40. Cammarota M, Bevilaqua LRM, Viola H, Kerr DS, Reichmann B, Teixeira V, et al. Participation of CaMKII in neuronal plasticity and memory formation. Cell Mol Neurobiol. 2002 Jun;22(3):259–67.
41. Campolongo P, Morena M, Scaccianoce S, Trezza V, Chiarotti F, Schelling G, et al. Novelty-Induced Emotional Arousal Modulates Cannabinoid Effects on Recognition Memory and Adrenocortical Activity. Neuropsychopharmacol. 2013 Jun;38(7):1276–86.
42. Carew TJ, Pinsker HM, Kandel ER. Long-term habituation of a defensive withdrawal reflex in aplysia. Science. 1972 Jan 28;175(4020):451–4.
43. Carter K, Lukowiak K, Schenk JO, Sorg BA. Repeated cocaine effects on learning, memory and extinction in the pond snail Lymnaea stagnalis. Journal of Experimental Biology. 2006 Nov 1;209(21):4273–82.
44. Chertkow-Deutsher Y, Cohen H, Klein E, Ben-Shachar D. DNA methylation in vulnerability to post-traumatic stress in rats: evidence for the role of the post-synaptic density protein Dlgap2. Int J Neuropsychopharmacol. 2010 Apr;13(3):347–59.
45. Choi KW, Smith RF, Buratowski RM, Quinn WG. Deficient protein kinase C activity in turnip, a Drosophila learning mutant. J Biol Chem. 1991 Aug 25;266(24):15999–15606.
46. Commins S, Cunningham L, Harvey D, Walsh D. Massed but not spaced training impairs spatial memory. Behavioural Brain Research. 2003 Feb;139(1–2):215–23.
47. Corning, WC, Dyal JA, Willows AO, Invertebrate learning: Vol. 2. Arthropods and gastropod mollusks. (Eds.). (1973). New York, NY, US: Plenum Press.
48. Costa-Mattioli M, Sossin WS, Klann E, Sonenberg N. Translational Control of Long-Lasting Synaptic Plasticity and Memory. Neuron. 2009 Jan;61(1):10–26.
49. Croll RP, Voronezhskaya EE, Hiripi L, Elekes K. Development of catecholaminergic neurons in the pond snail, Lymnaea stagnalis: II. Postembryonic development of central and peripheral cells. J Comp Neurol. 1999 Feb 15;404(3):297–309.
50. Croll RP. Insights into early molluscan neuronal development through studies of transmitter phenotypes in embryonic pond snails. Microsc Res Tech. 2000 Jun 15;49(6):570–8.

51. Crossley M, Lorenzetti FD, Naskar S, O'Shea M, Kemenes G, Benjamin PR, et al. Proactive and retroactive interference with associative memory consolidation in the snail Lymnaea is time and circuit dependent. Commun Biol. 2019 Dec;2(1):242.
52. Dalesman S, Lukowiak K. Effect of acute exposure to low environmental calcium on respiration and locomotion in Lymnaea stagnalis (L.). J Exp Biol. 2010 May;213(Pt 9):1471–6.
53. Darios F, Connell E, Davletov B. Phospholipases and fatty acid signalling in exocytosis. J Physiol (Lond). 2007 Dec 15;585(Pt 3):699–704.
54. De Caigny P, Lukowiak K. Crowding, an environmental stressor, blocks long-term memory formation in Lymnaea. J Exp Biol. 2008 Aug;211(Pt 16):2678–88.
55. De Zazzo J, Tully T. Dissection of memory formation: from behavioral pharmacology to molecular genetics. Trends Neurosci. 1995 May;18(5):212–8.
56. Dębiec J, Bush DEA, LeDoux JE. Noradrenergic enhancement of reconsolidation in the amygdala impairs extinction of conditioned fear in rats-a possible mechanism for the persistence of traumatic memories in PTSD. Depress Anxiety. marzo 2011;28(3):186–93.
57. Delcourt N, Thouvenot E, Chanrion B, Galéotti N, Jouin P, Bockaert J, et al. PACAP type I receptor transactivation is essential for IGF-1 receptor signalling and antiapoptotic activity in neurons. EMBO J. 2007 Mar 21;26(6):1542–51.
58. Disterhoft JF, Oh MM. Alterations in intrinsic neuronal excitability during normal aging. Aging Cell. 2007 Jun;6(3):327–36.
59. Disterhoft JF, Oh MM. Learning, aging and intrinsic neuronal plasticity. Trends Neurosci. 2006 Oct;29(10):587–99.
60. Dou J-T, Chen M, Dufour F, Alkon DL, Zhao W-Q. Insulin receptor signaling in long-term memory consolidation following spatial learning. Learn Mem. 2005 Dec;12(6):646–55.
61. Dröge W, Schipper HM. Oxidative stress and aberrant signaling in aging and cognitive decline. Aging Cell. 2007 Jun;6(3):361–70.
62. Dudai Y. The Neurobiology of Consolidations, Or, How Stable is the Engram? Annu Rev Psychol. 2004 Feb;55(1):51–86.
63. Dyakonova V, Hernádi L, Ito E, Dyakonova T, Zakharov I, Sakharov D. The activity of isolated snail neurons controlling locomotion is affected by glucose. Biophysics (Nagoya-shi). 2015;11:55–60.
64. Dyakonova VE, Sakharov DA. An isolated serotonergic neuron: the mechanism of excitation induced by enhanced synthesis of neurotransmitter. Dokl Biol Sci. 2001 Jun;378:230–2.
65. Elgersma Y, Sweatt JD, Giese KP. Mouse genetic approaches to investigating calcium/calmodulin-dependent protein kinase II function in plasticity and cognition. J Neurosci. 2004 Sep 29;24(39):8410–5.
66. Elliott CJ, Vehovszky A. Polycyclic neuromodulation of the feeding rhythm of the pond snail Lymnaea stagnalis by the intrinsic octopaminergic interneuron, OC. Brain Res. 2000 Dec 22;887(1):63–9.
67. Erspamer V. Active Substances in the Posterior Salivary Glands of Octopoda. I. Enteramine-like Substance. Acta Pharmacologica et Toxicologica. 1948;4(3–4):213–23.
68. Farooqui AA, Horrocks LA. Lipid peroxides in the free radical pathophysiology of brain diseases. Cell Mol Neurobiol. 1998 Dec;18(6):599–608.
69. Fei G, Guo C, Sun H-S, Feng Z-P. Chronic hypoxia stress-induced differential modulation of heat-shock protein 70 and presynaptic proteins. J Neurochem. 2007 Jan;100(1):50–61.
70. Feng ZP, Klumperman J, Lukowiak K, Syed NI. In vitro synaptogenesis between the somata of identified Lymnaea neurons requires protein synthesis but not extrinsic growth factors or substrate adhesion molecules. J Neurosci. 1997 Oct 15;17(20):7839–49.
71. Feng Z-P, Zhang Z, van Kesteren RE, Straub VA, van Nierop P, Jin K, et al. Transcriptome analysis of the central nervous system of the mollusc Lymnaea stagnalis. BMC Genomics. 2009 Sep 23;10:451.
72. Foster NL, Lukowiak K, Henry TB. Time-related expression profiles for heat shock protein gene transcripts (HSP40, HSP70) in the central nervous system of Lymnaea stagnalis exposed to thermal stress. Commun Integr Biol. 2015 Jun;8(3):e1040954.
73. Fromherz P, Offenhausser A, Vetter T, Weis J. A neuron-silicon junction: a Retzius cell of the leech on an insulated-gate field-effect transistor. Science. 1991 May 31;252(5010):1290–3.
74. Fromherz P, Stett A. Silicon-Neuron Junction: Capacitive Stimulation of an Individual Neuron on a Silicon Chip. Phys Rev Lett. 1995 Aug 21;75(8):1670–3.
75. Frost WN, Castellucci VF, Hawkins RD, Kandel ER. Monosynaptic connections made by the sensory neurons of the gill- and siphon-withdrawal reflex in Aplysia participate in the storage of long-term memory for sensitization. Proceedings of the National Academy of Sciences. 1 dicembre 1985;82(23):8266–9.
76. Fukaya T, Gondaira T, Kashiyae Y, Kotani S, Ishikura Y, Fujikawa S, et al. Arachidonic acid preserves hippocampal neuron membrane fluidity in senescent rats. Neurobiology of Aging. 2007 Aug;28(8):1179–86.
77. Fulton D, Kemenes I, Andrew RJ, Benjamin PR. A single time-window for protein synthesis-dependent long-term memory formation after one-trial appetitive conditioning. Eur J Neurosci. 2005 Mar;21(5):1347–58.
78. Gardzinski P, Lee DWK, Fei G-H, Hui K, Huang GJ, Sun H-S, et al. The role of synaptotagmin I C2A calcium-binding domain in synaptic vesicle clustering during synapse formation. J Physiol (Lond). 2007 May 15;581(Pt 1):75–90.
79. Geraerts WP. Neurohormonal control of growth and carbohydrate metabolism by the light green cells in Lymnaea stagnalis. Gen Comp Endocrinol. 1992 Jun;86(3):433–44.
80. Glanzman DL. New tricks for an old slug: the critical role of postsynaptic mechanisms in learning and memory in Aplysia. Prog Brain Res. 2008;169:277–92.
81. Goodman J, Packard MG. The influence of cannabinoids on learning and memory processes of the dorsal striatum. Neurobiol Learn Mem. 2015 Nov;125:1–14.
82. Ha TJ, Kohn AB, Bobkova YV, Moroz LL. Molecular characterization of NMDA-like receptors in Aplysia and Lymnaea: relevance to memory mechanisms. Biol Bull. 2006 Jun;210(3):255–70.
83. Haney J, Lukowiak K. Context learning and the effect of context on memory retrieval in Lymnaea. Learn Mem. 2001 Feb;8(1):35–43.
84. Harman D. Aging: a theory based on free radical and radiation chemistry. J Gerontol. 1956 Jul;11(3):298–300.
85. Hatakeyama D, Fujito Y, Sakakibara M, Ito E. Expression and distribution of transcription factor CCAAT/enhancer-binding protein in the central nervous system of Lymnaea stagnalis. Cell Tissue Res. 2004 Dec;318(3):631–41.
86. Hatakeyama D, Okuta A, Otsuka E, Lukowiak K, Ito E. Consolidation of long-term memory by insulin in Lymnaea is not brought about by changing the number of insulin receptors. Commun Integr Biol. 2013 May 1;6(3):e23955.
87. Hatakeyama D, Sadamoto H, Watanabe T, Wagatsuma A, Kobayashi S, Fujito Y, et al. Requirement of new protein synthesis of a transcription factor for memory consolidation: paradoxical changes in mRNA and protein levels of C/EBP. J Mol Biol. 2006 Feb 24;356(3):569–77.
88. Heni M, Kullmann S, Preissl H, Fritsche A, Häring H-U. Impaired insulin action in the human brain: causes and metabolic consequences. Nat Rev Endocrinol. 2015 Dec;11(12):701–11.
89. Hermann PM, Bulloch AG. Developmental plasticity of respiratory behavior in Lymnaea. Behav Neurosci. 1998 Jun;112(3):656–67.
90. Hermann PM, Lee A, Hulliger S, Minvielle M, Ma B, Wildering WC. Impairment of long-term associative memory in aging snails (Lymnaea stagnalis). Behav Neurosci. 2007 Dec;121(6):1400–14.
91. Hermitte G, Pedreira ME, Tomsic D, Maldonado H. Context shift and protein synthesis inhibition disrupt long-term habituation after spaced, but not massed, training in the crab Chasmagnathus. Neurobiol Learn Mem. 1999 Jan;71(1):34–49.
92. Hintzman D. Theoretical implications of the spacing effect. Theories in Cognitive Psychology; The Loyola Symposium. 1974 Jan 1;
93. Hongpaisan J, Alkon DL. A structural basis for enhancement of long-term associative memory in single dendritic spines regulated by PKC. Proceedings of the National Academy of Sciences. 2007 Dec 4;104(49):19571–6.
94. Hook SS, Means AR. Ca(2+)/CaM-dependent kinases: from activation to function. Annu Rev Pharmacol Toxicol. 2001;41:471–505.
95. Horrocks LA, Farooqui AA. Docosahexaenoic acid in the diet: its importance in maintenance and restoration of neural membrane function. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2004 Apr;70(4):361–72.
96. Hovland CI. Experimental studies in rote-learning theory. VI. Comparison of retention following learning to same criterion by massed and distributed practice. Journal of Experimental Psychology. 1940;26(6):568–87.
97. Hudmon A, Schulman H. Neuronal CA2+/calmodulin-dependent protein kinase II: the role of structure and autoregulation in cellular function. Annu Rev Biochem.

2002;71:473–510.
98. Hughes E, Shymansky T, Swinton E, Lukowiak KS, Swinton C, Sunada H, et al. Strain-specific differences of the effects of stress on memory in Lymnaea. J Exp Biol. 2017 01;220(Pt 5):891–9.
99. Inda MC, Delgado-García JM, Carrión AM. Acquisition, consolidation, reconsolidation, and extinction of eyelid conditioning responses require de novo protein synthesis. J Neurosci. 2005 Feb 23;25(8):2070–80.
100. Irvine EE, Vernon J, Giese KP. AlphaCaMKII autophosphorylation contributes to rapid learning but is not necessary for memory. Nat Neurosci. 2005 Apr;8(4):411–2.
101. Ito E, Kobayashi S, Kojima S, Sadamoto H, Hatakeyama D. Associative Learning in the Pond Snail, Lymnaea stagnalis. jzoo. 1999 Oct;16(5):711–23.
102. Ito E, Kojima S, Lukowiak K, Sakakibara M. From likes to dislikes: conditioned taste aversion in the great pond snail (Lymnaea stagnalis). Can J Zool. 2013 Mar 8;91(6):405–12.
103. Ito E, Yamagishi M, Hatakeyama D, Watanabe T, Fujito Y, Dyakonova V, et al. Memory block: a consequence of conflict resolution. Journal of Experimental Biology. 2015 Jun 1;218(11):1699–704.
104. Jenkner M, Müller B, Fromherz P. Interfacing a silicon chip to pairs of snail neurons connected by electrical synapses. Biological Cybernetics. 2001 Mar 23;84(4):239–49.
105. Joëls M, Pu Z, Wiegert O, Oitzl MS, Krugers HJ. Learning under stress: how does it work? Trends Cogn Sci (Regul Ed). 2006 Apr;10(4):152–8.
106. Jonas EA, Knox RJ, Kaczmarek LK, Schwartz JH, Solomon DH. Insulin receptor in Aplysia neurons: characterization, molecular cloning, and modulation of ion currents. J Neurosci. 1996 Mar 1;16(5):1645–58.
107. Jones JD. Aspects of respiration in Planorbis corneus L. and Lymnaea stagnalis L. (Gastropoda: Pulmonata). Comp Biochem Physiol. 1961 Dec;4:1–29.
108. Josselyn SA, Shi C, Carlezon WA, Neve RL, Nestler EJ, Davis M. Long-term memory is facilitated by cAMP response element-binding protein overexpression in the amygdala. J Neurosci. 2001 Apr 1;21(7):2404–12.
109. Kaang BK, Kandel ER, Grant SG. Activation of cAMP-responsive genes by stimuli that produce long-term facilitation in Aplysia sensory neurons. Neuron. 1993 Mar;10(3):427–35.
110. Kandel ER. The molecular biology of memory storage: a dialogue between genes and synapses. Science. 2001 Nov 2;294(5544):1030–8.
111. Kano T, Brockie PJ, Sassa T, Fujimoto H, Kawahara Y, Iino Y, et al. Memory in Caenorhabditis elegans is mediated by NMDA-type ionotropic glutamate receptors. Curr Biol. 2008 Jul 8;18(13):1010–5.
112. Karpinski BA, Morle GD, Huggenvik J, Uhler MD, Leiden JM. Molecular cloning of human CREB-2: an ATF/CREB transcription factor that can negatively regulate transcription from the cAMP response element. Proc Natl Acad Sci USA. 1992 Jun 1;89(11):4820–4.
113. Kashiyae Y, Kontani M, Kawashima H, Kiso Y, Kudo Y, Sakakibara M. Arachidonic acid enhances intracellular calcium levels in dentate gyrus, but not CA1, in aged rat. Neuroscience Research. 2009 Jun;64(2):143–51.
114. Kaul RA, Syed NI, Fromherz P. Neuron-Semiconductor Chip with Chemical Synapse between Identified Neurons. Phys Rev Lett. 2004 Jan 23;92(3):038102.
115. Kawai R, Kobayashi S, Fujito Y, Ito E. Multiple subtypes of serotonin receptors in the feeding circuit of a pond snail. Zool Sci. 2011 Jul;28(7):517–25.
116. Kemenes G, Benjamin PR. Appetitive learning in snails shows characteristics of conditioning in vertebrates. Brain Research. 1989 Jun;489(1):163–6.
117. Kemenes G, Benjamin PR. Lymnaea. Current Biology. 2009 Jan;19(1):R9–11.
118. Kemenes G, Kemenes I, Michel M, Papp A, Müller U. Phase-dependent molecular requirements for memory reconsolidation: differential roles for protein synthesis and protein kinase A activity. J Neurosci. 2006 Jun 7;26(23):6298–302.
119. Kemenes G, Staras K, Benjamin PR. In vitro appetitive classical conditioning of the feeding response in the pond snail Lymnaea stagnalis. J Neurophysiol. 1997 Nov;78(5):2351–62.
120. Kemenes G. Molecular and Cellular Mechanisms of Classical Conditioning in the Feeding System of Lymnaea. In: Handbook of Behavioral Neuroscience [Internet]. Elsevier; 2013 p. 251–64. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780124158238000204
121. Kemenes G., Hiripi L., Benjamin P. R. Behavioural and biochemical changes in the feeding system of Lymnaea induced by the dopamine and serotonin neurotoxins 6-hydroxydopamine and 5,6-dihydroxytryptamine. Philosophical Transactions of the Royal Society of London Series B: Biological Sciences. 1990 Sep 29;329(1254):243–55.
122. Kemenes I, Kemenes G, Andrew RJ, Benjamin PR, O'Shea M. Critical time-window for NO-cGMP-dependent long-term memory formation after one-trial appetitive conditioning. J Neurosci. 2002 Feb 15;22(4):1414–25.
123. Kemenes I, O'Shea M, Benjamin PR. Different circuit and monoamine mechanisms consolidate long-term memory in aversive and reward classical conditioning. Eur J Neurosci. 2011 Jan;33(1):143–52.
124. Kemenes I, Straub VA, Nikitin ES, Staras K, O'Shea M, Kemenes G, et al. Role of delayed nonsynaptic neuronal plasticity in long-term associative memory. Curr Biol. 2006 Jul 11;16(13):1269–79.
125. Kennedy CD, Houmes SW, Wyrick KL, Kammerzell SM, Lukowiak K, Sorg BA. Methamphetamine enhances memory of operantly conditioned respiratory behavior in the snail Lymnaea stagnalis. Journal of Experimental Biology. 2010 Jun 15;213(12):2055–65.
126. Kim B, Feldman EL. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Exp Mol Med. 2015 Mar;47(3):e149–e149
127. Kits KS, de Vries NJ, Ebberink RH. Molluscan insulin-related neuropeptide promotes neurite outgrowth in dissociated neuronal cell cultures. Neurosci Lett. 1990 Feb 16;109(3):253–8.
128. Klaassen LJ, Janse C, van der Roest M. Multiple synaptic connections of a single neuron change differentially with age. Neurobiol Aging. 1998 Aug;19(4):341–9.
129. Kobayashi S, Kojima S, Yamanaka M, Sadamoto H, Nakamura H, Fujito Y, et al. Operant Conditioning of Escape Behavior in the Pond Snail, Lymnaea stagnalis. jzoo. 1998 Oct;15(5):683–90.
130. Kodama E, Kuhara A, Mohri-Shiomi A, Kimura KD, Okumura M, Tomioka M, et al. Insulin-like signaling and the neural circuit for integrative behavior in C. elegans. Genes & Development. 1 novembre 2006;20(21):2955–60.
131. Koert CE, Spencer GE, van Minnen J, Li KW, Geraerts WP, Syed NI, et al. Functional implications of neurotransmitter expression during axonal regeneration: serotonin, but not peptides, auto-regulate axon growth of an identified central neuron. J Neurosci. 2001 Aug 1;21(15):5597–606.
132. Kojima S, Nanakamura H, Nagayama S, Fujito Y, Ito E. Enhancement of an inhibitory input to the feeding central pattern generator in Lymnaea stagnalis during conditioned taste-aversion learning. Neuroscience Letters. luglio 1997;230(3):179–82.
133. Kojima S, Yamanaka M, Fujito Y, Ito E. Differential Neuroethological Effects of Aversive and Appetitive Reinforcing Stimuli on Associative Learning in Lymnaea stagnalis. jzoo. 1996 Dec;13(6):803–12.
134. Korneev SA, Straub V, Kemenes I, Korneeva EI, Ott SR, Benjamin PR, et al. Timed and targeted differential regulation of nitric oxide synthase (NOS) and anti-NOS genes by reward conditioning leading to long-term memory formation. J Neurosci. 2005 Feb 2;25(5):1188–92.
135. Kotani S, Nakazawa H, Tokimasa T, Akimoto K, Kawashima H, Toyoda-Ono Y, et al. Synaptic plasticity preserved with arachidonic acid diet in aged rats. Neuroscience Research. 2003 Aug;46(4):453–61.
136. Kotani S, Sakaguchi E, Warashina S, Matsukawa N, Ishikura Y, Kiso Y, et al. Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. Neuroscience Research. 2006 Oct;56(2):159–64.
137. Kuzirian AM, Epstein HT, Gagliardi CJ, Nelson TJ, Sakakibara M, Taylor C, et al. Bryostatin Enhancement of Memory in *Hermissenda*. The Biological Bulletin. 2006 Jun;210(3):201–14.
138. Lapiedra O, Chejanovski Z, Kolbe JJ. Urbanization and biological invasion shape animal personalities. Glob Change Biol. febbraio 2017;23(2):592–603.
139. Lattal KM, Abel T. Different requirements for protein synthesis in acquisition and extinction of spatial preferences and context-evoked fear. J Neurosci. 2001 Aug 1;21(15):5773–80.
140. Li KW, Geraerts WP, Joosse J. Purification and sequencing of molluscan insulin-related peptide II from the neuroendocrine light green cells in Lymnaea stagnalis. Endocrinology. 1992 Jun;130(6):3427–32.
141. Lu YF, Kandel ER, Hawkins RD. Nitric oxide signaling contributes to late-phase LTP and CREB phosphorylation in the hippocampus. J Neurosci. 1999 Dec

1;19(23):10250–61.
142. Luck T, Luppa M, Briel S, Matschinger H, König H-H, Bleich S, et al. Mild cognitive impairment: incidence and risk factors: results of the leipzig longitudinal study of the aged. J Am Geriatr Soc. 2010 Oct;58(10):1903–10.
143. Lukowiak K, Ringseis E, Spencer G, Wildering W, Syed N. Operant conditioning of aerial respiratory behaviour in Lymnaea stagnalis. J Exp Biol. 1996;199(Pt 3):683–91.
144. Lukowiak K, Adatia N, Krygier D, Syed N. Operant conditioning in Lymnaea: evidence for intermediate- and long-term memory. Learn Mem. 2000 Jun;7(3):140–50.
145. Lukowiak K, Haque Z, Spencer G, Varshay N, Sangha S, Syed N. Long-term memory survives nerve injury and the subsequent regeneration process. Learn Mem. 2003 Feb;10(1):44–54.
146. Lukowiak K, Heckler B, Bennett TE, Schriner EK, Wyrick K, Jewett C, et al. Enhanced memory persistence is blocked by a DNA methyltransferase inhibitor in the snail Lymnaea stagnalis. J Exp Biol. 2014 Aug 15;217(Pt 16):2920–9.
147. Lukowiak K, Orr M, de Caigny P, Lukowiak KS, Rosenegger D, Han JI, et al. Ecologically relevant stressors modify long-term memory formation in a model system. Behav Brain Res. 2010 Dec 6;214(1):18–24.
148. Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE. The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain Cogn. 2007 Dec;65(3):209–37.
149. Mackintosh NJ. The psychology of animal learning. Oxford, England: Academic Press; 1974. xiv, 730. (The psychology of animal learning).
150. Mainardi M, Fusco S, Grassi C. Modulation of Hippocampal Neural Plasticity by Glucose-Related Signaling. Neural Plasticity. 2015;2015:1–10.
151. Malinow R, Schulman H, Tsien R. Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. Science. 1989 Aug 25;245(4920):862–6.
152. Martin KC, Michael D, Rose JC, Barad M, Casadio A, Zhu H, et al. MAP Kinase Translocates into the Nucleus of the Presynaptic Cell and Is Required for Long-Term Facilitation in Aplysia. Neuron. 1997 Jun 1;18(6):899–912.
153. McGaugh JL. Memory--a century of consolidation. Science. 2000 Jan 14;287(5451):248–51.
154. McGaugh JL. Time-Dependent Processes in Memory Storage. Science. 1966 Sep 16;153(3742):1351–8.
155. McGeoch JA. Forgetting and the law of disuse. Psychological Review. 1932;39(4):352–70.
156. McPartland JM. Phylogenomic and chemotaxonomic analysis of the endocannabinoid system. Brain Research Reviews. 2004 Apr 1;45(1):18–29.
157. Meester I, Ramkema MD, van Minnen J, Boer HH. Differential expression of four genes encoding molluscan insulin-related peptides in the central nervous system of the pond snail Lymnaea stagnalis. Cell Tissue Res. 1992 Jul;269(1):183–8.
158. Michel M, Kemenes I, Müller U, Kemenes G. Different phases of long-term memory require distinct temporal patterns of PKA activity after single-trial classical conditioning. Learn Mem. 2008 Sep;15(9):694–702.
159. Minami H, Dallenbach KM. The effect of activity upon learning and retention in the cockroach, Periplaneta americana. Am J Psychol. 1946 Jan;59:1–58.
160. Mita K, Yamagishi M, Fujito Y, Lukowiak K, Ito E. An increase in insulin is important for the acquisition conditioned taste aversion in Lymnaea. Neurobiol Learn Mem. 2014 Dec;116:132–8.
161. Mohammadi HS, Goudarzi I, Lashkarbolouki T, Abrari K, Elahdadi Salmani M. Chronic administration of quercetin prevent spatial learning and memory deficits provoked by chronic stress in rats. Behav Brain Res. 2014 Aug 15;270:196–205.
162. Moldow RL, Fischman AJ. Cocaine induced secretion of ACTH, beta-endorphin, and corticosterone. Peptides. settembre 1987;8(5):819–22.
163. Möller S, Saul N, Cohen AA, Köhling R, Sender S, Escobar HM, et al. Healthspan pathway maps in C. elegans and humans highlight transcription, proliferation/biosynthesis and lipids. bioRxiv. 2018 Aug 2;355131.
164. Montminy M. Transcriptional regulation by cyclic AMP. Annu Rev Biochem. 1997;66:807–22.
165. Morena M, Campolongo P. The endocannabinoid system: an emotional buffer in the modulation of memory function. Neurobiol Learn Mem. 2014 Jul;112:30–43.
166. Moroz LL, Winlow W, Turner RW, Bulloch AGM, Lukowiak K, Syed NI. Nitric oxide synthase-immunoreactive cells in the CNS and periphery of Lymnaea: NeuroReport. 1994 Jun;5(10):1277–80.
167. Morris RG, Anderson E, Lynch GS, Baudry M. Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. Nature. 1986 Mar 27;319(6056):774–6.
168. Müller U. The nitric oxide system in insects. Prog Neurobiol. 1997 Feb;51(3):363–81.
169. Murakami J, Okada R, Fujito Y, Sakakibara M, Lukowiak K, Ito E. Paired pulse ratio analysis of insulin-induced synaptic plasticity in the snail brain. J Exp Biol. 2013 May 15;216(Pt 10):1771–3.
170. Murakami J, Okada R, Sadamoto H, Kobayashi S, Mita K, Sakamoto Y, et al. Involvement of insulin-like peptide in long-term synaptic plasticity and long-term memory of the pond snail Lymnaea stagnalis. J Neurosci. 2013 Jan 2;33(1):371–83.
171. Muzzio IA, Talk AC, Matzel LD. Incremental redistribution of protein kinase C underlies the acquisition curve during in vitro associative conditioning in Hermissenda. Behav Neurosci. 1997 Aug;111(4):739–53.
172. Myers KM, Davis M. Behavioral and Neural Analysis of Extinction. Neuron. novembre 2002;36(4):567–84.
173. Nakamura H, Kobayashi S, Kojima S, Urano A, Ito E. PKA-Dependent Regulation of Synaptic Enhancement between a Buccal Motor Neuron and Its Regulatory Interneuron in Lymnaea stagnalis. jzoo. 1999 Jun;16(3):387–94.
174. Nelson TJ, Collin C, Alkon DL. Isolation of a G protein that is modified by learning and reduces potassium currents in Hermissenda. Science. 1990 Mar 23;247(4949 Pt 1):1479–83.
175. Nestler EJ, Hyman SE. Animal models of neuropsychiatric disorders. Nat Neurosci. 2010 Oct;13(10):1161–9.
176. Nestler EJ. Epigenetic mechanisms of drug addiction. Neuropharmacology. 2014 Jan;76 Pt B:259–68.
177. Niehof M, Manns MP, Trautwein C. CREB controls LAP/C/EBP beta transcription. Mol Cell Biol. 1997 Jul;17(7):3600–13.
178. Niki E. Free radical initiators as source of water- or lipid-soluble peroxyl radicals. Meth Enzymol. 1990;186:100–8.
179. Nikitin VP, Solntseva SV. Peculiarities of amnesia development during memory reconsolidation impairment induced by isolated or combined treatment with neurotransmitter receptor antagonists. Bull Exp Biol Med. 2013 May;155(1):6–10.
180. Olds JL, Anderson ML, McPhie DL, Staten LD, Alkon DL. Imaging of memory-specific changes in the distribution of protein kinase C in the hippocampus. Science. 1989 Aug 25;245(4920):866–9.
181. Orr MV, Lukowiak K. Electrophysiological and behavioral evidence demonstrating that predator detection alters adaptive behaviors in the snail Lymnaea. J Neurosci. 2008 Mar 12;28(11):2726–34.
182. Otsuka E, Matsunaga M, Okada R, Yamagishi M, Okuta A, Lukowiak K, et al. Increase in cyclic AMP concentration in a cerebral giant interneuron mimics part of a memory trace for conditioned taste aversion of the pond snail. Biophysics (Nagoya-shi). 2013;9:161–6.
183. Ottaviani E, Accorsi A, Rigillo G, Malagoli D, Blom JMC, Tascedda F. Epigenetic modification in neurons of the mollusc Pomacea canaliculata after immune challenge. Brain Res. 2013 Nov 6;1537:18–26.
184. Ottaviani E, Malagoli D, Franceschi C. Common evolutionary origin of the immune and neuroendocrine systems: from morphological and functional evidence to in silico approaches. Trends Immunol. 2007 Nov;28(11):497–502.
185. Park JH, Straub VA, O'Shea M. Anterograde signaling by nitric oxide: characterization and in vitro reconstitution of an identified nitrergic synapse. J Neurosci. 1998 Jul 15;18(14):5463–76.
186. Parvez K, Moisseev V, Lukowiak K. A context-specific single contingent-reinforcing stimulus boosts intermediate-term memory into long-term memory. Eur J Neurosci. 2006 Jul;24(2):606–16.
187. Pascale A, Govoni S, Battaini F. Age-related alteration of PKC, a key enzyme in memory processes: physiological and pathological examples. Mol Neurobiol. 1998 Feb;16(1):49–62.
188. Patel BA, Arundell M, Allen MC, Gard P, O'Hare D, Parker K, et al. Changes in the properties of the modulatory cerebral giant cells contribute to aging in the feeding system of Lymnaea. Neurobiol Aging. 2006 Dec;27(12):1892–901.

189. Pavlov (1927) PI. Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. Ann Neurosci. 2010 Jul;17(3):136–41.
190. Perazzona B, Isabel G, Preat T, Davis RL. The role of cAMP response element-binding protein in Drosophila long-term memory. J Neurosci. 2004 Oct 6;24(40):8823–8.
191. Perry CJ, Barron AB, Cheng K. Invertebrate learning and cognition: relating phenomena to neural substrate: Invertebrate learning and cognition. WIREs Cogn Sci. settembre 2013;4(5):561–82.
192. Pinna LA, Ruzzene M. How do protein kinases recognize their substrates? Biochim Biophys Acta. 1996 Dec 12;1314(3):191–225.
193. Pirger Z, Laszlo Z, Hiripi L, Hernadi L, Toth G, Lubics A, et al. Pituitary adenylate cyclase activating polypeptide (PACAP) and its receptors are present and biochemically active in the central nervous system of the pond snail Lymnaea stagnalis. J Mol Neurosci. 2010 Nov;42(3):464–71.
194. Pirger Z, Naskar S, László Z, Kemenes G, Reglődi D, Kemenes I. Reversal of age-related learning deficiency by the vertebrate PACAP and IGF-1 in a novel invertebrate model of aging: the pond snail (Lymnaea stagnalis). J Gerontol A Biol Sci Med Sci. 2014 Nov;69(11):1331–8.
195. Rajasethupathy P, Antonov I, Sheridan R, Frey S, Sander C, Tuschl T, et al. A role for neuronal piRNAs in the epigenetic control of memory-related synaptic plasticity. Cell. 2012 Apr 27;149(3):693–707.
196. Ramsey MM, Adams MM, Ariwodola OJ, Sonntag WE, Weiner JL. Functional characterization of des-IGF-1 action at excitatory synapses in the CA1 region of rat hippocampus. J Neurophysiol. 2005 Jul;94(1):247–54.
197. Ribeiro MJ, Schofield MG, Kemenes I, O'Shea M, Kemenes G, Benjamin PR. Activation of MAPK is necessary for long-term memory consolidation following food-reward conditioning. Learn Mem. 2005 Oct;12(5):538–45.
198. Ribeiro MJ, Serfozo Z, Papp A, Kemenes I, O'Shea M, Yin JCP, et al. Cyclic AMP response element-binding (CREB)-like proteins in a molluscan brain: cellular localization and learning-induced phosphorylation. Eur J Neurosci. 2003 Sep;18(5):1223–34.
199. Roberson ED, English JD, Adams JP, Selcher JC, Kondratick C, Sweatt JD. The mitogen-activated protein kinase cascade couples PKA and PKC to cAMP response element binding protein phosphorylation in area CA1 of hippocampus. J Neurosci. 1999 Jun 1;19(11):4337–48.
200. Roberts AC, Glanzman DL. Learning in Aplysia: looking at synaptic plasticity from both sides. Trends Neurosci. 2003 Dec;26(12):662–70.
201. Robinson L, McKillop-Smith S, Ross NL, Pertwee RG, Hampson RE, Platt B, et al. Hippocampal endocannabinoids inhibit spatial learning and limit spatial memory in rats. Psychopharmacology (Berl). 2008 Jul;198(4):551–63.
202. Roovers E, Vincent ME, van Kesteren E, Geraerts WP, Planta RJ, Vreugdenhil E, et al. Characterization of a putative molluscan insulin-related peptide receptor. Gene. 1995 Sep 11;162(2):181–8.
203. Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol Learn Mem. 2002 Nov;78(3):578–95.
204. Rosenegger D, Lukowiak K. The participation of NMDA receptors, PKC, and MAPK in the formation of memory following operant conditioning in Lymnaea. Mol Brain. 2010 Aug 31;3:24.
205. Rosenegger D, Parvez K, Lukowiak K. Enhancing memory formation by altering protein phosphorylation balance. Neurobiol Learn Mem. 2008 Oct;90(3):544–52.
206. Rosenegger D, Wright C, Lukowiak K. A quantitative proteomic analysis of long-term memory. Molecular Brain. 2010 Mar 23;3(1):9.
207. Rossato JI, Bevilaqua LRM, Lima RH, Medina JH, Izquierdo I, Cammarota M. On the participation of hippocampal p38 mitogen-activated protein kinase in extinction and reacquisition of inhibitory avoidance memory. Neuroscience. 2006 Nov 17;143(1):15–23.
208. Ruehle S, Rey AA, Remmers F, Lutz B. The endocannabinoid system in anxiety, fear memory and habituation. J Psychopharmacol. 2012 Jan;26(1):23–39.
209. Sadamoto H, Hatakeyama D, Kojima S, Fujito Y, Ito E. Histochemical study on the relation between NO-generative neurons and central circuitry for feeding in the pond snail, Lymnaea stagnalis. Neurosci Res. 1998 Sep;32(1):57–63.
210. Sadamoto H, Kitahashi T, Fujito Y, Ito E. Learning-Dependent Gene Expression of CREB1 Isoforms in the Molluscan Brain. Front Behav Neurosci. 2010;4:25.
211. Sadamoto H, Saito K, Muto H, Kinjo M, Ito E. Direct observation of dimerization between different CREB1 isoforms in a living cell. PLoS ONE. 2011;6(6):e20285.
212. Sadamoto H, Sato H, Kobayashi S, Murakami J, Aonuma H, Ando H, et al. CREB in the pond snail Lymnaea stagnalis: Cloning, gene expression, and function in identifiable neurons of the central nervous system. J Neurobiol. 2004 Mar;58(4):455–66.
213. Sakakibara M, Kawai R, Kobayashi S, Horikoshi T. Associative learning of visual and vestibular stimuli in Lymnaea. Neurobiol Learn Mem. 1998 Jan;69(1):1–12.
214. Sanchez-Mejia RO, Mucke L. Phospholipase A2 and arachidonic acid in Alzheimer's disease. Biochim Biophys Acta. 2010 Aug;1801(8):784–90.
215. Sandi C, Loscertales M, Guaza C. Experience-dependent Facilitating Effect of Corticosterone on Spatial Memory Formation in the Water Maze. European Journal of Neuroscience. aprile 1997;9(4):637–42.
216. Sandi C, Pinelo-Nava MT. Stress and Memory: Behavioral Effects and Neurobiological Mechanisms. Neural Plast. 2007. Available from:
217. Sangha S, McComb C, Lukowiak K. Forgetting and the extension of memory in Lymnaea. J Exp Biol. 2003a Jan;206(Pt 1):71–7.
218. Sangha S, Morrow R, Smyth K, Cooke R, Lukowiak K. Cooling blocks ITM and LTM formation and preserves memory. Neurobiol Learn Mem. 2003d Sep;80(2):130–9.
219. Sangha S, Scheibenstock A, Lukowiak K. Reconsolidation of a long-term memory in Lymnaea requires new protein and RNA synthesis and the soma of right pedal dorsal 1. J Neurosci. 2003b Sep 3;23(22):8034–40.
220. Sangha S, Scheibenstock A, Martens K, Varshney N, Cooke R, Lukowiak K. Impairing forgetting by preventing new learning and memory. Behav Neurosci. 2005 Jun;119(3):787–96.
221. Sangha S, Scheibenstock A, Mc Comb C, Lukowiak K, Intermediate and long-term memories of associative learning are differentially affected by transcription versus translation blockers in Lymnaea. Journal of Experimental Biology 2003c 206, 1605–1613
222. Schacter DL, Chiao JY, Mitchell JP. The seven sins of memory: implications for self. Ann N Y Acad Sci. 2003 Oct;1001:226–39.
223. Scheibenstock A, Krygier D, Haque Z, Syed N, Lukowiak K. The Soma of RPeD1 Must Be Present for Long-Term Memory Formation of Associative Learning in Lymnaea. Journal of Neurophysiology. 2002 Oct 1;88(4):1584–91.
224. Schmidt HD, McGinty JF, West AE, Sadri-Vakili G. Epigenetics and Psychostimulant Addiction. Cold Spring Harbor Perspectives in Medicine. 1 marzo 2013;3(3):a012047–a012047.
225. Schwaerzel M, Jaeckel A, Mueller U. Signaling at A-Kinase Anchoring Proteins Organizes Anesthesia-Sensitive Memory in Drosophila. J Neurosci. 2007 Jan 31;27(5):1229–33.
226. Sekiguchi T, Suzuki H, Yamada A, Mizukami A. Cooling-induced retrograde amnesia reflexes Pavlovian conditioning associations in Limax flavus. Neuroscience Research. gennaio 1994;18(4):267–75.
227. Selcher JC, Weeber EJ, Varga AW, Sweatt JD, Swank M. Protein kinase signal transduction cascades in mammalian associative conditioning. Neuroscientist. 2002 Apr;8(2):122–31.
228. Sharma SK, Bagnall MW, Sutton MA, Carew TJ. Inhibition of calcineurin facilitates the induction of memory for sensitization in Aplysia: requirement of mitogen-activated protein kinase. Proc Natl Acad Sci USA. 2003 Apr 15;100(8):4861–6.
229. Sharma SK, Carew TJ. The roles of MAPK cascades in synaptic plasticity and memory in Aplysia: facilitatory effects and inhibitory constraints. Learn Mem. 2004 Aug;11(4):373–8.
230. Shimizu E, Tang YP, Rampon C, Tsien JZ. NMDA receptor-dependent synaptic reinforcement as a crucial process for memory consolidation. Science. 2000 Nov 10;290(5494):1170–4.
231. Silva AJ, Kogan JH, Frankland PW, Kida S. CREB and memory. Annu Rev Neurosci. 1998;21:127–48.
232. Silva AJ. Molecular and cellular cognitive studies of the role of synaptic plasticity in memory. J Neurobiol. 2003 Jan;54(1):224–37.
233. Smit AB, Syed NI, Schaap D, van Minnen J, Klumperman J, Kits KS, et al. A glia-derived acetylcholine-binding protein that modulates synaptic transmission. Nature. 2001 May 17;411(6835):261–8.
234. Smit AB, Thijsen SF, Geraerts WP, Meester I, van Heerikhuizen H, Joosse J. Characterization of a cDNA clone encoding molluscan insulin-related peptide V of Lymnaea stagnalis. Brain Res Mol Brain Res. 1992 Jun;14(1–2):7–12.
235. Smit AB, Vreugdenhil E, Ebberink RH, Geraerts WP, Klootwijk J, Joosse J. Growth-controlling molluscan neurons produce the precursor of an insulin-related peptide.

Nature. 1988 Feb 11;331(6156):535–8.
236. Spencer GE, Syed NI, Lukowiak K. Neural changes after operant conditioning of the aerial respiratory behavior in Lymnaea stagnalis. J Neurosci. 1999 Mar 1;19(5):1836–43.
237. Spiteller G. Is lipid peroxidation of polyunsaturated acids the only source of free radicals that induce aging and age-related diseases? Rejuvenation Res. 2010 Feb;13(1):91–103.
238. Stankiewicz AM, Swiergiel AH, Lisowski P. Epigenetics of stress adaptations in the brain. Brain Res Bull. 2013 Sep;98:76–92.
239. Stefano G, Mantione K, Casares F, M. Kream R. Anaerobically functioning mitochondria: Evolutionary perspective on modulation of energy metabolism in Mytilus edulis. Invertebrate Survival Journal. 2015 Jan 1;12:22–8.
240. Straub VA, Grant J, O'Shea M, Benjamin PR. Modulation of serotonergic neurotransmission by nitric oxide. J Neurophysiol. 2007 Feb;97(2):1088–99.
241. Sugai R, Azami S, Shiga H, Watanabe T, Sadamoto H, Kobayashi S, et al. One-trial conditioned taste aversion in Lymnaea: good and poor performers in long-term memory acquisition. J Exp Biol. 2007 Apr;210(Pt 7):1225–37.
242. Sultana R, Butterfield DA. Role of oxidative stress in the progression of Alzheimer's disease. J Alzheimers Dis. 2010;19(1):341–53.
243. Sun M-K, Alkon DL. Synergistic effects of chronic bryostatin-1 and α-tocopherol on spatial learning and memory in rats. European Journal of Pharmacology. 2008 Apr;584(2–3):328–37.
244. Sunada H, Riaz H, de Freitas E, Lukowiak K, Swinton C, Swinton E, et al. Heat stress enhances LTM formation in Lymnaea: role of HSPs and DNA methylation. J Exp Biol. 2016 01;219(Pt 9):1337–45.
245. Sunada H, Takigami S, Lukowiak K, Sakakibara M. Electrophysiological characteristics of feeding-related neurons after taste avoidance Pavlovian conditioning in Lymnaea stagnalis. Biophysics (Nagoya-shi). 2014;10:121–33.
246. Sunada H, Watanabe T, Hatakeyama D, Lee S, Forest J, Sakakibara M, et al. Pharmacological effects of cannabinoids on learning and memory in Lymnaea. J Exp Biol. 2017 01;220(Pt 17):3026–38.
247. Swinton C, Swinton E, Shymansky T, Hughes E, Zhang J, Rothwell C, et al. Configural learning: a higher form of learning in Lymnaea. J Exp Biol. 2019 Feb 1;222(3):jeb190405.
248. Syed NI, Bulloch AG, Lukowiak K. In vitro reconstruction of the respiratory central pattern generator of the mollusk Lymnaea. Science. 1990 Oct 12;250(4978):282–5.
249. Syed NI, Ridgway RL, Lukowiak K, Bulloch AG. Transplantation and functional integration of an identified respiratory interneuron in Lymnaea stagnalis. Neuron. 1992 Apr;8(4):767–74.
250. Syed NI, Winlow W. Coordination of locomotor and cardiorespiratory networks of Lymnaea stagnalis by a pair of identified interneurones. J Exp Biol. 1991 Jul;158:37–62.
251. Szapiro G, Vianna MRM, McGaugh JL, Medina JH, Izquierdo I. The role of NMDA glutamate receptors, PKA, MAPK, and CAMKII in the hippocampus in extinction of conditioned fear. Hippocampus. 2003;13(1):53–8.
252. Takahashi T, Takigami S, Sunada H, Lukowiak K, Sakakibara M. Critical Period of Memory Enhancement during Taste Avoidance Conditioning in Lymnaea stagnalis. Skoulakis EMC, editor. PLoS ONE. 2013 Oct 3;8(10):e75276.
253. Takigami S, Sunada H, Lukowiak K, Kuzirian AM, Alkon DL, Sakakibara M. Protein kinase C mediates memory consolidation of taste avoidance conditioning in Lymnaea stagnalis. Neurobiology of Learning and Memory. 2014a May;111:9–18.
254. Takigami S, Sunada H, Lukowiak K, Sakakibara M. High voltage with little current as an unconditional stimulus for taste avoidance conditioning in Lymnaea stagnalis. Neurosci Lett. 2013 Oct 25;555:149–53.
255. Takigami S, Sunada H, Lukowiak K, Sakakibara M. Spaced taste avoidance conditioning in Lymnaea. Neurobiology of Learning and Memory. 2014b Jan;107:79–86.
256. Tan H, Ahmad T, Loureiro M, Zunder J, Laviolette SR. The role of cannabinoid transmission in emotional memory formation: implications for addiction and schizophrenia. Front Psychiatry. 2014;5:73.
257. Tascedda F, Malagoli D, Accorsi A, Rigillo G, Blom JMC, Ottaviani E. Molluscs as models for translational medicine. Med Sci Monit Basic Res. 2015 Apr 30;21:96–9.
258. Taylor BE, Lukowiak K. The respiratory central pattern generator of Lymnaea: a model, measured and malleable. Respir Physiol. 2000 Sep;122(2–3):197–207.
259. Ter Maat A. Egg laying in the hermaphrodite pond snail Lymnaea stagnalis. Prog Brain Res. 1992;92:345–60.
260. Terranova JP, Storme JJ, Lafon N, Péŕio A, Rinaldi-Carmona M, Le Fur G, et al. Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. Psychopharmacology (Berl). 1996 Jul;126(2):165–72.
261. Teskey ML, Lukowiak KS, Riaz H, Dalesman S, Lukowiak K. What's hot: the enhancing effects of thermal stress on long-term memory formation in Lymnaea stagnalis. J Exp Biol. 2012 Dec 15;215(Pt 24):4322–9.
262. Thomas GM, Huganir RL. MAPK cascade signalling and synaptic plasticity. Nat Rev Neurosci. 2004 Mar;5(3):173–83.
263. Totani Y, Aonuma H, Oike A, Watanabe T, Hatakeyama D, Sakakibara M, et al. Monoamines, Insulin and the Roles They Play in Associative Learning in Pond Snails. Front Behav Neurosci. 2019;13:65.
264. Tramutola A, Lanzillotta C, Barone E, Arena A, Zuliani I, Mosca L, et al. Intranasal rapamycin ameliorates Alzheimer-like cognitive decline in a mouse model of Down syndrome. Transl Neurodegener. 2018;7:28.
265. Tronson NC, Wiseman SL, Olausson P, Taylor JR. Bidirectional behavioral plasticity of memory reconsolidation depends on amygdalar protein kinase A. Nat Neurosci. 2006 Feb;9(2):167–9.
266. Tsien JZ, Huerta PT, Tonegawa S. The essential role of hippocampal CA1 NMDA receptor-dependent synaptic plasticity in spatial memory. Cell. 1996 Dec 27;87(7):1327–38.
267. Valjent E, Caboche J, Vanhoutte P. Mitogen-activated protein kinase/extracellular signal-regulated kinase induced gene regulation in brain: a molecular substrate for learning and memory? Mol Neurobiol. 2001 Jun;23(2–3):83–99.
268. van Minnen J, Smit AB, Joosse J. Central and peripheral expression of genes coding for egg-laying inducing and insulin-related peptides in a snail. Arch Histol Cytol. 1989;52 Suppl:241–52.
269. Wan H, Mackay B, Iqbal H, Naskar S, Kemenes G. Delayed intrinsic activation of an NMDA-independent CaM-kinase II in a critical time window is necessary for late consolidation of an associative memory. J Neurosci. 2010 Jan 6;30(1):56–63.
270. Wang H, Shimizu E, Tang Y-P, Cho M, Kyin M, Zuo W, et al. Inducible protein knockout reveals temporal requirement of CaMKII reactivation for memory consolidation in the brain. Proc Natl Acad Sci USA. 2003 Apr 1;100(7):4287–92.
271. Watson SN, Nelson MA, Wildering WC. Redox agents modulate neuronal activity and reproduce physiological aspects of neuronal aging. Neurobiol Aging. 2012 Jan;33(1):149–61.
272. Watson SN, Wright N, Hermann PM, Wildering WC. Phospholipase A_2: the key to reversing long-term memory impairment in a gastropod model of aging. Neurobiol Aging. 2013 Feb;34(2):610–20.
273. Wildering WC, van der Roest M, de Vlieger TA, Janse C. Age-related changes in junctional and non-junctional conductances in two electrically coupled peptidergic neurons of the mollusc Lymnaea stagnalis. Brain Res. 1991 Apr 26;547(1):89–98.
274. Winlow W, Syed NI. The respiratory central pattern generator of Lymnaea. Acta Biol Hung. 1992;43(1–4):399–408.
275. Xia S, Miyashita T, Fu T-F, Lin W-Y, Wu C-L, Pyzocha L, et al. NMDA receptors mediate olfactory learning and memory in Drosophila. Curr Biol. 2005 Apr 12;15(7):603–15.
276. Xia S-Z, Feng C-H, Guo A-K. Temporary Amnesia Induced by Cold Anesthesia and Hypoxia in Drosophila. Physiology & Behavior. ottobre 1998;65(4–5):617–23.
277. Yamaguchi M, Yoshida H. Drosophila as a Model Organism. Adv Exp Med Biol. 2018;1076:1–10.
278. Yamanaka M, Hatakeyama D, Sadamoto H, Kimura T, Ito E. Development of key neurons for learning stimulates learning ability in Lymnaea stagnalis. Neurosci Lett. 2000 Jan 7;278(1–2):113–6.
279. Yehuda S, Rabinovitz S, Lcarasso R, Imostofsky D. The role of polyunsaturated fatty acids in restoring the aging neuronal membrane. Neurobiology of Aging. 2002

Sep;23(5):843–53.
280. Yeoman MS, Pieneman AW, Ferguson GP, Ter Maat A, Benjamin PR. Modulatory role for the serotonergic cerebral giant cells in the feeding system of the snail, Lymnaea. I. Fine wire recording in the intact animal and pharmacology. J Neurophysiol. 1994 Sep;72(3):1357–71.
281. Yerkes RM, Dodson JD. The relation of strength of stimulus to rapidity of habit-formation. Journal of Comparative Neurology and Psychology. 1908;18(5):459–82.
282. Yin JCP, Wallach JS, Del Vecchio M, Wilder EL, Zhou H, Quinn WG, et al. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell. 1994 Oct 7;79(1):49–58.
283. Zeck G, Fromherz P. Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip. Proceedings of the National Academy of Sciences. 2001 Aug 28;98(18):10457–62.
284. Zhang G-R, Wang X, Kong L, Lu X-G, Lee B, Liu M, et al. Genetic Enhancement of Visual Learning by Activation of Protein Kinase C Pathways in Small Groups of Rat Cortical Neurons. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2005 Oct 1;25:8468–81.
285. Zovkic IB, Guzman-Karlsson MC, Sweatt JD. Epigenetic regulation of memory formation and maintenance. Learn Mem. 2013 Jan 15;20(2):61–74.

Figure Captions

Fig. 1 – Schematic representation of the molecular mechanisms implicated in memory formation in *Lymnaea.* LymCREB-driven transcription results downstream of:

- the activation of AMPc by LymPACAP, which, in turn, mediates almost all its actions through Lym-PKA and the subsequent activation of LymMAPK;
- the entrance of Ca2+ through NMDARs, that activates directly or indirectly numerous protein kinases, including PKC and LymCaKMII, together with LymNOS. LymNOS, for its part, promotes the synthesis of LymNO, which regulates LymCREB activation, acting via LymPKG.

After phosphorylation LymCREB1 initiated a cascade of altered gene activity and new protein synthesis, necessary for synaptic enhancement in memory consolidation, acting via LymC/EBP and IGF1. In contrast, LymCREB2 inhibited the function of LymCREB1 and the ratio of activator/repressor LymCREBs has been proposed to act as a "molecular switch" in determining whether LTM is formed. Even if LymPKA, LymNMDA receptors, LymCaMKII, LymCREB, and LymNOS/NO are selectively activated or upregulated, it seems likely that these and other signalling molecules are part of a synergistic effort and together contribute to the memory consolidation process, with none of them alone being sufficient for LTM

Adapted from Kemenes, 2013

Fig. 2 – Different time windows of the key molecular targets necessary for memory in snails, in the 0h to 6h (acquisition/early consolidation) and 24hr time window (late consolidation).

During the acquisition phase of memory LymPACAP, LymPKA, LymNO, LymGRIN, LymCaMKII and LymMAPK are activated. During the first hours of memory formation and consolidation (defined as early and intermediate term consolidation phase) the activation of LymPKA, LymNO, LymCamKII, LymMAPK, LymPKA and LymCREB occurs, together with the transcription and the synthesis of new mRNA and proteins respectively. LymPKA is also activated during the intermediate consolidation phase (5-6 hr), when the synthesis of new proteins occurs. Finally, in the last phase of memory consolidation, LymCaMKII is expressed.

Adapted from Kemenes, 2013

TABLE 1 Abbreviations of used Acronyms

AC	adenylate cyclase
AMPc	the homologous of Cyclic Adenosine Monophosphate
LymPACAP	the homologous of pituitary adenylate cyclase-activating polypeptide
LymPKA	the homologous of protein kinase A
LymCaKMII	the homologous of calcium calmodulin-dependent protein kinase II
LymMAPK	the homologous of mitogen-associated protein kinase
LymGRIN	the homologous of ionotropic glutamatergic NMDA receptors
LymNOS	the homologous of nitric oxide synthase
LymNO	the homologous of nitric oxide
LymPKG	the homologous of protein kinase G
LymCREB	the homologous of cAMP response element-binding protein
LymC/EBP	the homologous of CCAAT/enhancer binding protein
Lym-IGF2	the homologous of insulin growth factor 2
MIP II	Molluscan insulin-related peptide
5-HT	5-hydroxytryptamine - serotonin
DA	Dopamine
HSPs	Heat shock protein
LymCBrs	The homologous of cannabinoid receptors
PLA_2	Phospholipase A_2
FFAs	Free fatty acids
PUFAs	Poly-unsaturated fatty acids
CNS	Central nervous system
STM	Short-term memory
ITM	Intermediate-term memory
LTM	Long-term memory
CS	Conditioned stimulus
US	Unconditioned stimulus
CTA	Conditioned taste aversion
RPeD1	Right pedal dorsal 1 interneuron
CGCs	Cerebral giant cells
PTSD	Post-traumatic stress disorder

FIGURE 1.TIF

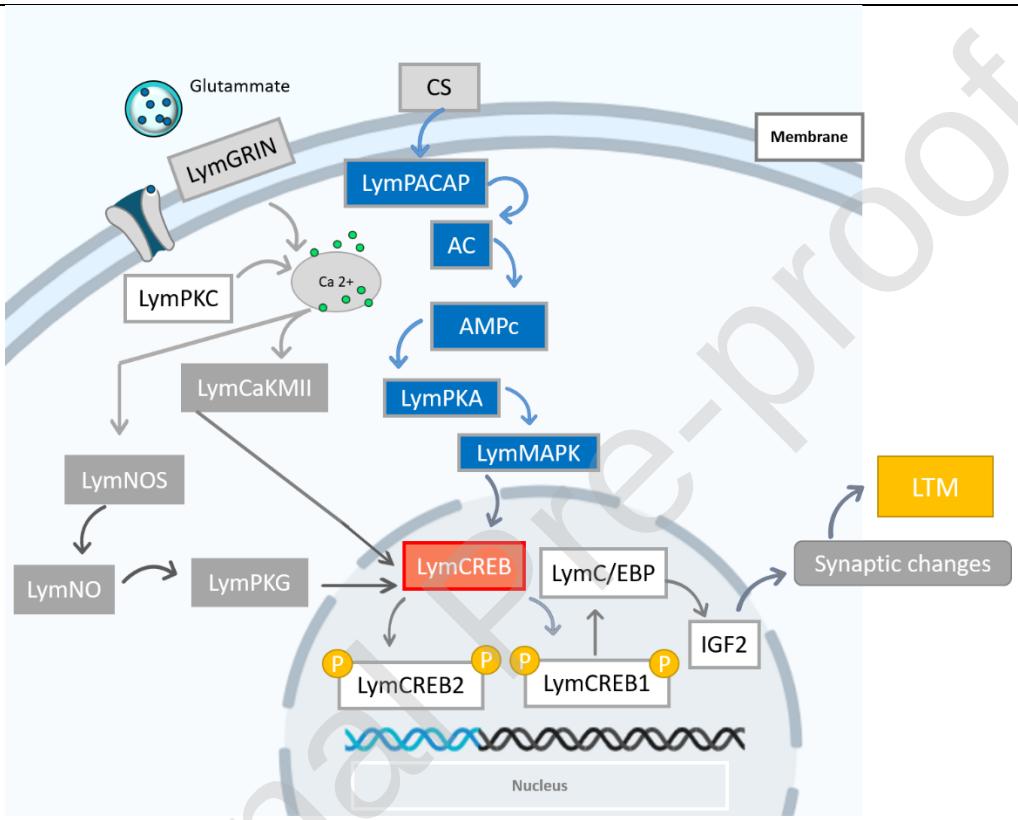

FIGURE 2.TIF