© 2023. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2023) 226, jeb244844. doi:10.1242/jeb.244844

COMMENTARY

CENTENARY ARTICLE

100 YEARS OF DISCOVERY

Invertebrates as models of learning and memory: investigating neural and molecular mechanisms

Veronica Rivi[1,*], Cristina Benatti[1,2], Giovanna Rigillo[3] and Joan M. C. Blom[2,3]

ABSTRACT

In this Commentary, we shed light on the use of invertebrates as model organisms for understanding the causal and conserved mechanisms of learning and memory. We provide a condensed chronicle of the contribution offered by mollusks to the studies on how and where the nervous system encodes and stores memory and describe the rich cognitive capabilities of some insect species, including attention and concept learning. We also discuss the use of planarians for investigating the dynamics of memory during brain regeneration and highlight the role of stressful stimuli in forming memories. Furthermore, we focus on the increasing evidence that invertebrates display some forms of emotions, which provides new opportunities for unveiling the neural and molecular mechanisms underlying the complex interaction between stress, emotions and cognition. In doing so, we highlight experimental challenges and suggest future directions that we expect the field to take in the coming years, particularly regarding what we, as humans, need to know for preventing and/or delaying memory loss.

This article has an associated ECR Spotlight interview with Veronica Rivi.

KEY WORDS: Cognitive function, Stress, Emotions, Evolution, Neuro-regeneration, Aging

The contributions of invertebrates to comparative neuroscience

What do the experiments that revealed the cellular components of the brain (Ramón y Cajal, 1894; Garcia-Lopez et al., 2010), described the mechanisms of nerve impulse transmission (Castellucci and Kandel, 1974; Hodgkin and Huxley, 1952; Katz, 1949, 2016), led to the identification of the neurotransmitter gamma-aminobutyric acid (Florey, 1991), and led to the characterization of the molecular basis of learning and memory have in common?

They have all marked the history of neuroscience, have earned numerous Nobel Prizes, and, not least, were all conducted in invertebrate organisms.

Although invertebrates possess small nervous systems, consisting of a limited number of neurons or ganglia, they are not limited in their ability to produce sophisticated and complex behaviors or even high-order forms of learning (see Glossary) (Preuss, 1995). Indeed, many invertebrates possess large neurons which facilitated microelectrode recordings and allowed the characterization of the neural and molecular basis of learning and memory in a comparative context (Kandel and Kupfermann, 1970).

The evolutionary process that prompted the diversity among species also promoted the conservation of numerous key physiological processes that are well preserved across taxa (Pembroke et al., 2021). By virtue of this, although invertebrates possess small brains and are phylogenetically distant from mammals, they have been and still are of fundamental importance in understanding basic neuroscience and in accelerating the pace at which mammalian studies can be translated to humans (Fig. 1) (Rivi et al., 2020). While maintaining the simple organization of the invertebrate nervous system, the behavioral repertoires and cognitive abilities of mollusks and arthropods have been shown to be highly comparable to those of mammals (Benjamin et al., 1985; Carew et al., 1981; Crow and Alkon, 1978, 1980; Lederhendler and Alkon, 1987; Strausfeld, 2012). This unique combination of simple nervous systems and complex behaviors provided a major contribution to the characterization of the conserved mechanisms by which memory is formed and stored. Thus, the results obtained in invertebrates have been translated first to mammalian models and then to humans.

Note that this Commentary is not intended to be an exhaustive review of the invertebrate model systems that have been used to study the behavioral, cellular and molecular mechanisms of learning and memory. Owing to space limitations, we have restricted our discussion to selected mollusks and insects (especially flies and bees) as well as crayfish and planaria, but other extremely innovative work on learning and memory mechanisms using models such as *Caenorhabditis elegans* and cephalopod mollusks is worth mentioning and has been recently reviewed by Rahmani and Chew (2021) and Schnell et al. (2021).

Groundbreaking theories on memory formation and storage

The ability to form memories has profound effects on an organism's life and survival (Nairne and Pandeirada, 2016). The knowledge of past experiences allows animals to plastically respond to present challenges and thus promote adaptation to ever-changing environments (Bisaz et al., 2014). Given the importance of memory, many attempts have been made to characterize the cellular and molecular processes involved in cognitive functions and to localize the physical trace of a memory, which is known as an engram (Dudai and Eisenberg, 2004).

The idea that memory is stored as lasting changes in the brain dates back at least to Plato and Aristotle's time (~2400 years ago), but its scientific articulation emerged in 1894 when Cajal first proposed that memory is stored as an anatomical change in the strength of neuronal connections (Bailey et al., 2000). For the following 50 years, little evidence was gathered to support this idea, until Hebb in 1949 proposed a model that memory is established through changes in the number and strength of synaptic connections between neurons (Hebb, 1949). This theory paved the way for studies performed in the 1960s and early 1970s aimed at investigating how changes in behavior

---

[1]Department of Life Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy. [2]Centre of Neuroscience and Neurotechnology, University of Modena and Reggio Emilia, 41125 Modena, Italy. [3]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, 41125 Modena, Italy.

*Author for correspondence (veronica.rivi@unimore.it)

V.R., 0000-0002-8413-4510; C.B., 0000-0003-0236-9525; G.R., 0000-0002-8853-4431; J.M.C.B., 0000-0002-4974-1964

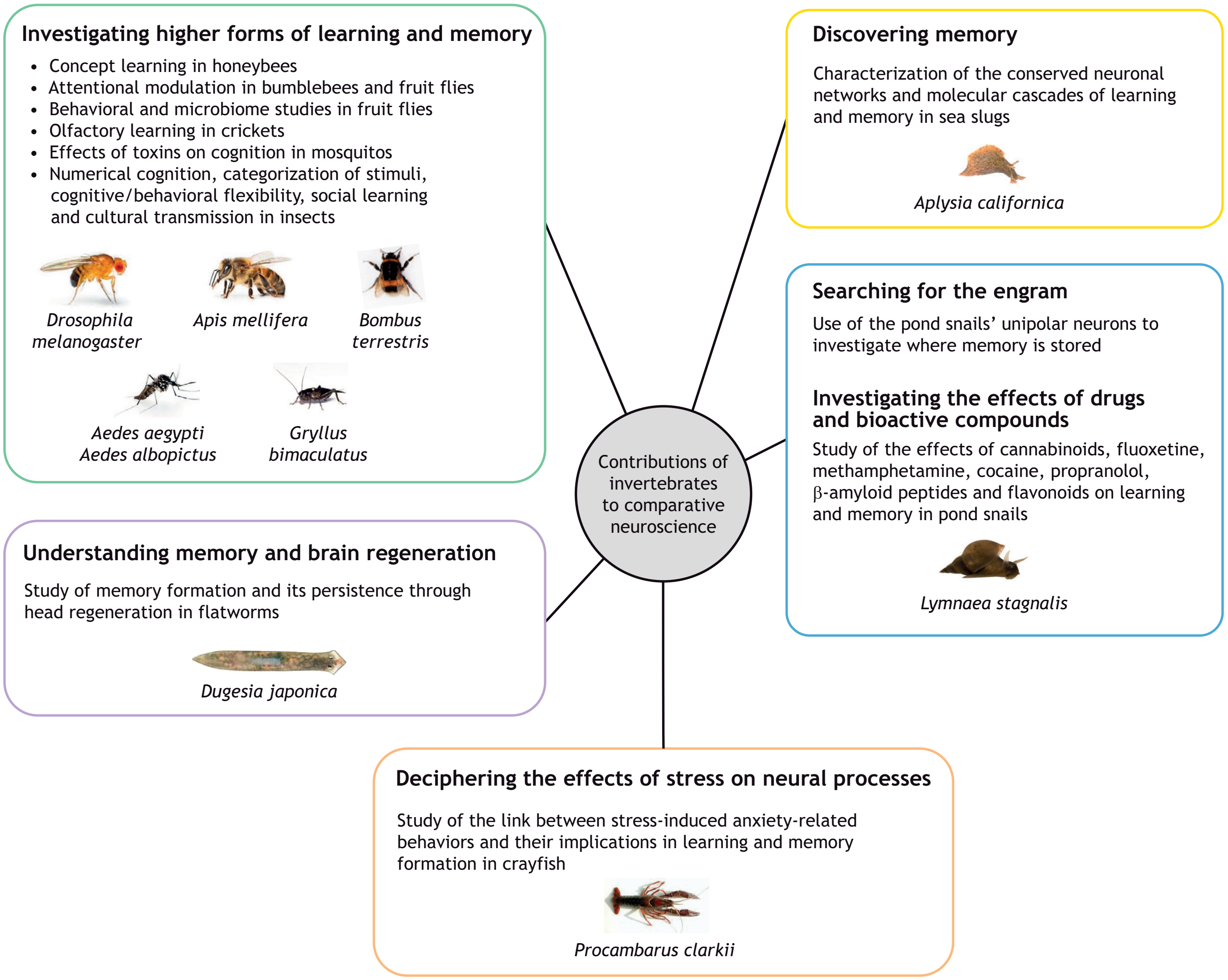

Fig. 1. Conceptual summary figure of the key contributions of invertebrates to the field of memory and learning.

resulting from training procedures reflect changes in the activity of specific neurons.

Discovering memory with the help of *Aplysia californica*

Whereas the majority of the scientific community was focused on characterizing the memory-related processes in rodent models, brilliant and revolutionary researchers such as Tauc and Kandel made the radical decision to focus their research on an invertebrate model, the sea slug *Aplysia californica*. They believed – correctly – that this model would make the study of the fundamental and conserved neuronal and molecular events associated with memory and learning easier. In the chapter 'Searching for an ideal system to study memory' of his 2007 book, Kandel (2007) defines his approach as 'reductionist', aspiring to replicate Pavlov's conditioning procedures in *A. californica*. Faria (2020) explains how, by taking advantage of the direct link between synaptic plasticity and behavior, Kandel characterized the neuronal networks and molecular cascades of learning and memory. These aspirations were later translated into results that earned Kandel, Carlsson and Greengard the Nobel Prize in Physiology or Medicine for their discoveries on the conserved mechanisms of synaptic plasticity (Carew et al., 1971; Kandel et al., 2014).

Over the past decades, this model contributed to new evidence about learning and memory processes and their alterations in pathological conditions (Abrams, 2012; Hawkins, 2013). For example, the molecular mechanisms that mediate the attention-like process in *Aplysia* have proved to be highly conserved, thus helping in characterization of attentional processes in mammals (Hawkins, 2013). *Aplysia* also provides a unique model to examine the effects of age on learning, memory and arousal. In fact, it has been demonstrated in *Aplysia* that aging: (1) impairs the long-term retention of habituation (see Glossary), (2) prevents the acquisition of sensitization in the siphon withdrawal reflex (see Glossary) and (3) reduces arousal, reflecting the age-dependent alterations of behavioral plasticity reported in vertebrates, including *Homo sapiens* (Bailey et al., 1983). Furthermore, as the molecular mechanisms underlying the effects of age on behavior share common features across phyla, the short lifespan of *Aplysia* and the simplicity of its central nervous system (CNS), represent important advantages for neuro-aging studies (Kron et al., 2020).

Inspired and encouraged by the early results obtained using *A. californica*, multitudes of neuroscientists all around the world exploited the 'simpler' nervous systems of invertebrates to define the synaptic and integrative properties of neural circuits that control

Glossary

Classical conditioning
Also known as 'Pavlovian conditioning', this form of learning consists of the temporal-contingent association between two stimuli: an initially neutral stimulus (the conditional stimulus, CS) and a biologically relevant stimulus (the unconditional stimulus, US). By the temporal and forward pairing of the CS with the US, the CS evokes a response that is similar to the response (i.e. behavior) that the US evoked (Bitterman, 2006; Pavlov, 1997).

Cognitive/behavioral flexibility
An organism's ability to appropriately and efficiently adjust its behavior according to a changing environment; most commonly measured with task switching and set-shifting tasks (Armbruster et al., 2012).

Conditioned taste aversion (CTA)
Learned association between the taste of a particular food and a negative stimulus such that the food is considered to be the cause of the aversive systemic effect. As a result of the learned association, there is a hedonic shift from positive to negative in the preference of that specific taste (Schier et al., 2019).

Founder effects
The reduction in genomic variability that occurs when a small group of individuals becomes separated from a larger population. Over time, the resulting new subpopulation will have genotypes and physical traits resembling the initial small, separated group and these may be very different from the original larger population (National Human Genome Research Institute).

Genetic drift
Mechanism of evolution characterized by random fluctuations in the frequency of a particular version of a gene (allele) in a population. Although it primarily affects small, isolated populations, the effects of genetic drift can be strong, sometimes causing traits to become overwhelmingly frequent or to disappear from a population (National Human Genome Research Institute).

Habituation
Learned decreased responsiveness to a stimulus with repeated presentation and is often adaptive in that it makes it less likely that individuals will respond to harmless stimuli. The counterpart to habituation is sensitization (see below) (Blumstein, 2016).

Higher forms of learning
Cognitive abilities that extend beyond 'simple' associative learning.

Operant conditioning
Form of learning that takes place through rewards (i.e. positive reinforcement) and/or punishments (i.e. negative reinforcement) for different behavioral patterns. The main basic principle of this form of learning is the association between an individual's behavior and the response or consequence to that particular behavior.

Sensitization
The increased responsiveness to a stimulus with repeated presentation (Blumstein, 2016).

Siphon withdrawal reflex
A behavioral paradigm developed by Kandel and colleagues, using a light tactile stimulus to the siphon as a conditioned stimulus (CS), which produces weak siphon and gill withdrawal, and a strong electric shock to the tail as the unconditioned stimulus (US), which produces a massive withdrawal reflex. The specific temporal pairing of the CS and US endows the CS with the ability to trigger enhanced withdrawal of both the siphon and the gill (Carew et al., 1981, 1983; Hawkins et al., 1989).

behaviors and they are conditioned (Nader, 2015). Subsequently, an increasing number of invertebrate models entered the learning and memory field.

Searching for the engram at (pond) snail's pace: the powerful model system *Lymnaea stagnalis*

In 1950, Lashley published 'In Search of the Engram', a famous document in which he summarized his theories on memory and the brain (Bruce, 2001; Dudai and Eisenberg, 2004). Since that time, many questions remain unsolved and neuroscientists are still searching for the engram, the site of memory storage.

Despite the unquestionable importance of synaptic plasticity for brain function, the exact role of cellular and connectivity modifications in learning processes remains obscure (Humeau and Choquet, 2019). Moreover, despite recent progress in developing techniques for identifying and manipulating engrams at the neuronal level, the neurobiological underpinnings of memory retrieval remain almost unexplored (Frankland et al., 2019).

Given these unresolved questions, molluscan unipolar neurons represent a unique platform to investigate where memory is stored. In fact, their primary neurites, which are the site where most of the synaptic interactions occur, can survive for long periods without their soma and still remain competent to synthesize new proteins (Lukowiak et al., 2003; Scheibenstock et al., 2002). This finding allowed researchers to distinguish between the sites (neurites or soma) in which memories are processed (Kandel, 1979; Lukowiak et al., 2003). Whereas intermediate term memory (ITM) is only dependent on new protein synthesis, long-term memory (LTM) depends on both altered gene activity and new protein synthesis (Sakakibara, 2008). Thus, by ablating the soma, it has been possible to demonstrate that memories are consolidated and stored in the neurons' soma (Lukowiak et al., 2000; Sangha et al., 2003; Scheibenstock et al., 2002). Experiments like these have been conducted extensively in another molluscan organism: the great pond snail *Lymnaea stagnalis*. The results obtained were extraordinary, making it a better model than its 'cousin' *Aplysia* for the search for the engram. The reasons for this are threefold. First, the neural circuits that mediate interesting and tractable behavior, such as feeding and aerial respiration, have been characterized (Benjamin, 1983, 2012; Lukowiak et al., 2006; McComb et al., 2003; Spencer et al., 1999; Straub et al., 2004; Syed et al., 1992a,b). Second, these behaviors can be classically and operantly conditioned (see Glossary) and, depending on the training procedure used, both ITMs and LTMs can be formed (Batabyal et al., 2021; Benatti et al., 2020, 2022; Kawai et al., 2004; Kemenes and Benjamin, 1994; Lukowiak et al., 1996; Rivi et al., 2020, 2021a,b, 2022b,c,d,e). Third, for aerial respiration, a single neuron, RPeD1 (right pedal dorsal 1), has proven to be a sufficient and necessary site for memory formation, consolidation, reconsolidation and extinction (Sangha et al., 2003; Scheibenstock et al., 2002). This is, so far, the only instance in both invertebrates and vertebrates where a single neuron meets both the sufficiency and necessity criteria for mediating different hierarchical aspects of memory. In fact, if the soma of RPeD1 is ablated and the primary neurite is left behind (allowing the organism to perform aerial respiration), learning occurs and ITMs are formed, but LTMs can no longer be demonstrated (Scheibenstock et al., 2002). However, if the soma of RPeD1 is ablated after LTM consolidation, memory is still present, suggesting that the soma of RPeD1 is not needed for the retention of LTMs (Scheibenstock et al., 2002). These results have laid the foundation for future genomic and proteomic studies aimed at elucidating the molecular events occurring in this neuron, giving a significant contribution to defining the engram. Experiments such as these cannot be carried out in mammal preparations, as the disruption of the neuronal soma usually causes the death of the entire cell.

A connectome and analysis of the adult *Drosophila* central brain

Another important challenge for neuroscience is the characterization of the neural circuits responsible for animal learning and behavior. The electron microscopy techniques available today, by enabling high-quality and multi-scale neuronal imaging, significantly advanced the understanding of brain-wide connectivity (Li et al.,

2020) and showed that the learned changes in behavior following conditioning are the result of changes in connection strength between neurons across multiple circuits (Abraham et al., 2019). Such studies aim to generate a complete 'map' of the chemical synapses between all neurons in order to study the effects of the perturbation of single cells on behavior and physiology (Josselyn and Frankland, 2018; Sehgal et al., 2018). However, the development of a comprehensive understanding of these circuits at a single neuronal level cannot be easily performed in mammals because of the complexity of their brains and behaviors (Eichler et al., 2017; Li et al., 2020).

On the other hand, the fruit fly *Drosophila melanogaster* has proved to be a leading candidate to study the comprehensive structure and function of the brain, and the mechanistic basis of learning, memory formation and complex behaviors (Scheffer and Meinertzhagen, 2021). This is, in part, due to the simplicity of its CNS (Raji and Potter, 2021). Furthermore, a century of work on fly genetics makes manipulation of its 'small' brain easier than in any other animal species (Sokolowski, 2001).

To date, scientists are on their way to compiling a connectome (i.e. a map of all neurons and their chemical synapses) of the mushroom body of *D. melanogaster* (Eichler et al., 2017). The mushroom body is a higher-order parallel fiber system that is essential for flies to form and retain associations between stimuli and reinforcement (Eichler et al., 2017). Its conserved neuronal architecture and important role in learning and memory (Devineni and Scaplen, 2022; Shinomiya et al., 2022; Simpson, 2009) allowed the reconstruction of a circuit map of the mushroom body. This map will guide future comparative studies aiming to provide a functional understanding of how flies learn, remember and forget. When, in 1850, Dujardin first described mushroom bodies of insects and compared them with the vertebrate cerebral cortex, he could hardly have imagined that nearly 150 years later it would be shown that these structures are indeed involved in mediating conserved cognitive functions or that 170 years later the study of connectomes would be established.

Investigating the memory-altering effects of drugs and compounds

The contribution made by invertebrates extends beyond the mere characterization of the 'where', 'how' and 'when' of memory formation, consolidation and loss. The applications of these models, in fact, extend on several fronts. For example, aquatic mollusks with their open circulatory systems allow the use of membrane-permeant drugs and compounds that can be easily absorbed. This has helped to unravel the complexity of various signaling pathways and provide new insights into how drugs and molecules can modulate different neuronal functions and behaviors (Fodor et al., 2020a,b; Rivi et al., 2020). In the past decades, many studies have demonstrated the memory-enhancing or -impairing effects induced by the exposure of organisms to drugs and compounds (Gho and Ganetzky, 1992; Monleón et al., 2008; Nakai et al., 2022; Søvik et al., 2018). In this context, *L. stagnalis* has been recognized as a useful organism to examine the effects of drugs such as cannabinoids, fluoxetine, methamphetamine, cocaine and propranolol as well as bioactive compounds, such as flavonoids, on learning and memory (Batabyal and Lukowiak, 2021; Benatti et al., 2017; Carter et al., 2006; Fernell et al., 2016; Il-Han et al., 2010; Ito et al., 2014; Kagan et al., 2022; Kennedy et al., 2010; Rivi et al., 2021a, 2022a; Swinton et al., 2018, 2020, 2021).

Furthermore, *L. stagnalis* has been used to study the effects of amyloid-β (Aβ) peptides on the snails' ability to form LTMs (Ford et al., 2015, 2017). Aβ peptides, in fact, are implicated in memory loss, neuronal impairment and neurodegeneration in Alzheimer's disease (Harrington, 2012) and Aβ1–42 oligomers have been identified as toxic fragments that likely affect LTM through synaptic plasticity pathways (Ford et al., 2015, 2017). These findings added an important piece to the puzzle for the global understanding of neurophysiological processes underlying aging and memory decline (Fodor et al., 2021).

The cognitive richness of insect behaviors and their higher forms of learning and memory

Another very important taxon for the study of memory and learning is insects. Over the past few years, several studies have described the numerous ways in which insects have contributed to answering outstanding questions related to complex behaviors. For example, the honeybee (*Apis mellifera*) has been critically important for characterization of circadian rhythms (Rubin et al., 2006); the worm *Caenorhabditis elegans*, the planarian *Dugesia japonica* and the tussock moth (*Eloria noyesi*) have been adopted for addiction research (Søvik and Barron, 2013); lobsters (*Homarus americanus*) and crickets (*Gryllus bimaculatus*) can be used for studying aggressive behaviors (Briones-Fourzán et al., 2015); and many insect species provide unique systems to investigate how early-life experience alters the brain and behavior (Westwick and Rittschof, 2021). Research on insects has revealed the existence of a variety of cognitive phenomena and higher forms of learning and memory (see Glossary) that were previously thought to be restricted to vertebrates and, sometimes, only to humans. We provide examples of these here.

Concept learning

Honeybees can rapidly master two abstract concepts simultaneously using spatial relationships (above/below and right/left) and then transfer their choices to unknown stimuli that offer the best match in terms of dual-concept availability (Avarguès-Weber et al., 2012).

Attentional modulation

Studies on bumblebee (*Bombus terrestris*) and honeybee color learning provided the first evidence of attentional processes in insects and demonstrated how these complex mechanisms can be modulated by experience (Dyer and Garcia, 2014; Gumbert, 2000). These animals, in fact, can be trained to distinguish between a rewarded and a non-rewarded color as well as between target and distractor stimuli (Menzel and Giurfa, 2001). A recent study in *D. melanogaster* demonstrated that, in the presence of competing percepts, attention can be switched from one attentional state to another one. This phenomenon – known as 'attentional rivalry' – seems to be evolutionarily conserved. As the slowing of rivalry rate is associated with heritable psychiatric disorders, such as bipolar disorder, perceptual rivalry in flies represents a powerful model for investigating the genetic and molecular influences on rivalry rate and may even shed light on human cognitive and behavioral dysfunction (Miller et al., 2012).

Furthermore, taking advantage of the tractability of *D. melanogaster* in both behavioral and microbiome studies (Wong et al., 2014), Silva et al. (2021) recently tested how the elimination of microorganisms affects the organism's behavior. The study demonstrated that microbiologically sterile (axenic) flies had a moderate reduction in memory performance. Moreover, axenic flies showed a tendency to sleep longer and had reduced sleep rebound after sleep deprivation (Silva et al., 2021).

As growing evidence suggests, neural circuits conserved between the *D. melanogaster* and mammalian brain control not only wakefulness and activity but also many aspects of interactions between organisms and their gut microbiome (Cryan et al., 2019; Ezenwa et al., 2012). Thus, this model organism offers a great opportunity to elucidate the mechanisms underlying microbiome-dependent traits, opening a new avenue for translational studies.

Olfactory learning

Shifting from flies to crickets (*Gryllus bimaculatus*), Matsumoto and Mizunami (2005) demonstrated that these organisms possess highly developed olfactory learning capabilities, which are characterized by fast acquisition, long retention and easy memory recall. Additional studies showed that this form of learning was established early during the evolution of hemimetabolous insects, representing a valid tool for new insights into the evolution of neuronal systems subserving olfactory learning in both vertebrates and invertebrates (Taylor et al., 2020).

Effect of toxins on cognition

Not least, insects can be used as models for investigating the effects of herbicides on cognitive functions (Aloizou et al., 2020). One of the most widespread herbicides in the world is glyphosate N-(phosphonomethyl)-glycine (Ait-Bali et al., 2020). A decline in learning and navigation abilities has been observed in honeybees fed with concentrations of glyphosate similar to those found in the environment (Bara et al., 2014). Moreover, in the mosquitoes *Aedes aegypti* and *Aedes albopictus*, exposure to this herbicide altered larval development time and sex ratio, as well as the expression of genes conferring resistance to insecticides (Bara et al., 2014). In 2018, Balgan, Lazzari and Guerreri showed that the exposure of *A. aegypti* larvae to a field-realistic dose of glyphosate had deleterious effects on habituation learning (Baglan et al., 2018). This study opened the way for future ecotoxicological studies using mosquito larvae as a bio-indicator to evaluate the impact of herbicides, pollutants and chemical compounds on cognition.

Further examples of cognitive phenomena seen in insects include numerical cognition (Pahl et al., 2013), categorization of stimuli (Benard et al., 2006), cognitive/behavioral flexibility (Loukola et al., 2017; see Glossary), social learning and cultural transmission (Alem et al., 2016).

Flatworms: masters of neuro-regeneration and much more

Planarians have recently become important model organisms in developmental and regenerative biology (Brown and Pearson, 2017). Their remarkable regenerative capacities – driven by an adult stem cell population – make them valid tools for investigating the molecular mechanisms behind neural repair and patterning. Furthermore, because of their rich behavioral repertoires and learning abilities, planarians are a potential tool for elucidating the dynamics of memory during brain regeneration. In 2013, Shomrat and Levin developed a system for investigating the dynamics of memory in a regenerating planarian's nervous system. For this purpose, they developed a computerized behavioral protocol to train flatworms in an environmental familiarization paradigm. As memory persisted for at least 14 days – a sufficiently long time for the nervous system to regenerate – they also demonstrated that trained, decapitated planarians exhibit memory retrieval after regenerating a new head (Shomrat and Levin, 2013). This study not only revealed LTM in planarians but also its persistence through head regeneration. The high tractability of this model system may shed light on the interface between body patterning and stored memories. Furthermore, future studies on these organisms may provide a great contribution to improving stem cell-derived treatments of degenerative brain disorders in human adults.

What can invertebrates teach us about a stressful and emotional world?

The studies on learning and memory in invertebrates find applications in our everyday life. We all live in a stressful world. Research over the last 40 years defined stress and the hormones and neurotransmitters released during and after a stressful event as major modulators of human learning and memory (Joëls et al., 2006; Vogel and Schwabe, 2016). Here, we define stress as a state that requires physiological and/or behavioral readjustment or modification to maintain the well-being of the organism (Lukowiak et al., 2014). The importance of invertebrate studies on stress relies on the fact that consistently with vertebrates, stress can alter adaptive behaviors, thereby either enhancing or diminishing learning and memory formation and/or recall.

As exemplified by the Yerkes–Dodson Law, too much or too little stress impedes LTM formation, while 'just the right amount' of stress enhances LTM (Teigen, 1994). Because LTM formation requires 'neuronal cost' (in terms of altered gene activity and new protein synthesis), organisms invest energy only for 'relevant' events. This relevancy is in part determined by the level of stress perceived. However, the same stimulus may be perceived as a stressor for one organism but not for another, or only at certain times and not at others in the same organism. Furthermore, populations or strains within a species may differ in their perception of, or response to, environmental stressors, showing – in turn – different adaptive behaviors and memory-forming potential (Lukowiak et al., 2014; Rivi et al., 2022f). Because stress has a broad definition, different stressors may have different biological consequences and, sometimes, opposite effects: some stressors block LTM formation, whereas others enhance it. This complex scenario is further complicated when multiple stressors are encountered. Although the exposure to different stressors may result in the same behavioral memory phenotype (i.e. memory enhancement or impairment), it is difficult to predict what the outcome will be regarding memory formation when a combination of stressors interact (Dalesman et al., 2013; Lukowiak et al., 2014). Thus, how combinations of stressors act is an emergent (i.e. basically unpredictable) property of how organisms perceive the stressors (Dalesman et al., 2013).

Given the complexity of vertebrate brains and behaviors, it is not too surprising that studies conducted in these models have led to sometimes contradictory results. Thus, studies using invertebrates may help researchers to decipher how stress affects memory at the behavioral, neuronal and molecular levels (Aonuma et al., 2018; Batabyal et al., 2022; Dalesman et al., 2013; Ito et al., 2015, 2017; Neckameyer and Nieto-Romero, 2015; Ottaviani and Franceschi, 1996; Rivi et al., 2022b; Soravia et al., 2021). For example, it has been demonstrated that different stress states resulting from different durations of food deprivation alter the ability of *L. stagnalis* to exhibit LTM (Ito et al., 2015). In particular, snails deprived of food for 1 day (a modest level of stress) before aversive classical conditioning (see Glossary) show optimal conditioned taste aversion (CTA; see Glossary) and LTM, whereas those starved for 5 days (high level of stress) before training do not show the memory phenotype. This is because severe food deprivation blocks the snails' ability to express memory, which is formed but overpowered by severe hunger (Ito et al., 2015). This study demonstrated that CTA-LTM is both dependent on the level of stress and the context in which memory is formed. In fact, CTA-LTM memory expression occurs only if severely food-deprived snails are given *ad libitum* access to food for 7 days after training and are tested for memory recall after 1 day of starvation, which recreates the context in which they were trained (Ito et al., 2017).

Stress not only affects learning and memory but is also strongly associated with emotions. The link between cognitive function, stress and emotional states in humans has been largely demonstrated (Tyng et al., 2017). However, invertebrate studies in this field are only in their very early stages. Strange as it may seem, invertebrates exhibit various cognitive, behavioral and physiological traits that indicate internal states evocative of what we consider emotions. Emotions are defined as transient central states triggered by environmental stimuli resulting from an integration of subjective experiences, cognitive evaluation, behavior, neurophysiology and motivation (Anderson and Adolphs, 2014). For example, fear is a motivational state aroused by specific stimuli that give rise to defensive behavior or escape, whereas anxiety is a fear-related negative emotion, induced by a threat/aversive stressor to wellbeing or survival, either actual or potential (Steimer, 2002). In 2014, by using a sub-aquatic dark-light plus maze (a modified version of the famous plus-maze apparatus used for studying anxiety in rodents, consisting of light and dark arms), Fossat and coworkers studied stress-induced anxiety-related behaviors in crayfish (*Procambarus clarkii*). The exposure of animals to electric shocks before the maze test severely decreased exploratory behavior and increased light avoidance, a reaction strikingly similar to that observed in vertebrates (Fossat et al., 2014). These results not only emphasize the ability of an invertebrate model to exhibit a state that is like a mammalian emotion but may also have implications for learning and memory research. In fact, fear, anxiety and stress are inter-related in memory loss and cognitive decline in mammals (Sinoff and Werner, 2003).

Another important aspect that makes invertebrates valid models to study the intricate relationship between emotions, stress and cognition is that some physiological traits of stress and emotions described in vertebrates, such as increased heart and ventilation rate, also occur in invertebrates (Even et al., 2012; Ložek et al., 2019; Orr et al., 2007; Renwrantz and Spielvogel, 2011). For example, one of the CTA training procedures characterized in *L. stagnalis* used a conditional stimulus that elicits the feeding behavior (Benjamin and Kemenes, 2010) paired with an aversive unconditional stimulus that induces the whole-body withdrawal response and inhibits feeding (Ito et al., 1999; Sadamoto et al., 2010). Kita and colleagues (2011) demonstrated that after training and memory formation, the appetitive stimulus no longer elicited feeding, but increased the heart rate, inducing a response that was similar to fear in mammals (Kita et al., 2011; Steimer, 2002).

Furthermore, the high level of conservation of neurochemicals across taxa may be useful to the study of stress, emotions, learning and memory, and their interactions in invertebrates. The role of biogenic amines in the regulation of all these processes has been largely demonstrated in mammals (Purves et al., 2001). However, invertebrate nervous systems contain corresponding biogenic amines that are structurally and functionally similar to mammalian neurotransmitters, neuromodulators and hormones (D'Aniello et al., 2020). For example, training pond snails in the presence of their predator effluent enhances LTM formation and allows the memory to be recalled under a broader range of challenges (Dalesman et al., 2006; Forest et al., 2016). This stressor-mediated memory enhancement is prevented by exposure to the serotonin blockers mianserin and methysergide, suggesting a serotonergic modulation activated by risk perception, which results in enhanced memory formation (Forest et al., 2016). These data are consistent with previous studies in humans correlating increased serotonin levels with enhanced responses to anxiety-related stimuli and memory consolidation (Meneses, 2015; Wong et al., 2005).

These results may pave the way for future investigations aimed at unveiling the conserved core mechanisms of the intricate relationship between stress, emotion and cognitive function among a diversity of species and model animal systems.

What's next? Future perspectives

As reported in this brief commentary, the use of invertebrates has significantly contributed to the characterization of the behavioral, neuronal and molecular mechanisms of memory formation, consolidation, reconsolidation and extinction. In addition, invertebrates can also be used to study the mechanisms through which some bioactive compounds (i.e. flavonoids), drugs (i.e. antidepressants, drugs of abuse, anti-inflammatory drugs, etc.), contexts and stressors affect (i.e. impair or enhance) memory.

We strongly believe that the use of invertebrates in applied neuroscience is only just beginning and that these organisms will provide a major contribution to answering the unsolved questions in neuroscience, revealing highly conserved characteristics between invertebrates and vertebrates, including humans.

Comparative studies for investigating the evolution of the neuronal connectomes

The study of the connectome in insects and mollusks will provide important comparative information on how some neuronal connections have been maintained during the evolution despite the immense diversity in brain size and complexity across taxa. For the advancement of research in the field of neuroscience in the near future, more emphasis should be given to studies conducted using wild animals (Gibbs et al., 1997; Greif and Yovel, 2019; Kagan and Lukowiak, 2019; Masek et al., 2014; Swinton et al., 2021). In fact, as laboratory-inbred colonies tend to be subject to founder effects and genetic drift (see Glossary), they may not reflect the full range of behavioral responses and cognitive functions of natural populations (Brekke et al., 2018). We strongly believe – in accordance with numerous studies from rodents – that the human physiological and pathological processes related to memory and learning conditions are far closer to that of wild animals than to those of inbred ones (Festing, 1976; Rivi et al., 2022b; Tuttle et al., 2018).

Invertebrates as valid tools for linking the 'why' and 'how' of aging and memory loss

Despite the extensive efforts in biomedical research, many aspects of neuro-aging are still not fully understood. Invertebrates such as mollusks (e.g. pond snails) and insects (e.g. flies) offer great advantages, including the accessibility and simplicity of their nervous system, which may contribute to the global understanding of neurophysiological processes underlying aging and memory decline at the genomic, neuronal and behavioral level (Fodor et al., 2021). For example, several relevant gero-protectors (i.e. molecules involved in protecting against aging) have been identified in *Lymnaea*, including gelsolin, presenilin, huntingtin, Parkinson's disease protein 7/protein deglycase DJ-1 and amyloid precursor protein (Fodor et al., 2021). These results strongly encourage future studies aimed at investigating the molecular, cellular and circuit mechanisms underlying the neurophysiological and neuropathological bases of aging and its effect on learning and memory abilities.

Gut microbiome and memory differences across taxa

There has been a growing interest in determining the mechanisms underlying the individual cognitive abilities across organisms

(Orr et al., 2009; Rivi et al., 2022b,c; Sunada et al., 2017). To date, only a few studies investigate the role (potentially) played by the gut microbiome as a driver of individual cognitive differences in natural populations of animals (Davidson et al., 2018). However, we believe that investigating the inter- and intra-specific variations in the gut microbiome will provide new insight into evolutionary and environmental mechanisms involved in cognitive functions across taxa. In fact, numerous studies demonstrated a major role of the microbiome composition in regulating neurotransmitter levels, the expression of neural receptors, synaptic plasticity and neurogenesis (Chen et al., 2021).

Molecular approaches for understanding the physical basis of memory

As previously reported, invertebrates such as *Lymnaea* can be extremely useful to decipher complex engram networks and provide a comprehensive map of engram circuits. The use of advanced tools for genome engineering is necessary for investigating the molecular mechanisms underpinnings of engram cells and their connections during memory formation and consolidation. Emerging in 2013, gene editing based on CRISPR-Cas9 technology represents a powerful strategy for efficiently manipulating key genes in multiple organisms, including invertebrates (Abe and Kuroda, 2019; Gratz et al., 2015; Jinek et al., 2012; Kohno et al., 2016; Martin et al., 2016; Sieber et al., 2021). Will the engram be found? Will it be discovered how to stop memory loss or keep certain memories unchanged over time? Will it be possible to erase negative experiences and their effects on memory? These questions will probably be found in the near future and invertebrates may be extremely useful to open new avenues of research and be part of the discoveries that will make us rethink many of our currently accepted beliefs.

Acknowledgements

We thank Stefan Galander and Charlotte Rutledge for the extraordinary editing work done on our manuscript, as well as the three anonymous reviewers for valuable comments.

Competing interests

The authors declare no competing or financial interests.

Funding

Consorzio Interuniversitario Biotecnologie (CIB, Trieste, Italy) provided a research grant to V.R. to work as a visiting researcher at the Hotchkiss Brain Institute (University of Calgary, AB, Canada).

References

Abe, M. and Kuroda, R. (2019). The development of CRISPR for a mollusc establishes the formin Lsdia1 as the long-sought gene for snail dextral/sinistral coiling. *Development* **146**, dev175976. doi:10.1242/dev.175976

Abraham, W. C., Jones, O. D. and Glanzman, D. L. (2019). Is plasticity of synapses the mechanism of long-term memory storage? *NPJ Sci. Learn.* **4**, 9. doi:10.1038/s41539-019-0048-y

Abrams, T. W. (2012). Studies on Aplysia neurons suggest treatments for chronic human disorders. *Curr. Biol.* **22**, R705-R711. doi:10.1016/j.cub.2012.08.011

Ait-Bali, Y., Ba-M'hamed, S., Gambarotta, G., Sassoè-Pognetto, M., Giustetto, M. and Bennis, M. (2020). Pre- and postnatal exposure to glyphosate-based herbicide causes behavioral and cognitive impairments in adult mice: evidence of cortical ad hippocampal dysfunction. *Arch. Toxicol.* **94**, 1703-1723. doi:10.1007/s00204-020-02677-7

Alem, S., Perry, C. J., Zhu, X., Loukola, O. J., Ingraham, T., Søvik, E. and Chittka, L. (2016). Associative mechanisms allow for social learning and cultural transmission of string pulling in an insect. *PLoS Biol.* **14**, e1002564. doi:10.1371/journal.pbio.1002564

Aloizou, A.-M., Siokas, V., Vogiatzi, C., Peristeri, E., Docea, A. O., Petrakis, D., Provatas, A., Folia, V., Chalkia, C., Vinceti, M. et al. (2020). Pesticides, cognitive functions and dementia: a review. *Toxicol. Lett.* **326**, 31-51. doi:10.1016/j.toxlet.2020.03.005

Anderson, D. J., Adolphs, R. (2014). A framework for studying emotions across species. *Cell* **157**, 187-200. doi:10.1016/j.cell.2014.03.003

Aonuma, H., Totani, Y., Kaneda, M., Nakamura, R., Watanabe, T., Hatakeyama, D., Dyakonova, V. E., Lukowiak, K. and Ito, E. (2018). Effects of 5-HT and insulin on learning and memory formation in food-deprived snails. *Neurobiol. Learn. Mem.* **148**, 20-29. doi:10.1016/j.nlm.2017.12.010

Armbruster, D. J. N., Ueltzhöffer, K., Basten, U. and Fiebach, C. J. (2012). Prefrontal cortical mechanisms underlying individual differences in cognitive flexibility and stability. *J. Cogn. Neurosci.* **24**, 2385-2399. doi:10.1162/jocn_a_00286

Avarguès-Weber, A., Dyer, A. G., Combe, M. and Giurfa, M. (2012). Simultaneous mastering of two abstract concepts by the miniature brain of bees. *Proc. Natl. Acad. Sci. USA* **109**, 7481-7486. doi:10.1073/pnas.1202576109

Baglan, H., Lazzari, C. and Guerrieri, F. J. (2018). Glyphosate impairs learning in *Aedes aegypti* mosquito larvae at field-realistic doses. *J. Exp. Biol.* **221**, jeb187518. doi:10.1242/jeb.187518

Bailey, C. H., Castellucci, V. F., Koester, J. and Chen, M. (1983). Behavioral changes in aging *Aplysia*: a model system for studying the cellular basis of age-impaired learning, memory, and arousal. *Behav. Neural Biol.* **38**, 70-81. doi:10.1016/S0163-1047(83)90399-0

Bailey, C. H., Giustetto, M., Huang, Y.-Y., Hawkins, R. D. and Kandel, E. R. (2000). Is heterosynaptic modulation essential for stabilizing Hebbian plasticity and memory. *Nat. Rev. Neurosci.* **1**, 11-20. doi:10.1038/35036191

Bara, J. J., Montgomery, A. and Muturi, E. J. (2014). Sublethal effects of atrazine and glyphosate on life history traits of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae). *Parasitol. Res.* **113**, 2879-2886. doi:10.1007/s00436-014-3949-y

Batabyal, A. and Lukowiak, K. (2021). Configural learning memory can be transformed from intermediate-term to long-term in pond snail *Lymnaea stagnalis*. *Physiol. Behav.* **239**, 113509. doi:10.1016/j.physbeh.2021.113509

Batabyal, A., Rivi, V., Benatti, C., Blom, J. M. C. and Lukowiak, K. (2021). Long-term memory of configural learning is enhanced via CREB upregulation by the flavonoid quercetin in *Lymnaea stagnalis*. *J. Exp. Biol.* **224**, jeb242761. doi:10.1242/jeb.242761

Batabyal, A., Chau, D., Rivi, V. and Lukowiak, K. (2022). Risk in one is not risk in all: snails show differential decision making under high- and low-risk environments. *Anim. Behav.* **190**, 53-60. doi:10.1016/j.anbehav.2022.05.013

Benard, J., Stach, S. and Giurfa, M. (2006). Categorization of visual stimuli in the honeybee *Apis mellifera*. *Anim. Cogn.* **9**, 257-270. doi:10.1007/s10071-006-0032-9

Benatti, C., Colliva, C., Blom, J., Ottaviani, E. and Tascedda, F. (2017). Transcriptional effect of serotonin in the ganglia of *Lymnaea stagnalis*. *Invertebr. Surviv. J.* **14**, 251-258.

Benatti, C., Rivi, V., Colliva, C., Radighieri, G., Tascedda, F. and Blom, J. M. C. (2020). Redefining operant conditioning of escape behaviour in *Lymnaea stagnalis*. *Invertebr. Surviv. J.* **17**, 129-137.

Benatti, C., Rivi, V., Alboni, S., Grilli, A., Castellano, S., Pani, L., Brunello, N., Blom, J. M. C., Bicciato, S. and Tascedda, F. (2022). Identification and characterization of the kynurenine pathway in the pond snail *Lymnaea stagnalis*. *Sci. Rep.* **12**, 15617.

Benjamin, P. R. (1983). Gastropod feeding: behavioural and neural analysis of a complex multicomponent system. *Symp. Soc. Exp. Biol.* **37**, 159-193.

Benjamin, P. R. (2012). Distributed network organization underlying feeding behavior in the mollusk *Lymnaea*. *Neural Syst. Circuits* **2**, 4. doi:10.1186/2042-1001-2-4

Benjamin, P. and Kemenes, G. (2010). *Lymnaea* learning and memory. *Scholarpedia* **5**, 4247. doi:10.4249/scholarpedia.4247

Benjamin, P. R., Elliott, C. J. H. and Ferguson, G. P. (1985). Neural network analysis in the snail brain. In *Model Neural Networks and Behavior* (ed. A. I. Selverston), pp. 87-108. Boston, MA: Springer US.

Bisaz, R., Travaglia, A. and Alberini, C. M. (2014). The neurobiological bases of memory formation: from physiological conditions to psychopathology. *Psychopathology* **47**, 347-356. doi:10.1159/000363702

Bitterman, M. E. (2006). Classical conditioning since Pavlov. *Rev. Gen. Psychol.* **10**, 365-376. doi:10.1037/1089-2680.10.4.365

Blumstein, D. T. (2016). Habituation and sensitization: new thoughts about old ideas. *Anim. Behav.* **120**, 255-262. doi:10.1016/j.anbehav.2016.05.012

Brekke, T. D., Steele, K. A. and Mulley, J. F. (2018). Inbred or outbred? Genetic diversity in laboratory rodent colonies. *G3* **8**, 679-686. doi:10.1534/g3.117.300495

Briones-Fourzán, P., Domínguez-Gallegos, R. and Lozano-Álvarez, E. (2015). Aggressive behaviour of spotted spiny lobsters (*Panulirus guttatus*) in different social contexts: the influence of sex, size, and missing limbs. *ICES J. Mar. Sci.* **72**, i155-i163. doi:10.1093/icesjms/fsu219

Brown, D. D. R. and Pearson, B. J. (2017). A brain unfixed: unlimited neurogenesis and regeneration of the adult planarian nervous system. *Front. Neurosci.* **11**, 289. doi:10.3389/fnins.2017.00289

Bruce, D. (2001). Fifty years since Lashley's In search of the Engram: refutations and conjectures. *J. Hist. Neurosci.* **10**, 308-318. doi:10.1076/jhin.10.3.308.9086

Carew, T. J., Castellucci, V. F. and Kandel, E. R. (1971). An analysis of dishabituation and sensitization of the gill-withdrawal reflex in *Aplysia*. *Int. J. Neurosci.* **2**, 79-98. doi:10.3109/00207457109146995

Carew, T. J., Walters, E. T. and Kandel, E. R. (1981). Classical conditioning in a simple withdrawal reflex in *Aplysia californica*. *J. Neurosci.* **1**, 1426-1437. doi:10.1523/JNEUROSCI.01-12-01426.1981

Carew, T. J., Hawkins, R. D. and Kandel, E. R. (1983). Differential classical conditioning of a defensive withdrawal reflex in *Aplysia californica*. *Science* **219**, 397-400. doi:10.1126/science.6681571

Carter, K., Lukowiak, K., Schenk, J. O. and Sorg, B. A. (2006). Repeated cocaine effects on learning, memory and extinction in the pond snail *Lymnaea stagnalis*. *J. Exp. Biol.* **209**, 4273-4282. doi:10.1242/jeb.02520

Castellucci, V. F. and Kandel, E. R. (1974). A quantal analysis of the synaptic depression underlying habituation of the gill-withdrawal reflex in *Aplysia*. *Proc. Natl. Acad. Sci. USA* **71**, 5004-5008. doi:10.1073/pnas.71.12.5004

Chen, Y., Xu, J. and Chen, Y. (2021). Regulation of neurotransmitters by the gut microbiota and effects on cognition in neurological disorders. *Nutrients* **13**, 2099. doi:10.3390/nu13062099

Crow, T. J. and Alkon, D. L. (1978). Retention of an associative behavioral change in *Hermissenda*. *Science* **201**, 1239-1241. doi:10.1126/science.694512

Crow, T. J. and Alkon, D. L. (1980). Associative behavioral modification in *Hermissenda*: cellular correlates. *Science* **209**, 412-414. doi:10.1126/science.209.4454.412

Cryan, J. F., O'Riordan, K. J., Cowan, C. S. M., Sandhu, K. V., Bastiaanssen, T. F. S., Boehme, M., Codagnone, M. G., Cussotto, S., Fulling, C., Golubeva, A. V. et al. (2019). The microbiota-gut-brain axis. *Physiol. Rev.* **99**, 1877-2013. doi:10.1152/physrev.00018.2018

Dalesman, S., Rundle, S. D., Coleman, R. A. and Cotton, P. A. (2006). Cue association and antipredator behaviour in a pulmonate snail, *Lymnaea stagnalis*. *Anim. Behav.* **71**, 789-797. doi:10.1016/j.anbehav.2005.05.028

Dalesman, S., Sunada, H., Teskey, M. L. and Lukowiak, K. (2013). Combining stressors that individually impede long-term memory blocks all memory processes. *PLoS One* **8**, e79561. doi:10.1371/journal.pone.0079561

D'Aniello, E., Paganos, P., Anishchenko, E., D'Aniello, S. and Arnone, M. I. (2020). Comparative neurobiology of biogenic amines in animal models in deuterostomes. *Front. Ecol. Evol.* **8**, 587036. doi:10.3389/fevo.2020.587036

Davidson, G. L., Cooke, A. C., Johnson, C. N. and Quinn, J. L. (2018). The gut microbiome as a driver of individual variation in cognition and functional behaviour. *Philos. Trans. R. Soc. B Biol. Sci.* **373**, 20170286. doi:10.1098/rstb.2017.0286

Devineni, A. V. and Scaplen, K. M. (2022). Neural circuits underlying behavioral flexibility: insights from *Drosophila*. *Front. Behav. Neurosci.* **15**, 821680. doi:10.3389/fnbeh.2021.821680

Dudai, Y. and Eisenberg, M. (2004). Rites of passage of the engram. *Neuron* **44**, 93-100. doi:10.1016/j.neuron.2004.09.003

Dujardin, F. (1850). Memoire sur le systeme nerveux des insects. *Ann. Sci. Nat. Zool.* **14**, 195-206.

Dyer, A. G. and Garcia, J. E. (2014). Color difference and memory recall in free-flying honeybees: forget the hard problem. *Insects* **5**, 629-638. doi:10.3390/insects5030629

Eichler, K., Li, F., Litwin-Kumar, A., Park, Y., Andrade, I., Schneider-Mizell, C. M., Saumweber, T., Huser, A., Eschbach, C., Gerber, B. et al. (2017). The complete connectome of a learning and memory centre in an insect brain. *Nature* **548**, 175-182. doi:10.1038/nature23455

Even, N., Devaud, J.-M. and Barron, A. B. (2012). General stress responses in the honey bee. *Insects* **3**, 1271-1298. doi:10.3390/insects3041271

Ezenwa, V. O., Gerardo, N. M., Inouye, D. W., Medina, M. and Xavier, J. B. (2012). Animal behavior and the microbiome. *Science* **338**, 198-199. doi:10.1126/science.1227412

Faria, M. A. (2020). The neurobiology of learning and memory – as related in the memoirs of Eric R. Kandel. *Surg. Neurol. Int.* **11**, 252. doi:10.25259/SNI_458_2020

Fernell, M., Swinton, C. and Lukowiak, K. (2016). Epicatechin, a component of dark chocolate, enhances memory formation if applied during the memory consolidation period. *Commun. Integr. Biol.* **9**, e1205772. doi:10.1080/19420889.2016.1205772

Festing, M. F. W. (1976). Phenotypic variability of inbred and outbred mice. *Nature* **263**, 230-232. doi:10.1038/263230a0

Florey, E. (1991). GABA: history and perspectives. *Can. J. Physiol. Pharmacol.* **69**, 1049-1056. doi:10.1139/y91-156

Fodor, I., Urbán, P., Kemenes, G., Koene, J. M. and Pirger, Z. (2020a). Aging and disease-relevant gene products in the neuronal transcriptome of the great pond snail (*Lymnaea stagnalis*): a potential model of aging, age-related memory loss, and neurodegenerative diseases. *Invert. Neurosci.* **20**, 9. doi:10.1007/s10158-020-00242-6

Fodor, I., Hussein, A. A., Benjamin, P. R., Koene, J. M. and Pirger, Z. (2020b). The unlimited potential of the great pond snail, *Lymnaea stagnalis*. *Elife* **9**, e56962. doi:10.7554/eLife.56962

Fodor, I., Svigruha, R., Kemenes, G., Kemenes, I. and Pirger, Z. (2021). The great pond snail (*Lymnaea stagnalis*) as a model of aging and age-related memory impairment: an overview. *J. Gerontol. Ser. A* **76**, 975-982. doi:10.1093/gerona/glab014

Ford, L., Crossley, M., Williams, T., Thorpe, J. R., Serpell, L. C. and Kemenes, G. (2015). Effects of Aβ exposure on long-term associative memory and its neuronal mechanisms in a defined neuronal network. *Sci. Rep.* **5**, 10614. doi:10.1038/srep10614

Ford, L., Crossley, M., Vadukul, D. M., Kemenes, G. and Serpell, L. C. (2017). Structure-dependent effects of amyloid-β on long-term memory in *Lymnaea stagnalis*. *FEBS Lett.* **591**, 1236-1246. doi:10.1002/1873-3468.12633

Forest, J., Sunada, H., Dodd, S. and Lukowiak, K. (2016). Training *Lymnaea* in the presence of a predator scent results in a long-lasting ability to form enhanced long-term memory. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **202**, 399-409. doi:10.1007/s00359-016-1086-z

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J.-P. and Cattaert, D. (2014). Comparative behavior. Anxiety-like behavior in crayfish is controlled by serotonin. *Science* **344**, 1293-1297. doi:10.1126/science.1248811

Frankland, P. W., Josselyn, S. A. and Köhler, S. (2019). The neurobiological foundation of memory retrieval. *Nat. Neurosci.* **22**, 1576-1585. doi:10.1038/s41593-019-0493-1

Garcia-Lopez, P., Garcia-Marin, V. and Freire, M. (2010). The histological slides and drawings of Cajal. *Front. Neuroanat.* **4**, 9. doi:10.3389/neuro.05.009.2010

Gho, M. and Ganetzky, B. (1992). Analysis of repolarization of presynaptic motor terminals in *Drosophila* larvae using potassium-channel-blocking drugs and mutations. *J. Exp. Biol.* **170**, 93-111. doi:10.1242/jeb.170.1.93

Gibbs, A. G., Chippindale, A. K. and Rose, M. R. (1997). Physiological mechanisms of evolved desiccation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **200**, 1821-1832. doi:10.1242/jeb.200.12.1821

Gratz, S. J., Rubinstein, C. D., Harrison, M. M., Wildonger, J. and O'Connor-Giles, K. M. (2015). CRISPR-Cas9 genome editing in *Drosophila*. *Curr. Protoc. Mol. Biol.* **111**, 31.2.1-31.2.20. doi:10.1002/0471142727.mb3102s111

Greif, S. and Yovel, Y. (2019). Using on-board sound recordings to infer behaviour of free-moving wild animals. *J. Exp. Biol.* **222**, jeb184689. doi:10.1242/jeb.184689

Gumbert, A. (2000). Color choices by bumble bees (*Bombus terrestris*): innate preferences and generalization after learning. *Behav. Ecol. Sociobiol.* **48**, 36-43. doi:10.1007/s002650000213

Harrington, C. R. (2012). The molecular pathology of Alzheimer's disease. *Neuroimaging Clin. N Am.* **22**, 11-22, vii. doi:10.1016/j.nic.2011.11.003

Hawkins, R. D. (2013). Possible contributions of a novel form of synaptic plasticity in *Aplysia* to reward, memory, and their dysfunctions in mammalian brain. *Learn. Mem.* **20**, 580-591. doi:10.1101/lm.031237.113

Hawkins, R. D., Lalevic, N., Clark, G. A. and Kandel, E. R. (1989). Classical conditioning of the *Aplysia* siphon-withdrawal reflex exhibits response specificity. *Proc. Natl. Acad. Sci. USA* **86**, 7620-7624. doi:10.1073/pnas.86.19.7620

Hebb, D. O. (1949). *The Organization of Behavior; A Neuropsychological Theory*. Oxford, England: Wiley.

Hodgkin, A. L. and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol.* **117**, 500-544. doi:10.1113/jphysiol.1952.sp004764

Humeau, Y. and Choquet, D. (2019). The next generation of approaches to investigate the link between synaptic plasticity and learning. *Nat. Neurosci.* **22**, 1536-1543. doi:10.1038/s41593-019-0480-6

Il-Han, J., Janes, T. and Lukowiak, K. (2010). The role of serotonin in the enhancement of long-term memory resulting from predator detection in Lymnaea. *J. Exp. Biol.* **213**, 3603-3614. doi:10.1242/jeb.048256

Ito, E., Kobayashi, S., Kojima, S., Sadamoto, H. and Hatakeyama, D. (1999). Associative learning in the pond snail, *Lymnaea stagnalis*. *Zool. Sci.* **16**, 711-723. doi:10.2108/zsj.16.711

Ito, E., Yamagishi, M., Takigami, S., Sakakibara, M., Fujito, Y. and Lukowiak, K. (2014). The Yerkes-Dodson law and appropriate stimuli for conditioned taste aversion in *Lymnaea*. *J. Exp. Biol.* **218**, 336-339. doi:10.1242/jeb.113266

Ito, E., Yamagishi, M., Hatakeyama, D., Watanabe, T., Fujito, Y., Dyakonova, V. and Lukowiak, K. (2015). Memory block: a consequence of conflict resolution. *J. Exp. Biol.* **218**, 1699-1704. doi:10.1242/jeb.120329

Ito, E., Totani, Y. and Oike, A. (2017). Necessity knows no law in a snail. *Eur. Zool. J.* **84**, 457-464. doi:10.1080/24750263.2017.1363303

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A. and Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816-821. doi:10.1126/science.1225829

Joëls, M., Pu, Z., Wiegert, O., Oitzl, M. S. and Krugers, H. J. (2006). Learning under stress: how does it work? *Trends Cogn. Sci.* **10**, 152-158. doi:10.1016/j.tics.2006.02.002

Josselyn, S. A. and Frankland, P. W. (2018). Memory allocation: mechanisms and function. *Annu. Rev. Neurosci.* **41**, 389-413. doi:10.1146/annurev-neuro-080317-061956

Kagan, D. and Lukowiak, K. (2019). Configural learning in freshly collected, smart, wild *Lymnaea*. *J. Exp. Biol.* **222**, jeb212886. doi:10.1242/jeb.212886

Kagan, D., Batabyal, A., Rivi, V. and Lukowiak, K. (2022). A change in taste: the role of microRNAs in altering hedonic value. *J. Exp. Biol.* **225**, jeb243840. doi:10.1242/jeb.243840

Kandel, E. R. (1979). Small systems of neurons. *Sci. Am.* **241**, 66-76. doi:10.1038/scientificamerican0979-66

Kandel, E. R. (2007). *In Search of Memory: The Emergence of a New Science of Mind.* New York: W. W. Norton.

Kandel, E. R. and Kupfermann, I. (1970). The functional organization of invertebrate ganglia. *Annu. Rev. Physiol.* **32**, 193-258. doi:10.1146/annurev.ph.32.030170.001205

Kandel, E. R., Dudai, Y. and Mayford, M. R. (2014). The molecular and systems biology of memory. *Cell* **157**, 163-186. doi:10.1016/j.cell.2014.03.001

Katz, B. (1949). Neuro-muscular transmission in invertebrates. *Biol. Rev.* **24**, 1-20. doi:10.1111/j.1469-185X.1949.tb00568.x

Katz, P. S. (2016). Evolution of central pattern generators and rhythmic behaviours. *Philos. Trans. R. Soc. B Biol. Sci.* **371**, 20150057. doi:10.1098/rstb.2015.0057

Kawai, R., Sunada, H., Horikoshi, T. and Sakakibara, M. (2004). Conditioned taste aversion with sucrose and tactile stimuli in the pond snail *Lymnaea stagnalis*. *Neurobiol. Learn. Mem.* **82**, 164-168. doi:10.1016/j.nlm.2004.06.003

Kemenes, G. and Benjamin, P. R. (1994). Training in a novel environment improves the appetitive learning performance of the snail, *Lymnaea stagnalis*. *Behav. Neural Biol.* **61**, 139-149. doi:10.1016/S0163-1047(05)80067-6

Kennedy, C. D., Houmes, S. W., Wyrick, K. L., Kammerzell, S. M., Lukowiak, K. and Sorg, B. A. (2010). Methamphetamine enhances memory of operantly conditioned respiratory behavior in the snail *Lymnaea stagnalis*. *J. Exp. Biol.* **213**, 2055-2065. doi:10.1242/jeb.042820

Kita, S., Hashiba, R., Ueki, S., Kimoto, Y., Abe, Y., Gotoda, Y., Suzuki, R., Uraki, E., Nara, N., Kanazawa, A. et al. (2011). Does conditioned taste aversion learning in the pond snail *Lymnaea stagnalis* produce conditioned fear? *Biol. Bull.* **220**, 71-81. doi:10.1086/BBLv220n1p71

Kohno, H., Suenami, S., Takeuchi, H., Sasaki, T. and Kubo, T. (2016). Production of knockout mutants by CRISPR/Cas9 in the European Honeybee, *Apis mellifera* L. *Zoolog. Sci.* **33**, 505-512. doi:10.2108/zs160043

Kron, N. S., Schmale, M. C. and Fieber, L. A. (2020). Changes in metabolism and proteostasis drive aging phenotype in Aplysia californica sensory neurons. *Front. Aging Neurosci.* **12**, 573764. doi:10.3389/fnagi.2020.573764

Lashley, K. S. (1950). *In search of the engram. In Physiological mechanisms in animal behavior. Society for Experimental Biology Symposium IV.* pp. 454-482. New York: Academic Press.

Lederhendler, I. I. and Alkon, D. L. (1987). Associatively reduced withdrawal from shadows in *Hermissenda*: a direct behavioral analog of photoreceptor responses to brief light steps. *Behav. Neural Biol.* **47**, 227-249. doi:10.1016/S0163-1047(87)90370-0

Li, F., Lindsey, J. W., Marin, E. C., Otto, N., Dreher, M., Dempsey, G., Stark, I., Bates, A. S., Pleijzier, M. W., Schlegel, P. et al. (2020). The connectome of the adult Drosophila mushroom body provides insights into function. *Elife* **9**, e62576. doi:10.7554/eLife.62576

Loukola, O. J., Solvi, C., Coscos, L. and Chittka, L. (2017). Bumblebees show cognitive flexibility by improving on an observed complex behavior. *Science* **355**, 833-836. doi:10.1126/science.aag2360

Ložek, F., Kuklina, I., Grabicová, K., Kubec, J., Buřič, M., Grabic, R., Randák, T., Císař, P. and Kozák, P. (2019). Behaviour and cardiac response to stress in signal crayfish exposed to environmental concentrations of tramadol. *Aquat. Toxicol.* **213**, 105217. doi:10.1016/j.aquatox.2019.05.019

Lukowiak, K., Ringseis, E., Spencer, G., Wildering, W. and Syed, N. (1996). Operant conditioning of aerial respiratory behaviour in *Lymnaea stagnalis*. *J. Exp. Biol.* **199**, 683-691. doi:10.1242/jeb.199.3.683

Lukowiak, K., Adatia, N., Krygier, D. and Syed, N. (2000). Operant conditioning in *Lymnaea*: evidence for intermediate- and long-term memory. *Learn. Mem.* **7**, 140-150. doi:10.1101/lm.7.3.140

Lukowiak, K., Sangha, S., Scheibenstock, A., Parvez, K., McComb, C., Rosenegger, D., Varshney, N. and Sadamoto, H. (2003). A molluscan model system in the search for the engram. *J. Physiol.* **97**, 69-76. doi:10.1016/j.jphysparis.2003.10.008

Lukowiak, K., Martens, K., Orr, M., Parvez, K., Rosenegger, D. and Sangha, S. (2006). Modulation of aerial respiratory behaviour in a pond snail. *Respir. Physiol. Neurobiol.* **154**, 61-72. doi:10.1016/j.resp.2006.02.009

Lukowiak, K., Sunada, H., Teskey, M., Lukowiak, K. and Dalesman, S. (2014). Environmentally relevant stressors alter memory formation in the pond snail *Lymnaea*. *J. Exp. Biol.* **217**, 76-83. doi:10.1242/jeb.089441

Martin, A., Serano, J. M., Jarvis, E., Bruce, H. S., Wang, J., Ray, S., Barker, C. A., O'Connell, L. C. and Patel, N. H. (2016). CRISPR/Cas9 mutagenesis reveals versatile roles of Hox genes in crustacean limb specification and evolution. *Curr. Biol.* **26**, 14-26. doi:10.1016/j.cub.2015.11.021

Masek, P., Reynolds, L. A., Bollinger, W. L., Moody, C., Mehta, A., Murakami, K., Yoshizawa, M., Gibbs, A. G. and Keene, A. C. (2014). Altered regulation of sleep and feeding contributes to starvation resistance in *Drosophila melanogaster*. *J. Exp. Biol.* **217**, 3122-3132. doi:10.1242/jeb.103309

Matsumoto, Y. and Mizunami, M. (2005). Formation of long-term olfactory memory in the cricket *Gryllus bimaculatus*. *Chem. Senses* **30**, i299-i300. doi:10.1093/chemse/bjh233

McComb, C., Meems, R., Syed, N. and Lukowiak, K. (2003). Electrophysiological differences in the CPG aerial respiratory behavior between juvenile and adult *Lymnaea*. *J. Neurophysiol.* **90**, 983-992. doi:10.1152/jn.00263.2003

Meneses, A. (2015). Serotonin, neural markers, and memory. *Front. Pharmacol.* **6**, 143. doi:10.3389/fphar.2015.00143

Menzel, R. and Giurfa, M. (2001). Cognitive architecture of a mini-brain: the honeybee. *Trends Cogn. Sci.* **5**, 62-71. doi:10.1016/S1364-6613(00)01601-6

Miller, S., Ngo, T. and Van Swinderen, B. (2012). Attentional switching in humans and flies: rivalry in large and miniature brains. *Front. Hum. Neurosci.* **5**, 188. doi:10.3389/fnhum.2011.00188

Monleón, S., Vinader-Caerols, C., Arenas, M. C. and Parra, A. (2008). Antidepressant drugs and memory: insights from animal studies. *Eur. Neuropsychopharmacol.* **18**, 235-248. doi:10.1016/j.euroneuro.2007.07.001

Nader, K. (2015). Reconsolidation and the dynamic nature of memory. *Cold Spring Harb Perspect. Biol.* **7**, a021782. doi:10.1101/cshperspect.a021782

Nairne, J. S. and Pandeirada, J. N. S. (2016). Adaptive memory: the evolutionary significance of survival processing. *Perspect. Psychol. Sci.* **11**, 496-511. doi:10.1177/1745691616635613

Nakai, J., Chikamoto, N., Fujimoto, K., Totani, Y., Hatakeyama, D., Dyakonova, V. E. and Ito, E. (2022). Insulin and memory in invertebrates. *Front. Behav. Neurosci.* **16**, 882932. doi:10.3389/fnbeh.2022.882932

Neckameyer, W. S. and Nieto-Romero, A. R. (2015). Response to stress in *Drosophila* is mediated by gender, age and stress paradigm. *Stress* **18**, 254-266. doi:10.3109/10253890.2015.1017465

Orr, M. V., El-Bekai, M., Lui, M., Watson, K. and Lukowiak, K. (2007). Predator detection in *Lymnaea stagnalis*. *J. Exp. Biol.* **210**, 4150-4158. doi:10.1242/jeb.010173

Orr, M. V., Hittel, K. and Lukowiak, K. (2009). 'Different strokes for different folks': geographically isolated strains of *Lymnaea stagnalis* only respond to sympatric predators and have different memory forming capabilities. *J. Exp. Biol.* **212**, 2237-2247. doi:10.1242/jeb.031575

Ottaviani, E. and Franceschi, C. (1996). The neuroimmunology of stress from invertebrates to man. *Prog. Neurobiol.* **48**, 421-440. doi:10.1016/0301-0082(95)00049-6

Pahl, M., Si, A. and Zhang, S. (2013). Numerical cognition in bees and other insects. *Front. Psychol.* **4**, 162. doi:10.3389/fpsyg.2013.00162

Pavlov, I. P. (1997). Excerpts from the work of the digestive glands. *Am. Psychol.* **52**, 936-940. doi:10.1037/0003-066X.52.9.936

Pembroke, W. G., Hartl, C. L. and Geschwind, D. H. (2021). Evolutionary conservation and divergence of the human brain transcriptome. *Genome Biol.* **22**, 52. doi:10.1186/s13059-020-02257-z

Preuss, T. M. (1995). Do rats have prefrontal cortex? The Rose-Woolsey-Akert program reconsidered. *J. Cogn. Neurosci.* **7**, 1-24. doi:10.1162/jocn.1995.7.1.1

Purves, D., Augustine, G. J., Fitzpatrick, D., Katz, L. C., Lamantia, A.-S., McNamara, J. O. and Williams, S. M. (eds) (2001). Thalamocortical interactions In *Neuroscience*, 2nd edn. Sunderland, MA: Sinauer Associates.

Rahmani, A. and Chew, Y. L. (2021). Investigating the molecular mechanisms of learning and memory using *Caenorhabditis elegans*. *J. Neurochem.* **159**, 417-451. doi:10.1111/jnc.15510

Raji, J. I. and Potter, C. J. (2021). The number of neurons in Drosophila and mosquito brains. *PLoS One* **16**, e0250381. doi:10.1371/journal.pone.0250381

Ramón y Cajal, S. (1894). La fine structure des centres nerveux. *Proc. R. Soc. Lond.* **55**, 444-468.

Renwrantz, L. and Spielvogel, F. (2011). Heart rate and hemocyte number as stress indicators in disturbed hibernating vineyard snails, *Helix pomatia*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **160**, 467-473. doi:10.1016/j.cbpa.2011.08.002

Rivi, V., Benatti, C., Colliva, C., Radighieri, G., Brunello, N., Tascedda, F. and Blom, J. M. C. (2020). *Lymnaea stagnalis* as model for translational neuroscience research: from pond to bench. *Neurosci. Biobehav. Rev.* **108**, 602-616. doi:10.1016/j.neubiorev.2019.11.020

Rivi, V., Batabyal, A., Juego, K., Kakadiya, M., Benatti, C., Blom, J. M. C. and Lukowiak, K. (2021a). To eat or not to eat: a Garcia effect in pond snails (*Lymnaea stagnalis*). *J. Comp. Physiol. A* **207**, 479-495. doi:10.1007/s00359-021-01491-5

Rivi, V., Benatti, C., Lukowiak, K., Colliva, C., Alboni, S., Tascedda, F. and Blom, J. M. C. (2021b). What can we teach Lymnaea and what can Lymnaea teach us? *Biol. Rev. Camb. Philos. Soc.* **96**, 1590-1602. doi:10.1111/brv.12716

Rivi, V., Batabyal, A., Benatti, C., Blom, J. M., Tascedda, F. and Lukowiak, K. (2022a). A flavonoid, quercetin, is capable of enhancing long-term memory formation if encountered at different times in the learning, memory formation, and memory recall continuum. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* **208**, 253-265. doi:10.1007/s00359-021-01522-1

Rivi, V., Batabyal, A., Benatti, C., Blom, J. M. C. and Lukowiak, K. (2022b). Nature versus nurture in heat stress induced learning between inbred and outbred populations of *Lymnaea stagnalis*. *J. Therm. Biol.* **103**, 103170. doi:10.1016/j.jtherbio.2021.103170

Rivi, V., Batabyal, A., Wiley, B., Benatti, C., Tascedda, F., Blom, J. M. C. and Lukowiak, K. (2022c). Fluoride affects memory by altering the transcriptional activity in the central nervous system of *Lymnaea stagnalis*. *Neurotoxicology* **92**, 61-66. doi:10.1016/j.neuro.2022.07.007

Rivi, V., Benatti, C., Actis, P., Tascedda, F. and Blom, J. M. C. (2022d). Behavioral and transcriptional effects of short or prolonged fasting on the memory performances of *Lymnaea stagnalis*. *Neuroendocrinology* **2022**, 1-17. doi:10.1159/000527489

Rivi, V., Batabyal, A., Benatti, C., Tascedda, F., Blom, J. M. C. and Lukowiak, K. (2022e). Aspirin reverts lipopolysaccharide-induced learning and memory impairment: first evidence from an invertebrate model system. *Naunyn Schmiedebergs Arch. Pharmacol.* **395**, 1573-1585.

Rivi, V., Batabyal, A., Benatti, C., Tascedda, F., Blom, J. M. and Lukowiak, K. (2022f). Too hot to eat: wild and lab-bred Lymnaea stagnalis differ in feeding response following repeated heat exposure. *Biol. Bull.* **243**, 38-43.

Rubin, E. B., Shemesh, Y., Cohen, M., Elgavish, S., Robertson, H. M. and Bloch, G. (2006). Molecular and phylogenetic analyses reveal mammalian-like clockwork in the honey bee (*Apis mellifera*) and shed new light on the molecular evolution of the circadian clock. *Genome Res.* **16**, 1352-1365. doi:10.1101/gr.5094806

Sadamoto, H., Kitahashi, T., Fujito, Y. and Ito, E. (2010). Learning-dependent gene expression of CREB1 isoforms in the molluscan brain. *Front. Behav. Neurosci.* **4**, 25. doi:10.3389/fnbeh.2010.00025

Sakakibara, M. (2008). Cellular and molecular aspects of short-term and long-term memory from molluscan systems. In *Novel Trends in Brain Science: Brain Imaging, Learning and Memory, Stress and Fear, and Pain* (ed. M. Onozuka and C.-T. Yen), pp. 131-148. Tokyo: Springer Japan.

Sangha, S., Scheibenstock, A. and Lukowiak, K. (2003). Reconsolidation of a long-term memory in *Lymnaea* requires new protein and RNA synthesis and the soma of right pedal Dorsal 1. *J. Neurosci.* **23**, 8034-8040. doi:10.1523/JNEUROSCI.23-22-08034.2003

Scheffer, L. K. and Meinertzhagen, I. A. (2021). A connectome is not enough – what is still needed to understand the brain of Drosophila? *J. Exp. Biol.* **224**, jeb242740. doi:10.1242/jeb.242740

Scheibenstock, A., Krygier, D., Haque, Z., Syed, N. and Lukowiak, K. (2002). The Soma of RPeD1 must be present for long-term memory formation of associative learning in *Lymnaea*. *J. Neurophysiol.* **88**, 1584-1591. doi:10.1152/jn.2002.88.4.1584

Schier, L. A., Hyde, K. M. and Spector, A. C. (2019). Conditioned taste aversion versus avoidance: a re-examination of the separate processes hypothesis. *PLoS One* **14**, e0217458. doi:10.1371/journal.pone.0217458

Schnell, A. K., Amodio, P., Boeckle, M. and Clayton, N. S. (2021). How intelligent is a cephalopod? Lessons from comparative cognition. *Biol. Rev. Camb. Philos. Soc.* **96**, 162-178. doi:10.1111/brv.12651

Sehgal, M., Zhou, M., Lavi, A., Huang, S., Zhou, Y. and Silva, A. J. (2018). Memory allocation mechanisms underlie memory linking across time. *Neurobiol. Learn. Mem.* **153**, 21-25. doi:10.1016/j.nlm.2018.02.021

Shinomiya, K., Nern, A., Meinertzhagen, I. A., Plaza, S. M. and Reiser, M. B. (2022). Neuronal circuits integrating visual motion information in *Drosophila melanogaster*. *Curr. Biol.* **32**, 3529-3544.e2. doi:10.1016/j.cub.2022.06.061

Shomrat, T. and Levin, M. (2013). An automated training paradigm reveals long-term memory in planarians and its persistence through head regeneration. *J. Exp. Biol.* **216**, 3799-3810. doi:10.1242/jeb.087809

Sieber, K., Saar, M., Opachaloemphan, C., Gallitto, M., Yang, H. and Yan, H. (2021). Embryo injections for CRISPR-mediated mutagenesis in the ant *Harpegnathos saltator*. *J. Vis. Exp.* **9**, 168. doi:10.3791/61930

Silva, V., Palacios-Muñoz, A., Okray, Z., Adair, K. L., Waddell, S., Douglas, A. E. and Ewer, J. (2021). The impact of the gut microbiome on memory and sleep in *Drosophila*. *J. Exp. Biol.* **224**, jeb233619. doi:10.1242/jeb.233619

Simpson, J. H. (2009). Mapping and manipulating neural circuits in the fly brain. *Adv. Genet.* **65**, 79-143. doi:10.1016/S0065-2660(09)65003-3

Sinoff, G. and Werner, P. (2003). Anxiety disorder and accompanying subjective memory loss in the elderly as a predictor of future cognitive decline. *Int. J. Geriatr. Psychiatry* **18**, 951-959. doi:10.1002/gps.1004

Sokolowski, M. B. (2001). Drosophila: genetics meets behaviour. *Nat. Rev. Genet.* **2**, 879-890. doi:10.1038/35098592

Soravia, C., Ashton, B. J., Thornton, A. and Ridley, A. R. (2021). The impacts of heat stress on animal cognition: implications for adaptation to a changing climate. *WIREs Clim. Change* **12**, e713. doi:10.1002/wcc.713

Søvik, E. and Barron, A. B. (2013). Invertebrate models in addiction research. *Brain Behav. Evol.* **82**, 153-165. doi:10.1159/000355506

Søvik, E., Berthier, P., Klare, W. P., Helliwell, P., Buckle, E. L. S., Plath, J. A., Barron, A. B. and Maleszka, R. (2018). Cocaine directly impairs memory extinction and alters brain DNA methylation dynamics in honey bees. *Front. Physiol.* **9**, 79. doi:10.3389/fphys.2018.00079

Spencer, G. E., Syed, N. I. and Lukowiak, K. (1999). Neural changes after operant conditioning of the aerial respiratory behavior in Lymnaea stagnalis. *J. Neurosci.* **19**, 1836-1843. doi:10.1523/JNEUROSCI.19-05-01836.1999

Steimer, T. (2002). The biology of fear- and anxiety-related behaviors. *Dialogues Clin. Neurosci.* **4**, 231-249. doi:10.31887/DCNS.2002.4.3/tsteimer

Straub, V. A., Styles, B. J., Ireland, J. S., O'Shea, M. and Benjamin, P. R. (2004). Central localization of plasticity involved in appetitive conditioning in *Lymnaea*. *Learn. Mem.* **11**, 787-793. doi:10.1101/lm.77004

Strausfeld, N. J. (2012). *Arthropod Brains: Evolution, Functional Elegance, and Historical Significance*. Harvard University Press.

Sunada, H., Totani, Y., Nakamura, R., Sakakibara, M., Lukowiak, K. and Ito, E. (2017). Two strains of *Lymnaea stagnalis* and the progeny from their mating display differential memory-forming ability on associative learning tasks. *Front. Behav. Neurosci.* **11**, 161. doi:10.3389/fnbeh.2017.00161

Swinton, E., De Freitas, E., Swinton, C., Shymansky, T., Hiles, E., Zhang, J., Rothwell, C. and Lukowiak, K. (2018). Green tea and cocoa enhance cognition in *Lymnaea*. *Commun. Integr. Biol.* **11**, 161. doi:10.1080/19420889.2018.1434390

Swinton, E., Shymansky, T., Swinton, C. and Lukowiak, K. (2020). Stress before training alters memory retrieval of a non-declarative memory in *Lymnaea*. *J. Exp. Biol.* **223**, jeb223727. doi:10.1242/jeb.223727

Swinton, C., Swinton, E., Phillips, I. and Lukowiak, K. (2021). A thermal stressor, propranolol and long-term memory formation in freshly collected *Lymnaea*. *J. Exp. Biol.* **224**, jeb242293. doi:10.1242/jeb.242293

Syed, N. I., Bulloch, A. G. and Lukowiak, K. (1992a). The respiratory central pattern generator (CPG) of Lymnaea reconstructed in vitro. *Acta Biol. Hung.* **43**, 409-419.

Syed, N. I., Ridgway, R. L., Lukowiak, K. and Bulloch, A. G. M. (1992b). Transplantation and functional integration of an identified respiratory interneuron in *Lymnaea stagnalis*. *Neuron* **8**, 767-774. doi:10.1016/0896-6273(92)90097-W

Taylor, J. E., Lau, H., Seymour, B., Nakae, A., Sumioka, H., Kawato, M. and Koizumi, A. (2020). An evolutionarily threat-relevant odor strengthens human fear memory. *Front. Neurosci.* **14**, 638. doi:10.3389/fnins.2020.00638

Teigen, K. H. (1994). Yerkes-Dodson: a law for all seasons. *Theory Psychol.* **4**, 525-547. doi:10.1177/0959354394044004

Tuttle, A. H., Philip, V. M., Chesler, E. J. and Mogil, J. S. (2018). Comparing phenotypic variation between inbred and outbred mice. *Nat. Methods* **15**, 994-996. doi:10.1038/s41592-018-0224-7

Tyng, C. M., Amin, H. U., Saad, M. N. M. and Malik, A. S. (2017). The influences of emotion on learning and memory. *Front. Psychol.* **8**, 1454. doi:10.3389/fpsyg.2017.01454

Vogel, S. and Schwabe, L. (2016). Learning and memory under stress: implications for the classroom. *NPJ Sci. Learn.* **1**, 1-10. doi:10.1038/npjscilearn.2016.11

Westwick, R. R. and Rittschof, C. C. (2021). Insects provide unique systems to investigate how early-life experience alters the brain and behavior. *Front. Behav. Neurosci.* **15**, 660464. doi:10.3389/fnbeh.2021.660464

Wong, D. T., Perry, K. W. and Bymaster, F. P. (2005). Case history: the discovery of fluoxetine hydrochloride (Prozac). *Nat. Rev. Drug Discov.* **4**, 764-774. doi:10.1038/nrd1821

Wong, A. C.-N., Dobson, A. J. and Douglas, A. E. (2014). Gut microbiota dictates the metabolic response of *Drosophila* to diet. *J. Exp. Biol.* **217**, 1894-1901. doi:10.1242/jeb.101725