OPEN BIOLOGY

royalsocietypublishing.org/journal/rsob

Research

Cite this article: Rivi V, Yakubets K, Pele G, Batabyal A, Blom JMC, Tascedda F, Benatti C, Lukowiak K. 2026 Exploring the role of stress sensitivity in memory formation: why do some animals learn while others do not? Lessons from *Lymnaea stagnalis*. *Open Biol.* **16**: 250283.
https://doi.org/10.1098/rsob.250283

Received: 4 August 2025
Accepted: 9 January 2026

Subject Areas:
molecular biology, neuroscience

Keywords:
configural learning, neuroplasticity, predator, intrastrain differences

Author for correspondence:
Cristina Benatti
e-mail: cristina.benatti@unimore.it

†Joint first authors

Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.8332594.

THE ROYAL SOCIETY PUBLISHING

Exploring the role of stress sensitivity in memory formation: why do some animals learn while others do not? Lessons from *Lymnaea stagnalis*

Veronica Rivi[1,†], Kate Yakubets[3,†], Grace Pele[3], Anuradha Batabyal[5,6], Johanna M. C. Blom[2], Fabio Tascedda[1,7], Cristina Benatti[2] and Ken Lukowiak[4]

[1]Department of Life Sciences, and [2]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia, Modena, Italy
[3]Department of Physiology and Pharmacology, and [4]Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta, Canada
[5]University of Calgary Cumming School of Medicine, Calgary, Alberta, Canada
[6]FLAME University, Pune, Maharashtra, India
[7]Consorzio Interuniversitario Biotecnologie, Trieste, Italy

VR, 0000-0002-8413-4510; KY, 0009-0003-6606-5376; AB, 0000-0002-3373-5519; JMCB, 0000-0002-4974-1964; FT, 0000-0002-3422-004X; CB, 0000-0003-0236-9525; KL, 0000-0001-9028-1931

The ability to learn and form memory is critical for survival, yet even genetically similar individuals can vary considerably in their cognitive performance. Using the pond snail *Lymnaea stagnalis*, we investigated how individual sensitivity to stress influences configural learning—a higher-order form of associative learning in which the simultaneous exposure to two contrasting stimuli, such as a predatory odour and an appetitive taste, results in the appetitive stimulus becoming associated with risk and evoking anti-predator behaviours. We used freshly collected, predator-naive snails from Margo Lake, Canada. While group-level data suggested the Margo strain failed to learn, individual-level analysis revealed that some snails successfully formed configural memories, while others did not. We hypothesized that this divergence reflects differences in individual (predator-related) stress responsiveness, which may modulate the engagement of memory-related molecular pathways. To test this, we measured expression levels of selected genes in the central ring ganglia. Snails that formed configural memories showed significantly higher expression of stress-responsive genes, components of the serotonin pathway and markers of neuroplasticity, along with increased endocannabinoid turnover. These findings suggest that individual variation in stress reactivity can drive adaptive differences in cognitive performance, offering new insights into the molecular and behavioural mechanisms underlying learning and memory.

1. Introduction

What causes the remarkable variation in learning and memory observed among individuals of the same species, even those sharing nearly identical genetic backgrounds? This question is central to understanding behavioural ecology, neurobiology and cognitive evolution [1–7]. Even when genetic differences are minimized, individuals can exhibit substantial variation in how they perceive, interpret and respond to environmental stimuli, highlighting the complexity of cognitive processes in natural populations [8–12].

This kind of intrastrain variability is especially intriguing because it likely reflects subtle divergences in neural processing, physiological state or gene regulation, rather than overt genetic differences [13–17]. Understanding the molecular underpinnings of such behavioural variation is crucial for uncovering how organisms fine-tune their cognitive strategies to meet specific environmental demands [18–20].

However, in mammalian systems, this task is further complicated by the structural complexity of the nervous system, genetic heterogeneity and limited experimental access to specific neural circuits [6,21]. In contrast, invertebrate models offer a powerful complementary tool [22–25] that enables high-resolution investigations of behaviour and gene expression under experimentally tractable and genetically controlled conditions [26].

Although individual cognitive variation remains relatively underexplored, a growing body of research across taxa has begun to reveal its ecological and evolutionary significance [27]. For example, zebrafish (*Danio rerio*) reared in standardized environments display marked differences in learning and memory, correlating with differential expression of genes involved in neural circuitry and stress reactivity [28–30]. These findings underscore the role of gene–environment interactions in determining individual cognitive trajectories, even in the absence of genetic variation. Similar trends have been observed in *Drosophila melanogaster*, where variation in dopaminergic and serotonergic gene expression has been linked to performance in associative learning tasks [31,32].

Building on these insights, the present study examines intrastrain variation in learning and memory using the freshwater snail *Lymnaea stagnalis* (Linnaeus 1758), a well-established model in neurobiology [33–37]. Thanks to its rich and well-characterized behavioural repertoire [34,38–44] and robust, reproducible paradigms for investigating diverse forms of learning and memory [45–49], it offers a valid model system to explore conserved molecular pathways underlying synaptic plasticity and stress regulation [50–53].

In behavioural assays designed to simulate ecologically relevant stressors, *Lymnaea* exhibits adaptive responses to a wide range of environmental challenges, including thermal fluctuations [54–57], low calcium levels [58], pollutants and toxins [59], drugs and bioactive compounds [60–62], food deprivation [63–66], overcrowding [67] and predator exposure [68,69]. Among these, predator-associated cues elicit particularly intriguing cognitive responses.

When a food stimulus (e.g. carrot slurry; C) is presented together with a predator-derived cue (e.g. crayfish effluent; CE), the appetitive taste is perceived as threatening, resulting in the suppression of feeding behaviour [70]. This response reflects configural learning (CL), a higher-order form of associative learning in which the snail encodes the combined stimulus in a configural unit and subsequently the appetitive or positive stimulus alone results in the response for the stress or aversive stimulus as the appetitive stimulus acquires a new meaning indicating 'aversion or stress' [1,70]. Such learning demonstrates a level of cognitive processing that extends beyond simple associations, enabling *Lymnaea* to flexibly reassess the valence of familiar cues within novel contexts [71].

In laboratory-inbred strains of *Lymnaea*, CL has been shown to produce memory traces lasting for at least 3 hours [70]. However, this memory can be strengthened under specific conditions. For example, long-term memory (LTM—lasting up to 24 hours) is formed when snails undergo a second training session within 7 days of the first or when exposed to cognitive enhancers, such as flavonoids [71–73]. Remarkably, even after more than 300 generations in a standardized, predator-free environment, these laboratory strains retain the capacity to detect predator cues and form configural associations [74]. In addition to this laboratory-inbred strain of *Lymnaea*, we found that two wild, predator-experienced snail populations collected from White Sand and Stony Lake (Canada) are also capable of forming CL [75]. These findings demonstrate that both wild and laboratory-reared snails possess the cognitive mechanisms required for higher-order learning. However, strain-specific differences have also emerged. A third wild population, collected from Margo Lake (i.e. a predator-free environment), responds to CE at the molecular level, upregulating the expression levels of genes associated with the serotonergic system (a pathway known to mediate antipredator responses) [69], yet appears not to form configural memories. Importantly, prior analyses of this population treated the strain as a behavioural aggregate, without considering individual variation. However, understanding how animals respond to complex environmental threats requires examining not only average behavioural tendencies across populations or strains but also the individual-level variation that underlies these responses.

Thus, in this study, we investigated whether individual-level differences in learning exist within a crayfish-naive population, and, if so, whether such differences correspond to distinct transcriptional differences in the central ring ganglia. Therefore, we performed experiments aimed at answering specific questions: (1) *Do some individuals within the Margo population form configural memories, despite the strain's average behavioural profile suggesting otherwise?* (2) *If so, are these behavioural differences reflected in distinct transcriptional profiles related to neuromodulation, synaptic plasticity and stress regulation?* For this purpose, we subjected freshly collected Margo snails to a CL paradigm and classified individuals as 'learners' or 'non-learners' based on their behavioural responses. We then conducted targeted gene expression analyses in snails' central ring ganglia. Candidate genes were selected for their established roles in cognitive processes, including synaptic plasticity [76,77]. These included heat shock protein 70 (LymHSP70), a conserved chaperone protein involved in cellular stress protection [52,78–80]; tryptophan hydroxylase (LymTPH) and serotonin transporter (LymSERT), orthologues of vertebrate genes that regulate serotonergic signalling [81–84]; glutamate (NMDA) receptor subunit 1 (LymGRIN1), an orthologue of the mammalian NMDA receptor subunit essential for glutamatergic synaptic plasticity [85,86]; purinergic receptor P2X (LymP2X), involved in stress-induced neuronal excitation [87,88]; and cAMP response element-binding protein 1 (LymCREB1), an evolutionarily conserved transcription factor critical for LTM formation [1,89,90].

Together, these candidate genes were selected to test the hypothesis that intrastrain differences in learning and stress responsiveness are associated with distinct transcriptional signatures in the central ring ganglia, reflecting modulation of neuromodulatory, synaptic plasticity, and stress-related pathways in learners versus non-learners.

Supporting this approach, across taxa, increased serotonergic tone is consistently associated with enhanced predator detection and learning [91–96]. If similar mechanisms apply to *Lymnaea*, learners (i.e. those more susceptible to the predator threat) may exhibit transcriptional effects reflecting elevated serotonergic signalling that may support encoding predator-induced fear and learning. Moreover, as stress responses involving heat shock proteins and purinergic receptors enhance neural excitability across species [96–100], learners might display elevated LymHSP70 and LymP2X expression levels, indicating heightened neural readiness during learning. Additionally, glutamatergic NMDA receptor activity and CREB-dependent transcription are conserved mechanisms critical for memory formation [97–103], raising the hypothesis that learners may exhibit increased LymGRIN1 and LymCREB1 expression, thereby facilitating synaptic plasticity and memory stabilization. Finally, given emerging evidence implicating endocannabinoid signalling in *Lymnaea* behavioural plasticity [53], we also included four genes that are orthologues of key enzymes involved in the synthesis and degradation of the endocannabinoids 2-arachidonoylglycerol and anandamide [104]: diacylglycerol lipase (LymDAGL) and monoacylglycerol lipase (LymMAGL), which synthesize and degrade 2-arachidonoylglycerol (2-AG), respectively [105]; and N-acyl phosphatidylethanolamine phospholipase D (LymNAPE-PLD) and fatty acid amide hydrolase (LymFAAH), which regulate anandamide metabolism [106]. In mammals, reduced endocannabinoid tone, mediated by increased degradation or decreased synthesis of key ligands, is associated with enhanced anxiety-like behaviour and improved threat discrimination [107,108]. If similar dynamics exist in *Lymnaea*, then learners may be characterized by upregulation of degradative enzymes (LymMAGL, LymFAAH) and downregulation of synthetic enzymes (LymDAGL, LymNAPE-PLD), consistent with reduced endocannabinoid signalling and heightened vigilance. This coordinated modulation may act to fine-tune serotonergic output and synaptic responsiveness during the encoding of aversive compound cues [109,110].

Together, these hypotheses provide a framework for laying the groundwork for future studies that aim to investigate the conserved mechanisms through which neuromodulatory and stress-related gene expression may contribute to individual differences in aversive learning. Thus, combining individual-level behavioural phenotyping with targeted gene expression analysis offers new insight into the molecular basis of cognitive variation in an ecologically relevant invertebrate model.

2. Methods

2.1. Snails

In this study, we used adult snails (shell lengths 20–25 mm) collected from Margo Lake, Saskatchewan, Canada (Saskatoon, Saskatchewan, 51°49′ N, 103°21′1.8″ W; elevation 526 m). Because all individuals share a common local genetic background and environmental history, we refer to the observed variation as *intrastrain*, enabling us to examine behavioural and molecular differences that likely reflect individual-level variation in memory performance. This contrasts with *interstrain* comparisons, such as between laboratory-reared and wild populations or between inbred and outbred lines, which involve greater genetic and environmental divergence and may obscure subtle within-population variability. After collection, the snails were housed at the University of Calgary under standardized laboratory conditions at 20 ± 1°C on a 16 h : 8 h light–dark cycle [58] and were fed romaine lettuce ad libitum.

2.2. Study design

To investigate whether individual snails from a genetically and environmentally homogeneous population exhibit differences in configural memory formation and whether these differences correlate with transcriptional profiles, we trained 15 naive adult snails from the Margo population using a CL paradigm.

Following a standardized memory test, individuals were behaviourally classified as ‘learners’ or ‘non-learners’.

Snails were then sacrificed, and their central ring ganglia were harvested for targeted gene expression analysis. In parallel, we included an untrained control group of eight Margo snails, housed under identical conditions, to assess baseline gene expression levels in the absence of a learning-related behavioural paradigm (figure 1). Sample sizes for behavioural and gene expression analyses were determined *a priori* using G*Power 3.1 (https://2054-4-10647044.it.softonic.com/) based on paired and RM-way ANOVA designs, respectively. For behavioural analyses, we assumed a two-tailed paired *t*-test with an expected large effect size (Cohen’s $d = 1.5$), $\alpha = 0.05$ and desired power of 0.8, which yielded a minimum of six individuals per group. For gene expression analyses, one-way ANOVAs with three groups (learners, non-learners and untrained controls) were used with an expected medium-to-large effect size ($f = 0.4$), $\alpha = 0.05$ and $1 - \beta = 0.8$, resulting in a minimum of 7–8 samples per group.

2.3. Configural learning training procedure and definition of learners and non-learners

To assess CL, we utilized a well-validated paradigm in which snails learnt to suppress feeding in response to an appetitive stimulus (C) when presented simultaneously with a predator cue (CE) [70]. Carrot slurry was prepared by blending approximately 600 g of peeled organic carrots in 500 ml of artificial pond water, followed by repeated straining to produce a particle-free solution [85]. This stimulus reliably elicits the feeding rasping response in *Lymnaea*, characterized by rhythmic scraping motions of the radula [111,112]. Snails were individually placed on a raised 14 cm petri dish with a mirror underneath to facilitate clear observation. After a 3 min acclimation period in either pond water or carrot slurry, rasping behaviour was recorded for 2 min and expressed as rasps per minute.

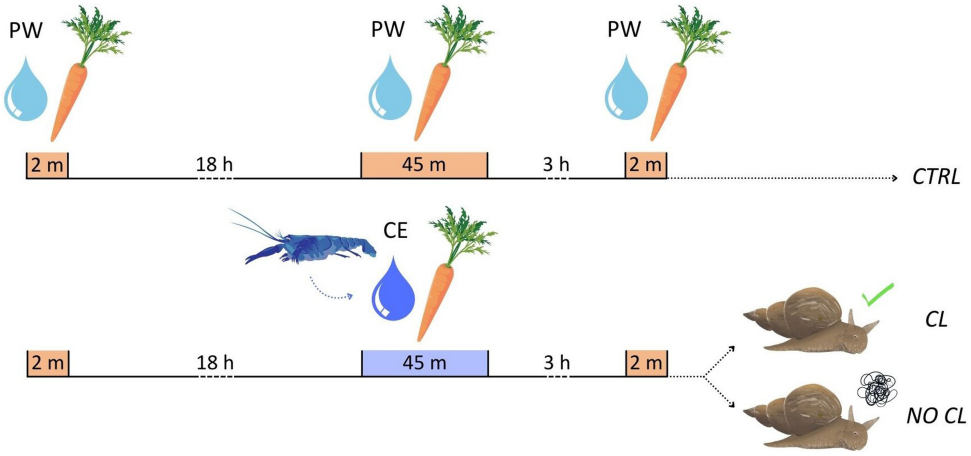

Figure 1. Study design. Snails were first exposed to a carrot slurry prepared by blending a carrot with artificial pond water and the feeding behaviour was recorded for 2 min. Eighteen hours later, control (CTRL) snails were re-exposed to the carrot slurry for 45 min, whereas trained snails were exposed for 45 min to a carrot slurry prepared with 500 ml of crayfish effluent (CE) in place of pond water. Three hours after this exposure, feeding behaviour was reassessed in carrot slurry made with artificial pond water, again for 2 min. Trained snails were classified as learners or non-learners based on changes in feeding behaviour following the CL procedure. Specifically, snails that showed a ≥20% reduction in rasping rate at C post 3 h compared to C pre were considered learners, while those with <20% reduction were classified as non-learners.

Snails were then returned to their home tanks, and after 18 hours, underwent the CL session: a 45 min exposure to carrot slurry prepared with 500 ml of CE (C + CE) instead of artificial pond water. CE was collected from a 70 l aquarium housing northern crayfish (*Faxonius virilis*) [113,114]. Three hours post-training, snails were retested with the carrot slurry (made with artificial pond water) and the feeding response was again measured. CL was quantified as the change in rasps per minute in carrot slurry between pre- and post-exposure to C + CE. Snails were classified as learners if they exhibited a ≥20% reduction in rasps per minute at 3 hours post-training relative to baseline; those with less than 20% reduction were classified as non-learners. This threshold was established through behavioural sensitivity analysis, which demonstrated that a 20% cut-off minimizes variability caused by noise while maintaining meaningful behavioural effects and statistical power (electronic supplementary material, figure S1).

A control group was exposed to carrot slurry prepared with pond water alone (i.e. no CE) for 45 min. All behavioural testing was conducted between 08.00 and 11.00, consistent with prior findings that *Lymnaea* exhibits peak learning performance during early photophase [114].

2.4. RNA extraction and reverse transcription

Following the completion of behavioural testing, snails were immediately anaesthetized by placing them on ice to minimize stress and metabolic activity before tissue collection [115]. Immediate sacrifice after the 3 hour memory test was essential, as memory formed by a single CL session is known to persist for approximately 3 hours. This timing allowed us to capture gene expression profiles during the early phase of memory formation (i.e. the 3 hour interval between the simultaneous exposure to predator cue and appetitive taste, and the subsequent memory test, when learners attribute a new 'meaning' to the carrot slurry following its simultaneous exposure to the CE) [69]. Delaying tissue collection could have resulted in the loss of these transient transcriptional signatures, due to either return to baseline or transition to later, less specific phases of plasticity. Additionally, since LTM (24 hour retention) in *Lymnaea* requires multiple spaced training sessions, our single-session design inherently limited memory duration. Thus, sampling beyond 3 hours would not have reflected stable memory but rather a post-consolidation or memory-decay state. Once anaesthetized, individuals were sacrificed, and the central ring ganglia were dissected and preserved in RNAlater solution (Invitrogen) to stabilize RNA from degradation. Samples were then processed at the University of Modena and Reggio Emilia (Italy). Total RNA was then extracted from each tissue using an RNeasy Mini Kit (Qiagen), adhering to the manufacturer's protocol, which included an on-column DNase I digestion step to eliminate any potential genomic DNA contamination. The quantity and purity of the extracted RNA were measured spectrophotometrically using a NanoDrop instrument (Thermo Fisher Scientific), ensuring that only high-quality RNA was used for downstream applications. RNA purity was evaluated by calculating the absorbance ratios at 260/280 and 260/230 nm, with acceptable ratios of approximately 2.0 and 2.0–2.2, respectively, confirming the absence of protein or organic contaminants. For complementary DNA (cDNA) synthesis, 200 ng of total RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), following the manufacturer's instructions.

2.5. Quantitative real-time polymerase chain reaction

Real-time polymerase chain reaction (PCR) was conducted using SYBR Green Master Mix (Bio-Rad). Each qPCR reaction contained 20 ng of cDNA template and was performed according to protocols previously described by [116] to ensure methodological consistency. The thermal cycling programme included an initial denaturation step at 95°C for 2 min, followed

by 40 amplification cycles consisting of 95°C for 15 s, 60°C for 30 s and 72 °C for 30 s. Cycle threshold (Ct) values were calculated using CFX Maestro™ software (Bio-Rad). Gene-specific primers were designed with the NCBI Primer-BLAST tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and synthesized by Merck KGaA (Darmstadt, Germany).

Each primer pair generated amplicons between 114 and 199 bp in length, with primer lengths of 19–23 nucleotides, melting temperatures between 58 and 62°C and GC content of 40–60%. Primer efficiency was determined by standard curve analysis and ranged from 95% to 105% across all primer pairs. The specificity of primers was confirmed by both dissociation curve analysis and agarose gel electrophoresis on a 2% gel, which ruled out the presence of non-specific products or primer-dimers. Specific forward and reverse primers targeting genes of interest were used at a final concentration of 300 nmol l^{-1}, with primer sequences carefully validated and reported in prior studies [53,87] and detailed in table 1. Ct values were calculated using CFX Maestro™ software (Bio-Rad), providing the raw data necessary for relative quantification.

To normalize gene expression levels and account for potential sample-to-sample variation, the stability of two candidate reference genes, *Lymnaea* elongation factor 1-alpha (LymEF1α) and *Lymnaea* tubulin (LymTUB), was rigorously evaluated using NormFinder software (https://moma.dk/normfinder-software) [117]. The arithmetic mean of these two reference genes was then used for normalization to improve accuracy. Relative gene expression was calculated using the $2^{-}\Delta\Delta$Ct method, employing untrained Margo snails as the calibrator group to establish baseline expression levels for comparison. Before statistical analysis, extreme outliers—defined as data points lying more than three times the interquartile range beyond the boxplot whiskers—were identified using the boxplot tool in SPSS (more than 3× the interquartile range outside the end of the interquartile box) and excluded to reduce the influence of anomalous values.

2.6. Statistical analyses

Normality of all datasets was assessed using the Kolmogorov–Smirnov one-sample test to confirm the appropriateness of parametric analyses. Behavioural data comparing the number of rasps elicited by carrot slurry before and after simultaneous exposure to appetitive taste and predator scent were analysed using paired *t*-tests. Differences in gene expression among learners, non-learners and untrained controls were analysed by one-way ANOVA followed by Tukey's *post hoc* tests, a robust method for detecting group-specific differences across discrete behavioural categories. Effect sizes for ANOVA were reported as *R*-squared (R^2), indicating the proportion of variance explained by group membership. In contrast, effect sizes for pairwise comparisons using independent *t*-tests were expressed as eta squared (η^2). To correct for multiple comparisons and minimize type I error, Tukey's multiple comparisons test was applied *post hoc*, with adjusted *p*-values reported alongside 95% confidence intervals for differences between group means. Both unadjusted *p*-values and adjusted *q*-values are presented for full transparency. All statistical analyses were performed using SPSS v. 26.0 (IBM Corp., Armonk, NY, USA) and graphical representations were generated with GraphPad Prism v. 10.00e (GraphPad Software, La Jolla, CA, USA).

3. Results

3.1. Intrastrain differences in predator sensitivity and configural learning memory

Initial analysis of the full cohort of Margo snails (n = 15) revealed no significant change in rasping frequency to carrot slurry following CL training, which involved simultaneous exposure to carrot slurry and CE (t = 1.62, d.f. = 14, p = 0.13, partial η^2 = 0.16) (figure 2). However, when individuals were classified based on behavioural performance, two distinct memory phenotypes emerged. Six snails showing a reduction in rasping frequency of ≥20% were categorized as learners (figure 2), while the remaining nine were classified as non-learners (figure 2). Separate analyses revealed that learners exhibited a significant decrease in rasping behaviour post-training (t = 3.57, d.f. = 5, p = 0.0016, partial η^2 = 0.72), consistent with CL, whereas non-learners showed no significant change (t = 0.69, d.f. = 8, p = 0.5, partial η^2 = 0.06).

3.2. Margo snails that form configural learning show transcriptional differences in stress responsivity, serotonergic activation and neuroplasticity

We observed a significant increase in LymHSP70 expression levels ($F_{2,20}$ = 7.2, p = 0.004, R^2: 0.42; figure 3A) in snails that successfully formed CL memory compared to those that failed to form memory (p = 0.011, q = 4.58) and untrained controls (p = 0.006, q = 4.94). Focusing on serotonergic targets, we found that memory-forming snails exhibited significantly higher expression levels of LymTPH ($F_{2,18}$ = 12.31, p = 0.0004, R^2: 0.57; figure 3B) and LymSERT ($F_{2,20}$ = 6.23, p = 0.008, R^2: 0.38; figure 3C) compared to the untrained controls (p = 0.002, q = 5.65 and p = 0.007, q = 4.82, respectively) and their non-learner counterparts (p = 0.0005, q = 6.59 and p = 0.03, q = 3.87, respectively). Similarly, the mRNA levels of LymP2X were significantly increased in learner snails ($F_{2,20}$ = 7.68, p = 0.003, R^2: 0.43; figure 3D), with expression levels higher compared to both controls (p = 0.007, q = 4.80) and non-learners (p = 0.005, q = 5.05). To further characterize the transcriptional correlates of memory formation, we investigated the expression of genes involved in long-term plasticity [49], focusing on LymGRIN1 and LymCREB. LymGRIN1 expression levels were significantly higher in learner snails ($F_{2,20}$ = 7.09, p = 0.005, R^2: 0.41; figure 3E), compared to those of both non-learners (p = 0.004, q = 5.19) and controls (p = 0.02, q = 4.13). Similarly, LymCREB1 was significantly elevated in the learner group ($F_{2,20}$ = 5.53, p = 0.012, R^2: 0.42; figure 3F), with *post hoc* analyses confirming higher expression relative to non-learners (p = 0.015, q = 4.39) and controls (p = 0.03, q = 3.97).

Table 1. The forward and reverse primer nucleotide sequences utilized in qRT-PCR, along with the accession number for each target and the size (bp) of the PCR product obtained through the amplification of cDNA (mRNA). The prefix 'Lym' refers to *Lymnaea stagnalis*, indicating these are putative homologues identified via sequence annotation in this species. This annotation convention explicitly states when genes represent orthologues based on homology to well-characterized genes in other species.

gene bank accession	target	product length (bp)	type sequence
DQ206432.1	*Lymnaea stagnalis* heat-shock protein 70, **LymHSP70**	199	5′– AGGCAGAGATTGGCAGGAT–3′
			5′–CCATTTCATTGTGTCGTTGC–3′
AF129815.1	*Lymnaea stagnalis* tryptophan hydroxylase, **LymTPH**	179	5′–AGGATACAGTCTACCGACAG–3′
			5′–TGAGTTCACGGAAAACTATT–3′
FX185022	*Lymnaea stagnalis* serotonin transporter, **LymSERT**	177	5′–ATACCGTACCTTGTCATGTT–3′
			5′–TGTTGTAGTACCAGGAGACA–3′
JX524180.1	*Lymnaea stagnalis* P2X receptor, **LymP2X**	150	5′–GGGATCGTCTTCGTGGTGA–3′
			5′–AGTTCCTGGCCTTCAACAGAT–3′
AY571900.1	*Lymnaea stagnalis* NMDA-type glutamate receptor, **LymGRIN1**	140	5′–AGAGGATGCATCTACAATTT–3′
			5′–CCATTTACTAGGTGAACTCC–3′
AB041522.1	*Lymnaea stagnalis* cAMP responsive element binding protein, **LymCREB1**	180	5′–AACCGCAGCAACCCTAACAA–3′
			5′–GTCAGCAGGGAATGGTCCTG–3′
FX181219.1	diacylglycerol lipase, **LymDAGL**	272	5′–CCATGTGACCCCACAATGGA–3′
			5′–TTTGTGCAGCCACGGAAAAC–3′
FX189644.1	monoglyceride lipase, **LymMAGL**	216	5′–TTCTGACGGATTGCCTGGTC–3′
			5′–ACTCAATGGGAGGTGCAGTG–3′
FX195512.1	*N*-acyl-phosphatidylethanolamine-hydrolysing phospholipase D, **LymNAPE-PLD**	312	5′–CCATGTTTCCCAGGGGTTCT–3′
			5′–GGCGGGCTAAGCTAAGTTGT–3′
FX195089.1	fatty acid amide hydrolase, **LymFAAH**	168	5′–CCCTCAAGACAAGACGCGAT–3′
			5′–CTAATGCGCCCCCTAGTTGT–3′
X15542.1	*Lymnaea stagnalis* beta-tubulin, **LymTUB**	127	5′–GAAATAGCACCGCCATCC–3′
			5′–CGCCTCTGTGAACTCCATCT–3′
DQ278441.1	*Lymnaea stagnalis* elongation factor 1-alpha, **LymEF1α**	150	5′–GTGTAAGCAGCCCTCGAACT–3′
			5′–TTCGCTCATCAATACCACCA–3′

3.3. Configural learning modulates the endocannabinoid system

Learner snails showed significantly higher expression levels of LymMAGL ($F_{2,20}$ = 7.81, p = 0.003, R^2: 0.44; figure 4B) and LymFAAH ($F_{2,20}$ = 11.12, p = 0.0006, R^2: 0.53; figure 4C), with LymMAGL expression being higher compared to both controls (p = 0.004, q = 5.18) and non-learners (p = 0.008, q = 4.73), while LymFAAH was also significantly elevated (p = 0.0008, q = 6.22 versus control; p = 0.002, q = 5.58 versus non-learners). Conversely, the expression of genes involved in endocannabinoid synthesis was significantly decreased. In particular, the expression levels of LymDAGL, responsible for 2-AG biosynthesis ($F_{2,20}$ = 3.68, p = 0.004; R^2: 0.28; p = 0.03, q = 3.82, figure 4A), and LymNAPE-PLD, which synthesizes AEA ($F_{2,20}$ = 7.40, p = 0.004; R^2: 0.43; figure 4D), were reduced in learners compared to non-learners (p = 0.03, q = 3.82 and p = 0.003, q = 5.4, respectively).

4. Discussion

This study reveals intrastrain variability among Margo snails in their ability to form configural memories, despite uniform genetic and environmental backgrounds. Although the entire cohort underwent identical CL training, no significant behavioural change was observed at the group level. However, when individuals were classified based on their memory performances, two distinct behavioural phenotypes, learners and non-learners, emerged. This individual variation emphasizes that learning and memory formation are not uniform but depend on intrinsic differences (such as stochastic developmental processes, epigenetic modifications or subtle microenvironmental influences) in how individuals perceive and integrate environmental cues—specifically, food and predator scent. This aligns with evidence from other model organisms, including inbred rodent strains (genetically nearly identical and largely homozygous at most loci) maintained under strictly controlled laboratory conditions [15]. It suggests that such variability is intrinsic and biologically significant. Based on this, we hypothesize that

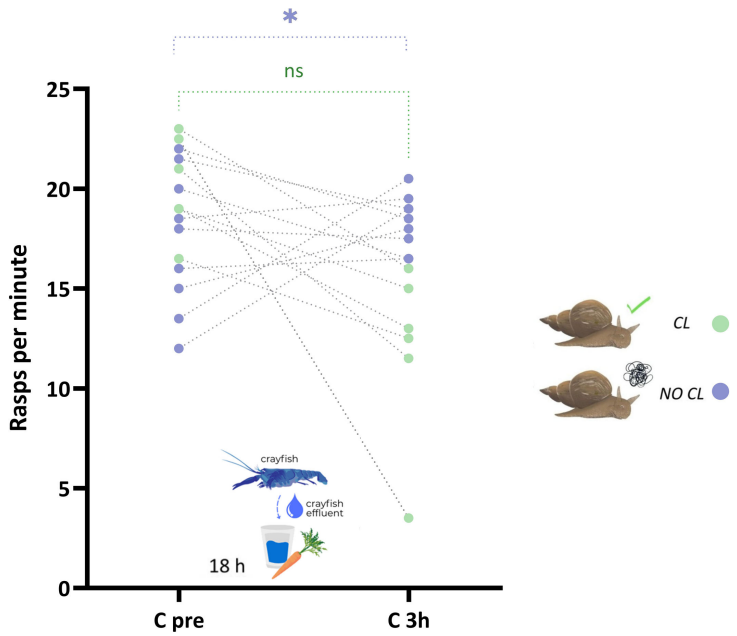

Figure 2. Intrastrain differences in predator sensitivity and CL memory. Margo snails ($n = 15$) were exposed to carrot slurry and the number of rasps was measured for 2 min (C pre). Eighteen hours later, snails were exposed simultaneously to the carrot slurry and the CE, and 3 hours later, the number of rasps was again recorded in carrot slurry made with snails' pond water (C 3 h). Margo snails that formed CL showed a significant reduction in the number of rasps ($n = 6$—green circles), whereas those that did not form CL showed no significant reduction in the number of rasps ($n = 9$—purple circles). Data were analysed using a paired *t*-test. *$p < 0.005$; ns = not significant as $p > 0.05$.

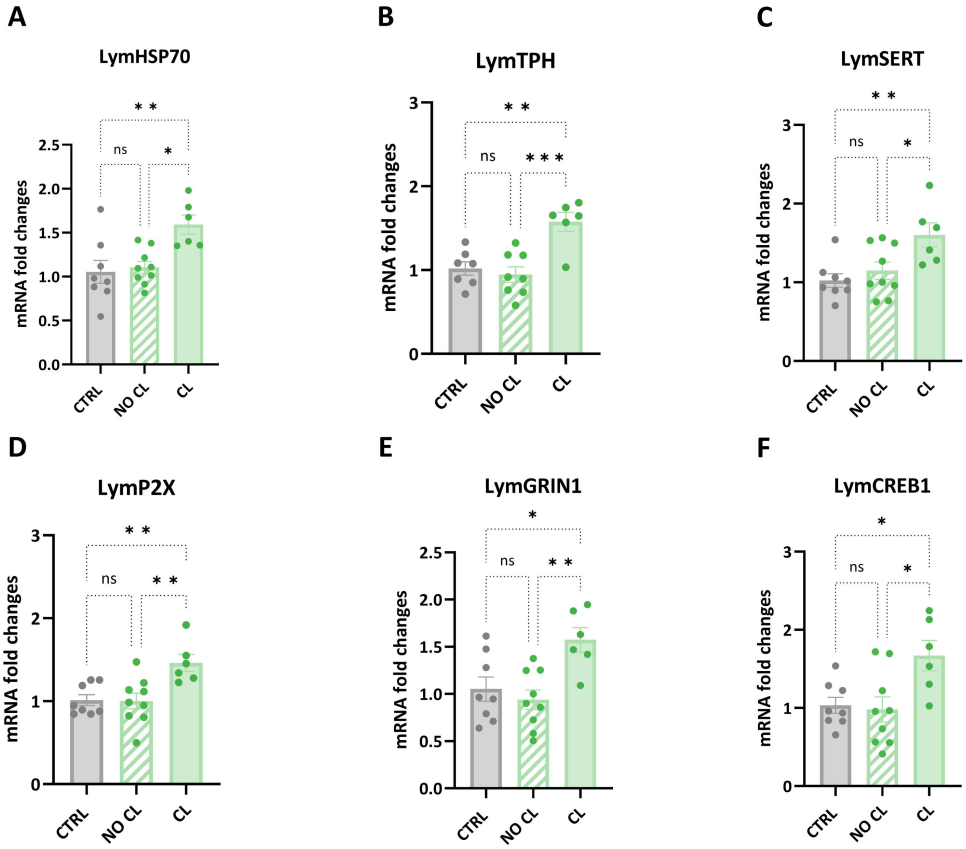

Figure 3. Transcriptional effects induced by the CL procedure on the expression levels of key targets involved in stress response and neuroplasticity in the central ring ganglia of *L. stagnalis*. After being simultaneously exposed to C + CE for 45 min, 6 snails formed CL memory (CL group, green bars), whereas 9 snails did not (NO CL group, diagonal green bars). Eight control snails (CTRL—grey bars) were exposed to C + PW for 45 min instead of C + CE (CTRL group, grey bars). Snails were sacrificed and RNA from the central ring ganglia was extracted and retrotranscribed. (A) LymHSP70, (B) LymTPH, (C) LymSERT, (D) LymP2X, (E) LymGRIN1 and (F) LymCREB1 mRNA expression in the ganglia were measured by qPCR. Data are represented as means ± s.e.m. and were analysed with one-way ANOVA followed by Tukey's *post hoc* test. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

individual differences in learning ability and stress sensitivity in snails are meaningful biological traits that are likely to persist across different genetic backgrounds and environmental contexts, even though their magnitude or expression may vary.

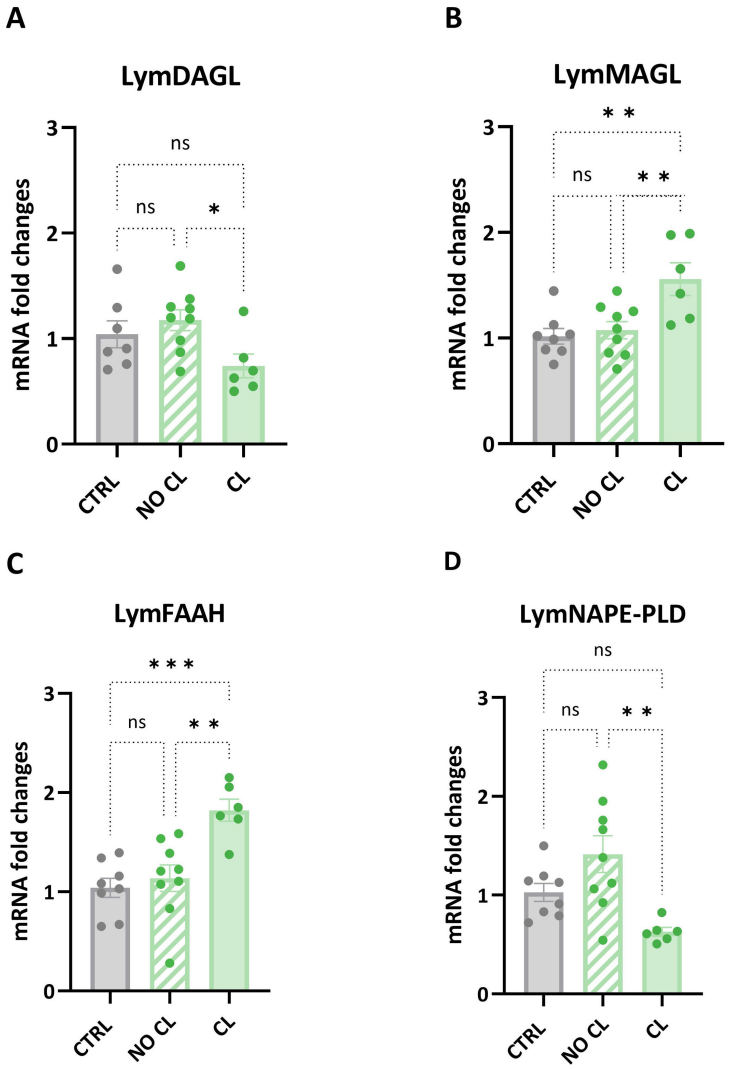

Figure 4. Transcriptional effects induced by the CL procedure on the expression levels of enzymes of the endocannabinoid system in the central ring ganglia of *L. stagnalis*. After being simultaneously exposed to C + CE for 45 min, 6 snails formed CL memory (CL group, green bars), whereas 9 snails did not (NO CL group, diagonal green bars). Eight control snails (CTRL—grey bars) were exposed to C + PW for 45 min instead of C + CE (CTRL group, grey bars). Snails were sacrificed and RNA from the central ring ganglia was extracted and retrotranscribed. (A) LymDAGL, (B) LymMAGL, (C) LymFAAH and (D) LymNAPE-PLD mRNA expression were measured by qPCR. Data are represented as means ± s.e.m. and were analysed with one-way ANOVA followed by Tukey's *post hoc* tests. ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

Therefore, future studies examining multiple populations and ecological conditions will be conducted to test this hypothesis and evaluate the broader applicability of our findings.

Building on our behavioural results, we next sought to determine whether such behavioural variability is accompanied by underlying molecular differences. Specifically, we asked: *What transcriptional changes underlie the formation of configural learning memory? How do gene expression patterns differ between individuals who successfully form these associations ('learners') versus those who do not ('non-learners')?* To address these questions, we employed a targeted candidate gene approach, selecting a curated panel of genes implicated in stress responsiveness, neuromodulation and memory formation [52,59,66,73,85,90,116,118–121]. While we cannot entirely exclude contributions from generalized stress responses, several aspects of our design support the interpretation that the transcriptional profiles observed here reflect early molecular events underlying configural memory formation [109,110].

First, all individuals experienced the same training protocol, minimizing the likelihood that differential expression stems from non-specific stress. Second, the timing of sampling aligns with established windows for memory-related gene expression in both invertebrates and vertebrates [122–125]. Third, our gene panel targets conserved regulators of synaptic plasticity and neuromodulation, rather than general stress markers, further supporting their relevance to learning and memory processes [80,96,126–131].

One of the most striking molecular features of memory-forming snails was the significant increase in LymHSP70 expression levels, suggesting that the perception of CE as a biologically meaningful stressor [132] is a necessary component for memory formation using this specific CL training procedure. This finding is consistent with studies in mammalian systems where HSP70 mRNA levels are significantly higher in learning-associated brain regions, such as the hippocampus, following exposure to novel or aversive experiences [133–136]. Additionally, our previous studies revealed that this target is a key player in enabling memory formation under different stressful conditions and behavioural procedures [49,52,53,90,137]. Here, increased LymHSP70 mRNA levels may reflect enhanced neuronal resilience and an increased readiness to process and integrate aversive cues, ultimately facilitating the association between predator scent and food.

The significant increase in LymTPH and LymSERT expression levels in learners versus non-learners further supports this hypothesis. These targets may increase serotonergic synthesis and recycling, suggesting an overall enhancement of serotonergic tone [82,138,139]. Serotonin is widely recognized as a neuromodulator of arousal, vigilance and associative learning [133,134,140], and its involvement in predator detection has been documented in numerous taxa [82,95,96,126,141]. Our finding that only memory-forming snails show heightened serotonergic gene expression suggests that the detection of predator cues and the integration of that signal into memory is gated by the sensitivity of the serotonergic system [69]. Thus, the perception of the CE as a risk signal is not uniform across individuals, and only those with sufficient serotonergic responsiveness appear capable of encoding the predator–food association. Interestingly, both LymTPH and LymSERT have previously been shown to increase in response to crayfish exposure, even in naive snails [69]. However, this is the first time we focused on the transcriptional effects induced by the CL procedure. In other words, previous studies aimed at investigating the transcriptional effects induced by CE alone; here, this stimulus was used as the aversive cue in the behavioural paradigm, allowing us to investigate intrastrain differences. Importantly, in the present study, only a subset of snails (i.e. those that formed CL) exhibited both increased expression of serotonergic genes and corresponding behavioural evidence of learning. This suggests that serotonergic gene upregulation may be an experience-dependent molecular change linked specifically to CL.

Complementing the serotonergic findings, learners also exhibited elevated expression of LymP2X. In vertebrates, P2X receptors have been linked to long-term potentiation, synaptic transmission and modulation of fear and anxiety responses [102,142]. In our study, LymP2X mRNA levels were significantly higher in learners' central ring ganglia, suggesting enhanced purinergic modulation of synaptic circuits involved in threat detection and associative learning. In addition to these modulatory systems, memory-forming snails exhibited elevated expression of LymGRIN1 and LymCREB1, key targets for synaptic plasticity and LTM formation [143,144]. These findings align well with established molecular cascades in vertebrates and suggest that *Lymnaea* may recapitulate fundamental aspects of memory biology.

Perhaps the most novel and unexpected aspect of our findings is the differential expression of genes involved in the endocannabinoid system. Critically, the endocannabinoid system, widely studied in vertebrates for its role in regulating fear, anxiety and stress, interacts directly with serotonergic pathways [110,129]. Cannabinoid receptors are expressed on serotonergic neurons, where endocannabinoids modulate serotonin release and receptor activity at both presynaptic and post-synaptic sites [110,145,146]. Depending on the context, this modulation can either enhance or inhibit serotonergic signalling, thereby influencing the expression and formation of CL memories [110,145,146]. Although the specific cellular mechanisms of endocannabinoid system function are not yet fully characterized in *Lymnaea*, our findings raise the hypothesis that endocannabinoid signalling may influence serotonin-mediated anti-predatory responses during learning and memory formation. Consistent with this idea, snails that exhibited CL showed increased expression of degradative enzymes (LymMAGL and LymFAAH), which metabolize 2-AG and anandamide, respectively, and decreased expression of synthetic enzymes (LymDAGL and LymNAPE-PLD), responsible for 2-AG and anandamide production [109]. In mammals, similar shifts, characterized by increased degradation or reduced synthesis of 2-AG and anandamide, are associated with increased anxiety-like behaviour and improved discrimination of threat-relevant stimuli [109,110,147]. We hypothesize that analogous processes may occur in *Lymnaea*, where reduced endocannabinoid tone in learners—which may alter serotonergic signalling [105,106,148–152,135]—could support heightened vigilance and experience-dependent plasticity under conditions of perceived threat, promoting LTM formation. This is consistent with our previous findings showing that pharmacological manipulation of the endocannabinoid system alters LTM following operant conditioning of aerial respiration [153], suggesting that endocannabinoid–serotonergic interactions provide a modulatory mechanism linking environmental stressors to memory encoding and consolidation in *Lymnaea*.

While these hypotheses are compelling, it is important to note that our study focused exclusively on transcriptional changes. Although the precise extent to which these transcriptional changes drive behavioural and memory outcomes remains to be determined, they offer testable hypotheses about the molecular mechanisms underlying inter-individual variability in learning. To fully assess the functional impact of these transcriptional responses on learning, memory consolidation and the modulation of neural circuits by stress, future studies will integrate protein measurements, enzymatic activity, pharmacological manipulations and electrophysiology.

Additionally, our molecular data suggest that CL memory formation may reflect a coordinated modulation of functionally distinct pathways, including serotonergic signalling, purinergic transmission and the endocannabinoid system, suggesting that these transcriptional effects may be modulated, at least in part, by broader transcriptomic programmes potentially controlled by master transcription factors or other global regulatory elements. However, in this study, we focused on specific genes with established roles in learning, neuromodulation and stress adaptation, rather than conducting a genome-wide survey. To fully elucidate the molecular architecture underlying CL and individual variability, future research should incorporate transcriptome-wide methods such as RNA sequencing. These approaches would enable the identification of co-regulated gene modules, uncover key transcriptional regulators and map the broader signalling networks engaged during predator cue processing and memory formation. Moreover, transcriptomic datasets allow for advanced multivariate analyses, such as principal component analysis, hierarchical clustering and gene co-expression network inference, that can reveal latent structure and functional organization within high-dimensional gene expression data [154].

Moreover, it is important to emphasize that our behavioural measure focused exclusively on feeding behaviour, which is shaped by a complex interplay of motivational, cognitive and physiological factors [155–158]. While reduced feeding in this context is regarded as a valid proxy for CL, it may also reflect modulations in arousal, stress responses or general motivational states. Consequently, future studies integrating complementary behavioural, physiological and neurobiological measures will disentangle the relative contributions of these factors.

Future research should explore a wider range of learning paradigms [45,62,115,159,160], as different stressors can selectively affect various cognitive processes. These results emphasize the importance of paradigm-specific assessments to fully

understand how individual differences in stress sensitivity impact learning and memory. Additionally, they underline the necessity of examining multiple behavioural contexts to capture the complexity and variability of cognitive responses to environmental challenges.

Additionally, in the current study, we did not quantify behaviour during the 45 min conditioning period, focusing instead on post-conditioning feeding responses as indicators of learning and memory. However, in ongoing unpublished experiments, we have begun to characterize behavioural dynamics during this critical exposure phase. Preliminary observations indicate that snails exposed to C+CE exhibit pronounced anti-predatory behaviours, including a significant decrease in rasping activity and increased time spent withdrawn inside their shells. Quantifying these dynamic anti-predatory behaviours in real time could provide valuable insights into how the balance between stress-induced inhibition and learning-related plasticity unfolds within individuals. Moreover, exploring individual differences in the magnitude or duration of these early stress responses may help clarify why some snails successfully form aversive associative memories while others do not. Thus, future studies integrating detailed behavioural monitoring during conditioning with concurrent molecular and neurophysiological measurements could illuminate the temporal interplay between stress and learning systems, ultimately advancing our understanding of how immediate environmental challenges shape cognitive outcomes at the individual level.

In the present study, we focused on a single post-training time point corresponding to intermediate-term memory, which relies on de novo protein synthesis and persists up to 3 hours [71,161]. While this approach allowed us to capture transcriptional changes associated with memory that requires protein synthesis, it does not provide information about earlier (i.e. short-term memory) or later (i.e. LTM) molecular events. Future studies examining multiple time points will be critical to elucidate the temporal progression of transcriptional responses, including how early gene expression patterns may prime neural circuits for plasticity and how late transcriptional changes consolidate memory. Such time-course analyses will also allow for the identification of transient versus sustained molecular signatures, and clarify how stress-sensitive pathways interact with memory formation across distinct temporal phases.

Finally, this study focused exclusively on the central ring ganglia, though future work will expand to peripheral tissues, including the buccal ganglia. The central ring ganglia contain the cerebral giant cells [34,115,162,163], which are well-established as necessary for both the formation and recall of LTM in *Lymnaea* [164]. Based on this pivotal role, we targeted our molecular analyses to the central ring ganglia and deliberately did not assay other ganglia. By concentrating on this region, we could capture the molecular signatures most directly linked to behavioural outcomes. Indeed, our results demonstrate that these transcriptional changes in the central ring ganglia track with individual learning performance and stress sensitivity, reinforcing their central role in memory-related plasticity. Thus, focusing on the central ring ganglia initially provides the most meaningful and biologically relevant insights into the molecular mechanisms underlying learning in *Lymnaea*. However, we plan to perform additional experiments to include the buccal ganglia, which are directly involved in feeding behaviours, as well as other peripheral ganglia, to determine whether the transcriptional changes observed in the central ring ganglia are conserved across neural regions. These analyses will allow us to evaluate region-specific versus systemic patterns of gene expression and examine potential interactions between central and peripheral circuits in mediating stress responsiveness and behavioural plasticity.

5. Conclusion

This study reveals that even within a genetically and environmentally uniform population of *Lymnaea*, individuals vary markedly in their ability to form configural memories—differences reflected at the transcriptional level by key targets involved in stress response, anti-predatory behaviour and synaptic plasticity. While our targeted approach highlights critical molecular players, it also opens the door for future research into broader, dynamic regulatory networks shaping learning at the individual level. The challenge ahead lies in unravelling how these molecular signatures translate into functional neural circuits and enduring behavioural adaptations. Exploring this intricate 'behavioural, molecular, and neural dance' promises to deepen our understanding not only of invertebrate cognition but also of fundamental principles governing learning and memory across all animals.

Ethics. Ethical approval was not required for experiments involving *Lymnaea stagnalis*, as this species is an invertebrate and not subject to regulations under current animal welfare legislation (e.g. EU Directive 2010/63/EU). Nonetheless, all procedures were conducted in accordance with the principles of ethical research and animal welfare. The study adhered to the 3Rs (Replacement, Reduction, and Refinement) to ensure responsible use of animals in research. The number of animals used was minimized without compromising scientific validity, and all efforts were made to ensure animal well-being. Snails were maintained under optimal conditions, including clean, oxygenated water, appropriate nutrition, and low-density housing, to reduce stress and promote natural behavior.

Data accessibility. Data files will be made available upon reasonable request to the corresponding author (cristina.benatti@unimore.it).

Supplementary material is available online [165].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. V.R.: conceptualization, data curation, formal analysis, methodology, visualization, writing—original draft; K.Y.: conceptualization, data curation, formal analysis, methodology, visualization, writing—review and editing; G.P.: conceptualization, data curation, investigation, writing—review and editing; A.B.: conceptualization, data curation, methodology, writing—review and editing; J.M.C.B.: funding acquisition, writing—review and editing; F.T.: funding acquisition, resources, writing—review and editing; C.B.: conceptualization, funding acquisition, supervision, writing—review and editing; K.L.: conceptualization, project administration, resources, supervision, validation, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. This research was supported by FAR2024_Ricerca diffusa, University of Modena and Reggio Emilia and the Natural Sciences and Engineering Research Council of Canada.

Acknowledgements. The authors would like to thank Dr Eleonora Daini for her assistance in creating the graphical abstract, which was produced using MindTheGraph and Canva. V.R. gratefully acknowledges support from the Company of Biologists through a Travelling Fellowship, which supported her research visit to the Hotchkiss Brain Institute in K.L.'s laboratory.

References

1. Batabyal A, Rivi V, Benatti C, Blom JMC, 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)
2. Roy B, Dwivedi Y. 2023 An insight into the sprawling microverse of microRNAs in depression pathophysiology and treatment response. *Neurosci. Biobehav. Rev*. **146**, 105040. (doi:10.1016/j.neubiorev.2023.105040)
3. Ferreira VHB, Lansade L, Calandreau L, Cunha F, Jensen P. 2023 Are domesticated animals dumber than their wild relatives? A comprehensive review on the domestication effects on animal cognitive performance. *Neurosci. Biobehav. Rev*. **154**, 105407. (doi:10.1016/j.neubiorev.2023.105407)
4. Leadbeater E, Watrobska C. 2025 What could evolve in the evolution of memory? *Phil. Trans. R. Soc. B* **380**, 20240109. (doi:10.1098/rstb.2024.0109)
5. Sih A, Del Giudice M. 2012 Linking behavioural syndromes and cognition: a behavioural ecology perspective. *Phil. Trans. R. Soc. B* **367**, 2762–2772. (doi:10.1098/rstb.2012.0216)
6. Rivi V, Benatti C, Rigillo G, Blom JMC. 2023 Invertebrates as models of learning and memory: investigating neural and molecular mechanisms. *J. Exp. Biol*. **226**, jeb244844. (doi:10.1242/jeb.244844)
7. Rivi V, Batabyal A, Pele G, Yakubets K, Dominici R, Blom JMC, Tascedda F, Benatti C, Lukowiak K. 2025 Intrastrain variability in memory formation of freshly collected *Lymnaea stagnalis*: the influence of stressor type on memory. *Comp. Biochem. Physiol. B Biochem. Mol. Biol*. **280**, 111140. (doi:10.1016/j.cbpb.2025.111140)
8. Hale R, Piggott JJ, Swearer SE. 2016 Describing and understanding behavioral responses to multiple stressors and multiple stimuli. *Ecol. Evol*. **7**, 38–47. (doi:10.1002/ece3.2609)
9. Lefebvre L, Sol D. 2008 Brains, lifestyles and cognition: are there general trends? *Brain Behav. Evol*. **72**, 135–144. (doi:10.1159/000151473)
10. Rosa MGP, Tweedale R. 2005 Brain maps, great and small: lessons from comparative studies of primate visual cortical organization. *Phil. Trans. R. Soc. B* **360**, 665–691. (doi:10.1098/rstb.2005.1626)
11. Seed A, Seddon E, Greene B, Call J. 2012 Chimpanzee 'folk physics': bringing failures into focus. *Phil. Trans. R. Soc. B* **367**, 2743–2752. (doi:10.1098/rstb.2012.0222)
12. Taylor AH, Hunt GR, Medina FS, Gray RD. 2008 Do New Caledonian crows solve physical problems through causal reasoning? *Proc. R. Soc. B* **276**, 247–254. (doi:10.1098/rspb.2008.1107)
13. Courret C, Larracuente AM. 2023 High levels of intra-strain structural variation in *Drosophila simulans* X pericentric heterochromatin. *Genetics* **225**, iyad176. (doi:10.1093/genetics/iyad176)
14. Jung SH *et al*. 2020 Strain differences in responsiveness to repeated restraint stress affect remote contextual fear memory and blood transcriptomics. *Neuroscience* **444**, 76–91. (doi:10.1016/j.neuroscience.2020.07.052)
15. Laudani S *et al*. 2023 Gut microbiota alterations promote traumatic stress susceptibility associated with p-cresol-induced dopaminergic dysfunctions. *Brain Behav. Immun*. **107**, 385–396. (doi:10.1016/j.bbi.2022.11.004)
16. Mozhui K *et al*. 2010 Strain differences in stress responsivity are associated with divergent amygdala gene expression and glutamate-mediated neuronal excitability. *J. Neurosci. Off. J. Soc. Neurosci*. **30**, 5357–5367. (doi:10.1523/JNEUROSCI.5017-09.2010)
17. Valcek A *et al*. 2025 Inter- and intra-bacterial strain diversity remains the 'elephant in the (living) room'. *NPJ Antimicrob. Resist*. **3**, 67. (doi:10.1038/s44259-025-00138-8)
18. Niemelä PT, Vainikka A, Forsman JT, Loukola OJ, Kortet R. 2013 How does variation in the environment and individual cognition explain the existence of consistent behavioral differences? *Ecol. Evol*. **3**, 457–464. (doi:10.1002/ece3.451)
19. Rowe C, Healy SD. 2014 Measuring variation in cognition. *Behav. Ecol*. **25**, 1287–1292. (doi:10.1093/beheco/aru090)
20. Sol D, Bateman-Neubert A, Noguer L, Taylor AH. 2025 The evolutionary puzzle of cognition: challenges and insights from individual-based studies. *Phil. Trans. R. Soc. B* **380**, 20240123. (doi:10.1098/rstb.2024.0123)
21. Abbondanza A *et al*. 2025 Dissection of neurochemical pathways across complexity and scale. *J. Neurochem*. **169**, e70160. (doi:10.1111/jnc.70160)
22. Gatto E, Loukola OJ, Agrillo C. 2022 Quantitative abilities of invertebrates: a methodological review. *Anim. Cogn*. **25**, 5–19. (doi:10.1007/s10071-021-01529-w)
23. Menzel R, Benjamin PR. 2013 Beyond the cellular alphabet of learning and memory in invertebrates. In *Handbook of behavioral neuroscience* (eds R Menzel, PR Benjamin), pp. 3–5. Amsterdam, The Netherlands: Academic Press. (doi:10.1016/B978-0-12-415823-8.00001-0)
24. Ottaviani E, Franceschi C. 1996 The neuroimmunology of stress from invertebrates to man. *Prog. Neurobiol*. **48**, 421–440. (doi:10.1016/0301-0082(95)00049-6)
25. Rivi V, Benatti C, Lukowiak K, Colliva C, Alboni S, Tascedda F, Blom JMC. 2021 What can we teach *Lymnaea* and what can *Lymnaea* teach us? *Biol. Rev. Camb. Philos. Soc*. **96**, 1590–1602. (doi:10.1111/brv.12716)
26. Krishnan N, Xu J. 2023 Editorial: Invertebrates as model organisms: opportunities and challenges in physiology and bioscience research. *Front. Physiol*. **14**, 1244594. (doi:10.3389/fphys.2023.1244594)
27. Thornton A, Lukas D. 2012 Individual variation in cognitive performance: developmental and evolutionary perspectives. *Phil. Trans. R. Soc. B* **367**, 2773–2783. (doi:10.1098/rstb.2012.0214)
28. DePasquale C, Kemerer N, White N, Yost M, Wolfkill J, Sturgill J, Li X. 2021 The influence of an enriched environment in enhancing recognition memory in zebrafish (*Danio rerio*). *Front. Vet. Sci*. **8**, 749746. (doi:10.3389/fvets.2021.749746)
29. Fontana BD, Moraes HS, Pretzel CW, Mohammed KA, Canzian J, Bonan CD, Parker MO, Rosemberg DB. 2025 Individual differences in boldness influence working memory and stress-induced repetitive behaviors in zebrafish. *Eur. J. Neurosci*. **62**, e70173. (doi:10.1111/ejn.70173)
30. Tran S, Gerlai R. 2013 Individual differences in activity levels in zebrafish (*Danio rerio*). *Behav. Brain Res*. **257**, 224–229. (doi:10.1016/j.bbr.2013.09.040)
31. Mery F, Kawecki TJ. 2002 Experimental evolution of learning ability in fruit flies. *Proc. Natl Acad. Sci. USA* **99**, 14274–14279. (doi:10.1073/pnas.222371199)
32. Smith MAY, Honegger KS, Turner G, de Bivort B. 2022 Idiosyncratic learning performance in flies. *Biol. Lett*. **18**, 20210424. (doi:10.1098/rsbl.2021.0424)
33. Amorim J, Abreu I, Rodrigues P, Peixoto D, Pinheiro C, Saraiva A, Carvalho AP, Guimarães L, Oliva-Teles L. 2019 *Lymnaea stagnalis* as a freshwater model invertebrate for ecotoxicological studies. *Sci. Total Environ*. **669**, 11–28. (doi:10.1016/j.scitotenv.2019.03.035)

34. Benjamin PR. 2012 Distributed network organization underlying feeding behavior in the mollusk *Lymnaea*. *Neural Syst. Circuits* **2**, 4. (doi:10.1186/2042-1001-2-4)
35. Fodor I, Hussein AA, Benjamin PR, Koene JM, Pirger Z. 2020 The unlimited potential of the great pond snail, *Lymnaea stagnalis*. *eLife* **9**, e56962. (doi:10.7554/elife.56962)
36. Kemenes G. 1997 In vivo neuropharmacological and in vitro laser ablation techniques as tools in the analysis of neuronal circuits underlying behavior in a molluscan model system. *Gen. Pharmacol. Vasc. Syst.* **29**, 7–15. (doi:10.1016/S0306-3623(96)00520-4)
37. Rivi V, Benatti C, Colliva C, Radighieri G, Brunello N, Tascedda F, Blom JMC. 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)
38. Crossley M, Staras K, Kemenes G. 2016 A two-neuron system for adaptive goal-directed decision-making in *Lymnaea*. *Nat. Commun.* **7**, 11793. (doi:10.1038/ncomms11793)
39. Davidson GL, Cienfuegos IA, Dalesman S. 2024 Antibiotic-altered gut microbiota explain host memory plasticity and disrupt pace-of-life covariation for an aquatic snail. *ISME J.* **18**, wrae078. (doi:10.1093/ismejo/wrae078)
40. Koene JM. 2006 Tales of two snails: sexual selection and sexual conflict in *Lymnaea stagnalis* and *Helix aspersa*. *Integr. Comp. Biol.* **46**, 419–429. (doi:10.1093/icb/icj040)
41. Koene JM *et al*. 2024 The genome of the simultaneously hermaphroditic snail *Lymnaea stagnalis* reveals an evolutionary expansion of FMRFamide-like receptors. *Sci. Rep.* **14**, 29213. (doi:10.1038/s41598-024-78520-1)
42. Lukowiak K, Martens K, Orr M, Parvez K, Rosenegger D, 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)
43. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2023 A novel behavioral display in *Lymnaea* induced by quercetin and hypoxia. *Biol. Bull.* **244**, 115–127. (doi:10.1086/725689)
44. Rivi V *et al*. 2025 First evidence of an anxiety-like behavior and its pharmacological modulation in a molluscan model organism, *Lymnaea stagnalis*. *Transl. Psychiatry* **15**, 177. (doi:10.1038/s41398-025-03399-z)
45. Benatti C, Rivi V, Colliva C, Radighieri G, Tascedda F, Blom JMC. 2020 Redefining operant conditioning of escape behaviour in *Lymnaea stagnalis*. *Invertebr. Surviv. J.* **17**, 129–137. (doi:10.25431/1824-307X/isj.v0i0.129-137)
46. Benjamin PR, Kemenes G. 2009 Invertebrate models to study learning and memory: *Lymnaea*. In *Encyclopedia of neuroscience* (ed. LR Squire), pp. 197–204. Oxford, UK: Academic Press. (doi:10.1016/B978-008045046-9.00804-4)
47. Ito E, Kojima S, Lukowiak K, Sakakibara M. 2013 From likes to dislikes: conditioned taste aversion in the great pond snail (*Lymnaea stagnalis*). *Can. J. Zool.* **91**, 405–412. (doi:10.1139/cjz-2012-0292)
48. Rivi V, Batabyal A, Juego K, Kakadiya M, Benatti C, Blom JMC, Lukowiak K. 2021 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)
49. Rivi V, Batabyal A, Benatti C, Sarti P, Blom JMC, Tascedda F, Lukowiak K. 2024 A translational and multidisciplinary approach to studying the Garcia effect, a higher form of learning with deep evolutionary roots. *J. Exp. Biol.* **227**, jeb247325. (doi:10.1242/jeb.247325)
50. Cristina B *et al*. 2022 Identification and characterization of the kynurenine pathway in the pond snail *Lymnaea stagnalis*. *Sci. Rep.* **12**, 15617. (doi:10.1038/s41598-022-19652-0)
51. Fodor I, Urbán P, Kemenes G, Koene JM, Pirger Z. 2020 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)
52. Foster NL, Lukowiak K, Henry TB. 2015 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.* **8**, e1040954. (doi:10.1080/19420889.2015.1040954)
53. Rivi V, Rigillo G, Batabyal A, Lukowiak K, Pani L, Tascedda F, Benatti C, Blom JMC. 2024 Different stressors uniquely affect the expression of endocannabinoid-metabolizing enzymes in the central ring ganglia of *Lymnaea stagnalis*. *J. Neurochem.* **168**, 2848–2867. (doi:10.1111/jnc.16147)
54. Fernell M, Rivi V, Batabyal A, Lukowiak K. 2021 The temperature sensitivity of memory formation and persistence is altered by cold acclimation in a pond snail. *J. Exp. Biol.* **224**, jeb242513. (doi:10.1242/jeb.242513)
55. Rivi V, Batabyal A, Benatti C, Blom JMC, Lukowiak K. 2022 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)
56. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JM, Lukowiak K. 2022 Too hot to eat: wild and lab-bred *Lymnaea stagnalis* differ in feeding response following repeated heat exposure. *Biol. Bull.* **243**, 38–43. (doi:10.1086/720948)
57. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2024 Hot and cold exposure triggers distinct transcriptional and behavioral responses in laboratory-inbred pond snails. *Water Biol. Secur.* **4**, 100315. (doi:10.1016/j.watbs.2024.100315)
58. Dalesman S, Lukowiak K. 2010 Effect of acute exposure to low environmental calcium on respiration and locomotion in *Lymnaea stagnalis* (L.). *J. Exp. Biol.* **213**, 1471–1476. (doi:10.1242/jeb.040493)
59. Rivi V, Batabyal A, Wiley B, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2022 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)
60. Batabyal A, Rivi V, Benatti C, Blom JMC, Tascedda F, Lukowiak K. 2024 Snails go on a fast when acetylsalicylic acid comes along with heat stress: a possible effect of HSPs and serotonergic system on the feeding response. *Comp. Biochem. Physiol. C Toxicol. Pharmacol.* **276**, 109805. (doi:10.1016/j.cbpc.2023.109805)
61. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2022 Aspirin reverts lipopolysaccharide-induced learning and memory impairment: first evidence from an invertebrate model system. *Naunyn Schmiedebergs Arch. Pharmacol.* **395**, 1573–1585. (doi:10.1007/s00210-022-02286-4)
62. Rivi V, Batabyal A, Benatti C, Blom JM, Tascedda F, Lukowiak K. 2023 Novel taste, sickness, and memory: lipopolysaccharide to induce a Garcia-like effect in inbred and wild strains of *Lymnaea stagnalis*. *Physiol. Behav.* **263**, 114137. (doi:10.1016/j.physbeh.2023.114137)
63. Kagan D, Rivi V, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2023 No food for thought: an intermediate level of food deprivation enhances memory in *Lymnaea stagnalis*. *J. Exp. Biol.* **226**, jeb245566. (doi:10.1242/jeb.245566)
64. Kita S *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)
65. Mita K, Yamagishi M, Fujito Y, Lukowiak K, Ito E. 2014 An increase in insulin is important for the acquisition conditioned taste aversion in *Lymnaea*. *Neurobiol. Learn. Mem.* **116**, 132–138. (doi:10.1016/j.nlm.2014.10.006)
66. Rivi V, Benatti C, Actis P, Tascedda F, Blom JMC. 2022 Behavioral and transcriptional effects of short or prolonged fasting on the memory performances of *Lymnaea stagnalis*. *Neuroendocrinology* **113**, 406–422. (doi:10.1159/000527489)
67. Lukowiak K, Sunada H, Teskey M, Lukowiak K, 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)

68. Batabyal A, Chau D, Rivi V, 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)
69. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2023 Prey populations with different predation histories show differences in behavioral and transcriptional effects under acute predation threat. *Neurobiol. Learn. Mem*. **203**, 107775. (doi:10.1016/j.nlm.2023.107775)
70. Swinton C, Swinton E, Shymansky T, Hughes E, Zhang J, Rothwell C, Kakadiya M, Lukowiak K. 2019 Configural learning: a higher form of learning in *Lymnaea*. *J. Exp. Biol*. **222**, jeb190405. (doi:10.1242/jeb.190405)
71. Batabyal A, 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)
72. Rivi V, Batabyal A, Lukowiak K. 2024 The multifaceted effects of flavonoids on neuroplasticity. *Restor. Neurol. Neurosci*. **42**, 93–111. (doi:10.3233/RNN-230150)
73. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2024 Quercetin, the new stress buster: investigating the transcriptional and behavioral effects of this flavonoid on multiple stressors using *Lymnaea stagnalis*. *Comp. Biochem. Physiol. Toxicol. Pharmacol*. **287**, 110053. (doi:10.1016/j.cbpc.2024.110053)
74. Dalesman S, Rundle SD, Cotton PA. 2007 Predator regime influences innate anti-predator behaviour in the freshwater gastropod *Lymnaea stagnalis*. *Freshw. Biol*. **52**, 2134–2140. (doi:10.1111/j.1365-2427.2007.01843.x)
75. Kagan D, Lukowiak K. 2019 Configural learning in freshly collected, smart, wild *Lymnaea*. *J. Exp. Biol*. **222**, jeb212886. (doi:10.1242/jeb.212886)
76. Schwabe L. 2025 Memory under stress: from adaptation to disorder. *Biol. Psychiatry* **97**, 339–348. (doi:10.1016/j.biopsych.2024.06.005)
77. Dhabhar FS. 2018 The short-term stress response—mother nature's mechanism for enhancing protection and performance under conditions of threat, challenge, and opportunity. *Front. Neuroendocrinol*. **49**, 175–192. (doi:10.1016/j.yfrne.2018.03.004)
78. Dokladny K, Lobb R, Wharton W, Ma TY, Moseley PL. 2010 LPS-induced cytokine levels are repressed by elevated expression of HSP70 in rats: possible role of NF-κB. *Cell Stress Chaperones* **15**, 153–163. (doi:10.1007/s12192-009-0129-6)
79. Jin J, Li Y, Zhou Z, Zhang H, Guo J, Wan F. 2020 Heat shock factor is involved in regulating the transcriptional expression of two potential Hsps (AhHsp70 and AhsHsp21) and its role in heat shock response of *Agasicles hygrophila*. *Front. Physiol.* **11**, 562204. (doi:10.3389/fphys.2020.562204)
80. Zatsepina OG *et al*. 2021 Hsp70 affects memory formation and behaviorally relevant gene expression in *Drosophila melanogaster*. *Cell Stress Chaperones* **26**, 575–594. (doi:10.1007/s12192-021-01203-7)
81. Aonuma H, Mezheritskiy M, Boldyshev B, Totani Y, Vorontsov D, Zakharov I, Ito E, Dyakonova V. 2020 The role of serotonin in the influence of intense locomotion on the behavior under uncertainty in the mollusk *Lymnaea stagnalis*. *Front. Physiol.* **11**, 221. (doi:10.3389/fphys.2020.00221)
82. Il-Han J, Janes T, 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)
83. Ivashkin E, Khabarova MY, Melnikova V, Nezlin LP, Kharchenko O, Voronezhskaya EE, Adameyko I. 2015 Serotonin mediates maternal effects and directs developmental and behavioral changes in the progeny of snails. *Cell Rep*. **12**, 1144–1158. (doi:10.1016/j.celrep.2015.07.022)
84. Lukowiak K, Martens K, Rosenegger D, Browning K, de Caigny P, Orr M. 2008 The perception of stress alters adaptive behaviours in *Lymnaea stagnalis*. *J. Exp. Biol*. **211**, 1747–1756. (doi:10.1242/jeb.014886)
85. Rivi V, Batabyal A, Lukowiak K, Benatti C, Rigillo G, Tascedda F, Blom JMC. 2023 LPS-induced Garcia effect and its pharmacological regulation mediated by acetylsalicylic acid: behavioral and transcriptional evidence. *Biology* **12**, 1100. (doi:10.3390/biology12081100)
86. Rivi V, Rigillo G, Alboni S, Koene JM, Pani L, Lukowiak K, Tascedda F, Blom JMC, Benatti C. 2025 Unraveling lipopolysaccharide-induced behavioral and molecular effects in *Lymnaea stagnalis*, an emerging model organism for translational neuroscience. *Int. Immunopharmacol*. **152**, 114418. (doi:10.1016/j.intimp.2025.114418)
87. Bavan S, Straub VA, Webb TE, Ennion SJ. 2012 Cloning and characterization of a P2X receptor expressed in the central nervous system of *Lymnaea stagnalis*. *PLoS One* **7**, e50487. (doi:10.1371/journal.pone.0050487)
88. Dong N *et al*. 2021 Ion channel profiling of the *Lymnaea stagnalis* ganglia via transcriptome analysis. *BMC Genomics* **22**, 18. (doi:10.1186/s12864-020-07287-2)
89. Bartsch D, Casadio A, Karl KA, Serodio P, Kandel ER. 1998 CREB1 encodes a nuclear activator, a repressor, and a cytoplasmic modulator that form a regulatory unit critical for long-term facilitation. *Cell* **95**, 211–223. (doi:10.1016/s0092-8674(00)81752-3)
90. Rivi V, Caruso G, Caraci F, Alboni S, Pani L, Tascedda F, Lukowiak K, Blom JMC, Benatti C. 2024 Behavioral and transcriptional effects of carnosine in the central ring ganglia of the pond snail *Lymnaea stagnalis*. *J. Neurosci. Res*. **102**, e25371. (doi:10.1002/jnr.25371)
91. Winberg S, Myrberg AA, Nilsson GE. 1993 Predator exposure alters brain serotonin metabolism in bicolour damselfish. *Neuroreport* **4**, 399–402. (doi:10.1097/00001756-199304000-00014)
92. Ross RS, Medrano P, Boyle K, Smolen A, Curran T, Nyhus E. 2015 Genetic variation in the serotonin transporter gene influences ERP old/new effects during recognition memory. *Neuropsychologia* **78**, 95–107. (doi:10.1016/j.neuropsychologia.2015.09.028)
93. Tortora F, Hadipour AL, Battaglia S, Falzone A, Avenanti A, Vicario CM. 2023 The role of serotonin in fear learning and memory: a systematic review of human studies. *Brain Sci*. **13**, 1197. (doi:10.3390/brainsci13081197)
94. Imlach AR, Ward DD, Vickers JC, Summers MJ, Felmingham KL. 2017 Association between the serotonin transporter gene polymorphism and verbal learning in older adults is moderated by gender. *Transl. Psychiatry* **7**, e1144. (doi:10.1038/tp.2017.107)
95. Adamec R, Burton P, Blundell J, Murphy DL, Holmes A. 2006 Vulnerability to mild predator stress in serotonin transporter knockout mice. *Behav. Brain Res*. **170**, 126–140. (doi:10.1016/j.bbr.2006.02.012)
96. Pimentel AFN, Carvalho T dos S, Lima F, Lima-Maximino M, Soares MC, Maximino C. 2019 Conditional approach as cooperation in predator inspection: a role for serotonin? *Behav. Brain Res*. **365**, 164–169. (doi:10.1016/j.bbr.2019.03.005)
97. Sriram K, Rodriguez-Fernandez M, Doyle FJ. 2012 A detailed modular analysis of heat-shock protein dynamics under acute and chronic stress and its implication in anxiety disorders. *PLoS One* **7**, e42958. (doi:10.1371/journal.pone.0042958)
98. Pauwels K, Stoks R, de Meester L. 2005 Coping with predator stress: interclonal differences in induction of heat-shock proteins in the water flea *Daphnia magna*. *J. Evol. Biol*. **18**, 867–872. (doi:10.1111/j.1420-9101.2005.00890.x)
99. Al-Aqil A, Zulkifli I, Hair Bejo M, Sazili AQ, Rajion MA, Somchit MN. 2013 Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. *Poult. Sci*. **92**, 33–40. (doi:10.3382/ps.2012-02446)
100. Domingos LB, Silva Júnior AF da, Diniz CRAF, Rosa J, Terzian ALB, Moraes Resstel LB. 2024 P2X7 receptors modulate acquisition of cue fear extinction and contextual background memory generalization in male mice. *Neuropharmacology* **261**, 110177. (doi:10.1016/j.neuropharm.2024.110177)

101. von Muecke-Heim IA, Ries C, Urbina L, Deussing JM. 2021 P2X7R antagonists in chronic stress-based depression models: a review. *Eur. Arch. Psychiatry Clin. Neurosci*. **271**, 1343–1358. (doi:10.1007/s00406-021-01306-3)
102. Pankratov YV, Lalo UV, Krishtal OA. 2002 Role for P2X receptors in long-term potentiation. *J. Neurosci*. **22**, 8363–8369. (doi:10.1523/jneurosci.22-19-08363.2002)
103. Alagar Boopathy LR, Jacob-Tomas S, Alecki C, Vera M. 2022 Mechanisms tailoring the expression of heat shock proteins to proteostasis challenges. *J. Biol. Chem*. **298**, 101796. (doi:10.1016/j.jbc.2022.101796)
104. Jang Y, Kim M, Hwang SW. 2020 Molecular mechanisms underlying the actions of arachidonic acid-derived prostaglandins on peripheral nociception. *J. Neuroinflammation* **17**, 1–27. (doi:10.1186/s12974-020-1703-1)
105. Matheson J, Zhou XMM, Bourgault Z, Le Foll B. 2021 Potential of fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL), and diacylglycerol lipase (DAGL) enzymes as targets for obesity treatment: a narrative review. *Pharm. Basel Switz*. **14**, 1316. (doi:10.3390/ph14121316)
106. Tevosian M *et al*. 2023 NAPE-PLD deletion in stress-TRAPed neurons results in an anxiogenic phenotype. *Transl. Psychiatry* **13**, 152. (doi:10.1038/s41398-023-02448-9)
107. Gallego-Landin I, García-Baos A, Castro-Zavala A, Valverde O. 2021 Reviewing the role of the endocannabinoid system in the pathophysiology of depression. *Front. Pharmacol*. **12**, 762738. (doi:10.3389/fphar.2021.762738)
108. Hill MN, Campolongo P, Yehuda R, Patel S. 2018 Integrating endocannabinoid signaling and cannabinoids into the biology and treatment of posttraumatic stress disorder. *Neuropsychopharmacology* **43**, 80–102. (doi:10.1038/npp.2017.162)
109. Lutz B, Marsicano G, Maldonado R, Hillard CJ. 2015 The endocannabinoid system in guarding against fear, anxiety and stress. *Nat. Rev. Neurosci*. **16**, 705–718. (doi:10.1038/nrn4036)
110. Haj-Dahmane S, Shen RY. 2011 Modulation of the serotonin system by endocannabinoid signaling. *Neuropharmacology* **61**, 414–420. (doi:10.1016/j.neuropharm.2011.02.016)
111. Kagan D, Batabyal A, Rivi V, 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)
112. Orr M, Lukowiak K. 2010 Sympatric predator detection alters cutaneous respiration in *Lymnaea*. *Commun. Integr. Biol*. **3**, 42–45. (doi:10.4161/cib.3.1.9634)
113. Orr MV, Hittel K, 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)
114. Wagatsuma A, Sugai R, Chono K, Azami S, Hatakeyama D, Sadamoto H, Itoi E. 2004 The early snail acquires the learning. Comparison of scores for conditioned taste aversion between morning and afternoon. *Acta Biol. Hung*. **55**, 149–155. (doi:10.1556/ABiol.55.2004.1-4.18)
115. Rivi V *et al*. 2025 Effects of the inhibition of miRNA biogenesis in the central ring ganglia of a widely used invertebrate model species, *Lymnaea stagnalis*. *Comp. Biochem. Physiol. C Toxicol. Pharmacol*. **297**, 110291. (doi:10.1016/j.cbpc.2025.110291)
116. Rivi V, Batabyal A, Benatti C, Tascedda F, Blom JMC, Lukowiak K. 2023 Comparison of behavioural and transcriptional responses to a heat stressor between freshly collected and an inbred strain of *Lymnaea*. *Can. J. Zool*. **101**, 904–912. (doi:10.1139/cjz-2023-0088)
117. Wang Q, Ishikawa T, Michiue T, Zhu BL, Guan DW, Maeda H. 2012 Stability of endogenous reference genes in postmortem human brains for normalization of quantitative real-time PCR data: comprehensive evaluation using geNorm, NormFinder, and BestKeeper. *Int. J. Leg. Med*. **126**, 943–952. (doi:10.1007/s00414-012-0774-7)
118. Azami S *et al*. 2006 Altered gene activity correlated with long-term memory formation of conditioned taste aversion in *Lymnaea*. *J. Neurosci. Res*. **84**, 1610–1620. (doi:10.1002/jnr.21045)
119. Roberts WA. 2006 Animal memory: episodic-like memory in rats. *Curr. Biol*. **16**, R601–R603. (doi:10.1016/j.cub.2006.07.001)
120. Rodríguez Peris L, Scheuber MI, Shan H, Braun M, Schwab ME. 2024 Barnes maze test for spatial memory: a new, sensitive scoring system for mouse search strategies. *Behav. Brain Res*. **458**, 114730. (doi:10.1016/j.bbr.2023.114730)
121. Marshall RES, Hurly TA, Sturgeon J, Shuker DM, Healy SD. 2013 What, where and when: deconstructing memory. *Proc. R. Soc. B* **280**, 20132194. (doi:10.1098/rspb.2013.2194)
122. Cavallaro S, D'Agata V, Manickam P, Dufour F, Alkon DL. 2002 Memory-specific temporal profiles of gene expression in the hippocampus. *Proc. Natl Acad. Sci. USA* **99**, 16279–16284. (doi:10.1073/pnas.242597199)
123. Tadi M, Allaman I, Lengacher S, Grenningloh G, Magistretti PJ. 2015 Learning-induced gene expression in the hippocampus reveals a role of neuron–astrocyte metabolic coupling in long term memory. *PLoS One* **10**, e0141568. (doi:10.1371/journal.pone.0141568)
124. Igaz LM, Bekinschtein P, Vianna MMR, Izquierdo I, Medina JH. 2004 Gene expression during memory formation. *Neurotox. Res*. **6**, 189–204. (doi:10.1007/BF03033221)
125. Simbriger K, Amorim IS, Lach G, Chalkiadaki K, Kouloulia S, Jafarnejad SM, Khoutorsky A, Gkogkas CG. 2021 Uncovering memory-related gene expression in contextual fear conditioning using ribosome profiling. *Prog. Neurobiol*. **197**, 101903. (doi:10.1016/j.pneurobio.2020.101903)
126. Huo Y, Fang Q, Shi YL, Zhang YH, Zhang JX. 2014 Chronic exposure to a predator or its scent does not inhibit male–male competition in male mice lacking brain serotonin. *Front. Behav. Neurosci*. **8**, 116. (doi:10.3389/fnbeh.2014.00116)
127. Kandel ER. 2012 The molecular biology of memory: cAMP, PKA, CRE, CREB-1, CREB-2, and CPEB. *Mol. Brain* **5**, 14. (doi:10.1186/1756-6606-5-14)
128. Fossat P, Bacqué-Cazenave J, De Deurwaerdère P, Delbecque JP, Cattaert D. 2014 Comparative behavior. Anxiety-like behavior in crayfish is controlled by serotonin. *Science* **344**, 1293–1297. (doi:10.1126/science.1248811)
129. Lafenêtre P, Chaouloff F, Marsicano G. 2007 The endocannabinoid system in the processing of anxiety and fear and how CB1 receptors may modulate fear extinction. *Pharmacol. Res*. **56**, 367–381. (doi:10.1016/j.phrs.2007.09.006)
130. Rivi V *et al*. 2023 The role of dopamine D3 receptors, dysbindin, and their functional interaction in the expression of key genes for neuroplasticity and neuroinflammation in the mouse brain. *Int. J. Mol. Sci*. **24**, 8699. (doi:10.3390/ijms24108699)
131. Brechbühl J, Moine F, Tosato MN, Sporkert F, Broillet MC. 2015 Identification of pyridine analogs as new predator-derived kairomones. *Front. Neurosci*. **9**, 253. (doi:10.3389/fnins.2015.00253)
132. Forest J, Sunada H, Dodd S, 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)
133. Kemenes I, Straub VA, Nikitin ES, Staras K, O'Shea M, Kemenes G, Benjamin PR. 2006 Role of delayed nonsynaptic neuronal plasticity in long-term associative memory. *Curr. Biol*. **16**, 1269–1279. (doi:10.1016/j.cub.2006.05.049)
134. Kemenes I, O'Shea M, Benjamin PR. 2011 Different circuit and monoamine mechanisms consolidate long-term memory in aversive and reward classical conditioning: long-term memory consolidation. *Eur. J. Neurosci*. **33**, 143–152. (doi:10.1111/j.1460-9568.2010.07479.x)
135. Domingos LB, Hott SC, Terzian ALB, Resstel LBM. 2018 P2X7 purinergic receptors participate in the expression and extinction processes of contextual fear conditioning memory in mice. *Neuropharmacology* **128**, 474–481. (doi:10.1016/j.neuropharm.2017.08.005)
136. Porto RR, Dutra FD, Crestani AP, Holsinger RMD, Quillfeldt JA, Homem de Bittencourt PI, de Oliveira Alvares L. 2018 HSP70 facilitates memory consolidation of fear conditioning through MAPK pathway in the hippocampus. *Neuroscience* **375**, 108–118. (doi:10.1016/j.neuroscience.2018.01.028)

137. Rivi V, Batabyal A, Benatti C, Blom JM, Tascedda F, Lukowiak K. 2021 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)
138. Totani Y, Aonuma H, Oike A, Watanabe T, Hatakeyama D, Sakakibara M, Lukowiak K, Ito E. 2019 Monoamines, insulin and the roles they play in associative learning in pond snails. *Front. Behav. Neurosci*. **13**, 65. (doi:10.3389/fnbeh.2019.00065)
139. Sunada H, Totani Y, Nakamura R, Sakakibara M, Lukowiak K, 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)
140. Aonuma H, Totani Y, Sakakibara M, Lukowiak K, Ito E. 2018 Comparison of brain monoamine content in three populations of *Lymnaea* that correlates with taste-aversive learning ability. *Biophys. Physicobiol*. **15**, 129–135. (doi:10.2142/biophysico.15.0_129)
141. Batabyal A, Lukowiak K. 2023 Tracking the path of predator recognition in a predator-naive population of the pond snail. *Behav. Ecol*. **34**, 125–135. (doi:10.1093/beheco/arac107)
142. Yamamoto K, Kosukegawa S, Kobayashi M. 2024 P2X receptor- and postsynaptic NMDA receptor-mediated long-lasting facilitation of inhibitory synapses in the rat insular cortex. *Neuropharmacology* **245**, 109817. (doi:10.1016/j.neuropharm.2023.109817)
143. Lau CG, Zukin RS. 2007 NMDA receptor trafficking in synaptic plasticity and neuropsychiatric disorders. *Nat. Rev. Neurosci*. **8**, 413–426. (doi:10.1038/nrn2153)
144. Bartsch D, Ghirardi M, Casadio A, Giustetto M, Karl KA, Zhu H, Kandel ER. 2000 Enhancement of memory-related long-term facilitation by ApAF, a novel transcription factor that acts downstream from both CREB1 and CREB2. *Cell* **103**, 595–608. (doi:10.1016/s0092-8674(00)00163-x)
145. Abrams JK, Johnson PL, Hay-Schmidt A, Mikkelsen JD, Shekhar A, Lowry CA. 2005 Serotonergic systems associated with arousal and vigilance behaviors following administration of anxiogenic drugs. *Neuroscience* **133**, 983–997. (doi:10.1016/j.neuroscience.2005.03.025)
146. Nocheva H, Stoynev N, Vodenicharov V, Krastev D, Krastev N, Mileva M. 2024 Cannabinoid and serotonergic systems: unraveling the pathogenetic mechanisms of stress-induced analgesia. *Biomedicines* **12**, 235. (doi:10.3390/biomedicines12010235)
147. Elphick MR. 2012 The evolution and comparative neurobiology of endocannabinoid signalling. *Phil. Trans. R. Soc. B* **367**, 3201–3215. (doi:10.1098/rstb.2011.0394)
148. Ashton JC, Dowie MJ, Glass M. 2017 The endocannabinoid system and human brain functions: insight from memory, motor, and mood pathologies. In *The endocannabinoid system* (ed. E Murillo-Rodríguez), pp. 115–186. London, UK: Academic Press. (doi:10.1016/B978-0-12-809666-6.00005-8)
149. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, Kathuria S, Piomelli D. 2002 Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl Acad. Sci. USA* **99**, 10819–10824. (doi:10.1073/pnas.152334899)
150. Gunduz-Cinar O, Hill MN, McEwen BS, Holmes A. 2013 Amygdala FAAH and anandamide: mediating protection and recovery from stress. *Trends Pharmacol. Sci*. **34**, 637–644. (doi:10.1016/j.tips.2013.08.008)
151. Kemble AM *et al*. 2022 A potent and selective inhibitor for the modulation of MAGL activity in the neurovasculature. *PLoS One* **17**, e0268590. (doi:10.1371/journal.pone.0268590)
152. Longaretti A, Forastieri C, Gabaglio M, Rubino T, Battaglioli E, Rusconi F. 2020 Termination of acute stress response by the endocannabinoid system is regulated through lysine-specific demethylase 1-mediated transcriptional repression of 2-AG hydrolases ABHD6 and MAGL. *J. Neurochem*. **155**, 98–110. (doi:10.1111/jnc.15000)
153. Sunada H, Watanabe T, Hatakeyama D, Lee S, Forest J, Sakakibara M, Ito E, Lukowiak K. 2017 Pharmacological effects of cannabinoids on learning and memory in *Lymnaea*. *J. Exp. Biol*. **220**, 3026–3038. (doi:10.1242/jeb.159038)
154. Solomon-Lane TK, Hofmann HA. 2019 Early-life social environment alters juvenile behavior and neuroendocrine function in a highly social cichlid fish. *Horm. Behav*. **115**, 104552. (doi:10.1016/j.yhbeh.2019.06.016)
155. Petrovich GD. 2011 Learning and the motivation to eat: forebrain circuitry. *Physiol. Behav*. **104**, 582–589. (doi:10.1016/j.physbeh.2011.04.059)
156. Petrovich GD. 2018 Lateral hypothalamus as a motivation-cognition interface in the control of feeding behavior. *Front. Syst. Neurosci*. **12**, 14. (doi:10.3389/fnsys.2018.00014)
157. Tierney AJ. 2020 Feeding, hunger, satiety and serotonin in invertebrates. *Proc. R. Soc. B* **287**, 20201386. (doi:10.1098/rspb.2020.1386)
158. Keen-Rhinehart E, Dailey MJ, Bartness T. 2010 Physiological mechanisms for food-hoarding motivation in animals. *Phil. Trans. R. Soc. B* **365**, 961–975. (doi:10.1098/rstb.2009.0225)
159. Lukowiak K, Ringseis E, Spencer G, Wildering W, 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)
160. Rivi V, Batabyal A, Benatti C, Blom JMC, Tascedda F, Lukowiak K. 2023 Investigating the interactions between multiple memory stores in the pond snail *Lymnaea stagnalis*. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol*. **210**, 91–102. (doi:10.1007/s00359-023-01649-3)
161. Braun MH, Lukowiak K. 2011 Intermediate and long-term memory are different at the neuronal level in *Lymnaea stagnalis* (L.). *Neurobiol. Learn. Mem*. **96**, 403–416. (doi:10.1016/j.nlm.2011.06.016)
162. Patel BA, Arundell M, Allen MC, Gard P, O'Hare D, Parker K, Yeoman MS. 2006 Changes in the properties of the modulatory cerebral giant cells contribute to aging in the feeding system of *Lymnaea*. *Neurobiol. Aging* **27**, 1892–1901. (doi:10.1016/j.neurobiolaging.2005.09.041)
163. Yeoman MS, Kemenes G, Benjamin PR, Elliott CJ. 1994 Modulatory role for the serotonergic cerebral giant cells in the feeding system of the snail, *Lymnaea*. II. Photoinactivation. *J. Neurophysiol*. **72**, 1372–1382. (doi:10.1152/jn.1994.72.3.1372)
164. Sunada H, Takigami S, Lukowiak K, Sakakibara M. 2014 Electrophysiological characteristics of feeding-related neurons after taste avoidance pavlovian conditioning in *Lymnaea stagnalis*. *Biophysics* **10**, 121–133. (doi:10.2142/biophysics.10.121)
165. Rivi V *et al*. 2026 Supplementary material from: Exploring the role of stress sensitivity in memory formation: why do some animals learn while others do not? Lessons from *Lymnaea stagnalis*. Figshare. (doi:10.6084/m9.figshare.c.8332594)