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Quercetin, the new stress buster: Investigating the transcriptional and behavioral effects of this flavonoid on multiple stressors using *Lymnaea stagnalis*

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ABSTRACT

Growing evidence suggests that a flavonoid-rich diet can prevent or reverse the effects of stressors, although the underlying mechanisms remain poorly understood. One common and abundant flavonoid found in numerous foods is quercetin. This study utilizes the pond snail *Lymnaea stagnalis*, a valid model organism for learning and memory, and a simple but robust learning paradigm—operant conditioning of aerial respiration—to explore the behavioral and transcriptional effects of different stressors on snails' cognitive functions and to investigate whether quercetin exposure can prevent stress effects on learning and memory formation. Our findings demonstrate that three different stressors—severe food deprivation, lipopolysaccharide injection (an inflammatory challenge), and fluoride exposure (a neurotoxic agent)—block memory formation for operant conditioning and affect the expression levels of key targets related to stress response, energy balance, and immune response in the snails' central ring ganglia. Remarkably, exposing snails to quercetin for 1 h before stress presentation prevents these effects at both the behavioral and transcriptional levels, demonstrating the potent stress-preventive properties of quercetin. Despite the evolutionary distance from humans, *L. stagnalis* has proven to be a valuable model for studying conserved mechanisms by which bioactive compounds like quercetin mitigate the adverse effects of various stressors on cognitive functions across species. Moreover, these findings offer insights into quercetin's potential for mitigating stress-induced physiological and cognitive impairments.

1. Introduction

Quercetin is the most abundant flavanol in the human diet, found in various plant-derived foods such as nuts, grapes, onions, broccoli, apples, and green tea (Anand David et al., 2016). Dietary quercetin consumption is associated with numerous health benefits, including antioxidant and anti-inflammatory effects, and protection against aging-related diseases such as cardiovascular pathologies, cancer, and neurodegenerative disorders (Amanzadeh et al., 2019; Chiang et al., 2023). Traditionally, these benefits have been attributed to its antioxidant and free-radical scavenging properties demonstrated in *in vitro* studies (Li et al., 2016a; Proshkina et al., 2016). However, the exact mechanisms by which quercetin exerts its *in vivo* effects remain unresolved (Broman-Fulks et al., 2012; Chiang et al., 2023; Li et al., 2016a; Nabavi et al., 2012).

The pond snail *Lymnaea stagnalis* represents a valuable model organism for comparative biochemistry and physiology for several reasons (Amorim et al., 2019; Fodor et al., 2020a; Lagadic and Caquet, 1998). First, it shares many conserved molecular pathways with mammals, including those learning and memory, aging, and metabolism (Dong et al., 2021; Fodor et al., 2021, 2020b; Murakami et al., 2013). Second, it offers easily reproducible yet solid experimental paradigms for studying the effects of stressors, drugs, and bioactive compounds both at the molecular and behavioral levels (Rivi et al., 2023b, 2023c, 2022a,

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2024). Third, *L. stagnalis* possesses a relatively simple nervous system lacking a blood-brain barrier and an open circulatory system (Rivi et al., 2020). These features, together with the ability of flavonols like quercetin to diffuse across the integument and easily reach the central nervous system, all serve to make *L. stagnalis* an attractive system for pharmacological studies (Gatto et al., 2022; Rivi et al., 2023f).

Previously, we demonstrated that quercetin enhances LTM formation for the operant conditioning of aerial respiration (Rivi et al., 2021a) and induces a significant upregulation of LymCREB1, a key neuroplasticity gene, in snails' central ring ganglia (Batabyal et al., 2021). Moreover, we found that quercetin blocks the upregulation of heat shock proteins 70 and 40 (HSPs) induced by acute heat shock exposure, providing the first evidence in a molluscan model that this flavonol can prevent the effects of an acute stressor both at the behavioral and molecular levels (Rivi et al., 2022b, 2021b).

In this study, we explored the behavioral and transcriptional effects of various ecologically relevant stressors on the learning and memory abilities of *Lymnaea* (Rivi et al., 2024a) and investigated whether quercetin could mitigate these effects. The selected stressors were: severe fasting (*i.e.*, five-day food deprivation regimen), an immune challenge (*i.e.*, lipopolysaccharide – LPS – injection), and exposure to fluoride, a neurotoxic compound.

These stressors not only affect *Lymnaea*'s homeostatic behaviors and cognitive abilities (Batabyal et al., 2024, 2022; Lukowiak et al., 2014; Rivi et al., 2022d; Rivi et al., 2024a; Wiley et al., 2022) but also parallels stressors that affect mammals, including humans. In particular, prolonged fasting can impair cognitive processes by depleting glucose levels critical for maintaining neuronal function (Papalini et al., 2017). Similarly, neuroinflammation contributes to cognitive impairment leading to reduced hippocampal neurogenesis, loss of synaptic connections, neuronal apoptosis, and impaired synaptic plasticity (Gopu et al., 2022). Moreover, the increasing levels of fluoride in aquatic ecosystems due to industrial discharge and agricultural runoff, not only pose toxicity risks to freshwater organisms like *Lymnaea*, but groundwater fluoride contamination also represents a growing global concern, with its toxic effects causing widespread adverse health outcomes in human populations (Dhara et al., 2022; Godebo et al., 2023; Gopu et al., 2022; Pankhurst et al., 1980).

To investigate whether exposure to these stressors would impair snails' learning and memory abilities and, crucially, whether quercetin treatment could prevent this impairment, we utilized a solid behavioral paradigm: the operant conditioning of aerial respiration (Lukowiak et al., 2000). It is well-established that in lab-inbred snails, such as those used in this study, a single 30-minute training session (TS) induces intermediate-term memory (ITM) that lasts around 3 h and depends on new protein synthesis (Lukowiak et al., 2000; Rivi et al., 2024, 2023f; Braun and Lukowiak, 2011; Dash and Moore, 2007; Sangha et al., 2003; Takahashi et al., 2013; Wood et al., 2021).

The effects of each stressor and the potential protective properties of quercetin were assessed independently through three distinct experiments.

In *Experiment 1* we first determine whether 5-day food-deprived snails learn and form ITM following operant conditioning of aerial respiration. If they do not form ITM we then will ask whether exposure to quercetin prevents the effects of fasting both at the transcriptional and behavioral levels. That is, would quercetin have neuroprotective effects? We focused on key targets for stress response and energy balance, including LymHSP70, LymMIP-II, LymMIPR, and LymNPY.

In *Experiment 2* we first determine whether quercetin treatment prevents the LPS-induced block of learning and memory formation following operant conditioning of aerial respiration both at the transcriptional and behavioral levels. That is, *does quercetin possess anti-inflammatory effects?* We focused on key targets for immune and stress response, including LymMDM, LymTLR4, LymAIF-1, and LymHSP70.

In *Experiment 3* we first determine whether exposure to fluoride for 45 min prevents the ability of snails to learn and form ITM following operant conditioning of aerial respiration and – if so – whether exposure to quercetin prevents these effects both at the transcriptional and behavioral levels, exerting antioxidant effects. We focused on LymHSP70, the serotonin transporter and rate-limiting enzyme (LymSERT and LymTPH, respectively), and LymCCO, as previous studies from rodents demonstrated that fluoride-exposed mice exhibit increased serotonin levels compared to non-exposed ones, suggesting that fluoride may be impacting behavioral outcomes through alterations in serotonin levels (Kupnicka et al., 2020; Lu et al., 2019).

This is the first study investigating whether quercetin would prevent the effects of ecologically relevant stressors at both the transcriptional and behavioral levels in an invertebrate model organism. By doing so, we aimed to uncover quercetin's potential neuroprotective role against stress-induced cognitive deficits.

2. Material and methods

2.1. Snails

In this study, we used a laboratory-inbred strain of *Lymnaea stagnalis* (W-strain) maintained at the University of Calgary's Biology Department. The snails were housed in artificial pond water made from deionized water, supplemented with Instant Ocean (0.25 g/L) and $CaCO_3$ to maintain calcium levels above 50 mg/L (Dalesman and Lukowiak, 2010). It needs to be noted that fluoride is not present in this artificial pond water (Wiley et al., 2022). Animals were kept at 20 ± 1 °C under a 16-hour light and 8-hour dark cycle. Six-month-old snails with shell lengths of 20–25 mm were used for the experiments.

2.2. Quercetin solution

We prepared a quercetin solution using quercetin (3,3′,4′,5,7-pentahydroxyflavone; Sigma Chemical Company, St Louis, MO, USA; >95 % purity) dissolved in 0.1 % dimethyl sulfoxide (DMSO – vehicle - V). Specifically, 50 μL of quercetin was dissolved in 500 mL of artificial pond water. This concentration has been effective in previous studies involving Lymnaea (Rivi et al., 2023d; Sunada et al., 2016).

2.3. Food deprivation

Snails that have been deprived of food for 5 days have been successfully used in learning and memory studies in *L. stagnalis* (Totani et al., 2023) previously based on protocols from our previous studies. Food-deprived snails were placed in clean tanks without access to food. Mortality rates did not exceed typical levels for the food-deprivation condition, with a maximum of one mortality per group.

2.4. LPS injection

Snails received an injection of 25 μg of *Escherichia coli*-derived LPS serotype O127 (L3129), equivalent to approximately 8 mg/kg into the abdominal cavity. The LPS solution was prepared by dissolving 625 μg of LPS in 1 mL of snail saline solution (containing 51.3 mM NaCl, 1.7 mM KCl, 5.0 mM MgCl2, 1.5 mM CaCl2, and 5.0 mM HEPES, with a pH of 7.9–8) (Rivi et al., 2024b). Control snails were injected with 40 μL of snail saline. Following injection, snails were placed in an upside-down position without immersion in artificial pond water for 10 min, in line with previous studies (Rivi et al., 2023e).

2.5. Fluoride solution

For our experiments, we used a low fluoride concentration of 0.3 mg/L, achieved by adding 1.4 mg of sodium fluoride to 2 l of artificial PW. This concentration has been effective in previous studies involving *L. stagnalis* (Wiley et al., 2022).

2.6. Operant conditioning of aerial respiration

Aerial respiration consists of the spontaneous opening and closing of the pneumostome, the respiratory orifice (Lukowiak et al., 1996). To increase the frequency of aerial respiration, a hypoxic environment was maintained by bubbling 100 % N2 into the test beaker for 20 min before and during the experiment (Moroz et al., 1993). A weak tactile stimulus to the pneumostome area served as the negative reinforcement when a snail attempted to open its pneumostome, causing it to close without triggering a full-body withdrawal response. Snails underwent one 0.5-hour TS and a 0.5-hour MT 3 h later. Snails were acclimated for 10 min in a 1-L beaker filled with 500 mL of hypoxic pond water before the TS and MT. During both the TS and MT, tactile stimuli were applied to the pneumostome area whenever the snails attempted to open it. Thus, we plot the number of attempted pneumostome openings in both TS and MT. As previously described (Rivi et al., 2023a, 2021c), when there is only a single TS; memory (*i.e.*, the number of attempted pneumostome openings in MT) is defined as a significant reduction in the number of attempted pneumostome openings between TS and MT.

2.7. Study design

In this series of experiments, we aimed to investigate the effects of various stressors and treatments on learning and memory formation in *L. stagnalis*, with a particular focus on assessing the protective effects of quercetin exposure. Each experiment targeted specific treatment conditions to evaluate both transcriptional and behavioral responses within the snails' central ring ganglia. To minimize potential bias, snails were randomly assigned to their respective treatment groups. The treatments were administered by an unblinded experimenter (Cristina Benatti), while all behavioral assessments were conducted by blinded experimenters (Veronica Rivi and Anuradha Batabyal), ensuring objectivity and rigor in the evaluation of results.

2.7.1. Experiment 1

In Experiment 1 (Fig. 1 and Fig. 2), we first investigated the transcriptional effects induced by 5 days of food deprivation, quercetin treatment, and their combination in the central ring ganglia of *L. stagnalis*.

For this purpose, 32 naïve snails were divided into 4 groups ($N = 8$ each):

- Control (CTRL) snails had *ad libitum* access to food and were exposed to the vehicle (in which quercetin was dissolved) for 1 h. They were sacrificed 3 h later.
- Snails of the 'Quercetin group (Q)' group had *ad libitum* access to food and were exposed to quercetin for 1 h. They were sacrificed 3 h later.
- Snails of the 'Food Deprivation (FD) group' were food-deprived for 5 days. On the 5th day, they were exposed to the vehicle for 1 h and sacrificed 3 h later.
- Snails of the 'FD_Q group' were food-deprived for 5 days. On the 5th day, they were exposed to quercetin for 1 h and sacrificed 3 h later.

Next, we tested the hypothesis that snails deprived of food for 5 days would not form learning and memory following operant conditioning of aerial respiratory behavior, while a 1-hour exposure to quercetin before the 30-minute TS and/or the MT for ITM would counteract the effects of food deprivation.

We used 40 naïve snails for this study, divided into 4 groups ($N = 10$ each) depending on the type of treatment and its occurrence:

- **Vehicle exposure before training**: 5-day food-deprived snails were exposed to the vehicle for 1 h before being trained for 30 min. ITM was tested 3 h post-training.
- **Vehicle exposure after training**: 5-day food-deprived snails were trained for 30 min and 1 h later were exposed to the vehicle for 1 h. ITM was tested 3 h post-training.
- **Quercetin exposure before training**: 5-day food-deprived snails were exposed to quercetin for 1 h, then subjected to the TS for 30 min. ITM was tested 3 h post-training.
- **Quercetin exposure after training**: 5-day food-deprived snails were exposed to quercetin for 1 h after being trained TS for 30 min. ITM was tested 3 h post-training.

2.7.2. Experiment 2

The aim of Experiment 2 (Fig. 3 and Fig. 4) was to investigate the anti-inflammatory properties of quercetin at both transcriptional and behavioral levels. First, we examined the transcriptional effects induced by LPS injection, quercetin treatment, and quercetin exposure before LPS injection in the central ring ganglia of *L. stagnalis*. For this purpose, 32 naïve snails were divided into four groups ($N = 8$ each):

- Control (CTRL) snails were exposed to the vehicle for 1 h, injected with snail saline immediately later, and sacrificed 3 h later.
- Snails of the 'Quercetin (Q) group' were exposed to quercetin for 1 h, injected with snail saline immediately later, and sacrificed 3 h later.
- Snails of the 'LPS group' were exposed to the vehicle for 1 h. Immediately later, were injected with LPS, and sacrificed 3 h later.
- Snails of the 'Q_LPS group' were exposed to quercetin for 1 h, injected with LPS immediately later, and sacrificed 3 h later.

Next, we tested the hypothesis that a 1-hour exposure to quercetin before the 30-minute TS and/or the MT for ITM would prevent the effects of LPS on cognition.

We used 60 naïve *L. stagnalis* for this study, divided into 6 groups ($N = 10$ each) depending on depending on the treatments and their occurrence:

- **Vehicle exposure and LPS injection before training**: Snails were exposed to the vehicle for 1 h, then were injected with LPS, and, 3 h later subjected to the TS for 30 min. ITM was tested 3 h post-TS.
- **Vehicle exposure and LPS injection after training**: Snails were first trained for 30 min, then were exposed to the vehicle for 1 h, before being injected with LPS. ITM was tested 3 h post-TS.
- **Quercetin exposure and LPS injection before training**: Snails were exposed to quercetin for 1 h, then were injected with LPS, and, 3 h later were subjected to the TS for 30 min. ITM was tested 3 h post-TS.
- **Quercetin exposure and LPS injection after training**: Snails were first trained for 30 min, then were exposed to quercetin for 1 h, before being injected with LPS. ITM was tested 3 h post-TS.
- **Vehicle exposure and saline injection before training**: Snails were exposed to the vehicle for 1 h, then were injected with snail saline, and, 3 h later subjected to the TS for 30 min. ITM was tested 3 h post-TS.
- **Vehicle exposure and saline injection after training**: Snails were first trained for 30 min, then were exposed to the vehicle for 1 h, before being injected with snail saline. ITM was tested 3 h post-TS.

2.7.3. Experiment 3

In Experiment 3 (Fig. 5 and Fig. 6), we first investigated the transcriptional effects induced by fluoride exposure for 45 min, quercetin treatment, and their combination in the central ring ganglia of *L. stagnalis*.

- Snails of the 'control (CTRL) group' ($N = 6$) were placed in artificial pond water for 45 min and, immediately later, in the vehicle for 1 h. Three hours later were sacrificed.

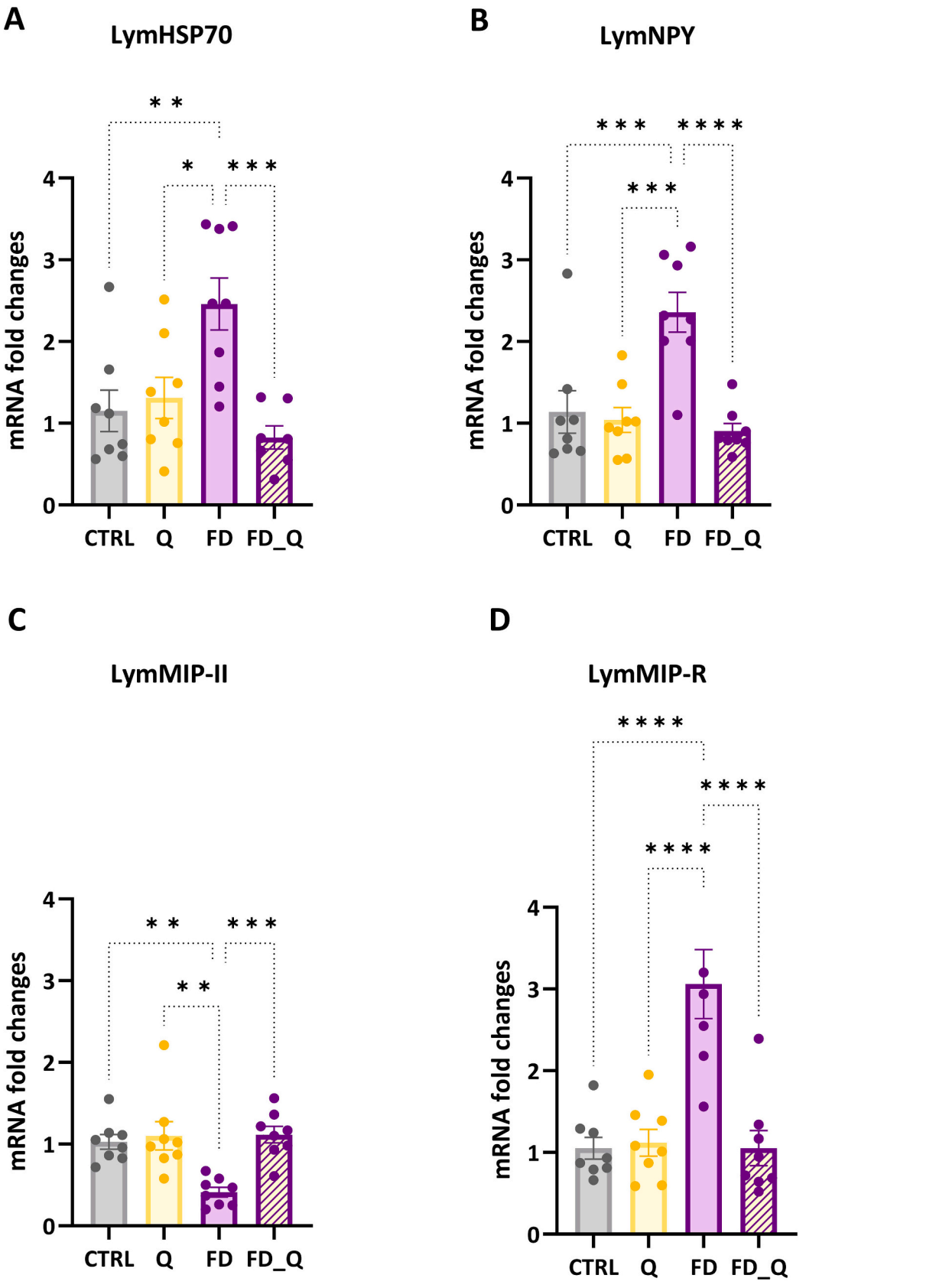

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Fig. 1. Transcriptional effects following five days of food deprivation, quercetin, and their combined presentation in *L. stagnalis* central ring ganglia. The expression of LymHSP70 (A), LymNPY (B), LymMIP-II (C), and LymMIP-R (D) was been measured in the central ring ganglia of *ad libitum* fed snails (grey bars), snails exposed to quercetin for 1 h (yellow bars), 5-day food deprived (purple bars), and snails both food deprived and exposed to quercetin (diagonal bars). The mRNA levels were analyzed by real-time PCR. Data are represented as means ± SEM and were analyzed with One-way ANOVA followed by Tukey *post hoc* analyses. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, * $p < 0.05$. $N = 8$ for each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

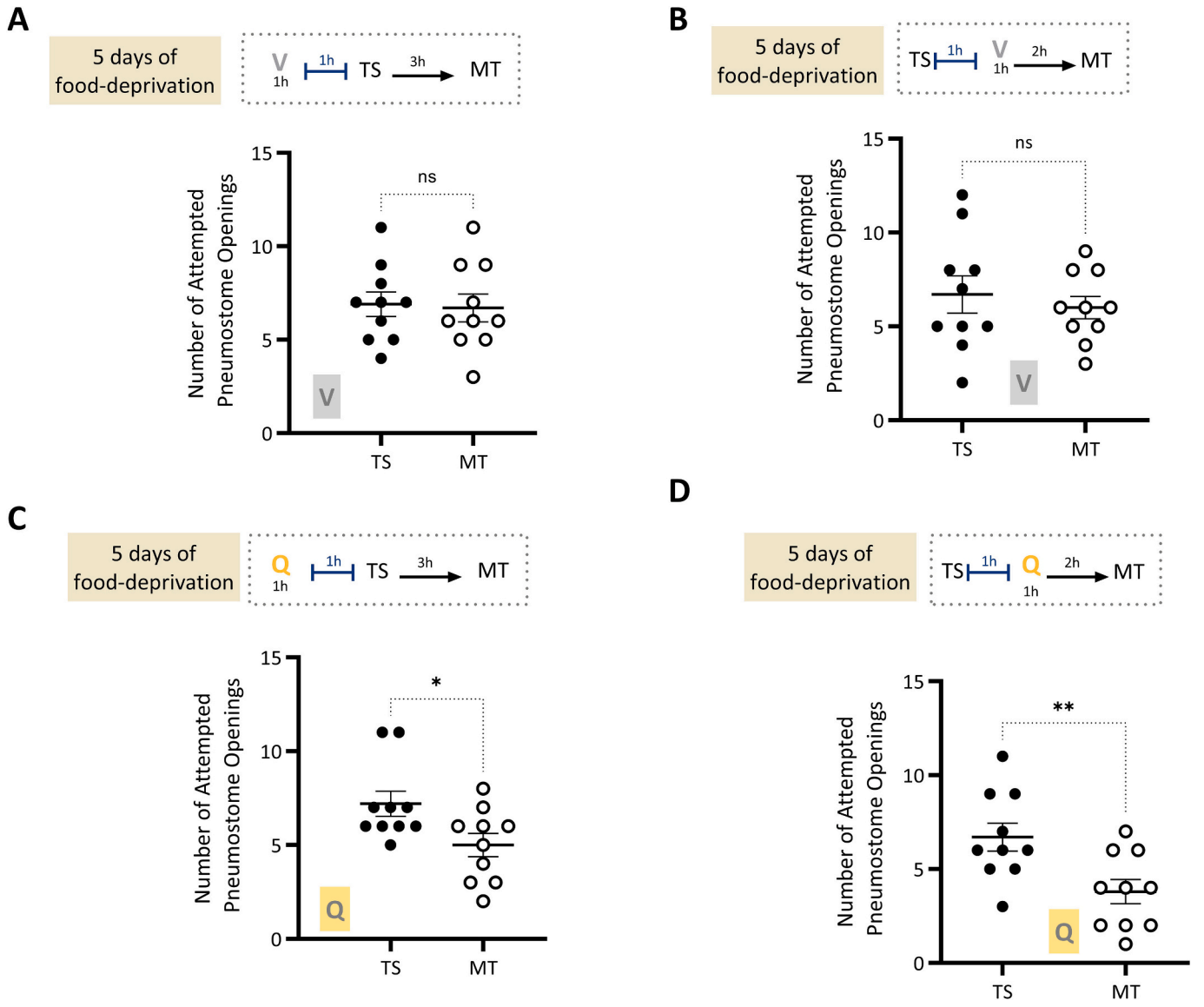

Fig. 2. Quercetin, food deprivation and cognitive ability. The timeline for each experiment is presented above the data. (A) Ten naïve snails were exposed to the vehicle (V) in which quercetin was dissolved (*i.e.*, DMSO – V) for 1 h. One hour later, snails were trained with a single 30-minute training session (TS-black circles) and a memory test (MT) was performed 3 h later to determine if ITM formed (MT– empty circles). ITM was not formed as there was no significant difference in the number of attempted openings between TS and MT. (B) Ten naïve snails were food-deprived for 5 days before being trained for 30 min (TS – black circles). One hour after training, snails were exposed to the vehicle (V) for 1 h, and ITM was tested 3 h post-TS (MT – empty circles). ITM was not formed as there was no significant difference in the number of attempted openings between TS and MT. (C) Ten naïve snails were food-deprived for 5 days before being exposed to quercetin (Q) for 1 h and, 1 h later, trained for 30 min (TS – black circles). Memory (MT-empty circles) was tested 3 h later. ITM formed as there was a significant decrease in the number of attempted openings between TS and MT. (D) Ten naïve snails were food-deprived for 5 days before being trained for 30 min (TS – black circles). One hour after training, snails were exposed to Q for 1 h, and memory (MT-empty circles) was tested 3 h later. ITM formed as there was a significant decrease in the number of attempted openings between TS and MT. Data are represented as means ± SEM and analyzed with a paired *t*-test. **p < 0.01, * p < 0.05, ns = not significant as $p > 0.05$.

- Snails of the 'Quercetin (Q) group' ($N = 8$) were placed in artificial pond water for 45 min and, then immediately later, to quercetin for 1 h. Three hours later were sacrificed.
- Snails of the 'fluoride (F) group' (N = 8) were exposed to the vehicle for 1 h and, then immediately, to fluoride-containing pond water for 45 min. Three hours later were sacrificed.
- Snails of the 'F_Q group' (N = 8) were exposed to quercetin for 1 h and, then immediately, to fluoride-containing pond water for 45

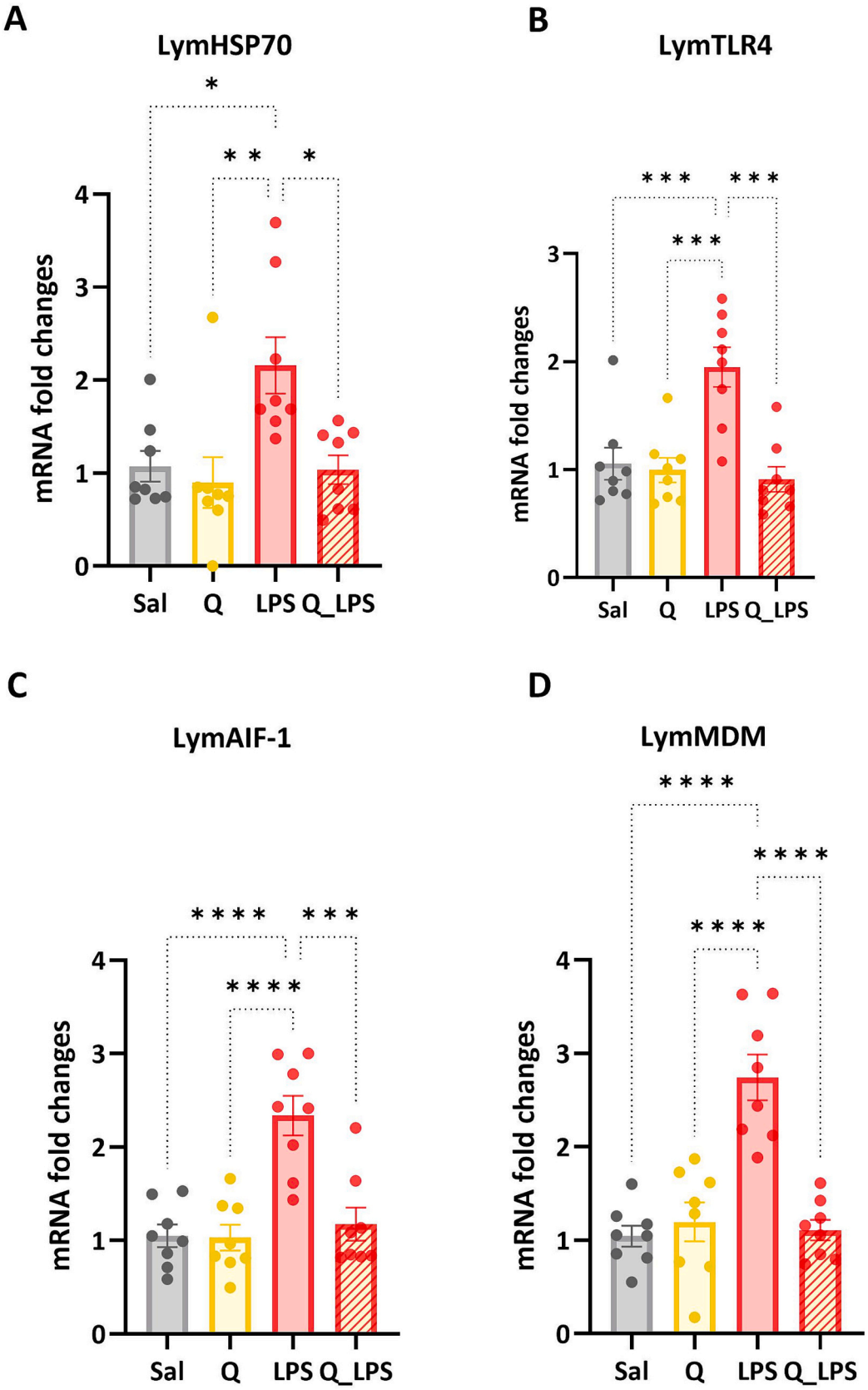

Fig. 3. Transcriptional effects following LPS-injection, quercetin and the exposure to quercetin before LPS in snails' central ring ganglia. The expression of levels of LymHSP70 (A), LymTLR4 (B), LymAIF-1 (C), and LymMDM (D) were measured in the central ring ganglia of saline-injected snails (grey bars), snails exposed to quercetin for 1 h (yellow bars), snails injected with LPS (red bars), and snails exposed to quercetin (diagonal bars). The mRNA levels were analyzed by real-time PCR. Data are represented as means ± SEM and were analyzed with One-way ANOVA followed by Tukey *post hoc* analyses. ****$p < 0.0001$, ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$. $N = 8$ for each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

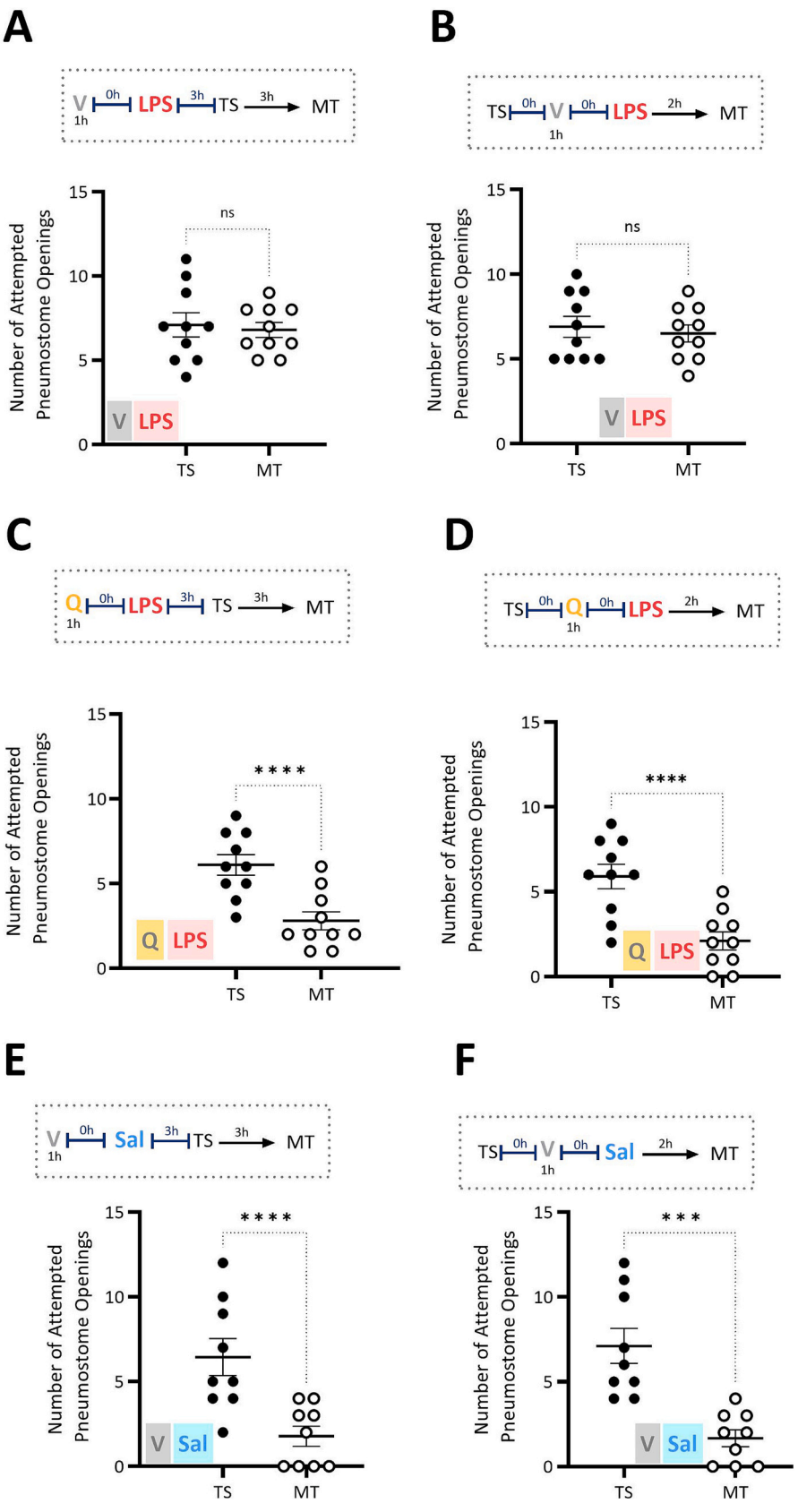

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Fig. 4. Quercetin, LPS-injection and cognitive ability. The timeline for each experiment is presented above the data. (A) Ten naïve snails were exposed to the vehicle (V) which quercetin was dissolved in (*i.e.*, DMSO – V) for 1 h and then injected with LPS. Three hours later, snails were trained with a single 30-min TS (closed circles) and ITM was tested 3 h later (MT-open circles). ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (B) Ten naïve snails were trained for 30 min (TS-closed circles) and then exposed to the vehicle (V) for 1 h before being injected with LPS. Three hours later memory was tested (MT-open circles). ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (C) Ten naïve snails were exposed to the quercetin (Q) for 1 h and then injected with LPS. Three hours later, snails were trained with a single 30-minute TS (closed circles) and memory was tested (MT-open circles) 3 h later. ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (D) Ten naïve snails were trained for 30 min (TS) and then immediately exposed to Q for 1 h before being injected with LPS. Memory was tested (MT-open circles) 3 h later. ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (E) Ten naïve snails were exposed to the vehicle (V) for 1 h and then injected with snail saline (Sal). Three hours later, snails were trained with a single 30-min TS (closed circles). Memory was tested (MT-open circles) 3 h later.
ITM formed as there was a significant decrease in the number of attempted pneumostome openings between TS and MT (F) Ten naïve snails were trained for 30 min (TS) and then exposed to the vehicle (V) for 1 h before being injected with snail saline (Sal). Memory was tested (MT-open circles) 3 h later. ITM formed as there was a significant decrease in the number of attempted pneumostome openings between TS and MT Data are represented as means ± SEM and analyzed with a paired t-test. ****$p < 0.0001$, ns = not significant as $p > 0.05$.

min. Three hours later were sacrificed. Thus, for this experiment, a total of 30 snails was used.

Next, we tested the hypothesis that snails exposed to fluoride-containing pond water for 45 min would not form learning and memory following operant conditioning of aerial respiration, while a 1-hour exposure to quercetin before the 30-minute TS and/or the MT for ITM would prevent the effects of fluoride.

For this purpose, we used 40 naïve snails for this study, divided into five groups ($N = 10$ each) depending on the type of treatment and its occurrence:

- **Vehicle exposure and Fluoride treatment before training**: snails were exposed to the vehicle for 1 h, then were exposed to fluoride-containing pond water for 45 min, before being subjected to the TS 3 h later. ITM was tested 3 h post-training.
- **Vehicle exposure and Fluoride treatment after training**: snails were trained for 30 min and, immediately later, were exposed to the vehicle for 1 h. After that, snails were exposed to fluoride-containing pond water for 45 min. ITM was tested 3 h post-training.
- **Quercetin exposure and Fluoride treatment before training**: snails were exposed to the quercetin for 1 h, then to fluoride-containing pond water for 45 min, before being subjected to the TS 3 h later. ITM was tested 3 h post-training.
- **Quercetin exposure and Fluoride treatment after training**: snails were trained for 30 min and, immediately later, were exposed to the quercetin for 1 h. After that, snails were exposed to fluoride-containing pond water for 45 min and ITM was tested 3 h post-training.

2.8. Total RNA extraction, reverse transcription, and real-time polymerase chain reaction

After being on ice for 10 min, snails were sacrificed. The central ring ganglia were dissected (buccal ganglia were excluded) and stored at −80 °C before analysis. Total RNA was extracted and treated with DNase using the GenElute™ Total RNA Miniprep Kit and DNASE70-On-Column DNase I Digestion Set (Merck Millipore). 200 ng of the total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher). qPCR was performed on 20 ng of mRNA using a Bio-Rad® CFX Connect™ Real-Time PCR Detection System with SYBR Green Master Mix (Bio-Rad). Cycle threshold values were determined using CFX Maestro™ Software (Bio-Rad). Specific primers were used at a final concentration of 300 nM (Table 1). Dissociation curve analysis and 2 % agarose gel electrophoresis confirmed the absence of nonspecific PCR products or primer dimers. mRNA levels of each target gene were normalized against the mean of two reference genes, elongation factor 1α and tubulin, as assessed using Normfinder® software (Wang et al., 2012), considering both intra- and intergroup variations.

The amplification efficiencies of the target and control genes were approximately equal. The $2-\Delta\Delta Ct$ method was used for quantitative analysis, with the control animals serving as the calibrators.

2.9. Statistical analysis

Behavioral data were analyzed using a paired Student's *t-test* to compare the number of attempted pneumostome openings recorded during the 0.5 h TS and the MT performed 3 h later.

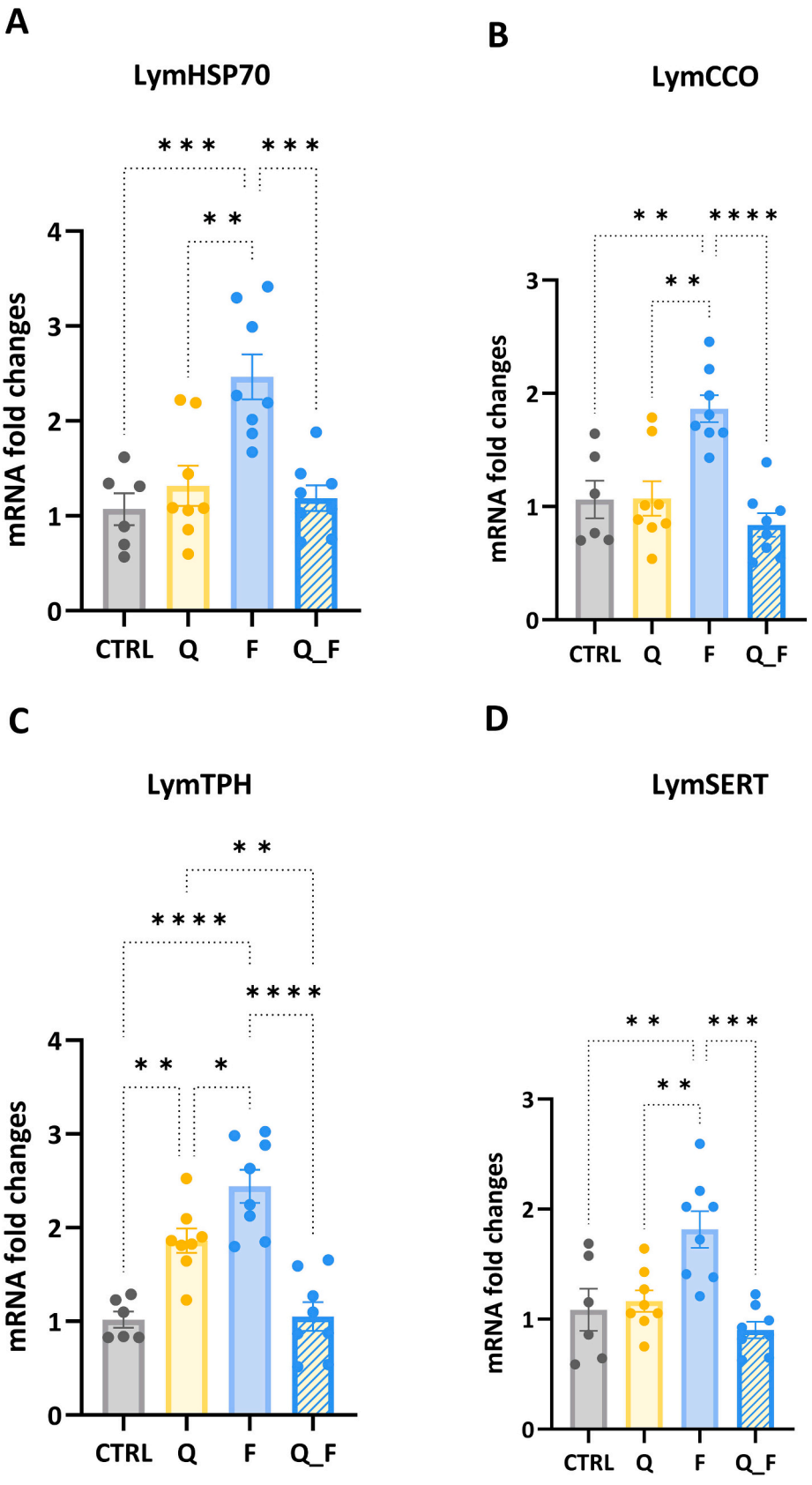
For gene expression analyses, first, we analyzed our data for normality assumption using Kolmogorov-Smirnov one-sample test for normality (K-S distance and P): all targets displayed a normal distribution. One-way ANOVA was used to compare the expression levels of each target and significant differences were determined by Tukey's *post hoc* test. All tests were defined as significant at $p < 0.05$. Data were presented as mean ± standard error (SEM). All statistical analyses were performed using SPSS v. 26.0 (IBM Corp., Armonk, NY, USA), while graphs were generated using GraphPad Prism v. 9.00e for MAC® (GraphPad Software, Inc., La Jolla, CA, USA).

3. Results

3.1. Experiment 1

The primary objective of Experiment 1 was to assess whether a period of 5 days without food, exposure to quercetin alone, and the combination of both conditions influenced the expression levels of selected targets involved in energy homeostasis and stress response in the central ring ganglia of *L. stagnalis*: the molluscan insulin peptide II (MIP-II) and its receptor (LymMIPR), the orexigenic neuropeptide Y (NPY), and the orthologue of HSP70 (Ito et al., 2015, 2013; Mita et al., 2014; Murakami et al., 2013; Rivi et al., 2022e).

In this study, One-way ANOVA revealed a significant main effect of the procedures on all selected targets: LymHSP70 [$F(3,28) = 6.26$, $P = 0.002$] (Fig. 1A), LymNPY [$F(3,28) = 11.39$, $P < 0.0001$] (Fig. 1B), LymMIP-II [$F(3,28) = 8.69$, $P = 0.0003$] (Fig. 1C), and LymMIP-R [$F(3,27) = 15.29$, $P < 0.0001$] (Fig. 1D). A Tukey's *post-hoc* analysis showed that the 5-day food deprivation (FD) significantly upregulated the expression levels of LymHSP70, LymNPY, and LymMIP-R compared to *ad libitum* fed control snails (LymHSP70: $p = 0.0054$, LymNPY: $p = 0.0009$, LymMIP-R: $p < 0.0001$) and those exposed to quercetin alone (LymHSP70: $p = 0.016$, LymNPY: $p = 0.0004$, LymMIP-R: $p < 0.0001$). Conversely, LymMIP-II expression was significantly downregulated by the 5-day food deprivation compared to control snails with *ad libitum* access to food and those exposed to quercetin alone ($p = 0.004$ and $p = 0.001$, respectively). Quercetin treatment alone did not significantly alter the expression levels of these targets compared to control snails. However, in the 5-day food-deprived snails, a 1-hour exposure to quercetin (*i.e.*, Q_FD group) prevented the upregulation of LymHSP70, LymNPY, and LymMIP-R, and the downregulation of LymMIP-II induced by food deprivation (Q_FD *vs* FD: LymHSP70: $p = 0.0007$, LymNPY: $p <$



(caption on next page)

Fig. 5. Transcriptional effects following exposure to fluoride, quercetin and the exposure to quercetin before fluoride in snails' central ring ganglia. The expression of levels of LymHSP70 (A), LymTLR4 (B), LymAIF-1 (C), and LymMDM (D) have been measured in the central ring ganglia of saline-injected snails (grey bars), snails exposed to quercetin for 1 h (yellow bars), snails injected with LPS (red bars), and snails exposed to quercetin (diagonal bars). The mRNA levels were analyzed by real-time PCR. Data are represented as means ± SEM and were analyzed with One-way ANOVA followed by Tukey *post hoc* analyses. ****p < 0.0001, ***p < 0.001, **p < 0.01, * p < 0.05. N = 8 for each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

0.0001=, LymMIP-II: p = 0.0009, LymMIP-R: p < 0.0001). At the behavioral level, we found that ITM for the operant conditioning of aerial respiration was not formed in 5-day food-deprived snails exposed to the vehicle (V) either before (Fig. 2A) or after (Fig. 2B) the single 30-minute training session as there was no significant reduction in the number of attempted pneumostome openings between TS and MT (paired *t*-test: $t = 1.02$, df = 9, $p = 0.33$ and. $t = 0.31$, df = 9, $p = 0.76$, respectively).

Control experiments – (Supplementary Fig. 1A) demonstrated that exposure to only the vehicle (*i.e.*, DMSO) did not affect the snails' ability to form ITM following a single 30-minute TS (One-Way ANOVA: [F (2, 37) = 8.22, $P = 0.001$]. That is, there was a significant reduction in the number of attempted pneumostome openings in the 3 h MT compared to TS (p = 0.004, Tukey's *post hoc*). Additionally, consistent with previous studies, a single 30-minute TS did not result in LTM formation, as there was no significant difference in the number of attempted pneumostome openings in the 24-hour MT compared to TS ($p = 0.002$, Tukey's *post hoc*).

On the other hand, a 1 h exposure to quercetin in 5-day food-deprived snails either before (Fig. 2C) or after (Fig. 2D) the 30-minute TS reversed the effects of food deprivation on memory formation, as there was a significant reduction in the number of attempted pneumostome openings between TS and MT, indicating that ITM was formed (paired *t*-test: $t = 2.79$, df = 9, $p = 0.02$ and $t = 4.20$, df = 9, p = 0.002, respectively). Control experiments (Supplementary Fig. 1B) demonstrated that the exposure to 1) PW; 2) the vehicle (V) or; 3) quercetin for 1 h before training did not alter the number of attempted pneumostome openings in TS between the 3 groups (PW, V, and Q) as no significant differences were found in the number of attempted pneumostome openings recorded in snails exposed to artificial pond water for 1 h (One-Way ANOVA: F (2, 27) = 0.17, $P = 0.84$].

3.2. Experiment 2

The main goal of Experiment 2 was to determine the transcriptional effects of a 1-hour exposure to quercetin before LPS injection on the expression levels of key stress and immune response targets in the central ring ganglia of *L. stagnalis*. In particular, we focused our attention on the expression levels of Toll-like receptor 4 (TLR4), molluscan defense molecule (MDM), allograft-inflammatory factor-1 (AIF-1), and HSP70 (Rivi et al., 2023e). A One-way ANOVA revealed a main effect of the procedures on all selected targets: LymHSP70 [F(3,28) = 6.26, $P = 0.002$] (Fig. 3A), LymTRL4 [F(3,28) = 11.40, $P < 0.0001$] (Fig. 3B), LymAIF-1 [F(3,28) = 14.43, $P < 0.0001$] (Fig. 3C), and LymMDM [F (3,27) = 20.88, $P < 0.0001$] (Fig. 3D). Specifically, LPS injection significantly upregulated the mRNA levels of these targets compared to both saline-injected snails (LymHSP70: $p = 0.13$, LymTRL4: $p = 0.0008$, LymAIF-1: $p < 0.0001$, and LymMDM: $p < 0.0001$) and snails exposed to quercetin alone (LymHSP70: $p = 0.003$, LymTRL4: $p = 0.0004$, LymAIF-1: p < 0.0001, and LymMDM: p < 0.0001). Notably, quercetin exposure alone did not affect the expression levels of the selected targets, as no significant differences were observed compared to control saline-injected snails. However, pre-exposure to quercetin for 1 h before LPS injection prevented the upregulation of these targets, with expression levels similar to those in the saline-injected and quercetin-exposed snails and significantly lower than those recorded in LPS-injected snails (LymHSP70: $p = 0.01$, LymTRL4: $p = 0.0001$, LymAIF-1: $p = 0.0002$, and LymMDM: p < 0.0001, Tukey's *post-hoc* test). At the behavioral level, we found that LPS blocked the ability of *L. stagnalis* to form ITM following operant conditioning of aerial respiration either when injected before (Fig. 4A) or after (Fig. 4B) the single 30-minute TS. There was no significant reduction in the number of attempted pneumostome openings between TS and MT ($t = 0.47$, df = 9, $p = 0.65$ and t = 0.47, df = 9, p = 0.65, respectively). However, exposing snails to quercetin for 1 h either 3 h before (Fig. 4C) or after (Fig. 4D) LPS injection reversed the negative effects of the immune challenge on memory formation, enabling snails to show ITM. In particular, we found a significant reduction in the number of attempted pneumostome openings in MT compared to TS, indicating that ITM was formed (paired *t*-test: $t = 9$, df = 9, $p < 0.0001$, and $t = 8.14$, df = 9, $p < 0.0001$, respectively). Control experiments led us to ascertain that the effects observed were not due to the injection *per se*, as injecting snails with saline either 3 h before (Fig. 4E) or after (Fig. 4F) TS did not affect the ability of snails to form ITM (paired t-test: $t = 11.34$, df = 9, p < 0.0001 and $t = 8.9$, df = 9, p < 0.0001, respectively).

3.3. Experiment 3

The first aim of Experiment 3 was to assess whether exposing *L. stagnalis* to fluoride for 45 min, to quercetin for 1 h, and the combination of both conditions would influence the expression levels of cytochrome *c* oxidase (CCO), a major regulatory enzyme of the electron transport chain in snails' central ring ganglia (Dong et al., 2021), HSP70, a key modulator for stress response (Multhoff, 2007; Zatsepina et al., 2021), and serotonin rate-limiting enzyme (tryptophane hydroxylase) and transporter (SERT) in the central ring ganglia of *L. stagnalis*. A One-way ANOVA revealed significant effects of the treatments on all the selected targets: LymHSP70 [F(3,26) = 10.98, P < 0.0001] (Fig. 5A), LymCCO [F(3,26) = 11.86, P < 0.0001] (Fig. 5B), LymTPH [F(3,26) = 21.56, P < 0.0001] (Fig. 5C), and LymSERT [F(3,26) = 9.30, $P = 0.0002$] (Fig. 5D). Tukey's *post-hoc* test demonstrated that fluoride exposure significantly upregulated the mRNA levels of these targets compared to control snails (LymHSP70: $p = 0.003$, LymCCO: $p = 0.002$, LymTPH: p < 0.0001, and LymSERT: $p = 0.005$) and those exposed to quercetin alone (LymHSP70: $p = 0.001$, LymCCO: p = 0.001, LymTPH: $p = 0.038$, and LymSERT: $p = 0.007$). Quercetin exposure alone did not affect the expression levels of the selected targets, with the sole exception of LymTPH, whose expression levels were significantly higher in quercetin-exposed snails compared to the control ones (p = 0.003). No significant differences were observed between LymHSP70, LymCCO, and LymSERT compared to control saline-injected snails. Interestingly, pre-exposure to quercetin for 1 h before fluoride exposure prevented the upregulation of these targets, with expression levels similar to those in the control and quercetin groups and significantly lower than those in LPS-injected snails (LymHSP70: $p = 0.0004$, LymCCO: p < 0.0001, LymTPH: p < 0.0001, and LymSERT: $p = 0.0002$).

At the behavioral level, we found that fluoride exposure for 45 min affected the ability of *L. stagnalis* to form ITM following operant conditioning of aerial respiration either when presented before (Fig. 6A) or after (Fig. 6B) a single 30-minute TS. That is, fluoride exposure prevented ITM formation as there was no significant reduction in the number of attempted pneumostome openings in MT compared to TS ($t = 0.84$, df = 9, $p = 0.42$ and $t = 0.18$, df = 9, $p = 0.86$, respectively). However, exposing snails to quercetin for 1 h either before (Fig. 6C) or after (Fig. 6D) fluoride treatment reversed fluoride's memory-blocking effects (*i.e.*, ITM formed here), as evidenced by a significant reduction

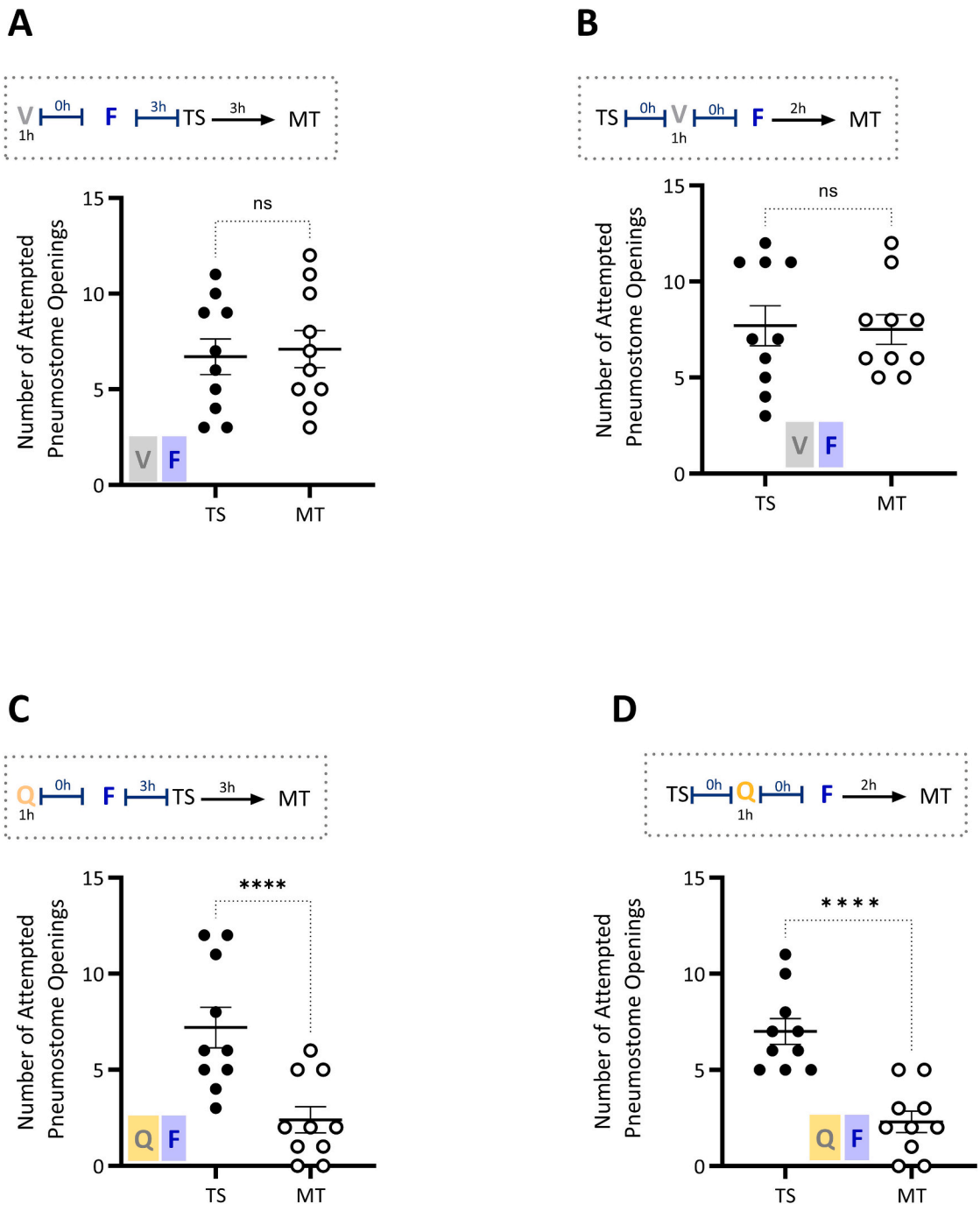


Fig. 6. Quercetin, fluoride exposure and cognitive ability. The timeline for each experiment is presented above the data. (A) Ten naïve snails were exposed to the vehicle (V) in which quercetin was dissolved (*i.e.*, DMSO – V) for 1 h and then exposed to fluoride (F) for 45 min, before being trained with a single 30-min TS (closed circles). ITM was tested 3 h later (MT-open circles). ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (B) Ten naïve snails were trained for 30 min (TS – closed circles) and then exposed to the vehicle (V) for 1 h before being exposed to fluoride (F) for 45 min. ITM was tested 3 h later (MT-open circles). ITM did not form as there was no significant difference in the number of attempted pneumostome openings between TS and MT. (C) Ten naïve snails were exposed to the quercetin (Q) for 1 h and then exposed to fluoride (F) for 45 min, before being trained with a single 30-min TS (closed circles). ITM was tested 3 h later (MT-open circles). ITM formed as there was significant decrease in the number of attempted pneumostome openings between TS and MT. (D) Ten naïve snails were trained for 30 min (TS – closed circles) and then exposed to Q for 1 h before being exposed to fluoride (F) for 45 min. ITM was tested 3 h later (MT-open circles). ITM formed as there was significant decrease in the number of attempted pneumostome openings between TS and MT. Data are represented as means ± SEM and analyzed with a paired t-test. ****$p < 0.0001$, ns = not significant as $p > 0.05$.

in the number of attempted pneumostome openings in MT compared to TS ($t = 11.53$, df = 9, $p < 0.0001$ and $t = 8.41$, df = 9, $p < 0.0001$, respectively).

4. Discussion

In this study, we investigated the molecular and behavioral effects of three ecologically relevant stressors on the memory abilities of *L. stagnalis* and the therapeutic potential of quercetin to reverse the

Table 1
The forward (FW) and reverse (RV) primer nucleotide sequences utilized in qRT-PCR are provided, along with the accession number of the PCR product obtained through the amplification of cDNA (mRNA).

Gene bank accession	Target	Type sequence
DQ206432.1	*Lymnaea stagnalis* heat-shock protein 70 LymHSP70	5′ - AGGCAGAGATTGGCAGGAT - 3′ 3′ - CCATTTCATTGTGTCGTTGC - 5′
X59302.1	*Lymnaea stagnalis* molluscan insulin-related peptide LymMIP II	5′ - CCAATCATCTTGCAGTTTA - 3′ 3′ - GTCGTCCAGATCTGTTTCT - 5′
X84994.1	*Lymnaea stagnalis* putative molluscan insulin-related peptide receptor LymMIPR	5′ - ATTGGAGACTTTGGTATGAC - 3′ 3′ - ACACTCCATCTTTGAGAGAC - 5′
AJ238276.1	*Lymnaea stagnalis* neuropeptide Y, LymNPY	5′ - ACTCTTGGTGTCACTGCTCG - 3′ 3′ - CTTGCGCCGTTTCTCTTTCC - 5′
AY577328.1	*Lymnaea stagnalis* Toll-like receptor 4 LymTLR4	5′ - GGAGGGTCAAGCATAAAGTGT - 3′ 3′ - CATCAAGGTCAACGCCAAT - 5′
U58769.1	*Lymnaea stagnalis* molluscan defense molecule precursor LymMDM	5′ - CGGGTACACACACAGATGGA - 3′ 3′ - TGACTGAACATTGGGCACAC - 5′
DQ278446.1	*Lymnaea stagnalis* allograft inflammatory factor LymAIF	5′ - CGTTTATGGTAAGCTGGAAAGA - 3′ 3′ - CTGGGAGCAAAGTCAAGCAT - 5′
AF129815.1	*Lymnaea stagnalis* tryptophan hydroxylase, LymTPH	5′ - AGGATACAGTCTACCGACAG - 3′ 3′ - TGAGTTCACGGAAAACTATT - 5′
FX185022	*Lymnaea stagnalis* serotonin transporter, LymSERT	5′ - ATACCGTACCTTGTCATGTT - 3′ 3′ - TGTTGTAGTACCAGGAGACA - 5′
FX185516.1	*Lymnaea stagnalis* cytochrome *c* oxidase subunit I LymCCO1	5′ - TCATAAAGATATTGGTACCTTG - 3′ 3′ - AAGCATGTGCTGTAACAATA - 5′
X15542.1	Snail, beta-tubulin LymTUB	5′ - GAAATAGCACCGCCATCC - 3′ 3′ - CGCCTCTGTGAACTCCATCT - 5′
DQ278441.1	*Lymnaea stagnalis* elongation factor 1-alpha LymEF1α	5′ - GTGTAAGCAGCCCTCGAACT - 3′ 3′ - TTCGCTCATCAATACCACCA - 5′

cognitive deficits resulting from specific stressors. In particular, we asked whether quercetin treatment induces antioxidant, anti-inflammatory, and energy-restoring effects by preventing or reversing the effects of fluoride, LPS, and food deprivation at both the molecular and behavioral levels.

The significant upregulation of the expression of levels of LymHSP70, LymNPY, and LymMIP-R and downregulation of LymMIP-II mRNA observed in *Experiment 1* suggest – albeit at the transcriptional level - a heightened stress response and altered energy regulation in the central ring ganglia of severely food-deprived snails. In particular, the upregulation of LymHSP70 - a heat shock protein that helps protect cells from stress - suggests that following 5 days of food deprivation snails are under significant physiological stress (Mayer and Bukau, 2005; Wang et al., 2012; Zatsepina et al., 2021), whereas the high expression levels of LymNPY, a neuropeptide involved in energy balance and feeding behavior, suggest an adaptive response to low energy levels consistent with data from mammals (Engström Ruud et al., 2020). The strong upregulation of LymMIP-R and downregulation of LymMIP-II in food-deprived snails – consistent with our previous studies (Rivi et al., 2022e) - may reflect a complex interplay in the stress response mechanisms under the 5-day food deprivation regime (Mita et al., 2014). Interestingly, quercetin treatment alone did not significantly alter the expression levels of these genes compared to control snails with *ad libitum* access to food, suggesting that quercetin does not independently affect these targets under normal conditions. However, in food-deprived snails, a 1-hour exposure to quercetin mitigated the effects of food deprivation on the selected targets, suggesting that quercetin has a protective effect against the molecular changes induced by food deprivation. These data show that quercetin can reverse gene activity caused by food deprivation.

Behaviorally, the 5-day food deprivation regimen impaired the cognitive ability of *L. stagnalis* as evidenced in their inability to form ITM following operant conditioning of aerial respiration. Similar results were found with food deprivation when snails were trained using a procedure to produce a Garcia effect and conditioned taste aversion (Nakai et al., 2022; Rivi et al., 2022e; Totani et al., 2019). However, quercetin exposure reversed the cognitive deficit associated with food deprivation. Thus, in these food-deprived snails quercetin restored the snails' cognitive ability (*i.e.*, they became competent to form ITM). These behavioral data are consistent with our molecular data, suggesting that quercetin mitigates the adverse effects of food deprivation on both gene expressions leading to the recovery of cognitive function. However, it remains to be determined whether quercetin's effects are beneficial for snails in the long run.

For example, the changes elicited by 5-days of food deprivation may cause behavioral changes leading to a better chance of survival in an environment lacking food. That is, the internal physiological changes induced by food deprivation could lead to a torpor-like, low-energy state condition similar to what is thought to happen in the wild when snails survive winter conditions (*i.e.*, ~6–7 months under the pond ice in the winter (Fernell et al., 2021). It might be feasible to ask this question using the experimental procedure used in the Fernell et al. (2021) study by placing snails without food into a 4 °C environment for at least 1 month following an injection of quercetin or vehicle. We plan to perform this experiment in the future using both inbred W-strain snails which have never experienced living in a pond that freezes for at least 6 months each year, and *L. stagnalis* collected from ponds where such freezing occurs. A third possibility is to use freshly collected snails just before freeze-up as their diet of pond macrophytes may yield interesting results compared to the offspring of those snails raised in the lab on romaine lettuce.

Experiment 2 demonstrated the anti-inflammatory effects of quercetin in snails injected with LPS, consistent with studies from other model organisms (Li et al., 2016a). The upregulation of LymHSP70, LymTRL4, LymAIF-1, and LymMDM. following LPS injection suggests an active immune response and increased stress response. In particular, LymHSP70's upregulation is consistent with its role in stress protection (Batabyal et al., 2024). LymTRL4, a Toll-like receptor, is involved in the immune response, and its increased expression indicates activation of immune signaling pathways. Moreover, the upregulation of LymAIF-1, an allograft inflammatory factor, and LymMDM, a macrophage-derived mediator, further highlights the immune activation induced by LPS (Hoek et al., 1996; Vizioli et al., 2020). Pre-exposure to quercetin for 1 h before LPS injection prevented the upregulation of these targets, with expression levels similar to those in control and quercetin-treated groups. This suggests that quercetin may exert anti-inflammatory and stress-mitigating properties, effectively normalizing the expression of stress and immune response genes in the presence of an immune challenge (Khan et al., 2018; Li et al., 2016b; Lv et al., 2024; Proshkina et al., 2016; Takashima et al., 2014; Tang et al., 2019; Xu et al., 2024).

At the behavioral level, consistent with previous studies, LPS injection impaired the snails' ability to form ITM for operant conditioning of aerial respiration (Rivi et al., 2022c), whereas quercetin exposure, either before or after LPS injection, reversed the cognitive impairments,

allowing ITM formation. This behavioral improvement mirrors the molecular data, underscoring quercetin's protective role against immune-induced cognitive deficits (Anand David et al., 2016; Broman-Fulks et al., 2012; Chiang et al., 2023; Rivi et al., 2021a).

Experiment 3 investigated the effects of fluoride exposure to snails reared in a fluoride-free environment (Wiley et al., 2022), quercetin alone, and their combination on the expression of genes involved in stress response and energy metabolism, specifically LymHSP70, LymCCO, LymTPH, and LymSERT. Fluoride exposure for 45 min significantly upregulated these genes, indicating a stress response. LymHSP70's increased expression suggests a stress-protective mechanism similar to that observed in the other experiments (Bittencourt et al., 2023; Rivi et al., 2022d; Zhao et al., 2017), whereas the upregulation of LymCCO, involved in cellular respiration, indicates metabolic stress. LymTPH and LymSERT, related to serotonin synthesis and transport (Gingrich and Hen, 2001), suggest alterations in neurotransmitter pathways due to fluoride exposure, consistent with previous studies from mammals (Bartos et al., 2022; Kinawy, 2019; Kumar et al., 2024; Lu et al., 2019).

Quercetin treatment prevented the fluoride-induced upregulation of these genes, indicating its potential to counteract fluoride-induced stress at the molecular level (Li et al., 2019). Interestingly, quercetin alone increased the expression of LymTPH, suggesting a modulatory effect on serotonin pathways independent of stress, requiring future studies. Behaviorally, fluoride exposure impaired ITM formation, similar to the other stressors and consistent with other behavioral procedures (Godebo et al., 2023; Gopu et al., 2022). Quercetin exposure before or after fluoride exposure reversed these impairments, highlighting its consistent protective effect across different types of stress.

Our results further remark on the high level of conservation of the effects of stress on learning and memory abilities from mammals to invertebrates, as well as the protective effects of flavonoids.

In particular, data obtained with food deprivation experiments are similar to stress responses that have been observed in rodents, where severe food deprivation similarly results in the upregulation of stress-related genes, including heat shock proteins like HSP70 and NPY, leading to cognitive impairments (Beck, 2006; Mattson, 2012; Moura et al., 2018). Moreover, quercetin has demonstrated protective effects in both snail and rodent models by reducing oxidative stress, and inflammation, and normalizing the expression of stress-related genes (Ghafouri-Fard et al., 2021). For example, quercetin has been shown to decrease the levels of ROS and increase antioxidant enzyme activities, which helps in maintaining cellular homeostasis and protecting against stress-induced neuronal damage. Additionally, studies from rodents, demonstrated that LPS administration induces a robust immune response characterized by increased expression of Toll-like receptors (*e.g.*, TRL4) and pro-inflammatory cytokines like TNF-α and IL-1β (Ciesielska et al., 2021; Dokladny et al., 2010; Kojima et al., 2004; Lu et al., 2008). These changes are associated with neuroinflammation and cognitive impairments (Choudhary et al., 2023; Dantzer and Kelley, 2007). Studies in rodents have also shown that quercetin can inhibit the activation of microglia and astrocytes (Lu et al., 2024), the key players in neuroinflammation, and reduce the levels of pro-inflammatory cytokines, thereby protecting against LPS-induced cognitive deficits (Yang et al., 2018). In rodents and zebrafish, fluoride exposure similarly affects cognitive functions (Dondossola et al., 2022; Grandjean, 2019), leading to oxidative stress, mitochondrial dysfunction, and alterations in neurotransmitter levels, particularly serotonin (Bittencourt et al., 2023; Lu et al., 2019).

Consistent with our data from snails, rodent studies have demonstrated that quercetin can reduce fluoride-induced oxidative stress and neuroinflammation by enhancing antioxidant defenses and modulating neurotransmitter systems (Nabavi et al., 2012). Quercetin's ability to prevent mitochondrial damage and maintain cellular energy production is important in counteracting fluoride's detrimental effects (Qi et al., 2022). The reversal of these cognitive impairments by quercetin demonstrates its potential role in preserving or restoring cognitive functions under stress. In rodents, similar cognitive impairments are observed under stress conditions. For example, food deprivation has been shown to impair spatial memory and learning in tasks like the Morris water maze and radial arm maze (Chiang et al., 2023).

5. Conclusions

Having shown that the results from our model organism align with findings from other model organisms such as rodents, suggests the conserved mechanisms through which different stressors affect learning and memory functions, as well as the conserved quercetin's protective effects against stress-induced molecular and cognitive impairments. The consistent efficacy of quercetin in mitigating stress responses and preserving cognitive functions highlights its potential for therapeutic applications in managing stress-related disorders, not only in snails but also potentially in other species, including humans. Thus, the results of this study pave the way for future studies aimed at delving deeper into the mechanisms through which quercetin exerts its protective effects, possibly exploring its interaction with specific signaling pathways and its long-term impact on gene expression and behavior. Additionally, dose-response studies and investigations into the effects of quercetin on other stress-related genes and behaviors in different species could help generalize its therapeutic potential and applications.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpc.2024.110053.

Ethics approval

Ethical approval is not required for research work with *L. stagnalis*; however, every effort was made to ameliorate the suffering of animals, ensuring adequate food, clean oxygenated water, and low-density housing conditions. The stressors used in this study have no long-term effects on snails (personal observation).

CRediT authorship contribution statement

Veronica Rivi: Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anuradha Batabyal:** Writing – review & editing, Validation, Methodology, Conceptualization. **Cristina Benatti:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Fabio Tascedda:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition. **Johanna Maria Catharina Blom:** Writing – review & editing, Supervision, Resources, Project administration. **Ken Lukowiak:** Writing – review & editing, Visualization, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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Data availability

If reasonably requested, Dr. Veronica Rivi can be contacted to obtain

the original materials.

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