

Supplementary Material

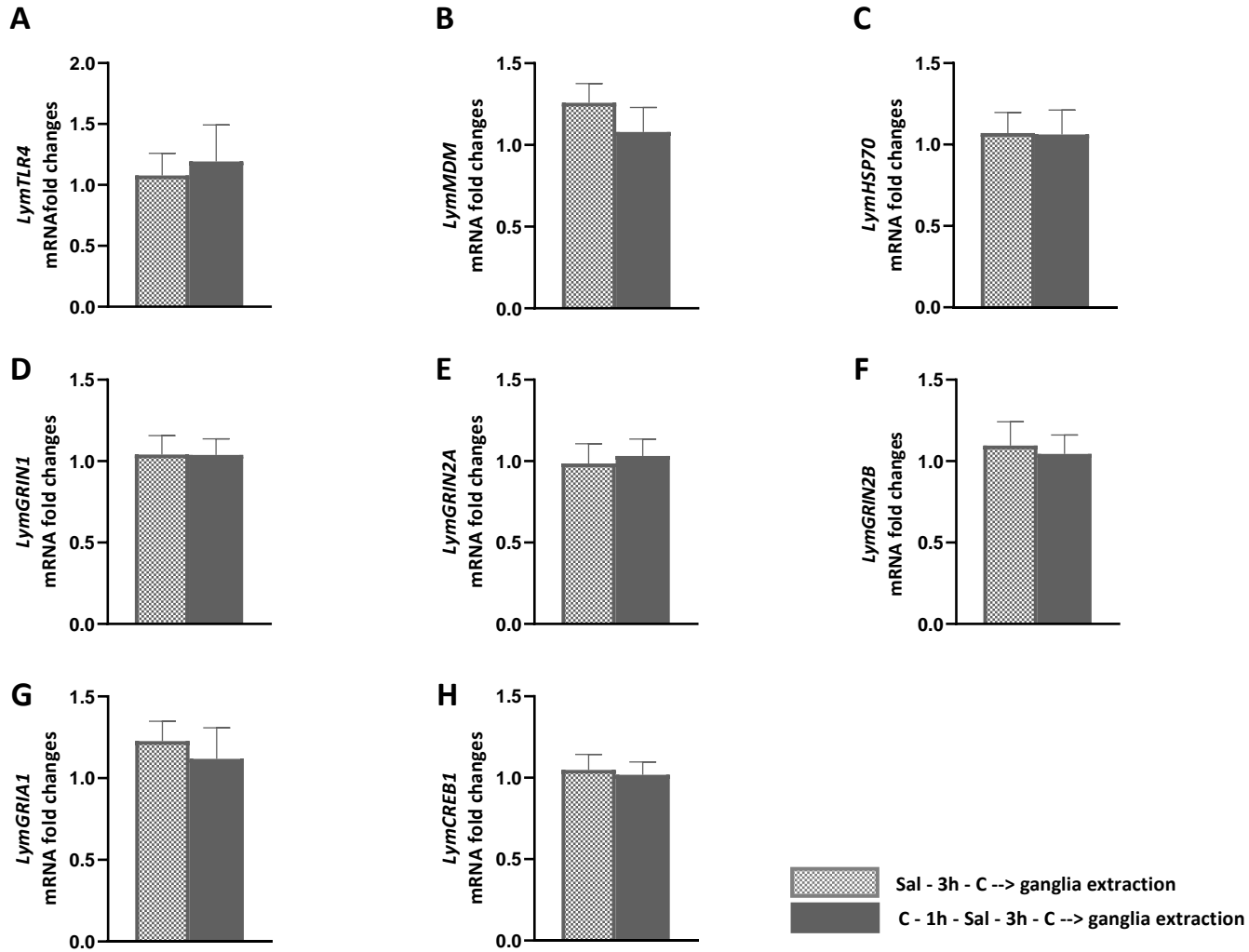


Figure S1. *LymTLR4*, *LymMDM*, *LymHSP70*, *LymGRIN1*, *LymGRIN2A*, *LymGRIN2B*, *LymGRIA1*, and *LymCREB1* expression levels showed no significant difference in snails exposed to carrot slurry 1 h before and 3 h after the snail saline injection (Saline group) and those of them exposed to carrot slurry only after the snail saline injection (Saline_C group). The expression of *LymTLR4* (A), *LymMDM* (B), *LymHSP70* (C), *LymGRIN1* (D), *LymGRIN2A* (E), *LymGRIN2B* (F), *LymGRIA1* (G), and *LymCREB1* (H) were measured in the central ring ganglia of snails injected with snail saline and then exposed for the first time to carrot slurry 3 h later (checkered bars) and those exposed to carrot slurry 1 h before and 3 h after being injected with snail saline (full dark grey bars). Immediately after the re-exposure to the carrot slurry for 2 min, snails of the Saline and Saline_C groups were sacrificed, the central ring ganglia were dissected, and the RNA was extracted and reverse-transcribed. No significant differences were found in the expression levels of these targets between the groups. The mRNA levels were analyzed by RT-qPCR. N = 8 for each group. Data are represented as means \pm SEM and were analyzed with unpaired *t*-test.

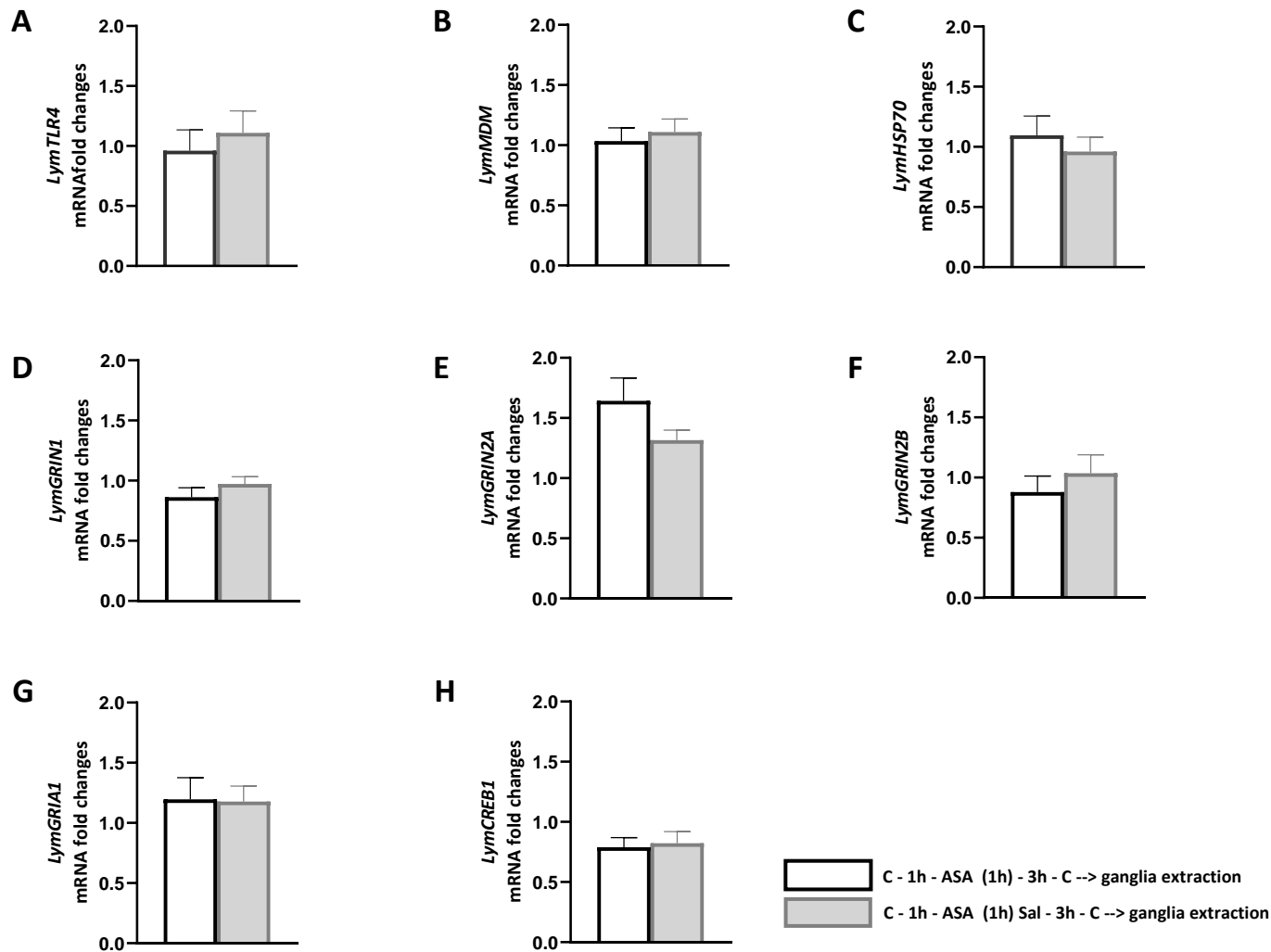


Figure S2. *LymTLR4*, *LymMDM*, *LymHSP70*, *LymGRIN1*, *LymGRIN2A*, *LymGRIN2B*, *LymGRIA1*, and *LymCREB1* expression levels showed no significant difference in snails of the ASA group and those of the ASA_Saline group. Snails of the 'ASA group' (white bars) were exposed to carrot slurry for 2 min and the number of rasps elicited by the carrot slurry was counted. One hour later, snails were exposed to ASA for 1 h. Three hours later, snails were re-exposed to carrot slurry for 2 min and the number of rasps elicited by the carrot slurry was again recorded. Snails of the 'ASA_Saline group' (full grey bars) were exposed to carrot slurry for 2 min and the number of rasps elicited by the carrot slurry was counted. One hour later, snails were exposed to ASA for 1 h and immediately after were injected with snail saline. Three hours later, snails were re-exposed to carrot slurry for 2 min and the number of rasps elicited by the carrot slurry was again recorded. Immediately after the re-exposure to the carrot slurry for 2 min, snails of the ASA and ASA_Saline groups were sacrificed, the central ring ganglia were dissected, and the RNA was extracted and reverse-transcribed. The expression of *LymTLR4* (A), *LymMDM* (B), *LymHSP70* (C), *LymGRIN1* (D), *LymGRIN2A* (E), *LymGRIN2B* (F), *LymGRIA1* (G), and *LymCREB1* (H) were measured and compared. No significant differences were found in the expression levels of these targets between the groups. The mRNA levels were analyzed by RT-qPCR. N = 8 for each group. Data are represented as means \pm SEM and were analyzed with unpaired *t*-test.

To identify and characterize the transcripts codifying for the glutamate ionotropic receptor NMDA type subunits 2A and 2B

(GRIN2A and GRIN2B) and the glutamate ionotropic receptor AMPA type subunit 1 (GRIA1) in *L. stagnalis*, we recurred to the contig annotation table generated by Benatti et al., (2022) [60] (Table S1).

Table S1. Contig annotation table. For each contig, the ID, the contig length (bp), and the RefSeq protein ID identified in *Biomphalaria glabrata* (a gastropod whose genome and transcriptome have been characterized and annotated) are reported with the corresponding gene definition. When available, the RefSeq protein ID identified in *Mus musculus* and *Homo sapiens* is also reported, together with the gene symbol.

<i>L. stagnalis</i> contigs				Homology association by BlastX				
Contig ID	Length (bp)	RNAseq TPM value	RefSeq protein ID <i>B. glabrata</i>	Gene symbol <i>B. glabrata</i>	RefSeq protein ID <i>M. musculus</i>	Gene symbol <i>M. musculus</i>	RefSeq protein ID <i>H. sapiens</i>	Gene symbol <i>H. sapiens</i>
FCFB012	247	3.080	XP_013076880	LOC106063108	NP_032196, XP_006521857	<i>Grin2a</i>	NP_001127879, NP_001127880, NP_000824, XP_016878662, XP_016878661, XP_011520763, XP_011520760	GRIN2A
FCFB011	214	3.048	XP_013088398	LOC106072555	XP_006505638, XP_006505637, XP_011239523, XP_011239522, XP_006505636, NP_032197, XP_017176885	<i>Grin2b</i>	XP_011518930, XP_011518931, XP_016874708, NP_000825	GRIN2B
FCFB011	134	10.009	XP_013069087	LOC106056784	NP_001034284	<i>Gria2</i>	XP_011535937, NP_001107655, NP_001244952, NP_001244951, XP_016864882, NP_001244949	GRIA1

This approach allowed us to identify one specific transcript per each glutamatergic receptor type: FX_180835 (*LymGRIN2A*), FX_180839 (*LmGRIN2B*), and FX_183516.1 (*LymGRIA1*). All transcripts resulted to be expressed (TPM > 0) in the publicly available *L. stagnalis* transcriptome (i.e., Transcriptome Shothgun Assembly).

To confirm whether these transcripts were expressed in the central ring ganglia of *L. stagnalis*, we performed qualitative PCR on each transcript using the set of primers listed in Table S2.

Table S2. Validated primers for sequencing. For each predicted target are reported the relative transcript FX_, the forward (FW), and reverse (RV) primers' sequences, with the corresponding position on the transcript.

Match on	Predicted target	Position on transcript		Primer sequence
FX180835	Putative <i>LymGRIN2A</i>	FW	2119	TAAGTTTAACTGGACGGACT
		RV	3645	ATAGCGTCCTTGACATTAGT
FX180839	Putative <i>LymGRIN2B</i>	FW	2439	CAGTATTGAGAACATGGTTG
		RV	4273	GGTTCCTTGATGGTTTATTA
FX183516.1	Putative <i>LymGRIA1</i>	FW	250	GGATCTTCTGACATCAAGTT
		RV	2477	CTGCAGAGAATGTACCAATA

We performed qualitative PCR using Dream Taq DNA polymerase (Thermo Scientific, Waltham, Massachusetts, United States) as previously described [60]. Primer sequences were designed by NCBI PrimerBLAST software (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>) (accessed on May, 31 st, 2023) and were synthesized by Merck KGaA (Darmstadt, Germany). PCR products were electrophoresed on agarose gel (2%), and DNA fragments were visualized by UV illumination to confirm the correct amplicon size. PCR products (600–800 bp) were purified using the High Pure PCR Product Purification Kit (Roche Diagnostics Corporation, Indianapolis, Indiana, USA) following the manufacturer's instructions, and were directly sequenced using the Sanger sequencing method. Sequencing was performed using ABI

PRISM 3130xl Genetic Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and BigDye Terminator v1.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, Massachusetts, USA). The sequence analysis of the PCR fragments was performed using Sequence Scanner Software 2.0 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and sequences were compared and aligned to the contigs of *L. stagnalis* using BLASTn (<https://blast.ncbi.nlm.nih.gov/Blastn/> (accessed on June 1st, 2023). The sequences generated by Sanger sequencing confirmed that the transcripts predicted in silico by the computational analysis uniquely matched their respective templates.

Moreover, we found that all transcripts contained an open reading frame (ORF) (Table S3) whose identity was further confirmed by aligning its sequence with the amino acid sequences of the corresponding enzyme from other invertebrate and vertebrate model organisms, like *B. glabrata*, *Aplysia californica*, *M. musculus*, and *H. sapiens* (Table S4). The amino acid sequences were predicted using the Open Reading Frame (ORF) Finder tool of NCBI (<https://www.ncbi.nlm.nih.gov/orffinder/> (accessed on June 1st, 2023).

Table S3. ORFs of the putative glutamatergic receptors in *L. stagnalis*. For each contig, the ID, the FX_ value corresponding to *L. stagnalis* Transcriptome Shotgun Assembly, the ORFs, and the predicted amino acid size (aa) are reported.

<i>Lymnaea stagnalis</i>			
TARGET	MATCH ON	ORF	aa size
<i>LymGRIN2</i> A	FX180835	MATSMEEENRVLILGGTGFIGRNLVDYLVRNKLARKIRVVDKVPQMAWLNPRHLESFENPAVEFCQ ANIINKASAEKVFCDDGGDFVINLAAETKHGQ SEPVYKEGIYRLSLNCAELAAMHKVKRFIELSTAQIYTHEKKPAREDAKSEPWTNLARHKLDVEKAL ENYVDLNYVILRPAIVYGIGDRNGLTPRLIGA IYKYTMQKMQLWTKDLKMNTVHVDDVRAIWHLCFHGNGRQVYNLTDKGDTTQGLISLVSEL FDINYDFVGAIFSNLARVRMTDIVDDINEKHMRPWS DACQRDQVSNTPLPIDQELLYNKHFLIDGSKIESTGFAYTIPKLEIGHLKEILDYITLGLMPRSLVS NEVFYTPTDVEAACLDHGADGDCGLEIDH TADQDSAHS	409
		MEMISNPMIDIPSHQPATCLKIKLLWVDSNNLLQMSSQRFHCDALHGNFPVPTTISRVEPSIDATI GRTCQCLKHAGAKPVLTSFTNIYSRAEKYSVQ DQQHDAKEKSPRNKISTTGSMEFLASCPEKRWNSFLSCRMCTHIGNLNAYFSFRFLSPERGALINVE RLPQERVCKVEKMNSASCRGSNIFYKTKLNQ KSSYSVTSNIHFIPKEITGNISHSEYSTERKSSPGIRTCVDCKASFMKTSDTPCVGRFRFESSDKSPKSLRS HSDSHANIWSLDTNHCSTPSTDTHAT SSNTTASFPTKPTRTFEQRWRPTKIVSDYPSRVSTLLSFVLVFLTLVASQTNGQNIKAGIHAIYPDRISMS KALSTELISTLYSVRRNLHVLRVSYDIE HVNTSLKNDDPKEILNLFCKGVFPHHVTILNINPLGMKRRSSNQYILELASYLGLPIISWDTEYIV GSQSLRTVQLAPTIEHQAKAMVSMLEYNWT	
		AFTIVTGPVSGSSQFVTSIENMVEQSQKNALKTPAHSRKESLNILSIINIRKPEDILPQLVKVKGSDTRVF LLHSASSMALDIIQTARTNLTDKDYVWI LTRTAIPNAREGPRSPVGLMGIDDFELTAMRQALKWGIWLNALADMAKTQGLLANMTIPPKE SCDSDKEPFWRDGEILYRHMLKVNLGEPQIKFN ENGLTQQTDLIIVNLQWIVGGKNKTEWKQVGRWTLHGLKMNEIVWPGESTVPPTGKPKRAFLRVA TLNELPYVIYRNLSSENGGCEEKSLPCQIYNRNDKK EITSNVTIAKCCAGLSMDLLKILSDQLNFDYEIKEVIDGKWGLINKTTNAWNLVKAALLNNEADIV MTAFKINPERASAVNFSVPYLETGIKIVLRD GAISPTAFLEPYDYASWSLILFVHATGSSILIFEWLSPYGLNRGHTPMRDHFKSLFRSFWLIWAMLF TSVQTDTPKGIASRFLANIWALFALVFLAS YTANLAAFMITKEEFYDLSGIQDYRLQNPYTMKPPFRYATIPNGSTEANIRANHKDMYNYMKAFNQ PDVDAGIAELKQGRIQAFIYDSSVLEYASRDPK	
<i>LymGRIN2</i> B	FX180839		1504

LymGRIA1	FX183516 .1	CRLVTVGNNRYAMTGYGVGFPPNNKPSRNPWIDKFNKHLKLQENGDMDLQKFWLAGACDTKEK GVSNRTLGLNFTSAFILLGSGVLLGLLILIFEHLKYFKCRKRLRKWDKCGCCALVSLSMGKSLQFKDY VDEAMSAYSSTRCKNP VCEIQIWKLRHQLDMAALLKIDHLQNLCEISGEFLPLMPSTVENEKRTHEHQQKPLAVRANGMQQ SQHYKTEKSNHRQGKKTASKLPSSQSAIDLPEVSF LRSLELRDGGNFIDKFEIYENYEKPDSSPVADHFSDFLLPPPPQGESPILLGSSACNDDTGGGGSSYGG DTFLGFDDFHSENAAGSRASVLAGRMAAMGS SPGNVLRRTPSYTSVAVGRDNGLDASDSSPNMTGRVRQYDGKTYVGVDSSTVT	945
		MMAKSHAKRIVVHSLIVMDQRFMIYCVIFALIGLHSTRSLERSVGLILQEDMPTKDTEYEQAQDFATM VVNSNLLNERRPGREMARVFPTLRNRTVFSN YKLGGTICGMANEGVMVIVGTSRASSFNTIQSYCQALQVPYILVTPSRPNPSDGYHYDLSVCPPIEAV MKVVANLSLADNKVFYVYDSDGLWRLQRMV QYFQMKDIMPRVLDVFRVDRISKAYVILRALDKLSEEKTIVLDLSTTQAYKIILEQIVDVGMNRENYH YILAGADAMDNLDRDFFSQFLYGGVEITAFQ FVNNQTDTYRKWQQMWEQYKDDFPNLFPLKTGSALMIDAVRALHEALMFEIPKQHTARTHPSTRC DMDNFKSSDVTSPNLNKNKYFQGLTGPLALKQG RRSEYSIDVYKLGFKQPMKQATWKSSDLMDGSKTFVPEVAIQNTTQRVTLIEPPFVMRIENRNGA PPVGNLDLEGYCIDLIEALARSEDFFEYQIYL TEEYGDKNETDGTWNGIIGQLINQERDIAVAPLTITQDRERVVDFSKPFMDTGISIMIKPKDKTKPGVF SFMDPLDTRVWLCAIGFLAVSGVLYFVGRF SPYEWNVSEDSTERTATTVFSISNTLWFLGALMQQGSDISRFSFSGRVIGSAWWFFTLIISSYTANLA AFLTIEKLVSISADDLVGHPTIKYGTKK TGSSWRFFEKSTVDTFVRMRKEMLNADVLFEYSEGVRKVRESKGYAFLLESAMNSYYSQQEPC DTMMVGDKLDNKGYGVAITYVNYPLRHINIAVL TLKEKGELIKLTQKWWFDKGCQGDQSVSKESTGTQSALTLNVSIGIFHILIGGLVLSMLTSSLEYLIQR KLIRRKNIKKATKNYTLVPPPPPPRRLCF GSAANRNGIEPVWENGMSDAAAAAAHEQQNLMSEDPDEYHNGT	

As shown in Table S4, the predicted protein had several amino acids very close to those of the human protein and the corresponding orthologue of several organisms extensively employed as standard models in preclinical studies.

Table S4. ORF homology table. Homology between the ORF of the putative glutamatergic receptors in *L. stagnalis* with orthologues from different organisms. For each contig, the ID, the amino acid (aa) size (bp), the FX_ value corresponding to *L. stagnalis* Transcriptome Shotgun Assembly, and the RefSeq protein ID identified in *B. glabrata*, *A. californica*, *M. musculus*, and *H. sapiens*, along with the corresponding gene definition and aa size, are reported.

<i>Lymanaea stagnalis</i>			<i>Biomphalaria glabrata</i>			<i>Aplysia californica</i>			<i>Mus musculus</i>			<i>Homo sapiens</i>		
TARGE T	MATC HON	aa size	Accession	Target	% identities	Accession	Target	% identities	Accession	Target	% identities	Accession	Target	% identities
LymGRI N2A	FX18083 5	409	XP_013076 878.1	Predicted Glutamate receptor ionotropic, NMDA 2A-like	51.12%	XP_01293 8956.1	NR2A	43.65 %	P35436.2	N-methyl-D- aspartate receptor 2A subunit	39.71%	Q12879. 1	N-methyl-D- aspartate receptor 2A subunit	32.48%
LymGRI N2B	FX18083 9	1504	XP_055875 497.1	Predicted Glutamate receptor ionotropic, NMDA 2B-like	68.75%	NP_00119 1485.1	NR2B	58.99 %	NP_00135 0679.1	N-methyl-D- aspartate receptor 2B subunit	33.92%	NP_0008 25.2	N-methyl-D- aspartate receptor 2B subunit	33.98%
LymGRI A1	FX18351 6.1	945	XP_013069 087.1	Predicted glutamate receptor-like	83.00%	NP_00119 ^e 1398.1	Glutamate receptor subunit protein GluR3	78.00 %	P23819.3	AMPA- selective glutamate receptor 1	39.00%	P42263.2	AMPA- selective glutamate receptor 3	36.97%

Candidate primers for quantitative RT-qPCR were designed with NCBI Primer-BLAST software (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>, accessed on June 1st, 2023) and synthesized by Merck KGaA (Darmstadt, Germany). Primers were designed to have a length of 19–23 nucleotides, a melting temperature between 58 and 60 °C, a GC content between 40 and 60%, and to generate an amplicon between 150 and 250 bp. All primers produced consistent results without amplifying off-target products or generating primer dimers (data available from the corresponding author upon request). Following amplification, each primer pair produced amplicons that yielded single bands at the correct size after electrophoresis in 2% agarose gels. No amplification was observed in cDNA from another pulmonated gastropod, *Pomacea canaliculate*, or controls that lacked reverse transcriptase in the RT-PCR (data available from the corresponding author upon request). Primer specificity was also checked by melt curve analysis (Figure S3). A single sharp peak with no primer-dimer was observed for all used primer pairs.

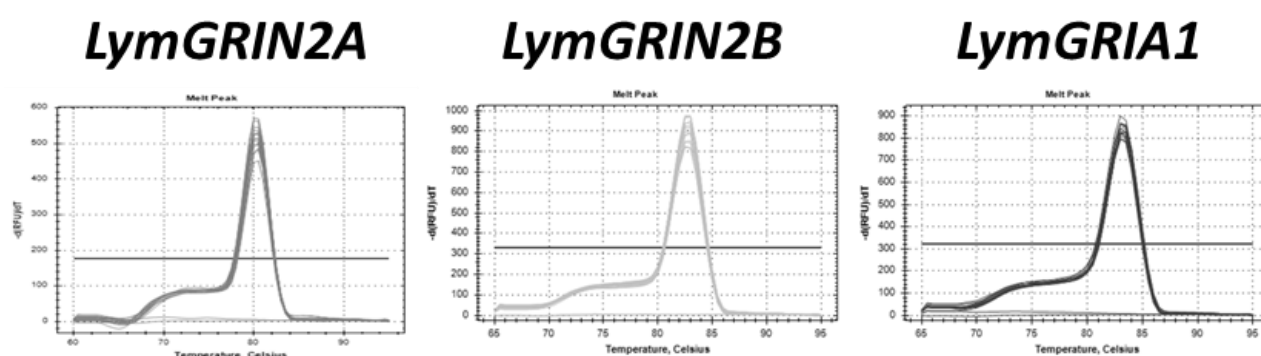


Figure S3. Melting curves analysis. For each target, a single sharp peak with no primer-dimer was observed.

Results showed that the RT-qPCR efficiency was between 97.98% and 105%, and the R² of primers was greater than 0.98. In addition, the mean C_q of *LymGRIN2A*, *LymGRIN2B*, and *LymGRIA* ranged from 19.16 to 25.23 for 20 ng of cDNA with moderately abundant mRNA levels in the ganglia of *L. stagnalis* (Table S5).

Table S5. Validated primers for gene expression analysis. For each enzyme of the KP, the relative transcript FX, the forward (FW) and reverse (RV) primers' sequences, with the corresponding size (bp), efficiency, R² score resulting from the validation experiments, and the Ct value obtained with 20 ng of cDNA are reported.

Transcript	Target	Size (bp)	Primer sequences	Efficiency	R ²	Ct value 20 ng
FX180835	<i>Lymnaea stagnalis</i> NMDA-type glutamate receptor subunit 2A <i>LymGRIN2A</i>	129 bp (3454-3583)	FW: GATCACCAAGGAT GATTACT	105%	0.99	25.23
			RV: CTTGGCTATATTCA AGTCTGT			
FX180839	<i>Lymnaea stagnalis</i> NMDA-type glutamate receptor subunit 2B <i>LymGRIN2B</i>	126 bp (4147-4273)	FW: GACTCCTCTGTTTT GGAATA	97.98%	0.98	19.16

			RV: GGTTCCTTGATGGT TTATTA			
			FW: AGACTGTTGTAGCT GTCCTT			
FX183516. 1	<i>Lymnaea stagnalis</i> AMPA-type glutamate receptor subunit 1 <i>LymGRIA1</i>	111 bp (1205- 1316)	<hr/> RV: ATAGCTATTGGATT TCTTGC	99.85%	0.99	20.32