Electronic Supplementary Material

An ancient FMRFamide-related peptide-receptor pair induces defense behavior in a brachiopod larva

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Table S1: Peptides that were tested in *Terebratalia transversa* larvae and the necessary concentrations to induce defense stance.

| peptide                  | batch 1                          | batch 2                          |
|--------------------------|-----------------------------------|-----------------------------------|
| DFLRFamide*              | 500 nM for full contraction       | 750 nM for full contraction       |
| AFLRFamide*              | 1 µM for full contraction         | 2 µM for full contraction         |
| FLRFamide**              | 3 µM for full contraction         | 3 µM for full contraction         |
| LRFamide**               | no contraction at 50 µM           | no contraction at 50 µM           |
| DFLRWamide               | 7.5 µM for full contraction       | 10 µM for full contraction        |
| AFLRWamide               | 20 µM for full contraction        | 20 µM for full contraction        |
| DFLRYamide               | 50 µM for full contraction        | 50 µM for full contraction        |
| AFLRYamide               | weak contraction in about 50% of the larvae at 50 µM | no contraction at 50 µM |
| DFLRLamide               | weak contraction in about 50% of the larvae at 50 µM | no contraction at 50 µM |
| AFLRLamide               | no contraction at 50 µM           | no contraction at 50 µM           |
| YMRFamide***             | 10 µM for full contraction        | 15 µM for full contraction        |
| NSDGlamide*              | no contraction at 50 µM           | no contraction at 50 µM           |
| TDKCVPVYlamide*          |                                   |                                  |
| AAKAPSSlamide*           |                                   |                                  |
| CYLYDCINamide*           |                                   |                                  |
| MDPSQFGYGlamide*         |                                   |                                  |
| YSLDGIGSGLlamide*        |                                   |                                  |
| LSDYYAWAAQTRLamide*     |                                   |                                  |
| GWamide                  |                                   |                                  |
| RGWamide(*)              |                                   |                                  |
| LGWamide**               |                                   |                                  |
| Flamide**                |                                   |                                  |
| KPIHYlamide**            |                                   |                                  |
| WQGMKMWamide***          |                                   |                                  |

* peptides predicted from *T. transversa* prepropeptide sequences
** shortened versions of peptides predicted from *T. transversa* prepropeptide sequences
*** peptides predicted from *Novocrania anomala* prepropeptide sequences
Figure S1: FLRFamide peptide and receptor expression in *T. transversa* larvae. A-C late larvae, *in situ* hybridization. Arrows indicate expression domains. A FLRFamide receptor expression. B ventral FLRFamide expression between apical and mantle lobe. (Same specimen as in C, with a focus on the ventral side.) C FLRFamide expression in apical lobe. D early larva with FLRFamide expression in apical lobe. E SEM picture of early larva. Scale bar = 30 µm.

Figure S2: Phylogeny of the *T. transversa*-FLRFamide receptor. Cladogram of neuropeptide GPCRs that showed connections in the clustermap to the *T. transversa* FLRFamide receptor (Figure 3). The dashed lines indicate receptor groups related to the *Terebratalia* FLRFamide receptor. Branches with filled circle at the end indicate a receptor that was deorphanized in a previous study.
Figure S3: Combined dose-response curves of the *T. transversa* FLRFamide receptor to AFLRFamide, DFLRFamide, FLRFamide and DFLRWamide. RLU relative luminescence.

**Terebratalia transversa** FLRFamide prepropeptide sequence

MNRSVLLAVAVASPLDHVSIGNYRTPCSDLTHFCYDLDSEFWPSYGSQEKDMIPKRGGGGEYGEPEGLSSFVDEPYRTGICKRDFLRFCKRRYDDNERSDQALEALNRVTDFLRFCKRDGLFCGSYNLRGYSDNSKRMRRSINKESDKPSSIESDVTNYKGFALLSKSMSAARKDAIKRAFLRFCRLQKEYQQDDKRRAFLRFCNTNGIIRDNPTSDTKEETNSETDNSAKTVTDNHVIDNVDSKRALRFCKKASCKRAFLRFCSEKMNISSSNKSSPLFGEKHTKRAFLRFCKSVRN

**Novocrania anomala** YMRFamide prepropeptide sequence

MAKEEAHALLAVDVIQLMLPSGASOEKEAKMNFPQLRDDLSSKLQQLDDIKVLTAVPDEKRFFDTPTRULSKYRLFRGRGIVDPPYHEPSDAVDGGFDFKHGFNRKHYLRFRGRGIDSNPHGYSNALEAGAMDGFEKNKRYLRFCKSVDYVTPETHEILKRSVKQKTPENGKEISPPKSTIKDKRYMRFCKKSIEDERKRMRFCKKSDDEAQKRRMYRFCKKDESPDE

signal peptide - cleavage site - C-terminal amidation site - predicted FLRFamide like neuropeptides

**Terebratalia GPCR sequences:**

**Terebratalia transversa** FLRFamide receptor (deorphanzied) (R1)

MAAAKEFGIRKRESYFKPYVINDDNLNTTITIRATTGGLPQHDTMTVSMLNCTLPNCTTNNTDNTNGGSPILADSQAQILITFYTLAILAAFNGIALLIIFSTGRSGRDLYTNYNLLALADLSMSVFICPFTFTPIIHYIWHQFGSAMCTIVLFLQTATVVVSVSTNMAIGRFLEVAPLRSRASKQVVVRIIPWNLASPNFVVAQTVDGNYQYCTEKPWKPGKQKLIIFILIFTYIIPLVAlITTYGIAMKLWRQARPGENARAREAQLOQSKRVIKMLFDTVILLFLGFVCLWHTFIVLNLDNPFLAQNAQDQREIAYICFHLMASNFSQPIIIYGFILNDFRADWFLIFVCLFCCSCKAYFKNHRMSYTRSPNARWQSSFSHLERRASARPILNGSTSSSSTDKHKHFNSFARSKTDTLALLVHPESKQRTFKRNGAKRSQPMALVVPVTVYNRSRDNTSVSMKLEGIEDPSTKENSIK

**Terebratalia transversa** orphan luqin GPCR homolog (R2)

FISMPFAITVLSITGNVLCFAVLRNQTMRSSSYYFILNLAVSDILMCAPEMCIPFTFVANVLLLDDWFPGHVMCPVNLFQAMAVFLSAFTLIVSLDRYVAIIFPFLARKKLOQVARQVXYROMHCYQVCEIWTDDNRFVYSLCILGQFFPLFVLVLYTITIIIGVWKKFPGFAFYRQRMKATRKKVMMMVYVIIICLPYHCITIRGVDITDFDYDEEHAPLTWVFVYKLAMNSCNNPIIFYMNKFRSFIFKTECLCCCKRISGRTCQESVKIKRNTYATNTTTEKSSKNGTNTVKTCCSSQRSQRTSGDELAMIDILPV

**Terebratalia transversa** orphan neuropeptide GPCR (R3)

LRHLTPLTILSLVGRHHFLKLKISKIYYWSRCINMSKELSIYEFENGFNRDKNTTPMDAGNDRTYSIGNIIAYTVIISLFGNVLQCVVYRNKLQTVTNNIFAVNAILLSLASSSNILPFTITTTRLDEWVLGFSFVCHFVFIPIMSVVYSTFTTLIALIDHQVIVYLVPLPKITKIYVQVGLVWTLITLALTAFTRYADIIIVHYRVEKCMKTYHPHSLHSITLITISIQYCLPFIISIMYGRKAIKLWSRGPLHGTQELHSHKTKKSMRLIVVVICIFGCLWPNLNYHITDFTKDLTRFHNSKAFACWHLAFSFVGYNPIFYLVNLDAFREKVKIHLCQSFRRDKSSIKHPGRADKKQPSLTTRTRSTIYSSKKVQKTDIIGKISPIDYQGIDMQDFKISKIDPQYTQLMQLQDVPMRDINQLTNAQYTERAYPKAMQDSESIPDERSLIEALRGAPHASEEDLLDIL*

**Terebratalia transversa** GPCR sequences:
**Terebratalia transversa** orphan neuropeptide GPCR, related to P. dumerilii NpY-4 and insect FMRFa receptor (R4)

MTCLRTMLENTSPTGKLSREITNMTAGLTGVQTYGNHSVCGHPDPSTD1IMFOFIANGIIGSIILVGVCVGNLIALIVLRHSMGFTSTYLSALAIFFDTI1LLLCPFLFSPLPITNNITWDSTYID1YPKMLVYPIPIILSQCTIETYTVAPTRQCYC1NWPKNKCLLSRTRQALIVITILILGSV1NYSPRIEFTFYQGCSLHTQVNLK1VPSFEGSDPFTRKVK1YFL1S1VFHPLVFLV1FL1KLLAK1VRSK1QLQ1KASTVKEN11

**Terebratalia GPCR cloning primer:**
[Non-gene specific adapters with restriction enzyme sites are given in brackets]

**R1:**
Forward: [ACAATAGAATCCGGCCACC]ATGCCGCTGCAAAGAGGTTTC
Reverse: [ACAATAGGCGCCGCCC]TAATCCCCAGACAGATGCTACC (includes partial 3' UTR)

**R2:**
Forward: [ACAATAGGATCCGGCCACC]CCATGGTTTCTGCAATCAC
Reverse: [ACAATAGGCGCCGCCC]GGCCTTGTGAATTTCTTGT (includes partial 3' UTR)

**R3:**
Forward: [ACAATAGGATCCGGCCACC]ATGTCAGGAGACTACTGATTACTT
Reverse: [ACAATAGGCGCCGCCC]CTGTGACATGAGATGATGAG (includes partial 3' UTR)

**R4:**
Forward: [ACAATAGGATCCGGCCACC]ATGATGAATACATACCTACTCGG
Reverse: [ACAATAGGCGCCGCCC]CTACACAAGATATTACCTTGGG

**Accession numbers of neuropeptide receptor reference sequences**
[Xboc.ma.tri.15475.1, X.bocki, in transcriptome SRX1343818] [Locus 45236.0_Transcript_10, N.westlandi, in transcriptome SRX1343819] [Msti.ma.tri.15359.1, M.stichopi, in transcriptome SRX1343814] [Msti.ma.tri.31113.1, M.stichopi, in transcriptome SRX1343814] [Msti.ma.tri.31092.1, M.stichopi, in transcriptome SRX1343814] [Msti.ma.tri.51813.1_Transcript_3/0, H.spinulosa, in transcriptome SRX1343820] [AKQ63063.1, P.dumerilii, Luqin receptor, deorphanized] [AKQ63064.1, P.dumerilii, FMRFamide receptor, deorphanized] [H9K8U7_APIME, H.sapiens, RYamide receptor, deorphanized] [F1R3V0_DANRE, D.rerio, enzyme (includes partial 3' UTR)]