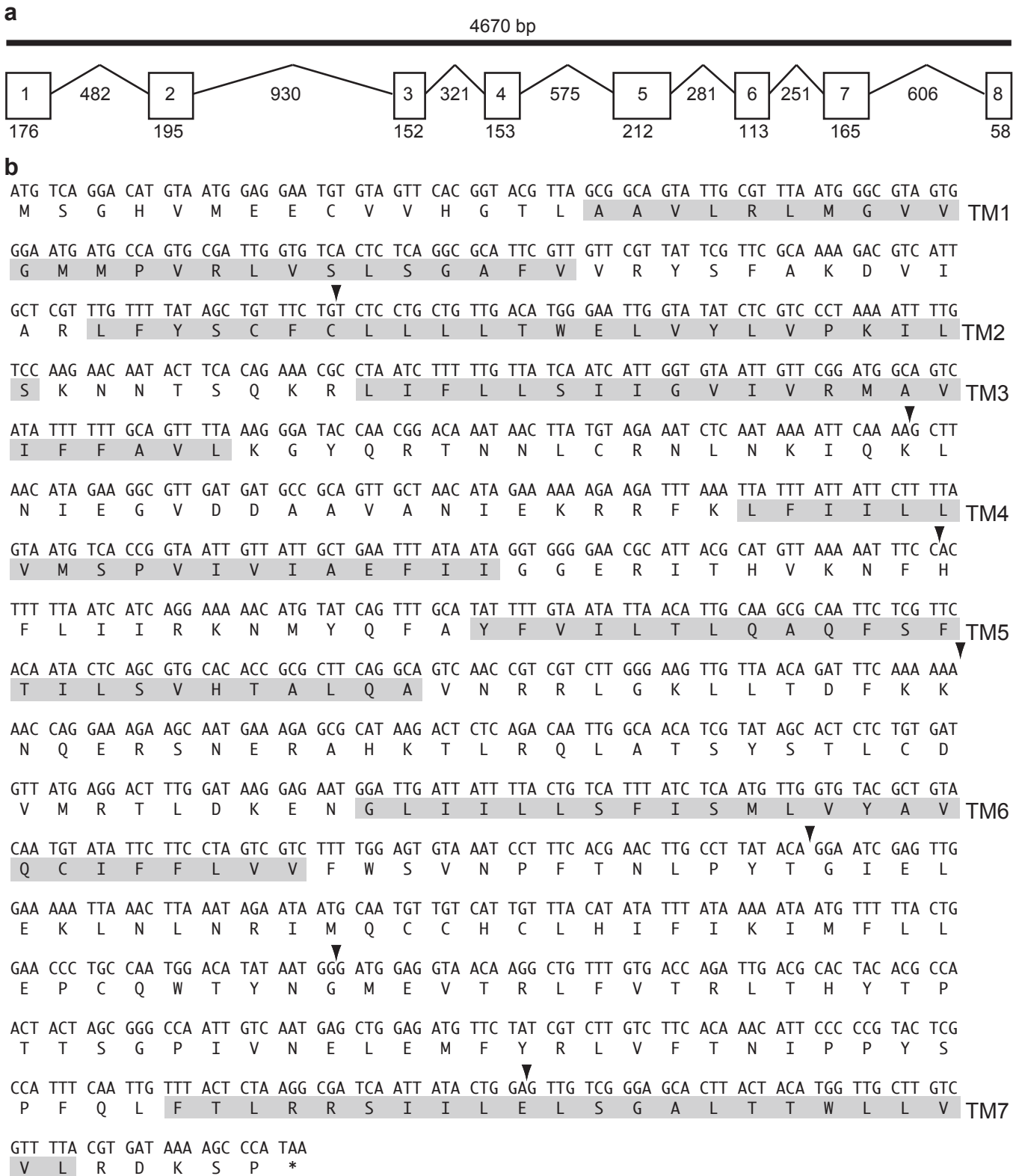
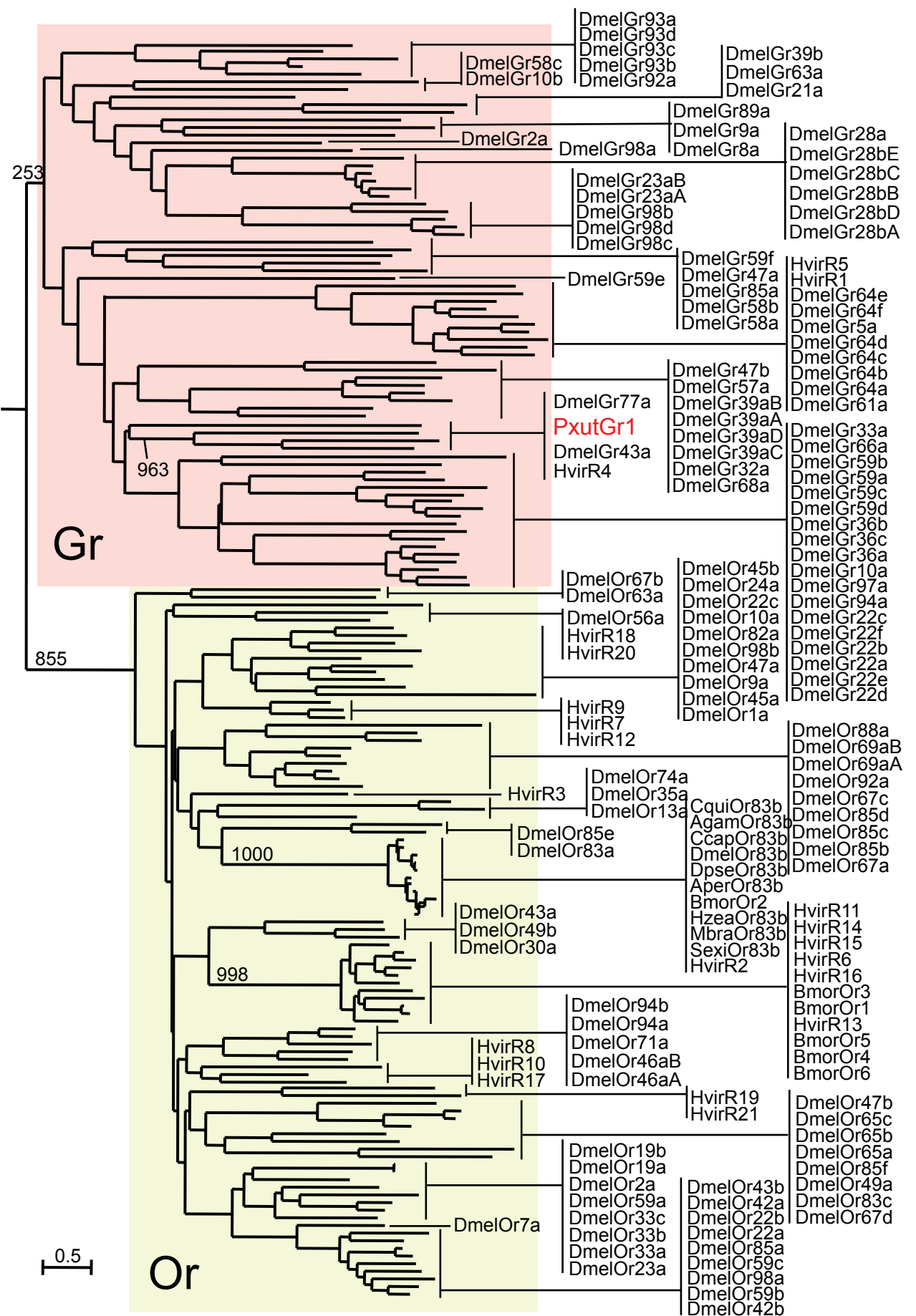
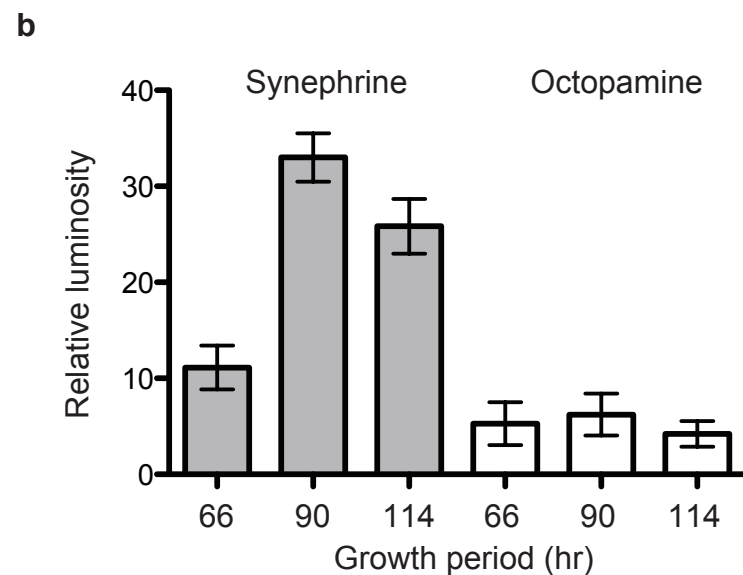
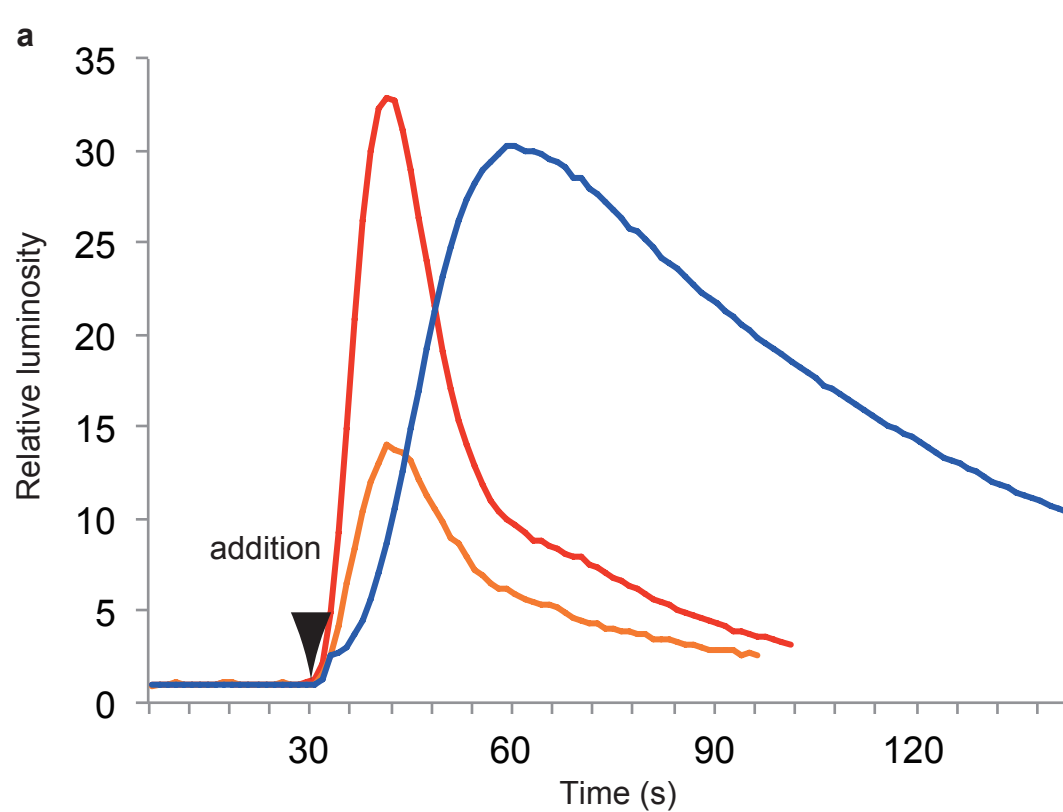




**Supplementary Figure S1: Structure of the *PxutGr1* gene and nucleotide and amino acid sequences of the coding region.**

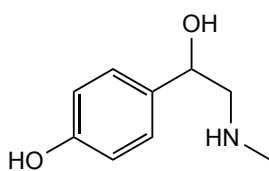
**a:** Schematic representation of the genomic structure of *PxutGr1*. The bold line shows the length in nucleotides from the initiation to the termination codons shown in B. White boxes indicate exon regions 1-8. The numbers of nucleotides (bp) for each exon and intron are denoted. **b:** Nucleotide and amino acid sequences of the *PxutGr1* coding region. Gray boxes (TM1-7) show transmembrane regions predicted by the TMHMM algorithm. Arrowheads indicate intron junctions.

**a**

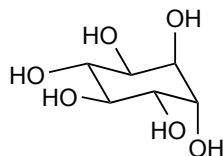


**Supplementary Figure S3: Effect of culture age of Sf9 cells on the response to syneprine and octopamine.**

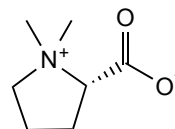
**a:** Sf9 cells were infected with baculovirus containing *Aequorin* and *PxutGr1* for various periods, and stimulated by syneprine as in Methods (orange: 66hr, red: 90hr, blue: 114hr.). The arrow indicates the time of pulse (2 seconds) of syneprine solution (30 mM). **b:** Experiments in (a) are repeated 6-8 times and presented as histograms with error bars. Stimulation by octopamine (30mM) was performed as with syneprine. Error bars represent Standard Deviation.



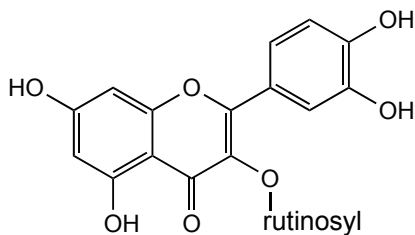
synephrine



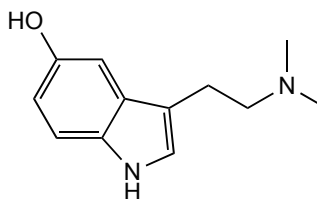
*chiro*-inositol



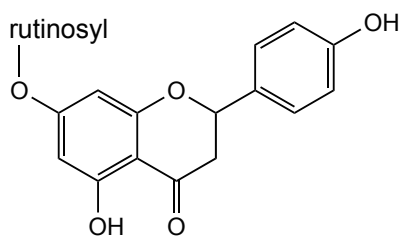
stachydrine



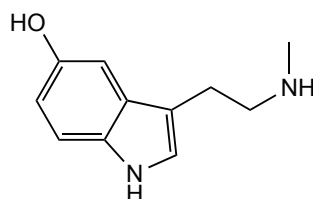
rutin



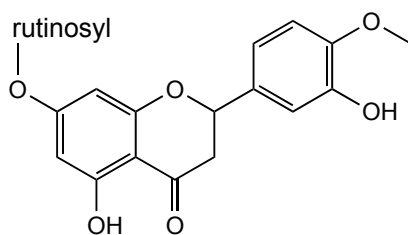
bufotenine



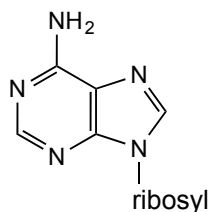
narirutin



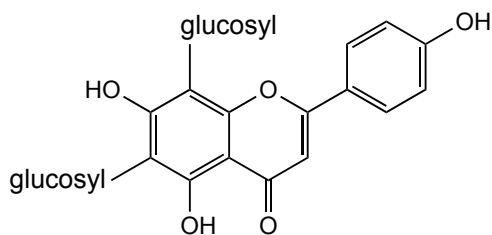
5-hydroxy-*N* $\omega$ -methyltryptamine



hesperidin

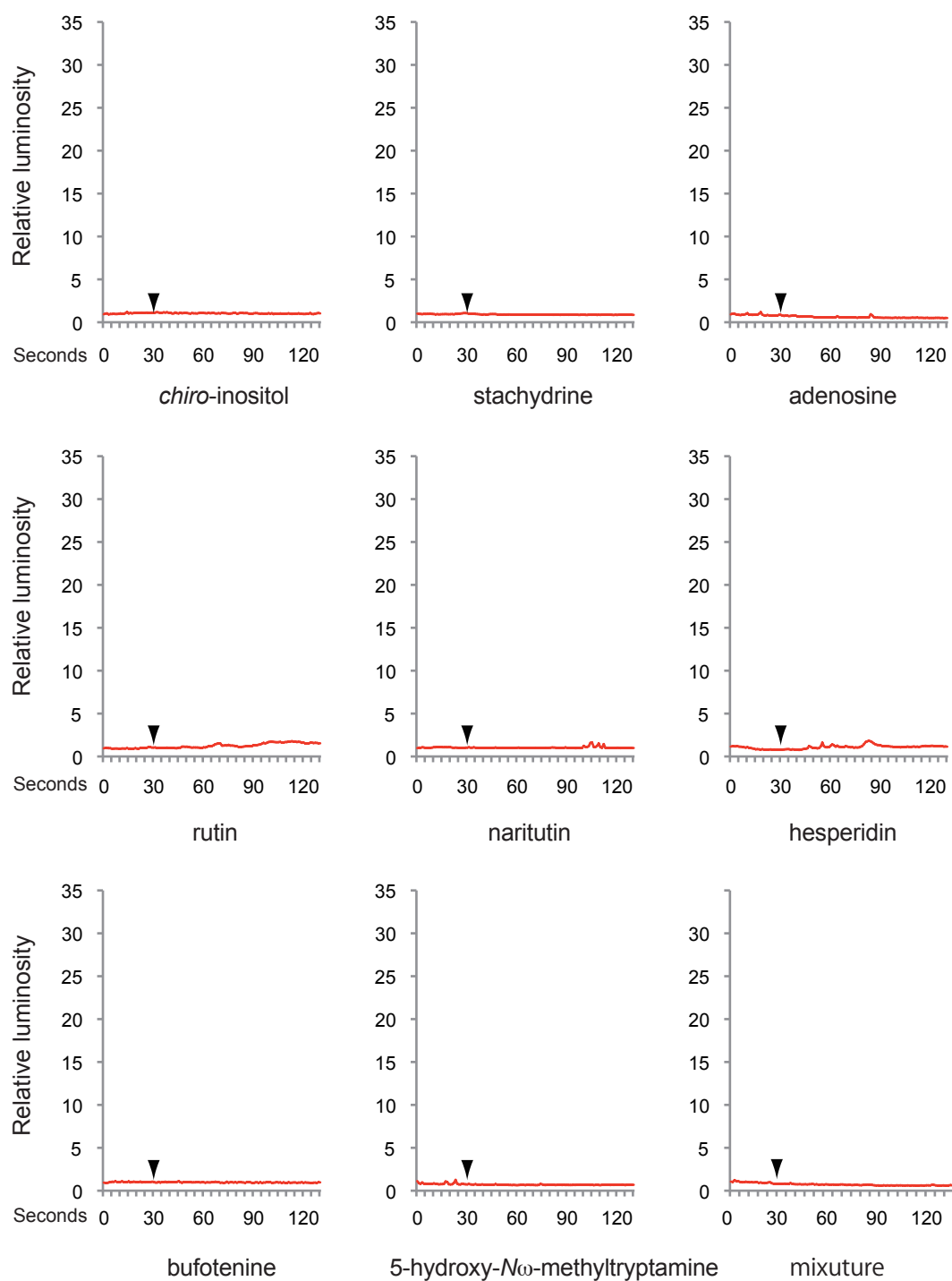


adenosine

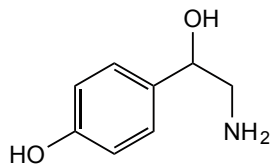


vicenine-2

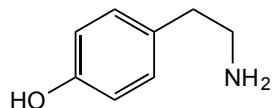
**Supplementary Figure S4: Structure of ten oviposition stimulants.**



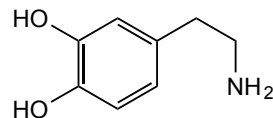
**Supplementary Figure S5: Response of Sf9 (PxutGr1/Aeq) cells to various oviposition stimulants.** The calcium imaging assay was performed as described in Methods using cultures 90 hrs after the viral infection. Arrowheads indicate addition of each compound for 2 seconds. No activity was observed for at least 120 seconds in any compounds tested. Other eight oviposition stimulants other than synephrine. Mixture is the mixture of the 8 compounds in this figure



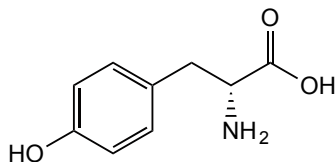
octopamine



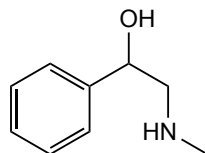
tyramine



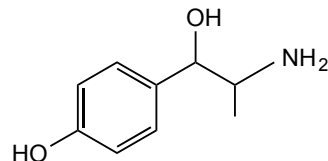
dopamine



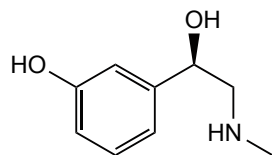
L-tyrosine



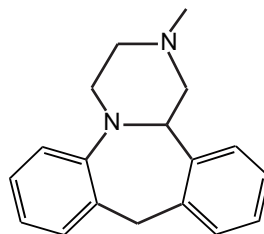
α-(Methylaminomethyl)  
benzyl alcohol



α-(1-Aminoethyl)-4-  
hydroxybenzyl alcohol

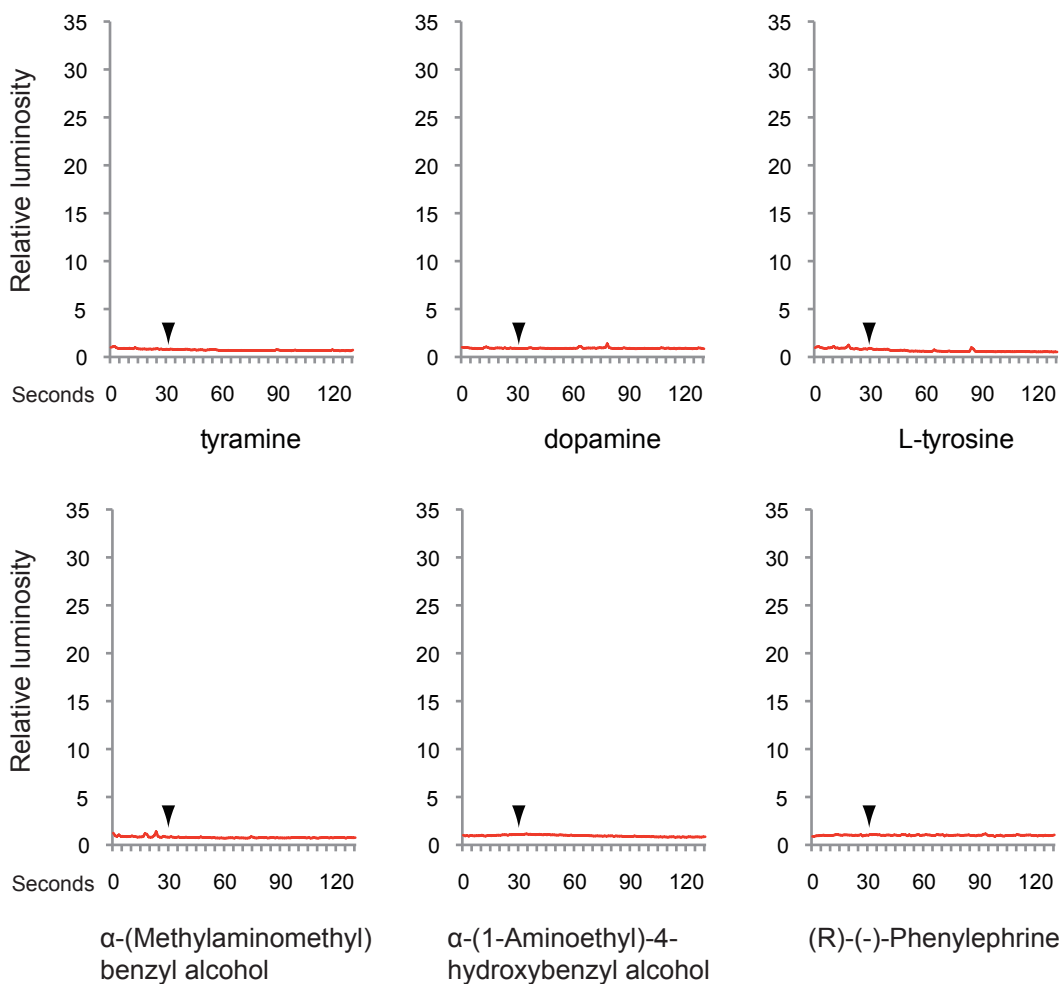


(R)-(-)-Phenylephrine



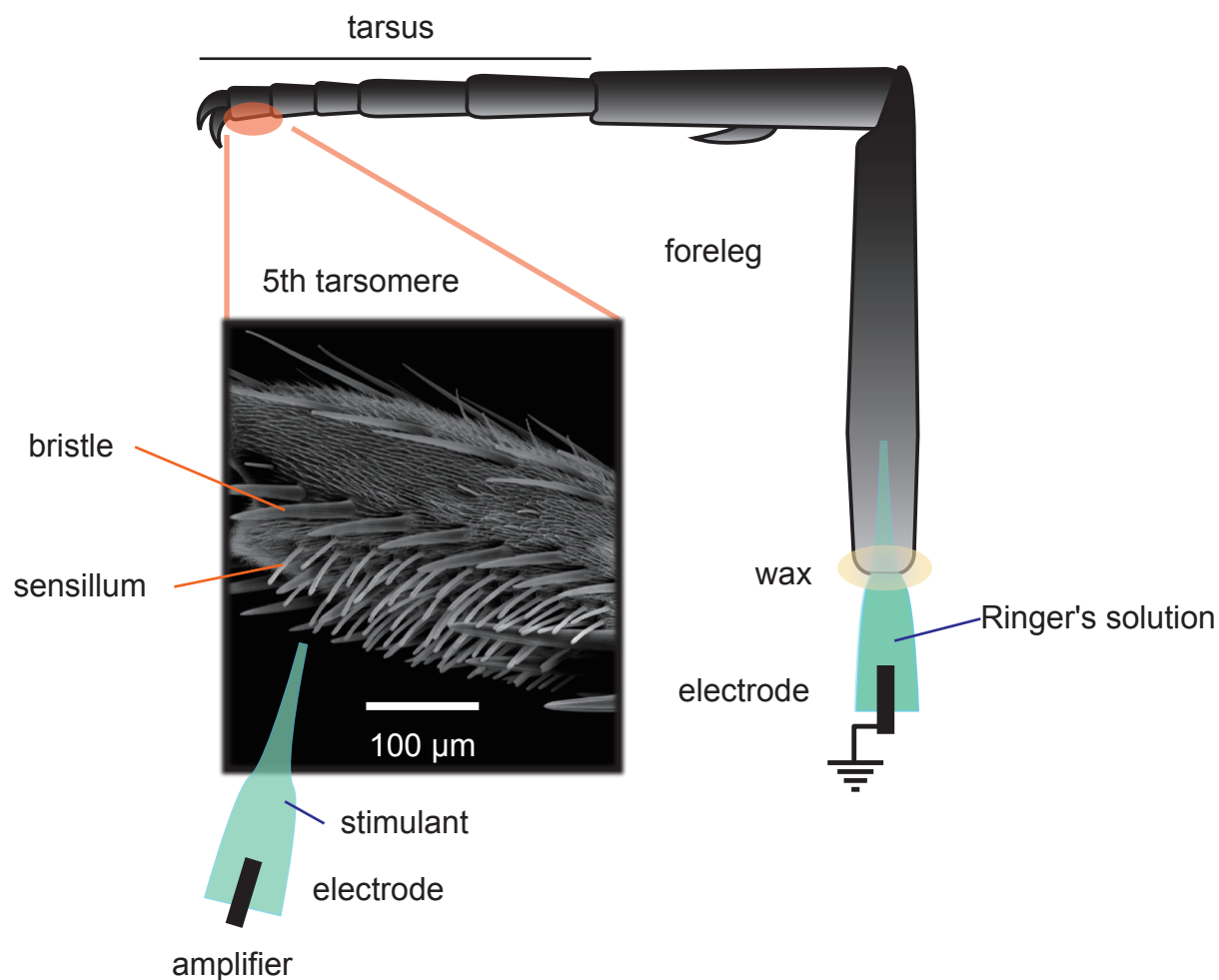
mianserin

**Supplementary Figure S6: Structure of compounds structurally related with synephrine and an antagonist, mianserin.**



**Supplementary Figure S7: Response of Sf9 (PxutGr1/Aeq) cells to various chemicals structurally related to synephrine.**

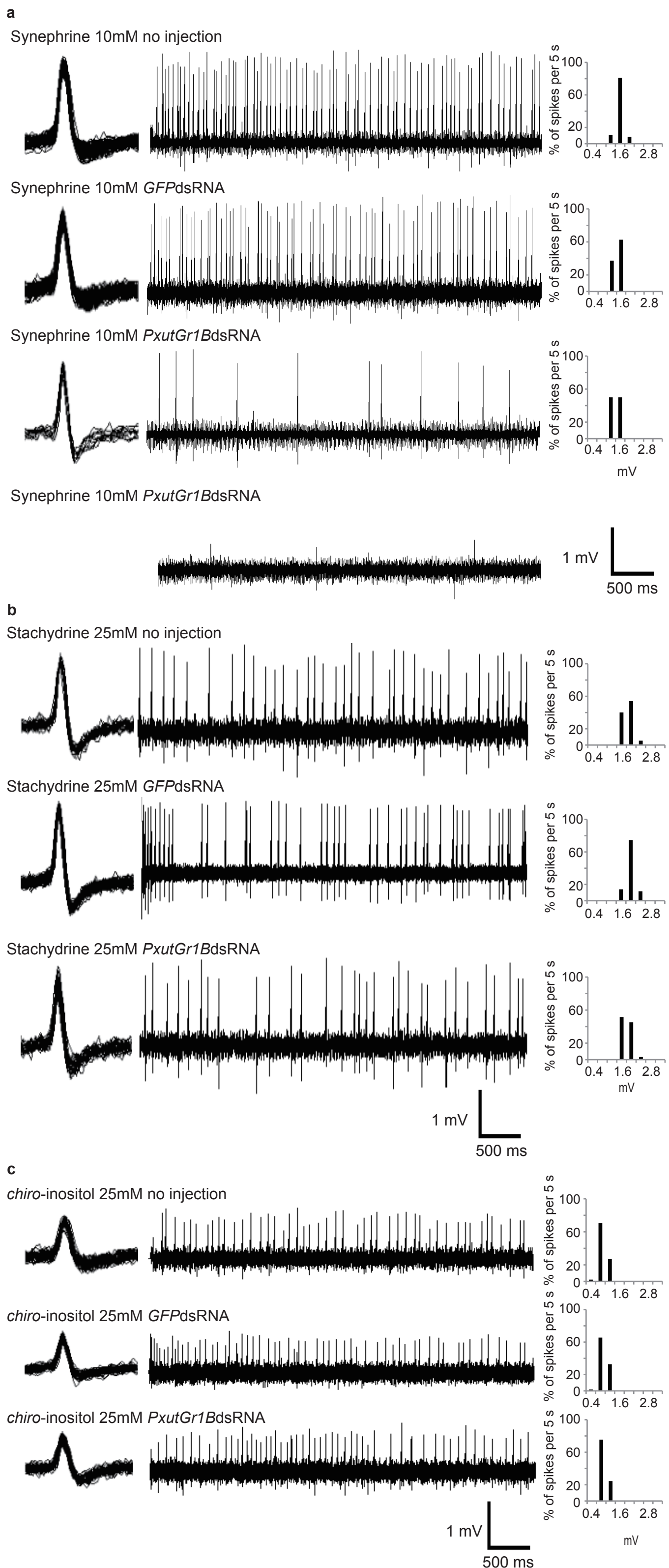
Arrowheads indicate addition of each compound for 2 seconds. No activity was observed for at least 120 seconds in any compounds tested.



**Supplementary Figure S8: A diagram of electrophysiological recording system with a SEM picture of 5th tarsomere.**

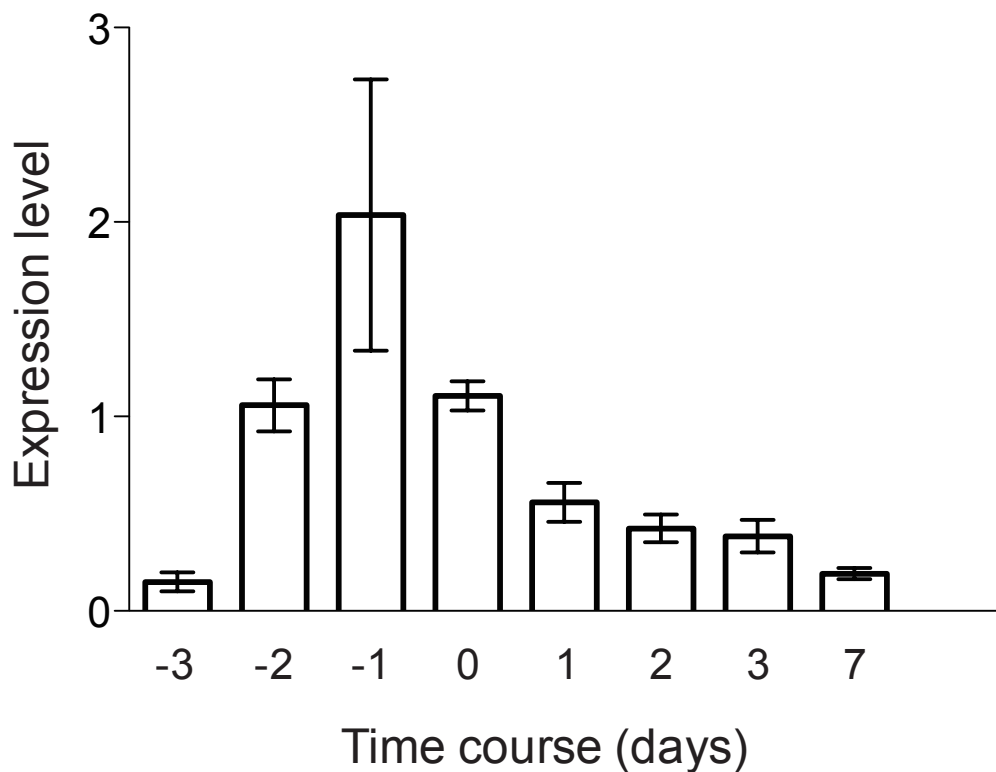
The system is described in Methods.





**Supplementary Figure S9: Recordings of electrophysiological responses to various stimulants: Effects of injection of dsRNA of *PxutGr1* and *GFP* (control).**

The response of each sensillum was determined for 5 seconds and analyzed as described in Methods. **a:** Responses to 10mM syneprhine. No variance was observed in non-injected and *GFPdsRNA* injected individuals. Since the responses by sensillum from *PxutGr1dsRNA* injected individuals were variable from sensillum to sensillum, typical two examples are shown. **b:** Responses to 25mM stachydrine. No effects were observed by injections within the statistic variations. **c:** Responses to 25mM *chiro*-inositol. No effects were observed by injections.



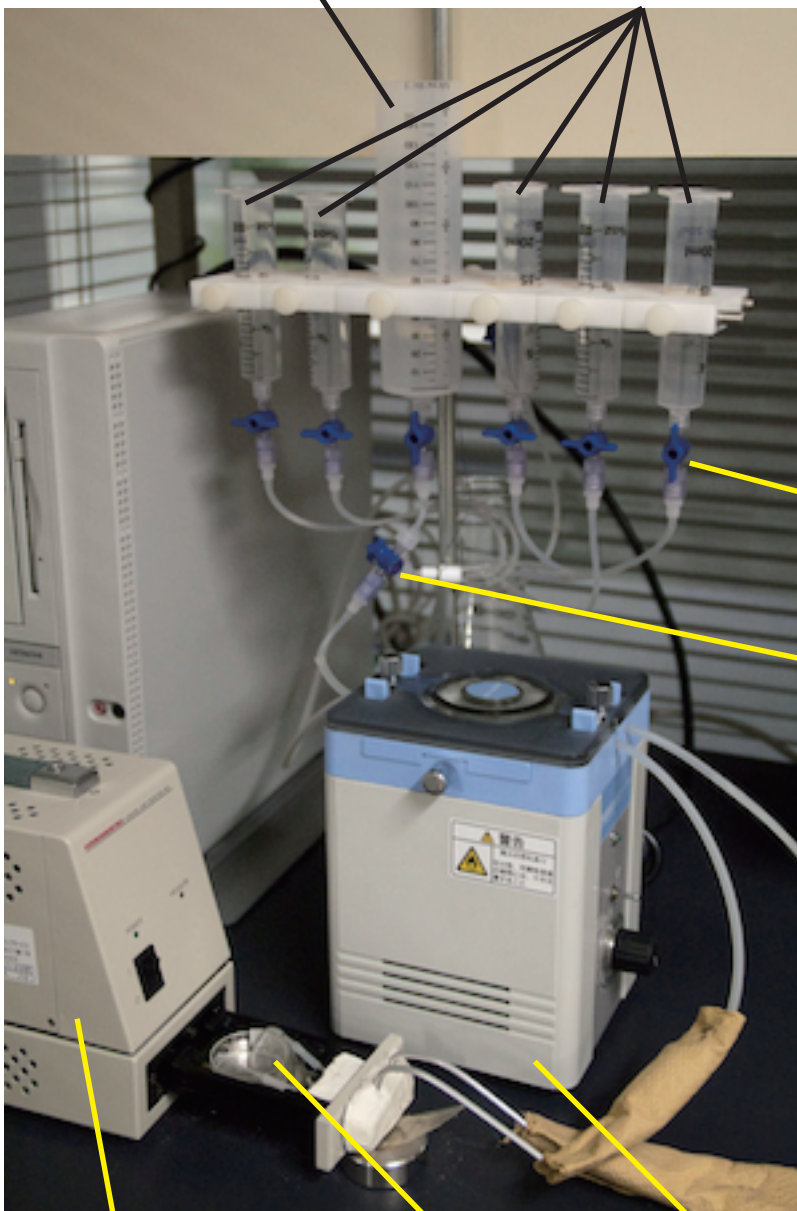
**Supplementary Figure S10: Developmental time course of the expression of *PxutGr1* in pupae and adults.**

RNA was prepared from a single foreleg tarsi cut off from pupae and adults and relative expression levels of *PxutGr1* were measured by quantitative RT-PCR as described in Methods. Each bar represents mean value of relative mRNA expression level with SD error bar from 3-7 experiments. The mean value of day 0 (the day of eclosion) was used as a reference to normalize.

**a**

Vessel for Ringer's solution with or without mianserin

Vessels for compound solutions



Cocks

3-way cock

Low level luminescent detector

Petri dish (Sf9 cells)

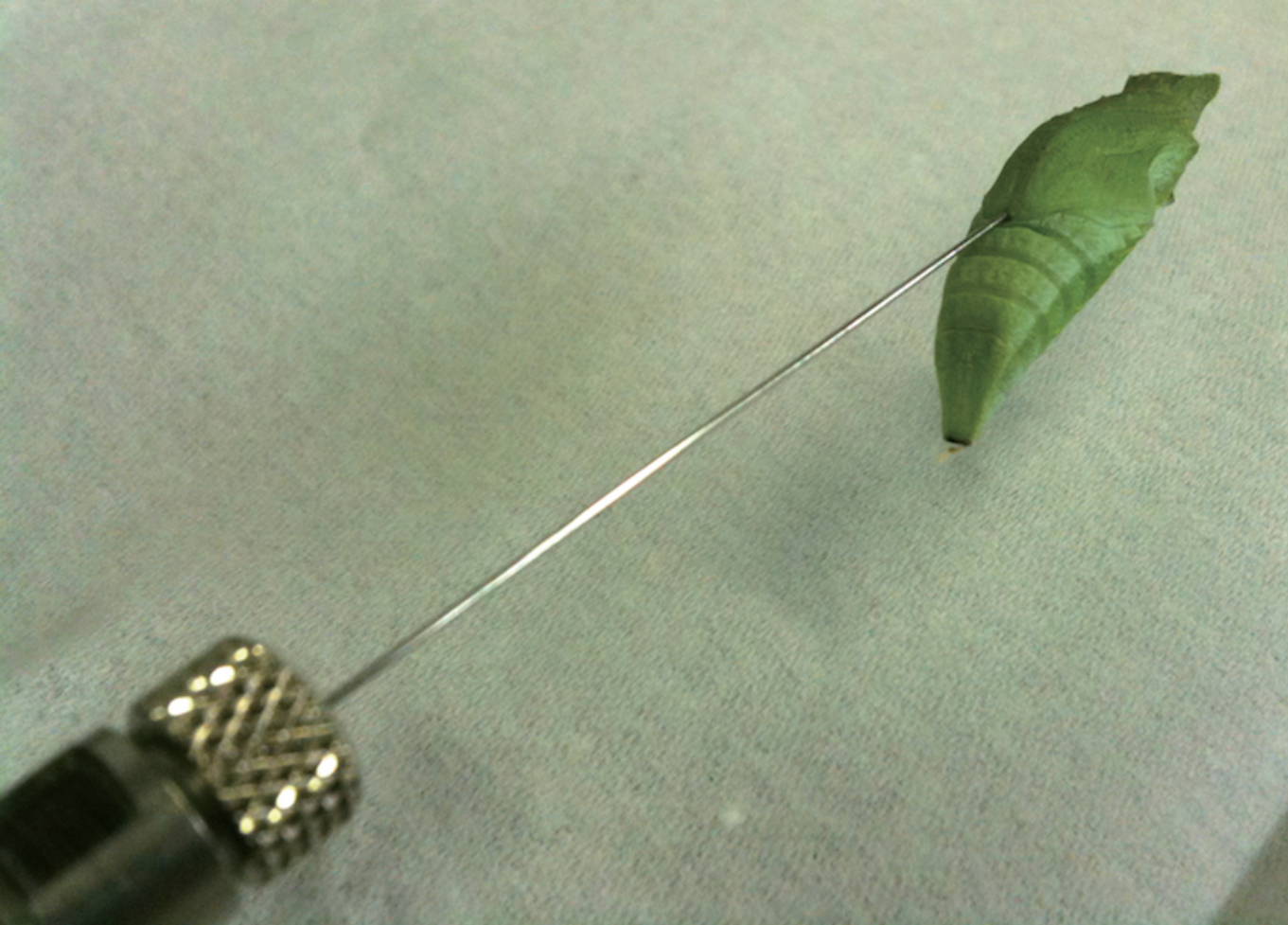
Peristaltic pump

**b**

**Supplementary Figure S11: Instrument used for the calcium imaging assay.**

**a:** An overall picture of the instrument. Parts showing their functions are indicated. **b:** The Petri-dish containing 2ml cell culture and a perfusion device are shown





**Supplementary Figure S12: Injection of water or dsRNA into pupae.**

10 $\mu$ l (1 $\mu$ g/ $\mu$ l) solution was injected using a capillary syringe into the ventral side of female pupae, at the junction between the thorax and abdomen.

| Supplementary Table S1 The list of primers. |                       |                                                                    |
|---------------------------------------------|-----------------------|--------------------------------------------------------------------|
| RT-PCR                                      | PxutGr1 full length   | ATGTCAGGACATGTAATGGAGGAATG                                         |
|                                             |                       | TTATGGGCTTTTATCACGTAAACGAC                                         |
|                                             | Ribosomal protein S9  | TGAAGATCATCGGTGAATATGGTCTC                                         |
|                                             |                       | TCTTCTTCGTCATCGTTAGCAGCAC                                          |
| Baculovirus expression                      | PxutGr1               | GGATCTCTAGACCACCATGTCAGGAC<br>ATGTAATGGAGGAATG                     |
|                                             |                       | GTTTATTAGGCCTTTAGTGGTGGTGGT<br>GGTGGTGTGGGCTTTTATCACGTAAA<br>ACGAC |
|                                             | Aequorin              | GATCCCCGGGCCACCATGACAAGCAA<br>ACAATACTCAGTCAAGCTTACACC             |
|                                             |                       | TTTATTACTAGTTTAGGGGACAGCTCC<br>ACCGTAGAGCTTTTCGCAAGCAGG            |
|                                             | PxutGr1dsA sense      | CAGATCTGAATTCCCACCGGAGGAAT<br>GTGTAGTTCACGGTACG                    |
|                                             |                       | TTATTATCCTCAGGTTTCTATGTTAGC<br>AACTGCGGCATC                        |
|                                             | PxutGr1dsA anti-sense | CTTTTATTACTAGTGGAGGAATGTGTA<br>GTTACAGGTACG                        |
|                                             |                       | CACAGATCCCCGGGCCACCTTTCTAT<br>GTTAGCAACTGCGGCATC                   |
|                                             | PxutGr1dsB sense      | CAGATCTGAATTCCCACCGCAGTTTTA<br>AAGGGATACCAACGGAC                   |
|                                             |                       | TTATTATCCTCAGGCGCTCTTTCATTG<br>CTTCTTTCCTGG                        |
|                                             | PxutGr1dsB anti-sense | CTTTTATTACTAGTGCAGTTTTAAAGG<br>GATACCAACGGAC                       |
|                                             |                       | CACAGATCCCCGGGCCACCCGCTCTT<br>TCATTGCTTCTTTCCTGG                   |
|                                             | PxutGr1dsC sense      | CAGATCTGAATTCCCACCTGGCAACA<br>TCGTATAGCACTCTCTG                    |
|                                             |                       | TTATTATCCTCAGGTAAACAATTGAAA<br>TGGCGAGTACGG                        |
|                                             | PxutGr1dsC anti-sense | CTTTTATTACTAGTTGGCAACATCGTA<br>TAGCACTCTCTG                        |
|                                             |                       | CACAGATCCCCGGGCCACCTAAACAA<br>TTGAAATGGCGAGTACGG                   |
|                                             | GFPds sense           | CAGATCTGAATTCCCACCCATGACTTT<br>TTCAAGTCTGCCATGC                    |
|                                             |                       | TTATTATCCTCAGGACCATGTGGTCTC<br>TCTTTTCGTTTG                        |
|                                             | GFPds anti-sense      | CTTTTATTACTAGTCATGACTTTTTCAA<br>GTCTGCCATGC                        |
|                                             |                       | CACAGATCCCCGGGCCACCACCATGT<br>GGTCTCTCTTTTCGTTTG                   |
| dsRNA preparation                           | PxutGr1 region A      | TAATACGACTCACTATAGGGAGAGCA<br>GTTTTAAAGGGATACCAACGGAC              |
|                                             |                       | TAATACGACTCACTATAGGGAGACGC<br>TCTTTCATTGCTTCTTTCCTGG               |
|                                             | PxutGr1 region B      | TAATACGACTCACTATAGGGAGAGGA<br>GGAATGTGTAGTTCACGGTACG               |
|                                             |                       | TAATACGACTCACTATAGGGAGATTTC<br>TATGTTAGCAACTGCGGCATC               |
| Quantitative RT-PCR                         | PxutGr1               | GACAAGGCTGTTTGTGACC                                                |
|                                             |                       | CACGTAAAACGAGAAGCAACC                                              |
|                                             | ribosomal protein S17 | GTAATAAAATTGCCGGATTTGC                                             |
|                                             |                       | GAAGCCCATTGATGTTGTAAAG                                             |