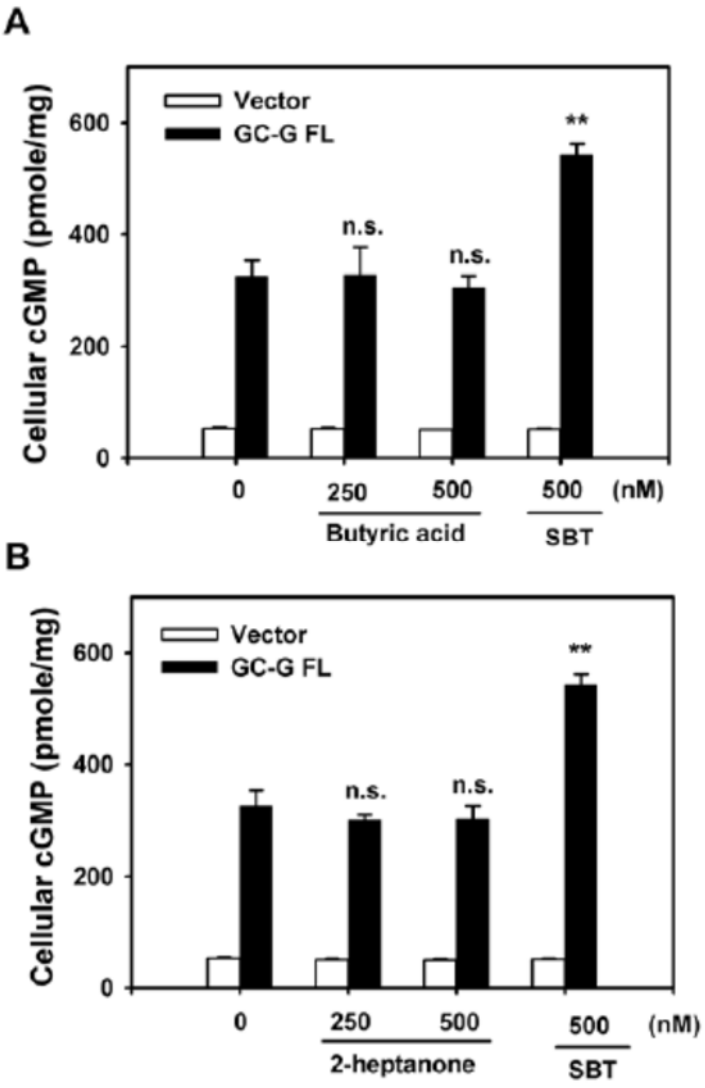
Guanylyl cyclase-G is an alarm pheromone receptor in mice

Ying-Chi Chao, Joerg Fleischer and Ruey-Bing Yang

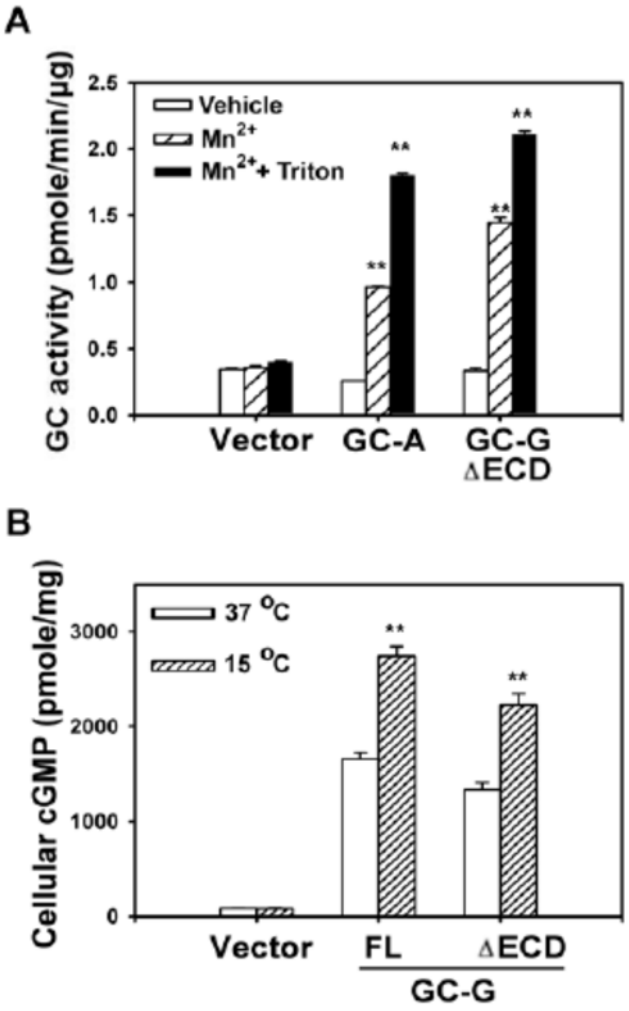
Appendix

Table of Contents



Appendix Figure S1: Butyric acid and 2-heptanone do not activate GC-G enzymatic activity.

(A, B) Two days after transient transfection with an empty vector or a plasmid coding for FLAG-tagged GC-G FL, HEK-293T cells were incubated for 20 min with the indicated concentrations of butyric acid (A), 2-heptanone (B) or SBT (A, B) at 37 °C and cellular cGMP concentration was measured. Data are mean ± SD from three experiments in triplicate. n.s., not significant; **P < 0.01.

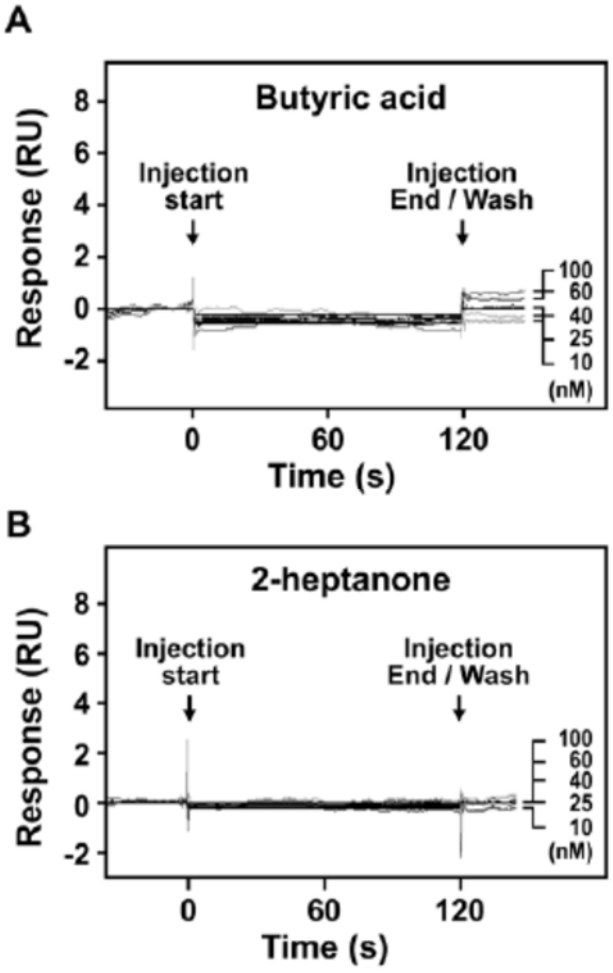


Appendix Figure S2: The ΔECD variant of GC-G is capable of stimulated cGMP synthesis.

(A) GC-G ΔECD has preserved its capacity to be activated by Mn^{2+}. Two days after transient transfection of HEK-293T cells with an empty vector or a plasmid encoding either the (FLAG-tagged) guanylyl cylase subtype GC-A or the (FLAG-tagged) ΔECD variant of GC-G, GC activity in cell membrane fractions was assayed for 20 min at 37 °C in the presence of 5 mM Mn^{2+} or 5 mM Mn^{2+} + 0.1% Triton X-100. All data are mean ± SD from three experiments in triplicate. **P < 0.01.

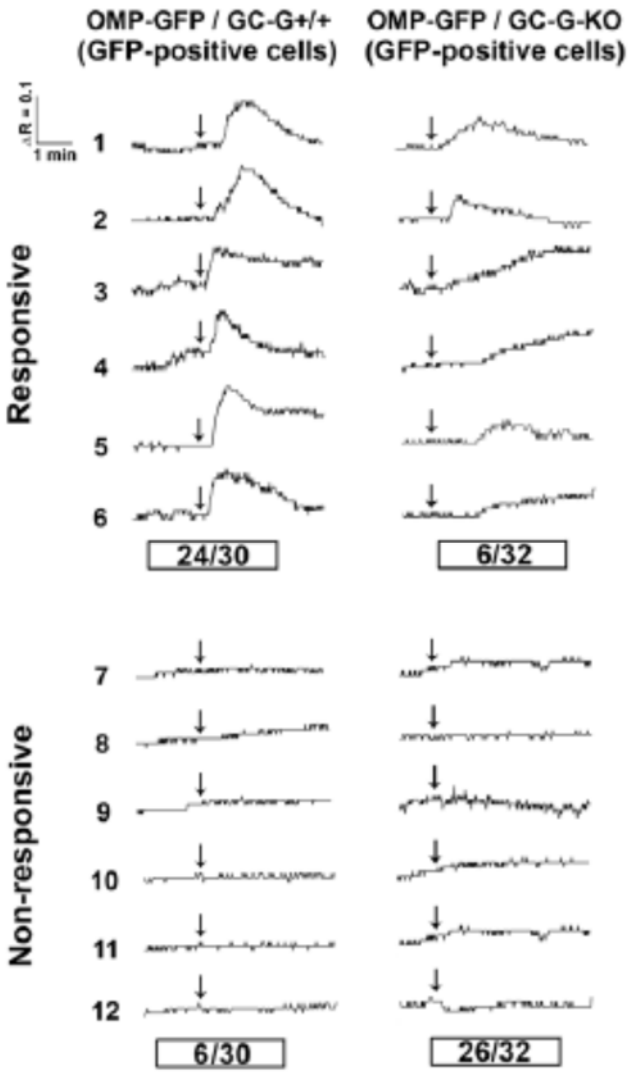
(B) Coolness stimulates intracellular cGMP accumulation in HEK-293T cells expressing the GC-G ΔECD mutant protein. Two days after transfection with an empty

vector or a plasmid coding either for (FLAG-tagged) GC-G FL or (FLAG-tagged) GC-G ΔECD, cells were exposed to the indicated ambient temperatures (37 or 15 °C) for 20 min before cellular cGMP concentration was determined. All data are mean ± SD from three experiments in triplicate. **P < 0.01.



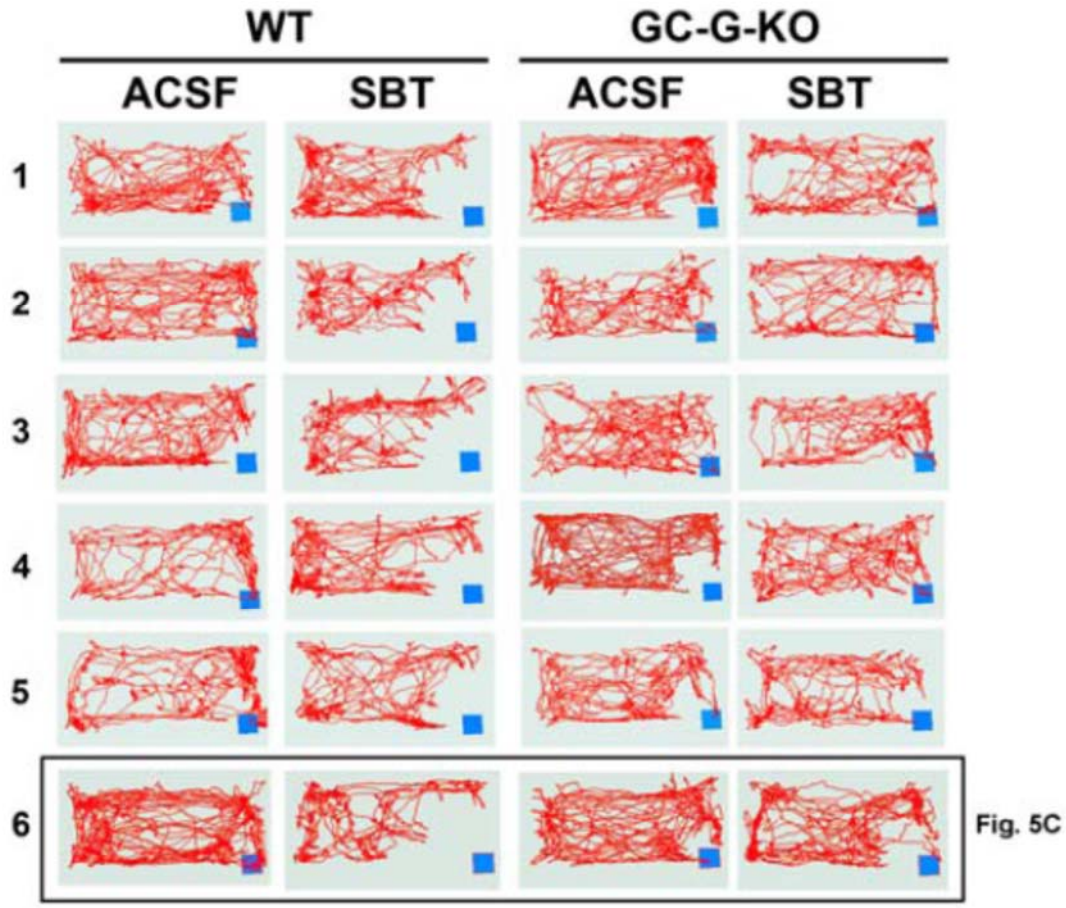
Appendix Figure S3: Butyric acid and 2-heptanone do not bind to the ECD of GC-G.

(A, B) Raw data from surface plasmon resonance (SPR) spectroscopy experiments upon injections with butyric acid (A) or 2-heptanone (B). The butyric acid and 2-heptanone concentrations used in these experiments are given. The black lines represent the global fit of the data to a 1:1 biomolecular interaction model. Responses are indicated in relative units (RU).



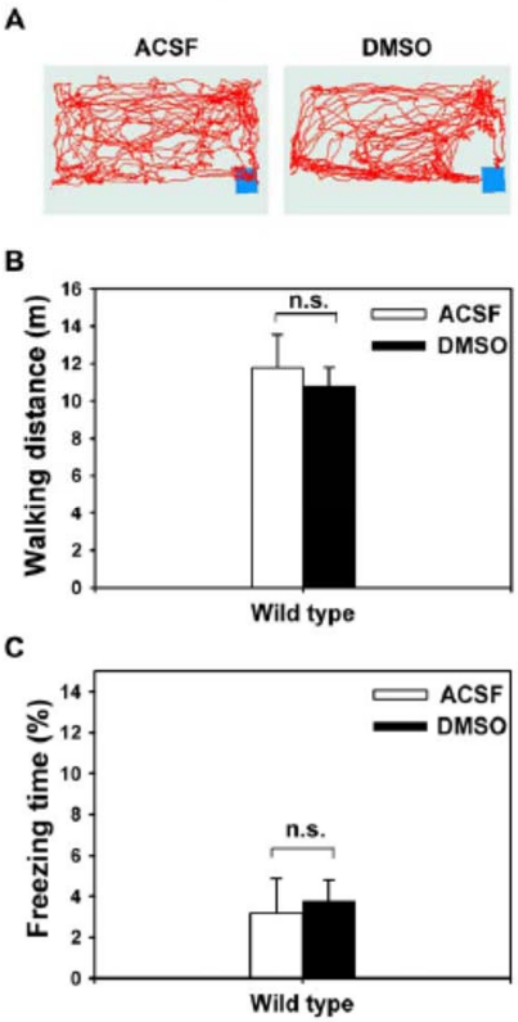
Appendix Figure S4: Representative ratiometric calcium transients following exposure to SBT (1 µM) in GFP-positive GG neurons from OMP-GFP/GC-G$^{+/+}$ and OMP-GFP/GC-G-KO pups.

Six responsive and six non-responsive GG neurons from OMP-GFP/GC-G$^{+/+}$ as well as OMP-GFP/GC-G-KO mouse pups are exemplarily shown (SBT-induced ΔR values greater than 0.05 were considered as responses). The onset of SBT application is indicated by arrows. SBT activated 24 of the 30 tested GFP-positive GG neurons from OMP-GFP/GC-G$^{+/+}$ mice while it only stimulated 6 of the 32 analyzed GFP-expressing GG neurons from OMP-GFP/GC-G-KO animals.



Appendix Figure S5: Walking traces of all WT and GC-G-KO mice used in the avoidance behavior tests.

Traces showing the trajectory of WT or GC-G-KO mice in the testing chamber during a 5-min session in the presence of SBT or ACSF (control). The position of the blotting paper soaked with either SBT or ACSF is indicated by the blue rectangle.



Appendix Figure S6: DMSO does not affect freezing behavior and walking distances.

(A) Examples of video frames tracking the trajectory of male WT mice (8-10 weeks old) exposed to a piece of blotting paper impregnated with 50 µl of 1% DMSO (diluted in ACSF) or 50 µl ACSF buffer (control).

(B-C) Analyses of video tracks revealed that DMSO did not significantly alter walking distance (B) or freezing time (C) as compared to ACSF. For each stimulus (DMSO or ACSF), 3 animals were analyzed.