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Supplementary Materials for

Differential Regulation by Cyclic Nucleotides of the CNGA4 and CNGB1b Subunits in Olfactory Cyclic Nucleotide–Gated Channels

Vasilica Nache, Thomas Zimmer, Nisa Wongsamitkul, Ralf Schmauder, Jana Kusch, Lisa Reinhardt, Wolfgang Bönigk, Reinhard Seifert, Christoph Biskup, Frank Schwede, Klaus Benndorf*

*To whom correspondence should be addressed. E-mail: Klaus.Benndorf@mti.uni-jena.de

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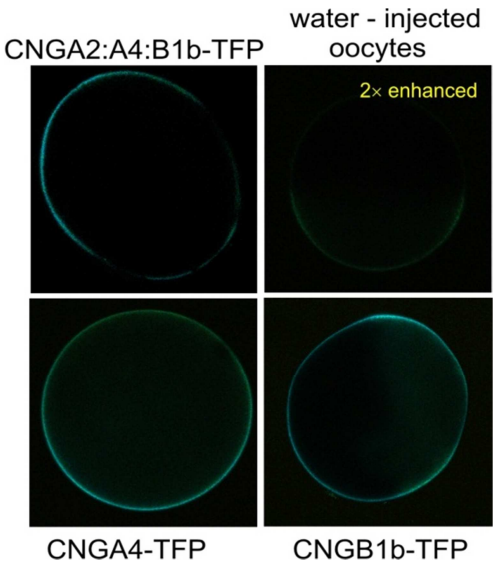
The PDF file includes:

SUPPLEMENTARY MATERIALS

A



B

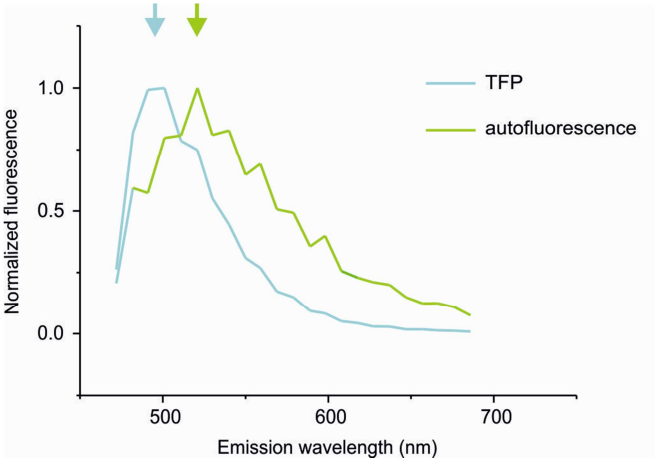


Figure S1. Spectrally coded confocal images of *Xenopus* oocytes injected with RNA encoding TFP-labeled subunits. (A) The circles are optical cross sections of whole *Xenopus* oocytes injected with the respective RNA (CNGA2:A4:B1b-TFP, CNGA4-TFP, CNGB1b-TFP). The teal color is generated by the TFP-labeled subunits, the green color by the autofluorescence. **(B)** Emission spectra of CNGA4-TFP and the autofluorescence arising from the oocyte. The emission spectra of the oocyte's autofluorescence (green) and TFP (teal) overlapped. To separate the signals, spectral imaging, combined with linear unmixing implemented in the confocal microscope software, was applied offline to separate the TFP signal from the autofluorescence.

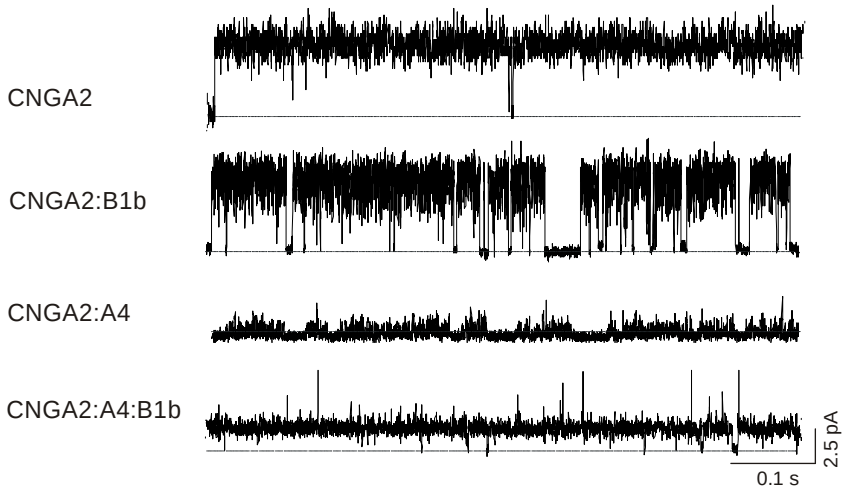


Figure S2. Representative single-channel currents of CNGA2, CNGA2:A4, CNGA2:B1b, and CNGA2:A4:B1b channels. The currents were measured at +100 mV in the presence of a saturating concentration of cGMP. In comparison with the homotetrameric CNGA2 channels (n = 5), both CNGA2:B1b and CNGA2:A4 channels showed a more flickery gating. For CNGA2:B1b channels (n = 4), the openings were shorter and the open channel noise was larger; whereas, for CNGA2:A4 channels (n = 4), the channel activity was not time resolved, resulting in apparently smaller single-channel current. In contrast, the CNGA2:A4:B1b channels (n = 4) showed a small and defined single-channel current amplitude.

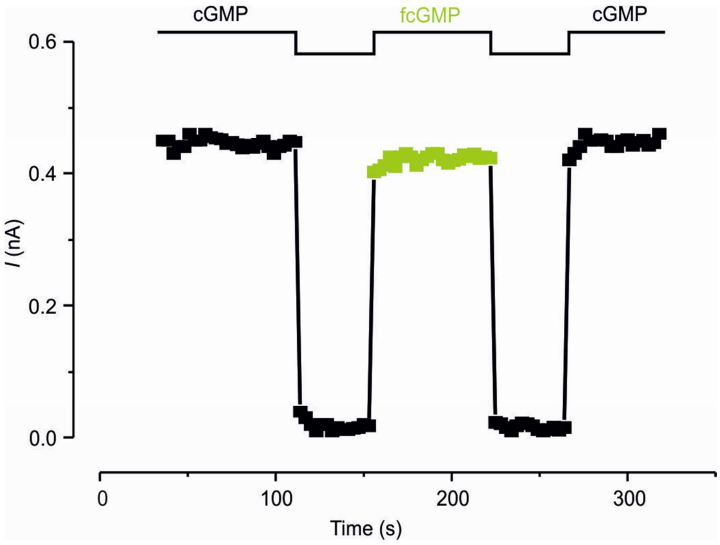


Figure S3. Rapid and reversible activation by fcGMP or cGMP of heterotetrameric olfactory channels. Representative CNGA2:A4:B1b current (*I*) measured in the absence and presence of either 17.5 µM fcGMP or cGMP at +10 mV. The channels are activated by cGMP and fcGMP with similar efficiency.

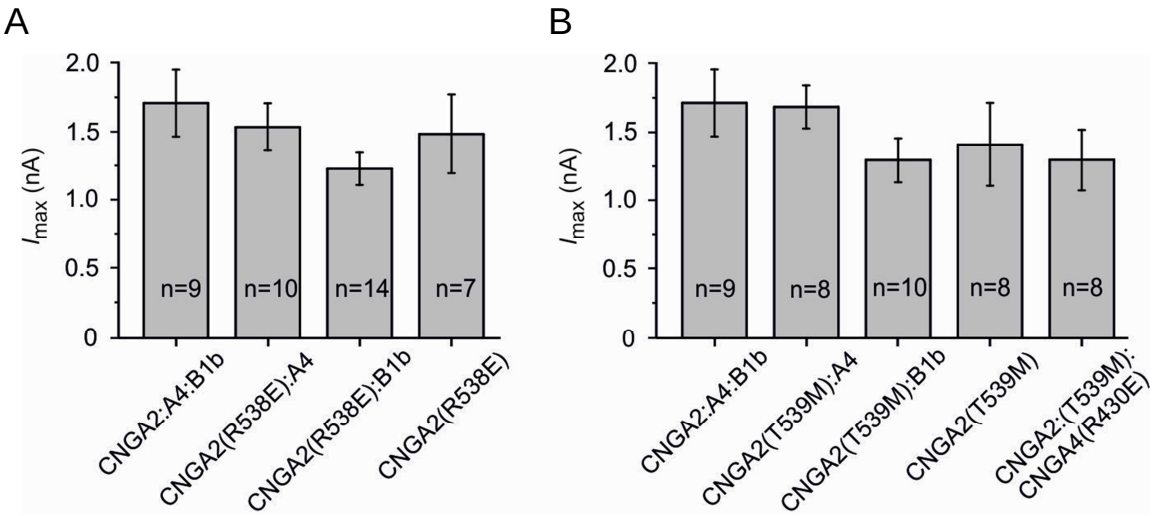


Figure S4. Maximum current generated by the different CNGA2(R538E)- and CNGA2(T539M)-containing channels. The bars indicate the maximal current obtained in the presence of a saturating ligand concentration as follows: (**A**) 100 µM cGMP for the CNGA2 and CNGA2:A4:B1b channels and 3 mM cGMP for the CNGA2(R538E)-containing constructs; (**B**) 25 µM cAMP for the CNGA2(T539M)-containing constructs and 1 mM cAMP for the CNGA2[T539M:A4(R430E)] channels.