Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/11/520/eaah3738/DC1

Supplementary Materials for

cAMPr: A single-wavelength fluorescent sensor for cyclic AMP

Christopher R. Hackley, Esteban O. Mazzoni, Justin Blau*

*Corresponding author. Email: justin.blau@nyu.edu

Published 6 March 2018, *Sci. Signal.* **11**, eaah3738 (2018)
DOI: 10.1126/scisignal.aah3738

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/11/520/eaah3738/DC1)

Movie S1 (.avi format). Time lapse of ES cells expressing cAMPr exposed to forskolin.
Movie S2 (.avi format). Time lapse of ES cells expressing cAMPr exposed to DMSO.
Movie S3 (.avi format). Time lapse of RA-differentiated neurons exposed to forskolin.
Movie S4 (.mov format). Time lapse of SAG-differentiated neurons exposed to forskolin.
Movie S5 (.avi format). Time lapse of SAG-differentiated neurons exposed to dopamine.
Movie S6 (.avi format). Time lapse of RA-differentiated neurons exposed to dopamine.
Movie S7 (.avi format). Time lapse of the response of DNs expressing cAMPr to activation of LN_vs by ATP shown in pseudocolor.

Movie S8 (.avi format). Time lapse of the response of LN_vs expressing cAMPr to activation by ATP shown in pseudocolor.
Movie S9 (.avi format). Time lapse of the response of LN_vs expressing cAMPr and RCaMP1h to carbachol.

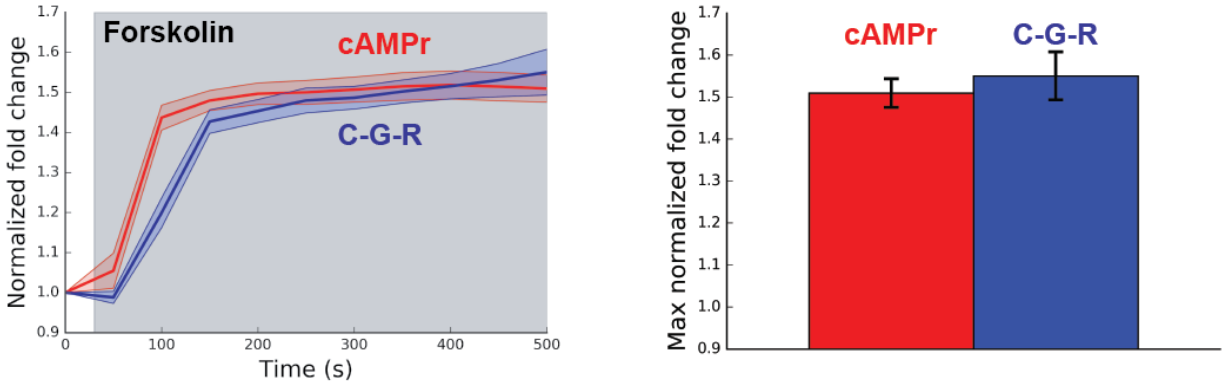

Fig. S1. Comparison of cAMPr and C-G-R in ES cells. Left: Average response of ES cell colonies from two independent experiments expressing cAMPr (red, n = 15 colonies) or C-G-R (blue, n = 13 colonies) to 40 µM forskolin in a 24-well plate. Solid lines represent the average fold-increase in fluorescence with the initial fluorescence value set to 1. Red and blue shading represent the SEM. The left of the gray shaded box indicates when forskolin was added to the wells; the medium in each well was not changed during the experiment. Right: Quantification of the maximal fold-change in cAMPr (red) and C-G-R (blue) fluorescence in response to 40 µM forskolin from the graphs (left). Error bars show the SD at the maximal time point (500 s). A two-sided unpaired *t* test revealed no statistically significant difference in the maximum changes between cAMPr and C-G-R ($P = 0.54$).

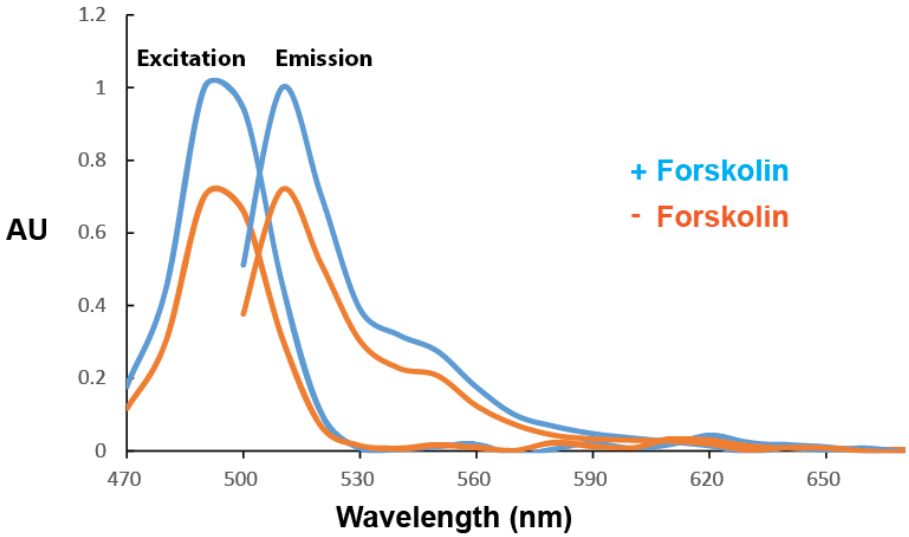

Fig. S2. cAMPr excitation and emission spectra in ES cells. Plots of the excitation and emission spectra collected from ES colonies expressing cAMPr with (blue) and without (orange) the application of 40 µM forskolin. Data are from 18 ES cell colonies. Data were collected on a Leica SP8 microscope using xyλ mode with 10-nm steps for both the excitation and emission measurements.

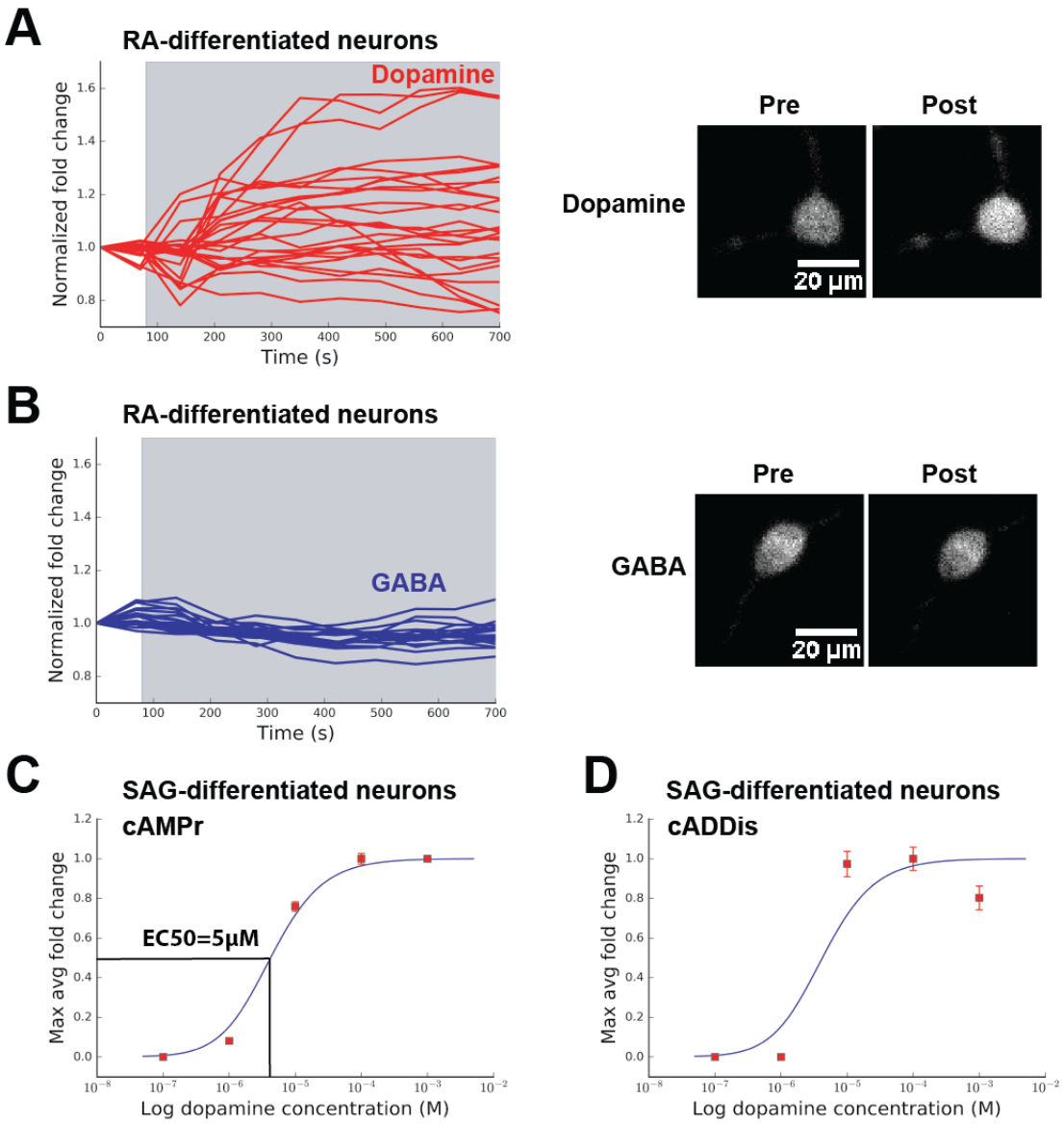

Fig. S3. Responses of RA- and SAG-differentiated neurons to neurotransmitters. (**A**) Left: Normalized fold-change in fluorescence of individual RA-differentiated neurons expressing cAMPr in response to 1 mM dopamine. The graph shows the individual traces of 16 neurons from two independent experiments. Right: Images of one RA-differentiated neuron expressing cAMPr whose fluorescence increased after the addition of 1 mM dopamine. (**B**) Left: Normalized fold-change in fluorescence of individual RA-differentiated neurons expressing cAMPr in response to 1 mM GABA. The graph shows the individual traces of 15 neurons from two independent experiments. Right: Images of one RA-differentiated neuron expressing cAMPr whose fluorescence did not change after the addition of 1 mM GABA. (**C**) Red squares show the average fold-change in cAMPr fluorescence in SAG neurons in response to the indicated concentrations of dopamine. The blue line was generated based on a theoretical response with a Hill coefficient of 1 and an EC_{50} value of 5 µM. Data are from 15 to 20 soma for each treatment. Two sample unpaired *t* tests showed statistical significant *P* values of $P < 0.05$ for all values except 1 mM vs. 100 µM when compared to the next highest value (1 mM vs. 100 µM: $P = 0.90$; 100 vs. 10 µM: $P < 0.05$; 10 vs. 1 µM: $P < 1 \times 10^{-8}$; 1 µM vs. 100 nM: $P < 0.01$). (**D**) Red squares show the average fold-change in cADDis fluorescence in SAG neurons in response to the indicated concentrations of dopamine. The blue line was generated based on a theoretical response with a Hill coefficient of 1 and an EC_{50} value of 5 µM. Data are from 16 to 20 soma for each treatment. Two sample unpaired *t* tests showed no statistically significant *P* values of $P < 0.05$ except for 10 vs. 1 µM when compared to the next highest value (1 mM vs. 100 µM: $P = 0.07$; 100 vs. 10 µM: $P = 0.90$; 10 vs. 1 µM: $P < 1 \times 10^{-7}$; 1 µM vs. 100 nM: $P = 0.75$).

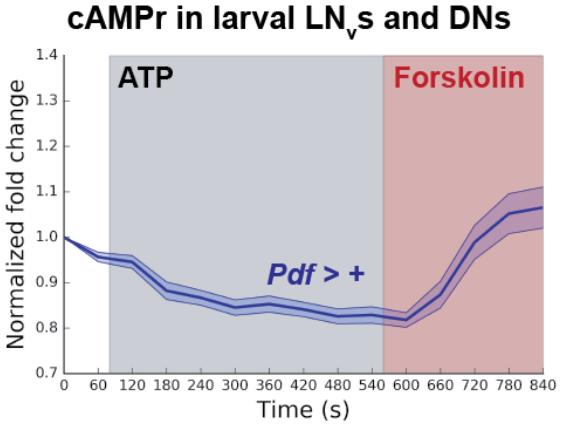

Fig. S4. ATP-treated larval clock neurons respond to forskolin. Graph shows a continuation of the control experiments shown in Fig. 5, D and E. Control larvae in which cAMPr was expressed in all clock neurons, but which did not express $P2X_2$, showed no increases in fluorescence in response to ATP (gray box), but exhibited increased fluorescence after the addition of forskolin (red box). Data are from 11 neurons from three different brains.

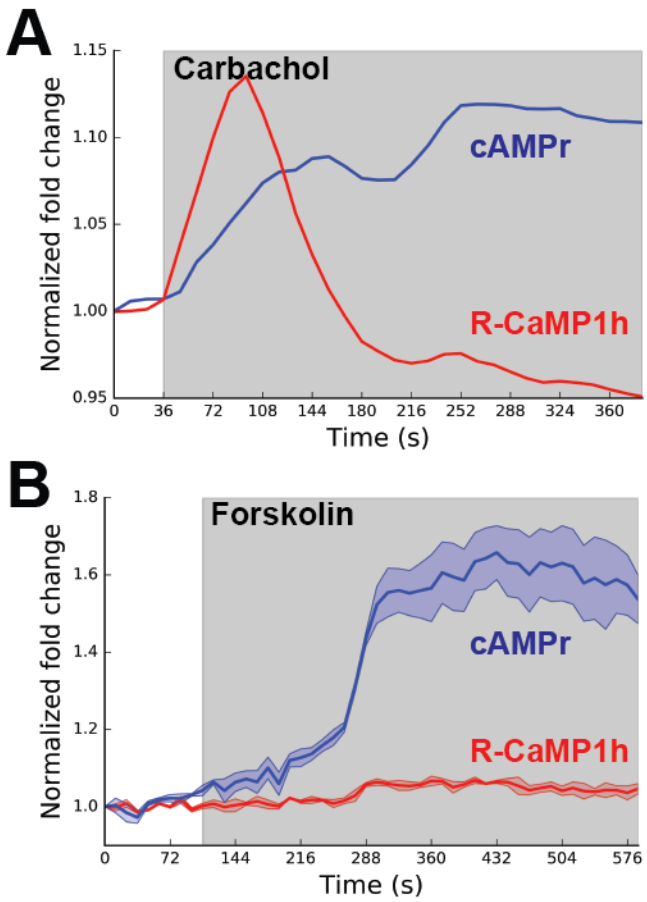

Fig. S5. Two-photon imaging of cAMPr and RCaMP1h in PDF-expressing LN_vs. (**A**) Quantification of fluorescence from cAMPr (blue) and RCaMP1h (red) in the cell bodies of PDF-expressing LN_vs in response to 100 µM carbachol (gray box) as described in Fig. 6, but instead imaged by two-photon excitation. Data are from four neurons from one larval brain. (**B**) Experiments were performed as described for (A) except that the brain was treated with 40 µM forskolin (gray box). Intracellular Ca^{2+} increased 3 to 5% in response to forskolin.

Table S1. Locomotor activity rhythms of adult flies in constant darkness. Locomotor activity of *Pdf-Gal4* and *UAS-cAMPr* parental control flies or flies expressing cAMPr in LN_vs (*Pdf-Gal4 / UAS-cAMPr; Pdf-Gal4 /* +) in complete darkness. A two-sided unpaired *t* test revealed no statistically significant difference between any two genotypes ($P > 0.5$).

Genotype	n rhythmic / total	Avg. period (h) ± SD	Avg. power ± SD
Pdf-Gal4 / +*; Pdf-Gal4 /* +	9/10	23.9 ± 0.2	468 ± 64
UAS-cAMPr / +	16/16	23.7 ± 0.4	438 ± 74
Pdf-Gal4 / UAS-cAMPr; Pdf-Gal4 / +	16/16	23.8 ± 0.2	730 ± 176

Table S2. Primers used for cloning.

Primer name	Primer sequence (5' to 3')
Epac1-F-plox	GCTTGATATCGAATTCCACCAAGCTTATGGTGAGCAAG
Epac1-R-plox	CGGGCTGCAGGAATTCGGCCGCTTACTTGTAC
PkaR_R113A-F	GATGCCGCGTCCTATGTTGCCAAGGTTATACCGAAAG
PkaR_R113A-R	CTTTCGGTATAACCTTGGCAACATAGGACGCGGCATC
PkaR_R226A-F	TGAAACTGTGGGGCATTGACGCTGACAGCTACAGAAGGATCCTC
PkaR_R226A-R	GAGGATCCTTCTGTAGCTGTCAGCGTCAATGCCCCACAGTTTCA
plox-C-G-R-F	GCTTGATATCGAATTCCACCATGGGCAACGCCGCCGCCGCCAAGAAG
plox-C-G-R-R	CGGGCTGCAGGAATTTTACATCTTCCGCTTTCTCAGCG
cpGFP-N4-F	ATACCCGGG**SNNSNNSNNSNN**AACGTCTATATCAAGGCCGAC
cpGFP-N4-R	ATAGCATGC**SNNSNNSNNSNN**GTTGTACTCCAGCTTGTGCC
C-G-R_pTorpe-F	ATATCTAGAGGCAACGCCGCCGCCGCCAAGAA
C-G-R_pTorpe-R	CGCAAGCTT**CTA**AAACTCAGTAAACTCCTTGCC
3'-PkaC-PG-cpGFP-5'-R	GTCGGCCTTGATATAGACGTTCCCGGGAAACTCAGTAAACTCCTTGCCACAC
5'-cpGFP-PG-PkaC-3'-F	GTGTGGCAAGGAGTTTACTGAGTTTCCCGGGAACGTCTATATCAAGGCCGAC
5'-PkaC-flanker-F-II	GAGAGACGCGGGAAGCAG
3'-cpGFP-PkaR-5'-R	TCGCCGCCGGCCCTTGCATGCGTTGTACTCCAGCTTGTGCC
5'-PkaR-cpGFP-3'-F	GGCACAAGCTGGAGTACAACGCATGCAAGGGCCGGCGGCGA
3'-PkaR-flanker-R	GTCTTCAAACTGGACTGGTTCC
Pka-C_Y204A-F	TTGTGTGGGACCCCTGAGGCCTTGGCCCCCGAGATTATC
Pka-C_Y204A-R	ATAATCTCGGGGGCCAAGGCCTCAGGGGTCCCACACAAG
pUASTTB-cAMPr-F	AGGGAATTGGGAATTCAAAATGGGCAACGCCGCCGCCGCCAAGAAG
pUASTTB-cAMPr-R	ATCTGTTAACGAATTTTACATCTTCCGCTTTCTCAGCGTGCTTC
vCHaT_C-G-R-F	TTCATCATGGCGGCCGCAATGGGCAACGCCGCCGCCGCCAAGAAG
vCHaT_C-G-R-R	CGCTCAGCTGGAATTCTTACATCTTCCGCTTTCTCAGCGTGCTTC

Movie S1. Time lapse of ES cells expressing cAMPr exposed to forskolin.
Movie S2. Time lapse of ES cells expressing cAMPr exposed to DMSO.
Movie S3. Time lapse of RA-differentiated neurons exposed to forskolin.
Movie S4. Time lapse of SAG-differentiated neurons exposed to forskolin.
Movie S5. Time lapse of SAG-differentiated neurons exposed to dopamine.
Movie S6. Time lapse of RA-differentiated neurons exposed to dopamine.
Movie S7. Time lapse of the response of DNs expressing cAMPr to activation of LN_vs by ATP shown in pseudocolor.
Movie S8. Time lapse of the response of LN_vs expressing cAMPr to activation by ATP shown in pseudocolor.
Movie S9. Time lapse of the response of LN_vs expressing cAMPr and RCaMP1h to carbachol. cAMPr shown in green and RCaMP1h in red.