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Endocannabinoid 2-arachidonoyl glycerol increases the transcription of *daf-7* in ASI neurons

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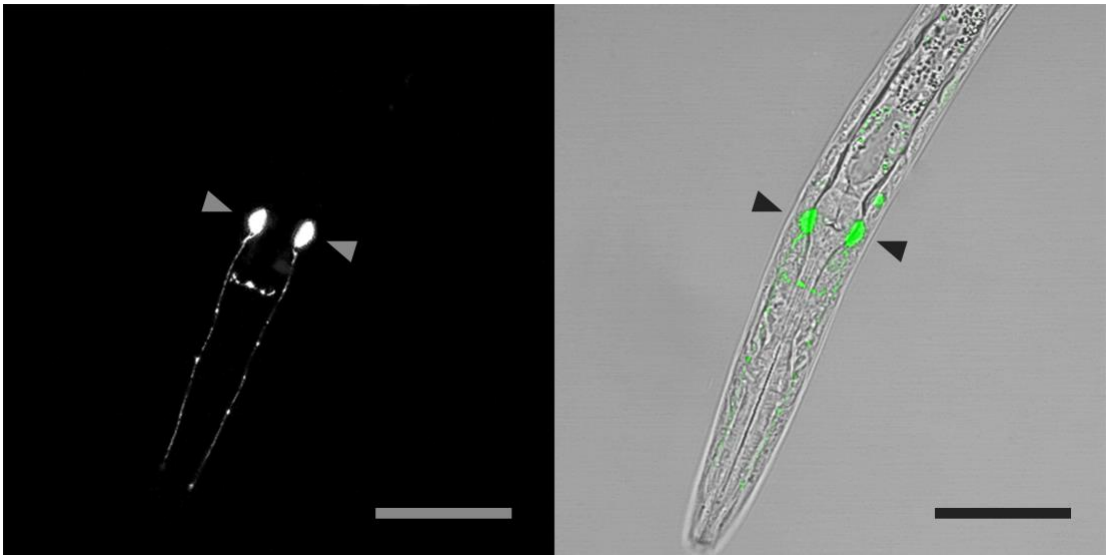

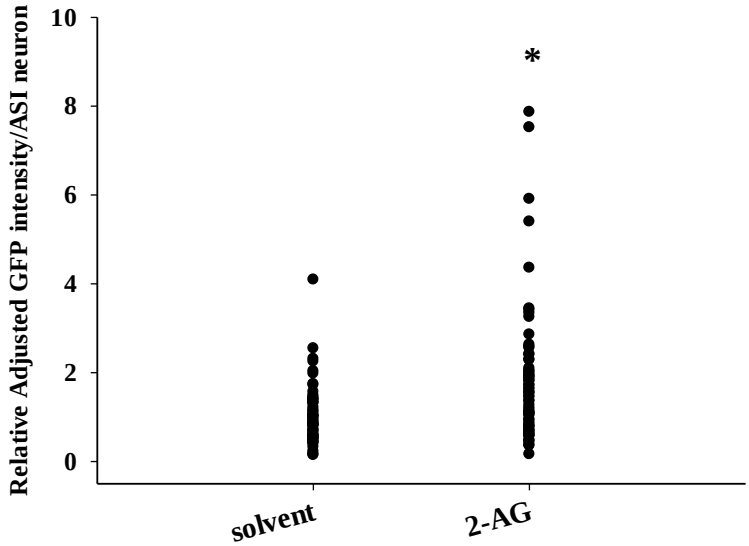

Description

daf-7 expression in ASI neurons is increased when postembryonic developmental stage L1 nematodes are grown in the presence of endocannabinoid 2-arachidonoyl glycerol (2-AG). Left upper panel: representative image of reporter strain FK181 *ksIs2 [Pdaf-7::GFP+rol-6(su1006)]* captured under GFP epifluorescence microscopy. Right upper panel: same individual showing bright field microscopy image together with GFP signal. Gray/black triangles mark ASI neurons expressing *daf-7*. Scale bars correspond to 0.03 mm. Lower panel: point plot of the adjusted GFP intensity/ASI neuron values gathered from worms treated either with the carrier solvent or with 2-AG. 2-AG treatment

significantly increases *daf-7* expression in ASI neurons. (*) indicates statistically significant difference with solvent control condition; the difference in the median values of the relative adjusted GFP fluorescence/ASI neuron between the two groups is greater than would be expected by chance; there is a statistically significant difference between solvent and 2-AG (Mann-Whitney Rank Sum Test, p=<0.001). The number of independent experiments carried out was three. The total number of ASI neurons analyzed was 70 for each condition.

Method
Worms were raised on solid NGM plates supplemented with bacteria and the corresponding supplement for each condition tested. *daf-7* expression was monitored following the protocol described by (Myers, 2012). Briefly, synchronized L1 larvae were fed with *Escherichia coli* HT115(DE3) supplemented with either 2-arachidonoyl glycerol (2-AG) 100 µM or with an equal volume of carrier solvent (acetonitrile) for 1,5 h at 20 ºC. GFP fluorescence was viewed with confocal microscopy in ASI neurons of L1 FK181 animals. L1 images were captured with Zeiss LSM880 scan head on an axio observer Z1 inverted microscope with a 60x 1.4 AN oil immersion objective. A laser line 488 nm of an argon ion laser was used for the excitation, the detection was done in a GaAsP spectral detector with a bandwidth between 508 and 588 nm. GFP intensity in ASI neurons was quantified using NIH Image J software. GFP intensity in each ASI cell body was subtracted from the intensity of a similarly sized background selection to get the adjusted GFP intensity /ASI neuron value.

Reagents
Strain FK181 *ksIs2 [Pdaf-7::GFP+rol-6(su1006)]*
2-arachidonoyl glycerol (Cayman Chemical Company, Item# 62160, CAS# 53847-30-6)
Acetonitrile (Merck, CAS# 75-05-08)

References
Myers, E. M. Gαo and Gαq regulate the expression of *daf-7*, a TGFβ-like gene, in *Caenorhabditis elegans*. PloS One. 2012; 7(7), e40368.PubMed PMID:22808145.

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