Figures and figure supplements

Antinociceptive modulation by the adhesion GPCR CIRL promotes mechanosensory signal discrimination

Sven Dannhäuser et al
Figure 1. Drosophila Cirl is expressed in proprioceptors and nociceptors. (A) The Cirl promoter drives Tomato photoprotein expression (magenta; dCirlpGAL4 > UAS-CD4::tdTomato) in type one larval pentascolopidial ChO (lch5) neurons and type 2 C4da nociceptors, identified by a GFP-ppk promoter fusion (green; ppk-CD4::tdGFP). Magnified view of (B) C4da and (C) ChO neurons. Shown are immunohistochemical stainings against the fluorescent proteins. Scale bars (A) 20 μm, (B,C) 10 μm.
A characteristic nocifensive 'corkscrew' roll of larvae upon mechanical stimulation with a von Frey filament (40 mN force). Quantification of nocifensive behaviour in different genotypes. Increased nocifensive responses were observed in dCirlKO and upon nociceptor-specific expression of an RNAi construct (ppk-GAL4 >UAS-dCirlRNAi). Cirl re-expression rescued the null mutant (dCirlKO ppk-GAL4 >UAS-dCirl) and Cirl overexpression (ppk-GAL4 >UAS-dCirl) reduced nocifensive responses. Raising animals at a higher temperature (29°C vs. 25°C) increases UAS/GAL4-dependent transgene expression (Duffy, 2002). Data are presented as mean and individual values (lower bar plot) and as the difference between means with 95% confidence intervals (upper dot plot). Asterisks denote level of significance, *p≤0.05, **p≤0.01, ***p≤0.001.
Figure 3. Potentiation of nociceptor function by cAMP. (A) Schematic illustration of cAMP production by bPAC. (B) Optogenetic assay. Stimulated and spontaneous nocifensive responses can be promoted and elicited, respectively, by bPAC activation in C4da neurons (blue labels, photostimulation). Larval behaviour was observed during 3 min illumination (~200 μW/mm² at 475 nm) followed by mechanical stimulation (40 mN von Frey filament). (C) Nocifensive behaviour of PDE mutants with ~73% (dunce) and ~35% (dunceML) residual cAMP hydrolysis rates (Davis and Kiger, 1981). (D) The adenylyl cyclase inhibitors SQ22536 and DDA (500 μM) reduce nocifensive responses to comparable levels in control and dCirlKO larvae. Data are presented as mean and individual values. Asterisks denote level of significance, ***p<0.001.
Figure 4. Cirl decreases the excitability of nociceptors. (A) Calcium imaging of C4da axon terminals expressing GCaMP6m (ppk-GAL4 >UAS-GCaMP6m) in semi-intact larval preparations. Representative baseline ($F_0$) and maximum calcium responses ($F_{\text{max}}$) are shown for control and CirlRNAi animals upon von Frey filament stimulation (45 mN). Scale bar, 10 µm. (B) Average calcium traces (arrow indicates stimulation) and quantification of the signals ($\Delta F/F_0$). CirlRNAi significantly elevates mechano-nociceptive responses of C4da neurons. (C) Nocifensive responses (red) elicited via ChR2XXM-mediated photostimulation of C4da neurons in control (ppk-GAL4 >UAS-chop2XXM) and dCirlKO larvae (dCirlKO ppk-GAL4 >UAS-chop2XXM). (D) Structure of the GPS region in Drosophila CIRL (Scholz et al., 2017). The Stachel sequence (light blue) is part of the GAIN domain (blue) contained in the CTF. Conserved, mutated amino acids required for receptor autoproteolysis at the GPS are shown in red ($-2$: dCirlH>A, $+1$: dCirlT>A). (E) Quantification of nocifensive behaviour in dCirlT>A and dCirlH>A receptor mutants. Data are presented as mean and individual values. Asterisks denote level of significance, **p<0.01, ***p<0.001. See also Figure 4—figure supplements 1 and 2.
Figure 4—figure supplement 1. Larval preparation for calcium imaging.
Figure 4—figure supplement 2. CIRL protein expression in mechanical nociceptors. (A,B) The genomic transgene dCirl^{N-RFP} (Scholz et al., 2017) reports low protein expression levels in C4da neurons (arrows). (B) Shown are confocal images of immunohistochemical stainings against RFP (A, black; B, magenta) and the membrane marker anti-HRP (horseradish peroxidase; B, green). Scale bar 25 μm.
Figure 5. Sensitization of nociceptors through chemotherapy-induced neuropathy. (A) Increased nocifensive behaviour following paclitaxel treatment (10 μM) is counteracted by overexpressing Cirl in nociceptors. (B) Example images of C4da neuron morphology upon paclitaxel administration and Cirl overexpression. Scale bars, 100 μm. (C, D) Morphometric quantification of dendritic complexity of C4da neurons in the different genotypes. Data are presented as mean and individual values. Asterisks denote level of significance, *p≤0.05, **p≤0.01, ***p≤0.001.
Figure 6. Neuropathy-induced mechanical allodynia correlates with decreased Cirl1 expression in mammalian non-peptidergic nociceptors. (A) Traumatic injury of the sciatic nerve (CCI, green) in Wistar rats results in mechanical allodynia after one week as measured by von Frey Hairs (paw withdrawal threshold) in comparison to the contralateral side (grey). (B, C) Quantification of Cirl1 (B) and Cirl3 (C) mRNA levels in subpopulations of rat DRG neurons via in situ hybridization (RNAscope). Shown are control conditions (naive DRGs, grey) and one week after injury (green) following the emergence of allodynia. Data are presented as mean and individual values. Asterisks denote level of significance, **p ≤ 0.01, ***p ≤ 0.001. (D, E) Example images of the RNAscope assay in DRG neurons. Shown are projections of confocal stacks stained against IB4 and labelled with probes against Cirl1 and Cirl3 under control conditions (D) and CCI (E). Scale bar 20 μm.
Figure 7. cAMP downregulation by Drosophila CIRL adjusts mechanosensory submodalities in opposite directions. Scheme summarizing how processing of different levels of mechanical force is bidirectionally modulated by CIRL’s downregulation of cAMP production. Whereas low threshold mechanosensory neurons (ChOs; gentle touch) are less responsive in Cirl mutants, high threshold mechanical nociceptors (C4da neurons; harsh touch) become sensitized.

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