Extended Data Figure 5 | Day 1 male mating defects involving spicule coordination, spicule circuit activation in *unc-25*, *unc-97*, and *nrx-1* mutant males, and spicule neuron or muscle activation induces DVB neurite outgrowth. a, Per cent average mating success (sperm transfer) for day 1 and 3 males during 5-min timed mating assays with 15 *unc-31(e928)* hermaphrodites (*n* is number of worms, data points represent average percentage for each replicate of multiple males). **b**, Quantification of attempts at spicule prodding during 5-min timed mating assay for day 1 and 3 males. **c**, Ratio of protraction:prodding attempts during 5-min timed mating assay for males at days 1 and 3. **d**–**f**, Confocal images of *lim-6^{int4}::wCherry* (**d**), total neurite length (**e**), and number or neurite junctions (**f**) of *unc-25(e156)*, *unc-25(e156);*Ex[*gar-3b::ChR2::yfp*], *unc-97(su110)*, *unc-97(su110);*Ex[*gar-3b::ChR2::yfp*], *nrx-1(wy778)*, and *nrx-1(wy778);*Ex[*gar-3b::ChR2::yfp*] males following activation at day 1 (488-nm light for 3 × 15 s every 45 min for 4.5 h). **g**–**i**, Confocal images (**g**) and quantification of total neurite outgrowth (**h**) and number of neurite junctions (**i**) in control, Ex[*unc-103E::ChR2::yfp*], and Ex[*unc-103F^{4}::ChR2::yfp*] worms after activation at day 1 with retinal (488-nm light for 3 × 5 s every 45 min for 4.5 h). **j**, **k**, Quantification of total neurite outgrowth (**j**) and number of neurite junctions (**k**) at day 1 in control, Ex[*lim-6^{int4}::ChR2::yfp*] (DVB), Ex[*unc-103E::ChR2::yfp*] (neuron-specific), and Ex[*unc-103F^{4}::ChR2::yfp*] (muscle-specific) males after activation but in the absence of retinal. **l**, Time to protraction of control and Ex[*lim-6^{int4}::ChR2::yfp*] males after day 1 activation in the absence of retinal. Dot represents one worm; magenta bar, median; boxes, quartiles; one-way ANOVA and post-hoc Tukey HSD, *P* values shown above plots, bold shows significance ($P < 0.05$), scale bars, 10 μm.

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