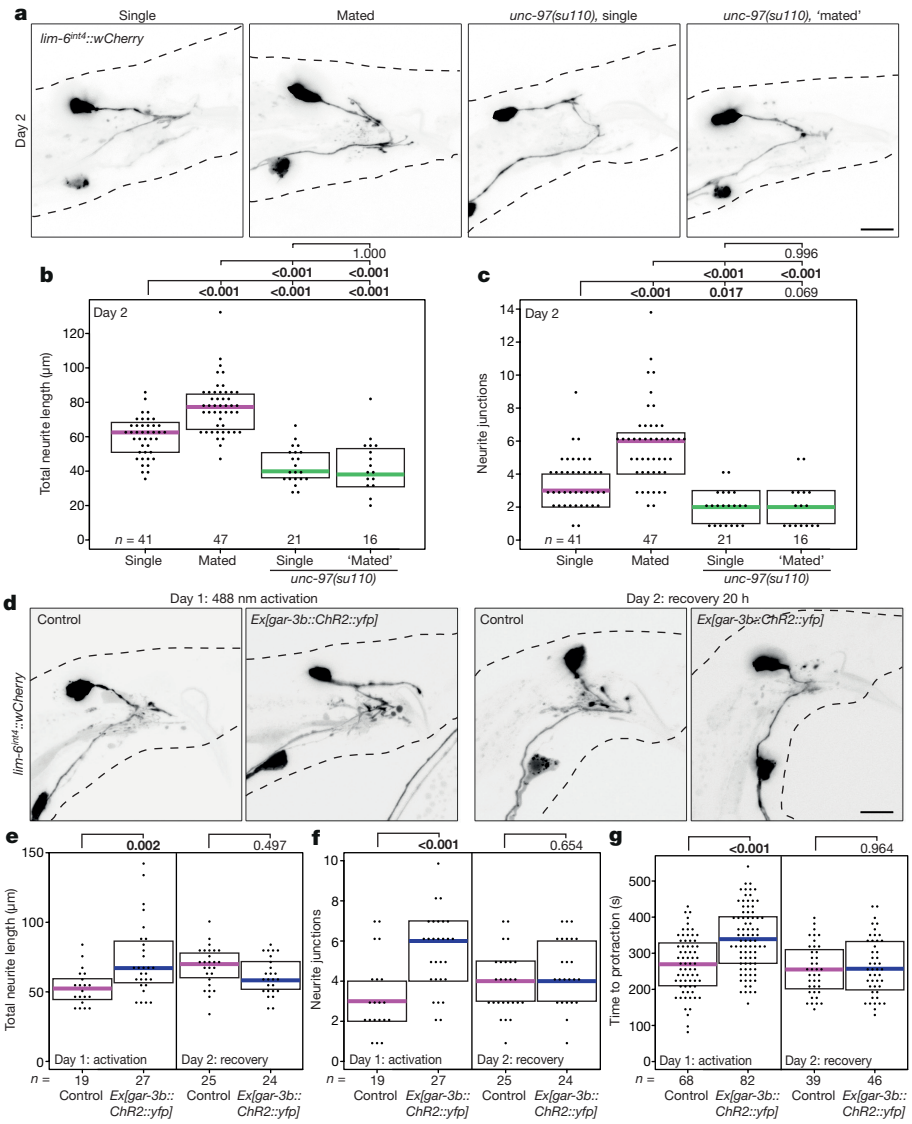

Figure 3 | DVB neurite outgrowth is experience-dependent, can be driven by circuit activity, and affects behaviour. **a**–**c**, Confocal images of *lim-6*^{int4}*::wCherry* (**a**), total neurite length (**b**), and number or neurite junctions (**c**) of males housed by themselves (single), with hermaphrodites (mated), *unc-97* mutant males housed by themselves (single), or with hermaphrodites ('mated') after 48 h. Controls and males expressing channelrhodopsin (Ex[*gar-3b::ChR2::yfp*]) activated at day 1 (488 nm light for 3 × 15 s every 45 min for 4.5 h) or recovered for 20 h (day 2). **d**–**g**, Confocal images of DVB (**d**; *lim-6*^{int4}*::wCherry*; scale bar,10 µm), and quantification of total neurite outgrowth (**e**), number of neurite junctions (**f**) and time to spicule protraction on aldicarb (**g**). 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 ($P < 0.05$).

mediated activation of postsynaptic DVB targets (spicule neurons and muscle) resulted in immediate protraction of spicules[16] (Fig. 2b, Supplementary Video 2). Repeated activation of the spicule protraction circuit caused a significant increase in DVB neurite length and junctions ($P = 0.002$ and $P < 0.001$, respectively; Fig. 3d–f, day 1), independent of GABA signalling (Extended Data Fig. 5d–f). Males exposed to repeated activation, but subsequently allowed to recover, had DVB neurites that were indistinguishable from those of controls, suggesting that neurite growth is dynamic and potentially reversible (Fig. 3d–f). Repeated activation of either spicule neurons or muscles separately demonstrated that activity in either can induce DVB neurite growth (Extended Data Fig. 5g–i).

We next tested whether activity-induced DVB neurites influence DVB neuron function and worm behaviour. We activated and recovered males in the same manner as above, and then used the aldicarb assay to analyse spicule protraction behaviour. Males at day 1 following repeated activation of the spicule protraction circuit showed a significant delay in the time to aldicarb-induced protraction ($P < 0.001$; Fig. 3g, day 1), implying that activity-induced neurites have a direct and immediate effect on DVB spicule function. Males that were exposed to repeated activation of the spicule protraction circuit but allowed to recover had spicule protraction indistinguishable from that of day 2 controls (Fig. 3g, day 2), indicating that induced behavioural changes are dynamic and repeated activation does not result in lasting protraction defects.

To test whether a reduction in circuit activity affects DVB neurites, we exposed males to exogenous GABA, expecting to silence the targets of GABAergic DVB signalling. This resulted in a reduction in DVB neurites (Extended Data Fig. 6a–c). To implicate spicule circuit inhibition more specifically, we silenced spicule protraction neurons and muscles with a histamine-gated chloride channel in day 5 males; this also reduced DVB neurites (Extended Data Fig. 6d–f). In summary, DVB neurites extend in response to the activity levels of the spicule protraction circuit, including postsynaptic targets of DVB.

Neurexin and neuroligin control DVB plasticity

DVB neurite outgrowth appears to be a form of morphological and functional plasticity that fine-tunes the excitatory and inhibitory balance for coordinated spicule protraction. Several synaptic molecules have been implicated in excitatory and inhibitory balance, including the synaptic adhesion molecule neurexin and its trans-synaptic binding partner neuroligin[24–27]. Males with a deletion allele of the single *C. elegans* neuroligin orthologue *nlg-1* show increased DVB neurite outgrowth at day 3 compared to controls (Fig. 4a–c). The increase in DVB neurite outgrowth at day 3 was rescued by GFP-tagged NLG-1 expressed under its own promoter (Extended Data Fig. 7a–c), which was localized in a punctate pattern in numerous neurons and muscles of the male tail (Extended Data Fig. 8). *nlg-1* mutants displayed a spicule protraction phenotype that matches the expected phenotypes observed upon increased DVB branching (Fig. 4d). Expression of NLG-1 in the