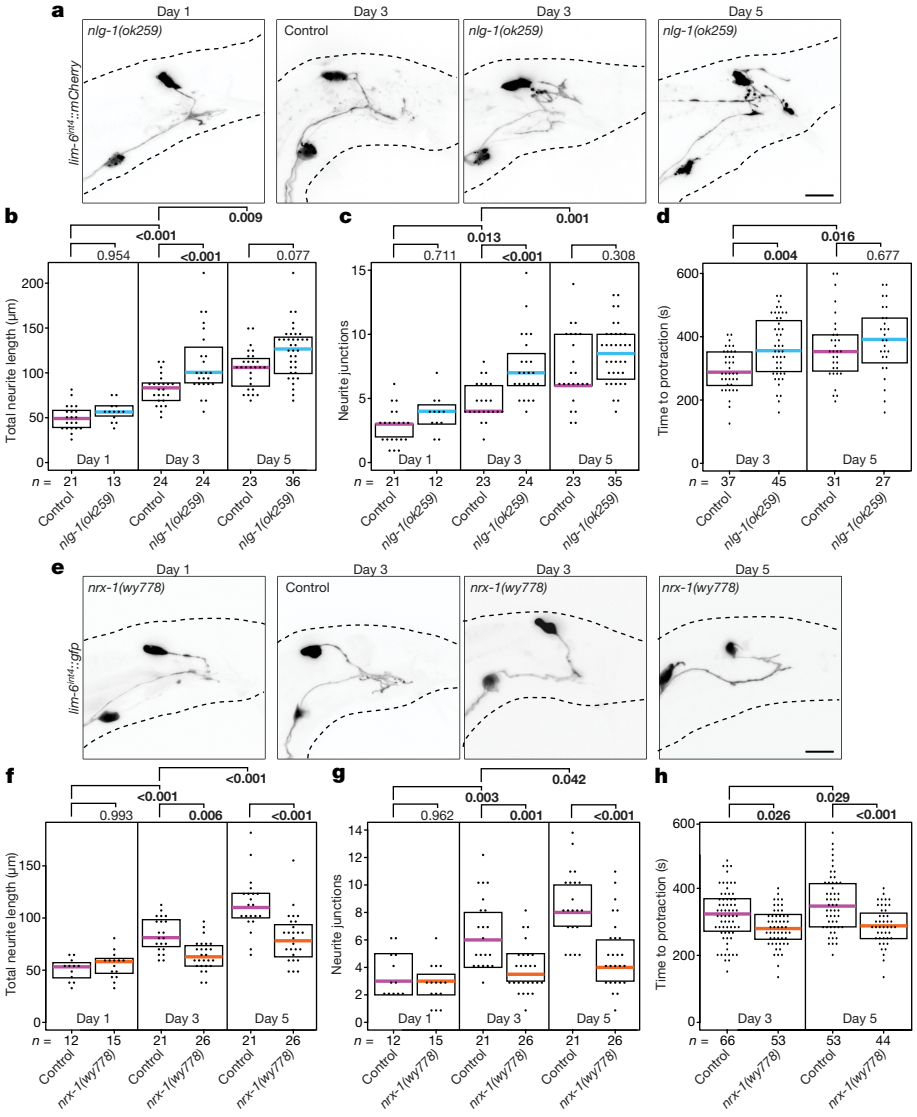

Figure 4 | Neuroligin and neurexin influence DVB neurite outgrowth and spicule protraction behaviour. a, Confocal images of DVB (*lim-6^{int4}::wCherry)* in *nlg-1*(*ok259*) mutant and control male worms. **b**, **c**, Quantification of total neurite outgrowth (**b**) and number of neurite junctions (**c**) in *nlg-1*(*ok259*) and control male worms. **d**, Time to aldicarb-induced spicule protraction in control and *nlg-1*(*ok259*) males. **e**, Confocal images of DVB (*lim-6^{int4}::gfp*) in *nrx-1*(*wy778*) mutant and control worms. **f**, **g**, Quantification of total neurite outgrowth (**f**) and number of neurite junctions (**g**) in *nrx-1*(*wy778*) and control worms. **h**, Time to aldicarb-induced spicule protraction in control and *nrx-1*(*wy778*). Scale bars, 10 µm. 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 ($P < 0.05$).

DVB neuron, the SPC, PCA and PCB neurons, or the SPC neuron and spicule muscles did not rescue the *nlg-1* mutant phenotype, whereas expression in the spicule protractor and anal depressor muscles or in the spicule retractor muscles did rescue the phenotype (Extended Data Fig. 7d, e), indicating that NLG-1 contributes to DVB neurite outgrowth by functioning in multiple postsynaptic DVB muscles. Silencing the spicule protraction circuit in *nlg-1* mutant males at day 5 with *gar-3b::HisCl1* or overnight exposure to exogenous GABA resulted in no significant reduction in DVB neurite branching (Extended Data Fig. 7f, g). These results suggest that the *nlg-1* mutant phenotype cannot be explained by indirect alteration of the spicule circuit or more global perturbations in activity as a result of loss of NLG-1.

Unexpectedly, males with a deletion allele of *nrx-1* (which encodes the *C. elegans* orthologue of neurexin)[28] displayed a significant reduction in neurite outgrowth at days 3 and 5, a phenotype opposite to the *nlg-1* mutant phenotype ($P = 0.006$ and $P < 0.001$, respectively; Fig. 4e–g). *nrx-1* mutants showed a corresponding decrease in time to aldicarb-induced spicule protraction (Fig. 4h). The *nrx-1* locus produces both a long and short isoform[29], and two long isoform-specific mutant alleles recapitulated the null phenotype (Extended Data Fig. 9a–c). Repeated channelrhodopsin-mediated activation of the spicule protraction circuit failed to induce DVB neurites in *nrx-1* mutants (Extended Data Fig. 5d–f), indicating that the *nrx-1* phenotype is not explained solely by reduced circuit activity that could be envisioned to result from loss of NRX-1.

NRX-1 is broadly expressed throughout the *C. elegans* nervous system[29]. Expression of the long isoform of NRX-1 in DVB using the *lim-6^{int4}* promoter resulted in rescue of the *nrx-1(wy778)* neurite outgrowth defect (Extended Data Fig. 9d, e). The long NRX-1 isoform still rescued the mutant phenotype even after deletion of the C-terminal PDZ binding motif, whereas the short NRX-1 isoform did not (Extended Data Fig. 9d, e). Overexpression of the long isoform of NRX-1 in wild-type male DVB neurons significantly increased DVB neurite length ($P = 0.047$) (Extended Data Fig. 9d, e), and when tagged with GFP, localized diffusely on the soma and neurites of DVB (Extended Data Fig. 9j). The reduction in time to aldicarb-induced spicule protraction in *nrx-1* mutants was rescued by expression of the long isoform of NRX-1 in DVB, but overexpression of NRX-1 in wild-type worms did not change time to spicule protraction compared with control wild-type males (Extended Data Fig. 9f). These results indicate that the long isoform of NRX-1 is required in DVB for neurite outgrowth, which may extend the gene's role beyond its canonical function at synapses. Varying the levels of NRX-1 in DVB directly alters the extent of neurite outgrowth, and loss of NRX-1 in DVB reduces inhibition onto the spicule protraction circuit so that spicule protraction occurs more rapidly.

The exuberant DVB neurite branching phenotype of *nlg-1* mutants is completely suppressed by loss of NRX-1, and the increase in DVB neurite branching observed upon NRX-1 overexpression is not further enhanced by loss of NLG-1 (Extended Data Fig. 9g–i). Furthermore,