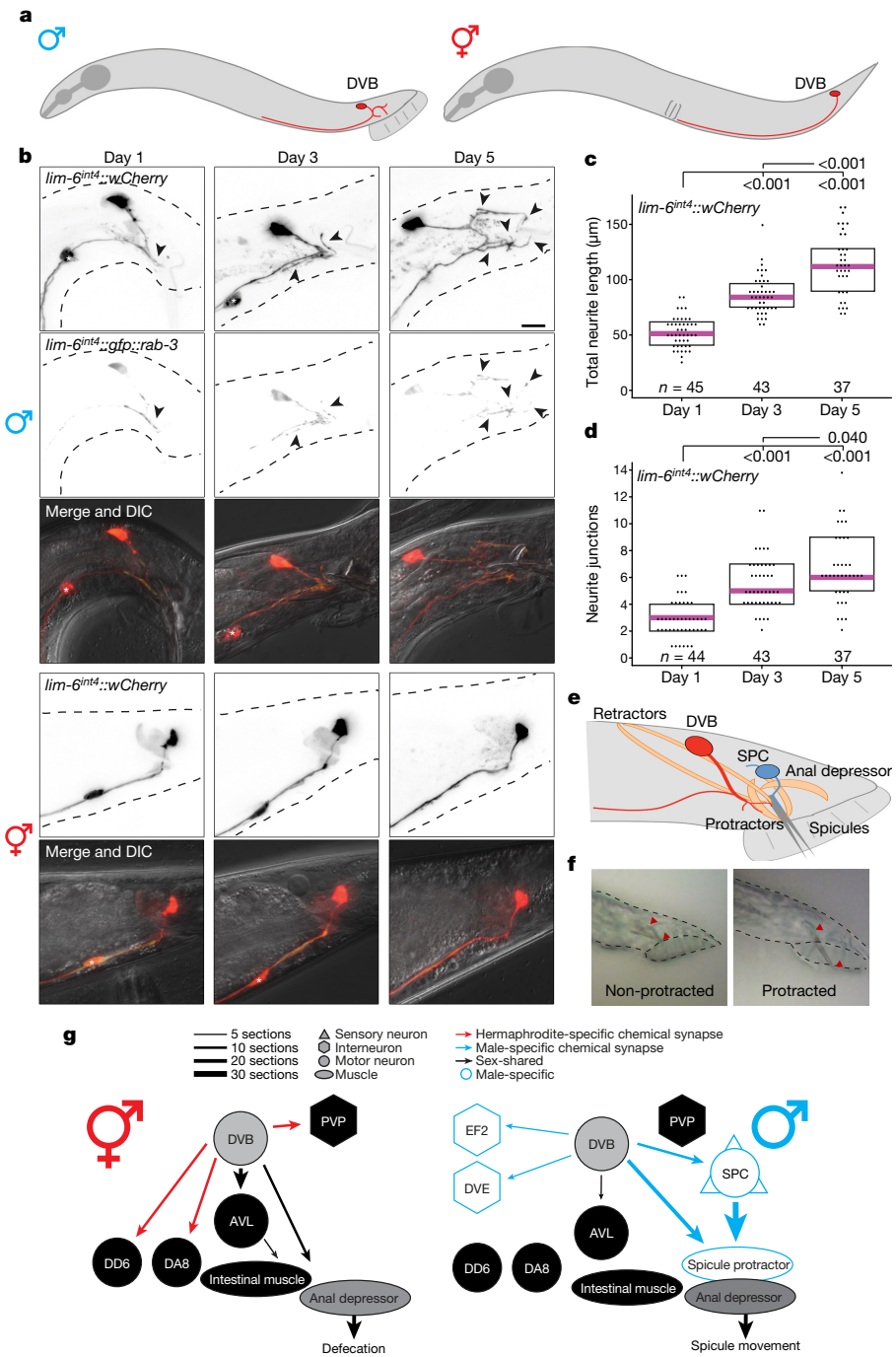

Figure 1 | Progressive neurite outgrowth of the GABAergic DVB neuron in adult males. **a**, DVB neuron schematic. **b**, DVB visualized with *lim-6*^{int4}*::wCherry* in adult males and hermaphrodites (asterisk, PVT neuron; arrowheads, DVB neurites, *n* as indicated in **c**). Presynaptic boutons visualized with presynaptic marker *lim-6*^{int4}*::gfp::rab-3*. DIC, differential interference contrast. **c**, **d**, DVB neurite outgrowth in males quantified by total neurite length (**c**) and number of neurite junctions (**d**). Dot represents one worm; magenta bar, median; boxes, quartiles. Comparison using one-way ANOVA and post-hoc Tukey HSD, *P* values shown above plots, bold shows significance ($P < 0.05$). **e**, Schematic of DVB and postsynaptic spicule-associated neurons and muscles in male tail. **f**, Sample images of males with non-protracted or protracted spicules (red triangles indicate base and tip of spicules; tail and male fan are outlined, demonstrating protracted spicules extending underneath male fan; $n > 10$). **g**, Connectivity of DVB at adult stage inferred from electron micrographs (sections indicate number of EM sections over which *en passant* synapses were observed)[8,11]. Behavioural output indicated for each sex. Scale bars, 10 μm.

spicule protraction at day 5 (aldicarb assay described below, Extended Data Fig. 4a–d), suggesting that GABA contributes to restriction of spicule protraction in later adulthood.

To further characterize the role of DVB in active spicule protraction, we used the acetylcholine esterase inhibitor aldicarb, which induces spicule protraction through the accumulation of acetylcholine at neuromuscular synapses onto spicule protractor muscles[13] (Fig. 2d). Aldicarb-induced spicule protraction took longer as males aged from day 1 to day 5 (Fig. 2e), during the same period as DVB neurite outgrowth. To directly test whether DVB is involved in this behavioural change, we combined laser ablation of DVB with aldicarb-induced spicule protraction. DVB ablation at day 1 resulted in slower spicule protraction in response to aldicarb than in control and mock-ablated males, again demonstrating that DVB input at day 1 has an excitatory effect on spicule protraction (Fig. 2f). DVB ablation at day 5 resulted in faster spicule protraction in response to aldicarb than in control and mock-ablated males, demonstrating a functional switch for DVB from an excitatory to an inhibitory input on spicule protraction (Fig. 2f). These results were confirmed using genetic 'ablation' of DVB (*lim-6* transcription factor mutant[14]; Fig. 2f). Together, our results confirm that DVB switches function in adulthood, and implicate DVB as the main contributor to the temporal change observed in spicule protraction and defecation behaviour.

To investigate how the switch of DVB function during DVB neurite outgrowth relates to changes in synaptic connectivity, we used trans-synaptic labelling (GRASP[15]) to visualize synapses between DVB and the spicule protraction neurons and muscles (Fig. 2g). The number of these specific synaptic connections increased from day 1 to 5 (Fig. 2h, i). We also visualized synapses between DVB and the spicule retractor muscles (Fig. 2j; Extended Data Fig. 4h); the number of these synapses decreased from day 1 to 5 (Fig. 2k, l). These results provide evidence that structural remodelling of axons and dendrites in