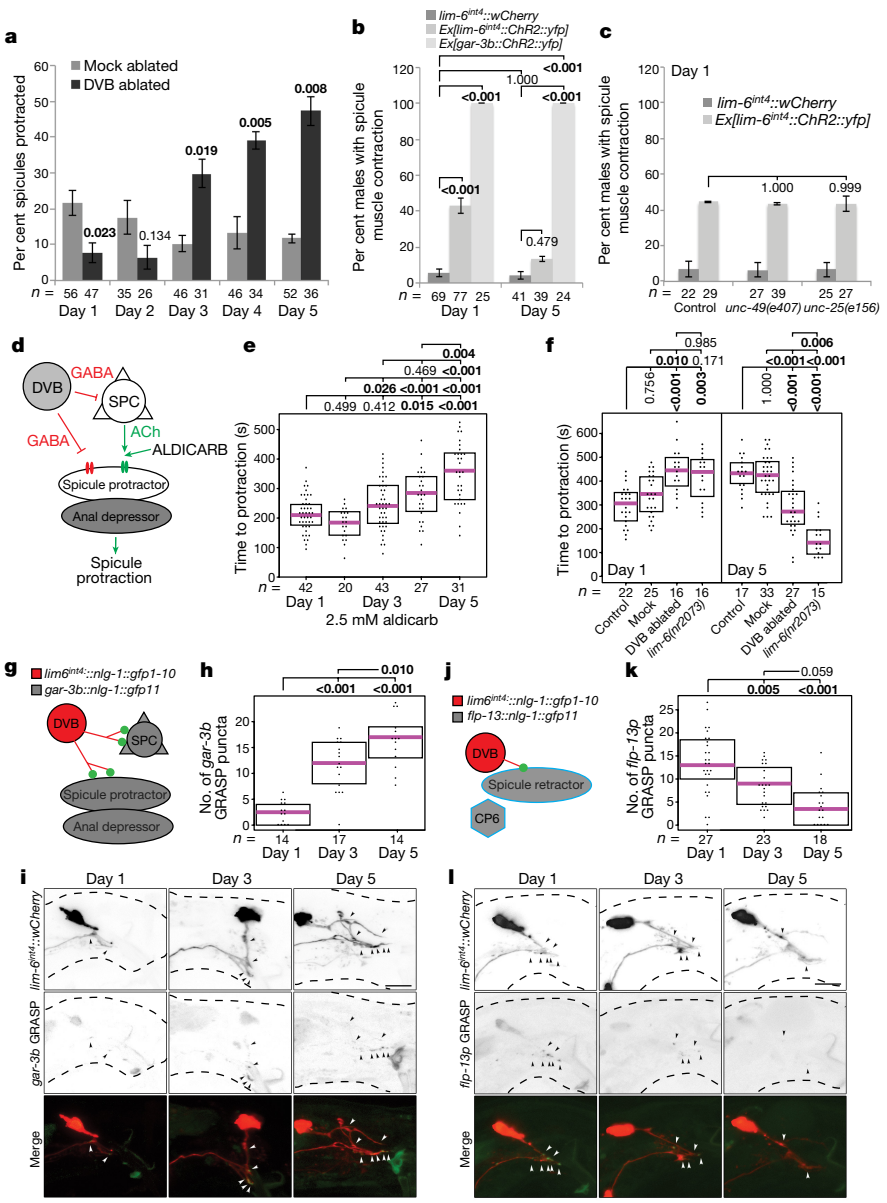

Figure 2 | DVB neuron undergoes a functional switch in adulthood resulting in dynamic behavioural output. a, Per cent of mock-ablated or DVB-ablated males with chronically protracted spicules 20 h after ablation at day indicated (mean ± s.e.m., two-tailed Student's *t*-test). **b**, Per cent of worms responding to 488-nm light with movement of spicules for control, Ex[*lim-6*^{int4}*::ChR2::yfp*], and Ex[*gar-3b::ChR2::yfp*] worms (mean ± s.e.m., one-way ANOVA and post-hoc Tukey HSD). **c**, Per cent of worms with or without Ex[*lim-6*^{int4}*::ChR2::yfp*] responding to blue light with spicule movement at day 1 in control, *unc-49(e407)*, and *unc-25(e156)* males (mean ± s.e.m., one-way ANOVA and post-hoc Tukey HSD). **d**, Diagram of GABA and acetylcholine input onto spicule muscles showing site of aldicarb action. **e**, Males on 5 mM aldicarb medium timed for spicule protraction for more than 5 s. Dot represents one worm; magenta bar, median; boxes, quartiles; one-way ANOVA and post-hoc Tukey HSD. **f**, Control, mock-ablated, DVB-ablated, or *lim-6(nr2073)* mutant males timed for aldicarb-induced spicule protraction 12 h after ablation. **g**, Diagram of synaptic connections labelled with *lim-6/gar-3* GRASP. **h**, **i**, Quantification (**h**) and confocal images (**i**) of *lim-6/gar-3* GRASP puncta. **j**, Diagram of synaptic connections labelled with *lim-6/flp-13* GRASP. **k**, **l**, Quantification (**k**) and confocal images (**l**) of *lim-6/flp-13* GRASP puncta. For **i**, **l**, arrowheads, location of GRASP puncta on DVB neurites; scale bars, 10 μm. *P* values shown above plots, bold shows significance ($P < 0.05$).

adulthood can rewire specific synaptic targets, supporting the notion that this remodelling can markedly alter connectivity within circuits and alter downstream behaviour.

Male spicule protraction into the hermaphrodite vulva is the most complex step of the male mating behaviour, involving coordination of cholinergic and GABAergic signalling[16–18]. The balance of excitatory and inhibitory signalling is crucial for successful spicule insertion, which must be further coordinated with changes in sex muscle excitability in early adulthood[13,19–21]. Day 1 and day 3 males are proficient at most steps of mating[20]; however, in five-minute timed mating assays, day 3 males were significantly more likely than day 1 males to successfully complete mating with sperm transfer ($P = 0.003$; Extended Data Fig. 5a). We scored the spicule-related steps of mating (spicule prodding and spicule protraction) and found that day 1 males showed more spicule prodding attempts overall and a lower ratio of protraction to prodding attempts compared with day 3 males (Extended Data Fig. 5b, c), indicating that day 1 males are less capable than day 3 males of transitioning from spicule prodding to spicule protraction. This suggests that the morphological and functional plasticity of DVB in males may fine-tune and coordinate the defecation and spicule protraction circuits to increase mating success.

DVB neurites are experience- and activity-dependent

To determine whether DVB plasticity occurs in response to experience, we tested whether the act of mating itself altered DVB neuron morphology by exposing males to hermaphrodites for the first 48 h of adulthood. Single males housed with hermaphrodites showed significant increases in DVB neurite length and junctions compared to males housed alone ($P < 0.001$; Fig. 3a–c). *C. elegans* males housed with other males or in isolation can engage in mating-like behaviours, which may include spicule protraction. To minimize mating sensory input and self-mating behaviour, we analysed DVB neurite outgrowth in *pkd-2* (cation channel) mutant males[22] and in genetically paralysed mutant males (*unc-97*)[23]. *pkd-2* mutant males have reduced DVB neurite outgrowth at day 3, whereas *unc-97* mutant males have almost no DVB neurites at day 3 (Extended Data Fig. 4e–g); however, they can protract spicules in response to aldicarb (data not shown) and their neurites can be ectopically induced (Extended Data Fig. 5d–f). Paralysed males also show no change in neurite outgrowth when housed with hermaphrodites for 48 h (Fig. 3a–c). These results demonstrate that DVB neurite outgrowth is experience-dependent and is potentially driven by spicule protraction and activity of the postsynaptic spicule protraction circuit.

We next investigated whether activity of the postsynaptic targets of DVB contributes to DVB neurite outgrowth. Channelrhodopsin-