mated plates were checked for fluorescent progeny to ensure successful mating had occurred, and then mated and non-mated (individually housed) males were subjected to confocal microscopy. *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 also analysed DVB neurite outgrowth in males with mutation in *pkd-2*[22] and in males genetically paralysed by a mutation in *unc-97*/LIMS1 (affects body wall muscle ultrastructure)[23].

Mating behaviour assay. Mating assays were based on procedures described previously[34,35]. Males were picked at the L4 stage and kept apart from hermaphrodites. One male was transferred to a plate covered with a fresh OP50 lawn containing 15 adult *unc-31(e928)* hermaphrodites. Day 1 males were counted as less than 18 h after L4 moult. Males were observed for 5 min from the time of first contact with a hermaphrodite or until they ejaculated, whichever came first. Males were scored for their ability to prod the vulva, protract spicules, and transfer sperm. Mating success was calculated as 100× the number of males that transferred sperm successfully divided by the total number of males tested. The number of attempts at prodding was calculated by summing attempts at prodding for each male. The protraction:prodding ratio was calculated by dividing the number of spicule protractions by the number of attempts at prodding for each male.

Synapse visualization. GRASP plasmid construction is described above. For visualization of synaptic connections between DVB and neurons and muscles downstream of DVB that form the spicule protraction circuit, we injected *lim-6*^{int4}*::nlg-1::gfp1-10* (pMH18) to label the presynaptic DVB, together with *gar-3b::nlg-1::gfp11* (pMH20) to label the postsynaptic SPC and spicule protractor muscles. Plasmids were injected together at 25 ng μl^{-1} with the coinjection marker *ttx-3::gfp* (also at 25 ng μl^{-1}) to generate extrachromosomal arrays. For visualization of synaptic connections between DVB and neurons and muscles downstream of DVB that express *flp-13*, we injected *lim-6*^{int4}*::nlg-1::gfp1-10* (pMH18) to label the presynaptic DVB together with *flp-13::nlg-1::gfp11* (pMH23) to label the postsynaptic spicule retractor muscles. Plasmids were injected together at 25 ng μl^{-1} with the coinjection marker *ttx-3::gfp* (also at 25 ng μl^{-1}) to generate extrachromosomal arrays. Synapses between DVB and spicule retractor muscles were not reported in electron microscopy of an 'old male'[36], possibly owing to the observed decrease in these synapses after day 1; alternatively, these synapses may have been characterized as one of several 'unknown' connections of DVB[8]. The *flp-13* promoter also labels CP6 in males, which has few synapses with DVB that were located in the electron micoscopy reconstruction anterior to the DVB neurites, and the branched parts of the axons of DVB and CP6 appear not to make contact (Extended Data Fig. 4h).

Spicule activation assay with channelrhodopsin. All-*trans* retinal was added to LB/OP50 medium and coated over the entire plate at a final concentration of 0.1 mM. We obtained strains expressing channelrhodopsin under the *gar-3b* promoter[17,30,37] labelling spicule protraction neurons and muscles, under the *unc-103E* promoter labelling spicule protractors and anal depressor muscles, and under the *unc-103F* promoter labelling spicule neurons SPC, PCA, and PCB[16] (gifts from L. R. Garcia). Worms were incubated overnight on retinal plates before all assays involving channelrhodopsin-containing strains. For the spicule protraction assay, male worms on retinal plates were individually subjected to 488-nm light for 10 s, three times with 30 s between trials, on a Nikon eclipse E400 microscope. Obvious spicule muscle contraction for any of the three trials was recorded as a response. Videos were recorded using a mounted Exo Labs Focus camera. For the activation protocol, male worms on retinal plates were subjected to alternating 488-nm light three times (15 s light/15 s dark) on a Leica M165 FC dissecting scope, repeated every 45 min for 4.5 h. Worms were then subjected to confocal microscopy or aldicarb behavioural assay. Controls for neurite outgrowth and aldicarb behaviour were performed on males under the same conditions but not exposed to the channelrhodopsin cofactor all-*trans* retinal (Extended Data Fig. 5j–l). For recovery, worms were placed in the dark for ~20 h after the activation protocol, then subjected to the same analysis. A small number of individual males subjected to confocal imaging before and after activation, or after activation and following recovery, demonstrated addition of neurites following activation, and removal of neurites following recovery; however, the difficulty of this analysis precluded quantification.

Neuronal silencing with histamine chloride channel (HisCl1). Control or transgenic worms were picked onto normal NGM plates seeded with OP50 at the L4 stage, then picked the evening before the indicated day of analysis onto 10 mM histamine or control plates with OP50 bacteria as a food source. For *gar-3b::HisCl1* silencing assays, males were left on histamine or control plates overnight then subjected to confocal microscopy the following morning. For *lim-6*^{int4}*::HisCl1* defecation analysis, males were picked onto histamine plates, allowed to adjust for 5 min and then analysed for defecation behaviour. Histamine plates were prepared as previously described[12].

Defecation assay. Males were placed on control or 10 mM histamine plates with food on the day of analysis, allowed to explore for 5 min, and then observed for 10–12 min on a low magnification Leica MZ8 light dissecting microscope. Expulsion steps were recorded for the time between consecutive expulsions, and the presence of spicule protraction within 3 s before or after expulsion. The percentage of expulsion steps associated with spicule protraction was calculated for each male. The time between consecutive expulsion steps was calculated by averaging all times recorded between consecutive expulsions for each male.

Exogenous GABA exposure. Males were picked onto normal NGM plates seeded with OP50 at the L4 stage, then picked before the day of analysis onto 30 mM GABA[10] or control plates seeded with OP50 and left overnight, and then subjected to confocal microscopy. For 3-day GABA exposure, males were picked onto 30 mM GABA or control plates seeded with OP50, left for 3 days and then subjected to confocal microscopy.

Measurement of fluorescence intensity. To quantify the fluorescence intensity of *nlg-1p::nlg-1::gfp*, a stack of images was acquired using confocal microscopy with the same acquisition parameters between samples (objective, pixel size, laser intensity, pinhole size, and PMT settings). The fluorescence intensity mean was obtained using ZEN Black software. For the dorsal spicule muscles, the muscles were outlined and the cross-section with the highest mean was recorded. Dorsal spicule muscles include the gubernacular retractor, gubernacular erector, anterior oblique, and anal depressor, which could be outlined easily, whereas the spicule protractor could not always be observed in males after day 1. For the pre-anal ganglion and DVB or background, a pre-defined circle was used to outline the region of interest, and the cross section with the highest mean was recorded. The ratio of fluorescence intensity was calculated by dividing the mean of the dorsal spicule muscles (arbitrary units) by the mean of the DVB or background (arbitrary units) or by dividing the mean of the pre-anal ganglion by the mean of the DVB or background (arbitrary units).

Cell-autonomous changes in sexual identity. We tested cell-autonomous changes in the sexual identity of DVB (*lim-6*^{int4} promoter) and muscles (*myo-3* promoter) by expressing either the cDNA of *fem-3* in hermaphrodites to masculinize each tissue, or the cDNA of *tra-2*$^{intracellular\ domain}$ in males to feminize each tissue[38–40]. In males with feminized DVB or muscles, we observed no suppression of DVB neurites, and in hermaphrodites with masculinized DVB or muscle, we observed no induction of DVB neurites.

Statistics and reproducibility. We performed two-tailed Student's *t*-test or one-way ANOVA with post-hoc Tukey HSD test using *R* and *RStudio*; *P* values are shown on each graph. No statistical methods were used to predetermine sample size, and the experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment. Number of independent biological replicates: Fig. 1b–d, 7; Fig. 2a–c, h–k, 3 or more; Fig. 2e, f, 2 or more; Figs 3a–g, 3 or more; Figs 4a–h, 3 or more; Extended Data Fig. 1a–c, 4 or more, Extended Data Fig. 1d, 2 or more; Extended Data Fig. 2a, b, 4 or more; Extended Data Fig. 2c–h, 2 or more; Extended Data Fig. 3a–c, 3 or more; Extended Data Fig. 3d–f, 2 or more; Extended Data Fig. 4a–c, h, 2 or more; Extended Data Fig. 4d–g, 3 or more; Extended Data Fig. 5a–f, 4 or more; Extended Data Fig. 5g–l, 2 or more; Extended Data Fig. 6a–f, 2 or more; Extended Data Fig. 7a–h, 3 or more; Extended Data Fig. 8a–c, 3 or more; Extended Data Fig. 8d–f, 2 or more; Extended Data Fig. 9b–i, 3 or more; Extended Data Fig. 9j, 2 or more; Extended Data Fig. 10a–c, 3 or more.

Data availability. The data that support the findings of this study are available from the corresponding author upon reasonable request.

30. LeBoeuf, B., Correa, P., Jee, C. & García, L. R. *Caenorhabditis elegans* male sensory-motor neurons and dopaminergic support cells couple ejaculation and post-ejaculatory behaviors. *eLife* **3,** e02938 (2014).
31. Oren-Suissa, M., Bayer, E. A. & Hobert, O. Sex-specific pruning of neuronal synapses in *Caenorhabditis elegans*. *Nature* **533,** 206–211 (2016).
32. Longair, M. H., Baker, D. A. & Armstrong, J. D. Simple Neurite Tracer: open source software for reconstruction, visualization and analysis of neuronal processes. *Bioinformatics* **27,** 2453–2454 (2011).
33. Fang-Yen, C., Gabel, C. V., Samuel, A. D., Bargmann, C. I. & Avery, L. Laser microsurgery in *Caenorhabditis elegans*. *Methods Cell Biol.* **107,** 177–206 (2012).
34. Garcia, L. R., LeBoeuf, B. & Koo, P. Diversity in mating behavior of hermaphroditic and male–female *Caenorhabditis* nematodes. *Genetics* **175,** 1761–1771 (2007).
35. Hilliard, M. A., Bargmann, C. I. & Bazzicalupo, P. *C. elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. *Curr. Biol.* **12,** 730–734 (2002).
36. Sulston, J. E., Albertson, D. G. & Thomson, J. N. The *Caenorhabditis elegans* male: postembryonic development of nongonadal structures. *Dev. Biol.* **78,** 542–576 (1980).
37. Correa, P., LeBoeuf, B. & García, L. R. C. *C. elegans* dopaminergic D2-like receptors delimit recurrent cholinergic-mediated motor programs during a goal-oriented behavior. *PLoS Genet.* **8,** e1003015 (2012).
38. White, J. Q. *et al.* The sensory circuitry for sexual attraction in *C. elegans* males. *Curr. Biol.* **17,** 1847–1857 (2007).
39. Lee, K. & Portman, D. S. Neural sex modifies the function of a *C. elegans* sensory circuit. *Curr. Biol.* **17,** 1858–1863 (2007).
40. Mowrey, W. R., Bennett, J. R. & Portman, D. S. Distributed effects of biological sex define sex-typical motor behavior in *Caenorhabditis elegans*. *J. Neurosci.* **34,** 1579–1591 (2014).