Glia-derived neurons are required for sex-specific learning in C. elegans

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Sex differences in behaviour extend to cognitive-like processes such as learning, but the underlying dimorphisms in neural circuit development and organization that generate these behavioural differences are largely unknown. Here we define at the single-cell level—from development, through neural circuit connectivity, to function—the neural basis of a sex-specific learning in the nematode Caenorhabditis elegans. We show that sexual conditioning, a form of associative learning, requires a pair of male-specific interneurons whose progenitors are fully differentiated glia. These neurons are generated during sexual maturation and incorporated into pre-existing sex-shared circuits to couple chemotactic responses to reproductive priorities. Our findings reveal a general role for glia as neural progenitors across metazoan taxa and demonstrate that the addition of sex-specific neuron types to brain circuits during sexual maturation is an important mechanism for the generation of sexually dimorphic plasticity in learning.

During sexual maturation, the nervous system undergoes sexually dimorphic changes that couple behaviour to reproductive needs1–5. Sex differences in behaviour extend beyond courtship and mating to cognitive-like processes such as learning that also enhance reproductive success6–7. However, the precise dimorphisms in neural circuit development and organization that generate these differences in behavioural plasticity are mainly unknown.

C. elegans is a sexually dimorphic species with hermaphrodites that can self-fertilize and males that can only cross-fertilize hermaphrodites. As a consequence, males display a repertoire of reproductive behaviours not performed by the hermaphrodite and these behaviours require sex differences in the nervous system that develop during sexual maturation8,9. The best described dimorphisms are the male-specific sensory and motor circuits required for mating. However, non-reproductive behaviours such as learning are also sexually dimorphic. Males, but not hermaphrodites, are capable of sexual conditioning, a process that involves associative learning and leads to a switch in chemotactic responses that facilitates effective mate finding10. Sexual conditioning requires sex-shared and male-specific sensory neurons10, but whether the circuit also contains sexually dimorphic interneurons for integration is not known.

The MCMs are a novel class of interneurons

We identified a previously unnoticed bilateral pair of neurons in the head of males that are required for sexual conditioning. We have termed them the MCMs (for mystery cells of the male) (Fig. 1). The identification of the MCMs is surprising given that the C. elegans anatomy, neuronal connectivity and developmental lineage have been characterized extensively8,9,11–14. We noted the presence of the MCMs when we were analysing the expression pattern of a reporter transgene for the neuropeptide pdf-1. In males, but not in hermaphrodites, we observed pdf-1 expression in a bilateral pair of cells located dorso-anterior to the pharyngeal metacorpus, a region devoid of neuronal cell bodies in hermaphrodites (Fig. 1a–c). This region is comprised of glial and epithelial cells, some of which project anteriorly to the nose11,15. Unlike any other cells in this region, we observed that the male’s pdf-1-positive cells send projections posteriorly into the nerve ring (Fig. 1b and Extended Data Fig. 1a, b). The nerve ring is the main concentrated neuropil of the nematode brain, where most sensory neuron–interneuron synapses and neural processing occur13. We found that the pdf-1-positive cells also express the pan-neuronal reporter rab-3, suggesting they may be neurons. rab-3 expression was first observed at the L4 stage, when sexual maturation begins (Fig. 1c). Through reporter gene analysis, and ultrastructural reconstruction, we found that the MCMs are indeed fully differentiated neurons. In addition to rab-3, these cells express a battery of neuronal genes required for both electrical and chemical communication (Fig. 1d, Extended Data Fig. 1c and Extended Data Table 1). These include components of the SNARE complex; Na+ and voltage-gated Ca2+ channel subunits; innexins; components of the machinery for neuropeptide secretion ric-19 and ida-1; and pdf-1. At the ultrastructural level, we observed dense-core vesicles in the MCM somata and projections, and clear-core vesicles at presynaptic densities (Fig. 1e). Together, these observations indicate that the MCMs are a novel class of male-specific interneurons.

We identified the MCMs in electron micrographs of serial sections through the male head and established their entire neural connectivity (Fig. 2 and Extended Data Table 2). The cell bodies send processes posteriorly in the amphid bundles, from which they diverge to enter the nerve ring, and from which they exit and extend into the ventral nerve cord (Extended Data Fig. 1b). In the nerve ring and ventral nerve cord we identified synaptic interactions with 24 neuron classes (Extended Data Fig. 1d and Extended Data Table 2). The bulk of the synaptic input (65%) is from three interneuron classes, two of which are sex-shared (AVF and PVQ) and one of which is male-specific (EF). These interneurons receive extensive sensory inputs from the male copulatory circuits in the tail and extend processes through the ventral nerve cord and into the nerve ring, where they connect to the MCMs both directly and through RIF (a sex-shared class of second-order interneurons that receive input from head.
The MCMs are newly identified male-specific neurons. A reporter transgene (rab-3::rfp) of an adult male (same region as in Figure 1) The MCMs are newly identified male-specific neurons. The MCMs in adult males of reporter transgenes for neuronal markers. MCMR (18 cells), Confocal projection of pdf-1::rfp expression in the head of a male. Confocal projection of pdf-1::rfp expression in the head of an adult male (same region as in a). Expression of the pan-neuronal reporter transgene rab-3::rfp (Ras GTPase) in the head of an hermaphrodite. The largest synaptic output is onto the AVB pre-motor interneurons that drive forward locomotion. These outputs are both direct (41%) and indirect via RIF (14%). Thus, the presence of the MCMs creates a series of male-specific disynaptic feed-forward triplet motifs connecting male-specific EF and sex-shared AVF, PVQ and RIF to AVB (Fig. 2). Such triplet motifs recur frequently in the C. elegans nervous system. There is also a small amount of reciprocal interaction between the MCMs and the male-specific, pheromone-sensing CEM head sensory neurons. The major input from mate-sensing circuits and their output onto second- and third-order interneurons make the MCMs ideally placed for the integration of mate-experience into circuits regulating behavioural plasticity to sensory stimuli.

The MCMs regulate sex-specific learning
We found that the MCMs are required for a male-specific switch in chemosensory behaviour induced by sexual conditioning. Sexual conditioning is a mate-experience-dependent process of behavioural modification that overrides the effects of starvation during chemosensory learning. This plasticity confers on males the ability to use recent environmental chemosensory cues as signals for effective mate finding, and reflects the adult male prioritization of sex over food. One chemosensory behaviour that is subject to sexual conditioning is salt-avoidance learning. C. elegans is normally attracted to salt but both males and hermaphrodites can learn to avoid salt when it is previously associated with an aversive stimulus such as starvation. Ablation of the MCMs at the late fourth larval (L4) stage with a laser microbeam resulted in males that failed to undergo a sexually conditioned switch, thus avoiding salt rather than being attracted to it, after conditioning with starvation, salt and mates (Fig. 3b). Importantly, MCM ablation did not disrupt salt attraction in non-conditioned males or salt avoidance learning after conditioning with salt and starvation in the absence of mates (Fig. 3b), indicating that the defects are specific to sexual conditioning and not due to inability to sense salt or starvation.

Sexual conditioning requires two types of mate inputs: a secreted pheromone synthesized by the enzyme DAF-22 and sensed through chemosensory amphid neurons and the male-specific CEM sensory neurons in the head, and a contact-dependent cue that is sensed through the male-specific ray neurons in the tail. MCM-ablated
males responded efficiently to both daf-22-dependent secreted pheromones (ascarosides) (Fig. 4a, b and Extended Data Fig. 2a) and contact-dependent hermaphrodite cues that initiate the mating sequence and inhibit male food-avoiding behaviour\(^2\). These results indicate that the MCMs are not required for the sensory detection of mates, salt, or starvation, but that they specifically regulate the integration of these cues to produce behavioural plasticity in a context- and sex-specific manner.

Having found a role for the MCMs in sexual conditioning, we next asked whether the MCMs are important regulators of other male-specific behaviours from the coordinated sensory-motor programmes of mating\(^24\) to other integrative behaviours such as the decision to leave food in search of mates\(22,23\). MCM ablation did not cause defects in any other male-specific behaviour tested or overall fertility (Fig. 4c, d and Extended Data Fig. 2b–f), demonstrating a surprisingly specific role for the MCMs in sexual conditioning.

To establish whether sexual conditioning is regulated through neuromodulation, we tested pdf-1 \(tm996\) mutants in the salt chemotaxis learning assay. The \(tm996\) deletion allele removes the minimal promoter and first exon of the pdf-1 gene and is likely to be a null\(^26,27\). We found that pdf-1 mutant males, like MCM-ablated males, failed to undergo sexual conditioning and avoided salt after conditioning with starvation, salt and mates (Fig. 3b). Loss of pdf-1, like loss of MCMs, did not disrupt salt sensation in naive animals or the ability to associate salt and starvation (Fig. 3b). pdf-1 mutants also responded to secreted and contact-dependent mate cues, although they were slightly, but significantly, less efficient than wild-type males at contact response (Fig. 4a, b and Extended Data Fig. 2b–f). Together, these results tentatively suggest that the MCMs may modulate the circuits that regulate chemosensory plasticity through neuropeptide secretion.

### The MCMs arise from glia

Next, we sought to establish the developmental mechanisms by which these male-specific neurons arise. Unlike any other neurons in *C. elegans* and other invertebrates, which arise from epithelial or undifferentiated blast cells\(8,11,12,28\), we found that the MCMs arise from glial cells. Glia are specialized cells with projections that ensheath the cilia of the sensory neurons with which they are associated, and provide structural and functional support to these neurons\(29\). As neuronal gene expression in the MCMs begins during sexual maturation, we
examined the expression of an rnr-1::gfp reporter, which labels cells in S-phase\(^9\), to determine whether the MCMs are born at this stage via an undescribed cell division in the male head. In the head region, we observed strong expression only in males (Fig. 5a). Expression was observed in a bilateral pair of cells at the late L3 stage, corresponding to the amphid socket (AMso) glial cells, and perduring in two bilateral pairs of cells at the early L4 stage, corresponding to the two AMso and the MCMs (Fig. 5b and Extended Data Fig. 3a). The lack of expression in the hermaphrodite head is consistent with the lack of AMso glial cell division in this sex\(^12\).

Ablation of one of the bilateral pair of AMso glial cells at the L3 stage in males resulted in a consistent loss of the MCM on the opercular side (Extended Data Table 3). Importantly, the MCM was not lost when the AMso was ablated at the mid-L4 stage, after the MCM was born (Extended Data Table 3). Together this demonstrates that the AMso glial cells are the MCM progenitors.

The AMso glial cells are born during embryogenesis from the same neurogenic competence of the AMso glial cells is specified extrinsically (Extended Data Fig. 4a). In order to establish whether sex-specific neurogenic competence of the AMso glial cells is specified extrinsically

**AMso plasticity is intrinsically regulated**

The AMso glial cells are born during embryogenesis from the same fully described lineages in both sexes (Extended Data Fig. 4a)\(^11\), but retain their plasticity only in males, re-entering the cell cycle during sexual maturation. We found that the AMso developmental program occurred according to the genetic sex of the AMso cells and not according to that of the rest of their lineage or the animal. Sexual dimorphism in *C. elegans* is regulated by a genetic pathway that converges on the Ci/GLI-like zinc-finger transcription factor *tra-1*, which inhibits male development and activates hermaphrodite development\(^11,32\). Consistent with a role for the sex-determination pathway in MCM specification, 22/22 *tra-1(e1488)* sex-transformed XX pseudo-males produced MCMs (data not shown). The sister cells of the AMso are the male-specific CEM sensory neurons, which undergo cell death during embryogenesis in hermaphrodites\(^11\) (Extended Data Fig. 4a). In order to establish whether sex-specific neurogenic competence of the AMso glial cells is specified extrinsically

**Figure 5 | The MCMs originate from a male-specific cell division of the AMso glial cells.** Lateral views of animals oriented anterior to the left and dorsal to the top. a, Expression of the S phase reporter transgene rnr-1::gfp in whole animals at the L4 stage (a) and in the head of L3 and L4 males (b). The male-specific division in the head is indicated by dashed circles in a. The rest of expression corresponds to developing reproductive structures. c, Differential interference contrast (DIC) and fluorescent images of the AMso cell body with RFP-labelled histones during cell division. d, WormAtlas diagram depicting the morphology and position of the AMso and the MCM in the male head at the L3 and L4 stages. e, Head of animals with sex-reversal genetic manipulations of AMso. Feminization by expression of the *grl-2::tra-1(ic)::SL2::mCherry* transgene *oleEx23*. Masculinization by expression of the *grl-2::fem-3::SL2::mCherry* transgenes *oleEx18* and *oleEx24*.

**Figure 6 | The male AMso cells are fully differentiated glia before and after the division that generates the MCM neuron.** Lateral views of animals oriented anterior to the left and dorsal to the top. a, AMso glial cell (green, *grl-2::gfp*) and amphid sensory neuron dendrites (red, *pdf-1::rfp*) in an L3 hermaphrodite and L3 and L4 males. b, Diagram of the AMso glial cell and the distal end of its projection to the nose, where it ensheaths the cilia of sensory dendrites. c, Expression in AMso and perdurance in MCM of reporter transgenes for glial markers in L3, L4 and adult males. *ptr-10* (Patch-receptor); *itr-1* (IP3 receptor); *grl-2* (Hedgehog-like/Ground-related).
or intrinsically, we expressed sex-reversing transgenes under cell-restricted promoters to uncouple the genetic sex of the AMso glial cells from that of the rest of the animal. We used the *gri-2* and *ztf-16* promoters, which drive expression in the AMso cells after they are born and one or two other non-overlapping cell types in the head in larval animals \(^3\),\(^5\),\(^6\). Masculinization of the AMso cells in hermaphrodites, by expression of a *fem-3* transgene \(^3\),\(^5\),\(^6\), resulted in AMso cell division and MCM neuronal differentiation (Fig. 5e, Extended Data Fig. 4b and Extended Data Table 4). Conversely, feminization of AMso cells in males, by overexpression of the *tra-2* intracellular domain (*tra-2IC*), \(^3\),\(^5\),\(^6\), resulted in lack of AMso cell division and lack of MCMs (Fig. 5e, Extended Data Fig. 4c and Extended Data Table 4). Masculinizing transgenes in males or feminizing transgenes in hermaphrodites had no effect on AMso development (Fig. 5e). These results indicate that the competence of the AMso glial cell to become a neural progenitor is specified intrinsically, and extend the battery of sexual dimorphisms regulated by the sex-determination pathway to the intrinsic properties of glia.

### The male AMso cells are fully differentiated glia

We found that the male AMso glial cells are fully differentiated specialized cells before they divide, indicating that the production of the MCMs from AMso involves a complete switch in terminal cell fate (Fig. 6). In hermaphrodites and adult males, the AMso cells are specialized glial cells that form a hole in the cuticle through which most amphid neurons contact the outside world \(^1\), (Fig. 6a, b). Hermaphroditic AMso cells also express a battery of glial-cell markers \(^5\),\(^8\). We observed that at the L3 stage, before their division, the male AMso cells display the same characteristics as the AMso of hermaphrodites and adult males: they project to the nose where they form a socket that ensheaths amphid neuron cilia (Fig. 6a, b), and they express AMso markers (Fig. 6c and Extended Data Table 5). Following AMso division, one daughter retains the AMso identity and the other becomes the MCM, which loses molecular and structural characteristics of glia (Extended Data Fig. 5) and, as described above, acquires a neuronal identity. Similar complete switches in terminal cell fates have been described as transdifferentiation events in *C. elegans* and other systems \(^1\),\(^3\),\(^9\)–\(^1\).

### Discussion

The production of the MCMs from AMso glial cells through a division that results in self-renewal and neuronal differentiation is equivalent to a snapshot of the reiterated neuronal differentiation events arising from radial glia cell divisions during vertebrate neurogenesis \(^4\). However, the extent to which vertebrate neural progenitors are fully differentiated glia remains unclear and, in particular, it is not known whether the support and progenitor functions of glia can be integrated within one cell \(^3\),\(^5\),\(^8\). Our results provide the first example of neurons arising from glia in a non-vertebrate organism and demonstrate that fully differentiated, functional glia can retain neural progenitor properties during normal development.

In *C. elegans* the best described transdifferentiation event is the direct conversion of the rectal epithelial cell *Y* into a neuron \(^9\),\(^4\). Other cell fate switches in *C. elegans* and other systems, which are not direct but require cell division, have also been suggested to be transdifferentiation events \(^1\),\(^0\),\(^1\). The complete cell fate switch we observe suggests that the production of neurons from glia may be a process of natural transdifferentiation. It will be interesting to determine whether direct and indirect transdifferentiations of this kind occur through similar molecular mechanisms.

Our findings provide a direct link between developmental and anatomical sexual dimorphism in higher-order processing areas of the brain and sexually dimorphic behavioural plasticity during learning. We have shown that sex differences in learning to require the production, late in development, of a male-specific class of cephalic interneurons that were not previously known to exist. These interneurons are incorporated into pre-existing, sex-shared circuits allowing the male to change behavioural priorities according to its new reproductive needs. The MCMs function to confer salience to previous mate-experience during male navigation. Based on their connectivity, we suggest that these interneurons may function in other chemosensory-plasticity behaviours that may be subject to sexual conditioning. The production of more neurons in males is also a mechanism to generate cognitive sexual dimorphism in the song-learning system of songbirds, where neural progenitors have a glial identity \(^4\). However, whether these neurons are a male-specific class and whether their progenitors are intrinsically sexually dimorphic is not known. Our findings indicate that the addition of sex-specific neurons is an effective way of remodelling brain circuits during sexual maturation and reveal a general, possibly ancient role for glia as neural progenitors to assemble circuits for higher-order processing.

**Online Content** Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper, references unique to these sections appear only in the online paper.

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METHODS

No statistical methods were used to predetermine sample size. No samples were excluded from analysis. For behavioural assays, investigators were blinded to the genotype or surgery of the animals during experiments and outcome assessment. Experiments testing response to secreted pheromones were randomized.

Strains. *him-5(e1490)* was used as the wild-type background strain. For behavioural assays, investigators were blinded to the genotype or surgery of the animals during experiments and outcome assessment. Experiments testing response to secreted pheromones were randomized.

Response to mate pheromones. Animals were isolated as L4 the night before the assay. For the assay, single males were placed with 30 mates (hermaphrodites) in a 20 mm food lawn. Males were tested until they responded to a mate or for three minutes, whatever happened first. A male was scored as responding to mate contact if it placed its tail ventral down on the mate's body and initiated the mating sequence by backing along the mate's body to make a turn. The response efficiency was calculated by dividing a response by the total number of contacts made with the mate before responding. If a male did not respond within three minutes, it was scored as having 0 efficiency. Males were scored blindly. The Mann–Whitney U–test was used for statistical analysis.

Turning and location of vulva. Single males (isolated the night before as L4) were tested on a 10 μl lawn of food (E. coli OP50) with 30 unc-51 hermaphrodites for 3 min. Upon response to contact, up to seven turns were scored as good or bad. Good turns: sharp ventral bend around the tip of the body of the mate, followed by uninterrupted scanning along the other side of the mate's body. Bad turns: stutter (stops moving backwards before turning and moves forward before continuing with backward move); wide (tail positioned in a loose bend when turning around the tip of the mate); swim off (continues backing after reaching the end of the mate's body and loses contact); or interrupted (after turning, the male does not continue backing along the mate's body). The proportion of good turns out of total turns was scored.

For location of vulva efficiency, the number of encounters with the vulva until it was first located was scored. The assay finished when the male located the vulva or after three minutes, whatever happened first. Location efficiency was scored as 1 divided by the total number of encounters.

The Mann–Whitney U–test was used for statistical analysis.

Fertility assays. A single rab-3::rfp transgenic male was left to mate with two unc-51 hermaphrodites on a 15-μl lawn of food (E. coli OP50) for 24 h and then removed. The hermaphrodites were transferred to a fresh plate every 24 h for a total of three days. The proportion of cross-progeny from total progeny was scored for three ablated individuals and three controls. t-test was used for statistical analysis.

Food-leaving and retention assays. As described in ref. 27.

Electron microscopy and serial reconstruction. Four samples were fixed by chemical fixation or high-pressure freezing and freeze substitution as previously described49. Ultrathin sections were cut using a RMC Powertome XL, collected onto grids, and imaged using either a Philips CM10 TEM or Zeiss Supra 40 FE-SEM. Sections were elastically aligned28 and volumetrically reconstructed using TrakEM2 (ref. 51). The MCM cell bodies were identified in the EM sections based on position and morphology and in comparison to similar hermaphrodite sections. This identity was further confirmed by the identification of a posterior projection that no other cell with its soma in the same region is known to project posteriorly. This was followed by serial tracing of the projections to establish their morphology and connectivity. Synaptic connectivity and skeleton diagrams were determined using Ealign21. Circuit diagrams of connectivity were generated using Cytoscape25. We estimated the anatomical strength of synaptic connectivity between two neurons by summing the number of serial sections where we counted synaptic connections.
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Extended Data Figure 1 | The MCMs are newly identified male-specific neurons. a, WormAtlas-style diagram depicting the morphology and position of one of the bilateral pair of MCM neurons in the head of a male worm and its projection within the nerve ring and along the ventral cord. b, Volumetric reconstruction of the MCML cell body and projection based on tracing of serial EM sections. c, Co-expression of transgenes for neuronal markers in the rab-3-positive cells identified as MCMs (indicated with dashed red circles). All photographs are lateral views of animals oriented anterior to the left and dorsal to the top except for ric-19, which are dorsal views. Transgenes are listed in Extended Data Table 1. pdf-1 (neuropeptide pigment dispersing factor); snb-1 (synaptobrevin); ida-1 (tyrosine phosphatase-like receptor, orthologue of mammalian phogrin); ric-19 (rab-2 effector); nca-1 (NALCN Na⁺ channel subunit); ccb-1 (voltage-gated Ca²⁺ channel subunit); unc-36 (voltage-gated Ca²⁺ channel subunit); inx-3 (gap junction innexin). D, dorsal; L, left; R, right; V, ventral. d, Diagram of the neurons that directly connect to and from the MCMs. Triangles, sensory neurons; octagons, interneurons and unidentified neurons. The thickness of the arrows is proportional to the anatomical strength of the connections (Extended Data Table 2).
Extended Data Figure 2 | The MCMs are not required for other male-specific behaviours. **a**, Response of intact and MCM-ablated males (inIts179(ida-1::gfp);him-8(e1489) and otIs356(rab-3::rfp)him-5(e1490)) to dilutions of ascaroside pheromones (Ascr). Graphs represent Tukey box plots of logarithmic transformations of the data; n, number of independent events (that is, entry in scoring region). t-test with Bonferroni correction was used for statistical analysis. ***P < 0.001; **P < 0.01; *P < 0.05; n.s., no statistically significant difference (P ≥ 0.05). **b**, Response efficiency to mate contact of intact, MCM-ablated and pdf-1(tm1996) mutant males measured as the proportion of responses out of total contacts with an hermaphrodite. Intact and MCM-ablated animals were inIts179(ida-1::gfp);him-8(e1489). Wild-type animals were him-5(e1490). A response indicates that the male placed its tail ventral down on the mate’s body and backed along it to make a turn. For **b–e**, n, number of individual animals tested. Error bars indicate s.e.m. Mann–Whitney U-test was used for statistical analysis. *P < 0.05; n.s., no statistically significant difference (P ≥ 0.05). **c**, **d**, Proportion of good turns (**c**) and location of vulva efficiency (**d**) of intact and MCM-ablated males (inIts179(ida-1::gfp);him-8(e1489) and otIs356(rab-3::rfp)him-5(e1490)). Wild-type animals were him-5(e1490). A response indicates that the male placed its tail ventral down on the mate’s body and backed along it to make a turn. For **c–d**, n, number of individual animals tested. Error bars indicate s.e.m. ***P < 0.001; n.s., no statistically significant difference (P ≥ 0.05). **e**, Fertility (measured as proportion of cross-progeny) of intact and MCM-ablated males (otIs356(rab-3::rfp)him-5(e1490)). For **e**, n, number of individual animals tested. Error bars indicate s.e.m. Mann–Whitney U-test was used for statistical analysis. *P < 0.05; n.s., no statistically significant difference (P ≥ 0.05). **f**, Mate-searching behaviour, measured as P_L values (probability of leaving food per hour) in the absence or presence of mates, of intact and MCM-ablated males (otIs356(rab-3::rfp)him-5(e1490)). n, number of individual animals tested. Two independent population assays were performed on different days. Maximum likelihood statistical analysis was used to compare P_L values. Error bars indicate s.e.m. ***P < 0.001; n.s., no statistically significant difference (P ≥ 0.05).
Extended Data Figure 3 | The MCMs arise from a division of the AMso glial cell. All photographs are lateral views of animals oriented anterior to the left and dorsal to the top. a, Fluorescent photographs showing the two cells expressing rnr-1::gfp co-labelled with the glial marker ptr-10::rfp and the neuronal marker rab-3::rfp in the head of males at the early and late L4 stages. The AMso and MCM cell bodies are indicated with dashed lines. b, Fluorescent images of the AMso cell body and its projection at two time points during cell division. Photos are overexposed for visualization of the projection, indicated by arrows. The chromosomes are labelled with a histone::rfp transgene, and the AMso cell body is indicated by dashed lines.
Extended Data Figure 4 | AMso plasticity is regulated by AMso genetic sex.

a, Diagram of the AMso and MCM lineage. b, c, Proportion of individuals with MCMs in control animals and animals expressing sex-reversing transgenes in AMso. b, AMso masculinization with grl-2::fem-3::SL2::mCherry transgenes (oleEx18 and oleEx24). c, AMso feminization with grl-2::tra-2IC::SL2::mCherry transgenes (oleEx19 and oleEx23) and ztf-16::tra-2IC::SL2::mCherry transgene oleEx22. MCM cell fate was identified with ida-1::gfp or rab-3::yfp reporter transgenes. In the head, the grl-2 promoter drives expression in AMso and the excretory duct and pore cells, and the ztf-16 glial enhancer drives expression in the AMso and amphid sheath glia. # indicates an independent transgenic array line for each manipulation. \( \chi^2 \) test was used for statistical analysis; ***P < 0.001; n.s., no statistical significant difference (\( P \geq 0.05 \)); \( n \) = number of animals scored.
Extended Data Figure 5 | The MCMs lose molecular and structural characteristics of glia after birth. a, Proportion of MCMs with presence of the glial marker ptr-10::myrRFP or the neuronal marker ida-1::gfp at different stages after MCM birth. b, Electron micrograph of a cross-section of an adult male head showing the MCM and AMso cell body ultrastructure. Neighbouring tissues are colour coded following WormAtlas (http://www.wormatlas.org/colorcode.htm). Purple (pharynx), muscle (green), hypodermis (light cream), AMso (amphid socket, pink). The dendrites of the amphid neurons (amphid bundle) are not colored.
### Extended Data Table 1 | Reporter transgenes for neuronal markers tested for MCM expression

| protein/function         | gene       | array                                                                 | MCM expression |
|--------------------------|------------|-----------------------------------------------------------------------|----------------|
| Neuronal GTPase          | rab-3      | otls291 (rab-3prom:: NLS YFP+rol-6), otls356(rab-3prom::rfp)           | + 62/62        |
| Neuronal GEF             | rgef-1     | evls111 (rGEFprom:: GFP)                                              | +              |
| Neuropeptide             | pdf-1      | myEx696 (pdf-1prom::RFP+unc-122::GFP)                                 | +              |
| Phogrin/Exocytosis       | ida-1      | lns179(ida-1prom::GFP)                                                | + 59/59        |
| Neuropeptide exocytosis  | ric-19     | otEx6173 (ric-19prom6::NLS-TagRFP) (gift from Hobert lab)             | +              |
| Syntaxin                 | unc-64     | otEx4553 (fosmid-based transcriptional reporter) (gift from Hobert lab) | +              |
| Synaptobrevin            | snb-1      | otEx5422 (fosmid-based translational reporter) (gift from Hobert lab)  | +              |
| Synaptotagmin            | snt-1      | otEx5924( fosmid-based transcriptional reporter) (gift from Hobert lab) | +              |
| Na\(^{+}\) channel subunit | nca-1  | hpEx528 (nca-1prom::GFP) (gift from Zhen lab)                        | +              |
| Ca\(^{2+}\) channel subunit | ccb-1 | sEX10836(T28F2.5prom::GFP+pCeh361)                                      | +              |
| Ca\(^{2+}\) channel subunit | unc-36 | sEx12293(C50C3.9prom::GFP+pCeh361)                                      | +              |
| Innexin                  | inx-3      | zwEx103 [inx-3prom::GFP +lin-15(+)]                                   | +              |
| VGLUT                    | eat-4      | otls388 (fosmid-based reporter)                                      | –              |
| Choline transporter      | cho-1      | otls323 (fosmid-based reporter)                                      | –              |
| VGAT                     | unc-47     | otls348[unc-47prom(300bp)::mCHOPT1::unc54-3'UTR), pha-1(+)]          | –              |
| Intraflagellar transport (marker for ciliated neurons) | osm-6   | mnls17(osm-6prom::osm-6::GFP)                                        | –              |
| Fatty acid hydroxylase (marker for the XXX cell) | daf-9 | dhls59(daf-9prom::GFP)                                                | –              |
| Octopamine synthesis     | tbh-1      | nlS107[tbh-1prom::GFP+lin-15(+)]                                      | –              |
| Neuropeptide             | flp-21     | nly80 (flp-21prom::GFP)                                               | –              |
| Nematocin receptor       | ntr-1      | lstEx33 [ntr-1prom::gfp]                                              | –              |
| Ca\(^{2+}\) channel subunit | cca-1   | sEx14060(C54D2.5aprom::GFP+pCeh361)                                    | –              |
| K\(^{+}\) channel subunit | unc-103 | rGE[unc-103prom::YFP + pBX1]                                          | –              |
| Innexin                  | inx-12     | zwEx112 [inx-12prom::GFP +lin-15(+)]                                  | –              |
| Innexin                  | inx-13     | zwEx113 [inx-13prom::GFP +lin-15(+)]                                  | –              |
| Innexin                  | inx-5      | zwEx105 [inx-5prom::GFP +lin-15(+)]                                   | –              |

+ indicates presence; – indicates absence. For integrated arrays, quantification is indicated. At least 15 animals were examined for each transgene. This Table cites refs 54–68.
### Extended Data Table 2 | MCM connectivity

| type     | presynaptic | postsynaptic | #synapses | #EM sections |
|----------|-------------|--------------|-----------|--------------|
| chemical | AVF         | MCM          | 16        | 61           |
| chemical | AVJ         | MCM          | 1         | 2            |
| chemical | AVM         | MCM          | 1         | 1            |
| chemical | BDU         | MCM          | 3         | 7            |
| chemical | CEM         | MCM          | 2         | 5            |
| chemical | CEP         | MCM          | 1         | 1            |
| chemical | EF          | MCM          | 6         | 35           |
| chemical | MCM         | ADA          | 1         | 3            |
| chemical | MCM         | ALM          | 2         | 3            |
| chemical | MCM         | AQR          | 2         | 3            |
| chemical | MCM         | AVB          | 29        | 61           |
| chemical | MCM         | AVF          | 1         | 2            |
| chemical | MCM         | AVH          | 1         | 2            |
| chemical | MCM         | BDU          | 1         | 3            |
| chemical | MCM         | CEM          | 1         | 1            |
| chemical | MCM         | CEM          | 5         | 8            |
| chemical | MCM         | CEP          | 1         | 1            |
| chemical | MCM         | EF           | 2         | 3            |
| chemical | MCM         | MCM          | 2         | 6            |
| chemical | MCM         | PVC          | 1         | 2            |
| chemical | MCM         | PVN          | 3         | 3            |
| chemical | MCM         | PVU          | 1         | 1            |
| chemical | MCM         | RIC          | 2         | 3            |
| chemical | MCM         | RID          | 1         | 3            |
| chemical | MCM         | RIF          | 11        | 23           |
| chemical | MCM         | RIM          | 3         | 7            |
| chemical | MCM         | RIPR         | 3         | 6            |
| chemical | MCM         | RMD          | 1         | 2            |
| chemical | MCM         | SIA          | 1         | 2            |
| chemical | MCM         | SMB          | 2         | 3            |
| chemical | MCM         | unk1        | 2         | 4            |
| chemical | MCM         | unk10       | 1         | 2            |
| chemical | MCM         | unk11       | 1         | 2            |
| chemical | MCM         | unk12       | 1         | 1            |
| chemical | MCM         | unk13       | 1         | 1            |
| chemical | MCM         | unk2        | 1         | 3            |
| chemical | MCM         | unk3        | 1         | 2            |
| chemical | MCM         | unk6        | 1         | 1            |
| chemical | MCM         | unk7        | 3         | 7            |
| chemical | MCM         | unk8        | 4         | 4            |
| chemical | MCM         | unk9        | 1         | 2            |
| chemical | PVC         | MCM          | 1         | 3            |
| chemical | PVQ         | MCM          | 1         | 4            |
| chemical | PVS         | MCM          | 1         | 2            |
| chemical | RIC         | MCM          | 1         | 1            |
| chemical | RIF         | MCM          | 10        | 20           |
| chemical | RIM         | MCM          | 3         | 3            |
| chemical | RMG         | MCM          | 1         | 2            |
| chemical | SMB         | MCM          | 2         | 3            |
| chemical | unk14      | MCM          | 3         | 15           |
| chemical | unk15      | MCM          | 1         | 5            |
| chemical | unk16      | MCM          | 1         | 1            |
| chemical | unk4       | MCM          | 2         | 3            |
| chemical | unk5       | MCM          | 1         | 1            |

*unk refers to neurons whose identity has not been confirmed unambiguously in the EM serial sections.*
## Extended Data Table 3 | Cell ablations of candidate MCM progenitors

| ablated cell           | stage | animals with loss of MCM/total |
|------------------------|-------|---------------------------------|
| AMso                   | L3    | 6/7                             |
| AMso                   | L4    | 0/3                             |
| H0 seam cell           | L1    | 1/16                            |
| H1 seam cell           | L1    | 0/2                             |
| H0+H1                  | L1    | 0/1                             |
| Hyp1 (lateral nucleus) | L1    | 0/4                             |
| H0+Hyp1 (lateral nucleus) | L1   | 0/1                             |
**Extended Data Table 4 | Mosaic analysis of sex-transformation arrays, scoring the presence of MCMs**

| sex reversal          | array                           | array in AMso and other cells | array in other cells only |
|-----------------------|---------------------------------|-------------------------------|---------------------------|
| Masculinised hermaphrodites | oleEx18 (gri-2prom::fem-3:SL2::mCherry) | 89% (n=28)                   | 0% (n=22)                 |
| Feminised males       | oleEx19 (gri-2prom::tra-2IC::SL2::mCherry) | 0% (n=39)                    | 100% (n=7)                |
| Feminised males       | oleEx22 (ztf-16prom::tra-2IC::SL2::mCherry) | 2.5% (n=40)                  | 87.5% (n=16)              |

*Expression of oleEx18 (gri-2::fem-3::SL2::mCherry) in the head was observed in AMso, excretory duct and pore cells and sometimes in the pharynx and/or hypodermis. Expression of oleEx19 (gri-2::tra-2IC::SL2::mCherry) in the head was observed in AMso, excretory duct and pore cells and sometimes in the hypodermis. Expression of oleEx22 (ztf-16::tra-2IC::SL2::mCherry) in the head was observed in AMso and sometimes in the amphid sheath and/or a neuron in the nerve ring.*
| protein/function | gene | array | glial subtype expression | AMso expression |
|------------------|------|-------|--------------------------|----------------|
| Patched (PTCHD3) related receptor | *ptr-10* | *nsls108[ptr-10prom::myristyl-Rfp]* | All glia | + |
| Hedgehog-like/Ground-related | *grf-2* | *sEx12852[T16G1.8prom::GFP+pCeh361]* | AMso and PHso | + |
| Inositol trisphosphate receptor | *itr-1* | *jwEx51( itr1promB::GFP+rol-6) and nsEx1153 [F16F9.3prom::mCherry+itr-1prom::CFP+rol-6(su1006)]* | AMso | + |
| C2H2 zinc-finger transcription factor | *ztf-16* | *oleEx22[ ztf-16enhancer::tra-2i::SL2cherry+elt-2::GFP] * | AMso, AMsh, PHso | + |
| Basic helix-loop-helix transcription factor | *hlh-17* | *leEx1713[ hlh-17prom::GFP+unc-119(+)]* | CEPsh | – |
| Caspr (Neurexin superfamily) | *itx-1* | *otEx[W03D8.6prom::GFP+rol-6]* | OL and IL glia | – |

+ indicates presence; – indicates absence. At least 15 animals were examined for each transgene. AMso, amphid socket; PHso, phasmid socket; AMsh, amphid sheath; PHsh, phasmid sheath; CEPsh, cephalic sensilla sheath; OLL, outer labial; IL, inner labial. This Table cites refs 34, 35, 69–72.