Male pheromones modulate synaptic transmission at the *C. elegans* neuromuscular junction in a sexually dimorphic manner

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Abstract The development of functional synapses in the nervous system is important for animal physiology and behaviors, and its disturbance has been linked with many neurodevelopmental disorders. The synaptic transmission efficacy can be modulated by the environment to accommodate external changes, which is crucial for animal reproduction and survival. However, the underlying plasticity of synaptic transmission remains poorly understood. Here we show that in *Caenorhabditis elegans*, the male environment increases the hermaphrodite cholinergic transmission at the neuromuscular junction (NMJ), which alters hermaphrodites’ locomotion velocity and mating efficiency. We identify that the male-specific pheromones mediate this synaptic transmission modulation effect in a developmental stage-dependent manner. Dissection of the sensory circuits reveals that the AWB chemosensory neurons sense those male pheromones and further transduce the information to NMJ using cGMP signaling. Exposure of hermaphrodites to the male pheromones specifically increases the accumulation of presynaptic CaV2 calcium channels and clustering of postsynaptic acetylcholine receptors at cholinergic synapses of NMJ, which potentiates cholinergic synaptic transmission. Thus, our study demonstrates a circuit mechanism for synaptic modulation and behavioral flexibility by sexual dimorphic pheromones.

Introduction Faithful synaptic transmission is essential for animal physiology and behaviors. The disturbance of synaptic transmission has been linked with several neurodevelopmental disorders, including autism.
spectrum disorders (ASD). In the past decades, researchers have identified numerous genes encoding synaptic proteins that are linked with neurodevelopmental disorders, and their mutations cause the dysregulated synaptic transmission in human diseases (Homoyzogosity Mapping Consortium for Autism et al., 2016; Autism Sequencing Consortium et al., 2019; Geisheker et al., 2017; Iossifov et al., 2012; Lee et al., 2019; Morrow et al., 2008; Neale et al., 2012; C Yuen et al., 2017), including SHANK3, NRXN, and NLGN for autism (Chen et al., 2020; Lee et al., 2015; Levinson and El-Husseini, 2005; Orefice et al., 2019; Sudhof, 2008), MECP2 for Rett’s syndrome (Chao et al., 2007; Orefice et al., 2019), FMR1 for Fragile X syndrome (Olmos-Serrano et al., 2010), and UBE3 for Angelman syndrome (Judson et al., 2016; Wallace et al., 2012).

The process of synaptogenesis occurs in the early postnatal developmental period and can be modulated by the environment. The effects of synaptic modulation could persist until adulthood and cause a lifelong impact. Various environmental contexts can modulate synaptic transmission and behaviors through experience-dependent plasticity, which provides a critical and conserved mechanism to generate animal behavior diversity and adaption. Among the environmental contexts, social interaction, such as the density of the conspecifics sharing the same habitat, represents one of the most important environmental conditions that modulate animal physiology and behaviors to meet the ever-changing environment and internal needs (Chen and Hong, 2018). For example, social isolation of rats during the critical period of adolescence enhances long-term potentiation of NMDA receptor-mediated glutamatergic transmission in the ventral tegmental area (Whitaker et al., 2013). Besides that, maternal separation has been found to have a profound lifelong influence on animal models at a mature stage of life. It causes habenula hyperexcitability, AMPA receptors delivery, and synaptic plasticity defects in the developing barrel cortex (Miyazaki et al., 2012; Tchenio et al., 2017). However, the underlying mechanism on how social interaction modulates synaptic transmission remains elusive.

There are many ways in which social interaction can influence neural development. Pheromone effects between conspecifics are strong drivers that modulate behaviors and alter physiology, allowing appropriate responses to particular social environments (Liberles, 2014). These effects are often sexually dimorphic. Mouse pups elicit parental care behaviors in virgin females, for instance, but promote infanticidal behaviors in virgin males through pheromonal compounds (submandibular gland protein C and hemoglobins) and physical traits (Isogai et al., 2018). In Caenorhabditis elegans (C. elegans), a family of glycolipids called ascarosides function as the pheromones to mediate social interactions. Males and hermaphrodites secrete several ascarosides in different amounts that elicit sexual dimorphic responses (Butcher et al., 2007; Edison, 2009; Greene et al., 2016; Srinivasan et al., 2008; Srinivasan et al., 2012). For example, the male-enriched ascr#10 induces attraction behavior in hermaphrodites, but causes aversion behavior in males (Izrayelit et al., 2012). However, it remains unclear whether and how specific pheromone-mediated effects are involved in neurodevelopmental processes, including synaptogenesis and synaptic transmission.

Here, we show that the male environment increases the cholinergic synaptic transmission at the neuromuscular junction (NMJ) in C. elegans hermaphrodites, decreasing hermaphrodite’s locomotion activity and promoting mating efficiency. The male-specific pheromones (ascarosides) mediate these effects in a sexually dimorphic manner. Such ascaroside-mediated modulation of the cholinergic synaptic transmission is developmental stage dependent. We further used various neuron-type-specific ablation experiments to confirm that these male-specific pheromone signals are received and processed by the AWB chemosensory neuron pair in hermaphrodites. Upon reception, AWB neurons transduce the information to the NMJ using cGMP signaling. Furthermore, we used multiple reporter fusion constructs to show that the male-specific pheromones cause increased calcium channel accumulation and acetylcholine receptor (AChR) clustering at cholinergic synapses. Collectively, our work elucidates how individuals sense and adapt to the social environment, providing insights into how pheromones regulate the development and function of the nervous system.
Results

The male environment modulates synaptic transmission at the hermaphrodite NMJ

C. elegans has two sexes: XX hermaphrodites and XO males. The somatically female hermaphrodites can produce hermaphrodite progeny by self-fertilization (although rare males are generated through spontaneous X chromosome loss), whereas in the presence of males, they are also able to mate with males to give rise to equal ratios of hermaphrodites and males (Figure 1A). Hermaphrodites generated by self-fertilization or by crossing share the same genetic background but develop in distinct environments (i.e., in the presence or absence of males). Therefore, it provides an excellent system to study how social interaction modulates the establishment and maintenance of synaptic transmission during development. We selected the C. elegans NMJ as a model to examine the male environment’s effects on synaptic transmission. The C. elegans NMJ includes body-wall muscles that receive synaptic inputs from both excitatory cholinergic and inhibitory GABAergic motor neurons (Richmond and Jorgensen, 1999). The coordination of excitatory and inhibitory innervations guarantees C. elegans sinusoidal movement. In the presence of acetylcholinesterase inhibitors such as aldicarb, the breakdown of acetylcholine is prevented, and acetylcholine accumulates over time at synapses. As a result, worms become paralyzed due to hyper-excitation (Mahoney et al., 2006). The timing of the paralysis is influenced by the inhibitory innervations from GABAergic neurons that counteract acetylcholine’s excitatory effect and delay paralysis. The percentage of paralyzed worms over time can be used as a measurement of excitatory versus inhibitory synaptic transmission ratio (E/I ratio) at the NMJ. As a result, the alteration of sensitivity to aldicarb reflects the changes in NMJ synaptic transmission (Vashlishan et al., 2008).

To determine whether the NMJ synaptic transmission differs between hermaphrodites generated through self-fertilization versus crossing, we applied aldicarb to young adult hermaphrodites and examined the percentage of paralyzed animals. We found that around 39.8% of hermaphrodites from self-fertilization were paralyzed after 70 min’ exposure to aldicarb. In contrast, almost all of the hermaphrodites from crossing were paralyzed (Figure 1B), indicating that hermaphrodites obtained by crossing are more sensitive to aldicarb. Thus, the NMJ E/I ratio is increased in crossed hermaphrodites than those obtained by self-fertilization.

There are three possible explanations for the observed differences in NMJ synaptic transmission in crossed hermaphrodites: first, it could be a parental inheritance effect, such as RNA transgenerational transmission (Alcazar et al., 2008; Rechavi et al., 2011); second, it could be caused by direct contact with males (Shi and Murphy, 2014); third, male metabolites secreted into the environment could modulate hermaphrodite development. To rule out the potential effects of parental inheritance and male contact, we directly exposed hermaphrodites from self-fertilization to medium conditioned by either the male or the hermaphrodite environment since egg stage. The conditioned medium was prepared by collecting cultures of him-5 mutants containing around 40% males (male-conditioned medium) or wild-type hermaphrodites alone (hermaphrodite-conditioned medium). Both conditioned media contain metabolites secreted by 30,000 young adult worms during 3 hr cultivation (Figure 1C). After growing hermaphrodites in the conditioned medium, we found that the hermaphrodites cultured in the male-conditioned medium became paralyzed earlier than those in the hermaphrodite-conditioned medium (80.31% vs. 61.11% paralyzed after 70 min’ exposure to aldicarb) (Figure 1D). This result suggests that the effect of the male environment on hermaphrodite NMJ is mediated by male-secreted metabolites. In the following experiments, we directly used the male-conditioned medium and hermaphrodite-conditioned medium unless otherwise specified.

We then analyzed muscle excitability as another independent measure of synaptic transmission changes at the NMJ. Previous work has shown that the body-wall muscle at the C. elegans NMJ receives both excitatory and inhibitory inputs from cholinergic and GABAergic neurons, respectively (Richmond and Jorgensen, 1999). When the excitatory and inhibitory synaptic transmission ratio increases at the NMJ, the excitability of muscle cells should increase. To verify the increased excitability of the body-wall muscle, we expressed the genetically encoded calcium indicator GCaMP3 in muscle cells (under the myo-3 promoter) and the channelrhodopsin variant Chrimson in VB and DB motor neurons (under the acr-5 promoter) (Figure 1E; Tian et al., 2009). Fluorescence changes reflect calcium influx and excitability in the GCaMP3-expressing cells. We found that the baseline GCaMP3 fluorescence is higher in hermaphrodites grown in the male-conditioned medium.

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Figure 1. The male excretome increases cholinergic synaptic transmission at hermaphrodite NMJ. (A) Schematic illustration of C. elegans reproduction. Hermaphrodites with two X chromosomes generate all hermaphrodite progeny via self-fertilization. While hermaphrodites are crossed with males that have a single X chromosome, an equal ratio of hermaphrodite and male offspring are generated. (B) Time course analysis of 1.4 mM aldicarb-induced paralysis in hermaphrodites generated from hermaphrodite self-fertilization (black, Self-fertilization) and hermaphrodite-male crossing (orange, Crossed). (C) Schematic illustration of conditioned medium preparation. 30,000 young-adult wild-type (WT) and him-5 mutant worms were collected and incubated in 1 ml ddH₂O for 3 hr. Metabolites secreted by hermaphrodites and males were collected and used to make hermaphrodite-conditioned (Herm-cond) and male-conditioned medium (Male-cond). (D) Time course analysis of Aldicarb-induced paralysis in hermaphrodites cultured in hermaphrodite-conditioned medium (black, Herm-cond) and male-conditioned medium (orange, Male-cond). (E) Schematic illustration showing calcium current recording at the C. elegans NMJ. Chrimson driven by the acr-5 promoter was expressed specifically in cholinergic motor neurons, and GCaMP3 under the myo-3 promoter was expressed in the body-wall muscle. (F–G) Chrimson-evoked calcium transients in body-wall muscle were analyzed using GCaMP3 as a calcium indicator. For adult hermaphrodites cultured in hermaphrodite-conditioned medium (black, Herm-cond) or male-conditioned medium (orange, Male-cond), the averaged responses (F) and the averaged and individual relative increase in GCaMP3 fluorescence intensity ΔF/F (G) are shown. The gray shadings in F indicate the SEM of GCaMP3 responses. The dashed line indicates when the illumination with nominal wavelength at 640 nm for Chrimson activation was applied. (H–J) Endogenous acetylcholine transmission was assessed by recording mEPSCs from body muscles of wild-type adult hermaphrodites cultured in hermaphrodite-conditioned or male-conditioned medium. Representative mEPSC traces (H), the mean mEPSC rates (I), and the mean mEPSC amplitudes (J) are shown. (K–M) Endogenous GABA transmission was assessed by recording mIPSCs from body muscles of wild-type adult hermaphrodites cultured in hermaphrodite-conditioned or male-conditioned medium. Representative mIPSC traces (K), the mean mIPSC rate (L), and the mean mIPSC amplitude (M) are shown. The data for individual animal.
compared with those grown in the hermaphrodite-conditioned medium, suggesting relatively higher resting muscle excitability (Figure 1—figure supplement 1). Moreover, we excited the VB and DB cholinergic motor neurons via optogenetic activation of Chrimson with red light (wavelength at 640 nm) (Klapoetke et al., 2014) and observed significantly increased GCaMP3 fluorescence intensity potentiation (assessed as ΔF/F) in hermaphrodites grown in the male-conditioned medium (Figure 1F–G). These results indicate that the male excretome environment causes increased excitatory and inhibitory synaptic transmission ratio and muscle excitability at the NMJ of hermaphrodites.

The acetylcholine transmission rate at the NMJ is potentiated by the male excretome environment

The increased E/I ratio could be caused by either increased cholinergic transmission or decreased GABAergic transmission. To distinguish between these two possibilities, we analyzed spontaneous miniature excitatory postsynaptic currents (mEPSCs) and miniature inhibitory postsynaptic currents (mIPSCs) at the NMJ. We found that the mEPSC frequency was significantly increased in hermaphrodites from male-conditioned medium compared to those from hermaphrodite-conditioned medium (Figure 1H,I), but the mEPSC amplitude was not changed (Figure 1H,J). When we examined inhibitory postsynaptic currents, we detected no significant differences in mIPSC frequency and amplitude between hermaphrodites from male- or hermaphrodite-conditioned medium (Figure 1K–M). The electrophysiology data suggest that potentiation of acetylcholine transmission rate mainly contributes to the observed increase in the E/I ratio at the NMJ of hermaphrodites in the male excretome environment.

The male excretome environment increases the hermaphrodite NMJ synaptic transmission during the juvenile stage

To delineate whether there are any critical developmental windows for the observed synaptic transmission modulation by the male environment, we transferred hermaphrodites to male-conditioned medium at a series of different developmental stages (egg, L1 [24 hr after egg], L2–L3 [36 hr after egg], and mid-L4 [48 hr after egg]). We then measured synaptic transmission in young adults with the aldicarb assay (Figure 2A). The hermaphrodites transferred to the male-conditioned medium at the egg, L1, and L2–L3 stage presented significantly increased sensitivity to aldicarb when they grow into young adult (Figure 2B, Figure 2—figure supplement 1A–C, 92.5% vs. 55.1% at 70 min for egg stage, 46.9% vs. 24.9% for the L1 stage, and 71.3% vs. 14.0% for L2–L3 stage). In contrast, we observed no differences in sensitivity to aldicarb between hermaphrodites transferred to male-conditioned medium at the mid-L4 stage and those from hermaphrodite-conditioned medium (Figure 2B, Figure 2—figure supplement 1D, 15.9% vs. 14.0% at 70 min). Those data suggest that exposure to the male excretome environment in L3–L4 stage is critical for modulation of the NMJ synaptic transmission in hermaphrodites.

To study whether the sustained male environment is required to maintain the cholinergic synaptic transmission potentiation at NMJ, we removed hermaphrodites from male-conditioned medium out of the male environment at L4 (48 hr after egg) and young adult (60 hr after egg). 24 and 12 hr later, we performed the aldicarb assay (Figure 2A,C, Figure 2—figure supplement 1E). We found that hermaphrodites leaving the male excretome environment at the young adult stage still showed an increased sensitivity to aldicarb compared with those in the hermaphrodite-conditioned medium (66.3% vs. 42.0% at 70 min). The effect was comparable to that in hermaphrodites sustained in the male environment (78.0% vs. 42.0%) (Figure 2C), suggesting that the maintenance of the elevated...
Figure 2. The male excretome modulates the hermaphrodite NMJ synaptic transmission in a developmental-stage-dependent manner. (A) Schematic illustration of the life cycles of *C. elegans* and the time when the hermaphrodite-conditioned medium (dashed black lines) or male-conditioned medium (solid orange lines) was applied. (B) The percentage of animals paralyzed on 1.4 mM aldicarb at 70 min were plotted for hermaphrodites cultured in male-conditioned medium (orange) starting from the egg stage, L1 stage, L2–L3 stage, and mid-L4 stage. Hermaphrodites cultured in hermaphrodite-conditioned medium (black) served as controls. (C) The percentage of animals paralyzed on 1.4 mM aldicarb at 70 min were plotted for hermaphrodites cultured in male-conditioned medium from the egg stage to the mid-L4 stage (L4 out) and young adult stage (Adult out); hermaphrodites cultured in hermaphrodite-conditioned medium (black) or male-conditioned medium (orange) served as controls. (D–F) Time course analysis of aldicarb-induced paralysis in hermaphrodites cultured in hermaphrodite-conditioned medium (black) and male-conditioned medium (orange) in CB4856 (D), AB3 (E), and TR389 (F) strains. (G) The percentage of animals paralyzed on 1.4 mM aldicarb at 80 min were plotted for N2 hermaphrodites cultured in N2 hermaphrodite (N2 herm-cond)-, N2 male (N2 Male-cond)-, TR389 hermaphrodite (TR389 Herm-cond)- or TR389 male (TR389 Male-cond)-conditioned medium. (H) Locomotion behavior analysis of single adult hermaphrodite cultured in N2 hermaphrodite (Herm-cond)-, TR389 male (TR389 Male-cond)-, and N2 male (Male-cond)-conditioned medium. The averaged and individual crawling locomotion velocities were plotted. (I) Measurement of hermaphrodite mating efficiency cultured in N2 hermaphrodite-, TR389 male-, and N2 male-conditioned medium. In B–I, *p<0.05, ***p<0.001, ns not significant, two-way ANOVA with post hoc Sidak multiple comparisons for (B–C) and (G), two-way ANOVA comparing all of the time points for (D–F), one-way ANOVA with post hoc Dunnett multiple comparisons for (H and I).
cholinergic synaptic transmission rate at the hermaphrodites NMJ does not require a sustained male excretome environment in adults. In contrast, we observed that the hermaphrodite leaving the male-conditioned medium at the mid-L4 stage presented similar aldicarb sensitivity to those from the hermaphrodite-conditioned medium (37.7% vs. 42.0% at 70 min) (Figure 2C, Figure 2—figure supplement 1E). Taken together, these data support the notion that the male environment exposure at a critical period (the L3–L4 stage) is required for the modulation of hermaphrodites NMJ cholinergic synaptic transmission.

The aforementioned experiments were carried out using the Bristol N2 strain. To determine whether the male excretome environment’s effect is conserved in other *C. elegans* strains, we studied several natural variations, including the Australian strain AB3, the Hawaiian strain CB4856, and the Madison strain TR389. We observed that the male-conditioned medium accelerated animal paralysis in the CB4856 (Figure 2D, 59.7% vs. 20.7% after 60 min of aldicarb) and the AB3 strains (Figure 2E, 52.8% vs. 28.6% after 80 min of aldicarb), but not in the TR389 strain (Figure 2F, 68.46% vs. 60.99% after 130 min of aldicarb). Thus, although the effect of the male environment does exist in other natural *C. elegans* strains, exceptions do exist, as in the TR389 strain.

Two possibilities could account for this lack of a modulator effect: TR389 males may not be able to secrete the modulator ascarosides; alternatively, TR389 hermaphrodites cannot sense and respond to the modulator ascarosides. To distinguish between these two possibilities, we grew Bristol N2 hermaphrodites in the TR389 male-conditioned medium and compared their synaptic transmission by aldicarb sensitivity with those maintained in N2 and TR389 hermaphrodite-conditioned medium. The three groups presented similar sensitivity to aldicarb (Figure 2G). In contrast, TR389 hermaphrodites grown in the N2 male-conditioned medium showed significantly increased sensitivity to aldicarb compared to those in the N2 hermaphrodite-conditioned medium (Figure 2—figure supplement 2). Thus, TR389 males appear unable to secrete the modulator ascarosides.

**The male excretome environment alters hermaphrodite locomotion and promotes mating efficiency**

As mentioned above, the coordination of excitatory and inhibitory innervations at NMJ guarantees *C. elegans* sinusoidal movement. To study whether the altered cholinergic synaptic transmission impacts body-bend amplitude and coordination of animal movement, we compared the locomotion of hermaphrodites from male- or hermaphrodite-conditioned medium. We observed that males had higher body curvature and locomotor velocity than hermaphrodites (Figure 2—figure supplement 3A–B), consistent with previous studies (Mowrey et al., 2014). We did not observe body-bend curvature differences in hermaphrodites from male- and hermaphrodite-conditioned medium (Figure 2—figure supplement 3C–D). However, the locomotor velocities of hermaphrodites from male-conditioned medium are significantly lower than those from hermaphrodite-conditioned medium (Figure 2H). In contrast, the TR389 male-conditioned medium did not show similar effects (Figure 2H). This supports the notion that the altered NMJ synaptic transmission by the male excretome affects hermaphrodite locomotion. It is possible that the disturbance of excitatory and inhibitory synaptic transmission balance at NMJ compromise locomotion activity.

Communications between conspecifics modulate behaviors and alter physiology to allow appropriate responses to particular social environments. To study the physiological significance of male...
excretome modulation, we tested its effect on hermaphrodite’s egg-laying behaviors and mating abilities. We calculated the brood size of hermaphrodites from hermaphrodite- and male-conditioned medium, and observed no significant differences between the two groups (Figure 2—figure supplement 4). Then we measured the mating efficiency with males in hermaphrodites from male- and hermaphrodite-conditioned medium. Two young adult stage hermaphrodites from male- or hermaphrodite-conditioned medium were cultured with two young-adult males for 24 hr. Successful mating was scored when more than three male progenies were generated in the mating plate. The results showed that hermaphrodites from TR389 male-conditioned medium had higher mating efficiency compared to those from hermaphrodite-conditioned medium (Figure 2I), which is consistent with previous research that the male environment reduces hermaphrodite exploration and increases mating behaviors (Aprison and Ruvinsky, 2019a; Aprison and Ruvinsky, 2019b). Interestingly, we found that N2 male-conditioned medium showed a significant further increase of hermaphrodite mating efficiency than the TR389 male-conditioned medium (Figure 2I). We speculate that the N2 males secrete additional metabolites to modulate locomotion and mating efficiency in hermaphrodites.

Male-specific ascarosides mediate the modulatory effect of the male excretome environment on the hermaphrodite NMJ synaptic transmission

To identify the additional metabolites secreted by the N2 males, we focused on searching the male pheromones. In C. elegans, ascarosides are known to function as pheromones to mediate social interactions and modulate development (Butcher et al., 2007; Butcher et al., 2009; Ludewig et al., 2019; Ludewig and Schroeder, 2013; Srinivasan et al., 2008; Wu et al., 2019). We hypothesized that the observed effects of the male environment on hermaphrodite cholinergic synaptic transmission at the NMJ may be mediated by male-specific ascarosides. Ascarosides are derivatives of 3,6-dideoxysugar ascarylose, and their biosynthesis requires several dehydrogenases, including DAF-22, which β-oxidizes and shortens long-chain fatty acids to generate bioactive medium- and short-chain ascarosides (Figure 3A; Butcher et al., 2009; von Reuss et al., 2012; Zhou et al., 2018). Therefore, most of the active short- and medium-chain ascarosides are absent from the metabolomes of daf-22 mutants (Butcher et al., 2009; von Reuss et al., 2012; Zhou et al., 2018).

To test whether the effect of the male environment on hermaphrodite NMJ synaptic transmission is mediated by ascarosides, we grew hermaphrodites in daf-22 conditioned medium and compared their aldicarb sensitivity to hermaphrodites grown in the wild-type conditioned medium. We found that the hermaphrodites grown in the daf-22 male-conditioned medium exhibited similar aldicarb sensitivity with those grown in daf-22 hermaphrodite-conditioned medium (Figure 3B, 43.7% vs. 43.7% at 70 min). The inability of the daf-22 male environment to modulate hermaphrodite NMJ synaptic transmission suggests that male-specific ascarosides do contribute to the observed modulatory effects on synaptic transmission.

The pheromone effects are often sexually dimorphic. To study whether the male-specific ascarosides also modulate male NMJ synaptic transmission, we compared the aldicarb sensitivity of males grown in the hermaphrodite- and male-conditioned media. We did not observe aldicarb sensitivity differences in males from hermaphrodite- and male-conditioned medium (Figure 3—figure supplement 1A). Since males can secrete those modulatory pheromones themselves, we took advantage of daf-22 mutant males that have the defects in pheromone production. We also found that daf-22 males from the male-conditioned medium did not show any significant differences in aldicarb sensitivity compared to those from the hermaphrodite-conditioned medium (Figure 3C). In contrast, the daf-22 hermaphrodites showed higher aldicarb sensitivity from the male-conditioned medium compared to those from the hermaphrodite-conditioned medium (Figure 3—figure supplement 1B), suggesting daf-22 mutation did not alter the modulatory effect of male-conditioned medium on hermaphrodites. These results indicate that male-specific ascarosides cannot modulate synaptic transmission in males, suggesting a sexually dimorphic effect of those male-specific ascarosides.

The C. elegans ascarosides comprise a complex mixture of ascaroside derivatives that vary according to their side chains; there are saturated, α,β-unsaturated (e.g., α,β double-bond), and β-hydroxylated (e.g., β-hydroxylated side chain) derivatives. Hermaphrodites and males are known to accumulate distinct types and quantities of these various ascarosides (Butcher et al., 2009; von Reuss et al., 2012). To identify the ‘modulator ascarosides’ that function in the observed
Figure 3. Ascarosides in the male environment modulate hermaphrodite NMJ synaptic transmission. (A) Proposed model of peroxisomal β-oxidation enzymes ACOX-1, MAOC-1, DHS-28, and DAF-22 in ascaroside side-chain biosynthesis. (B) The percentage of animals paralyzed on 1.4 mM aldicarb at 70 min were plotted for N2 hermaphrodites cultured in hermaphrodite (N2 Herm-cond)-, male (N2 Male-cond)-, daf-22 mutants herm (daf-22 Herm-cond)-, or daf-22 mutant male (daf-22 Male-cond)-conditioned medium. (C) The percentage of animals paralyzed on 0.5 mM aldicarb at 100 min were plotted for daf-22 mutant males cultured in hermaphrodite (N2 Herm-cond)-, male (N2 Male-cond)-, daf-22 mutants herm (daf-22 Herm-cond)-, or daf-22 mutant male (daf-22 Male-cond)-conditioned medium. (D) Schematic illustration of excretome preparation for UPLC-MS. Around 30,000 freshly starved worms were cultured in S medium supplemented with concentrated OP50 for 7 days. The excretomes were collected by centrifugation, filtration, and lyophilized extraction, followed by UPLC-MS analysis. (E) β-hydroxylated ascaroside profiles in excretomes obtained from N2 hermaphrodites (N2 herm excretome), N2 mixed-gender animals of him-5 mutants (N2 male excretome), and TR389 mixed-gender animals (TR389 male excretome). (F) The percentage of animals paralyzed on 1.4 mM aldicarb at 90 min were plotted for β-oxidation mutants (acox-1.1, maoc-1, and dhs-28). (G) The percentage of animals paralyzed on 1.4 mM aldicarb at 70 min were plotted for N2 and dhs-28 mutant hermaphrodites cultured in hermaphrodite-conditioned medium (Herm-cond), male-conditioned medium (Male-cond). In (B–C), (E–G), *p<0.05, **p<0.01, ***p<0.001, ns not significant, two-way ANOVA with post hoc Sidak multiple comparisons for (B–C) and (F–G), one-way ANOVA with post hoc Dunnett multiple comparisons for (E).

The online version of this article includes the following source data and figure supplement(s) for figure 3:

Source data 1.
Figure supplement 1. The male environment cannot modulate NMJ synaptic transmission in males.
Figure supplement 1—source data 1.
Figure supplement 2. UPLC-MS analysis of excretome from animal cultures.
Figure supplement 2—source data 1.
modulation of the hermaphrodite NMJ synaptic transmission, we first analyzed the TR389 strain, which recalls appearing unable to secrete the modulator ascarosides.

To further determine the identity of the modulator ascarosides, we used ultra-performance liquid chromatography–mass spectrometry (UPLC-MS) analyses to compare the excretomes among N2 hermaphrodite cultures (containing N2 hermaphrodites only), N2 male cultures, daf-22 male cultures, and TR389 male cultures (all of the male cultures contains around 35% males) (Figure 3D). We collected and analyzed culture media samples with UPLC-MS and found that ascr#10 was enriched in both the N2 male and TR389 male cultures relative to the N2 hermaphrodite cultures (Figure 3—figure supplement 2A), consistent with previous reports (Izrayelit et al., 2012). We also observed that the daf-22 male cultures lacked most of the short- and medium-chain ascarosides, and accumulated the long-chain ascarosides (Figure 3—figure supplement 2B), confirming the role of DAF-22 in dehydrogenating and shortening ascaroside side chains.

Next, reasoning that the modulator ascarosides should be enriched in N2 male-conditioned culture, we compared the UPLC-MS profiles of N2 male cultures with the N2 hermaphrodites and the TR389 male cultures. The medium-chain β-hydroxylated ascarosides were substantially increased in the N2 male cultures compared to the N2 hermaphrodite cultures and TR389 male cultures. Specifically, the significantly enriched β-hydroxylated ascarosides in N2 males included C13, C14, and C15 ascarosides (Figure 3E). Notably, we detected no significant changes between the N2 and TR389 male cultures for saturated ascarosides (Figure 3—figure supplement 2C). These results implicate that the medium-chain β-hydroxylated ascarosides may act as the male modulator ascarosides.

Pursuing this with a genetic approach, we acquired a mutant of the known ascaroside synthesis enzyme DHS-28; previous analysis of the dhs-28 mutant hermaphrodite metabolome has shown that these animals accumulate β-hydroxylated medium-chain ascarosides (Butcher et al., 2009; von Reuss et al., 2012). We conducted aldicarb assays to compare the E/I ratios of dhs-28 cultures with those of N2 hermaphrodites grown in the hermaphrodite-conditioned medium. As expected, dhs-28 mutant hermaphrodites were more sensitive to aldicarb compared with N2 hermaphrodites (Figure 3F). We also tested hermaphrodites of other known ascaroside synthesis mutants, including maoc-1 and acox-1.1 – which are known to accumulate saturated and α,β-unsaturated side-chain ascarosides – but found that maoc-1 mutants present slightly increase of aldicarb sensitivity than the wild type, and acox-1.1 mutants were indifferent from the wild type for their sensitivity to aldicarb (Figure 3F). Furthermore, we examined the male ascaroside effects on dhs-28 mutants, which could accumulate β-hydroxylated medium-chain ascarosides themselves. The result showed that dhs-28 hermaphrodites cannot be modulated by male ascarosides by presenting comparable aldicarb sensitivity when in hermaphrodite- and male-conditioned medium (Figure 3G). These experiments with ascaroside biosynthesis mutants establish that environmental enrichment of β-hydroxylated medium-chain ascarosides increases the hermaphrodite NMJ E/I ratio, thereby supporting that these specific ascarosides may function as NMJ cholinergic synaptic transmission modulators.

**AWB sensory neurons are involved in sensing the modulator ascarosides and transmit signals to the NMJ through cGMP signaling**

Pheromone signals in the environment are detected and integrated by chemosensory neural circuits (Ludewig and Schroeder, 2013; Srinivasan et al., 2008). In *C. elegans*, there are 11 pairs of chemosensory neurons that can respond to pheromone signals (ASE, AWC, AWA, AWB, ASH, ASI, ADF, AGS, ASJ, ASK, and ADL). To identify the specific chemosensory neurons sensing the modulator ascarosides, we used a miniSOG (mini Singlet Oxygen Generator)-induced genetic ablation strategy. miniSOG is an engineered fluorescent protein that can generate singlet oxygen upon blue light illumination. Targeting miniSOG to mitochondria can lead to singlet oxygen accumulation in mitochondria, which induces rapid and efficient cell death (Qi et al., 2012). To examine the genetic ablation efficiency, we coexpressed mCherry and miniSOG in the chemosensory neurons under the control of the flp-21 promoter and quantified the miniSOG ablation efficiency based on the percentage of live neurons labeled by mCherry before and after induction of cell death. To optimize the ablation protocol, we tested continuous blue light stimulation at a power of 57 mW/cm² over different periods (Figure 4—figure supplement 1A). We found that 15 min’ stimulation resulted in complete loss of mCherry signals in around 47.8% of neurons and a dramatic reduction of mCherry signals in 26.1% of neurons, whereas stimulation for 30 min or 50 min led to complete loss of mCherry signals in almost 80% of neurons and faint residual expression of mCherry signals in 20% of neurons.
We screened all the 11 pairs of chemosensory neurons based on miniSOG-induced genetic ablation of hermaphrodites at the late L1 stage (Figure 4A). We grew hermaphrodites in male-conditioned or hermaphrodite-conditioned medium following ablation of each specific chemosensory neuron type, and measured their sensitivity to aldicarb. Ablation of the AWB (str-1 promoter driving miniSOG) neuron pair in hermaphrodites blocked the increased sensitivity to aldicarb following exposure to the male-conditioned medium (Figure 4B,C, 45.6% vs. 43.4% at 80 min). In contrast, the increased aldicarb sensitivity in the male-conditioned medium remained when any other chemosensory neurons were ablated (Figure 4A, Figure 4—figure supplement 2A–G). To further confirm the requirement of AWB in sensing the modulator ascarosides, we compared the locomotion of AWB-ablated hermaphrodites from male- and hermaphrodite-conditioned medium. Our data showed that ablation of AWB neurons decreased the locomotion velocity in hermaphrodites from the hermaphrodite-conditioned medium. In addition, ablation of AWB neurons blocked the decreased velocity by the modulator ascarosides (Figure 4D). These results support that AWB neurons in hermaphrodites are necessary for the effects of male-specific modulator ascarosides on NMJ synaptic transmission.

We also tested whether activation of AWB neurons is sufficient to modulate NMJ synaptic transmission. We specifically expressed the channelrhodopsin variant CHIEF in AWB neurons and administered blue light illumination in the presence of all-trans retinal (ATR) to activate AWB neurons throughout the L4 stage. We observed decreased aldicarb sensitivity in animals fed with ATR (ATR+ light- vs. ATR– light–). Nevertheless, hermaphrodites with activated AWB neurons during the L4 stage showed higher sensitivity to aldicarb than controls without blue light activation (ATR+ light+ vs. ATR+ light–) (Figure 4E). This effect is absent in the groups lacking ATR (ATR– light+ vs. ATR– light–) (Figure 4E). In contrast, activation of ASJ/ASI neurons or other amphid wing neurons like AWA and AWC cannot increase aldicarb sensitivity, and the hermaphrodites with AWA neurons activation even present slightly decreased aldicarb sensitivity (Figure 4—figure supplement 3). These findings confirm that activation of the AWB chemosensory neuron pair in hermaphrodites is sufficient to modulate the NMJ synaptic transmission.

In order to test whether AWB neurons directly sense those modulator ascarosides, we monitored intracellular \( \text{Ca}^{2+} \) dynamics upon male excretomes stimulation by expressing the calcium indicator GCaMP6f in AWB neurons. We found that the AWB neurons elicited a rapid and robust calcium transient responding to the male excretomes. However, no responses were detected by stimulation with the hermaphrodite excretomes (Figure 4F–H). Collectively, the data support that AWB neurons directly respond to the male-specific modulator ascarosides.

We next explored which signaling molecules in AWB neurons mediate their responsivity to the modulator ascarosides. In C. elegans, most chemical odors are perceived upon their binding to specific G-protein coupled receptors (GPCRs) located in chemosensory neurons; these receptors subsequently activate downstream signaling cascades (Bargmann, 2006; Li and Liberles, 2015; Liberles, 2014). The G protein ODR-3 and the cGMP-gated channels TAX-2 have been implicated in chemosensory signal transduction in AWB neurons. We first examined the NMJ E/I ratio in tax-2 and odr-3 mutant hermaphrodites. We observed no differences in sensitivity to aldicarb for tax-2 mutant hermaphrodites upon exposure to the male-conditioned or hermaphrodite-conditioned media (Figure 4I). In the odr-3 mutants, we even observed a decreased aldicarb sensitivity in hermaphrodites from male-conditioned medium (Figure 4J), suggesting that the ability to mediate the downstream signaling effects of modulator ascarosides and increase NMJ E/I ratio is disrupted in these mutants (Figure 4I,J). Further supporting this, complementing TAX-2 expression in AWB neurons rescued the increased aldicarb sensitivity phenotype of hermaphrodites grown in the male-conditioned medium (Figure 4I). Expression of TAX-2 in ASI and ASJ neurons had no such rescue effect (Figure 4I). The increased sensitivity to aldicarb was also rescued by ODR-3 complementation in AWB neurons (Figure 4J). Together, these results establish that the cGMP signaling pathway in AWB chemosensory neurons transmits male-specific modulator ascaroside signals to the NMJ.
Figure 4. AWB neurons sense the modulator ascarosides. (A) The table lists all of the chemosensory neurons examined in the screen, the neuron-specific promoters used to drive miniSOG expression, and the impact of neuron ablation on sensing of modulator ascarosides. (B) Representative images showing that mCherry-labeled AWB neurons were specifically ablated by blue light-induced miniSOG activation. Scale bar, 40 μm. (C) The percentage of animals paralyzed on 1.4 mM aldicarb at 80 min were plotted for hermaphrodites expressing miniSOG in AWB neurons (str-1 promoter) with and without blue-light-induced ablation. Black: cultured in hermaphrodite-conditioned medium; Orange: cultured in male-conditioned medium. (D) Locomotion behavior analysis of single adult worm from AWB-ablated hermaphrodites in hermaphrodite- and male-conditioned medium. The averaged crawling locomotion velocities were plotted. (E) The percentage of animals paralyzed on 1.4 mM aldicarb at 90 min were plotted for hermaphrodites with AWB neurons optogenetically activated during the L4 stage. The channelrhodopsin variant CHIEF was expressed in AWB chemosensory neurons driven by the str-1 promoter. The blue light was turned on to excite AWBs in transgenetic animals fed with or without ATR. (F) Top: snapshots of GCaMP6f fluorescent signals of an AWB neuron before, during, and after addition of male excretome. Scale bar, 10 μm. Bottom: the calcium trace showing the AWB neuron activated by male excretome. (G) Curves and average intensities of Ca^{2+} signals evoked by the hermaphrodite or male excretome in the soma of AWB with GCaMP6f expression. The shaded box represents the addition of the hermaphrodite or male excretome. (H) Scatter diagram and quantification of the Ca^{2+} change. Each point represents Ca^{2+} peak value from one animal. (I) The percentages of animals paralyzed on 1.4 mM aldicarb at 80 min were plotted for tax-2(p691) mutant hermaphrodites and TAX-2 expression restored in AWB or ASJ/ASI neurons cultured in hermaphrodite- (black) and male-conditioned medium (orange). (J) The percentages of animals paralyzed on 1.4 mM aldicarb at 80

Figure 4 continued on next page

Qian et al. eLife 2021;10:e67170. DOI: https://doi.org/10.7554/eLife.67170

12 of 29

Neuroscience
Excitatory postsynaptic receptor clustering is increased in hermaphrodites exposed to the male environment

The steps of the synaptic transmission process include presynaptic vesicle fusion, neurotransmission, and neurotransmitter binding to postsynaptic receptors. The increased cholinergic synaptic transmission rate at the hermaphrodite NMJ induced by the modulator ascarosides could reflect changes in any of these steps. We first examined whether any NMJ synaptic structures were altered, specifically by labeling cholinergic synapses via expression of a RAB-3-GFP fusion protein in DA and DB cholinergic motor neurons (using the *unc-129* promoter) (Colavita et al., 1998). DA and DB neurons are known to receive synaptic inputs in the ventral nerve cord and to form NMJs with the body-wall muscle in the dorsal nerve cord, and this results in the formation of puncta comprising presynaptic RAB-3 proteins that can be observed at DA/DB axon terminals in the dorsal cord (Colavita et al., 1998).

We observed that puncta fluorescence intensities and densities were comparable in hermaphrodites grown in either hermaphrodite- or male-conditioned medium (Figure 5A), which suggested that the excitatory synapse structures were unaltered by the presence of modulator ascarosides. We next labeled the GABAergic motor neuron terminals by expressing RAB-3 fused with RFP under the *unc-25* promoter (Jin et al., 1999). Similar to the excitatory cholinergic synapses, the puncta fluorescence intensities and densities in the inhibitory GABAergic synapses did not differ between hermaphrodites from male-conditioned or hermaphrodite-conditioned medium (Figure 5B), which collectively suggest that neither excitatory nor inhibitory synapse structures are affected by the modulator ascarosides.

We then examined the extent of excitatory and inhibitory postsynaptic receptor localization in hermaphrodites by analyzing the subcellular distributions of nicotinic acetylcholine receptors (nAchRs; excitatory) and GABA_A receptors (inhibitory). A single-copy transgenic insertion technique was applied to express fluorescence reporter fusion variants of two known nAchR subunit proteins (UNC-29-RFP and ACR-16-RFP) or a GABA_A receptor subunit (UNC-49-mCherry) under the control of a muscle-specific promoter. At cholinergic synapses, the hermaphrodites from the male-conditioned medium had a slight but significant increase in puncta signal intensities for the nAchRs compared to those from the hermaphrodite-conditioned medium (Figure 5C,D). However, the GABA_A R intensities were not changed (Figure 5E). Both the nAchRs and GABA_A R densities were unaltered (Figure 5C–E). Thus, the male-specific modulator ascarosides are involved in increased postsynaptic receptor abundance at excitatory synapses in hermaphrodites.

Presynaptic CaV2 calcium channel localization at NMJ cholinergic synapses is increased in hermaphrodites exposed to the male environment

Next, we examined if the process of presynaptic neurotransmission is regulated based on the fact that the mEPSC frequency was increased in hermaphrodites by the male-specific pheromones (Figure 1H–J). N-type voltage-gated calcium channels (CaV2) are required for the presynaptic calcium influx process that underlies both excitatory and inhibitory neurotransmission (Liu et al., 2018; Tong et al., 2017). Therefore, we inspected CaV2 calcium channel localization and abundance at...
Figure 5. Modulator ascarosides promote postsynaptic AchR synaptic localization. (A) The puncta fluorescence intensities and densities marked by the excitatory synaptic GFP::RAB-3 (under unc-129 promoter) in dorsal nerve cord axons were unaltered by modulator ascarosides. Representative images (top panel), mean puncta intensities and puncta density (bottom panel) are shown for hermaphrodites grown in hermaphrodite- or male-conditioned medium. (B) The puncta fluorescence intensities and densities marked by the inhibitory synaptic RFP::RAB-3 (under unc-25 promoter) were unaltered in Figure 5 continued on next page.
hermaphrodites cultured in male-conditioned medium. Representative images (top), mean puncta intensities and puncta density (bottom) are shown. (C–E) The muscle-specific ACR-16::RFP, UNC-29::RFP, and UNC-49::RFP fluorescence intensities and densities in hermaphrodites cultured in hermaphrodite- and male-conditioned medium. Representative images, mean puncta intensities and puncta density are shown separately. Scale bars, 5 μm. **p<0.005, ***p<0.001, ns not significant, unpaired Student’s t-test.

The online version of this article includes the following source data for figure 5:

Source data 1.

presynaptic elements in hermaphrodites grown in either hermaphrodite- or male-conditioned medium. To visualize endogenous CaV2 at excitatory or inhibitory synapses separately, we utilized the split GFP complementary system (Cabantous et al., 2005; Kamiyama et al., 2016). In C. elegans, UNC-2 encodes the CaV2 calcium channel α subunit, and we used CRISPR/Cas9 system to insert a sequence coding for seven GFP11 fragments at the C-terminus of UNC-2/CaV2. In parallel, the GFP 1–10 fragment was constitutively expressed in DA and DB cholinergic motor neurons under the control of the unc-129 promoter or in the GABAergic motor neurons under the control of the unc-47 promoter (Figure 6A). In this way, we were able to monitor the endogenous localization of CaV2 channels at excitatory and inhibitory synapses. To validate the correct subcellular localization, we co-expressed the presynaptic marker UNC-57/Endophilin fused with mCherry. The CaV2-GFP fusion protein formed fluorescent puncta largely co-localized with UNC-57/Endophilin in dorsal cord axons (Figure 6B,C, Pearson correlation coefficient 0.7808 ± 0.022 for DA/DB cholinergic motor terminals, and 0.7880 ± 0.0175 for GABAergic motor neuron terminals), confirming that CaV2-splitGFP is localized correctly at presynaptic elements. We further found that UNC-57/endophilin fluorescence intensities and densities were indistinguishable in hermaphrodites from the hermaphrodite- and male-conditioned medium (Figure 6—figure supplement 1). This result is consistent with RAB-3-GFP imaging results and support that the presynaptic structure is not altered by male pheromone (Figure 5A,B).

Comparison of the CaV2 puncta fluorescence intensities revealed a significant increase at cholinergic synapses of hermaphrodites from male-conditioned medium compared to those from the hermaphrodite-conditioned medium (Figure 6D). A slight but notable increase in densities was also observed (Figure 6D). In contrast, we detected no significant differences in CaV2 puncta fluorescence intensities and densities at GABAergic synapses (Figure 6E).

To further confirm that CaV2 is the synaptic target of modulator ascarosides, we compared the cholinergic synaptic transmission and locomotion velocity in unc-2 hermaphrodites from male- and hermaphrodite-conditioned medium. The mEPSC rate and locomotion velocity in the unc-2 mutant were decreased compared to those in the wild type (Figure 7A,B), which is correlated with the requirement of CaV2 in mediating presynaptic transmission. Furthermore, we found that the male-specific ascarosides no longer increase mEPSC rates in the unc-2 hermaphrodites (Figure 7A–C). Similarly, the locomotion velocity was not changed in unc-2 hermaphrodites from male-condition medium compared to those from hermaphrodite-condition medium (Figure 7D), which suggests that unc-2 mutation blocks the effects of the male-specific modulator ascarosides on NMJ synaptic transmission. These findings collectively indicate that the male-specific modulator ascarosides may promote the accumulation of CaV2 calcium channels at excitatory cholinergic synapses, accounting for the potentiated cholinergic synaptic transmission at NMJ.

Discussion

In this study, we have revealed a novel mechanism through which the male environment modulates the NMJ synaptic transmission, locomotion behavior, and mating efficiency in hermaphrodites. We show that the male environment effects are mediated based on exposure to male-specific pheromones at a specific developmental stage in hermaphrodites (the entire L3–L4 stage). We further demonstrate that hermaphroditic sense and process these male-specific pheromones by AWB chemosensory neurons using the cGMP signaling. At the hermaphroditic NMJ, presynaptic calcium channel localization and postsynaptic acetylcholine receptor clustering are elevated by exposure to male-specific pheromones, resulting in an increased cholinergic synaptic transmission. Our results provide mechanistic details of how environmental factors alter neuronal development and physiology,
Figure 6. Modulator ascarosides increase the abundance of excitatory presynaptic CaV2 calcium channels at the NMJ. (A) Schematic illustration of split GFP experimental design. Seven copies of the splitGFP 11 were inserted into the C-terminal of unc-2 genomic loci by CRISPR-Cas9 system. The splitGFP1-10 was expressed in B-type cholinergic and GABAergic motor neurons by unc-129 and unc-47 promoters. The unc-57-mCherry under the same promoter was separated with splitGFP1-10 by SL2 and was also used as a coexpressed presynaptic marker. (B, C) Presynaptic UNC-2::splitGFP (green) and UNC-57::mCherry (red) were co-localized in the dorsal nerve cord at both excitatory (B) and inhibitory (C) synapses. Representative images (top, scale bar, 10 μm) and linescan curves (bottom) are shown. For linescan curves, the mCherry signals were plotted on the left Y-axis, while the splitGFP signals were plotted on the right. one arbitrary fluorescence intensity unit equals 100 gray value. (D, E) The puncta fluorescence intensities and densities of UNC-2::splitGFP in B-type motor neurons (D) and GABAergic motor neurons (E) of hermaphrodites cultured in hermaphrodite- or male-conditioned medium. Representative images (scale bar, 5 μm), mean puncta intensities, and puncta densities are shown. In D and E, *p<0.05, **p<0.01, ns not significant, unpaired Student’s t-test.

The online version of this article includes the following source data and figure supplement(s) for figure 6:

Source data 1.

Figure supplement 1. Excitatory and inhibitory synapse structures are not affected by the modulator ascarosides.

Figure 6 continued on next page
presenting insights to better understand the associations between dysregulated neurodevelopment and various psychiatric diseases.

C. elegans NMJ as a model to study synaptogenesis

Here, we used the C. elegans NMJ as a model to study synaptic transmission, and our work underscore C. elegans as a useful model to study synaptic transmission in vivo. The motor circuit of C. elegans relies on a precise balance between cholinergic excitation and GABAergic inhibition of body-wall muscles to generate precise locomotion activities (Richmond and Jorgensen, 1999). Both our and others’ studies have identified mechanisms of synaptogenesis and synaptic transmission that are

Figure 7. CaV2 calcium channel is the synaptic target of the modulator ascarosides. (A–C) Endogenous acetylcholine transmission was assessed by recording mEPSCs from body muscles of wild-type N2 and unc-2 mutant adult hermaphrodites cultured in hermaphrodite- or male-conditioned medium. Representative mEPSC traces (A), the mean mEPSC rates (B), and the mean mEPSC amplitudes (C) are shown. The data for wild type (N2) is the same as in Figure 1H–J. (D) Locomotion behavior analysis of the single wild-type and unc-2 mutant hermaphrodite in hermaphrodite- and male-conditioned medium. The averaged and individual locomotion velocities were plotted. In (B)–(D), *p<0.05, **p<0.001, ns not significant, one-way ANOVA with post hoc Dunnett multiple comparisons.

The online version of this article includes the following source data for figure 7:

Source data 1.
shared by the C. elegans NMJ and the mammalian central nervous system (Dolphin and Lee, 2020; Hata et al., 1993; Ogawa et al., 1998; Pevsner et al., 1994; Richmond et al., 1999; Rizo and Süss-hof, 2012). In the worm motor circuit and the mammalian brain, acetylcholine is an excitatory neurotransmitter while GABA is an inhibitory neurotransmitter. Moreover, the clustering of acetylcholine receptors and GABA receptors at synapses is observed in C. elegans and vertebrates (Maro et al., 2015; Poulopoulos et al., 2009; Tong et al., 2015; Tu et al., 2015).

It is also highly notable that many autism-linked synaptic proteins, including Neuroligins and Neurexins, have been shown to function with conserved roles in NMJ synaptogenesis and synaptic transmission (Hart and Hobert, 2018; Hu et al., 2012; Kurshan et al., 2018; Philbrook et al., 2018; Tong et al., 2017): Neuroligins and Neurexins form trans-synaptic complex and regulate synaptic transmission in both mammalian central nervous system and C. elegans NMJ (Hu et al., 2012; Kurshan et al., 2018; Tong et al., 2017). Neuroligins are required for postsynaptic GABA_A-receptor clustering and inhibitory synaptic transmission (Maro et al., 2015; Poulopoulos et al., 2009; Tong et al., 2015; Tu et al., 2015). While Neurexins undergo ectodomain shedding by ADAM10 protease (Borcel et al., 2016; Tong et al., 2015), bind to presynaptic CaV2 calcium channel α2δ subunits, and regulate calcium channel activity (Luo et al., 2020; Tong et al., 2017). Thus, the mechanisms we identified here in the C. elegans NMJ may provide new insights into how synaptic transmission is maintained in the mammalian brain.

**Sexual dimorphic modulation on NMJ synaptic transmission**

We show that a previously unknown circuit comprised of AWB chemosensory neurons regulates NMJ synaptic transmission in C. elegans. Interestingly, the male-enriched pheromones increase the acetylcholine transmission specifically in hermaphrodites but not in males, suggesting sexual dimorphism in the regulation of NMJ synaptic transmission. This could be mediated by sex-specific neuronal circuits that are composed of either sex-specific or sex-shared neurons to process and transmit male pheromone signals to NMJ. A C. elegans male has 385 neurons, whereas a hermaphrodite has 302 neurons. The majority of male-specific neurons are localized in the male tail and are involved in the complex mating behaviors. There are several hermaphrodite-specific neurons in the nervous system, including VC and HSN motor neurons, which are mainly required for reproductive functions (Banerjee and Hallem, 2018; Emmons, 2018; García and Portman, 2016). On the other hand, several sex-shared neurons, including motor neurons, AWA, AWC, and ASK chemosensory neurons, DVA mechanosensory neurons, as well as AVA interneurons, could contribute to sex-specific neural circuits by mediating attraction and aversion behaviors (Banerjee and Hallem, 2018; Bayer and Hobert, 2018; Cook et al., 2019; Fagan et al., 2018; Mowrey et al., 2014; Narayan et al., 2016; Wan et al., 2019). Our results identified that AWB chemosensory neurons mediate a sexually dimorphic modulation of NMJ synaptic transmission. Further studies will be required to unravel the downstream neural circuits, including interneurons and premotor neurons, that function to process the modulator ascaroside signals to modulate NMJ synaptic transmission. Another possibility for this sexual dimorphic modulation is from sexually dimorphic hormone signaling pathways, such as vasoressin/oxytocin and their receptors (Garrison et al., 2012).

Our data show that pheromones modulating hermaphrodite NMJ synaptic transmission are enriched in N2 males. Previous studies have reported various male-specific ascarosides, including ascr#10 and indole containing ascarosides (IC-ascarosides, especially icas#3 and icas#9). However, our data indicate that ascr#10 and indole IC-ascarosides are unlikely the modulator ascarosides. First, ascr#10 levels are comparable in N2 and TR389 males. Second, previous work has established that ASI and ASK sensory neurons are required for hermaphrodites to sense ascr#10 and IC-ascarosides (Aprison and Ruvinsky, 2017; Dong et al., 2016), whereas we find that ASI and ASK neurons are dispensable for hermaphrodites to sense the modulator ascarosides. In contrast, our UPLC-MS data strongly suggest that the medium-chain β-hydroxylated ascarosides (C13, C14, and C15) may mediate this effect. Although we provided extensive genetic evidence, we have not experimentally confirmed that these specific ascarosides are sufficient to modulate hermaphrodite NMJ synaptic transmission.

Previous studies by Brunet and Murphy labs have shown that male pheromone exposure affects animal health and shortens hermaphrodite life span (Maures et al., 2014; Shi and Murphy, 2014; Shi et al., 2017). Here our data suggest that it might be different mechanisms to modulate longevity and NMJ synaptic transmission. In previous research, they found that exposure of hermaphrodite to
male pheromones at the beginning of their life (day 1) or sexual maturity (day 4) had a similar effect on hermaphrodites’ life span. However, we showed that L3–L4 is a critical developmental stage for modulation of hermaphrodite NMJ synaptic transmission by male pheromones (Figure 2A–C). Distinct male-specific pheromones may mediate the effects on longevity and NMJ. Further studies should be carried out to identify the specific ascaroside pheromones in males.

Our work demonstrates that early pheromone environment exposure has a long-term effect on synaptic transmission. We suspect that the observed effects may be mediated through endocrine signaling pathways, such as DAF-7/TGF-β and DAF-2/insulin, which are known to drive both epigenetic and transcriptional changes. In this light, recent studies have shown how pheromone exposure can inhibit learning behavior by disrupting the balance between two insulin-like peptides, ins-16 and ins-4 (Wu et al., 2019). Further studies are required to characterize whether endocrine system components like insulin signaling molecules are involved in regulating synaptic transmission in response to male-specific ascarosides.

**Presynaptic calcium channels as neuromodulation targets**

Our results show that modulator pheromones regulate hermaphrodite NMJ cholinergic transmission by altering the presynaptic localization of calcium channel CaV2 at cholinergic synapses. These results support that CaV2 calcium channels can be viewed as potential targets for environmental modulation of the synaptic transmission. At synapses, CaV2 channels are known to form large signaling complexes in the presynaptic nerve terminal that are responsible for calcium influx and neurotransmitter release (Dolphin and Lee, 2020). Numerous studies have verified causal relationships for calcium channel mutations and polymorphisms in neuropsychiatric diseases, including ASD (Nanou and Catterall, 2018; Zamponi, 2016). Our previous studies identified a synaptic retrograde signal mediated by autism-linked proteins that regulate CaV2 presynaptic localization to alter excitatory synaptic transmission (Tong et al., 2017). Here, we present the important evidence that the presynaptic calcium channel CaV2 could also be a target of social interaction modulation to shift the synaptic excitation and inhibition balance. These results support the idea that changes in presynaptic calcium channel localization could be impactful in some forms of ASD.

How might changes in chemosensory neuron activity contribute to presynaptic calcium channel localization? Our results suggest that it is not a general change of CaV2 expression levels because we observed increased presynaptic localization at cholinergic synapses but not at GABAergic synapses. We suspect that the specific synaptic recruitment of CaV2 is somehow potentiated by the modulator ascarosides. Previous studies have suggested that protein interactions are required for cell-surface localization of calcium channels as well as their docking at the active zone. It is therefore possible that pre-synapse specific proteins that are only present at cholinergic synapses may act downstream of the chemosensory circuits to regulate the surface localization of CaV2 channels.

Collectively, our findings reveal a novel mechanism through which pheromones in the environment modulate synaptogenesis and synaptic transmission in the nervous system. Beyond suggesting that calcium channels may be a shared target for both genetic and environmental modulation during development, our study lays a foundation for studies into the signaling and cell-specific functions underlying neurodevelopmental dysfunction.

**Materials and methods**

| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Strain, strain background (C. elegans) | C. elegans strains used in this study are listed in Supplementary file 1 | | | |
| Strain (Escherichia coli) | OP50 | CGC | RRID:WB-STRAIN.OP50 | |
| Sequence-based reagent | Sequence information is listed in Supplementary file 2 | | | |

**Key resources table**

**Materials and methods**

Qian et al. eLife 2021;10:e67170. DOI: https://doi.org/10.7554/eLife.67170

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|-----------------------------------|-------------|---------------------|-------------|------------------------|
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**Materials and methods**

Qian et al. eLife 2021;10:e67170. DOI: https://doi.org/10.7554/eLife.67170

19 of 29
| Reagent type (species) or resource | Designation | Source or reference | Identifiers | Additional information |
|-----------------------------------|-------------|---------------------|-------------|------------------------|
| Recombinant DNA reagent           | pPD49.26    | Addgene (Andrew Fire) | [link](https://www.addgene.org/1686/) |                         |
| Recombinant DNA reagent           | pPD95.75    | Addgene (Andrew Fire) | [link](https://www.addgene.org/1494/) |                         |
| Recombinant DNA reagent           | pCFJ910     | Addgene (Erik Jorgensen) | [link](https://www.addgene.org/44481/) |                         |
| Recombinant DNA reagent           | C214527     | Yingchuan B. Qi Qi et al., 2012 | | Plasmid: Punc-17β::tomm-20N::miniSOG |
| Recombinant DNA reagent           | quan0071    | Quan Wen Xu, T et al., 2018 | | Plasmid: Pacr-5::chrism |
| Recombinant DNA reagent           | pSG368      | Shangbang Gao Gao, S et al., 2018 | | Plasmid: GCaMP6f |
| Commercial assay or kit            | PureLink HiPure Plasmid Miniprep Kit | Invitrogen | Cat#: K210002 |                         |
| Commercial assay or kit            | QIAprep Spin Miniprep Kit | Qiagen | Cat#: 27106 |                         |
| Commercial assay or kit            | PrimeSTAR Max DNA Polymerase | Takara | Cat#: R045A |                         |
| Commercial assay or kit            | hyPerFUsion high-fidelity DNA polymerase | ApexBio | Cat#: K1032 |                         |
| Commercial assay or kit            | Hieff CLoone TM Plus One Step Cloning Kit | Yeasen | Cat#: 10911ES62 |                         |
| Chemical compound, drug           | Aldicarb | ApexBio | Cat#: B4778 |                         |
| Chemical compound, drug           | All-trans-Retinal | Sigma | Cat#: R2500 |                         |
| Chemical compound, drug           | Geneticin, G418 Sulfate | GOLDBIO | Cat#: G-418–1 |                         |
| Chemical compound, drug           | 2,3-Butanedione monoxide | Sigma | Cat#: B0753 |                         |
| Chemical compound, drug           | Polybead Microspheres 0.10 μm | Polysciences | Cat#: 00876–15 |                         |
| Chemical compound, drug           | Fluospheres carboxylat | Life Science | Cat#: F8813 |                         |
| Software, algorithm                | ImageJ | NIH | [link](https://imagej.nih.gov/ij/download.html) |                         |
| Software, algorithm                | Igor pro 6.3 | WaveMetrics | [link](https://www.wavemetrics.com/products/igo/ro/igo/ro.htm) |                         |
| Software, algorithm                | GraphPad Prism 8 | GraphPad | [link](https://www.graphpad.com/scientific-software/prism/) |                         |
| Software, algorithm                | MATLAB | MathWorks | [link](https://www.mathworks.com/products/matlab.html?ts_tid=hp_products_matlab) |                         |
| Software, algorithm                | MetaMorph | Molecular Devices | [link](https://www.moleculardevices.com/systems/metamorph-research-imaging/metamorph-microscopy-automation-and-image-analysis-software) |                         |
| Software, algorithm                | WormLab | MBF Bioscience | [link](https://www.mbfbioscience.com/wormlab) |                         |
Contact for reagent and resource sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact Xia-Jing Tong (tongxj@shanghaitech.edu.cn).

Experimental model and subject details

Animals

*C. elegans* were maintained under standard conditions at 20˚C on plates made from nematode growth medium (NGM). *E. coli* OP50 was used as a food source for all experiments except where HB101 *E. coli* was utilized for electrophysiology study. A description of all alleles can be found at [http://www.wormbase.org/#012-34-5](http://www.wormbase.org/#012-34-5). Animals were obtained from Bristol variety N2 strain unless specially indicated. Transgenic animals were prepared by microinjection, and integrated transgenes were isolated following UV irradiation or by miniMos insertion.

Plasmids

All worm expression vectors were modified versions of pPD49.26 (A. Fire) or miniMos vector pCFJ910. Standard methods and procedures were utilized for all of the plasmids. A 3.1 kb *ceh-36* promoter was used for expression in ASE and AWC chemosensory neurons. A 3 kb *odr-10* promoter was used for expression in AWA chemosensory neurons. A 3 kb *str-2* promoter was used for expression in AWB chemosensory neurons. A 3 kb *str-1* promoter was used for expression in ASI chemosensory neurons. A 3 kb *srb-6* promoter was used for expression in ADF, ADL, and ASH chemosensory neurons. A 3 kb *gpa-4* promoter was used for expression in ASI chemosensory neurons. A 3.9 kb *sra-7* promoter was used for expression in ASK chemosensory neurons. A 4.1 kb *flp-21* promoter was used for expression in the majority of the chemosensory neurons. A 4.3 kb *acr-5* promoter was used for expression in DB and VB motor neurons. A 2.4 kb *myo-3* myosin promoter was used for expression in body muscles. For rescue experiments, TAX-2 (F36F2.5.1), ODR-3 (C34D1.3.1), and ACR-16 (F25G6.3) were amplified from the N2 cDNA library using gene-specific primers.

Generation of single-copy insertion allele by homologous recombination

The xjSI0002 allele encoding RFP-tagged ACR-16 minigene under the muscle-specific *myo-3* promoter was generated by miniMOS ([Frøkjær-Jensen et al., 2014](frøkjær2014)). The RFP sequence was inserted between the third and the fourth transmembrane segment of ACR-16.

Aldicarb assay

The aldicarb assay was performed as previously described ([Vashlishan et al., 2008](vashlishan2008)). Aldicarb (Apex-Bio) was dissolved in ethyl alcohol and added to NGM at a final concentration of 1.4 mM (Testing hermaphrodites) or 0.5 mM (Testing males). These plates (35 mm) were seeded with 75 µl OP50 and allowed to dry overnight before use. More than 20 animals at the young adult stage (otherwise indicated) were picked on an aldicarb plate for aldicarb assay. Animals were scored as paralyzed when they did not respond to the platinum wire prodding. The paralyzed animals were counted every 10 or 15 min. At least three double-blind replicates for each group were tested.

Preparation of conditioned media

Hermaphroditic- and male-secreted metabolites were collected according to a previous publication ([Srinivasan et al., 2008](srinivasan2008)). Synchronized *C. elegans* (WT [N2], *him-5* [N2], WT [TR389], *him-5* [TR389], *daf-22* [N2], and *daf-22; him-5* [N2]) with a density of 10,000 worms/plates (90 mm, three plates) were grown on the nematode growth medium (NGM) agarose (seeded with *E. coli* strain OP50) at 20˚C. There were 43.07 ± 0.77%, 39.26 ± 1.55%, and 37.29 ± 1.28% males in *him-5* (N2), *daf-22; him-5* (N2), and *him-5* (TR389) strains, respectively. After worms reached the young adult stages, they were collected and washed three times with M9 buffer to remove bacteria. To further remove the bacteria in the gut, the worms were then placed in M9 buffer in a shaker (150 rpm) at 20˚C for 30 min and rinsed three times with ddH2O. Subsequently, worm-secreted metabolites were collected by incubating the worms in ddH2O in a shaker (150 rpm) for 3 hr with a density of 30,000 worms/ml. Afterward, the worms were removed by settling on ice for 5 min. The metabolites were filtered
through 0.22 μm filters, aliquoted, and stored at −80°C. For conditioned medium preparation, 10 μl metabolites mixed with 90 μl OP50 E. coli were spread on a 35 mm NGM plate. Plates were allowed to dry overnight before use.

**Calcium imaging**

Muscle calcium responses were measured by detecting fluorescence intensity changes of GCaMP3. *C. elegans* expressing GCaMP3 in the body-wall muscle (P*myo-3::GCaMP3) and Chrimson in the DB and VB neurons (Pacr-5::Chrimson) were used for calcium imaging experiments. Young adult animals fed with 1.6 mM ATR (in 100 μl E. coli OP 50) were immobilized on 10% agarose pads by polystyrene microbeads. Fluorescence images were captured using a Nikon 60 × 1.4 NA objective on a Nikon spinning-disk confocal system (Yokogawa CSU-W1) at 10 ms per frame. We used wide-field illumination with a nominal wavelength at 640 nm for Chrimson activation. The GCaMP signals were captured using 488 nm laser excitation and were analyzed by ImageJ software.

Calcium responses in the soma of AWB sensory neurons were measured by detecting fluorescence intensity changes of GCaMP6f. A home-made microfluidic device was used for calcium imaging as previously described (Liu et al., 2019; Zou et al., 2018). Briefly, a young adult animal was rinsed by M9 buffer and loaded into a home-made microfluidic device with its nose exposed to buffer under laminar flow. Diluted metabolites of N2 hermaphrodite and him-5 mutants was delivered using a programmable automatic drug feeding equipment (MPS-2, InBio Life Science Instrument Co. Ltd, Wuhan, China). For Ca$^{2+}$ fluorescence imaging in AWB, the neurons were exposed under fluorescent excitation light for 30 s before recording, to eliminate the light-evoked calcium transients. During the recording process, for the first 5 s, we gave the M9 solution and then switched hermaphrodite excretome or male excretome for 30 s, removing extract liquid from 35 s and washing for 25 s. The AWB neurons were imaged with a 60× objective (Olympus) and EMCCD camera (Andor iXonEM) at 100 ms/frame. The imaging sequences were subsequently analyzed using Image-Pro Plus6 (Media Cybernetics, Inc, Rockville, MD).

**Adult locomotion analysis**

To analyze adult locomotor behavior, young adult worms were washed with M9 buffer before transferred to the unseeded NGM plate and allowed to recover for 5 min. Animal locomotion was recorded at a rate of 10 frames per second for 1 min. The mean body-bend amplitude and crawling locomotion velocity were analyzed by WormLab. All the assays were done at 25°C.

**Mating behaviors**

Mating efficiency was assessed as previously described (Yin et al., 2017). Briefly, two young adult stage hermaphrodites from male- or hermaphrodite-conditioned medium were cultured with two young-adult males for 24 hr. Successful mating was scored when more than three male progenies were generated in the mating plate. Mating efficiency was obtained by calculating the percentage of successful mating in more than 15 plates.

**Liquid culture and mass spectrometric analysis**

The crude pheromone extracts were prepared according to a previously published protocol (Zhang et al., 2013). N2 wild-type, him-5 mutant, daf-22; him-5 mutant, or TR389 him-5 mutant worms were grown for two generations on 60 mm NGM plate seeded with *E. coli* OP50 bacteria. Worms from four plates were washed by M9 buffer and cultured in 50 ml S complete medium (100 mM NaCl, 50 mM KPO$_4$, 3 mM CaCl$_2$, 3 mM MgSO$_4$, 5 μg/ml cholesterol, 10 mM potassium citrate, 50 μM disodium EDTA, 25 μM FeSO$_4$, 10 μM MnCl$_2$, 10 μM ZnSO$_4$, 1 μM CuSO$_4$) at 20°C and 200 rpm. The animals were cultured with shaking at 200 rpm for 7 days (around 30,000 worms/50 ml). 25x Concentrated *E. coli* OP50 bacteria were supplemented every day (0.3 ml for day 1, 1 ml for days 2–5, and 2 ml for days 6–7). After 7 days, the supernatant medium containing metabolites was collected by centrifugation (3000 g, 10 min). Then the supernatant media were frozen at −80°C, lyophilized, and extracted with methanol for UPLC-MS analysis. UPLC-MS was performed using a Sciex TripleTOF 6600 system. Chromatographic separations were achieved using a Waters Acquity UPLC BEH C18 column (1.7 μm, 2.1 × 10 mm), with a flow rate of 0.4 ml/min. Data acquisition and processing were performed by Analyst and Peakview (Sciex).
Genetic ablation with miniSOG

The genetically encoded photosensitizer, miniSOG, was used to ablate specific neurons as previously described (Qi et al., 2012). Synchronized Late L1 larva animals (24 hr after egg hatching at 20˚C) expressing miniSOG under specific promoters were exposed to wide-field blue light (460 nm) at an intensity of 57 mW/cm² for 30 min, and then animals were grown in the 20˚C incubator before experiments. The ablation efficiency was measured by comparing mCherry fluorescent signal with and without blue light stimulation.

Optogenetic activation of chemosensory neurons

To prepare the plates for optogenetic activation of neurons, 1.6 mM of all-trans-retinal (ATR, 100 mM dissolved in ethanol) or ethanol (control) was mixed with OP50 E. coli culture and spotted on 35 mm NGM plates. Plates were allowed to dry for 24 hr before usage. Transgenic worms with channelrhodopsin variant CHIEF expressed in ASJ/ASI, AWA, AWB, or AWC chemosensory neurons were grown overnight on the NGM plates. Animals at L4 larval stages received 100 ms pulse stimulation of blue light (460 nm wavelength, 2.4 mW/mm² power) for 10 min (five times) until the animals entered the adult stage.

Fluorescent microscopy imaging

For quantitative analysis of fluorescence intensities and densities, images were captured using a 100× (NA = 1.4) objective on an Olympus microscope (BX53). Young adult worms were immobilized with 30 μg/μl 2,3-butanedione monoxime. The maximum intensity of dorsal cord projections of Z-series stacks was obtained by Metamorph software (Molecular Devices). Line scans were analyzed in Igor Pro (WaveMetrics) using a custom script (Dittman and Kaplan, 2006). The mean fluorescence intensities of reference FluoSphere microspheres (0.5 μm, ThermoFisher Scientific) were measured during each experiment controlled for changes in illumination intensities. To assess the synaptic accumulation of fluorescent proteins, we used the calculation of ΔF/F as (F_puncta – F_axon)/F_axon. And we also counted the density of fluorescent puncta.

Electrophysiology

Electrophysiology was conducted on dissected C. elegans as previously described (Hu et al., 2012). Worms were superfused in an extracellular solution containing 127 mM NaCl, 5 mM KCl, 26 mM NaHCO₃, 1.25 mM NaH₂PO₄, 20 mM glucose, 1 mM CaCl₂, and 4 mM MgCl₂, bubbled with 5% CO₂, 95% O₂ at 22˚C. Whole-cell recordings were carried out at −60 mV for mEPSCs, and 0 mV for mIPSCs. The internal solution contained 105 mM CH₃O₃SCs, 10 mM CsCl, 15 mM CsF, 4 mM MgCl₂, 5 mM EGTA, 0.25 mM CaCl₂, 10 mM HEPES, and 4 mM Na₂ATP. The solution was adjusted to pH 7.2 using CsOH.

Statistics

All data were reported as mean ± SEM (standard error of the mean). Statistical analyses were performed using GraphPad Prism (version 8). We calculated p-values by two-way ANOVA (Figures 1B, D and 2D–F, Figure 2—figure supplement 1A–E, Figure 2—figure supplement 2, Figure 2—figure supplement 4, Figure 4—figure supplement 2A–G, Figure 4—figure supplement 3A–C), two-way ANOVA with post hoc Sidak multiple comparisons (Figures 2B–C, G, 3B–C, F–G, 4C, E, and I–J, Figure 3—figure supplement 1A–B), one-way ANOVA with post hoc Dunnett multiple comparisons (Figures 2H–I, 3E, 4D, and 7B–D, Figure 3—figure supplement 2A–C), and unpaired Student’s t-test (Figures 1F–G, I–J, L–M, 4H, 5A–E, and 6D–E, Figure 1—figure supplement 1B, Figure 2—figure supplement 3A and C, Figure 6—figure supplement 1A,B). In all figures, p-values are denoted as *<0.05, **<0.01, ***<0.001.

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Author contributions

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Additional files
Supplementary files
• Supplementary file 1. C. elegans strains used in this study.
• Supplementary file 2. Sequence information.
• Transparent reporting form

Data availability
All data generated or analysed during this study are included in the manuscript and supporting files. Source data files have been provided. For other information (such as primers), we have included them in the methods.

References
Alcazar RM, Lin R, Fire AZ. 2008. Transmission dynamics of heritable silencing induced by double-stranded RNA in Caenorhabditis elegans. Genetics 180:1275–1288. DOI: https://doi.org/10.1534/genetics.108.089433, PMID: 18757930
Aprison EZ, Ruvinsky I. 2017. Counteracting ascarosides act through distinct neurons to determine the sexual identity of C. elegans Pheromones. Current Biology 27:2589–2599. DOI: https://doi.org/10.1016/j.cub.2017.07.034, PMID: 28844464
Aprison EZ, Ruvinsky I. 2019a. Coordinated behavioral and physiological responses to a social signal are regulated by a shared neuronal circuit. Current Biology 29:4108–4115. DOI: https://doi.org/10.1016/j.cub.2019.10.012, PMID: 31708394
Aprison EZ, Ruvinsky I. 2019b. Dynamic regulation of Adult-Specific functions of the nervous system by signaling from the reproductive system. Current Biology 29:4116–4123. DOI: https://doi.org/10.1016/j.cub.2019.10.011
Autism Sequencing Consortium, Doan RN, Lim ET, De Rubeis S, Betancur C, Cutler DJ, Chiocchetti AG, Overman LM, Soucy A, Goetze S, Freitag CM, Walsh CA, Buxbaum JD, Yu TW. 2019. Recessive gene disruptions in autism spectrum disorder. Nature Genetics 51:1092–1098. DOI: https://doi.org/10.1038/s41588-019-0433-8, PMID: 31209396
Banerjee N, Hallem E. 2018. Sexual dimorphisms: how Sex-Shared neurons generate Sex-Specific behaviors. Current Biology 28:R254–R256. DOI: https://doi.org/10.1016/j.cub.2018.01.066, PMID: 29558638
Bargmann CI. 2006. Chemosensation in C. elegans. WormBook 25:1–29. DOI: https://doi.org/10.1895/wormbook.1.123.1
Bayer EA, Hobert O. 2018. Past experience shapes sexually dimorphic neuronal wiring through monoaminergic signalling. Nature 561:117–121. DOI: https://doi.org/10.1038/s41586-018-0452-0, PMID: 30150774
Borcel E, Palczynska M, Krzisch M, Dimitrov M, Ulrich G, Toni N, Fraering PC. 2016. Shedding of neurexin 3β ectodomain by ADAM10 releases a soluble fragment that affects the development of newborn neurons. Scientific Reports 6:39310. DOI: https://doi.org/10.1038/srep39310, PMID: 27991559
Butcher RA, Fujita M, Schroeder FC, Clardy J. 2007. Small-molecule pheromones that control dauer development in Caenorhabditis elegans. Nature Chemical Biology 3:420–422. DOI: https://doi.org/10.1038/nchembio.2007.3, PMID: 17558398
Butcher RA, Ragains JR, Li W, Ruvkun G, Clardy J, Mak HY. 2009. Biosynthesis of the Caenorhabditis elegans dauer pheromone. PNAS 106:1875–1879. DOI: https://doi.org/10.1073/pnas.0810338106, PMID: 19174521
C Yuen RK, Merico D, Bookman M, L Howe J, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z, Pellecchia G, Buchanan JA, Walker S, Marshall CR, Uddin M, Zarrei M, Deneault E, D’Abate L, Chan AJ, Koyanagi S, et al. 2017. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nature Neuroscience 20:602–611. DOI: https://doi.org/10.1038/nn.4524, PMID: 28263302
Cabantous S, Terwilliger TC, Waldo GS. 2005. Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. Nature Biotechnology 23:102–107. DOI: https://doi.org/10.1038/nbt1044, PMID: 15580262

Chao HT, Zoghbi HY, Rosenmund C. 2007. MeCP2 controls excitatory synaptic strength by regulating glutamatergic synapse number. Neuron 56:58–65. DOI: https://doi.org/10.1016/j.neuron.2007.08.018, PMID: 17920015

Chen Q, Deister CA, Gao X, Guo B, Lynn-Jones T, Chen N, Wells MF, Liu R, Goad MJ, Dimidchstein J, Feng S, Shi Y, Liao W, Lu Z, Fishell G, Moore CI, Feng G. 2020. Dysfunction of cortical GABAergic neurons leads to sensory hyper-reactivity in a Shank3 mouse model of ASD. Nature Neuroscience 23:520–532. DOI: https://doi.org/10.1038/s41593-020-0598-6

Chen P, Hong W. 2018. Neural circuit mechanisms of social behavior. Neuron 98:16–30. DOI: https://doi.org/10.1016/j.neuron.2018.02.026

Colavita A, Krishna S, Zheng H, Padgett RW, Culotti JG. 1998. Pioneer axon guidance by UNC-129, a C. elegans TGF-beta. Science 281:706–709. DOI: https://doi.org/10.1126/science.281.5377.706, PMID: 9685266

Cook SJ, Jarell TA, Brittin CA, Wang Y, Bloniarz AE, Yakovlev MA, Nguyen KCQ, Tang LT, Mayer EA, Duerr JS, Bülow HE, Hobert O, Hall DH, Emmons SW. 2019. Whole-animal connectomes of 7225 genes and functional domains. Science 366:349–369. DOI: https://doi.org/10.1126/science.1224896, PMID: 30550786

Dittman JS, Kaplan JM. 2006. Factors regulating the abundance and localization of synaptobrevin in the plasma membrane. PNAS 103:1139–11404. DOI: https://doi.org/10.1073/pnas.0600784103, PMID: 16844789

Dolphin AC, Lee A. 2020. Presynaptic calcium channels: specialized control of synaptic neurotransmitter release. Nature Reviews Neuroscience 21:213–229. DOI: https://doi.org/10.1038/s41583-020-0278-2, PMID: 32161339

Dong C, Dolke F, von Reuss SH. 2016. Selective MS screening reveals a sex pheromone in Caenorhabditis briggsae and species-specificity in indole ascorside signalling. Organic & Biomolecular Chemistry 14:7217–7225. DOI: https://doi.org/10.1039/c3ob01230b, PMID: 27381649

Edison AS. 2009. Caenorhabditis elegans pheromones regulate multiple complex behaviors. Current Opinion in Neurobiology 19:378–388. DOI: https://doi.org/10.1016/j.conb.2009.07.007, PMID: 19665885

Emmons SW. 2018. Neural circuits of sexual behavior in Caenorhabditis elegans. Annual Review of Neuroscience 41:349–369. DOI: https://doi.org/10.1146/annurev-neuro-070815-014056, PMID: 29709211

Fagan KA, Luo J, Lagoy RC, Schroeder FC, Albrecht DR, Portman DS. 2018. A Single-Neuron chemosensory switch determines the valence of a sexually dimorphic sensory behavior. Current Biology 28:902–914. DOI: https://doi.org/10.1016/j.cub.2018.02.029, PMID: 29526590

Frejkaer-Jensen C, Davis MW, Sarov M, Taylor J, Filbotte S, LaBella M, Pozniakovsky A, Moerman DG, Jorgensen EM. 2014. Random and targeted transgene insertion in Caenorhabditis elegans using a modified Mos1 transposon. Nature Methods 11:529–534. DOI: https://doi.org/10.1038/nmeth.2889, PMID: 24820376

Garcia LR, Portman DS. 2016. Neural circuits for sexually dimorphic and sexually divergent behaviors in Caenorhabditis elegans. Current Opinion in Neurobiology 38:46–52. DOI: https://doi.org/10.1016/j.conb.2016.02.002, PMID: 26929998

Garrison JL, Macosko EZ, Bernstein S, Pokala N, Albrecht DR, Bargmann CI. 2012. Oxytocin/vasopressin-related peptides have an ancient role in reproductive behavior. Science 338:540–543. DOI: https://doi.org/10.1126/science.1226201, PMID: 23112335

Geisheker MR, Heymann G, Wang T, Coe BP, Turner TN, Steissman HAF, Hoekzema K, Kvarnung M, Shaw M, Friend K, Liebelt J, Barnett C, Thompson EM, Haan E, Guo H, Andersen BM, Nordgren A, Lindstrand A, VandeWey G, Alberti A, et al. 2017. Hotspots of missense mutation identify neurodevelopmental disorder genes and functional domains. Nature Neuroscience 20:1043–1051. DOI: https://doi.org/10.1038/nn.4589, PMID: 28628100

Greene JS, Brown M, Dobosiewicz M, Ishida IG, Macosko EZ, Zhang X, Butler RA, Cline DJ, McGrath PT, Bargmann CI. 2016. Balancing selection shapes density-dependent foraging behaviour. Nature 539:254–258. DOI: https://doi.org/10.1038/nature19848, PMID: 27799655

Hart MP, Hobert O. 2018. Neurexin controls plasticity of a mature, sexually dimorphic neuron. Nature 553:165–170. DOI: https://doi.org/10.1038/nature25192, PMID: 29323291

Hata Y, Slaughther CA, Südhof TC. 1993. Synaptic vesicle fusion complex contains unc-18 homologue bound to syntaxin. Nature 366:347–351. DOI: https://doi.org/10.1038/366347a0, PMID: 8247129

Homozgyosity Mapping Consortium for Autism, Doan RN, Bae Bl, Cubelos B, Chang C, Hossain AA, Al-Saad S, Mukaddes NM, Oner O, Al-Saffar M, Balkhy S, Gascon GG, Nieto M, Walsh CA. 2016. Mutations in human accelerated regions disrupt cognition and social behavior. Cell 167:341–354. DOI: https://doi.org/10.1016/j.cell.2016.08.071, PMID: 27667684

Hu Z, Hom S, Kudze T, Tong XJ, Choi S, Aramuni G, Zhang W, Kaplan JM. 2012. Neurexin and neurexin-like mediate retrograde synaptic inhibition in C. elegans. Science 337:980–984. DOI: https://doi.org/10.1126/science.1224896, PMID: 22859820

Iossifov I, Ronemus M, Levy D, Wang Z, Hakker I, Rosenbaum J, Yamrom B, Lee YH, Narzisi G, Leotta A, Kendall J, Grabowska E, Ma B, Marks S, Rodgers L, Stepanky A, Troge J, Andrews P, Bekritsky M, Pradhan K, et al. 2012. De novo gene disruptions in children on the autism spectrum. Neuron 74:285–299. DOI: https://doi.org/10.1016/j.neuron.2012.04.009, PMID: 22542183

Isogai Y, Wu Z, Love MI, Ahn MH, Bambah-Mukku D, Hua V, Farrell K, Dulac C. 2018. Multisensory logic of infant-Directed aggression by males. Cell 175:1827–1841. DOI: https://doi.org/10.1016/j.cell.2018.11.032, PMID: 30550786
Izrayelit Y, Sinivasan J, Campbell SL, Jo Y, von Reuss SH, Genoff MC, Sternberg PW, Schroeder FC. 2012. Targeted metabolomics reveals a male pheromone and sex-specific ascorbic biosynthesis in Caenorhabditis elegans. ACS Chemical Biology 7:1321–1325. DOI: https://doi.org/10.1021/cb300169c, PMID: 22662967

Jin Y, Jorgensen E, Hartwieg E, Horvitz HR. 1999. The Caenorhabditis elegans gene unc-25 encodes glutamic acid decarboxylase and is required for synaptic transmission but not synaptic development. The Journal of Neuroscience 19:539–548. DOI: https://doi.org/10.1523/JNEUROSCI.19-02-0539.1999, PMID: 9880574.

Judson MC, Wallace ML, Sidorov MS, Burette AC, Gu B, van Woerden GM, King IF, Han JE, Zylka MJ, Elgersma Y, Weinberg RJ, Philpot BD. 2016. GABAergic Neuron-Specific loss of Ube3a causes angelman Syndrome-Like EEG abnormalities and enhances seizure susceptibility. Neuron 90:56–69. DOI: https://doi.org/10.1016/j.neuron.2016.02.040, PMID: 27021170

Kamiyama D, Sekine S, Barsi-Rhney B, Hu J, Chen B, Gilbert LA, Ishikawa H, Leonetti MD, Marshall WF, Weissman JS, Huang B. 2016. Versatile protein tagging in cells with split fluorescent protein. Nature Communications 7:11046. DOI: https://doi.org/10.1038/ncomms11046, PMID: 26988139

Klapoetke NC, Murata Y, Kim SS, Pulver SR, Birdsey-Benson A, Cho YK, Morimoto TK, Chuong AS, Carpenter EJ, Tian Z, Wang J, Xie Y, Yan Z, Zhang Y, Chow BY, Surek B, Melkonian M, Jayaraman V, Constantine-Paton M, Wong GK, et al. 2014. Independent optical excitation of distinct neural populations. Nature Methods 11:338–346. DOI: https://doi.org/10.1038/nmeth.2836, PMID: 24509633

Kurshan PT, Merrill SA, Dong Y, Ding C, Hammarlund M, Bai J, Jorgensen EM, Shen K. 2018. γ-Neurexin and frizzled mediate parallel synapse assembly pathways antagonized by receptor endocytosis. Neuron 100:150–166. DOI: https://doi.org/10.1016/j.neuron.2018.09.007, PMID: 30269993

Lee J, Chung C, Ha S, Lee D, Kim DY, Kim H, Kim E. 2015. Shank3-mutant mice lacking exon 9 show altered excitation/inhibition balance, enhanced rearing, and spatial memory deficit. Frontiers in Cellular Neuroscience 9:94. DOI: https://doi.org/10.3389/fncel.2015.00094, PMID: 25852484

Lee C, Kang EY, Gandal MJ, Eskin E, Geschwind DH. 2019. Profiling allele-specific gene expression in brains from individuals with autism spectrum disorder reveals preferential minor allele usage. Nature Neuroscience 22:1521–1532. DOI: https://doi.org/10.1038/s41593-019-0461-9, PMID: 31455884

Levinson JN, El-Husseini A. 2005. Building excitatory and inhibitory synapses: balancing neuroligin partnerships. Neuron 48:171–174. DOI: https://doi.org/10.1016/j.neuron.2005.09.017, PMID: 16242398

Li Q, Liberles SD. 2015. Aversion and attraction through olfaction. Current Biology 25:R120–R129. DOI: https://doi.org/10.1016/j.cub.2014.11.044, PMID: 25649823

Liberles SD. 2014. Mammalian pheromones. Annual Review of Physiology 76:151–175. DOI: https://doi.org/10.1146/annurev-physiol-021113-170334, PMID: 23988175

Liu H, Li L, Wang W, Gong J, Yang X, Hu Z. 2018. Spontaneous vesicle fusion is differentially regulated at cholinergic and GABAergic synapses. Cell Reports 22:2334–2345. DOI: https://doi.org/10.1016/j.celrep.2018.02.023, PMID: 29490270

Liu H, Qin LW, Li R, Zhang C, Al-Sheikh U, Wu ZX. 2019. Reciprocal modulation of 5-HT and octopamine regulates pumping via feedforward and feedback circuits in C. elegans. PNAS 116:7107–7112. DOI: https://doi.org/10.1073/pnas.1819261116, PMID: 30872487

Ludewig AH, Aryukhin AB, Aprison EZ, Rodrigues PR, Pulido DC, Burkhardt RN, Panda O, Zhang YK, Gudivanda P, Ruvinsky I, Schroeder FC. 2019. An excerted small molecule promotes C. elegans reproductive development and aging. Nature Chemical Biology 15:838–845. DOI: https://doi.org/10.1038/s41589-019-0321-7, PMID: 31320757

Ludewig AH, Schroeder FC. 2013. WormBook: the online review of C. elegans Biology. WormBook 10:1–22. DOI: https://doi.org/10.1895/wormbook.1.155.1

Luo FJ, Scip A, Jiang M, Sudhof TC. 2020. Neurexins cluster Ca2+ channels within the presynaptic active zone. Embo Journal 39:e103208. DOI: https://doi.org/10.15252/embj.2019103208

Mahoney TR, Luo S, Nonet ML. 2006. Analysis of synaptic transmission in Caenorhabditis elegans using an aldicarb-sensitivity assay. Nature Protocols 1:1772–1777. DOI: https://doi.org/10.1038/nprot.2006.281, PMID: 17487159

Mare GS, Gao S, Olechewir AM, Hung WL, Liu M, Özkan E, Zhen M, Shen K. 2015. MADD-4/Punctin and neurexin organize C. elegans GABAergic Postsynapses through Neuroligin. Neuron 86:1420–1432. DOI: https://doi.org/10.1016/j.neuron.2015.05.015, PMID: 26028574

Maures TJ, Booth LN, Benayoun BA, Izrayelit Y, Schroeder FC, Brunet A. 2014. Males shorten the life span of C. elegans hermaphrodites via secreted compounds. Science 343:541–544. DOI: https://doi.org/10.1126/science.1244160, PMID: 24292626

Miyazaki T, Takase K, Nakajima W, Tada H, Ohyu D, Sano A, Goto T, Hirase H, Malinow R, Takahashi T. 2012. Disrupted cortical function underlies behavior dysfunction due to social isolation. Journal of Clinical Investigation 122:2690–2701. DOI: https://doi.org/10.1172/JCI63060, PMID: 22706303

Morrow EM, Yoo S-Y, Flavell SW, Kim T-K, Lin Y, Hill RS, Mukaddes NM, Balkhy S, Gascon G, Hashmi A, Al-Saad S, Ware J, Joseph RM, Greenblatt R, Gleason D, Ertelt JA, Apse KA, Bodel J, Partlow JN, Barry B, et al. 2008. Identifying autism loci and genes by tracing recent shared ancestry. Science 321:218–223. DOI: https://doi.org/10.1126/science.1157657

Mowrey WR, Bennett JR, Portman DS. 2014. Distributed effects of biological sex define sex-typical motor behavior in Caenorhabditis elegans. Journal of Neuroscience 34:15579–15591. DOI: https://doi.org/10.1523/JNEUROSCI.4352-13.2014, PMID: 24478342

Nanou E, Catterall WA. 2018. Calcium channels, synaptic plasticity, and neuropsychiatric disease. Neuron 98:466–481. DOI: https://doi.org/10.1016/j.neuron.2018.03.017, PMID: 29723500
Qian et al. eLife 2021;10:e67170. DOI: https://doi.org/10.7554/eLife.67170

Narayan A, Venkatacharav V, Durak O, Reilly DK, Bose N, Schroeder FC, Samuel AD, Srinivasan J, Sternberg PW. 2016. Contrasting responses within a single neuron class enable sex-specific attraction in Caenorhabditis elegans. PNAS 113:E1392–E1401. DOI: https://doi.org/10.1073/pnas.1600786113, PMID: 26903633

Neale BM, Koo Y, Liu L, Ma’ayan A, Samocha KE, Sabo A, Lin CF, Stevens C, Wang LS, Makarov V, Polak P, Yoon S, Maguire J, Crawford EL, Campbell NG, Geller ET, Valladares O, Schaffer C, Liu H, Zhao T, et al. 2012. Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485:242–245.

Ogawa H, Harada S, Sassa T, Yamamoto H, Hosono R. 1998. Functional properties of the unc-64 gene encoding a Caenorhabditis elegans syntaxin. Journal of Biological Chemistry 273:2192–2198. DOI: https://doi.org/10.1074/jbc.273.4.2192

Olmos-Serrano JL, Paluszkiwicz SM, Martin BS, Kaufmann WE, Corbin JG, Huntsman MM. 2010. Defective GABAergic neurotransmission and pharmacological rescue of neuronal hyperexcitability in the amygdala in a mouse model of fragile X syndrome. Journal of Neuroscience 30:9929–9938. DOI: https://doi.org/10.1523/JNEUROSCI.1714-10.2010, PMID: 20660275

Orefice LL, Mosko JR, Morency DT, Wells MF, Tasnim A, Mozeika SM, Ye M, Chirila AM, Emanuel AJ, Rankin G, Fane RM, Lehtinen MK, Feng G, Ginty DD. 2019. Targeting peripheral somatosensory neurons to improve Tactile-Related phenotypes in ASD models. Cell 178:867–886. DOI: https://doi.org/10.1016/j.cell.2019.07.024, PMID: 31938341

Pevsner J, Hsu SC, Braun JE, Calakos N, Ting AE, Bennett MK, Scheller RH. 1994. Specificity and regulation of a synaptic vesicle docking complex. Neuron 13:353–361. DOI: https://doi.org/10.1016/0896-6273(94)90352-2, PMID: 8060516

Phillbrook A, Ramachandran S, Lambert CM, Olivier D, Florman J, Alkema MJ, Lemons M, Francis MM. 2018. Neurexin directs partner-specific synaptic connectivity in C. elegans. eLife 7:e35692. DOI: https://doi.org/10.7554/eLife.35692, PMID: 30039797

Poulopoulos A, Aramuni G, Meyer G, Soykan T, Hoon M, Papadopoulos T, Zhang M, Paarmann I, Fuchs C, Harvey K, Jedlicka P, Schwarzer SW, Betz H, Harvey RJ, Brose N, Zhan W, Varoqueaux F. 2009. Neuroligin 2 drives postsynaptic assembly at Perisomatic inhibitory synapses through gephyrin and collybistin. Neuron 63:628–642. DOI: https://doi.org/10.1016/j.neuron.2009.08.023, PMID: 19755106

Qi YB, Garren EJ, Shu X, Tsien RY, Jin Y. 2012. Photo-inducible cell ablation in Caenorhabditis elegans using the genetically encoded singlet oxygen generating protein miniSOG. PNAS 109:7499–7504. DOI: https://doi.org/10.1073/pnas.1012495109, PMID: 22532663

Richmond JE, Davis WS, Jorgensen EM. 1999. UNC-13 is required for synaptic vesicle fusion in C. elegans. Nature Neuroscience 2:959–964. DOI: https://doi.org/10.1038/14755, PMID: 10526333

Richmond JE, Jorgensen EM. 1999. One GABA and two acetylcholine receptors function at the C. elegans neuromuscular junction. Nature Neuroscience 2:791–797. DOI: https://doi.org/10.1038/12160, PMID: 10461217

Rizzo J, Südhof TC. 2012. The membrane fusion enigma: snares, Sec1/Munc18 proteins, and their Accomplices— Guilty as Charged? Annual Review of Cell and Developmental Biology 28:279–308. DOI: https://doi.org/10.1146/annurev-cellbio-101011-155818

Shi C, Runnels AM, Murphy CT. 2017. Mating and male pheromone kill Caenorhabditis males through distinct mechanisms. eLife 6:e23493. DOI: https://doi.org/10.7554/eLife.23493, PMID: 28290982

Shi C, Murphy CT. 2014. Mating induces shrinking and death in Caenorhabditis mothers. Science 343:536–540. DOI: https://doi.org/10.1126/science.1242958, PMID: 24356112

Srinivasan J, Kaplan F, Ajedrini R, Zachariah C, Alborn HT, Teal PE, Malik RU, Edison AS, Sternberg PW, Schroeder FC. 2008. A blend of small molecules regulates both mating and development in Caenorhabditis elegans. Nature 454:1115–1118. DOI: https://doi.org/10.1038/nature07168, PMID: 18650807

Srinivasan J, von Reuss SH, Bose N, Zaslaver A, Mahanti P, Ho MC, O’Doherty OG, Edison AS, Sternberg PW, Schroeder FC. 2012. A modular library of small molecule signals regulates social behaviors in Caenorhabditis elegans. PLOS Biology 10:e1001237. DOI: https://doi.org/10.1371/journal.pbio.1001237, PMID: 22253572

Südhof TC. 2008. Neuroligins and neurexins link synaptic function to cognitive disease. Nature 455:903–911. DOI: https://doi.org/10.1038/ Nature07456, PMID: 18923512

Tchenio A, Lecca S, Valentinova K, Mammel M. 2017. Limiting huberal hyperactivity ameliorates maternal separation-driven depressive-like symptoms. Nature Communications 8:1135. DOI: https://doi.org/10.1038/s41467-017-01192-1, PMID: 29074844

Tian L, Hires SA, Mao T, Huber D, Chiappe ME, Chalasani SH, Petreanu L, Akerboom J, McKinney SA, Schreiter ER, Bargmann CI. 2012. Caenorhabditis elegans neurons use genetically encoded singlet oxygen generating protein miniSOG. Nature 485:1407–1409. DOI: https://doi.org/10.1038/nmeth.1398

Tong XJ, Hu Z, Liu Y, Anderson D, Kaplan JM. 2015. A network of autism linked genes stabilizes two pools of synaptic GABA(A) receptors. eLife 4:e09648. DOI: https://doi.org/10.7554/eLife.09648, PMID: 26575289

Tong XJ, López-Soto EJ, Li L, Liu H, Nedelcu D, Lipscombe D, Hu Z, Kaplan JM. 2017. Retrograde synaptic inhibition is mediated by α-Neurexin binding to the α28 subunits of N-Type calcium channels. Neuron 95:326–340. DOI: https://doi.org/10.1016/j.neuron.2017.06.018, PMID: 28695945

Tu H, Pina-Lucárre B, Ji T, Jospin M, Bessereau JL. 2015. C. elegans Punctin clusters GABA(A) Receptors via neuroligin binding and UNC-40/DCC recruitment. Neuron 86:1407–1419. DOI: https://doi.org/10.1016/j.neuron.2015.05.013, PMID: 26028575
Vashlishan AB, Madison JM, Dybbs M, Bai J, Sieburth D, Ch’ng Q, Tavazoie M, Kaplan JM. 2008. An RNAi screen identifies genes that regulate GABA synapses. Neuron 58:346–361. DOI: https://doi.org/10.1016/j.neuron.2008.02.019, PMID: 18466746

don Reuss SH, Bose N, Srinivasan J, Yim JJ, Judkins JC, Sterneberg PW, Schroeder FC. 2012. Comparative metabolomics reveals biogenesis of Ascarosides, a modular library of small-molecule signals in C. elegans. Journal of the American Chemical Society 134:1817–1824. DOI: https://doi.org/10.1021/ja210202y, PMID: 22239548

Wallace ML, Burette AC, Weinberg RJ, Philpot BD. 2012. Maternal loss of Ube3a produces an excitatory/inhibitory imbalance through neuron type-specific synaptic defects. Neuron 74:793–800. DOI: https://doi.org/10.1016/j.neuron.2012.03.036, PMID: 22681684

Wan X, Zhou Y, Chan CM, Yang H, Yeung C, Chow KL. 2019. SRD-1 in AWA neurons is the receptor for female volatile sex pheromones in C. elegans males. EMBO Reports 20:e46288. DOI: https://doi.org/10.15252/embr.201846288, PMID: 30792215

Whitaker LR, Degoulet M, Morikawa H. 2013. Social deprivation enhances VTA synaptic plasticity and drug-induced contextual learning. Neuron 77:335–345. DOI: https://doi.org/10.1016/j.neuron.2012.11.022, PMID: 23352169

Wu T, Duan F, Yang W, Liu H, Caballero A, Fernandes de Abreu DA, Dar AR, Alcedo J, Ch’ng Q, Butcher RA, Zhang Y. 2019. Pheromones modulate learning by regulating the balanced signals of two insulin-like peptides. Neuron 104:1095–1109. DOI: https://doi.org/10.1016/j.neuron.2019.09.006, PMID: 31676170

Yin JA, Gao G, Liu XJ, Hao ZQ, Li K, Kang XL, Li H, Shan YH, Hu WL, Li HP, Cai SQ. 2017. Genetic variation in glia-neuron signalling modulates ageing rate. Nature 551:198–203. DOI: https://doi.org/10.1038/nature24463, PMID: 29120414

Zamponi GW. 2016. Targeting voltage-gated calcium channels in neurological and psychiatric diseases. Nature Reviews Drug Discovery 15:19–34. DOI: https://doi.org/10.1038/nrd.2015.5, PMID: 26542451

Zhang X, Noguez JH, Zhou Y, Butcher RA. 2013. Analysis of ascarosides from Caenorhabditis elegans using mass spectrometry and NMR spectroscopy. Methods in Molecular Biology 1068:71–92. DOI: https://doi.org/10.1007/978-1-62703-619-1_6, PMID: 24014355

Zhou Y, Wang Y, Zhang X, Bhar S, Jones Lipinski RA, Han J, Feng L, Butcher RA. 2018. Biosynthetic tailoring of existing ascaroside pheromones alters their biological function in C. elegans. eLife 7:e33286. DOI: https://doi.org/10.7554/eLife.33286, PMID: 29863473

Zou W, Fu J, Zhang H, Du K, Huang W, Yu J, Li S, Fan Y, Baylis HA, Gao S, Xiao R, Ji W, Kang L, Xu T. 2018. Decoding the intensity of sensory input by two glutamate receptors in one C. elegans interneuron. Nature Communications 9.5. DOI: https://doi.org/10.1038/s41467-018-06819-5