GENETICS, 2023, 223(4), iyad017
https://doi.org/10.1093/genetics/iyad017
Advance Access Publication Date: 8 February 2023
Investigation

OXFORD GENETICS

The *Caenorhabditis elegans* innexin INX-20 regulates nociceptive behavioral sensitivity

Aditi H. Chaubey,[1] Savannah E. Sojka,[1] John O. Onukwufor,[2] Meredith J. Ezak,[1] Matthew D. Vandermeulen,[1] Alexander Bowitch,[1] Anežka Vodičková,[3] Andrew P. Wojtovich,[2,3] Denise M. Ferkey[1,]*

[1]Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY 14260, USA
[2]Department of Pharmacology and Physiology, University of Rochester Medical Center, Rochester, NY 14642, USA
[3]Department of Anesthesiology and Perioperative Medicine, University of Rochester Medical Center, Rochester, NY 14642, USA

*Corresponding author. Department of Biological Sciences, University at Buffalo, State University of New York, Buffalo, NY 14260, USA. Email: dmferkey@buffalo.edu

Abstract

Organisms rely on chemical cues in their environment to indicate the presence or absence of food, reproductive partners, predators, or other harmful stimuli. In the nematode *Caenorhabditis elegans*, the bilaterally symmetric pair of ASH sensory neurons serves as the primary nociceptors. ASH activation by aversive stimuli leads to backward locomotion and stimulus avoidance. We previously reported a role for guanylyl cyclases in dampening nociceptive sensitivity that requires an innexin-based gap junction network to pass cGMP between neurons. Here, we report that animals lacking function of the gap junction component INX-20 are hypersensitive in their behavioral response to both soluble and volatile chemical stimuli that signal through G protein-coupled receptor pathways in ASH. We find that expressing *inx-20* in the ADL and AFD sensory neurons is sufficient to dampen ASH sensitivity, which is supported by new expression analysis of endogenous INX-20 tagged with mCherry via the CRISPR-Cas9 system. Although ADL does not form gap junctions directly with ASH, it does so via gap junctions with the interneuron RMG and the sensory neuron ASK. Ablating either ADL or RMG and ASK also resulted in nociceptive hypersensitivity, suggesting an important role for RMG/ASK downstream of ADL in the ASH modulatory circuit. This work adds to our growing understanding of the repertoire of ways by which ASH activity is regulated via its connectivity to other neurons and identifies a previously unknown role for ADL and RMG in the modulation of aversive behavior.

Keywords: innexin, INX-20, gap junction, cGMP, behavior, chemosensation, nociception, avoidance, *C elegans*

Introduction

External chemical cues provide information that drives *Caenorhabditis elegans* attraction to or avoidance of a particular environment (Bargmann 2006; Ferkey *et al.* 2021). However, the appropriateness of a behavioral response is context-dependent, reflective of both an animal's life history and its present internal state. Thus, the signal transduction pathways that mediate chemosensation are subject to modulatory inputs and regulation. For example, *C. elegans* sensitivity to aversive stimuli correlates with feeding status, such that wild-type animals are more likely to respond to noxious cues when they are well fed than they are upon food deprivation (Chao *et al.* 2004; Ferkey *et al.* 2007; Wragg *et al.* 2007; Harris *et al.* 2009; Ezcurra *et al.* 2011; Krzyzanowski *et al.* 2016). This behavioral reprioritization likely reflects the need to balance avoiding potentially dangerous situations with the central requirement to find food. Diminishing aversive responses when starved may serve to increase the likelihood that an animal risks entry into a new environment that could potentially provide a food source.

Across species, nociceptive sensory systems detect harmful stimuli and allow for the initiation of protective behavioral responses. In *C. elegans*, the ASH nociceptors are the primary sensory neurons used to detect aversive stimuli. The ASH neurons are considered "polymodal" since they detect a broad range of aversive stimuli, including tastants, odorants, ions, heavy metals, detergent SDS, extreme pHs, osmotic stress, and mechanosensory stimulation (nose touch) (Bargmann *et al.* 1990; Kaplan and Horvitz 1993; Troemel *et al.* 1995; Hart *et al.* 1999; Sambongi *et al.* 1999; Troemel 1999; Sambongi *et al.* 2000; Hilliard *et al.* 2002, 2004, 2005; Yoshida *et al.* 2012; Chatzigeorgiou *et al.* 2013; Sassa and Maruyama 2013; Taniguchi *et al.* 2014; Tran *et al.* 2017; Liu *et al.* 2018). Among these, bitter tastants (e.g. quinine) and the odorant 1-octanol are thought to signal through G protein-coupled receptors (GPCRs) (Fukuto *et al.* 2004; Ezak *et al.* 2010), although the identity of their receptors is not yet known.

Following GPCR activation by a ligand, heterotrimeric G proteins (composed of Gα, Gβ, and Gγ subunits) transduce the signals to intracellular effectors. While G protein-coupled signaling can be inhibited directly at the level of receptors via phosphorylation of GPCRs by G protein-coupled receptor kinases (GRKs) (Pitcher *et al.* 1998; Bunemann and Hosey 1999; Ferguson 2001; Pierce and Lefkowitz 2001; Fukuto *et al.* 2004; Wood *et al.* 2012; Wood and Ferkey 2016; Komolov and Benovic 2018), the downstream G protein subunits can also be regulated. Regulator of G protein signaling (RGS) GTPase-activating proteins can dampen Gα signaling by binding to Gα subunits and stabilizing the transition state for GTP hydrolysis, thus accelerating their intrinsic GTPase activity (Ross and Wilkie 2000; Hollinger and Hepler 2002; Willars 2006).

Received: September 03, 2022. **Accepted:** February 01, 2023
© The Author(s) 2023. Published by Oxford University Press on behalf of the Genetics Society of America. All rights reserved. For permissions, please e-mail: journals.permissions@oup.com

We previously identified a role for the *C. elegans* cGMP-dependent protein kinase (PKG) EGL-4 in the negative regulation of nociceptive signaling within the ASH neurons (Krzyzanowski *et al.* 2013). EGL-4 likely dampens ASH sensitivity by phosphorylating and activating RGS-2 and RGS-3, which then downregulate G protein-coupled sensory signaling in ASH (Krzyzanowski *et al.* 2013). Thus, animals lacking EGL-4 function are hypersensitive to dilute concentrations of stimuli that signal through G protein-coupled pathways (bitter tastants and 1-octanol) (Krzyzanowski *et al.* 2013). As a PKG, EGL-4 requires cGMP binding for activation (Krzyzanowski *et al.* 2013), the source of which is (at least in part) the guanylyl cyclase ODR-1 (Krzyzanowski *et al.* 2016). Our previous work further suggested that, upon food deprivation, cGMP produced by ODR-1 in the AWB, AWC, and ASI sensory neurons moves through a gap junction network from these neurons to ASH to dampen nociceptive sensitivity (Krzyzanowski *et al.* 2016).

While a gap junction-based network has been proposed to modulate ASH signaling, there is still limited knowledge as to the extent of the network or the innexins (*in*vertebrate analogs of the con*nexins*) involved. INX-4 functions in ASH and, like *egl-4* loss-of-function (lof) (Krzyzanowski *et al.* 2013), *inx-4(lof)* animals are hypersensitive to dilute quinine (Krzyzanowski *et al.* 2016). Thus, INX-4 may serve to let cGMP into ASH. Similarly, loss of INX-18 or INX-19 function leads to quinine hypersensitivity; INX-18 is required in ASK (which forms gap junctions with ASH), while INX-19 function is required in both ASK and ASH (Voelker *et al.* 2019). Both *inx-18(lof)* and *inx-19(lof)* animals showed diminished cGMP reporter (Woldemariam *et al.* 2019) fluorescence in ASH, suggesting that gap junctions between ASK and ASH are important for transporting cGMP into ASH (Voelker *et al.* 2019). Although *inx-20(lof)* animals also showed behavioral hypersensitivity to dilute quinine (Krzyzanowski *et al.* 2016), its previously reported expression pattern was limited primarily to pharyngeal tissue (Altun *et al.* 2009), so its site of action for modulating ASH signaling was not pursued.

Here, we provide evidence that INX-20 is expressed in and functions in the ADL and AFD sensory neurons to modulate ASH-mediated quinine response. ADL connects to ASH indirectly, via gap junctions with the RMG interneurons and the ASK sensory neurons. Like *inx-20(lof)* mutants, animals lacking either ADL, RMG, or ASK are also hypersensitive to dilute quinine. This is the first evidence that these neurons are part of the network that regulates ASH nociceptive sensitivity (Krzyzanowski *et al.* 2016), and it identifies a neuronal role for INX-20 in modulating *C. elegans* chemosensory behavior.

Materials and methods

C. elegans culture

Strains were maintained under standard conditions on NGM agar plates seeded with OP50 *Escherichia coli* bacteria (Brenner 1974).

Behavioral assays

Well-fed young adult *C. elegans* animals grown at 20°C were used for analysis, and all behavioral assays were performed on at least three separate days, in parallel with controls. Response to the soluble aversive tastants was scored as the percentage of animals that initiated backward locomotion within 4 s of encountering a drop of the stimulus placed on the agar plate in front of a forward moving animal (Hilliard *et al.* 2002, 2004; Fukuto *et al.* 2004; Ezak *et al.* 2010; Krzyzanowski *et al.* 2013, 2016). We note that our studies use a "wet drop" that animals enter, not a "dry drop," and each animal is tested only once. Tastants were dissolved in M13 buffer, pH 7.4 (Wood 1988). Response to 1-octanol was scored as the amount of time it took an animal to initiate backward locomotion when presented with a hair dipped in 1-octanol (Troemel *et al.* 1995; Hart *et al.* 1999; Fukuto *et al.* 2004; Ferkey *et al.* 2007; Ezak *et al.* 2010; Likhite *et al.* 2015; Krzyzanowski *et al.* 2016). All animals were tested 30 min after transfer to NGM plates lacking bacteria ("off food"). For heat shock experiments, animals were raised to young adulthood and then shifted to 33°C for 2 h. They were allowed to recover for 4 h at 20°C prior to assaying. All data are presented as the mean ± standard error of the mean (SEM).

Statistical analysis

One-way ANOVA with Tukey's Honestly Significant Difference (HSD) was used for all statistical analyses, except Figs. 1 and 5c, for which the Student's two-tailed t-Test was used. The ANOVA statistics were computed using GraphPad Prism 7.0. In all figures, * denotes $P < 0.05$, ** denotes $P < 0.01$, *** denotes $P < 0.001$, and **** denotes $P < 0.0001$. ns denotes $P \geq 0.05$.

Plasmid construction

For a list of plasmids and a description of their construction, including primers used, see Supplemental Information.

Transgenic strain generation

Germline transformations were performed as previously described (Mello *et al.* 1991). For *inx-20* rescue experiments, 25 ng/µl of pJM67 *elt-2::gfp* plasmid (Fukushige *et al.* 1998) was used as the co-injection marker, along with 50 ng/µl of the rescuing plasmid. Cell-specific RNA interference (RNAi) knockdown experiments were performed as previously described (Esposito *et al.* 2007), except *inx-1*, *inx-7*, and *inx-8*. As fusion PCRs were unsuccessful for these three genes, a noncoding fragment of each was instead subcloned into pPD49.26 in either the sense or antisense orientations under the control of the *nlp-56* promoter. 50 ng/µl of pJM67 *elt-2::gfp* plasmid (Fukushige *et al.* 1998) was co-injected with ∼50 ng/µl of each PCR fusion product (Esposito *et al.* 2007) or 50 ng/µl each of the sense and antisense plasmids. See Supplemental Information for a list of primers used. Genetic ablation experiments were performed as previously described (Chelur and Chalfie 2007). 50 ng/µl of *unc-122p::rfp* (Miyabayashi *et al.* 1999) (Addgene #8938) was co-injected with 75 ng/µl of *mCasp* plasmid constructs. For the blue light-inducible guanylyl cyclase (BlgC) experiments, 15 ng/µl of pJM67 *elt-2::gfp* plasmid (Fukushige *et al.* 1998) was used as the co-injection marker, along with 20 ng/µl of the cyclase plasmid and 65 ng/µl of pUC19 (Yanisch-Perron *et al.* 1985).

Strains

For a list of strains used in this study, see Supplemental Information.

CRISPR-Cas9 genome editing of *inx-20*

Homology-directed genome editing with CRISPR-Cas9 (Paix *et al.* 2015) was used to fuse mCherry to the carboxyl terminus (C-terminus) of INX-20 prior to the translational stop codon/3′ UTR of *inx-20*. Briefly, mCherry was amplified from pCFJ104 (Fwd: ACACCAGCTCCTCAATTCCTTCGACCTCCAAGCAGtaGAA TGGCaTCAGCTGCGAATGTAggagcatcgggagcctcaggagcatcgATGG TCTCAAAGGGTGAAG, Rev: ggtatcaggaaaacaacaaaatattgaaatta TTACTTATACAATTCATCCATGCCACC) resulting in a PCR repair template that contained 35 bp of homology to either side of the Cas9 cut site and encoded a nine-amino acid flexible linker region. Cas9 was purified, as previously described (Trewin *et al.* 2019). The C-terminus of INX-20 was targeted with the crRNA CATTC GCAGCTGAAGCCATT (Dharmacon), and edits were selected

using the *dpy-10* co-CRISPR approach (Paix *et al.* 2015; Trewin *et al.* 2019). A mix containing 25 mM KCl, 7.5 mM HEPES, 1 µg/µl tracrRNA, 0.8 µg/µl *inx-20* crRNA, 50 ng/µl *dpy-10* ssODN, 0.16 µg/µl *dpy-10* crRNA, 2.5 µg/µl Cas9, and 180 ng/µl *inx-20::linker::mCherry* repair template was injected into the germline of adult *C. elegans*. F1 progeny were screened for *dpy-10* edits and mCherry fluorescence. The CRISPR allele was confirmed using PCR amplification (Fwd: AGGTCTGCGACGGAAAACAT; Rev: GCGGATTTC TTTTGTGCTTTGTG), sequenced, and outcrossed to N2 to remove the Dpy phenotype.

Neuronal identification

Animals carrying *inx-20(jbm47[inx-20::linker::mCherry])*, which tags endogenous INX-20 with mCherry, were crossed to animals carrying integrated transgenes marking selected neurons. ADL was marked by *otIs646* (*srh-127p::gfp*), and AFD was marked by *oyIs18* (*gcy-8p::gfp*). Images were obtained using a Zeiss Axio Imager Z1 microscope [using a 40× Plan-NEO oil objective (AFD) or 63× Plan-APO oil objective (ADL), epi-fluorescence, and DIC optics], high-resolution AxioCam MRm digital camera, and Zeiss AxioVision software.

Results

The INX-20 innexin gap junction component regulates sensitivity to G protein-coupled stimuli

We previously found that animals lacking INX-20 function are hypersensitive in response to a dilute concentration (1 mM) of the bitter tastant quinine; significantly more *inx-20 lof* animals responded to dilute quinine than wild-type animals (Krzyzanowski *et al.* 2016) (and Fig. 1a). *inx-20(lof)* animals were also hypersensitive to dilute concentrations of an additional bitter tastant, amodiaquine (Fig. 1b). As in mammals, *C. elegans* detect bitter compounds via G protein-coupled receptor pathways (Hilliard *et al.* 2004; Chandrashekar *et al.* 2006; Palmer 2007). The aversive volatile odorant 1-octanol also activates G protein-coupled signaling (Roayaie *et al.* 1998; Fukuto *et al.* 2004). To determine whether INX-20 regulates 1-octanol avoidance, animals were assayed for their time to reverse when presented with a range of 1-octanol concentrations. In response to dilute 1-octanol (30 and 10%), *inx-20(lof)* animals responded better than wild-type animals (Fig. 1c).

C. elegans also avoid soluble stimuli, including the heavy metal copper and the detergent SDS, that are not thought to signal through G protein-coupled receptors (Bargmann *et al.* 1990; Sambongi *et al.* 1999; Hilliard *et al.* 2002, 2005). To assess whether INX-20 modulates response to these compounds, animals were tested for their avoidance response across a range of concentrations for each. In all cases, the response of *inx-20(lof)* animals was similar to that of wild-type animals (Fig. 1, d, e). We conclude that INX-20 regulates response to a subset of G protein-coupled chemosensory responses, including the bitter tastants quinine and amodiaquine and the aversive odorant 1-octanol, but does not regulate nociceptive sensitivity in general.

INX-20 function in adult animals is sufficient to regulate behavioral sensitivity

inx-20 expression was previously seen to begin at the threefold larval stage and continue through adulthood (Altun *et al.* 2009). To assess when INX-20 function is required to modulate quinine sensitivity, the *inx-20* cDNA was placed under the control of a heat shock inducible promoter (Stringham *et al.* 1992) and introduced into *inx-20(lof)* animals. Induction of *inx-20* expression by heat shock in adult animal stages, after developmental cell fate specification and neuronal connectivity are complete, returned the behavioral response to dilute quinine to wild-type levels when assayed 4 h later (Fig. 2a). Transgenic animals that were not heat shocked remained hypersensitive, similar to *inx-20(lof)* animals (Fig. 2a). These results demonstrate that INX-20 function in adult animal stages is sufficient to modulate behavioral sensitivity to dilute quinine.

INX-20 functions in sensory neurons to regulate quinine sensitivity

Assessment of the *inx-20* pattern of expression is complicated by the fact that *inx-20* lies within an operon. Using only a short upstream promoter sequence to drive GFP expression (co-injected with a corresponding cosmid containing *inx-20* genomic sequence to allow for in vivo homologous recombination), *inx-20* was previously reported to be expressed in just a few cells of the alimentary canal: the pm1, pm2, and pm8 cells of the pharynx, the intestinal–rectal valve, and the pharyngeal epithelium (weakly); no neuronal expression was observed (Altun *et al.* 2009). Furthermore, no pharyngeal phenotype has been reported for *inx-20(lof)* animals. To determine whether INX-20 functions in pharyngeal cells to regulate quinine sensitivity, the *ceh-34* (pm1, pm2) and *hmgr-1* (pm8) promoters (Hirose *et al.* 2010; Ranji *et al.* 2014) were used in combination to restore *inx-20* cDNA expression, and animals were assayed for response to 1 mM quinine. However, *inx-20(lof)* animals expressing these constructs remained hypersensitive (Fig. 2b). This suggests that INX-20 is likely expressed and functions in cells not identified by the previous expression analysis.

The gap junction components INX-4 (ASH), INX-18 (ASK), and INX-19 (ASH/ASK) have been shown to function in sensory neurons to regulate quinine sensitivity (Krzyzanowski *et al.* 2016; Voelker *et al.* 2019). To assess whether INX-20 might also have a neuronal role in modulating aversive behavior, despite the lack of reported expression there, we used the pan-neural *rab-3* promoter (Nonet *et al.* 1997; Frokjaer-Jensen *et al.* 2008) to express *inx-20* cDNA in *inx-20(lof)* animals. Expression using this promoter dampened the *inx-20(lof)* hypersensitive response such that transgenic animals responded comparable to wild-type animals (Fig. 2c). Furthermore, *inx-20* cDNA expression in just the sensory neurons, using the *osm-5* promoter (Haycraft *et al.* 2001) also returned the quinine sensitivity of *inx-20(lof)* animals to wild-type levels (Fig. 2d). Combined, these data suggest that INX-20 functions in adult sensory neurons to regulate behavioral response to quinine.

INX-20 functions in the ADL and AFD sensory neurons to regulate quinine sensitivity

The *C. elegans* Neuronal Gene Expression Map & Network (CeNGEN) project aims to report the complete transcriptional profile of the entire *C. elegans* nervous system at single-neuron resolution (Hammarlund *et al.* 2018). We consulted the publicly available CeNGEN site (https://cengen.shinyapps.io/CengenApp/) to see if this approach might have revealed additional sites of *inx-20* expression missed in the original GFP analysis. CeNGEN identified the ADL sensory neurons as having the highest expression of *inx-20* mRNA of any cell type. Of the next nine cell types listed, PQR and PHC also showed expression, although at lower levels (∼3.5-fold and ∼10.6-fold lower, respectively). RIR, ASI, AFD, and AWC^{OFF} neurons showed very low expression, ranging from ∼20- to 40-fold lower than the level seen in ADL.

ADL has not previously been examined for a role in the modulation of quinine sensitivity. However, among the neurons listed

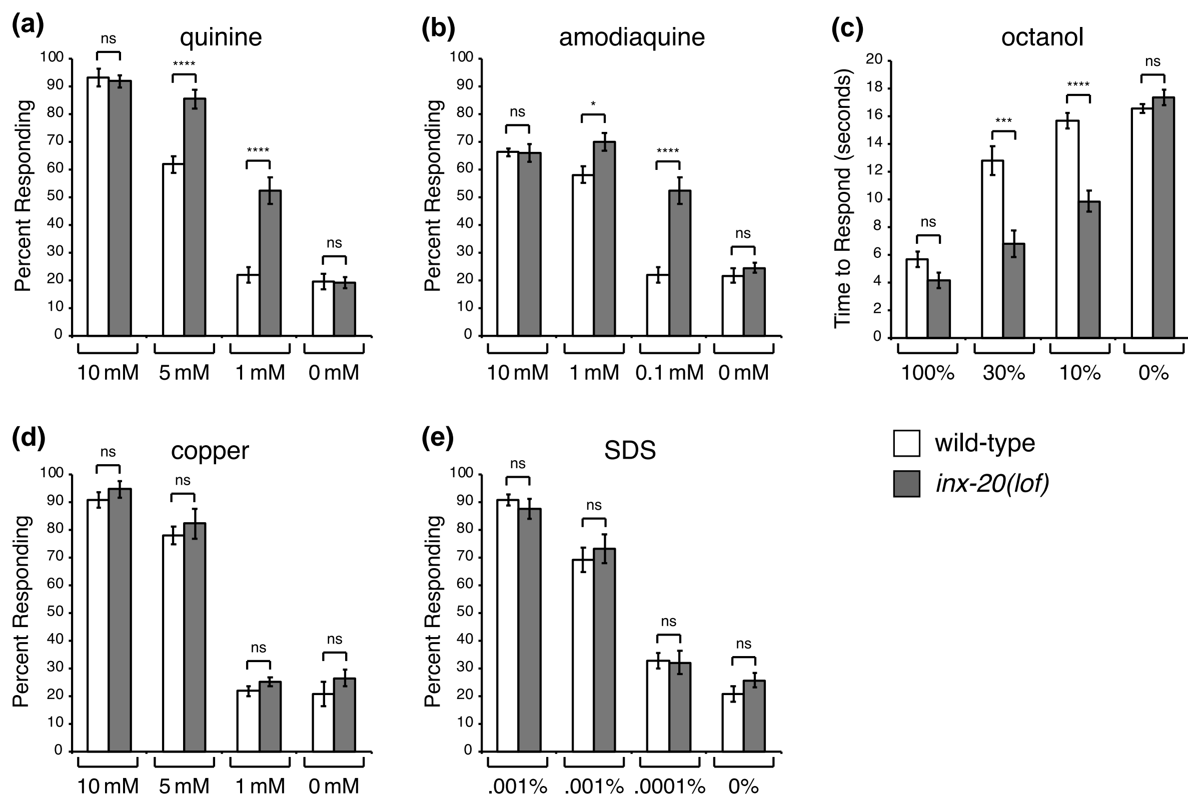

Fig. 1. INX-20 does not regulate ASH sensitivity in general. Animals lacking INX-20 function are hypersensitive to dilute concentrations of the bitter tastants quinine (a) and amodiaquine (b). The percentage of animals responding is shown in both panels. *inx-20(lof)* animals are also hypersensitive to dilute concentrations of the volatile odorant octanol (c). The time to response is shown. *inx-20(lof)* animals respond similarly to wild-type animals to both copper (d) and SDS (e), across a range of concentrations ($P > 0.2$ at each). The percentage of animals responding is shown. $n > 40$ for each. Error bars represent the standard error of the mean (SEM). Allele used: *inx-20(ok426)*. WT = the N2 wild-type strain. lof = loss-of-function. ns = not significant.

above, AFD and ASI were previously implicated in the modulation of quinine sensitivity, playing a major and minor role, respectively (Krzyzanowski *et al.* 2016). To examine possible sites of INX-20 function in this context, we used the cell-specific RNAi approach of Esposito *et al.* (Esposito *et al.* 2007) to knock down *inx-20* expression in the ADL, AFD, and ASI sensory neurons (Fig. 3a). Knockdown of *inx-20* in either ADL or AFD resulted in more animals responding to dilute quinine relative to wild-type animals, although neither reached the level of response seen in *inx-20(lof)* animals. However, simultaneous knockdown of *inx-20* in both ADL and AFD resulted in quinine hypersensitivity comparable to *inx-20(lof)* animals. Knockdown in ASI had no effect.

As a complimentary approach, to determine whether INX-20 function in either ADL, AFD, or ASI would be sufficient to dampen the quinine hypersensitivity of *inx-20(lof)* animals, we used cell-specific promoters to express the *inx-20* cDNA in each of these cells (Fig. 3b). Expression in either ADL or AFD significantly dampened *inx-20(lof)* hypersensitivity. *inx-20(lof)* animals expressing *inx-20* cDNA in just ADL (or in both ADL and AFD simultaneously) responded comparable to wild-type animals (Fig. 3b). Expression in ASI had no effect. Combined, these results suggest that gap junctions that include INX-20 subunits function in the ADL and AFD neurons to regulate behavioral response to the bitter tastant quinine.

INX-20 is expressed in the ADL and AFD sensory neurons

In an attempt to visualize endogenous INX-20 expression, we used the CRISPR-Cas9 homology-directed genome editing approach optimized for *C. elegans* (Paix *et al.* 2015) to fuse mCherry to the C-terminus of endogenous INX-20. Gross whole animal examination revealed bright fluorescence marking expression at the posterior end of the terminal bulb of the pharynx and at the posterior end of the intestine (Supplemental Fig. 1), consistent with reported expression in pm8 and the intestinal–rectal valve (Altun *et al.* 2009), respectively. To look for additional sites perhaps having lower level expression, animals expressing INX-20::mCherry were crossed to animals carrying integrated transgenes marking ADL (*srh-127p::gfp*) or AFD (*gcy-8p::gfp*). Consistent with the cell-specific RNAi and rescue experiments described above, INX-20::mCherry expression was observed in both neurons (Fig. 4). Although expression levels were quite low, it was observed in both ADLs in 67/90 animals; expression was not seen in 23 animals. Expression was observed in both AFDs in 91/120 animals; expression was seen in only 1 AFD in 2 animals, and expression was not seen in the remaining 27 animals. We conclude that INX-20 is expressed in ADL and AFD, albeit at very low levels, likely near the threshold of detection. We also note that discrete puncta, which have been observed for several other innexins, were not observed here.

The ADL, RMG, and ASK neurons modulate quinine sensitivity

To further examine the role of the ADL sensory neurons in modulating quinine sensitivity, we examined animals in which these cells were genetically ablated via cell-specific expression (Taniguchi *et al.* 2014) of mouse caspase-1 (*srh-281p::mCasp1*) (Hamakawa *et al.* 2015). Animals lacking ADL were hypersensitive to dilute quinine, responding similarly to *inx-20(lof)* animals

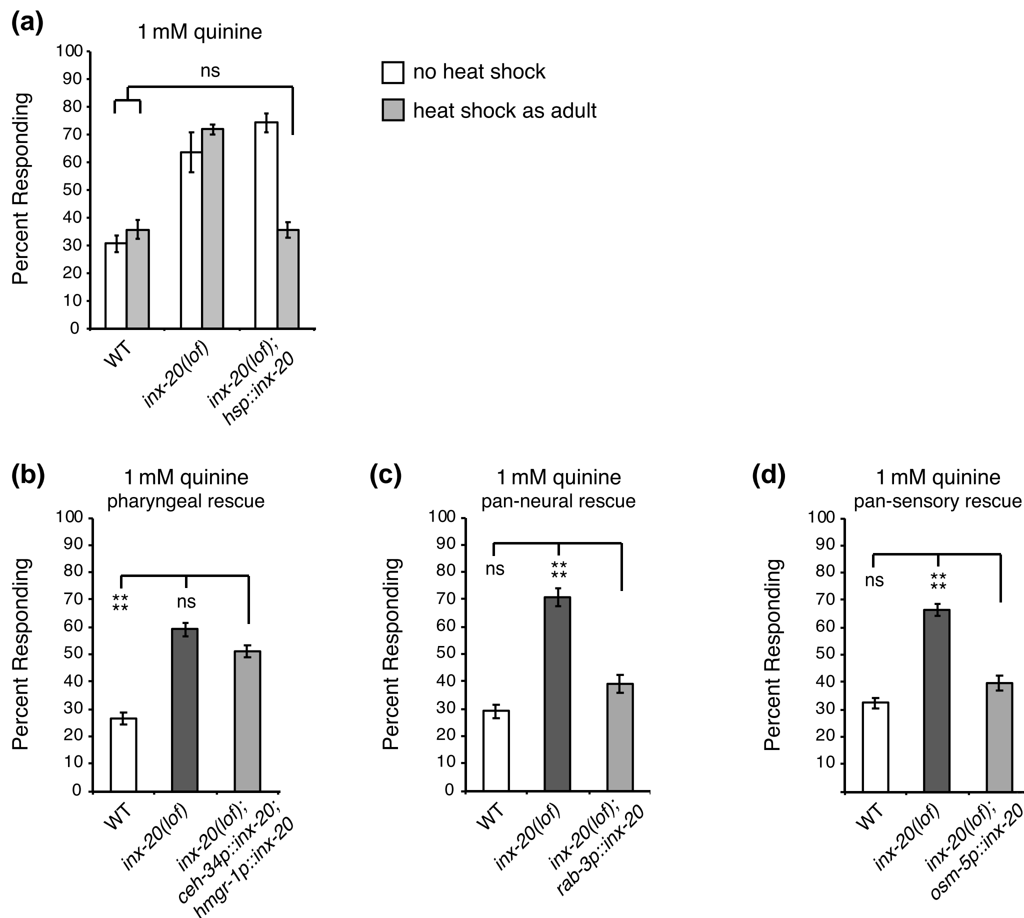

Fig. 2. INX-20 functions in adult sensory neurons. a) INX-20 functions in adult animals to regulate behavioral sensitivity. Adult *inx-20(lof)* animals expressing *inx-20* cDNA under the control of a heat shock inducible promoter (*hsp*) (Stringham *et al.* 1992) were tested without heat shock (white bars) or 4 h after heat shock treatment (gray bars). While *inx-20(lof)* animals have a hypersensitive response to dilute (1 mM) quinine, heat shock-induced expression of *inx-20* in adult *inx-20(lof)* animals abolished this hypersensitivity and returned response to the degree seen in wild-type animals ($P > 0.9$ when compared to wild-type animals with or without heat shock treatment). b) *inx-20* expression in pharyngeal cells using the *ceh-34* (pm1, pm2) and *hmgr-1* (pm8) promoters (Hirose *et al.* 2010; Ranji *et al.* 2014) was not sufficient to rescue the behavioral hypersensitivity of *inx-20(lof)* animals ($P > 0.07$ when compared to *inx-20(lof)* animals). c) Pan-neural expression of *inx-20* with the *rab-3* promoter (Nonet *et al.* 1997; Frokjaer-Jensen *et al.* 2008) was sufficient to eliminate behavioral sensitivity and returned response to levels comparable to wild-type animals ($P < 0.001$ when compared to *inx-20(lof)*, $P > 0.1$ when compared to wild-type). d) Expression of *inx-20* in just the sensory neurons, using the *osm-5* promoter (Haycraft *et al.* 2001), was also sufficient to rescue hypersensitivity and return response to wild-type levels ($P < 0.001$ when compared to *inx-20(lof)*, $P > 0.2$ when compared to wild-type). The percentage of animals responding is shown. Error bars represent the standard error of the mean. The combined data of ≥ 3 independent lines and $n \geq 90$ transgenic animals are shown in each panel. Allele used: *inx-20(ok426)* loss-of-function. WT = the N2 wild-type strain. lof = loss-of-function. ns = not significant.

(Fig. 5a). We consulted the *C. elegans* WormWiring project (https://wormwiring.org) for the most current annotations of the original electron micrograph series reported by White *et al.* (1986) and saw that ADL does not form gap junction connections directly with the ASH neurons that detect quinine. Instead, ADL connects indirectly to ASH via the interneuron RMG and the sensory neuron ASK (White *et al.* 1986; Cook *et al.* 2019). To determine whether RMG regulates quinine response, we selectively (Taylor *et al.* 2019; Lorenzo *et al.* 2020) ablated this pair of neurons in wild-type animals (by expressing *nlp-56p::mCasp1*). Loss of the RMG interneurons also resulted in significant behavioral hypersensitivity (Fig. 5a). While ASK aids in the detection of 10 mM quinine (Hilliard *et al.* 2004), gap junction connections between ASK and ASH were previously shown to dampen ASH response to dilute (1 mM) quinine (Voelker *et al.* 2019). We found that ablation of ASK (by expressing *srbc-66p::mCasp1*) also resulted in hypersensitivity to 1 mM quinine (Fig. 5a), consistent with ASK dampening the dilute quinine response.

To determine whether RMG and ASK lie downstream of INX-20 function in ADL, we expressed *srh-220p::inx-20* to restore INX-20 in the ADL neurons of *inx-20(lof)* animals or *inx-20(lof)* animals lacking RMG (via *nlp-56p::mCasp1* expression) or ASK (via *srbc-66p::mCasp1* expression) or both. While expression of *inx-20* cDNA in ADL returned quinine response to wild-type levels in *inx-20(lof)* animals with both of these neuron pairs, animals lacking RMG or ASK remained somewhat hypersensitive despite *inx-20* expression in ADL (Fig. 5b). However, in both cases there was a partial decrease in the level of hypersensitivity compared to *inx-20(lof)* animals. In contrast, simultaneous loss of both RMG and ASK completely blocked the ability of ADL-expressed *inx-20* cDNA to dampen quinine hypersensitivity. The response frequency of these animals remained comparable to *inx-20(lof)* animals (Fig. 5b).

To assess whether cGMP generation in ADL is sufficient to dampen quinine sensitivity, we used the *srh-220* promoter (McCarroll *et al.* 2005) to express a blue light-inducible guanylyl

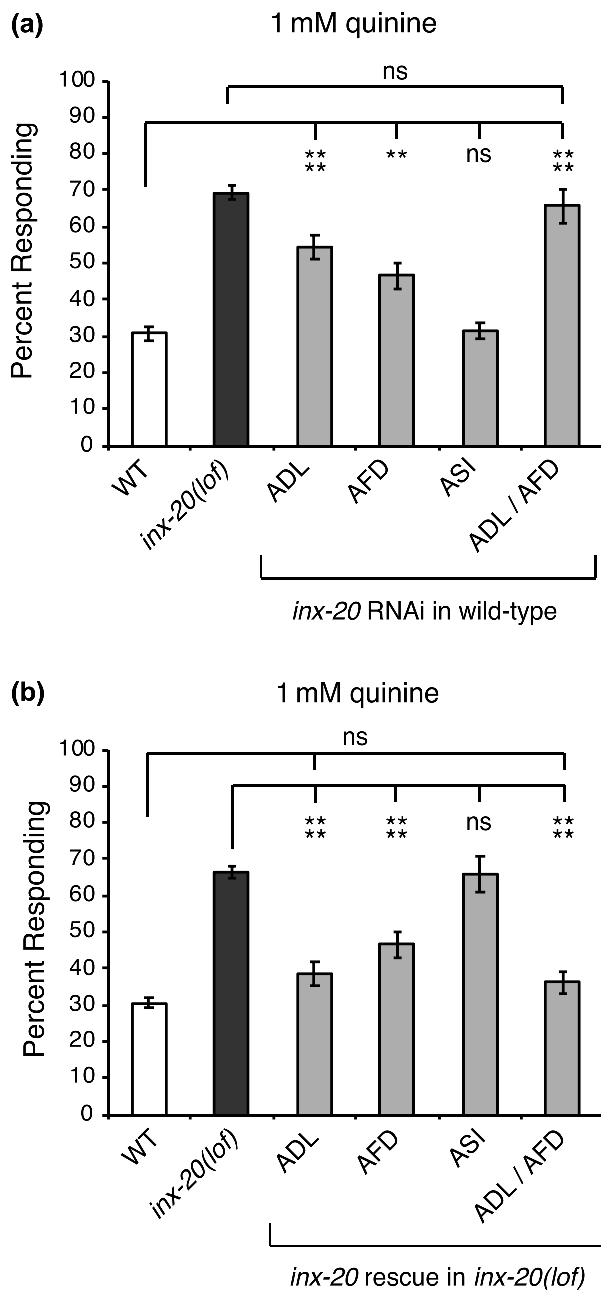

Fig. 3. INX-20 functions in the ADL and AFD sensory neurons. a) The *srh-220* (ADL) (McCarroll *et al.* 2005), *gcy-8* (AFD) (Yu *et al.* 1997), or *gpa-4* (ASI) (Jansen *et al.* 1999) promoters were used to co-express a noncoding fragment of *inx-20* in the sense and antisense orientations in otherwise wild-type animals. RNAi knockdown of *inx-20* in either ADL or AFD leads to behavioral hypersensitivity to dilute (1 mM) quinine ($P < 0.0001$ and $P < 0.01$, respectively, when compared to wild-type), while knockdown in ASI had no effect ($P > 0.9$). Simultaneous knockdown in both ADL and AFD leads to hypersensitivity comparable to *inx-20(lof)* animals ($P > 0.9$). b) The *srh-220* (ADL) (McCarroll *et al.* 2005), *gcy-8* (AFD) (Yu *et al.* 1997), or *gpa-4* (ASI) (Jansen *et al.* 1999) promoters were used to express *inx-20* cDNA in *inx-20(lof)* animals. Expression in ADL fully rescued quinine hypersensitivity ($P > 0.2$ when compared to wild-type animals), while expression in AFD partially rescued hypersensitivity ($P < 0.001$ when compared to either *inx-20(lof)* or wild-type animals). Simultaneous rescue in both ADL and AFD fully rescued quinine hypersensitivity ($P > 0.6$ when compared to wild-type animals). Expression of *inx-20* cDNA in ASI had no effect ($P > 0.9$ when compared to *inx-20(lof)* animals). The percentage of animals responding is shown. The combined data of ≥ 3 independent lines and $n \geq 90$ transgenic animals are shown. Allele used: *inx-20(ok426)* loss-of-function. WT = the N2 wild-type strain. lof = loss-of-function. ns = not significant.

cyclase (BlgC) (Ryu *et al.* 2010) in the ADL neurons of animals lacking the blue–violet light receptor LITE-1 (Edwards *et al.* 2008). When assayed 10 min after a 30-s exposure to blue light, animals expressing BlgC in the ADL sensory neurons displayed a 28% decrease in the percentage of animals responding to 5 mM quinine (Fig. 5c), while animals that were not preexposed to blue light displayed wild-type sensitivity. Blue light exposure did not dampen response in animals lacking INX-20 function. To determine whether RMG and/or ASK are required for ADL-generated cGMP to dampen quinine sensitivity, these neurons were ablated alone or in combination. Loss of either partially blocked the ability *srh-220p::BlgC* to dampen quinine response in *lite-1(lof)* animals; loss of RMG resulted only in an 18% decrease in percent responding, while loss of ASK resulted in a 16% decrease. However, simultaneous ablation of both RMG and ASK completely blocked the ability of ADL-expressed BlgC to dampen quinine response (Fig. 5c). Combined, these results suggest that gap junction connections between ADL and both RMG/ASK are important for passing cGMP to modulate ASH-mediated behavioral responses.

In addition to the gap junction connections described above, ADL also forms direct chemical synapses onto ASH (https://wormwiring.org), although it is not presynaptic to either RMG or ASK (White *et al.* 1986; Cook *et al.* 2019). UNC-13 and SNB-1 (synaptobrevin) proteins are required for synaptic vesicle fusion and neurotransmitter release at synapses (Nonet *et al.* 1998; Richmond *et al.* 1999; Tokumaru and Augustine 1999; Calahorro and Izquierdo 2018). To rule out a role for traditional chemical signaling from ADL in the modulation of quinine sensitivity, we used cell-specific RNAi (Esposito *et al.* 2007) to knock down *unc-13* or *snb-1* and block synaptic transmission from ADL because null mutants for both are lethal. Animals in which either *unc-13* or *snb-1* was knocked down in ADL did not show increased sensitivity to dilute quinine (Fig. 5d). Although it is possible that the degree of *unc-13* and *snb-1* knockdowns were not sufficient to fully disrupt synaptic transmission, these results suggest that ADL influences ASH-mediated response to quinine due to gap junction connections with RMG and ASK, and not via vesicular synaptic transmission.

The RMG interneurons utilize INX-7 to modulate quinine sensitivity

CeNGEN reports low-level expression of five innexins in the RMG interneurons: *unc-7*, *unc-9*, *inx-1*, *inx-7*, and *inx-8*. Among these, *inx-7* shows the highest level of expression. To determine whether any of these innexins function in RMG to modulate quinine response, we individually knocked down each in wild-type animals, using the *nlp-56* promoter. Only RMG knockdown of *inx-7* resulted in behavioral hypersensitivity to dilute (1 mM) quinine (Fig. 5e), suggesting that INX-7 contributes to RMG's role in dampening response to dilute quinine.

Discussion

Behavioral plasticity—the ability to adapt and fine-tune behavior to changes in the external environment or internal physiological changes—is critical for animal survival and can result from changes in broad brain areas or at specific neuronal connections. Chemical synapses utilize vesicular neurotransmitter release at synaptic clefts between cells. Gap junction channels are formed through the association of transmembrane connexin (vertebrate) or innexin (invertebrate) proteins. Sometimes referred to as electrical synapses, gap junctions are physical connections between cells that allow for direct cytoplasmic communication (Cheung

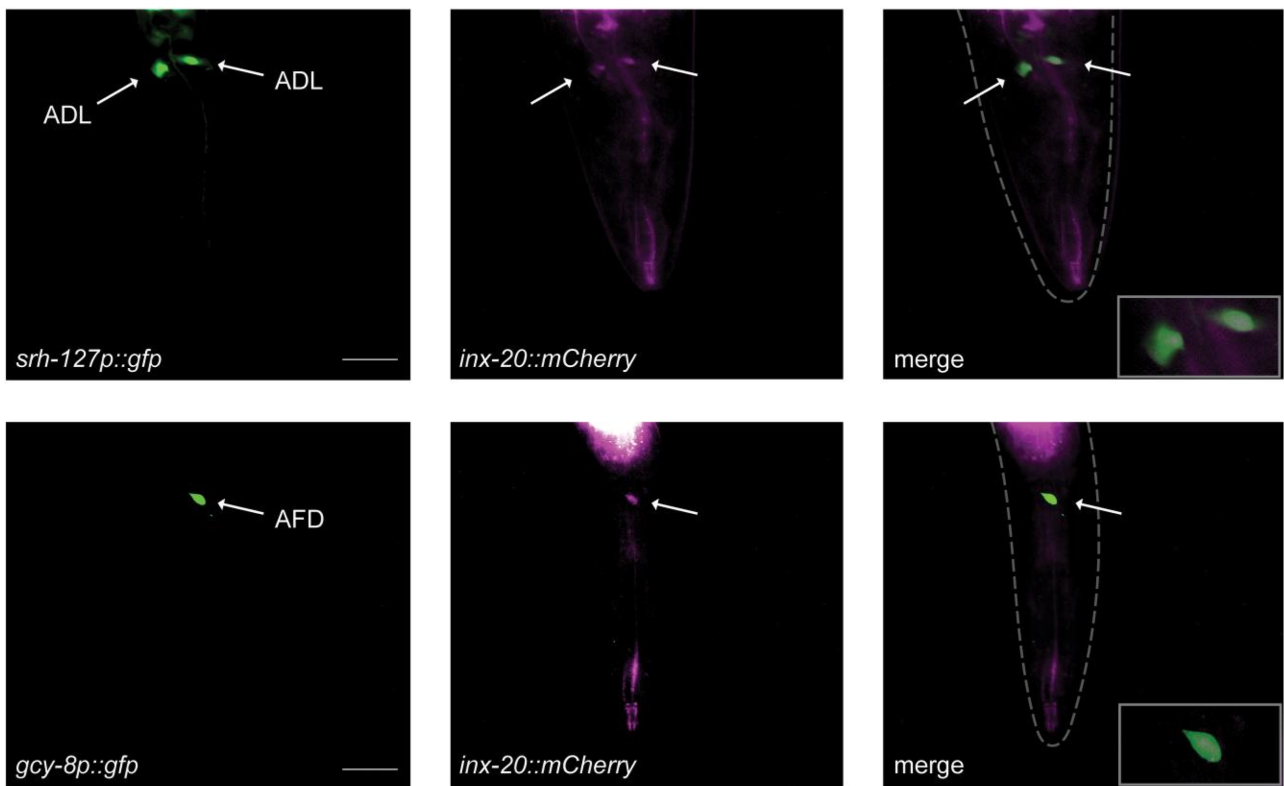

Fig. 4. INX-20::mCherry expression is seen in ADL and AFD. CRISPR-Cas9-mediated genome editing, optimized for *C. elegans* (Paix *et al.* 2015), was used to generate INX-20::mCherry. The integrated transgenes *otIs646* (Masoudi *et al.* 2018) (*srh-127p::gfp*) and *oyIs18* (Satterlee *et al.* 2001) (*gcy-8p::gfp*) were used to mark ADL and AFD, respectively. Weak INX-20::mCherry expression was observed in both neurons. Only one AFD is in the focal plane here. Insets show a zoomed in view of the soma where colocalization is observed. Scale bar = 25 μm in both panels.

et al. 2014; Palacios-Prado *et al.* 2014). However, it is now appreciated that their role extends beyond electrical coupling of cells and that a variety of ions and small molecules can pass through these channels in different cell types (Anderson and Albertini 1976; Saez *et al.* 1989; Kirchhoff *et al.* 1998; Goldberg *et al.* 1999; Simon 1999; Norris *et al.* 2009; Vaccari *et al.* 2009; Mao *et al.* 2013; Shuhaibar *et al.* 2015).

The *C. elegans* genome contains 25 genes that encode innexins. Of these, 16 were reported to be expressed exclusively in neurons (Altun *et al.* 2009), suggesting that nervous system connectivity in *C. elegans* relies heavily on a gap junction network for communication. Indeed, innexins have many developmental and signaling roles in the *C. elegans* nervous system (Jin *et al.* 2020). An updated wiring diagram of the *C. elegans* nervous system reported the anatomical identification of 890 gap junctions (Varshney *et al.* 2011), although the innexin components that make up specific individual connections remain largely uncharacterized. Furthermore, the varied roles of gap junctions in dynamic circuitry usage and information flow through the nervous system are not well understood.

We previously described a role for gap junctions in the feeding state-dependent modulation of ASH-mediated nociceptive responses (Krzyzanowski *et al.* 2016). While cGMP functions in ASH to dampen calcium signaling and nociceptive sensitivity, it is produced in neighboring neurons that are indirectly connected to ASH via a gap junction network (Krzyzanowski *et al.* 2013, 2016; Voelker *et al.* 2019). The gap junction component INX-4 functions in ASH and is required for cGMP entry into ASH (Krzyzanowski *et al.* 2016). INX-19 is also important for the diffusion of cGMP from ASK to ASH and is present on both sides of ASK–ASH gap junctions (Voelker *et al.* 2019). Although INX-18 also functions in ASK, its primary role there appears to be in promoting proper localization of INX-19 (Voelker *et al.* 2019). However, INX-18 is found in some gap junctions that contain INX-19, and it may interact with innexins besides INX-19 in ASH (Voelker *et al.* 2019). Here, we report a neuronal role for INX-20, which was not previously known to be expressed in, or function in, the nervous system. Cell-specific RNAi knockdown and cell-specific rescue experiments both revealed a role for INX-20 in the ADL and AFD sensory neurons in modulating quinine response sensitivity.

Although the ADL sensory neurons are best known for their role in pheromone detection, they do also play a minor role in the detection of several aversive chemical stimuli—both those that signal through G protein-coupled pathways and those that do not (Bargmann 2006; Ferkey *et al.* 2021). However, ADL does not appear to serve as a direct quinine detector. Neuronal ablation experiments showed that ASH is the major quinine-detecting neuron, and ASK also contributes but has a more minor role that is only revealed when both neurons are ablated in combination. ADL ablation, alone or in combination with ASH ablation, did not show a significant reduction in quinine avoidance (Hilliard *et al.* 2004).

By examining a lower concentration of quinine than was used by Hilliard *et al.* (2004), we found that instead of directly mediating quinine avoidance, ADL serves a circuit-level modulatory role in this aversive response. When presented with 1 mM quinine, wild-type animals respond on average only about 30% of the time (Figs. 1–3 and 5). However, animals in which ADL has been ablated were hypersensitive, responding twice as frequently (Fig. 5a). Thus, our results suggest that ADL is important for dampening quinine sensitivity and avoidance. ADL is indirectly connected to ASH via gap

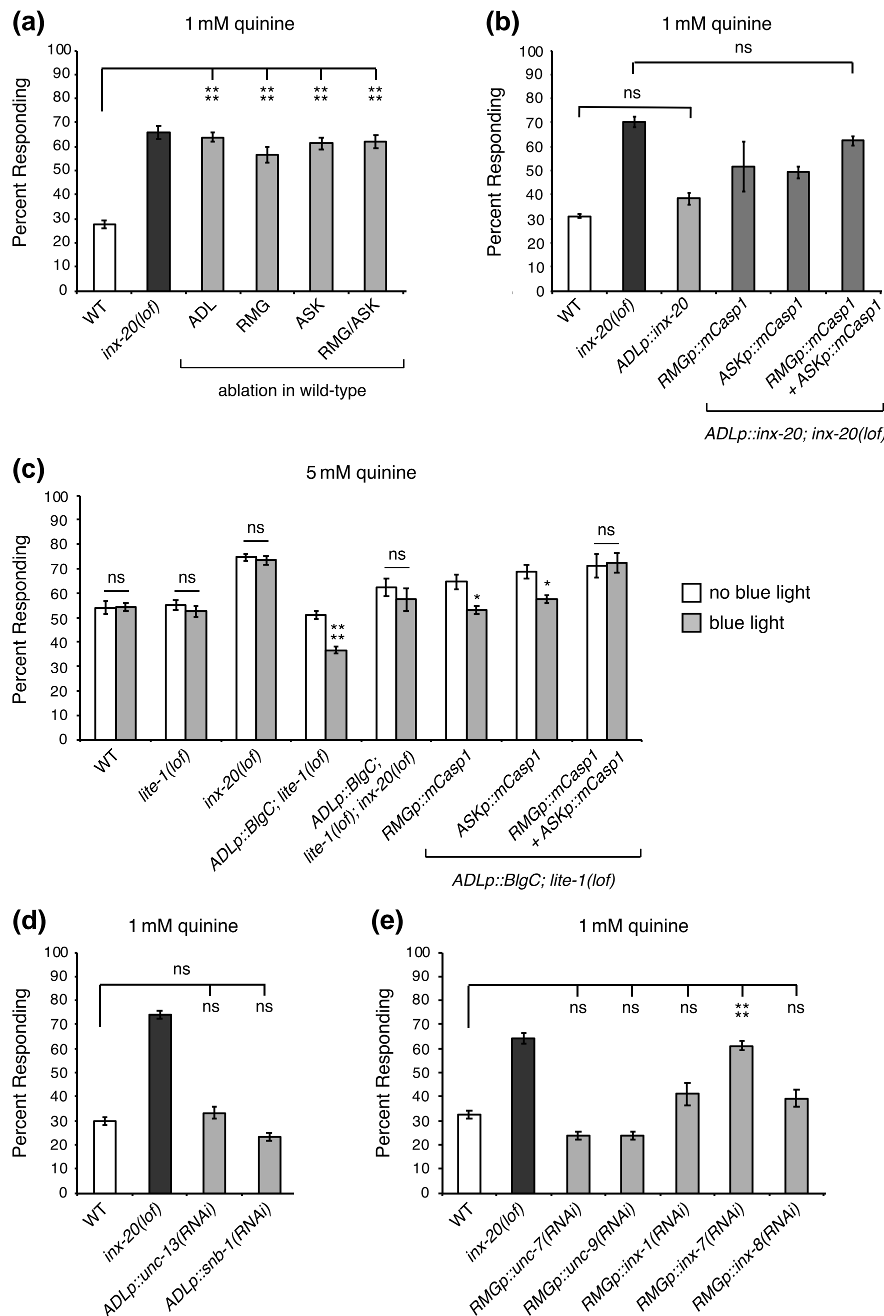

Fig. 5. The ADL and RMG neurons modulate quinine sensitivity. The ADL sensory neurons do not form gap junction connections directly with ASH. However, they are connected to ASH via gap junctions with RMG and ASK. a) Genetic ablation of either the ADL, RMG, or ASK neurons in otherwise wild-type animals resulted in behavioral hypersensitivity to dilute (1 mM) quinine ($P < 0.0001$ for each when compared to wild-type animals). The *srh-281p* (ADL) (Taniguchi *et al.* 2014; Hamakawa *et al.* 2015), *nlp-56p* (RMG) (Taylor *et al.* 2019; Lorenzo *et al.* 2020), and *srbc-66* (ASK) (Kim *et al.* 2009) promoters were used to express mouse caspase-1 (mCasp1). b) Expression of *srh-220p::inx-20* (ADL rescue) fully rescued quinine hypersensitivity of *inx-20(lof)* animals ($P > 0.08$ when compared to wild-type), while *srh-220p::inx-20* only partially rescued quinine response in animals lacking RMG (*nlp-56p::mCasp1*) or ASK (*srbc-66p::mCasp1*) ($P < 0.0001$ for either ablation when compared to either wild-type or *inx-20(lof)* animals). Simultaneous ablation of both RMG and ASK fully blocked ADL rescue (*srh-220p::inx-20*) of quinine hypersensitivity ($P > 0.2$ when compared to *inx-20(lof)* animals). c) The ADL-selective *srh-220* promoter (McCarroll *et al.* 2005) was used to drive expression of a blue light-inducible guanylyl cyclase (BlgC) (Ryu *et al.* 2010). Adult animals expressing BlgC were tested without blue light exposure (white bars) or after a 30-s exposure (gray bars). *lite-1(lof)* animals responded to 5 mM quinine similarly to wild-type animals ($P > 0.9$). Transgenic *lite-1(lof)* animals expressing BlgC in ADL displayed a 28% decrease in percent responding following blue light exposure ($P < 0.0001$). Loss of *inx-20* blocked this diminution ($P > 0.1$). Ablation of either RMG or ASK only partially blocked the ability of BlgC to dampen quinine response ($P < 0.03$ each). ADL-generated cGMP had no effect on quinine response in animals lacking both RMG and ASK ($P > 0.8$). d) UNC-13- and SNB-1-dependent synaptic signaling from ADL does not modulate quinine sensitivity. The *srh-220p* (ADL) (McCarroll *et al.* 2005) promoter was used to co-express a noncoding fragment of either *unc-13* or *snb-1* in both the sense and antisense orientations in otherwise wild-type animals. RNAi knockdown of neither *unc-13* nor *snb-1* in the ADL neurons resulted in behavioral hypersensitivity to dilute (1 mM) quinine ($P > 0.1$ when compared to wild-type animals for each transgene). e) The *nlp-56* (RMG) promoter was used to co-express a noncoding fragment of *unc-7*, *unc-9*, *inx-1*, *inx-7*, or *inx-8* in the sense and antisense orientations in otherwise wild-type animals. RNAi knockdown of *inx-7* lead to behavioral hypersensitivity to dilute (1 mM) quinine ($P < 0.0001$ when compared to wild-type animals). The percentage of animals responding is shown. The combined data of ≥ 3 independent lines and $n \geq 90$ transgenic animals are shown in each panel. Alleles used: *inx-20(ok426)* and *lite-1(xu492)* loss-of-function. WT = the N2 wild-type strain. lof = loss-of-function. ns = not significant.

Fig. 6. Model for INX-20 modulation of ASH-mediated nociceptive signaling. The innexin INX-20 functions primarily in the ADL sensory neurons but also plays a minor role in AFD, to decrease *C. elegans* behavioral sensitivity to aversive stimuli that signal through G protein-coupled receptor pathways in ASH. Loss of INX-20 function leads to behavioral hypersensitivity to these stimuli (e.g. quinine and 1-octanol). Based on the re-annotated wiring diagram (WormWiring.org), ADL is connected to ASH indirectly via gap junction connections with RMG and ASK (White *et al.* 1986; Cook *et al.* 2019). Our working model is that cGMP produced by a yet unidentified guanylyl cyclase in ADL moves through gap junction connections from ADL, through RMG and ASK, to the ASH nociceptors. We previously proposed that cGMP produced by the transmembrane guanylyl cyclase ODR-1 in the AWB/AWC/ASI sensory neurons (Krzyzanowski *et al.* 2016) moves through ADF/AFD/AIA to ASH. Once in ASH, cGMP activates the cGMP-dependent protein kinase EGL-4, which likely directly phosphorylates the regulator of G protein signaling proteins RGS-2 and RGS-3, stimulating their activity (Krzyzanowski *et al.* 2013) to downregulate Gα proteins. The site of action for each innexin shown to modulate ASH sensitivity is shown: INX-20 (ADL, AFD—this study); INX-7 (RMG—this study); INX-18 [ASK—(Voelker *et al.* 2019)]; INX-19 [ASK, ASH—(Voelker *et al.* 2019)]; INX-4 [ASH—(Krzyzanowski *et al.* 2016)]. The guanylyl cyclase that might function in ADL is not known. Magenta coloration indicates this study's additions to the modulatory circuit: the ADL and RMG neurons, INX-20 function in ADL and AFD, and INX-7 function in RMG. We have also shown that RMG and ASK are downstream of *inx-20* function in ADL.

junctions with the RMG interneurons and the ASK sensory neurons (Fig. 6), and ablation of either neuron pair similarly resulted in hypersensitivity to dilute quinine (Fig. 5a). We also found that both RMG and ASK are required for ADL-expressed INX-20 to dampen the quinine hypersensitivity of *inx-20(lof)* animals (Fig. 5b) and for cGMP ectopically generated in ADL (by BlgC) to dampen quinine response (Fig. 5c). This suggests an interesting setup such that ASK appears to serve as both a detector of higher concentrations of quinine (Hilliard *et al.* 2004) and a modulator (inhibitor) of response to dilute quinine (Fig. 5).

Several innexins are expressed in RMG and could form heterotypic channels with the innexins expressed in ADL, ASK, and/or ASH; UNC-7a expression has been reported in RMG by GFP reporter analysis (Altun *et al.* 2009), and transcriptional profiling showed *unc-9*, *inx-1*, *inx-7*, and *inx-8* expression in RMG (CeNGEN: https://cengen.shinyapps.io/CengenApp/) (Hammarlund *et al.* 2018). We found that RNAi knockdown of *inx-7* in RMG resulted in quinine hypersensitivity, suggesting that this innexin functions in RMG to dampen response to dilute quinine. While individual knockdown of the other innexins did not affect behavioral response to quinine, it is possible that some or all of them also could act in a combinatorial manner.

In addition to its broad spectrum of chemosensory responses (Bargmann 2006; Ferkey *et al.* 2021), *C. elegans* are extremely thermosensitive—able to detect temperature changes of 0.01°C or less over a > 10°C temperature range (Luo *et al.* 2006; Ramot *et al.* 2008). The AFD sensory neurons have been established as the primary regulators of thermosensory behaviors in *C. elegans* (Mori and Ohshima 1995; Luo *et al.* 2006; Wang *et al.* 2013; Goodman and Sengupta 2018), although they also detect the gas CO_2 (Bretscher *et al.* 2011) and possibly magnetic fields (Vidal-Gadea *et al.* 2015). There is no evidence to date that they detect chemosensory ligands. However, we previously showed that AFD negatively regulates quinine avoidance, likely serving as an intermediary neuron in the passage of cGMP from the AWC chemosensory neurons to ASH (Krzyzanowski *et al.* 2016). Thus, INX-20 may allow passage of cGMP either into AFD from other neurons (e.g. AWC) or out of AFD (e.g. to ASH) (Fig. 6). INX-4 functions in ASH and is required for ectopically produced cGMP to enter ASH (Krzyzanowski *et al.* 2016). Thus, one possibility is that INX-20 (in AFD) and INX-4 (in ASH) come together to form a heterotypic gap junction connection between these cells that allows for cGMP movement between them.

In summary, we have identified a neuronal role for INX-20 in the non-cell-autonomous regulation of quinine behavioral sensitivity (Fig. 6). While the ASH (major) and ASK (minor) neurons detect quinine (Hilliard *et al.* 2004), INX-20 functions in the ADL and AFD sensory neurons to dampen quinine response. Although we do not know which other innexins INX-20 physically interacts with to form functional channel connections, we propose that, like INX-4 and INX-19 (and possibly INX-18) (Krzyzanowski *et al.* 2016; Voelker *et al.* 2019), INX-20 is part of a gap junction network that serves to pass cGMP to the ASH nociceptors. Our work also identifies a previously unknown modulatory role for the ADL and RMG neurons (as well as the innexin INX-7) in modulating chemosensation.

In animals ranging from invertebrates to mammals, chemosensory responses and appetitive behaviors are modulated by nutritional state (Critchley and Rolls 1996; Dietrich and Horvath 2009; Magni *et al.* 2009; Savigner *et al.* 2009; Niki *et al.* 2010; Shin and Egan 2010; Sengupta 2013; Komuniecki *et al.* 2014; Ryan *et al.* 2014). In *C. elegans*, responses to ASH-detected nociceptive stimuli are diminished when animals are removed from food (Chao *et al.* 2004; Ferkey *et al.* 2007; Wragg *et al.* 2007; Harris *et al.* 2009; Ezcurra *et al.* 2011; Krzyzanowski *et al.* 2016). This context-dependent diminution of protective avoidance behaviors may allow starved animals to enter new environments, even in the face of potential dangers, so that they have an increased chance of finding new food sources. However, an animal's immediate feeding status is not the only relevant factor influencing the appropriateness of a behavioral response. As a pheromone sensor, ADL activation may serve as a proxy for increasing population density and, by extension, dwindling food availability. By distributing the modulation of ASH sensitivity among sensory neurons that detect qualitatively different types of stimuli in the environment (AWB, AWC, ASI, and ADL)—but that all signal information about an animal's current and/or future likelihood of well-being—behavioral responses can be tuned to maximize fitness and survival. While high sensitivity to noxious stimuli can certainly be advantageous, context-dependent responses rely on the integration of diverse sets of environmental information to modulate neuronal function.

Data availability

Plasmids and strains are available upon request. All data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

Supplemental material available at GENETICS online.

Acknowledgments

We thank Noelle L'Etoile for valuable discussions and are grateful to Doug Portman, Takaaki Hirotsu, and Yuichi Iino for reagents. Some strains used in this study were obtained from the *Caenorhabditis* Genetics Center, which is funded in part by the National Institutes of Health—Office of Research Infrastructure Programs (P40 OD010440).

Funding

This work was supported by the National Institutes of Health (R01DC015758 to DMF and R01NS092558 to APW).

Conflicts of interest

None declared.

Literature cited

Altun ZF, Chen B, Wang ZW, Hall DH. High resolution map of *Caenorhabditis elegans* gap junction proteins. Dev Dyn. 2009;238(8):1936–1950. doi:10.1002/dvdy.22025.

Anderson E, Albertini DF. Gap junctions between the oocyte and companion follicle cells in the mammalian ovary. J Cell Biol. 1976;71(2):680–686. doi:10.1083/jcb.71.2.680.

Bargmann CI. *Chemosensation in C. elegans*. In: Community TCR, editors. WormBook. WormBook; 2006. doi:10.1895/wormbook.1.123.1.

Bargmann CI, Thomas JH, Horvitz HR. Chemosensory cell function in the behavior and development of *Caenorhabditis elegans*. Cold Spring Harb Symp Quant Biol. 1990;55(0):529–538. doi:10.1101/sqb.1990.055.01.051.

Brenner S. The genetics of *Caenorhabditis elegans*. Genetics. 1974;77(1):71–94. doi:10.1093/genetics/77.1.71.

Bretscher AJ, Kodama-Namba E, Busch KE, Murphy RJ, Soltesz Z, Laurent P, de Bono M. Temperature, oxygen, and salt-sensing neurons in *C. elegans* are carbon dioxide sensors that control avoidance behavior. Neuron. 2011;69(6):1099–1113. doi:10.1016/j.neuron.2011.02.023.

Bunemann M, Hosey MM. G-protein coupled receptor kinases as modulators of G-protein signalling. J Physiol. 1999;517(1):5–23. doi:10.1111/j.1469-7793.1999.0005z.x.

Calahorro F, Izquierdo PG. The presynaptic machinery at the synapse of *C. elegans*. Invert Neurosci. 2018;18(2):4. doi:10.1007/s10158-018-0207-5.

Chandrashekar J, Hoon MA, Ryba NJ, Zuker CS. The receptors and cells for mammalian taste. Nature. 2006;444(7117):288–294. doi:10.1038/nature05401.

Chao MY, Komatsu H, Fukuto HS, Dionne HM, Hart AC. Feeding status and serotonin rapidly and reversibly modulate a *Caenorhabditis elegans* chemosensory circuit. Proc Natl Acad Sci U S A. 2004;101(43):15512–15517. doi:10.1073/pnas.0403369101.

Chatzigeorgiou M, Bang S, Hwang SW, Schafer WR. *tmc-1* encodes a sodium-sensitive channel required for salt chemosensation in *C. elegans*. Nature. 2013;494(7435):95–99. doi:10.1038/nature11845.

Chelur DS, Chalfie M. Targeted cell killing by reconstituted caspases. Proc Natl Acad Sci U S A. 2007;104(7):2283–2288. doi:10.1073/pnas.0610877104.

Cheung G, Chever O, Rouach N. Connexons and pannexons: newcomers in neurophysiology. Front Cell Neurosci. 2014;8:348. doi:10.3389/fncel.2014.00348.

Cook SJ, Jarrell TA, Brittin CA, Wang Y, Bloniarz AE, Yakovlev MA, Nguyen KCQ, Tang LT, Bayer EA, Duerr JS, *et al*. Whole-animal connectomes of both *Caenorhabditis elegans* sexes. Nature. 2019;571(7763):63–71. doi:10.1038/s41586-019-1352-7.

Critchley HD, Rolls ET. Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol. 1996;75(4):1673–1686. doi:10.1152/jn.1996.75.4.1673.

Dietrich MO, Horvath TL. Feeding signals and brain circuitry. Eur J Neurosci. 2009;30(9):1688–1696. doi:10.1111/j.1460-9568.2009.06963.x.

Edwards SL, Charlie NK, Milfort MC, Brown BS, Gravlin CN, Knecht JE, Miller KG. A novel molecular solution for ultraviolet light detection in *Caenorhabditis elegans*. PLoS Biol. 2008;6(8):e198. doi:10.1371/journal.pbio.0060198.

Esposito G, Di Schiavi E, Bergamasco C, Bazzicalupo P. Efficient and cell specific knock-down of gene function in targeted *C. elegans* neurons. Gene. 2007;395(1-2):170–176. doi:10.1016/j.gene.2007.03.002.

Ezak MJ, Hong E, Chaparro-Garcia A, Ferkey DM. *Caenorhabditis elegans* TRPV channels function in a modality-specific pathway to regulate response to aberrant sensory signaling. Genetics. 2010;185(1):233–244. doi:10.1534/genetics.110.115188.

Ezcurra M, Tanizawa Y, Swoboda P, Schafer WR. Food sensitizes *C. elegans* avoidance behaviours through acute dopamine signalling. EMBO J. 2011;30(6):1110–1122. doi:emboj201122 [pii] 10.1038/emboj.2011.22.

Ferguson SS. Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev. 2001;53(1):1–24.

Ferkey DM, Haspel G, Dionne HM, Hess HA, Suzuki H, Schafer WR, Koelle MR, Hart AC. *C. elegans* G protein regulator RGS-3 controls sensitivity to sensory stimuli. Neuron. 2007;53(1):39–52. doi:10.1016/j.neuron.2006.11.015.

Ferkey DM, Sengupta P, L'Etoile ND. Chemosensory signal transduction in *Caenorhabditis elegans*. Genetics. 2021;217(3). doi:10.1093/genetics/iyab004.

Frokjaer-Jensen C, Davis MW, Hopkins CE, Newman BJ, Thummel JM, Olesen SP, Grunnet M, Jorgensen EM. Single-copy insertion of transgenes in *Caenorhabditis elegans*. Nat Genet. 2008;40(11):1375–1383. doi:10.1038/ng.248.

Fukushige T, Hawkins MG, McGhee JD. The GATA-factor *elt-2* is essential for formation of the *Caenorhabditis elegans* intestine. Dev Biol. 1998;198(2):286–302.

Fukuto HS, Ferkey DM, Apicella AJ, Lans H, Sharmeen T, Chen W, Lefkowitz RJ, Jansen G, Schafer WR, Hart AC. G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*. Neuron. 2004;42(4):581–593. doi:10.1016/s0896-6273(04)00252-1.

Goldberg GS, Lampe PD, Nicholson BJ. Selective transfer of endogenous metabolites through gap junctions composed of different connexins. Nat Cell Biol. 1999;1(7):457–459. doi:10.1038/15693.

Goodman MB, Sengupta P. The extraordinary AFD thermosensor of *C. elegans*. Pflugers Arch. 2018;470(5):839–849. doi:10.1007/s00424-017-2089-5.

Hamakawa M, Uozumi T, Ueda N, Iino Y, Hirotsu T. A role for ras in inhibiting circular foraging behavior as revealed by a new method

for time and cell-specific RNAi. BMC Biol. 2015;13(1):6. doi:10.1186/s12915-015-0114-8.

Hammarlund M, Hobert O, Miller DM III, Sestan N. The CeNGEN project: the complete gene expression map of an entire nervous system. Neuron. 2018;99(3):430–433. doi:10.1016/j.neuron.2018.07.042.

Harris GP, Hapiak VM, Wragg RT, Miller SB, Hughes LJ, Hobson RJ, Steven R, Bamber B, Komuniecki RW. Three distinct amine receptors operating at different levels within the locomotory circuit are each essential for the serotonergic modulation of chemosensation in *Caenorhabditis elegans*. J Neurosci. 2009;29(5):1446–1456. doi:10.1523/JNEUROSCI.4585-08.2009.

Hart AC, Kass J, Shapiro JE, Kaplan JM. Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. J Neurosci. 1999;19(6):1952–1958. doi:10.1523/JNEUROSCI.19-06-01952.1999.

Haycraft CJ, Swoboda P, Taulman PD, Thomas JH, Yoder BK. The *C. elegans* homolog of the murine cystic kidney disease gene Tg737 functions in a ciliogenic pathway and is disrupted in *osm-5* mutant worms. Development. 2001;128(9):1493–1505. doi:10.1242/dev.128.9.1493.

Hilliard MA, Apicella AJ, Kerr R, Suzuki H, Bazzicalupo P, Schafer WR. *In vivo* imaging of *C. elegans* ASH neurons: cellular response and adaptation to chemical repellents. Embo J. 2005;24(1):63–72. doi:10.1038/sj.emboj.7600493.

Hilliard MA, Bargmann CI, Bazzicalupo P. *C. elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. Curr Biol. 2002;12(9):730–734. doi:10.1016/s0960-9822(02)00813-8.

Hilliard MA, Bergamasco C, Arbucci S, Plasterk RH, Bazzicalupo P. Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in *Caenorhabditis elegans*. Embo J. 2004;23(5):1101–1111. doi:10.1038/sj.emboj.7600107.

Hirose T, Galvin BD, Horvitz HR. Six and Eya promote apoptosis through direct transcriptional activation of the proapoptotic BH3-only gene *egl-1* in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 2010;107(35):15479–15484. doi:10.1073/pnas.1010023107.

Hollinger S, Hepler JR. Cellular regulation of RGS proteins: modulators and integrators of G protein signaling. Pharmacol Rev. 2002;54(3):527–559. doi:10.1124/pr.54.3.527.

Jansen G, Thijssen KL, Werner P, van der Horst M, Hazendonk E, Plasterk RH. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. Nat Genet. 1999;21(4):414–419. doi:10.1038/7753.

Jin EJ, Park S, Lyu X, Jin Y. Gap junctions: historical discoveries and new findings in the *Caenorhabditis elegans* nervous system. Biol Open. 2020;9(8). doi:10.1242/bio.053983.

Kaplan JM, Horvitz HR. A dual mechanosensory and chemosensory neuron in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A. 1993;90(6):2227–2231. doi:10.1073/pnas.90.6.2227.

Kim K, Sato K, Shibuya M, Zeiger DM, Butcher RA, Ragains JR, Clardy J, Touhara K, Sengupta P. Two chemoreceptors mediate developmental effects of dauer pheromone in *C. elegans*. Science. 2009;326(5955):994–998. doi:10.1126/science.1176331.

Kirchhoff S, Nelles E, Hagendorff A, Kruger O, Traub O, Willecke K. Reduced cardiac conduction velocity and predisposition to arrhythmias in connexin40-deficient mice. Curr Biol. 1998;8(5):299–302. doi:10.1016/s0960-9822(98)70114-9.

Komolov KE, Benovic JL. G protein-coupled receptor kinases: past, present and future. Cell Signal. 2018;41:17–24. doi:10.1016/j.cellsig.2017.07.004.

Komuniecki R, Hapiak V, Harris G, Bamber B. Context-dependent modulation reconfigures interactive sensory-mediated microcircuits in *Caenorhabditis elegans*. Curr Opin Neurobiol. 2014;29:17–24. doi:10.1016/j.conb.2014.04.006.

Krzyzanowski MC, Woldemariam S, Wood JF, Chaubey AH, Brueggemann C, Bowitch A, Bethke M, L'Etoile ND, Ferkey DM. Aversive behavior in the nematode *C. elegans* is modulated by cGMP and a neuronal gap junction network. PLoS Genet. 2016;12(7):e1006153. doi:10.1371/journal.pgen.1006153.

Krzyzanowski MC, Brueggemann C, Ezak MJ, Wood JF, Michaels KL, Jackson CA, Juang BT, Collins KD, Yu MC, L'Etoile ND, *et al.* The *C. elegans* cGMP-dependent protein kinase EGL-4 regulates nociceptive behavioral sensitivity. PLoS Genet. 2013;9(7):e1003619. doi:10.1371/journal.pgen.1003619.

Likhite N, Jackson CA, Liang MS, Krzyzanowski MC, Lei P, Wood JF, Birkaya B, Michaels KL, Andreadis ST, Clark SD, *et al.* The protein arginine methyltransferase PRMT5 promotes D2-like dopamine receptor signaling. Sci Signal. 2015;8(402):ra115. doi:10.1126/scisignal.aad0872.

Liu Z, Kariya MJ, Chute CD, Pribadi AK, Leinwand SG, Tong A, Curran KP, Bose N, Schroeder FC, Srinivasan J, *et al.* Predator-secreted sulfolipids induce defensive responses in *C. elegans*. Nat Commun. 2018;9(1):1128. doi:10.1038/s41467-018-03333-6.

Lorenzo R, Onizuka M, Defrance M, Laurent P. Combining single-cell RNA-sequencing with a molecular atlas unveils new markers for *Caenorhabditis elegans* neuron classes. Nucleic Acids Res. 2020;48(13):7119–7134. doi:10.1093/nar/gkaa486.

Luo L, Clark DA, Biron D, Mahadevan L, Samuel AD. Sensorimotor control during isothermal tracking in *Caenorhabditis elegans*. J Exp Biol. 2006;209(23):4652–4662. doi:10.1242/jeb.02590.

Magni P, Magni P, Dozio E, Ruscica M, Celotti F, Masini MA, Prato P, Broccoli M, Mambro A, More M, *et al.* Feeding behavior in mammals including humans. Ann N Y Acad Sci. 2009;1163(1):221–232. doi:10.1111/j.1749-6632.2008.03627.x.

Mao GK, Li JX, Bian FH, Han YY, Guo M, Xu BS, Zhang MJ, Xia GL. Gap junction -mediated cAMP movement between oocytes and somatic cells. Front Biosci (Elite Ed). 2013;E5(2):755–767. doi:10.2741/e656.

Masoudi N, Tavazoie S, Glenwinkel L, Ryu L, Kim K, Hobert O. Unconventional function of an achaete-scute homolog as a terminal selector of nociceptive neuron identity. PLoS Biol. 2018;16(4):e2004979. doi:10.1371/journal.pbio.2004979.

McCarroll SA, Li H, Bargmann CI. Identification of transcriptional regulatory elements in chemosensory receptor genes by probabilistic segmentation. Curr Biol. 2005;15(4):347–352. doi:10.1016/j.cub.2005.02.023.

Mello CC, Kramer JM, Stinchcomb D, Ambros V. Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. Embo J. 1991;10(12):3959–3970. doi:10.1002/j.1460-2075.1991.tb04966.x.

Miyabayashi T, Palfreyman MT, Sluder AE, Slack F, Sengupta P. Expression and function of members of a divergent nuclear receptor family in *Caenorhabditis elegans*. Dev Biol. 1999;215(2):314–331. doi:10.1006/dbio.1999.9470.

Mori I, Ohshima Y. Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature. 1995;376(6538):344–348. doi:10.1038/376344a0.

Niki M, Yoshida R, Takai S, Ninomiya Y. Gustatory signaling in the periphery: detection, transmission, and modulation of taste information. Biol Pharm Bull. 2010;33(11):1772–1777. doi:10.1248/bpb.33.1772.

Nonet ML, Saifee O, Zhao H, Rand JB, Wei L. Synaptic transmission deficits in *Caenorhabditis elegans* synaptobrevin mutants. J Neurosci. 1998;18(1):70–80. doi:10.1523/JNEUROSCI.18-01-00070.1998.

Nonet ML, Staunton JE, Kilgard MP, Fergestad T, Hartwieg E, Horvitz HR, Jorgensen EM, Meyer BJ. *Caenorhabditis elegans rab-3* mutant

synapses exhibit impaired function and are partially depleted of vesicles. J Neurosci. 1997;17(21):8061–8073. doi:10.1523/JNEUROSCI.17-21-08061.1997.

Norris RP, Ratzan WJ, Freudzon M, Mehlmann LM, Krall J, Movsesian MA, Wang H, Ke H, Nikolaev VO, Jaffe LA. Cyclic GMP from the surrounding somatic cells regulates cyclic AMP and meiosis in the mouse oocyte. Development. 2009;136(11):1869–1878. doi:10.1242/dev.035238.

Paix A, Folkmann A, Rasoloson D, Seydoux G. High efficiency, homology-directed genome editing in *Caenorhabditis elegans* using CRISPR-Cas9 ribonucleoprotein complexes. Genetics. 2015;201(1):47–54. doi:10.1534/genetics.115.179382.

Palacios-Prado N, Huetteroth W, Pereda AE. Hemichannel composition and electrical synaptic transmission: molecular diversity and its implications for electrical rectification. Front Cell Neurosci. 2014;8:324. doi:10.3389/fncel.2014.00324.

Palmer RK. The pharmacology and signaling of bitter, sweet, and umami taste sensing. Mol Interv. 2007;7(2):87–98. doi:10.1124/mi.7.2.9.

Pierce KL, Lefkowitz RJ. Classical and new roles of β-arrestins in the regulation of G-protein-coupled receptors. Nat Rev Neurosci. 2001;2(10):727–733. doi:10.1038/35094577.

Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. Annu Rev Biochem. 1998;67(1):653–692. doi:10.1146/annurev.biochem.67.1.653.

Ramot D, MacInnis BL, Goodman MB. Bidirectional temperature-sensing by a single thermosensory neuron in *C. elegans*. Nat Neurosci. 2008;11(8):908–915. doi:10.1038/nn.2157.

Ranji P, Rauthan M, Pitot C, Pilon M. Loss of HMG-CoA reductase in *C. elegans* causes defects in protein prenylation and muscle mitochondria. PLoS One. 2014;9(6):e100033. doi:10.1371/journal.pone.0100033.

Richmond JE, Davis WS, Jorgensen EM. UNC-13 is required for synaptic vesicle fusion in *C. elegans*. Nat Neurosci. 1999;2(11):959–964. doi:10.1038/14755.

Roayaie K, Crump JG, Sagasti A, Bargmann CI. The Gα protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in *C. elegans* olfactory neurons. Neuron. 1998;20(1):55–67. doi:10.1016/s0896-6273(00)80434-1.

Ross EM, Wilkie TM. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem. 2000;69(1):795–827. doi:10.1146/annurev.biochem.69.1.795.

Ryan DA, Miller RM, Lee K, Neal SJ, Fagan KA, Sengupta P, Portman DS. Sex, age, and hunger regulate behavioral prioritization through dynamic modulation of chemoreceptor expression. Curr Biol. 2014;24(21):2509–2517. doi:10.1016/j.cub.2014.09.032.

Ryu MH, Moskvin OV, Siltberg-Liberles J, Gomelsky M. Natural and engineered photoactivated nucleotidyl cyclases for optogenetic applications. J Biol Chem. 2010;285(53):41501–41508. doi:10.1074/jbc.M110.177600.

Saez JC, Connor JA, Spray DC, Bennett MV. Hepatocyte gap junctions are permeable to the second messenger, inositol 1,4,5-trisphosphate, and to calcium ions. Proc Natl Acad Sci U S A. 1989;86(8):2708–2712. doi:10.1073/pnas.86.8.2708.

Sambongi Y, Nagae T, Liu Y, Yoshimizu T, Takeda K, Wada Y, Futai M. Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in *Caenorhabditis elegans*. Neuroreport. 1999;10(4):753–757. doi:10.1097/00001756-199903170-00017.

Sambongi Y, Takeda K, Wakabayashi T, Ueda I, Wada Y, Futai M. *Caenorhabditis elegans* senses protons through amphid chemosensory neurons: proton signals elicit avoidance behavior. Neuroreport. 2000;11(10):2229–2232. doi:10.1097/00001756-200007140-00033.

Sassa T, Maruyama IN. A G-protein alpha subunit, GOA-1, plays a role in *C. elegans* avoidance behavior of strongly alkaline pH. Commun Integr Biol. 2013;6(6):e26668. doi:10.4161/cib.26668.

Satterlee JS, Sasakura H, Kuhara A, Berkeley M, Mori I, Sengupta P. Specification of thermosensory neuron fate in *C. elegans* requires *ttx-1*, a homolog of *otd/Otx*. Neuron. 2001;31(6):943–956. doi:10.1016/s0896-6273(01)00431-7.

Savigner A, Duchamp-Viret P, Grosmaitre X, Chaput M, Garcia S, Ma M, Palouzier-Paulignan B. Modulation of spontaneous and odorant-evoked activity of rat olfactory sensory neurons by two anorectic peptides, insulin and leptin. J Neurophysiol. 2009;101(6):2898–2906. doi:10.1152/jn.91169.2008.

Sengupta P. The belly rules the nose: feeding state-dependent modulation of peripheral chemosensory responses. Curr Opin Neurobiol. 2013;23(1):68–75. doi:10.1016/j.conb.2012.08.001.

Shin YK, Egan JM. Roles of hormones in taste signaling. Results Probl Cell Differ. 2010;52:115–137. doi:10.1007/978-3-642-14426-4_10.

Shuhaibar LC, Egbert JR, Norris RP, Lampe PD, Nikolaev VO, Thunemann M, Wen L, Feil R, Jaffe LA. Intercellular signaling via cyclic GMP diffusion through gap junctions restarts meiosis in mouse ovarian follicles. Proc Natl Acad Sci U S A. 2015;112(17):5527–5532. doi:10.1073/pnas.1423598112.

Simon AM. Gap junctions: more roles and new structural data. Trends Cell Biol. 1999;9(5):169–170. doi:10.1016/s0962-8924(99)01547-0.

Stringham EG, Dixon DK, Jones D, Candido EP. Temporal and spatial expression patterns of the small heat shock (*hsp16*) genes in transgenic *Caenorhabditis elegans*. Mol Biol Cell. 1992;3(2):221–233. doi:10.1091/mbc.3.2.221.

Taniguchi G, Uozumi T, Kiriyama K, Kamizaki T, Hirotsu T. Screening of odor-receptor pairs in *Caenorhabditis elegans* reveals different receptors for high and low odor concentrations. Sci Signal. 2014;7(323):ra39. doi:10.1126/scisignal.2005136.

Taylor SR, Santpere G, Reilly M, Glenwinkel L, Poff A, McWhirter R, Xu C, Weinreb A, Basavaraju M, Cook SJ, *et al.* Expression profiling of the mature *C. elegans* nervous system by single-cell RNA-sequencing. bioRxiv 737577. https://doi.org/10.1101/737577.2019, preprint: not peer reviewed.

Tokumaru H, Augustine GJ. UNC-13 and neurotransmitter release. Nat Neurosci. 1999;2(11):929–930. doi:10.1038/14710.

Tran A, Tang A, O'Loughlin CT, Balistreri A, Chang E, Coto Villa D, Li J, Varshney A, Jimenez V, Pyle J, *et al. C. elegans* avoids toxin-producing *Streptomyces* using a seven transmembrane domain chemosensory receptor. Elife. 2017;6. doi:10.7554/eLife.23770.

Trewin AJ, Bahr LL, Almast A, Berry BJ, Wei AY, Foster TH, Wojtovich AP. Mitochondrial reactive oxygen species generated at the Complex-II matrix or intermembrane space microdomain have distinct effects on redox signaling and stress sensitivity in *Caenorhabditis elegans*. Antioxid Redox Signal. 2019;31(9):594–607. doi:10.1089/ars.2018.7681.

Troemel ER. Chemosensory signaling in *C. elegans*. Bioessays. 1999;21(12):1011–1020. doi:10.1002/(SICI)1521-1878(199912)22:1<1011::AID-BIES5>3.0.CO;2-V.

Troemel ER, Chou JH, Dwyer ND, Colbert HA, Bargmann CI. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. Cell. 1995;83(2):207–218. doi:10.1016/0092-8674(95)90162-0.

Vaccari S, Weeks JL II, Hsieh M, Menniti FS, Conti M. Cyclic GMP signaling is involved in the luteinizing hormone-dependent meiotic maturation of mouse oocytes. Biol Reprod. 2009;81(3):595–604. doi:10.1095/biolreprod.109.077768

Varshney LR, Chen BL, Paniagua E, Hall DH, Chklovskii DB. Structural properties of the *Caenorhabditis elegans* neuronal network. PLoS Comput Biol. 2011;7(2):e1001066. doi:10.1371/journal.pcbi.1001066.

Vidal-Gadea A, Ward K, Beron C, Ghorashian N, Gokce S, Russell J, Truong N, Parikh A, Gadea O, Ben-Yakar A, *et al.* Magnetosensitive neurons mediate geomagnetic orientation in *Caenorhabditis elegans*. Elife. 2015;4. doi:10.7554/eLife.07493.

Voelker L, Upadhyaya B, Ferkey DM, Woldemariam S, L'Etoile ND, Rabinowitch I, Bai J. INX-18 and INX-19 play distinct roles in electrical synapses that modulate aversive behavior in *Caenorhabditis elegans*. PLoS Genet. 2019;15(10):e1008341. doi:10.1371/journal.pgen.1008341.

Wang D, O'Halloran D, Goodman MB. GCY-8, PDE-2, and NCS-1 are critical elements of the cGMP-dependent thermotransduction cascade in the AFD neurons responsible for *C. elegans* thermotaxis. J Gen Physiol. 2013;142(4):437–449. doi:10.1085/jgp.201310959.

White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. Phil Trans R Soc Lond. 1986;314(1165):1–340. doi:10.1098/rstb.1986.0056.

Willars GB. Mammalian RGS proteins: multifunctional regulators of cellular signalling. Semin Cell Dev Biol. 2006;17(3):363–376. doi:10.1016/j.semcdb.2006.03.005.

Woldemariam S, Nagpal J, Hill T, Li J, Schneider MW, Shankar R, Futey M, Varshney A, Ali N, Mitchell J, *et al.* Using a robust and sensitive GFP-based cGMP sensor for real-time imaging in intact *Caenorhabditis elegans*. Genetics. 2019;213(1):59–77. doi:10.1534/genetics.119.302392.

Wood WB. *The Nematode Caenorhabditis elegans*. In: Cold Spring Harbor Laboratory. New York: Cold Spring Harbor; 1988. 667pp.

Wood JF, Ferkey DM. GRK Roles in *C. elegans*. In: Gurevich VV, Gurevich EV, Tesmer JJG, editors. G Protein-Coupled Receptor Kinases. New York (NY): Humana Press; 2016. p. 283–299. doi:10.1007/978-1-4939-3798-1.

Wood JF, Wang J, Benovic JL, Ferkey DM. Structural domains required for *Caenorhabditis elegans* G protein-coupled receptor kinase 2 (GRK-2) function *in Vivo*. J Biol Chem. 2012;287(16):12634–12644. doi:10.1074/jbc.M111.336818.

Wragg RT, Hapiak V, Miller SB, Harris GP, Gray J, Komuniecki PR, Komuniecki RW. Tyramine and octopamine independently inhibit serotonin-stimulated aversive behaviors in *Caenorhabditis elegans* through two novel amine receptors. J Neurosci. 2007;27(49):13402–13412. doi:10.1523/JNEUROSCI.3495-07.2007.

Yanisch-Perron C, Vieira J, Messing J. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene. 1985;33(1):103–119. doi:10.1016/0378-1119(85)90120-9.

Yoshida K, Hirotsu T, Tagawa T, Oda S, Wakabayashi T, Iino Y, Ishihara T. Odour concentration-dependent olfactory preference change in *C. elegans*. Nat Commun. 2012;3(1):739. doi:10.1038/ncomms1750.

Yu S, Avery L, Baude E, Garbers DL. Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. Proc Natl Acad Sci U S A. 1997;94(7):3384–3387. doi:10.1073/pnas.94.7.3384.

Editor: K. Kim