Correction

NEUROSCIENCE

The authors note that Figs. 2 and 3 appeared incorrectly. The corrected figures and their legends appear below.

Fig. 2. A functional map of the sensory system reveals a hierarchical sparse code. Rows correspond to individual neurons and columns to stimuli. Each stimulus was tested for an ON and OFF response, and at least five worms were tested for each stimulus. Neural activity as indicated by Ca²⁺ imaging is color-coded: blue, decrease; green, no response; red, increase. The dopaminergic neurons anterior deirid neuron class E (ADE), cephalic neurons (CEP), and postdeirid neuron class E (PDE) are a subgroup of the mechanosensory neurons.
Fig. 3. Sparse coding; only a small fraction of the neurons responds to each of the stimuli. Blue circles denote the fraction of neurons that changed their activity at each condition. The order of the circles matches the order of the conditions shown in Fig. 2. Pairs of blue circles correspond to on/off responses, except for the case of pH 5 or 9. Black circles denote the results simulating signal propagation in the network. The four circles correspond to whether one, two, three, or four sensory neurons are directly activated by the stimulus (simulations).
Hierarchical sparse coding in the sensory system of *Caenorhabditis elegans*

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Animals with compact sensory systems face an encoding problem where a small number of sensory neurons are required to encode information about its surrounding complex environment. Using *Caenorhabditis elegans* worms as a model, we ask how chemical stimuli are encoded by a small and highly connected sensory system. We first generated a comprehensive library of transgenic worms where each animal expresses a genetically encoded calcium indicator in individual sensory neurons. This library includes the vast majority of the sensory system in *C. elegans*. Imaging from individual sensory neurons while subjecting the worms to various stimuli allowed us to compile a comprehensive functional map of the sensory system at single neuron resolution. The functional map reveals that despite the dense wiring, chemosensory neurons represent the environment using sparse codes. Moreover, although anatomically closely connected, chemo- and mechano-sensory neurons are functionally segregated. In addition, the code is hierarchical, where few neurons participate in encoding multiple cues, whereas other sensory neurons are stimulus specific. This encoding strategy may have evolved to mitigate the constraints of a compact sensory system.

Results and Discussion

To measure sensory system activity in a single neuron resolution, we generated a library of transgenic worms, where each strain encodes the calcium indicator GCaMP3 (circularly permuted green fluorescent protein-calmodulin-M13 peptide version 3) in individual types of sensory neurons. The library, comprising 19 strains, includes the vast majority of the sensory system: It contains 15 types of chemosensory neurons representing 34 individual neurons and 11 types of mechanosensory neurons representing 24 individual neurons. The full list of the neurons as well as the description of the transgenic lines is found in Fig. 1 and Table S1.

We next measured activity of each of the sensory neurons in response to several chemical cues using a microfluidic device, the “Olfactory chip” (15). We chose to assay volatile and soluble attractants (Isoamyl alcohol, diacetyl, NaCl, pH 9, and *Escherichia coli* supernatant) or repellents (1 M Glycerol, pH 5), all of which are well-known stimulants of *C. elegans* (13, 16, 17). For each stimulus, we assayed neural activity following stimulus presentation (ON step) and removal (OFF step). In addition, we assayed the response of sensory neurons to blue light (485 nm), a known aversive stimulus to *C. elegans* worms (18, 19). This large-scale single neuron resolution analysis was then compiled into a comprehensive functional map of the sensory system (Fig. 2).

In our analyses, we considered neurons to be activated only if their GCaMP fluorescent signal was increased by at least 20% or decreased by at least 15% during the 7 s following the ON or OFF step, respectively. We set the threshold to 20%, as control measurements, in which the ON/OFF steps included switching between two streams containing the same buffer solution (no stimulus), showed a typical variability of ~10%. Moreover, to determine if a neuron genuinely responded to a given stimulus, we

Significance

We investigated how a numerically and spatially compact nematode nervous system encodes information about the world. A library of transgenic worms expressing a genetically encoded calcium indicator in each type of sensory neuron was constructed and used to assay neural activity in response to various chemical stimuli to compile a functional map of a sensory system. We find that the sensory system uses hierarchical sparse coding, a strategy that mitigates the limited size and the shallow structure of the neural network. Also, this is a timely study that significantly adds to the communal effort and enthusiasm in obtaining functional maps of the connectome.

Author contributions: A.Z. and P.W.S. designed research; A.Z., I.L., O.S., S.G., and L.Y. performed research; A.Z. contributed new reagents/analytic tools; A.Z. analyzed data; and A.Z. and P.W.S. wrote the paper.

The authors declare no conflict of interest.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1423656112/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1423656112
considered the rise and fall in neural activity only during the first 7 s following the change. This strict criterion precludes the possibility of assigning spontaneous neural activity as a true response.

Strikingly, we found that only a small fraction of the chemosensory neurons is activated in each of the conditions tested (Fig. 2). A minimal fraction of ~5% of the chemosensory system is activated in response to a single volatile cue (e.g., isoamyl alcohol, IAA), and as many as 40% of the chemosensory neurons are activated when presented with a rich complex stimulus (14). In single types of sensory neurons (Q–V) mechanosensory neurons, (W–X) phasmid chemosensory neurons. Full details of the transgenic lines are given in Table S1.

![Diagram of C. elegans neural network](image_url)

Fig. 1. A comprehensive library of transgenic animals expressing GCaMP3 (14) in single types of sensory neurons. (A–D) Individual chemosensory neurons with left and right distinction. (E–N) Chemosensory neurons types in which both right and left neurons are tagged. Because the left and right neurons are located on different focal planes, usually one of them can be observed in each of the images. (Q and P) Dopaminergic neurons. (Q–V) Mechanosensory neurons. (W–X) Phasmid chemosensory neurons. Full details of the transgenic lines are given in Table S1.

comes in direct contact with the flowing stimulus (in all previous assays described, the tip of the nose was protruding out to contact the stimulus). In both orientations, either heads or tails forward, we did not detect a response from these neurons.

In addition to detecting neural activity from the vast majority of the chemosensory neurons, we also successfully detected calcium signals in mechanosensory neurons in which we expressed Channelrhodopsin (Fig. 4). Together, these widely observed responses exclude the possibility that sparseness could result from impaired activity of the GCaMP-expressing neurons.

The small fraction of activated neurons may come as a surprise given the high connectivity of the network. For example, Majewska and Yuste calculated that when looking at the graph of neurons connected by gap junctions only, over 90% of the neurons are coupled either directly or indirectly, via any number of coupled neurons (11). Moreover, a recent assembly of the network assigned many more gap junctions and synapses (12), so the connectivity may be even higher than estimated. Indeed, simulations of signal propagation in the network suggest that the vast majority of the neurons are expected to participate in stimulus encoding (Figs. 3 and Fig. S1; see Materials and Methods for a detailed description of the simulations). Our experimental findings, however, reveal that encoding is sparse (Figs. 2 and 3).

There are several advantages to using a sparse encoding strategy. It allows storing a greater number of representations as well as newly acquired memories (5, 20), and it is also energy efficient (21). This parsimony is particularly relevant given that C. elegans worms frequently face dire conditions (22) with limited resources and consequently evolved various strategies to alleviate energy deficits (23).

The compiled functional map (Fig. 2) also reveals a functional hierarchy: Few neurons respond to most chemical cues tested, whereas other neurons are more stimulus-specific. This functional hierarchy cannot be explained by network anatomy as the neurons at the top of the hierarchy are not hubs of the network but rather have an average number of synaptic partners (Fig. S2). Of particular interest are the amphid wing cell C (AWC) chemosensory neurons, which respond to most stimuli (Fig. 2). Moreover, we found that AWC also mildly responds to the change in the flow direction in the absence of a chemical stimulus (Fig. S3). This moderate activation (~20%), however, is significantly lower than the activation observed in response to the different chemical stimuli, indicating that the ubiquitous response to those chemicals is genuine (Fig. S3).

This ubiquitous response has functional behavioral significance, as genetically ablated AWC worms (24) show impaired chemotactic behavior to a variety of chemical stimuli (17), including stimuli assayed in this study (Fig. S4), and therefore play a key role in the correct encoding of many different stimuli.

Sparseness and functional hierarchy are design features common in neural systems with several layers of information processing (5, 11). Revealing these features of the C. elegans nervous system at the sensory level itself suggests that signal processing and integration may be already implemented at the sensory level itself or at the interface between the sensory and the interneuron level. Indeed, sensory neurons have been shown to be specialized to compute and temporally differentiate chemosensory cues (25, 26). Moreover, the structure of the neural network is shallow: We analyzed the C. elegans neural network and found that the average shortest path from each of the chemosensory neurons (at the sensory periphery) to the motor neurons (the most downstream elements in the nervous system) is 3.5 ± 0.8 synapses. Thus, signal integration at the sensory periphery could be particularly beneficial in the case of C. elegans given the small size and shallow structure of its nervous system. Indeed, the chemosensory neuron AWC\textsuperscript{ON} (“ON” denotes expression of the str-2 gene, which encodes a seven transmembrane receptor) was shown to act as an interneuron downstream of the primary salt-sensing
and C. elegans chemotactic behavior can be controlled by manipulating a single pair of amphid interneuron class Y (AIY) interneurons that are postsynaptic to AWC (28).

In addition, the functional map suggests that the chemosensory system is functionally segregated from the mechanosensory system: None of the 11 mechanosensory neuron types, comprising 24 individual mechanosensory neurons (out of 30 in total), was activated upon chemical stimulation (Fig. 2). This functional segregation is probably bidirectional, as stimulating key mechanosensory neurons did not elicit a response in AWC ON, the chemosensory neuron at the top of the functional hierarchy (Fig. 4). The functional segregation cannot be predicted based on the available connectome alone, as the two sensory modalities are anatomically intertwined, suggesting that activity of a neuron from one modality is also likely to activate a neuron from the other modality. Thus, although the anatomical proximity and the intertwined wiring may suggest a potential cross-talk between the two modalities, our functional dynamics data demonstrate that the two modalities are functionally separated despite the possibility of massive neuropeptide modulation.

The compiled functional map (Fig. 2) consists of several stimuli, each tested at a single concentration. It would be interesting to perform an in-depth analysis to quantitate how each of the neurons responds to varying concentrations of the stimuli using dedicated high-throughput microfluidic devices as has been done for amphid wing neuron class A (AWA) (29). Modulating stimulus concentration may somewhat change the ensemble of encoding sensory neurons, often to include the amphid sensilla neuron class H (ASH) polymodal neuron if high concentrations of a known chemoattractant are used (e.g., NaCl and diacetyl) (30, 31). However, addition of a few more neurons to the encoding ensemble is unlikely to change the observed sparse response, as these stimuli are encoded using less than 30% (5–30%) of the sensory neurons.

We addressed the encoding problem by looking at the population of responding neurons. A more sophisticated and fine-tuned encoding may lie in the relative response time among the different neurons. Because neural response latencies are expected to be very short (presumably less than a second), accurate high-temporal measurements should be made in animals expressing GCaMP in several neurons. In this regard, it will be interesting to elucidate which neurons are the primary direct sensors to the chemical cue and which are secondary, postsynaptic neurons. Addressing these questions will be possible by crossing with neural transmission mutants, such as unc-31 or unc-13.

Here we have shown that the C. elegans sensory system represents environmental stimuli using sparse codes, a strategy that...
We used GCaMP3 (14) for the construction of the mechanosensory system is functionally segregated from the chemosensory system. (A) The transgenic animal expressing Channelrhodopsin (ChR2) and GCaMP3 in mechanosensory neurons and GCaMP3 in AWC, syEx1211[mec-4::ChR2-mCherry, mec-4::GCaMP3, str-2::GCaMP3; pha-1::PHA-1]; pha-1(e2123ts); him-5(e1490), strain PS6421. (B) Light-induced activation of the mechanosensory neurons. Activation of Channelrhodopsin and calcium imaging begin at time point zero, when light (480 nm) is turned on. (C) Light activation of the mechanosensory neurons does not elicit activity in AWC, a neuron that is at the top of the functional hierarchy of the chemosensory system.

Fig. 4. The mechanosensory system is functionally segregated from the chemosensory system. (A) The transgenic animal expressing Channelrhodopsin (ChR2) and GCaMP3 in mechanosensory neurons and GCaMP3 in AWC, syEx1211[mec-4::ChR2-mCherry, mec-4::GCaMP3, str-2::GCaMP3; pha-1::PHA-1]; pha-1(e2123ts); him-5(e1490), strain PS6421. (B) Light-induced activation of the mechanosensory neurons. Activation of Channelrhodopsin and calcium imaging begin at time point zero, when light (480 nm) is turned on. (C) Light activation of the mechanosensory neurons does not elicit activity in AWC, a neuron that is at the top of the functional hierarchy of the chemosensory system.

Materials and Methods

Library Construction. We used GCaMP3 (14) for the construction of the comprehensive library. Promoters used to drive expression in individual sensory neurons are given in Table S1. All constructs were fusion PCR products injected into a pha-1 background (either e2123 or a double mutant of pha-1(e2123); him-5(e1490), PS6421). In general, we injected a mix of 10-50 ng/µL of the fusion PCR product together with 70-80 ng/µL of the pha-1 rescue construct. In a few cases where we observed defective progeny, we generated transgenic animals by injecting lower concentrations of the fusion PCR (1-5 ng/µL). The full list of the strains generated in this study is given in Table S1.

Calcium Imaging. To apply the various chemical stimuli in an ON/OFF manner while immobilizing the worms for calcium imaging, we used the “olfactory chamber” (15). The chemical stimuli included 10−4 IAA, 10−4 diacetyl, 50 mM NaCl, 1 M Glycerol, pH 5 and pH 9, and supernatant from a 3-d-old E. coli (strain OP 50) culture. We used two inverted fluorescence microscope setups: (i) Zeiss Axiovert equipped with an IXON397 EMCCD camera (Andor) and (ii) Olympus IX83 equipped with a Evolved EMCCD camera (Photometrics).

Exposure time ranged from 100 ms to 500 ms depending on the intensity of the signal in the various neurons. In a typical experiment, we subjected the worm to the control stream for 10 s and then switched to the stimulus stream (an ON step). An OFF step was done by switching the stimulus stream to the control stream. Importantly, for each tested condition, we replaced the microfluidic chip as well as all of the connecting tubes with new, unused ones. This ensured that no traces of contaminating cues will be flowing together with the tested stimulus. Moreover, to prevent possible accumulation of contaminating bacteria, we replaced all of the tubing setup every 2-3 d, and excessively washed the chips periodically even if we repeated the experiment with the same stimulus.

A minimum of five measurements was performed for each neuron per condition. In cases where the signal (∆F/F) was low and close to background level, we increased the number of neurons assayed. We determined that a neuron was activated (increased calcium levels) or inhibited (decreased calcium levels) if ∆F/F > 20% or ∆F/F < 15%, respectively. This threshold is based on control experiments (without applying stimuli) in which fluorescence fluctuated by ~10%. To determine whether a neuron responded to the stimulus and to avoid accounting for stochastic calcium changes, we considered only the change in the first 7 s following the switch in the condition. When imaging from neurons sensitive to the blue light (480 nm), we first allowed the worms to adapt to the light for 2–3 min before switching between the chemical streams. These neurons included ASH, amphid sensillum neuron class K (ASK), amphid sensillum neuron class J (ASJ), and inner labial neuron class 2 (IL2).

To test whether activation of selected mechano-sensory neurons activate AWC, the sensory neuron at the top of the functional hierarchy (Fig. 2), we generated a transgenic worm (PS6421) expressing Channelrhodopsin (ChR2–mCherry) and GCaMP3 in a subset of mechano-sensory neurons as well as GCaMP3 in AWC, syEx1211[mec-4::ChR2-mCherry, mec-4::GCaMP3, str-2::GCaMP3; pha-1(e2123ts); him-5(e1490)]. L4 hermaphroditic worms were grown on OP 50 supplemented with 200 µM of all-transretinal (Sigma) for 1–2 d in the dark (34). Blue light (480 nm) was used to activate channelrhodopsin, in which case
GCaMP signal was clearly elevated in the mechano-sensory neurons. To image AWCCON and the distant mechanosensory neurons simultaneously, we used a 20x magnification (Fig. 4A).

Network-Wide Simulations of Signal Propagation. We simulated signal propagation in the network to estimate the fraction of neurons expected to change their activity in response to activation of individual chemosensory neurons. Although network connectivity is available (9, 12), the sign of the vast majority of the synapses (e.g., excitatory/inhibitory) is unknown. To overcome this limitation, we used a brute force approach simulating signal flow in tens of thousands of randomly generated networks where network connectivity was left intact, but synapses were randomly assigned as excitatory or inhibitory with varying probabilities. This approach ensures that the simulations will cover a broad space of the possible networks. The aim of these simulations is merely to estimate the fraction of neurons with changed activity rather than predicting the neural ensemble encoding a given stimulus.

The simulations were performed by initially activating one, two, three, or four sensory neurons at a time (Fig. 3) and then propagating the signal in the network over discrete time points in an analog fashion: At each time point, an excitatory synapse added one activity unit to the postsynaptic neuronal activity, and gap junctions were enabled to transmit neural activity with a probability of 0.5, as gap junctions can be rectifying synapses (Fig. 4B). We found that the AWC neurons are specialized for temporal differentiation. (Fig. 3 and Fig. 5).

Chemotaxis Assays. We found that the AWCCON chemosensory neuron responds to a broad panel of chemical cues. To test whether this ubiquitous response also has functional behavioral significance, we performed chemotaxis assays of AWCCON genetically ablated worms (24). Although worms' attraction to IAA is known to be mediated by AWCCON neurons, diacetyl and NaCl that are sensed by AWA and ASE neurons, respectively, are not known as AWCCON-mediated chemotactants (13, 35, 36). We therefore compared intact worms to AWCCON genetically ablated worms for their potential to be attracted to these cues (Fig. S4).

We used a standard protocol for the chemotaxis assays, where worms were placed at the center of a plate and a stimulus and control are spotted on two opposing sides two centimeters from the worms. NaCl was spotted on the agar 20 h and 4 h before the assay to generate sharp gradients according to ref. 37. This spotting protocol generates sharper gradients that drop by approximately an order of magnitude 1 cm away from the source. Two microfilters with the corresponding concentrations of IAA and diacetyl were spotted on the plate lid immediately before the assay. In general, we used concentrations that are higher than the ones used in the microfluidic calcium imaging experiments to generate effective sharp gradients along the trajectories of the worms. In addition, effective sensing and chemotaxis along gradients may require higher concentrations of the stimulus as opposed to the lower concentrations required to elicit a response in a switch-like ON/OFF manner as used in the microfluidic experiments. Chemotaxis Index was calculated using the standard formula (Nctrl – Nst) (NON – Nst) after 3–5 min from the beginning of the assay.

ACKNOWLEDGMENTS. We thank Piali Sengupta for sharing the genetically ablated AWCCON strain (PY7502). The research leading to these results has received funding from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013) and European Research Council Grant Agreement 336803. Initial stages of this research were supported by the Caltech Center for Biological Circuit Design. P.W.S. is an investigator of the Howard Hughes Medical Institute, which supported this work.