Ammonium-Acetate Is Sensed by Gustatory and Olfactory Neurons in Caenorhabditis elegans

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Abstract

Background: Caenorhabditis elegans chemosensation has been successfully studied using behavioral assays that treat detection of volatile and water soluble chemicals as separate senses, analogous to smell and taste. However, considerable ambiguity has been associated with the attractive properties of the compound ammonium-acetate (NH₄Ac). NH₄Ac has been used in behavioral assays both as a chemosensory neutral compound and as an attractant.

Methodology/Main Findings: Here we show that over a range of concentrations NH₄Ac can be detected both as a water soluble attractant and as an odorant, and that ammonia and acetic acid individually act as olfactory attractants. We use genetic analysis to show that NaCl and NH₄Ac sensation are mediated by separate pathways and that ammonium sensation depends on the cyclic nucleotide gated ion channel TAX-2/TAX-4, but acetate sensation does not. Furthermore we show that sodium-acetate (NaAc) and ammonium-chloride (NH₄Cl) are not detected as Na⁺ and Cl⁻ specific stimuli, respectively.

Conclusions/Significance: These findings clarify the behavioral response of C. elegans to NH₄Ac. The results should have an impact on the design and interpretation of chemosensory experiments studying detection and adaptation to soluble compounds in the nematode Caenorhabditis elegans.

Introduction

Animals rely on sensory information to respond appropriately to a variety of beneficial and harmful environmental conditions. One such response is chemotaxis, in which an animal samples a continuously changing chemical environment and generates movement toward an attractant [1]. Despite its simple nervous system, the nematode Caenorhabditis elegans is able to chemotax to a large number of different attractants including cations and anions, amino acids, alkaline pH, cyclic nucleotides and many volatile organic odorants [1–3]. C. elegans chemotaxis offers an appealing system to study how the nervous system processes and integrates sensory information with a limited number of neurons.

Chemical compounds that are attractive to C. elegans have been classified in several different kinds of behavioral assays. Ward [3] assayed water soluble chemoattraction in radial gradients of attractant. Attraction to anions or cations alone was tested by pairing the tested ion with a counter-ion (ammonium or acetate) that was not attractive under these conditions. These experiments showed that anions (Cl⁻, Br⁻, I⁻) and cations (Na⁺, Li⁺, K⁺, Mg²⁺) are attractive when peak gradient concentrations are 2–20 mM [3]. Similar results were seen in an alternative assay in which worms choose between two streams of liquid containing different attractants. In this assay, weak attraction to ammonium and acetate ions could also be detected [4]. Later, Bargmann and colleagues studied water soluble and odorant chemotaxis in detail [1,2]. By ablating ciliated amphid sensory neurons with a laser beam, these studies identified the sensory neurons necessary for detecting attractants. They found that water soluble chemotaxis is mediated primarily by the pair of ASE neurons with a minor contribution from ADF, ASG, ASI and ASK [1]. Chemotaxis to odorants is mediated by two other pairs of neurons: AWG and AWA [2]. Thus, C. elegans has senses equivalent to taste and smell.

The distinction between taste and smell in C. elegans has a morphological correlate. The amphid sensory sensillum contains twelve pairs of sensory neurons, eight of which are directly exposed to the environment. The exposed neurons mainly sense water soluble chemicals. However, there is at least one exception to this; the exposed ADL neurons are important for the avoidance of the odorant 1-octanol [5,6]. The four pairs of neurons that are not directly exposed to the environment participate in odorant (AWA, AWB, AWG) and temperature sensation (AFD).

Wicks et al. [7], and Jansen et al. [8], studied attraction to water soluble chemicals with another behavioral assay, the quadrant assay. In this assay, two diagonally opposed quadrants of a plate are filled with an attractive chemical whereas the two remaining quadrants...
have no attractant. Under these assay conditions, NH$_4$Ac is a poor attractant at low concentration (1 mM) but a potent attractant at high concentration (75 mM) [8]. Thus, the attractive properties of NH$_4$Ac depend on concentration and the choice of behavioral assay.

Here we show that NH$_4$Ac is detected both as a water soluble attractant and as an odorant, and that ammonia and acetic acid individually act as olfactory attractants. We use genetic analysis to show that NaCl and NH$_4$Ac sensation are mediated by separate pathways and that ammonium sensation depends on the cyclic nucleotide gated ion channel TAX-2/TAX-4, but acetate sensation does not. Mutant analysis shows that NH$_4$Ac is detected by exposed and non-exposed sensory neurons. Furthermore we show that NaAc and NH$_4$Cl do not constitute Na$^+$ and Cl$^-$ specific stimuli under these experimental conditions. Our results clarify NH$_4$Ac chemosensation and its molecular basis.

**Results**

Ammonia and acetic acid are volatile attractants

We previously reported that chemotaxis to the peak of an NH$_4$Ac gradient was intact in animals that could not detect NaCl, suggesting that separate pathways exist for detecting these attractants [9]. Because a solution of NH$_4$Ac has a characteristic smell, we hypothesized that NH$_4$Ac could be detected by an odorant pathway. We assayed odorant chemotaxis (Fig. 1A) by spotting the attractant either on the plate or the lid immediately before the assay. In both conditions wild-type animals accumulated at the attractant source (Fig. 1B, C). Attraction could be toward acetate/acetic acid and ammonium/ammonia or to only one of these compounds. To test this, we assayed attraction to acetic acid and ammonia separately by spotting the attractants on the lid. Both compounds were attractive (Fig. 1D). Thus, C. elegans can sense ammonium and acetate as distinct attractants.

Ammonium-acetate chemotaxis depends on ciliated neurons

To identify signaling pathways that mediate NH$_4$Ac sensation we performed chemotaxis assays with well-characterized mutants. We used three types of mutants: (1) cilium structure mutants which have defects in the sensory endings of ciliated sensory neurons, (2) sensory transduction mutants which lack
components necessary for signal transduction, and (3) neuron specification mutants which lack transcription factors that are necessary for the correct development and function of specific neurons. Neuron specification mutants can be helpful in identifying candidate cells for functions such as chemosensation. However, experimental results obtained by this approach should be interpreted with caution, because factors such as developmental compensation and residual function of the impaired cells cannot be ruled out.

(1) Cilium structure mutants. Depending on the cells affected, cilium structure mutants display impaired chemotaxis to water soluble attractants (osm-3) or to both odorants and water soluble attractants (che-2 and che-3) [2,7,10]. osm-3 is expressed only in sensory neurons with exposed cilia [11] and osm-3 mutants do not exhibit structural defects in non-exposed cilia [12]. In our assays, two osm-3 mutants (p802 and mn391) showed significantly reduced chemotaxis to NH₄Ac compared to wild-type (Fig. 2), implicating exposed ciliated neurons in chemotaxis to NH₄Ac. However, osm-3(p802) chemotaxed significantly better than the negative control in both water soluble and odorant assays (osm-3(p802) vs. negative control, p<0.05). Thus, osm-3(p802) chemotaxis was only partially impaired. One way to interpret this is that both exposed and non-exposed ciliated neurons are involved in normal NH₄Ac chemotaxis. Alternatively, only the exposed neurons are involved, but osm-3 mutants do not completely eliminate their function. Mutants in che-2 and che-3 eliminated chemotaxis to NH₄Ac (Fig. 2, che-2, che-3 vs. neg. control, p<0.05). These mutants affect both exposed and non-exposed cilia, suggesting that both classes of neurons are involved in sensing NH₄Ac. However, because these mutants also have more severe structural defects than osm-3.

Figure 2. Genetic analysis of chemotaxis to NH₄Ac presented in water soluble or odorant form. (A) Water soluble chemotaxis assays. Chemotaxis index is plotted vs. strain for assays in which radial gradients of NH₄Ac were established by diffusion in the agar. (B) Odorant NH₄Ac assays. Chemotaxis index is plotted vs. strain for assays in which a droplet of NH₄Ac (10 μL, 7.5 M) was suspended from the lid of the plate. In A and B, each bar represents the mean of at least 8 independent assays; n.d. means no data. Wild type (neg. con) is a negative control assay with no attractant on plate. Statistics: * p<0.05 and ** p<0.01 in a one way ANOVA and Dunnet’s post test comparing all means to the wild-type (N2) mean; # p<0.05 in a one-way ANOVA with Dunnet’s post test comparing che-2(e1033), che-3(e1124), and osm-3(p802) to the negative control.

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(2) Sensory transduction mutants. These mutants show a variety of phenotypes, from broad defects in many sensory modalities to defects in the response to a single odorant [13]. The cyclic nucleotide gated cation channel TAX-2/TAX-4 is an example of a protein that is necessary for many sensory processes. TAX-2/TAX-4 is widely expressed in sensory neurons. Consistent with this, tax-2 and tax-4 mutant animals are defective in soluble and odorant chemotaxis as well as thermotaxis [14,15]. Guanylyl cyclase daf-11 mutants have phenotypes similar to tax-2 and tax-4 mutants [16,17]. This suggests that DAF-11 activity generates the cGMP which gates TAX-2/TAX-4 channels. In our assays, tax-2 and daf-11 null mutants were impaired for water soluble and odorant chemotaxis to NH4Ac (Fig. 2). DAF-11 is likely to function as a heterodimer with another guanylyl cyclase, ODR-1 [18]. ODR-1 is expressed in non-exposed neurons (AWC and AWB) and exposed neurons (ASI, ASJ and ASK). odr-1 mutants are defective in AWC and AWB-mediated olfaction but chemotaxis to soluble compounds detected by non-exposed neurons has not been well-studied [19]. In NH4Ac chemotaxis assays odr-1 mutants have significant defects only in odorant assays (Fig. 2B). The G-alpha subunit ODR-3 is mainly involved in sensing odors and noxious stimuli whereas NaCl sensation is normal [20]. Consistent with this, odr-3 mutants showed significantly reduced chemotaxis to NH4Ac only in the odorant assay (Fig. 2B). These results show that NH4Ac sensation depends on G-protein signaling pathways.

(3) Neuron specification mutants. These mutants lack transcription factors which are necessary for correct cell specification [21]. che-1 has lost all ASE specific expression [22,23] and odr-7 has impaired AWA function and morphology [24]. Neither che-1 nor odr-7 null mutants showed defects in either type of chemotaxis assay to NH4Ac. Thus, perturbing ASE or AWA function through a combination of loss of sensory transduction and deletion in the promoter region and first exon of ceh-36 abolishes its expression in only four pairs of neurons: ASE, AQR, ASH, ASJ, ASI, AWB, and PQR [14]. However, as noted previously, che-1; odr-1 double mutants had no defect in chemotaxis to NH4Ac (Fig. 2A, B). Thus, we interpret che-1 attraction to NH4Cl as chemotaxis to ammonium only, and che-1 attraction to NaAc as chemotaxis to acetate only. Accordingly, we use the che-1 strain as our positive control for chemotaxis to these compounds. tax-2(p678) and tax-2(sa1205) chemotaxis to ammonium was significantly reduced compared to che-1(p679) (Fig. 3B). In contrast, acetate chemotaxis was not significantly impaired for either tax-4 or tax-2 (Fig. 3C). We furthermore tested two additional alleles of tax-2(p671 and p691) which were both in agreement with tax-2(sa1205) (data not shown). Thus, ammonium sensation depends on TAX-2/TAX-4, but acetate is detected by a TAX-2/TAX-4 independent pathway.

We used tax-2(p694) as an alternative way to examine which cells are involved in detecting ammonium. In wild-type animals, TAX-2 is expressed in AWC, AFD, ASE, ASG, ASJ, AQR, BAG, ASK, ASI, AWB, and PQR [14]. However, tax-2(p694) has a deletion in the promoter region and first exon of tax-2 that abolishes its expression in only four pairs of neurons: ASE, AQR, AFD, and BAG [14]. tax-2(p694) had no defect in chemotaxis to ammonium (Fig. 3B), indicating that these four cells were not necessary for ammonium sensation.

The residual ammonium and acetate chemotaxis ability of che-1(p679), in which ASE neurons are defective, implies that at least one additional sensory neuron is required for chemotaxis to these compounds. To determine whether this additional sensory function resides among the exposed or non-exposed class of neurons, we tested oem-3(p802), in which all exposed cilia are defective, but non-exposed cilia are intact [12]. oem-3(p802) was completely defective in sensing NaCl (Fig. 3A) and thus should give ammonium or acetate specific responses in NH4Cl and NaAc assays, respectively. We found that oem-3(p802) chemotaxis to ammonium and acetate was reduced relative to che-1(p679) (Fig. 3B, C). We conclude that some of the residual ammonium and acetate sensory function likely resides among the exposed chemoexcitatory neurons other than ASE. We did not examine further which other exposed neurons might be responsible for the residual responses to ammonium or acetate, though the ADF, ASI, and ASG neurons implicated in NH4Cl sensation by laser ablation experiments are possible candidates [1].

NaAc and NH4Cl are not Na⁺ and Cl⁻ specific stimuli

NaAc and NH4Cl have been used as approximations for sodium and chloride specific stimuli in chemotaxis assays [1,27–29] under the assumption that ammonium and acetate are relatively unattractive to worms [3]. However, as noted above, whether or not NH4Ac is attractive depends on assay method. We used che-1, tax-2(p694) and ceh-36 mutants in water soluble chemotaxis assays

[7,10,12], it does not exclude the possibility that only exposed neurons are involved (but see below).
to determine the attractive properties of ammonium and acetate ions under our conditions. None of the mutants chemotaxed to NaCl whereas all but ceh-36 chemotaxed normally to NH4Ac and exhibited significant chemotaxis to NaAc and NH4Cl (Fig. 4). Because che-1 and tax-2(p694) mutants are completely defective in sensing chloride (see also Fig. 3A) but can still sense ammonium (Fig. 3B), these results indicate that NH4Cl, a putative chloride-specific stimulus, is in fact a combination of attraction to both chloride and ammonium under these experimental conditions. Similarly, chemotaxis to NaAc in wild-type animals is a combination of attraction to Na+ and acetate.

Although ammonium and acetate were attractive under our conditions, we did find special conditions under which NaAc acts as a Na+–only stimulus. In discrimination assays, in which a NaAc gradient is formed on top of a high uniform background concentration of NH4Cl, wild-type animals were able to locate the gradient peak (Fig. 4, NaAc/NH4Cl). In contrast, the NaCl-chemotaxis defective mutants che-1(p679), che-1(ot66), and tax-2(p694) were unable to locate the gradient peak. This difference between wild-type and NaCl-chemotaxis defective mutants indicates that under conditions of high background NH4Cl, wild-type worms are orienting to Na+ but not acetate.

Under the opposite conditions, in which NH4Cl chemotaxis was tested against a high background of NaAc, the NaCl-chemotaxis defective mutants were not different from wild-type. Thus, these conditions do not provide a Cl2–only stimulus.

We also tested two alleles of ceh-36 (ks86 and ky646) to compare with mutants that have left/right bilateral asymmetries in ASE tested under similar or identical conditions [9,28]. ceh-36(ks86) and ceh-36(ky646) were completely impaired in chemotaxis to all attractants, including NaCl (Figure 4). This is in contrast to ASE/L/ASER mutants [9,28], which show relatively subtle defects in chemotaxis to NaAc and NH4Cl, but no defects to NaCl. These results suggest that although ASE neurons express most cell specific markers in the ceh-36 background [22,25,26], normal ASE function is abolished.

Figure 3. Genetic analysis of ammonium and acetate signal transduction pathways. (A–C) Water soluble chemotaxis assays for NaCl, NH4Cl, and NaAc. In each case, radial gradients of attractant were established by diffusion in the agar. Each bar represents the mean of at least 4 independent assays. Statistics: * p<0.05 and ** p<0.01 in a one-way ANOVA and Dunnet’s post test comparing all mutants to the che-1(p679), which serves as a control in that it has normal chemotaxis to NH4Ac but no chemotaxis to NaCl; $ p<0.01 in a one sample t-test comparing the observed mean to a mean of zero.

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Discussion

Ammonium-acetate is an attractive odorant

*C. elegans* chemosensation has been successfully studied with behavioral assays that treat volatile and water soluble chemotaxis as separate senses, analogous to smell and taste. This approach has been successful, partly because distinct groups of sensory neurons mainly mediate responses only to odorants or to water soluble compounds. In several water soluble chemotaxis assays ammonium and acetate have been used either as neutral counter-ions or as attractants [1,7–9,28,29]. Here we show that ammonium and acetate are attractive in both water soluble and odorant chemotaxis assays. Over a range of concentrations, NH$_4$Ac spotted on a plate or on the lid above the plate was attractive to *C. elegans*. Furthermore, NH$_3$ and acetic acid alone were attractive odorants, leading to accumulation of animals near the source of either. It should be pointed out that NH$_4$Ac was used at high concentrations in the odorant assays, probably above the normal range encountered naturally. It is also unlikely that the odorant properties of NH$_4$Ac affect the response of *C. elegans* in the standard chemotaxis assay developed by Bargmann and colleagues [1] in which a small point source of attractant is applied the day prior to chemotaxis assays; the local concentration during the chemotaxis assay would not be expected to be high enough to elicit odorant attraction, though it might affect response as a soluble attractant as we have shown. In contrast, the “quadrant assay” developed by Plasterk and colleagues is very different-half of the plate contains attractant at uniform high concentration [7]. Under these experimental conditions it is likely that odorant responses can contribute to NH$_4$Ac attraction.

Ammonium and acetate can also be detected as water soluble compounds absorbed into the agar; animals were attracted to the peak of a shallow gradient of water soluble NH$_4$Ac where no focal odorant source would be expected because NH$_4$Ac has diffused into the agar over a wide area. Furthermore, we used che-1 animals to test the assumption that NaAc and NH$_4$Cl are equivalent to Na$^+$ and Cl$^-$ specific stimuli. It is clear that this is not a valid assumption under these experimental conditions. On the contrary, we have shown that a significant part of chemotaxis to NaAc and NH$_4$Cl is to acetate and ammonium ions (Figure 4).

Chemotaxis to NH$_4$Ac appears to conflict with previous findings from our laboratory [28]. The fact that we now find NH$_4$Ac to be attractive whereas Pierce-Shimomura *et al*. [28] did not was unexpected because the peak concentration and the spatial extent of the NH$_4$Ac gradients were almost identical in the two studies. However, there were three significant differences between the studies. First, in the new assays, worms were immobilized at the gradient peak whereas in our earlier study worms were free to leave the peak, and frequently did so (J. Pierce-Shimomura, personal communication), possibly because of sensory adaptation. Second, we counted the number of worms reaching the peak, whereas Pierce-Shimomura *et al*. recorded dwell time at the peak. Because dwell time would be reduced by worms leaving the peak, the Pierce-Shimomura assay was probably less sensitive than the present assay. Finally, we performed the assays for 60 minutes on animals started 30 mm from the peak of attractant whereas Pierce-Shimomura *et al*. performed the assays for 30 minutes on animals started 20 mm from the peak of attractant.

Figure 4. Genetic analysis of the relative ionic contributions to water soluble chemotaxis assays. Attractants and uniform background compositions are indicated below each group of bars; the no-background conditions are indicated by “-” and n.d. means no data. Each bar represents the mean of at least 8 independent assays. Statistics: * p<0.05 and ** p<0.01 in a one way ANOVA and Dunnet’s post test comparing all means to the wild-type (N2) mean.

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Shimomura et al. assayed single animals for 20 minutes placed 11 mm from the peak.

One question is whether the NH$_4$Ac water soluble and odorant assays measure qualitatively different behaviors or are merely quantitatively different measures of the same behavior. Our assays (see Figure 2) are consistent with either possibility. The odorant assay may simply be a more sensitive assay that can reveal the weaker defects of such mutants as odr-3, odr-7, and odr-7 odr-1 that were not detectable in water soluble assays. Because we found no mutants that were normal in odorant assays and specifically defective in water soluble assays, we cannot conclude that the two assays measure qualitatively different senses. Whether NH$_4$Ac is defective in water soluble assays, we cannot conclude that the two mutants that were normal in odorant assays and specifically were not detectable in water soluble assays. Because we found no such combination of volatile and water soluble cues.

Several cells and distinct pathways detect ammonium and acetate

Our results suggest that NH$_4$Ac sensation is distributed over several neurons. Interestingly, impaired ASE and AWC specification in the ceh-36 mutant completely disrupts NH$_4$Ac chemotaxis. Odorant specific mutations odr-3 and odr-7 odr-1 which perturb both AWA and AWC disrupt chemotaxis to NH$_4$Ac when it is placed on the lid. These mutations do not disrupt water soluble chemotaxis to NH$_4$Ac, which probably reflects the bias of the assays—in the lid assay the olfactory sensory component is more heavily weighted. This is supported by additional data: a high uniform background of NaCl perturbs water soluble chemotaxis more than chemotaxis to odorants spotted on the lid (Fig. S2).

At the level of sensory neurons, there is enough redundancy that only mutations affecting at least two of the three pairs of neurons ASE, AWC, and AWA disrupt chemotaxis. However, double mutants of che-1 with odr-7 or odr-1 indicate that this “two of three” model is not correct in its simplest form. One caveat to these experiments is that the mutants used may not completely eliminate the function of the cell. For example, odr-1 mutations eliminate a single signal transduction component in AWC and should not affect other possible odr-1 independent signal transduction pathways in this cell. The che-1 and odr-7 mutations lack proper terminal differentiation of ASE and AWA. However, in both cases, the cell is not eliminated and may still be capable of some sensation, possibly as a result of acquiring certain features of another sensory neuron. There is some evidence that the default olfactory neuron cell fate resembles AWC [30]. Thus, the effects of the che-1 and odr-7 mutations may be less severe than complete elimination of the cell. Additionally, experiments with odr-3 indicate that exposed sensory neurons other than ASE may also be involved in sensing ammonium and acetate. Therefore, single animal experiments using cell specific laser ablations or calcium imaging are needed to fully characterize the cells involved in detecting NH$_4$Ac.

Genetic analysis showed that NH$_4$Ac and NaCl chemotaxis are separable processes. che-1 mutants are unable to chemotax to NaCl yet show wild-type chemotaxis to NH$_4$Ac. Effectively, this made it possible for us to use Na$^+$ and Cl$^-$ as neutral counter-ions for acetate and ammonium ions, respectively. Interestingly, ammonium sensation depends on the TAX-2/TAX-4 channel, but acetate sensation does not. TAX-2/TAX-4 independent sensory pathways are well described in the AWA neurons, where OSM-9 (a TRP-like channel) is necessary for sensory transduction [31]. However, we think it unlikely that attraction to acetate is exclusively mediated by AWA since odr-7 mutants show wild-type chemotaxis to NH$_4$Ac. Also, oom-9 mutants are not defective in chemotaxis to acetic acid (data not shown).

We also assayed the tax-2(p694) mutant which has lost TAX-2 expression in ASE, AQR, AFD, and BAG [14]. tax-2(p694) has normal AWC function but impaired ASE and AFD function [14]. tax-2(p694) chemotaxis to ammonium is not reduced and thus ASE is not necessary for ammonium sensation. Since che-36 chemotaxis is completely impaired, we think it likely that AWC is involved in detecting ammonium in water soluble chemotaxis assays. Cyclic nucleotide dependent signaling in AWC depends on at least two G-alpha subunits, ODR-3 and GPA-3, and two downstream guanyl cyclases, ODR-1 and DAF-11 [32]. odr-3, odr-1, def-11, and tax-2 all show similarly reduced chemotaxis to NH$_4$Ac in the odorant assay. Our results are consistent with the interpretation that this reduction is due to a selective loss of ammonium sensation.

Water soluble NH$_4$Cl chemotaxis has mainly been ascribed to ASE based on the careful analysis of chemotaxis after ablation of all the exposed ciliated neurons, alone or in combination [1]. Comparison of ablated animals with the cilium structure mutant che-2 [35] (which lacks exposed and non-exposed ciliated neurons), shows that there is a residual response to NH$_4$Cl after ablation of ASE (and any of the other exposed neurons). This observation is therefore consistent with a possible role for AWC in ammonium sensation.

Sensory pathways and taste adaptation

Worms pre-exposed to a compound often have reduced chemotaxis to the same compound, a process termed adaptation. Jansen et al. [8] showed that for water soluble compounds this process is partly salt specific. However, not all salts produce adaptation, and cross-adaptation is limited to certain salts. For example, pre-exposure to NH$_4$Ac does not cause adaptation to NH$_4$Ac or cross-adaptation to NaCl. Adaptation to NaCl appears to be a complex process involving multiple cells and molecular pathways [33]. Our results suggest that multiple sensory cells (ASE, AWC, AWA, and possibly others), at least two separate pathways (TAX-2/TAX-4 dependent and independent), and both the worm equivalents of taste and smell are involved in detecting ammonium and acetate. This could explain the rather complex pattern of partial adaptation and cross-adaptation. Specifically, the lack of cross-adaptation between NH$_4$Ac and NaCl is not surprising given that the two salts are sensed by separate pathways.

It is clear that some chemosensory specificity is concentration dependent. For example, odr-10 mutants which lack the putative diacetyl receptor are unable to locate the peak of low concentrations of diacetyl, but show wild-type chemotaxis to higher concentrations [34]. Similar mechanisms may be important for water soluble chemotaxis. Thus, for chemotaxis to NaCl in gradients with high peak concentrations (25-800 mM), ablation of ASE does not completely eliminate chemotaxis. Under these experimental conditions, ADF, ASG, and ASI are important for NaCl chemotaxis [1]. However, in a modified chemotaxis assay with shallower gradients (peak approx. 10 mM), ablation of ASE eliminates NaCl chemotaxis ([35] and J. Pierce-Shimomura, personal communication). Also, the data presented in this study in a similar shallow
gradient showed that two alleles of che-1 were completely defective in chemotaxis to NaCl, even though che-1 appears not to be expressed in ADF, ASG or ASI [23]. It is interesting that the che-36 loss of function alleles are not only completely impaired for NH4Ac chemotaxis but also for NaCl chemotaxis. che-36 loss of function mutations affect the expression of ASE specific markers only weakly [25,26] and have been proposed by Lanjun et al. [26] to mainly affect bilateral asymmetry in the ASE neurons. Our results favor the interpretation by Koga & Ohshima that CEH-36 is necessary for ASE function.

Materials and Methods

Strains and genetics

All strains were derived from the wild-type N2 strain and grown under standard conditions at room temperature on nematode growth medium seeded with the Escherichia coli strain OP50 [36]. The following mutant strains were used: che-36(k836) X, che-36(k864) X, che-1(a066) I, che-1(p679) I, che-2(a1033) X, che-3(e1124) I, daf-11(sa195) V, odr-1(sa1936) X, odr-3(a2150) V, odr-7(ky4) X, odr-3(mn391) IV, odr-3(p802) IV, tax-2(p671) II, tax-2(p691) II, tax-2(p694) II, tax-2(sa1203) II, tax-4(p678) III and the double mutants syi140 I che-3(ky646) X, odr-7(ky4) odr-1(n1936) X. Putative null alleles: che-3(ky646), che-1(p679), che-2(k1033), che-3(e1124), daf-11 (sa195), odr-7(ky4), odr-3 (p802), tax-2 (sa1205) and tax-4 (p678) have nonsense mutations in the genes and are putative null alleles [7,10,15,16,23,24,26,37], J. Kemner, personal communication. che-1(a066) has a deletion of part of the promoter and beginning of gene and is a putative null allele [22]. Loss of function alleles: odr-3(a2150) and odr-3(mn391) have late nonsense mutations [20,37], che-36(k864) has a missense mutation [25] and odr-1(n1936) has a splice donor mutation [19], tax-2(p694) has a deletion in the promoter region and first exon of tax-2 that abolishes its expression in only four pairs of neurons: ASE, AQR, AFD, and BAG [14].

Chemotaxis assays

The chemotaxis assay was based on assays developed by Bargmann and Horvitz [1] and Pierce-Shimomura et al. [28]. Assays were performed on 10 cm plates containing 20 g/L agar, 5 mM potassium phosphate (pH = 6.0), 1 mM CaCl2, and 1 mM MgSO4 (“standard plates”). Assay plates for discrimination assays additionally contained 50 mM NaAc, pH = 6.0 or 100 mM NH4Cl, pH = 6.0. Different background concentrations of NH4Cl and NaAc were used because animals showed poor chemotaxis to NH4Cl in 100 mM NaAc [9]. We also tested the effect of assay plate composition in accordance with other published chemotaxis assays: “Jansen” (20 g/L agar, 5 mM potassium-phosphate (pH = 6.6), 1 mM CaCl2, 1 mM MgSO4 [8]), “Ward” (15 g/L agarose, 10 mM HEPES (pH = 7.2), 0.25% Tween 20 [3]) and “Pierce” (17 g/L agar, 2 mM NH4Cl, 1 mM CaCl2, 1 mM MgSO4, 25 mM potassium-phosphate (pH = 6.5) [28]). Please see figure S3.

Water soluble chemotaxis assays: Radial gradients were formed by placing 10 µL of 2.5 M attractant or ddH2O (control) at diametrically opposed locations on the plate (see Fig. 1A). The attractant was allowed to diffuse for 14–16 hours at room temperature. To increase the steepness of the gradient, 4 to 5.5 hours prior to the chemotaxis assay, an additional 4 µL of attractant or ddH2O was added to the attractant and control spots, respectively. The peak of the gradient was estimated to be on the order of 10 mM with a fall-off to less than 1 mM at 20 mm from the peak, based on a diffusion model assuming no borders [28]. Attractants NaCl, NH4Ac, NH4Cl, and NaAc (Sigma, MO, USA) were dissolved in ddH2O to a concentration of 2.5 M and adjusted to pH = 6.0 with either ammonium-hydroxide or acetic acid.

Odorant chemotaxis assays: Attractant solution was placed on the lid above the “attractant spot” and ddH2O placed above the “negative control spot” immediately before the assay (see Fig. 1A). Attractants for odorant assays were dissolved in ddH2O at a concentration of 7.5 M. NH4Acetate used directly on the plate was adjusted to pH = 6.0. For the dose-response curve in Fig. 1B, the odorant was placed directly on the plate immediately prior to placing worms on the plate. For dose-response odorant chemotaxis assays (Fig. 1B and C) odorant concentration was kept constant and different volumes of attractant were placed on the assay plate.

For both types of assay, synchronized unstarved adult animals were rinsed off culture plates with S basal for odorant assays and sterile ddH2O for water soluble chemotaxis assays. To remove bacteria and other potential attractants, animals were subsequently washed twice with 10 mL ddH2O and pelleted loosely in a table top centrifuge. Animals were transferred using glass Pasteur pipettes. The rinse and wash procedure took ~15–25 minutes. Before placing animals on assay plates, sodium azide (2.0–2.5 µL, 0.25 M) was pipetted onto the plate at the attractive spot and the negative control spot to immobilize animals reaching either spot. The azide immobilized animals within a radius of ~10 mm. Animals were transferred to the center of the plate in a droplet of ~50 µL ddH2O. Excess ddH2O was removed with filter paper.

Chemotaxis assays were performed at room temperature for 60 minutes and assay plates were subsequently placed in a refrigerator (5°C) to prevent further movement of the animals. Results were quantified by counting worms that reached the attractant spot (zone A), the negative control spot (zone C), or the remainder of the plate (zone B), as shown in Fig. 1A. Animals that were found in the inner circle at the end of the assay period were counted but not included in the count of total number of animals, because most of these animals were injured, dead, or had burrowed in the agar. Chemotaxis index (C.I.) was calculated as (A−C)/(A+C). The theoretical range of the index was 1.0 (complete attraction) to −1.0 (complete repulsion). There were usually ~150 worms per plate; plates with less than 30 worms were not counted. In general, two assays with the same attractant were performed in parallel with the two plates oriented in opposite directions to minimize the influence of extraneous cues.

We did note one qualitative difference between chemotaxis toward NH4Ac or acetic acid and the other compounds. Animals were attracted to NH4Ac and acetic acid yet never reached the peak of the gradient; instead, animals were paralyzed a small distance away. Also, when stored at 5°C, the animals appeared to decompose faster on plates containing NH4Ac or acetic acid than on plates containing the other attractants. The high concentration of acetate was not sufficient in itself to paralyze the animals, because nematodes reached the attractant peak on plates without azide. Thus acetate appears to sensitize worms to the effect of azide.

Statistics

Means represent data pooled from assays run on at least three different days; error bars are s.e.m.. Methods for specific statistical comparisons are given in the figure legends.

Supporting Information

Figure S1 NH4Ac odorant chemotaxis of double mutants. (A) che-1(p679); odr-7(ky4) double mutant chemotaxis. (B) che-1(p679); odr-1(a1936) double mutant chemotaxis. Only four assays were performed and therefore no statistical analysis has been performed on these experiments.
**Figure S2 Effects of salts in plate on NH4Ac chemotaxis.** (A) N2 water soluble chemotaxis to NH4Ac with normal chemotaxis plates (background “blank”), 50 mM Na-acetate or 100 mM NH4Cl or 100 mM NaCl added to chemotaxis plate. (B) N2 odor-lid chemotaxis to volumes of 7.5 M NH4Ac spotted on lid on standard chemotaxis plates (background “blank”) or 100 mM NaCl (background “NaCl”). Statistics: Each data point represents the mean of at least 5 independent assays, error bars represent SEM. Statistics: (C) and (D) One-way ANOVA and Tukey’s multiple comparisons test between all pairs of columns.

**Figure S3 Effect of plate composition on NH4Ac chemotaxis.** (A) N2 odor chemotaxis to 10 µL 7.5 M NH4Ac spotted on plate before assay. Four different types of chemotaxis plates were used (see Materials and Methods) There is no statistical difference between means. (B) N2 odor-lid chemotaxis to 10 µL 7.5 M NH4Ac spotted on lid. The effect of plate composition is small, except for “Ward” background, which is statistically different from all other backgrounds. Worms moved very poorly on agarose plates and it is not clear if the low chemotaxis index represents a lack of NH4Ac sensation or a movement defect. Statistics: Each data point represents the mean of at least 5 independent assays, error bars represent SEM. Statistics: (C) and (D) One-way ANOVA and Tukey’s multiple comparisons test between all pairs of columns.

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**Author Contributions**
Conceived and designed the experiments: CF SL MA. Performed the experiments: CF MA. Analyzed the data: CF. Contributed reagents/materials/analysis tools: CF SL. Wrote the paper: CF SL MA.

**References**
1. Bargmann CI, Horvitz HR (1991) Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in C. elegans. Neuron 7: 729–742.
2. Bargmann CI, Hartwig E, Horvitz HR (1993) Odorant-selective genes and neurons mediate olfaction in C. elegans. Cell 74: 515–527.
3. Ward S (1975) Chemotaxis by the nematode Caenorhabditis elegans: identification of attractants and analysis of the response by use of mutants. Proc Natl Acad Sci U S A 70: 817–821.
4. Dusenbery DB (1974) Analysis of chemotaxis in the nematode Caenorhabditis elegans by countercurrent separation. J Exp Zool 188: 41–47.
5. Chao MY, Komatsu H, Fukuto HS, Dionne HM, Hart AC (2004) Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. Proc Natl Acad Sci U S A 101: 15312–15317.
6. Troemel ER, Kimmel BE, Bargmann CI (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell 91: 161–169.
7. Wicks SR, de Vries CJ, van Luenen HG, Plasterk RH (2000) CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in Caenorhabditis elegans. Dev Biol 221: 295–307.
8. Jansen G, Winkvold D, Plasterk RH (2002) The G-protein gamma subunit gpc-1 of the nematode C. elegans is involved in taste adaptation. EMBO J 21: 966–994.
9. Chang S, Johnston RJ Jr, Frokjaer-Jensen C, Lockery S, Hobert O (2004) MicroRNAs act sequentially and asymmetrically to control chemosensory lateralization in the nematode, N. columbiana. Genetics 167: 783–789.
10. Fujiwara M, Ishihara T, Katsura I (1999) A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of C. elegans sensory cilia. Development 126: 4839–4848.
11. Tabash M, Siddiqui ZK, Nishikawa K, Siddiqui SS (1995) Exclusive expression of C. elegans osm-3 kinase gene in chemosensory neurons open to the external environment. J Mol Biol 247: 377–389.
12. Perkins LA, Hedgecock EM, Thomson JN, Caloni JG (1986) Mutant sensory cilia in the nematode Caenorhabditis elegans. Dev Biol 117: 436–447.
13. Mori I (1999) Genetics of chemotaxis and thermotaxis in the nematode Caenorhabditis elegans. Annu Rev Genet 33: 399–422.
14. Coburn CM, Bargmann CI (1998) A putative cyclic nucleotide-gated channel is required for sensory development and function in C. elegans. Neuron 17: 695–704.
15. Komatsu H, Mori I, Rhee JS, Akaike N, Ohshima Y (1996) Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in C. elegans. Neuron 7: 707–718.
16. Birulytė BA, Link EM, Vowels JJ, Tian H, Colacurcio PL, et al. (2000) A transmembrane guanylyl cyclase (DAF-11) and Hip90 (DAF-21) regulate a common set of chemosensory behaviors in caenorhabditis elegans. Genetics 155: 95–104.
17. Vowels JJ, Thomas JH (1994) Multiple chemosensory defects indaf-11 anddaf-21 mutants of Caenorhabditis elegans. Genetics 138: 303–316.
18. Morton DB (2004) Invertebrates yield a plethora of atypical guanylyl cyclases. Mol Neurobiol 29: 97–116.
19. L’Etoile ND, Bargmann CI (2000) Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron 25: 575–586.
20. Rosayre K, Crump JG, Sagasti A, Bargmann CI (1998) The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron 20: 55–67.
21. Lanjuin A, Sengupta P (2004) Specification of chemosensory neuron subtype identities in Caenorhabditis elegans. Curr Opin Neurobiol 14: 22–30.
22. Chang S, Johnston RJ Jr, Hobert O (2003) A transcriptional regulatory cascade that controls left/right asymmetry in chemosensory neurons of C. elegans. Genes Dev 17: 2123–2133.
23. Uchida O, Nakano H, Koga M, Ohshima Y (2003) The C. elegans chem-1 gene encodes a zinc finger transcription factor required for specification of the ASE chemosensory neurons. Development 130: 1215–1224.
24. Sengupta P, Colbert HA, Bargmann CI (1994) The C. elegans gene odr-7 encodes an olfactory-specific member of the nuclear receptor superfamily. Cell 79: 971–980.
25. Koga M, Ohshima Y (2004) The C. elegans ceh-36 gene encodes a putative homomodomain transcription factor involved in chemosensory functions of ASE and AWC neurons. J Mol Biol 336: 579–587.
26. Lanjuin A, VanHoven MK, Bargmann CI, Thompson JK, Sengupta P (2003) Otx/od homobox genes specify distinct sensory neuron identities in C. elegans. Dev Cell 3: 621–633.
27. Ortiz CO, Eichberger JF, Poy SL, Frokjaer-Jensen C, Lockery S, et al. (2006) Searching for neuronal left/right asymmetry: genomewide analysis of olfactory receptor-type guanylyl cyclases. Genetics 175: 131–149.
28. Pierce-Shimomura JT, Faumont S, Gaston MR, Pearson BJ, Lockery SR (2001) The homeobox gene lim-6 is required for distinct chemosensory representations in C. elegans. Nature 410: 694–698.
29. Sakai S, Yamamoto M, Ino Y (2001) Plasticity of chemoreception revealed by paired presentation of a chemotactant and starvation in the nematode Caenorhabditis elegans. J Exp Biol 204: 1757–1764.
30. Sagasti A, Hobert O, Troemel ER, Ruskun G, Bargmann CI (1999) Alternative olfactory neuron fates are specified by the LIM homeobox gene lim-4. Genes Dev 13: 1794–1806.
31. Colbert HA, Smith TL, Bargmann CI (1997) OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in Caenorhabditis elegans. J Neurosci 17: 8259–8269.
32. Lans H, Rademakers S, Jansen G (2004) A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in Caenorhabditis elegans. EMBO J 23: 312–322.
33. Sengupta P, Zhou JH, Bargmann CI (1996) The odr-10 gene encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacyl glycerol. Cell 94: 899–909.
34. Pierce-Shimomura JT, Morse TM, Lockery SR (1999) The fundamental role of pirovetu genes in Caenorhabditis elegans chemotaxis. J Neurosci 19: 9537–9569.
35. Brenner S (1974) The genetics of Caenorhabditis elegans. Genetics 77: 71–94.
36. Snow JJ, Oo G, Gummerson AL, Walker MR, Zhou HM, et al. (2004) Two antero- and post-oculofugal transport motors cooperate to build sensory cilia on C. elegans neurons. Nat Cell Biol 6: 1109–1113.