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Rapid Communication

Chemotaxis behavior toward an odor is regulated by constant sodium chloride stimulus in *Caenorhabditis elegans*

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ABSTRACT

We studied the chemotaxis behavior of *Caenorhabditis elegans* toward a chemoattractant in the presence of background sensory stimulus. Chemotaxis toward an odor butanone was greater in the presence of sodium chloride (NaCl) than that without NaCl. By contrast, chemotaxis toward NaCl was not affected by a butanone background. The salt-sensing ASE neuron-deficient *che-1(p674)* mutants and worms with ASE genetically ablated showed high chemotaxis toward butanone, regardless of the presence of a NaCl background. Therefore, in wild-type worms, information from ASE in the absence of NaCl suppresses butanone chemotaxis, while the suppression is removed in the presence of NaCl.

Animals receive multiple sensory inputs and the information flow may interact mutually in the nervous system to determine behavior. *Caenorhabditis elegans* has a relatively simple nervous system, and is widely used to study nervous system interactions. A simple behavioral assay to examine the interaction between two sensory signals is the use of a signal stimulus for chemotaxis and a constant sensory stimulus (background). This scheme has been employed in a variety of studies, and includes sodium (or chloride) ion chemotaxis on a chloride (or sodium) ion background (Pierce-Shimomura et al., 2001), benzaldehyde chemotaxis on a butanone background (Wes and Bargmann, 2001), and lysine chemotaxis on a chloride ion background (Shingai et al., 2005). Nevertheless, there is limited evidence of an enhancement of chemotaxis behavior by the background using this scheme. To investigate the sensory integration that affects behavior, we focused on olfactory and gustatory stimuli. Butanone, isoamyl alcohol, and sodium chloride are attractants for *C. elegans*. *C. elegans* has three pairs of olfactory sensory neurons, including the AWC neuron pair. AWC neurons consist of AWC^{ON} and AWC^{OFF}, which express several different receptor genes, and the differences between the two neurons are determined randomly in the developmental process (Troemel et al., 1999). Butanone is only sensed by AWC^{ON}, while isoamyl alcohol is sensed by both AWC^{ON} and AWC^{OFF}. In the present study, we examined the effect of the NaCl background on the chemotaxis behavior toward butanone in *C. elegans*.

Strains were maintained at 20 °C on a lawn of *Escherichia coli* OP50 using the standard method (Brenner, 1974). Wild-type N2 Bristol, PR674 *che-1(p674)*, CB1034 *che-1(1034)*, QS3 *qrIs1*[*sra-9::mCasp1, sra-9::gfp, acd-3::gfp*] IV, OH8585 *otIs4[gcy-7::gfp]; otEx3822*[*ceh-36*:: CZ-caspase3(p17); gcy-7::caspase3(p12)-NZ; myo-3::mCherry], and OH8593 *ntIs1[gcy-5p::gfp] V; otEx3830[ceh-36::CZ-caspase3(p17); gcy-5::caspase3(p12)-NZ; myo-3::mCherry]* were obtained from the Caenorhabditis Genetics Center. Transgenic worms were generated by germline transformation using the microinjection method (Mello et al., 1991). The recCaspase3 (Chelur and Chalfie, 2007) was expressed using the *gcy-5* and *gcy-7* promoters for genetic ablation of ASE and the *srh-142* promoter for ADF. For ablation of ASI neurons, mouse caspase1 (mCasp1) (Yoshida et al., 2012) was expressed using the *daf-7* promoter. For *che-1(p674)* rescue experiments, the wild-type genomic *che-1* region was amplified by polymerase chain reaction (PCR) using the primers 5′-TGTCGGAACTGTCGGAAGAATCAAGC and 3′-TCTTCATTTCTCTCCCACTCATCCAC. Transgenic worms carrying this PCR product were generated by germline transformation (50 ng/μL).

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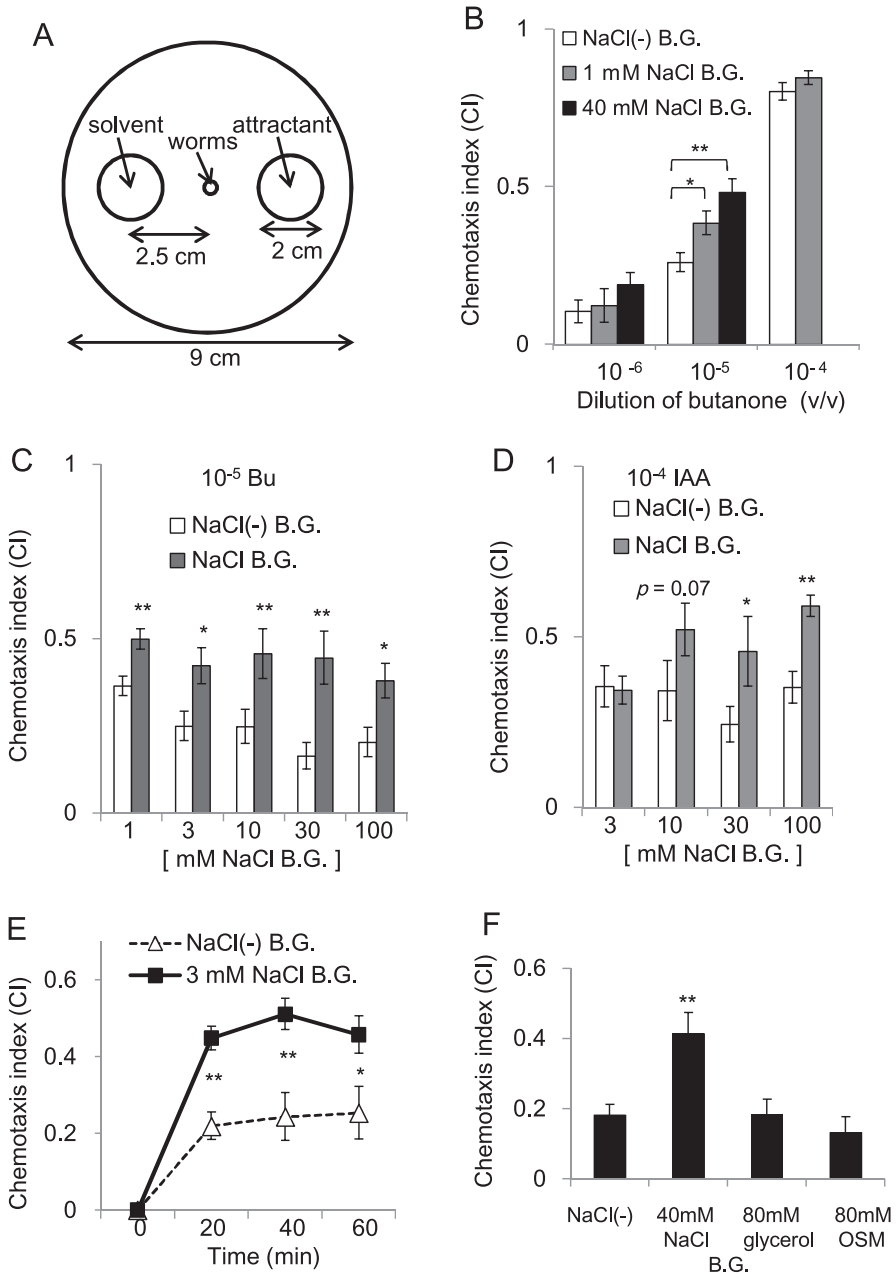

Fig. 1. Chemotaxis toward AWC-sensed odors in wild-type worms with and without a NaCl background. (A) Configuration of assay plate. (B) Chemotaxis index (CI) for butanone at different dilutions in the absence (NaCl(−)) and presence of a NaCl background. (C) CI for butanone with different concentrations of the NaCl background. (D) CI for isoamyl alcohol with different concentrations of the NaCl background. (E) Time course of CI for 10^5-fold dilution of butanone. (F) CIs for 10^5-fold dilution of butanone in the NaCl-free background without adjusting osmolarity to the 40 mM NaCl background, in the NaCl-free background with adjusted osmolarity by adding 80 mM glycerol, and with adjusted osmolarity by 40 mM glucose and 40 mM sucrose (80 mM OSM). Sixteen to forty plates were used to obtain each CI average, apart for (E) which used six plates. Error bars indicate SEM: $^*p < 0.05$, $^{**}p < 0.01$ indicate statistical difference between with and without a NaCl background in all figures.

The chemotaxis assay toward odor was performed on a 9-cm plate (Fig. 1A) containing 10 mL of agar (1 mM $MgSO_4$, 5 mM KH_2PO_4, pH 6.0 adjusted with KOH, 1.5% (w/v) agar, and NaCl at the indicated concentration). As a control, an assay without a NaCl background was performed in each experiment. One-and-a-half microliters of odor, first diluted in ethanol at 10^2-fold and then diluted in water, was spotted 2.5 cm from the center of the plate, and 1.5 μL of solvent was spotted at the opposite side of the plate. One microliter of 0.5 M sodium azide was spotted at the odor source and solvent. Odor and sodium azide were spotted just before placement of the worms. Well-fed young adult worms were collected and washed with wash buffer (1 mM $MgSO_4$, 5 mM KH_2PO_4, pH 6.0, 0.005% (v/v) Tween 20), and transferred to the center of the assay plate. Because ASI-deficient worms tended to be dauer larvae, the transgenic dauer larvae were picked and cultured for 2 days at 20 °C for recovery, and then assayed. In the assay for chemotaxis toward NaCl with a butanone or isoamyl alcohol background, 7 μL NaCl solution was placed on the assay plate at 15 h and 3 h before the start of the assay, and 1.5 μL odor was randomly placed at six locations on the inside of the assay plate lid. The lid was frequently rotated during the assay to keep the concentration of butanone in the space of the plate uniform. The total number of worms (N), the numbers of worms at the attractant source (A) and solvent (B) at 60 min, otherwise stated explicitly, were counted. The chemotaxis index (CI) was calculated by $CI = (A - B)/N$. A paired t-test was used for statistical analysis, unless stated otherwise.