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Developmental changes in chemotactic response and choice of two attractants, sodium acetate and diacetyl, in the nematode *Caenorhabditis elegans*

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Abstract

The chemotactic behavior of the nematode *Caenorhabditis elegans* to chemical attractants, water-soluble sodium acetate and odorant diacetyl, was investigated using nematodes at various developmental stages to examine the effects of postembryonic development on chemotactic response and spontaneous locomotion. The chemotactic responses to attractants increased as development progressed, and the largest responses to either 1.0 M sodium acetate or 0.1% diacetyl were seen at the young adult (YA) or day adult (A1) stage, respectively. Responses to the chemicals declined thereafter in-line with increasing age. The chemotaxis indices for attractants correlated with activity of spontaneous locomotion ($p<0.01$), suggesting that a change in spontaneous locomotion is one of the factors involved with the change in chemotactic responses during development. We also investigated the effect of aging on attractant choice by the simultaneous presentation of 0.6 M sodium acetate and 0.1% diacetyl. In the presence of both attractants, the fraction of larval animals at the sodium acetate location was greater than that at the diacetyl location ($p<0.05$). The fractions of YA animals that gathered at either location were almost identical, whereas the fraction of adult animals at the diacetyl location was greater than that at the sodium acetate location ($p<0.05$). The patterns of attractant choice of the long-lived *daf-2* mutants and short lifespan *mev-1* mutants showed the same tendency as those of wild type nematodes in the presence of both attractants. These results suggest that a change in the neuronal mechanisms controlling attractant choice and preference occurs during developmental progression.
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1. Introduction

The free-living soil nematode, *Caenorhabditis elegans*, is a popular model organism for the study of development and behavior. Its genome has been successfully mapped (The *C. elegans* Sequencing Consortium, 1998), and the simplistic nature of nematode neuroanatomy, containing only 302 neurons, has resulted in the description of a complete neuroanatomical map (White et al., 1986). Therefore, it should be possible to closely predict the relationships between behavior, neurons and genes using the nematode *C. elegans*.

The nematode demonstrates chemotactic behavior to various chemicals, such as ions (e.g., Na^+ and Cl^-), amino acids, alcohols, and other substances (Bargmann and Horvitz, 1991; Bargmann et al., 1993), all of which are produced by food bacteria and thus act as natural attractants (Ward, 1973). These chemicals are detected by chemosensory neurons in the head sensory organ amphid and the tail organ phasmid (Ward, 1973; Bargmann and Horvitz, 1991; Hilliard et al., 2002), and play a significant role in mediating chemotactic behavior. In our previous study, we have investigated chemotactic responses of young adult (YA) nematodes, which were always used in our chemotaxis assay experiment, to the simultaneous presentation of sodium acetate and diacetyl in order to examine the effect of multiple attractants on chemotactic behavior (Matsuura et al., 2004). The results indicated that the nematode recognized sodium acetate as a stronger attractant than diacetyl. Our question is whether this preference for one attractant over another is consistent during development.

The nematode is an excellent animal model for aging studies, because of its short reproductive cycle and the existence of

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many different kinds of lifespan mutants. Recently, the age-related decline of chemotaxis to diacetyl has been investigated using N2 and *daf-2* mutants, and revealed that a decline in muscle tissue integrity was correlated with onset of age-related behavioral deficits (Glenn et al., 2004). The effect of aging on spontaneous locomotion and nematode behavior, such as non-associative and associative learning, has also been examined. Beck and Rankin used 4-day, 7-day and 12-day post-hatching nematodes, and revealed that both spontaneous and reflexive movements to mechanical stimuli diminished in-line with increasing age (Beck and Rankin, 1993). In terms of isothermal tracking, which is an associative learning behavior, the nematode showed the highest ability at the YA stage, and this ability declined with change in locomotor activity according to aging (Murakami and Murakami, 2005). These observations suggest that the acquisition of nematode behavior is linked to the change in spontaneous locomotion. However, there have been no observations regarding the effect of postembryonic development on chemotaxis and attractant choice in the nematode.

In the present study, we carried out two types of experiments. The first study was investigations of the chemotactic responses of the nematode to various concentrations of attractants and spontaneous locomotion at each developmental stage, to examine the effects of development on chemotaxis and spontaneous locomotion. The second study examined the effect of development on attractant choice by simultaneously presenting sodium acetate and diacetyl, using wild type (N2) animals, *daf-2* and *mev-1* mutants.

Reduction-of-function mutations in *daf-2* mutants extend their lifespan (Kenyon et al., 1993; Larsen et al., 1995). The *mev-1* mutants have a short lifespan as a result of a hypersensitive response to oxidative stress (Honda et al., 1993). These mutants were used to confirm that the tendency of change in preference for attractant was identical throughout the nematode.

2. Materials and methods

2.1. Animals

C. elegans wild type strain (Bristol N2), long-lived *daf-2* (*m577*) and short lifespan *mev-1* (*kn1*) mutants were obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota. The nematodes were grown and maintained on nematode growth medium (NGM) agar plates (3 g/L NaCl, 2.5 g/L polypeptone, 5 mg/L cholesterol, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 25 mM KH_2PO_4 (pH 6.0) and 17 g/L agar) in an incubator at 20 °C with the OP50 strain of *Escherichia coli*. All experiments were performed during the daytime at 20 °C to prevent the influence of circadian rhythm (Saigusa et al., 2002).

For the assays we used synchronously-staged nematodes of various developmental stages. To obtain them, thirty gravid nematodes were moved to a fresh NGM plate, allowing the animals to lay eggs for 3 h, then removed from the plate (Matsuura et al., 2004; Shingai et al., 2005). Identification of each larval stage was determined by period after hatching, length (size) and characteristics of the body. In our observation, N2 animals reached second larval (L2), third larval (L3) and forth larval (L4) stages approximately 20 h, 30 h and 40 h after hatching at 20 °C, respectively. However, these timings were postponed by an environmental condition. Therefore, the identification of larval stage was mainly determined based on length (size) and characteristics of the body (Sulston and Horvitz, 1977). For instance, L4 stage animals have a crescent-shaped structure around the vulva, but this structure disappeared for YA. N2 animals develop into YA hermaphrodites approximately 75 h after removal of gravid nematodes at 20 °C (Matsuura et al., 2004; Shingai et al., 2005). The developmental timing during larval stages of *daf-2* mutants was almost the same as that for N2 animals (Huang et al., 2004), and that of *mev-1* mutants was delayed approximately 1 day in comparison with that of the N2 and *daf-2* mutants.

The coding for the adult stages, e.g., A1, A2 and A14, indicates the period of development after becoming YA hermaphrodites. For example, A1 and A7 refer to nematodes 24 h and 7 days after reaching the YA stage, respectively. After reaching the YA stages, nematodes were moved to a fresh NGM plate every day to prevent the lack of food *E. coli* and contamination of other stages nematodes.

2.2. Chemotaxis assays using a single attractant (study 1)

2.2.1. The relationship between chemotaxis and spontaneous locomotion

We used 9-cm-diameter tissue culture dishes for chemotaxis assays containing 1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0) and 17 g/L agar, omitting NaCl. In our previous study, animals at the YA stage showed the highest level of attraction to 1.0 M sodium acetate (CH_3COONa) and 0.1% diacetyl (Matsuura et al., 2004). Therefore, we used the same concentrations of these attractants for the present investigation.

Sodium acetate (7 µL) was placed onto the surface of an assay plate (test location; location A in Fig. 1A) for 15–18 h and again 3 h before the start of the experiment in order to obtain a concentration gradient. The assay plate was then placed in an incubator at 20 °C. To anesthetize the animals, 1 µL of 0.5 sodium azide was spotted at the same location as that of sodium acetate shortly before the experiment. As a control, 1 µL of 0.5 M sodium azide was also spotted 4 cm away from the test location (control location; location B in Fig. 1A). For the assay of diacetyl, 1.5 µL of diacetyl dissolved in 99.5% ethanol, and 1 µL of 0.5 M sodium azide, were spotted at the test location (location A in Fig. 1A) shortly before the experiment. As a control, 1.5 µL of 99.5% ethanol and 1 µL of 0.5 M sodium azide were spotted at the control location (location B in Fig. 1A).

All nematodes were washed three times with buffer (1 mM $CaCl_2$, 1 mM $MgSO_4$, 5 mM KH_2PO_4 (pH 6.0), and 0.05% Tween 20) prior to use after removal from NGM plates containing bacteria as food (Matsuura et al., 2004, 2005). To avoid an effect of population density on chemotactic behavior (Matsuura et al., 2005), approximately 30 nematodes were placed at two original locations (C and D), equidistant (2.8 cm) from the test and control spots, using a micropipette (Fig. 1A).

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