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·Original Article·

Modulation of the assay system for the sensory integration of 2 sensory stimuli that inhibit each other in nematode *Caenorhabditis elegans*

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Abstract: **Objective** To perform the modulation of an assay system for the sensory integration of 2 sensory stimuli that inhibit each other. **Methods** The assay system for assessing the integrative response to 2 reciprocally-inhibitory sensory stimuli was modulated by changing the metal ion barrier. Moreover, the *hen-1*, *ttx-3* and *casy-1* mutants having known defects in integrative response were used to evaluate the modulated assay systems. Based on the examined assay systems, new genes possibly involved in the sensory integration control were identified. **Results** In the presence of different metal ion barriers and diacetyl, locomotion behaviors, basic movements, pan-neuronal, cholinergic and GABAergic neuronal GFP expressions, neuronal development, structures of sensory neurons and interneurons, and stress response of nematodes in different regions of examined assay systems were normal, and chemotaxis toward different concentrations of diacetyl and avoidance of different concentrations of metal ions were inhibited. In the first group, most of the nematodes moved to diacetyl by crossing the barrier of Fe^{2+}, Zn^{2+}, or Mn^{2+}. In the second group, almost half of the nematodes moved to diacetyl by crossing the barrier of Ag^{+}, Cu^{2+}, Cr^{2+}, or Cd^{2+}. In the third group, only a small number of nematodes moved to diacetyl by crossing the barrier of Pb^{2+} or Hg^{2+}. Moreover, when nematodes encountered different metal ion barriers during migration toward diacetyl, the percentage of nematodes moving back and then turning and that of nematodes moving straight to diacetyl were very different. With the aid of examined assay systems, it was found that mutations of *fsn-1* that encodes a F-box protein, and its target *scd-2* that encodes a receptor tyrosine kinase, caused severe defects in integrative response, and the sensory integration defects of *fsn-1* mutants were obviously inhibited by *scd-2* mutation. **Conclusion** Based on the nematode behaviors in examined assay systems, 3 groups of assay systems were obtained. The first group may be helpful in evaluating or identifying the very subtle deficits in sensory integration, and the third group may be useful for the final confirmation of sensory integration defects of mutants identified in the first or the second group of assay systems. Furthermore, the important association of sensory integration regulation with stabilization or destabilization of synaptic differentiation may exist in *C. elegans*.

Keywords: sensory integration; paired stimuli; assay system; metal ion barrier; *C. elegans*

1 Introduction

Multi-sensory integration, one of the steps of information processing, is essential for the discrimination of different environmental stimuli and generation of behavioral

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responses in animals. Besides in primates and human beings, multi-sensory integration is also observed in the central nervous system of invertebrates despite their simple nervous systems[1-4]. Nematode *Caenorhabditis elegans* (*C. elegans*) is a widely used invertebrate animal model, having a simple and well-described nervous system with 302 neurons in adult hermaphrodites[5]. The functions of its sensory neurons and the molecular mechanisms underlying sensory transduction have also been well elucidated[6,7]. *C. elegans* has a sensory organ called amphid containing 12 left-right homologous pairs of sensory neurons, which are involved in generating behaviors such as thermotaxis toward cultivation temperature and chemotaxis toward chemical stimuli[8,9]. The arrangement of known sensory neurons and the measurable behavioral outputs make *C. elegans* a suitable tool for investigating multi-sensory integration in the nervous system[10,11].

For *C. elegans*, several assay systems have already been established to study the mechanism of sensory integration, by examining the chemotaxis behavior toward concentration gradients of 2 distinct chemosensory stimuli[12-14]. For example, Mutsuura *et al.* have established an assay system to investigate the possible integration of multiple attractant stimuli, such as sodium acetate and diacetyl[13]. Ishihara *et al.* have established an assay system with 2 sensory stimuli that inhibit each other, in which animals have to cross a Cu^{2+} barrier to reach an attractive odorant, diacetyl[12]. Recently, Adachi *et al.* have further investigated the phase-dependent preference for thermosensation and chemosensation during simultaneous presentation assay, and found that *C. elegans* worms prefer the temperature at first, and the chemoattractant sodium chloride thereafter[11]. By using the assay system that examines the integrative response to 2 reciprocally-inhibitory sensory stimuli as mentioned above, Ishihara *et al.* identified that helix-loop-helix protein 1 (HEN-1), a secretory protein with an LDL receptor motif, and abnormal Thermo Taxis family member (TTX-3), a LIM-type transcriptional factor, regulate the sensory integration of nematodes[12]. Besides, mutations of *casy-1* gene that encodes an ortholog of calsyntenins/alcadeins, and *nlg-1* gene that encodes a postsynaptic cell adhesion protein Neuroligin, can also cause deficits in integrative response to 2 sensory signals[15,16].

Currently, *C. elegans* has served as an important animal model in the field of neurotoxicological research, including neurotoxicity assessment for various metals[17,18]. Exposure to high concentrations of heavy metals has been shown to cause noticeable axonal degeneration and neuronal loss in nerve cords, together with significant reductions in the relative size and fluorescent intensity of AVL, RMEs, and RIS neurons, suggesting that neurodegeneration and structure abnormality may occur in GABAergic motor neurons after heavy metal exposure[19,20]. Exposure to high concentrations of metal ions also induces severe deficits in chemotaxis and thermotaxis, probably through altering the morphology or development of chemosensory and thermosensory neurons and damaging the molecular basis of functioning of chemosensory and thermosensory neurons in nematodes[21,22]. Moreover, exposure to metal ions at relatively high concentrations can result in severe deficits in associative learning and memory behaviors[23-25]. Among various metal ions examined, exposure to lead (Pb^{2+}) and mercury (Hg^{2+}) ions usually causes more severe neurotoxic effects than exposure to other metal ions does[20,25].

In the assay system that examines the sensory interaction of 2 reciprocally-inhibitory sensory stimuli, Cu^{2+} serves as an aversive stimulus[12]. Due to the variations in the neurotoxicity of different metal ions and in the extent to which nematodes take aversive action in the presence of different metal ion barriers, we assume that the sensory interaction assay systems with different characteristics can be obtained through replacing the Cu^{2+} ion barrier by other types of metal ion barriers. Thus, in the present study, the assay system with 2 sensory stimuli that inhibited each other was modified by changing the metal ion barrier. The modified assay system was further used for screening of genes involved in sensory integration.

2 Materials and methods

2.1 Chemicals Ferric sulfate, zinc sulfate, silver nitrate, copper acetate, cadmium chloride, chromium chloride, lead

nitrate, mercury chloride, and manganese chloride were all purchased from Sigma-Aldrich (St. Louis, MO, USA) and served as metal ion barriers.

2.2 Preparation of nematode cultures Nematodes used in the present study included wild-type Bristol (N2), JC2154 [*hen-1(tm501)*], FK134 [*ttx-3(ks5)*], RB888 [*casy-1(ok739*)], RB783 [*scd-2(ok565)*], VC980 [*fsn-1(gk429)*], *fsn-1(gk429);scd-2(ok565)*, NW1229 [*F25B3.3::GFP*] (a maker of pan-neuronal GFP expression)[26], LX929 [*vsIs48*[*Punc-17::GFP*]] (a maker of a synaptic vesicle acetylcholine transporter to label cholinergic neurons)[27], EG1285 [*oxIs12* [*unc-47::GFP*]] (a marker of vesicular GABA transporter that labels GABAergic neurons)[28], ST66 [*ncIs17* [*hsp-16.2::eGFP*]], DA1288 [*gcy-7::GFP*] (a marker that labels ASE sensory neurons)[29], CX3260 [*kyIs37* [*odr-10::GFP*]] (a marker that labels AWA sensory neurons)[30], HA3 [*nuIs11* [*osm-10::GFP*]] (a marker that labels ASH sensory neurons)[31], OH3701 [*otIs173* [*ttx-3::GFP*]] (a marker that labels AIY interneurons)[32], VM182 [*akEx51* [*glr-2::GFP*]] (a marker that labels AIA interneurons)[33], and BC12233 [*sEx12233*[*C13G5.1::GFP*]] (a marker that labels AIZ interneurons)[34]. All the single mutants used in the present study had a loss-of-function mutation. Nematodes were maintained on nematode growth medium (NGM) plates seeded with *E. coli* OP50 at 20 ºC as described previously[35]. Gravid nematodes were washed off the plates into centrifuge tubes, and were lysed with a bleaching mixture (containing 0.45 mol/L NaOH and 2% HOCl). Age-synchronous populations of young adult nematodes were obtained as reported previously[36].

2.3 Behavioral assays Locomotion behaviors were assessed as previously described[18]. Briefly, the examined nematodes were picked onto a second plate and allowed 1 min for recovery. Head thrash, which was defined as a change in the direction of bending at the mid body, was counted for 1 min. Body bend was counted for 20 s, and a body bend was defined as a change in the direction of the part of the nematodes corresponding to the posterior bulb of the pharynx along the y axis, assuming that the nematode was traveling along the x axis. Two basic movements, forward sinusoidal movement (forward turns) and reversal movement (backward turns) with a 30-s interval were also observed. Fifty nematodes were examined in each assay system, and 3 independent experiments were performed.

To evaluate the aversive responses of nematodes to different metal ion barriers, a specific metal ion barrier was first prepared in the middle of a 9-cm diameter assay plate, and then nematodes were placed 3.5-cm away from the metal ion barrier on one side (Fig. 1A). After 90 min, the animals on the original side and on the opposite side were counted, respectively. The aversive responses of nematodes to different metal ion barriers were evaluated by percentage of the number of worms on the opposite site to the total number of worms on both sides.

Avoidance of metal ion can also be analyzed by the quadrant assay as described previously[37]. The assay agar plate was partitioned into 4 parts, of which 2 parts contained metal ion. Whether nematodes would move into the metal-containing parts was tested. Chemotaxis towards the volatile chemoattractant diacetyl was assayed as described[38,39]. Before the washed nematodes were placed onto the assay plate, about 1 μL diluted diacetyl was spotted directly onto the surface of the assay plate, together with sodium azide to anaesthetize the nematodes. As a control, sodium azide was also spotted at a position 4 cm away from the center of the diacetyl gradient. Approximately 100 nematodes were placed equidistant from these 2 spots. Temperature was maintained at 20 °C. After 30 min, nematodes around each spot were counted. The chemotaxis index (CI) was calculated: CI = $(N_+ - N_-)$/(total number of nematodes on the plate), where N_+ indicated the number of nematodes within 1.5 cm of the center of the diacetyl gradient and N_- indicated the number of nematodes within 1.5 cm of the control spot. Three independent experiments were performed.

2.4 Assessment of the integrative response to olfactory (diacetyl) and gustatory (metal ion) stimuli Briefly, 25 μL ferric sulfate, zinc sulfate, silver nitrate, copper acetate, cadmium chloride, chromium chloride, lead nitrate, mercury chloride, or manganese chloride solution was spread on the middle of a 9-cm diameter assay plate [10 mmol/L HEPES

(pH 7.0), 1 mmol/L $MgSO_4$, 1 mmol/L $CaCl_2$, 50 mmol/L NaCl, 2% agar] [12]. After 18 h, 2 μL diluted diacetyl was spotted on one side of the plate divided by the metal ion barrier, and then approximately 50 nematodes were placed 3.5-cm away from the metal ion barrier on another side of the plate. After 90 min, the nematodes on each side were counted. The index was calculated as the percentage of the number of nematodes on the odorant side to the total number of animals. The behavioral changes after starvation was analyzed on young adults starved on NGM plates without bacteria for 5 h. At least 6 independent experiments were performed.

2.5 Analysis of GABAergic neuronal loss Neuronal loss was analyzed as previously described[40]. The GABA motor neurons were visualized by *oxIs12* maker[28]. The numbers of ventral and dorsal cord gaps were quantified to reflect the axonal degeneration. In addition, neuronal loss was evaluated by comparing the number of cell bodies of motor neurons in the nervous system of nematodes. The images were photographed and examined on the same day to avoid the effects of light source variance on fluorescence intensity.

2.6 Analysis of stress response The stress response was analyzed basically as previously described[41]. The expression of heat shock protein 16 (HSP-16), a classical stress protein, can be induced by an array of environmental stressors including heavy metal ion exposure, heat shock, and oxidative stress[42]. Here the transgenic line of *ncIs17* (*hsp-16.2::eGFP*) was employed to investigate the possible induction of stress responses, because it was reasoned that the stress response can be indicated by HSP-16 expression[42]. To analyze the change patterns of *hsp-16.2* expression, the examined transgenic nematodes were allowed to settle for 10 min, and then pipetted onto an agar pad on a glass slide, mounted and observed for the fluorescent signals with a fluorescent microscope. Fluorescence was recorded and color images were captured with Magnafire® software (Olympus, Irving, TX, USA). The fluorescence levels were measured using ImageJ Software (NIH Image) by determining the average pixel intensity in the intestine of each animal.

2.7 Genetics Double mutant construct *fsn-1(gk429);scd-2(ok565)* was carried out using standard genetic protocols. The homozygous double mutant nematodes were identified by PCR screen for F3 progeny of *fsn-1* and *scd-2* mutants.

2.8 Statistical analysis All data were expressed as means±SEM and analyzed with one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. $P < 0.05$ was indicated as statistically significant. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA, USA).

3 Results

3.1 Evaluation of nematode aversive responses to different metal ion barriers The aversive abilities of wild-type N2 nematodes to different barriers were first evaluated. Nine kinds of metal ions were selected, including Fe^{2+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Cd^{2+}, Cr^{2+}, Pb^{2+}, Hg^{2+}, and Mn^{2+}, at concentrations of 0.1, 1, 10, and 100 mmol/L. As shown in Fig. 1B, wild-type N2 nematodes exhibited the most severe aversive responses to Pb^{2+} and Hg^{2+} barriers. In addition, wild-type N2 nematodes had less severe aversive responses to Ag^{+}, Cu^{2+}, Cd^{2+}, and Cr^{2+} barriers. In contrast, wild-type N2 nematodes exhibited only moderate aversive responses to Fe^{2+}, Zn^{2+} and Mn^{2+} barriers. Therefore, these 9 metal ions can be divided into 3 types according to the different aversive responses of nematodes.

3.2 Modulated assay system for assessing the integrative response to 2 sensory signals Besides the aversive responses to different heavy metal ions, *C. elegans* also shows chemotaxis toward odorants like diacetyl[38]. In the present modulated assay system, nematodes would have to cross a specific metal ion barrier to reach an attractant odorant, diacetyl (Fig. 2A). In the presence of 100 mmol/L metal ion barrier and 1×10^{-2} diacetyl dilution, nematodes exhibited 3 types of performance (Fig. 2B). For the first type, approximately 72.3%, 75.6%, and 75.4% nematodes reached the attractant odorant by crossing the barriers of Fe^{2+}, Zn^{2+}, and Mn^{2+}, respectively. For the second type, approximately 44.5%, 53.4%, 55% and 51.2% nematodes reached the attractant diacetyl by crossing the barriers of

Cu^{2+}, Cd^{2+}, Cr^{2+}, and Ag^{+}, respectively. For the third type, only approximately 28.4% and 28.6% nematodes reached the attractant odorant by crossing the barriers of Hg^{2+} and Pb^{2+}, respectively.

Moreover, these assay systems were further modulated to evaluate the responses to various concentrations of diacetyl and metal ion quantitatively (Fig. 2C,D). As observed previously[12], in the absence of metal ion barrier, the sensory integration index increased depending on the diacetyl concentration. In contrast, in the presence of Fe^{2+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Cd^{2+}, Cr^{2+}, Pb^{2+}, Hg^{2+}, or Mn^{2+} ion barrier, chemotaxis toward different concentrations of diacetyl was noticeably inhibited, as compared with that in the absence of metal ion barrier. Besides, the chemotaxis showed a tendency of enhancement with the increase in diacetyl concentration (Fig. 2C). Furthermore, in the absence of diacetyl, the sensory integration index decreased, as compared with that in the presence of diacetyl. It also showed a tendency of decrease with the increase in the metal ion concentration (Fig. 2D). In contrast, in the presence of diacetyl, the avoidance of different concentrations of different metal ions was obviously suppressed (Fig. 2D). Therefore, these assay systems could be explored to investigate the integrative response to 2 sensory signals, since the observed sensory integration behaviors in these assay systems depended on the concentrations of both diacetyl and metal ions.

3.3 Formation of different sensory integration performance is closely associated with the altered behavioral performance in the presence of different metal ion barriers To further examine the possible factors influencing the formation of different sensory integration performance of nematodes in assay systems with different metal ion barriers, the locomotion behaviors of nematodes were investigated in different assay systems. As shown in Fig. 3, head thrash and body bends of nematodes were analyzed in different regions (region A, the diacetyl side; region B, the original side), and no obvious defects in head thrash or body bend were observed in nematodes in different regions of the assay system. This suggested that the observed different performance of nematodes in assay systems with different metal ion barriers was not due to the alteration of locomotion behaviors of nematodes.

Moreover, basic movements of nematodes in different regions of examined assay systems were examined (Fig. 3). Similarly, no obvious defects in forward turn or backward turn were observed, suggesting that the observed differences in nematode performance were also not due to the

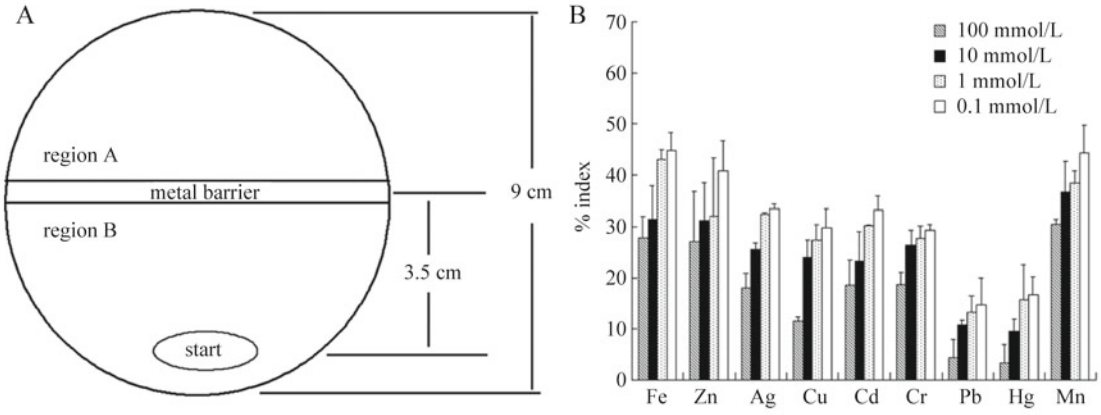

Fig. 1 Evaluation of aversive responses of wide-type nematodes to different metal ion barriers. A: To evaluate the aversive response of nematodes to metal ion barrier, a specific metal ion barrier was first prepared in the middle of a 9-cm diameter assay plate, and then nematodes were placed 3.5-cm away from the metal ion barrier on one side. The numbers of animals on the original side (B) and the opposite side (A) were scored. The index was calculated as A / (A+B) × 100%. B: Different aversive responses of nematodes to different metal ion barriers. Wild-type N2 nematodes had the most severe aversive responses to Pb^{2+} and Hg^{2+} barriers, strong aversive responses to Ag^{+}, Cu^{2+}, Cd^{2+} and Cr^{2+} barriers, and only moderate aversive responses to Fe^{2+}, Zn^{2+} and Mn^{2+} barriers.

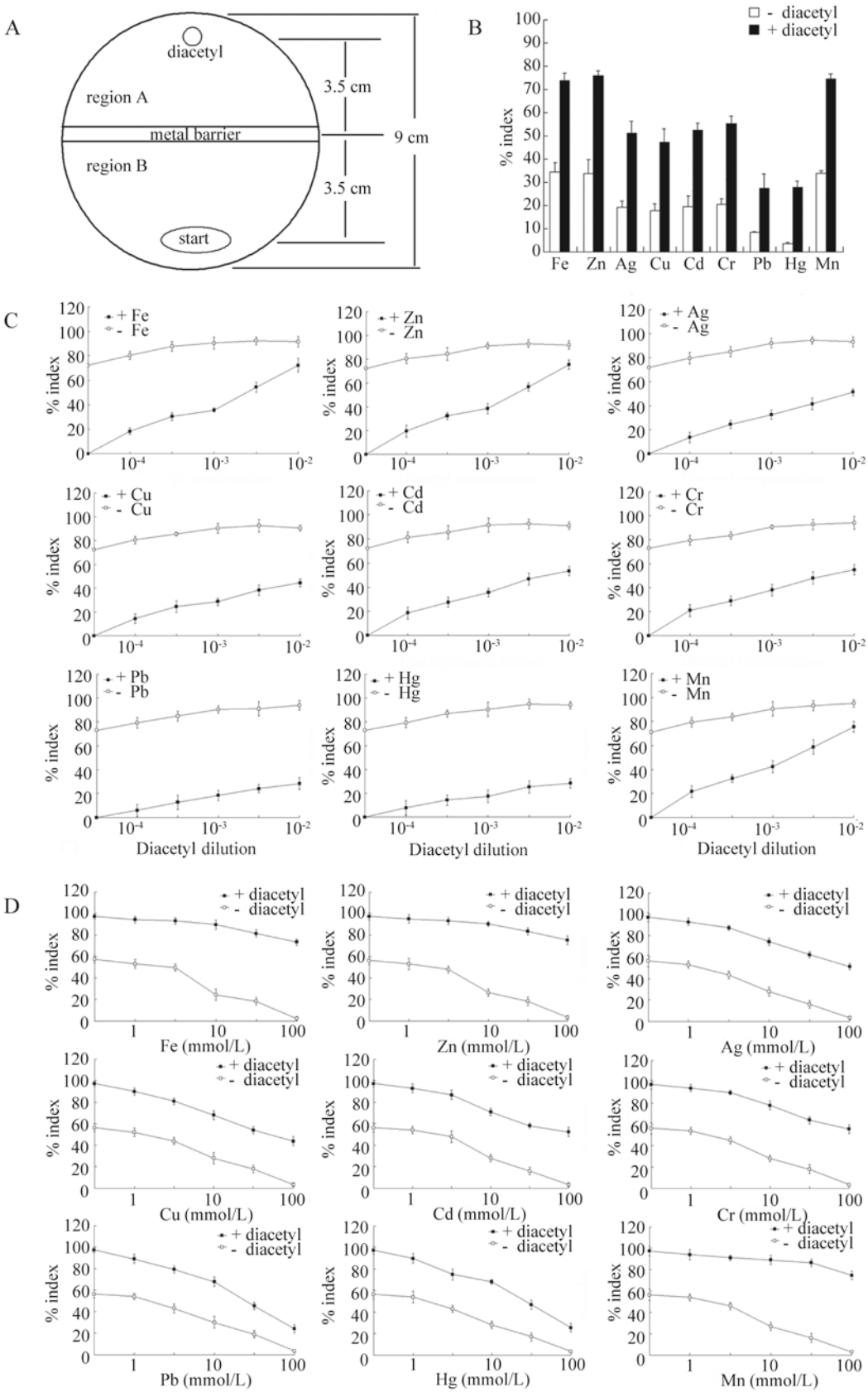
A
diacetyl
region A
metal barrier
region B
start
3.5 cm
9 cm
3.5 cm
B
- diacetyl
+ diacetyl
% index
Fe Zn Ag Cu Cd Cr Pb Hg Mn
C
+ Fe
- Fe
+ Zn
- Zn
+ Ag
- Ag
+ Cu
- Cu
+ Cd
- Cd
+ Cr
- Cr
+ Pb
- Pb
+ Hg
- Hg
+ Mn
- Mn
% index
Diacetyl dilution
D
+ diacetyl
- diacetyl
% index
Fe (mmol/L)
Zn (mmol/L)
Ag (mmol/L)
Cu (mmol/L)
Cd (mmol/L)
Cr (mmol/L)
Pb (mmol/L)
Hg (mmol/L)
Mn (mmol/L)

Fig. 2 Modulated assay systems for assessing the integrative responses to 2 sensory signals. A: A schematic of the assay system. A specific metal ion barrier was first prepared in the middle of a 9-cm diameter assay plate. After 18 h, diacetyl was spotted on one side of the plate, and then nematodes were placed 3.5-cm away from the metal ion barrier on another side of the plate. The numbers of animals on the original side (B) and the odorant side (A) were scored. The index was calculated as A / (A+B) × 100%. B: Sensory integration assay of wild-type N2 nematodes in assay systems with different metal ion barriers (100 mmol/L), in the presence or absence of diacetyl. C: Response indices of wild-type N2 nematodes to diacetyl with (+) or without (-) 100 mmol/L metal ion. In the presence of the metal ion barrier, chemotaxis toward different concentrations of diacetyl was noticeably inhibited, as compared with that in the absence of metal ion barrier. Besides, the chemotaxis showed a tendency of enhancement with the increase in diacetyl concentration. D: Response indices of wild-type N2 nematodes to metal ion with (+) or without (-) diluted diacetyl. In the absence of diacetyl, the response index decreased, as compared with that in the presence of diacetyl. It also showed a tendency of decrease with the increase in the metal ion concentration.

alteration of basic movements of nematodes in different regions of examined assay systems.

Again, the neuronal development of nematodes in different regions of examined assay systems was examined. No abnormal morphological pan-neuronal GFP expression was observed in nematodes in different regions of examined assay systems (data not shown). Similarly, no abnormal morphological alteration in cholinergic or GABAergic neurons was detected in nematodes in different regions of examined assay systems, compared with control (data not shown). Strains of NW1229, LX929, and EG1285 showed similar sensory integration behaviors as wild-type N2 nematodes in examined assay systems (data not shown). Moreover, there was no noticeable increase in neuronal loss or in dorsal and ventral cord gaps, of GABAergic motor neurons in nematodes in different regions of examined as-

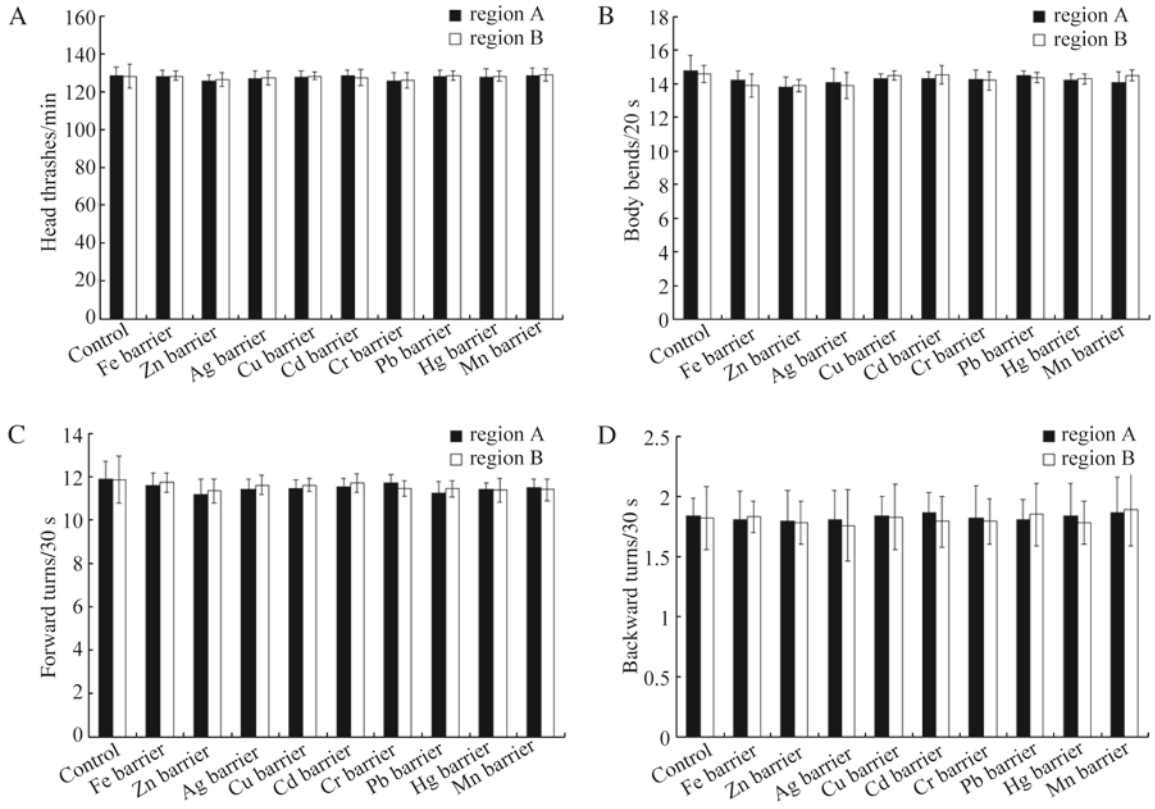

Fig. 3 Locomotion behaviors and basic movements of nematodes on the original side (region B) and on the opposite side (region A) of the sensory integration assay system. A: Head thrashes of nematodes in different regions of sensory integration assay systems. B: Body bends of nematodes in different regions of sensory integration assay systems. C: Forward turns of nematodes in different regions of sensory integration assay systems. D: Backward turns of nematodes in different regions of sensory integration assay systems.

say systems, compared with control (Fig. 4A–C). The patterns of series of sensory neurons (ASE, AWA, and ASH) and interneurons (AIY, AIA, and AIZ) were observed based on the putative neuronal circuit of the integrative response to 2 sensory signals which was raised by Ishihara *et al.*[12], and no noticeable morphological alterations of these sensory neurons and interneurons were detected in nematodes in different regions of examined assay systems, as compared with the control (data not shown). Strains of DA1288, CX3260, HA3, OH3701, VM182, and BC12233 also showed similar interaction behaviors as wild-type N2 nematodes in the examined assay systems (data not shown).

One possible explanation for the altered integration of sensory signals in different assay systems is the induction of stress response. Thus, the stress responses of nematodes in different regions of examined assay systems were further examined, and no obvious induction of stress responses was observed in nematodes in different regions of examined assay systems compared with control (Fig. 4D).

Furthermore, the behavioral performance of nematodes in different assay systems was investigated (Fig. 5). When encountering 100 mmol/L Fe^{2+}, Zn^{2+}, Ag^{+}, Cu^{2+}, Cd^{2+}, Cr^{2+}, Pb^{2+}, Hg^{2+}, or Mn^{2+} during migration toward 1×10^{-2} diacetyl dilution, approximately 75.6%, 77.4%, 89.2%, 90.3%, 89.3%, 88%, 96.7%, 97.2%, and 78% nematodes moved back and then turned, respectively, and approximately 16.5%, 14.8%, 5.3%, 4.7%, 6.4%, 6.7%, 1.2%, 1.4%, and 15.4% nematodes moved straight toward the odorant, respectively. Therefore, the altered behavioral performance of nematodes in assay systems may be closely associated with the formation of different sensory integration in the presence of different metal ion barriers.

3.4 Sensory integration phenotypes of *hen-1*, *ttx-3* and *casy-1* mutants in the examined assay systems Previous studies have demonstrated that mutations of *hen-1*, *ttx-3* and *casy-1* may result in deficits in sensory integration[12,15].

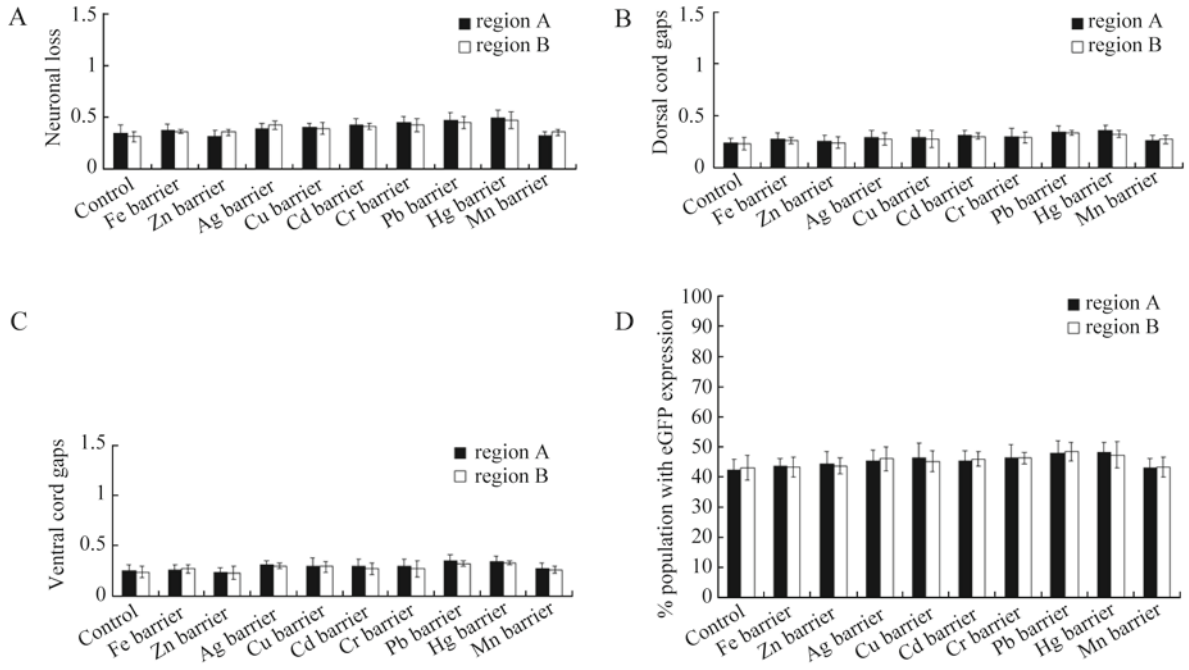

Fig. 4 Neuronal loss and stress response of nematodes in different regions of sensory integration assay systems. A: Neuronal loss of GABAergic motor neurons of nematodes in different regions of sensory integration assay systems. The strain of *oxIs12* [*Punc-47::GFP; lin-15(+)*] was used to visualize GABAergic motor neurons. B: Dorsal cord gaps of GABAergic motor neurons of nematodes in different regions of sensory integration assay systems. C: Ventral cord gaps of GABAergic motor neurons of nematodes in different regions of sensory integration assay systems. D: Stress response of neurons in nematodes in different regions of sensory integration assay systems. Transgenic line of *ncIs17* (*hsp-16.2::eGFP*) was used to investigate the possible induction of stress response.

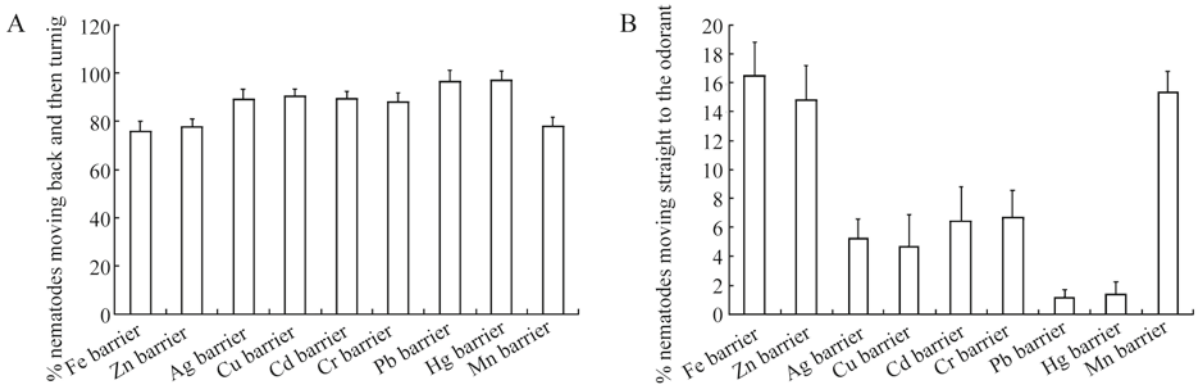

Fig. 5 Behavioral performance of nematodes in sensory integration assay systems. A: The percentage of nematodes that moved back and then turned in different sensory integration assay systems. B: The percentage of nematodes that moved straight to the odorant in different sensory integration assay systems.

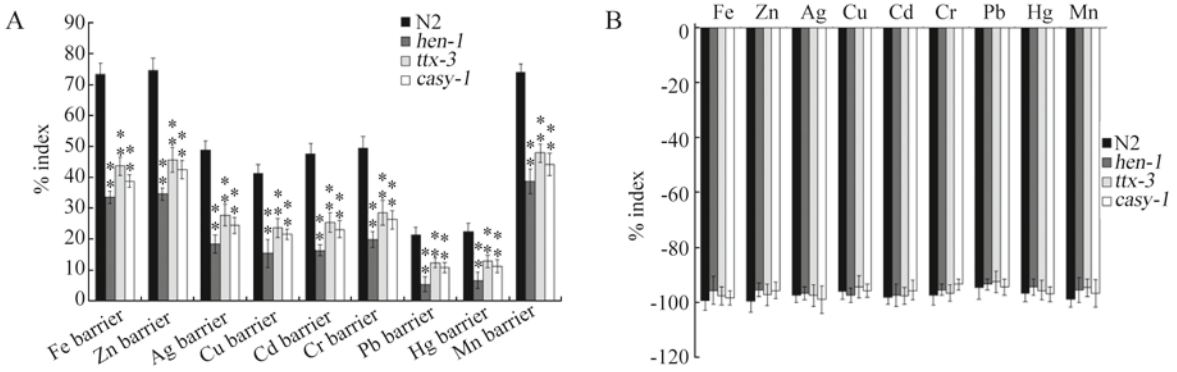

Fig. 6 Sensory integration analysis of *hen-1*, *ttx-3* and *casy-1* mutants in assay systems with various metal ion barriers. A: Sensory integration phenotypes of *hen-1*, *ttx-3* and *casy-1* mutants in assay systems. All the 3 mutants showed deficits in the sensory integration phenotypes in the assay systems with different metal ion barriers, compared with wild-type N2. The integrative response indices of *hen-1*, *ttx-3*, and *casy-1* mutants in the presence of Fe^{2+}, Zn^{2+}, or Mn^{2+} ion barrier were higher than those in the presence of Ag^{+}, Cu^{2+}, Cd^{2+}, or Cr^{2+} barrier. B: The quadrant assay revealed no obvious deficits in avoidance of different metal ions (100 mmol/L) in *hen-1*, *ttx-3* and *casy-1* mutants, as compared with the wild-type N2. $^{**}P < 0.01$ *vs* wild-type N2.

In the present study, the sensory integration phenotypes of these mutants in examined assay systems were further investigated. As shown in Fig. 6, all the 3 mutants showed deficits in the sensory integration phenotypes in the assay systems with different metal ion barriers, compared with wild-type N2 ($P < 0.01$). Nevertheless, the integrative response indices of *hen-1*, *ttx-3*, and *casy-1* mutant nematodes in the presence of Fe^{2+}, Zn^{2+}, or Mn^{2+} ion barrier were higher than those in the presence of Ag^{+}, Cu^{2+}, Cd^{2+}, or Cr^{2+} barrier. Moreover, the integrative response indices of *hen-1*, *ttx-3*, and *casy-1* mutant nematodes in the presence of Pb^{2+} or Hg^{2+} barrier were obviously lower than those in the presence of Ag^{+}, Cu^{2+}, Cd^{2+}, or Cr^{2+} ion barrier. Besides, the quadrant assay on these examined mutants revealed no obvious deficits in avoidance of different metal ions.

3.5 A F-box protein FSN-1 and its target SCD-2 regulate the sensory integration in *C. elegans* The assay systems with Fe^{2+}, Cu^{2+}, or Hg^{2+} ion barrier, which represented the 3 different sensory integration assay systems, were

further selected for screening the genes possibly involved in sensory integration. Examination of a series of mutants revealed that mutations of *fsn-1* and its target gene *scd-2*, which encode a F-box protein and a receptor tyrosine kinase, respectively, resulted in severe deficits in sensory integration. In the assay system with Fe^{2+}, Cu^{2+}, or Hg^{2+} ion barrier, both the *fsn-1* and the *scd-2* mutants showed deficits in sensory integration (Fig. 7A). Interestingly, in the

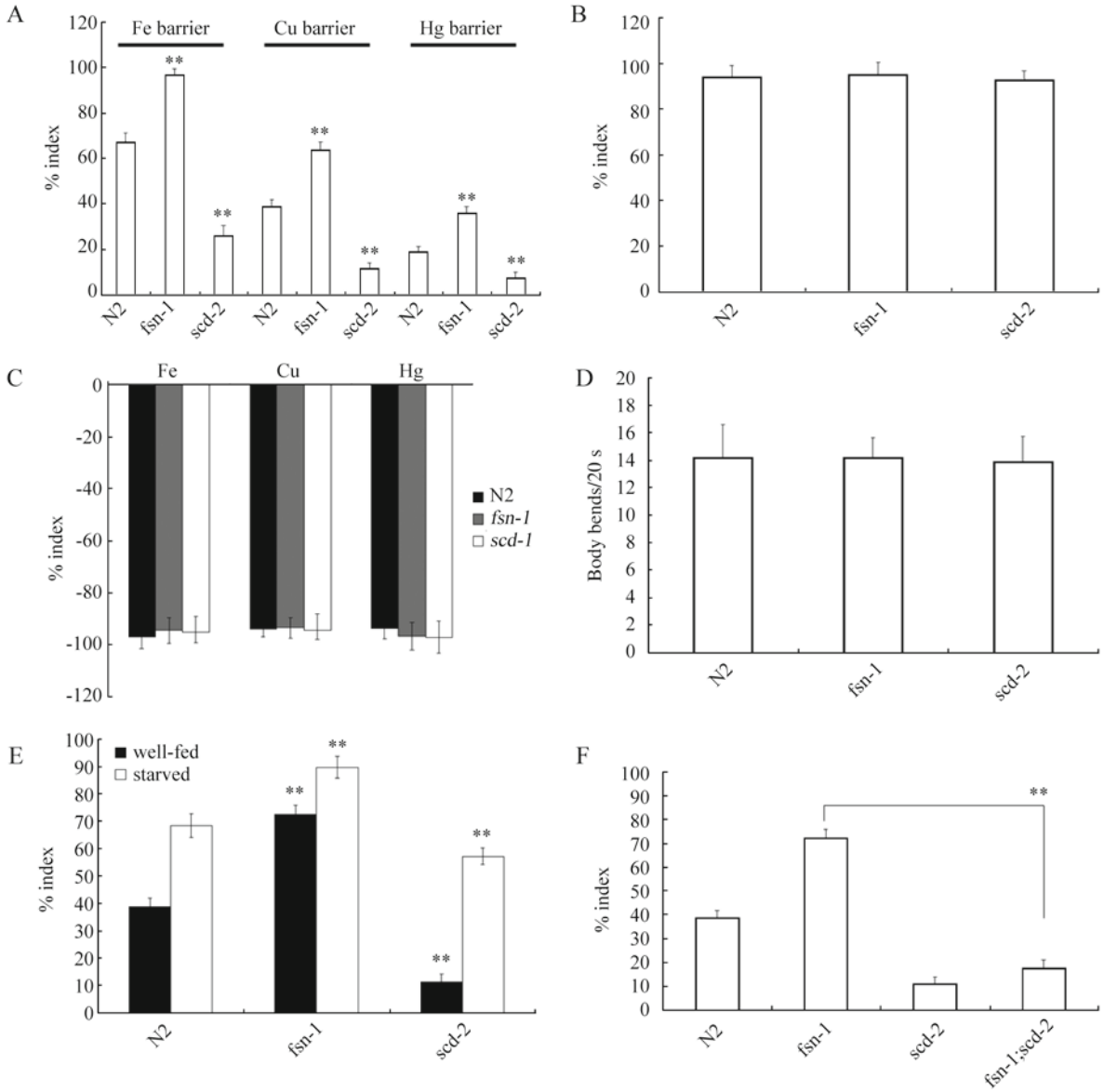

Fig. 7 Mutations of *fsn-1* and *scd-2* result in deficits in sensory integration. A: Sensory integration phenotypes of *fsn-1* and *scd-2* mutants. In the systems with Fe^{2+}, Cu^{2+}, or Hg^{2+} ion barrier, the integrative response index of *fsn-1* mutant was much higher than that of wild-type N2, while the integrative response index of *scd-2* mutant was much lower than that of wild-type N2. B: There was no alteration of chemotaxis toward diacetyl in *fsn-1* or *scd-2* mutant, as compared with wild-type N2. C: The quadrant assay revealed that there was no alteration of avoidance of Fe^{2+}, Cu^{2+} or Hg^{2+} ion barrier (100 mmol/L) in *fsn-1* or *scd-2* mutants, as compared with wild-type N2. D: Wild-type N2, *fsn-1* and *scd-2* mutants showed similar locomotion behaviors. E: Sensory integration assay of wild-type N2, *fsn-1* and *scd-2* mutants in well-fed or starved condition. F: Genetic interaction of *fsn-1* with *scd-2* in regulating the sensory integration. The sensory integration phenotype of *fsn-1* mutants could be suppressed by the mutation of *scd-2* gene. $^{**}P < 0.01$.

systems with Fe^{2+}, Cu^{2+}, or Hg^{2+} ion barrier, the integrative response index of *fsn-1* mutant was much higher than that of wild-type N2, while the integrative response index of *scd-2* mutant was much lower than that of wild-type N2 (Fig. 7A). Besides, mutation of *fsn-1* or *scd-2* did not induce obvious alteration of chemotaxis toward diacetyl or avoidance of Fe^{2+}, Cu^{2+}, or Hg^{2+} ion barrier (Fig. 7B, C). It has been indicated that *fsn-1* mutants have a slightly reduced body length and normal locomotion[43]. Here it was further demonstrated that both *fsn-1* and *scd-2* mutants showed a normal phenotype of locomotion behavior as revealed by body bend (Fig. 7D). In addition, like wild-type N2 nematodes, both *fsn-1* and *scd-2* mutants changed their sensory integration behaviors after starvation (Fig. 7E), suggesting that the phenotypes of *fsn-1* and *scd-2* mutants are not due to the insensitivity to starvation.

Moreover, the possible genetic interaction of *fsn-1* with *scd-2* in regulating the sensory integration was investigated (Fig. 7F). Results showed that the sensory integration phenotype of *fsn-1* mutants could be suppressed by the mutation of *scd-2* gene.

4 Discussion

In the present study, we tried to perform the modulation of sensory integration assay system based on the assay system established by Ishihara *et al.*[12], by changing the metal ion barrier[12]. Our data suggest that the assay system with selected metal ion barrier could be used to investigate the capacity of sensory integration of nematodes. Several lines of evidence have been raised to support the usefulness of these assay systems. First, the locomotion behaviors and basic movements of nematodes in different regions of assay systems were normal. Second, there was no obvious abnormality in morphological pan-neuronal, cholinergic or GABAergic neuronal GFP expression in nematodes in different regions of examined interaction assay systems. Third, no noticeable deficits in neuronal development were found in nematodes in different regions of assay systems. Fourth, there were no noticeable structural alterations of a series of sensory neurons or interneurons in nematodes in different regions of assay systems. Besides the ASE, AWA, and ASH sensory neurons and AIY, AIA, and AIZ interneurons, other sensory neurons such as ASI, ADF, ASG, ASJ, AWB, AWC, and AFD also displayed normal morphological patterns (unpublished data). Finally, we did not detect obvious stress responses in nematodes located at different regions of assay systems. More importantly, we observed that the chemotaxis toward different concentrations of diacetyl was inhibited in the presence of different metal ion barriers, while the avoidance of different concentrations of different metal ions was suppressed in the presence of diacetyl. These data suggest that besides Cu^{2+}, a series of other metal ions can also serve as metal ion barriers in the interaction assay system. In addition, our data further support the validity of the sensory integration assay system established by Ishihara *et al.*[12].

Moreover, we observed that the different sensory integration phenotypes of wild-type N2 nematodes in different assay systems may be largely associated with their behavioral performance in the presence of different metal ion barriers. When nematodes encountered different metal ion barriers during migration toward diacetyl, the percentage of nematodes that moved back and then turned and the percentage of nematodes moving straight to the odorant were very different. At least one possibility may help explain this finding, that is, wild-type N2 nematodes have different aversive abilities to different metal ion barriers, which is also consistent with the previous observations on toxicity of different metal ions on nematodes[19-22, 25].

Based on the sensory integration behaviors of nematodes in different assay systems with different metal ion barriers, these assay systems are summarized into 3 groups. In the first group, most of the examined wild-type N2 nematodes moved to diacetyl by crossing the barrier of Fe^{2+}, Zn^{2+}, or Mn^{2+}. In the second group, almost half of the examined wild-type N2 nematodes moved to diacetyl by crossing the barrier of Ag^{+}, Cu^{2+}, Cr^{2+}, or Cd^{2+}. In the third group, less wild-type N2 nematodes moved to diacetyl by crossing the barrier of Pb^{2+} or Hg^{2+}. The characteristics of the 3 groups of sensory integration assay systems largely correspond to the characteristics of 3 types of aversive abilities of nematodes to different metal ion barriers.

Among these 3 groups, the second group represents the most common assay system as described previously[12]. The first group may be helpful in evaluating or identifying very subtle deficits in sensory integration of examined mutants. In contrast, the third group may be useful for the final confirmation of sensory integration defects of mutants identified in the first or the second group of assay systems. Thus, the assay system for assessing the integrative response to paired stimuli described here and the system basically established by Ishihara *et al.*[12] will facilitate the identification of mutants defective in sensory integration[12]. By using the *hen-1*, *ttx-3* and *casy-1* mutants, we further demonstrated that *hen-1*, *ttx-3* and *casy-1* mutants exhibited more severe sensory integration defects in the first group of assay systems than in the second group. Similarly, these mutants exhibited more severe sensory integration defects in the second group of assay systems than in the third group.

Ishihara *et al.*[12] have proposed a putative neuronal circuit for the sensory integration control of nematodes, although in this neuronal circuit, only the function of AIY interneuron has been proven[12]. In such a neuronal circuit, the ADL, ASH, and ASE sensory neurons of nematodes are included because of their roles in chemotaxis or in avoidance of Cu^{2+} [12,44]. In the putative neuronal circuit for the integrative response to 2 sensory signals assayed in the present study, the sensory neuron for diacetyl is AWA[38], and the sensing of Cd^{2+} and Cu^{2+} by externally exposed ADL, ASE, and ASH sensory neurons elicits avoidance response in *C. elegans*[45]. Nevertheless, it still remains unclear which sensory neuron accounts for the avoidance response of nematodes to metal ions employed in the first and the third groups of assay systems. The integrative response to 2 sensory signals may be also regulated by amphid interneurons AIA, AIB, AIY, and AIZ[12]. Thus, further identification of neurons responsible for the avoidance response of nematodes to metal ions such as Fe^{2+}, Zn^{2+}, Mn^{2+}, Pb^{2+} and Hg^{2+} will be helpful in revealing the relatively complete neuronal circuit for the first and the third groups of sensory integration assay systems.

Furthermore, by using the assay systems with Fe^{2+}, Cu^{2+}, or Hg^{2+} barrier, we identified that the *fsn-1* and *scd-2* mutants showed severe sensory integration deficits. In *C. elegans*, FSN-1 is required for the restriction and/or maturation of synapses in presynaptic neurons, and SCD-2 may serve as a downstream target for FSN-1 to stabilize the synaptic formation[43]. The amount and localization of SCD-2 are negatively regulated by FSN-1, and mutation of *scd-2* suppresses the synaptic defects of *fsn-1*[43]. Interestingly, we found that the sensory integration defects of *fsn-1* mutants were obviously inhibited by *scd-2* mutation. Therefore, the possible association of sensory integration regulation with stabilization or destabilization of synaptic differentiation in *C. elegans* may be an important question, and solving this question needs further investigations.

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References:

[1] Joiner MA, Griffith LC. Visual input regulates circuit configuation in courtship conditioning of *Drosophila melanogaster.* Learn Memory 2000, 7: 32–42.

[2] Miller EK, Cohen JD. An integrative theory of prefrontal cortex function. Annu Rev Neurosci 2001, 24: 167–202.

[3] Koechlin E, Hyafil A. Anterior prefrontal function and the limits of human decision-making. Science 2007, 318: 594–598.

[4] Zhang K, Guo J, Peng Y, Xi W, Guo A. Dopamine-mushroom body circuit regulates saliency-based decision-making in *Drosophila.* Science 2007, 316: 1901–1904.

[5] White JG, Southgate E, Thomson JN, Brenner S. The structure of the nervous system of the nematode *Caenorhabditis elegans*. Philos Tran Royal Soc B: Biol Sci 1986, 314: 1–340.

[6] Bargmann CI. Genetic and cellular analysis of behavior in *C. elegans.* Annu Rev Neurosci 1993, 16: 47–71.

[7] Bargmann CI, Kaplan JM. Signal transduction in the *Caenorhabditis elegans* nervous system. Annu Rev Neurosci 1998, 21: 279–308.

[8] Bargmann CI, Mori I. Chemotaxis and thermotaxis. In *C. elegans* II. Edited by: Riddle DL, Blumenthal T, Meyer BJ, Priess JR. New York: Cold Spring Harbor Laboratory Press, 1997: 717–737.

[9] Mori I. Genetics of chemotaxis and thermotaxis in the nematode *Caenorhabditis elegans*. Annu Rev Genet 1999, 33: 399–422.

[10] Ye HY, Ye BP, Wang DY. Learning and learning choice in the nematode *Caenorhabditis elegans*. Neurosci Bull 2006, 22: 355–360.

[11] Adachi R, Osada H, Shingai R. Phase-dependent preference of thermosensation and chemosensation during simultaneous presentation assay in *Caenorhabditis elegans.* BMC Neurosci 2008, 9: 106.

[12] Ishihara T, Iino Y, Mohri A, Mori I, Gengyo-Ando K, Mitani S, *et al*. HEN-1, a secretory protein with an LDL receptor motif, regulates sensory integration and learning in *Caenorhabditis elegans*. Cell 2002, 109: 639–649.

[13] Matsuura T, Oikawa T, Wakabayashi T, Shingai R. Effects of simultaneous presentation of multiple attractants on chemotactic response of the nematode *Caenorhabditis elegans.* Neurosci Res 2004, 48: 419–429.

[14] Lin L, Wakabayashi T, Oikawa T, Sato T, Ogurusu T, Shingai R. *Caenorhabditis elegans* mutants having altered preference of chemotaxis behavior during simultaneous presentation of two chemoattractants. Biosci Biotechnol Biochem 2006, 70: 2754–2758.

[15] Ikeda DD, Duan Y, Matsuki M, Kunitomo H, Hutter H, Hedgecock EM, *et al.* CASY-1, an orthlog of calsyntenins/alcadeins, is essential for learning in *Caenorhabditis elegans*. Proc Natl Acad Sci U S A 2008, 105: 5260–5265.

[16] Hunter JW, Mullen G, McManus JR, Heatherly JM, Duke A, Rand JB. Neuroligin-deficient mutants of *C. elegans* have sensory processing deficits and are hypersensitive to oxidative stress and mercury toxicity. Dis Model Mech 2010, 3: 366–376.

[17] Leung MC, Williams PL, Benedetto A, Au C, Helmcke KJ, Aschner M, *et al. Caenorhabditis elegans*: an emerging model in biomedical and environmental toxicology. Toxicol Sci 2008, 106: 5–28.

[18] Wang DY, Xing XJ. Assessment of locomotion behavioral defects induced by acute toxicity from heavy metal exposure in nematode *Caenorhabditis elegans.* J Environ Sci 2008, 20: 1132–1137.

[19] Du M, Wang DY. The neurotoxic effects of heavy metal exposure on GABAergic system in nematode *Caenorhabditis elegans.* Environ Toxicol Pharmacol 2009, 27: 314–320.

[20] Xing XJ, Rui Q, Du M, Wang DY. Exposure to lead and mercury in young larvae induces more severe deficits in neuronal survial and synaptic function than in adult nematodes. Arch Environ Contam Toxicol 2009, 56: 732–741.

[21] Xing XJ, Du M, Xu XM, Rui Q, Wang DY. Exposure to metals induces morphological and functional alteration of AFD neurons in nematode *Caenorhabditis elegans*. Environ Toxicol Pharmacol 2009, 28: 104–110.

[22] Xing XJ, Du M, Zhang YF, Wang DY. Adverse effects of metal exposure on chemotaxis towards water-soluble attrabtants regulated by ASE sensory neurons in nematode *Caenorhabditis elegans.* J Environ Sci 2009, 21: 1684–1694.

[23] Wang DY, Wang Y. Nickle sulfate induces numerous defects in *Caenorhabditis elegans* that can also be transferred to progeny. Environ Pollut 2008, 151: 585–592.

[24] Ye HY, Ye BP, Wang DY. Trace administration of vitamin E can retrieve and prevent UV-irradiation- and metal exposure-induced memory deficits in nematode *Caenorhabditis elegans*. Neurobiol Learn Memory 2008, 90: 10–18.

[25] Zhang YF, Ye BP, Wang DY. Effects of metal exposure on associative learning behavior in nematode *Caenorhabditis elegans.* Arch Environ Contam Toxicol 2010, 59: 129–136.

[26] AltunGultekin Z, Andachi Y, Tsalik EL, Pilgrim D, Kohara Y, Hobert O. A regulatory cascade of three homeobox genes, *ceh-10, ttx-3* and *ceh-23*, controls cell fate specification of a defined interneuron class in *C. elegans.* Development 2001, 128: 1951–1969.

[27] Chase DL, Pepper JS, Koelle MR. Mechanism of extrasynaptic dopamine signaling in *Caenorhabditis elegans.* Nat Neurosci 2004, 7: 1096–1103.

[28] McIntire SL, Reimer RJ, Schuske K, Edwards RH, Jorgensen EM. Identification and characterization of the vesicle GABA transporter. Nature 1997, 389: 870–876.

[29] 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: 3384–3387.

[30] Sengupta P, Chou JH, Bargmann CI. *odr-10* encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell 1996, 84: 899–909.

[31] 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: 63–72.

[32] Hobert O, Mori I, Yamashita Y, Honda H, Ohshima Y, Liu Y, *et al.* Regulation of interneuron function in the *C. elegans* thermoregulatory pathway by the *ttx-3* LIM homeobox gene. Neuron 1997, 19: 345–357.

[33] Chalasani SH, Chronis N, Tsunozaki M, Gray JM, Ramot D, Goodman MB, *et al.* Dissecting a circuit for olfactory behavior in *Canorhabditis elegans.* Nature 2007, 450: 63–70.

[34] Mckay SJ, Johnsen R, Khattra J, Asano J, Baillie DL, Chan S, *et al.* Gene expression profiling of cells, tissues, and developmental stages of the nematode *C. elegans.* Cold Spring Harb Symb Quant Biol 2003, 68: 159–170.

[35] Brenner S. The genetics of *Caenorhabditis elegans*. Genetics 1974, 77: 71–94.

[36] Donkin S, Williams PL. Influence of developmental stage, salts and food presence on various end points using *Caenorhabditis elegans* for aquatic toxicity testing. Environ Toxicol Chem 1995, 14: 2139–2147.

[37] Wicks SR, de Vries CJ, van Luenen HG, Plasterk RH. CHE-3, a cytosolic dynein heavy chain, is required for sensory cilia structure and function in *Caenorhabditis elegans*. Dev Biol 2000, 221: 295–307.

[38] Bargmann CI, Hartwieg E, Horvitz HR. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. Cell 1993, 74: 515–527.

[39] Saeki S, Yamamoto M, Iino Y. Plasticity of chemotaxis revealed by paired presentation of a chemoattractant and starvation in the nematode *Caenorhabditis elegans*. J Exp Biol 2001, 204: 1757–1764.

[40] Kraemer BC, Zhang B, Leverenz JB, Thomas JH, Trojanowski JQ, Schellenberg GD. Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy. Proc Natl Acad Sci U S A 2003, 100: 9980–9985.

[41] Wang DY, Liu PD, Yang YC, Shen LL. Formation of a combined Ca/Cd toxicity on lifespan of nematode *Caenorhabditis elegans*. Ecotoxicol Environ Safe 2010, 73: 1221–1230.

[42] Chu KW, Chow KL. Synergistic toxicity of multiple heavy metals is revealed by a biological assay using a nematode and its transgenic derivative. Aquat Toxicol 2002, 61: 53–64.

[43] Liao EH, Hung W, Abrams B, Zhen M. An SCF-like ubiquitin ligase complex that controls presynaptic differentiation. Nature 2004, 430: 345–350.

[44] 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: 2229–2232.

[45] Sambongi Y, Nagae T, Liu Y, Yoshimizu T, Takeda K, Wada Y, *et al*. Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in *Caenorhabditis elegans*. Neuroreport 1999, 10: 753–757.

秀丽线虫对两种相互抑制刺激信号进行感觉整合分析系统的改进

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摘要：目的 本研究旨在对秀丽线虫针对两种相互抑制刺激信号进行感觉信号整合的分析体系进行改进。**方法** 在分析体系中，尝试通过改变金属离子墙类型来改变动物对两种相互抑制刺激信号的整合。此外，用3种已经被证实有感觉信号整合缺陷的*hen-1*、*ttx-3*和*casy-3*突变体评估调整后的分析体系。借助新建立的分析体系，进一步鉴定可能参与动物感觉信号整合的基因。**结果** 在分析系统不同区域，秀丽线虫的运动行为、基本运动能力、神经发育与应激反应均正常。各种浓度的金属离子墙能抑制线虫对不同浓度丁二酮的趋向性。此外，各种浓度的丁二酮也能抑制线虫对不同浓度金属离子的规避性。大多数的秀丽线虫越过Fe^{2+}、Zn^{2+}或Mn^{2+}墙趋向丁二酮（第1类）；约有一半数量的秀丽线虫会越过Ag^{+}、Cu^{2+}、Cr^{2+}或Cd^{2+}墙趋向丁二酮（第2类）；只有很少数量的秀丽线虫会越过Pb^{2+}或Hg^{2+}墙趋向丁二酮（第3类）。此外，在向丁二酮趋向过程中，当秀丽线虫遇到不同金属离子墙时，其做出向后运动而后趋向于丁二酮的比例与其直接趋向于丁二酮运动的比例呈现出差异。借助于建立的分析体系，可观察到编码F-box蛋白的*fsn-1*基因的突变体以及编码其靶点酪氨酸激酶受体的*scd-2*基因的突变体均表现出严重的感觉信号整合缺陷，且*fsn-1*突变体的感觉信号整合缺陷可显著被*scd-2*突变抑制。**结论** 基于模型中的动物感觉信号整合行为，本研究中使用的分析系统可分为3类。第1类分析系统可能有助于评估或鉴定突变体中微弱的感觉信号整合缺陷，而第3类分析系统可能有助于进一步确认第1类及第2类分析系统鉴定的感觉信号整合缺陷。此外，秀丽线虫中突触组装分化的稳定或去稳定化可能与感觉信号整合的调控存在密切的联系。

关键词：感觉信号整合；成对刺激信号；分析系统；金属墙；秀丽线虫