Effects of Ionizing Radiation on Locomotory Behavior and Mechanosensation in *Caenorhabditis elegans*

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Nematode/Behavior/Locomotory rate/Dopaminergic pathway/Oxidative stress

Locomotory behavior (motility) and mechanosensation are of vital importance in animals. We examined the effects of ionizing radiation (IR) on locomotory behavior and mechanosensation using a model organism, the nematode *Caenorhabditis elegans*. Bacterial mechanosensation in *C. elegans* induces the dopamine-mediated slowing of locomotion in the presence of bacteria (food), known as the basal slowing response. We previously reported an IR-induced reduction of locomotory rate in the absence of food. In the present study, we observed a similar IR-induced reduction of locomotory rate in the *cat-2* mutant, which is defective in bacterial mechanosensation. The dose response pattern of the locomotory rate in the presence of food was relatively flat in wild-type animals, but not in *cat-2* mutants. This suggests that the dopamine system, which is related to bacterial mechanosensation in *C. elegans*, might have a dominant effect on locomotory rate in the presence of food, which masks the effects of other stimuli. Moreover, we found that the behavioral responses of hydrogen peroxide-exposed wild-type animals are similar to those of IR-exposed animals. Our findings suggest that the IR-induced reduction of locomotory rate in the absence of food is mediated by a different pathway from that for bacterial mechanosensation, at least partially through IR-produced hydrogen peroxide.

INTRODUCTION

Locomotory behavior (motility) and mechanosensation are of vital importance in animals. Animals (including humans) are exposed to ionizing radiation (IR) from natural and artificial sources, such as radon-222, cosmic rays, and nuclear accidents such as at Chernobyl. We investigated the effects of IR on locomotory behavior and mechanosensation using a model organism.

The nematode *Caenorhabditis elegans* (*C. elegans*) is an well-studied model organism for which the simple nervous system in adults, genetic information, and behavioral paradigms have been well described. Sawin et al. reported a decrease in the locomotory rates of well-fed adult *C. elegans* on a bacterial lawn (food) compared with those on plates lacking bacteria and defined this response as the “basal slowing response”. The presence of bacteria is perceived by mechanosensory stimulation via eight dopaminergic sensory neurons. The *cat-2* gene encodes a tyrosine hydroxylase (TH), an enzyme required for the biosynthesis of endogenous dopamine. TH-deficient *cat-2* mutants have reduced dopamine levels (about 55% of wild-type animals) where dopamine is synthesized in a TH-independent manner, and show a defect in the basal slowing response. We previously reported that acute exposure to IR reduced the locomotory rates of *C. elegans* in the absence of food. These results raise the question of whether IR reduces the locomotory rate in the presence of food as well as in the absence of food. Slow locomotion of *C. elegans* in the presence of food is mediated by a dopamine system for mechanosensation, and dysfunction of a dopamine system in mammals following IR exposure inhibits behavior.
suspect the possible involvement of disorder of a dopamine system on radiation response in *C. elegans*. Thus, we examined the effect of IR on the basal slowing response regarding to mechanosensation in *cat-2* mutants that are deficient in TH for the synthesis of dopamine. The present study provides basic insights into the effects of IR on the function or integration in the nervous system of *C. elegans*.

**MATERIALS AND METHODS**

**Strains and culture**

The *C. elegans* wild-type Bristol N2,7) CB1112 *cat-2(e1112)II* mutant8) and the *Escherichia coli* HB101 strain, were obtained from the Caenorhabditis Genetics Center. Using standard methods,7) animals were grown at 20°C on 6-cm plates containing 10 ml of nematode growth medium (NGM) agar spread with *E. coli* HB101 (Fig. 1a). Well-fed adults were used in all experiments.

**Sample preparation and treatments**

Animals were collected from the plates and washed twice with S basal buffer (S-buffer).3) More than 100 animals in a few drops of S-buffer were transferred to an NGM plate with a fresh bacterial lawn (Transfer 1 in Fig. 1b). Excess fluids were absorbed with Kimwipes (CRECIA, Tokyo, Japan), and the plates were sealed with Parafilm (Pechiney Plastic Packaging, Chicago, IL) to avoid drying. Animals were placed on a plate for at least 1 h (including irradiation time), and exposed to ⁶⁰Co γ-rays at a dose rate of 32 Gy/min at room temperature (Fig. 1c). Control animals were sham-irradiated and handled in parallel with the test animals, except for irradiation. In the case of dopamine rescue experiments, a fresh solution of dopamine hydrochloride was prepared at a concentration of 2 mM in M9 buffer,3) and 400 μl of the solution was added to NGM plates with a bacterial lawn (Fig. 1c). The plates were allowed to dry at room temperature for 1 h, and more than 100 animals were placed on the plates 1 h before irradiation. For locomotory rate assays, 6-cm plates of fresh NGM agar with or without a ring-shaped bacterial lawn of *E. coli* (Fig. 1c) were prepared.3) We transferred fifty or more animals to the assay plates in a drop of S-buffer (Fig. 1d and 1e). If the assay plate contained

<table>
<thead>
<tr>
<th>Operation</th>
<th>Method</th>
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<tbody>
<tr>
<td>(a) Incubation</td>
<td>3-4 d</td>
</tr>
<tr>
<td>(b) Transfer 1</td>
<td>~ 1 h</td>
</tr>
<tr>
<td>(c) Irradiation</td>
<td>3-45 min</td>
</tr>
<tr>
<td>(d) Transfer 2</td>
<td>5 min ~</td>
</tr>
<tr>
<td>(e) Assay</td>
<td>collect, wash, and drop</td>
</tr>
<tr>
<td>(f) Data analysis</td>
<td>dopamine</td>
</tr>
</tbody>
</table>

**Fig. 1.** Outline of the experimental procedures. (a) Incubation. *C. elegans* were grown at 20°C on NGM plates spread with *E. coli*. (b) Transfer 1. Before irradiation, animals were transferred to plates with a fresh bacterial lawn. (c) Irradiation. Animals were exposed to γ-rays, and control animals were handled in parallel except for irradiation. For the dopamine rescue experiment, we used plates with a bacterial lawn and 2 mM dopamine. (d) Transfer 2. After irradiation, animals were transferred to two pairs of assay plates (food(−) and food(+)) conditions in the paired experiment. In the “food(−)” condition, animals were placed on plates without bacteria. On the other hand, plates with a ring-shaped bacterial lawn were used for the “food(+))” condition. Exposure to hydrogen peroxide was carried out in this step. (e) Locomotory rate assay. Body bends of five animals were manually counted on each plate in every paired experiment. (f) Data analysis. The normalized locomotory rate was calculated using the presented equation.

a ring-shaped bacterial lawn, animals were transferred to the clear zone at the center of the ring. For exposure to hydrogen peroxide (H$_2$O$_2$), we used S-buffer containing H$_2$O$_2$ at the “Transfer 2” step (Fig. 1d). The total exposure time was 8 min.

Estimation of ionizing radiation dose
For the irradiation, plates were placed horizontally like a culture condition, and the standard deviation for the absorbed dose was evaluated by assuming uniformly distributed animals on the plate based on a previously described method. We used polymer-alanine dosimeters (Hitachi Cable, Tokyo, Japan) for dose estimation. The estimated dose were 303 ± 18, 506 ± 21 and 905 ± 37 Gy for wild-type; and 274 ± 11, 455 ± 18 and 892 ± 36 Gy for cat-2 mutants.

Locomotory rate assay
To reduce starvation, we handled four plates (two pairs of plates with and without a ring-shaped bacterial lawn) at the same time (Fig. 1e). The experiment was independently performed at each dose. Five min after Transfer 2, the locomotory rate was measured using body bends (BB), which is defined as the number of bends in the anterior body region at 20-s intervals. The BB values of five animals on each plate were manually counted and averaged. The plates with and without a ring-shaped bacterial lawn are referred to hereafter as “food(+)” and “food(–)”, respectively.

Data analysis
In each paired experiment, the average locomotory rates (measured using BB) of animals on each plate were normalized by the average locomotory rate of non-irradiated control animals in the food(–) condition (Fig. 1f). Using such normalized values, we evaluated the frequency distribution of locomotory rates of wild-type animals in the food(–) and food(+) conditions. Therefore, the presence of the locomotory rate in the food(–) condition (BB > 0) is needed to evaluate the basal slowing response. We examined the frequency distribution of locomotory rates of exposed animals in the food(–) condition and those in the food(+) condition. Therefore, the presence of the locomotory rate in the food(–) condition (BB > 0) is needed to evaluate the basal slowing response. The error bars indicate the standard error of the mean (SEM). The variation between the non-irradiated and irradiated data sets was tested by one-way ANOVA, and significance by unpaired t tests with a Bonferroni modification for multiple comparisons of data. We used a two-way factorial ANOVA to find significant main effects for dose and strain between wild-type and cat-2 mutant animals or a interaction term. A value of p < 0.05 was considered to be significant. SYSTAT software (Systat Software, Inc., CA, USA) was used for statistical analyses.

RESULTS

Inhibition of the locomotory rate following high-dose exposure to IR
To investigate the postirradiation locomotory rate at the higher-dose than the previous reports, we first analyzed the frequency distribution of locomotory rates of wild-type animals exposed to 1420-Gy IR were not moving, but non-moving animals were not seen at the other doses (Table 1). In addition, the proportion of very slow-moving (1 ≤ BB ≤ 3) animals increased up to 13% at 1420 Gy, whereas fewer than 2% of animals moved slowly at doses of less than 905 Gy. Increasing population of non-moving and very slow-moving animals at 1420 Gy decreased the mode value of the distribution, compared with an averaged locomotory rate (Table 1). This suggests that slowing of locomotion (the basal slowing response) in normal-moving (BB > 3) animals is underestimated at 1420 Gy. Therefore, in the following experiments, we irradiated animals with less than approximately 900 Gy to evaluate the IR-induced effects on locomotory rate in food(–) and food(+) conditions.

Dose response of the locomotory rate in wild-type and cat-2 mutant animals
To investigate the effects of IR on the locomotory rate of animals in food(–) and food(+) conditions, wild-type and cat-2 mutant animals were transferred to the “Transfer 2” step (Fig. 1d). The total exposure time was 8 min.

Table 1. Effects of IR on the frequency distributions (%) of body bends of wild-type animals in the food(–) condition. n indicates the number of animals analyzed. The value in shaded line shows the percent of non-moving animals, and the bold-type value shows the mode value at each dose.

<table>
<thead>
<tr>
<th>Body bends /20s</th>
<th>Dose n</th>
<th>0 Gy 185</th>
<th>905 Gy 50</th>
<th>1420 Gy 30</th>
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<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>1– 3</td>
<td>0.5</td>
<td>2.0</td>
<td>13.3</td>
<td></td>
</tr>
<tr>
<td>4– 6</td>
<td>0.0</td>
<td>6.0</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>7– 9</td>
<td>1.1</td>
<td>8.0</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
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<td>1.6</td>
<td>12.0</td>
<td>36.7</td>
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<tr>
<td>13–15</td>
<td>5.9</td>
<td>30.0</td>
<td>3.3</td>
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<tr>
<td>16–18</td>
<td>9.7</td>
<td>18.0</td>
<td>6.7</td>
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</tr>
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<td>19–21</td>
<td>20.0</td>
<td>12.0</td>
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<td>22–24</td>
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<td>6.0</td>
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<td>28–30</td>
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<td>31–33</td>
<td>3.8</td>
<td>0.0</td>
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cat-2 mutant animals were irradiated with graded doses of γ-rays. The locomotory rate was measured using BBs in food(–) and food(+) conditions, and the values were normalized (see materials and methods). The tests of the dose response pattern of the locomotory rate in the food(–) condition (solid lines in Figs. 2A and 2B) were followed by using a two-way ANOVA. We found that the main effect for dose was significant ($F_{(3,126)} = 121.331; p = 0$), but both of the main effect for strain and the interaction term were not significant ($F_{(1,126)} = 0.797; p = 0.374$ for strain, $F_{(3,126)} = 1.405; p = 0.244$ for interaction). It indicates that the dose response pattern of the locomotory rate in food(–) between wild-type and cat-2 mutant animals varied as a function of dose. The normalized locomotory rate of wild-type animals in food(–) was significantly reduced by IR in a dose-dependent manner, as previously reported5) (the solid line in Fig. 2A; $p < 0.05$). In addition, cat-2 mutants in our assays showed a weak defect in the reduction of locomotory rate (0.72 at the dose 0 Gy in Fig. 2B), which was rescued to the same level as wild-type animals by exogenous 2 mM dopamine (0.57 at 0 Gy in cat-2 mutant). Irradiation tests of the locomotory rate in food(+) condition (dashed lines in Figs. 2A and 2B) followed a two-way ANOVA except for 905 Gy. There were significant main effects for dose ($F_{(2,107)} = 3.935; p = 0.022$ and strain ($F_{(1,107)} = 6.295; p = 0.014$), and significant interaction term ($F_{(2,107)} = 7.038; p = 0.001$). It indicates that the dose response pattern varies not only as a function of dose but also with strains (wild-type and cat-2 mutant animals). In wild-type animals, irradiation with 905 Gy, but not with 303 or 506 Gy, reduced the locomotory rate in the food(+) condition by approximately 10% (the dashed line in Fig. 2A). On the other hand, cat-2 mutants showed an IR-dependent significant decrease in locomotory rate in the food(+) condition (the dashed line in Fig. 2B; $p < 0.05$). 35% decrease in the locomotory rate in food(+) was observed in cat-2 mutants irradiated with 455 Gy. These results show a significant difference in the dose response pattern of the locomotory rate in food(+) between wild-type and mutant animals except for a maximum dose. Also, the dose response pattern of cat-2 mutants in food(+) was very similar to that in the food(–) condition, except in animals irradiated with 892-Gy IR (the dashed line in Fig. 2B). Compared with the dopamine rescue at the dose of 0 Gy, the locomotory rates of cat-2 mutants irradiated with 455 Gy was not altered by exogenous 2 mM dopamine ($p = 0.749$). These data indicate

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**Fig. 2.** Effects of IR on the locomotory rate and the basal slowing response. Dose response of the normalized locomotory rate in wild-type (A) and cat-2 mutant (B) animals. The averaged locomotory rates in BB of n independent experiments in non-irradiated wild-type animals in the food(–) condition were 22.1 (n = 11) at 303 Gy, 20.4 (n = 10) at 506 Gy, and 23.7 (n = 10) at 905 Gy. The averaged locomotory rates in BB in non-irradiated cat-2 mutants in the food(–) condition were 20.7 (n = 11) at 274 Gy, 21.4 (n = 14) at 455 Gy, and 21.3 (n = 11) at 892 Gy. The error bars represent the SEM. The asterisk indicates significant differences between irradiated and non-irradiated animals ($p < 0.05$).
Radicals cause the production of reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$). In addition, exposure to H$_2$O$_2$ induces a decrease in the tap response (tapping the body of animals) in *Caenorhabditis elegans* of animals, which masks the effect of IR.

**Effects of H$_2$O$_2$ exposure in wild-type animals**

We showed IR-induced modulation of locomotory rate in both food(–) and food(+) conditions, but the initial events underlying this phenomenon are still unclear. It is well known that exposure to IR results in the formation of free radicals, for example, OH • or H •. The reactions of free radicals cause the production of reactive oxygen species (ROS), including hydrogen peroxide (H$_2$O$_2$). In addition, exposure to H$_2$O$_2$ induces a decrease in the tap response (tapping the plate induces a switch from forward to backward movement of animals) in *C. elegans* and alteration of synaptic plasticity in the mammalian hippocampus. Thus, we explored the potential effectiveness of H$_2$O$_2$ in the inhibition of *C. elegans* motility.

Wild-type animals were exposed to graded doses of H$_2$O$_2$ (0.1, 0.5, 1.0 mM) during the “Transfer 2” step (Fig. 1d), and the locomotory rates in the food(–) and food(+) conditions were evaluated. The locomotory rates in the food(–) condition, but not in the food(+) condition, were significantly decreased in H$_2$O$_2$-exposed animals ($p < 0.05$) (Fig. 3). The dose responses of locomotory rate in both food(–) and food(+) conditions were similar to those in IR-exposed animals (Fig. 2A), supporting the possibility that IR-induced inhibition of *C. elegans* motility was caused by IR-produced H$_2$O$_2$.

**DISCUSSION**

In this study, we examined the effects of IR on locomotory behavior and bacterial mechanosensation in *C. elegans* and found the following: (1) IR reduces locomotory rates in the food(–) condition via a different pathway from the CAT-2 dependent dopaminergic pathway (Figs. 2A and 2B), (2) the dose response pattern of the locomotory rate in the food(+) condition was relatively flat, suggesting that bacterial mechanosensation is the dominant mechanism regulating locomotory rate (Figs. 2A and 2B), and (3) H$_2$O$_2$ formation induced by IR is a potential cause of the inhibition of *C. elegans* motility (Fig. 3). These findings are discussed in the following paragraphs.

The dopamine system in the central nervous system of mammals plays an important role in behavior and learning. Dysfunction of this system following IR exposure induces inhibition of motility and a decline in learning behavior. It was reported that an acute IR exposure inhibited motility in rats, and that this inhibited motility was associated with the decreased neurotransmission in the rat brain, e.g., dopamine or sodium channels. In addition, it is well known that acute IR exposure induces a disorder of a dopamine system, and causes a conditioned taste aversion. In *C. elegans*, the dopamine system is also important in behavioral plasticity, for example, the basal slowing response and the tap response. By contrast, we here reported that the IR-induced reduction of locomotory rate in the food(–) condition was controlled by a different mechanism from the dopamine system. In addition, exogenous 2 mM dopamine did not affect the locomotory rates of *C. elegans* mutants irradiated with 455 Gy in the food(–) condition ($p = 1$; data not shown). This finding supports a novel idea that the dopaminergic pathway responsible for CAT-2 is not related to the IR-induced reduction of locomotory rate in food(–), the nature of which is unclear (left panel in Fig. 4).

As shown in Figs. 2A and 2B, there is no effect of IR on the locomotory rates of wild-type animals in the food(+) condition, that is, the so-called “floor effect”, where no more inhibition is possible or detectable. In this case, the inhibitory effects of bacterial mechanostimulation are so strong that they mask the effects of IR. In addition, if damage to muscle cells was the cause of the IR-induced reduction of the locomotory rate in the food(–) condition, the IR-induced reduction would be reflected in the dose response of the locomotory rate in the food(+) condition. However, this was not the case for the locomotory rate in the food(+) condition. Thus, the basal slowing response of *C. elegans* may be the dominant response, masking the effects of other stimuli on locomotory rate. Moreover, the IR-induced reduction of the locomotory rate in *cat-2* mutants was stopped at the same level as the locomotory rate of wild-type animals in the food(+) condition. This might be caused by the pseudo-
response that IR induced the activation of dopamine receptors and/or other related factors (“X” in the right panel in Fig. 4) for signal transmission of bacterial mechanostimulation. Taken together, the neural circuit for locomotion control shown in Fig. 4 might regulate the level of the locomotory rate after integrating entire internal signals and external signals (including IR and food). Further research elucidating the floor effect and the pseudo-response hypothesis will be needed.

Hydrogen peroxide (H₂O₂) is a reactive oxygen species (ROS) produced by ionizing irradiation, and alters synaptic plasticity. Our data demonstrated that exposure to 0.1–1.0 mM H₂O₂ induced a decrease in the locomotory rate and the floor effect on the basal slowing response (Fig. 3). Therefore, H₂O₂ arising from IR exposure may affect locomotory behavior. However, the quantity of H₂O₂ produced by IR is well known to be less than that of the present exposure experiment. For ⁶⁰Co γ radiation at neutral pH and room temperature, the G value of H₂O₂ is equal to 0.68, indicating that irradiation at a dose of 500 Gy produces 0.04 mM H₂O₂. While we used short-time exposure (8 min) to H₂O₂ to reduce starvation, 2-hr incubation with H₂O₂ is used in tests of survival. Thus, the final internal body concentration of H₂O₂ might be less than that of the previous report, that is, less than the concentration of the soaking buffer used in the present study. Taken together, H₂O₂ arising from IR might be one of the causes of the change in the locomotory rate following acute IR exposure in C. elegans (Fig. 4).

Internal dopamine also produces ROS, and the dopamine-dependent oxidative stress is toxic to neurons. Dopamine in C. elegans is probably degraded not by monoamine oxidase A (MAO-A) but by autooxidation, because C. elegans does not have a homolog of MAO-A. Intracellular autooxidation of dopamine generates H₂O₂ and dopamine-quinone. The latter leads to structural modifications of proteins and reduced levels of glutathione. The dopamine-derived H₂O₂ may decrease the locomotory rate of C. elegans as shown in Fig. 3. In our experiments, exogenous 2 mM dopamine significantly decreased the locomotory rate of non-irradiated cat-2 mutants in the food(+) condition, that is the rescue of the basal slowing response. In this case, if the decrease in the locomotory rate is caused by the dopamine-dependent H₂O₂, the non-irradiated wild-type animals exposed to exogenous 2 mM dopamine also could show the decrease in the locomotory rate. However, we did not observed the significant decrease in the normalized locomotory rate of exogeneous 2 mM dopamine exposed wild-type animals 0.08 nM and 0.045 nM assuming that the whole body is water, respectively. It may mean that exposure to exogenous 2 mM dopamine induces the increase of the internal dopamine at the sub-nM order concentration, thereby allowing cat-2 mutants to do the basal slowing response. On the other hand, dopamine is autoxidized according to the following scheme: Dopamine + O₂⁻ + 2H⁺ → Dopamine semiquinone + H₂O₂. These findings suggest that the dopamine level needed for the basal slowing response, or in C. elegans.
is sub-nM order, and H$_2$O$_2$ derived from the autoxidation of dopamine is also sub-nM order. Taken together, the concentration of dopamine-dependent H$_2$O$_2$ should be much lower than that of IR-dependent H$_2$O$_2$ (0.04 mM).

In conclusion, we found that the IR-induced reduction of the locomotory rate in *C. elegans* was not mediated by CAT-2. In addition, bacterial mechanosensation in *C. elegans* might be the dominant mechanism regulating locomotory rate, masking the effects of other stimuli such as IR. Our future experiments will focus on the molecular mechanisms underlying the reduction of locomotory rate following IR exposure and the dominant effect of mechanosensation.

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