Serotonin Deficiency Shortens the Duration of Forward Movement in Caenorhabditis elegans

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Serotonin has been implicated in numerous behaviors in a wide variety of animals. We examined the effect of serotonin deficiency, induced by genetic perturbations and cell ablations, on the duration of Caenorhabditis elegans forward movement. Mutants with defective serotonin biosynthesis or worms with ablated serotonergic neurons showed a markedly decreased duration of forward movement, suggesting involvement of this neuromodulator in the regulation of the duration of worm locomotion.

Key words: Caenorhabditis elegans; locomotion; serotonin; laser ablation

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Serotonin is an important neurotransmitter in both vertebrates1) and invertebrates.2,3) Dysfunction of the serotonergic system has been implicated in numerous aspects of animal behaviors, including fear, mood, and aggression.1) Serotonin is also involved in the regulation of rhythmic motor actions of animals such as facial whisker movement in rats,4) swimming in leeches3,5) and locomotory behavior of the nematode Caenorhabditis elegans.6,7)

C. elegans locomotion is simple: under standard laboratory conditions, they move on the surface of agarose gel with their bodies bending periodically. It has been reported that in the presence of exogenous serotonin, the worms become sluggish.5,7) On the other hand, defects in the serotonin-mediated signal transduction pathway cause hyperactivity in their locomotion (the frequency of body bending/unit time increases in mutants).7) Recently, we have analyzed C. elegans locomotion by measuring the duration of forward movement, and have identified the chemosensory neuronal pathway regulating duration.8) During the course of our previous study, we revealed a function of a serotonergic chemosensory neuron ADF in promoting forward movement (or suppressing backward movement), suggesting serotonergic modulation of the duration of forward movement. To better understand the effect of serotonin on the regulation of worm forward movement, we observed the locomotory behavior of mutants defective in the biosynthesis of the monoamine transmitter.

Wild-type C. elegans ver. Bristol N2, and tph-1(mg280) [tryptophan hydroxylase], cat-4(e1141) [abnormal catecholamine distribution], bas-1(ad446) [biogenic amine synthesis related] and egl-1(n487) [egg laying defective] mutants were grown in standard nematode growth medium (NGM) agar plates at 20 °C, as described previously,9) except for the use of 10 mM PIPES-KOH (pH 6.6) instead of potassium phosphate buffer. All the worms used in the behavioral analyses were young adults. Worm locomotory behavior was analyzed as described previously.8,10) A single well-fed young adult worm grown on an NGM agar plate seeded with E. coli (OP50) was transferred to a freshly prepared unseeded assay plate (1.5% agarose, 10 mM PIPES-KOH [pH 6.6], 51 mM NaCl, 1 mM MgSO4, 1 mM CaCl2, 0.005% Tween 20; allowed to dry for 10 min with the lid removed immediately before use) using a platinum pick. Worm locomotion was recorded at 20 °C for at least 70 min. Worm locomotory behaviors during the 0–10 min period (immediately after transfer to an unseeded plate) and the 50–60 min period (50 min after transfer to an unseeded assay plate) were classified into forward and backward movement, turn through the use of video images. The statistical significance of differences between the wild-type and mutant (or cell-ablated, drug-treated) worms was determined using Mann-Whitney’s U-test. The average duration of the forward movement of each worm was taken as a single data point for calculation.

C. elegans locomotory behavior consists of four simple events: forward and backward movement, turning by deep-bending (omega-shaped turns), and rest.11) Well-fed wild-type C. elegans show highly frequent backward movement, resulting in short-duration forward movement (less than 20 s) immediately after transfer to an unseeded plate (pivoting,8) area-restricted search behavior,12) or local search behavior13). The duration of forward movement increased with time (approximately
200 s on average, 50 min after food withdrawal), establishing a distinct locomotory state, traveling8) (or long range dispersal13)) (Fig. 1, wild-type). To elucidate the effect of serotonin depletion on the regulation of the duration of forward movement, the tph-1(mg280) mutant, lacking only one tryptophan hydroxylase gene (and thus lacking serotonin biosynthesis14)), was examined for its locomotion. The mutant appeared defective in the long-duration forward movement characteristic of wild-type C. elegans (Fig. 1AB). Consistently, the duration of forward movement of the mutant during a 50–60 min period decreased significantly. Similar defects were observed in two other serotonin-deficient mutants, cat-4(e1141) and bas-1(ad446) (Fig. 1C).

Serotonin is required for appropriate neuronal cell migration in both mammalian8) and C. elegans.16) Hence, it was reasonable to consider that these serotonin-deficient mutants exhibit short-duration forward movement because of their abnormal neuronal circuit. However, we were able to restore the short duration of forward movement of the cat-4(e1141) mutant, which lacks a biosynthetic pathway for both serotonin and dopamine,17) to a level comparable to that of the wild-type by brief pre-treatment with exogenous serotonin or dopamine (Fig. 2). We also treated the bas-1(ad446) mutant with these transmitters and obtained essentially same results as for cat-4(e1141) (data not shown). These results suggest that the short-duration forward movement during the 50–60 min period in these mutants were observed as a consequence of a defect in serotonergic signaling, but not of an abnormal neuronal circuit. Mutants have been treated with neurotransmitters, as reported by Sawin et al.18) Freshly prepared serotonin (50 mM in H2O, serotonin creatinine sulfate complex; Sigma-Aldrich, St. Louis, MO) and/or dopamine (50 mM in H2O, dopamine hydrochloride; Sigma-Aldrich) were added to sterilized NGM agar solution immediately before pouring the solution to the plate to obtain a final concentration of 2 mM. Concentrated E. coli (OP50) culture was spread onto the plate as a food source. Only freshly prepared culture plates were used.

Taking these results together, serotonin affects not only the body-bending rate,6,7) but also the duration of forward movement. Our results also suggest a contribution of dopamine to this behavioral alteration, but we did not further investigate this contribution in this study.
Recently, Hills et al. \(^{12}\) reported that multiple dopaminergic neurons (ADE, PDE and CEP) are involved in the modulation of the frequency of directional reversals (almost equivalent to the modulation of the duration of forward movement).

A young adult \(C.\) \textit{elegans} hermaphrodite has 6 classes of 11 serotonergic neurons.\(^{6,18,19}\) Previous studies indicated that both the NSM and HSN serotonergic neurons had functions in the modulation of locomotory behavior.\(^{18,20}\) We assayed worms in which HSN and NSM neurons were eliminated to determine whether these two classes of serotonergic neurons contribute to the duration of forward movement (Table 1). The \textit{egl-1(n487)} mutant lacks HSN neurons genetically because of inappropriate cell degeneration.\(^{19}\) NSM neurons were laser-ablated at the L2 larval stage, as described\(^{21}\) and the worms operated were cultured for 2 days before assay. The \textit{egl-1(n487)} mutant showed short-duration forward movement of about 50% of wild-type animals during the 50–60 min period, to the same extent as for ADF ablation. The laser ablation of NSM neurons shortened the duration of forward movement during the 50–60 min period. Moreover, NSM-ablated worms showed a significantly short duration of forward movement immediately after transfer worms to an unseeded plate (0–10 min period). These results suggest that endogenous serotonin released from these neurons is involved in the regulation of the duration of forward movement.

Several recent studies analyzing worm locomotory behaviors\(^{12,13}\) have referred the transition from a locomotory state with short-duration forward movement (pivoting,\(^{8}\) area-restricted search\(^{12}\) or local-search\(^{13}\)) to that with long-duration forward movement (traveling\(^{8}\) or long-range dispersal\(^{13}\)) as a change in foraging strategy in \(C.\) \textit{elegans}. Upon removal from food, worms seek food in their vicinities first, then seek it in distant areas after prolonged food-deprivation. Serotonin is thought to be involved in food-related signaling in \(C.\) \textit{elegans}.\(^{6,7,14,18}\) It became of interest to see how the serotonergic pathway affects the duration of the forward movement. Detailed molecular genetic and behavioral studies are required to clarify the mechanism underlying the serotonergic modulation of locomotory behavior.

### Table 1. Forward Movement of Worms Lacking Specific Neurons

<table>
<thead>
<tr>
<th>Neuron</th>
<th>Average Duration (s)</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Wild-type</td>
<td>0–10 min: 12.8 ± 0.8 s, 50–60 min: 196.2 ± 35.0 s</td>
<td>This study</td>
</tr>
<tr>
<td>ADF (–)</td>
<td>0–10 min: 10.7 ± 0.7 s, 50–60 min: 86.5 ± 15.3(^{16}) s</td>
<td>8</td>
</tr>
<tr>
<td>NSM (–)</td>
<td>0–10 min: 8.7 ± 0.5(^{16}) s, 50–60 min: 108.5 ± 13.6(^{16}) s</td>
<td>This study</td>
</tr>
<tr>
<td>egl-1(n487)</td>
<td>0–10 min: 12.8 ± 0.9 s, 50–60 min: 82.3 ± 19.2(^{16}) s</td>
<td>This study</td>
</tr>
</tbody>
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\(^{1}\)Average duration ± SEM
\(^{2}\)Significantly different from wild-type, \(p < 0.05\), \(\ast \ p < 0.01\)

\(^{3}\)HSN (–)\(^{20}\)

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### References


