Serotonin Promotes $G_\alpha$-Dependent Neuronal Migration in Caenorhabditis elegans

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Summary

Background: The directed migration of neurons during development requires attractive and repulsive cues that control the direction of migration as well as permissive cues that potentiate cell motility and responsiveness to guidance molecules.

Results: Here, we show that the neurotransmitter serotonin functions as a permissive signal for embryonic and postembryonic neuronal migration in the nematode C. elegans. In serotonin-deficient mutants, the migrations of the ALM, BDU, SDQR, and AVM neurons were often foreshortened or misdirected, indicating a serotonin requirement for normal migration. Moreover, exogenous serotonin could restore motility to AVM neurons in serotonin-deficient mutants as well as induce AVM-like migrations in the normally nonmotile neuron PVM; this indicates that serotonin was functioning as a permissive cue to enable neuronal motility. The migration defects of serotonin-deficient mutants were mimicked by ablations of serotonergic neuroendocrine cells, implicating humoral release of serotonin in these processes. Mutants defective in $G_\alpha$ and $G_\beta$ signaling, or in N-type voltage-gated calcium channels, showed migration phenotypes similar to serotonin-deficient mutants, and these molecules appeared to genetically function downstream of serotonin in the control of neuronal migration.

Conclusions: Thus, serotonin is important for promoting directed neuronal migration in the developing C. elegans nervous system. We hypothesize that serotonin may promote cell motility through G protein-dependent modulation of voltage-gated calcium channels in the migrating cell.

Introduction

The regulation and guidance of cell movement is critical for normal development of the nervous system. In particular, the outgrowth and navigation of axonal growth cones and the motility and direction of migrating neurons depend on spatially and temporally regulated guidance molecules [1, 2]. In some cases, these molecules function as positional cues for guiding the direction of cell movement; examples include the netrin, semaphorin, and slit/robo families of diffusible ligands [3–6]. In other cases, such as with laminin and NCAMs, molecules can serve as permissive cues that promote motility per se or potentiate a response to positional cues [7–9]. Although recently a great deal of progress has been made in understanding the mechanisms controlling the motility and guidance of axon growth cones, the mechanisms underlying the guidance of migratory neurons are less well understood.

One way to obtain information about the process of neuronal migration is by using a genetically tractable animal such as the nematode Caenorhabditis elegans. C. elegans has a simple, well-characterized nervous system consisting of 302 identified neurons, and it is extremely amenable to classical and molecular genetic studies [10]. Moreover, because individual migrating cells can be specifically labeled in transgenic animals expressing green fluorescent protein under the control of a cell-type-specific promoter, it is straightforward to assay cell migration in wild-type and mutant animals in vivo.

A number of C. elegans neurons and neuroblasts undergo directed migration in embryogenesis or during larval development. Among the best studied of these are the Q neuroblasts and their descendents (Figure 1) [11]. QL and QR are bilateral homologs found, respectively, in the left and right midbody region in the C. elegans L1 larva. During the first larval molt, QR migrates from the midbody to the anterior body region. During this anterior migration, QR undergoes cell divisions that give rise to the neuroblast QR.pa, which in turn divides to produce AVM, a mechanosensory cell, and the interneuron SDQR. AVM then migrates ventrally and projects an axon into the ventral nerve cord, while SDQR migrates dorsally and projects an axon into the dorsal sublateral nerve. In contrast, QL migrates in a posterior direction toward the tail. After undergoing a pattern of cell divisions identical to that of QR, the QL.pa neuroblast divides to produce the touch neuron PVM and the interneuron SDQL; however, unlike their right-side homologs, these neurons do not migrate from their birthplace on the lateral midline [12].

Many genes have been identified whose products are important for guidance of the Q cells and/or their descendents. Many of these genes affect the migrations of the neuroblasts (i.e., QR/L, QR/L.p, and QR/R.p) that undergo long anterior- or posterior-directed migrations before dividing to generate neurons [13–17]. A second set of genes, including the netrin guidance molecule unc-6 and its receptors unc-5 and unc-40, control the migration and axonal guidance of one QR descendant in particular, SDQR [18]. Mutations in these genes lead to a ventral displacement of the SDQR cell body and cause the SDQR axon to project into the ventral nerve cord rather than the dorsal sublateral nerve. Finally, a third group of genes, including the calcium channels unc-2 [19] and egl-19 [20], and the CaMII kinase unc-43 [21], affect the migration of the AVM and SDQR cell bodies. Although mutations in egl-19 affect guidance of the AVM axon as well as its cell body, mutations in unc-2 and unc-43 lead to specific defects in cell migration. In each of these mutants, the ventral migration of AVM frequently does not occur or is misdirected, suggesting that calcium influx in the migrating AVM neuron promotes cell motility and/or response to guidance cues [22]. However, the signals that might control such a...
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(A) Embryonic migrations. ALML/R and BDUL/R are generated by mitotic divisions of the AB.arppaapp and AB.arpppapp neuroblasts in the embryonic head. In each case, during late embryogenesis, the anterior daughter (ALM) undergoes a long posterior and dorsal migration, while the posterior daughter (BDU) undergoes a shorter posterior migration.

(B) QR and QL lineages. During the L1/L2 molt, QR migrates anteriorly, undergoing two divisions to generate the QR.pa neuroblast. QR.pa divides to produce AVM, a touch neuron, and SDQR, an interneuron. In contrast, QL migrates posteriorly, undergoing divisions that produce the QL.pa neuroblast. QL.pa divides to produce PVM, a touch neuron, and SDQL, an interneuron.

(C) Postembryonic migrations of the Q descendant neurons. After AVM and SDQR arise from division of QR.pa, SDQR migrates dorsally and anteriorly and projects an axon into the sublateral nerve. AVM migrates ventrally and projects an axon into the ventral nerve cord. QL.pa produces PVM and SDQL, whose cell bodies do not undergo any further migrations.

calcium influx in the developing neuron have not been identified.

In this study, we demonstrate that the neurotransmitter serotonin promotes directed migration of several migrating neurons, including AVM and SDQR. We also show that exogenous serotonin can induce migration in the PVM neurons that normally do not migrate, indicating that serotonin is a permissive signal that can trigger cell motility. Finally, we present evidence that serotonin’s effect on neuronal migration is mediated through a signaling pathway involving the trimeric G proteins Go, Gq, and Gs and the voltage-gated calcium channel UNC-2.

Results and Discussion

Serotonin-Deficient Mutants Exhibit Postembryonic Cell Migration Defects

To determine whether serotonin affected neuronal migration, we first assayed the positioning of the postembryonic touch receptor neuron AVM and its sister cell SDQR in serotonin-deficient mutants. Specifically, the final positions of the AVM and SDQR cell bodies were determined in mutants carrying a loss-of-function mutation in one of four genes: tph-1 [23] (which encodes tryptophan hydroxylase), cat-4, bas-1 [24] (genes required for AA-decarboxylase activity), or cat-1 [25] (which encodes the vesicular monoamine transporter). Previous studies had shown that serotonin levels are significantly reduced in bas-1 mutants and undetectable in tph-1 and cat-4 mutants [23, 24], while cat-1 mutants produce serotonin but fail to transport it to neuronal processes [25]. We observed that mutations in all four genes caused defects in the proper positioning of AVM and SDQR (Figure 2, Table 2). Specifically, although AVM and SDQR adopted final positions in the animal’s anterior body region, they were frequently found at positions that were dorsal, posterior, or even anterior to their wild-type locations. Interestingly, a dopamine-deficient mu-
In addition to the postembryonically migrating neurons AVM and SDQR, several other neurons undergo directed migrations during embryogenesis. For example, ALM and BDU are bilaterally symmetric sister neurons that are generated in the head region of the embryo. During late embryogenesis, the BDU neurons migrate a short distance to lateral positions just posterior to the head, while the ALM neurons migrate a longer distance to dorsal and more posterior positions (Figure 1). To determine whether serotonin affected the guidance of these migrations, we assayed the final positions of ALM and BDU in tph-1, cat-1, bas-1, and cat-4 mutant animals. We observed that the cell bodies of both ALM and BDU were often found anterior to their normal positions in both tph-1 and cat-4 mutants (Table 2). To determine whether serotonin affected the guidance of these cells’ axons, we generated tph-1 mutant strains carrying either a mec-4::GFP transgene (which labels the ALM neurons) or a slt-1::GFP transgene (which labels the BDU neurons) [28]. In both cases, the guidance of the labeled neurons’ axons was normal in the tph-1 mutant background, and the expression of both marker transgenes was not significantly altered (data not shown). Thus, serotonin appeared to be specifically required to effectively guide the embryonic migrations of the ALM and BDU cell bodies, but not their axons.

We also examined the effect of serotonin deficiency on a nonneuronal cell migration. During postembryonic development, the gonadal distal tip cells undergo complex directed migrations that determine the morphology and orientation of the mature gonad [29]. We observed that, in tph-1 deletion mutants, the distal tip cell migrations were completely normal (75/75 animals tested). Thus, the effect of serotonin on cell migration may be specific for developing neurons.

Involvement of Serotonergic Neuroendocrine Cells in Neuronal Migration

We next wished to identify the source of serotonin that facilitates neuronal migration in wild-type animals. Only a small number of C. elegans neurons express tryptophan hydroxylase, the essential enzyme for serotonin biosynthesis; in the embryo and L1 larva, these include the two NSM neurosecretory cells and the two ADF chemosensory neurons [23]. To determine if either of these two cell types might be the source of the serotonin involved in promoting neuronal migration, we performed cell-specific laser ablations of these cells immediately after hatching and assessed the effect of the ablation on the postembryonic AVM and SDQR migrations. We found that ablation of the NSMs alone resulted in a significant (penetrance = 20%–25%) migration defect (Table 1). Likewise, ablation of the ADFs alone also caused a significant defect in AVM and SDQR migration (penetrance = 45%–50%). Ablation of all four neurons resulted in a migration defect that, while no greater in penetrance than that of the ADF singly ablated animals, was essentially identical in penetrance to the tph-1 deletion mutant. Thus, the NSM and ADF neurons together appeared sufficient to account for serotonin’s effects on neuronal migration during postembryonic development.

Exogenous Serotonin Restores Directed Cell Migration

Since endogenous serotonin appeared to be important for promoting motility in migrating neurons, we reasoned that application of exogenous serotonin might rescue the migration defects of serotonin-deficient mutants. Since the egg shell surrounding the embryo is not serotonin permeable, we assayed whether exogenous serotonin added to the growth medium could rescue the postembryonic migration defects in AVM and SDQR. We observed that exogenous serotonin indeed rescued the AVM and SDQR cell body positioning defects of tph-1, cat-1, cat-4, and bas-1 mutant animals (Figure 4), supporting the hypothesis that serotonin can potentiate...
directed migration of these neurons. Interestingly, exogenous serotonin also often induced PVM, the normally sessile left-side homolog of AVM, to migrate in wild-type animals (Figure 4). PVM's site of birth is relatively distant from the location of the NSM and ADF neurons, and it is therefore probably exposed to lower levels of endogenous serotonin than AVM during normal development. Thus, the ability of exogenous serotonin to promote ectopic PVM migration suggests that serotonin may function as a permissive signal that can promote AVM and PVM motility.

**GOA-1 Functions Downstream of Serotonin in Guiding Neuronal Migration**

What signaling molecules mediate serotonin’s influence on neuronal migration? Most serotonin receptors are coupled to G protein-mediated signaling pathways; thus, we reasoned that serotonin’s effects on neuronal
migration might require the activity of one or more G proteins. The C. elegans genome contains 20 genes encoding distinct G protein α subunits; viable loss- or reduction-of-function alleles have been identified for 17 of these [30, 31]. Among these strains, mutants carrying loss-of-function mutations in the Gα homolog goa-1 and the Gβ homolog egl-30 displayed the most penetrant (approximately 30% for AVM) misplacement phenotypes (Figure 5). The effects of goa-1 and egl-30, like serotonin, were specific to cell body migration, since the AVM axon entered the ventral cord in the correct location in mutant animals even when the cell body from which the axon originated was misplaced (data not shown). Interestingly, whereas a gain-of-function allele of egl-30 caused significant misplacement of all four neurons, a goa-1 gain-of-function allele showed no misplacement phenotype for AVM, SDQR, or BDU (Table 2). Thus, Gα, like serotonin, appeared to play a permissive rather than an instructive role in the migrations of these three neurons.

Together, these data suggested that serotonin’s effect on AVM, SDQR, and BDU cell migration might be mediated by a Gα-dependent signal transduction pathway. To further examine this possibility, we analyzed the cell migration phenotypes of tph-1; goa-1 double mutants. In a double mutant carrying loss-of-function mutations in both tph-1 and goa-1, we observed that, in these animals, the migration defects (assayed with respect to AVM, SDQR, and BDU) showed the same penetrance as in the tph-1 single mutant (Table 3), implying that GOA-1 affected the same aspect of the AVM misplacement phenotypes (Figure 5). The effects of goa-1 and egl-30, like serotonin, SDQR/BDU migration process as serotonin.

In principle, GOA-1 could mediate either serotonin release or serotonin response during neuronal migration. To distinguish between these possibilities, we analyzed a double mutant carrying a tph-1 deletion and a goa-1 gain-of-function allele. In this double mutant, we observed a substantially lower penetrance of AVM, SDQR, and BDU than in the tph-1 single mutant. Thus, the dominant, constitutively activating goa-1 mutation suppressed the cell migration phenotypes of the tph-1 deletion mutant, implying that Gα functioned downstream of serotonin in promoting directed migration of these neurons. We also found that exogenous serotonin failed to rescue the migration defects of tph-1; goa-1 double mutants.
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Figure 5. Misplacement Phenotypes of *goa-1*, *egl-30*, and *unc-2* Mutant Animals

(A) The positions adopted by ALM, BDU, AVM, and SDQR in *egl-30(n686)*, *goa-1(n1134)*, and *unc-2(mu74)* mutant animals are summarized (*n* = 100 for *egl-30*; *n* = 200 for *unc-2* and *goa-1*). Left is anterior; up is dorsal. Wild-type positions are indicated by darkened circles. Arrows represent embryonic migrations to wild-type position for ALM and BDU, and migration from the final division of QR.pa into wild-type positions of AVM and SDQR.

(B) AVM and SDQR migration defects are not rescued by exogenous serotonin in *goa-1(n1134)* and *unc-2(mu74)* mutants. Mutant animals were allowed to develop on exogenous serotonin (7.5 mM 5-HT). The numbers of animals tested (no drug/on serotonin) were: *goa-1(n1134)*, *n* = 200/*n* = 100; *unc-2(mu74)*, *n* = 200/*n* = 100.

The effect of a *goa-1* loss-of-function mutant, as would be predicted if serotonin functioned upstream of *goa-1* (Figure 5). These results suggested that serotonin might induce neuronal migration through activation of Go signaling pathways in the migrating cell.

To further test this possibility, we examined whether expression of active GOA-1 in the migrating neurons under a cell-type-specific promoter could rescue the migration defect of a *goa-1* recessive mutant. For these experiments, we chose the *egl-46* promoter, which was reported to direct expression in a small set of neurons that includes the developing AVM and SDQR neurons [32]. We generated transgenic lines expressing an *egl-46* reporter construct (see the Experimental Procedures) and observed reliable expression of the transgene in the developing AVM and SDQR neurons; in contrast,
expression of the transgene was never observed in NSM or ADF. Thus, if GOA-1 functioned within the migrating neurons, an egl-46::goa-1(dm) transgene might be expected to rescue the AVM/SDQR migration defects of a goa-1(n1134) mutant. In fact, we found that this was so; out of 29 independent F1 animals transformed with the transgene, AVM was misplaced in only 2 and SDQR was misplaced in only 3 (Table 3). Thus, these results support the hypothesis that GOA-1 acts within the migrating neurons to promote directed motility and provided additional evidence that GOA-1 is involved in serotonin response rather than serotonin release.

The UNC-2 Calcium Channel May Function Downstream of Serotonin and Go

The neuronal migration phenotypes of tph-1, egl-30, and goa-1 loss-of-function mutants were quite similar to those previously observed for the calcium channel gene unc-2 (Figure 5). For example, mutations in all four genes caused similarly penetrant AVM, SDQR, ALM, and BDU migration defects, and all four genes specifically affected the migrations of the neuronal cell bodies but not their axons. It therefore seemed plausible that goa-1 and serotonin might participate in the same pathway as unc-2 in promoting neuronal migration. To investigate this possibility, we constructed double mutants carrying mutations in unc-2 and either goa-1 or tph-1. When we analyzed tph-1(mg280); unc-2(mu74) and goa-1(n1134); unc-2(mu74) double mutants, we found that the penetrance of the migration defects in the double mutants was comparable to their penetrance in the single mutants (Table 3). These results therefore suggested that all three genes functioned in the same pathway to promote directed cell migration.

To assess whether unc-2 functioned upstream or downstream of goa-1 with respect to neuronal migration, we also analyzed the phenotype of a double mutant carrying a goa-1 gain-of-function allele and an unc-2 null allele. We observed that the unc-2 mutation was largely epistatic to goa-1: the migration defects in the double mutant were equally penetrant to those of the unc-2 single mutant for BDU and SDQR, and they were comparably penetrant for AVM. Furthermore, we also observed that the migration defects of the unc-2 mutant animals were not rescued by exogenous serotonin, suggesting that the migration defects of unc-2 mutant animals are not a result of a deficiency in serotonin release (Figure 5). Taken together, our results suggest that unc-2 functions downstream of serotonin and Gq in the control of AVM, SDQR, and BDU migration.

Conclusions

We have shown here that serotonin is an important regulator of both embryonic and postembryonic neuronal migrations in the nematode C. elegans. In serotonin-deficient animals, the migrating neurons ALM, BDU, AVM, and SDQR were frequently mispositioned as a result of shortened or misdirected migrations. Not only could exogenous serotonin rescue these migration defects, it could also induce PVM, a normally nonmotile homolog of AVM, to undergo ventral and/or anterior migration. In developing embryos and first-stage larvae, serotonin is present exclusively in neurons and neurosecretory cells located in the head. Thus, serotonin may normally serve as a permissive cue that confers motility and/or responsiveness to localized guidance molecules on migrating neurons in the anterior of the animal.

The migration defects of serotonin-deficient mutants were phenocopied by ablation of the NSM and ADF neurons; thus, the serotonin that promotes neuronal mi-

Table 2. Penetrance of Migration Defects in Serotonin-Deficient and G Protein Mutants

<table>
<thead>
<tr>
<th>Strain Genotype</th>
<th>AVM</th>
<th>SDQR</th>
<th>ALM</th>
<th>BDU</th>
<th>n</th>
</tr>
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<tr>
<td>N2</td>
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<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>200</td>
</tr>
<tr>
<td>tph-1(mg280)</td>
<td>43%</td>
<td>23%</td>
<td>40%</td>
<td>50%</td>
<td>200</td>
</tr>
<tr>
<td>cat-4(e1141)</td>
<td>31%</td>
<td>15%</td>
<td>34%</td>
<td>24%</td>
<td>100</td>
</tr>
<tr>
<td>bas-1(0446)</td>
<td>30%</td>
<td>12%</td>
<td>13%</td>
<td>18%</td>
<td>100</td>
</tr>
<tr>
<td>cat-1(e1111)</td>
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<td>12%</td>
<td>17%</td>
<td>6%</td>
<td>100</td>
</tr>
<tr>
<td>cat-2(e1112)</td>
<td>6%</td>
<td>10%</td>
<td>6%</td>
<td>4%</td>
<td>50</td>
</tr>
<tr>
<td>egl-30(n686)</td>
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<td>26%</td>
<td>25%</td>
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<td>100</td>
</tr>
<tr>
<td>egl-30(n676dm)</td>
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<td>30%</td>
<td>22%</td>
<td>12%</td>
<td>100</td>
</tr>
<tr>
<td>goa-1(n1134f)</td>
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<td>27%</td>
<td>19%</td>
<td>24%</td>
<td>200</td>
</tr>
<tr>
<td>goa-1(sy19dm)</td>
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<tr>
<td>egl-8(n1791)</td>
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<td>14%</td>
<td>29%</td>
<td>10%</td>
<td>85</td>
</tr>
<tr>
<td>dkg-1(n662)</td>
<td>24%</td>
<td>30%</td>
<td>49%</td>
<td>1%</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 3. Double Mutant Analysis of tph-1, goa-1, and unc-2 Migration Phenotypes

<table>
<thead>
<tr>
<th>Strain Genotype</th>
<th>AVM</th>
<th>SDQR</th>
<th>ALM</th>
<th>BDU</th>
<th>n</th>
</tr>
</thead>
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<td>goa-1(n1134f)</td>
<td>32%</td>
<td>27%</td>
<td>19%</td>
<td>24%</td>
<td>200</td>
</tr>
<tr>
<td>goa-1(n1134f) + egl-46::goa-1(Q205L) transgene*</td>
<td>7% *</td>
<td>10% *</td>
<td>20%</td>
<td>24%</td>
<td>29 *</td>
</tr>
<tr>
<td>goa-1(sy19dm)</td>
<td>0%</td>
<td>0%</td>
<td>37%</td>
<td>0%</td>
<td>150</td>
</tr>
<tr>
<td>tph-1(md280)</td>
<td>43%</td>
<td>23%</td>
<td>40%</td>
<td>50%</td>
<td>200</td>
</tr>
<tr>
<td>unc-2(mu74)</td>
<td>34%</td>
<td>23%</td>
<td>41%</td>
<td>16%</td>
<td>200</td>
</tr>
<tr>
<td>goa-1(n1134f); unc-2(mu74)</td>
<td>36%</td>
<td>29%</td>
<td>42%</td>
<td>29%</td>
<td>200</td>
</tr>
<tr>
<td>tph-1(md280); unc-2(mu74)</td>
<td>38%</td>
<td>34%</td>
<td>33%</td>
<td>29%</td>
<td>200</td>
</tr>
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<td>goa-1(n1134f); tph-1(mg280)</td>
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<td>49%</td>
<td>42%</td>
<td>29%</td>
<td>100</td>
</tr>
<tr>
<td>tph-1(md280); goa-1(sy19dm)</td>
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<td>10%</td>
<td>39%</td>
<td>6%</td>
<td>200</td>
</tr>
<tr>
<td>unc-2(mu74); goa-1(sy19dm)</td>
<td>21%</td>
<td>21%</td>
<td>25%</td>
<td>17%</td>
<td>200</td>
</tr>
</tbody>
</table>

Statistically different from untransformed; *p < 0.001, *p < 0.02.

Statistically different from untransformed; *p < 0.001, *p < 0.02.

Statistically different from untransformed; *p < 0.001, *p < 0.02.
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Experimental Procedures

Strains and General Methods

Strains were grown and assayed at room temperature (22°C) unless stated otherwise. Animals were grown on standard nematode growth media (NGM) with Escherichia coli strain OP50 as a food source. For drug experiments, 5-hydroxytryptamine (creatinine sulfate complex, Sigma) was added to NGM at 7.5 mM. The mutant strains used in this study are: goa-1(n1134J), egl-30(m686J), egl-30(sy67dmJ), syIs9[goa-1(Q2OS5J)]dms, tph-1[mg280], cat-2(e111J), bas-1(ad466)III, cat-4(e1147)J, unc-2(m74)X, and cat-1(e111))]dms. The egl-30(dms) and goa-1(dms) strains were kind gifts from Carol Bastiani and Jane Mendel in the Sternberg lab. The egl-30 dominant allele contains two egl-30 mutations, (md168) and an intragenic revertant allele causing an amino acid change of R334 to W. The GFP strain used in the ablations analysis, pha-1(e2123ts); him-5(e1490)[pha-1(1) tph-1:GFP], was provided by Rene Garcia. ZdsIs4[p mec-4::GFP], a kind gift from the Driscoll lab, carries a transcrip
tional fusion of the mec-4 promoter to GFP.

Microscopy

Nematodes were mounted on 2% agarose pads with 40 mM NaCl for anesthesia. The positions of AVM, SDQR, ALM, and BDU were determined by using Nomarski optics at the end of L1 and early L2 stage. At this stage, the hypodermal V cells have divided once and are stationary markers that can be used to score the position of the neuron cell bodies. The positions of AVM and ALM axons were visualized by fluorescence microscopy in L4 worms carrying the mec-4::GFP array.

Treatment with Exogenous Serotonin

To assay the effect of serotonin on postembryonic migrations, newly hatched first stage larvae (L1s) were transferred to serotonin plates and grown to the second (L2) or fourth (L4) larval stage. The position of PVM was scored in L4 animals with GFP fluorescence from the mec-4::GFP array. The positions of SDDL, PVD, and PDE were scored at the same stage with Nomarski optics. AVM and SDQR were scored in L2 with Nomarski optics.

Construction of Double Mutants

The ZdsIs4[p mec-4::GFP] integrated array was crossed into the tph-1(mg280), goa-1(n1134J), egl-30(m686J), and goa-1(sy9d) genetic backgrounds by using standard methods. Double mutants containing the tph-1(mg280) allele along with a mutation in goa-1, egl-30, or unc-2 were constructed by first crossing the single mutants and then identifying animals homozygous for goa-1, egl-30, or unc-2 in the F2 generation based on their visible uncoordinated phenotypes. Homozygosity for tph-1 was then confirmed by PCR analysis of the F3 self-progeny of these animals.

Analysis of the QR.pa Cell Lineage

Animals were staged by isolating larvae that hatched within a 30-
minute time window. These staged larvae were then grown for 7–8 hr at 20°C. Worms were then mounted on 2% agarose pads and observed for 3–4 hr under Nomarski optics.

Ablations of Serotonergic Neurons

Laser ablations were performed on newly hatched L1 animals by using a standard protocol [49]. Neurons were identified for ablation by using a tph-1::GFP marker, which is restricted mainly to the ADF and NSM neurons at the L1 stage. After the ablation, worms were allowed to recover until they reached the L2 larval stage, during which the final positions of AVM and SDQR were scored. Individuals in which GFP was still visible in the target neuron after the ablation
were excluded from the analysis. Mock-ablated animals were ani-
imals transferred to agar pads and anesthetized in parallel to the
animals that underwent laser ablation. The ablated animals showed
a statistically significant increase in misplaced neurons relative to
mock-treated animals (p < 0.02 for AVM in NSM-ablated compared to
mock-ablated animals, p < 0.01 for all other ablations).

**goa-1 Transgenic Rescue**

A 3-kb egl-46 genomic fragment containing the first eight amino
acids of EGL-46 was fused to goa-1 genomic DNA containing the
activating gain-of-function mutation, syl5[goa-1( Q205L)] [32, 50],
and to the fluorescent GFP derivative YC2.3 [51]. This fragment has
been reported to direct expression of GFP in the following neurons
during the L1 and L2 larval stages: the developing neurons of the Q
lineages (AVM, SDQR, PVM, and SDQL); up to 10 neurons in the
head, including the PLPs but not the NSMs or ADFs; the HSNs; and
11 ventral cord motorneurons [32]. A transgenic line expressing the
egl-46::YC2.3 transgene (AQ743; genotype: lin-15(n765; ljEx19[egl-
46::YC2.3; lin-15(+)])) was confirmed to reliably express fluorescent
protein in the developing AVM and SDQR neurons (but never in ADF
or NSM; n > 25). The egl-46::goa-1( Q205L) PCR product was gel
purified and cojected with the myo-2:YC2.1 construct [48]. F1 L2
larvae with glowing pharynges were scored for migration defects.
Mosaic animals (detected by partial expression of the GFP marker
in the pharynx and pharyngeal nervous system) were not included
in the analysis. The number of mispositioned AVM and SDQR neu-
rons in animals expressing the pegr-46::goa-1( Q205L) transgene
was significantly different from uninfected animals according to the
z test (p < 0.001 for AVM, p < 0.02 for SDQR).

**Acknowledgments**

We would like to thank Rene Garcia, Carol Bastiani, Jane Mendel,
Ji Ying Sze, Cynthia Kenyon, and the Caenorhabditis Genetics Cen-
ter for strains; members of our lab for discussions; and Rex Kerr,
Massimo Hilliard, and Christian Frekjaer-Jensen for comments on
the manuscript. This work was supported by grants from the Na-
tional Institutes of Health (DA12891) and the Human Frontiers Sci-
cence Program (to W.R.S.) and a training grant from the National
Institutes of Health (to K.S.K.).

Received: May 30, 2002
Revised: August 22, 2002
Accepted: August 23, 2002
Published: October 15, 2002

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