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Natural Variation in a Neuropeptide Y Receptor Homolog Modifies Social Behavior and Food Response in *C. elegans*

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Summary

Natural isolates of *C. elegans* exhibit either solitary or social feeding behavior. Solitary foragers move slowly on a bacterial lawn and disperse across it, while social foragers move rapidly on bacteria and aggregate together. A loss-of-function mutation in the *npr-1* gene, which encodes a predicted G protein–coupled receptor similar to neuropeptide Y receptors, causes a solitary strain to take on social behavior. Two isoforms of NPR-1 that differ at a single residue occur in the wild. One isoform, NPR-1 215F, is found exclusively in social strains, while the other isoform, NPR-1 215V, is found exclusively in solitary strains. An NPR-1 215V transgene can induce solitary feeding behavior in a wild social strain. Thus, isoforms of a putative neuropeptide receptor generate natural variation in *C. elegans* feeding behavior.

Introduction

Innate social behaviors play a role in courtship and mating, in maternal behaviors, in aggression toward intruders, and in more complex interactions involving larger communities of animals (Lorenz, 1950; Wilson, 1975). The simplest social interaction, aggregation of members of a species, can be observed in many kinds of animals and even in social bacteria and social amoebae. Social interactions vary both within and across species, making them an attractive subject for behavioral studies.

Part of the natural variation in behavior within a species is due to genetic differences between individuals. For example, different inbred strains of mice exhibit aggressive, maternal, and sexual behaviors that are characteristic to a particular strain (Crawley et al., 1997), and studies of identical human twins suggest a genetic component to personality traits like shyness (McGue and Bouchard, 1998). However, the genes that contribute to natural behavioral variability are largely unknown. Genetically altered strains of mice or flies can exhibit specific behavioral deficits because of single gene mutations (Brown et al., 1996; Ryner et al., 1996; Finley et al., 1997; Monaghan et al., 1997), but the standard mutational approach may not provide good models for understanding natural behavioral variation. An alternative approach that is more open to potential complexity and quantitative effects is the direct genetic analysis of natural heterogeneity. Indeed, studies of foraging behavior and circadian rhythms in wild *Drosophila* strains have revealed genes that contribute to natural variation in these responses (Osborne et al., 1997; Sawyer et al., 1997). This approach is also being taken to map natural behavioral differences in inbred mice (Melo et al., 1996; Finley et al., 1997; Wehner et al., 1997) and genetic influences on human behavior (Brunner et al., 1993; Karayiorgou et al., 1997).

The nematode *C. elegans* is a good organism in which to study natural variation in behavior because of its simple nervous system, powerful genetics, and the availability of many wild strains isolated from different parts of the world (Hodgkin and Doniach, 1997; Bargmann and Kaplan, 1998). Here, we investigate genetic variability in a social feeding behavior of *C. elegans*. Wild-type hermaphrodites from the laboratory strain N2 are solitary in plentiful food; they aggregate into swarms or clumps when food is limiting but disperse when food is absent. A regulated swarming response has been observed in many other nematode species in culture and in the natural environment, suggesting that this is a common type of nematode behavior (McBride and Hollis, 1966; Croll, 1970). Different wild strains of *C. elegans* exhibit differences in this foraging behavior, so that some wild strains of *C. elegans* aggregate even in plentiful food (R. Cassada, L. Avery, personal communication). In addition, hermaphrodites from the N2 strain aggregate in plentiful food if they are mutant for certain genes involved in dauer formation, a developmental program controlled by food and pheromone cues (Thomas et al., 1993). Dauer formation is controlled by a small group of sensory neurons, and the clumping phenotype of dauer mutants suggests that a sensory input contributes to aggregation.

Here, we show that variation in responses to food and other animals in wild strains of *C. elegans* is due to natural variation in *npr-1*, a predicted G protein–coupled receptor in the NPY receptor family.

Results

Wild Strains of *C. elegans* Vary in Their Response to Food and Other Animals

Wild strains of *C. elegans* exhibit one of two foraging behaviors on a lawn of *E. coli* bacteria (Table 1; Hodgkin and Doniach, 1997; R. Cassada, personal communication). Some wild strains like the English strain N2 are solitary foragers. These strains disperse across a bacterial lawn and browse alone (Figures 1A and 1C). Other wild strains like the German strain RC301 and the English strain CB4932 are social foragers and aggregate together on food to form clumps (Figures 1B and 1D; the clumping phenotype is abbreviated Clp). A clump can contain from three to several hundred worms. We quantitated this simple social behavior in the German strain RC301 by measuring the percentage of animals in a population that clumps at any one time. More than 50% of RC301 animals were in clumps, compared to less than 2% of N2 animals (Figure 1E).

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Table 1. Behavior of Natural Isolates of *C. elegans*

Standard Name	Source	Clp-Bor	Speed (μm/s) −Food	Speed (μm/s) +Food
npr-1(ad609)	—	+	225	183
CB4856	Hawaii	+	196	203
AB3	Adelaide, Australia	+	230	191
CB4858	Pasadena, California	+	233	190
CB4857	Claremont, California	+	261	189
CB4854	Altadena, California	+	268	184
AB1	Adelaide, Australia	+	211	182
CB4852	England?	+	228	178
KR314	Vancouver, Canada	+	275	170
CB4932	Taunton, England	+	202	169
CB4855	Palo Alto, California	+	228	168
RC301	Freiburg, Germany	+	240	156
CB4853	Altadena, California	+	169	149
TR389	Madison, Wisconsin	−	228	117
CB3191	Altadena, California	−	203	115
N2	Bristol, England	−	232	109
TR403	Madison, Wisconsin	−	170	82
CB4555	Pasadena, California	−	157	75

The geographical origin of each wild strain is indicated together with its behavioral phenotype. The *npr-1(ad609)* mutant is included for comparison. Clp and Bor (clumping and bordering) behaviors were scored qualitatively in all strains. The average speed of animals in the presence ($n > 25$) and absence ($n > 46$) of food is also given; the SEM is smaller than 10 μm/s for each value.

Social and solitary wild strains exhibited other behavioral differences, particularly in their responses to food. Clumps of social animals tended to burrow into the agar medium (Hodgkin and Doniach, 1997), while solitary strains such as N2 burrowed far less. Solitary strains dispersed evenly across the *E. coli* lawn (Figure 1C), while social strains accumulated where bacteria was thickest, usually at the edges of the lawn (Figure 1D; R. Cassada, L. Avery, personal communication). The tendency of social worms to accumulate at the edge of the bacterial lawn is called bordering (abbreviated Bor; R. Cassada, personal communication; Hodgkin and Doniach, 1997). More than 95% of the social RC301 worms accumulated at the border, but only about 15% of the solitary N2 worms bordered (Figure 1F); the distribution of N2 was approximately that predicted by random dispersal across the bacterial lawn. Bacteria can be made to grow more thickly in specific regions of the lawn by adding glycerol to those regions. Under these circumstances, social foragers accumulated at the regions with abundant bacteria even if these were not at the edge of the lawn (data not shown).

The preference of social strains for thicker parts of the bacterial lawn was not sufficient to explain their clumping behavior; at low nematode density it was clear that social animals did not distribute evenly on thick parts of the lawn but instead aggregated into dense groups. This observation suggests that animals clump due to mutually attractive stimuli, but the nature of the stimulus is unknown. Clumping did not occur in the absence of *E. coli*, suggesting that food plays an important role in social behavior.

Since N2 animals clump when food is limiting, the clumping behavior of social wild strains could be due to poor perception of food. To examine this possibility, eight wild social strains were characterized for their chemotaxis responses to a panel of odorants. Although some variation was observed between different strains, the overall chemotaxis responses of the social strains were similar to those of N2, indicating that the social strains did not have a significant decrease in olfactory sensitivity (data not shown).

Isolated Social Animals Move Twice as Quickly as Solitary Animals on Food

When we used a videotracking system to follow animals and analyze their movement, we found that individual animals from social and solitary strains differed in their locomotion even in the absence of other animals. Animals from all solitary wild strains moved more slowly on food than animals from social wild strains (Table 1). By contrast, both solitary and social strains moved at a similar rapid speed in the absence of bacteria. Food induced a roughly 2-fold inhibition of movement in solitary but not social strains (Table 1), so that on average, social animals moved almost twice as quickly as solitary animals on food. However, social animals changed their locomotory pattern when they joined a clump. When moving on food, social animals made long forays at speeds of about 190 μm/s. When they joined a clump, social animals dramatically reduced their speed and reversed frequently to stay in the clump.

Mutations in *npr-1* Convert a Solitary Strain into a Social Strain

To gain more insight into genetic components of social behavior, we characterized EMS-induced mutations in the solitary strain N2 that caused N2 to exhibit social behaviors. Three such mutations, *n1353*, *ad609*, and *ky13*, were kindly provided by L. Avery, S. Hekimi, and H. Colbert, respectively. All of these mutants exhibited a striking clumping behavior that was stronger than that of the dauer-constitutive clumping mutants (Thomas et al., 1993), and none of the mutants appeared to have defects in dauer formation. Interestingly, these chemically induced mutant strains behaved like the social wild strains in all respects. Like the social wild strains, the EMS-induced social mutants aggregated together on bacteria, accumulated where bacteria were thickest, and burrowed into the agar (Figure 1 and data not shown). The average speed of the mutants was indistinguishable from wild-type N2 animals in the absence of food, but in the presence of food the social mutants moved twice as quickly as N2 (Figure 2A). These results suggest that single gene mutations can give rise to all of the behavioral differences characteristic of wild social and solitary strains. Genetic analysis indicated that the three alleles were recessive for all phenotypes, failed to complement one another, and mapped to the same position on the left arm of the X chromosome (Figure 2B and data not shown; see Experimental Procedures), suggesting that they were due to lesions in a single gene, called *npr-1* (for *n*euro*p*eptide receptor *r*esemblance, see below). The phenotypes caused by the *ky13* and *ad609* alleles were not altered over a deficiency that deletes the *npr-1* locus (Figure 2B).

Since mutations in *npr-1* altered several aspects of

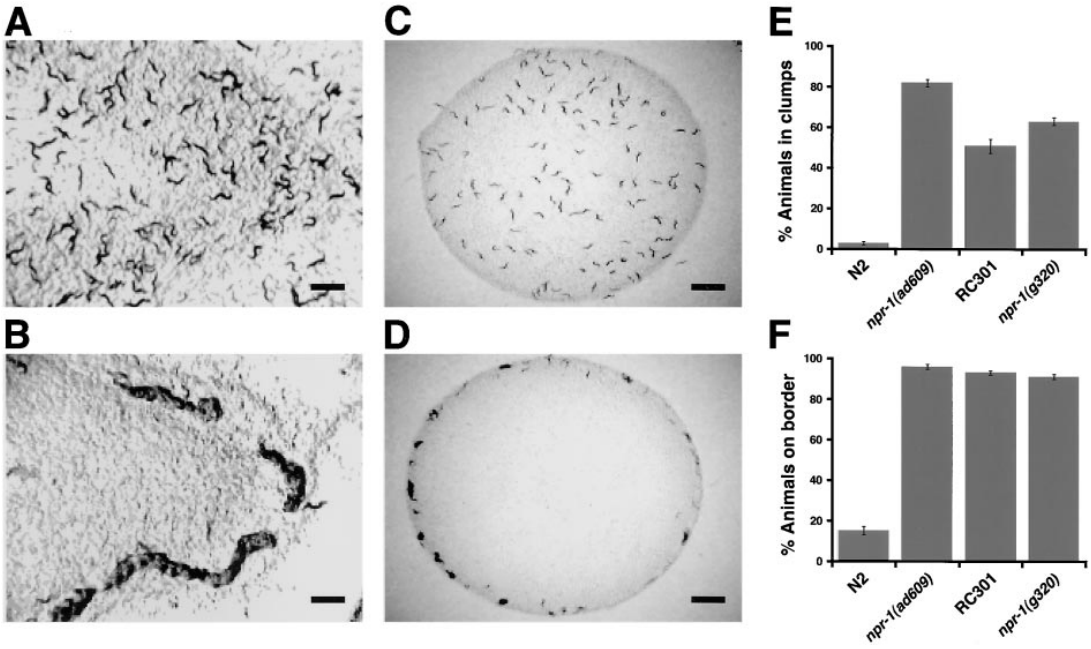

Figure 1. Solitary and Social Behavior

(A) and (C) Solitary animals disperse evenly across a bacterial lawn and browse alone.

(B) and (D) Social animals aggregate together into clumps and accumulate at the edge of the lawn where bacteria are thickest.

Animals in (A) and (C) are from the N2 wild strain; animals in (B) and (D) are of genotype *npr-1(ad609)*. Wild social strains behave like *npr-1(ad609)* mutants. The scale bars represent 1 mm in (A) and (B) and 2.5 mm in (C) and (D).

(E) Percentage of animals aggregated in clumps. A clump is defined as three or more animals that are in contact along at least half their length.

(F) Percentage of animals that accumulate at the border. The border is a ring ~2 mm wide at the outer edge of a circular lawn where bacteria grow more thickly. Lawns used in these assays were approximately 25 mm in diameter (typical assays are shown in [C] and [D]). For (E) and (F), each data point represents the average of at least eight independent assays using ~120 well-fed adult animals per assay (error bars equal the SEM). The genotype of each strain tested is indicated under the bar graph. N2 and RC301 are solitary and social wild strains, respectively. *npr-1(ad609)* is an EMS allele from N2; *npr-1(g320)* (strain CX4057) was isolated from RC301 following repeated backcrosses with N2.

C. elegans behavior, it seemed possible that some of the phenotypes of the social *npr-1* mutants might be indirect effects of another behavioral change. For example, animals that had experienced aggregation might subsequently exhibit rapid movement on bacteria. To test this possibility, we allowed single eggs of genotype *npr-1(ad609)* to grow to adulthood in isolation and then recorded their locomotion on food. The adults raised in isolation were just as active as *npr-1(ad609)* worms grown in a population (181 μm/s; n = 7; SEM = 14), moving twice as quickly on food as wild-type N2 grown in isolation (78 μm/s; n = 8; SEM = 24). The increased locomotory activity of *npr-1* mutants is therefore not a consequence of clumping. Conversely, high locomotor activity might cause clumping and/or bordering. To test this possibility, we examined the effects of the *npr-1* mutation in sluggish mutant backgrounds. *npr-1* mutations induced distinct clumping and bordering behaviors in uncoordinated mutants such as *unc-25* and *unc-29*, indicating that these behaviors do not require hyperactivity. Moreover, hyperactivity is not sufficient to generate clumping or bordering: several hyperactive mutants including *goa-1* (Mendel et al., 1995; Segalat et al., 1995) do not clump or border.

The N2 wild strain and the *npr-1* mutant could differ either in the production of a signal that induced clumping or in the response to a clumping signal from other animals. These possibilities were examined by mixing animals of different genotypes. A group of *npr-1* animals marked with the *lon-2* mutation was mixed with either wild-type N2 animals or unmarked *npr-1* mutant animals and allowed to clump on a plate. While *npr-1* mutants would join a group of *lon-2 npr-1* animals, wild-type N2 animals would not join the group (n = 20). These results demonstrate that N2 and *npr-1* mutants differ in their response to other animals. It remains possible that *npr-1* and N2 animals also differ in production of a clumping signal, but that possibility has not been tested.

The behavior of social wild strains is very similar to that of *npr-1* mutants. This observation suggested that variation in *npr-1* could account for the behavioral differences between social and solitary wild strains. To ask whether this was the case, we constructed congenic inbred strains that had sequences from the wild social strain RC301 around the *npr-1* locus but had N2 sequences at all other loci by extensive backcrossing of RC301 by N2 (Leon Avery, personal communication; see Experimental Procedures). Six out of six independently outcrossed strains exhibited strong social behavior, similar to social behavior in the original RC301 strain (Figures 1 and 2). Since the outcrossing was performed without reference to the social phenotype, these results

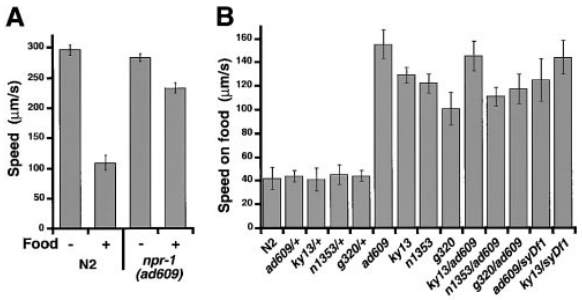

Figure 2. Isolated *npr-1* Mutant Animals Are Hyperactive on Food

(A) Movement of isolated N2 and *npr-1(ad609)* animals in the presence and absence of food. The presence or absence of food is indicated below each bar.

(B) Genetic characterization of *npr-1* alleles. The three *npr-1* mutants studied, *ad609*, *ky13*, *n1353*, and the wild allele *g320* are all hyperactive on food compared to N2 animals. The *g320* allele was isolated from the social strain RC301 after repeated backcrossing with the solitary N2 strain. For (A), each data point represents the average speed of at least 24 animals scored during more than 72 min of recording; for (B), at least 4 animals were analyzed, representing more than 12 min of recordings. Error bars are SEM for both (A) and (B). Animals were not clumped during the recordings. The low values for speed in the presence of food in (B) (compared to other assays shown) are due to a thicker bacterial lawn: thicker food induces a stronger slowing response.

show that a single region of the RC301 genome linked to *npr-1* was sufficient to generate a social behavioral phenotype. This region of the RC301 genome has been given the allele name *g320*.

Further mapping data indicated that *g320* mapped to a location close to *npr-1* (see Experimental Procedures). The clumping, bordering, and locomotory phenotypes of the *g320* allele, which were all recessive, were not complemented by alleles of *npr-1* (Figure 2B and data not shown). These results suggest that the natural variation in foraging behavior seen between N2 and RC301 could be caused by variation at the *npr-1* locus. The backcrossed *npr-1(g320)* strains were less social and less hyperactive than the EMS-induced *npr-1* strains (Figures 1E and 2B), suggesting that the EMS-induced mutations were more disruptive of *npr-1* function than the natural *g320* allele.

npr-1 Encodes a Member of the Neuropeptide Y Receptor Family

The *npr-1* gene was cloned from the solitary N2 strain by genetic mapping followed by rescue of the social and hyperactive behavioral phenotypes of *npr-1(ad609)* mutants (Figure 3A). In this and subsequent transgenic experiments, we present data for rescue of the hyperactive phenotype; however, in all instances rescue of hyperactivity coincided with rescue of the clumping and bordering phenotypes. Rescuing activity was narrowed down to a 9 kb fragment of cosmid F10A4 (pM4, Figure 3A). Data from the *C. elegans* genome project suggested that this region contained a single predicted open reading frame, C39E6.6, which encodes a seven transmembrane domain receptor. This receptor most closely resembles members of the neuropeptide Y receptor (NPY receptor) family (Figures 3B and 3C). A frameshift mutation that changed the reading frame of this receptor after transmembrane domain 6 abolished rescuing activity (pM5, Figure 3A). These results suggest that *npr-1* is encoded by the neuropeptide Y receptor–like gene.

To confirm the identity of the *npr-1* gene, we sequenced the NPY receptor–like open reading frame in the three EMS-induced *npr-1* mutants. The allele *ky13* had a C to T transition that introduces a stop codon after the first transmembrane domain; this allele is therefore a putative null mutation of *npr-1*. The allele *n1353* had a glycine to aspartate substitution in transmembrane domain 3 of the receptor, and the allele *ad609* was associated with two substitutions: a threonine to isoleucine change in transmembrane domain 2, and a threonine to alanine change in transmembrane domain 4 (Figure 3C).

Outside *C. elegans*, *npr-1* is most closely related to the mammalian NPY receptors Y1, Y4, and Y6; for example, it shares 37% amino acid identity with Y4 in the transmembrane regions (Figure 3C). Y4 is one of five subtypes of NPY receptors from various mammalian species that define the NPY receptor family (Blomqvist and Herzog, 1997). Different NPY receptors from humans show between 30% and 51% amino acid identity overall and 40%–62% identity in the transmembrane regions (Figure 3C; Blomqvist and Herzog, 1997). As is typically observed for seven transmembrane domain receptors, similarity is less striking in the loop regions. The predicted intracellular C-terminal tail of *npr-1* is unusually long compared to that of NPY receptors, and NPR-1 ends in the sequence TNV, a potential match to the S/TXV motif that binds PDZ domain–containing proteins and clusters some receptors (Kornau et al., 1997).

The *C. elegans* genome includes at least six potential members of the NPY receptor family besides *npr-1* (*C. elegans* Sequencing Consortium, and our unpublished results). Like *npr-1*, these receptors showed highest homology to NPY receptors in the transmembrane regions. The closest relative to *npr-1* is *npr-2*, a receptor found on cosmid T05A1, which shares 46% amino acid identity with *npr-1* over a 384 amino acid stretch.

To examine *npr-1* expression, we fused the coding region for the green fluorescent protein (GFP) to the *npr-1* gene 24 amino acids after the seventh predicted transmembrane domain. This reporter construct, which truncates the C-terminal tail of NPR-1 at position 366, restores solitary behavior to *npr-1(ad609)* mutants (Figure 3A). NPR-1:GFP is expressed in neurons, including neurons in the head, the ventral nerve cord, and the preanal ganglion (Figure 4 and data not shown). Thus, *npr-1* is likely to function in neurons to regulate behavior.

Natural Variation in the Response of *C. elegans* to Food and Other Animals Is Associated with a Single Amino Acid Substitution in *npr-1*

The clumping allele *g320* derived from the wild strain RC301 behaved genetically like an allele of *npr-1*: it had the same phenotypes as alleles of *npr-1*, it mapped to the *npr-1* region, and it failed to complement *npr-1* mutations and a deficiency of the *npr-1* region. Since the social behavior associated with the *g320* allele was recessive to N2 (Figure 2B), we asked whether the N2

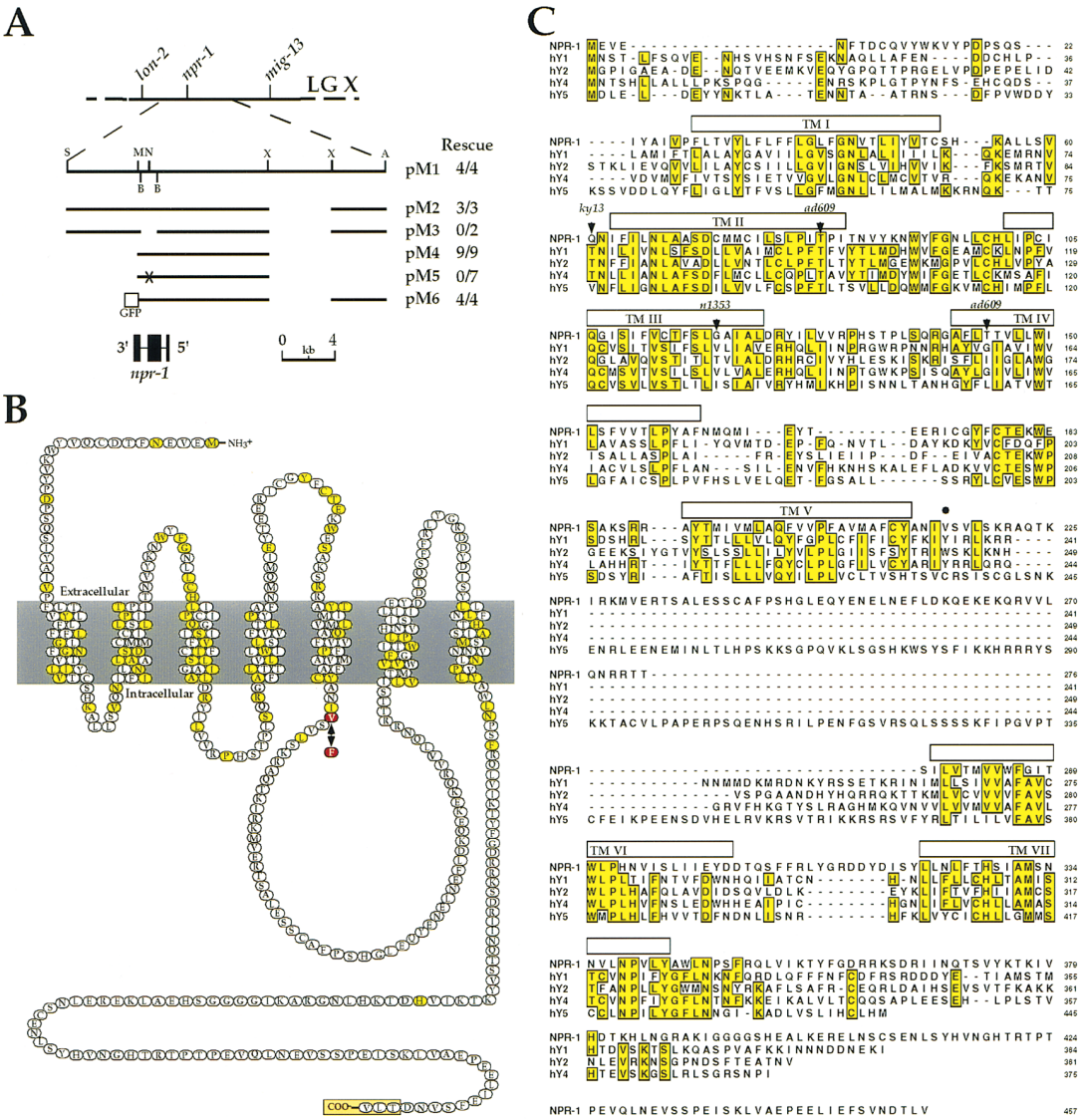

Figure 3. Cloning and Sequence Analysis of *npr-1*

(A) The location of *npr-1* on linkage group X (LGX) is indicated at the top. Subclones from the *lon-2/mig-13* interval were tested for rescue of the hyperactivity and clumping phenotypes of *npr-1(ad609)*. For each experiment, the number of independent transformed lines rescued is given as a fraction of the total number of lines analyzed. Rescue was scored if animals moved with speeds that were not significantly different from N2 controls (typical results are shown in Figure 5A). The structure of the *npr-1* gene is shown at the bottom. Boxes represent exons. pM5 has a frameshift mutation that truncates NPR-1 after transmembrane domain 6; the location of the frameshift is indicated by an X. In pM6, the open reading frame for GFP (indicated by the box) is fused in frame to the *npr-1* open reading frame. A, ApaI; B, BamHI; M, MluI; N, NcoI; S, SacII; X, XhoI.

(B) Predicted amino acid sequence and transmembrane topology of *npr-1*. The topology is based on that predicted for other members of the G protein–coupled receptor family, with the N-terminal region extracellular. The membrane is represented by the shaded box. Letters correspond to the deduced primary sequence of NPR-1. Highlighted in yellow are residues that are identical between *npr-1* and at least two human NPY receptors (see [C]). Amino acid residue 215 at the N-terminal region of intracellular loop 3 is valine in solitary wild strains and phenylalanine in social wild strains (indicated by the red balls and double headed arrow). The orange box at the C terminus highlights a potential match to the motif S/TXV that binds PDZ domain–containing proteins and clusters some receptors. The predicted *npr-1* sequence obtained from cDNAs is identical to the predicted gene C39E6.6 (Genbank U49944, PID:g1208818).

(C) Alignment of NPR-1 with human NPY receptors. Yellow boxes indicate residues that are identical in at least three of the five receptors. Amino acids are numbered beginning at the first methionine. The seven predicted transmembrane domains are indicated by open boxes above the sequence. Arrowheads indicate residues mutated in EMS alleles of *npr-1*: Q61Stop in *ky13*; T83I and T144A in *ad609*; and G118D in *n1353*. Residue 215, which is valine in wild solitary strains and phenylalanine in wild social strains, is marked by a black dot. The GFP fusion pM6 truncates the C-terminal tail of NPR-1 at residue 366.

version of the *npr-1* gene could transform RC301 from social to solitary behavior. Strikingly, when the N2-derived *npr-1* clone was introduced into RC301, the resulting transgenic strains were solitary and behaved indistinguishably from N2 (Figure 5A). Two other social wild strains, the Australian strain AB3 and the Hawaiian

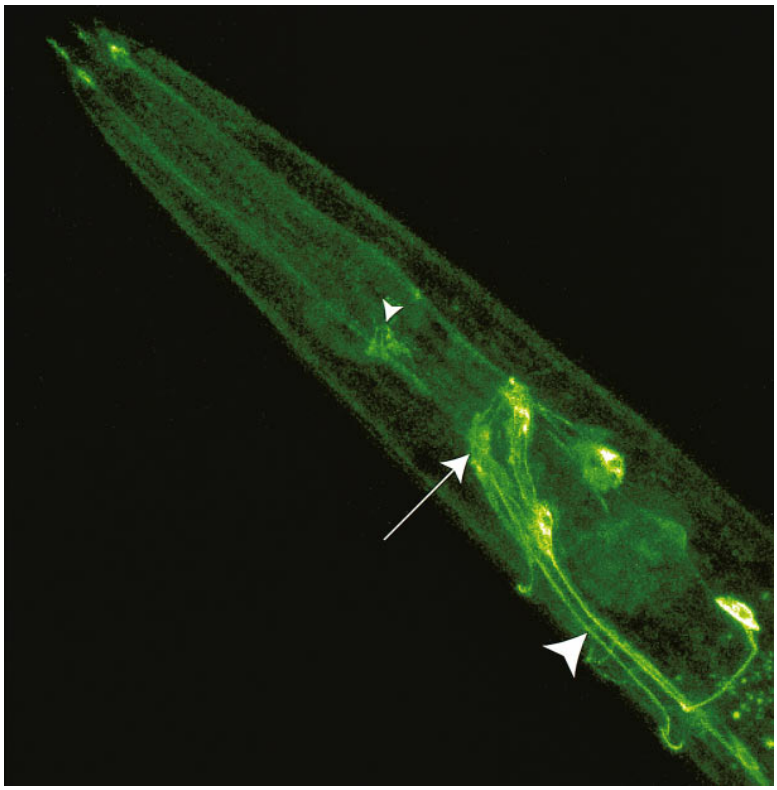

Figure 4. NPR-1 Is Expressed in Neurons

Confocal projection of an adult hermaphrodite showing *npr-1:GFP* expression in neurons in the head (ventro-lateral view; anterior is top left). GFP expression is driven from construct pM6, which encodes C-terminally truncated NPR-1 tagged at its C terminus with GFP (See Figure 3A). The animal shown is *npr-1(ad609)* but is restored to solitary behavior by the rescuing pM6 construct. Expression is concentrated in the nerve ring, the major process bundle of the head (arrow). Several neuronal cell bodies are visible; since this staining is widespread and faint, it has not been characterized in detail. Neuronal processes of the ventral nerve cord (large arrowhead) and pharynx (small arrowhead) also express GFP.

strain CB4856, were also converted to solitary behavior by transgenic expression of N2-derived *npr-1* (Figure 5A and data not shown). These data suggest that natural variation at *npr-1* might contribute to the behavioral differences between wild social and solitary strains.

To identify the difference between the *g320* and the N2 alleles of *npr-1*, we sequenced the *npr-1* open reading frame from the wild strain RC301. RC301 and N2 differed at only one base pair in the *npr-1* coding region, resulting in a valine (N2) to phenylalanine (*g320*) substitution at position 215 close to transmembrane domain 5 in the third intracellular loop of the receptor (Figures 3B and 5B). All other *npr-1* exons were identical between these two strains, suggesting that the change at residue 215 could alter *npr-1* function.

We then sequenced *npr-1* in 15 other wild strains of *C. elegans* isolated from all over the world. Eleven of these are social strains similar to RC301, while four are solitary strains similar to N2 (Table 1; Hodgkin and Doniach, 1997). Like RC301, all 11 social strains had a phenylalanine at position 215, while the four solitary strains and N2 all had a valine at this position (Figure 5B). The region sequenced included all 1.4 kb of exon sequences and 300 bp of intron sequences. All other *npr-1* coding sequences were identical across the 17 strains, but the presence of other polymorphisms confirmed that the strains represent independent isolates and not multiple isolates of the same strain. Within the *npr-1* locus, the two social strains CB4856 and CB4932 both had an insertion of approximately 50 bp in intron 1, and CB4932 had an additional two changes, a deletion of a single T residue in intron 3, and a silent codon substitution in exon 3. Since the polymorphisms detected in *npr-1* were all in social strains, we used polymorphisms described previously (Egilmez et al., 1995) to confirm that two of the solitary strains, CB4555 and TR403, were different from one another and from N2. Thus, despite their geographically disparate origins, the wild strains displayed a perfect correlation between NPR-1 215F and social behavior, and NPR-1 215V and solitary behavior.

NPR-1 215F and NPR-1 215V Have Different Biological Activities

To prove that the single amino acid difference between the natural social and solitary *npr-1* alleles altered receptor function, we specifically substituted phenylalanine for valine at *npr-1* codon 215 in pM4, the N2-derived *npr-1* rescuing clone. When this clone, *npr-1* V215F, was injected into *npr-1* mutants at standard concentrations (20 ng/μl), it failed to restore them to solitary behavior (Figure 6A). The V215F clone was also injected into N2 animals at high concentration (200 ng/μl) to ask whether overexpression would convert the solitary N2 strain to a social strain. Ten independent N2 lines that were transgenic for *npr-1* V215F retained solitary behavior, indicating that *npr-1* V215F could not induce social behavior. These results are consistent with the observation that the solitary *npr-1* 215V allele is dominant to the social *npr-1* 215F allele (Figure 2B).

If NPR-1 V215F represents a less active version of the protein than NPR-1 215V, high copy expression of V215F might restore solitary behavior to *npr-1* mutants. To test this hypothesis, the *npr-1* V215F clone and the parent pM4 *npr-1* 215V clone were compared for their ability to restore solitary behavior to *npr-1(ad609)* mutants after they were injected at three concentrations: 20 ng/μl, 60 ng/μl, and 200 ng/μl. Increasing the concentration of *npr-1* clone injected in this protocol is likely to lead to

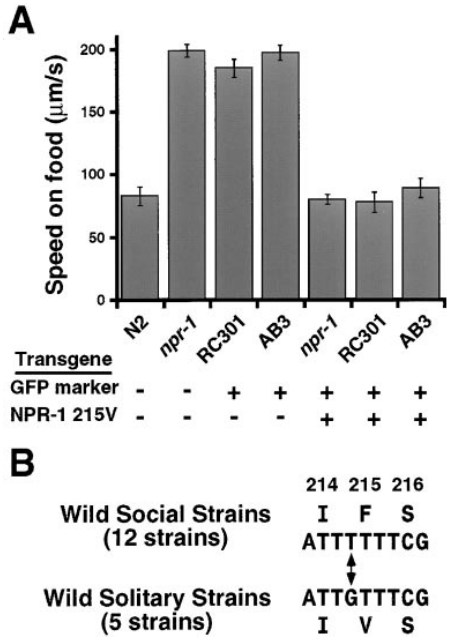

Figure 5. The NPR-1 215V Allele Is Sufficient for Solitary Foraging Behavior

(A) An NPR-1 215V transgene induces solitary foraging behavior in social strains. The genotype of each strain is indicated under the bar. Also indicated is whether animals tested carried extrachromosomal transgenes of NPR-1 215V and/or a GFP marker. N2 is an English wild solitary strain, while AB3 and RC301 are wild social strains from Australia and Germany. The *npr-1* allele used was *ad609*. Each data point represents the average speed of at least 27 animals, each of which was recorded for 3 min (error bars are SEM). The GFP marker is plasmid pTG96, which expresses GFP in most somatic tissues (Gu et al., 1998; see Experimental Procedures).

(B) NPR-1 differs at a single residue between social and solitary wild strains. The sequence of NPR-1 in social and solitary strains is indicated for the region encoding amino acids 214 to 216. All social wild strains encode phenylalanine at position 215 of NPR-1 (TTT), while all solitary wild strains encode valine (GTT). NPR-1 protein is otherwise identical between wild strains.

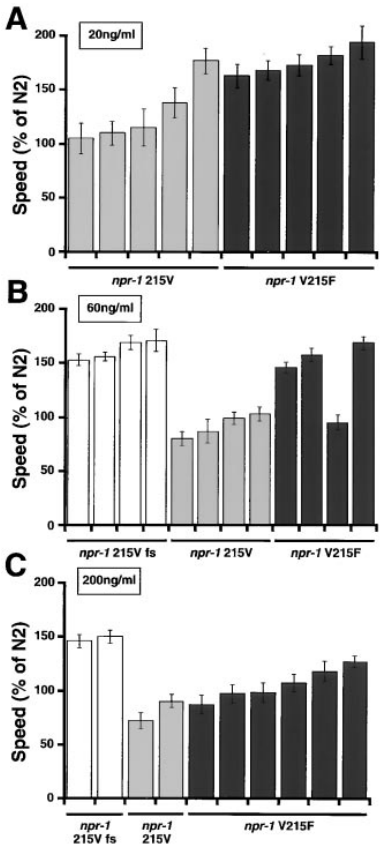

Figure 6. NPR-1 215F Represses Social Foraging Behavior More Weakly than NPR-1 215V

Movement of *npr-1(ad609)* animals transgenic for *npr-1* constructs injected at 20 ng/μl (A), 60 ng/μl (B), and 200 ng/μl (C). Data points are standardized to the speed of N2, which is 100%. Bars represent independent transgenic lines: open bars, lines carrying *npr-1* 215V fs (fs, frameshift), encoding a frameshifted, nonfunctional *npr-1* clone (plasmid pM5, Figure 3A); light shaded bars, lines carrying *npr-1* 215V (plasmid pM4, Figure 3A), a rescuing clone derived from N2; dark shaded bars, lines carrying *npr-1* V215F (plasmid pM4 with an engineered V215F substitution). Each data point represents the average speed of at least 16 animals, each of which was recorded for 3 min (error bars are SEM).

increasing *npr-1* overexpression in transgenic animals (Roayaie et al., 1998). The control *npr-1* 215V construct restored solitary foraging behavior to *npr-1(ad609)* mutants at all three concentrations injected (10/11 transgenic lines examined were rescued; Figure 6). In contrast, the *npr-1* V215F construct did not restore solitary behavior to *npr-1(ad609)* mutants when injected at 20 ng/μl (Figure 6A) and only occasionally rescued when injected at 60 ng/μl (Figure 6B). However, when injected at 200 ng/μl, NPR-1 V215F frequently did restore solitary foraging behavior to *npr-1(ad609)* mutants (Figure 6C). These results indicate that NPR-1 215F is functionally different from NPR-1 215V: NPR-1 215V efficiently inhibits social foraging behavior, but NPR-1 215F can only do so weakly.

Discussion

Different Wild *C. elegans* Strains Exhibit Distinct Behavioral Patterns

Natural isolates of *C. elegans* fall into two groups that exhibit distinctive patterns of foraging behavior. The laboratory strain N2 and four other strains are solitary on a lawn of *E. coli*, disperse evenly across the bacterial lawn, and slow their movement on *E. coli*. RC301 and 11 other strains accumulate in clumps, remain at the edge of a bacterial lawn, move rapidly on a lawn of *E. coli*, and slow their movement when they join a clump. Remarkably, this entire constellation of behavioral differences can be attributed to alteration of a single gene, the predicted neuropeptide receptor gene *npr-1*. Null mutations in *npr-1* cause the solitary N2 strain to exhibit all characteristics of wild social strains, and introduction of the N2 *npr-1* allele into several wild social strains converts all of their behaviors into the solitary N2 behavior pattern.

A single nucleotide change that alters a valine to a phenylalanine in *npr-1* is associated with the behavioral differences between all 17 social and solitary wild strains. We suggest that this mutation arose only once. The 5 solitary strains came from England, California, and Wisconsin, while the 12 social strains came from England, Germany, Australia, Hawaii, California, and British

Columbia. Thus, irrespective of whether the social or the solitary isoform of *npr-1* is ancestral, strains bearing the new isoform must have spread from their origin to several continents. In at least one instance, social and solitary wild strains were isolated from the same sod of earth (Hodgkin and Doniach, 1997). The spread of social and solitary strains to different parts of the globe, and their coexistence in at least some locations, suggests that the two foraging strategies provide selective advantages under different environmental conditions.

The difference between the social and solitary strains is only apparent in the presence of a bacterial lawn. When bacteria become limiting, all of the strains form swarms or clumps, and when bacteria are absent, all of the strains are solitary and active. These results indicate that different food environments may favor solitary or social behavior. In the laboratory, *C. elegans* is routinely maintained on a diet of *E. coli* (Brenner, 1974), but bacteria in the wild vary greatly in the chemicals that they produce (Dainty et al., 1985; Zechman and Labows Jr., 1985) and in their attractiveness to *C. elegans* (Andrew and Nicholas, 1976; Grewal and Wright, 1992). If the presence of particular bacterial metabolites normally regulates the transition between social and solitary behavior, the preferred behavior could be different on different bacterial species. It is likely that behaviors we observed represent a narrow view of a more complex behavioral choice.

Clumping behavior, and the related phenomena of swarming and synchronized movements, have been described in plant parasitic nematodes, marine nematodes, and various rhabditid species (McBride and Hollis, 1966; Croll, 1970), suggesting that these behaviors are ancient. The significance of swarming is unknown, but for some nematodes, it has been shown to be species specific, nonsexual, and density dependent (Hollis, 1960, 1962).

Several different models could explain the diverse behavioral phenotypes of *npr-1* mutants. In one model, *npr-1* activity could repress a swarming response, perhaps by inhibiting sensory neurons that detect pheromones or other neurons in a pheromone response circuit. Food could stimulate production of an *npr-1* ligand; as food levels dropped, the solitary N2 strain would derepress the swarming response and clump. The less potent social *npr-1* allele could favor clumping even in plentiful food. The effect of *npr-1* on the movement of individual animals might be an intrinsic part of the swarming response, or it could be part of a distinct pathway by which the receptor modulates movement. Resolution of these models awaits identification of the cells in which *npr-1* acts, and the cells that are the source of *npr-1* ligands. Interestingly, mild clumping is induced in the N2 strain by mutations in the dauer pathway, a sensory response that is regulated by food and pheromone (Thomas et al., 1993). It may be that some of the neurons that regulate dauer formation also participate in the social response.

Natural variation of foraging behavior in *Drosophila* (Sokolowski, 1980; Osborne et al., 1997; Sokolowski et al., 1997) bears striking parallels to the natural variation we observe in *C. elegans*. Wild *Drosophila* larvae from "sitter" strains are less active than wild "rover" larvae in the presence of food. In the absence of food, sitters and rovers are indistinguishable. This difference in locomotory behavior is reminiscent of that observed between *C. elegans* social (∼roving) and solitary (∼sitting) strains, although *Drosophila* sitter and rover strains are not known to have different social behaviors. As in *C. elegans*, the behavioral differences between *Drosophila* rover and sitter strains are largely due to variation at a single locus. The *foraging* gene encodes a cyclic guanosine monophosphate (cGMP)-dependent protein kinase, and sitter strains have reduced levels of this kinase compared to rover strains (Osborne et al., 1997). The evolutionary significance of the different foraging strategies in *Drosophila* wild strains has been explored using competition experiments (Sokolowski et al., 1997). When both rovers and sitters are grown together for many generations at high population densities (crowded conditions), rover strains outcompete sitter strains, while conversely, under low population density, sitter strains outcompete their rover rivals. This result suggests that when food is abundant the less energetic sitter strategy is at a selective advantage, and when food is scarce, rovers that actively seek new food patches are at a selective advantage. Perhaps analogous selections operate on *C. elegans* social-rover and solitary-sitter strains at different population densities.

A Neuropeptide Y Receptor Homolog Regulates Choice between Social and Solitary Behavior

npr-1 encodes a predicted seven transmembrane receptor of the neuropeptide Y receptor family. A null mutation in *npr-1* converts the solitary N2 wild strain into a social strain. This result indicates that *npr-1* activity represses social behavior or activates solitary behavior.

Neuropeptide Y receptors are typical G protein–coupled receptors that activate heterotrimeric guanine nucleotide–binding proteins (G proteins) to modulate the activity of enzymes, ion channels, and second messenger pathways within the cell (Strader et al., 1994; Gudermann et al., 1997). The neuropeptide Y (NPY) receptor family includes at least six receptor subtypes that are thought to mediate the activity of neuropeptide Y, peptide YY, and pancreatic polypeptide (Blomqvist and Herzog, 1997). The physiological effects of neuropeptide Y include the regulation of food consumption, mood, anxiety, memory retention, hippocampal excitability, and blood pressure (Wahlestedt and Reis, 1993; Heilig and Widerlov, 1995; Baraban et al., 1997). Peptide YY and pancreatic polypeptide act mostly in the periphery, where they regulate pancreatic or gastrointestinal function. It is interesting that the nematode *npr-1* gene also acts in the response to food.

The similarity to mammalian NPY receptors suggests that *npr-1* has a neuropeptide ligand, but there are no obvious NPY homologs in the *C. elegans* genome. However, there are other predicted neuropeptides that might encode *npr-1* ligands (Rosoff et al., 1993; Brownlee et al., 1994; Maule et al., 1996).

The two versions of NPR-1 observed in 17 wild strains of *C. elegans* differ only at amino acid position 215. The solitary valine 215 isoform has an activity that is

dominant to the social phenylalanine 215 form based on two criteria. First, a cross between N2 and a strain that is homozygous for the natural 215F *g320* allele but otherwise isogenic to N2 generates solitary heterozygous progeny. Second, expression of *npr-1* 215V from a transgene is sufficient to make a wild social strain behave like a solitary strain, but an *npr-1* 215F transgene did not alter the behavior of a wild solitary strain. The importance of residue 215 was confirmed in experiments in which codon 215 in the N2 *npr-1* clone was mutated from valine to phenylalanine. Expression of *npr-1* 215V rescued the EMS-induced *npr-1(ad609)* mutation, but expression of *npr-1* V215F had the same effect only in high-copy transgenic lines that probably overexpress the receptor.

Residue 215 is in the third intracellular loop of the predicted NPR-1 protein in a region that is important for G-protein coupling in many seven transmembrane receptors. Some studies suggest that this residue contributes to the specificity of G-protein coupling (Bluml et al., 1994b; Burstein et al., 1996; Gudermann et al., 1997). In particular, an aromatic amino acid at a position corresponding to residue 215 of *npr-1* has been shown to be critical for muscarinic receptor-mediated activation of phospholipase C (Bluml et al., 1994a; Burstein et al., 1996); a receptor with a valine at this position cannot activate Gq. Amino acid substitutions at position 215 of *npr-1* might therefore be expected to alter the strength or the specificity of G-protein coupling.

It is likely that the social *npr-1* 215F variant obtained from the wild encodes a functional G-protein receptor, albeit one that is different from *npr-1* 215V. Clumping behavior is substantially weaker in RC301 or the outcrossed wild social strain *npr-1(g320)* than in *npr-1(null)* mutants, indicating that *npr-1* 215F is still capable of repressing social behavior. Comparative sequence analysis is also consistent with a function for *npr-1* 215F: most members of the NPY receptor family, including the Y1, Y2, and Y4 receptors (which have been cloned from many species), have an aromatic residue at the position corresponding to residue 215 in *npr-1* (Figure 3C). Even *npr-2*, the closest *C. elegans* homolog of *npr-1*, has a phenylalanine at this position.

Neuropeptides are present throughout the brain and are often released together with classical neurotransmitters. In most circumstances, their G protein–coupled receptors do not act in classical fast neurotransmission but rather in processes that regulate the probability of a neuronal response over longer time periods. Interventions that enhance or decrease neuropeptide function can have potent and long-lasting effects on choice of behavior patterns. We suggest that changes in neuropeptide pathways could be a widespread mechanism for generating natural variation in behavior.

Experimental Procedures

Strains

All nematodes were grown at 20°C under standard conditions (Brenner, 1974). Wild strains of *C. elegans* were obtained from the *Caenorhabditis* Genetics Center and are described in Hodgkin and Doniach (1997). All three *npr-1* mutants were obtained following ethylmethanesulfonate mutagenesis of the canonical wild strain N2. *ad609* was isolated by Siegfried Hekimi, *n1353* by Leon Avery, and *ky13* by Heather Colbert. The *npr-1* strains used in this study were DA609 *npr-1(ad609)*, DA508 *npr-1(n1353)* (both obtained from Leon Avery), and CX3048 *npr-1(ky13)*. All these strains were backcrossed twice with N2. DA650, a derivative of RC301 that had been outcrossed ten times with N2 by Leon Avery, was used as a starting stock to outcross *npr-1(g320)* further (see below). Other strains used were as follows: MT2211, *dpy-3(e27) unc-2(e55)* X; CF333, *lon-2(e678) mig-13(mu31) dpy-8(e130)* X; CX2780, *unc-6(n102) dpy-7(e88)* X; and PS1032, *unc-2(e55) lon-2(e678)/syDf1* X. Strains generated in this work were CX4055, *npr-1(ad609) lin-15(n765ts)* X; CX4056, *lon-2(e678) npr-1(ad609* X ; and CX4057, *npr-1(g320)* X.

Behavioral Assays

Bordering and clumping behaviors were quantitated simultaneously. NGM plates containing 2.1% agar were seeded 2 days before the assay with 200 μl of *E. coli* OP50 in LB medium. Micropipetting this amount of bacteria resulted in a circular lawn about 25 mm in diameter. Approximately 120 well-fed adult worms from uncrowded plates were picked onto this lawn and left at 20°C for 3 hr. After 3 hr, clumping behavior was measured by calculating the fraction of animals that were in contact with two or more other animals along at least 50% of their body length. At the same time, bordering behavior was measured by calculating the fraction of animals that resided within 2 mm of the edge of the bacterial lawn.

Movement speed was measured using a computerized video-tracking system with a digital CCTV camera (Javelin Ultrachip) mounted onto a Leica M3Z dissecting microscope, connected to a MacIntosh Power PC 8100/100AV equipped with Radius Videovision (Radius, Inc.). Videos of worms were captured onto the computer using Adobe Premiere software and analyzed using the DIAS software program (Soll, 1995), which can track the movement of up to 50 animals. Speed was calculated as instantaneous speed between one frame and the next, or as average speed over a longer period of time. Video recordings were carried out at room temperature, which varied from 20°C–23°C.

Locomotion assays were performed on NGM media with 5% of the regular amount of bactopeptone (Sulston and Hodgkin, 1988). Agar plates were allowed to dry for a week before being used. For measuring nematode speed in the presence of bacterial food, agar plates were seeded with a small drop of *E. coli* OP50 2 days before the assay. Between five and seven well-fed young adult hermaphrodites from uncrowded plates were placed on the plate and allowed to recover undisturbed for 4 min on the microscope stage. Behavior was then recorded for 4 min at two frames per second. For measuring nematode speed in the absence of food, about 100 adult animals were picked to an unseeded agar plate, allowed to crawl around the plate for 10 min to remove adhering food, and picked to a fresh locomotion assay plate. Animals were then processed as described above. More nematodes had to be used for assays in the absence of food because animals spent only short periods of time in the recording area if food was absent.

Genetics

To establish that *npr-1* alleles were recessive, we measured speed and qualitatively assessed clumping behavior in non-Lon heterozygotes made by crossing *npr-1* mutant males with *lon-2(e678)* X hermaphrodites. Positive controls were worms homozygous for each allele, and the non-Lon progeny of the cross *ad609* × *lon-2 ad609*. N2 was the negative control. Noncomplementation between *npr-1* alleles was established in the same experiment by measuring speed and assessing the clumping phenotype of *trans*-heterozygous strains. *trans*-heterozygotes were generated by the following crosses: *ky13* × *lon-2(e678) ad609*; *n1353* × *lon-2(e678) ad609*, *g320* × *lon-2(e678) ad609*.

npr-1 was mapped between *lon-2* and *mig-13* on the X chromosome: *ad609* × *lon-2(e678) mig-13(mu31) dpy-8(e130)* X, 1/16 Lon non Dpy recombinants was Clp and *mig-13*(+); *ky13* × *lon-2(e678) mig-13(mu31) dpy-8(e130)* X, 2/41 Lon non Dpy recombinants were Clp and *mig-13*(+). To establish that *ad609*, *ky13*, and *g320* were not complemented by *syDf1*, a deletion which fails to complement *lon-2*, we constructed hemizygous strains by crossing *npr-1* mutant males with *unc-2(e55) lon-2(e678)/syDf1* X hermaphrodites. Non-Lon animals that laid 1/4 dead eggs and failed to have Lon progeny

must be of genotype *npr-1/syDf1*. These hemizygous strains clumped and were hyperactive, indicating that *syDf1* does not complement *ad609*, *ky13*, or *g320*.

npr-1 X maps 0.02 cM away from *lon-2* X. To establish that the social phenotype of the RC301 wild strain was linked to *npr-1*, we outcrossed the RC301 derivative DA650 ten times with the N2-derived strain *lon-2(e678)* to generate six independent strains that retain RC301 sequences around the *npr-1* locus but bear N2 DNA at unlinked loci. These strains were made by the following scheme. Males from the DA650 social strain (which are non Lon) were mated with *lon-2(e678)* hermaphrodites (outcross 1). Non-Lon hermaphrodite progeny from this cross were mated with *lon-2(e678)* males (outcross 2). The non-Lon male progeny from this cross were then mated with *lon-2(e678)* hermaphrodites as in outcross 1. This outcrossing strategy was pursued to the tenth outcross, when the X chromosome carrying the wild-type *lon-2(+)* chromosome derived from RC301 was made homozygous.

The social phenotype of RC301 was mapped to the *npr-1* region by the following crosses: *g320* (from strain DA650) × *unc-6(n102) dpy-7(e88)* X, 7/7 Dpy non-Unc recombinants were Clp; *g320* × *dpy-3(e27) unc-2(e55)* X, 9/9 Dpy non-Unc recombinants were Clp.

Molecular Biology

General molecular manipulations followed standard protocols (Sambrook et al., 1989). The sequence of the *npr-1* genomic region was established by the *C. elegans* Sequencing Consortium (Wilson et al., 1994). *npr-1* cDNA was obtained by RT-PCR and confirmed the gene structure of C39E6.6 predicted by Genefinder. Molecular changes in the *npr-1* gene associated with wild strains and with *npr-1* mutants were identified by sequencing PCR amplified products of the *npr-1* gene using the fmol sequencing kit (Promega).

npr-1 plasmids were derived from cosmid F10A4, which overlaps the sequenced cosmids F43C9 and C39E6 (Wilson et al., 1994). Details of plasmid construction are available on request.

Transgenic Strains

Germline transformation was carried out as described (Mello et al., 1991). The *lin-15* clone pJMZ (50 ng/μl) (Clark et al., 1994) was used as a coinjection marker, except for rescue of wild strains (see below). Strains were either *lin-15(n765ts)* or *npr-1(ad609) lin-15(n765ts)* X. For rescue of wild strains, plasmid pTG96 (Gu et al., 1998) was used as a coinjection marker. pTG96 expresses GFP throughout somatic tissues of *C. elegans*. Unless otherwise specified, test DNA was injected at 50–100 ng/μl. Transgenic lines were identified by rescue of the *lin-15(n765ts)* multivulva phenotype at 20°C or by GFP fluorescence. The social/solitary phenotype of transgenic lines was determined by measuring the speed of animals in locomotion assays and by examining whether the transgenic animals clumped and bordered. At least four transgenic lines were examined for each tested clone.

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GenBank Accession Numbers

The GenBank accession number for cosmid C39E6, which contains *npr-1*, is U49944; the PID for NPR-1 is g1208818.