C. elegans food choice exhibits effort discounting-like behavior

Jonathan R. M. Millet, Serge Faumont, Aaron B. Schatz, Amanda M. White, Kathy D. Chicas-Cruz, Shawn R. Lockery*

Institute of Neuroscience, 1254 University of Oregon, Eugene OR 97403, USA

*Correspondence: shawn@uoregon.edu

ABSTRACT

Cost-benefit decisions are ubiquitous in both human and animal behavior. Economists have developed formal models of cost-benefit decision-making by focusing on discounting behavior, the devaluation of a reward based on the costs associated with it. The phylogenetic limits of discounting behavior remain unknown. Here, we provide evidence that the nematode *C. elegans* exhibits behavior closely resembling effort discounting. Given a choice between food options that are easy or difficult to consume, worms devalue the latter in a manner predicted by economic models. We identified a plausible mechanism for this behavior based on differential rates of leaving food patches and demonstrated that this mechanism is disrupted by deficits in dopamine signaling, as in rodents. Together, these results establish *C. elegans* as a potential invertebrate model for discounting behavior and set new phylogenetic bounds on this type of cost-benefit decision-making.

INTRODUCTION

Cost-benefit decisions are ubiquitous in animal behavior, driven by universal evolutionary pressures to maximize survival and reproductive success. Foraging, mate selection, parental investment, and territoriality each demand a balance between costs, such as energy expenditure and risk, and benefits such as resources, mates, and offspring. A better understanding of cost-benefit decision-making, including its biological basis, would advance a variety of fields, including economics, psychology, neuroscience, and behavioral ecology.

Economic theorists have developed formal models of cost-benefit decision-making by focusing on *discounting* behavior (Samuelson, 1937; Mazur and Coe, 1987). In this context, discounting refers to the tendency of individuals to *devalue* a reward based on the costs associated with it. Costs commonly investigated include delay, risk, and effort. In general, as cost increases, perceived or subjective value decreases. For example, in the case of delay discounting, receiving a guaranteed payment of $35 after 30 days is worth less than receiving $25 today if a person accepts the smaller immediate reward. In risk discounting, participants might choose to receive $50 for certain rather than a 50% chance of receiving $100. In effort discounting, participants might choose to receive $1 with no effort rather than $5 after repeatedly squeezing a stiff handgrip (Stanek and Richter, 2021).

Studies of discounting are not limited to humans. The literature on discounting in rodents is particularly rich, with multiple paradigms for all three forms of discounting (Bailey, Simpson and Balsam, 2016a; Salamone *et al.*, 2018; Gray *et al.*, 2019; Castrellon *et al.*, 2021). In models of delay discounting, for example, mice choose between low-reward and high-reward nose pokes, where the higher reward arrives after a delay (Isles *et al.*, 2004). Risk discounting can be modeled by giving subjects a choice between a low-probability lever yielding a large reward (4 food pellets) and a high-probability lever yielding a small reward (1 food pellet) 100% of the time (St. Onge, Chiu and Floresco, 2010). Effort discounting can be modeled by giving rodents a choice between low-reward (2 food pellets) and high-reward (4 food pellets) arms of a T-maze

where a physical barrier must be climbed to obtain the higher reward (Salamone, Cousins and Bucher, 1994). However, behaviorists have also observed discounting in several species of nonhuman apes, monkeys, lemurs, jays, chickens, pigeons, honeybees, and other animals (Isles *et al.*, 2004; Green, Myerson and Calvert, 2010; Hayden, 2016).

The phylogenetic limits of discounting behavior are unknown. We therefore asked whether foraging decisions in the nematode worm *C. elegans* exhibit discounting-like behavior. *C. elegans* is an omnivorous bacterivore. Worms swallow bacteria through rhythmic contractions of the pharynx, a tube-shaped muscular organ that delivers bacterial particles to the gut; each pharyngeal contraction is called a pump. *C. elegans* primarily inhabits rotting plant material such as decaying fruits and stems (Frézal and Félix, 2015). Its natural habitat contains thousands of different bacterial species (Samuel *et al.*, 2016). Each species has a characteristic nutritional quality, defined in terms of the growth rate of individual worms cultured on that species (Avery and Shtonda, 2003; Samuel *et al.*, 2016). *C. elegans* food preferences are sensitive not only to nutritional quality but also to food density in a manner that satisfies quantitative economic criteria for choices based on subjective value (Katzen *et al.*, 2023). *C. elegans* foraging behavior has been used to investigate the neuronal and genetic basis of exploration-exploitation trade-offs (Bendesky *et al.*, 2011; Milward *et al.*, 2011), a classical example of cost-benefit decision-making. In the laboratory, worms feed on patches of bacteria grown on agarose plates. They leave and re-enter a patch many times. Leaving rates are low on high-quality lawns but increase if the bacterial food is depleted or is difficult to ingest (Shtonda and Avery, 2006; Bendesky *et al.*, 2011; Milward *et al.*, 2011; Olofsson, 2014; Scheer and Bargmann, 2023). This response pattern suggests that leaving promotes exploration to find a better food source.

The fact that *C. elegans* exhibits subjective-value based choice and cost-benefit feeding decisions suggested to us that it might also exhibit discounting behavior. Of the various forms of discounting, we focused on effort discounting as it uniquely does not require learned instrumental responses, like lever pressing for rewards, which are not feasible in *C. elegans*. To investigate discounting behavior, we baited the arms of a miniature T-maze with selected

densities of a single species of bacteria. One arm contained normal bacteria, while the other arm contained a bacterial preparation in which the cells had been elongated, which we predicted would make them more effortful to ingest and less preferred by *C. elegans*.

We found that *C. elegans* exhibits choice behavior that closely resembles effort discounting. Given a choice between equal densities of normal and elongated bacteria, worms prefer normal bacteria. This preference can be reversed by presenting elongated bacteria at a higher density than normal bacteria, indicating that high food density compensates for greater effort, as in rodent models. Furthermore, worms can be made indifferent to the two food options by adjusting relative food densities. It is therefore possible to measure the extent of devaluation and accurately predict novel indifference points. Preference for normal bacteria can be attributed to an increased patch-leaving rate on elongated food. Additionally, typical levels of discounting require intact dopamine signaling. Our results expand the repertoire of cost-benefit behaviors in *C. elegans* and demonstrate that lower invertebrates are capable of effort discounting-like behavior.

RESULTS

Elongated bacteria require more effort to eat

We conceptualized feeding effort in *C. elegans* as the amount of pharyngeal muscle activity required to ingest a given quantity of food. The starting point of our experiments was to make bacteria more effortful to consume. Computer simulations of pharyngeal mechanics predict that large bacterial cells are swallowed less efficiently than small cells (Avery and Shtonda, 2003). Consistent with this result, worms develop more slowly when cultured on large bacteria, possibly because they are acquiring nutrients less efficiently (Avery and Shtonda, 2003). These findings led to the hypothesis that larger bacteria require more effort to eat, necessitating more pharyngeal pumps per unit of food consumed. To test this hypothesis, we artificially increased the size of an easily ingested bacterium, *Comamonas* spp., using the antibiotic cephalexin. In Gram-negative bacteria such as *Comamonas*, cephalexin interferes with bacterial cell septation during mitosis, resulting in elongated bacterial filaments that nevertheless remain edible (Millet *et al.*, 2022) (Figure 1—figure supplement 1).

Pharyngeal pumping rates in normal and elongated bacteria were measured in the reference strain N2 by placing individual worms in a microfluidic channel filled with a bacterial suspension and fitted with electrodes that record the electrical signals associated with each pharyngeal contraction (Lockery *et al.*, 2012). Bacteria were washed with cephalexin-free buffer before being fed to worms. Pumping frequencies in normal and elongated bacteria were equivalent (Figure 1A; Table 1, row 1) indicating that worms expend approximately the same amount of effort per unit time feeding on normal and elongated food. To test the hypothesis that elongation interferes with food absorption, we measured nutrient uptake in worms cultured on normal or elongated bacteria, using fat stores as a proxy for carbohydrate and lipid consumption. This was done by staining fat stores with the specific label Oil-Red-O (Soukas *et al.*, 2009) and quantifying the density of staining after a two-day culture period extending from the L1 to the L4 stage. We found that fat levels were significantly lower in worms cultured on elongated food (Figure 1B2; Table 1, row 2). Given that pumping rate was unchanged in the presence of normal versus elongated bacteria (Figure 1A), the lower fat stores observed in

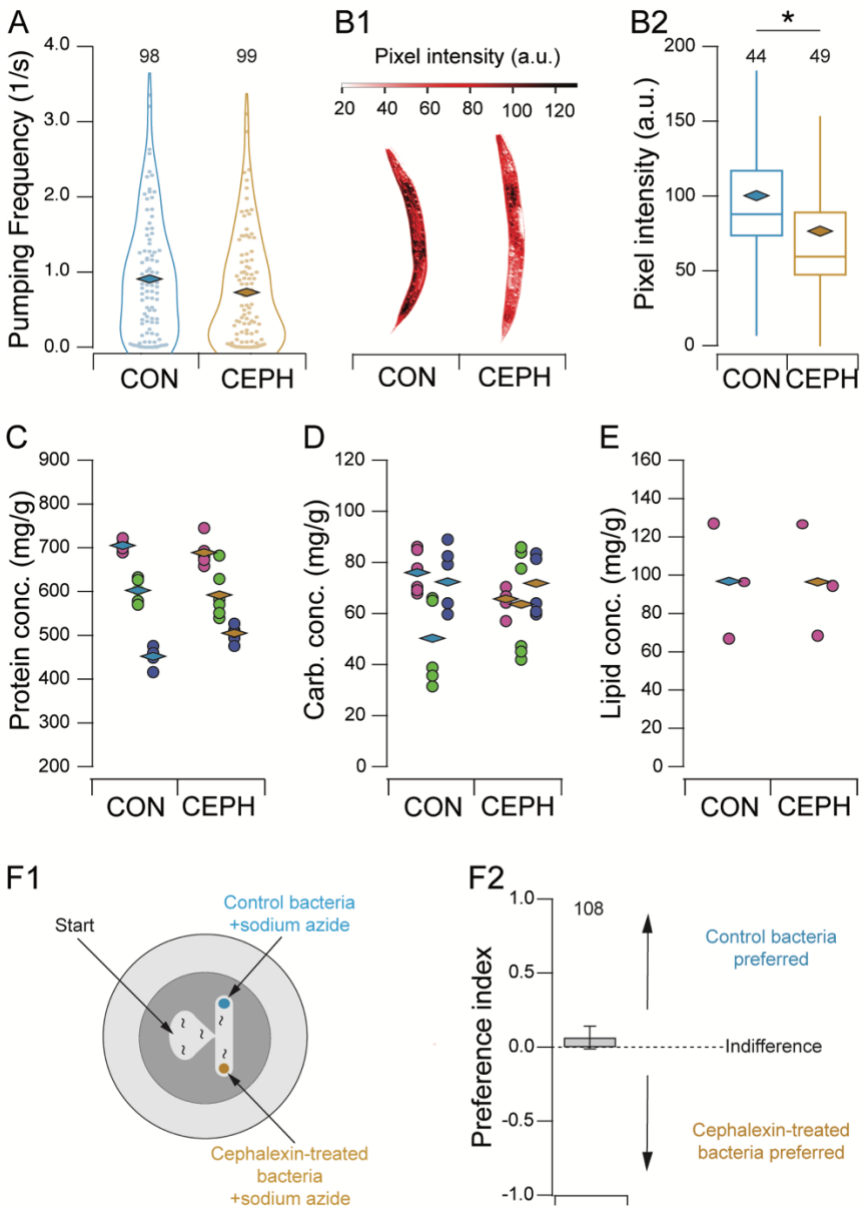
worms fed elongated bacteria suggest that the amount of nutrition incorporated per pump is lower in worms feeding on elongated versus normal bacteria. This supports our hypothesis that elongation impairs food transport, requiring longer feeding bouts and thus increased muscular activity to consume an equivalent amount of food.

Our effort-discounting paradigm assumes that the normal and elongated bacteria are equivalent except for the amount of effort required to consume them. For example, elongation could affect nutrient content or olfactory attractiveness of bacteria. *C. elegans* food preferences are sensitive to the nutrient content of bacteria (Feng *et al.*, 2025). We therefore asked whether cephalexin treatment affects nutrient content. We focused on key macronutrients: carbohydrates, lipids, and proteins. For protein and carbohydrates, we used colorimetric assays (Materials and Methods; Figure 1C,D). For lipids, we used phase separation to harvest and measure lipid content (Materials and Methods; Figure 1E). In all three tests, we detected no differences between control and cephalexin-treated bacteria (Table 1, rows 3-5). We conclude that cephalexin treatment does not alter bacterial nutritional composition.

Worms are attracted to particular species of bacteria by the characteristic mixture of volatile organic compounds each species emits (Worthy *et al.*, 2018). Thus, another concern is that cephalexin treatment might alter the odors produced by bacteria thereby changing their attractiveness. To control for this possibility, we performed a T-maze assay (Materials and Methods) in which patches of normal and elongated bacteria were placed at the ends of each respective arm. The patches contained equal volumes of bacteria at an optical density (OD) of 1.0. Patches were spiked with sodium azide, a paralytic agent, to trap worms once they reached a patch. Worms were placed at the base of the T-maze and allowed to explore it for 60 minutes after which the number of worms in each patch was counted. A food preference index ($-1 \leq I \leq 1$) was calculated, where positive and negative values indicate preference for normal and elongated bacteria respectively, and $I \cong 0$ denotes indifference between the two options (Materials and Methods). We found that worms under these conditions were indifferent (Figure

161 1F2; Table 1, row 6). This result indicates that cephalexin treatment does not alter the relative
162 attractiveness of normal or elongated bacteria.

Figure 1



163 **Figure 1. Establishment of an effort discounting paradigm in C. *elegans*.**
164 A. Pharyngeal pumping frequency in suspensions of normal (CON) and elongated (CEPH) bacteria.
165 Symbols (box and whisker plot): *filled circles*, single-worm data points; *diamonds*, means;
166 *numbers*, sample size. B1. Quantification of Oil-Red-O staining of lipid in worms cultured on
167 normal (CON) or elongated (CEPH) bacteria. Median worms from each condition are shown.
168 B2. Mean pixel intensity of Oil-Red-O staining of lipid in worms cultured on normal (CON) or
169 elongated (CEPH) bacteria. *Diamonds*, means; *numbers*, sample size.

C-D. Protein and carbohydrate in worms cultured on normal (CON) and elongated bacteria (CEPH). Columns of data points of the same color are biological replicates run in parallel. Symbols: *filled circles*, technical replicates (n = 6); *diamonds*, means of technical replicates. E. Lipid concentration in worms cultured on normal (CON) and elongated bacteria (CEPH). Symbols: *filled circles*, biological replicates (n = 3); *diamonds*, means of biological replicates. F1. T-maze assay for relative attractiveness of normal and elongated bacteria. Food patches contained sodium azide as a paralytic agent. F2. Mean preference index in the T-maze assay. Error bars, ± 95% CI.

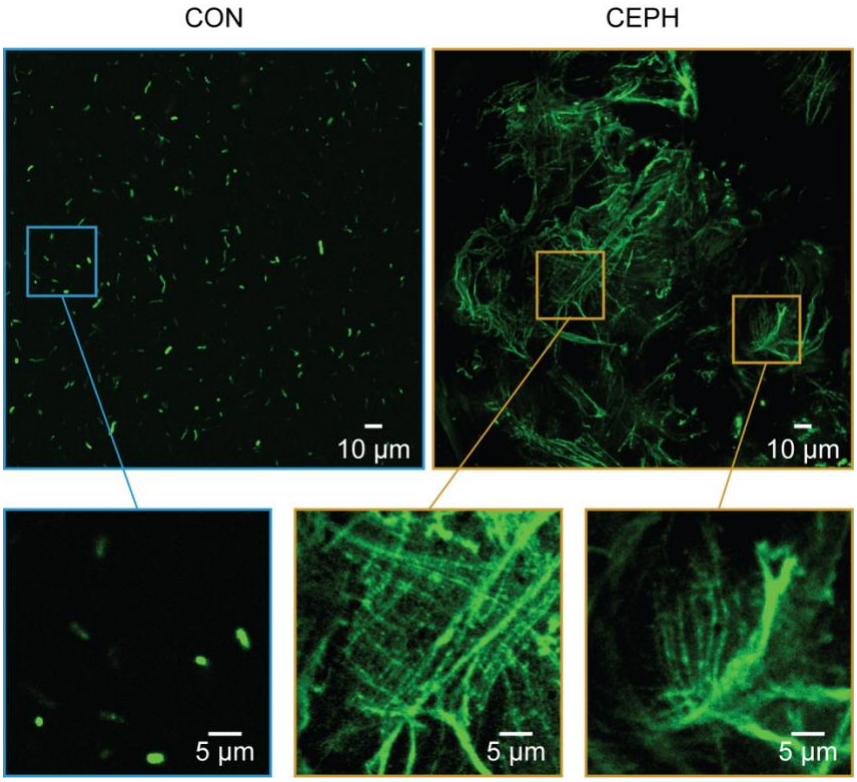


Figure 1—figure supplement 1. Cephalexin-treated bacteria.
Representative images of control (CON) and cephalexin-treated bacteria (CEPH) labeled with the fluorescent dye BacLight Green. The contrast in CON and CEPH images was optimized separately for each image. After treatment with cephalexin, *Comamonas* formed long filaments.

186 **Worms devalue food that requires more effort to eat**

187 Having demonstrated that elongated bacteria require more effort to eat but are equivalent to
188 normal bacteria in terms of nutrition and attractiveness, we next asked whether *C. elegans*
189 exhibits a preference for the normal, easier-to-eat bacteria – evidence of effort discounting-like
190 behavior. We tested this by measuring food preference in the T-maze assay using the same
191 methodology as in Figure 1F but without the paralytic agent, allowing individual worms to
192 sample both patches. One arm of the T-maze was baited with normal bacteria at an OD of 1.0,
193 while the other arm contained elongated bacteria at ODs of 1.0, 1.5, or 2.0. Worms explored the
194 maze for 60 minutes and the food preference index was computed based on worm counts at
195 the end of this period. There was a significant overall effect of optical density of elongated
196 bacteria on preference (Figure 2B; Table 1, row 7). When normal and elongated bacteria were at
197 the same concentration (OD 1), worms preferred normal bacteria (I = 0.10; Figure 2B; Table 1,
198 row 8). This demonstrates that worms do, in fact, devalue elongated food. When normal and
199 elongated bacteria were at OD 1.0 and 2.0, respectively, worms preferred elongated bacteria (I
200 = -0.17; Figure 2B; Table 1, row 9). This suggests that, in the worm's valuation system, higher
201 food density compensates for greater effort. This outcome was not a foregone conclusion. For
202 instance, it was possible that no amount of elongated food would be sufficient to make worms
203 prefer it. Our results are consistent with findings in rodents, where high-effort options are
204 chosen when the food reward density is sufficiently high (Floresco, Tse and Ghods-Sharifi, 2008).
205 When the optical densities of normal and elongated food were 1.0 and 1.5, respectively, worms
206 were indifferent between the two options (I = 0.03; Figure 2B; Table 1, row 10). Indifference
207 points are also observed in rodent studies of effort discounting (Salamone, Cousins and Bucher,
208 1994; van den Bos *et al.*, 2006; Ostrander *et al.*, 2011). Overall, we conclude that in feeding
209 decisions, worms take into account both relative effort and relative food density.

210

211 **An economic model predicts novel indifference points**

212 Indifference points are significant because, by definition, the presented options have equal
213 perceived value, allowing for the quantification of devaluation. This quantification is only
214 possible at an indifference point. For example, consider a hypothetical human scenario in which

a participant is asked to choose between receiving $20 immediately or $20 with the added cost of climbing 20 flights of stairs. Most participants would choose the immediate $20. Given a choice between $20 immediately and $100 at the cost of climbing 20 flights of stairs, some participants might choose the $100 option. However, suppose that when given a choice between $20 immediately and $80 at the cost of climbing 20 flights of stairs, a particular participant is indifferent between the two options. For this person, under these circumstances, we can conclude that the $20 option is perceived as equal in value to the $80 option. In other words, the $80 option has been discounted (devalued) to $20.

Formally, at an indifference point,

$$V(E) = d(E)V_0$$

where E represents effort, V_0 represents the original value of the reward before devaluation and $V(E)$ is the discounted value of V_0. The quantity $d(E)$ is the discount factor, which, in economic models of discounting, is a function of E. From the above equation,

$$d(E) = \frac{V(E)}{V_0}$$

with $0 \leq d(E) \leq 1$; in the above example $d(E)$ = 20/80 or 1/4. In economic models, $d(E)$ is inversely related to E but the precise form of $d(E)$ varies between models (Figure 2—figure supplement 1).

In our experiments, E is determined by the degree of bacterial elongation. Although this quantity is unknown, it remained unchanged because we used the same cephalexin exposure protocol across experiments (see Materials and Methods). It is analogous to the fixed effort of climbing 20 flights of stairs in the hypothetical example. Assuming a linear relationship between optical density and perceived value, we can determine $d(E)$ from the indifference point shown in Figure 2B, where V_0 = OD 1.5 and $V(E)$ = OD 1.0, yielding $d(E)$ = 2/3. In the most basic

models of discounting, $d(E)$ is independent of V_0. Assuming independence, other values of V_0 can be inserted into equation 1 to predict novel indifference points. For instance, setting V_0 = OD 3 predicts indifference at $V(E)$ = OD 2, and setting V_0 = OD 0.75 predicts indifference at $V(E)$ = OD 0.5. That is, any pair of optical densities (normal versus elongated) in a 2:3 ratio should be an indifference point. We tested these predictions by measuring preference in T-mazes baited with normal and elongated food at OD ratios (normal:elongated) of 2:3 and 0.5:0.75, finding indifference as predicted (Figure 2C, Table 1, rows 11-14). The fact that standard discounting models correctly predict indifference points reinforces the conclusion that *C. elegans* exhibits discounting-like behavior.

Figure 2

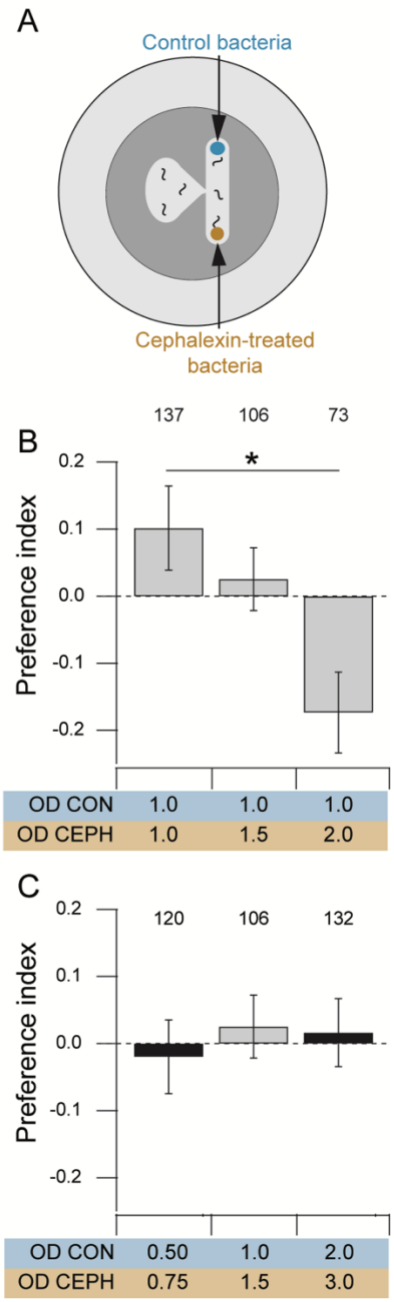


Figure 2. Food preference in T-maze assays.
A. T-maze assay for preference for normal (Control) versus elongated bacteria (cephalexin).
B. Effect on preference of raising the optical density of elongated bacteria (CEPH). Mean preference index is shown for assays in which three different optical densities (OD) of elongated bacteria (CEPH) were paired with normal bacteria (CON) at OD 1.0. Worms are indifferent when elongated bacteria are at OD 1.5. *Numbers*, sample size. Error bars ± 95% CI. C. Tests of predicted indifference points. Mean preference index is shown for three different pairs of

optical densities of normal (CON) and elongated (CEPH) bacteria. The grey bar is the indifference point found in panel B. The black bars are novel indifference points found by scaling the optical density of elongated bacteria by the discount factor of 2/3 obtained from the grey bar. *Numbers*, sample size. Error bars ± 95% CI.

Figure 2—figure supplement 1

Model	Equation	Discount factor
Hyperbolic	$V(E) = \frac{V_0}{1 + \alpha E}$	$\frac{1}{1 + \alpha E}$
Generalized hyperbolic	$V(E) = \frac{V_0}{(1 + \alpha E)^s}$	$\frac{1}{(1 + \alpha E)^s}$
Exponential	$V(E) = \frac{V_0}{e^{\alpha E}}$	$\frac{1}{e^{\alpha E}}$

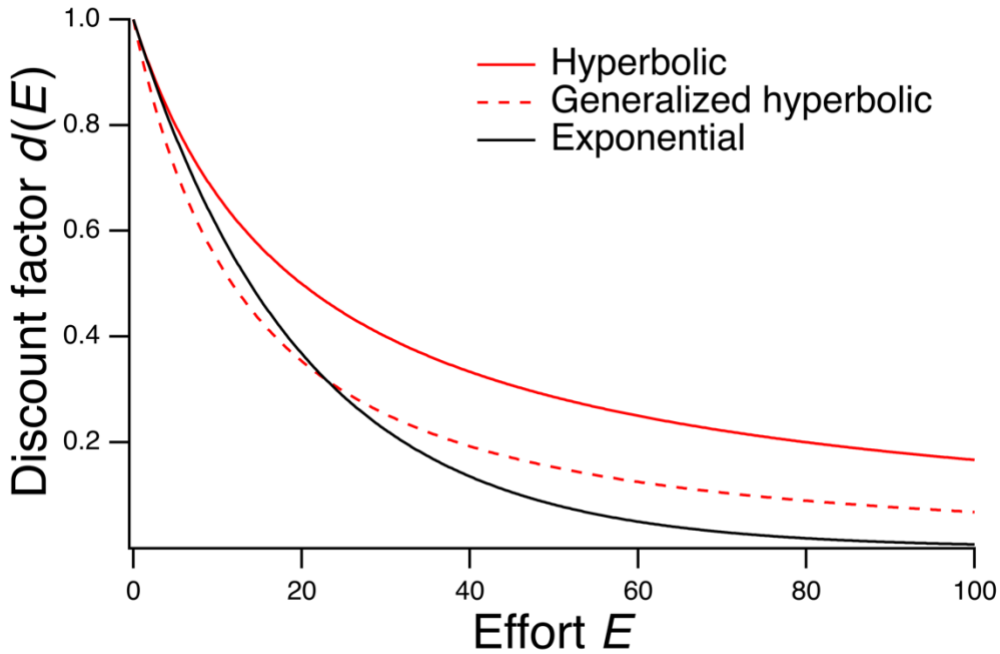


Figure 2—figure supplement 1. Standard models of effort discounting.
There are three main models of effort discounting, each with a unique equation relating effort and reward. V_0 is the value of the reward if it were given effort-free. E is the level of effort required to obtain the effortful reward. $V(E)$ is the discounted value of V_0 under conditions of indifference, given level of effort E. The quantity α is a positive constant representing the chooser's sensitivity to effort. The quantity s in the generalized hyperbolic model is the shape parameter. Discount factors are constant at fixed E. In the graph, V_0 = 100, α = 0.05, and s = 1.5.

Effort discounting-like behavior is based on assessment of the local food environment

We next investigated the behavioral mechanism through which effort influences food preference, examining how worms interact with patches of normal versus elongated food. We studied these interactions at the microscopic level of individual worms to explain behavior at the macroscopic level of T-maze assays. This was done by performing food-patch leaving assays (Scheer and Bargmann, 2023) using either normal or elongated bacteria. Individual worms were transferred to a 1 cm diameter, nematode growth medium-filled arena containing a small patch (3–4 mm diameter) of normal or elongated bacteria (OD 1.0) at its center. The patch size was comparable to those in the T-maze assay. Each worm was video recorded for 15 minutes while it entered and left the patch multiple times (Figure 3A).

For analysis, we took a kinetic approach to investigate the rates at which worms stochastically changed their position relative to the food patch. This approach is both simple, as it does not require assigning specific locomotory states at particular times to individual worms, and powerful, as it forms a bridge between the microscopic and macroscopic behavior of a stochastic system, thereby elucidating underlying dynamics. Accordingly, we defined three kinetic states based on occupancy of specific zones in the arena (Figure 3B):

1. State F, *On Food*: both head and tail of the worm were in contact with the food patch.
2. State B, *At Border*: either the head or tail was in contact with the food patch, but not both.
3. State O, *Off Food*: neither head nor tail was in contact with the food patch.

This system contains four state transitions: F→B, B→O, O→B, and B→F. Behavior was analyzed in terms of state probabilities ($P_{\mathrm{F}}, P_{\mathrm{B}}, P_{\mathrm{O}}$) and rate constants ($k_{\mathrm{FB}}, k_{\mathrm{BO}}, k_{\mathrm{OB}}, k_{\mathrm{BF}}$). State probability was defined as the fraction of total observation time the worm was in each state. Rate constants were defined as the number of transitions divided by the total time spent in the state of origin. Rate constants determine the probability per unit time of transitioning from one state to another and thus reflect the underlying physical causes of state transitions.

We observed a variety of changes in kinetic parameters on elongated versus normal food. P_{B} and P_{O} increased at the expense of P_{F}, consistent with decreased preference for elongated food in T-maze assays (Figure 3C; Table 1, rows 15,16). The rate constants exhibited two key features. First, in control animals, between adjacent states, the mean rate constants leading to patch entry (O→B→F transitions) were greater than the mean rate constants leading to patch leaving (F→B→O transitions). In particular, $k_{\mathrm{BF}} > k_{\mathrm{FB}}$ and $k_{\mathrm{OB}} > k_{\mathrm{BO}}$ (Figure 3, D1 vs. D3 and D2 vs. D4; Table 1, rows 17-23). This indicates a greater propensity to enter food patches than to leave them, which is consistent with efficient foraging. Second, changes in rate constants brought about by elongated food exhibited a distinctive pattern: the rates for patch leaving ($k_{\mathrm{FB}}, k_{\mathrm{BO}}$) were increased whereas the rates for patch entry (k_{OB}, k_{BF}) were unchanged (Figure 3, D1 and D2 vs. D3 and D4; Table 1, rows 24-28). Thus, worms exhibited a heightened propensity to leave elongated food, consistent with previous observations (Scheer and Bargmann, 2023). The fact that rates for patch entry were unaffected by elongated bacteria rules out a mechanism in which worms, exiting patches of elongated food (which is relatively undesirable), become reluctant to re-enter them. Finally, we found that speed on elongated food increased, whereas speed off food remained unchanged (Figure 3E; Table 1, rows 29-33). Applying the kinetic data of individual worms to the population level in T-maze assays suggests that effort discounting-like behavior could result from feeding decisions based on the local food environment rather than a direct comparison of patch contents across the arms of the T-maze.

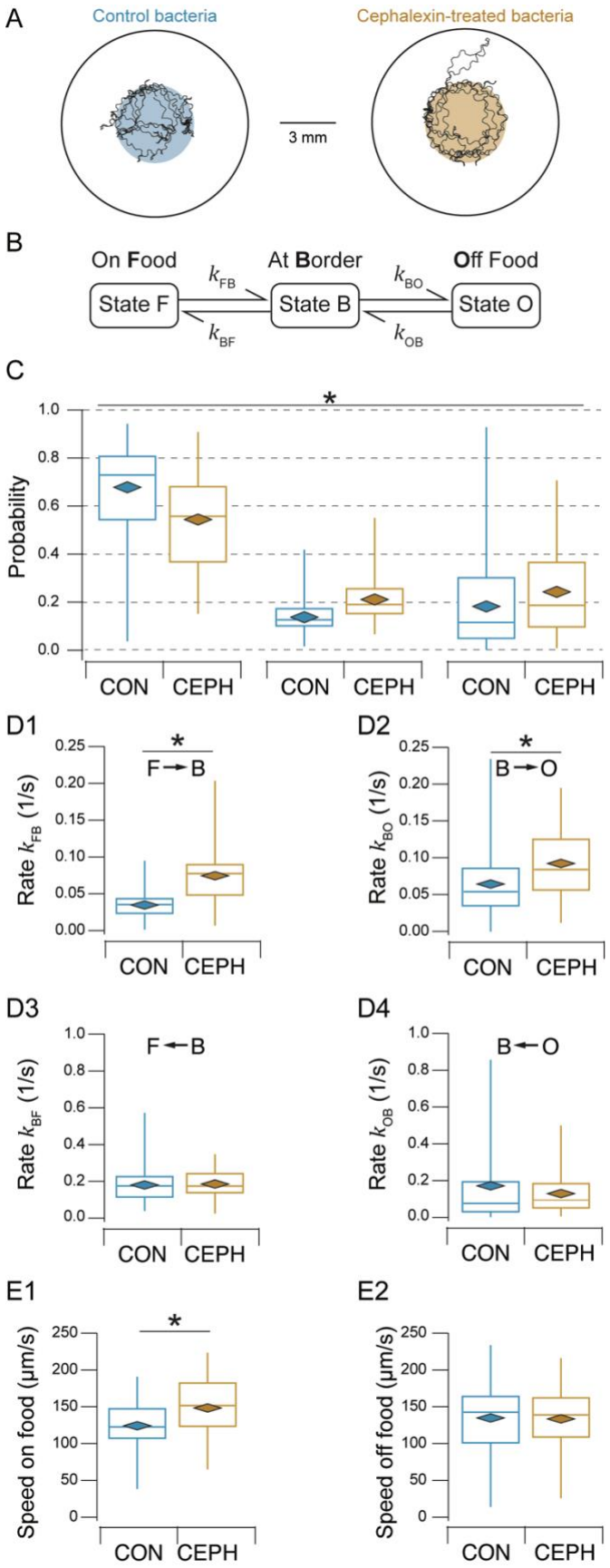

A
Control bacteria
Cephalexin-treated bacteria
3 mm
B
On Food
At Border
Off Food
State F
State B
State O
k_{FB}
k_{BF}
k_{BO}
k_{OB}
C
Probability
CON
CEPH
D1
Rate k_{FB} (1/s)
F→B
D2
Rate k_{BO} (1/s)
B→O
D3
Rate k_{BF} (1/s)
F←B
D4
Rate k_{OB} (1/s)
B←O
E1
Speed on food (µm/s)
E2
Speed off food (µm/s)

Figure 3. Kinetic analysis of food-patch leaving assays.
A. Food-patch leaving assays for normal (control) and elongated (cephalexin-treated) bacteria. *Open circles*, arenas; *colored circles*, food. A representative track of a single worm is superimposed on each diagram. B. Three-state kinetic model of behavior in the assay. Each state represents the indicated zone in the arena. C. Probability of states F, B, and O for normal (CON) and elongated (CEPH) bacteria. Significance was assessed using compositional statistics as described in Figure 3—figure supplement 1. D. Rate constants for normal (CON) and elongated (CEPH) bacteria. E. Locomotion speed for normal (CON) and elongated (CEPH) bacteria on or off food.

Figure 3—figure supplement 1

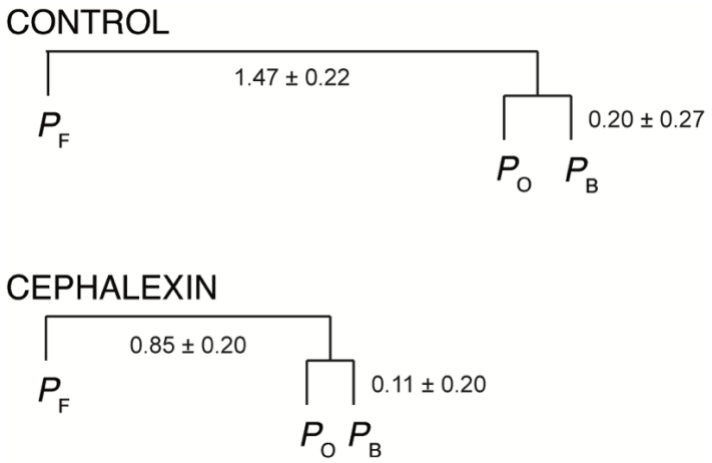


Figure 3—figure supplement 1. Statistical test of the effect of cephalexin on state probabilities in Figure 3C.

State probabilities are an instance of *compositional data*, meaning data that sum to a constant (1 in this case). Such data require special treatment because a decrease in one variable means an increase in at least one other variable. Accordingly, data were first subjected to an isometric log ratio transform (see Materials and Methods). The transformation quantifies the change in *balance* between subsets of the data. Balances are indicated by the numbers in the figure. Here, the control balance between P_{F} and the reciprocal probabilities P_{B} and P_{O} (1.47) is decreased by cephalexin treatment (0.85). A *t*-test on individual worm balances revealed that P_{F} significantly decreased while the joint P_{B} and P_{O} data significantly increased. In contrast, a similar approach showed that the balance between P_{B} and P_{O} was not significantly affected. This indicates that P_{B} and P_{O} increased by similar amounts.

345

346 **Intact dopamine signaling is required for effort discounting-like behavior**

347 A salient aspect of effort discounting in rodents is its sensitivity to changes in midbrain
348 dopamine signaling (Salamone *et al.*, 2018). Increased signaling promotes preference for high-
349 value, high-effort rewards, whereas decreased signaling promotes preference for low-value,
350 low-effort rewards (Bailey, Simpson and Balsam, 2016b; Salamone *et al.*, 2018; Hart and
351 Izquierdo, 2019); in other words, increased dopamine signaling results in greater effort
352 tolerance. Dopamine signaling is a candidate mechanism for the regulation of effort
353 discounting-like behavior in *C. elegans* because it affects how worms respond to the presence
354 and absence of food as they enter or leave food patches. Worms slow down when they
355 encounter a food patch, a response that requires intact dopamine signaling (Sawin,
356 Ranganathan and Horvitz, 2000). When worms exhaust their food supply or are removed from a
357 food patch, they engage in a bout of frequent, high-angled turns called area-restricted search
358 (Hills, Brockie and Maricq, 2004). This response also requires dopamine signaling.

359

360 To test whether dopamine signaling is involved in effort discounting-like behavior in *C. elegans*,
361 we examined food preference in a variety of mutants with altered dopamine signaling. The gene
362 *cat-2* encodes a tyrosine hydroxylase essential to the main synthesis pathway for dopamine; *cat-*
363 *2* mutants are commonly used to reduce dopamine signaling in *C. elegans*. The gene *dat-1*
364 encodes a dopamine transporter that mediates the reuptake of dopamine into presynaptic
365 neurons; *dat-1* mutants are used to increase dopamine signaling. The effects of reduced
366 dopamine signaling can also be investigated in dopamine receptor mutants. *C. elegans* has four
367 genes encoding dopamine receptors with homology to dopamine receptors in mammals. Of
368 these, *dop-1*, *dop-2*, and *dop-3* are the most thoroughly characterized to date. *dop-1* is a DRD1
369 homolog, whereas *dop-2* and *dop-3* are DRD2 homologs. The genes *dop-1* and *dop-3* are
370 required for normal responses to the presence of food (Chase, Pepper and Koelle, 2004); *dop-2*
371 is required for increased levels of activity in the absence of food (Bastien *et al.*, 2024). We found
372 a significant overall effect of strain on food preference measured in the T-maze assay when both
373 foods were at OD 1.0 (Figure 4; Table 1, row 34). Post hoc testing showed that *cat-2*, *dat-1*, *dop-*

374 *1*, and *dop-2* were indistinguishable from N2, whereas *dop-3* mutants were significantly
375 different from N2 (Table 1, row 36-40). In particular, they were indifferent between the two food
376 options, indicative of decreased effort discounting-like behavior (Table 1, row 41). We conclude
377 that fully intact dopamine signaling is required for normal effort discounting-like behavior in *C.*
378 *elegans*, as in rodents. However, the fact that impaired dopamine signaling rendered worms
379 indifferent between the food options suggests that decreased dopamine signaling increases
380 tolerance for high-effort food, in contrast to its effect in rodents. The absence of an effect of the
381 *cat-2* and *dat-1* mutations is puzzling in view of the requirement for *dop-3*. One possibility is
382 that residual dopamine in the *cat-2* mutant (Sanyal *et al.*, 2004) is sufficient for normal effort
383 discounting-like behavior and that normal levels of dopamine signaling are saturating, such that
384 *dat-1* mutations have no effect.

Figure 4

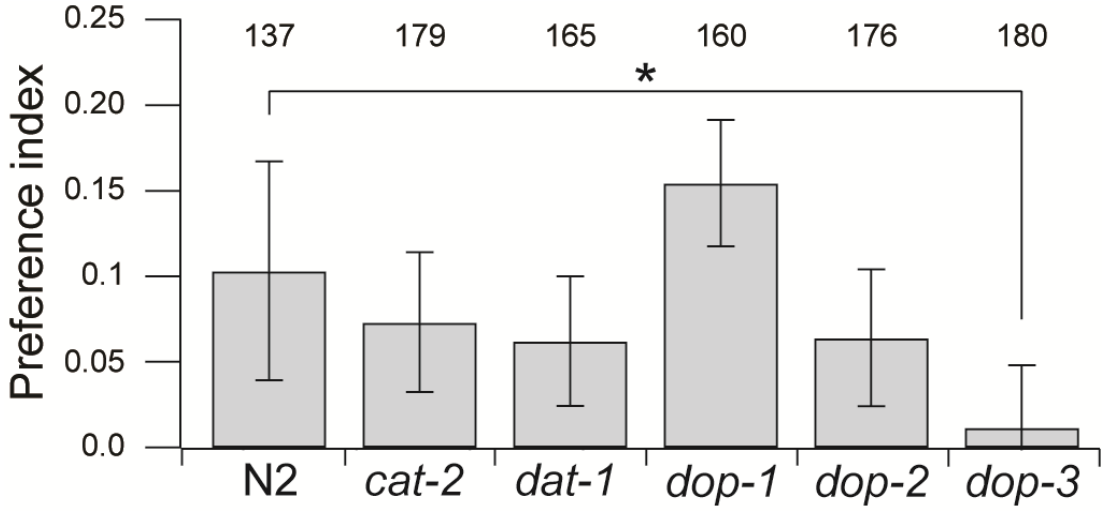
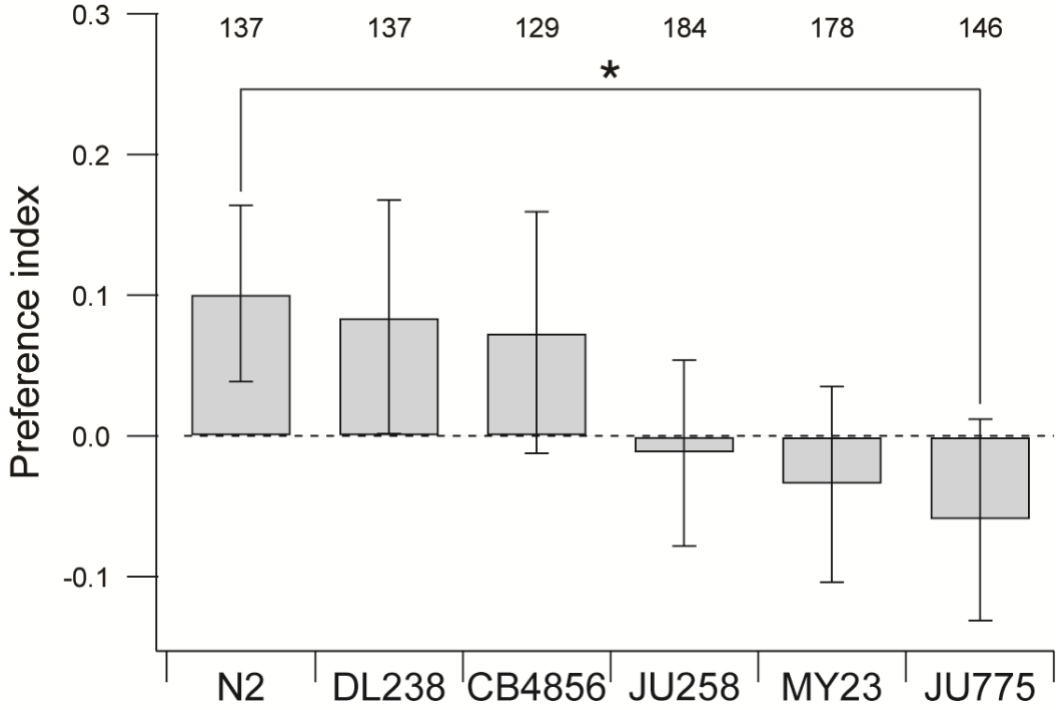


Figure 4. Effect of dopamine signaling mutations on preference for normal versus elongated bacteria. Data are mean preferences measured in T-maze assays. Dopamine signaling was reduced by a nonsense mutation in *cat-2* or by deletions of three dopamine receptor genes (*dop*). Dopamine signaling was increased by a deletion in *dat-1*. *Numbers*, sample size. Error bars ± 95% CI.

Effort discounting-like behavior may not be an adaptation to the laboratory environment

Finally, we considered whether effort discounting-like behavior may reflect adaptation to the laboratory environment. The reference strain used in this study, N2, has been cultured for over 50 years in the laboratory setting, where it is typically fed small, easily consumed bacteria, such as *E. coli* strain OP50. Consequently, it may have lost the ability to consume larger bacteria efficiently. To address this question, we tested whether any natural isolate strains would prefer normal over elongated bacteria. Accordingly, we performed T-maze assays on five such strains with normal and elongated food at OD 1.0. Natural isolate strains proved to be challenging to work with as they frequently escaped the maze or burrowed in the substrate. These traits reduced the number of countable worms per assay, leading to considerable variance in the data. Nevertheless, there was a significant overall effect of strain on mean preference index (Figure 5; Table 1, row 42). Post hoc testing revealed that only the strain JU775 had a preference index that was significantly different from N2 (Table 1, row 48) and that JU775's preference was not distinguishable from zero (Table 1, row 49). Thus, JU775 was indifferent between normal and elongated food. On the other hand, mean preference of DL238 was both different from zero (Table 1, row 50) and indistinguishable from N2 (Table 1, row 44). That is, DL238 behaved like N2. We conclude that N2's preference is probably not an adaptation to the laboratory environment. Between DL238 and JU775, there was a range of different responses, suggesting the possibility of genetic variation in preference for normal versus elongated food. This could reflect differences in effort tolerance or ease of consumption of elongated bacteria.

Figure 5



414

415 **Figure 5. Preference for normal versus elongated bacteria in N2 and five wild-isolate strains.**
416 Data are mean preferences measured in T-maze assays. N2 and DL238 are significantly different
417 from zero. *Numbers*, sample size. Error bars ± 95% CI.
418

DISCUSSION

Effort discounting is a form of cost-benefit decision-making with implications for economics, psychology, neuroscience, behavioral ecology, and other fields. To investigate effort discounting in *C. elegans*, worms were given a choice between two patches of the same species of bacteria. One patch contained normal bacteria, whereas the other patch contained elongated bacteria, which we showed to be more effortful to consume yet similar to normal bacteria in nutrition and attractiveness. When the two types of bacteria were presented at equal density, worms preferred normal bacteria. Three lines of evidence suggest that this preference is analogous to the devaluation of a reward based on increased effort to obtain it: (1) Preference can be reversed by increasing the density of the effortful option. (2) The relative density of normal and effortful food can be titrated to an indifference point. (3) Widely used economic models of discounting correctly predict novel indifference points in *C. elegans*. Points (1) (Floresco, Tse and Ghods-Sharifi, 2008) and (2) (Salamone, Cousins and Bucher, 1994; van den Bos *et al.*, 2006; Ostrander *et al.*, 2011), together with the requirement for intact dopamine signaling (Salamone *et al.*, 2018), establish similarities to effort discounting in other animal models. The demonstration of effort discounting-like behavior in a lower invertebrate sets a new phylogenetic boundary on discounting behavior.

Relationship to previous work

An increase in patch-leaving frequency in response to elongated bacteria has been reported previously (Scheer and Bargmann, 2023). There are some significant differences between that study and ours: (1) *E. coli* was used instead of *Comamonas*; (2) bacteria were elongated with the antibiotic aztreonam which renders food inedible to *C. elegans* (Gruninger, Gualberto and Garcia, 2008; Ben Arous, Laffont and Chatenay, 2009), whereas the cephalexin treatment we used resulted in elongated but edible food (Millet *et al.*, 2022); (3) worms were video-recorded for 40 minutes instead of 15 minutes; (4) food-patch leaving was defined using different behavioral criteria; (5) patch-entry rates were not considered. Nevertheless, our results confirm and extend the previous findings by showing that the effect of elongated food on patch-leaving

447 rate is robust across bacterial species, varied degrees of edibility of elongated food, and
448 differences in details of behavioral analysis.
449
450 **Utility of economic modeling**
451 Although *C. elegans* patch-leaving behavior has been described in detail before, placing it in an
452 explicit economic context is new. This context provided a quantitative framework that enriched
453 our study in two respects. First, it gave us a metric – the discount factor – for quantifying the
454 extent to which effortful food was devalued by the worm. We observed that worms were
455 indifferent when normal and elongated bacteria were at ODs 1.0 and 1.5, respectively, yielding a
456 discount factor of 2/3. Second, this framework made testable predictions. At the level of effort
457 determined by our cephalexin exposure protocol, any combination of optical densities of
458 normal and elongated food in a 2:3 ratio should be an indifference point. This prediction was
459 verified by measuring preference under conditions in which the optical density of elongated
460 food was both higher and lower than the original value of 1.5. This result shows that standard
461 models of effort discounting may apply to *C. elegans*. It will now be interesting to test whether
462 the discount factor is sensitive to effort, as assumed in many discounting models (Figure 1—
463 figure supplement 1). This could be done by identifying indifference points when effort is
464 manipulated by changing cephalexin exposure conditions to increase or decrease the extent of
465 elongation.
466
467 **A model of effort-discounting like behavior**
468 We propose a model of effort discounting that builds upon previous studies of foraging in *C.*
469 *elegans*. Once worms left the starting point of the T-maze, they were free to move back and
470 forth between food patches at the end of each arm, encountering one patch then the other in
471 serial fashion. Thus, our procedure was a foraging assay. In a simple model to explain the food
472 preferences we observed, worms make an on-line assessment of food value in the current patch
473 which in turn alters patch-leaving dynamics, increasing the exit rates from cephalexin-treated
474 patches as shown in Figure 3. The decision of whether to stay or leave a patch of food while
475 foraging has been well studied in *C. elegans*. (Shtonda and Avery, 2006; Bendesky *et al.*, 2011;

Milward *et al.*, 2011; Olofsson, 2014; Scheer and Bargmann, 2023). These studies point to a similar mechanism. The key advance here is the demonstration that the worm's foraging decisions are consistent with formal effort-discounting models under our conditions.

Stay-or-leave decision making based on assessment of current conditions has been observed in the context of effort discounting in other organisms. One procedure widely used in rodent studies is the *progressive ratio task* (Salamone *et al.*, 2018). In this task, animals can either lever-press for a preferred food or consume a less preferred food that is freely available nearby. However, the number of lever presses required to obtain preferred food increases as a function of the cumulative number of lever presses until the effort-cost of obtaining preferred food becomes too high and the animal switches to a freely available food. This is a foraging assay in the sense that the lever and the freely available food are patches, and the animal decides whether or not to leave the "lever" patch. Our assay also has parallels in the human effort-discounting literature. In one example (Bonnelle *et al.*, 2015), participants are presented with a series of virtual apple trees. They can see how many apples are in the current tree and how much effort (squeezing a handgrip) is required to gather them. Their task is to decide whether or not to gather apples based on the perceived cost and benefit. Here, the trees are equivalent to patches and the decision to gather apples or not is analogous to a stay-leave decision in patch foraging. Thus, stay-or-leave decision making may be a conserved solution to effortful foraging tasks.

Role of dopamine signaling in effort discounting-like behavior

Fully intact dopamine signaling is required for normal effort discounting-like behavior in *C. elegans*. This finding is broadly consistent with the known requirements of dopamine signaling for foraging behavior in *C. elegans*, as our T-maze assay is essentially a foraging assay. *C. elegans* dopamine receptors DOP-1, DOP-2, and DOP-3 are required for normal responses to the presence or absence of food (Chase and Koelle, 2007; Suo, Culotti and Van Tol, 2009; Bastien *et al.*, 2024). Dopamine itself is required for area-restricted search in which worms, suddenly

finding themselves outside a food patch, engage in a period of frequent locomotory reorientations in an attempt to relocate the food (Hills, Brockie and Maricq, 2004).

However, the relationship between dopamine signaling and effort discounting-like behavior in *C. elegans* remains an area for future research. For example, although mutations in the dopamine pathway cause a range of locomotor phenotypes (Chase, Pepper and Koelle, 2004; McDonald *et al.*, 2007; Ezak and Ferkey, 2010) and may affect metabolism (De Barros *et al.*, 2014), only *dop-3* displays a phenotype in our effort-discounting assay, suggesting a specific role of *dop-3* in effort-discounting in *C. elegans*. Understanding the precise role of *dop-3* and other components of the dopamine signaling pathway in effort discounting will require more sensitive behavioral assays as well as other technical approaches, including cell-specific rescues. The fact that decreased dopamine signaling in the *dop-3* mutant rendered worms indifferent between the food options suggests that decreased dopamine signaling *increases* tolerance for high-effort food, in contrast to its effects in rodents. This discrepancy may reflect a true difference in the function of dopamine in effort discounting in *C. elegans* and rodents, or methodological differences. In particular, manipulations of dopamine signaling in rodent studies mainly involve acute experiments using pharmacological agents, whereas we studied worms with chronic, lifelong alterations in dopamine signaling.

In summary, our study provides strong evidence that *C. elegans* exhibits effort discounting-like behavior. By demonstrating that worms devalue effortful food like higher organisms, we extend the phylogenetic boundary of this behavior. The application of an economic framework allowed us to quantify this effect and make testable predictions, confirming its robustness. Future research can now exploit the utility of *C. elegans* as a genetically tractable system to explore how environmental factors and genetic variations influence effort-based decision-making. Such an approach could provide deeper insights into the fundamental principles and molecular basis of cost-benefit decisions.

TABLES

533

Row	Figure	Test	Effect or comparison tested	Units of replication or sampling	Number of replicates	Statistic	Statistic value	DF 1 or combined DF	*p*	Effect size metric	Effect size
1	1A	Mann-Whitney	Cephalexin	Worms	99, 98	*U*	4178	–	9.23E-02	Rank-biserial correlation (r)	0.13 (small)
2	1B2	Mann-Whitney	Cephalexin	Worms	44, 49	*U*	618	–	**3.20E-04**	Rank-biserial correlation (r)	0.43 (moderate)
3	1C	*t*-Test	Cephalexin	Biol. Replicate	3, 3	*t*	0.10	2	9.28E-01	Cohen's d	0.08 (small)
4	1D	*t*-Test	Cephalexin	Biol. Replicate	3, 3	*t*	0.10	2	9.31E-01	Cohen's d	0.08 (small)
5	1E	*t*-Test	Cephalexin	Tech. Replicate	3, 3	*t*	0.12	2	9.91E-01	Cohen's d	0.01 (small)
6	1F2	*t*-Test	Mean not = 0	Assay plates	108	*t*	1.65	104	3.56E-01	Cohen's d	0.16 (small)
7	2B	1-way ANOVA	OD ratios	Assay plates	137, 106, 73	*F*	19.07	2	**1.52E-08**	Eta squared	0.11 (moderate)
8	2B	*t*-Test	Mean not = 0	Assay plates	137	*t*	3.20	136	**1.69E-03**	Cohen's d	0.27 (small)
9	2B	*t*-Test	Mean not = 0	Assay plates	73	*t*	1.99	72	**2.06E-07**	Cohen's d	0.67 (large)
10	2B	*t*-Test	Mean not = 0	Assay plates	106	*t*	1.08	105	2.82E-01	Cohen's d	0.10 (small)
11	2C	1-way ANOVA	OD ratios	Assay plates	120, 106, 132	*F*	0.83	2	4.37E-01	Eta squared	0.00 (negligible)
12	2C	*t*-Test	Mean not = 0	Assay plates	120	*t*	0.71	119	4.80E-01	Cohen's d	0.06 (small)
13	2C	*t*-Test	Mean not = 0	Assay plates	106	*t*	1.08	105	2.82E-01	Cohen's d	0.10 (small)
14	2C	*t*-Test	Mean not = 0	Assay plates	132	*t*	0.64	131	5.22E-01	Cohen's d	0.06 (small)
15	3C	*t*-Test	P_F vs (P_O, P_B)	Single worm	66,60	*t*	4.22	123.57	**4.62E-05**	Cohen's d	0.75 (medium)
16	3C	*t*-Test	P_O vs P_B	Single worm	66,60	*t*	0.57	116.99	5.73E-01	Cohen's d	0.10 (small)
17	3D	1-way ANOVA	Transition type	Single worm	66,60	*F*	24.71	3	**4.22E-14**	Eta squared	0.22 (large)
18	3D	Tukey	k_{FB}, k_{BF}	Single worm	66,60	*q*	3.68	122	**<1.00E-07**	Cohen's d	2.199391 (large)
19	3D	Tukey	k_{BO}, k_{OB}	Single worm	66,60	*q*	3.68	122	**4.30E-06**	Cohen's d	0.68 (medium)
20	3D	Tukey	k_{BO}, k_{BF}	Single worm	66,60	*q*	3.68	122	**6.00E-07**	Cohen's d	1.60 (large)
21	3D	Tukey	k_{OB}, k_{BF}	Single worm	66,60	*q*	3.68	122	9.77E-01	Cohen's d	0.051 (negligible)
22	3D	Tukey	k_{FB}, k_{BO}	Single worm	66,60	*q*	3.68	122	4.86E-01	Cohen's d	0.91 (large)
23	3D	Tukey	k_{OB}, k_{FB}	Single worm	66,60	*q*	3.68	122	**0**	Cohen's d	0.88 (large)
24	3D	MANOVA	Cephalexin	Single worm	66,60	*F*	16.44	4121	**8.77E-11**	Eta squared (partial)	0.35 (large)
25	3D1	*t*-Test	Cephalexin	Single worm	66,60	*t*	7.38	79.39	**1.36E-10**	Cohen's d	1.36 (large)
26	3D2	*t*-Test	Cephalexin	Single worm	66,60	*t*	3.32	118.69	**1.18E-03**	Cohen's d	0.60 (medium)
27	3D3	*t*-Test	Cephalexin	Single worm	66,60	*t*	0.28	123.25	7.83E-01	Cohen's d	0.05 (negligible)
28	3D4	*t*-Test	Cephalexin	Single worm	66,60	*t*	1.38	95.7	1.70E-01	Cohen's d	0.24 (small)
29	3E	2-way ANOVA	Cephalexin	Single worm	66,60	*F*	5.24	1	**2.29E-02**	Eta squared	0.02 (small)
30	3E	2-way ANOVA	Food	Single worm	66,60	*F*	0.70	1	4.00E-01	Eta squared	0.00 (negligible)
31	3E	2-way ANOVA	Cephalexin × Food	Single worm	66,60	*F*	6.66	1	**1.10E-02**	Eta squared	0.03 (small)
32	3E	*t*-Test	Cephalexin	Single worm	66,60	*t*	3.68	115.91	**4.59E-04**	Cohen's d	0.66 (medium)
33	3E	*t*-Test	Cephalexin	Single worm	66,60	*t*	0.19	123.94	8.47E-01	Cohen's d	0.034 (negligible)
34	4	1-way ANOVA	Strain	Assay plates	137, 179, 165, 160, 175, 180	*F*	7.69	3	**4.71E-05**	Eta squared	0.03 (small)
35	4	Dunnett test				*q*	2.50	649			
36			N2 vs *cat-2*	Assay plates	137, 179	*q*	0.97		7.72E-01		
37			N2 vs *dat-1*	Assay plates	137, 164	*q*	1.30		5.37E-01		
38			N2 vs *dop-1*	Assay plates	137, 180	*q*	-1.61		3.33E-01		
39			N2 vs *dop-2*	Assay plates	137, 176	*q*	1.25		5.71E-01		
40			N2 vs *dop-3*	Assay plates	137, 160	*q*	2.78		**1.92E-02**	Cohen's d	0.50 (medium)
41	4	*t*-Test	Mean not = 0	Assay plates	180	*t*	0.65	159	5.14E-01	Cohen's d	0.05 (small)
42	5	1-way ANOVA	Strain	Assay plates	137, 137, 129, 184, 178, 146	*F*	3.3	5	**5.83E-03**	Eta squared	0.018 (small)
43	5	Dunnett test				*q*	2.51	905			
44			N2 vs DL238	Assay plates	137, 137	*q*	0.31		9.98E-01		
45			N2 vs CB4856	Assay plates	137, 129	*q*	0.51		9.81E-01		
46			N2 vs JU258	Assay plates	137,184	*q*	2.26		9.42E-02		
47			N2 vs MY23	Assay plates	137, 178	*q*	2.46		5.81E-02		
48			N2 vs JU775	Assay plates	137, 146	*q*	3.03		**1.12E-02**	Cohen's d	0.68 (medium)
49	5	*t*-Test	Mean not = 0	Assay plates	146	*t*	0.36	145	1.03E-01	Cohen's d	0.13 (small)
50	5	*t*-Test	Mean not = 0	Assay plates	137	*t*	2.02	136	**4.56E-02**	Cohen's d	0.17 (small)

534 **Table 1.** Statistical information. Bold *p* values denote significant effects. Note that the Tukey and
535 Dunnett tests are multiple comparison tests and their *p*-values do not need to be adjusted for
536 multiple comparisons.
537

538 **MATERIALS AND METHODS**

539 **Key resources table**
540

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Strain (*E. coli*)	OP50	CGC[1]	RRID:WB-STRAIN:WBStrain00041969	Worm maintenance
Strain, strain background (*Comamonas* spp.)	DA1877	CGC	RRID:WB- STRAIN:WBStrain00040995	Behavioral testing
Strain (C. elegans)	N2	CGC	RRID:WB- STRAIN:WBStrain00000001	All WT assays
Strain (C. elegans)	*cat-2(e1112)*	CB	CB1112	Behavioral testing
Strain (C. elegans)	*dat-1(ok157)*	CGC	RM2702	Behavioral testing
Strain (C. elegans)	*dop-1(vs101)*	CGC	LX636	Behavioral testing
Strain (C. elegans)	*dop-2(vs105)*	CGC	LX702	Behavioral testing
Strain (C. elegans)	*dop-3(vs106)*	CGC	LX703	Behavioral testing
Drug	cephalexin hydrate	Thermo Fisher	J6317206	Bacteria elongation
Dye	Oil-Red-O	Sigma-Aldrich	00625	Lipid storage analysis
Dye	BacLight Green	Thermo Fisher	B-35000	Bacteria visualization
Software algorithm	Igor Pro	Wavemetrics	Version 9.05	Behavioral testing, image analysis
Software	R	R Core Team (2024)	Version 4.4.2 (2024-10-31)	Statistical analysis
Software	WormLab	MBF Bioscience	Version 2024	Video analysis

541
542 [1]*Caenorhabditis* Genetics Center.
543

544 **Bacteria culture**

545 Two bacterial species were used: *E. coli* (OP50) used for worm culture, and *Comamonas* spp.
546 (DA1877) for assays. Bacterial cultures were grown in liquid lysogeny broth (LB) under sterile

547 conditions at 37°C with agitation. For all assays, DA1877 was grown for one day, then "Control"
548 cultures were solubilized with M9 buffer (3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, 1mL 1 mol/L $MgSO_4$,
549 H_2O to 1L); "cephalexin" cultures were supplemented with a solution of M9 and cephalexin
550 hydrate 97% to a concentration of 450 µmol/L and incubated for 2 hours. Cultures then
551 underwent three rounds of concentration by centrifugation, followed by rinsing with 10 mL of
552 M9 buffer before being resuspended to their final concentration. Concentration was defined as
553 optical density at 600 nm (OD), as measured with a cell density meter (Laxco, DSM, Bothell, WA,
554 USA). All measurements were performed on samples diluted into the linear range of the
555 instrument (OD 0.1–1.0).
556
557 **Worm maintenance & synchronization**
558 All strains were maintained at 20°C on 5 cm plates containing nematode growth medium (NGM)
559 seeded with *Escherichia coli* OP50 bacteria as a food source (Brenner, 1974). For
560 synchronization, 20 young adult worms were transferred t
561 o fresh 5 cm NGM plates. Adults were removed from the plates after laying eggs for 3 hours.
562 The plates were maintained at 20°C until worms reached the young adult stage.
563
564 **Pharyngeal pumping assay**
565 Pharyngeal pumping was measured electrophysiologically using the ScreenChip microfluidic
566 system (InVivo Biosystems, Eugene, OR, USA). Synchronized young adults were preincubated
567 with control or cephalexin-treated bacteria at OD 1 for 20 minutes before being transferred into
568 the reservoir of a microfluidic device fitted with electrodes. Individual worms were moved one
569 at a time from the reservoir into the recording channel and given one to two minutes to
570 acclimate. Voltage transients associated with pharyngeal pumping were recorded for five
571 minutes; such recordings are called electropharyngeograms. All recordings were performed
572 within 90 minutes of the worms being loaded into the microfluidic reservoir. Mean pumping
573 frequency was extracted using custom code written in Igor Pro (WaveMetrics, Lake Oswego, OR,
574 USA).
575

576 **Food-preference assay**

577 T-mazes (Levichev *et al.*, 2023) were constructed by cutting masks from sheets of 2 mm thick
578 ethylene-vinyl acetate foam (Darice Craft Foam, Strongsville, OH, USA) using a fabric-cutting
579 machine (Cricut, South Jordan, UT, USA). Masks were placed on blank 5 cm NGM plates. Maze
580 arms were baited with 4.5 µL of various bacterial suspensions as described in the text.
581 Synchronized young adults were washed by five rounds of centrifugation (30 sec, 300 g)
582 followed by aspiration of the supernatant, then deposited by liquid transfer at the starting point
583 of the maze. A transparent plastic disc was placed over the maze to eliminate air currents.
584 Twelve plates were placed on a flatbed scanner (EPSON, model J221B, Los Alamitos, CA, USA)
585 and imaged after an elapsed time of 60 minutes. Imaged worms were counted manually. The
586 food preference index was computed as $I = (n_{\mathrm{N}} - n_{\mathrm{L}})/(n_{\mathrm{N}} + n_{\mathrm{L}})$, where n_{N} and n_{L} are the
587 number of worms in the normal and elongated patches, respectively. In some experiments, a
588 paralytic agent (sodium azide, NaN_3, 3 µL at 20 mM) was added to each food patch to prevent
589 animals from leaving the patch of food after reaching it. Sodium azide diffuses through the agar
590 over time, and its action is not instantaneous. To account for these effects, all worms within 5
591 mm of the end of the maze arm, rather than only those on food, were counted when calculating
592 the preference index.

593

594 **Patch-leaving assay**

595 Custom 8-well plates (25 mm × 45 mm) were fabricated from acrylic plastic. Wells (diameter: 10
596 mm; depth: 6 mm) were filled with NGM agar. Bacterial suspension (2 µL, OD 1) was placed at
597 the center of each well, forming a patch 3-4 mm in diameter. Synchronized one-day-old adult
598 worms were cleaned by transferring them to an unseeded NGM plate for several minutes. One
599 worm was transferred to each well using a pick and placed at the center of the patch. The well
600 plate was immediately placed on a stage 8 cm above a diffuse white-light source and imaged (2
601 or 5 frames per second, 23.4 µm/pixel) for 15 minutes using a video camera (AmScope, 1080P,
602 Irvine, CA, USA) fitted with a telephoto lens (Nikon USA, AF Micro Nikkor 60 mm, Melville, NY,
603 USA). The distance between the lens and the stage was 50 cm. Worm tracks and locomotion
604 parameters were extracted using WormLab software (MBF Bioscience, Williston, VT, USA) and

605 analyzed using custom routines in Igor Pro (WaveMetrics, Lake Oswego, OR, USA). To define the
606 worm's position with respect to the patch, its margin was traced manually.
607
608 **Bacterial composition analysis**
609 Bacteria were grown under sterile conditions at 37°C with agitation in 500 mL of LB overnight,
610 pelleted (5,000 rpm, 3 minutes), washed with water, and re-pelleted. Pellets were stored at -
611 80°C prior to analysis. Bacterial stocks were weighed and then lysed with CelLytic B Plus kit
612 (Sigma-Aldrich, CB0050, Saint Louis, MO, USA) on the day of the assay.
613
614 Protein. Protein was assayed with the Pierce BCA Protein Assay kit (Thermo Fisher Scientific,
615 A55864, Waltham, MA, USA). Readings were taken with a spectrophotometer (NanoDrop,
616 Thermo Fisher Scientific, ND-2000, Waltham, MA, USA) using the bicinchoninic acid assay
617 setting.
618
619 Carbohydrate. Carbohydrate was measured with the Abbexa Total Carbohydrate Assay Kit
620 (Abbexa, abx298986, Cambridge, UK). Spectrophotometer readings were made at OD 540 nm.
621
622 Lipid. Lipid was quantified using the method of Aggeler *et al*. (Aggeler, Then and Ghosh, 1987).
623 We prepared 100 µL of cell lysate and added: 500 µL methanol, 250 µL dichloromethane, and
624 100 µL distilled H_2O. The mixture was vortexed before adding an additional 375 µL of distilled
625 water. The solution was vortexed again to form an emulsion and then centrifugated at 4800 g
626 for 10 minutes. The bottom layer of solution was transferred to a pre-weighed tube and then
627 weighed again after the lipid layer dried. The difference in weight was used to measure the
628 quantity of lipid per 100 µL of cell lysate.
629
630 **Bacteria visualization**
631 Control and cephalexin-treated bacteria were prepared as described above. BacLight Green dye
632 dissolved in DMSO was added to the bacteria at a final concentration of 0.1 µM. Following a 15-
633 minute incubation, bacteria were mounted between a microscope slide and a coverslip, then

634 imaged on a spinning disk confocal microscope (Nikon Eclipse Ti-2E with Yokogawa CSU-W1;
635 objective CFI60 Plan Fluor 40x Oil Immersion, N.A. 1.3; Melville, NY, U.S.A.).
636
637 **Fat storage analysis**
638 Fat storage was assessed using the procedures of Soukas *et al*. (Soukas *et al.*, 2009) and Stuhr
639 et al. (Stuhr and Curran, 2020). A 0.5% isopropanol solution of Oil-Red-O (Sigma-Aldrich, Oil-
640 Red-O powder 00625, Saint Louis, MO, USA) was prepared several days before the assay.
641 Bacterial cultures (Control and cephalexin) were prepared as described above and adjusted to
642 OD 3 before being loaded onto fresh NGM plates. Seventeen young adult worms were placed
643 on these plates and allowed to lay eggs for 5 hours before being removed. The plates were
644 incubated at 20°C for two days. L4 larvae were harvested and washed in PBS + 0.01% Triton X-
645 100. Worms were fixed with a 60% isopropanol solution and incubated with the Oil-Red-O
646 solution for two hours on a rotating plate. Worms were washed twice with PBS + Triton 0.01%
647 solution, first for 30 minutes, then for one hour, on a rotating plate. Stained worms were
648 imaged on a microscope slide at 32×, and staining intensity was quantified using custom
649 routines in Igor Pro (WaveMetrics, Lake Oswego, OR, USA).
650
651 **Statistical analysis**
652 General. Statistical details for each test (figure panel, statistical test, effect or comparison
653 tested, definition of units of replication, number of replicates, statistic value, degrees of
654 freedom, *p*-value, and effect size) are compiled in Table 1. These details are cited in the text
655 using the notation "Table 1, row *n*," where *n* is the row number in the table. For all
656 comparisons, the centers of distributions were their arithmetic mean or median. For dispersion
657 measures, we used 95% confidence intervals. The following statistical tests were employed
658 according to the experimental design: We used *t*-tests to determine whether the mean differed
659 from zero. To assess differences in pairs of means, we used *t*-tests and, when the data were not
660 normally distributed (according to the Shapiro-Wilk test), the Mann-Whitney U test. For
661 comparisons of three or more means we used 1-way or 2-way ANOVA with *post hoc* tests.
662

State probabilities. State probabilities are an instance of *compositional data*, meaning data that sum to 1 (or 100%), which requires special treatment. For analysis, we subjected these data to the isometric log ratio transform (Aitchison, 1986; Egozcue *et al.*, 2003) using the "compositions" package in R (van den Boogaart and Tolosana-Delgado, 2008). Following transformation, *t*-tests were used to infer statistical significance (Figure 3—figure supplement 1).

Box plots. The box represents the first and third quartiles of the data, with a horizontal line representing the median. The whiskers extend to the maximum and minimum values following the removal of extreme outliers. These were defined as points with values below $Q1 - 3 \times IQR$ or above $Q1 + 3 \times IQR$, where $Q1$ is the first quartile, $Q3$ the third quartile and IQR is the interquartile range $Q3 - Q1$. A diamond symbol represents the mean.

Number of replicates. In the case of *assay plates*, a replicate is unique cohort of worms on an assay plate, such as a T-maze. In the case of *worms*, a replicate is a unique animal tested once. Nutrient replicates (Figure 1C–E) were biological or technical, as indicated in Table 1. The minimum sample size for the T-maze assays was based on pilot experiments that demonstrated the ability to detect moderate to small effects with 10–30 replicates per experimental condition. Previously published electropharyngeogram data (Katzen *et al.*, 2023) showed that mutants or treatments could be distinguished with approximately 20 replicates; however, to ensure the detection of small effect sizes across experimental conditions, larger sample sizes were used.

Effect size. For *t*-tests we used Cohen's *d*. For Mann-Whitney U tests, we used the rank-biserial correlation with $r = 2\,U/(n_1 n_2) - 1$, where U is the Mann-Whitney statistic and n_1 and n_2 are group sizes. For 1-way ANOVA, we used eta squared with $\eta^2 = SS_{\text{between}}/SS_{\text{total}}$ where SS_{between} is the sum of squares between groups and SS_{total} is the total sum of squares. For 2-way ANOVA we used $\eta^2 = SS_{\text{effect}}/SS_{\text{total}}$ where SS_{effect} is the sum of squares for a factor or interaction. For MANOVA we used partial eta squared with $\eta_p^2 = SS_{\text{effect}}/(SS_{\text{effect}} + SS_{\text{error}})$ where SS_{error} is sum of squares for error.

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COMPETING INTERESTS

S.R.L. has a competing financial interest in InVivo Biosystems, Inc., which produces the ScreenChip system utilized in the pharyngeal pumping assay. The other authors have no competing interests to declare.

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