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C. elegans food choice exhibits effort discounting-like behavior

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This **important** work develops the C. elegans as a model organism for studying effort-based discounting by asking the worms to choose between patches of easy and hard to digest bacteria. The authors provide **convincing** evidence that the nematodes are effort discounting. They also provide **solid** evidence of involvement of dopamine in the food preference and that the finding is not restricted to lab-acclimated strains.

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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;

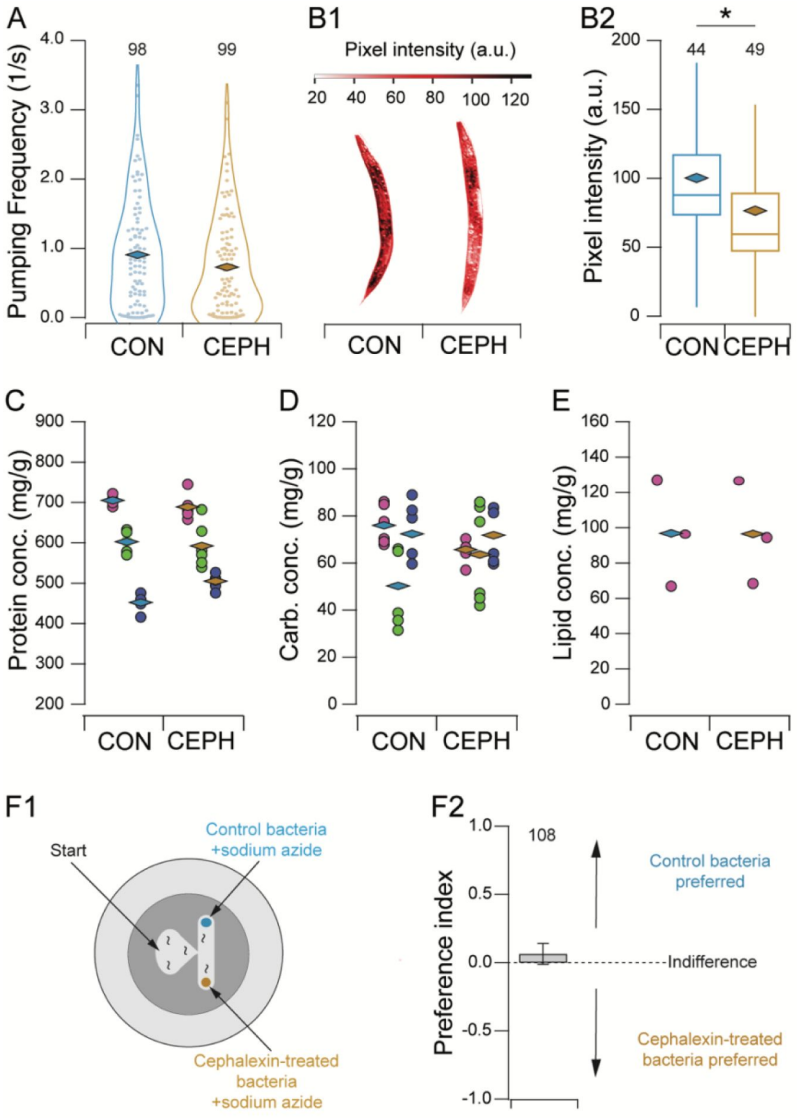


Figure 1.

Establishment of an effort discounting paradigm in *C. elegans*.

A. Pharyngeal pumping frequency in suspensions of normal (CON) and elongated (CEPH) bacteria. Symbols (box and whisker plot): *filled circles*, single-worm data points; *diamonds*, means; *numbers*, sample size. B1. Quantification of Oil-Red-O staining of lipid in worms cultured on normal (CON) or elongated (CEPH) bacteria. Median worms from each condition are shown. B2. Mean pixel intensity of Oil-Red-O staining of lipid in worms cultured on normal (CON) or 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.

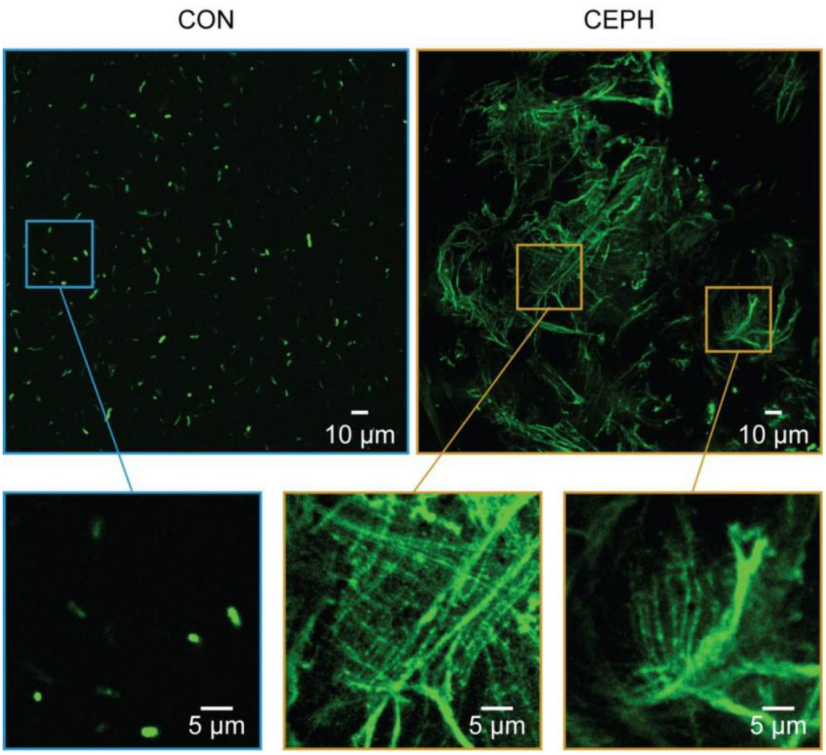


Figure 1––figure supplement 1.

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.

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)

Table 1.

Statistical information.

Bold *p* values denote significant effects. Note that the Tukey and Dunnett tests are multiple comparison tests and their *p*-values do not need to be adjusted for multiple comparisons.

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

Worms devalue food that requires more effort to eat

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

An economic model predicts novel indifference points

Indifference points are significant because, by definition, the presented options have equal perceived value, allowing for the quantification of devaluation. This quantification is only 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

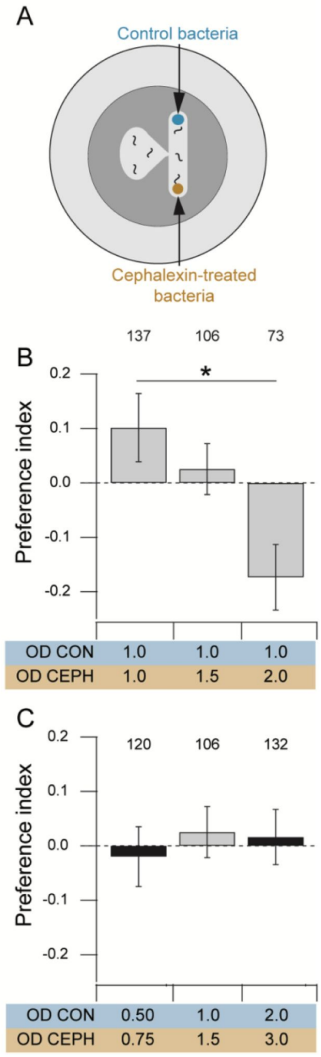


Figure 2.

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.

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	$V(E) = \frac{V_0}{e^{\alpha E}}$	$\frac{1}{e^{\alpha E}}$

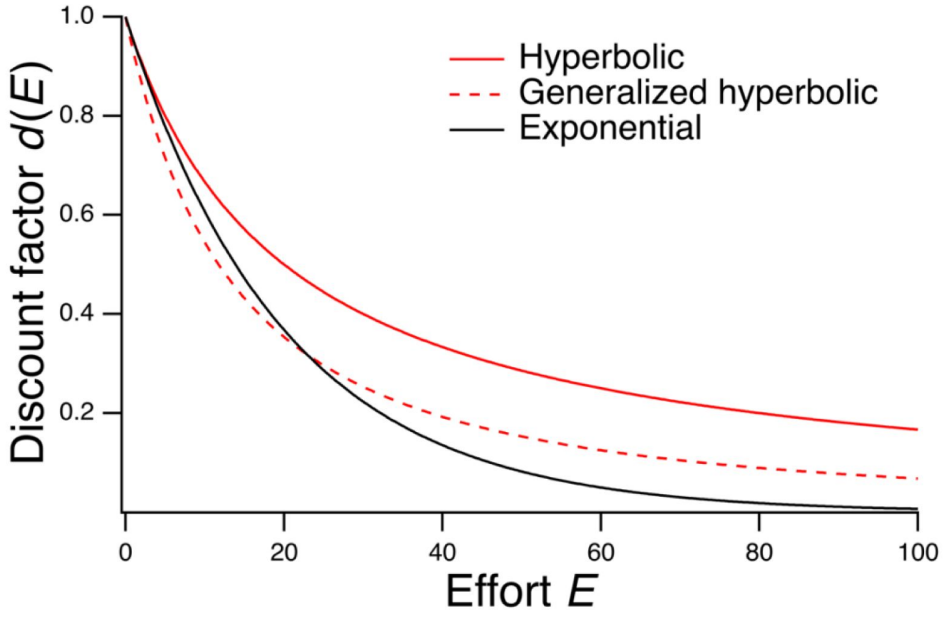


Figure 2--figure supplement 1.

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.

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.

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**):

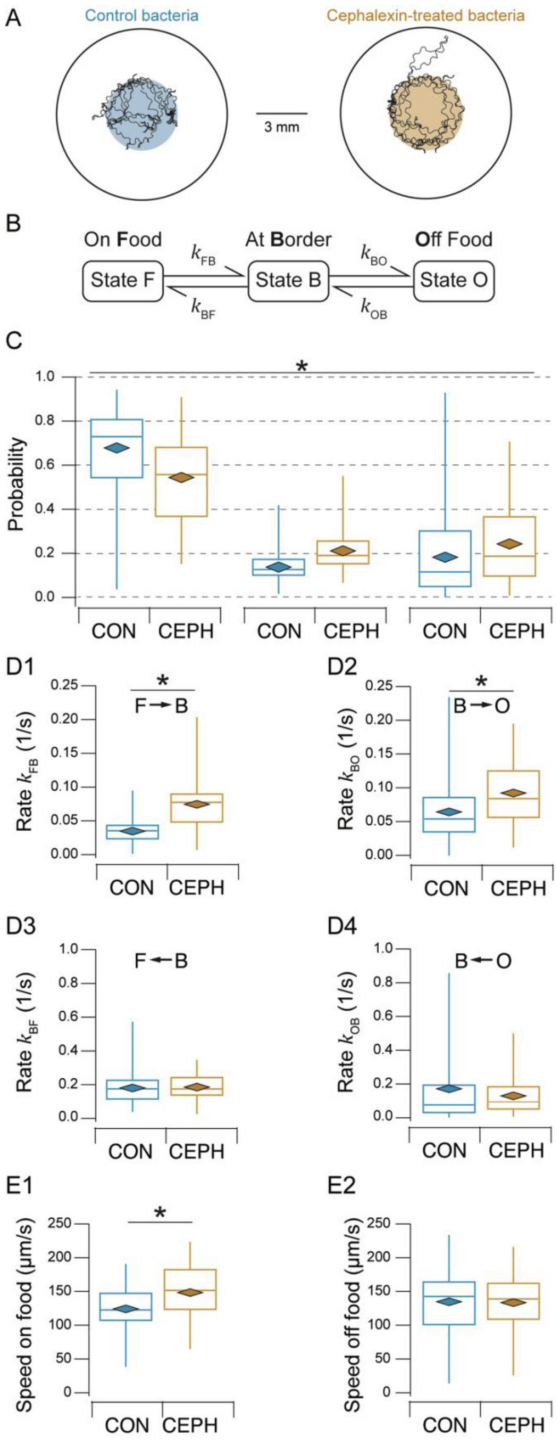


Figure 3.

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.

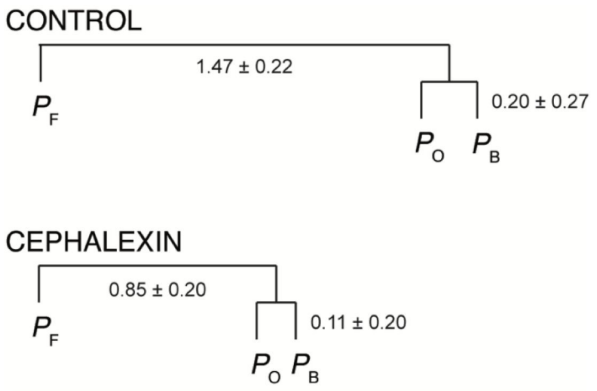


Figure 3--figure supplement 1.

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.

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_F, P_B, P_O) and rate constants (k_{FB}, k_{BO}, k_{OB}, k_{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, $k_{BF} > k_{FB}$ and $k_{OB} > k_{BO}$ (**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_{FB}, k_{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.

Intact dopamine signaling is required for effort discounting-like behavior

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

To test whether dopamine signaling is involved in effort discounting-like behavior in *C. elegans*, we examined food preference in a variety of mutants with altered dopamine signaling. The gene *cat-2* encodes a tyrosine hydroxylase essential to the main synthesis pathway for dopamine; *cat- 2*

mutants are commonly used to reduce dopamine signaling in *C. elegans*. The gene *dat-1* encodes a dopamine transporter that mediates the reuptake of dopamine into presynaptic neurons; *dat-1* mutants are used to increase dopamine signaling. The effects of reduced

dopamine signaling can also be investigated in dopamine receptor mutants. *C. elegans* has four genes encoding dopamine receptors with homology to dopamine receptors in mammals. Of these, *dop-1*, *dop-2*, and *dop-3* are the most thoroughly characterized to date. *dop-1* is a DRD1 homolog, whereas *dop-2* and *dop-3* are DRD2 homologs. The genes *dop-1* and *dop-3* are required for normal responses to the presence of food (Chase, Pepper and Koelle, 2004); *dop-2* is required for increased levels of activity in the absence of food (Bastien et al., 2024). We found a significant overall effect of strain on food preference measured in the T-maze assay when both foods were at OD 1.0 (**Figure 4**; **Table 1, row 34**). Post hoc testing showed that *cat-2*, *dat-1*, *dop-1*, and *dop-2* were indistinguishable from N2, whereas *dop-3* mutants were significantly different from N2 (**Table 1, row 36**-40). In particular, they were indifferent between the two food options, indicative of decreased effort discounting-like behavior (**Table 1, row 41**). We conclude that fully intact dopamine signaling is required for normal effort discounting-like behavior in *C. elegans*, as in rodents. However, the fact that impaired dopamine signaling rendered worms indifferent between the food options suggests that decreased dopamine signaling increases tolerance for high-effort food, in contrast to its effect in rodents. The absence of an effect of the *cat-2* and *dat-1* mutations is puzzling in view of the requirement for *dop-3*. One possibility is that residual dopamine in the *cat-2* mutant (Sanyal et al., 2004) is sufficient for normal effort discounting-like behavior and that normal levels of dopamine signaling are saturating, such that *dat-1* mutations have no effect.

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.

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

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.

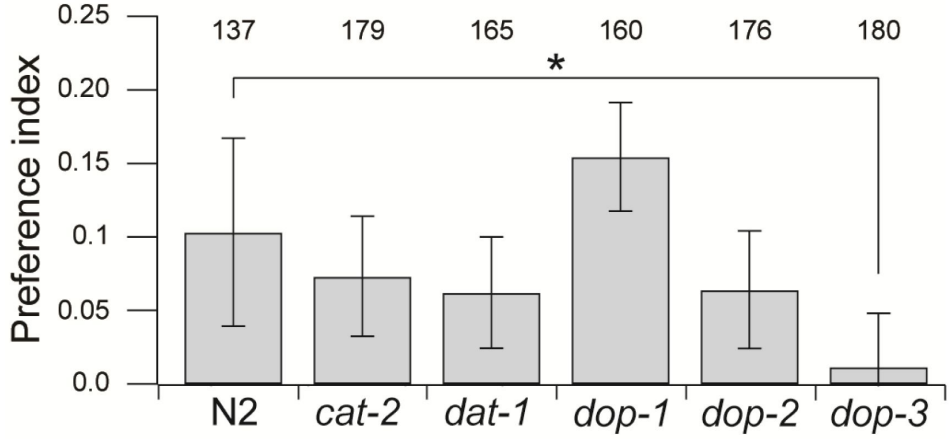
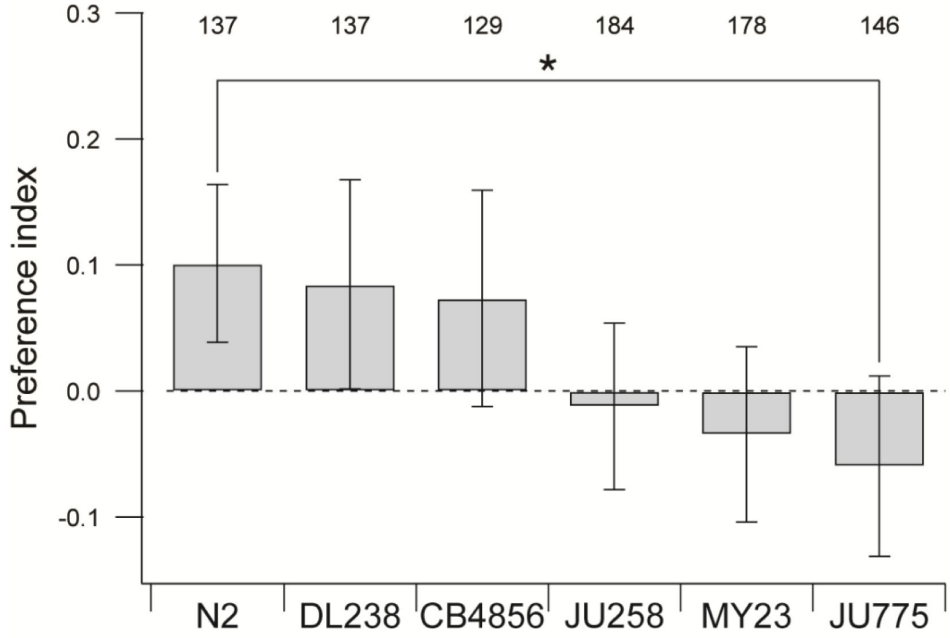


Figure 5.

Preference for normal versus elongated bacteria in N2 and five wild-isolate strains.

Data are mean preferences measured in T-maze assays. N2 and DL238 are significantly different from zero. *Numbers*, sample size. Error bars ± 95% CI.



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 rate is robust across bacterial species, varied degrees of edibility of elongated food, and differences in details of behavioral analysis.

Utility of economic modeling

Although *C. elegans* patch-leaving behavior has been described in detail before, placing it in an explicit economic context is new. This context provided a quantitative framework that enriched our study in two respects. First, it gave us a metric – the discount factor – for quantifying the extent to which effortful food was devalued by the worm. We observed that worms were indifferent when normal and elongated bacteria were at ODs 1.0 and 1.5, respectively, yielding a discount factor of 2/3. Second, this framework made testable predictions. At the level of effort determined by our cephalexin exposure protocol, any combination of optical densities of normal and elongated food in a 2:3 ratio should be an indifference point. This prediction was verified by measuring preference under conditions in which the optical density of elongated food was both higher and lower than the original value of 1.5. This result shows that standard models of effort discounting may apply to *C. elegans*. It will now be interesting to test whether the discount factor is sensitive to effort, as assumed in many discounting models (**Figure 1–– figure supplement 1**). This could be done by identifying indifference points when effort is manipulated by changing cephalexin exposure conditions to increase or decrease the extent of elongation.

A model of effort-discounting like behavior

We propose a model of effort discounting that builds upon previous studies of foraging in *C. elegans*. Once worms left the starting point of the T-maze, they were free to move back and forth between food patches at the end of each arm, encountering one patch then the other in serial fashion. Thus, our procedure was a foraging assay. In a simple model to explain the food preferences we observed, worms make an on-line assessment of food value in the current patch which in turn alters patch-leaving dynamics, increasing the exit rates from cephalexin-treated patches as shown in **Figure 3**. The decision of whether to stay or leave a patch of food while 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.

Materials and methods

Key resources table

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

[1]*Caenorhabditis* Genetics Center.

Bacteria culture

Two bacterial species were used: *E. coli* (OP50) used for worm culture, and *Comamonas* spp. (DA1877) for assays. Bacterial cultures were grown in liquid lysogeny broth (LB) under sterile conditions at 37°C with agitation. For all assays, DA1877 was grown for one day, then “Control” cultures were solubilized with M9 buffer (3g KH_2PO_4, 6g Na_2HPO_4, 5g NaCl, 1mL 1 mol/L $MgSO_4$, H_2O to 1L); “cephalexin” cultures were supplemented with a solution of M9 and cephalexin hydrate 97% to a concentration of 450 µmol/L and incubated for 2 hours. Cultures then underwent three rounds of concentration by centrifugation, followed by rinsing with 10 mL of M9 buffer before being resuspended to their final concentration. Concentration was defined as optical density at 600 nm (OD), as measured with a cell density meter (Laxco, DSM, Bothell, WA, USA). All measurements were performed on samples diluted into the linear range of the instrument (OD 0.1–1.0).

Worm maintenance & synchronization

All strains were maintained at 20°C on 5 cm plates containing nematode growth medium (NGM) seeded with *Escherichia coli* OP50 bacteria as a food source (Brenner, 1974). For synchronization, 20 young adult worms were transferred t o fresh 5 cm NGM plates. Adults were removed from the plates after laying eggs for 3 hours. The plates were maintained at 20°C until worms reached the young adult stage.

Pharyngeal pumping assay

Pharyngeal pumping was measured electrophysiologically using the ScreenChip microfluidic system (InVivo Biosystems, Eugene, OR, USA). Synchronized young adults were preincubated with control or cephalexin-treated bacteria at OD 1 for 20 minutes before being transferred into the reservoir of a microfluidic device fitted with electrodes. Individual worms were moved one at a time from the reservoir into the recording channel and given one to two minutes to acclimate. Voltage transients associated with pharyngeal pumping were recorded for five minutes; such recordings are called electropharyngeograms. All recordings were performed within 90 minutes of the worms being loaded into the microfluidic reservoir. Mean pumping frequency was extracted using custom code written in Igor Pro (WaveMetrics, Lake Oswego, OR, USA).

Food-preference assay

T-mazes (Levichev et al., 2023) were constructed by cutting masks from sheets of 2 mm thick ethylene-vinyl acetate foam (Darice Craft Foam, Strongsville, OH, USA) using a fabric-cutting machine (Cricut, South Jordan, UT, USA). Masks were placed on blank 5 cm NGM plates. Maze arms were baited with 4.5 µL of various bacterial suspensions as described in the text.

Synchronized young adults were washed by five rounds of centrifugation (30 sec, 300 g) followed by aspiration of the supernatant, then deposited by liquid transfer at the starting point of the maze. A transparent plastic disc was placed over the maze to eliminate air currents.

Twelve plates were placed on a flatbed scanner (EPSON, model J221B, Los Alamitos, CA, USA) and imaged after an elapsed time of 60 minutes. Imaged worms were counted manually. The food preference index was computed as $I = (n_N - n_L)/(n_N + n_L)$, where n_N and n_L are the number of worms in the normal and elongated patches, respectively. In some experiments, a paralytic agent (sodium azide, NaN_3, 3 µL at 20 mM) was added to each food patch to prevent animals from leaving the patch of food after reaching it. Sodium azide diffuses through the agar over time, and its action is not instantaneous. To account for these effects, all worms within 5 mm of the end of the maze arm, rather than only those on food, were counted when calculating the preference index.

Patch-leaving assay

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

Bacterial composition analysis

Bacteria were grown under sterile conditions at 37°C with agitation in 500 mL of LB overnight, pelleted (5,000 rpm, 3 minutes), washed with water, and re-pelleted. Pellets were stored at - 80°C prior to analysis. Bacterial stocks were weighed and then lysed with CelLytic B Plus kit (Sigma-Aldrich, CB0050, Saint Louis, MO, USA) on the day of the assay.

Protein

Protein was assayed with the Pierce BCA Protein Assay kit (Thermo Fisher Scientific, A55864, Waltham, MA, USA). Readings were taken with a spectrophotometer (NanoDrop, Thermo Fisher Scientific, ND-2000, Waltham, MA, USA) using the bicinchoninic acid assay setting.

Carbohydrate

Carbohydrate was measured with the Abbexa Total Carbohydrate Assay Kit (Abbexa, abx298986, Cambridge, UK). Spectrophotometer readings were made at OD 540 nm.

Lipid

Lipid was quantified using the method of Aggeler et al. (Aggeler, Then and Ghosh, 1987). We prepared 100 µL of cell lysate and added: 500 µL methanol, 250 µL dichloromethane, and 100 µL distilled H_2O. The mixture was vortexed before adding an additional 375 µL of distilled water. The solution was vortexed again to form an emulsion and then centrifugated at 4800 g for 10 minutes. The bottom layer of solution was transferred to a pre-weighed tube and then weighed again after the lipid layer dried. The difference in weight was used to measure the quantity of lipid per 100 µL of cell lysate.

Bacteria visualization

Control and cephalexin-treated bacteria were prepared as described above. BacLight Green dye dissolved in DMSO was added to the bacteria at a final concentration of 0.1 µM. Following a 15-minute incubation, bacteria were mounted between a microscope slide and a coverslip, then imaged on a spinning disk confocal microscope (Nikon Eclipse Ti-2E with Yokogawa CSU-W1; objective CFI60 Plan Fluor 40x Oil Immersion, N.A. 1.3; Melville, NY, U.S.A.).

Fat storage analysis

Fat storage was assessed using the procedures of Soukas et al. (Soukas et al., 2009) and Stuhr et al. (Stuhr and Curran, 2020). A 0.5% isopropanol solution of Oil-Red-O (Sigma-Aldrich, Oil- Red-O powder 00625, Saint Louis, MO, USA) was prepared several days before the assay.

Bacterial cultures (Control and cephalexin) were prepared as described above and adjusted to OD 3 before being loaded onto fresh NGM plates. Seventeen young adult worms were placed on these plates and allowed to lay eggs for 5 hours before being removed. The plates were incubated at 20°C for two days. L4 larvae were harvested and washed in PBS + 0.01% Triton X- 100. Worms were fixed with a 60% isopropanol solution and incubated with the Oil-Red-O solution for two hours on a rotating plate. Worms were washed twice with PBS + Triton 0.01% solution, first for 30 minutes, then for one hour, on a rotating plate. Stained worms were imaged on a microscope slide at 32×, and staining intensity was quantified using custom routines in Igor Pro (WaveMetrics, Lake Oswego, OR, USA).

Statistical analysis

General

Statistical details for each test (figure panel, statistical test, effect or comparison tested, definition of units of replication, number of replicates, statistic value, degrees of freedom, *p*-value, and effect size) are compiled in **Table 1**. These details are cited in the text using the notation "**Table 1**, row *n*," where *n* is the row number in the table. For all comparisons, the centers of distributions were their arithmetic mean or median. For dispersion measures, we used 95% confidence intervals. The following statistical tests were employed according to the experimental design: We used *t*-tests to determine whether the mean differed from zero. To assess differences in pairs of means, we used *t*-tests and, when the data were not normally distributed (according to the Shapiro-Wilk test), the Mann-Whitney U test. For comparisons of three or more means we used 1-way or 2-way ANOVA with *post hoc* tests.

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 $\eta^2 = SS/(SS + SS)$ where SS_{error} is sum of squares for error.

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Additional files

Data

Additional information

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Reviewer #1 (Public review):

Summary:

Millet et al. show that C. elegans systematically prefers easy-to-eat bacteria but will switch its choice when harder-to-eat bacteria are offered at higher densities, producing indifference points that fit standard economic discounting models. Detailed kinetic analysis reveals that this bias arises from unchanged patch-entry rates but significantly elevated exit rates on effortful food, and dop-3 mutants lose the preference altogether, implicating dopamine in effort sensitivity. These findings extend effort-discounting behavior to a simple nematode, pushing the phylogenetic boundary of economic cost-benefit decision-making.

Strengths:

Extends the well-characterized concept of effort discounting into C. elegans, setting a new phylogenetic boundary and opening invertebrate genetics to economic-behavior studies.

Elegant use of cephalexin-elongated bacteria to manipulate "effort" without altering nutritional or olfactory cues, yielding clear preference reversals and reproducible indifference points.

Application of standard discounting models to predict novel indifference points is both rigorous and quantitatively satisfying, reinforcing the interpretation of worm behavior in economic terms.

The three-state patch-model cleanly separates entry and exit dynamics, showing that increased leaving rates-rather than altered re-entry-drive choice biases.

Demonstrates that _dop-3_ mutants lose normal effort discounting, firmly tying monoaminergic signaling to this behavior and paralleling vertebrate findings.

Demonstration of discounting in wild strain (solid evidence).

Weaknesses:

Only _dop-3_ shows an effect, whereas _cat-2_/_dat-1_ do not, leaving the broader role of dopamine synthesis and reuptake ambiguous.

With only five wild isolates tested, and only one clearly showing clear evidence of preference for the easy to eat bacteria, it's hard to conclude that effort discounting isn't a lab-strain artifact or how broadly it varies in natural populations.

https://doi.org/10.7554/eLife.106792.2.sa3

Reviewer #2 (Public review):

Summary:

Here Millet et al. adapted a t-maze paradigm for use in C. elegans to understand whether nematodes exhibit effort discounting behaviors comparable to other species. C. elegans worms were reliably sensitive to how effortful the food was to consume, allowing for the application of standard economic models of decision-making to be applied to their behavior. The authors then demonstrated the necessity of dopamine signaling for this behavior, identifying dop-3 mutants in particular as insensitive to effort. Together, this work establishes a new model system for the study of discounting behavior in cost-benefit decision-making.

Strengths:

The question is well-motivated and the approach taken here is novel; it is uncommon for worms to undergo such behavioural procedures (although this lab has previously been integral to pushing the extent of the complexity of behaviours studied in C. elegans). The authors are careful in their approach to altering and testing the properties of the elongated bacteria. Similarly, they go to some effort to understand what exactly is driving behavioural choices in this context, both through application of simple standard models of effort discounting and a kinetic analysis of patch leaving. The comparisons to various dopamine mutants further extends the translational potential of their findings. I also appreciate the comparison to natural isolate strains as the question of whether this behaviour may be driven by some sort of strain-specific adaptation to the environment is not regularly addressed in mammalian counterparts to this work.

Weaknesses:

The authors have now addressed concerns about whether the mechanisms underlying the choice behavior here are generalizable to other organisms. Specifically, their work speaks to foraging-inspired effort discounting paradigms in rodents and humans in which the decision is whether to stay or leave a given resource, rather than to simultaneous decision-making across two options in a T-maze.

The dopamine results are interesting but still difficult to interpret. As the authors discuss, the lack of an effect in the cat-2 and dat-1 mutants is surprising given the effect in the dop-3

mutants. Understanding what exactly the role of dop-3 is here therefore requires further study.

https://doi.org/10.7554/eLife.106792.2.sa2

Reviewer #3 (Public review):

Summary:

The authors establish a behavioral task to explore effort discounting in C. elegans. By using bacterial food that takes longer to consume, the authors show that for equivalent effort, as measured by pumping rate, animals obtain less food, as measured by fat deposition.

The authors formalize the task by applying a neuroeconomic decision making model that includes, value, effort, and discounting. They use this to estimate the discounting C. elegans apply based on ingestion effort by using a population level 2-choice T-maze.

They then analyze the behavioral dynamics of individual animals transitioning between on-food and off-food states. Harder to ingest bacteria led to increased food patch leaving.

Finally, they examined a set of mutants defective in different aspects of dopamine signaling, as dopamine plays a key role in discounting in vertebrates and regulates certain aspects of C. elegans foraging.

In their response to the first set of reviews, the authors take care to ensure their task is analogous to at least some of those used in mammals and make changes to the text to better clarify some of their conclusions. My view is the same--that this is an interesting paper for methodological and scientific reasons that brings an important theoretical framework to bear on C. elegans foraging behavior. While I think the mutant results are somewhat unsatisfying, this is not the principal contribution of the work.

Strengths:

The behavioral experiments and neuroeconomic analysis framework are compelling and interesting and make a significant contribution to the field. While these foraging behaviors have been extensively studied, few include clearly articulated theoretical models to be tested.

Demonstrating that C. elegans effort discounting fits model predictions and has stable indifference points is important for establishing these tasks as a model for decision making.

Weaknesses:

The dopamine experiments are harder to interpret. The authors point out the perplexing lack of an effect of dat-1 and cat-2. dop-3 leads to general indifference. I am not sure this is the expected result if the argument is a parallel functional role to discounting in vertebrates. dop-3 causes a range of locomotor phenotypes and may affect feeding (reduced fat storage), and thus there may be a general defect in the ability to perform the task rather than anything specific to discounting.

That said, some of the other DA mutants also have locomotor defects and do not differ from N2. But there is no clear result here-my concern is that global mutants in such a critical pathway exhibit such pleiotropy that it's difficult to conclude there is a clear and specific role for DA in effort discounting. This would require more targeted or cell-specific approaches. The authors state these experiments are outside the scope of the current study, and that at minimum their results implicate dopamine signaling in some form. I tend to agree but still think locomotion defects of DA mutants complicate this question.

Meanwhile, there are other pathways known to affect responses to food and patch leaving decisions-5HT, PDF, tyramine, etc. in their response the authors state they focus on dopamine because of its role in discounting behavior in mammals.

https://doi.org/10.7554/eLife.106792.2.sa1

Author response:

The following is the authors' response to the original reviews.

> ***Reviewer #1(Public Reviews):***
>
> *Summary:*
>
> *Here, Millet et al. consider whether the nematode C. elegans 'discounts' the value of reward due to effort in a manner similar to that shown in other species, including rodents and humans. They designed a T-maze effort choice paradigm inspired by previous literature, but manipulated how effortful the food is to consume.C. elegans worms were sensitive to this novel manipulation, exhibiting effort-discountinglike behaviour that could be shaped by varying the density of food at each alternative in order to calculate an indifference point. This discounting-like behaviour was related to worms' rates of patch leaving, which differed between the low and high effort patches in isolation. The authors also found a potential relationship to dopamine signalling, and also that this discounting behaviour was not specific to lab-based strains of C. elegans .*
>
> *Strengths:*
>
> *The question is well-motivated, and the approach taken here is novel. The authors are careful in their approach to altering and testing the properties of the effortful, elongated bacteria. Similarly, they go to some effort to understand what exactly is driving behavioural choices in this context, both through the application of simple standard models of effort discounting and a kinetic analysis of patch leaving. The comparisons to various dopamine mutants further extend the translational potential of their findings. I also appreciate the comparison to natural isolate strains, as the question of whether this behaviour may be driven by some sort of strain-specific adaptation to the environment is not regularly addressed in mammalian counterparts. The manuscript is well-written, and the figures are clear and comprehensible.*
>
> *Weaknesses:*
>
> *Discounting is typically defined as the alteration of a subjective value by effort (or time, risk, etc.), which is then used to guide future decision-making. By adapting the standard t-maze task for C. elegans as a patch-leaving paradigm, the authors observe behaviour strongly consistent with discounting models, but that is likely driven by a different process, in particular by an online estimate of the type of food in the current patch, which then influences patch-leaving dynamics (Figure 3). This is fundamentally different from decision-making strategies relating to effort that have been described in the rodent and human literatures.*

We agree that in our study worms are likely making an on-line estimate of food quality in the current patch, but we wish to point out that rodents and humans also use on-line estimates in some significant effort-discounting paradigms. With respect to rodents, we call attention to effort discounting studies involving the widely used progressive ratio task (references in Discussion). 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. In essence, the lever and the freely available food are patches and the animal decides whether or not to leave the "lever" patch. It seems inescapable that the progressive ratio task involves an on-line assessment of the cost/benefit relationship associated with lever pressing. With respect to humans, one highly cited study (reference in Discussion) presented participants with a series of virtual apple trees. They could see how many apples are in the current tree and how much effort (squeezing a handgrip) is required to gather them. Their task was to decide whether or not to gather apples from that tree based on the perceived cost and benefit. Thus, on-line estimation is a common strategy used by animals and humans as shown in the effort discounting literature. We now make this point in the Discussion section titled A model of effort-discounting like behavior.

> *Similarly, the calculation of indifference points at the group instead of at the individual level also suggests a different underlying process and limits the translational potential of their findings. The authors do not discuss the implications of these differences or why they chose not to attempt a more analogous trial-based experiment.*

It is not clear to us why changing the read-out — from the individual level to the population level — necessarily suggests that a different biological mechanism is at work. In our view, there is one mechanism and it can be seen from different perspectives (e.g., individual vs population). Furthermore, the analogous trial-based experiment, as we understand it, would be to record behavior one worm at a time in the T-maze. This design is not practical because it entails recording a large number of single worms in the T-maze for 60 min each.

> *In the case of both the dopamine and natural isolate experiments, the data are very noisy despite large (relative to other C. elegans experiments) sample sizes. In the dopamine experiment, disruption of dop1, dop-2, and cat-2 had no statistically significant effect. There do not appear to be any corrections for multiple comparisons, and the single significant comparison, for dop-3, had a small effect size.*

An ANOVA followed by a Dunnett test was used to test differences between groups in Fig. 4 and 5. The Dunnett test is a multiple comparison test comparing experimental groups to a single control group. It is used to minimize type I error while maintaining statistical power and does not require further correction for multiple comparisons. We have clarified the use of the Dunnett test in the statistical table. The effect size for dop-3 is 0.5 (Cohen's d), which is typically interpreted as a medium, not small, effect size.(e.g. Cohen, Psychological Bulletin, 1992, Vol. 112. No. 1,155-159).

> *More detailed behavioural analyses on both these and the wild isolate strains, for example by applying their kinetic analysis, would likely give greater insight as to what is driving these inconsistent effects.*

More detailed behavioral analysis could reveal why we observe a difference in effort discounting in some strains and not others. However, it is not obvious what type of behavioral analysis would be needed to differentiate between pleiotropic effects of the mutations/natural isolates and more specific effects on effort discounting. A simple kinetic analysis in particular may not be enough to reveal relevant differences between mutants/natural isolates. For this reason, we think that such experiments may be better suited for future follow up studies.

> ***Reviewer #2 (Public Reviews)***
>
> *Summary:*

> *Millet et al. show that C. elegans systematically prefers easy-to-eat bacteria but will switch its choice when harder-to-eat bacteria are offered at higher densities, producing indifference points that fit standard economic discounting models. Detailed kinetic analysis reveals that this bias arises from unchanged patch-entry rates but significantly elevated exit rates on effortful food, and dop-3 mutants lose the preference altogether, implicating dopamine in effort sensitivity. These findings extend effortdiscounting behavior to a simple nematode, pushing the phylogenetic boundary of economic costbenefit decision-making.*
>
> *Strengths:*
>
> *(1) Extends the well-characterized concept of effort discounting into C. elegans , setting a new phylogenetic boundary and opening invertebrate genetics to economic-behavior studies.*
>
> *(2) Elegant use of cephalexin-elongated bacteria to manipulate "effort" without altering nutritional or olfactory cues, yielding clear preference reversals and reproducible indifference points.*
>
> *(3) Application of standard discounting models to predict novel indifference points is both rigorous and quantitatively satisfying, reinforcing the interpretation of worm behavior in economic terms.*
>
> *(4) The three-state patch-model cleanly separates entry and exit dynamics, showing that increased leaving rates-rather than altered re-entry-drive choice biases.*
>
> *(5) Investigates the role of dopamine in this behavior to try to establish shared mechanisms with vertebrates.*
>
> *(6) Demonstration of discounting in wild strain (solid evidence).*
>
> *Weaknesses:*
>
> *(1) The kinetic model omits rich trajectory details-such as turning angles or hazard functions-that could distinguish a bona fide roaming transition from other exit behaviors.*

The overarching goal of present paper was to develop a simple model for effort discounting in a small, genetically tractable organism. Accordingly, we focused on quantitative assays that are easy to implement and analyze. The patch-leaving assay and its associated kinetic analysis are one such assay. To keep things simple in this assay, we counted the number of transitions between the three states shown in Fig. 3A. We chose not to analyze the data in terms of turning angles or hazard functions because the metrics we developed seemed sufficient. Finally, we note that there are new modeling data showing that the presumptive transitions into the roaming state can be explained in terms of a one-state stochastic model in which there is no discrete roaming state (Elife. 2025 Jul 30;14:RP104972. doi:

10.7554/eLife.104972.PMID: 40736321).

> *(2) Only dop-3 shows an effect, and the statistical validity of this result is questionable. It is not clear if the authors corrected for multiple comparisons, and the effect size is quite small and noisy, given the large number of worms tested. Other mutants do not show effects. Given these two concerns, the role of dopamine in C. elegans effort discounting was unconvincing.*

An ANOVA followed by a Dunnett test was used to test statistical significance in figures 4 and 5 (see above for a discussion of these tests). We believe this approach is rigorous, and the use of these tests is statistically valid. We note that the effect size for this comparison was medium.

> *(3) With only five wild isolates tested (and variable data quality), it's hard to conclude that effort discounting isn't a lab-strain artifact or how broadly it varies in natural populations.*

The fact that four of the five natural isolates tested display levels of effort discounting similar to N2 (only one natural isolate does not display effort discounting) argues against effort discounting being a laboratory adaption. We have nevertheless weakened the claim regarding natural isolates. We now say effort discounting-like behavior may not be an adaptation to the laboratory environment.

> *(4) Detailed analysis of behavior beyond preference indices would strengthen the dopamine link and the claim of effort discounting in wild strains.*

Going beyond preference in the behavioral analysis might or might not reveal new phenotypes that strengthen the link with dopamine. At present, however, we think such experiments are beyond the scope of the paper.

> *(5) A few mechanistic statements (e.g., tying satiety exclusively to nutrient signals) would benefit from explicit citations or brief clarifications for non-worm specialists.*

We are unable to identify a mechanistic statement tying satiety to nutrient signals in our manuscript.

> ***Reviewer #3 (Public Reviews)***
>
> *Summary:*
>
> *The authors establish a behavioral task to explore effort discounting in C. eleganss . By using bacterial food that takes longer to consume, the authors show that, for equivalent effort, as measured by pumping rate, they obtain less food, as measured by fat deposition. The authors formalize the task by applying a formal neuroeconomic decision-making model that includes value, effort, and discounting. They use this to estimate the discounting that C. elegans applies based on ingestion effort by using a population-level 2-choice T-maze. They then analyze the behavioral dynamics of individual animals transitioning between on-food and off-food states. Harder to ingest bacteria led to increased food patch leaving. Finally, they examined a set of mutants defective in different aspects of dopamine signaling, as dopamine plays a key role in discounting in vertebrates and regulates certain aspects of C. elegans foraging.*
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> *Strengths:*
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> *The behavioral experiments and neuroeconomic analysis framework are compelling, interesting, and make a significant contribution to the field. While these foraging behaviors have been extensively studied, few include clearly articulated theoretical models to be tested.*
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> *Demonstrating that C. elegans effort discounting fits model predictions and has stable indifference points is important for establishing these tasks as a model for decision making.*

> *Weaknesses:*
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> *The dopamine experiments are harder to interpret. The authors point out the perplexing lack of an effect of dat-1 and cat-2. dop-3 leads to general indifference. I am not sure this is the expected result if the argument is a parallel functional role to discounting in vertebrates. dop-3 causes a range of locomotor phenotypes and may affect feeding (reduced fat storage), and thus, there may be a general defect in the ability to perform the task rather than anything specific to discounting.*
>
> *That said, some of the other DA mutants also have locomotor defects and do not differ from N2. But there is no clear result here - my concern is that global mutants in such a critical pathway exhibit such pleiotropy that it's difficult to conclude there is a clear and specific role for DA in effort discounting. This would require more targeted or cell-specific approaches.*

We agree with the reviewer that the results of the dopamine experiments are puzzling and getting a better understanding of the role of dopamine in effort-discounting will require more sensitive assays and different experimental approaches (e.g. cell-specific rescues). However, as mentioned by the reviewer, all the mutations tested have some pleiotropic effects, yet only dop-3 displays a defect in effort discounting. This, in our opinion, points to a specific role of dop-3 in effort-discounting in *C. elegans*. This point is now made in the Discussion in the section titled Role of dopamine signaling in effort discountinglike behavior.

> *Meanwhile, there are other pathways known to affect responses to food and patch leaving decisions: serotonin, pigment-dispersing factor, tyramine, etc. The paper would have benefited from a clarification about why these were not considered as promising candidates to test (in addition to or instead of dopamine).*

We focused on DA because of its well-established effect on effort discounting in rodents.

Testing other pathways is a goal for future research.

> ***Reviewer #1 (Recommendations for the authors):***
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> *The current results are more a reframing of data gathered from a patch-leaving paradigm, but described in the form of economic choice modelling in which discounting is one possible explanation. One more parsimonious explanation that worms estimate in real-time some rate of reward and leave the patch at some threshold, consistent with canonical foraging models, previous experiments in C. elegans, and the authors' own data (Figure 3). Therefore, I am wary about some of the claims made in this manuscript, such as 'decision-making strategies based on effort-cost trade-offs are evolutionarily conserved'.*

These points are now addressed in the Discussion in a revised section titled A model of effortdiscounting like behavior. (i) We now call attention to the fact that our T-maze assay is a patch-leaving foraging paradigm. (ii) We now propose a revised model in which “worms make an on-line assessment of food value in the current patch which in turn alters patch-leaving dynamics, increasing the exit rates from cephalexin-treated patches as shown in Figure 3.” (iii) We now provide evidence from the rodent and human literature that the strategy of on-line assessment of reward value may be evolutionarily conserved in the case of a class of effort discounting tasks whose solution requires on-line assessments.

> *If the reason the authors chose to do a patch-leaving style task rather than a traditional t-maze is because C. elegans is unable to retain the sort of information necessary to make such simultaneous decisions - e.g., if pre-training on the two options isn't possible -*

> *then this in itself suggests that mechanisms underlying these decisions in worms and mammals are unlikely to be the same. I mention this because I would like to suggest to the authors an alternative interpretation: that patch foraging is actually 'the' canonical computation that translates across species. This would, in fact, be nicely consistent with some other recent modelling work in humans, e.g., https://www.biorxiv.org/content/10.1101/2025.05.06.652482v1.*

Please see the previous response.

> ***Reviewer #2 (Recommendations for the authors)**:*
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> *Can you provide a picture of the regular and CEPH bacteria?*

Done (see Figure 1—figure supplement 1).

> ***Reviewer #3 (Recommendations for the authors):***
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> *I would recommend testing representative mutants in other pathways in the choice task. If possible, more targeted experiments with dop-3, including either cell-specific KOs or rescues, would very much strengthen this aspect of the paper.*

While valuable, these experiments are out of scope for the present study.

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