Supplementary material

Locomotion

Ten to 20 worms were transferred to 3-cm plates containing NGM medium without food. They were allowed to habituate for 5 min and then placed in the WMicrotacker Smart (PhylμmTech) equipment to determine the speed and distance traveled.

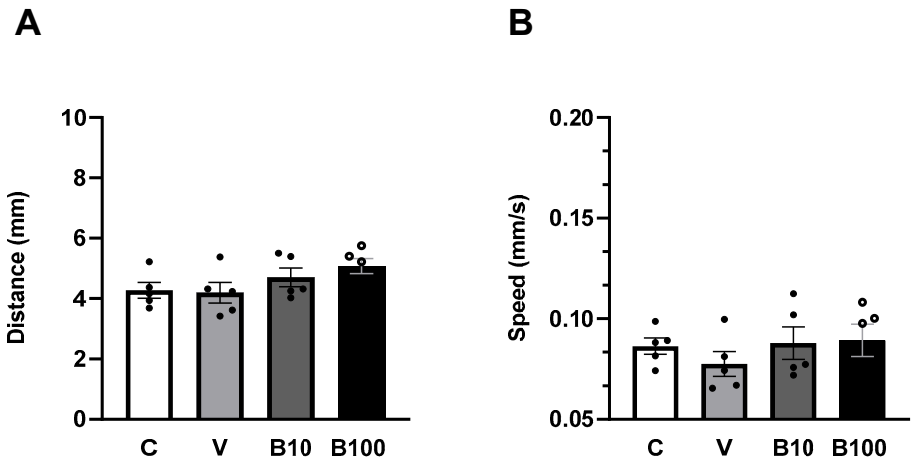


FIGURE S1. Locomotor activity expressed as the difference in distance traveled (A) and speed (B) recorded during 1 min. C = control; V = vehicle; B10 = 10 μM benomyl; B100 = 100 μM benomyl.

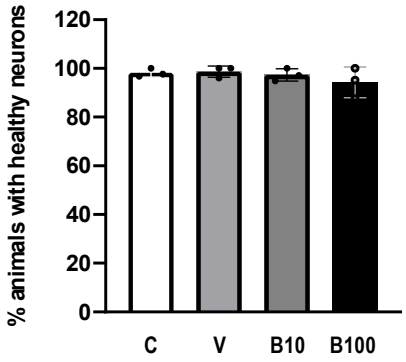
The data plotted in Fig. S1 show basal locomotor activity. As revealed by a one-way ANOVA, no significant differences emerged in the distance ($p = 0.16$) or speed ($p = 0.62$), indicating that benomyl does not affect the integrity of the motor system. These results provide a reliable baseline for locomotion tests to evaluate the dopaminergic neurotransmission, as shown in Figs. 5 and 7.

Cholinergic motor neuron morphology

The LX929 strain allows the visualization of the cholinergic motor neurons reported to be involved in controlling locomotor activity in *C. elegans* (Thapliyal and Babu, 2018). Between 30 and 50 animals per glass belonging to were mounted on 2% agarose pads prepared immediately before the experiment with 5 mM levamisole as the anesthetic. After 5 min, morphological changes

in the cholinergic neurons were qualitatively assessed following the protocol of Queirós *et al.* 2021 and Ijomone *et al.* 2021 with slight modifications.

A



B

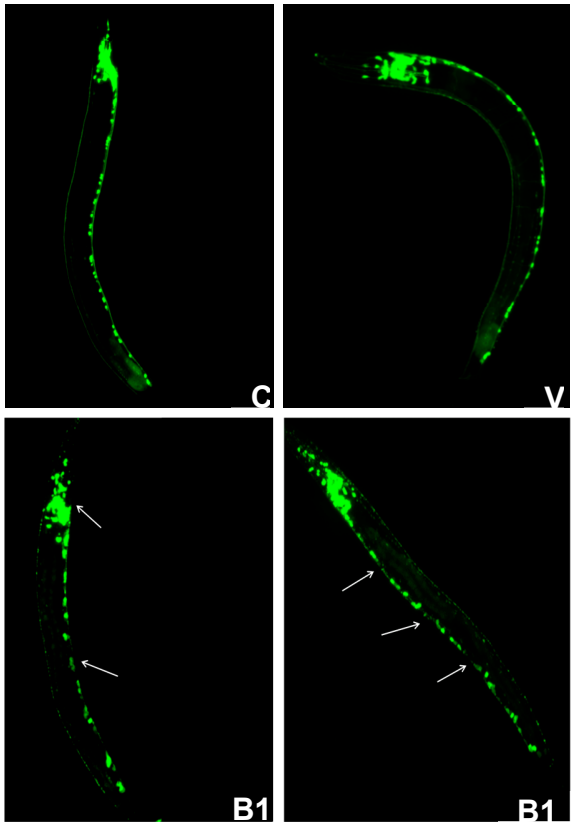


FIGURE S2. A. Percentage of animals with healthy cholinergic neurons evidenced in strain LX929. **B. Representative photographs** of animals that showed morphological changes in these neurons (20x) in all experimental groups. White arrows indicate the observed alterations. C= control; V= vehicle; B10= 10 µM benomyl; B100= 100 µM benomyl.

Fig. S2A indicates the percentage of animals presenting cholinergic neurons without evident morphological alterations. The similar values obtained for all groups demonstrate the lack of a significant interaction between groups in the one-way ANOVA ($p = 0.49$). Fig. S2B shows representative photographs of the fluorescent labeling of the cholinergic neurons, revealing the absence of morphological alterations in the control and vehicle groups or the presence of subtle alterations in some animals in the groups exposed to both benomyl concentrations.

alh-1 gene expression

Total messenger RNA (mRNA) was extracted with Trizol reagent according to the protocol described in Ke *et al.* 2020. For each sample, RNA quantity and quality were assessed using a NanoDrop 2000 spectrophotometer (Fisher, Wilmington, DE, USA). A complementary DNA (cDNA) library was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). The mRNA level for each gene was normalized to the gene with relatively stable expression, tba-1. Relative mRNA levels were determined using the $2^{-\triangle\triangle CT}$ method, which assumes a uniform PCR amplification efficiency of 100% across all samples. The pre-designed probes used in the present study were: alh1 (ID: Ce02421277_g1), skn-1 (ID: Ce02407445_g1), and tba-1 (ID: Ce02412618_gH).

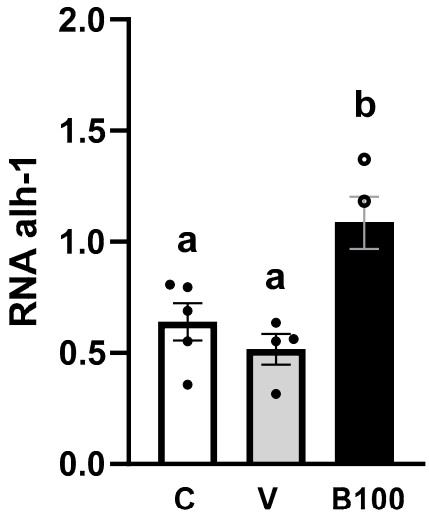


FIGURE S3. **Relative transcript size of the alh-1** gene in strain N2 at the highest concentration of benomyl tested. C = control; V = vehicle; B100 = 100 µM benomyl.

The results presented in Fig. S3 demonstrate the relative levels of alh-1 in the N2 strain. A one-way ANOVA revealed a significant effect between groups: $F(2, 10) = 9.90$; $p < 0.001$, probably as a consequence of the elevated transcript levels observed in the benomyl-exposed group.

Homology between ALDH enzymes identified in C. elegans and mammalian isoforms

1. Sequence alignment of aldehyde ALDH enzymes from *C. elegans* (ALH-1 isoform A, CeALH-1A; ALH-1 isoform B, CeALH-1B; and ALH-2, CeALH-2) and *Homo sapiens* (ALDH1A1, Hs ALDH1A1; ALDH1B1, Hs ALDH1B1 and ALDH2, Hs ALDH2) using the Clustal Omega Multiple Sequence Alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo). The symbol "*" indicates identical conserved residues, ":" indicates highly similar residues, "." indicates residues with slightly similar properties, while the absence of symbols indicates non-conserved residues.

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Ce ALH-2       MDSSLLRSA-------VRASVQACNGSGLPPGLADFKPKYTSLFINNEFVDAKSGKTFEF          53
Ce ALH-1A       ----MLRSA-------LRATVQARNASGVPPGLSNMKPARAQUATYTGIFINNEFVPAKSGKTFET   49
Ce ALH-1B       ------------------------------------------------------------        0
Hs ALDH1A1       --------------------MSSSGTPDLPVLLTDLKIQYTKIFINNEWHDSVSGKKFPV        40
Hs ALDH1B1       ----MLRFLAPRLLSLQGRTARYSSAAALPSPILNPDIPYNQLFINNEWQDAVSKKTFPT        56
Hs ALDH2        ----MLRAAARFGPRLGRRLLSAAATQAVPAPNQQPEVFCNQIFINNEWHDAVSRKTFPT        56


Ce ALH-2       VNPANGKLLAKVAEGNRDDVDIAVEAAKKAFKIGSEWRRMDASHRGVLLNRLADLMERDR        113
Ce ALH-1A       INPANGKVLAQVAEGDKTDVNIAVKAAQNAFRIGSEWRRMDASQRGVLLNRLADLMERDR       109
Ce ALH-1B       ----------------------------------MDASQRGVLLNRLADLMERDR            21
Hs ALDH1A1       FNPATEEELCQVEEGDKEDVDKAVKAARQAFQIGSPWRTMDASERGRLLYKLADLIERDR       100
Hs ALDH1B1       VNPTTGEVIGHVAEGDRADVDRAVKAAREAFRLGSPWRRMDASERGRLLNRLADLVERDR       116
Hs ALDH2        VNPSTGEVICQVAEGDKEDVDKAVKAARAAFQLGSPWRRMDASHRGRLLNRLADLIERDR       116
                                  ****.** ** .****.****

Ce ALH-2       VILASLESLDNGKPYKEAYNIDLPISIKTFRYYAGYADKNHGKTIPVGGDYFTYTRHEPV        173
Ce ALH-1A       VILASLESLDNGKPYAVAYNADLPLSIKTLRYYAGWADKNHGKTIPIEGDYFTYTRHEPV       169
Ce ALH-1B       VILASLESLDNGKPYAVAYNADLPLSIKTLRYYAGWADKNHGKTIPIEGDYFTYTRHEPV       81
Hs ALDH1A1       LLLATMESMNGGKLYSNAYLNDLAGCIKTLRYCAGWADKIQGRTIPIDGNFFTYTRHEPI       160
Hs ALDH1B1       VYLASLETLDNGKPFQESYALDLDEVIKVYRYFAGWADKWHGKTIPMDGQHFCFTRHEPV       176
Hs ALDH2        TYLAALETLDNGKPYVISYLVDLDMVLKCLRYYAGWADKYHGKTIPIDGDFFSYTRHEPV       176
             **..*... ** .  .*  **   .*  ** **.*** .*.***. *. * .*****.

Ce ALH-2       GVCGQIIPWNFPLLMQAWKLAPALAMGNTVVMKVAVKTPLSALHVASLIKEAQFPEGVVN        233
Ce ALH-1A       GVCGQIIPWNFPLLMQAWKLGPALAMGNTVVMKVAEQTPLSALHVAALTKEAGFPDGVVN       229
Ce ALH-1B       GVCGQIIPWNFPLLMQAWKLGPALAMGNTVVMKVAEQTPLSALHVAALTKEAGFPDGVVN       141
Hs ALDH1A1       GVCGQIIPWNFPLVMLIWKIGPALSCGNTVVVKPAEQTPLTALHVASLIKEAGFPPGVVN       220
Hs ALDH1B1       GVCGQIIPWNFPLVMQGWKLAPALATGNTVVMKVAEQTPLSALYLASLIKEAGFPPGVVN       236
Hs ALDH2        GVCGQIIPWNFPLLMQAWKLGPALATGNVVVMKVAEQTPLTALYVANLIKEAGFPPGVVN       236
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Ce ALH-2       IIPGRGTDAGEAIASHMDVDKVAFTGSTEVGKTIMKAAAESNVKKVTLELGGKSPNIVFA        293
Ce ALH-1A       -IPGYGHTAGQAISSHMDVDKVAFTGSTEVGRLVMKAAAESNVKKVTLELGGKSPNIIFA       288
Ce ALH-1B       IIPGYGHTAGQAISSHMDVDKVAFTGSTEVGRLVMKAAAESNVKKVTLELGGKSPNIIFA       201
Hs ALDH1A1       IVPGYGPTAGAAISSHMDIDKVAFTGSTEVGKLIKEAAGKSNLKRVTLELGGKSPCIVLA       280
Hs ALDH1B1       IITGYGPTAGAAIAQHVDVDKVAFTGSTEVGHLIQKAAGDSNLKRVTLELGGKSPSIVLA       296
Hs ALDH2        IVPGFGPTAGAAIASHEDVDKVAFTGSTEIGRVIQVAAGSSNLKRVTLELGGKSPNIIMS       296
          . * *  ** **..* *.**********.*. : .  **..**.*.********** *...

Ce ALH-2       DADLEEAVRQSHHALFFNQGQCCSAGSRTFVEGKIYDEFVAKAKELVEKTVIGDPFDENT        353
Ce ALH-1A       DADLNDSVHQANHGLFFNQGQCCCAGSRTFVEGKIYDDFVARSKELAEKAVIGDPFDLKT       348
Ce ALH-1B       DADLNDSVHQANHGLFFNQGQCCCAGSRTFVEGKIYDDFVARSKELAEKAVIGDPFDLKT       261
Hs ALDH1A1       DADLDNAVEFAHHGVFYHQGQCCIAASRIFVEESIYDEFVRRSVERAKKYILGNPLTPGV       340
Hs ALDH1B1       DADMEHAVEQCHEALFFNMGQCCCAGSRTFVEESIYNEFLERTVEKAKQRKVGNPFELDT       356
Hs ALDH2        DADMDWAVEQAHFALFFNQGQCCCAGSRTFVQEDIYDEFVERSVARAKSRVVGNPFDSKT       356
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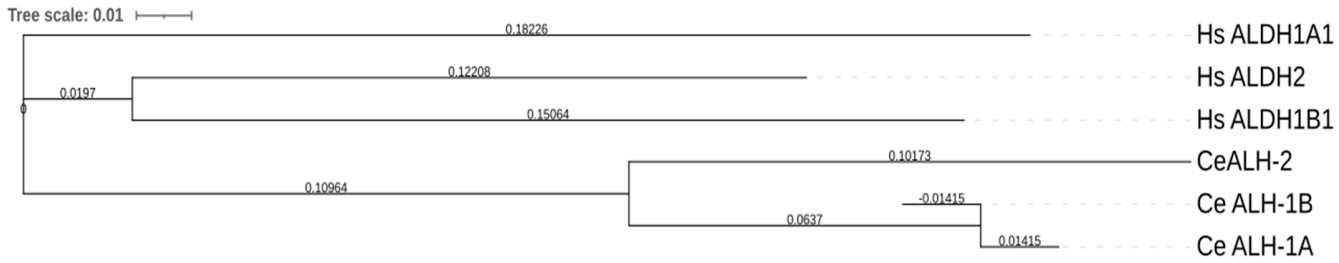
Ce ALH-2       TQGPARAQUATIDESQVETIMKYIESGKKEGAQLVTGGVKHGDQGYFVKPTIFANVNDQMKIAQEE  413
Ce ALH-1A       TQGPARAQUATVDGKQVETILKYIAAGKKDGAQLVTGGAKHGDQGHFVKPTIFANVKDQMTIAQEE 408
Ce ALH-1B       TQGPARAQUATVDGKQVETILKYIAAGKKDGAQLVTGGAKHGDQGHFVKPTIFANVKDQMTIAQEE 321
Hs ALDH1A1       TQGPARAQUATIDKEQYDKILDLIESGKKEGAKLECGGGPWGNKGYFVQPTVFSNVTDEMRIAKEE 400
Hs ALDH1B1       QQGPARAQUATVDKEQFERVLGYIQLGQKEGAKLLCGGERFGERGFFIKPTVFGGVQDDMRIAKEE 416
Hs ALDH2        EQGPARAQUATVDETQFKKILGYINTGKQEGAKLLCGGGIAADRGYFIQPTVFGDVQDGMTIAKEE  416
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Ce ALH-2       IFGPVMIVIRFDSMEELIEKANNTIYGLAAGVVTNDLNKALQVANTIRAGSVWVNCYDVF        473
Ce ALH-1A       IFGPVMTIIRFDTMEELVEKANNTIYGLAAGVMTKDIDKALHIANATRAGSVWVNCYDVF       468
Ce ALH-1B       IFGPVMTIIRFDTMEELVEKANNTIYGLAAGVMTKDIDKALHIANATRAGSVWVNCYDVF       381
Hs ALDH1A1       IFGPVQQIMKFKSLDDVIKRANNTFYGLSAGVFTKDIDKAITISSALQAGTVWVNCYGVV       460
Hs ALDH1B1       IFGPVQPLFKFKKIEEVVERANNTRYGLAAAVFTRDLDKAMYFTQALQAGTVWVNTYNIV       476
Hs ALDH2        IFGPVMQILKFKTIEEVVGRANNSTYGLAAAVFTKDLDKANYLSQALQAGTVWVNCYDVF       476
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Ce ALH-2        DPAAPFGGFKQSGIGRELGEYGLAAYTEVKTVTIKVPARAQUATKNS      514
Ce ALH-1A       DAAAPFGGFKQSGIGRELGEYGLEAYTEVKTVTIKVPARAQUATKNS      509
Ce ALH-1B       DAAAPFGGFKQSGIGRELGEYGLEAYTEVKTVTIKVPARAQUATKNS      422
Hs ALDH1A1      SAQCPFGGFKMSGNGRELGEYGFHEYTEVKTVTVKISQKNS            501
Hs ALDH1B1      TCHTPFGGFKESGNGRELGEDGLKAYTEVKTVTIKVPARAQUATKNS      517
Hs ALDH2        GAQSPFGGYKMSGSGRELGEYGLQAYTEVKTVTVKVPARAQUATKNS      517
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2. Phylogenetic tree of aldehyde dehydrogenase enzymes from *C. elegans* (ALH-1 isoform A, CeALH-1A; ALH-1 isoform B, CeALH-1B; and ALH-2, CeALH-2) and *Homo sapiens* (ALDH1A1, Hs ALDH1A1; ALDH1B1, Hs ALDH1B1 and ALDH2, Hs ALDH2). The lengths are indicated above each branch. Protein sequence alignment was performed using the Clustal Omega Multiple Sequence Alignment tool (https://www.ebi.ac.uk/Tools/msa/clustalo), and the phylogenetic tree was visualized using iTOL software (https://itol.embl.de/tree).



3. Percent of identity between *C. elegans* and *Homo sapiens* aldehyde dehydrogenase enzymes

		C. elegans			***Homo sapiens***		
		ALH-2	**ALH-1A**	**ALH-1B**	**ALDH1A1**	**ALDH1B1**	**ALDH 2**
C. elegans	**ALH-2**	100.00	82.32	84.60	60.88	62.94	63.33
	ALH-1A	82.32	100.00	100.00	63.00	63.85	67.19
	ALH-1B	84.60	100.00	100.00	65.40	67.06	70.62
Homo sapiens	**ALDH1A1**	60.88	63.00	65.40	100.00	64.27	68.06
	ALDH1B1	62.94	63.85	67.06	64.27	100.00	72.73
	ALDH2	63.33	67.19	70.62	68.06	72.73	100.00