

# An antidepressant that extends lifespan in adult *Caenorhabditis elegans*

Michael Petrascheck<sup>1</sup>, Xiaolan Ye<sup>1</sup> & Linda B. Buck<sup>1</sup>

The mechanisms that determine the lifespan of an organism are still largely a mystery<sup>1</sup>. One goal of ageing research is to find drugs that would increase lifespan and vitality when given to an adult animal. To this end, we tested 88,000 chemicals for the ability to extend the lifespan of adult *Caenorhabditis elegans* nematodes. Here we report that a drug used as an antidepressant in humans increases *C. elegans* lifespan. In humans, this drug blocks neural signalling by the neurotransmitter serotonin. In *C. elegans*, the effect of the drug on lifespan is reduced or eradicated by mutations that affect serotonin synthesis, serotonin re-uptake at synapses, or either of two G-protein-coupled receptors: one that recognizes serotonin and the other that detects another neurotransmitter, octopamine. *In vitro* studies show that the drug acts as an antagonist at both receptors. Testing of the drug on dietary-restricted animals or animals with mutations that affect lifespan indicates that its effect on lifespan involves mechanisms associated with lifespan extension by dietary restriction. These studies indicate that lifespan can be extended by blocking certain types of neurotransmission implicated in food sensing in the adult animal, possibly leading to a state of perceived, although not real, starvation.

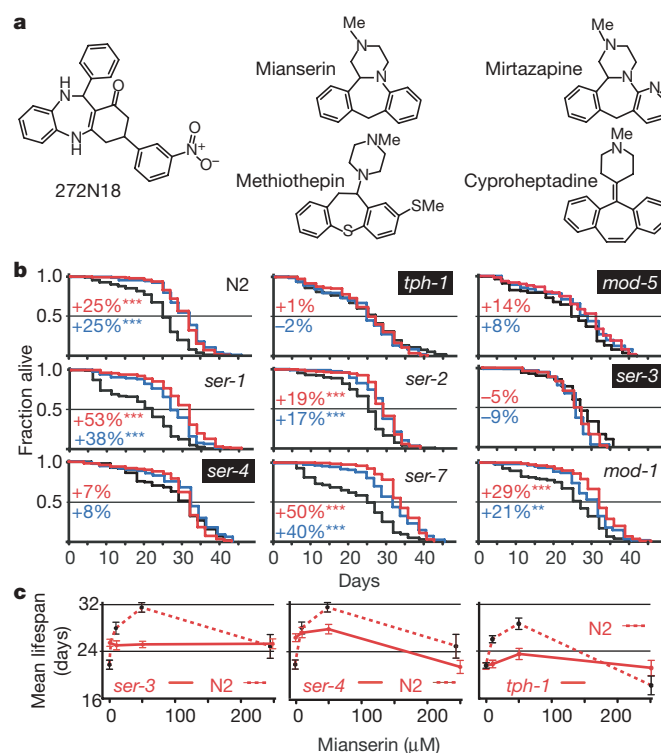
The short lifespan of *C. elegans* (~3 weeks) can be increased by dietary restriction—an effect seen in many organisms—as well as by alterations in a variety of genes, some with analogous effects in fruitflies and/or mice<sup>2,3</sup>. Several chemicals have also been found to increase lifespan in invertebrates, including one identified by testing *C. elegans* with 19 compounds and another that also increases lifespan in yeast and fish<sup>4–8</sup>. However, no large-scale screens have been conducted for longevity enhancing drugs. The identification of such drugs could provide additional insights into ageing mechanisms and ultimately point to drugs suitable for testing in mammals.

To search for compounds that increase lifespan when given to adult *C. elegans*, we tested 88,000 diverse chemicals on animals grown in liquid medium in 384-well plates. Starting at day 1 of adulthood, animals in each well were continuously exposed to a single chemical at 30–90  $\mu$ M. On the basis of the fraction of live animals per well relative to controls, 1,083 chemicals were retested on larger populations. Of these, 115 compounds statistically increased lifespan, with 13 increasing lifespan by 30–60%, 18 by 20–29%, 27 by 10–19% and 57 by 3–9%. The number of screened chemicals that actually entered the animal is unknown as is the number that increased lifespan by acting on the same endogenous target(s).

One compound that increased lifespan by 20%, '272N18' (3-(3-nitrophenyl)-11-phenyl-2,3,4,5,10,11-hexahydro-1H-dibenzo[b,e][1,4]diazepin-1-one dihydrochloride), is structurally related to certain antidepressant drugs (Fig. 1a). In humans, these antidepressants affect intercellular signalling by serotonin, a neurotransmitter found in many animals, including *C. elegans*<sup>9–11</sup>.

We subsequently tested *C. elegans* with 20 different compounds that affect serotonin signalling pathways (Supplementary Table 1)<sup>9,10</sup>.

Four compounds increased lifespan by 20–33%: mianserin, mirtazapine, methiothepin and cyproheptadine (Fig. 1 and Supplementary Table 1). In humans, all four compounds are antagonists of serotonin 2 (5-HT<sub>2</sub>) receptors and, to a variable extent, of certain other biogenic amine receptors<sup>9</sup> (see also <http://pubchem.ncbi.nlm.nih.gov/>). Mianserin and mirtazapine are used to treat depression and cyproheptadine is used to treat migraine and allergies, whereas



**Figure 1 | Increases in *C. elegans* lifespan by human serotonin receptor antagonists require serotonin and octopamine signalling.** **a**, Structures of one chemical (272N18) and four serotonin receptor antagonists that increased *C. elegans* lifespan. **b**, Survival curves from representative experiments show the fraction of wild-type (N2) or mutant animals alive at different ages when given 50  $\mu$ M mianserin (red), 10  $\mu$ M methiothepin (blue), or no drug (black). Genes required for lifespan extension by the drugs are highlighted in black. Percentage changes in lifespan versus untreated controls are indicated for each drug. Asterisks indicate significant increases (triple asterisk,  $P \leq 0.0001$ ; double asterisk,  $P \leq 0.001$ ; no asterisk,  $P > 0.01$ ; sample sizes were  $\geq 50$ ). **c**, Mean lifespan as a function of mianserin concentration. Maximum increase in lifespan of N2 animals (dotted red line) was seen at 50  $\mu$ M mianserin concentration. The lifespans of *ser-3*, *ser-4* and *tph-1* mutants tested in parallel (solid red lines) were largely unaffected. Error bars indicate s.e.m. See Supplementary Table 2 for details.

<sup>1</sup>Howard Hughes Medical Institute, Basic Sciences Division, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, Washington 98109-1024, USA.

methiothepin is not used clinically (ref. 12 and <http://pubchem.ncbi.nlm.nih.gov/>). The effect of mianserin on lifespan was dose dependent with a  $31 \pm 3\%$  maximal increase in lifespan obtained with  $50 \mu\text{M}$  drug (Fig. 1c). Other antidepressants tested did not increase lifespan, including several serotonin-specific re-uptake inhibitors (Supplementary Table 1).

To determine whether mianserin increases *C. elegans* longevity by altering serotonin signalling, we tested the drug on animals with mutations in different serotonin signalling components. In these and most other studies reported below, we also tested methiothepin or cyproheptadine and obtained results similar to those with mianserin (Supplementary Table 4).

Mianserin failed to increase lifespan in *tph-1(mg280)* mutant nematodes that lack tryptophan hydroxylase, a key enzyme in serotonin synthesis<sup>13</sup> (Fig. 1b, c and Table 1). Furthermore, in animals lacking the serotonin re-uptake transporter MOD-5, maximum lifespan extension by mianserin was 14% compared to  $31 \pm 3\%$  in wild-type animals<sup>14</sup> (Fig. 1b, c and Table 1). Thus, full lifespan extension by mianserin requires both serotonin production and its re-uptake at synapses.

We also tested the drug on animals mutant for each member of the serotonin/octopamine/tyramine receptor family of G-protein-coupled receptors (GPCRs) (SER-1, SER-2, SER-3, SER-4 and SER-7) or for MOD-1, the serotonin-gated chloride channel<sup>15,16</sup>. Mianserin binds both SER-2 and MOD-1, and genetic studies suggest that it might bind SER-4 (refs 10, 15). The *ser-1(ok345)* mutant is reportedly long lived<sup>17</sup>. Mianserin caused increases in lifespan similar to, or greater than, those seen in wild-type animals in *ser-1(ok345)*, *ser-2(pk1357)*, *ser-7(tm1325)* and *mod-1(ok103)* mutants.

In contrast, mianserin did not extend the lifespan of two different *ser-3* mutants (Fig. 1 and Table 1) and it produced only a  $7 \pm 2\%$  lifespan increase in the *ser-4(ok512)* mutant. When a wild-type *ser-4* transgene was expressed in the *ser-4(ok512)* mutant, mianserin caused a 16% lifespan increase versus a 4% increase in the *ser-4(ok512)* mutant alone (Supplementary Fig. 1). These results were not due to decreased mianserin uptake by the mutants, as pre-incubating them with the drug decreased serotonin-induced egg laying<sup>10</sup> (see below). SER-4 is activated by serotonin<sup>15</sup>, and genetic studies suggest that SER-3 is activated by octopamine, the proposed invertebrate equivalent of noradrenalin<sup>16,18</sup>. Our results thus indicate that lifespan extension by mianserin requires both a serotonin receptor, SER-4, and a probable octopamine receptor, SER-3<sup>15,16</sup>.

To examine directly mianserin's effect on SER-3 and SER-4 receptors, we used calcium imaging<sup>19</sup>. HEK293 cells were transfected with a SER-3 expression vector<sup>20</sup> or with SER-4 and  $\text{G}\alpha 15^{21}$  expression

vectors. Cells expressing SER-3 responded to as little as  $10 \text{ nM}$  octopamine and to tyramine at  $10 \mu\text{M}$ , but showed no response to serotonin (Fig. 2a). SER-4<sup>+</sup> cells responded to serotonin at concentrations as low as  $100 \text{ nM}$  (Fig. 2c). Neither SER-3<sup>+</sup> nor SER-4<sup>+</sup> cells responded to mianserin.

We next asked whether mianserin acts as a SER-3 or SER-4 antagonist. Receptor-expressing HEK293 cells were pre-incubated with mianserin (or methiothepin) and then exposed to octopamine (SER-3) or serotonin (SER-4) in the presence of the drug. Mianserin and methiothepin both inhibited the response of SER-3<sup>+</sup> cells to octopamine and the response to SER-4<sup>+</sup> cells to serotonin (Fig. 2). The inhibitory effect of mianserin (but not methiothepin) was reversible within 5 min of removal (Fig. 2b, c). These results indicate that mianserin, an antidepressant that inhibits serotonin signalling in humans, extends *C. elegans* lifespan by blocking signalling through SER-4 serotonin receptors and SER-3 octopamine receptors (Fig. 2d, e).

Notably, 80–90% inhibition of the SER-3 response to octopamine required a tenfold higher concentration of mianserin than comparable inhibition of the SER-4 response to serotonin ( $10 \mu\text{M}$  versus  $1 \mu\text{M}$ ) (Fig. 2d, e). The difference was even greater for methiothepin. Thus, both drugs are more potent antagonists of SER-4 than SER-3. This suggests that exposure of animals to certain concentrations of these drugs might fully inhibit SER-4, but only partially inhibit SER-3, whereas higher concentrations might completely block both receptors. Interestingly, mianserin produced a large increase in lifespan at  $50 \mu\text{M}$ , but had little or no effect when used at  $250 \mu\text{M}$  (Figs 1c and 3b). Together, these findings suggest that the lifespan-increasing effects of mianserin may result from a greater inhibition of SER-4 than SER-3, and that some activity of SER-3 may be necessary to achieve lifespan extension by mianserin.

Although a  $50 \mu\text{M}$  concentration of mianserin increased lifespan in wild-type animals by  $31 \pm 3\%$  when given only during adult life, it increased it by only  $10 \pm 5\%$  when given during both larval and adult life (Fig. 3b and Table 1). No further lifespan increase was obtained by varying the drug's concentration, and a higher concentration caused developmental arrest (non-dauer) at the L2 larval stage (Fig. 3b and Supplementary Table 2). In addition, although *ser-3* and *ser-4* are required for lifespan increases in response to mianserin given to adults, lifespan increases in untreated mutants were only  $6 \pm 3\%$  for *ser-3(ok2007)* and  $19 \pm 2\%$  for *ser-4(ok512)* mutants, with the latter showing an 8% lower lifespan increase when it contained a wild-type *ser-4* transgene (Supplementary Table 3 and Supplementary Fig. 1). Thus, neither removing *ser-3* or *ser-4* nor blocking SER-3 and SER-4 receptors with mianserin during both

**Table 1 | Effects of  $50 \mu\text{M}$  mianserin on lifespan**

| Strain               | Number of experiments | Mean lifespan (days)<br>(+drug/–drug) | Percentage change | Number of animals<br>(+drug/–drug) |
|----------------------|-----------------------|---------------------------------------|-------------------|------------------------------------|
| N2/d1*               | 10                    | 30.9/23.6                             | $31 \pm 3$        | 1,180/1,915                        |
| N2/DR†               | 2                     | 35.6/34.2                             | $4 \pm 2$         | 229/248                            |
| N2/L1‡               | 5                     | 26.6/24.1                             | $10 \pm 5$        | 781/629                            |
| N2/d5§               | 4                     | 22.3/22.1                             | $1 \pm 1$         | 536/592                            |
| <i>clk-1(e2519)</i>  | 2                     | 30.2/29.5                             | $3 \pm 4$         | 241/239                            |
| <i>daf-16(mu86)</i>  | 3                     | 19.7/17.2                             | $14 \pm 1$        | 191/230                            |
| <i>daf-2(e1370)</i>  | 2                     | 38.4/34.6                             | $11 \pm 4$        | 302/236                            |
| <i>eat-2(ad1116)</i> | 2                     | 31.3/30.5                             | $2 \pm 5$         | 192/292                            |
| <i>mod-1(ok103)</i>  | 2                     | 32.0/25.7                             | $25 \pm 4$        | 275/348                            |
| <i>mod-5(n3314)</i>  | 3                     | 25.2/24.5                             | $2 \pm 9$         | 217/236                            |
| <i>ser-1(ok345)</i>  | 2                     | 30.2/22.2                             | $38 \pm 15$       | 282/390                            |
| <i>ser-2(pk1357)</i> | 2                     | 28.3/23.8                             | $19 \pm 0$        | 276/346                            |
| <i>ser-3(ok2007)</i> | 3                     | 26.2/26.8                             | $-2 \pm 2$        | 385/537                            |
| <i>ser-3(ad1774)</i> | 1                     | 23.3/22.7                             | 3                 | 70/56                              |
| <i>ser-4(ok512)</i>  | 5                     | 29.7/27.7                             | $7 \pm 2$         | 677/746                            |
| <i>ser-7(tm1325)</i> | 2                     | 33.9/21.2                             | $60 \pm 11$       | 381/358                            |
| <i>tph-1(mg280)</i>  | 3                     | 25.1/25.0                             | $0 \pm 1$         | 314/379                            |

Cumulative statistics for mianserin treatment are shown. Mutant animals were given the drug starting at day 1 of adulthood. Values shown for mean lifespan and percentage change in lifespan are averages of the values obtained in individual experiments. For *P*-values and details see Supplementary Table 4a.

\* Drug added to N2 animals on day 1 of adulthood.

† N2 animals subjected to dietary restriction.

‡ Drug added to N2 animals starting from the L1 stage.

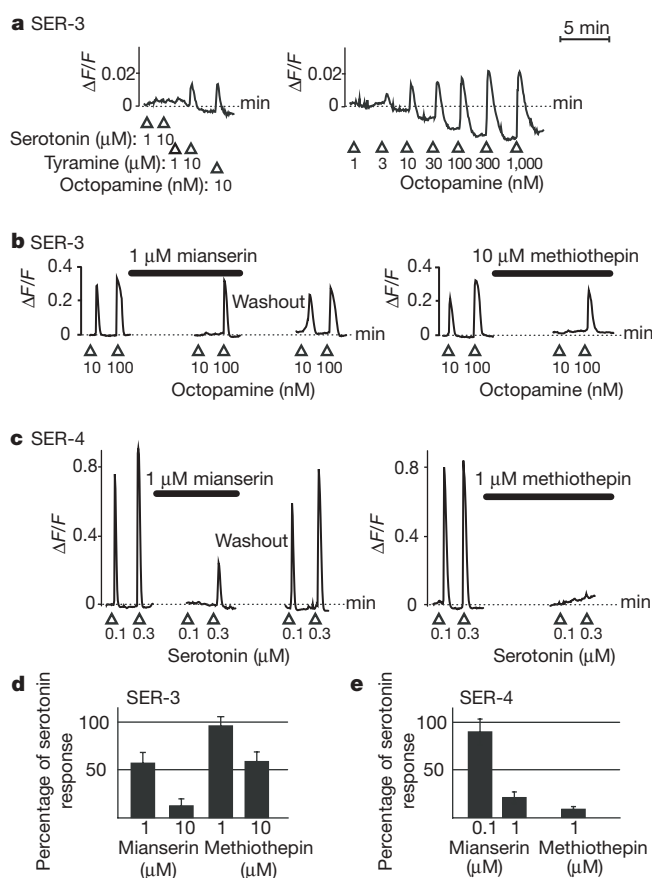
§ Drug added to N2 animals on day 5 of adulthood.

larval and adult life recapitulates the lifespan-increasing effects of mianserin given only during adulthood. One possible explanation is that SER-3 and/or SER-4 loss of function in larvae induces compensatory mechanisms that alter the animal's physiology and, thereby, mianserin's effects on adults. Age-associated differences in loss of function phenotypes have previously been seen in mice<sup>22</sup>.

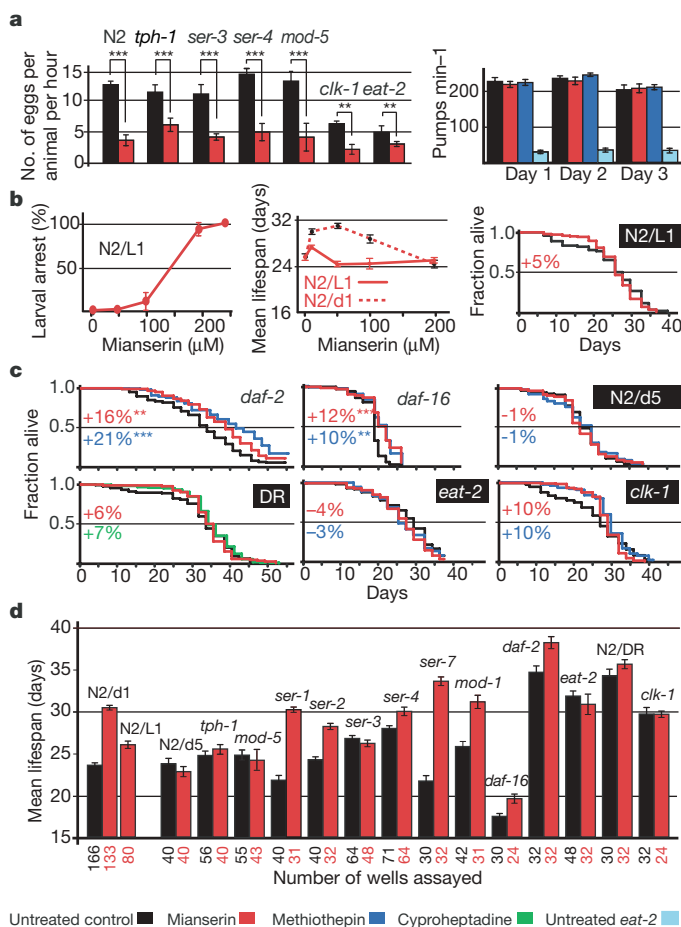
Does mianserin increase longevity via processes previously linked to ageing in *C. elegans*? We next tested mianserin on animals that have altered lifespans as a result of dietary restriction or because of mutations in genes encoding DAF-2 or DAF-16, two components of the IGF-1/insulin signalling pathway<sup>2,3</sup>, CLK-1, a mitochondrial protein<sup>23</sup>, or EAT-2, an ion channel subunit required for pumping food into the pharynx<sup>24</sup>.

Our results indicate that lifespan extension by mianserin involves ageing mechanisms associated with dietary restriction. The combination of mianserin and dietary restriction increased lifespan only  $4 \pm 2\%$  more than dietary restriction alone (Fig. 3c, d and Table 1). Furthermore, consistent with reported links between dietary restriction ageing mechanisms and increased lifespan in *eat-2(ad1116)* and

*clk-1(e2519)* mutants<sup>24</sup>, mianserin increased the lifespan of these two mutants by only  $2 \pm 5\%$  and  $3 \pm 4\%$ , respectively (Fig. 3c, d and Table 1). As in *tph-1(mg280)*, *mod-5(n3314)*, *ser-3(ok2007)* and *ser-4(ok512)* mutants, mianserin reduced serotonin-induced egg laying in both of these mutants, confirming drug uptake (Fig. 3a, left).



**Figure 2 | Mianserin and methiothepin are antagonists of SER-3 octopamine and SER-4 serotonin receptors.** HEK293 cells expressing SER-3 or SER-4 were monitored by calcium imaging for responses to potential ligands and drugs. In **a–c**, time is shown on the x axis and change in emitted fluorescence ( $\Delta F/F$ ) on the y axis. Responses are shown for single cells in **b** and **c** and for groups of cells in **a**, **d** and **e**. **a**, SER-3<sup>+</sup> cells showed calcium increases in response to 10 nM octopamine and 10 μM tyramine, but not to serotonin at 1 or 10 μM. EC<sub>50</sub> values for octopamine and tyramine were 24 nM and 26 μM, respectively. **b**, Mianserin and methiothepin (solid bars) inhibited the response of SER-3<sup>+</sup> cells to 10 nM but not 100 nM octopamine, an effect that was reversible for mianserin. **c**, SER-4<sup>+</sup> cells responded to 0.1 and 0.3 μM serotonin (EC<sub>50</sub> ~0.1 μM). Mianserin and methiothepin inhibited these responses, an effect that was reversible for mianserin. **d**, **e**, Bar graphs show the extent to which mianserin and methiothepin antagonized responses of SER-3<sup>+</sup> cells to 10 nM octopamine (**d**) or SER-4<sup>+</sup> cells to 300 nM serotonin (**e**). Responses are shown as percentages of responses seen in the absence of the drugs. Error bars indicate s.e.m.



**Figure 3 | Lifespan extension by mianserin and dietary restriction are related.** Mianserin was tested for the ability to extend the lifespan of various ageing mutants or animals subjected to dietary restriction. **a**, Left: pre-exposure to mianserin reduced serotonin-induced egg laying even in strains in which it failed to increase lifespan. Animals were (red bars) or were not (black bars) pre-incubated with mianserin before exposure to serotonin (4–6 experiments per condition, double asterisk,  $P \leq 0.001$ , triple asterisk,  $P \leq 0.0001$ , one-way analysis of variance test). Right: pharyngeal pumps per minute on different days after receiving mianserin (red), methiothepin (blue), or control diluent (black), or in control *eat-2* mutants deficient in pumping (light blue). **b**, Mianserin had different effects when given to animals as both larvae and adults rather than as adults only. Left: exposure to increasing concentrations of mianserin starting from the L1 stage led to progressive larval arrest. Middle: animals that did reach adulthood when exposed to mianserin as larvae (solid red line, N2/L1) showed little increase in lifespan in response to mianserin exposure as adults compared to animals exposed to the drug only as adults (dotted red line, N2/d1). Mean lifespan in days is shown as a function of increasing mianserin concentration for animals that reached adulthood. Right: survival curve for animals exposed to 50 μM mianserin starting from the larval L1 stage. All controls were assayed in parallel. Animals in which mianserin failed to increase lifespan are highlighted in black in **b** and **c**. **c**, Survival curves representing a typical experiment show the fraction of animals alive at different adult ages (in days) when animals were exposed to mianserin (red), methiothepin (blue), or control diluent (black). Percentage change and  $P$  values in **b** and **c** are indicated as in Fig. 1. (Sample sizes,  $\geq 50$ ). DR, dietary restriction. **d**, Mean lifespan in days for mianserin-treated and untreated control animals (cumulative). Mean lifespan and s.e.m. were calculated by averaging the lifespans of identically treated wells (10–15 animals per well). The number of wells assayed for each condition is indicated underneath each bar. Error bars indicate s.e.m. See Supplementary Tables 2–4 for details.



Although serotonin is involved in pharyngeal pumping<sup>11,25</sup>, two findings indicate that mianserin does not reduce food intake. First, although *eat-2(ad1116)* mutants showed an 80–90% decrease in pharyngeal pumping, pumping rates were the same in mianserin-treated and untreated animals (Fig. 3a). Second, dietary restriction can increase lifespan when initiated as late as day 10 of adulthood<sup>26</sup>, whereas exposure to mianserin beginning on day 5 had no effect on lifespan (Fig. 3c, d and Table 1). These results indicate that, although mianserin extends lifespan through mechanisms associated with dietary restriction, it does not do so by decreasing food intake.

Mianserin increased the lifespan of *daf-2(e1370)* and *daf-16(mu86)* mutants by  $11 \pm 4\%$  and  $14 \pm 1\%$ , respectively, suggesting that the two genes might also influence mianserin-induced lifespan extension (Fig. 3c, d). However, these results do not compare to the marked results obtained with dietary-restricted animals or *eat-2(ad1116)* and *clk-1(e2519)* mutants. These findings contrast with a requirement for *daf-16* for increased reproductive period (reproductive longevity) in *tph-1(mg280)* mutants. In our studies, untreated *tph-1(mg280)* mutants showed only a  $4 \pm 5\%$  increase in lifespan compared to wild-type animals, further distinguishing the effects of serotonin signalling on reproductive longevity versus lifespan<sup>13</sup>.

We found that *C. elegans* lifespan is increased by giving adult animals mianserin, a drug used as an antidepressant in humans. This effect requires the presence of serotonin as well as two neurotransmitter receptors: the SER-4 serotonin receptor and the SER-3 octopamine receptor. Similar to its antagonistic action on human serotonin receptors, mianserin inhibits both SER-4 and SER-3. Serotonin and octopamine are thought to serve as physiological antagonists that signal the presence of food (serotonin) versus starvation (octopamine) in *C. elegans*<sup>11,16,25</sup>. It may be that these two neurotransmitters exist in a dynamic equilibrium that is tipped in the direction of a starvation response by mianserin, possibly because of the greater inhibitory effect of mianserin on SER-4 than SER-3. In this way, mianserin might potentially create a 'perceived' state of starvation that, despite adequate food intake, would activate mechanisms of lifespan extension downstream of dietary restriction. Interestingly, one side effect of mianserin in humans is increased appetite, suggesting a possible evolutionary link between appetite and lifespan in *C. elegans* and humans<sup>27</sup>.

## METHODS SUMMARY

**Lifespan assay.** Lifespan was assessed in liquid medium<sup>5,28</sup> at 20 °C in 384- or 96-well plates. Age-synchronized *C. elegans* were distributed (seeded) in wells as L1 larvae (10–15 (96-well plates) or 6–12 (384-well plates) animals per well) together with *Escherichia coli* OP50. To prevent self-fertilization, 5-fluoro-2'-deoxyuridine (Sigma) was added 42–45 h after seeding (0.12 mM final). Unless otherwise specified, drugs were added 68 h after seeding, which corresponded to day 1 of adult life and of the lifespan assay. The fraction of animals alive was scored on the basis of body movement.

**Statistical analysis.** Comparisons and *P*-value calculations were made between treated and untreated animals of the same strain using the log-rank test (Mantel-Haenszel).

**Calcium imaging.** HEK293 cells were transfected with expression plasmids<sup>21</sup> encoding SER-4 or Gα15 and SER-3. Potential ligands were applied for 4 s, with 2 min separating different applications. Fluorescence emission was determined every 4 s. To test the effects of mianserin and methiothepin, cells were first exposed to a ligand and responses were recorded. Drugs were then applied for 5 min, after which responses to the ligands were tested again. Inhibition of responses by mianserin or methiothepin was calculated by the following equation: percentage inhibition =  $100 \times ((\text{response in the presence of inhibitor}) / (\text{uninhibited response}))$ .

**Full Methods** and any associated references are available in the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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**Supplementary Information** is linked to the online version of the paper at [www.nature.com/nature](http://www.nature.com/nature).

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## METHODS

**Strains and genetics.** All strains were maintained at 20 °C as described in ref. 29. *Caenorhabditis elegans* strains used were: Bristol strain (N2), CB4876 *clk-1(e2519)* III, GR1321 *tph-1(mg280)* II, CF1038 *daf-16(mu86)* I, CB1370 *daf-2(e1370)* III, DA1116 *eat-2(ad1116)* II, AQ866 *ser-4(ok512)* III, *ser-4(ok512);yzEx205[ser-4(+); pRF4(rol-6(su1006))]*, OHO313 *ser-2(pk1357)* X, DA1814 *ser-1(ok345)* X, MT9668 *mod-1(ok103)* V, MT9772 *mod-5(n3314)* I, CB1370 *daf-2(e1370)* III, DA2100 *ser-7(tm1325)* X, RB1631 *ser-3(ok2007)* I, and VN11 *ser-3(ad1774);tZIs3[cre::gfp, lin-15(+)]*.

**Lifespan assay.** Lifespans were assessed in liquid medium<sup>5,28</sup> (S-complete medium with 50 µg ml<sup>-1</sup> carbenicillin and 0.1 µg ml<sup>-1</sup> fungizone) in 384- or 96-well plates containing, respectively, 60 µl or 150 µl total volume, 6–12 or 10–15 nematodes, and 9 mg ml<sup>-1</sup> or 6 mg ml<sup>-1</sup> freshly prepared *E. coli* OP50 per well. Age-synchronized nematodes were seeded as L1 larvae and plates were sealed with tape (Nunc) to prevent evaporation. To prevent self-fertilization, 5-fluoro-2'-deoxyuridine (0.12 mM final) (Sigma) was added 42–45 h after seeding. Drugs were added 68 h after seeding (day 1 of adult life) unless otherwise specified. Day 1 of the lifespan assay started 68 h after seeding the animals into plates.

The fraction of animals alive per well was scored microscopically on the basis of movement. Before counting, each plate was put onto a plate rotator for 1–2 min. Strong microscope light (visual or ultraviolet) effectively stimulated movement even in old animals. Using this assay, *daf-16*, *eat-2* and *clk-1* ageing mutants showed alterations in lifespan similar to those reported using standard conditions (agar plates)<sup>2,3,24</sup>.

**Dietary restriction.** Animals were grown in liquid medium in the presence of food until day 3 of adulthood, when food was reduced 25-fold by serial dilution<sup>26</sup>.

**Statistical analysis.** STATA8 software was used for analysis. Comparisons and *P* value calculations were made between treated and untreated animals of the same strain using the log-rank test (Mantel–Haenszel). We observed the death of 98.6% of the animals (excluding screen). Animals that were still alive at the end of an experiment (1.4%) were analysed as alive up to this point with unknown time of death (censoring). Wells containing more than 19 animals were excluded from the analysis.

**Pharyngeal pumping.** Animals were grown in liquid medium at 20 °C (with or without drug). After the animals were transferred to bacteria-coated agar plates (with or without drug) and then left for 30 min, the grinder movements within a 10-s interval were counted. Animals were assayed on three consecutive days after drug treatment. Numbers of animals tested on the three days were: controls, *n* = 67/67/58; mianserin, *n* = 57/68/57; methiothepin, *n* = 26/27/24.

**Egg-laying assay.** The egg-laying assay was performed as described previously<sup>10</sup> but using 50 µM mianserin instead of 20 µM. Numbers of animals tested (with/without mianserin): N2 (64/54); *clk-1(e2519)* (26/26); *eat-2(ad1116)* (38/29); *mod-5(n3314)* (40/40); *ser-3(ok2007)* (39/29); *ser-4(ok512)* (48/38); *tph-1(mg280)* (47/40).

**Expression vectors.** The *ser-3* or *ser-4* coding region was amplified by PCR from cDNA prepared from *C. elegans* RNA, and then cloned into the pcDNA3.1(-) expression vector (Invitrogen) to give the SER-3 (pMP513#6) or SER-4 (pMP509#6) expression vector. In pMP509#6, sequence encoding the first 20 amino acids of bovine rhodopsin was added to the 5' end of the *ser-4* coding region to potentially enhance cell surface expression<sup>20</sup>.

**Calcium imaging.** A total of  $4 \times 10^5$  HEK293 cells were seeded into individual wells of 6-well plates containing coverslips coated with poly D-lysine, and then transfected with pMP509#6 (SER-4, 200 ng per well) and a Gα15 expression plasmid<sup>21</sup> (150 ng per well), or with pMP513#6 (SER-3, 200 ng per well), using lipofectamine according to the manufacturer's instructions (Invitrogen, catalogue number 1514-015). After 24 h, cells were loaded with the calcium indicator calcium 3 (Molecular Devices) in HBSS/20 mM HEPES (Gibco) for 1 h before imaging. Calcium imaging was done on coverslips in a perfusion chamber mounted on an inverted microscope (Olympus Ix70) using a 10×/0.3 NA objective (Olympus UplanFI) to maximize the number of imaged neurons. During imaging, cells were continuously perfused with HBSS and intermittently exposed to HBSS containing ligands and/or drugs. Ligands were applied for 4 s, with 2 min separating different applications. Fluorescence emission was determined every 4 s using a CCD camera (Hamatsu C4742-95-10NR) and a standard filter set (high Q filter set (R.P.I.): 470/40 excitation filter; 495 nm low-pass filter dichroic mirror; 525/50 nm emissions filter). To test the drugs mianserin and methiothepin, cells were first exposed to a ligand and responses were recorded. Drugs were added to the perfusion buffer and continuously applied for 5 min, after which responses to the ligands were tested again. The perfusion buffer was changed back to pure HBSS for a 5 min washout, after which ligands were applied again. Image analysis was done using Metafluor software (Molecular Devices). Fluorescent signals were normalized using the following equation:  $\Delta F/F = (F_t - F_0)/F_0$ . Inhibition of responses by mianserin or methiothepin was calculated by the following equation: percentage inhibition =  $100 \times ((\text{response in the presence of inhibitor})/(\text{uninhibited response}))$ .

**Drug preparation.** Mianserin, methiothepin and cyproheptadine (Sigma) were freshly prepared each time and dissolved in water at 50× final concentration before use.

29. Brenner, S. The genetics of *Caenorhabditis elegans*. *Genetics* **77**, 71–94 (1974).