Metabotropic GABA signalling modulates longevity in *C. elegans*

Lei Chun1,2, Jianke Gong1,2, Fengling Yuan1, Bi Zhang1,2, Hongkang Liu1, Tianlin Zheng1, Teng Yu1, X.Z. Shawn Xu2 & Jianfeng Liu1

The nervous system plays an important but poorly understood role in modulating longevity. GABA, a prominent inhibitory neurotransmitter, is best known to regulate nervous system function and behaviour in diverse organisms. Whether GABA signalling affects aging, however, has not been explored. Here we examined mutants lacking each of the major neurotransmitters in *C. elegans*, and find that deficiency in GABA signalling extends lifespan. This pro-longevity effect is mediated by the metabotropic GABA\(_B\) receptor GBB-1, but not ionotropic GABA\(_A\) receptors. GBB-1 regulates lifespan through G protein-PLC\(\beta\) signalling, which transmits longevity signals to the transcription factor DAF-16/FOXO, a key regulator of lifespan. Mammalian GABA\(_B\) receptors can functionally substitute for GBB-1 in lifespan control in *C. elegans*. Our results uncover a new role of GABA signalling in lifespan regulation in *C. elegans*, raising the possibility that a similar process may occur in other organisms.

1 College of Life Science and Technology, Collaborative Innovation Center for Brain Science, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China. 2 Department of Molecular and Integrative Physiology, Life Sciences Institute, University of Michigan, Ann Arbor, Michigan 48109, USA. Correspondence and requests for materials should be addressed to J.L. (email: jflu@mail.hust.edu.cn) or to X.Z.S.X. (email: shawnxu@umich.edu).
Aging is a complex physiological process modulated by a multitude of genetic and environmental factors. Recent work has revealed an important role of the nervous system in modulating aging. For example, in insulin/IGF-1 signalling, one of the best characterized longevity-regulatory pathways, the DAF-2 insulin/IGF-1 receptor primarily acts in the nervous system to regulate lifespan in Caenorhabditis elegans. Interestingly, work in mice revealed a similar mechanism. Environmental factors also affect aging by impinging on the nervous system. It is critical for the function and development of the nervous system and plays a key role in aging. For example, in insulin/IGF-1 signalling plays an important role in this process.

GABA is the primary inhibitory neurotransmitter in the mammalian brain. It is critical for the function and development of the nervous system and plays a key role in modulating longevity. GABA acts through both ionotropic and metabotropic receptors. As ion channels, ionotropic GABA \(_A\) receptors mediate the acute, fast actions of GABA. By contrast, metabotropic GABA \(_B\) receptors are G protein-coupled receptors (GPCRs) that execute the slow, long-lasting effects of GABA.

C. elegans is a popular model organism widely utilized to study the biology of aging, featuring a short generation time and life-span, as well as conserved genetic mechanisms that regulate longevity. C. elegans has also been widely used as a genetic model for neurobiology. Although C. elegans possesses a relatively small nervous system, it produces all the major neurotransmitters (for example, ACh, glutamate, GABA and biogenic amines) and their cognate receptors, all of which are encoded by evolutionarily conserved gene families. While the role of neurotransmitters in controlling behaviour and nervous system development and function has been extensively characterized, little is known about their role in aging.

Here we interrogated the role of neurotransmitters in lifespan control in C. elegans and found that GABA regulates lifespan through GABA\(_B\) receptor signalling. We further identified a genetic pathway that cell nonautonomously transmits longevity signals from the GABA\(_B\) receptor in motor neurons to the transcription factor FOXO/DAF-16 in the intestine. Our results identify a novel function of GABA beyond its conventional role in modulating animal behaviour and nervous system function and development.

**Results**

**Deficiency in GABA signalling extends lifespan.** The nervous system of C. elegans hermaphrodites is relatively simple, making it convenient to characterize the role of neurotransmission in lifespan control. We first assayed the lifespan of unc-13 mutant worms that are defective in synaptic transmission. These mutant animals are long lived (Fig. 1a and Supplementary Table 1), consistent with previous results. The fact that unc-13 mutant animals are long-lived indicates that deficiency in neurotransmitter signalling extends lifespan. To ascertain which neurotransmitter(s) may contribute to this effect, we examined mutant animals defective in each of the major neurotransmitters, including unc-17 (ACh), eat-4 (glutamate), cat-2 (dopamine), tph-1 (serotonin), tdc-1 (tyramine), tbh-1 (octopamine) and unc-25 (GABA) (Fig. 1b–h). Among these

---

**Figure 1 | Loss of GABA signalling promotes lifespan.** (a) unc-13 mutant worms are long-lived. UNC-13 is required for the release of neurotransmitters (log-rank test, \(P < 0.001\), \(n = 74–82\) for different genotypes). (b–g) Loss of octopamine (b), dopamine (c), glutamate (d), tyramine (e), serotonin (f) or acetylcholine signalling (g) modestly affect lifespan (log-rank test, \(P = 0.755\), \(P = 0.811\), \(P = 0.371\), \(P = 0.046\), \(P = 0.069\) and \(P = 0.912\), respectively. \(n = 44–114\) for different genotypes). (h) Loss of GABA signalling extends lifespan (log-rank test, \(P < 0.001\), \(n = 57–85\) for different genotypes). All lifespan assays were carried out at 20 °C and were repeated at least twice. 5-Fluoro-2'-deoxyuridine (FUDR) was included in assays involving unc-13 and unc-17 mutant worms, which are defective in egg-laying. Please see Supplementary Table 1 for detailed statistical analysis of lifespan data.
mutants, unc-25 worms lived the longest life, showing the most pronounced phenotype (Fig. 1h), while others only had modest or no effect (Fig. 1b–g). While these data by no means exclude a role of other neurotransmitters, it reveals an important function of GABA signalling in lifespan control.

**Loss of the GABA\(_B\) receptor gene gbb-1 extends lifespan.** GABA acts through both ionotropic and metabotropic receptors. The *C. elegans* genome encodes two metabotropic GABA\(_B\) receptor genes, gbb-1 and gbb-2, which are highly homologous to their mammalian counterparts. We found that gbb-1 but not gbb-2 mutants were long-lived (Fig. 2a). gbb-1;gbb-2 double mutant was indistinguishable from gbb-1 single mutant (Fig. 2a), indicating a specific role of gbb-1 in regulating lifespan. By contrast, mutant worms lacking unc-49, which encodes the sole evolutionarily conserved GABA\(_A\) receptor family member, were normally lived (Fig. 2b). These results identify a role for the GABA\(_B\) receptor gene gbb-1 in lifespan control.

gbb-1 is enriched in the nervous system but is also found to be expressed in other tissues such as muscle and intestine. We therefore asked where gbb-1 acts to regulate lifespan. Using tissue-specific promoters, we found that expression of gbb-1 cDNA in neurons, but not in muscle or intestine, rescued gbb-1 lifespan phenotype (Fig. 3a). Thus, gbb-1 appears to act in neurons to regulate lifespan.

We crossed the gbb-1 neuronal transgene into wild-type background and found that it rendered worms short-lived (Fig. 3b). Thus, overexpression of gbb-1 shortens lifespan. This is consistent with our mutant data that loss of gbb-1 extends lifespan, providing further evidence that gbb-1 regulates longevity.

**GBB-1-dependent lifespan regulation requires DAF-16/FOXO.** We wondered how gbb-1 controls lifespan. Various genetic and environmental factors regulate lifespan by converging on a subset of transcription factors. We therefore examined the major transcription factors known to regulate lifespan, and found that RNAi of daf-16 completely suppressed the lifespan-extending phenotype of gbb-1 mutant worms (Fig. 4a), while RNAi of other transcription factors such as hsf-1, skn-1 and pha-4 did not (Fig. 4b–d). Thus, daf-16 is required for the function of gbb-1, suggesting that gbb-1 signals to daf-16 to regulate lifespan.

**Gi/o-PLC\(_\beta\) transmits longevity signals from GBB-1 to DAF-16.** DAF-16 is best known to act downstream of insulin/IGF-1 signalling to regulate lifespan. Does GBB-1 genetically interact with insulin/IGF-1 signalling? To test this, we assayed the lifespan of gbb-1;daf-2 double mutant. daf-2 encodes the sole worm insulin/IGF-1 receptor homologue. The double mutant exhibited a lifespan significantly longer than both gbb-1 and daf-2 single mutants (Supplementary Fig. 1), suggesting that gbb-1 and daf-2 may act in different pathways. However, as a reduction-of-function allele of daf-2 was used here (daf-2 nulls are lethal), we did not further test this model. Thus, we do not rule out the possibility that gbb-1 and daf-2 may act in the same or overlapping pathways.

Then how does GBB-1 signal to DAF-16? As a GPCR, GBB-1 is unlikely to communicate with DAF-16 directly. We then sought to identify genes that transduce longevity signals from GBB-1 to DAF-16. As the GABA\(_B\) receptor is known to be coupled to Gi/o-PLC\(_\beta\) signalling, we examined this pathway. The worm genome encodes one Go orthologue, goa-1 (refs 38,39), and at least a dozen Gi-related genes. However, unlike gbb-1, goa-1 mutant worms are not long-lived (Supplementary Fig. 2), suggesting the presence of functional redundancy from other Gi/genes. To overcome this difficulty, we attempted to block Gi/o signalling using PTX (Pertussis toxin) by expressing it as a transgene in worm neurons. We found that inactivation of Gi/o by PTX extended lifespan (Fig. 5a). This PTX-dependent lifespan extension also required daf-16, as it can be blocked by daf-16 RNAi (Fig. 5a), suggesting that Gi/o may act upstream of DAF-16 to regulate lifespan. Further, the PTX transgene also suppressed the short-lived phenotype caused by overexpression of gbb-1 (Fig. 5b), suggesting that Gi/o acts downstream of gbb-1. These data support our model that Gi/o acts downstream of GBB-1 but upstream of DAF-16.

The GABA\(_B\) receptor is known to pass signals from Gi/o heterotrimeric G proteins to PLC\(_\beta\) (refs 10,37). The worm genome encodes one PLC\(_\beta\) homologue, EGL-8 (refs 41,42). Similar to gbb-1 mutant and PTX-transgenic worms, egl-8 mutant worms were long-lived (Fig. 5c), consistent with the model that egl-8 acts in the pathway. Importantly, the long-lived phenotype associated with egl-8 mutant worms was suppressed by daf-16 (Fig. 5c), suggesting that egl-8 acts upstream of daf-16. Furthermore, loss of egl-8 suppressed the short-lived phenotype caused by overexpression of gbb-1 (Fig. 5d), suggesting that egl-8 acts downstream of gbb-1. A prior study also showed that
EGL-8 regulates lifespan in a DAF-16-dependent manner.43 These results together support the model that G protein-
PLCβ signalling transduces longevity signals from GBB-1 to
DAF-16.

DKF-2/PKD acts upstream of DAF-16 to regulate lifespan.
DAF-16 is best known to be regulated by kinases1. We next set
out to identify kinases that act downstream of PLCβ but upstream
of DAF-16. Protein kinase C (PKC), a group of kinases activated
by DAG and/or Ca2+, is a primary downstream target of PLCβ.
The worm genome encodes four PKC homologues: tpa-1, pkc-1,
pkc-2 and pkc-3. However, none of these genes, when mutated or
knocked down, gave rise to a long-lived phenotype as did egl-8
and gbb-1 (Supplementary Fig. 3a–d). These data suggest that
PKC is unlikely to mediate the effect of EGL-8/PLCβ in longevity
signalling.

Figure 3 | gbb-1 acts in neurons to regulate lifespan. (a) Transgenic expression of gbb-1 in neurons (log-rank test, \(P = 0.198\)), but not in the intestine (log-
rank test, \(P < 0.001\)) or muscles (log-rank test, \(P < 0.001\)) suppresses the long-lived phenotype of gbb-1 mutant worms (log-rank test, \(P < 0.001\)). The rgef-1,
ges-1 and myo-3 promoters were used to drive gbb-1 cDNA expression in neurons, intestine and muscles, respectively61–63 (\(n = 47–63\) for different genotypes).
(b) Overexpression of gbb-1 cDNA in neurons shortens lifespan (log-rank test, \(P = 0.001\), \(n = 53–72\) for different genotypes). Each plasmid DNA listed above
was injected at a concentration of 50 ng \(\mu\)l\(^{-1}\). All lifespan assays were carried out at 20°C and were repeated at least three times. Please see Supplementary
Table 1 for detailed statistical analysis of lifespan data.

EGL-8 regulates lifespan in a DAF-16-dependent manner43. These results together support the model that G protein-
PLCβ signalling transduces longevity signals from GBB-1 to
DAF-16.

Figure 4 | GBB-1-dependent lifespan regulation requires the FOXO transcription factor DAF-16. (a) daf-16 RNAi fully suppresses the long-live phenotype
of gbb-1 mutant worms (log-rank test, \(P = 0.502\), \(n = 78–91\) for different genotypes). (b–d) RNAi of pha-4 (b, log-rank test, \(P < 0.001\)), skn-1 (c, log-
rank test, \(P < 0.001\)) or hsf-1 (d, log-rank test, \(P < 0.001\)) fail to suppress the long-lived phenotype of gbb-1 mutant worms (\(n = 79–94\) for different genotypes).
For RNAi experiments, NGM plates included carbenicillin (25 \(\mu\)g \(\mu\)l\(^{-1}\)) and IPTG (1 mM). HT115 bacteria-carrying vector or RNAi plasmid were seeded on
RNAi plates 2 days before the experiment. Worms were fed RNAi bacteria from the egg stage. All RNAi lifespan assays were carried out at 20°C, and were
repeated at least three times. Please see Supplementary Table 1 for detailed statistical analysis of lifespan data.

DKF-2/PKD acts upstream of DAF-16 to regulate lifespan.
DAF-16 is best known to be regulated by kinases1. We next set
out to identify kinases that act downstream of PLCβ but upstream
of DAF-16. Protein kinase C (PKC), a group of kinases activated
by DAG and/or Ca2+, is a primary downstream target of PLCβ.
The worm genome encodes four PKC homologues: tpa-1, pkc-1,
pkc-2 and pkc-3. However, none of these genes, when mutated or
knocked down, gave rise to a long-lived phenotype as did egl-8
and gbb-1 (Supplementary Fig. 3a–d). These data suggest that
PKC is unlikely to mediate the effect of EGL-8/PLCβ in longevity
signalling.
In addition to PKC, DAG can also activate another type of kinases, protein kinase D (PKD), in both a PKC-dependent and -independent manner. Two PKD homologues are found in C. elegans: *dkf-1* and *dkf-2* (ref. 45). We thus considered a potential role of these two PKD genes. *dkf-1* mutant worms were short-lived (Supplementary Fig. 3e–f), suggesting that it is probably not involved. By contrast, worms lacking *dkf-2* were long-lived (Fig. 6a), a phenotype similar to *gbb-1* and *egl-8* mutants. A previous study also showed that loss of *dkf-2* extends lifespan and does so by regulating DAF-16 (ref. 45). Indeed, we found that the long-lived phenotype of *dkf-2* mutants was completely suppressed by loss of *gbb-1* (Fig. 6b), consistent with the notion that *dkf-2* acts downstream of *gbb-1* to regulate lifespan.

To obtain further evidence that *dkf-2* regulates *daf-16*, we examined whether loss of *dkf-2* can promote *daf-16* function. We found that the mRNA levels of *daf-16* target genes, such as *mtl-1*, *sod-3* and *dod-3*, were upregulated in *dkf-2* mutant background (Fig. 6c). We also assayed the protein level of SOD-3::GFP fusion, a commonly used reporter for *daf-16* activity, and found that it was upregulated in *dkf-2* worms (Fig. 6d). Thus, *dkf-2* appears to regulate *daf-16* function, providing additional evidence supporting that DFK-2 acts upstream of *daf-16* to control lifespan.

**DKF-2 acts downstream of EGL-8 to regulate lifespan.** We then asked whether *dkf-2* functions in the *gbb-1* pathway to regulate lifespan. *dkf-2:gbb-1* double mutant exhibited a lifespan similar to single mutants (Fig. 6a), suggesting that they act in the same pathway. To gather additional evidence, we first rescued *dkf-2* long-lived phenotype by expressing wild-type *dkf-2* cDNA as a transgene in neurons in *dkf-2* mutant worms (Fig. 6e). In addition, this *dkf-2* transgene modestly shortened lifespan in wild-type background (Fig. 6f and Supplementary Table 1). Importantly, this transgene suppressed the long-lived phenotype of *gbb-1*, as well as *egl-8* mutant worms (Fig. 6g–h), providing additional evidence supporting that DFK-2 acts downstream of GBB-1 and EGL-8/PLCβ to regulate lifespan.

**Neuron-to-intestine signalling transmits longevity signals.** Apparently, the aforementioned genetic pathway, which transmits longevity signals from GBB-1 to DAF-16, does not inform where the pathway operates. Our data showed that GBB-1 acts in neurons to regulate lifespan (Fig. 3a), yet DAF-16 is best known to function in the intestine. This raises the question whether GBB-1 signals DAF-16 through neuron-to-intestine signalling. However, DAF-16 also functions in neurons, which confounds our data interpretation. To clarify this issue, we interrogated the site of action of DAF-16 in the GBB-1 pathway. We found that transgenic expression of *daf-16* cDNA in the intestine but not in neurons rescued the lifespan phenotype of *gbb-1;daf-16* double mutant (Fig. 7a), indicating that DAF-16 acts in the intestine in the GBB-1 pathway.
GBB-1 is broadly expressed in the nervous system. We further asked in which groups of neurons GBB-1 regulates lifespan. Transgenic expression of gbb-1 cDNA in sensory neurons under the osm-6 promoter did not rescue gbb-1 mutant phenotype (Fig. 7c), nor did expression of gbb-1 in subsets of interneurons using the glr-1 promoter (Fig. 7b). By contrast, restoring gbb-1 in ventral cord motor neurons through the acr-2 promoter fully rescued the gbb-1 longevity phenotype (Fig. 7d). Thus, GBB-1 can act in ventral cord motor neurons to regulate lifespan. This suggests a motor neuron-to-intestine signalling axis that transmits longevity signals from GBB-1 to DAF-16.

Rat GABA<sub>B</sub> receptor can functionally substitute for GBB-1. We expressed the rat GABA<sub>B</sub> receptor, GB1/GB2 (reft 47–49), as a transgene in gbb-1 mutant background using a neuron-specific promoter, and found that the transgene rescued the long-lived phenotype of gbb-1 mutant worms (Fig. 8a). This suggests that mammalian GABA<sub>B</sub> receptor can functionally substitute for worm GBB-1 in regulating lifespan.

Unlike worm GBB-1, the mammalian GABA<sub>B</sub> receptor has been extensively characterized pharmacologically. We thus took this advantage by testing some known antagonists of the GABA<sub>B</sub> receptor such as CGP36216 (ref. 52) and SCH50911 (ref. 53). These chemicals did not have a notable effect on lifespan.

Figure 6 | The PKD homologue DKF-2 acts downstream of G protein-PLC<i>β</i> signalling but upstream of DAF-16 to regulate lifespan. (a) dkf-2 acts in the gbb-1 pathway to regulate lifespan. dkf-2 mutant worms were long-lived (log-rank test, P < 0.001). dkf-2: gbb-1 double mutant showed a similar lifespan to single mutants (log-rank test, P = 0.919), suggesting that they are in the same pathway (n = 61–72 for different genotypes). (b) Loss of daf-16 fully suppresses the long-lived phenotype of dkf-2 mutant worms (log-rank test, P = 0.114, n = 72–82 for different genotypes). (c) qPCR analysis of DAF-16 target genes. qPCR reactions were run in triplicates for each genotype. Each experiment was repeated three times. Error bars, s.e.m. *P < 0.05 (analysis of variance (ANOVA) with Bonferroni test). (d) Quantification of SOD-3::GFP fluorescence intensity. SOD-3::GFP is encoded by the transgene mls84 (ref. 46). The left panels show representative images (selected from 10 similar images). Image quantification was performed as previously described. Error bars, s.e.m. **P < 0.001 (t-test). Scale bar, 100 μm. (e) Overexpression of dkf-2 in neurons fully suppresses the long-lived phenotype of dkf-2 mutant worms. Pgeg-1 is a neuron-specific promoter (log-rank test, P = 0.143, n = 77–98 for different genotypes). (f) Overexpression of dkf-2 modestly shortens lifespan (log-rank test, P = 0.005, n = 58–69 for different genotypes). (g) Overexpression of daf-16 suppresses the long-lived phenotype of gbb-1 mutant worms (log-rank test, P = 0.462, n = 44–72 for different genotypes). (h) Overexpression of daf-16 suppresses the long-lived phenotype of egl-8 mutant worms (log-rank test, P = 0.967, n = 50–98 for different genotypes). All lifespan assays were carried out at 20 °C and were repeated at least twice. FUDR was included in assays involving egl-8 mutant worms, which show a defect in egg laying. Please see Supplementary Table 1 for detailed statistical analysis of lifespan data.
assays were carried out at 20°C using gbb-1 mutant worms. The long-lived phenotype of gbb-1 mutants suppresses the short-lived phenotype of neurons and the intestine, respectively (opposite to that observed in gbb-1 null mutants). The regulatory role of GABA and GABAB receptor in lifespan regulation is consistent with its role in regulating longevity. A GABAB receptor generally mediates the slow, long-lasting actions of neurotransmitters. In the current study, we identified a new role of GABA signalling in aging. Interestingly, the effect of GABA on longevity is mediated by GABAB receptor rather than GABA A receptor. As a GPCR, GABA B receptor can functionally substitute for worm GBB-1 in lifespan control; furthermore, antagonists of mammalian GABA B receptor can extend the lifespan of extended-life Drosophila mutants. The identity of this neurotransmitter(s), however, remains elusive. By characterizing mutant strains defective in each of the major neurotransmitters in C. elegans, we found that GABA signalling plays an important role in regulating lifespan. To the best of our knowledge, this represents the first comprehensive analysis examining the role of neurotransmitters in lifespan control in any organism. Our results uncovered a genetic pathway that transmits longevity signals from GABA via G protein-PLCβ signalling to modulate aging in this organism. On the other hand, mammalian GABA B receptor can functionally substitute for its worm homologue in lifespan control; furthermore, antagonists of mammalian GABA B receptor can extend the lifespan of transgenic worms expressing this receptor, raising the possibility that GABA and GABAB receptor may play a role in aging in other organisms. It will be interesting to test this hypothesis in future studies.

Recent studies have uncovered an increasingly important role of the nervous system in longevity. Neurons regulate lifespan in a cell autonomous manner presumably through neurotransmission. Aside from neuroendocrine signalling, neurotransmitters are also believed to play a key role in this process. For example, endoplasmic reticulum (ER) stress in neurons affects lifespan through neurotransmitter rather than neuroendocrine signalling. The identity of this neurotransmitter(s), however, remains elusive. By characterizing mutant strains defective in each of the major neurotransmitters in C. elegans, we found that GABA signalling plays an important role in regulating lifespan. To the best of our knowledge, this represents the first comprehensive analysis examining the role of all neurotransmitters in lifespan control in any organism.
the FOXO transcription factor DAF-16, which is a key regulator of lifespan (Fig. 8f). Specifically, this genetic pathway consists of GBB-1, G protein, EGL-8/PLCβ, DKF-2/PKD and DAF-16/FOXO (Fig. 8f). It is interesting that PKD rather than PKC transmits signals from PLCβ in our case, although PKC plays an important role in temperature-dependent lifespan regulation in C. elegans56. One remaining question concerns how DKF-2 regulates DAF-16. The simplest model would be that DKF-2 phosphorylates DAF-16. However, we did not detect such phosphorylation in in vitro kinase assays (J.G., X.Z.S.X. and J.L., unpublished data). This suggests that DKF-2 likely regulates DAF-16 indirectly. Consistent with this model, we found that GBB-1 acts in motor neurons while DAF-16 functions in the intestine. Expression of DKF-2 in neurons is also sufficient to rescue daf-16 lifespan phenotype. Other components in the GBB-1 genetic pathway are also best known to act in the nervous system41,42. This reveals a motor neuron-to-intestine signalling axis that transmits longevity signals from GBB-1 to DAF-16. Recent studies have pointed to a critical role of such neuron-to-intestine signalling in lifespan control55,57. However, exactly how longevity signals are transmitted between neurons and intestinal cells remains a difficult question to address. Future efforts are needed to unravel the underlying mechanisms. Despite the observation that loss of GABA signalling seems to elicit the most pronounced effect on lifespan among all the major neurotransmitters, this does not necessarily indicate that other neurotransmitters do not have a role in lifespan control. Previous studies have demonstrated that worms experience a decline in dopamine and serotonin levels with age58. Octopamine has also been reported to play a key role in mediating CREB-regulated transcription coactivator (CRTC)-dependent lifespan regulation in neurons57. These neurotransmitters all bind to multiple types of receptors, including both GPCRs and ion channels. It is possible that their receptors have opposing effects on longevity, with some promoting lifespan and others inhibiting it, which may account for the modest effect resulting from a complete loss of
these neurotransmitters. It is also possible that these neurotransmitters may play a more prominent role in controlling lifespan under certain specific physiological conditions. Our studies will encourage others to investigate how the nervous system regulates lifespan through neurotransmitter signaling, an interesting but poorly understood question in the biology of aging.

**Methods**

**Genetics and molecular biology.** Wild-type: N2. **Quantification of SOD-3::GFP fluorescence intensity** was performed on an Olympus BX51 upright microscope as described previously. Images were quantified with MetaMorph Molecular Devices Inc.) and analysed with ImageJ (NIH). **Lifespan assay.** Lifespan studies were performed on 60-mm nematode growth medium (NGM) plates at 20°C as previously described. For each lifespan assay, 70–110 worms were included and transferred every other day to fresh NGM plates. **Microinjections** were performed using standard protocols. Each plasmid DNA listed above was microinjected into young adults and transgenic lines were tested for lifespan to confirm the results. For simplicity and clarity, only the data from one transgenic line were shown.

**References**

1. Kenyon, C. J. The genetics of ageing. *Nature* **464**, 504–512 (2010).
2. Fontana, L., Partridge, L. & Longo, V. D. Extending healthy life span—from yeast to humans. *Science* **328**, 321–326 (2010).
3. Allen, E. N., Ren, J., Zhang, Y. & Alcedo, J. Sensory systems: their impact on *C. elegans* survival. *Neurosci. Biobehav. Rev.* **26**, 15–25 (2014).
4. Jeong, D. E., Artan, M., Seo, K. & Lee, S. J. Regulation of lifespan by chemoSENSory and thermoSENSory systems: findings in invertebrates and their implications in mammalian aging. *Front. Genet.* **3**, 218 (2012).
5. Wolkow, C. A., Kimura, K. D., Lee, M. S. & Ruvkun, G. Regulation of *C. elegans* life-span by insulin-like signaling in the nervous system. *Science* **290**, 147–150 (2000).
6. Taguchi, A., Wartschow, L. M. & White, M. F. Brain IRS2 signaling coordinates life span and nutrient homeostasis. *Science* **317**, 369–372 (2007).
7. Apley, J. & Kenyon, C. Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. *Nature* **402**, 804–809 (1999).
8. Alcedo, J. & Kenyon, C. Regulation of *C. elegans* longevity by specific gustatory and olfactory neurons. *Neuron* **41**, 45–55 (2004).
9. Bettler, B., Kaupmann, K. & Bowery, N. GABAB receptors: drugs meet clones. *Curr. Opin. Neurobiol.* **8**, 345–350 (1998).
10. Bettler, B., Kaupmann, K., Mosbachker, J. & Gassmann, M. Molecular structure and physiological functions of GABA(B) receptors. *Physiol. Rev.* **84**, 835–867 (2004).
11. D’Souza, M. S. & Markou, A. The 'stop' and 'go' of nicotine dependence: role of GABA and glutamate. *Cold Spring Harb. Perspect. Med.* **3**, doi:10.1101/cshperspect.a012146 (2013).
12. Vighiani, M., Terunuma, M. & Moss, S. J. The dynamic modulation of GABA(A) receptor trafficking and its role in regulating the plasticity of inhibitory synapses. *Physiol. Rev.* **91**, 1009–1022 (2011).
13. McCarthy, M. M., Auger, A. P. & Perrot-Sinal, T. S. Getting excited about GABA and sex differences in the brain. *Trends Neurosci.* **25**, 307–312 (2002).
14. Koella, W. P. GABA systems and behavior. *Adv. Biochem. Psychopharmacol.* **29**, 11–21 (1981).
15. Gassmann, M. & Bettler, B. Regulation of neuronal GABA(B) receptor functions by subunit composition. *Nat. Rev. Neurosci.* **13**, 380–394 (2012).
16. Xu, C., Zhang, W., Rondard, P., Pin, J. P. & Liu, J. Complex GABAB receptor complexes: how to generate multiple functionally distinct units from a single receptor. *Front. Pharmacol.* **5**, 12 (2014).
17. Bormann, J. The 'ABC' of GABA receptors. *Trends Pharmacol. Sci.* **21**, 16–19 (2000).
18. Hosie, A. M., Aronstein, K., Sattelle, D. B. & ffrench-Constant, R. H. Molecular biology of insect neuronal GABA receptors. *Trends Neurosci.* **20**, 578–583 (1997).
19. Kaupmann, K. et al. Expression cloning of GABA(B) receptors uncovers similarity to metabotropic glutamate receptors. *Nature* **386**, 239–246 (1997).
20. Jones, K. A. et al. GABA(B) receptors function as a heteromeric assembly of the subunits GABA(B)R1 and GABA(B)R2. *Nature* **396**, 674–679 (1999).
21. Kaupmann, K. et al. GABA(B)-receptor subtypes assemble into functional heteromeric complexes. *Nature* **396**, 683–687 (1999).
22. White, J. H. et al. Heteromeric GABA receptors are required for the formation of a functional GABA(B) receptor. *Nature* **396**, 679–682 (1998).
23. Bargmann, C. I. Neurobiology of the *Caenorhabditis elegans* genome. *Science* **282**, 2028–2033 (1998).
24. de Bono, M. & Maricq, A. V. Neuronal substrates of complex behaviors in *C. elegans*. *Annu. Rev. Neurosci.* **28**, 451–501 (2005).
25. Manzullo, I. N. & Brenner, S. A phorbol ester/diacylglycerol-binding protein encoded by the unc-13 gene of *Caenorhabditis elegans*. *Proc. Natl Acad. Sci. USA* **88**, 5729–5733 (1991).
26. Richmond, J. E., Davis, W. S. & Jorgensen, E. M. UNC-13 is required for synaptic vesicle fusion in *C. elegans*. *Nat. Neurosci.* **2**, 959–964 (1999).
27. Munoz, M. J. & Riddle, D. L. Positive selection of *C. elegans* mutants with increased stress resistance and longevity. *Genetics* **163**, 171–180 (2003).
28. Alfonso, A., Grundahl, K., Duer, J. S., Han, H. P. & Rand, J. B. The *Caenorhabditis elegans* unc-17 gene: a putative vesicular acetylcholine transporter. *Science* **261**, 617–619 (1999).
29. See, J. Y., Victor, M., Loer, C., Shi, Y. & Ruvkun, G. Food and metabolic signalling defects in the *Caenorhabditis elegans* serotonin-synthesis mutant. *Nature* **403**, 560–564 (2000).
30. Lints, R. & Emmons, S. W. Patternning of dopaminergic neurotransmitter identity among *Caenorhabditis elegans* ray sensory neurons by a TGFbeta family signaling pathway and a Fox gene. *Development* **126**, 5819–5831 (1999).
31. Alkema, M. J., Hunter-Ensor, M.,Ringstad, N. & Horvitz, H. R. Tyramine functions independently of octopamine in the *Caenorhabditis elegans* nervous system. *Neuron* **46**, 247–260 (2005).
32. Lee, R. Y., Sawin, E. R., Chalfie, M., Horvitz, H. R. & Avery, L. EAT-4, a homolog of a mammalian sodium-dependent inorganic phosphate transporter...
cotransporter, is necessary for glutamatergic neurotransmission in Caenorhabditis elegans. J. Neurosci. 19, 159–167 (1999).
33. Jin, Y., Jorgensen, E., Hartweg, E. & Horvitz, H. R. The Caenorhabditis elegans gene unc-25 encodes glutamic acid decarboxylase and is required for synaptic transmission but not synaptic development. J. Neurosci. 19, 539–548 (1999).
34. Dittman, J. S. & Kaplan, J. M. Behavioral impact of neurotransmitter-activated G-protein-coupled receptors: muscarinic and GABAB receptors regulate Caenorhabditis elegans locomotion. J. Neurosci. 28, 7104–7112 (2008).
35. Bamber, B. A., Beg, A. A., Twyman, R. E. & Jorgensen, E. M. The Caenorhabditis elegans unc-49 locus encodes multiple subunits of a heteromultimeric GABA receptor. J. Neurosci. 19, 5348–5359 (1999).
36. Kimura, K. D., Tissenbaum, H. A., Liu, Y. & Ruvkun, G. daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. Science 277, 942–946 (1997).
37. Tu, H. et al. GABA receptor activation protects neurons from apoptosis via IGF-1 receptor transactivation. J. Neurosci. 30, 749–759 (2010).
38. Mendel, J. E. et al. Participation of the protein Go in multiple aspects of behavior in C. elegans. Science 267, 1652–1655 (1995).
39. Segalat, L., Elkes, D. A. & Kaplan, J. M. Modulation of serotonin-controlled behaviors by Go in Caenorhabditis elegans. Science 267, 1648–1651 (1995).
40. Roayaie, K., Crump, J. G., Sagasti, A. & Bargmann, C. I. The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron 20, 55–67 (1998).
41. Lackner, M. R., Nurrius, S. J. & Kaplan, J. M. Facilitation of synaptic transmission by EGL-30 Gqalpha and EGL-8 PLCbeta: DAG binding to UNC-19 is required to stimulate acetylcholine release. Neuron 24, 335–346 (1999).
42. Miller, G., Emerson, M. D. & Rand, J. B. Gqalpha and diacylglycerol kinase negatively regulate the Gqalpha pathway in C. elegans. Neuron 24, 323–333 (1999).
43. Ching, Q., Sieburth, D. & Kaplan, J. M. Profiling synaptic proteins identifies regulators of insulin secretion and lifespan. PLoS Genet. 4, e1000283 (2008).
44. McKinsey, T. A. Derepression of pathological cardiac genes by members of the CaM kinase superfamily. Cardiovasc. Res. 73, 667–677 (2007).
45. Feng, H., Ren, M., Chen, L. & Rubin, C. S. Properties, regulation, and in vivo functions of a novel protein kinase D: Caenorhabditis elegans DKF-2 links diacylglycerol second messenger to the regulation of stress responses and lifespan. J. Biol. Chem. 282, 31273–31288 (2007).
46. Lihina, N., Berman, J. R. & Kenyon, C. Tissue-specific activities of C. elegans DAF-16 in the regulation of lifespan. Cell 115, 489–502 (2003).
47. Rondard, P. et al. Functioning of the dimeric GABA(B) receptor extracellular domain revealed by glycan wedge scanning. EMBO J. 27, 1321–1332 (2008).
48. Galvez, T. et al. AllostERIC interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. EMBO J. 20, 2152–2159 (2001).
49. Bin, J. P. et al. Activation mechanism of the heterodimeric GABA(B) receptor. Biochem. Pharmacol. 68, 1565–1572 (2004).
50. Coute, A., Moss, S. J. & Pangalos, M. N. GABAB receptors: a new paradigm in G protein signaling. Mol. Cell Neurosci. 16, 296–312 (2000).
51. Comps-Agrar, L. et al. The oligomeric state sets GABAB receptor signalling efficacy. EMBO J. 30, 2336–2349 (2011).
52. Jang, I. et al. CGP 36216 is a selective antagonist at GABAB3 presynaptic receptors in rat brain. Eur. J. Pharmacol. 415, 191–195 (2001).
53. Forest, W. Chemistry and pharmacology of GABAB receptor ligands. Adv. Pharmacol. 58, 19–62 (2010).
54. Enell, L. E., Kapan, N., Soderberg, J. A., Kahas, L. & Nassel, D. R. Insulin signaling, lifespan and stress resistance are modulated by metabotropic GABA receptors on insulin producing cells in the brain of Drosophila. PLoS ONE 5, e13570 (2010).
55. Taylor, R. C. & Dillin, A. XBP-1 is a cell-nonautonomous regulator of stress resistance and longevity. Cell 153, 1435–1447 (2013).
56. Xiao, R. et al. A genetic program promotes C. elegans longevity at cold temperatures via a thermosensitive TRP channel. Cell 152, 806–817 (2013).
57. Burkewitz, K. et al. Neuronal CRT-1 governs systemic mitochondrial metabolism and lifespan via a catecholamine signal. Cell 160, 842–855 (2015).
58. Yin, J. A., Liu, X. J., Yuan, J., Jiang, J. & Cai, S. Q. Longevity manipulations differentially affect serotonin/dopamine level and behavioral deterioration in aging Caenorhabditis elegans. J. Neurosci. 34, 3947–3958 (2014).
59. Hsu, A. L., Feng, Z., Hsieh, M. Y. & Xu, X. Z. Identification by machine vision of the rate of motor activity decline as a lifespan predictor in C. elegans. Neurobiol. Aging 30, 1498–1503 (2009).
60. Liu, J. et al. Functional aging in the nervous system contributes to age-dependent motor activity decline in C. elegans. Cell Metab. 18, 392–402 (2013).
61. Aamodt, E. I., Chung, M. A. & McGhee, J. D. Spatial control of gut-specific gene expression during Caenorhabditis elegans development. Science 252, 579–582 (1991).
62. Altun-Gultekin, Z. et al. A regulatory cascade of three homeobox genes, ceh-10, ttx-3 and ceh-23, controls cell fate specification of a defined interneuron class in C. elegans. Development 128, 1951–1969 (2001).
63. Fire, A. & Waterston, R. H. Proper expression of myosin genes in transgenic nematodes. EMBO J. 8, 3419–3428 (1989).
64. Cao, P. et al. Light-sensitive coupling of rhodopsin and melanopsin to G(0) and G(1) signal transduction in Caenorhabditis elegans. FASEB J. 26, 480–491 (2012).

Acknowledgements
We thank Charles Rubin and Ao-Lin Hsu for providing strains, Zhaoyang Feng for the PTX plasmid and Lingxiu Xu for technical assistance. Some strains were obtained from the CGC and Knockout Consortiums in the USA and Japan. This work was supported by the NSFC (31130028, 31225011 and 31420103909 to J.L.), the Program of Introducing Talents of Discipline to the Universities from the Ministry of Education (1538029 to J.L.), the Ministry of Science and Technology of China (2012CB51800 to J.L.), the Natural Science Foundation of Hebei Province (2014CFA010 to J.L.) and the NIA (Z.X.Z.X.).

Author contributions
LC. performed the experiments and analysed the data. J.G. and B.Z. initiated the project. J.G., F.Y., H.L., T.Z. and T.Y. performed some experiments. Z.X.Z.X. and J.L. supervised the project. C.L., Z.X.Z.X. and J.L. wrote the manuscript.

Additional information
Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at http://www.nature.com/reprintsandpermissions/

How to cite this article: Chun, L. et al. Metabotropic GABA signalling modulates longevity in C. elegans. Nat. Commun. 6:8828 doi: 10.1038/ncomms9828 (2015).