LETTER

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A gut microbial factor modulates locomotor behaviour in *Drosophila*

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While research into the biology of animal behaviour has primarily focused on the central nervous system, cues from peripheral tissues and the environment have been implicated in brain development and function[1]. There is emerging evidence that bidirectional communication between the gut and the brain affects behaviours including anxiety, cognition, nociception and social interaction[1–9]. Coordinated locomotor behaviour is critical for the survival and propagation of animals, and is regulated by internal and external sensory inputs[10,11]. However, little is known about how the gut microbiome influences host locomotion, or the molecular and cellular mechanisms involved. Here we report that germ-free status or antibiotic treatment results in hyperactive locomotor behaviour in the fruit fly *Drosophila melanogaster*. Increased walking speed and daily activity in the absence of a gut microbiome are rescued by mono-colonization with specific bacteria, including the fly commensal *Lactobacillus brevis*. The bacterial enzyme xylose isomerase from *L. brevis* recapitulates the locomotor effects of microbial colonization by modulating sugar metabolism in flies. Notably, thermogenetic activation of octopaminergic neurons or exogenous administration of octopamine, the invertebrate counterpart of noradrenaline, abrogates the effects of xylose isomerase on *Drosophila* locomotion. These findings reveal a previously unappreciated role for the gut microbiome in modulating locomotion, and identify octopaminergic neurons as mediators of peripheral microbial cues that regulate motor behaviour in animals.

Coordinated locomotion is required for fundamental activities of life such as foraging, social interaction and mating, and involves the integration of multiple contextual factors including the internal state of the animal and external sensory stimuli[10,11]. The intestine represents a major conduit for exposure to environmental signals that influence host physiology, and is connected to the brain through both neuronal and humoral pathways. Recent studies have shown that the intestinal microbiome regulates developmental and functional features of the nervous system[1,2], although the effects of gut bacteria on the neuromodulators and neuronal circuits involved in locomotion remain poorly understood. As central mechanisms of locomotion, including sensory feedback and neuronal circuits that integrate these modalities, are shared in lineages spanning arthropods and vertebrates[11–13], we used the fruit fly *D. melanogaster* to explore host–microbiome interactions that contribute to locomotor behaviour. Locomotion was examined in the presence (conventional; Conv) and absence (axenic; Ax) of commensal bacteria. In comparison to conventionally reared animals, axenic female adult flies showed increased walking speed and daily activity (Fig. 1a, b, g). *Drosophila* locomotion is characterized by a pattern of intermittent periods of pauses and activity bouts[11,14], during the latter of which the average speed of the fly is above a set threshold of 0.25 mm s^{-1}. An increase in average speed may be related to changes in temporal patterns, including the number and/or duration of walking bouts[14]. In axenic flies, the average length of walking bouts was higher and the average pause length was lower than in conventionally reared flies, whereas the number of bouts was the same for the two groups (Fig. 1c–f). These data reveal that the microbiota modulates walking speed and temporal patterns of locomotion in *Drosophila*.

The microbial community of *D. melanogaster* contains 5–20 bacterial species[15,16]. In laboratory-raised flies, two of the dominant species are *L. brevis* and *Lactobacillus plantarum*[15]. Specific bacteria in this community affect distinct features of *Drosophila* physiology, and even closely related microbial taxa can have different biological influences on the host[15,17,18]. Accordingly, we examined whether locomotor performance was affected differently by individual bacterial species. Despite similar levels of colonization (Extended Data Fig. 1a), mono-association with *L. brevis*—but not *L. plantarum*—starting at eclosion was sufficient to correct changes in speed and daily activity in axenic flies (Fig. 1a, b, g and Extended Data Fig. 1b–e). Varying the strain of *L. brevis* or the host diet did not alter bacterial influences on host speed (Extended Data Fig. 1c–e), and *L. brevis* could largely restore temporal patterns of locomotion (Fig. 1c–f and Extended Data Fig. 1f). Detailed gait analysis revealed that flies associated with *L. brevis* showed comparable locomotor coordination to that of conventionally reared flies (Fig. 1h and Extended Data Fig. 1g). Furthermore, axenic flies that were colonized with a 1:1 mixture of *L. brevis* and *L. plantarum* showed similar changes in walking speed to flies associated with *L. brevis* alone (Extended Data Fig. 1h).

To investigate whether the effects of microbial exposure depend on host developmental stage, we mono-colonized flies at 3–5 days post-eclosion (Extended Data Fig. 2a), when the development of the gastrointestinal tract and remodelling of the nervous system are complete[19–21]. Colonization with *L. brevis* alone in fully developed animals decreased locomotor speed and average walking bout length to levels similar to those of flies treated immediately after eclosion (Extended Data Fig. 2b–e). Changes in locomotion are likely to be independent of bacterial effects on host development, as conventionally reared flies treated after eclosion with broad spectrum antibiotics exhibited similar walking speeds to animals born under axenic conditions (Extended Data Fig. 2f). Administration of antibiotics increased fly locomotion in two wild-type lines (Extended Data Fig. 2g). Furthermore, colonization with *L. brevis*, but not *L. plantarum*, after the removal of antibiotics reduced locomotor behaviour to levels similar to conventionally reared flies (Fig. 1i and Extended Data Fig. 2h–l). From these data, we conclude that locomotion is modulated by select bacterial species of the *Drosophila* microbiome, and is mediated by active signalling rather than developmental influences.

Gut bacteria secrete molecular products that regulate aspects of host physiology, including immunity and feeding behaviour[22,23]. To explore how microbes influence locomotion, we administered either cell-free supernatant (CFS) collected from bacterial cultures or heat-killed bacteria to axenic flies. CFS alone from *L. brevis* (*L.b* CFS) significantly reduced hyperactivity in axenic flies whereas heat-killed bacteria did not (Fig. 2a and Extended Data Fig. 3a–e), demonstrating a requirement for metabolically active *L. brevis* for modulation of locomotion.

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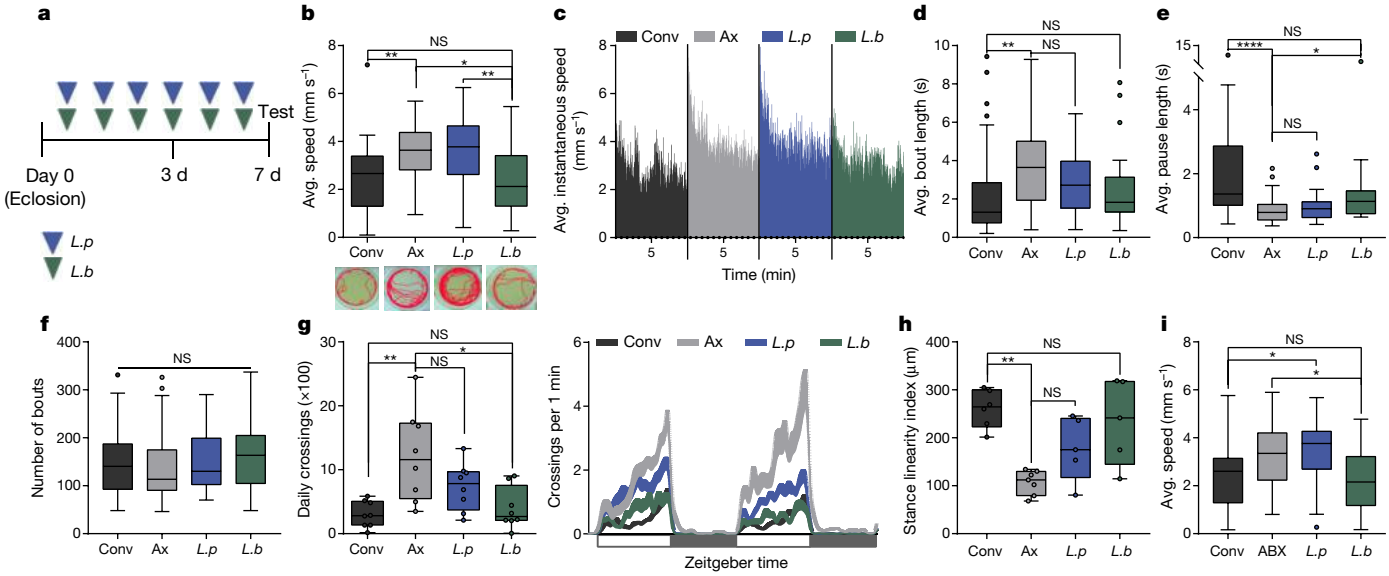

Fig. 1 | Select gut bacteria modulate locomotor behaviour in flies. **a**, Experimental design unless otherwise stated. In brief, female Oregon^R flies were either left untreated or treated with a bacterial culture or bacterial-derived factors through application to the fly medium (40 µl) daily for 6 days. **b**–**f**, Analysis of average speed (**b**; below are representative individual traces), average instantaneous speed (**c**; dashes show 5-min mark for each group), average bout length (**d**), average pause length (**e**) and number of bouts (**f**) of locomotion in conventional (Conv), axenic (Ax), and *L. plantarum* (*L.p*) or *L. brevis* (*L.b*) mono-associated flies over a 10-min period. **b**, Conv, $n = 36$ flies; Ax, $n = 36$; *L.p*, $n = 35$; *L.b*, $n = 36$. **c**, Conv, $n = 23$; Ax, $n = 29$; *L.p*, $n = 23$; *L.b*, $n = 21$. **d**–**f**, Conv, $n = 32$; Ax, $n = 36$; *L.p*, $n = 22$; *L.b*, $n = 20$. **g**, Daily activity of virgin female Oregon^R flies over a 2-day light–dark (12:12 h) cycle period, starting at time 0. $n = 8$ flies per condition. **h**, Stance linearity index for each group. Conv, $n = 6$; Ax, $n = 7$; *L.p*, $n = 5$; *L.b*, $n = 5$. **i**, Average speed of untreated conventional flies and conventional flies treated with antibiotics (ABX) for 3 days, following which flies were either left naive or colonized with *L.p* or *L.b*. Conv, $n = 25$; ABX, $n = 29$; *L.p*, $n = 24$; *L.b*, $n = 35$. Box-and-whisker plots show median and interquartile range (IQR); whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. For specific *P* values, see Supplementary Information. Kruskal–Wallis and Dunn's post hoc tests were used for statistical analysis.

L. brevis produces uracil[18], a molecule that affects the host immune response and may affect locomotion[22]. However, administration of physiological levels of uracil to axenic flies did not alter walking speed (Extended Data Fig. 3f). We next investigated whether immunity or feeding behaviour altered microbial-mediated locomotion. Depletion of the microbiome in immune-deficient (IMD) and Toll-knockout flies using antibiotics resulted in similar increases in walking speed compared to wild-type flies treated with antibiotics (Extended Data Fig. 4a, b). There were no differences in the expression of antimicrobial peptides or the dual oxidase gene *Duox* in axenic flies treated with *L.b* CFS compared with untreated axenic flies (Extended Data Fig. 4c). Moreover, although food intake may be influenced by bacterial species and can inhibit locomotor behaviour[23–25], there was no significant change in the amount of food ingested by flies treated with *L.b* CFS compared to untreated controls (Extended Data Fig. 4d,e).

Bacterial metabolism of amino acids and carbohydrates is associated with changes in host behaviour[6,8]; however, it is not known whether bacterial metabolic enzymes influence host locomotion. We used biochemical analysis of *L.b* CFS and comparative functional analysis of bacterial strains[26–28], and determined that bacterial locomotor effects are mediated via proteinaceous molecule(s) present in specific bacteria, including *L. brevis* and *Escherichia coli* (Extended Data Fig. 5a–e). Subsequently, a screen of *E. coli* strains containing single-gene mutations related to amino acid and carbohydrate metabolism identified xylose isomerase (Xi) as a candidate factor for modulation of locomotor behaviour (Extended Data Fig. 5f). Xi catalyses the reversible isomerization of certain sugars, including the conversion of D-glucose to D-fructose[29], and is present only in *L. brevis* and *E. coli* out of the bacterial strains tested (Extended Data Fig. 5e). Administration of His-tagged Xi from *L. brevis* (Xi*) reduced locomotor behaviour in axenic flies to levels similar to those in axenic flies treated with *L.b* CFS and conventionally reared flies (Fig. 2b, c and Extended Data Fig. 5g, h). The addition of His-tagged L-arabinose isomerase (Ai*), an enzyme that is not differentially expressed among the bacteria tested, did not influence

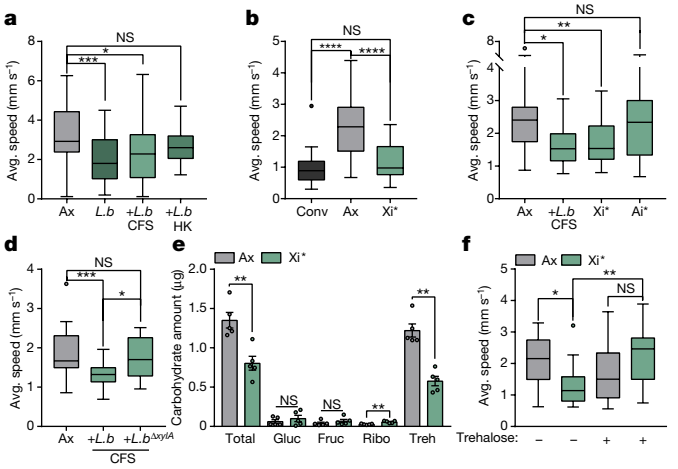

Fig. 2 | Xylose isomerase from *L. brevis* alters host locomotion. **a**–**c**, Average speed of conventional, axenic, *L.b* mono-associated, *L.b* CFS-treated axenic, heat-killed (HK) *L.b*-treated axenic, Xi* (100 µg ml^{-1})-treated axenic and Ai* (100 µg ml^{-1})-treated axenic flies. **a**, Ax, $n = 57$ flies; *L.b*, $n = 42$; *L.b* CFS, $n = 36$; *L.b* HK, $n = 24$. **b**, Conv, $n = 17$; Ax, $n = 45$; Xi*, $n = 29$. **c**, Ax, $n = 31$; *L.b* CFS, $n = 12$; Xi*, $n = 28$; Ai*, $n = 13$. **d**, Average speed of axenic flies and axenic flies treated with CFS from wild-type *L.b* or *xylA* mutant *L.b* (*L.b*$^{\Delta xylA}$) strains. Ax, $n = 28$; *L.b* CFS, $n = 29$; *L.b*$^{\Delta xylA}$ CFS, $n = 18$. **e**, Carbohydrate levels (mean ± s.e.m.) in axenic and Xi*-treated flies. $n = 5$ samples per condition. **f**, Average speed of axenic flies and Xi*-treated axenic flies left untreated or supplemented with trehalose (10 mg ml^{-1}) for 3 days before testing. Ax, $n = 16$; Xi*, $n = 18$; Ax + Treh, $n = 16$; Xi* + Treh, $n = 17$. Box-and-whisker plots show median and IQR; whiskers show 1.5 × IQR of the lower and upper quartiles. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. For specific *P* values, see Supplementary Information. Kruskal–Wallis and Dunn's (**a**–**d**, **f**) or Mann–Whitney *U* (**e**) post hoc tests used for statistical analysis. Gluc, glucose; Fruc, fructose; Ribo, ribose; Treh, trehalose.

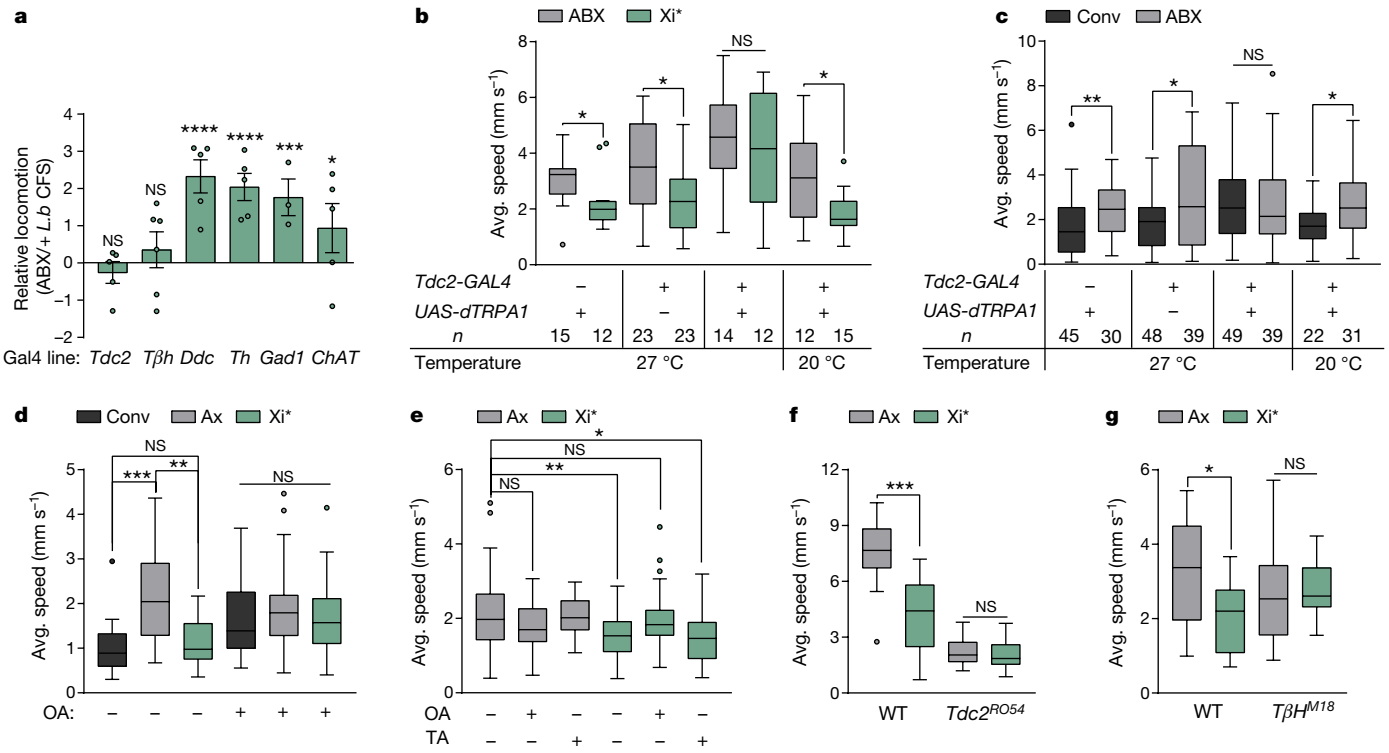

Fig. 3 | Octopamine mediates Xi-induced changes in locomotion. **a**, Difference in average speed (mean ± s.e.m.) for each GAL4 line crossed with *UAS–dTRPA1* at 27 °C. Each point denotes an independent trial. *Tdc2*, *Ddc*, *Th* (also known as *Ple*), *ChAT*, $n = 5$ trials; *Tβh*, $n = 6$; *Gad1*, $n = 3$. **b**, **c**, Average speed with or without thermogenetic activation. **d**, **e**, Average speed of flies left untreated or supplemented with octopamine (OA) or tyramine (TA) daily for 3 days. **d**, Conv, $n = 13$; Ax, $n = 33$; Xi*, $n = 21$; Conv + OA, $n = 29$; Ax + OA, $n = 27$; Xi* + OA, $n = 32$. **e**, Ax, $n = 58$; Ax + OA, $n = 13$; Ax + TA, $n = 10$; Xi*, $n = 54$; Xi* + OA, $n = 46$; Xi* + TA, $n = 27$. **f**, **g**, Average speed of antibiotic-treated wild-type (WT), *Tdc2*-null mutants ($Tdc2^{RO54}$) or *Tβh*-null mutants ($Tβh^{M18}$) left untreated (ABX) or treated with Xi* daily for 3 days. **f**, WT (w+): ABX, $n = 12$; Xi*, $n = 17$; $Tdc2^{RO54}$: ABX, $n = 19$; Xi*, $n = 17$. **g**, WT (Canton-S): $n = 15$; $Tβh^{M18}$: ABX, $n = 11$; Xi*, $n = 12$. Box-and-whisker plots show median and IQR; whiskers show 1.5 × IQR of the lower and upper quartiles. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. For specific *P* values, see Supplementary Information. Mann–Whitney *U* post hoc tests following a two-way ANOVA (**a**–**c**), Kruskal–Wallis and Dunn's post hoc tests (**d**, **e**), or Mann–Whitney *U* post hoc tests (**f**, **g**) were used for statistical analysis. *Ddc*, DOPA decarboxylase; *Th*, tyrosine hydroxylase; *Gad1*, glutamate decarboxylase 1; *ChAT*, choline acetyltransferase.

speed in axenic flies (Fig. 2c). Furthermore, we generated a chromosomal deletion of the xylose isomerase gene *xylA* in *L. brevis*, and found that the mutant strain lacked the ability to modulate host speed and daily activity (Fig. 2d and Extended Data Fig. 5g). No changes in survival or intestinal cellular apoptosis occured at the time of motor testing (Extended Data Fig. 5i, j). In addition, treatment with Xi* did not significantly alter sleep in axenic flies (Extended Data Fig. 6). Neither the addition of predicted products of Xi (D-fructose, D-glucose, D-xylose and D-xylulose) alone nor treatment with Xi inactivated by EDTA[29] reduced walking speed in axenic flies (Extended Data Fig. 7a–c). Carbohydrate analysis of whole flies showed that flies given Xi* had increased levels of ribose and reduced levels of trehalose compared to axenic controls (Fig. 2e), with no differences in these sugars in the fly medium (Extended Data Fig. 7d). Whereas EDTA-treated Xi* did not significantly alter trehalose levels, flies treated with this still displayed heightened levels of ribose compared to axenic controls (Extended Data Fig. 7e). In addition, similar to previous findings[30], conventionally reared and *L. brevis*-colonized flies showed reduced levels of trehalose compared to axenic flies (Extended Data Figs. 7f, g). Administration of trehalose alone reversed the effects of the microbiota on host speed, whereas supplementation with arabinose or ribose did not (Fig. 2f and Extended Data Fig. 7h–k). Collectively, these results demonstrate that Xi from *L. brevis* is sufficient to control locomotion in *Drosophila*, probably via modulation of key carbohydrates such as trehalose.

Specific neuronal pathways regulate complex behaviours in animals[31–33], and can be modulated by peripheral inputs such as intestinal and circulating carbohydrate levels[34]. To explore the involvement of various neuronal subsets in bacteria-induced motor behaviour, we used the thermosensitive cation channel *Drosophila* TRPA1 (dTRPA1) to activate neuronal populations that have been implicated in locomotion[35], via a repertoire of GAL4-driver lines. In combination with *UAS–dTrpA1* at the activity-inducing temperature (27 °C), activation of only two GAL4 lines—tyrosine decarboxylase (Tdc2) and tyramine beta-hydroxylase (Tβh), both of which label octopaminergic neurons—overrode modulation of locomotion by *L. brevis* (Fig. 3a and Extended Data Fig. 8). Accordingly, activation of Tdc-expressing cells abrogated the effects of Xi* treatment and removed the difference between conventionally reared and antibiotic-treated flies (Fig. 3b, c and Extended Data Fig. 9). The ability of *L. brevis* to decrease locomotion, however, was not changed by the activation of dopaminergic, serotoninergic, γ-aminobutyric acid (GABA)ergic or cholinergic neurons (Fig. 3a and Extended Data Fig. 8e–h). The administration of octopamine to conventionally reared, Xi*-, or *L.b* CFS-treated flies increased host walking speed to levels similar to that of axenic flies (Fig. 3d, e and Extended Data Fig. 10a). Furthermore, levels of *Tdc2* and *Tβh* (also known as *Tbh*) transcripts were lower in RNA extracted from the heads of Xi*- and *L.b* CFS-treated flies than in RNA from axenic flies (Extended Data Fig. 10b, c). As Tdc and Tβh are important for octopamine synthesis, these results further link octopamine to Xi-induced locomotor effects. Octopamine and tyramine are involved in multiple aspects of host physiology, including metabolism and behaviour, and have opposing roles in regulating certain motor behaviours[36–44]. Whereas administration of tyramine did not influence walking speed in axenic flies treated with Xi* or *L.b* CFS (Fig. 3e and Extended Data Fig. 10d), antibiotic-treated flies carrying a null allele for Tdc ($Tdc2^{RO54}$) no longer displayed differences in locomotion upon supplementation with Xi*

(Fig. 3f), suggesting that tyramine has an indirect role in this process. Limiting the expression of a transgene for diphtheria toxin (*DTI*) to octopaminergic and tyraminergic neurons outside the ventral nerve cord[39,45] resulted in equivalent average speeds between antibiotic and Xi*-treated flies (Extended Data Fig. 10e), implicating neurons in the supra-oesophageal and the sub-oesophageal zones in microbial effects on motor behaviour. Octopamine signalling is necessary for locomotor changes, as axenic flies treated with mianserin—an octopamine receptor antagonist—and antibiotic-treated flies carrying a null allele for Tβh ($T\beta H^{M18}$) or expressing Tβh RNAi no longer responded to treatment with Xi* or *L.b* CFS (Fig. 3g and Extended Data Fig. 10f–h). Similar results were also found when conventionally reared flies were compared to antibiotic-treated groups (Extended Data Fig. 10i–k). We conclude that defined products of the microbiome, and specifically Xi, negatively regulate octopaminergic pathways to modulate *Drosophila* locomotion (Extended Data Fig. 10l).

The microbiome influences neurodevelopment, regulates behaviour and contributes to various neurological and neuropsychiatric disorders. We have shown that gut bacteria modulate locomotion in female *Drosophila*. Depletion of the gut microbiota increases host exploratory behaviour, and the commensal bacterium *L. brevis* is sufficient to regulate locomotion. In addition, we have established that Xi from *L. brevis* corrects the locomotor phenotypes of axenic flies, a process that is mediated by trehalose and octopamine signalling in the host. However, further work is needed to identify the exact neurons and neuronal mechanisms involved, including potential changes in firing patterns. It will also be important to clarify sex-specific aspects of these microbial effects on locomotion[30,46]. Notably, germ-free mice show hyperactivity similar to that of axenic *Drosophila*, and specific bacteria have been shown to decrease locomotor activity in mice[1,47,48], although the neuronal pathways implicated in mammalian systems have yet to be identified. The mammalian counterpart of octopamine, noradrenaline, modulates locomotion[31,49], potentially implicating adrenergic circuitry as a conserved pathway that is co-opted by the microbiome in flies and mammals. In addition to motor behaviour, octopamine signalling has been linked to sugar metabolism, and trehalose serves as a major energy source for *Drosophila*[36]. Xylose isomerase may therefore facilitate adrenergic regulation of host physiology by orchestrating metabolic homeostasis, perhaps by altering internal energy storage, although additional work is needed to define how the microbiome mediates interactions between sugar metabolism and octopamine signalling. The inextricable link between metabolic state and locomotion suggests that peripheral influences on metabolism may signal via neuronal pathways to modulate physical activity. As animals have become metabolically intertwined with their microbiomes, perhaps it is not surprising that a fundamental trait such as locomotion is influenced by host–microbe symbiosis.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0634-9.

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METHODS

Fly stocks and rearing. We obtained Canton-S (#64349), *Imd*$^{-/-}$ (#55711), *Ti*$^{-/-}$ (#30652), *UAS-dTrpA1* (#26264), *Tdc2-GAL4* (#52243), *Tβh-GAL4* (#48332), *Th-GAL4* (#8488), *Ddc-GAL4* (#7009), *Gad1-GAL4* (#51630), *ChAT-GAL4* (#60317), *Elav-GAL4* (#46655), *UAS-Tβh*^{RNAi} (#27667), *UAS-DTI* (#25039) and *pBDPG4U-GAL4* (#68384) lines from Bloomington *Drosophila* Stock Center at Indiana University. Other fly stocks used were Oregon^{R} (kindly provided by A. A. Aravin and K. Fejes Tóth), *Tβh*^{M18} (kindly provided by M. H. Dickinson)[50], *Tdc2*^{R054} and *tsh-GAL80* (both kindly provided by D. J. Anderson)[51,52]. To minimize the effect of genetic background on behaviours, mutant fly lines were outcrossed for at least three generations onto a wild-type background.

Flies were cultured at 25 °C and 60% humidity on a 12-h light:12-h dark cycle and kept in vials containing fresh fly medium made at California Institute of Technology consisting of cornmeal, yeast, molasses, agar and p-hydroxy-benzoic acid methyl ester. Other dietary compositions used were created by altering this standard diet or the Nutri-Fly 'German Food' formula (Genesee Scientific) and were calculated using previously published nutritional data[53]. Axenic flies were generated using standard methods[18,54–58]. In brief, embryos from conventional flies were washed in bleach, ethanol and sterile PBS before being cultivated on fresh irradiated medium[54]. Axenic stocks were maintained through the application of an irradiated diet supplemented with antibiotics (500 μg/ml ampicillin, Putney; 50 μg/ml tetracycline, Sigma; 200 μg/ml rifamycin, Sigma) for at least one generation. For experiments, virgin female flies were collected shortly after eclosion and placed at random into vials (10–15 flies per vial) containing irradiated medium without antibiotics. Vials were changed every 3–4 days using sterile methods. The antibiotic-supplemented diet was given to conventional flies shortly after eclosion to generate antibiotic-treated (ABX) flies. Both antibiotic-treated and axenic flies were tested for contaminants by plating animal lysates on Man, Rogosa and Sharpe (MRS, BD Biosciences), mannitol (25 g/l mannitol, Sigma; 5 g/l yeast extract, BD Biosciences; 3 g/l peptone, BD Biosciences), and Luria–Bertani (LB, BD Biosciences) nutrient agar plates.

Bacterial strains. *L. brevis*^{EW}, *L. plantarum*^{WJL} and *A. pomorum* were obtained from laboratory-reared flies in the laboratory of W.-J. Lee (Seoul National University)[18,56,58]. *L. brevis*^{Bb14} (ATCC, #14869) and *L. brevis*$^{P-2}$ (ATCC, #27305) were isolated from human faeces and fermented beverages, respectively. *E. coli*^{K12} (CGSC, #7636) was grown in LB broth and *E. coli*$^{\Delta tyrA}$ (CGSC, #9131), *E. coli*$^{\Delta trpC}$ (CGSC, #10049), *E. coli*$^{\Delta manX}$ (CGSC, #9511), *E. coli*$^{\Delta treA}$ (CGSC, #9090), and *E. coli*$^{\Delta xylA}$ (CGSC, #10610)[28] were grown in LB broth supplemented with kanamycin (50 μg/ml). *L. brevis* and *L. plantarum* cultures were grown overnight in a standing 37 °C incubator in MRS broth (BD Biosciences). For mono-associations, fresh stationary phase bacterial cultures (OD_{600} = 1.0, 40 μl) were added directly to fly vials. Associations with two bacteria were performed in a 1:1 mixture. For heat-killed bacteria experiments, fresh cultures of *L. brevis* (OD_{600} = 1.0) were washed three times in sterile PBS, incubated at 100 °C for 30 min, and cooled to room temperature before being administered to flies. All treatments were supplied daily through application to the fly medium (40 μl) for 6 days following eclosion.

Bacterial supernatant preparations. Cell-free supernatants (CFS) of specified bacterial strains were collected from bacterial cultures (OD_{600} = 1.0) by centrifuging at 13,000*g* for 10 min and subsequent filtration through a 0.22-μm sterile filter (Millipore). CFS was dialysed in MilliQ water with a 3.5-kDa membrane (Thermo Scientific) overnight at 4 °C to generate *L.b* CFS and *L.p* CFS samples. Each of these treatments was supplied daily by application to the fly medium (40 μl) for 6 days following eclosion.

Heat and enzymatic treatment of *L.b* CFS. For heat-inactivation experiments, freshly prepared *L.b* CFS samples were incubated at 100 °C for 30 min and cooled to room temperature before being adminstered to flies. For proteinase K (PK) and trypsin (Typ) treatment, overnight dialysis of CFS was performed in Tris-HCl (pH 8 for PK and pH 8.5 for Typ) after which samples were treated with PK (100 μg/ml, Invitrogen) or Typ (0.05 μg/ml, Sigma) at 37 °C for 24 or 7 h, respectively. A proteinase inhibitor cocktail (Sigma) was added to stop the reaction and subsequently removed through overnight dialysis (Thermo Scientific) at 4 °C in MilliQ water. Aliquots of the samples were run on a 4–20% Tris-glycine gel (Invitrogen) to confirm protein cleavage. Controls followed the same protocol except for the addition of proteinase K or trypsin. For amylase digests, 20 μl of 100 mU/ml amylase (Sigma) was added to either freshly prepared *L.b* CFS or a PBS control for 30 min. and inhibited by lowering the pH to 4.5. Each of these treatments was supplied daily through application to the fly medium (40 μl) for 6 days following eclosion.

Production of His-tagged proteins (Xi* and Ai*). An expression plasmid for the production of His-tagged Xi from *L.b* (Xi*) was constructed by amplification of its gene and cloning of the resulting PCR product into the pQE30 cloning vector (Qiagen) using SLIC ligation. The following primer sequences were used for the construct: 5′-CGCATCACCATCACCATCACGGATCTTACTTGCTCAACGTATCGATGATGTAA-3′ and 5′-GGGGTACCGAGCTCGCATGCGGATCATGACTGAAGAATACTGGAAAGGC-3′. The conformation of the resulting plasmid was verified and it was transformed into *E. coli* (Turbo, NEB). This strain was then grown in LB medium containing ampicillin (100 μg/ml) and chloramphenicol (25 μg/ml) with shaking at 220 rpm at 37 °C for 1 h before the addition of 0.1 mM IPTG. After 4 h of shaking at 220 rpm at 37 °C, cells were pelleted and lysed using lysozyme (Sigma) and bead beating with matrix B beads (MP Biomedicals) for 45 s. Supernatant was collected after centrifugation and the Xi* protein purified through metal affinity purification under native conditions using HisPur Ni-NTA Spin Columns (Thermo Scientific). Protein purification was verified through western blot using an anti-6×His tag antibody (Abcam) and quantified using a Pierce BCA Protein Assay kit (Thermo Scientific) after which protein was stored at −20 °C. Expression and purification of His-tagged L-arabinose isomerase from *L.b* (Ai*) was performed under the same conditions and the following primer sequences were used for the construct: 5′-GGGGTACCGAGCTCGCATGCGGATCATGTTATCAGTTCCAGATTATGAATTTTGG-3′ and 5′-CGCATCACCATCACCATCACGGATCCTTACTTGATGAACGCCTTTGTCAT-3′. For EDTA treatment[29], purified Xi* was combined with 5 mM EDTA for 44 h at 4 °C and subsequently dialysed before administering to flies through application to the fly medium (40 μl) for 6 days following eclosion.

Generation of *xylA* deletion mutant (Δ*xylA*). Approximately 1-kb DNA segments flanking the region to be deleted were PCR-amplified using the following primers: 5′-ATTCCAATACTACCACTAGCAACGACATCCGTAAAGT-3′; 5′-AATTCGAGCTCGGTACCCGGGGATCCACAATCAGAATTGATCGCGGCAAC-3′; 5′-TCGTTGCTAGTGGTAGTATTGGAATCCTAAACCAGATTTCTTATCTTGATG-3′; 5′-GCCTGCAGGTCGACTCTAGAGGATCCCGCAAGTCTAGTGCGGCT-3′. The forward primers were designed using to be partially complementary at their 5′ ends by 25 bp. The fused PCR product was cloned into the BamHI site of the *Lactobacilli* vector pGID023 and mobilized into *L.b*. Colonies selected for erythromycin (Erm) resistance, indicating integration of the vector into the host chromosome, were re-plated onto MRS + Erm and subsequently passaged over 5 days and plated onto MRS + Erm. Colonies selected for Erm resistance were passaged again in MRS alone over 3 days and plated on MRS. The resulting colonies were plated in replica on MRS and MRS + Erm. Erm-sensitive colonies were screened by PCR to distinguish wild-type revertants from strains with the desired mutation.

Drug treatments. Axenic flies were either left untreated or treated with *L.b* CFS or Xi* for 3 days after eclosion. After switching to new irradiated fly medium, groups of axenic flies were treated by application to the fly medium (40 μl) with octopamine (OA, 10 mg/ml, Sigma), tyramine (TA, 10 mg/ml, Sigma), L-dopa (1 mg/ml, Sigma) or mianserin (2 mg/ml) every day for 3 days before testing, similar to previously published methods[33,37].

Bacterial load quantification. Intestines dissected from surface-sterilized 7-day-old adult female flies were homogenized in sterile PBS with ~100 μl matrix D beads using a bead beater. Lysate dilutions in PBS were plated on MRS agar plates and enumerated after 24 h at 37 °C.

Locomotion assays. Locomotor behaviour was assayed by three previously established methods: the *Drosophila* activity monitoring system (DAMS, Trikinetics)[59,60], video-assisted tracking[61–63] and gait analysis[64].

Activity measurements. Seven-day-old individual female flies were cooled on ice for 1 min and transferred into individual vials (25 × 95 mm) containing standard irradiated medium. Tubes were then inserted and secured into *Drosophila* activity monitors (DAMS, Trikinetics) and kept in a fly incubator held at 25 °C. Flies were allowed to acclimate to the new environment for 1 day before testing and midline crossing was sampled every minute. Average daily activity was calculated from the 2 days tested and actograms were generated using Actogram]J[60]. Sleep was defined as a 5 min bout of inactivity, as previously described[65].

Video-assisted tracking. Individual female flies were cooled on ice for 1 min before being introduced under sterile conditions into autoclaved arenas (3.5-cm diameter wells), which allowed free movement but restricted flight. After a 1-h acclimation period, arenas were placed onto a light box and recorded from above for a period of 10 min at 30 frames per s. All testing took place between ZT 0 and ZT 3 (ZT, Zeitgeber time; lights are turned on at ZT 0 and turned off at ZT 12) and both acclimation and testing occurred at 25 °C unless otherwise stated. Videos were processed using Ethovision software or the Caltech FlyTracker (http://www.vision.caltech.edu/Tools/FlyTracker/).

Bout analysis was performed using custom Python scripts (available upon request). The velocity curve was smoothed from the acquired video at 30 frames per s using a 15-s moving average window. A minimum walking speed of 0.25 mm/s was given, below which flies were moving but not walking ('pause bouts') and above which they were designated as walking ('walking bouts'). Lengths were measured as time between bout onset and offset.

Gait analysis. Experiments used an internally illuminated glass surface with frustrated total internal refraction to mark the flies' contact with the glass[64]. The movement of the flies and their contact were recorded with a high-frame-rate camera, and videos were quantified using the FlyWalker software package. For further details of the parameters, see ref. [64]. All groups consisted of 7-day-old female flies and were tested at room temperature.

Feeding assays. Female flies were collected at the same time as described for locomotor assays. Flies were transferred regularly onto fresh food until day 7, upon which the flies were starved for 2 h and subsequently transferred for 30 min to irradiated standard fly medium dyed with FD&C Blue no. 1 (Sigma) at a final concentration of 0.5 g dye per 100 g food. Flies were allowed to feed on the food (3–4 biological replicates and 7 flies per replicate) at 25 °C after which they were decapitated and their bodies collected. Each replicate was homogenized in 150 μl of PBS/0.05% Triton X-100 and centrifuged at 5,000*g* for 1 min to remove debris. Absorbance for all groups was measured together at 630 nm and the amount of food consumed was estimated from a standard curve of the same dye solution. The manual feeding (MAFE) assay was performed as previously described[66,67]. In brief, individual flies were introduced into a 200-μl pipette tip, which was cut to expose the proboscis. Flies were first water-satiated and presented with 100 mM sucrose delivered in a fine graduated capillary (VWR). After flies were unresponsive to 10 food stimuli, the assay was terminated and the total volume of food was calculated.

Measurement of life span. Adult female flies were transferred under sterile conditions to irradiated fly medium every 4–5 days. Survival in three or more independent cohorts containing 15–25 flies each was monitored over time.

Apoptosis assay. Midguts from 7-day-old female flies were dissected in PBS containing 0.1% Triton X-100 and the apoptosis assay was performed as previously described[18,56]. The percentage of apoptotic cells was determined by dividing the number of apoptotic cells by the total number of cells in each section and multiplying by 100.

Measurement of carbohydrate levels. Fly (5 flies per sample) and fly medium (0.1 g per sample) samples were homogenized in TE buffer (10 mM Tris, pH = 8, 1 mM EDTA) using a bead beater for 45 s followed by centrifugation at 7,000*g* for 3 min. The supernatant was heat-treated for 30 min at 72 °C before being stored at −80 °C before subsequent clean-up steps before running on high-performance anion exchange chromatography with pulsed amperometric detection.

One hundred microlitres of fly or fly medium homogenate in TE buffer was diluted with 200 μl UltraPure distilled water (Invitrogen) and sonicated to obtain a uniform solution. Samples were centrifuged at 2,000 rpm for 15 s to precipitate insoluble material. One hundred microlitres of the sample was filtered through a pre-washed Pall Nanosep 3K Omega centrifugal device (MWCO 3KDa, Sigma-Aldrich) for 15 min at 14,000 rpm and 7 °C. The filtrate was dried on Speed Vac. The dry sample was reconstituted in 300 μl UltraPure water and loaded onto a pre-washed Dionex OnGuard IIH 1cc cartridge. The flow through and 2 × 1 ml elution with Ultrapure water were collected in the same tube and lyophilized.

Monosaccharide analysis was done using a Dionex CarboPac PA1 column (4 × 250 mm) with PA1 guard column (4 × 50 mm); flow rate, 1 ml/min; pulsed amperometric detection with gold electrode. The elution gradient was as follows: 0–20 min, 19 mM sodium hydroxide; 20–50 min, 0–212.5 mM sodium acetate gradient with 19 mM sodium hydroxide; 50–65 min, 212.5 mM sodium acetate with 19 mM sodium hydroxide; 65–68 min, 212.5–0 mM sodium acetate with 19 mM sodium hydroxide; 68–85 min, 19 mM sodium hydroxide.

Trehalose, arabinose, galactose, glucose, mannose, xylose, fructose, ribose, sucrose and xylulose were used as standards. The monosaccharides were assigned based on the retention time and quantified using Chromeleon 6.8 chromatography data system software. In Extended Data Fig. 7f, g, measurements of trehalose levels were performed following the same isolation procedure and subsequently processed using a Trehalose Assay Kit (Megazyme) according to the manufacturer's instructions.

For experiments in which flies were treated with trehalose, arabinose or ribose, groups of axenic or axenic flies previously treated with Xi* were given trehalose, arabinose or ribose (10 mg/ml, Sigma) through application to the fly medium (40 μl) every day for 3 days before testing.

RNA isolation and quantitative real-time PCR. Heads (20 flies per sample) or decapitated bodies (5 flies per sample) were dissected on ice and immediately processed using an Arcturus PicoPure RNA isolation kit (Applied Biosystems) or a standard TRIzol-chloroform protocol (Thermo Fisher). One microgram of RNA was reverse transcribed using iScript cDNA Synthesis Kit, according to the manufacturer's protocol, (Bio-Rad) and diluted to 10 ng/μl based on the input concentration of total RNA.

Previously published primer pairs were used to target immune-related gene transcripts[18,68]. Other primer sequences used include *Tdc2* (F: GGTCTGCCGG ACCACTTTC, R: CACTCCGATGCGGAAGTCTG), *Tβh* (F: GCTTATCCGA CACAAAGCTGC, R: GAAAGCATTCTGCAAGTGGAA), *Ddc* (F: TGGGAT GAGCACACCATCTTG, R: GTAGAAGGGAATCAAACCCTCG), *Tph* (also known as *Trh*) (F: TGTTTTCGCCCAAGGATTCGT, R: CACCAGGTT TATGTCATGCTTCT). All primers were synthesized by Integrated DNA Technologies. Real-time PCR for the house-keeping genes *Rp49* and *RpL32* was used to ensure that input RNA was equal among all samples. Real-time PCR was performed on cDNA using an ABI PRISM 7900 HT system (Thermo Fisher) according to the manufacturer's instructions.

Data reporting and statistical analysis. No statistical methods were used to predetermine sample size. Sample size was based on previous literature in the field and experimenters were not blinded as almost all data acquisition and analysis were automated. After eclosion, virgin female flies with the same genotype were sorted into groups of 10–15 flies per vial at random. All flies in each vial were given the same treatment regime. For each experiment, the experimental and control flies were collected, treated and tested at the same time. A Mann–Whitney *U* test or Kruskal–Wallis test and Dunn's post hoc test were used for statistical analysis of behavioural data and carbohydrate analysis. Comparisons with more than one variant were first analysed using two-way ANOVA. An unpaired two-sided Student's *t*-test or a one-way ANOVA followed by a Bonferroni post hoc test were used for statistical analysis of quantitative RT–PCR results and CFU analysis. All statistical analysis was performed using Prism Software (GraphPad, version 7). *P* values are indicated as follows: $****P < 0.0001$; $***P < 0.001$; $**P < 0.01$; and $*P < 0.05$. See Supplementary Information for more details on statistical tests and exact *P* values for each figure. For boxplots, lower and upper whiskers represent 1.5 × IQR of the lower and upper quartiles, respectively; boxes indicate lower quartile, median, and upper quartile, from bottom to top. When all points are shown, whiskers represent range and boxes indicate lower quartile, median, and upper quartile, from bottom to top. Bar graphs are presented as mean ± s.e.m.

Code availability. Custom code for bout analysis is available from the corresponding authors upon request.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

All datasets generated are available from the corresponding authors upon request.

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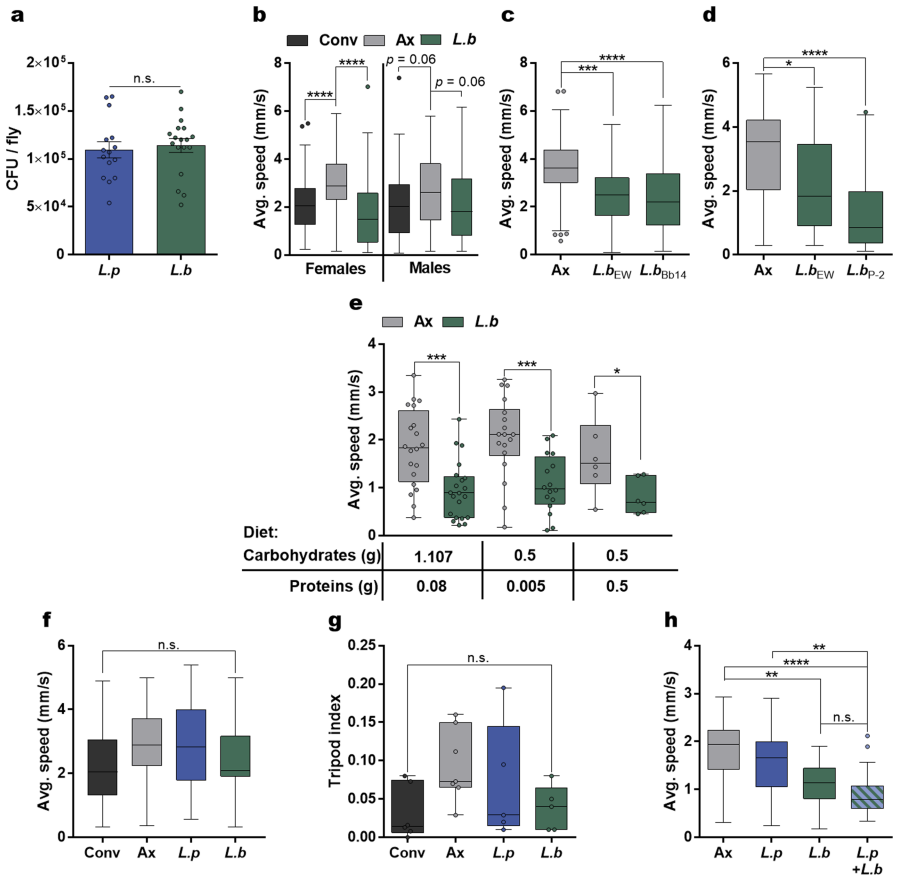
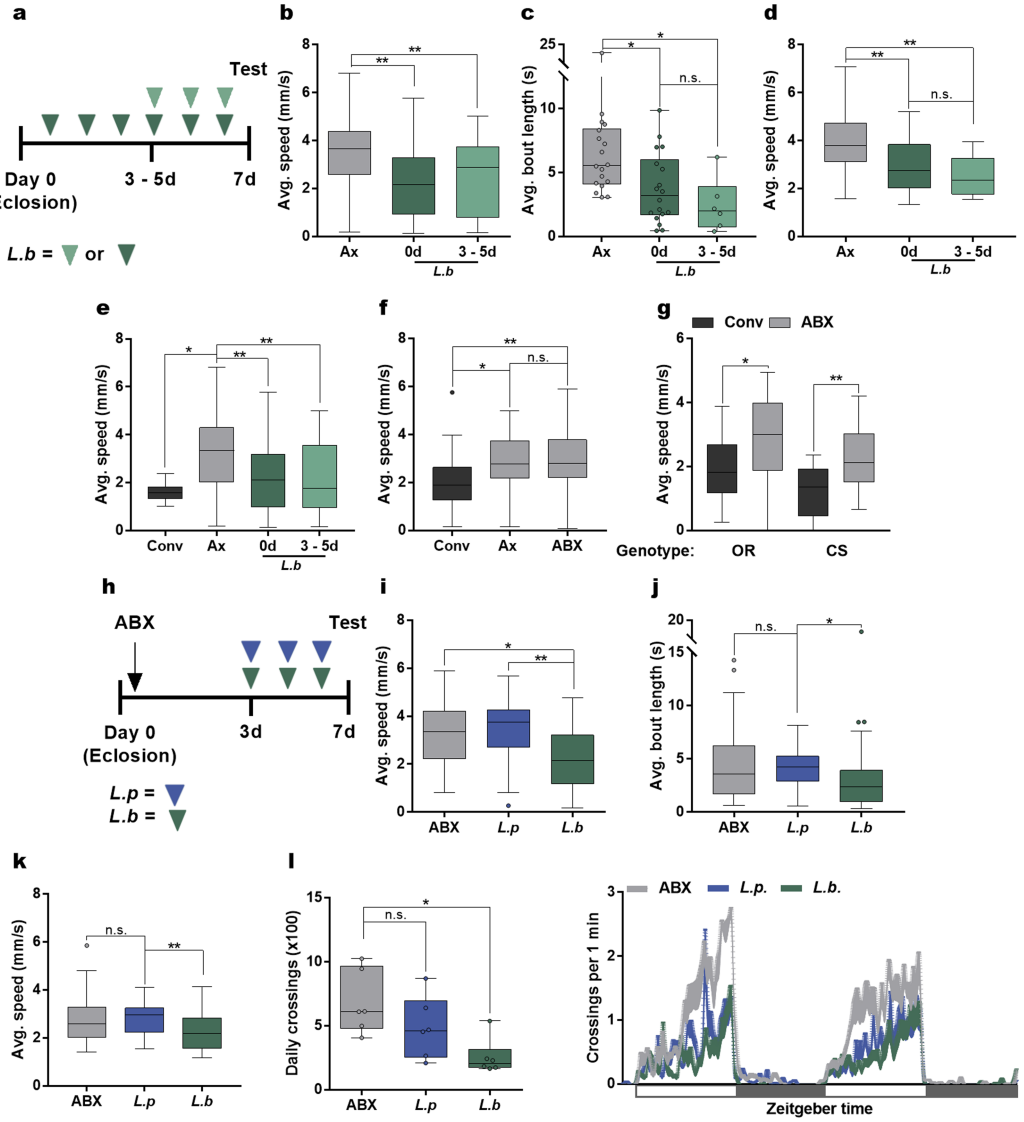
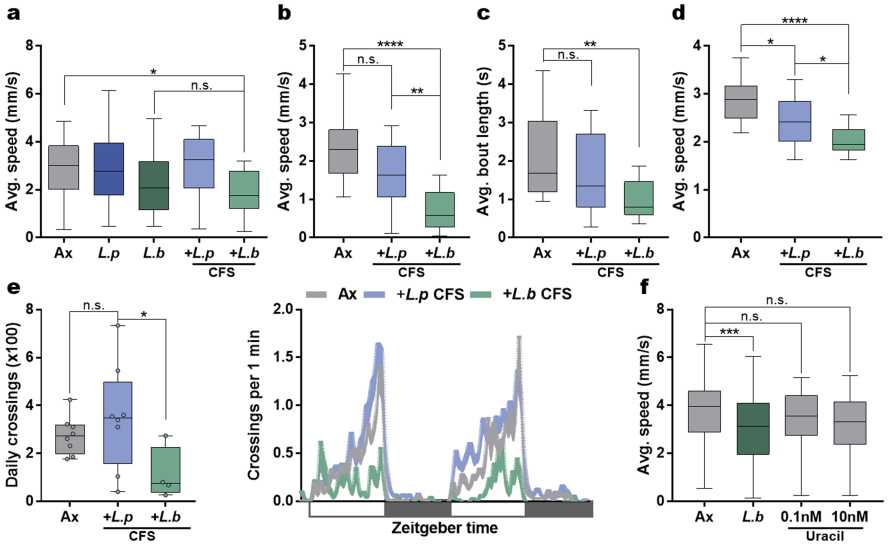


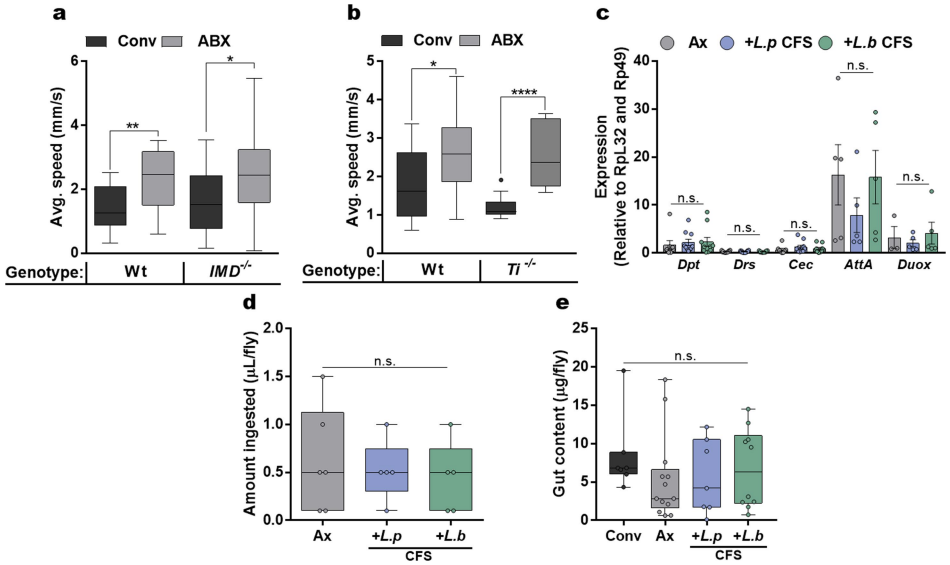
Extended Data Fig. 1 | Effects of colonization level, bacterial strain, and host diet on *L. brevis* modulation of locomotion. a, Colony-forming units (CFU) per individual fly (mean ± s.e.m.) for *L.p* or *L.b* mono-associated flies. *L.p*, $n = 15$; *L.b*, $n = 18$. **b**, Average speed of Conv, Ax and *L.b* mono-associated female or male flies. Females: Conv, $n = 90$; Ax, $n = 92$; *L.b*, $n = 89$; Males: Conv, $n = 100$; Ax, $n = 100$; *L.b*, $n = 95$. **c**, **d**, Average speed of Ax flies or flies mono-associated with *L.b* strains EW, Bb14 or P-2. **c**, Ax, $n = 58$; *L.b* EW, $n = 57$; *L.b* Bb14, $n = 57$. **d**, Ax, $n = 45$; *L.b* EW, $n = 28$; *L.b* P-2, $n = 42$. **e**, Average speed of Ax or *L.b* mono-associated flies raised on different diet compositions from eclosion until day 7. Diet 1 (left): Ax, $n = 20$; *L.b*, $n = 21$; diet 2 (middle): Ax, $n = 18$; *L.b*, $n = 16$; diet 3 (right): Ax, $n = 6$; *L.b*, $n = 6$. **f**, Average speed during walking bouts for Conv, Ax, *L.p* and *L.b* groups. Conv, $n = 23$; Ax, $n = 35$; *L.p*, $n = 22$; *L.b*, $n = 22$. **g**, Tripod index for Conv, Ax, *L.p* and *L.b* groups. Conv, $n = 6$; Ax, $n = 7$; *L.p*, $n = 5$; *L.b*, $n = 5$. **h**, Average speed of Ax flies or flies mono-associated with *L.p* or *L.b* alone or in combination (1:1). Ax, $n = 18$; *L.p*, $n = 24$; *L.b*, $n = 24$; *L.p* + *L.b*, $n = 24$. Box-and-whisker plots show median and IQR; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Specific *P* values are in the Supplementary Information. Unpaired Student's *t*-test (**a**), Kruskal–Wallis and Dunn's (**b**–**d**, **f**–**h**), or Mann–Whitney *U* (**e**) post hoc tests were used for statistical analysis. Data are representative of at least three independent trials for each experiment.



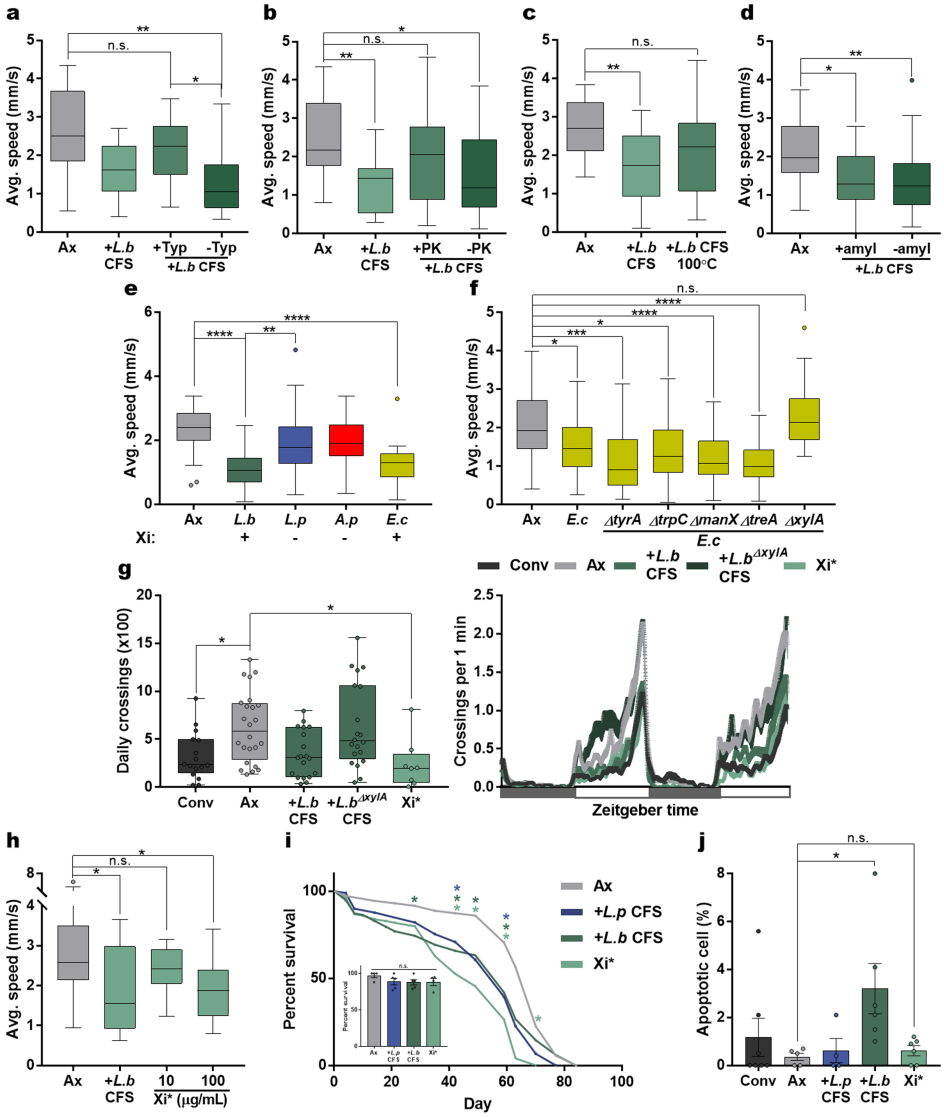
Extended Data Fig. 2 | Post-eclosion microbial signals decrease host locomotion. a, Experimental design (**b**–**e**) in which Ax flies were associated with *L.b* either directly after (day 0, dark green arrows) or 3–5 days after (light green arrows) eclosion. **b**–**d**, Average speed (**b**), average bout length (**c**) and average speed during walking bouts (**d**) of Ax flies and flies mono-associated with *L.b* at either day 0 or day 3–5. **b**, Ax, $n = 46$; *L.b* 0 d, $n = 47$; *L.b* 3–5 d, $n = 43$. **c**, Ax, $n = 18$; *L.b* 0 d, $n = 18$; *L.b* 3–5 d, $n = 6$. **d**, Ax, $n = 36$; *L.b* 0 d, $n = 36$; *L.b* 3–5 d, $n = 12$. **e**, Average speed of Conv flies, Ax flies and flies mono-associated with *L.b* at either day 0 or day 3–5. Conv, $n = 11$; Ax, $n = 53$; *L.b* 0 d, $n = 53$; *L.b* 3–5 d, $n = 52$. **f**, Average speed of Conv, Ax and Conv flies treated with antibiotics for 3 days after eclosion (ABX). Conv, $n = 32$; Ax, $n = 36$; ABX, $n = 36$. **g**, Average speed of Oregon^R (OR) and Canton-S (CS) Conv flies and Conv flies treated with antibiotics for 3 days after eclosion (ABX). OR: Conv, $n = 20$; ABX, $n = 22$; CS: Conv, $n = 12$; ABX, $n = 17$. **h**, Experimental design (**i**–**l**) in which conventionally reared flies were treated with antibiotics (ABX, black arrow) for 3 days following eclosion. All flies were subsequently placed on irradiated medium either without supplementation (ABX) or associated with *L.p* (blue arrows) or *L.b* (green arrows) for the 3 days before testing. **i**–**k**, Average speed (**i**), average bout length (**j**) and average speed during walking bouts (**k**) calculated for ABX, *L.p*- and *L.b*-associated flies. **i**, ABX, $n = 29$; *L.p*, $n = 24$; *L.b*, $n = 35$. **j**, ABX, $n = 36$; *L.p*, $n = 30$; *L.b*, $n = 35$. **k**, ABX, $n = 42$; *L.p*, $n = 30$; *L.b*, $n = 35$. **l**, Daily activity of ABX, *L.p* and, *L.b* groups (virgin female Oregon^R flies) over a 2-day 12 h light:12 h dark cycle period, starting at time 0. White boxes represent lights on and grey boxes represent lights off. $n = 6$ per condition. Box-and-whisker plots show median and IQR; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$. Specific *P* values are in the Supplementary Information. Kruskal–Wallis and Dunn's (**b**–**f**, **i**–**l**) or Mann–Whitney *U* (**g**) post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment.



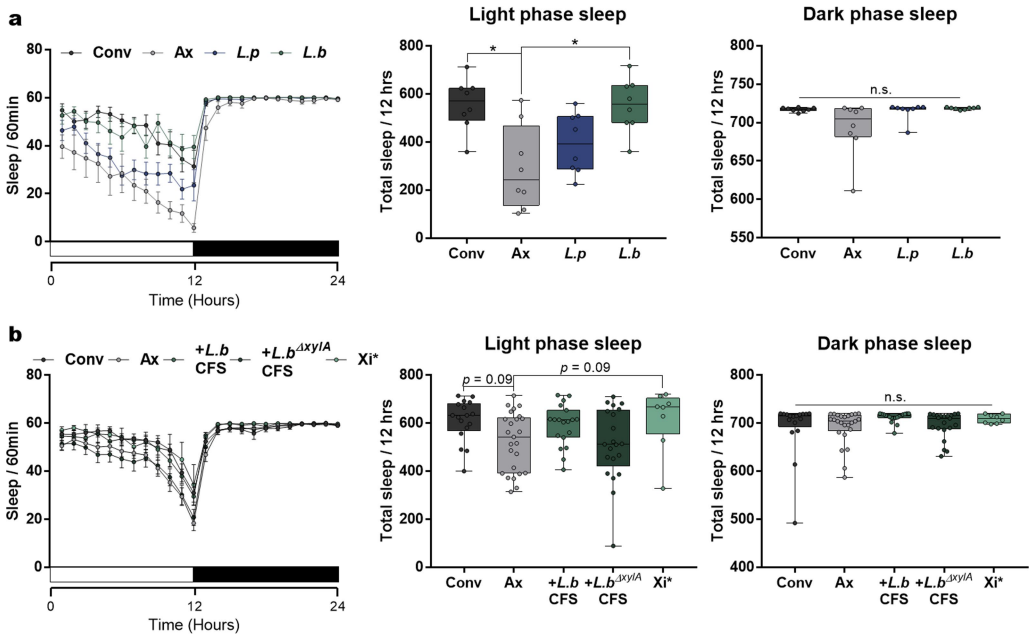
Extended Data Fig. 3 | Bacterial-derived products from *L. brevis* alter locomotion. a, Average speed of Ax flies, *L.p* or *L.b* mono-associated flies, and Ax flies treated with CFS from *L.p* or *L.b*. Ax, $n = 45$; *L.p*, $n = 17$; *L.b*, $n = 42$; *L.p* CFS, $n = 17$; *L.b* CFS, $n = 16$. **b**–**e**, Average speed (**b**), average bout length (**c**), average speed during walking bouts (**d**) and daily activity (**e**) of Ax flies and Ax virgin female Oregon^R flies treated with CFS from *L.p* or *L.b*. White boxes represent lights on and grey boxes represent lights off. **b**, Ax, $n = 23$; *L.p* CFS, $n = 20$; *L.b* CFS, $n = 20$. **c**, Ax, $n = 23$; *L.p* CFS, $n = 20$; *L.b* CFS, $n = 17$. **d**, Ax, $n = 22$; *L.p* CFS, $n = 21$; *L.b* CFS, $n = 17$. **e**, Ax, $n = 8$; *L.p* CFS, $n = 8$; *L.b* CFS, $n = 4$. **f**, Average speed of Ax, *L.b* mono-associated and Ax uracil-treated flies. Ax, $n = 96$; *L.b*, $n = 88$; 0.1 nM uracil, $n = 41$; 10 nM uracil, $n = 18$. Box-and-whisker plots show median and IQR ; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Specific *P* values are in the Supplementary Information. Kruskal–Wallis and Dunn's post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment.



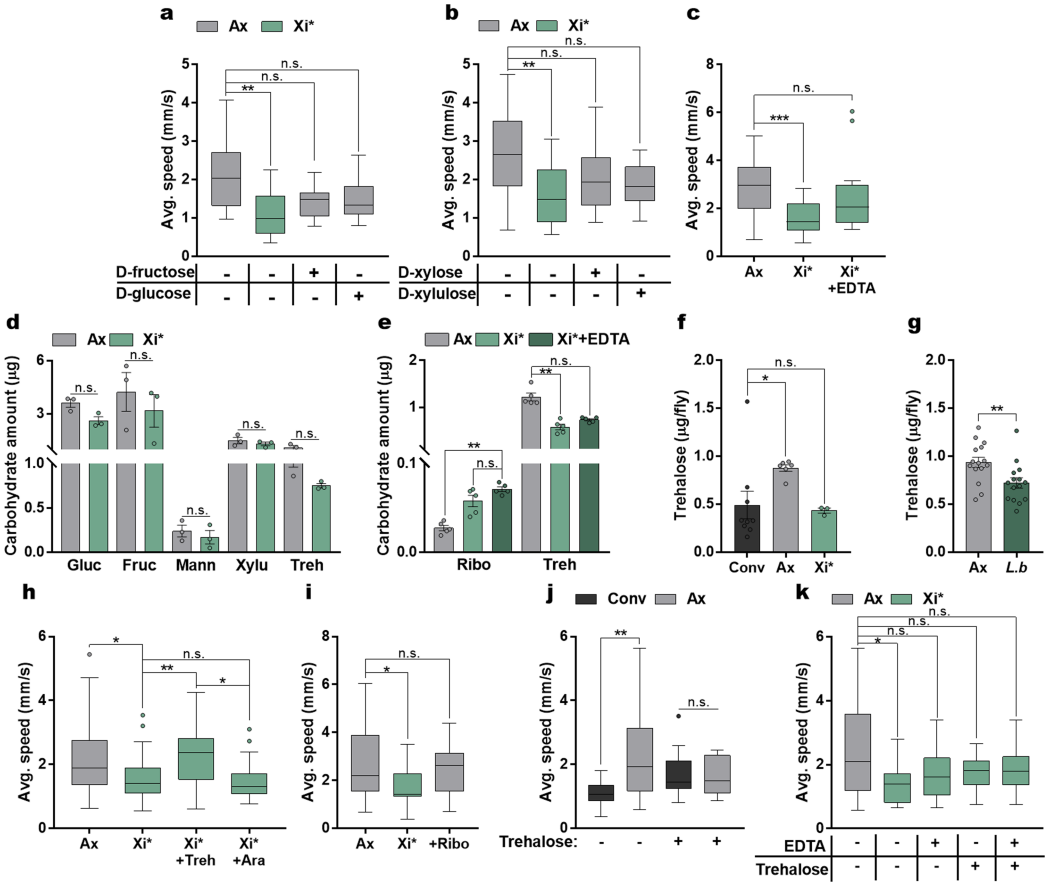
Extended Data Fig. 4 | Locomotor phenotypes are independent of food intake, anti-microbial peptides, and the immune deficiency (IMD) and Toll pathways. a, Average speed of wild-type background (Oregon^{R}, Wt) and $Imd^{-/-}$ flies placed on either medium alone or medium supplemented with antibiotics (ABX) following eclosion. Wt: Conv, $n = 16$; ABX, $n = 17$; $IMD^{-/-}$: Conv, $n = 24$; ABX, $n = 25$. **b**, Average speed of wild-type background (Canton-S, Wt) and $Ti^{-/-}$ flies placed on either medium alone or medium supplemented with antibiotics (ABX) following eclosion. Wt: Conv, $n = 15$; ABX, $n = 17$; $Ti^{-/-}$: Conv, $n = 10$; ABX, $n = 11$. **c**, qRT–PCR of immune-related transcripts (mean ± s.e.m.) in Ax and Ax *L.p* or *L.b* CFS-treated flies. *Dpt* (also known as *DptA*): Ax, $n = 8$; *L.p* CFS, $n = 10$; *L.b* CFS, $n = 10$; *Drs*: Ax, $n = 10$; *L.p* CFS, $n = 10$; *L.b* CFS, $n = 10$; *Cec* (also known as *CecA1*): Ax, $n = 8$; *L.p* CFS, $n = 10$; *L.b* CFS, $n = 10$; *AttA*: Ax, $n = 5$; *L.p* CFS, $n = 5$; *L.b* CFS, $n = 5$; *Duox*: Ax, $n = 3$; *L.p* CFS, $n = 5$; *L.b* CFS, $n = 5$. **d**, Amount ingested by Ax and Ax *L.p* or *L.b* CFS-treated flies over 10 trials during MAFE assay. Ax, $n = 6$; *L.p* CFS, $n = 5$; *L.b* CFS, $n = 6$. **e**, Intestinal content measured through supplementing the diet of Conv, Ax, and *L.p*- or *L.b*-CFS-treated Ax flies with blue food dye. Conv, $n = 7$; Ax, $n = 13$; *L.p* CFS, $n = 7$; *L.b* CFS, $n = 10$. Box-and-whisker plots show median and IQR; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{****}P < 0.0001$. Specific *P* values are in the Supplementary Information. Mann–Whitney *U* (**a, b**), one-way ANOVA and Bonferroni (**c**), and Kruskal–Wallis and Dunn's (**d, e**) post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment. *Dpt*, diptericin; *Drs*, drosomycin; *Cec*, cecropin; *AttA*, attacin-A; *Duox*, dual oxidase.



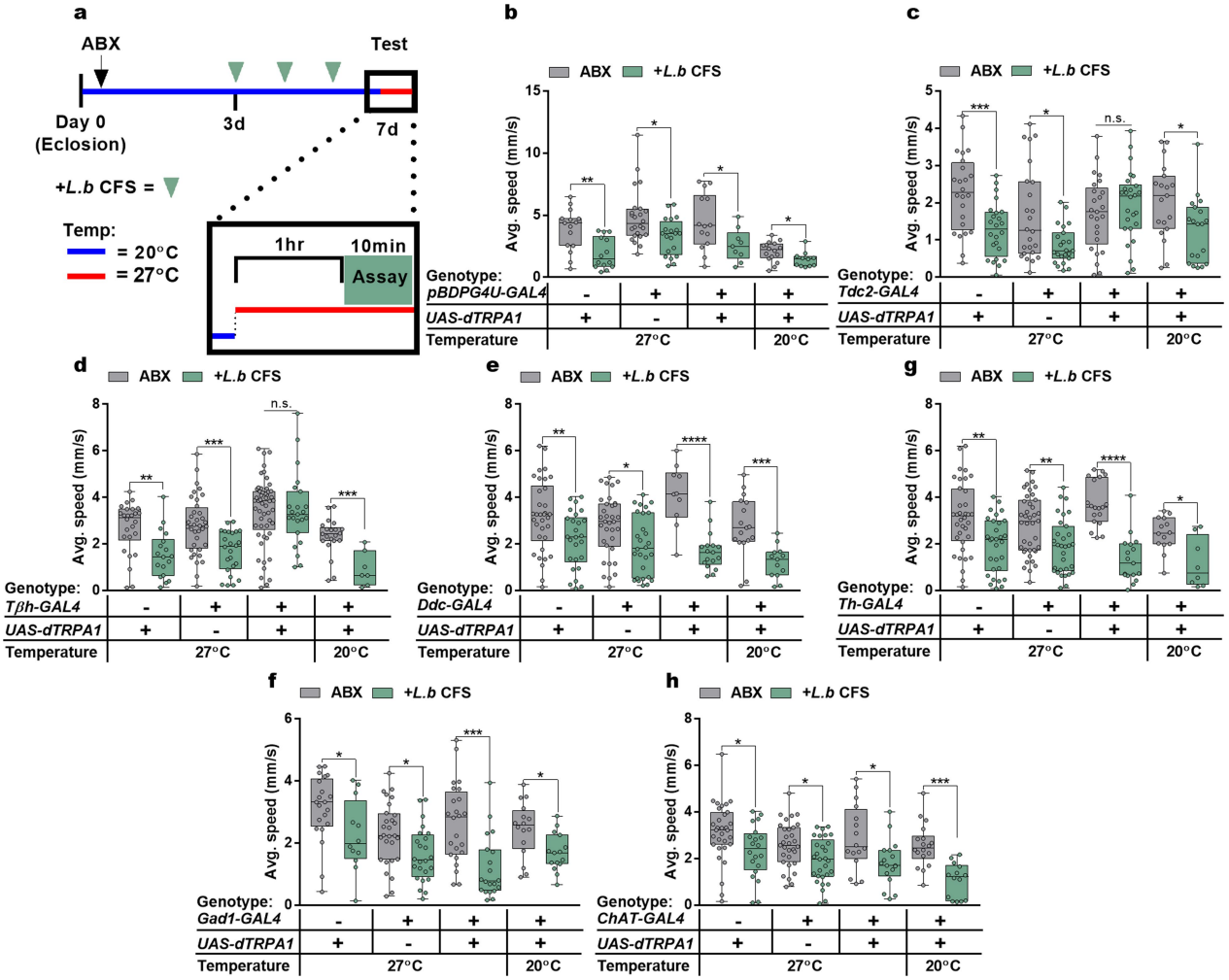
Extended Data Fig. 5 | Modulation of locomotion by the bacterial enzyme, xylose isomerase. a–c, Average speed of Ax flies or Ax flies treated with unaltered, protease-treated (Typ, trypsin; PK, proteinase-K) or heat-treated (100 °C) *L.b* CFS. **a**, Ax, $n = 18$; *L.b* CFS, $n = 18$; +Typ, $n = 17$; –Typ, $n = 17$. **b**, Ax, $n = 23$; *L.b* CFS, $n = 18$; +PK, $n = 23$; –PK, $n = 23$. **c**, $n = 18$. **d**, Average speed of Ax flies treated with amylase-treated PBS (Ax), amylase-treated *L.b* CFS (+ amyl *L.b* CFS) or unaltered *L.b* CFS (–amyl *L.b* CFS). Ax, $n = 30$; +amyl, $n = 17$; –amyl, $n = 30$. **e**, Average speed of Ax flies or flies mono-associated with *L.b*, *L.p*, *A. pomorum* (*A.p*), or *E. coli* (*E.c*). Below shows the presence (+) or absence (–) of Xi based on NCBI Blastn (*xylA* locus) and Blastp (Xi) results. Ax, $n = 30$; *L.b*, $n = 30$; *L.p*, $n = 29$; *A.p*, $n = 30$; *E.c*, $n = 18$. **f**, Average speed of Ax flies and flies mono-associated with either WT *E.c* or single gene knockout strains of *E.c* (Δ*tyrA*, Δ*trpC*, Δ*manX*, Δ*treA*, Δ*xylA*). Ax, $n = 65$; *E.c*; $n = 52$; $E.c^{\Delta tyrA}$, $n = 18$; $E.c^{\Delta trpC}$, $n = 17$; $E.c^{\Delta manX}$, $n = 45$; $E.c^{\Delta treA}$, $n = 46$; $E.c^{\Delta xylA}$, $n = 20$. **g**, Daily activity of Conv, Ax and Ax virgin female Oregon^R flies treated with *L.b* CFS, $L.b^{\Delta xylA}$ CFS or Xi* over a two-day 12 h light:12 h dark cycle period, starting at time 0. White boxes represent lights on and grey boxes represent lights off. Conv, $n = 16$; Ax, $n = 24$; *L.b* CFS, $n = 19$; $L.b^{\Delta xylA}$ CFS, $n = 20$; Xi*, $n = 8$. **h**, Average speed of Ax flies and Ax flies treated with *L.b* CFS or Xi*. Ax, $n = 16$; *L.b* CFS, $n = 11$; 10 μg/ml Xi*, $n = 12$; 100 μg/ml Xi*, $n = 14$. **i**, Lifespan measurements for Ax flies and Ax flies treated with *L.p* CFS, *L.b* CFS, or Xi*. Asterisks represent significance at the time point measured by Kruskal–Wallis and Dunn's post hoc test. Inset image shows survival at day 7 (mean ± s.e.m.). Ax, $n = 4$ groups; *L.p* CFS, $n = 5$ groups; *L.b* CFS, $n = 5$ groups; Xi*, $n = 4$ groups. **j**, Percentage of apoptotic cells (mean ± s.e.m.) in the intestines of Conv flies, Ax flies and Ax flies treated with *L.p* CFS, *L.b* CFS or Xi*. Conv, $n = 7$; Ax, $n = 5$; *L.p* CFS, $n = 4$; *L.b* CFS, $n = 6$; Xi*, $n = 6$. Box-and-whisker plots show median and IQR; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Specific *P* values are in the Supplementary Information. Kruskal–Wallis and Dunn's (**a–i**) or log-rank (**i**) post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment.



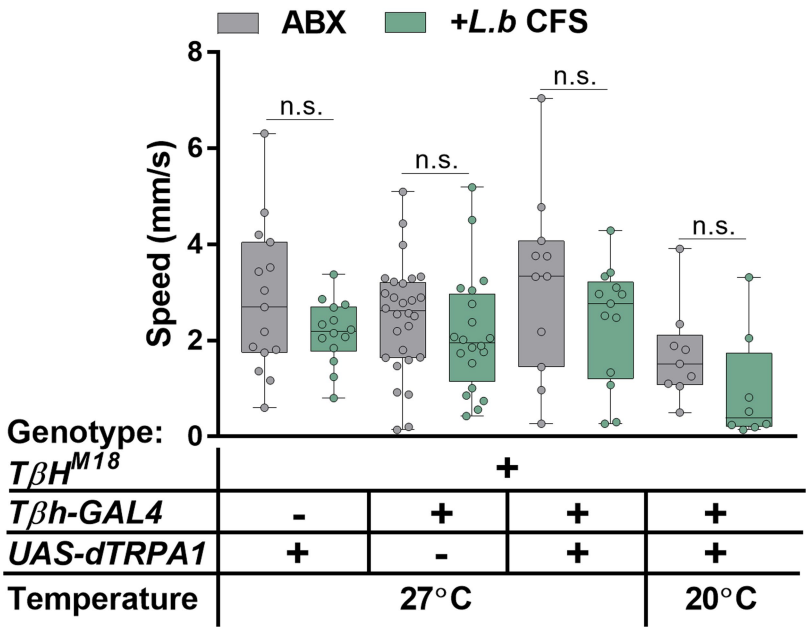
Extended Data Fig. 6 | Sleep analysis for mono-colonized flies and flies treated with bacterial factors. a, Twenty-four-hour sleep profiles (mean ± s.e.m.) of Conv, Ax, *L.p*- and *L.b*-colonized virgin female Oregon^R flies with the number of sleep bouts in 60-min time windows and total sleep in the light or dark phase. $n = 8$ flies per condition. **b**, Twenty-four-hour sleep profiles (mean ± s.e.m.) of Conv, Ax, *L.b* CFS, *L.b*$^{\Delta xylA}$ CFS and Xi* treated Ax virgin female Oregon^R flies with the number of sleep bouts in 60-min time windows and total sleep in the light or dark phase. Conv, $n = 17$; Ax, $n = 25$; *L.b* CFS, $n = 19$; *L.b*$^{\Delta xylA}$ CFS, $n = 21$; Xi*, $n = 8$. Box-and-whisker plots show median and IQR; whiskers show range. $^*P < 0.05$. Specific *P* values are in the Supplementary Information. Kruskal–Wallis and Dunn's post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment.



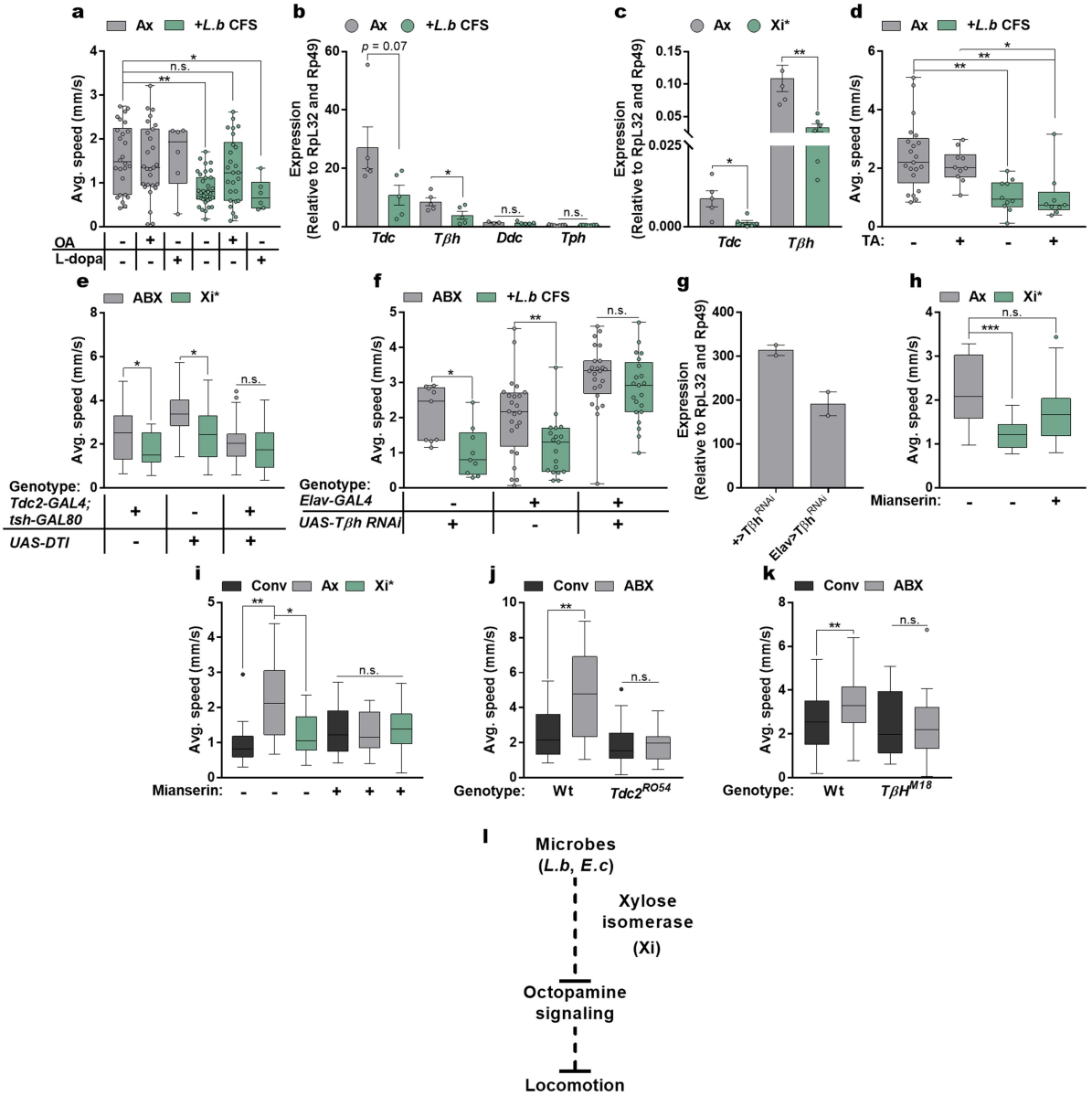
Extended Data Fig. 7 | Xylose isomerase activity and key carbohydrates are involved in Xi-mediated changes in locomotion. a, **b**, Average speed of Ax flies and Ax flies treated with Xi* or 100 µg/ml of D-fructose, D-glucose, D-xylose or D-xylulose. **a**, Ax, $n = 16$; Xi*, $n = 13$; D-fructose, $n = 13$; D-glucose, $n = 15$. **b**, Ax, $n = 26$; Xi*, $n = 21$; D-xylose, $n = 22$; D-xylulose, $n = 18$. **c**, Average speed of Ax flies and Ax flies treated with Xi* or Xi* inactivated by 5 mM EDTA. Ax, $n = 21$; Xi*, $n = 16$; Xi* + EDTA, $n = 18$. **d**, Carbohydrate levels (mean ± s.e.m.) in Ax and Xi*-treated fly medium. Each sample is from 0.1 g fly medium and represents a separate vial. $n = 3$ samples per condition. **e**, Carbohydrate levels (mean ± s.e.m.) in Ax, Xi*, and EDTA-treated Xi* flies. Each sample contains five flies. $n = 5$ samples per condition. **f**, Trehalose levels (mean ± s.e.m.) in Conv, Ax, and Xi*-treated flies. Conv, $n = 9$ samples; Ax, $n = 6$ samples; Xi*, $n = 3$ samples. **g**, Trehalose levels (mean ± s.e.m.) in Ax and *L.b*-colonized flies. $n = 15$ samples per condition. **h**, Average speed of Ax and Xi*-treated flies supplemented with either trehalose (Treh, 10 mg/ml) or arabinose (Ara, 10 mg/ml) for 3 days before testing. Ax, $n = 40$; Xi*, $n = 40$; Xi* + Treh, $n = 39$; Xi* + Ara, $n = 18$. **i**, Average speed of Ax flies and Xi*- or ribose (Ribo, 10 mg/ml)-treated flies. Ax, $n = 29$; Xi*, $n = 25$; Ribo, $n = 12$. **j**, Average speed of Conv and Ax flies supplemented with trehalose (Treh, 10 mg/ml) for 3 days before testing. Conv, $n = 15$; Ax, $n = 22$; Conv + Treh, $n = 18$; Ax + Treh, $n = 15$. **k**, Average speed of Ax and Xi* or EDTA-treated Xi* Ax flies subsequently left untreated or supplemented with trehalose (Treh, 10 mg/ml) for 3 days before testing. Ax, $n = 27$; Xi, $n = 19$; Xi + EDTA, $n = 24$; Xi + Treh, $n = 19$; Xi + EDTA + Treh, $n = 25$. Box-and-whisker plots show median and IQR; whiskers show 1.5 × IQR of the lower and upper quartiles. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Specific P values are in the Supplementary Information. Kruskal–Wallis and Dunn's (**a**–**c**, **e**, **f**, **h**–**k**) or Mann–Whitney U (**d**, **g**) post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment. Gluc, glucose; Fruc, fructose; Mann, mannose; Xylu, xylulose; Treh, trehalose; Ribo, ribose.



Extended Data Fig. 8 | Thermogenetic activation of neuromodulator-GAL4 lines. a, Experimental design in which Conv flies (Canton-S) were treated with antibiotics (ABX, black arrow) for 3 days following eclosion. All flies were subsequently placed on irradiated medium either without supplementation or treated with *L.b* CFS (green arrows) for 3 days. One hour before and during testing, flies were either exposed to 27 °C (red line) to facilitate thermogenetic activation or kept at 20 °C (blue line). **b**–**h**, Average speed of flies previously treated with antibiotics and subsequently left untreated (ABX) or treated with *L.b* CFS for 3 days before testing. **b**, UAS: ABX, $n = 15$; *L.b* CFS, $n = 14$; GAL4: ABX, $n = 24$; *L.b* CFS, $n = 20$; GAL4> UAS (27 °C): ABX, $n = 14$; *L.b* CFS, $n = 9$; GAL4> UAS (20 °C): ABX, $n = 16$; *L.b* CFS, $n = 11$. **c**, UAS: ABX, $n = 24$; *L.b* CFS, $n = 24$; GAL4: ABX, $n = 24$; *L.b* CFS, $n = 23$; GAL4> UAS (27 °C): ABX, $n = 25$; *L.b* CFS, $n = 26$; GAL4> UAS (20 °C): ABX, $n = 19$; *L.b* CFS, $n = 19$. **d**, UAS: ABX, $n = 26$; *L.b* CFS, $n = 18$; GAL4: ABX, $n = 36$; *L.b* CFS, $n = 24$; GAL4> UAS (27 °C): ABX, $n = 53$; *L.b* CFS, $n = 23$; GAL4> UAS (20 °C): ABX, $n = 21$; *L.b* CFS, $n = 7$. **e**, UAS: ABX, $n = 34$; *L.b* CFS, $n = 26$; GAL4: ABX, $n = 34$; *L.b* CFS, $n = 28$; GAL4> UAS (27 °C): ABX, $n = 10$; *L.b* CFS, $n = 17$; GAL4> UAS (20 °C): ABX, $n = 17$; *L.b* CFS, $n = 13$. **f**, UAS: ABX, $n = 36$; *L.b* CFS, $n = 30$; GAL4: ABX, $n = 40$; *L.b* CFS, $n = 31$; GAL4> UAS (27 °C): ABX, $n = 19$; *L.b* CFS, $n = 17$; GAL4> UAS (20 °C): ABX, $n = 14$; *L.b* CFS, $n = 8$. **g**, UAS: ABX, $n = 21$; *L.b* CFS, $n = 12$; GAL4: ABX, $n = 28$; *L.b* CFS, $n = 24$; GAL4> UAS (27 °C): ABX, $n = 24$; *L.b* CFS, $n = 20$; GAL4> UAS (20 °C): ABX, $n = 16$; *L.b* CFS, $n = 15$. **h**, UAS: ABX, $n = 31$; *L.b* CFS, $n = 20$; GAL4: ABX, $n = 31$; *L.b* CFS, $n = 29$; GAL4> UAS (27 °C): ABX, $n = 16$; *L.b* CFS, $n = 17$; GAL4> UAS (20 °C): ABX, $n = 18$; *L.b* CFS, $n = 14$. Box-and-whisker plots show median and IQR; whiskers show range. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Specific *P* values are in the Supplementary Information. Mann–Whitney *U* post hoc tests following a two-way ANOVA were used for statistical analysis. Data are representative of at least two independent trials for each experiment.



Extended Data Fig. 9 | Activation of octopaminergic neurons in flies carrying a null allele for Tβh (*Tβh*^{M18}). Average speed of flies previously treated with antibiotics and subsequently left untreated (ABX) or treated with *L.b* CFS for 3 days before testing. UAS: ABX, $n = 15$; *L.b* CFS, $n = 14$; GAL4: ABX, $n = 28$; *L.b* CFS, $n = 20$; GAL4> UAS (27 °C): ABX, $n = 11$; *L.b* CFS, $n = 13$; GAL4> UAS (20 °C): ABX, $n = 9$; *L.b* CFS, $n = 8$. Box-and-whisker plots show median and IQR; whiskers show range. Specific *P* values are in the Supplementary Information. Mann–Whitney *U* post hoc tests following a two-way ANOVA were used for statistical analysis. Data are representative of at least two independent trials.



Extended Data Fig. 10 | Octopamine mediates *L. brevis*- and Xi-induced changes in locomotion. **a**, Average speed of Ax and *L.b* CFS-treated Ax flies left untreated or supplemented with octopamine (OA, 10 mg/ml) or L-dopa (1 mg/ml) for 3 days. Ax, $n = 26$; Ax + OA, $n = 27$; Ax + L-dopa, $n = 6$; *L.b* CFS, $n = 35$; *L.b* CFS + OA, $n = 26$; *L.b* CFS + L-dopa, $n = 6$. **b**, RT–qPCR (mean ± s.e.m.) for transcripts from heads of Ax and *L.b* CFS-treated Ax flies. *Tdc2*: $n = 5$; *Tβh*, $n = 5$; *Ddc*: Ax, $n = 3$; *L.b* CFS, $n = 5$; *Tph*: $n = 7$. **c**, qRT–PCR (mean ± s.e.m.) for transcripts from heads of Ax or Xi*-treated Ax flies. Ax, $n = 5$ samples; Xi*, $n = 6$ samples. **d**, Average speed of Ax and *L.b* CFS-treated Ax flies left untreated or supplemented with tyramine (TA, 10 mg/ml) for 3 days. Ax, $n = 21$; Ax + TA, $n = 10$; *L.b* CFS, $n = 10$; *L.b* CFS+TA, $n = 9$. **e**, Average speed of control lines and flies expressing *DTI* in octopaminergic and tyraminergic neurons outside the ventral nerve cord. All flies were previously treated with antibiotics and subsequently left untreated (ABX) or treated with Xi* for 3 days before testing. GAL4;GAL80: Ax, $n = 25$; Xi*, $n = 18$; UAS: Ax, $n = 26$; Xi*, $n = 21$; GAL4> UAS: Ax, $n = 39$; Xi*, $n = 23$. **f**, Average speed of control lines and flies expressing *Tβh* RNAi in all neurons. All flies were previously treated with antibiotics and subsequently left untreated (ABX) or treated with *L.b* CFS for 3 days before testing. UAS: $n = 9$; GAL4: Ax, $n = 24$; *L.b* CFS, $n = 19$; GAL4> UAS: Ax, $n = 24$; *L.b* CFS, $n = 21$. **g**, *Tβh* mRNA measured from heads of flies previously treated with antibiotics. Error bars represent range. $n = 2$ samples per condition. **h**, Average speed of Ax and Xi*-treated Ax flies left untreated or supplemented with mianserin (Mian; 2 mg/ml) for 3 days. Ax, $n = 14$; Xi*, $n = 15$; Xi* + Mian, $n = 15$. **i**, Average speed of Conv, Ax and Xi*-treated Ax flies left untreated or supplemented with mianserin (2 mg/ml) for 3 days. Conv, $n = 13$; Ax, $n = 28$; Xi*, $n = 24$; Conv + Mian, $n = 27$; Ax + Mian, $n = 22$; Xi* + Mian, $n = 22$. **j**, Average speed of wild-type background (w+, Wt) and Tdc2-null mutants ($Tdc2^{RO54}$) either left untreated or after treatment with antibiotics for 3 days following eclosion. Wt Conv, $n = 13$; Wt ABX, $n = 21$; $Tdc2^{RO54}$ Conv, $n = 28$; $Tdc2^{RO54}$ ABX, $n = 34$. **k**, Average speed of wild-type background (Canton-S, Wt) and *Tβh*-null mutants ($Tβh^{M18}$) either left untreated or after treatment with antibiotics for 3 days following eclosion. Wt Conv, $n = 38$; Wt ABX, $n = 42$; $Tβh^{M18}$ Conv, $n = 25$; $Tβh^{M18}$ ABX, $n = 33$. **l**, Model of bacterial modulation of host locomotion. Box-and-whisker plots show median and IQR; whiskers show either 1.5 × IQR of the lower and upper quartiles or range. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Specific *P* values are in the Supplementary Information. Kruskal–Wallis and Dunn's (**a**, **d**, **h**, **i**), unpaired Student's *t*-test (**b**, **c**) or Mann–Whitney *U* (**e**, **f**, **j**, **k**) post hoc tests were used for statistical analysis. Data are representative of at least two independent trials for each experiment. *Tdc*, tyrosine decarboxylase; *Tβh*, tyramine beta-hydroxlyase; *Ddc*, DOPA decarboxylase; *Tph*, tryptophan hydroxylase.

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☐ Initial submission ☐ Revised version ☒ Final submission

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Experimental design

1. Sample size

Describe how sample size was determined.

Sample size was chosen based on preliminary experiments and literature in the field.

2. Data exclusions

Describe any data exclusions.

No data was excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All attempts at replication were successful under the experimental conditions defined.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

After eclosion, virgin female flies with the same genotype were sorted into groups of 10-15 flies per vial at random. All flies in each vial were administered with the same treatment regime. For each experiment, the experimental and control flies were collected, treated, and tested at the same time.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was not performed as almost all data acquisition and analysis was automated.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Daily activity measurements were taken using Drosophila activity monitors (DAMS, Trikinetics) and actograms were generated using the ActogramJ software. For video-assisted tracking experiments, Ethovision or the Caltech FlyTracker (http://www.vision.caltech.edu/Tools/FlyTracker/) software were used to process videos. Bout analysis was subsequently performed using simple custom python scripts, which are available upon request. Gait analysis was performed using the FlyWalker software package (http://biooptics.markalab.org/FlyWalker/). All statistical analysis was performed using Prism Software (GraphPad, version 7).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibodies were used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	No eukaryotic cell lines were used.
b. Describe the method of cell line authentication used.	No eukaryotic cell lines were used.
c. Report whether the cell lines were tested for mycoplasma contamination.	No eukaryotic cell lines were used.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	For our studies, we used the model organism, Drosophila melanogaster. Majority of the experiments used 7-day-old adult virgin female flies, except for Extended Data Figure 1b in which 7-day-old male flies were also used. The fly lines used include: Canton-S (#64349), Imd-/- (#55711), UAS-dTrpA1 (#26264), Tdc2-GAL4 (#52243), Tβh-GAL4 (#48332), Th-GAL4 (#8488), Ddc-GAL4 (#7009), Gad1-GAL4 (#51630), ChAT-GAL4 (#60317), Elav-GAL4 (#46655), UAS-TβhRNAi (#27667), UAS-DTI (#25039), and pBDPG4U-GAL4 (#68384) lines from Bloomington Drosophila Stock Center at Indiana University. Other fly stocks used were OregonR (kindly provided by A. A. Aravin and K. Fejes Tóth), TβHM18 (kindly provided by M. H. Dickinson) Tdc2R054, and tsh-GAL80 (kindly provided by D. J. Anderson). To minimize the effect of genetic background on behaviors, mutant fly lines were outcrossed for at least three generations onto a wild-type background.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	This study did not involve human research participants.