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A gut microbial factor modulates locomotor behaviour in *Drosophila*

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Sample Size and Statistics

Figure 1b – i

Analysis – (1b, 1i): Average speed calculated over the 10 min. testing period. (1d): Average bout length calculated over the 10 min. testing period. (1e): Average pause length calculated over the 10 min. testing period. (1f): Number of bouts over 10 min. testing period. (1g): Daily crossings sampled every min. per day. (1h): Stance linearity index, see ref. 64 for calculations

All are biological replicates assessed with a two-tailed test and data are representative of at least 3 independent trials for each experiment.

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
1b	Conv	1	36	Kruskal-Wallis test	1 v 2	0.0070
	Ax	2	36	(P < 0.0001)	1 v 3	0.0024
	L.p	3	35		1 v 4	0.9999
	L.b	4	36		2 v 3	0.9999
					2 v 4	0.0112
					3 v 4	0.0040
1d	Conv	1	32	Kruskal-Wallis test	1 v 2	0.0014
	Ax	2	36	(P = 0.0026)	1 v 3	0.2111
	L.p	3	22		1 v 4	0.9999
	L.b	4	20		2 v 3	0.9999
					2 v 4	0.2814
					3 v 4	0.9999
1e	Conv	1	32	Kruskal-Wallis test	1 v 2	< 0.0001
	Ax	2	36	(P < 0.0001)	1 v 3	0.0034
	L.p	3	22		1 v 4	0.9999
	L.b	4	20		2 v 3	0.9999
					2 v 4	0.0335
					3 v 4	0.3321
1f	Conv	1	32	Kruskal-Wallis test		
	Ax	2	36	(P = 0.6685)		
	L.p	3	22			
	L.b	4	20			
1g	Conv	1	8	Kruskal-Wallis test	1 v 2	0.0100
	Ax	2	8	(P = 0.0042)	1 v 3	0.1980
	L.p	3	8		1 v 4	0.9999
	L.b	4	8		2 v 3	0.9999
					2 v 4	0.0335
					3 v 4	0.4716
1h	Conv	1	6	Kruskal-Wallis test	1 v 2	0.0063
	Ax	2	7	(P = 0.0057)	1 v 3	0.8248
	L.p	3	5		1 v 4	0.9999
	L.b	4	5		2 v 3	0.6907
					2 v 4	0.0508
					3 v 4	0.9999

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
1i	Conv	1	25	Kruskal-Wallis test	1 v 2	0.2846
	Ax	2	29	(P = 0.0016)	1 v 3	0.0470
	L.p	3	24		1 v 4	0.9999
	L.b	4	35		2 v 3	0.9999
					2 v 4	0.0453
					3 v 4	0.0047

Figure 2a – f

Analysis – (2a – d, 2f): Average speed. (2e): Carbohydrate analysis of whole fly homogenates measured using HPAEC-PAD. Each sample contains 5 flies.

All are biological replicates assessed with a two-tailed test and data are representative of at least 2 independent trials for each experiment, except for Figure 2e in which one trial was performed.

Figure	Condition	Measurement	#	Sample Size	Statistical Test	Comparison	P-value
2a	Ax		1	57	Kruskal-Wallis	1 v 2	0.0004
	L.b		2	42	test	1 v 3	0.0294
	L.b CFS		3	36	(P = 0.0004)	1 v 4	0.9999
	L.b HK		4	24		2 v 3	0.9999
						2 v 4	0.1188
						3 v 4	0.8799
2b	Conv		1	17	Kruskal-Wallis	1 v 2	< 0.0001
	Ax		2	45	test	1 v 3	0.9999
	Xi*		3	29	(P < 0.0001)	2 v 3	< 0.0001
2c	Ax		1	31	Kruskal-Wallis	1 v 2	0.0253
	L.b CFS		2	12	test	1 v 3	0.0048
	Xi*		3	28	(P = 0.0014)	1 v 4	0.9999
	Ai*		4	13		2 v 3	0.9999
						2 v 4	0.2720
						3 v 4	0.2177
2d	Ax		1	28	Kruskal-Wallis	1 v 2	0.0002
	L.b		2	29	test	1 v 3	0.9056
	$L.b^{\Delta xylA}$		3	18	(P = 0.0003)	2 v 3	0.0424
2e	Ax	Total		5	Mann-Whitney		0.0079
	Xi*			5	test		
	Ax	Gluc		5	Mann-Whitney		0.5000
	Xi*			5	test		
	Ax	Fruc		5	Mann-Whitney		0.2222
	Xi*			5	test		
	Ax	Ribo		5	Mann-Whitney		0.0079
	Xi*			5	test		
	Ax	Treh		5	Mann-Whitney		0.0079
	Xi*			5	test		

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
2f	Ax	1	16	Kruskal-Wallis test	1 v 2	0.0465
	Xi*	2	18	(P = 0.0057)	1 v 3	0.8352
	Ax+Treh	3	16		1 v 4	0.9999
	Xi*+Treh	4	17		2 v 3	0.9999
					2 v 4	0.0090
					3 v 4	0.3024

Figure 3a – g

Analysis – (3a): Difference in average speed between flies calculated for each GAL4 line crossed with *UAS-dTRPA1* tested at 27˚C. Each point represents an independent trial, containing between 10 - 15 flies per group. Tdc2/Ddc/Th/ChAT, n = 5; Tβh, n = 6; Gad1, n = 3. For further details and graphs of each GAL4 line, see Extended Data Fig. 8. (3b – g): Average speed. For 3b and c, statistical significance was found for both variables (microbial status x genotype) calculated by Two-way ANOVA. In 3c, there is a significant interaction between microbial status and genotype (P = 0.0437). When examined separately, the Kruskal-Wallis test on only Conv flies across genotypes was significant (P = 0.0030) and the Kruskal-Wallis test on only ABX flies was not significant (P = 0.8426).
All are biological replicates assessed with a two-tailed test and data are representative of at least 2 independent trials for each experiment.

Figure	Condition	Genotype	Sample Size	Statistical Test	P-value
3b	ABX	*UAS-TrpA1*	15	Mann-	0.0139
	Xi*		12	Whitney test	
	ABX	*Tdc2-GAL4*	23	Mann-	0.0177
	Xi*		23	Whitney test	
	ABX	*GAL4>UAS*	14	Mann-	0.6308
	Xi*	*(27°C)*	12	Whitney test	
	ABX	*GAL4>UAS*	12	Mann-	0.0238
	Xi*	*(20°C)*	15	Whitney test	
3c	Conv	*UAS-TrpA1*	45	Mann-	0.0033
	ABX		30	Whitney test	
	Conv	*Tdc2-GAL4*	48	Mann-	0.0263
	ABX		39	Whitney test	
	Conv	*GAL4>UAS*	49	Mann-	0.4929
	ABX	*(27°C)*	39	Whitney test	
	Conv	*GAL4>UAS*	22	Mann-	0.0143
	ABX	*(20°C)*	31	Whitney test	

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
3d	Conv	1	13	Kruskal-Wallis test	1 v 2	0.0008
	Ax	2	33	(P < 0.0001)	1 v 3	0.9999
	Xi*	3	21		2 v 3	0.0015
	Conv+OA	4	29		4 v 5	0.9999
	Ax+OA	5	27		4 v 6	0.9999
	Xi*+OA	6	32		5 v 6	0.9999

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
3e	Ax	1	58	Kruskal-Wallis	1 v 2	0.9999
	Ax+OA	2	13	test	1 v 3	0.9999
	Ax+TA	3	10	(P = 0.0008)	1 v 4	0.0047
	Xi*	4	54		1 v 5	0.9999
	Xi*+OA	5	46		1 v 6	0.0351
	Xi*+TA	6	27		4 v 5	0.0980
					4 v 6	0.9999
					5 v 6	0.2695

Figure	Condition	Genotype	Sample Size	Statistical Test	P-value
3f	ABX	Wt (w+)	12	Mann-	0.0002
	Xi*		17	Whitney test	
	ABX	Tdc^{R023}	19	Mann-	0.4517
	Xi*		17	Whitney test	
3g	ABX	Wt (CS)	15	Mann-	0.0128
	Xi*		15	Whitney test	
	ABX	$T\beta h^{M18}$	11	Mann-	0.5254
	Xi*		12	Whitney test	

Extended Data Figure 1a – h

Analysis – (1a): Colony forming units. (1b – e, h): Average speed. (1f): Average speed calculated only within walking bouts. (1g): Tripod index: see ref. 64 for calculations.
All are biological replicates assessed with a two-tailed test.

Figure	Condition	Sample Size	Statistical Test	P-value
1a	*L.p*	15	Unpaired	0.6800
	L.b	18	Student's T-test	

Figure	Condition	Sex	#	Sample Size	Statistical Test	Comparison	P-value
1b	Conv	F	1	90	Kruskal-Wallis	1 v 2	< 0.0001
	Ax	F	2	92	test	1 v 3	0.0251
	L.b	F	3	89	(P < 0.0001)	2 v 3	< 0.0001
	Conv	M	4	100	Kruskal-Wallis	4 v 5	0.0669
	Ax	M	5	100	test	4 v 6	0.9999
	L.b	M	6	95	(P = 0.0302)	5 v 5	0.0664

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
1c	Ax	1	58	Kruskal-Wallis	1 v 2	0.0008
	L.b EW	2	57	test	1 v 3	< 0.0001
	L.b Bb14	3	57	(P < 0.0001)	2 v 3	0.9999
1d	Ax	1	45	Kruskal-Wallis	1 v 2	0.0463
	L.b EW	2	28	test	1 v 3	< 0.0001
	L.b P-2	3	42	(P < 0.0001)	2 v 3	0.0348
1e	Diet 1 Ax	1	20	Mann-Whitney	1 v 2	0.0006
	Diet 1 *L.b*	2	21	test		
	Diet 2 Ax	3	18	Mann-Whitney	3 v 4	0.0004
	Diet 2 *L.b*	4	16	test		
	Diet 3 Ax	5	6	Mann-Whitney	5 v 6	0.0411
	Diet 3 *L.b*	6	6	test		
1f	Conv	1	23	Kruskal-Wallis	1 v 4	0.9999
	Ax	2	35	test		
	L.p	3	22	(P = 0.1731)		
	L.b	4	22			
1g	Conv	1	6	Kruskal-Wallis	1 v 4	0.9999
	Ax	2	7	test		
	L.p	3	5	(P = 0.1358)		
	L.b	4	5			
1h	Ax	1	18	Kruskal-Wallis	1 v 2	0.9999
	L.p	2	24	test	1 v 3	0.0090
	L.b	3	24	(P < 0.0001)	1 v 4	< 0.0001
	L.p+*L.b*	4	24		2 v 3	0.2590
					2 v 4	0.0037
					4 v 5	0.9631

Extended Data Figure 2b – l

Analysis – (2b, e – g, i): Average speed. (2c, j): Average bout length. (2d, k): Average speed within walking bouts. (2l): Daily crossings.

All are biological replicates assessed with a two-tailed test.

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
2b	Ax	1	46	Kruskal-Wallis	1 v 2	0.0032
	L.b 0d	2	47	test	1 v 3	0.0096
	L.b 3-5d	3	43	(P = 0.0015)	2 v 3	0.9999
2c	Ax	1	18	Kruskal-Wallis	1 v 2	0.0295
	L.b 0d	2	18	test	1 v 3	0.0126
	L.b 3-5d	3	6	(P = 0.0041)	2 v 3	0.8985
2d	Ax	1	36	Kruskal-Wallis	1 v 2	0.0063
	L.b 0d	2	36	test	1 v 3	0.0019
	L.b 3-5d	3	12	(P = 0.0004)	2 v 3	0.6410

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
2e	Conv	1	11	Kruskal-Wallis	1 v 2	0.0127
	Ax	2	53	test	1 v 3	0.9999
	L.b 0d	3	53	(P = 0.0004)	1 v 4	0.9999
	L.b 3-5d	4	52		2 v 3	0.0065
					2 v 4	0.0056
					3 v 4	0.9999
2f	Conv	1	32	Kruskal-Wallis	1 v 2	0.0122
	Ax	2	36	test	1 v 3	0.0091
	ABX	3	36	(P = 0.0038)	2 v 3	0.9999
2g	Conv (OR)	1	20	Mann-Whitney	1 v 2	0.0265
	ABX (OR)	2	22	test		
	Conv (CS)	3	12	Mann-Whitney	3 v 4	0.0093
	ABX (CS)	4	17	test		
2i	ABX	1	29	Kruskal-Wallis	1 v 2	0.9999
	L.p	2	24	test	1 v 3	0.0203
	L.b	3	35	(P = 0.0011)	2 v 3	0.0019
2j	ABX	1	36	Kruskal-Wallis	1 v 2	0.9999
	L.p	2	30	test	1 v 3	0.0698
	L.b	3	35	(P = 0.0203)	2 v 3	0.0350
2k	ABX	1	42	Kruskal-Wallis	1 v 2	0.9999
	L.p	2	30	test	1 v 3	0.0607
	L.b	3	35	(P = 0.0079)	2 v 3	0.0094
2l	ABX	1	6	Kruskal-Wallis	1 v 2	0.8385
	L.p	2	6	test	1 v 3	0.0148
	L.b	3	6	(P = 0.0109)	2 v 3	0.2507

Extended Data Figure 3a – f

Analysis – (3a, b, f): Average speed. (3c): Average bout length. (3d): Average speed within walking bouts. (3e): Daily crossings.

All are biological replicates assessed with a two-tailed test.

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
3a	Ax	1	45	Kruskal-Wallis	1 v 2	0.9999
	L.p	2	17	test	1 v 3	0.3301
	L.b	3	42	(P = 0.01)	1 v 4	0.9999
	L.p CFS	4	17		1 v 5	0.0445
	L.b CFS	5	16		3 v 5	0.9999
					4 v 5	0.0906
3b	Ax	1	23	Kruskal-Wallis	1 v 2	0.1287
	L.p CFS	2	20	test	1 v 3	< 0.0001
	L.b CFS	3	20	(P < 0.0001)	2 v 3	0.0025

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
3c	Ax	1	23	Kruskal-Wallis	1 v 2	0.5833
	L.p CFS	2	20	test	1 v 3	0.0010
	L.b CFS	3	17	(P = 0.0016)	2 v 3	0.0696
3d	Ax	1	22	Kruskal-Wallis	1 v 2	0.0231
	L.p CFS	2	21	test	1 v 3	< 0.0001
	L.b CFS	3	17	(P < 0.0001)	2 v 3	0.0493
3e	Ax	1	8	Kruskal-Wallis	1 v 2	0.9999
	L.p CFS	2	8	test	1 v 3	0.2535
	L.b CFS	3	4	(P = 0.0469)	2 v 3	0.0472
3f	Ax	1	96	Kruskal-Wallis	1 v 2	0.0004
	L.b	2	88	test	1 v 3	0.9999
	0.1 Uracil	3	41	(P = 0.0009)	1 v 4	0.4509
	10 Uracil	4	18			

Extended Data Figure 4a – e

Analysis – (4a, b): Average speed. (4c): Expression of gene transcripts, samples from whole body homogenates. (4d): Amount ingested. (4e): Gut content.
All are biological replicates assessed with a two-tailed test.

Figure	Condition	Measurement	#	Sample Size	Statistical Test	Comparison	P-value
4a	Wt Conv		1	16	Mann-	1 v 2	0.0032
	Wt ABX		2	17	Whitney test		
	IMD Conv		3	24	Mann-	3 v 4	0.0168
	IMD ABX		4	25	Whitney test		
4b	Wt Conv		1	15	Mann-	1 v 2	0.0299
	Wt ABX		2	17	Whitney test		
	Ti Conv		3	10	Mann-	3 v 4	< 0.0001
	Ti ABX		4	11	Whitney test		
4c	Ax	*Dpt*	1	8	One-way		
	L.p CFS		2	10	ANOVA test		
	L.b CFS		3	10	(P = 0.8386)		
	Ax	*Drs*	4	10	One-way		
	L.p CFS		5	10	ANOVA test		
	L.b CFS		6	10	(P = 0.9441)		
	Ax	*Cec*	7	8	One-way		
	L.p CFS		8	10	ANOVA test		
	L.b CFS		9	10	(P = 0.5100)		
	Ax	*AttA*	10	5	One-way		
	L.p CFS		11	5	ANOVA test		
	L.b CFS		12	5	(P = 0.4687)		
	Ax	*Duox*	13	3	One-way		
	L.p CFS		14	5	ANOVA test		
	L.b CFS		15	5	(P = 0.6952)		

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
4d	Ax	1	6	Kruskal-Wallis		
	L.p CFS	2	5	test		
	L.b CFS	3	5	(P = 0.8626)		
4e	Conv	1	7	Kruskal-Wallis		
	Ax	2	13	test		
	L.p CFS	3	7	(P = 0.4236)		
	L.b CFS	4	10			

Extended Data Figure 5a – j

Analysis – (5a – f, h): Average speed. (5g): Daily crossings. (5i): Percent survival. Each sample consists of 15 – 25 flies. (5j): Percentage of apoptotic cells.
All are biological replicates assessed with a two-tailed test.

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
5a	Ax	1	18	Kruskal-Wallis	1 v 2	0.0566
	L.b CFS	2	18	test	1 v 3	0.9999
	+ Typ	3	17	(P = 0.0008)	1 v 4	0.0021
	- Typ	4	17		3 v 4	0.0181
5b	Ax	1	23	Kruskal-Wallis	1 v 2	0.0081
	L.b CFS	2	18	test	1 v 3	0.5168
	+PK	3	23	(P = 0.0037)	1 v 4	0.0139
	-PK	4	23		3 v 4	0.9999
5c	Ax	1	18	Kruskal-Wallis	1 v 2	0.0081
	L.b CFS	2	18	test	1 v 3	0.1867
	+100°C	3	18	(P = 0.0102)	2 v 3	0.7709
5d	Ax	1	30	Kruskal-Wallis	1 v 2	0.0170
	+amyl	2	17	test	1 v 3	0.0036
	-amyl	3	30	(P = 0.0016)	2 v 3	0.9999
5e	Ax	1	30	Kruskal-Wallis	1 v 2	< 0.0001
	L.b	2	30	test	1 v 3	0.2185
	L.p	3	29	(P < 0.0001)	1 v 4	0.9140
	A.p	4	30		1 v 5	< 0.0001
	E.c	5	18		2 v 3	0.0074
5f	Ax	1	65	Kruskal-Wallis	1 v 2	0.0183
	E.c	2	52	test	1 v 3	0.0003
	Δ*tyrA*	3	18	(P < 0.0001)	1 v 4	0.0300
	Δ*trpC*	4	17		1 v 5	< 0.0001
	Δ*manX*	5	45		1 v 6	< 0.0001
	Δ*treA*	6	46		1 v 7	0.9999
	Δ*xylA*	7	20			

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
5g	Conv	1	16	Kruskal-Wallis	1 v 2	0.0432
	Ax	2	24	test	2 v 3	0.0640
	L.b CFS	3	19	(P = 0.0027)	2 v 4	0.9999
	Δ*xylA* CFS	4	20		2 v 5	0.0134
	Xi	5	8			
5h	Ax	1	16	Kruskal-Wallis	1 v 2	0.0274
	L.b CFS	2	11	test	1 v 3	0.9999
	10 Xi	3	12	(P = 0.0167)	1 v 4	0.0263
	100 Xi	4	14			
5i	Ax	1	4	Kruskal-Wallis		
	L.p CFS	2	5	test		
	L.b CFS	3	5	(P = 0.4117)		
	Xi	4	4			
5j	Conv	1	7	Kruskal-Wallis	1 v 2	0.9999
	Ax	2	5	test	2 v 3	0.9999
	L.p CFS	3	4	(P = 0.0383)	2 v 4	0.0343
	L.b CFS	4	6		2 v 5	0.9999
	Xi	5	6			

Extended Data Figure 6a – b

Analysis – (6a, b): Sleep analysis.
All are biological replicates assessed with a two-tailed test.

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
6a Light Phase	Conv	1	8	Kruskal-Wallis	1 v 2	0.0193
	Ax	2	8	test	2 v 3	0.9999
	L.p	3	8	(P = 0.0036)	2 v 4	0.0193
	L.b	4	8			
6a Dark Phase	Conv	1	8	Kruskal-Wallis		
	Ax	2	8	test		
	L.p	3	8	(P = 0.0585)		
	L.b	4	8			
6b Light Phase	Conv	1	17	Kruskal-Wallis	1 v 2	0.0911
	Ax	2	25	test	2 v 3	0.4850
	L.p CFS	3	19	(P = 0.0314)	2 v 4	0.9999
	L.b CFS	4	21		2 v 5	0.0932
	Xi	5	8			
6b Dark Phase	Conv	1	17	Kruskal-Wallis		
	Ax	2	25	test		
	L.p CFS	3	19	(P = 0.1477)		
	L.b CFS	4	21			
	Xi	5	8			

Extended Data Figure 7a – k

Analysis – (7a – c, h – k): Average speed. (7d – e): Carbohydrate analysis measured using HPAEC-PAD. (7f – g): Trehalose levels in whole fly homogenate measured using a Megazyme Kit.
All are biological replicates assessed with a two-tailed test.

Figure	Condition	Measurement	#	Sample Size	Statistical Test	Comparison	P-value
7a	Ax		1	16	Kruskal-	1 v 2	0.0021
	Xi		2	13	Wallis test	1 v 3	0.1866
	+Fruc		3	13	(P = 0.0041)	1 v 4	0.1992
	+Gluc		4	15			
7b	Ax		1	26	Kruskal-	1 v 2	0.0026
	Xi		2	21	Wallis test	1 v 3	0.3686
	+Xylose		3	22	(P = 0.0048)	1 v 4	0.1780
	+Xylulose		4	18			
7c	Ax		1	21	Kruskal-	1 v 2	0.0003
	Xi		2	16	Wallis test	1 v 3	0.2565
	Xi+EDTA		3	18	(P = 0.0005)	2 v 3	0.0959
7d	Ax	Gluc	1	3	Mann-	1 v 2	0.1000
	Xi		2	3	Whitney test		
	Ax	Fruc	3	3	Mann-	3 v 4	0.4000
	Xi		4	3	Whitney test		
	Ax	Mann	5	3	Mann-	5 v 6	0.7000
	Xi		6	3	Whitney test		
	Ax	Xylu	7	3	Mann-	7 v 8	0.4000
	Xi		8	3	Whitney test		
	Ax	Treh	9	3	Mann-	9 v 10	0.1000
	Xi		10	3	Whitney test		
7e	Ax	Ribo	1	5	Kruskal-	1 v 2	0.0908
	Xi		2	5	Wallis test	1 v 3	0.0047
	Xi+EDTA		3	5	(P = 0.0004)	2 v 3	0.9601
	Ax	Treh	4	5	Kruskal-	4 v 5	0.0027
	Xi		5	5	Wallis test	4 v 6	0.1431
	Xi+EDTA		6	5	(P = 0.0001)	5 v 6	0.5373
7f	Conv	Treh	1	9	Kruskal-	1 v 2	0.0161
	Ax		2	6	Wallis test	1 v 3	0.9999
	Xi		3	3	(P = 0.0083)		
7g	Ax	Treh	1	15	Mann-	1 v 2	0.0059
	L.b		2	15	Whitney test		
7h	Ax		1	40	Kruskal-	1 v 2	0.0284
	Xi		2	40	Wallis test	1 v 3	0.9999
	Xi+Treh		3	39	(P = 0.0004)	1 v 4	0.0825
	Xi+Ara		4	18		2 v 3	0.0028
						3 v 4	0.0160

Figure	Condition	#	Sample Size	Statistical Test	Comparison	P-value
7i	Ax	1	29	Kruskal-Wallis test (P = 0.0286)	1 v 2	0.0350
	Xi	2	25		1 v 3	0.9999
	+Ribo	3	12			
7j	Conv	1	15	Kruskal-Wallis test (P = 0.0045)	1 v 2	0.0022
	Ax	2	22		1 v 3	0.0914
	Conv+Treh	3	18		3 v 4	0.9999
	Ax+Treh	4	15			
7k	Ax	1	27	Kruskal-Wallis test (P = 0.0499)	1 v 2	0.0361
	Xi	2	19		1 v 3	0.8516
	Xi+EDTA	3	24		1 v 4	0.9999
	Xi+Treh	4	19		1 v 5	0.9999
	Xi+EDTA+Treh	5	25			

Extended Data Figure 8b – h

Analysis – (8b – h): Average speed.

All are biological replicates assessed with a two-tailed test.

Figure	Condition	Genotype	Sample Size	Statistical Test	P-value
8b	ABX	*UAS-TrpA1*	15	Mann-Whitney test	0.0027
	L.b CFS		14		
	ABX	*pBDPG4U-GAL4*	24	Mann-Whitney test	0.0127
	L.b CFS		20		
	ABX	*GAL4>UAS (27°C)*	14	Mann-Whitney test	0.0456
	L.b CFS		9		
	ABX	*GAL4>UAS (20°C)*	16	Mann-Whitney test	0.0390
	L.b CFS		11		
8c	ABX	*UAS-TrpA1*	24	Mann-Whitney test	0.0008
	L.b CFS		24		
	ABX	*Tdc2-GAL4*	24	Mann-Whitney test	0.0252
	L.b CFS		23		
	ABX	*GAL4>UAS (27°C)*	25	Mann-Whitney test	0.4265
	L.b CFS		26		
	ABX	*GAL4>UAS (20°C)*	19	Mann-Whitney test	0.0233
	L.b CFS		19		
8d	ABX	*UAS-TrpA1*	26	Mann-Whitney test	0.0012
	L.b CFS		18		
	ABX	*Tβh-GAL4*	36	Mann-Whitney test	0.0003
	L.b CFS		24		
	ABX	*GAL4>UAS (27°C)*	53	Mann-Whitney test	0.5668
	L.b CFS		23		
	ABX	*GAL4>UAS (20°C)*	21	Mann-Whitney test	0.0006
	L.b CFS		7		

Figure	Condition	Genotype	Sample Size	Statistical Test	P-value
8e	ABX	*UAS-TrpA1*	34	Mann-Whitney test	0.0089
	L.b CFS		26		
	ABX	*Ddc-GAL4*	34	Mann-Whitney test	0.0157
	L.b CFS		28		
	ABX	*GAL4>UAS (27°C)*	10	Mann-Whitney test	< 0.0001
	L.b CFS		17		
	ABX	*GAL4>UAS (20°C)*	17	Mann-Whitney test	0.0004
	L.b CFS		13		
8f	ABX	*UAS-TrpA1*	36	Mann-Whitney test	0.0016
	L.b CFS		30		
	ABX	*Th-GAL4*	40	Mann-Whitney test	0.0041
	L.b CFS		31		
	ABX	*GAL4>UAS (27°C)*	19	Mann-Whitney test	< 0.0001
	L.b CFS		17		
	ABX	*GAL4>UAS (20°C)*	14	Mann-Whitney test	0.0103
	L.b CFS		8		
8g	ABX	*UAS-TrpA1*	21	Mann-Whitney test	0.0330
	L.b CFS		12		
	ABX	*Gad1-GAL4*	28	Mann-Whitney test	0.0120
	L.b CFS		24		
	ABX	*GAL4>UAS (27°C)*	24	Mann-Whitney test	0.0001
	L.b CFS		20		
	ABX	*GAL4>UAS (20°C)*	16	Mann-Whitney test	0.0135
	L.b CFS		15		
8h	ABX	*UAS-TrpA1*	31	Mann-Whitney test	0.0153
	L.b CFS		20		
	ABX	*ChAT-GAL4*	31	Mann-Whitney test	0.0179
	L.b CFS		29		
	ABX	*GAL4>UAS (27°C)*	16	Mann-Whitney test	0.0207
	L.b CFS		17		
	ABX	*GAL4>UAS (20°C)*	18	Mann-Whitney test	< 0.0001
	L.b CFS		14		

Extended Data Figure 9

Analysis – Average speed.

All are biological replicates assessed with a two-tailed test.

Figure	Condition	Genotype	Sample Size	Statistical Test	P-value
9	ABX	*UAS-TrpA1*	15	Mann-	0.3314
	L.b CFS	*Tβh*^{M18}	14	Whitney test	
	ABX	*Tβh-GAL4*	28	Mann-	0.2235
	L.b CFS	*Tβh*^{M18}	20	Whitney test	
	ABX	*GAL4>UAS*	11	Mann-	0.2284
	L.b CFS	*Tβh*^{M18} *(27°C)*	13	Whitney test	
	ABX	*GAL4>UAS*	9	Mann-	0.0745
	L.b CFS	*Tβh*^{M18} *(20°C)*	8	Whitney test	

Extended Data Figure 10a – k

Analysis – (10a, d, e, f, h – k): Average speed. (10b – c): Expression of gene transcripts, samples from head homogenates.

All are biological replicates assessed with a two-tailed test.

Figure	Condition	Measurement	#	Sample Size	Statistical Test	Comparison	P-value
10a	Ax		1	26	Kruskal-	1 v 2	0.9999
	Ax+OA		2	27	Wallis test	1 v 3	0.9999
	Ax+L-dopa		3	6	(P = 0.0004)	1 v 4	0.0012
	*L.b*CFS		4	35		1 v 5	0.9965
	*L.b*CFS+OA		5	26		1 v 6	0.0405
	*L.b*CFS+L-dopa		6	6			
10b	Ax	*Tdc*	1	5	Unpaired	1 v 2	0.0754
	L.b CFS		2	5	t-test		
	Ax	*Tβh*	3	5	Unpaired	3 v 4	0.0426
	L.b CFS		4	5	t-test		
	Ax	*Ddc*	5	3	Unpaired	5 v 6	0.6179
	L.b CFS		6	5	t-test		
	Ax	*Tph*	7	7	Unpaired	7 v 8	0.8425
	L.b CFS		8	7	t-test		
10c	Ax	*Tdc*	1	5	Unpaired	1 v 2	0.0122
	Xi		2	6	t-test		
	Ax	*Tβh*	3	5	Unpaired	3 v 4	0.0036
	Xi		4	6	t-test		
10d	Ax		1	21	Kruskal-	1 v 2	0.9999
	Ax+TA		2	10	Wallis test	1 v 3	0.0030
	L.b CFS		3	10	(P < 0.0001)	1 v 4	0.0019
	L.b CFS+TA		4	9		2 v 4	0.0296

Figure	Condition	Genotype	#	Sample Size	Statistical Test	Comparison	P-value
10e	Ax	*Tdc2-GAL4;*	1	25	Mann-Whitney test	1 v 2	0.0216
	Xi	*Tsh-GAL80*	2	18			
	Ax	*UAS-DTI*	3	26	Mann-Whitney test	3 v 4	0.0151
	Xi		4	21			
	Ax	*GAL4;GAL80*	5	39	Mann-Whitney test	5 v 6	0.3173
	Xi	*>UAS*	6	23			
10f	Ax	*UAS-TβhRNAi*	1	9	Mann-Whitney test	1 v 2	0.0106
	L.b CFS		2	9			
	Ax	*Elav-GAL4*	3	24	Mann-Whitney test	3 v 4	0.0040
	L.b CFS		4	19			
	Ax	*GAL4>UAS*	5	24	Mann-Whitney test	5 v 6	0.1703
	L.b CFS		6	21			
10h	Ax		1	14	Kruskal-Wallis test (P = 0.0010)	1 v 2	0.0007
	Xi		2	15		1 v 3	0.5040
	Xi+Mianserin		3	15		2 v 3	0.0593
10i	Conv		1	13	Kruskal-Wallis test (P = 0.0015)	1 v 2	0.0010
	Ax		2	28		1 v 3	0.9999
	Xi		3	24		2 v 3	0.0234
	Conv+Mian		4	27		4 v 5	0.9999
	Ax+Mian		5	22		4 v 6	0.9999
	Xi+Mian		6	22		5 v 6	0.9999
10j	Conv	Wt (w+)	1	13	Mann-Whitney test	1 v 2	0.0093
	ABX		2	21			
	Conv	Tdc^{R023}	3	28	Mann-Whitney test	3 v 4	0.6889
	ABX		4	34			
10k	Conv	Wt (CS)	1	38	Mann-Whitney test	1 v 2	0.0100
	ABX		2	42			
	Conv	$Tβh^{M18}$	3	25	Mann-Whitney test	3 v 4	0.7435
	ABX		4	33			