

Supplementary Materials

Microplastic exposure disturbs sleep structure, reduces lifespan, and decreases ovary size in *Drosophila melanogaster*

Wei Yan^{1,2,#}, Zi-Jie Li^{1,#}, Zi-Yi Lin^{1,2}, Shu-Qin Ji¹, William Ka Fai Tse³, Zhi-Qiang Meng^{1,2,4}, Chang Liu^{1,2,*}, Lei Li^{1,2,5,*}

¹Shenzhen-Hong Kong Institute of Brain Science, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518000, China

²CAS Key Laboratory of Brain Connectome and Manipulation, the Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518000, China

³Laboratory of Developmental Disorders and Toxicology, Center for Promotion of International Education and Research, Faculty of Agriculture, Kyushu University, Fukuoka, Fukuoka 819-0395, Japan

⁴Shenzhen Key Laboratory of Drug Addiction, the Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518000, China

⁵Guangdong Provincial Key Laboratory of Brain Connectome and Behavior, Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong 518000, China

#Authors contributed equally to this work

*Corresponding authors, E-mail: chang.liu3@siat.ac.cn; saralilei@siat.ac.cn

Supplementary Table S1. One-Way ANOVA analysis.

One-Way ANOVA	Figure	Test	DFn, DFd	F	P	Post-hoc Comparison	n1	n2	Mean Diff./Mean rank Diff.	Adjusted P-value	Summary	
Quantitative analysis of trypan blue-colored proportion of the gut section	Figure 2C	One-way ANOVA	2,15	1.03	0.0174	Control VS 1×MP	6	7	-26.4	0.0285	*	
						Control VS 10×MP	6	5	-29.95	0.0224	*	
Quantitative analysis of the survival rate at time points	Figure 3B	D 7	One-way ANOVA	2,21	2.364	0.1186	Control VS 1×MP	8	8	10.83	0.1947	ns
							Control VS 10×MP	8	8	12.5	0.1167	ns
	D 14	Kruskal-Wallis test	3,24	5.772	0.0558	Control VS 1×MP	8	8	5	0.3024	ns	
						Control VS 10×MP	8	8	8.313	0.0341	*	
	D 21	One-way ANOVA	2,21	4.286	0.0275	Control VS 1×MP	8	8	9.583	0.1862	ns	
						Control VS 10×MP	8	8	15.83	0.0169	*	
Quantitative analysis of changes in max episode length	Figure 4C	LP	One-way ANOVA	2,118	3.953	0.0218	Control VS 1×MP	37	41	-102.2	0.0133	*
							Control VS 10×MP	37	43	-37.5	0.6145	ns
	DP	Kruskal-Wallis test	3,121	2.845	0.2411	Control VS 1×MP	37	41	9.253	0.4892	ns	
						Control VS 10×MP	37	43	-3.271	>0.9999	ns	
Ovary size evaluation	Figure 6D	0 Week	One-way ANOVA	2,33	7.769	0.0017	Control VS 1×MP	12	12	-13 752	0.0587	ns
							Control VS 10×MP	12	12	9 944	0.2181	ns
	1 Week	One-way ANOVA	2,30	11.57	0.0002	Control VS 1×MP	10	11	49 389	0.0014	**	
						Control VS 10×MP	10	12	58 088	0.0002	***	
	2 Week	Kruskal-Wallis test	3,34	6.628	0.0364	Control VS 1×MP	10	12	6.617	0.2414	ns	
						Control VS 10×MP	10	12	10.95	0.0205	*	
	3 Week	One-way ANOVA	2,33	4.98	0.0129	Control VS 1×MP	12	12	34 420	0.009	**	
						Control VS 10×MP	12	12	9 239	0.838	ns	
4 Week	One-way ANOVA	2,33	8.32	0.0012	Control VS 1×MP	12	12	37 217	0.0005	***		
					Control VS 10×MP	12	12	17 351	0.1323	ns		
Female fecundity assay on fertilization ability or reproductive capacity	Figure 7C, D	Virgin flies	One-way ANOVA	2,45	1.233	0.301	Control VS 1×MP	16	16	5.454	0.269	ns
							Control VS 10×MP	16	16	3.901	0.563	ns
	Mated flies	One-way ANOVA	2,45	0.9201	0.4058	Control VS 1×MP	16	16	-6.381	0.4055	ns	
						Control VS 10×MP	16	16	-4.95	0.6427	ns	

Supplementary Table S2. T-test analysis.

T test		Test	Df	T/U	P-value	Summary	
Female fecundity assay during the development experiment	Figure 7B						
	D 1	Control VS 1×MP	Unpaired <i>t</i> test	10	$T=0.005525$	0.9957	ns
	D 2	Control VS 1×MP	Mann Whitney test	–	$U=17$	0.9372	ns
	D 3	Control VS 1×MP	Unpaired <i>t</i> test	10	$T=1.650$	0.13	ns
	D 4	Control VS 1×MP	Unpaired <i>t</i> test	10	$T=1.639$	0.1322	ns
	D 5	Control VS 1×MP	Unpaired <i>t</i> test	10	$T=0.5304$	0.6074	ns
	D 6	Control VS 1×MP	Unpaired <i>t</i> test	10	$T=0.3132$	0.7606	ns

–: Not available