Supplementary Information

Pharmacological rescue of isolation-induced social and neurochemical deficits in *Drosophila melanogaster*

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1 Supplementary Figures

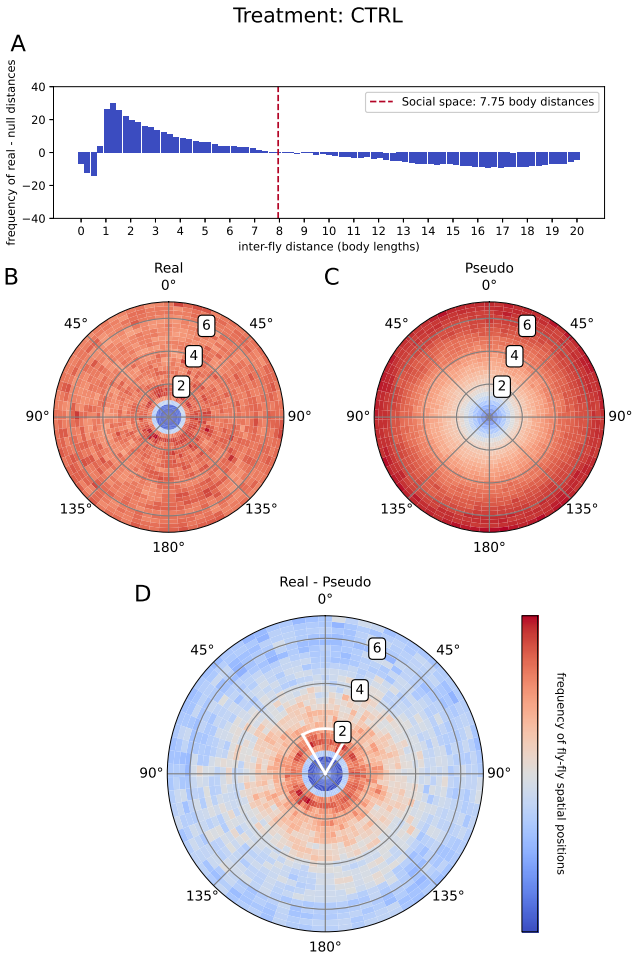

Figure S1: Analysis of social space and relative spatial positioning in the control (CTRL) group. A) Difference in the frequency of inter-individual distances between observed (real) and randomized (pseudo) fly distributions. Distances are expressed in body lengths. The red dashed line denotes the outer boundary of the group's social space, defined mathematically as the distance threshold (7.75 body lengths) where the subtracted real and pseudo frequency crosses zero (transitioning from spatial preference to random chance). B) Polar density heatmap depicting the spatial distribution (distance and angle) of neighboring flies relative to a focal fly centered at the origin (facing 0°) in the observed experimental data. C) Polar density heatmap of the pseudo (null) model, generated via randomized bootstrapping of fly positions. This represents the expected spatial distribution if flies were moving independently without social interactions. D) Difference polar heatmap (Real minus Pseudo) isolating the true social positioning preferences. Warm colors indicate spatial zones where flies accumulate significantly more often than expected by chance (attraction/preference), while cool colors indicate zones occupied less frequently than chance (avoidance). (If applicable, the white outlined sector represents the specific distance and angle criteria used to classify directed social interactions).

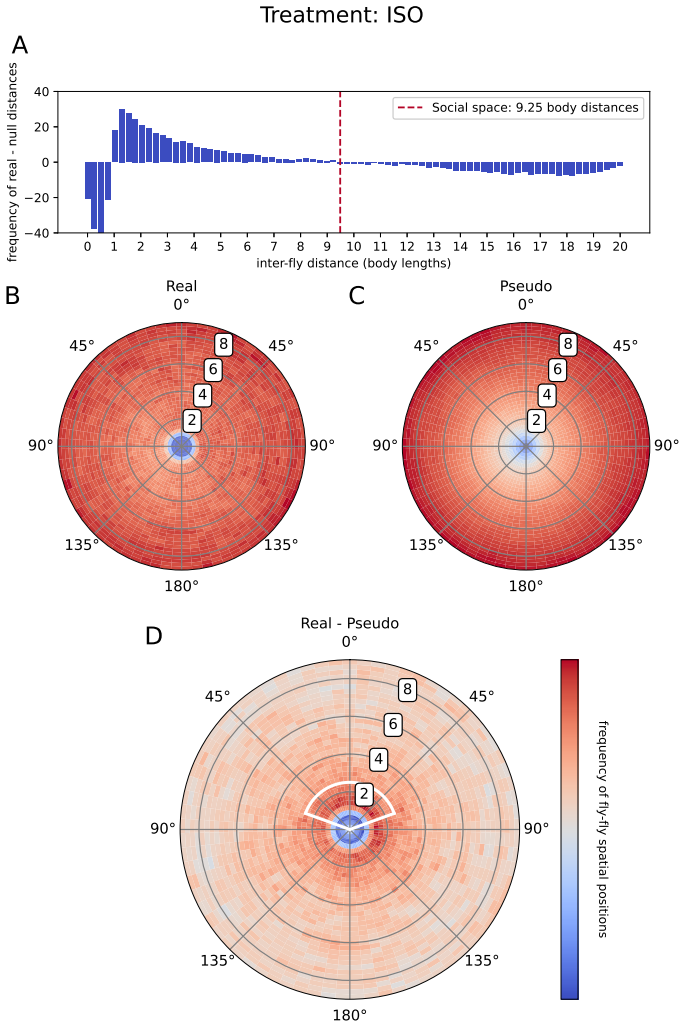

Figure S2: Analysis of social space and relative spatial positioning in the ISO treatment group. Plotting conventions and panel descriptions are identical to those described in Fig. S1. For flies treated with ISO, the outer boundary of the social space shifted to 9.25 body lengths. Compared to the CTRL group, flies exhibited an increased angle of interaction, indicating a broader angular distribution of neighboring individuals around the focal fly. This change is visible in the heatmap shown in the D) plot, which shows altered spatial preference patterns relative to the control condition.

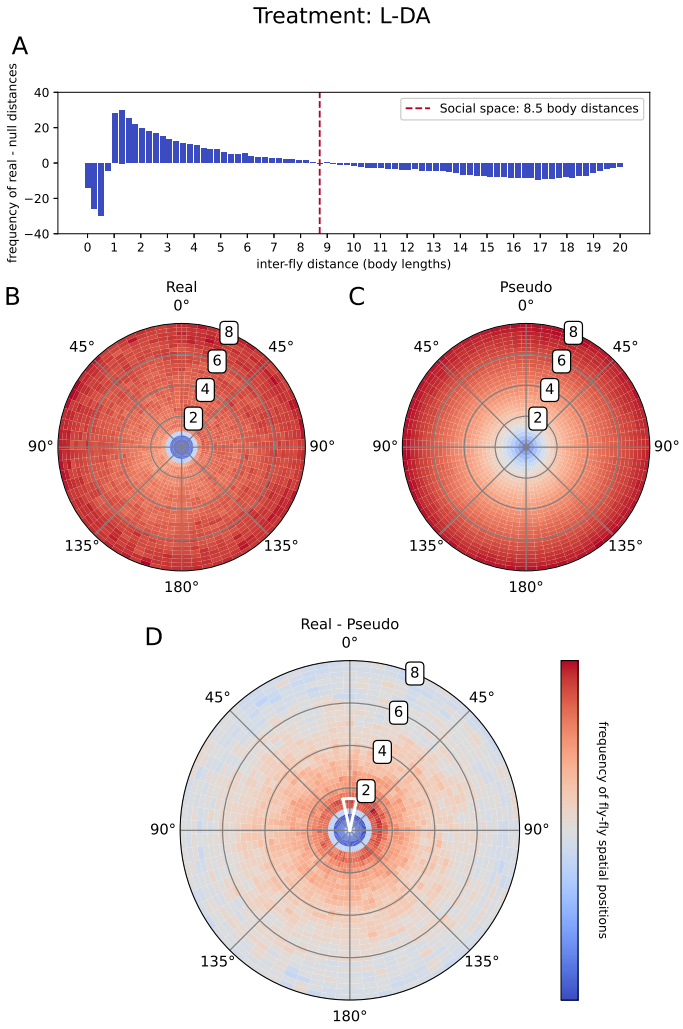

Figure S3: Analysis of social space and relative spatial positioning in the L-DA treatment group. Plots are generated as described in Figure 1. In the L-DA group, the social space boundary was established at 8.5 body lengths.

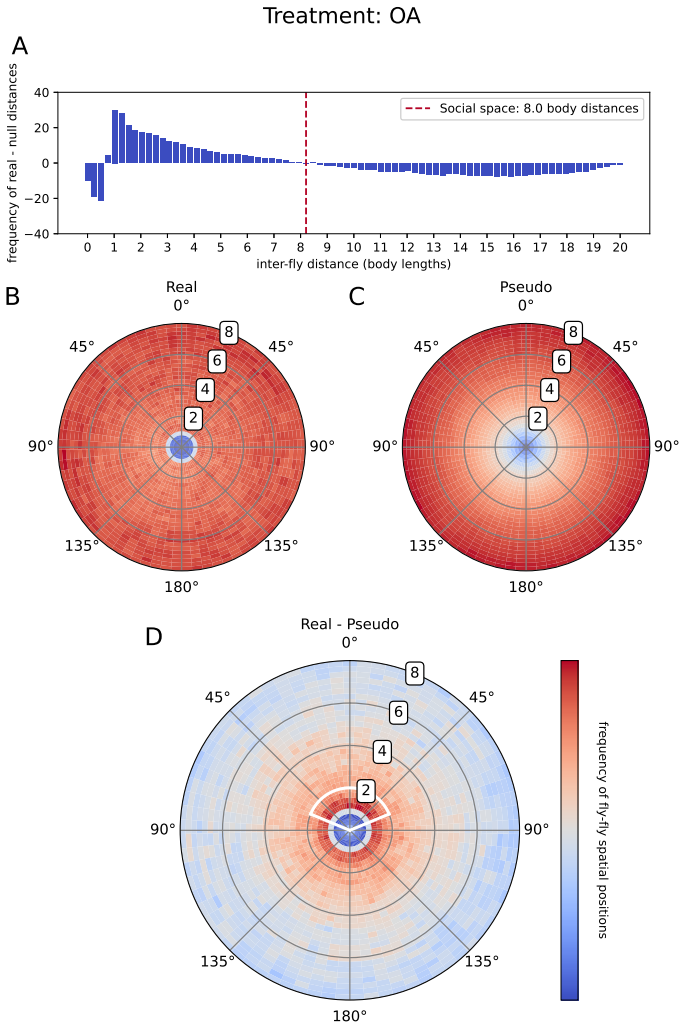

Figure S4: Analysis of social space and relative spatial positioning in the OA treatment group. Plots are generated as described in Figure 1. In the OA group, the social space boundary was established at 8 body lengths.

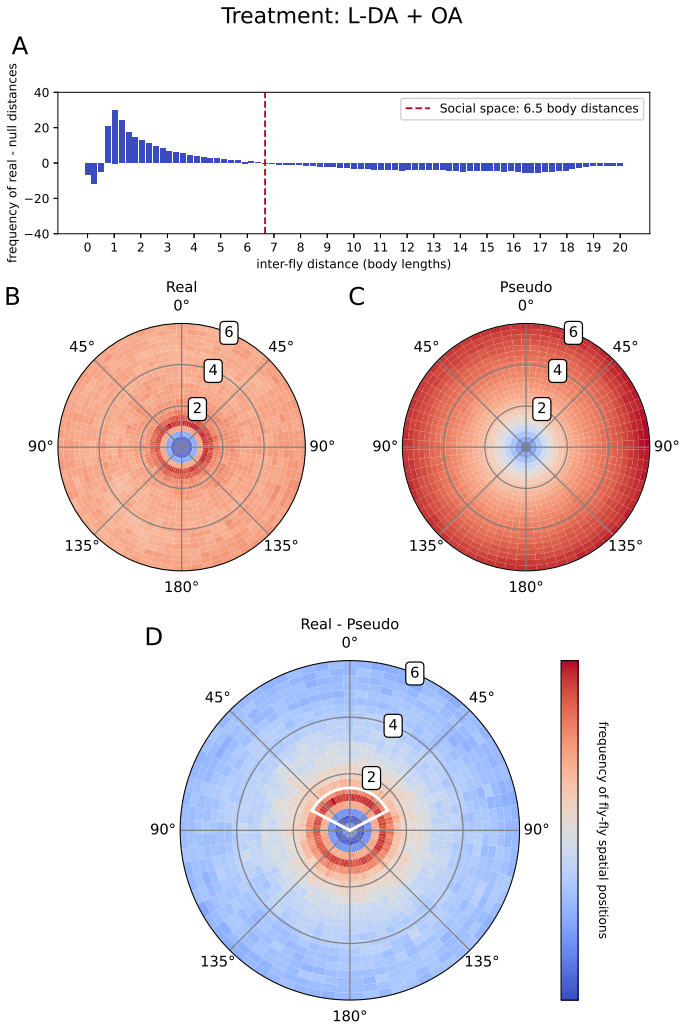

Figure S5: Analysis of social space and relative spatial positioning in the L-DA + OA treatment group. Plots are generated as described in Figure 1. In the L-DA + OA group, the social space boundary was established at 6.5 body lengths.

2 Supplementary Tables

Table S1: Comparison of experimental parameters with previous studies.

Parameter	Our method	Jezovit et al.[1]	Liu et al.[2]	Bentzur et al.[3]
Fly strain	Canton S	Canton S	Canton S	Canton S
Age when isolated	3–5 days	After eclosion	10 days	After eclosion
Length of isolation	5 days	3 days	1–6 days	3 days
Age when recorded	10 days	3 days	10–16 days	3–4 days
Number of flies in arena	12	12	16	10
Arena diameter	61 mm	60 mm	70 mm	245 mm
Movement restrictions	2D	2D	2D	2D
Arena height	3 mm	2 mm	3.5 mm	6 mm
Arena ceiling coated	Yes	Not specified	Yes	Thermally controlled
Transfer to arena	Aspiration	Aspiration	Cold anesthesia	Live transfer
Habituation in arena	15 min	15 min	1 min	1 min
Time of day recording	10:00 AM	Not specified	10:00–12:00 AM	Not specified
Recording duration	20 min	30 min	60 min	15 min
Recording conditions	Light	Dark	Dark	Dark or light
Tracking software	FlyTracker	Ctrax	FlyTracker	Ctrax
Data representation	Z-score	Z-score	Measurement values	Z-score

Table S2: MIDSNA Checklist.

Category	Parameter	Study-Specific Details
I. Experimental and Data Collection		
Experimental	Subject details	• **Species/Strain:** *Drosophila melanogaster*, Canton S (wild-type). • **Composition:** Groups of 12 all-male flies. • **Age:** 3–5 days old at start of treatment. • **Rearing:** Incubator at 25 °C, 70% humidity, 12:12 light–dark cycle.
Setup	Pre-experimental	Flies assigned to one of five conditions (5-day treatment): • **1. Control (CTRL):** Group-housed (12/vial), standard medium. • **2. Isolated (ISO):** Individual glass tubes, standard medium. • **3. ISO + L-DA:** Individual; medium + 5 mg/mL L-DOPA. • **4. ISO + OA:** Individual; medium + 10 mg/mL Octopamine. • **5. ISO + L-DA + OA:** Individual; medium + 5 mg/mL L-DOPA & 10 mg/mL Octopamine.
	Behavioral arena	• **Dimensions:** Circular arenas (61 mm diameter). • **Conditions:** Room temp, consistent lighting. Cleaned with water/ethanol between trials.
	Recording protocol	• **Habituation:** 15 min. **Duration:** 20 min recording. • **Time:** ≈ 10:00 AM (minimize circadian effects). • **Tracking:** FlyTracker[4] for trajectory extraction (manually verified).
II. Network Construction		
Interaction	Criteria method	**Fixed Criteria Approach.** Parameters manually set based on published criteria[5,6].
Definition	Parameters	Interaction defined if *all* three met: • **Distance:** < 2.5 body lengths. • **Angle:** Face-to-face angle $< 160°$. • **Time:** Duration ≥ 0.6 seconds.
	Aggregation model	**Static Network Model.** Aggregated over 20 min to create one Social Interaction Network (SIN) per replicate.
	Edge characteristics	**Weighted, Directed Edges.** From fly i to j: • **1. Count:** Total discrete interaction events. • **2. Duration:** Total time (seconds) spent interacting.
III. Core Analysis and Reporting		
Standardized	Core metrics set	• **Network-level:** Global Efficiency, Assortativity, Global Clustering Coefficient, Density. • **Node-level:** Betweenness Centrality, Closeness Centrality, Degree Centrality (Strength).
Metrics	Data availability	Scripts available on GitHub[7]. Edge lists/trajectories will be deposited in a public repository upon publication.

Table S3: LC–MS/MS parameters for neurotransmitter quantification.

Standard	**Pol**	**Prec.** (m/z)	**Frag.** (V)	**Prod.** (m/z)	**CE** (V)	**Slope** (a)	**Int.** (b)	$\mathbf{R^2}$	**Range** (μg/mL)
Dopamine	+	153.9	60	118.6 90.8	10 20	127063.16	70.39	0.9977	0.0001–1
Octopamine	+	153.9	60	136.9 90.8	4 24	602853.37	176.23	0.9977	0.0001–0.1
L-DOPA	+	197.7	70	180.9 151.8	4 8	20022.95	29.61	0.9940	0.0001–10
L-glutamine	+	147.2	70	129.8 83.9	4 14	50998.23	97.27	0.9916	0.0001–1
Tyramine	+	137.9	60	120.9 90.9 77.0	4 22 30	468141.16	330.99	0.9955	0.0001–1

3 Definitions and Mathematical Formulation of Network Metrics

Table S4: Complex network measures and their relevance in the *D. melanogaster* context.

Complex Network Measure	***D. melanogaster* Context**
Degree Centrality (D_c) is the node's degree k_i (number of connections) normalized by the maximum possible degree ($N-1$). $$D_c(i) = \frac{k_i}{N-1}$$	Indicates how socially connected a fly is. A high value suggests frequent interactions with many different flies.
Node Strength (Time) quantifies the cumulative duration of interactions for a node by summing the time-weights of all its edges. $$S_{\text{time}}(i) = \sum_{j \in N(i)} w_{\text{time}}(i,j)$$	Indicates the total time a fly spent interacting socially. High strength signifies prolonged social engagement.
Node Strength (Count) quantifies the total number of interactions for a node by summing the count-weights of all its edges. $$S_{\text{count}}(i) = \sum_{j \in N(i)} w_{\text{count}}(i,j)$$	Reflects how frequently a fly engages in social interactions. High strength means a high number of individual interaction events.
Closeness Centrality (C_c) is the reciprocal of the average shortest path distance from the node to all other nodes. $$C_c(i) = \frac{N-1}{\sum_{j \neq i} d(i,j)}$$	Measures how efficiently a fly can socially reach all other flies in the network. A high value indicates the fly is centrally located.
Clustering Coefficient (C_i) quantifies the degree to which a node's neighbors are connected to each other. $$C_i = \frac{2E_i}{k_i(k_i-1)}$$ *where E_i is the number of edges between neighbors of i, and k_i is degree.*	Indicates the cliquishness of a fly's social circle. A high coefficient suggests a fly is part of a tightly-knit group.
Betweenness Centrality (B_c) quantifies how often a node acts as a bridge along the shortest path between two others. $$B_c(i) = \sum_{j \neq k \neq i} \frac{\sigma_{jk}(i)}{\sigma_{jk}}$$ *where σ_{jk} is total shortest paths from j to k, and $\sigma_{jk}(i)$ are those passing through i.*	Identifies flies that act as crucial connectors between different social groups. A high value suggests the fly is important for maintaining social cohesion.

Table S5: Complex networks terminology and its relevance in the *D. melanogaster* context.

Complex Network Definition	***D. melanogaster* Context**
Node (vertex) is the basic element of the network, representing one object.	A single fruit fly.
Edge (link) represents a connection between two nodes.	A social interaction between two flies.
Network is a collection of nodes connected by edges.	All flies and their interactions from a single experiment.
Directed edge is a connection with a direction from one node to another.	A social interaction where the initiator is known (e.g., fly A approached fly B).
Edge weight is a numerical value assigned to an edge, indicating the strength or intensity of the connection.	A descriptive value, such as the duration of an interaction or the total number of interactions between two flies.
Time as edge weight is the total duration of interactions between two nodes.	The cumulative time two flies spent in social interaction during the experiment.
Count as edge weight is the total number of interactions between two nodes.	The number of times two flies had a social interaction during the experiment.
Global level analysis examines the overall structure and connectivity of the entire network.	Reveals broad patterns of social behavior and organization within the entire fly population.

Supplementary References

1. Jezovit, J.A., Alwash, N. & Levine, J.D. Using flies to understand social networks. *Front. Neural Circuits* **15**, 755093 (2021).

2. Liu, G. *et al.* A simple computer vision pipeline reveals the effects of isolation on social interaction dynamics in *Drosophila*. *PLoS Comput. Biol.* **14**, e1006410 (2018).

3. Bentzur, A. *et al.* Early life experience shapes male behavior and social networks in *Drosophila*. *Curr. Biol.* **31**, 486–501 (2021).

4. Eyjolfsdottir, E. *et al.* Detecting social actions of fruit flies in *Computer Vision – ECCV 2014: 13th European Conference, Zurich, Switzerland, September 6–12, 2014, Proceedings, Part II 13*. (Springer), pp. 772–787 (2014).

5. Wice, E.W. & Saltz, J.B. Selection on heritable social network positions is context-dependent in *Drosophila melanogaster*. *Nat. Commun.* **12**, 3357 (2021).

6. Petrovic, M. *Drosophila* social network analysis (2023).