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Dissociation of affective and neuroimmune recovery under plantscapes and behavioral load in a mouse depression model

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Abstract

Background

Rapid urbanization and reduced contact with natural settings are paralleled by rising rates of major depressive disorder (MDD). Naturalistic “plantscapes” have emerged as a promising, non-pharmacological intervention, yet their independent and combined effects with behavior remain unclear.

Methods

64 female mice were used in this study, which employed a 2×2×2 factorial design for an 8-week intervention. The variables included environment (plant landscape vs. white wall control), housing condition (group housing vs. Solitary housing), and activity condition (activity participation vs. non-participation). Weekly assessments included Open Field Test (OFT) and Sucrose Preference Test (SPT). Terminal endpoints included serum interleukin-1β (IL-1β) levels, ileal 16S rRNA profiling for α/β-diversity, and immunofluorescence staining to assess levels of brain-derived neurotrophic factor (BDNF) and ionized calcium-binding adapter molecule 1 (Iba1). Linear Mixed Models (LMMs) were used to analyze longitudinal behavioral data. Multivariable Linear Regression Models (MLRM) were employed to analyze terminal physiological outcomes and identify significant predictors.

Results

The intervention period had the largest impact on behavioral recovery in mice, explaining 49.6% and 43.3% of the variance in total distance traveled and sucrose preference, respectively (partial $\eta^2 = 0.496, 0.433$). Behavioral recovery exhibited a clear time dependent effect, with significant improvement mainly observed after Week 4 ($p < 0.05$). Both plantscape exposure and non-participation in activity were associated with higher locomotor distance and sucrose preference than the white-wall and activity-participation conditions, respectively (all $p \leq 0.003$). Environment and housing jointly explained 45.7% of the variance in IL-1β (model: $F = 7.861$, $p = 0.001$); group housing halved IL-1β relative to isolation ($p < 0.05$), and plantscape reduced levels by an additional 30–40% under solitary conditions. Plantscape increased Shannon and Chao-1 indices ($p < 0.05$) and segregated β-diversity from the control group, while the solitary enriched plantscape combination tripled the abundance of beneficial *Bacteroides* ($p = 0.002$).Activity is the only significant factor affecting BDNF levels ($t = 2.259$, $p = 0.035$), with the activity participation group showing significantly higher BDNF levels ($p = 0.049$).

Conclusions

The intervention period had the greatest impact on behavioral recovery, with improvements becoming most pronounced after Week 4. Both plantscape and non-activity also made significant contributions. Activity was a key factor in neurofunctional recovery, promoting neuroplasticity by increasing BDNF levels. Plantscape intervention supported neurobiological recovery by improving gut microbiota and reducing inflammation. Group housing and social interaction further enhanced immune regulation and emotional recovery. These findings suggest that eco-social interventions combining nature exposure and social interaction are effective strategies for treating depression.

1 Introduction

Major depressive disorder (MDD) has emerged as the leading cause of disability worldwide, paralleling rapid urbanization and diminishing everyday contact with nature. Urban growth therefore coincides with an alarming rise in depression prevalence. Chronic stress is a principal aetiological factor, and approximately 15% of patients attempt suicide [1]. Alarmingly, the incidence of depression is shifting toward increasingly younger cohorts [2]. Therapeutic landscape design, an emergent branch of landscape architecture, offers an environmental route to mitigate this trend. Meta-analyses show that regular exposure to green space lowers perceived stress, enhances mood, and strengthens psychological resilience [3, 4].

Two dominant theories, i.e. Kaplan’s Attention Restoration Theory (ART) and Ulrich’s Stress Reduction Theory (SRT), describe complementary pathways through which natural settings promote recovery. ART suggests that natural settings facilitate the restoration of directed attention, while SRT posits that such environments redirect focus away from stressors and promote emotional equilibrium [5, 6]. Convergent empirical evidence supports both frameworks, demonstrating robust psychophysiological benefits of nature exposure and its promise as a significant depression intervention. Nevertheless, reported effect sizes vary substantially across studies. Landscape typology appears to moderate outcomes, e.g. Deng et al. identified forests as most restorative, whereas Shu et al. reported superior affective benefits in open grasslands [7, 8]. Precise biological and behavioral mechanisms underlying these restorative effects remain poorly characterized. Evolutionary and neurophysiological studies are required to disentangle environment-health links across habitat types.

A further limitation is inconsistent exposure duration across trials. Most investigations rely on brief interventions that capture only transient mood shifts. For instance, Jin et al. used a 10-min park video, and Wang et al. delivered weekly 8-min VR sessions, both of which yielded short-lived gains [9, 10]. Such brevity precludes insight into dose-response relationships and sustained adaptation. Human trials are further confounded by uncontrolled lifestyle factors and heterogeneous personal attributes. These confounds erode internal validity, hamper long-term observation, and compromise translational guidance for real-world landscape design. Indoor “plantscapes” have therefore attracted interest as scalable, non-pharmacological interventions for depression and anxiety. Yet cognitive impairment and low motivation in clinical populations pose ethical and logistical hurdles to tightly controlled human experiments [11, 12].

Rodent models offer a tractable alternative. Mice, which share high genetic and neurobiological homology with humans, are ideal for controlled experiments [13, 14]. They permit rigorous variable control and longitudinal monitoring of depressive phenotypes, therapeutic response, and outcomes [15, 16]. Such

models enhance reproducibility and advance understanding of depression pathophysiology and environmental therapeutics [17, 18]. In pre-clinical depression research, the canonical non-pharmacological stimulus is the enriched environment (EE), cages equipped with running wheels, tunnels or rotating toys, have been shown to support neuroplasticity, enhance cognitive performance, and regulate emotional states in animal models [19]. In mice, EE attenuate anxiety and depression-like behaviors by down-regulating hippocampal inflammation and boosting neurotrophic signalling [20, 21]. Like plantscapes, these toys provide heightened sensory and exploratory stimulation; such cross-species parallels underscore the translational validity of rodent models for environmental therapy research [22, 23]. Like EE, behavioural context likewise modulates environmental benefits. Gehl categorized human outdoor behaviors into necessary, spontaneous, and social activities [24]. The latter two categories capture voluntary engagement and environmental preference [25, 26]. Exercise performed in natural settings yields larger affective gains than indoor equivalents [27]. Even solitary immersion in nature can evoke emotional benefits comparable to interpersonal connection [28]. These observations motivate systematic investigation of how behavioural context and environmental typology interact to affect depressive phenotypes.

Addressing this gap will inform evidence-based therapeutic landscape design. Here, we assess the independent and interactive effects of environment, social housing, and voluntary activity on psychological and physiological recovery in depressed mice. The 2 × 2 × 2 factorial design is carried out across environment (plantscape vs. white wall), housing (group vs. solitary), and activity (activity participation vs. non-participation). Our goal is to derive actionable principles for therapeutic landscape design. The study pursues three objectives: (1) Isolate key drivers of behavioral and inflammatory recovery; (2) Quantify recovery gains attributable to plantscape exposure; (3) Determine how voluntary activity and social context modulate these effects.

2 Methods and materials

2.1 Experimental animals and grouping

A cohort of 64 SPF female Kunming mice (6–8 weeks old, 18–22 g) were purchased from the Xi'an Jiaotong University Animal Center (license SCXK-Shaanxi-2018-001). All procedures were approved by the Northwest A&F University Ethics Committee and complied with the ARRIVE 2.0 guidelines. Animals acclimated for seven days in a controlled room (25 ± 1°C, 50 ± 5% relative humidity, 12 h light/dark), with autoclaved water and standard chow available ad libitum. Cages, bottles and corncob bedding were sanitized weekly. After acclimation, mice were block-randomized by body-weight into eight groups (n = 8) within a 2 × 2 × 2 factorial design that crossed environment (plantscape vs. white wall), housing condition (group housing vs. solitary housing), and activity condition (activity participation vs. non-participation).In the group housing condition, 4 mice were housed per cage, with 2 cages per group; in the solitary housing condition, 1 mouse was housed per cage, with 8 cages per group. All cages were placed in the designated environment type of their respective groups and subjected to the corresponding activity interventions. Enriched cages contained a stainless-steel running wheel and a rattan ball; sedentary cages lacked enrichment (Fig. 1,Table 1).

Table.1 Experimental Grouping

	Environmental Types	Housing Condition	Activity Condition
Group	Plantscape (P)	Solitary Housing (S)	Activity Participation (A)
	White wall (W)	Group Housing (G)	Non-Participation (N)
Note: PSA: n = 8 (8 cages), PGA: n = 8 (2 cages), PSN: n = 8 (8 cages), PGN: n = 8 (2 cages), WSA: n = 8 (8 cages), WGA: n = 8 (2 cages), WSN: n = 8 (8 cages), WGN: n = 8 (2 cages).			

2.2 Experimental procedures

2.2.1 Depression induction

Depression-like states were induced by daily intraperitoneal injections of reserpine (1.5 mg/kg; 14 d). Body mass was recorded every three days to titrate dosage and monitor health.

2.2.2 Environmental intervention

Two intervention rooms were constructed. The white-wall control presented only blank surfaces, eliminating external sensory cues (Fig. 2). The indoor plantscape (2.2 × 5.3 m) housed 30 green-leaf species (13 shrubs, 15 herbaceous plants and 2 climbers) selected for low volatility and shade tolerance (Fig. 3). Aromatic or flowering taxa were excluded. Vegetation was arranged to mimic natural stratification at murine scale. Both rooms were maintained under identical temperature and humidity and were physically isolated to prevent cross-contamination. Cages occupied distinct positions within each room and were rotated daily to equalize exposure. The exposure-testing cycle (see below) continued for 8 weeks.

Figure. 2 Different intervention environments and plant arrangement for indoor natural plantscape

2.2.3 Behavioral testing

All tests were conducted between 14:00 and 18:00 h by a single blinded experimenter.

Open-field test (OFT): Locomotor activity and anxiety-like behavior were recorded in a 50 × 50 × 50 cm acrylic arena. After 1 h room habituation, each mouse was placed in the center and video-tracked for 5 min; total distance travelled was the primary endpoint. The arena was sanitized with 75% ethanol between trials. OFT was performed weekly for nine sessions.

Sucrose-preference test (SPT): To quantify anhedonia, mice underwent 48 h of training with two bottles (1% sucrose vs. water) whose positions were alternated every 24 h. After 12 h water deprivation, a 12 h test determined volume intake; SPT was carried out weekly for nine sessions.

$$Sucrose\ Preference\ Index = \frac{Sucrose\ Intake}{Sucrose\ Intake + Pure\ Water\ Intake} \quad (1)$$

2.2.4 Physiological assessments

Each experimental group consisted of 8 mice. However, for terminal physiological sampling, only a subset of animals was selected. This was done to minimize the potential confounding effects of repeated sampling and ensure that the sample size remained sufficiently large for behavioral analysis. The selected animals were representative of the group, ensuring that biological variability was adequately captured while adhering to ethical guidelines for animal welfare. This approach was supplemented by the longitudinal behavioral data, which provided sufficient statistical power to assess the effects of the interventions.

(1)Inflammatory cytokine assay

At study termination, 0.2–0.3 mL of blood was collected via ocular puncture under isoflurane. Samples clotted for 2 h at room temperature and were centrifuged (3000 rpm, 15 min, 4°C). Serum was aliquoted and stored at -80°C. Interleukin-1β (IL-1β) was quantified in double replicate using a commercial ELISA kit.

(2)Gut microbiota profiling

Ileal contents were harvested for DNA extraction. V3-V4 16S-rRNA amplicons were generated with primers 341F/805R and sequenced on an Illumina MiSeq (Kunming Heke Biotechnology). α-Diversity (Shannon, Chao1) and β-diversity (Bray-Curtis, PCoA) were computed in QIIME 2 and R 4.3.1, and group differences were assessed by ANOSIM and PERMANOVA (999 permutations).

(3)Immunofluorescence staining

Dual-label immunofluorescence for BDNF and the microglial marker Iba1 was performed on 30-μm hippocampal sections (overnight primary incubation at 4°C, 1 h secondary at room temperature); nuclei were counterstained with DAPI and imaged under an epifluorescence microscope.

The entire experimental procedure is illustrated in Fig. 4.

2.3 Reagents and equipment

Reserpine stock solution (1000 mg/L in methanol, batch 2253016) was obtained from Shanghai Anpu Laboratory Technology Co. The OFT arena and automated video-tracking system were supplied by Shanghai Xinruan Information Technology Co.

2.4 Statistical analysis

Statistical analyses were performed in SPSS 27.0 and R 4.3.1. Data are expressed as mean ± SE. Longitudinal behavioral data from the open field test and the sucrose preference test were analyzed using Linear Mixed Models (LMM) to systematically evaluate the longitudinal effects of environmental type, activity condition, and housing condition on behavioral recovery in depressed mice. To account for repeated measures, group-housed mice are clustered within cages (2 cages/group); solitary-housed mice each constitute a single-mouse cage (8 cages/group). We therefore modeled Mouse ID as a random intercept, and additionally modeled Cage ID as a random intercept to account for potential clustering in group-housed conditions. Fixed effects included environment, activity, housing, intervention period, and their interactions. When significant fixed effects were detected, post hoc pairwise comparisons were conducted based on estimated marginal means to further identify the sources of significant main effects for environment type, activity condition, and intervention period. To control for multiplicity across pairwise tests, p values and confidence intervals were adjusted using the Sidak method (family-wise error rate $\alpha = 0.05$).

Terminal physiological outcomes were analyzed using Multivariable Linear Regression Models (MLR), with environment type, activity condition, and housing condition included as independent variables to identify significant predictors of physiological alterations. When the overall model was significant, post hoc analyses were subsequently performed to determine group-wise differences.The PERMANOVA (Permutational Multivariate Analysis of Variance) was used to assess the effect of the grouping factor on the Bray-Curtis dissimilarity matrix. The analysis was conducted using the adonis2 function in R, with 999 permutations. Graphs were generated in GraphPad Prism 10, and significance was set at $\alpha = 0.05$.

2.5 Ethics Statement

All animal experiments were conducted in accordance with the relevant guidelines and regulation and all procedures approved by the Institutional Animal Care and Use Committee (IACUC) of Northwest A&F University (IACUC 2024 – 1113).

3 Results

3.1 Psychological outcomes

3.1.1 Determinants of behavioral recovery

Longitudinal behavioral data were analyzed using linear mixed-effects models to examine the effects of environment, activity condition, housing condition, and intervention period on behavioral recovery. Using total distance traveled in the open field test as the outcome measure, the results showed significant main effects of environment and activity condition on total distance traveled ($p < 0.001$, Table. 2). A significant main effect of intervention period was also observed ($p < 0.001$,Table. 2), indicating a general increase in locomotor activity across the 8-week intervention period.

Effect size analyses further demonstrated that intervention period accounted for a large proportion of the variance in total distance traveled (partial η^2= 0.496,Table. 2), whereas activity condition exhibited a large effect (partial η^2=0.111), and environment showed a small effect (partial η^2= 0.044). In addition, no significant interactions were detected between intervention period and environment, activity condition, or housing condition ($p > 0.05$,Table. 2), suggesting that these interventions primarily enhanced overall locomotor activity rather than altering the temporal trajectory of behavioral recovery.

The sucrose preference test was evaluated weekly using the same linear mixed-effects modeling approach. The results revealed significant main effects of environment ($p = 0.003$,Table. 2) and activity condition ($p < 0.001$,Table. 2) on the sucrose preference index. Housing condition did not significantly affect sucrose preference ($p = 0.832$,Table. 2). A significant main effect of intervention period was also detected ($p < 0.001$), reflecting a gradual recovery of hedonic behavior over the course of the intervention. Effect size estimates indicated that intervention period exerted a large effect on sucrose preference (partial η^2= 0.433,Table.2), while activity condition also showed a large effect (partial η^2 = 0.155), and environment demonstrated a moderate effect (partial η^2= 0.082). In addition, no significant interactions were observed between intervention period and any intervention factors ($p > 0.05$), suggesting stable additive effects of environmental and activity interventions across the intervention period.

Table 2
Tests of Fixed Effects for Total Distance and Sucrose Preference Test(SPT)

Source	Numerator df	Denominator df		F		P		partial η^2	
		Total distance	SPT	Total distance	SPT	Total distance	SPT	Total distance	SPT
Intercept	1	319.738	102.262	3965.428	12152.689	< 0.001***	< 0.001***	0.925	0.992
Environment	1	319.738	102.262	14.628	9.173	< 0.001***	0.003**	0.044	0.082
Activity condition	1	319.738	102.262	40.083	18.745	< 0.001***	< 0.001***	0.111	0.155
Housing condition	1	319.738	102.262	1.481	0.045	0.225	0.832	0.005	< 0.001
Intervention period	8	319.815	103.512	39.269	9.878	< 0.001***	< 0.001***	0.496	0.433
Environment ×Activity condition	1	319.738	102.262	0.075	0.048	0.784	0.828	< 0.001	< 0.001
Environment ×Housing condition	1	319.738	102.262	0.002	0.06	0.962	0.807	< 0.001	0.001
Activity condition×Housing condition	1	319.738	102.262	0.686	0.072	0.408	0.789	0.002	0.001
Environment ×Intervention period	8	319.928	102.888	1.047	0.97	0.4	0.464	0.026	0.070
Activity condition× Intervention period	8	319.754	101.513	0.757	0.294	0.641	0.967	0.019	0.023
Housing condition× Intervention period	8	319.899	102.535	0.689	1.389	0.701	0.21	0.017	0.098
Environment ×Activity condition× Housing condition	1	319.738	102.262	1.602	0.004	0.207	0.948	0.005	< 0.001
*p < 0.05; **p < 0.01; ***p < 0.001									

3.1.2 Environmental modulation of recovery

Comparisons across different environments showed that mice housed in the landscape environment exhibited significantly greater total locomotor distance than those in the white-wall environment ($p < 0.001$,Table 2,Fig. 5a), indicating that landscape intervention markedly enhanced overall locomotor activity in depressed mice. In parallel, compared with the white-wall environment, the landscape environment also significantly increased the sucrose preference index ($p = 0.003$,Table 2,Fig. 5b).

3.1.3 Effect of voluntary activity

Comparisons based on activity condition revealed that mice in the non-activity group exhibited significantly greater total locomotor distance and a higher sucrose preference index than those in the activity group ($p < 0.001$,Table 2, Fig. 5c,Fig. 5d).

3.1.4 Intervention Period as the Primary Driver of Behavioral Recovery

Pairwise contrasts indicate that both locomotor distance and sucrose preference show minimal separation among Weeks 1–3(p>0.01,Table 3,Fig. 5e), followed by a marked improvement from Week 4 onward and a tendency to plateau during the late phase(p < 0.001,Table 3,Fig. 5e), consistent with a strong main effect of intervention period and the absence of period-by-intervention interactions in the mixed model.

Table.3 Comparison of Total Distance Traveled Between Different Intervention Period

(I) Intervention Period	(J) Intervention Period	Mean Difference (I–J)		Std. Error		df		P		95% CI of the Difference	
		Total distance	SPT	Total distance	SPT	Total distance	SPT	Total distance	SPT	Total distance	SPT
Baseline	Week 4	-10978.633	-9.082	960.13	2.513	320	104	<0.001***	0.017*	-14068.189.-7889.077	-17.32, -0.84
Week 1	Week 4	-4207.657	-9.166	960.13	2.513	320	104	0.001***	0.015*	-7297.212, -1118.101	-17.404,-0.9
Week 2	Week 4	-2408.721	-6.646	960.13	2.513	320	104	0.367	0.29	-5498.277,680.834	-14.884,1.59
Week 3	Week 4	-3151.170	-2.883	960.13	2.513	320	104	0.04*	1	-6240.726, -61.614	-11.121,5.35
*p < 0.05; **p < 0.01; ***p < 0.001											

3.2 Physiological Outcomes

3.2.1 Determinants of serum IL-1β

The multivariable regression model for serum IL-1β was highly significant (F = 7.861, p = 0.001, R^2 = 45.7%,Table 4). Both environmental context (p = 0.010,Table 4) and housing condition (p < 0.001,Table 4) emerged as independent predictors, whereas activity enrichment did not reach significance (p = 0.646,Table 4). The largest standardized β belonged to housing, indicating that social isolation is the dominant driver of systemic inflammation in this model.

Table 4
Linear Regression Analysis Results for Physiological Measurements

	Unstandardized Coefficients		Standardized coefficient	t	p	Collinearity Diagnosis	
	B	Standard Error	Beta			B	Standard Error
Constant	0.037	0.004	-	9.644	0.000***	-	-
Environment	0.011	0.004	0.385	2.768	0.010**	1.000	1.000
Housing Condition	-0.015	0.004	-0.552	-3.963	0.000***	1.000	1.000
Activity Condition	0.002	0.004	0.065	0.465	0.646	1.000	1.000
R^2	0.457						
Adjust R^2	0.399						
F	F (3,28) = 7.861,p = 0.001***						
D-W	1.780						
*p < 0.05; **p < 0.01; ***p < 0.001							

3.2.2 Environmental modulation of inflammation

Continuous exposure to the indoor plantscape markedly reduced circulating IL-1β relative to the white-wall control. The effect was most pronounced in solitary cohorts (SN and SA), where mean concentrations dropped by 32% (p = 0.033) and 41% (p = 0.001), respectively (Table 5,Fig. 6a). These data confirm that multisensory environmental enrichment can blunt the pro-inflammatory milieu associated with depressive phenotypes.

Table 5
Comparison of Interleukin-1β Levels in Mice under Different Environmental Interventions

Group		Mean Difference (P-W)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
SN	White Wall(W)	-0.016	0.007	0.033*	-0.03	-0.001
	Natural Plantscape(P)					
SA	White Wall(W)	-0.025	0.007	0.001***	-0.039	-0.011
	Natural Plantscape(P)					
GN	White Wall(W)	0.001	0.007	0.929	-0.014	0.015
	Natural Plantscape(P)					
GA	White Wall(W)	-0.003	0.007	0.695	-0.017	0.012
	Natural Plantscape(P)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different intervention environments (*p < 0.05; **p < 0.01; ***p < 0.001).

3.2.3 Markers impact of social housing

Across rooms and activity levels, group-housed mice exhibited consistently lower IL-1β than solitary counterparts. In the non-enriched white-wall (WN) and enriched white-wall (WA) groups, social housing halved IL-1β (p = 0.013 and p < 0.001; Table 6,Fig. 6b). Thus, affiliative contact exerts a robust anti-inflammatory effect that is additive to, but stronger than, environmental enrichment.

Table 6
Comparison of IL-1β Levels in Mice Under Different Housing Conditions

Group		Mean Difference (S-G)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
PN	Solitary Housing(S)	0.002	0.007	0.755	-0.012	0.017
	Group Housing(G)					
PA	Solitary Housing(S)	0.009	0.007	0.198	-0.005	0.024
	Group Housing(G)					
WN	Solitary Housing(S)	0.018	0.007	0.013*	0.004	0.033
	Group Housing(G)					
WA	Solitary Housing(S)	0.031	0.007	<0.001***	0.017	0.046
	Group Housing(G)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different Housing Condition (*p < 0.05; **p < 0.01; ***p < 0.001).

3.2.4 Gut-microbiota diversity

α-diversity mirrored the inflammatory profile. For the Shannon index, regression showed significant effects of environment (p = 0.032) and housing (p = 0.036) but not activity (p > 0.05; F = 3.409, p = 0.031;Table 7). Group-housed mice with non-participation (GN) displayed the largest gain in diversity (p = 0.011; Table 8,Fig. 6c), whereas solitary-enriched mice in plantscape (PA) retained higher diversity than their group-housed counterparts (p = 0.014; Table 9,Fig. 6d).

Table 7
Results of Linear Regression Analysis for Shannon Index

	Unstandardized Coefficients		Standardized coefficient	t	p	Collinearity Diagnosis	
	B	Standard Error	Beta			B	Standard Error
Constant	3.483	0.173	-	20.168	0.000***	-	-
Environment	0.389	0.173	0.364	2.253	0.032*	1.000	1.000
Housing Condition	-0.381	0.173	-0.357	-2.209	0.036*	1.000	1.000
Activity Condition	0.090	0.173	0.084	0.522	0.606	1.000	1.000
R^2	0.268						
Adjust R^2	0.189						
F	F (3,28) = 3.409,p = 0.031*						
D-W	1.602						
*p < 0.05; **p < 0.01; ***p < 0.001							

Table 8
Comparison of the Shannon Index of Mice Subjected to Various Environments

Group		Mean Difference (P-W)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
SN	White Wall(W)	0.455	0.328	0.178	-0.222	1.132
	Natural Plantscape(P)					
SA	White Wall(W)	0.43	0.328	0.202	-0.247	1.107
	Natural Plantscape(P)					
GN	White Wall(W)	0.902	0.328	0.011*	0.225	1.58
	Natural Plantscape(P)					
GA	White Wall(W)	-0.231	0.328	0.489	-0.908	0.447
	Natural Plantscape(P)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different intervention environments (*p < 0.05; **p < 0.01; ***p < 0.001).

Table 9
Comparison of the Shannon Index of Mice Subjected to Various Housing Condition

Group		Mean Difference (S-G)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
PN	Solitary Housing(S)	-0.002	0.328	0.994	-0.68	0.675
	Group Housing(G)					
PA	Solitary Housing(S)	0.872	0.328	0.014*	0.195	1.549
	Group Housing(G)					
WN	Solitary Housing(S)	0.445	0.328	0.188	-0.232	1.122
	Group Housing(G)					
WA	Solitary Housing(S)	0.211	0.328	0.526	-0.466	0.889
	Group Housing(G)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different Housing Condition (*p < 0.05; **p < 0.01; ***p < 0.001).

Chao-1 richness was driven predominantly by environment (p < 0.001) and, to a lesser extent, by activity (p = 0.022; F = 10.918, p < 0.001;Table 10). Plantscape exposure increased richness in SN, SA and GA cohorts (p = 0.018, 0.006 and 0.025; Table 11,Fig. 6e). No significant differences were observed between groups when activity was used as a variable (Fig. 6f).

Table 10
Results of Linear Regression Analysis for Chao-1 Index

	Unstandardized Coefficients		Standardized coefficient	t	p	Collinearity Diagnosis	
	B	Standard Error	Beta			B	Standard Error
Constant	838.512	17.155	-	48.877	0.000***	-	-
Environment	88.483	17.155	0.662	5.158	0.000***	1.000	1.000
Housing Condition	-8.445	17.155	-0.063	-0.492	0.626	1.000	1.000
Activity Condition	-41.708	17.155	-0.312	-2.431	0.022*	1.000	1.000
R^2	0.539						
Adjust R^2	0.490						
F	F (3,28) = 10.918,p = 0.000***						
D-W	1.581						

*p < 0.05; **p < 0.01; ***p < 0.001.

Table 11
Comparison of the Chao-1 Index of Mice Subjected to Various Environments

Group		Mean Difference (P-W)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
SN	White Wall(W)	91.824	36.155	0.018*	17.204	166.445
	Natural Plantscape(P)					
SA	White Wall(W)	109.504	36.155	0.006**	34.884	184.125
	Natural Plantscape(P)					
GN	White Wall(W)	65.923	36.155	0.081	-8.697	140.544
	Natural Plantscape(P)					
GA	White Wall(W)	86.681	36.155	0.025*	12.06	161.301
	Natural Plantscape(P)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different intervention environments (*p < 0.05; **p < 0.01; ***p < 0.001).

β-Diversity analysis (PCoA + ANOSIM) revealed a clear segregation between the control (C-WGN) and plantscape (E-PGN) clusters (R = 0.448, p = 0.03), signifying distinct microbial community structures when activity was restricted under group housing. Other group pairs showed substantial overlap, indicating fewer ecological differences (Fig. 7e). The PERMANOVA results indicate that the grouping factor did not have a statistically significant effect on the Bray-Curtis dissimilarity matrix (p = 0.125). The model explained 25.68% of the total variation (R^2 = 0.257), while 74.32% of the variation remained unexplained in the residuals(Table 12).

Table 12
PERMANOVA Results

Source	Df	Sum of Squares	R^2	F	Pr(> F)
Model	7	1.344	0.257	1.1845	0.125
Residual	24	3.890	0.743		
Total	31	5.233	1		

3.2.5 Gut-microbiota taxonomic composition

At the phylum level, Firmicutes and Bacteroidetes dominated (> 30% each) across all groups; Verrucomicrobiota exceeded 1% only in WSN, WSA, PGN, PSN and PSA (Fig. 7a;7b). Phylum-level relative abundances did not differ significantly. Genus-level profiling identified nine dominant taxa (> 1%, Fig. 7c; 7d), among which *Bacteroides* varied significantly among groups (p = 0.025;Table 13). Housing condition independently predicted *Bacteroides* abundance (p = 0.019;Table 14) : solitary- enriched mice (PA) exhibited a three-fold increase over group-enriched peers (p = 0.002; Table 15,Fig. 7f). This pattern suggests that the combination of isolation and plantscape enrichment may preferentially support beneficial, SCFA-producing genera linked to affective health.

Table 13
ANOVA Results for *Bacteroides* Relative Abundance

Between Groups	Sum of Squares	Degrees of Freedom	Mean Square	F	p
	220.942	**7**	**31.563**	**2.868**	**0.025***
Within Groups	264.145	24	11.006		
Total	485.088	31			
$p < 0.05$ ** $p < 0.01$*** $p < 0.001$					

Table 14
Results of Linear Regression Analysis for *Bacteroides* Relative Abundance

	Unstandardized Coefficients		Standardized coefficient	t	p	Collinearity Diagnosis	
	B	Standard Error	Beta			B	Standard Error
Constant	4.893	1.258	-	3.888	0.001***	-	-
Environment	0.321	1.258	0.041	0.255	0.801	1.000	1.000
Housing Condition	-3.141	1.258	-0.403	-2.496	0.019*	1.000	1.000
Activity Condition	2.517	1.258	0.323	2.000	0.055	1.000	1.000
R^2	0.269						
Adjust R^2	0.190						
F	F (3,28) = 3.431,p = 0.030*						
D-W	1.813						

$p < 0.05$ ** $p < 0.01$*** $p < 0.001$.

Table 15
Comparison of the *Bacteroides* Relative Abundance of Mice Subjected to Various Housing Condition

Group		Mean Difference (S-G)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
PN	Solitary Housing(S)	1.218	2.346	0.608	-3.624	6.059
	Group Housing(G)					
PA	Solitary Housing(S)	8.168	2.346	0.002**	3.327	13.01
	Group Housing(G)					
WN	Solitary Housing(S)	1.254	2.346	0.598	-3.588	6.096
	Group Housing(G)					
WA	Solitary Housing(S)	1.923	2.346	0.42	-2.919	6.764
	Group Housing(G)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different Housing Condition (*p < 0.05; **p < 0.01; ***p < 0.001).

3.2.6 Impact of Activity Factors on BDNF

The linear regression model showed that activity is the only significant factor influencing BDNF levels, suggesting that activity may affect neuroplasticity by promoting the expression of neurotrophic factors. Neither environment nor living condition had a significant effect on BDNF levels. The F-test results indicate that the model is significant overall (F = 3.111, p = 0.049; Table 16), meaning at least one independent variable affects BDNF. Specifically, activity (t = 2.259, p = 0.035; Table 16) was found to have a significant positive effect on BDNF levels. There was a significant difference between the activity participation and non-participation groups in the landscape group living environment (p = 0.049; Table 17, Fig. 9a), where the activity participation group exhibited higher BDNF levels than the non-participation group. The linear regression model with IBA1 as the dependent variable was not overall significant, and the main effects of each variable were also not significant (p>0.05).

Table 16
Results of Linear Regression Analysis for Average fluorescence intensity of BDNF staining

	Unstandardized Coefficients		Standardized coefficient	t	p	Collinearity Diagnosis	
	B	Standard Error	Beta			B	Standard Error
Constant	12.325	1.224	-	10.072	0.000***	-	-
Environment	-2.454	1.224	-0.37	-2.006	0.059	1	1
Housing Condition	-0.558	1.224	-0.084	-0.456	0.653	1	1
Activity Condition	2.764	1.224	0.417	2.259	0.035*	1	1
R^2	0.318						
Adjust R^2	0.216						
F	F (3, 20) = 3.111, p = 0.049*						
D-W	2.842						

*p < 0.05; **p < 0.01; ***p < 0.001.

Table 17
Comparison for Average fluorescence intensity of BDNF staining under Different Activity Interventions

Group		Mean Difference (N-A)	SE	Distinctiveness	95%CI of Difference	
					Lower Limit	Upper Limit
PS	Non-Participation(N)	-3.511	2.519	0.182	-8.852	1.83
	Activity Participation(A)					
PG	Non-Participation(N)	-5.372	2.519	0.049*	-10.713	-0.031
	Activity Participation(A)					
WS	Non-Participation(N)	-2.5	2.519	0.336	-7.841	2.841
	Activity Participation(A)					
WG	Non-Participation(N)	0.328	2.519	0.898	-5.013	5.669
	Activity Participation(A)					

Data are shown as the mean ± standard error. *Denotes statistical difference between the mice in different Activity Condition (*p < 0.05; **p < 0.01; ***p < 0.001).

4 Discussion

Our data showed that activity condition is a key factor in behavioral and neural recovery, with activity participation promoting neuroplasticity through increased BDNF levels, whereas non-participation in activity, rather than the mere presence of vegetation, dominated behavioral recovery. The results from different intervention periods show that behavioral recovery exhibits clear time dependent effects, with improvements becoming more pronounced and stable over time, supporting the importance of sustained intervention rather than short-term effects. Additionally, immersive plant landscapes provided extra benefits, especially under isolation conditions. Compared to activity and environment, social contact had a stronger effect on modulating inflammation, but had a more limited impact on emotional behavior. Overall, these findings emphasize the match between behavior, environment, and intervention duration, providing a more refined theoretical framework for understanding depressive recovery.

4.1 Psychological restoration

4.1.1 Relative weight of environment versus activity

Linear mixed effects models revealed that activity condition had a stronger impact on behavioral outcomes than environmental conditions, with larger effect sizes observed in both open-field and sucrose preference tests. This suggests that behavioral load plays a more decisive role in emotional recovery in reserpine-induced depressive mice, compared to environmental characteristics. While landscape intervention significantly improved locomotion and sucrose preference, its contribution was smaller than that of activity conditions. These findings indicate that environmental enrichment alone is insufficient for behavioral recovery, acting more as a supportive factor than a dominant driver of change[29, 30]. Our findings therefore support a “behavior first” hierarchy in which the opportunity for low demand engagement or rest dictates affective gain, with greenery providing an additive, but not overriding, contribution.

The superior performance of non-participation in activity mice suggests that, under chronic stress, additional motor or cognitive load can negate the benefits of activity. According to ART, natural scenes deliver effortless “soft fascination” that restores directed attention [31, 32]. When voluntary exercise is layered

onto an already complex setting, the combined attentional demand may exceed depressed animals' depleted cognitive resources, consistent with reports that interactive environments sometimes overload vulnerable individuals [32].

Importantly, the absence of significant interaction effects between environment and activity suggests that these factors operate largely independently, contributing additively to behavioral outcomes. Overall, these results emphasize that the effectiveness of environmental interventions depends on their compatibility with behavioral demands, favoring low load, restorative environments over activity intensive designs in depressive states.

4.1.2 Social housing and added value of plantscape immersion

Group housing did not enhance behavioral recovery, mirroring clinical observations that people with MDD often withdraw socially and can perceive group contexts as stressful[33]. Experimental isolation paradigms further show that both isolation and overcrowding can elevate anxiety in mice, implying a U-shaped social-density curve rather than a simple "more is better" effect [34]. Hence, social milieu must be titrated to the individual's affective tolerance.

Despite the overriding influence of activity condition, plantscape interventions still conferred significant behavioral gains, corroborating SRT's prediction of autonomic down regulation in visually rich natural settings[35, 36]. The enclosed, multi layered vegetation also fits Prospect-Refuge principles, which enhance perceived safety and exploratory choice [37]. Such spatial qualities may explain why the green room exceeded the white wall control even when activity was held constant. Comparable enhancements in exploratory drive have been documented after prolonged nature interventions in humans and in other murine depression models, supporting an evolutionarily conserved preference for biophilic cues [38]. The present data thus add to a growing body of evidence for cross species consistency in landscape perception and its restorative sequelae.

4.1.3 Cumulative Effects of Long Term Intervention on Behavioral Recovery in Depressive Mice

Behavioral recovery outcomes consistently showed significant main effects of the intervention period, indicating that behavioral improvements in depressive mice progressed with increasing intervention duration. Post hoc comparisons revealed a gradual improvement in behavioral measures over the course of the intervention, with the most pronounced changes emerging after several weeks of intervention. This pattern suggests that the benefits of environmental and activity interventions are not immediate but accumulate over time, supporting the notion that behavioral recovery is a progressive process requiring sustained intervention[39].

Interestingly, the lack of immediate effects during the early phase of intervention is consistent with findings that depressive-like behaviors, particularly anhedonia, require prolonged intervention to be effectively alleviated. Research on environmental enrichment and activity conditions suggests that while short-term interventions may improve certain aspects of depression, long term intervention is necessary to induce substantial changes in reward processing and behavior[39, 40]. Therefore, our findings highlight the critical importance of intervention duration in achieving meaningful recovery from depressive-like states, underscoring time and sustained intervention as key determinants of the effectiveness of depression-related treatment strategies.

4.2 Physiological therapy

4.2.1 Environment and housing: distinct routes to IL-1β control

Visual "soft fascination" reduces cognitive load and cortisol, priming the immune system for anti-inflammatory signalling [41]. Olfactory inputs reinforce this effect: monoterpenes such as limonene and α-pinene exhibit intrinsic neuro-protective and anti-inflammatory properties in vitro and in vivo [42]. Plants also emit negative air ions that modulate immune tone and reduce fatigue [43]. Together, these cues converge on NF-κB and other cytokine cascades, explaining the 30–40% IL-1β reduction we observed. However, quantifying dose-response relationships for each cue remains an open task. Group-housed mice showed about 50% lower IL-1β. Affiliative behaviors such as grooming dampen Hypothalamic-Pituitary-Adrenal (HPA) axis hyperactivity and blunt corticosterone spikes [44]. Parallel human studies link social support to faster surgical recovery and heightened safety perception, underscoring a conserved mechanism [45].

In short, serum IL-1β was shaped by both factors, but social isolation was the stronger driver, matching evidence that only two weeks of single housing markedly elevates neuro-inflammation in mice [46]. Housing alters locomotion, exploration, and aggression behaviors tightly coupled to fast neuro-immune signaling [46]. By contrast, green enrichment relies on slower sensory and neuroplastic pathways; enriched cages or forest bathing paradigms consistently down-regulate pro-inflammatory genes but require sustained exposure to reach maximal effect [41, 47]. Because the mental-health benefit of nature is often mediated by social ties, future trials should test eco-social double interventions that merge biophilic design with curated social spaces. Yet social overstimulation can divert attention from restorative scenery, so "social dose" must be calibrated to individual tolerance.

4.2.2 Plantscapes remodel gut-microbiota diversity

Exposure to the plantscape increased Shannon and Chao-1 indices, aligning with the biodiversity hypothesis, i.e. richer environmental microbiota seed a more resilient gut ecosystem [45, 48, 49]. White wall rooms mimic "microbial deprivation" typical of sealed indoor spaces, with attendant immunological risks [50]. Diversity gains coincided with enrichment of butyrate-producing taxa, e.g. *Lachnospiraceae, Butyricicoccus, Oscillospiraceae* etc.; butyrate potently inhibits microglial NF-κB activation and other inflammatory signals [51], linking microbiome changes to the observed drop in IL-1β. Housing exerted a countervailing effect: solitary mice displayed higher α-diversity, likely because reduced microbe transfer lowers competitive exclusion, allowing rare taxa to colonize [50]. Stable feeding and watering in single cages further support complex interaction networks.

Although phylum structure remained stable, solitary-plantscape mice tripled the abundance of *Bacteroides*, a key short-chain-fatty-acid (SCFA) producer linked to metabolic and mental health [52]. Social competition in group cages can elevate corticosterone and thin the mucus layer, restricting *Bacteroides* [44]. Targeted SCFA or *Bacteroides* supplementation may therefore benefit socially stressed populations, and exposure to plantscapes may promote the

production of these SCFAs and *Bacteroides*, potentially enhancing metabolic and gut microbiota resilience. There would summarize a clinical translation: combining low-arousal plantscapes with calibrated social contact offers a two-tier “green prescription” capable of reducing systemic inflammation, reshaping the microbiome, and ultimately easing depressive pathology.

4.2.3 Interaction of Activity, Environment, and Neuroimmune Responses

Activity conditions is the only significant factor influencing BDNF levels, suggesting that activity conditions may enhance neuroplasticity by promoting the expression of neurotrophic factors. However, the effects of environment and living conditions on BDNF were not found to be significant. This finding suggests that, although visual and emotional stimuli from the environment can provide a context for recovery, the actual neurobiological changes may depend more on the individual's active behaviors, particularly activity [53]. In existing literature, BDNF is considered a key factor in neuroplasticity and learning and memory, and activity is thought to promote brain adaptability and repair processes by increasing BDNF expression [54]. Specifically, activity can enhance the expression of neurotrophic factors, such as BDNF, in the hippocampus, improving neuronal connectivity and plasticity, thereby facilitating neural recovery [55].

Regarding neurobiological mechanisms, the effects of exercise may involve the upregulation of BDNF, a mechanism supported by multiple studies [53, 55]. Exercise activates the NR2B receptor, further promoting BDNF transcription and activating the downstream PI3K/Akt signaling pathway, ultimately fostering neuronal growth and survival [56]. Additionally, exercise may indirectly influence BDNF expression by reducing neuroinflammation. Depression patients typically exhibit elevated levels of neuroinflammation, and exercise helps regulate immune responses, reducing the production of inflammatory factors, thus maintaining the health of the nervous system [54].

However, our study found that not engaging in activities promoted better behavioral recovery. This phenomenon may reflect the balance between behavioral recovery and physiological recovery. The no activity group likely performed better in short-term behavioral tests due to lower inflammation and less physical exertion, while the activity group enhanced neurobiological recovery and adaptability by increasing BDNF levels, despite having poorer short-term behavioral performance [32]. This suggests that activity helps to enhance neuroplasticity by promoting the expression of neurotrophic factors, while behavioral recovery may be more influenced by immune responses, emotions, and short-term physical factors [57].

4.2.4 Gut-Brain Axis and Behavioral Regulation: The Interplay of Microbiota in Emotional Recovery

This study reveals how plant landscape interventions affect gut microbiota diversity, particularly through the increased abundance of Bacteroidetes phylum, which in turn influences SCFA levels[52]. SCFAs are known to play a crucial role in regulating neuroinflammation and behavioral performance. The gut microbiota interacts with the brain via the gut-brain axis, influencing the activity of the neuroimmune system, which is critical for mood and behavior regulation[50]. Specifically, butyrate is known to downregulate the NF-κB pathway in microglial cells, inhibiting neuroinflammatory responses, thereby regulating the brain's immune response and promoting brain recovery by enhancing neuroplasticity[54]. SCFAs enter the brain through the bloodstream, crossing the blood-brain barrier, suppressing the overactivation of microglial cells, reducing the production of pro-inflammatory factors, and lowering brain inflammation. Plant landscape interventions promoted gut microbiota diversity and enriched SCFA-producing bacteria, thereby reducing the concentrations of inflammatory factors, especially IL-1β, in both the blood and the brain. IL-1β not only damages neurons but also directly suppresses BDNF expression and signaling[44]. High levels of inflammation decrease BDNF levels in brain regions such as the hippocampus, inhibiting neurogenesis and synaptic plasticity, which are key mechanisms underlying cognitive and emotional disorders in depression.

Notably, this study observed significant differences in how different living conditions affected gut microbiota. Mice in the single-housing group exhibited higher gut microbiota diversity and greater sensitivity to the regulation of neuroimmune responses[54]. This finding aligns with existing studies suggesting a complex bidirectional feedback relationship between living density and microbiota composition changes. The social environment may influence the gut microbiota's structure and function by modulating microbial competition, which in turn affects behavior and emotional states.

Changes in the gut microbiota may regulate brain neurotransmitters and immune responses via gut-brain signaling pathways, directly influencing behavioral outcomes. SCFAs transmit signals to the brain through the vagus nerve, affecting the activity of the cortical areas, hippocampus, and prefrontal cortex, which are closely associated with emotional regulation, memory, and cognitive function[52]. Research has shown that SCFAs not only suppress neuroinflammation but also enhance the brain's antioxidant capacity and immune response, which is crucial for alleviating depressive symptoms and promoting behavioral recovery.

4.2.5 Limitations and Future Directions

Although the present 2 × 2 × 2 design provides a rare integrative look at behavior, inflammation, neuroplasticity and the microbiome, several caveats delimit its translational reach. First, the plantscape variable was treated as a binary “green vs. white” contrast; we neither manipulated canopy structure, spatial complexity nor species richness, each of which modulates psychological restoration and immune tone in humans [30, 35, 36]. A next step is a factorial vegetation study that orthogonally varies horizontal structure, vertical layering and species composition, thereby isolating the specific botanical cues that drive anti-depressive gain. Second, the “activity” factor was operationalized with only a running wheel and rattan ball; enrichment literature shows that the behavioral palette (tunnels, nesting, climbable meshes, multisensory objects) produces graded neuroplastic and affective responses [43, 55]. Future work should employ a stimulus library that spans aerobic, cognitive and socially facilitated play, and report individual usage logs to separate mere provision from actual engagement. Addressing these gaps will clarify dose–response relationships and enable precision “green prescriptions” that couple designable botanical parameters with tailored social and behavioral programmes.

5 Conclusion

This study reveals that in the reserpine-induced depressive mouse model, the primary driver of emotional recovery is behavioral load, rather than simple environmental intervention. Although plant landscape intervention contributes to reducing systemic inflammation by enhancing gut microbiome diversity and promoting SCFA production, the core of emotional and behavioral recovery lies in activity, especially its role in promoting neuroplasticity. Activity participation showed a significant advantage in increasing BDNF levels, whereas the non-activity group performed better in behavioral tests, suggesting that behavioral recovery and neurobiological recovery are regulated by different mechanisms and do not always synchronize. Changes in living environment also significantly affect immune responses. Cohabitation significantly reduced IL-1β levels, suggesting that social contact influences neuroimmune responses via the gut-brain axis, facilitating emotional recovery. Moreover, the results of different intervention periods indicated that behavioral recovery was time dependent, with the most pronounced improvements observed after several weeks of sustained intervention. This emphasizes that prolonged exposure to interventions is essential for significant behavioral and emotional recovery, and supports the notion that recovery is a progressive and cumulative process.

In conclusion, this study advocates for a dual "green prescription" strategy to promote recovery in patients with depression, combining natural plant landscapes with individualized social interactions. Specifically, low-intensity natural landscapes can serve as passive recovery spaces, helping to stabilize mood and create favorable conditions for neuroplasticity by improving gut microbiome and reducing inflammation. Meanwhile, moderate social interactions, by optimizing the intensity and frequency of contact, can further promote emotional recovery.

Declarations

Declaration of interests

The authors declare that they have no competing interests.

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Author Contribution

Conceptualization, L.Q. and T.G.; methodology, L.Q.,T.Z. and Y.P.; validation, L.Q., T.Z., Y.P. and T.G.; formal analysis, L.Q.,T.Z. and Y.P.; investigation, T.Z.,Y.P.,T.H.,F.L.,J.L.,Y.M and K.M.; resources, L.Q. and T.G.; data curation, T.Z. and Y.P.; Writing—Original draft preparation, T.Z. and F.L.; Writing—Review and editing, T.G; visualization, T.Z. and F.L.; supervision, L.Q. and T.G.; project administration, L.Q. and T.G.; funding acquisition, L.Q. and T.G. All authors have read and agreed to the published version of the manuscript.

Data Availability

The datasets generated and/or analysed during the current study are available in the NCBI BioProject repository under accession number PRJNA1435878. The raw 16S rRNA sequencing data will be publicly accessible from March 12, 2026, via the following link: https://www.ncbi.nlm.nih.gov/sra/PRJNA1435878

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Figures

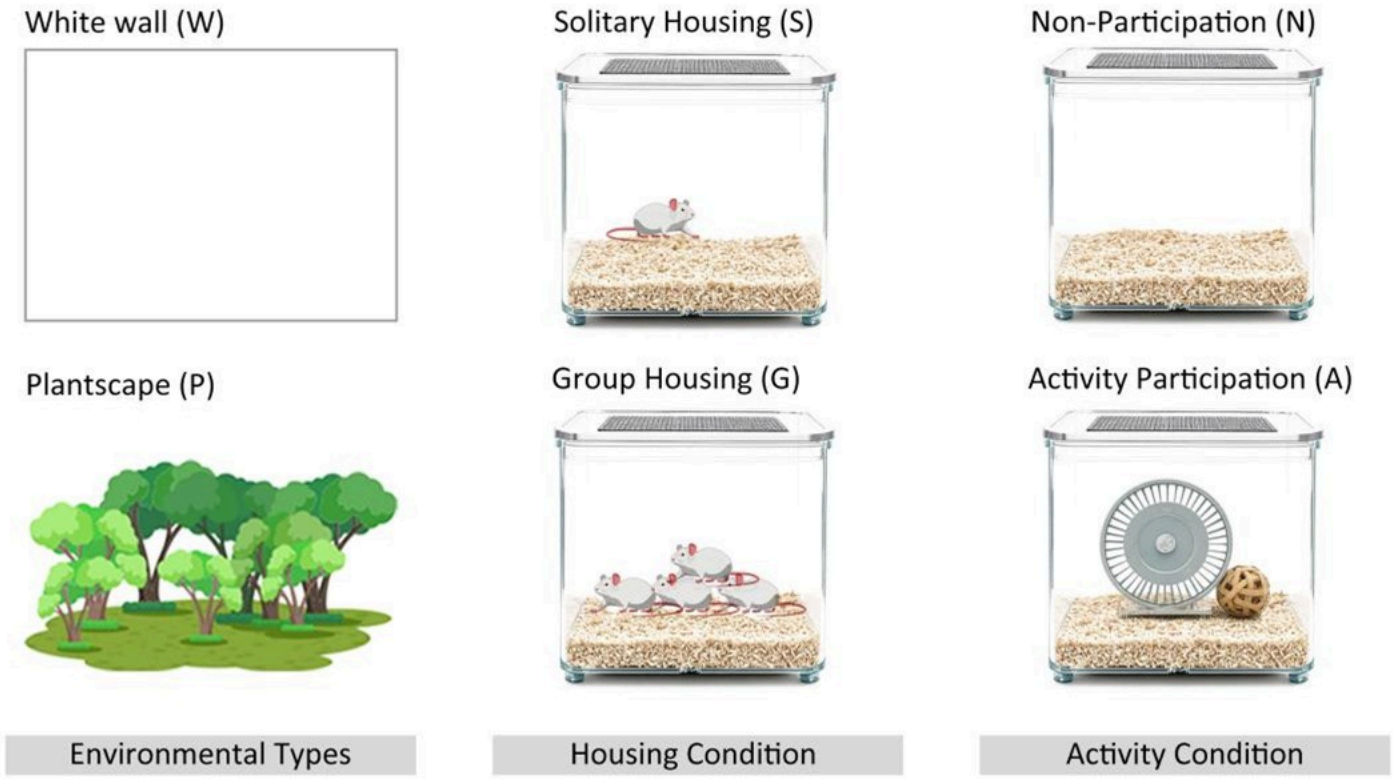


Figure 1

Experimental grouping.

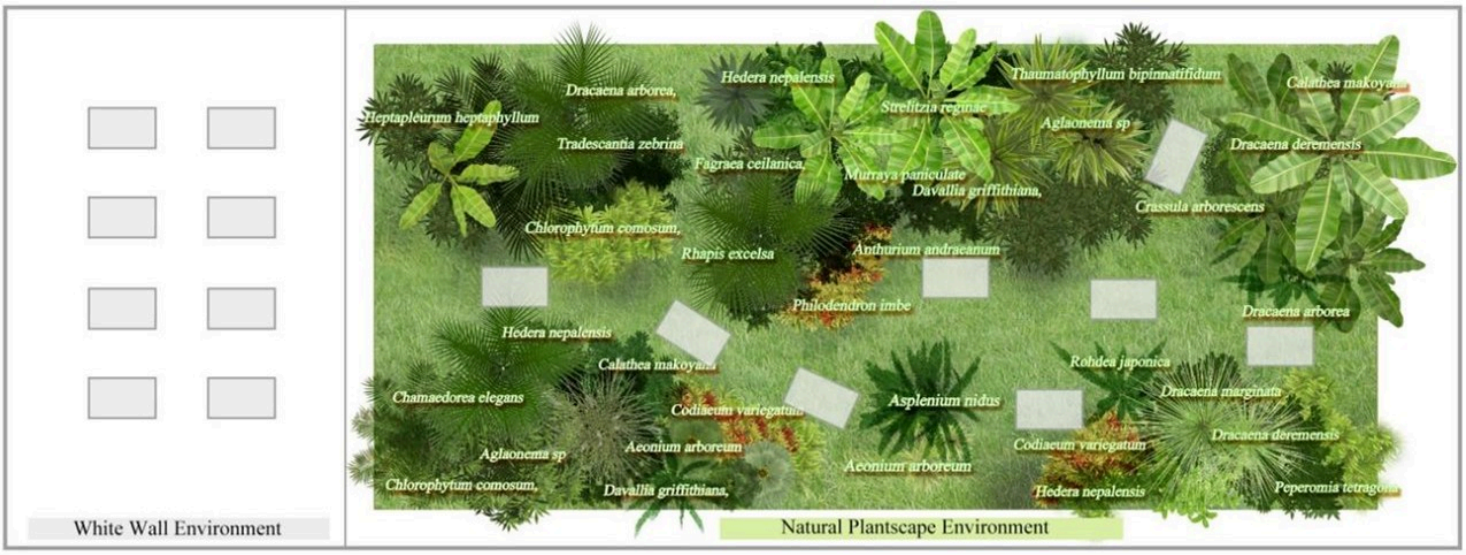


Figure 2

Different intervention environments and plant arrangement for indoor natural plantscape



Figure 3

The indoor plantscape: an overall and detailed view

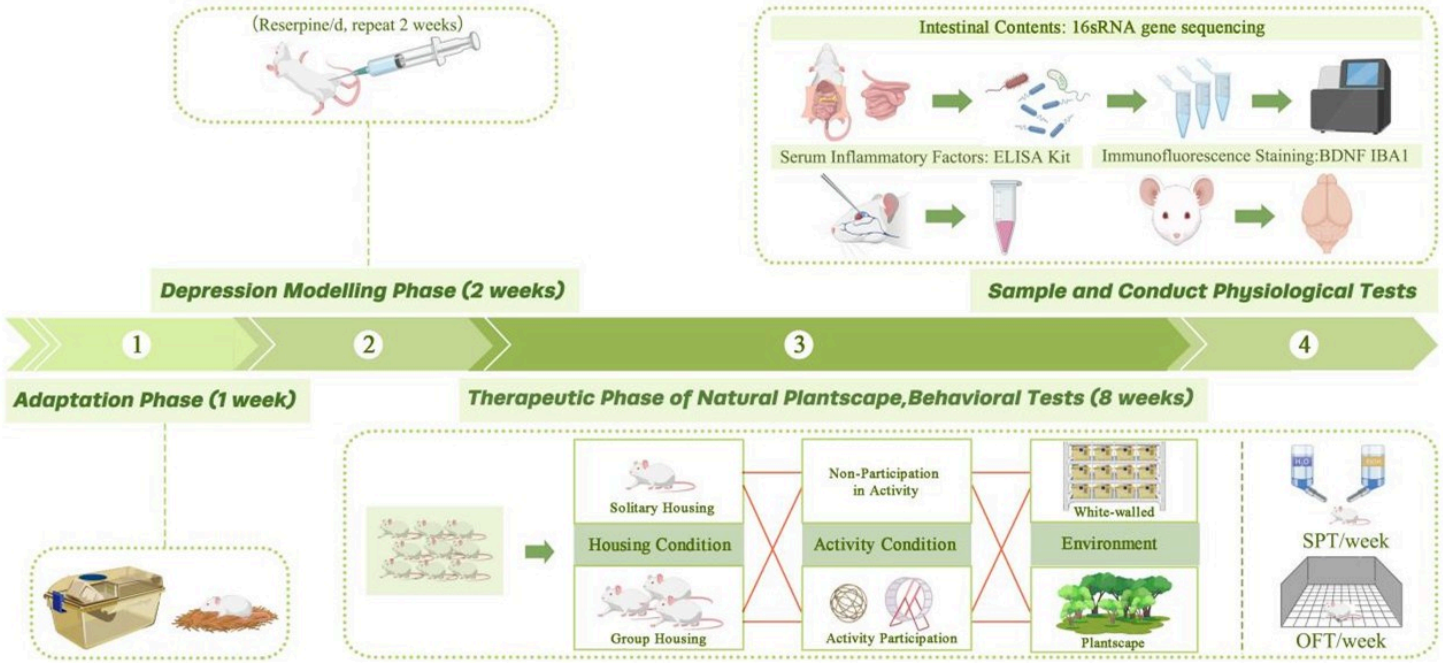


Figure 4

Experimental flowchart

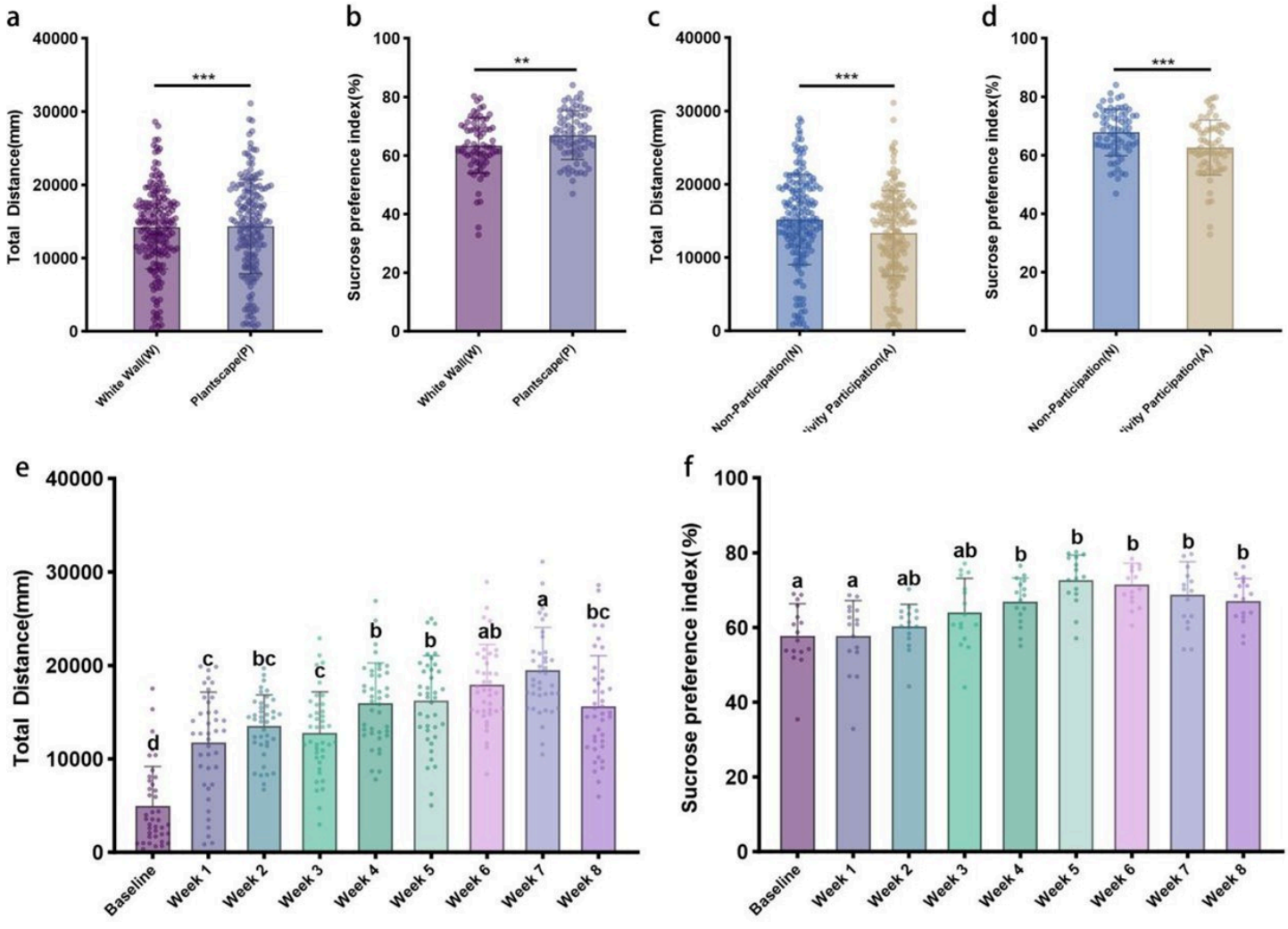


Figure 5

Psychological outcomes differences in mice under different environments, activity conditions, and intervention periods. a. Comparison of total distance traveled between different environments; b. Comparison of sucrose preference test between different environments; c. Comparison of total distance traveled between different activity conditions; d. Comparison of sucrose preference test between different activity conditions; e. Comparison of total distance traveled between different intervention period; f. Comparison of sucrose preference test between different intervention period. Data are shown as the mean ± standard error.*p<0.05; **p<0.01; ***p<0.001. Different lowercase letters (a-d) indicate statistically significant differences between groups or time points ($p < 0.05$), whereas the same letter indicates no significant difference.

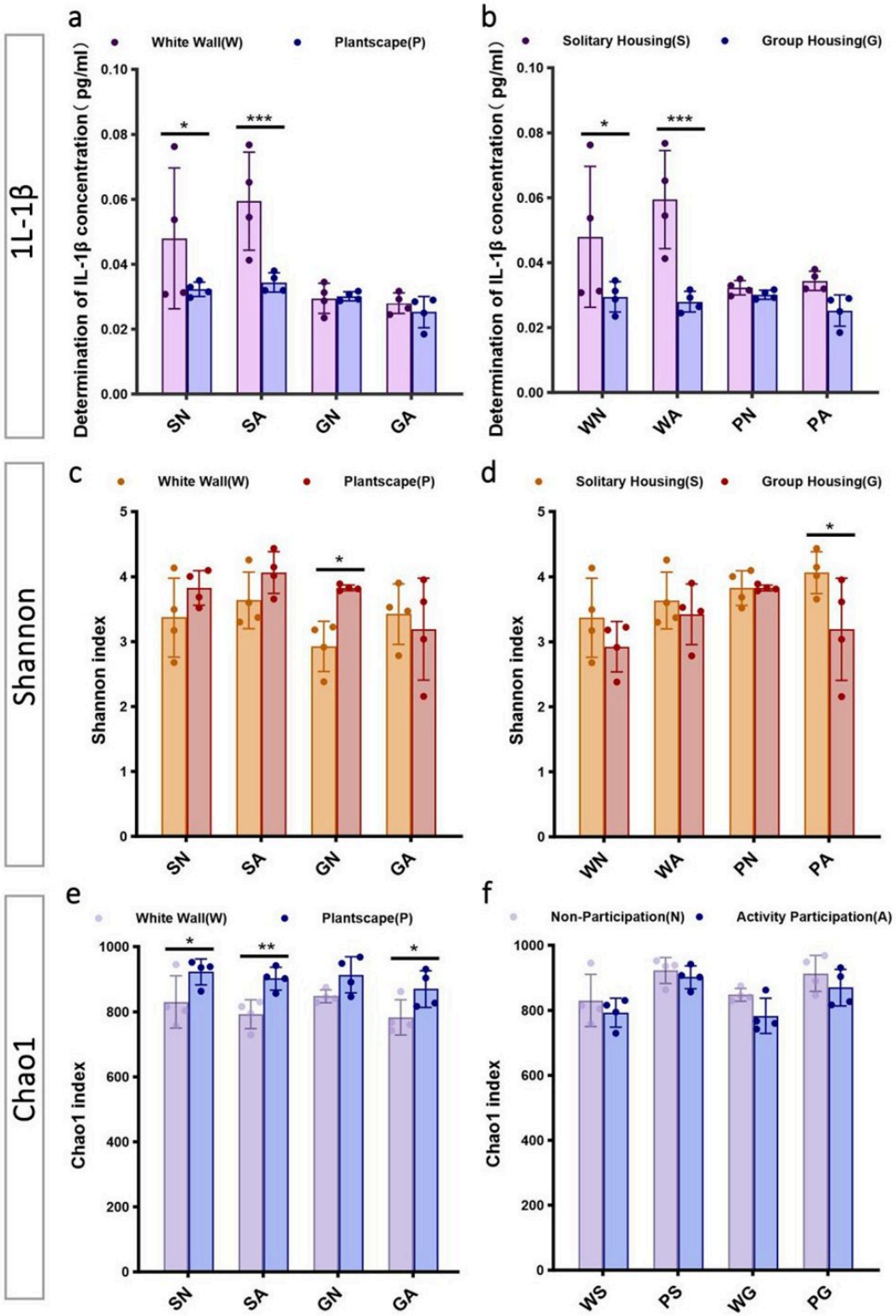


Figure 6

Physiological outcomes differences in mice under different environmental and different activityinterventions. a. IL-1β levels under different environments; b. IL-1β levels under different housing conditions;c. Shannon index under different environments; d. Shannon index under different housing conditions; e. Chao-

1 index under different environments; f. Chao-1 index under different activity conditions. Data are shown as the mean ± standard error.*p<0.05; **p<0.01; ***p<0.001.

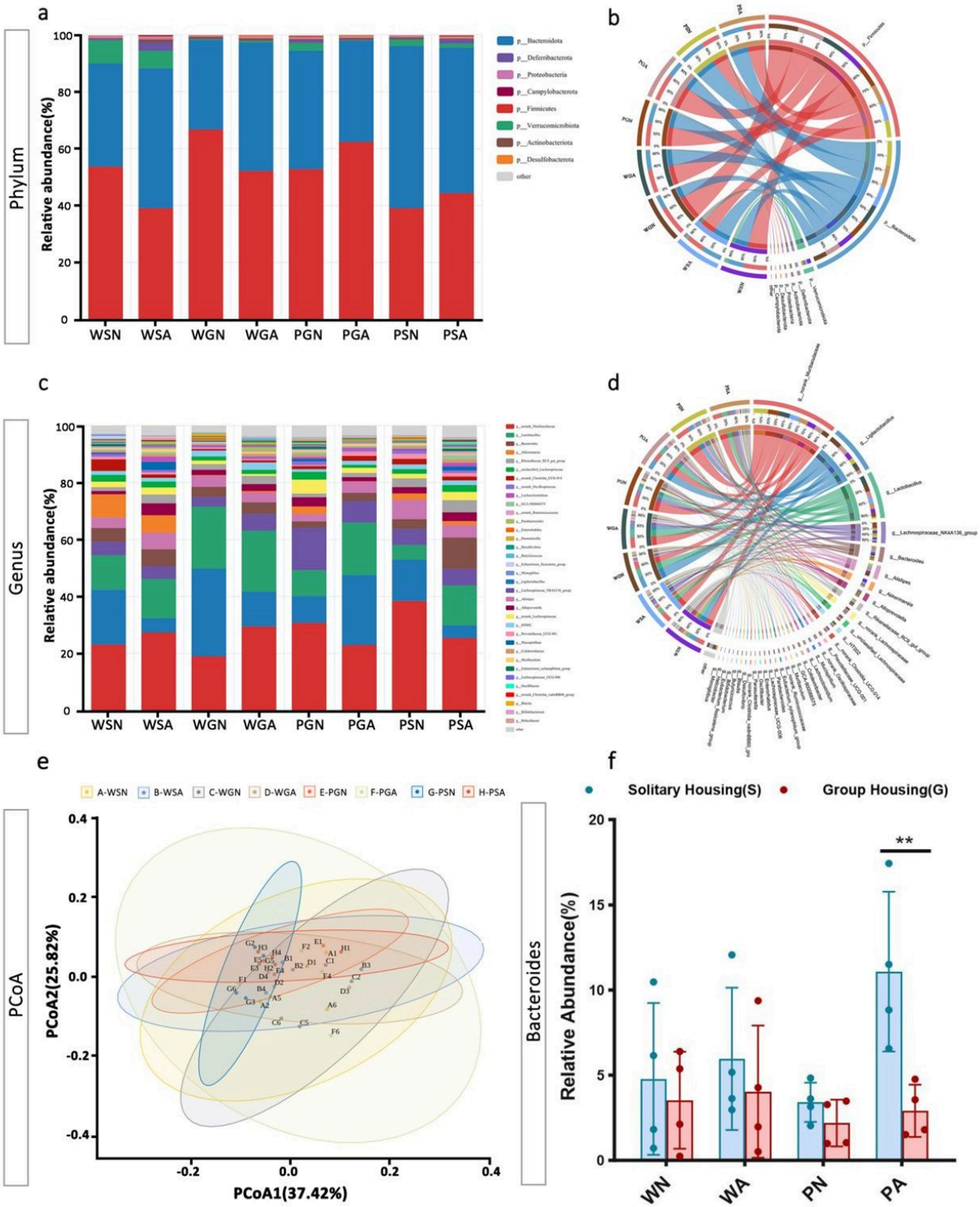


Figure 7

Gut Microbiota result. a. Phylum level histogram; b. Phylum level circos plot; c. Genus level histogram; d. Genus level circos plot; e. Gut microbiota β diversity; f. Relative abundance of *Bacteroides* under different housing conditions. **Data are shown as the mean ± standard error.***p<0.05; **p<0.01; ***p<0.001.

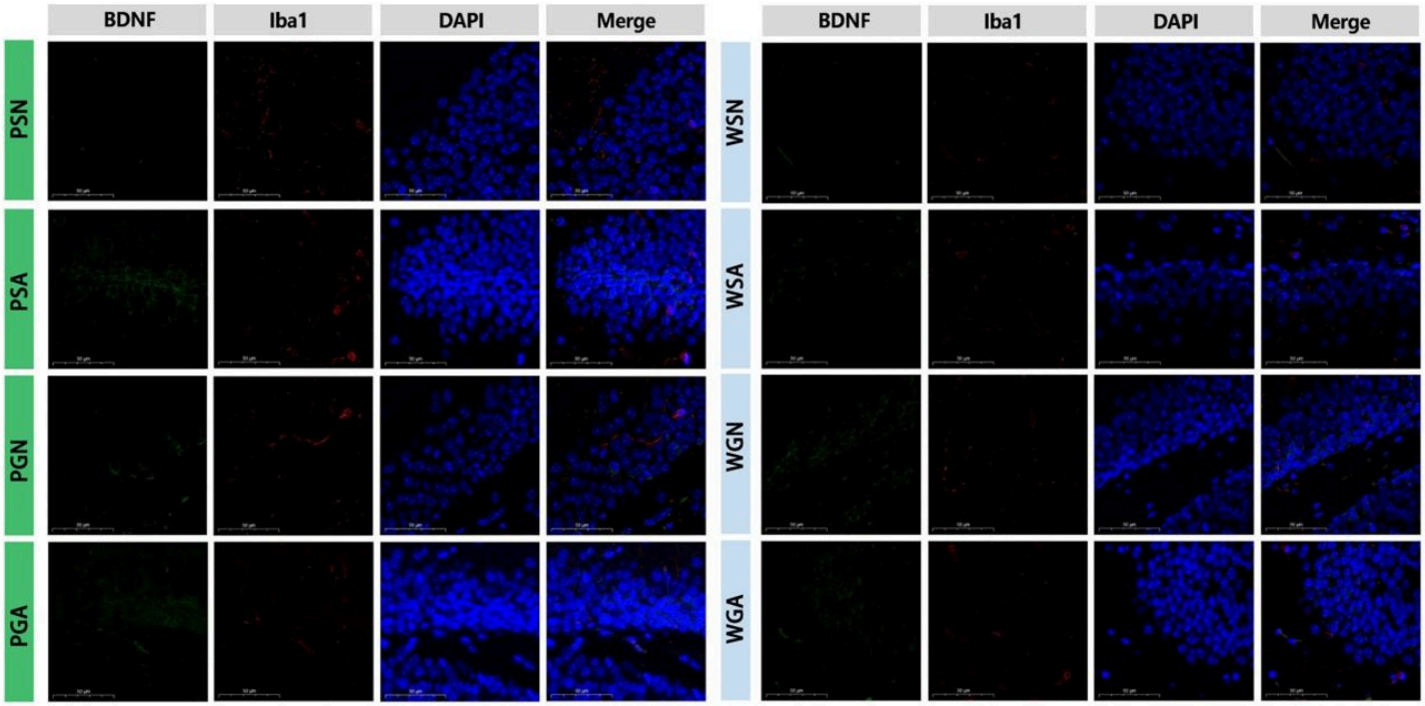


Figure 8

Representative immunofluorescence staining in the hippocampal dentate gyrus (scale bar: 500 μm).

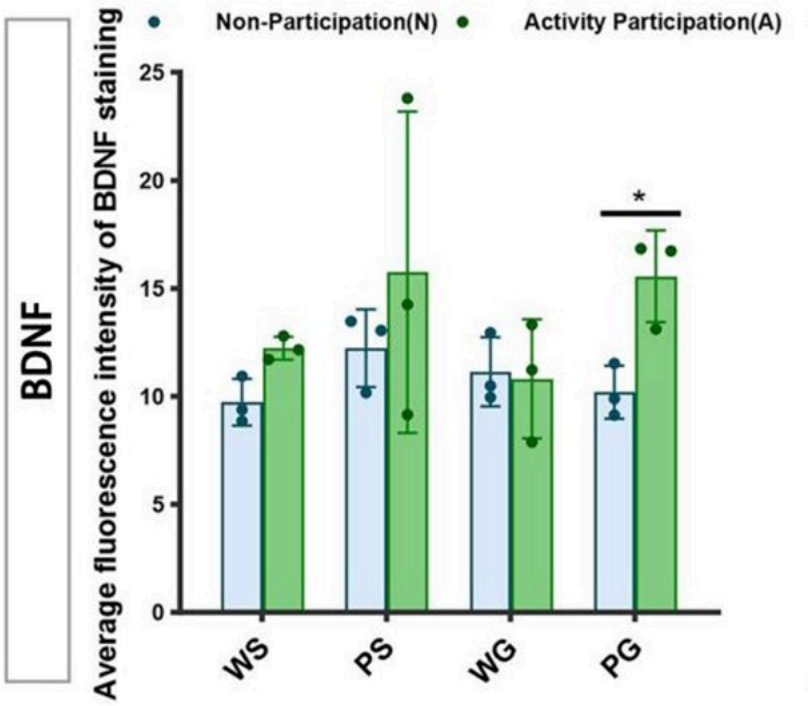


Figure 9

Comparison of BDNF mean fluorescence intensity under different activity conditions. **Data are shown as the mean ± standard error.** *p<0.05; **p<0.01; ***p<0.001.