Lack of social touch increases anxiety and alters social behaviors in mice

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Abstract

The importance of social interactions has been reported in different animal species. During the pandemic, although people can communicate through other sensory cues, social touch is mostly prohibited under different levels of social distance policies, which inspired us to explore the necessity of physical contact in mouse social interaction. In this study, we first conducted a long-term observation showing that pair-housed mice in a standard laboratory cage spent nearly half the day in

direct physical contact with each other. Furthermore, isolation experiments demonstrated that, even with access to other sensations, prevention of social touch for one month significantly induced anxiety levels, changed social behaviors and increased interleukin-6 cytokine in the hippocampus and the serum of mice. Our study demonstrated the necessity of social touch for the maintenance of mental health in mice. This information could have important implications for human social interactions, especially the social policies during a pandemic crisis.

Introduction

Humans, like other social animals, require constant social interactions to maintain physical and mental health. During the COVID-19 pandemic, most countries have, by necessity, issued orders such as social distancing, social quarantines or even stay-at-home rules to prevent the spread of the coronavirus. These restrictions not only affect daily life but also cause potential health concerns. Research has already suggested the influence of social isolation on mental illness, including anxiety and depression, along with an increase in suicide risk[1-3]. Numerous studies have also indicated that social disengagement is closely linked to physiological problems such as cardiovascular diseases, cognitive decline, disability and mortality[4]. After the emergence of the pandemic, these issues imposed further complexity and burdens on the health system.

The impact of social isolation has also been studied extensively in other animal species[3]. In rats and mice, social isolation causes a variety of behavioral changes, including hyperlocomotion, anxiety,

38 impulsivity, and learning and memory deficits[5]. Socially isolated mice also displayed abnormal social
39 behaviors, including increased aggression, reduced sexual preference and weakened social memory[6].
40 These abnormal behaviors are frequently associated with elevated serum concentrations of stress
41 hormones including corticosterone, catecholamine and corticotropin-releasing factor. Currently, due to
42 its striking effect, social isolation has been widely used as a standard method to induce stress for basic
43 or translational research in these rodent models[7].

44 Multiple social signals are exchanged during social interactions. While some social modalities,
45 such as olfactory and auditory cues, have been studied extensively and suggested to be important in
46 alleviating social isolation syndrome in mice[8,9], the specific influences of social touch on animal health
47 and physiology have rarely been addressed. In fact, during the COVID-19 pandemic, people can still
48 exchange visual or auditory social signals by virtual meeting or phone. However, physical contact,
49 such as hugging or handshaking, is strictly prohibited. These social restrictions inspired us to ask
50 whether social touch may play a crucial role in maintaining the mental health. To address this issue, in
51 this study, we evaluated the importance of physical touch by establishing a divided housing
52 environment in which mice could freely exchange visual, olfactory and auditory cues but could not
53 physically contact each other. Mice placed in these divided housing conditions showed increased
54 anxiety and altered social behavior compared to mice in standard pair or single housing. Concurrently,
55 we also observed elevated interleukin-6 (IL-6) in mice from divided housing conditions. Our study
56 therefore demonstrated a critical role of physical contact in maintaining mouse psychological health

conditions. This information highlights the importance of social touch in human interaction and may have some potential implications for the planning of social regulations during the pandemic period.

Results

Male mice spent half the day in social touch.

In order to examine the proportion of social touch time in the daily interactions of mice, the animals were housed in pairs, and all interactions were video recorded for 24 hours. The videos were analyzed based on the activity of the mice and all physical contact between them (Figure 1A). As expected, mice engaged in different activities and behavioral patterns between the light and dark periods. In the dark, mice mostly displayed exploratory behaviors without direct contact (both moving–no contact) (Figure 1B). In the light period, the activity levels of the mice were decreased, and physical contact was increased (no movement–contact) (Figure 1C). Importantly, in both the light and dark periods, there was a very minimal amount of time during which both mice were inactive and were not in physical contact (no movement–no contact), suggesting that mice mostly huddle together during their resting time. In summary, our 24-hour observation showed that mice spent half the day in physical contact with each other (Figure 1D), implying a strong motivation for social touch in their daily interactions.

Prevention of social touch increased anxiety-like behaviors.

Next, to investigate the necessity of social touch for mouse mental health, we housed two mice in a divided cage. Mice in this cage were separated by a transparent plate with holes, which allowed olfactory, visual, and auditory communications but prevented direct physical contact (Figure 2A). After one month of this housing arrangement, we examined these mice for behavior related to psychological symptoms, and compared with mice in single-housed and standard pair-housed conditions (Figure 2B).

Anxiety levels were evaluated by the open field test and the light-dark box test (Figure 3A). First, the open-field test showed that there was a significant decrease in activity in single-housed mice but no difference between pair-housed and split-housed animals (Figure 3B). Surprisingly, while there was no difference between pair- and single-housed animals, split-housed mice spent significantly less time in the center of the arena than pair-housed or single-housed mice (Figure 3C). In the light-dark box test, split-housed mice also spent significantly less time in the light chamber than pair-housed or single-housed animals (Figure 3D). The number of transitions between the two chambers was lower in the split-housed group than in the pair-housed group (Figure 3E). Together, the two experiments indicated increased anxiety levels in split-housed mice.

We also conducted the tail suspension and forced swim tests to evaluate depression levels. In terms of latency to the first immobility and total immobility time, we did not detect differences among the three groups in either assay (Figure 4A-D), suggesting that lack of physical touch raises anxiety but may not lead to depression in mice.

Physical isolation modulated social behaviors.

A previous study using the tube test and the open-field test showed higher anxiety levels in dominant mice than in subordinate mice[10]. We therefore applied the tube test to compare social dominance through pairwise interactions. Consistent to previous finding, we found a higher win rate (reflecting a more dominant social rank), in split-housed mice than in pair- or single-housed mice (Figure 5A). There was no difference between mice housed in paired and single conditions.

Social behaviors were also examined among these three groups using a standard intruder assay with Balbc intruders. Although there was no detectable difference in total social time (Figure 5B), we observed that mice in the split-housed group showed a significantly shorter latency than those in the single-housed group to interact with intruders (Figure 5C), implying stronger social motivation after physical isolation.

Physical isolation increased IL-6 in the hippocampus and serum of mice in divided cages.

Neuroinflammation in the hippocampus has been suggested as one of the mechanisms causing anxiety [11]. To investigate the potential inflammation in the hippocampus, we conducted quantitative PCR to quantify genes related to inflammation among these three groups (Figure S1). Our results indicated that there were higher expression levels of cytokine IL-6 and Allograft inflammatory factor 1 (Iba-1), in split-housed mice than pair-housed mice (Figure 6A). Since the relationship between cytokines, especially IL-6, and anxiety has been largely studied, not only in the hippocampus but also

in the serum[12,13], we further applied multiplex cytokine assay to measure the serum cytokine levels and found higher level of IL-6 in split- than single-housed mice (Figure 6B). Unexpectedly, the analysis also showed lower levels of IL-12p40 and IL-17A in split-housed mice than in single-housed mice (Figure 6B and Table S2).

Discussion

Male mice are known to be territorial and do not share space with other males in their natural environment[14]. Our long-term observation under laboratory conditions, however, demonstrated that male mice with the option of having their own space actually preferred to stay close to each other most of the time. While this result may not be surprising, these data present new quantitative evidence to demonstrate a preference for social contact in mice and imply the important role of physical interaction in their daily lives.

Through a physical isolation experiment, we further showed that deprivation of social touch could significantly increase anxiety-like behaviors in mice. Unexpectedly, our experiment did not detect a significant difference between single-housed and pair-housed mice in either anxiety- or depression-like behavior. Although it is generally believed that anxiety and depression can be induced by social isolation, this relationship has not always been consistent in mouse studies[8,9,15,16]. Failure to detect the difference might be due to the shorter isolation time or larger isolation space in our experimental designs. However, even there was no phenotypic distinction in single-housed mice, our experiments

133 based on the open-field test and the light-dark box test still detected increased anxiety-like behaviors
134 in split-housed mice. This increased anxiety was further supported by social dominance as measured
135 by the tube test[10]. The most surprising finding in this study was that, in contrast to previous studies[8,9],
136 divided housing caused even higher anxiety levels than single housing. These results implied that
137 sensing other social signals without direct physical interaction may produce an even more stressful
138 environment than complete social isolation. For example, animals may release threat messages in
139 visual, auditory or even olfactory forms, which can normally be alleviated by tactile interaction.
140 Alternatively, since other sensory cues may stimulate strong motivation for social interaction, which
141 is supported by the reduced social latency of split-housed mice, the restriction of physical contact could
142 potentially cause more undesirable pressure or stress. While our data demonstrated that social touch is
143 critical in regulating behavioral performance and maintaining normal health, the question of how social
144 signals other than touch can cause harmful influences remains to be explored.

145 Neuroinflammation involving cytokine IL-6 has been reported to induce anxiety in both mice and
146 human[11,12]. Our results from qPCR and multiplex assay both showed higher levels of IL-6 in mice in
147 divided housing condition. Although the differences were only significant between split-and pair-
148 housed in the hippocampus and significant between split- and single-housed in the serum, the trends
149 overall supported our observations in behavioral experiments. Surprisingly, multiplex assay also
150 detected reduced levels of IL-12p40 and IL-17A in the split-housed groups. Reductions in IL-12 levels
151 in serum have been reported in patients with mental diseases and in rodent models under stress[17-19].

IL-17A, however, is generally believed to be positively correlated with anxiety levels[20,21], which is contrary to our results. Our data, therefore, only partially support the potential role of cytokines in the increased level of anxiety in the split-housed group of mice.

Conclusion

Through long-term observation and manipulation of housing conditions, our study suggests that physical touch not only plays a significant role in daily interactions between mice but also functions as a crucial environmental factor for the maintenance of mental health. This finding has important implications for social interactions in humans. During a pandemic, although people can still communicate with each other via other sensory cues, social policy might need to tolerate at least minimal physical interactions to alleviate stress from social restrictions. Even in a more general sense, the power of social touch should never be underestimated. A simple hug is not merely about an emotional connection; it could also have a substantial impact on health.

Methods

Mice C57BL/6J and BALB/cByJ 8-week old male mice were purchased from the National Laboratory Animal Center in Taiwan. Mice were housed in a controlled animal room with a 12-h light/dark cycle (0700–1900 hr). Except for the long-term observations, all behavioral tests were conducted during the light period. All animal procedures were in compliance with institutional guidelines established and

171 approved by the Institutional Animal Care and Use Committee of National Tsing Hua University.

172

173 **Long-term observation** Mice were paired-housed in a standard cage under video recording from the
174 top for 24 hours. The videos were analyzed manually using Behavioral Observation Research
175 Interactive Software (BORIS). According to the mouse activity and physical contact, the behaviors
176 were classified into six categories: both moving-contact, both moving-no contact, single moving-
177 contact, single moving-no contact, no moving-contact, and no moving-no contact (Figure 1A).

178

179 **Isolation housing conditions** After 3-week habituation, mice were randomly allocated into three
180 housing conditions: (a) pair-housed cage; (b) split-housed cage; and (c) single-housed cage (Figure
181 2A). Mice in split-housed cage were separated by a transparent plate to prevent physical contact.
182 Multiple 0.5cm diameter holes on the plates allow freely exchange of olfactory, visual, and auditory
183 cues. Mice were kept in the housing conditions for one month before behavioral assays.

184

185 **Open field test** The Open field test was based on a previous study[22]. A mouse was placed into a
186 clean 50 cm x 50 cm open field apparatus for 6 minutes. Animal movement was filmed from
187 overhead, and the open field apparatus was cleaned with 70% ethanol between each trial. Videos
188 were evaluated using SMART VIDEO TRACKING Software (Panlab) to determine the total moving
189 distance to present the activity and total time in the box center (25 cm x 25 cm) to present the anxiety

level.

Light-Dark box assay The Light-Dark box assay was based on a previous study[23]. The light-dark box was made of opaque Plexiglas (50 cm x 25 cm white open chamber for the light box, 50 cm x 25 cm x 30 cm black closed chamber for the dark box). The chambers were connected by a 5.5 cm x 4.5 cm door in the middle of the wall separating the two chambers. Animals were placed in the middle of the light chamber for 6 minutes. Videos were evaluated using SMART VIDEO TRACKING Software (Panlab) to determine the total time in the light box to present the anxiety level. The apparatus was cleaned with 70% ethanol between each trial.

Standard forced swimming test The Forced swimming test was based on a previous study[24]. The container [11.5 cm (R)× 17.5 cm (H)] was filled with water that the animals could neither step on the bottom nor escape from the top. When the mice were placed in the container, the recording time started and the behavior were recorded by the digital camera for six minutes. After the test, mice were removed from the water, dried with an infrared lamp in their homecage. Videos were analyzed by SMART VIDEO TRACKING Software (Panlab) for the last five minutes to avoid unstable immobility behavior in the first minute. Floating was defined as immobility behavior or minimal movement for maintaining balance in the water. All mice were first time swimmers and none were used for multiple forced swimming test.

209

210 **Tail suspension test** Tail suspension test was based on a previous study[25]. The end of the mouse tail
211 was fastened on the bar 45cm above the desk by the tape, allowing the mice freely struggling and
212 waving the limbs. The behavior was recorded by digital camera for 6 minutes and analyzed for the
213 last five minutes by SMART VIDEO TRACKING Software (Panlab) to avoid unstable immobility
214 behavior in the first minute. Immobility behavior was defined as any passive behavior included
215 slightly swaying. All mice were tested only once and none were used for multiple tail suspension
216 test.

217

218 **Tube test** The tube test was based on a previous study[26]. A clear Plexiglas tube (3.75 cm diameter, 60
219 cm length) was used for the test. Two mice were simultaneously released at opposite ends of the tube
220 and then ran toward the middle. When a mouse retreated and set all four paws outside the tube, the
221 test trial was over and that mouse was considered as the loser. The win rates between two mice were
222 based on 4 consecutive trials, with each mouse starting at an alternative end of the tube for each trial.
223 The interior of the tube was cleaned after every pairwise with 70% ethanol.

224

225 **Intruder assay** The intruder assay was based on a previous study[27]. BALB/cByJ males were used as
226 intruders. To minimize intruders' aggression, the olfaction of intruders was ablated by injection of
227 2,6-dichlorobenzonitrile (dichlorobenil, Sigma D57558) at a concentration of 50mg/mL and a dose

of 100 ug/g of body weight for three times, every other day. For the assay, one intruder was introduced into the home cage of resident male for 10 minutes. For split- and pair-housed groups, cagemates and dividers were removed before the assay. The behaviors were digitally recorded and analyzed to obtain the latency time and total time for social interactions.

RNA extraction, cDNA synthesis and Quantitative Real-Time PCR Total RNA was extracted from hippocampus using RNeasy Mini Kit (Qiagen), including a DNase (Qiagen) treatment. cDNA of mRNA was generated by ReverTra Ace Set RT cDNA Synthesis Mix (Purigo) using oligo dT as primer. The qPCR reactions were performed under qTOWER^3 real-time PCR system (Analytik Jena) using the KAPA SYBR FAST Universal qPCR Kit (KAPA Biosystems, MA) following the manufactures protocol. The relative expression was calculated using the ΔCT method and *GAPDH* as normalization control. Primer sequences used for qPCR are shown in Table S1.

Measurement of serum cytokines Mouse blood was collected into blood collection tube (BD Vacutainer) from the Jugular Vein, then centrifuged at 1,000xg for 10 min. The plasma was stored at −80°C until analyzed. The cytokine levels in serum were measured by Bio-Plex Mouse cytokine Group I, 23-plex assay kit (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. The measured molecules included Eotaxin, G-CSF, GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 (p40), IL-12 (p70), IL-13, IL-17A, KC, MCP-1 (MCAF),

MIP-1α, MIP-1β, RANTES and TNF-α. In brief, 50ul serum sample was incubated with 50ul washed antibody-coupled beads in each well of the flat bottom plate for 30 minutes at room temperature. After wash to remove unbound materials, the beads were incubated with 25 ul biotinylated detection antibodies for 30 minutes. After washing away the unbound biotinylated antibodies, the beads were incubated with 50 ul streptavidin-PE for 10 minutes at room temperature. The beads were then resuspended in 125 ul assay buffer and read on the Bio-Plex suspension array system. The data were analyzed using Bio-Plex Manager software version 6.0.

Statistics All statistics were completed using GraphPad Prism 6.0 software. Except for the long-term observation, we removed maxima and minima values for each group in all experiments in order to exclude potential outliers. Welch's ANOVA followed by Dunnett's test were applied to all multiple comparisons. Pairwise comparisons in tube test were tested by Wilcoxon signed-rank test. All data is represented as mean +/- standard error of mean (S.E.M.).

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266 Conservation Research Center (to T-H. K.) and the Brain Research Center (to T-H Kuo).

267

268 **Author contributions in this study**

269 K-T Lin, and T-H Kuo designed the experiments; Y-K Ma, C-L L, Y-H C, C-H C, Y-S S performed

270 the experiments; Y-K Ma, P-Y Z, Y-H C, C-C C analyzed the data, K-T Lin and T-H Kuo wrote the

271 manuscript.

272

273 **Competing interests**

274 The authors declare no competing financial interests.

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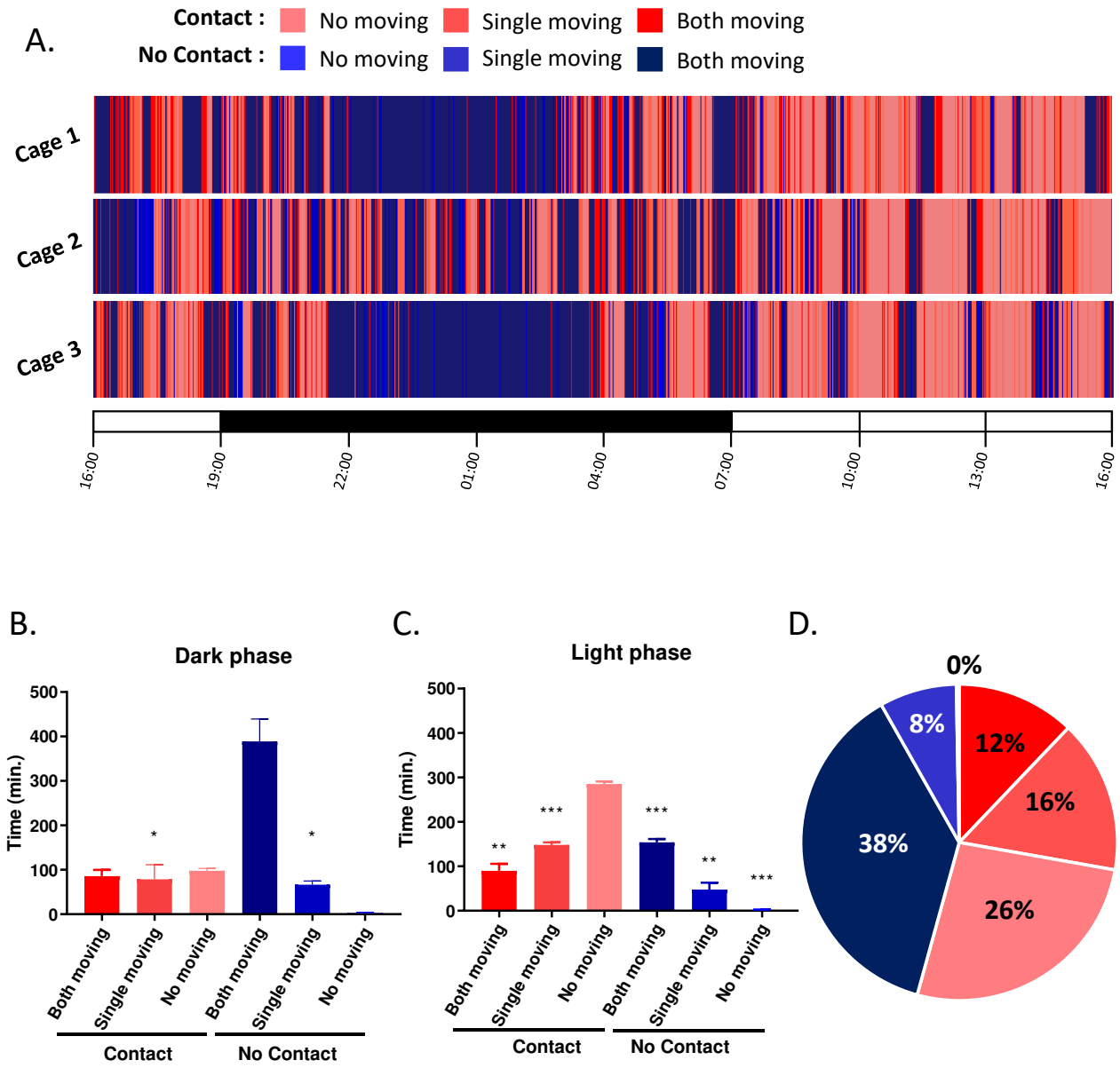

277

278 **Figure 1. Mice spent half a day physically contacting each other.** (A) A raster plot showing physical
279 interactions of pair-housed mice in 24 hours. (B) The total time for specific interactions between two
280 mice in the dark phase (Welch's ANOVA followed by Dunn's test, p values were determined by the
281 comparison to Both moving-No contact). (C) The total time for specific interactions between two mice
282 in the light phase (Welch's ANOVA followed by Dunn's test, p values were determined by the
283 comparison to No moving-Contact). (D) The percentage of time for specific interactions between two
284 mice in 24 hours. * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; n=3 pairs; Mean ± S.E.M.

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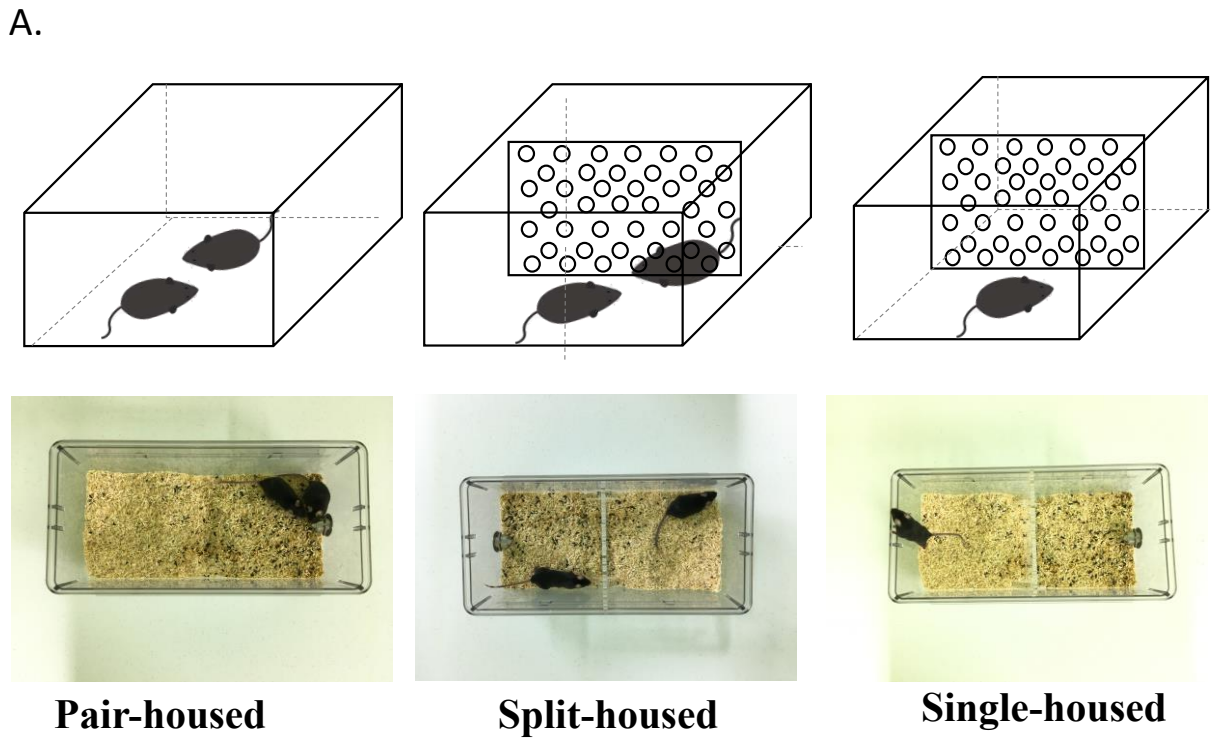

286

287 **Figure 2. The three different housing conditions and the experimental schedule.** (A) Male mice

288 were housed in three different conditions: pair, split, and single housing. (B) Schematic diagram of the

289 experimental schedule.

290

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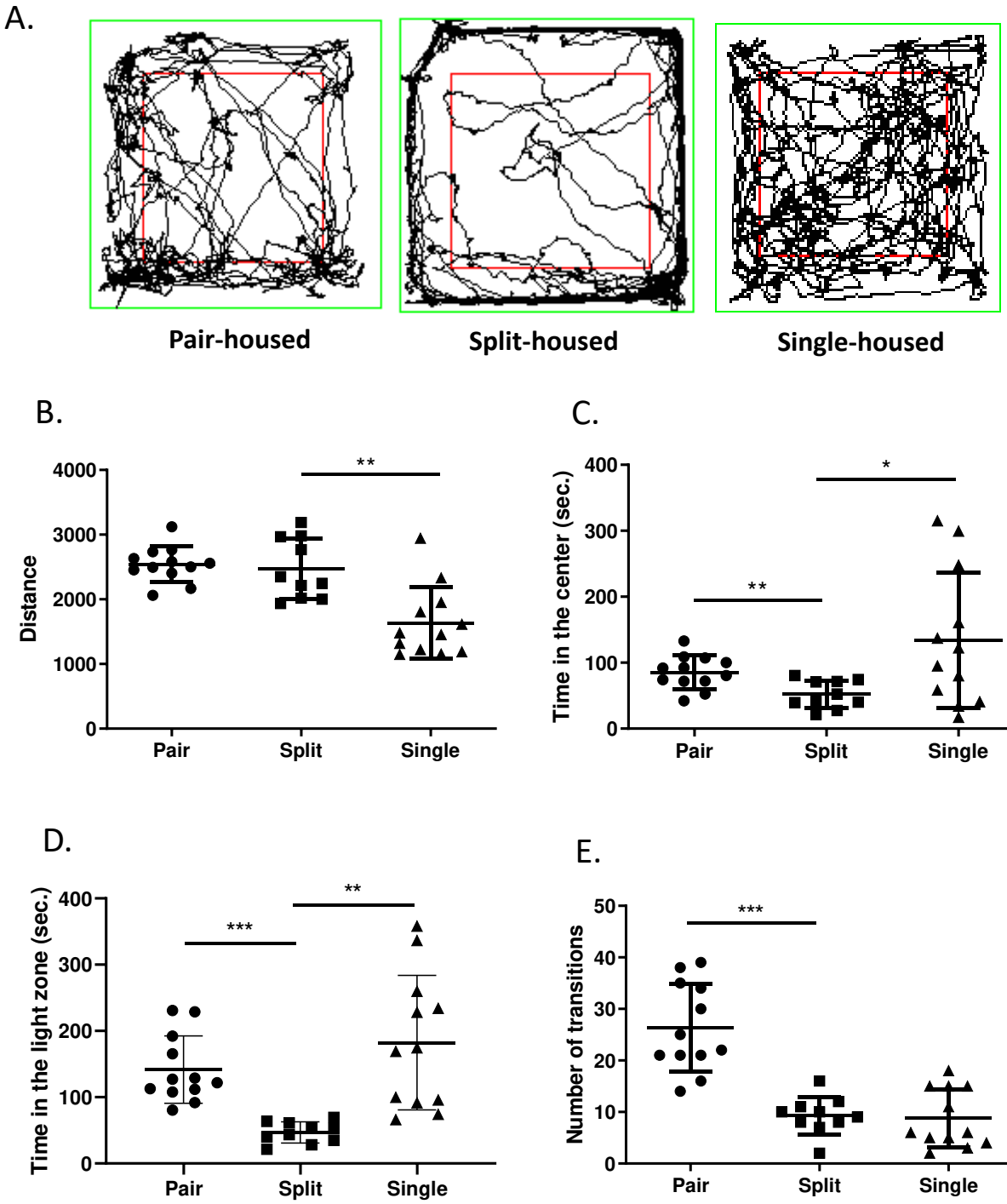


292

293 **Figure 3. Mice housed in divided cages showed increased anxiety-like behavior.** (A)
294 Representative traces of mice in the open field. (B) Travel distances in the open-field test. (C) Time
295 spent in the inner zone in the open-field test. (D) Time spent in the light zone during the light-dark box
296 test. (E) The number of transitions between the light and dark zones. Welch's ANOVA followed by
297 Dunn's test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; n=10-12 per group; Mean ± S.E.M.

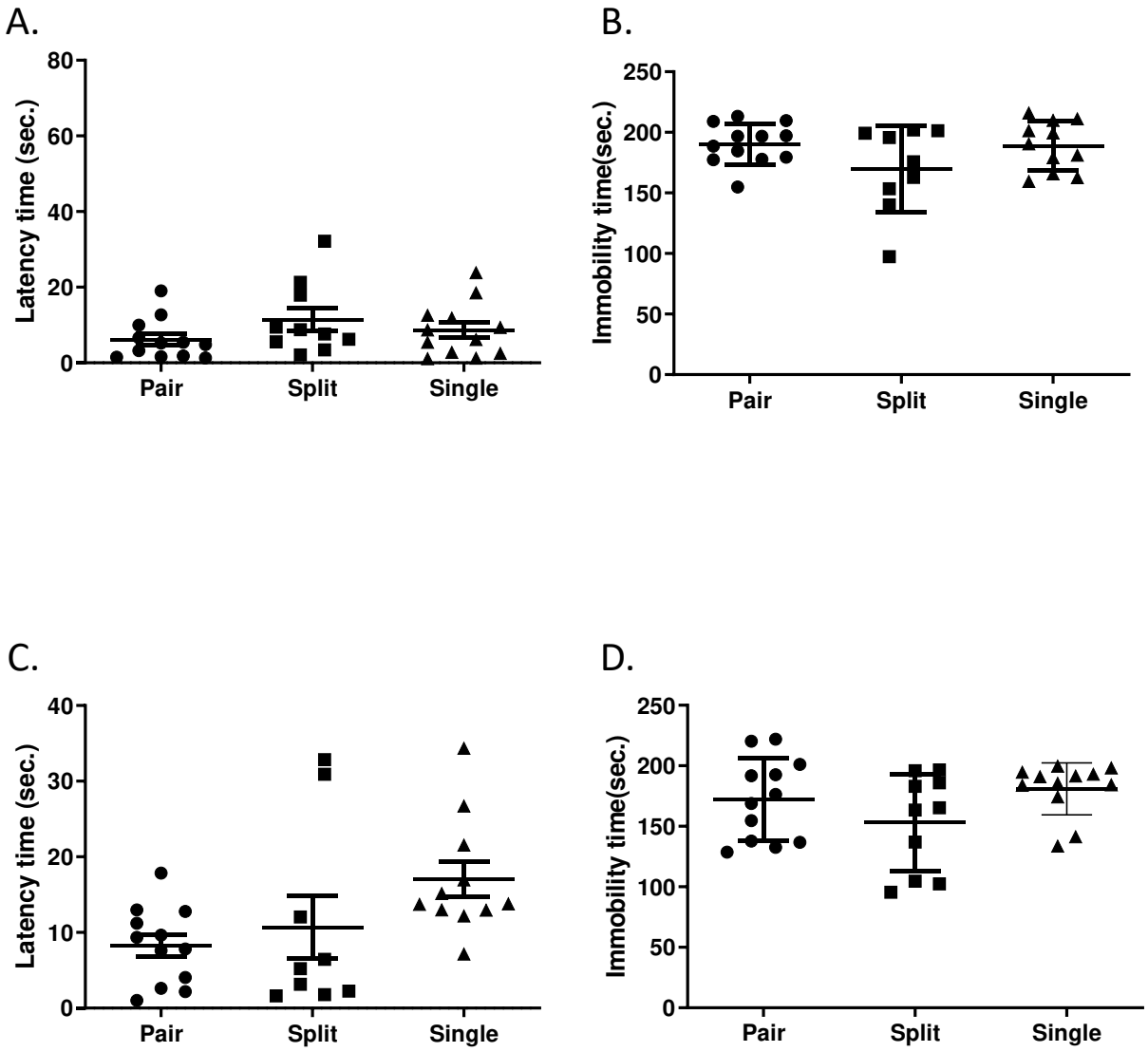
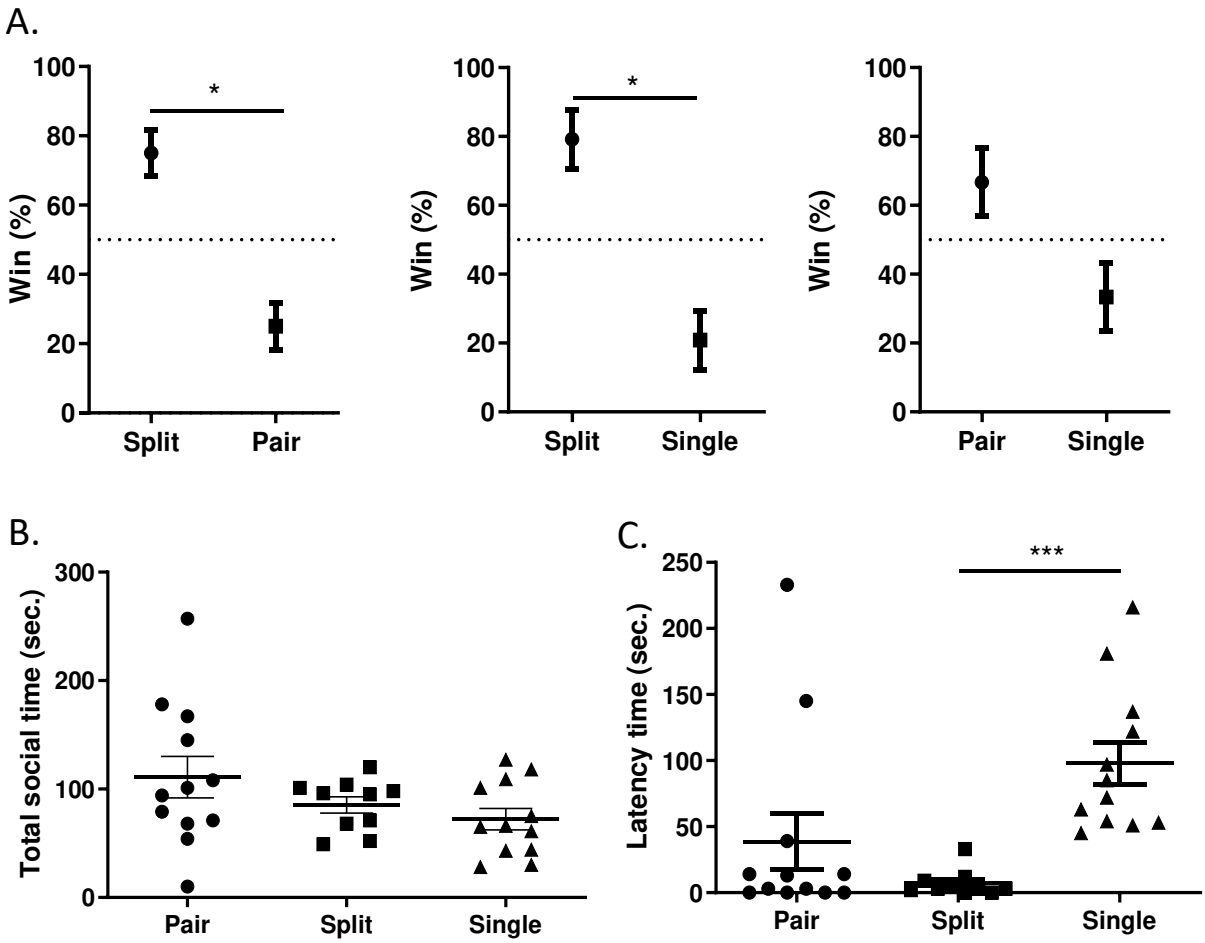


Figure 4. Depression-like behavior was similar among mice housed in different conditions. (A) Latency to the first bout of immobility in the forced swim test. (B) The total immobility time in the forced swim test. (C) Latency to the first bout of immobility in the tail suspension test. (D) The total immobility time in the tail suspension test. Welch's ANOVA followed by Dunn's test; n=10-12 per group; Mean ± S.E.M.



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310

311 **Figure 5. Mice housed in divided cage showed changes in social behaviors.** (A) Social dominance

312 evaluated by pairwise comparison in the tube test (Wilcoxon signed-rank test, n=10-12 pairs). (B) The

313 total duration of social interaction in the intruder assay. (C) The latency to physical interaction in the

314 intruder assay. Welch's ANOVA followed by Dunn's test; * = $p < 0.05$, *** = $p < 0.001$; n=10-12 per

315 group; Mean ± S.E.M.

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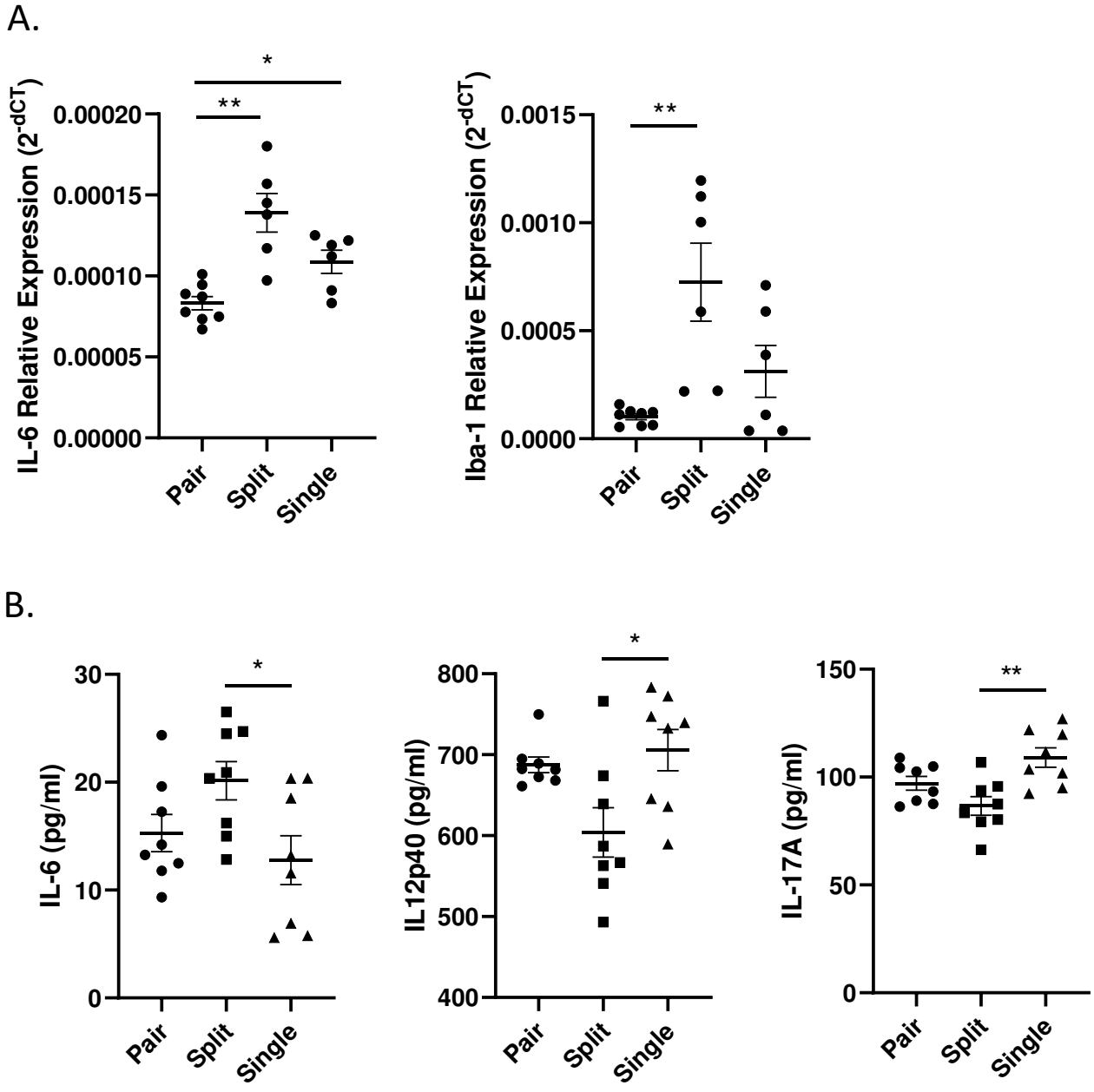

321

322 **Figure 6. There were higher levels of cytokine IL-6 in the hippocampus and the serum of mice**
323 **housed in divided cages.** (A) The expression levels of cytokine IL-6 and Iba-1 in hippocampus (n =
324 6-8 per group) (B) The serum concentrations of cytokines IL-6, IL-12p and IL-17A. (n = 8 per group).
325 Welch's ANOVA followed by Dunn's test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; Mean ± S.E.M.

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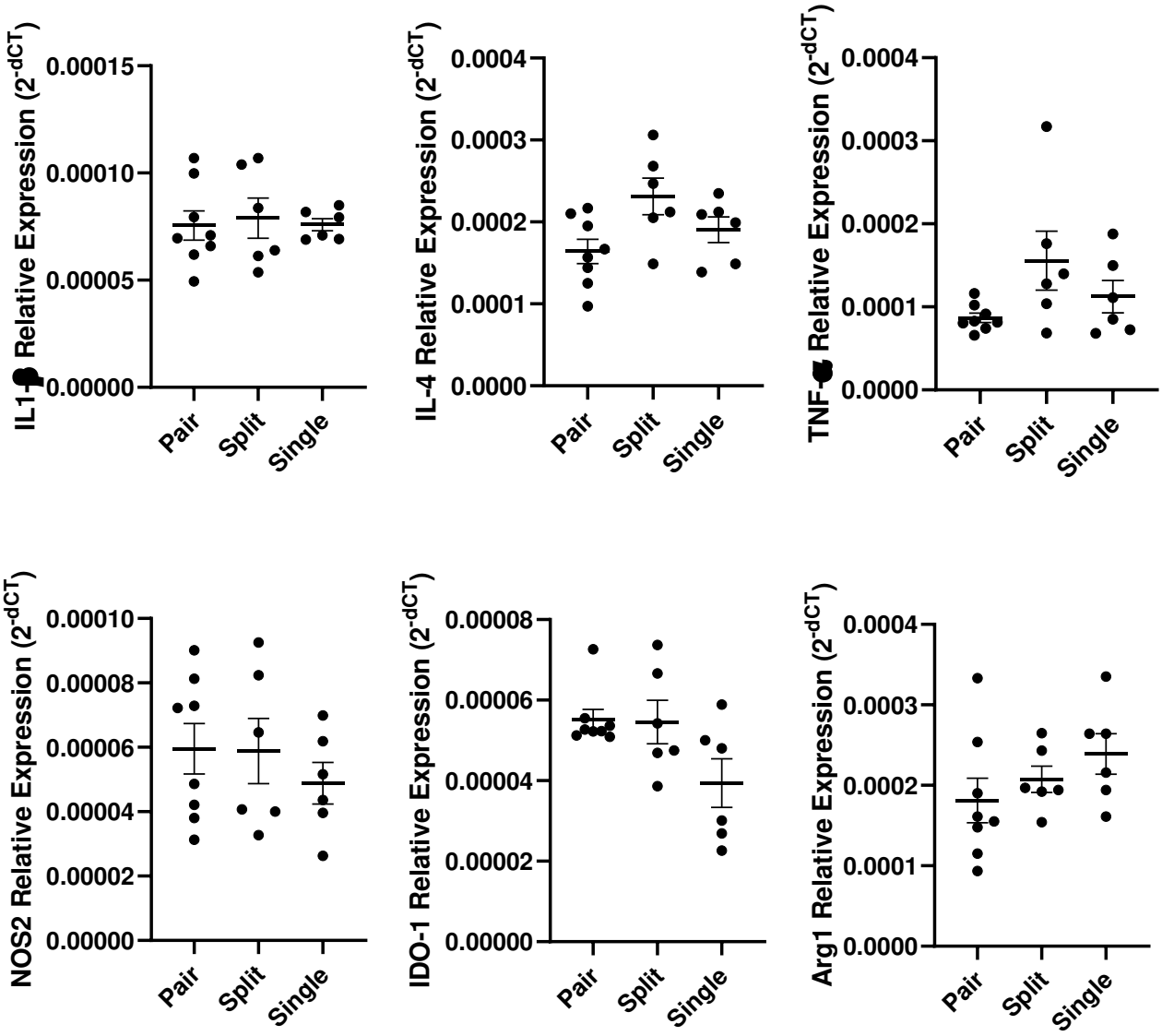

Figure S1. The cytokines showing no difference in hippocampus. The expression levels of cytokine IL-1β, IL-4, TNF-α, NOS2, IDO-1, and Arg1. Welch's ANOVA followed by Dunn's test; n = 6-8 per group; Mean ± S.E.M.

Table S1. Primer used for quantitative PCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
TNFα	*ATCTCATACCAGGAGAAAGTCAACCT*	*TGGGCTCATACCAGGGTTTG*
IL-1β	*GCCACCTTTTGACAGTGATGAG*	*AAGGTCCACGGGAAAGACAC*
IL-6	*TACCACTTCACAAGTCGGAGGC*	*CTGCAAGTGCATCATCGTTGTTC*
IL-4	*AGGTCACAGGAGAAGGGACG*	*ACTCTCTGTGGTGTTCTTCGT*
Iba1	*GGAAAGTCAGCCAGTCCTCC*	*TCCACATCAGCTTCTGTTGAAAT*
Arg1	*GTACATTGGCTTGCGAGACG*	*CGGCCTTTTCTTCCTTCCCAG*
IDO-1	*GCAGACTGTGTCCTGGCAAACT*	*AGAGACGAGGAAGAAGCCCTTG*
Nos2	*AAGGCCACATCGGATTTCAC*	*TGTTCCTCTATTTTTGCCTCTTTAAAG*
Gapdh	*GGCAAATTCAACGGCACAGT*	*GGGTCTCGCTCCTGGAAGAT*

Table S2. Serum cytokine levels (pg/ml)

	Pair	Split	Single	P value
IL-1 α	14.08±1.47	16.29±1.94	12.14±1.3	p=0.2425
IL-1β	64.87±7.67	80.15±9.55	65.24±10.58	p=0.4343
IL-2	74.63±5.45	75.09±3.82	61.71±8.82	p=0.2740
IL-3	65.14±2.71	60.35±2.35	64.89±5.62	p=0.6234
IL-4	72.91±5.6	71.25±3.41	69.23±9.82	p=0.9299
IL-5	69.71±10.53	74.91±11.11	57.21±9.73	p=0.4825
IL-6	15.29±1.72	20.13±1.78	12.78±2.25	p=0.0419*
IL-9	108.14±10.95	114.6±9.6	103.55±13.24	p=0.7908
IL-10	220.79±16.7	223.81±17.54	176.77±25.48	p=0.2137
IL-12(p40)	687.45±9.74	603.82±30.49	705.66±25.38	p=0.0180*
IL-12(p70)	596.28±61.13	653.19±22.66	623.2±65.85	p=0.7571
IL-13	120.1±12.78	148.75±17.84	131.62±9.27	p=0.3569
IL-17A	97.1±3.19	86.65±4.34	109.05±4.56	p=0.0037**
Eotaxin	1096.99±50.87	999.2±98.59	1163.88±103.89	p=0.4280
G-CSF	386.42±17.31	371.74±10.35	423.84±24.07	p=0.1444
GM-CSF	133.27±6.09	137.18±4.66	122.17±12.21	p=0.4413
IFN-γ	212.78±20.07	228.69±15.48	209.88±19.28	p=0.7421
KC	77.76±6.8	88.86±5.56	76.62±5.22	p=0.2912
MCP-1	445.41±53.98	527.7±46.74	424.14±42.18	p=0.2932
MIP-1 α	26.24±2.61	27.35±2.84	25.6±2.85	p=0.9041
MIP-1 β	219.92±11.28	227.45±6.51	226.66±11.71	p=0.8467
RANTES	123.2±8.53	118.59±6.3	125.37±4.27	p=0.7626
TNF-α	562.54±68.21	569.78±78.71	503.15±81.86	p=0.7977

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Figures

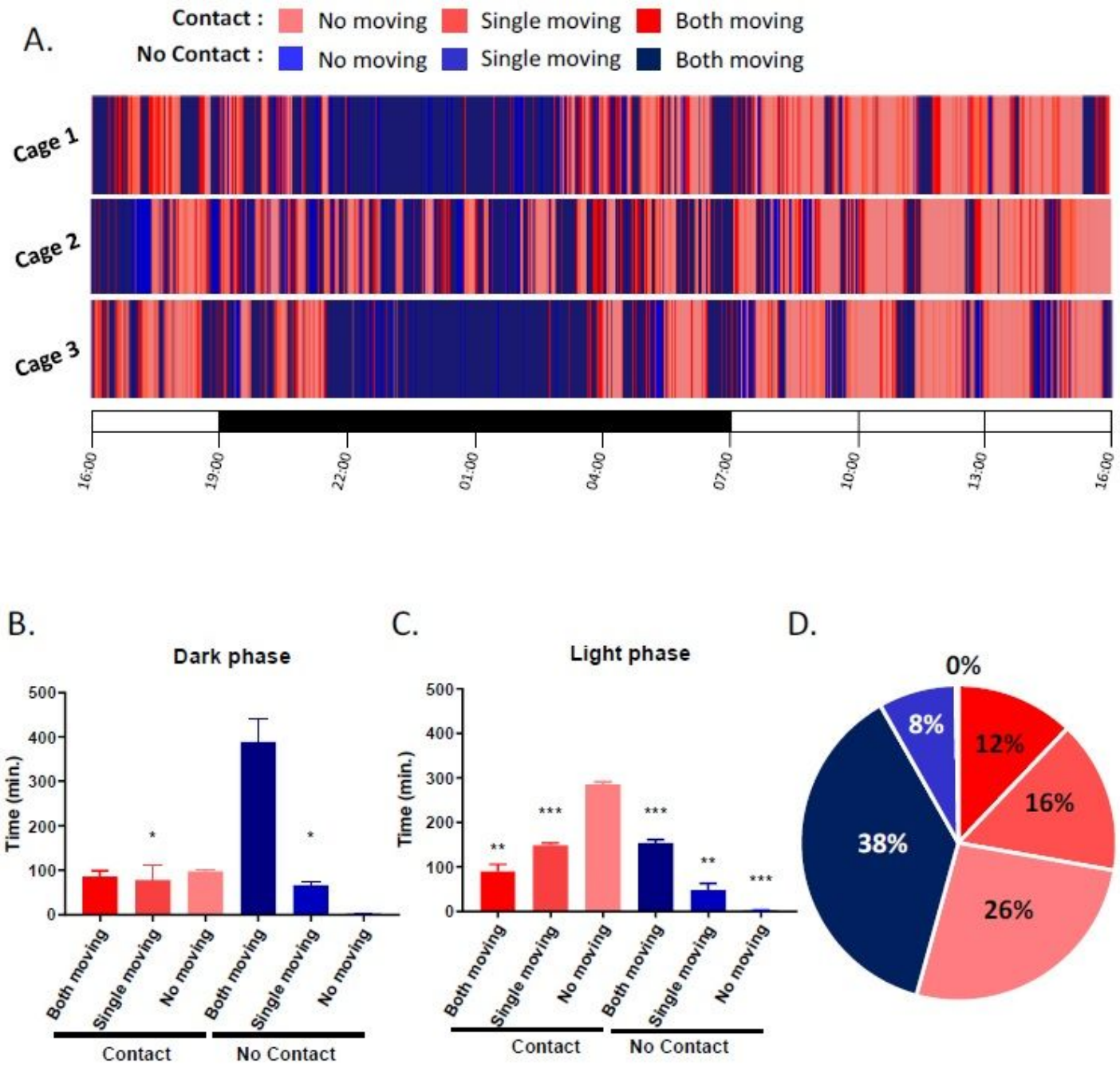

Figure 1

Mice spent half a day physically contacting each other. (A) A raster plot showing physical interactions of pair-housed mice in 24 hours. (B) The total time for specific interactions between two mice in the dark phase (Welch's ANOVA followed by Dunn's test, p values were determined by the comparison to Both moving-No contact). (C) The total time for specific interactions between two mice in the light phase (Welch's ANOVA followed by Dunn's test, p values were determined by the comparison to No moving-

Contact). (D) The percentage of time for specific interactions between two mice in 24 hours. * = p < 0.05, ** = p < 0.01, *** = p < 0.001; n=3 pairs; Mean ± S.E.M.

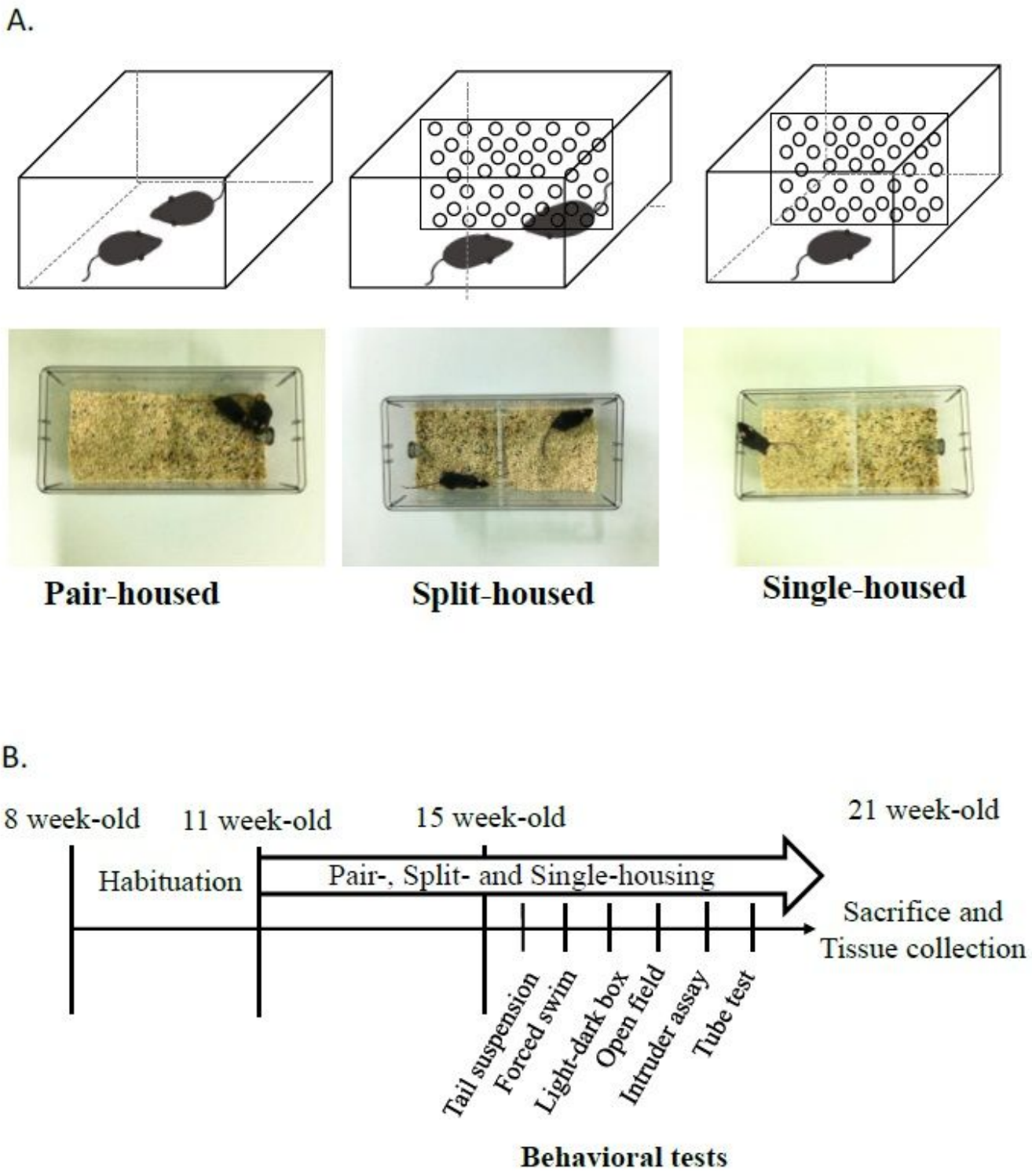

Figure 2

The three different housing conditions and the experimental schedule. (A) Male mice were housed in three different conditions: pair, split, and single housing. (B) Schematic diagram of the experimental schedule.

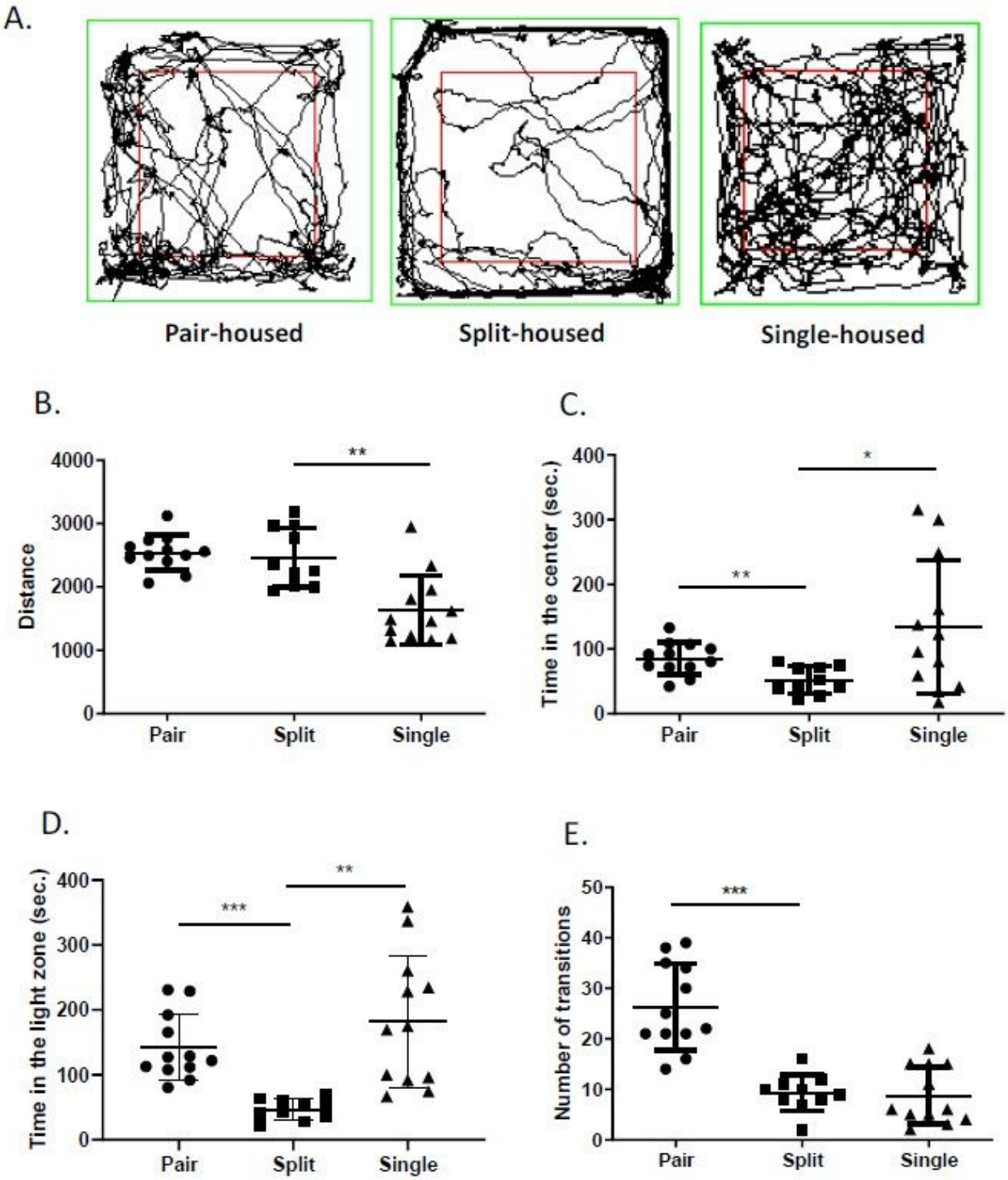

Figure 3

Mice housed in divided cages showed increased anxiety-like behavior. (A) Representative traces of mice in the open field. (B) Travel distances in the open-field test. (C) Time spent in the inner zone in the open-field test. (D) Time spent in the light zone during the light-dark box test. (E) The number of transitions between the light and dark zones. Welch's ANOVA followed by Dunn's test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; n=10-12 per group; Mean ± S.E.M.

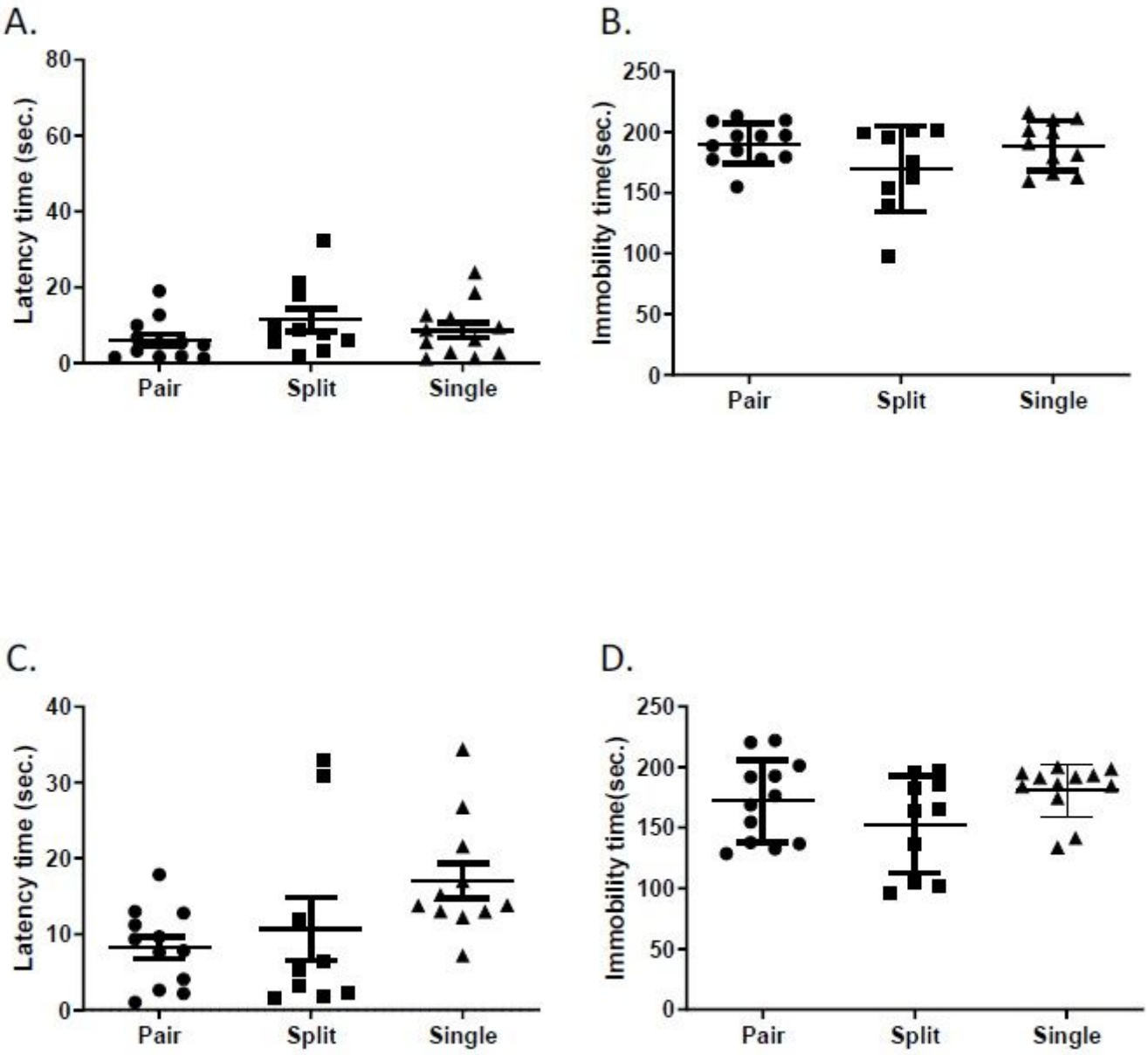

Figure 4

Depression-like behavior was similar among mice housed in different conditions. (A) Latency to the first bout of immobility in the forced swim test. (B) The total immobility time in the forced swim test. (C) Latency to the first bout of immobility in the tail suspension test. (D) The total immobility time in the tail suspension test. Welch's ANOVA followed by Dunn's test; n=10-12 per group; Mean ± S.E.M.

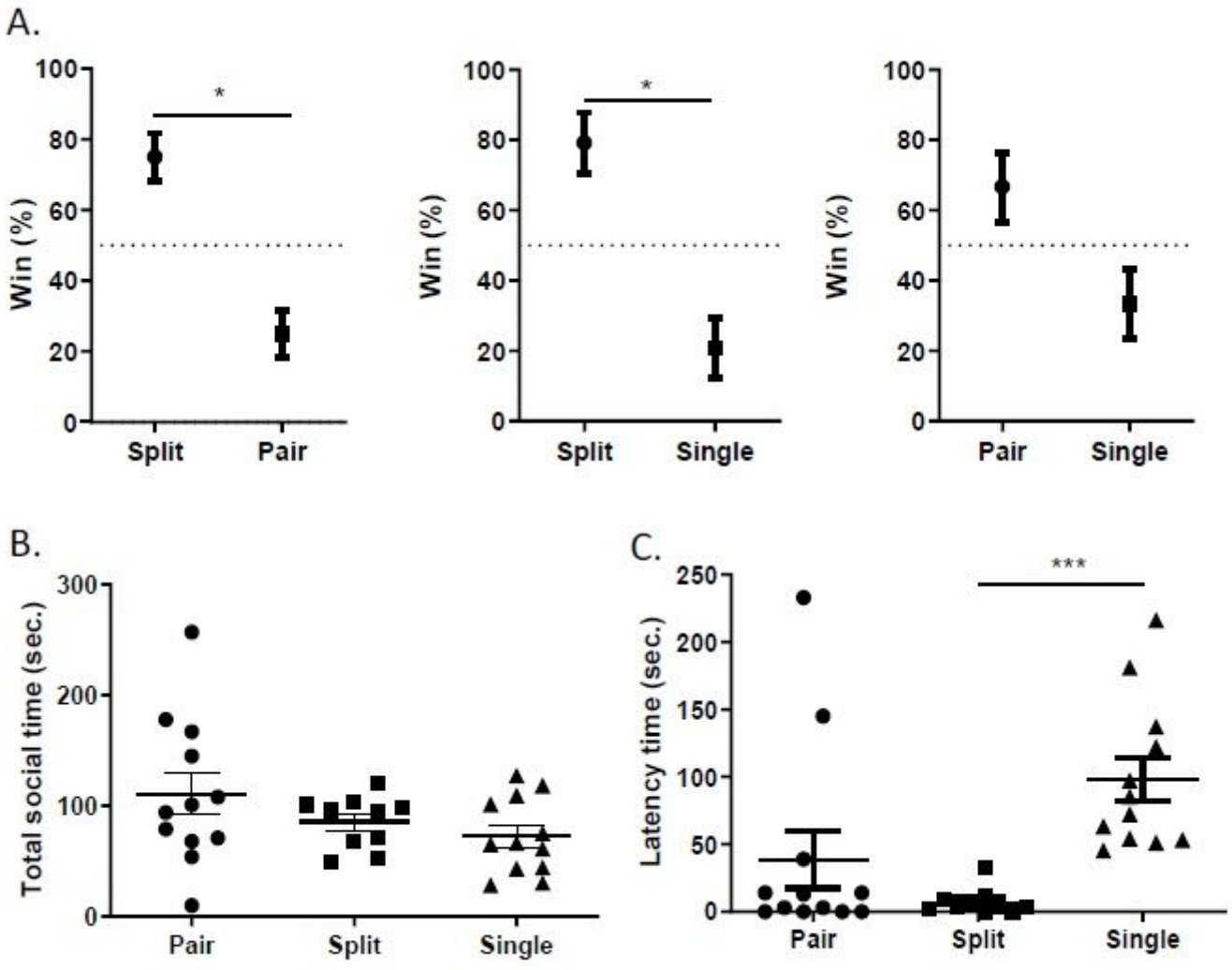

Figure 5

Mice housed in divided cage showed changes in social behaviors. (A) Social dominance evaluated by pairwise comparison in the tube test (Wilcoxon signed-rank test, n=10-12 pairs). (B) The total duration of social interaction in the intruder assay. (C) The latency to physical interaction in the intruder assay. Welch's ANOVA followed by Dunn's test; * = $p < 0.05$, *** = $p < 0.001$; n=10-12 per group; Mean ± S.E.M.

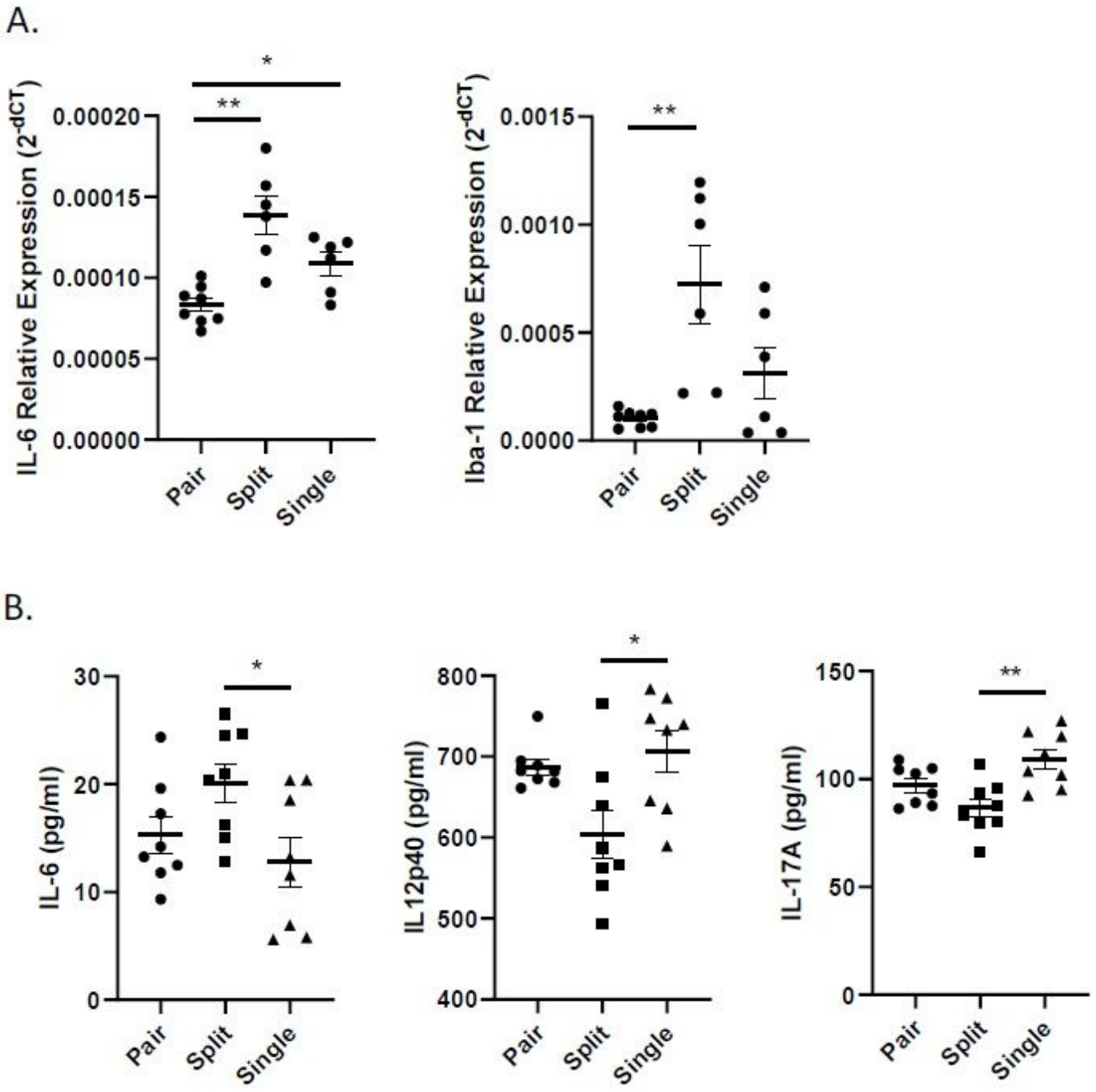

Figure 6

There were higher levels of cytokine IL-6 in the hippocampus and the serum of mice housed in divided cages. (A) The expression levels of cytokine IL-6 and Iba-1 in hippocampus (n = 6-8 per group) (B) The serum concentrations of cytokines IL-6, IL-12p and IL-17A. (n = 8 per group). Welch's ANOVA followed by Dunn's test; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$; Mean ± S.E.M.