Social isolation attenuates long-term behavioral and inflammatory effects of acute illness

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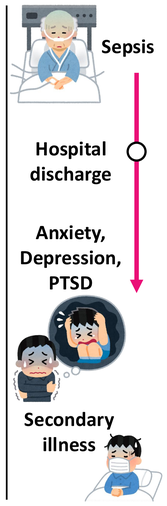
INTRODUCTION

- Critical illness survivors often experience long-term **mortality** and susceptibility to **secondary infection**. After physiologic recovery, up to one-sixth of critical illness survivors may experience **neuropsychiatric sequelae**, including anxiety and social withdrawal[5]. Animal studies have linked some of these sequelae to immune reprogramming and persistent inflammation in illness survivors[2]. Our lab demonstrated that the cytokine **lipocalin-2 (LCN2)** is highly upregulated in murine survivors of acute infection and mediates long-term anxiety-like behavior[7].
- Independently, **social isolation** is also linked to poor health outcomes, including anxiety and depression. Animal studies similarly show that social isolation alters **anxiety-like, depressive-like, and social behaviors**[4,6]. Social isolation alters inflammatory pathways, a potential mechanism for its adverse behavioral effects[1]. On the other hand, animal studies suggest a potential beneficial effect of social isolation to enhance bacterial clearance[3].
- How social isolation influences the long-term inflammatory and behavioral sequelae of acute illness is not well understood.

Objectives and Hypothesis:

The goal of this study was to test the hypothesis that social isolation during recovery from acute infection in mice modulates long-term anxiety-like and social behaviors, as well as systemic inflammatory response to a secondary immune challenge.



METHODS

Animals:

Adult (10-12 weeks) C57BL/6J mice (n=40, half female) received from Jackson Laboratories in groups of 5.

Illness Induction and Recovery:

Cecal slurry (CS) is a murine model of acute infection that induces polymicrobial peritonitis. Cecal slurry was prepared from the cecal contents of C57BL/6J mice.

Mice received intraperitoneal injection of either CS or saline (VEH) (n=20 each) on Day 0. For resuscitation, all mice received intraperitoneal injections of 25 mg/kg metronidazole + 75 mg/kg ceftriaxone and subcutaneous injections of 1 mL 37ºC saline starting 5 hours after CS , twice a day for 5 days. Body temperature and weight were recorded at every injection session.

Social Isolation Procedure:

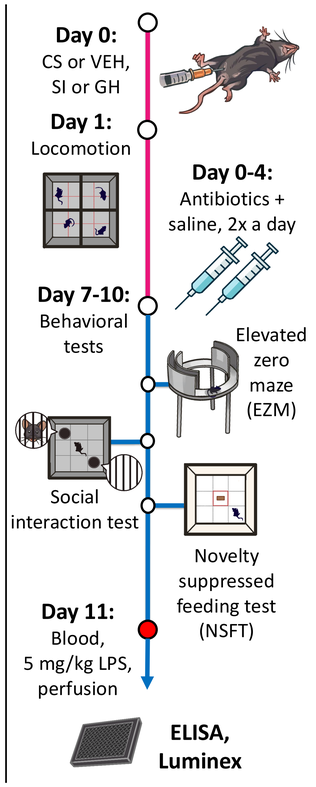
At the time of CS or VEH injection, mice were either maintained in group housing (GH) or socially isolated (SI) into single cages (n=20 each). All mice from the same original cage received the same CS or VEH treatment.

Molecular Assays:

Plasma samples were collected just prior to and 3 hours after a single 5 mg/kg lipopolysaccharide (LPS) injection on Day 11 and used to assess the effect of SI and CS on circulating cytokine levels after a secondary insult.

ELISA – lipocalin-2 (LCN2)

Luminex – interferon-gamma (IFNg), CXC motif chemokine ligand 1 (CXCL-1), interleukin-12 (IL-12), granulocyte-macrophage colony stimulating factor (GM-CSF), P-selectin, CC motif chemokine 11 (CCL-11)



RESULTS

SI during CS recovery does not alter patterns of weight or temperature change induced by CS

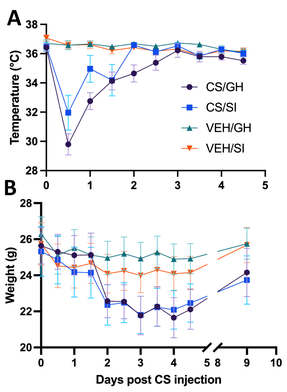


Figure 1. **(A)** Body temperature and **(B)** weight throughout CS recovery.

CS induces acute locomotion deficits that are attenuated by SI

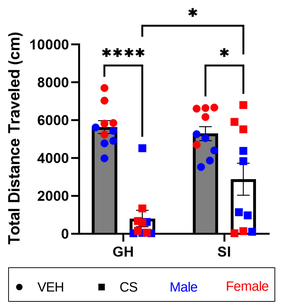


Figure 2. Distance traveled in the locomotion test on Day 1.

SI attenuates CS-induced reduction in exploration in the EZM

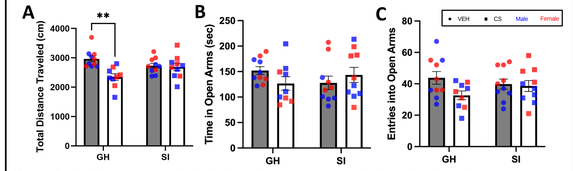


Figure 3. **(A)** Distance traveled, **(B)** time in open arms, and **(C)** entries into the open arms.

SI increases anxiety-like behavior in the NSFT

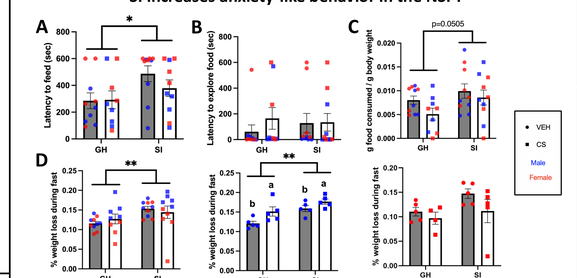


Figure 4. Latency to **(A)** feed and **(B)** explore the food pellet in the arena. **(C)** Normalized food intake during home cage trial. **(D)** Percent weight loss during prior 24 hour fast.

SI mitigates social preference in the social interaction test

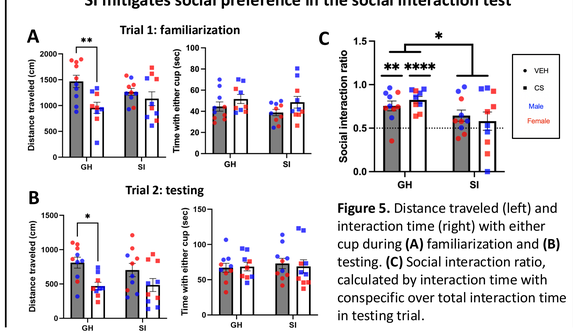


Figure 5. Distance traveled (left) and interaction time (right) with either cup during **(A)** familiarization and **(B)** testing. **(C)** Social interaction ratio, calculated by interaction time with conspecific over total interaction time in testing trial.

Acute immune challenge with LPS exaggerates increase in circulating LCN2 in CS mice, which is attenuated by SI

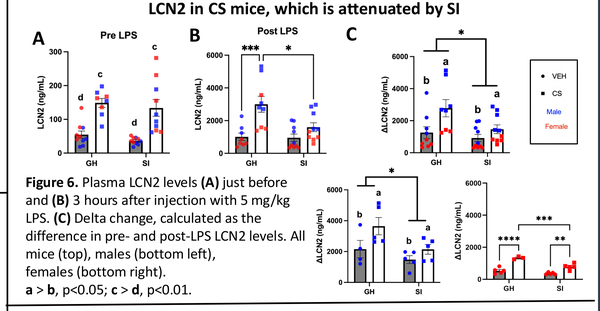


Figure 6. Plasma LCN2 levels **(A)** just before and **(B)** 3 hours after injection with 5 mg/kg LPS. **(C)** Delta change, calculated as the difference in pre- and post-LPS LCN2 levels. All mice (top), males (bottom left), females (bottom right).
a > b, p<0.05; **c > d**, p<0.01.

CS alters the inflammatory response to an LPS rechallenge

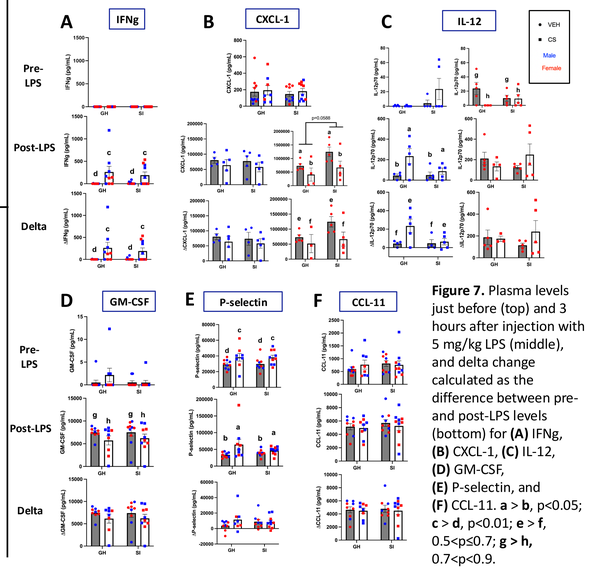


Figure 7. Plasma levels just before (top) and 3 hours after injection with 5 mg/kg LPS (middle), and delta change calculated as the difference between pre- and post-LPS levels (bottom) for **(A)** IFNg, **(B)** CXCL-1, **(C)** IL-12, **(D)** GM-CSF, **(E)** P-selectin, and **(F)** CCL-11. **a > b**, p<0.05; **c > d**, p<0.01; **e > f**, 0.5<p≤0.7; **g > h**, 0.7<p<0.9.

CONCLUSIONS

Summary

- SI protected against both **acute and chronic locomotor changes** induced by CS.
- SI **increased anxiety-like behavior** and **decreased social preference** independently of CS
- Prior CS exposure induced **persistent systemic elevation of LCN2** and **potentiated the LCN2 response** to a secondary immune challenge, an effect that was **attenuated by SI**.

Conclusions

- SI experienced concurrently with infection may **acutely diminish illness severity** in mice, conferring a biologically adaptive effect.
- SI **modulates social and affective behaviors independently** of its additional effect to rescue other CS-induced behavioral changes.
- The **LCN2 pathway may be uniquely involved** in the long-term effects of SI on behavior and its attenuation of CS-related behavioral changes.

ACKNOWLEDGEMENTS

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REFERENCES

1. Al Omran AJ, Shao AS, Watanabe S, Zhang Z, Zhang J, Xue C, Watanabe J, Davies DL, Shao XM, Liang J (2022) Social isolation induces neuroinflammation and microglia overactivation, while dihydromyricetin prevents and improves them. Journal of Neuroinflammation 19:2.
2. Denstaedt SJ, Spencer-Segal JL, Newstead M, Laborc K, Zeng X, Standiford TJ, Singer BH (2020) Persistent neuroinflammation and brain specific immune priming in a novel survival model of murine pneumosepsis. Shock 54:78–86.
3. Hamilton A, Rizzo R, Brod S, Ono M, Perretti M, Cooper D, D'Acquisto F (2022) The immunomodulatory effects of social isolation in mice are linked to temperature control. Brain, Behavior, and Immunity 102:179–194.
4. Ieraci A, Mallei A, Popoli M (2016) Social Isolation Stress Induces Anxious-Depressive-Like Behavior and Alterations of Neuroplasticity-Related Genes in Adult Male Mice. Neural Plast 2016:6212983.
5. Prescott HC, Angus DC (2018) Enhancing Recovery From Sepsis. JAMA 319:62–75.
6. Smolensky I, Zajac-Bakri K, Mallien AS, Gass P, Guzman R, Inta D (2024) Effects of single housing on behavior, corticosterone level and body weight in male and female mice. Laboratory Animal Research 40:35.
7. Yang SE, Johnston C, Laborc K, Kounelis-Wuillaume S, Schwieterman W, Hill AR, Spencer-Segal JL (2023) THU117 Lipocalin-2 As A Mediator Of Negative Affect After Sepsis. Journal of the Endocrine Society 7:bvad114.1195.