Prior episode of colitis impairs contextual fear memory

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Abstract
Accumulating evidence has shown that intestinal inflammations in inflammatory bowel disease (IBD) also drive pathological responses in organs outside the intestine, including the brain. Previous studies using the dextran sodium sulfate (DSS)-induced colitis model have shown that colonic inflammation contributes to the development of anxiety- and depression-related behaviors; however, little is known about whether memory function is affected. Here, we subjected male and female C57BL/6J mice to DSS-induced colitis for 6 days, followed by Pavlovian conditioned fear (CF) tests 15 days after the start of inflammation, when local colonic inflammation has receded. The contextual and cued CF tests were used to assess associative fear memory. We found that DSS-induced colitis led to significant impairment in contextual fear memory in both male and female mice; on the other hand, auditory cued fear memories were comparable between control and DSS-treated mice. There were marked signs of astrogliosis in the hippocampal regions 17 days (D17) after colitis induction. Furthermore, molecular characterization of hippocampi showed marked but transient increases in the expression of inflammatory genes Nfkb, Trem2 (microglial marker), GFAP (astrocyte marker), Il1b, and S100a8 in DSS-treated mice. While the expression of Nfkb, Trem2, and GFAP showed a peak on day 10, the S100a8 expression was high on days 10 and 17 and subsided on day 42. Interestingly, expression of Bdnf remained elevated in the times assessed (D10, 17, 42). Together, these results demonstrated that DSS-induced colitis could induce prolonged neuroinflammation and impaired contextual fear memory.

Keywords: Inflammatory bowel disease, Ulcerative colitis, Conditioned fear, Contextual fear memory

Main text
The prevalence of inflammatory bowel disease (IBD), a chronic inflammatory condition of the gastrointestinal tract, continues to rise [1]. IBDs, including Crohn's disease and ulcerative colitis, are chronic conditions that cycle between periods of active flare and remission. In addition to primary pathologies affecting the intestine, IBD has been linked to neuroinflammation and affects emotional functions including depression and anxiety [2].

To model IBD in rodents, dextran sulfate sodium (DSS)-induced colitis has been widely used, which elicits intestinal pathologies similar to human ulcerative colitis [3]. Previous studies have shown that DSS-induced colon inflammation led to increased brain excitability and neuroinflammatory phenotype, including the transcriptional increase of pro-inflammatory genes, and infiltration of monocytes and neutrophils [4–6]. While DSS-induced colitis has been shown to affect stress-related behaviors and increase anxiety- and depression-like behaviors [5–8], little is known about whether a prior episode of colitis affects memory function.

In the present study, we subjected male and female C57BL/6J mice to DSS-induced colitis for 6 days, followed by Pavlovian conditioned fear (CF) tests 15 days after the start of colitis induction (Fig. 1a), when local colonic inflammation had receded. The contextual and cued fear conditioning tests are widely used to evaluate associative fear learning and memory [9]. Given the
importance of the gut microbiome in contributing to the pathogenesis of ulcerative colitis, experimental mice were maintained on semi-purified OpenSource diets D12450J (Research Diets Inc.) to ensure consistency and reproducibility of the induced disease course. Mice received normal drinking water (control group) or DSS (2%) (MP Biomedicals; 36–50 kDa) in drinking water for 6 consecutive days to induce acute colitis, then switched back to normal drinking water. All mice were assessed for body weight, fecal consistency, and macroscopic fecal blood scores; detailed methods are described in Additional file 1. Experimental procedures were approved by the animal care committee of Texas A&M University.

Mice given 2% DSS for 6 days exhibited significant disease activities (composite score of fecal consistency and macroscopic fecal blood scores) and recovered gradually after cessation of DSS (Fig. 1b). Female C57BL/6J mice developed more severe disease symptoms at days 4–6 compared to male mice, but recovered to similar levels after cessation of DSS. On training day, mice received a mild foot shock conditioned with tone minus percent freezing during the tone presentation. 24 h later, mice were tested for contextual fear recall and placed in the same chamber for 5 min. Freezing behavior is used as an index of fear memory recall. Some male mice showed cued fear response below the 5% threshold and were excluded from data analysis, while all female mice responded. Overall, freezing behaviors for auditory cues were comparable between control and DSS-treated mice (Fig. 1d).

It has been shown that contextual information is encoded by neurons in the hippocampus and conveyed directly to the amygdala, which generates conditioned fear responses [10, 11]. Next, we assessed astrogliosis as an indication of neuroinflammation [12], performing immunostaining of the astroglial protein GFAP (glial fibrillary acidic protein) on brain tissues collected on day 17. Astroglisoi was determined by increased GFAP expression with hypertrophic morphology (enlarged cytoplasmic area and thickness of processes) [12]. We found increased GFAP-positive cells with hypertrophic morphology, resembling reactive astrocytes, in the hippocampus of DSS-treated mice (Fig. 1e). To further explore the temporal changes of the neuroinflammatory response to colonic inflammation, we collected hippocampi from control and DSS-treated mice at day 10, 17 and 42 after the colitis induction. Quantitative PCR data demonstrated that expression of inflammatory genes *Nfkb*, *Trem2*, *Gfap*, *Il1b*, *S100a8*, and *Bdnf* in the hippocampus collected from control and DSS-treated mice on Day 10, 17, and 42 (respectively; n = 4 per group). One-way ANOVA followed by Tukey’s multiple comparisons test, *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. All data were presented as mean±SD, except for 1b where disease scores were presented as mean±SEM, for clarity.
In conclusion, our study showed that contextual memory function was negatively impacted by an episode of colitis, with prolonged neuroinflammation in the hippocampal regions. Further work is required to determine mechanistically the interactions between innate neuroinflammatory response and neurons encoding
specific inputs to hippocampal-amygdala neurocircuit to affect contextual fear memory [11]. Of note, clinical functional MRI data showed that patients with active-stage ulcerative colitis exhibited decreased hippocampal/para-hippocampal activity that correlated with memory loss [14]. Overall, our data suggest that in addition to clinical management of the symptoms of IBD, other strategies to monitor and reduce neuroinflammation may need to be considered to prevent potential progression to chronic disease conditions such as dementia or neurodegenerative diseases, given that IBD patients are at increased risk for neurodegenerative diseases including Parkinson’s disease and dementia [15].

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13041-022-00961-4.

Additional file 1. Experimental Methods.

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Author contributions

CSW designed and supervised the experiments. CSW and VE performed the experiments and data analysis. CSW wrote the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

The detailed methods are described in the Additional file 1. All data and materials are available from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

All animal work was performed in compliance with the Institutional Animal Care and Use Committee (IACUC) at Texas A&M University (protocol approval numbers 2019-0273, 2019-0444).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. GBD Inflammatory Bowel Disease Collaborators. The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Gastroenterol Hepatol. 2020;5(1):17–30.
2. Mikocka-Walus A, Knowles SR, Keefet L, Graff L. Controversies revisited: a systematic review of the comorbidity of depression and anxiety with inflammatory bowel diseases. Inflamm Bowel Dis. 2016;22(3):752–62.
3. Czarnecki P, Parigi SM, Sorini C, Diaz OE, Das S, Gagliani N, et al. Conserved transcriptomic profile between mouse and human colitis allows unsupervised patient stratification. Nat Commun. 2019;10(1):2892.
4. Talley S, Valiauga R, Anderson L, Cannon AR, Choudhry MA, Campbell EM. DSS-induced inflammation in the colon drives a proinflammatory signature in the brain that isameliorated by prophylactic treatment with the S100A9 inhibitor paquimodium. J Neuroinflamm. 2021;18(1):263.
5. Nyuuki KD, Cluny NL, Swain MG, Sharkey KA, Pittman QJ. Altered brain excitability and increased anxiety in mice with experimental colitis: consideration of hyperalgesia and sex differences. Front Behav Neurosci. 2018;12:58.
6. Gadotti VM, Andonegui G, Zhang Z, MD’Aloha S, Baggio CH, Chen L, et al. Neuroimmune responses mediate depression-related behaviors following acute colitis. iScience. 2019;16:12–21.
7. Reichmann F, Hassan AM, Farzi A, Jain P, Schuligoi R, Holzer P. Dextran sulfate sodium-induced colitis alters stress-associated behaviour and neuropeptide gene expression in the amygdala-hippocampus network of mice. Sci Rep. 2015;5:9970.
8. Gadotti VM, Zamponi GW. Anxiolytic effects of the flavonoid luteolin in a mouse model of acute colitis. Mol Brain. 2019;12(1):114.
9. Maren S. Neurobiology of Pavlovian fear conditioning. Annu Rev Neurosci. 2001;24:897–931.
10. Xu C, Krabbe S, Grunendamm J, Botta P, Fadok JP, Osakada F, et al. Distinct hippocampal pathways mediate dissociable roles of context in memory retrieval. Cell. 2016;167(4):961–72.
11. Kim WB, Cho JH. Encoding of contextual fear memory in the hippocampal-amygdala circuit. Nat Commun. 2020;11(1):1382.
12. Pelny M, Pelka M. Astrocyte reactivity and reactive astrogliosis: costs and benefits. Physiol Rev. 2014;94(4):1077–98. 13. Lodeiro M, Puerta E, Ismail MA, Rodriguez-Rodriguez P, Ronnback A, Codita A, et al. Aggregation of the inflammatory S100A8 preceeds Abeta plaque formation in transgenic APP mice: positive feedback for S100A8 and Abeta productions. J Gerontol A Biol Sci Med Sci. 2017;72(3):219–28.
14. Fan W, Zhang S, Hu J, Liu B, Wen L, Gong M, et al. Aberrant brain function in active-stage ulcerative colitis patients: a resting-state functional MRI study. Front Hum Neurosci. 2019;13:107.
15. Zhang B, Wang HE, Bai YM, Tsai SJ, Su TP, Chen TJ, et al. Inflammatory bowel disease is associated with higher dementia risk: a nationwide longitudinal study. Gut. 2021;70(1):85–91.

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