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Sepsis survivor mice exhibit a behavioral endocrine syndrome with ventral hippocampal dysfunction

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ABSTRACT

Severe acute stressors are known to trigger mood disorders in humans. Sepsis represents one such stressor, and survivors often suffer long term from psychiatric morbidity. We hypothesized that sepsis leads to lasting changes in neural circuits involved in stress integration, altering affective behavior and the stress response. To investigate this hypothesis, sepsis was induced in male C57Bl/6 mice using cecal ligation and puncture (CLP), and control mice underwent sham surgery. Mice recovered from acute illness within 2 weeks, after which they exhibited increased avoidance behavior and behavioral despair compared with sham, with behavioral changes observed more than 5 weeks after recovery. Sepsis survivors also showed evidence of enhanced hypothalamic-pituitary-adrenal (HPA) axis activity, with increased corticosterone after a novel stressor and increased adrenal weight. In the brain, sepsis survivor mice showed decreased stress-induced cfos mRNA and increased glucocorticoid receptor immunoreactivity specifically in the ventral hippocampus, a brain region known to coordinate emotional behavior and HPA axis activity. We conclude that murine sepsis survivors exhibit a behavioral neuroendocrine syndrome of negative affective behavior and HPA axis hyperactivity, which could be explained by ventral hippocampal dysfunction. These findings could contribute to our understanding of the human post-intensive care syndrome.

1. Introduction

While major depression and other mood disorders are highly heterogeneous with numerous triggers (Akil et al., 2018), an understudied area is the role of severe medical illness in triggering these disorders. Sepsis, a severe systemic response to infection, affects more than 14 million patients in the world every year (Prescott et al., 2019). Though mortality from sepsis is high, the majority of patients survive. Unfortunately, the majority of survivors suffer from long-term physical, cognitive, and emotional morbidity (Prescott and Angus, 2018). Indeed, more than one third of survivors will suffer long-term from negative emotion, with psychiatric diagnoses including anxiety, depression, and post-traumatic stress disorder (Davydow et al., 2013a, 2013b; Jackson et al., 2014). New and persistent psychiatric illness in the growing global population of sepsis survivors is thus a major public health concern without effective strategies for prevention or treatment.

In the mouse, sterile sepsis induction with LPS injection, as well as the induction of polymicrobial sepsis with cecal ligation and puncture (CLP), led to anxiety-like behavior in survivors (Anderson et al., 2016, 2015; Calsavara et al., 2013; Neves et al., 2018). This suggests that mice also suffer from lasting negative affect after sepsis, and further study of this phenomenon may provide some insight into the mechanisms of neuropsychiatric morbidity in murine and human survivors.

We hypothesized that sepsis, like other acute and chronic stressors, leads to lasting changes in neural circuits involved in stress integration, measurable as changes in affective behavior and the stress response. While the effects of acute and persistent critical illness on the hypothalamic-pituitary-adrenal (HPA) axis have been well characterized (Téblick et al., 2019), alterations in HPA axis functioning in critical illness survivors have not been well studied, and their relationship to psychiatric symptoms is unknown.

To investigate this hypothesis, we studied murine survivors of CLP or sham surgery to establish their affective and endocrine phenotype. We found that after recovery from their acute illness, sepsis survivor mice displayed negative affective behavior, including avoidance in the open field and immobility in the tail suspension test. Sepsis survivor

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mice also displayed evidence of HPA axis hyperactivity. To begin to understand which neural circuits might be altered in sepsis survivors that could contribute to their behavioral and endocrine phenotype, we measured swim-induced cfos as a reflection of neural activity in multiple candidate brain regions and found decreased cfos mRNA specifically in the ventral hippocampus in sepsis survivors. The ventral hippocampus is known to coordinate both emotional behavior and the stress response, and therefore changes in its function could mediate the behavioral endocrine syndrome in sepsis survivor mice. Because this brain region is responsive to glucocorticoids, we analyzed glucocorticoid receptor (GR) immunoreactivity in sepsis survivors and found an increase in GR immunolabeling in the ventral hippocampus.

Our data suggest that sepsis survivor mice exhibit ventral hippocampal dysfunction alongside a phenotype involving known functions of this brain region, affective behavior and HPA axis regulation. The unique sensitivity of the ventral hippocampus to the lasting effects of sepsis and other acute inflammatory stressors is novel, and could underlie components of the human post-intensive care syndrome.

2. Methods and materials

2.1. Animals

Young adult 7–9 week old C57BL6 male mice were obtained from the Jackson Laboratory. Animals were group-housed on a 14:10 light/dark cycle with free access to food and water throughout the experiments. All experiments were approved by the University of Michigan Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Cecal ligation and puncture

Mice were anesthetized with ketamine and xylazine. Under aseptic conditions, a 1 − 2 cm laparotomy was performed. The cecum was ligated with a silk suture, and punctured through-and-through with a 19 gauge needle. The incision was closed with surgical clips. Sham mice underwent laparotomy, and the cecum was removed from the abdominal cavity and replaced without puncture. All mice were treated with imipenem/cilastatin (0.5 mg per mouse) and 0.5 mL normal saline immediately after surgery. The University of Michigan policy for humane endpoints was followed. Mortality from CLP was < 20 % (Fig. 1B).

2.3. Power calculations

Based on our experience with expected variability in behavior among mice, we calculated that we would require at least 8 animals per group to detect a 30 % decrease in the time-in-center variable in the CLP group with 80 % power. For the behavior experiments, CLP groups were larger than sham groups to accommodate unexpected variability in mortality and the possibility of increased behavioral variability in the CLP group.

For the histology experiments, we calculated that we would require at least 4 animals per group to detect a 50 % change in any histologic endpoint (cfos mRNA, GR immunolabeling) with 80 % power, assuming a standard deviation of 25 % of the sham group mean.

2.4. Behavior

Locomotion testing was performed over 5-minute sessions using VersaMax locomotion chambers (Omnitech Electronics, Columbus, OH). For open field testing, the mouse was allowed to freely explore a novel square open field 72 cm on each side and 36 cm high over 5 min. The forced swim test was conducted over 2 consecutive days, each day consisting of a 6-minute swim in beakers of 22 °C water. Analysis was based on the second day. The tail suspension test was performed by securing each mouse to a horizontal metal rod by its tail with tape, so that the mouse was suspended 20 cm above the table surface for 6 min. Small cardboard tubes were used to cover the tail to prevent tail climbing. Behavior tracking and data analysis was performed using VersaMax (Omnitech Electronics, Columbus, OH) or Ethovision 11.5 (Noldus, Inc., Leesburg, VA) software. Different mice were used for behavior (open field/tail suspension) and blood collection experiments so that the stress of repeated blood collection would not interfere with behavioral testing.

2.5. Blood collection

Mice were warmed for 1 − 2 min under an infrared lamp to dilate the tail veins. With the assistance of a tail vein restrainer (Braintree Scientific, Cambridge, MA), the tail vein was identified, and the area cleaned with ethanol and incised using a razor blade. Blood was collected using Microvette collection tubes (Braintree Scientific, Cambridge, MA) within 60 s of tail vein incision, and pressure was applied to ensure hemostasis prior to returning the mouse to the home cage.

2.6. Tissue collection

Mice were euthanized 30 min after the completion of forced swimming by rapid decapitation and trunk blood was collected. Brains were removed and immediately snap frozen on dry ice. Brains were stored at − 80 °C until future use. Adrenal glands were dissected, fat removed, and weighed.

2.7. Hormone assays

All blood samples were centrifuged at 4000 rpm for 10 min. The supernatant was collected and frozen at − 20 °C until further use. Corticosterone was measured using the Corticosterone DetectX Enzyme Immunoassay kit (Arbor Assays, Ann Arbor, MI).

2.8. In situ hybridization

The cfos probe was a 661 nucleotide probe against the mouse cfos mRNA coding region developed in our laboratory (NCBI reference sequence NM_010234, nucleotides 508–1175). Specificity was confirmed using the sense probe. To make the labeled riboprobe, a reaction mixture consisting of linearized plasmid, transcription buffer, [^{35}S]UTP, [^{35}S]ATP, 150 CTP and GTP, dithiothreitol, RNase inhibitor, and Polymerase was incubated at 37 °C for 90 min, then with added DNase for 15 min at room temperature. The probes were separated from unincorporated nucleotides using a Biorad Micro Bio-Spin 6 Chromatography column, diluted in water with dithiothreitol (DTT), and counted on a beta counter. The probes were diluted in hybridization buffer (containing 50 % formamide, 10 % dextran sulfate, 3 × SSC, 50 mm sodium phosphate buffer, pH 7.4, 1× Denhardt's solution, 0.1 mg/mL yeast tRNA, and 10 mm DTT) to yield 2 × 10^6 dpm/70 µl.

Sections (20 µm) were cut on a cryostat and direct mounted onto Superfrost Plus slides (Fisher Scientific). Sections were fixed in 4% paraformaldehyde for 1 h, then washed 5 times in 2X SSC (300 mM NaCl/30 mM sodium citrate, pH 7.2). They were then placed in a solution containing acetic anhydride (0.25 %) in triethanolamine (0.1 M), pH 8.0, for 10 min, rinsed in distilled water, dehydrated with graded ethanol washes, and air dried. Sections were then hybridized at 55 °C overnight with the labeled cRNA probes. Following hybridization, coverslips were removed and the slides were washed 5 times in 2X SSC, incubated for 1 h in RNaseA at 37 °C, washed in descending concentrations of SSC and incubated in 0.1X SSC at 60 C for 60 min. Slides were then rinsed in distilled H_2O, dehydrated through graded ethanol washes, air-dried, and exposed to film for 20 days.

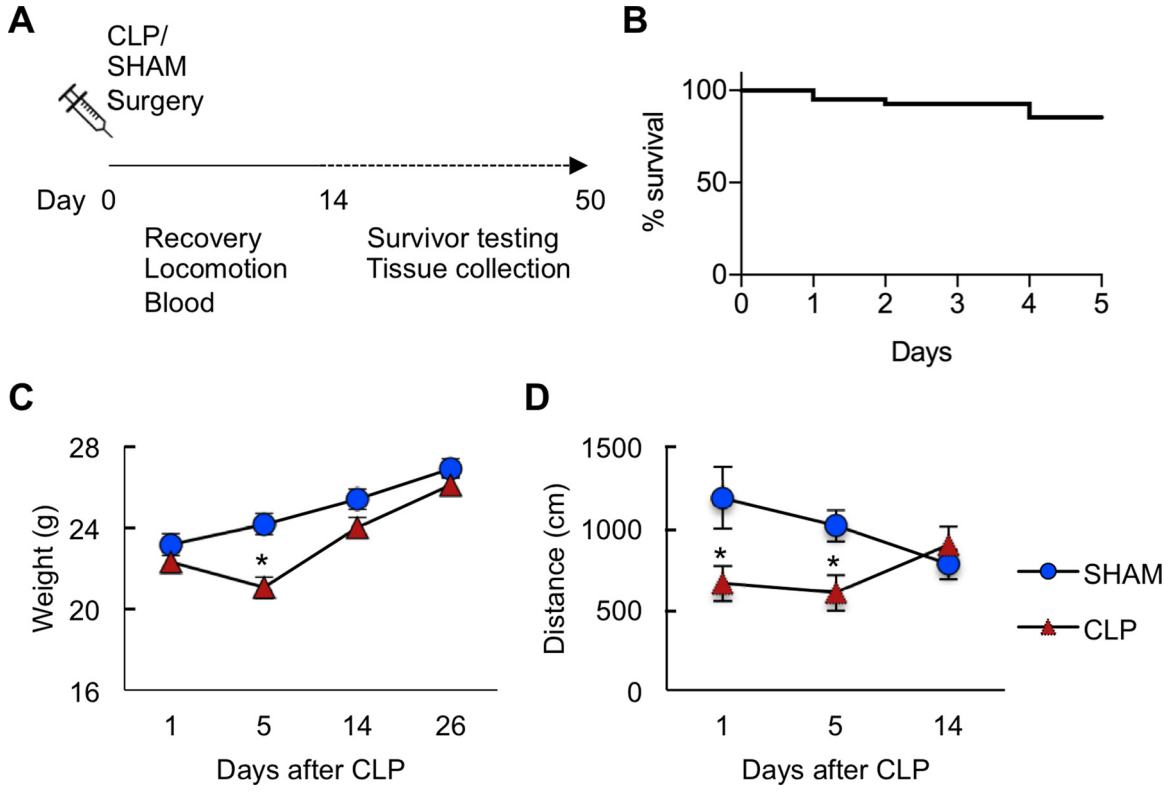

Fig. 1. CLP induces acute illness, and survivors recover within 14 days. A, schematic of the experimental design; B, survival curve showing 85 % mortality in the CLP group through day 5 (N = 41). Weight (C) and locomotion (D) decrease after CLP, with recovery to sham level by 14 days. (*p < 0.05, N = 10 per group).

Films were developed and digitized using a ScanMaker 1000XL Pro flatbed scanner (Microtek) using Silverfast MicroTekIT8 software (version 6.6.2r4a). Images were analyzed using ImageJ Analysis Software for Mac (National Institutes of Health). Optical density measurements were collected for each brain region from the left and right sides of the brain. Background measurements, taken for each section and hemisphere separately, were from white matter with an absence of visible cfos labeling. For hippocampus, selections were taken from each subregion using the selection brush tool at a fixed width. Background values were subtracted from each measurement to yield a relative optical density measurement. For each of the other areas, a fixed- size and shape region of interest was used. Optical density values were corrected for background, then multiplied by the area sampled to produce the Integrated Optical Density (IOD) measurement. Measurements from the same brain region across multiple sections were averaged to produce a single data point for each brain region per animal. The data presented is from the following number of sections per animal: 4 for dorsal hippocampus, 2 for intermediate hippocampus, 3 for ventral hippocampus, 3 for paraventricular hypothalamus, 5–6 for prefrontal cortex, and 4 for amygdala.

2.9. Immunofluorescence

Mounted sections were fixed with 4 % paraformaldehyde for 10 min. After several rinses with Tris buffered saline (TBS), sections were blocked and permeabilized in blocking buffer (10 % NGS, 3 % BSA, 1 % glycine, 0.4 % Triton-X in TBS) for 1 h. The sections were incubated with monoclonal rabbit anti-glucocorticoid antibody (1:400; D8H2, Cell Signaling, Danvers, MA, US) in blocking buffer overnight at 4 °C. The next day, the slides were rinsed with TBS three times for 10 min each, followed by incubation with blocking buffer for 30 min. Then sections were incubated with Alexa 488 conjugated polyclonal goat anti-rabbit antibody (1:400; A-11034, Invitrogen, Carlsbald, CA, US) in TBS blocking buffer for 2 h. The slides were washed three times with TBS, and mounted with coverslip using Vectashield mounting medium containing 4′-6-diamino-2-phenylindole (Vector laboratories, Burlingame, CA).

2.10. Confocal imaging

Images were acquired on an Olympus FluoView 1000 confocal microscope using 10x/0.4 NA objective at 488 nm excitation (2048 × 2048 resolution) using the same light conditions and acquisition parameters for every animal and section. Optical density of labeling in each subregion was quantified using ImageJ. Background values from an adjacent area with minimal visible labeling were subtracted, and values for each subregion were averaged over several sections for each animal. No adjustments in brightness, contrast, or other imaging characteristics were made to representative group images used for figures.

2.11. Data analysis

For quantification of in situ hybridization and immunohistochemistry, the experimenter performing the quantification was blinded to the experimental conditions until the quantification was completed. Tissue sections from immunohistochemistry containing obvious artifact or very poor quality were not analyzed. These exclusions were made prior to data analysis, and the experimenter adjudicating this was blinded to experimental group. Statistical analyses were conducted using Prism Graphpad 8, with $p < 0.05$ considered significant. Sham and CLP groups were compared using Student's *t*-tests for behavior, corticosterone, and adrenal weights. For in situ hybridization, cfos mRNA optical density was compared between SHAM and CLP for each analyzed brain region using *t*-test (PVN) or two-way ANOVA (all other regions). We used the False Discovery Rate (FDR) method to correct for multiple comparisons (two-stage step-up method of Benjamini, Krieger and Yekutieli) with a desired FDR of 5%, and report the original p-values and FDR-adjusted p values (as the q value).

Two-way ANOVA was used to compare basal and stress corticosterone (with repeated measures testing where appropriate) and for GR immunoreactivity. Graphs were made in Prism and Microsoft Excel (Version 15/31) and figures were assembled in Microsoft Powerpoint (Version 15.31) and Adobe Photoshop CC 2018.

3. Results

3.1. Physiologic recovery from CLP occurs by day 14

To establish the timeline for physical illness and recovery after CLP, weight loss and locomotion were used to measure sickness behaviors of anorexia and lethargy, respectively. Fig. 1A shows the outline of the experiments. Overall survival from CLP was 85 %, with all deaths occurring within 5 days of surgery (Fig. 1B). There were no deaths in the sham group. CLP mice exhibited significant weight loss after surgery, but began to regain weight after day 5 and were indistinguishable from sham by day 14 (Fig. 1C). CLP animals also exhibited lethargy after CLP, with significantly less locomotion in the testing chamber on days 1 and 5 after surgery, but not at day 14 (Fig. 1D). Thus, mice exhibit sickness behavior after CLP that recovers to sham levels by day 14.

3.2. Sepsis survivor mice show increased negative affective behaviors

After physiologic recovery from sepsis, mice underwent tests of affective behavior (Fig. 2). The novel open field test was used to assay avoidance behavior by measuring total exploratory activity and the time in the center of a novel, brightly lit open field. Fourteen days after surgery, CLP survivor mice explored the open field less and spent less time in the center compared with sham mice (Fig. 2A and B). This difference in exploratory behavior was not due to ongoing sickness behavior, as there was no difference in exploration in the locomotion test in a familiar context (Fig. 1D). In contrast, there was no significant difference in open field exploration between sham and CLP mice 50 days after surgery (Fig. 2C and D). At this time point, mice also underwent a tail suspension test. In this test, immobility time is considered a measure of "behavioral despair," and decreases in response to antidepressant treatment. (Can et al., 2012) CLP mice spent significantly more time immobile than sham mice 50 days after surgery, suggesting increased behavioral despair in sepsis survivor mice.

3.3. Sepsis survivor mice show increased hypothalamic-pituitary-adrenal axis activity

The increase in avoidance behavior and behavioral despair seen here in sepsis survivor mice are similar to those seen after acute or chronic stress in other rodent models, which are associated with long-lasting disturbances in function of the hypothalamic-pituitary-adrenal (HPA) axis (Armario et al., 2008; McEwen et al., 2015). We measured basal- and swim stress-induced plasma corticosterone levels to determine the effect of CLP on the activity of the HPA axis (Fig. 3). Comparison of corticosterone levels days 1 and 5 following surgery showed a significant effect of time ($F(1, 59) = 7.5$, $p = 0.0079$), with no effect of surgery ($F(1,59) = 0.6$, $p = 0.44$), indicating that sham and CLP surgeries elevated circulating corticosterone levels to a similar extent (Fig. 3A). At 14 days and 50 days after surgery, repeated-measures ANOVA revealed a main effect of stress on corticosterone levels ($F(1,14) = 56.35$, $p < 0.0001$ for 14 days; $F(1,16) = 196.6$, $p < 0.0001$ for 50 days), indicating that stress elevated corticosterone levels as expected (Fig. 3B). At 50 days, there was no significant effect of surgery or interaction between stress and surgery on corticosterone levels ($F(1,16) = 1.5$, $p = 0.23$ for surgery; $F(1,16) = 0.74$, $p = 0.40$ for interaction). In contrast, at 14 days, there was no main effect of surgery ($F(1,14) = 4.5$, $p = 0.052$) but a significant interaction between stress

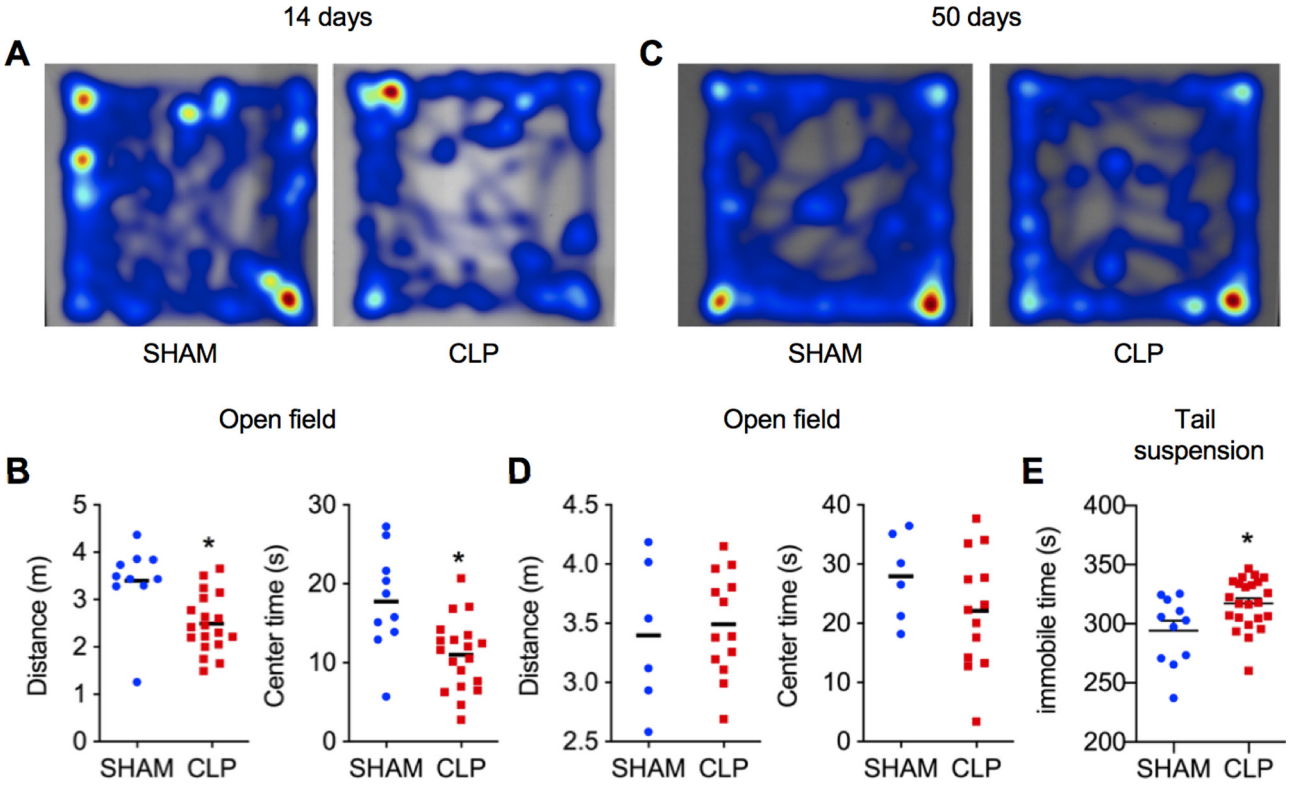

Fig. 2. CLP survivor mice show increased anxiety-like behavior and despair. 14 days after surgery, heat maps (A) with quantification (B) show decreased distance traveled and time spent in the center of a novel open field for CLP mice compared with sham (N = 10 for sham, 19 for CLP). *p < 0.05. 50 days after surgery, heat maps (C) and (D) quantification show no significant difference in behavior in the open field between CLP and sham (N = 6 for sham, 13 for CLP). 50 days after surgery, CLP mice show increased immobility in the tail suspension test (E). N = 11 for sham and 23 for CLP.

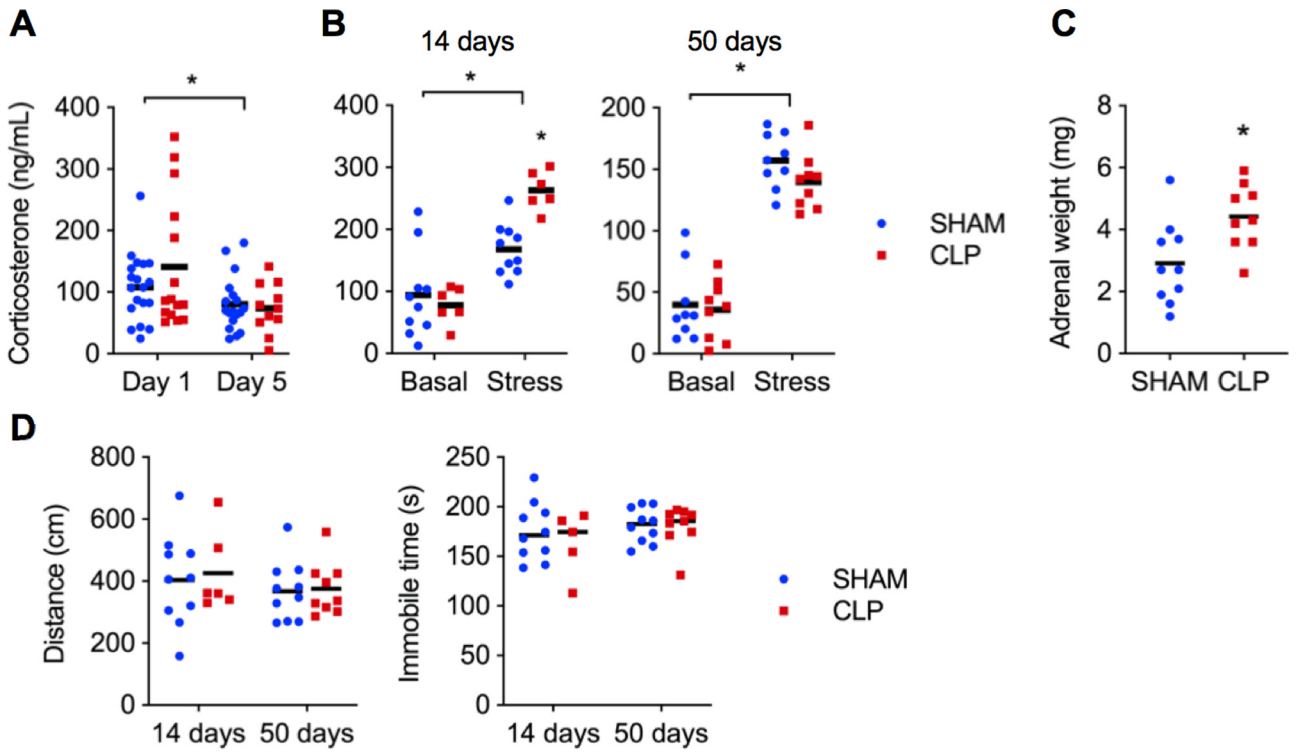

Fig. 3. CLP survivor mice show HPA axis dysfunction after CLP. A, CLP and sham mice show increased corticosterone 1 day after surgery with resolution by 5 days (*$p < 0.01$; N = 19 for sham, 15 for CLP). B, CLP survivor mice show a greater increase in corticosterone after swim stress compared with sham 14 days but not 50 days after surgery (*$p < 0.001$ compared to sham; N = 10 for sham and 6 for CLP at 14 days; N = 10 for sham, 9 for CLP at 50 days). C, 50 days after surgery, CLP survivor mice have increased adrenal weights compared to sham (*$p < 0.05$; N as in B). D, There was no difference in swimming behavior between the groups at either time point (N as in B).

and surgery ($F(1,14) = 10.7$, $p = 0.006$). Post-hoc testing showed that corticosterone levels in CLP survivors after stress were significantly higher than all other groups, including the stress sham group ($p < 0.005$ for all). Thus, corticosterone levels 30 min after swimming are higher in CLP survivor mice at 14 days but not 50 days, compared to sham. At 50 days, despite having similar basal and stress-induced corticosterone as sham, 50-day CLP survivor mice had increased adrenal weights (Fig. 3C), suggesting chronic activation of the HPA axis in very long-term CLP survivors. There was no significant difference between sham and CLP survivor mice in immobility or swimming distance in the forced swim test at this time point (Fig. 3D). Therefore, different behavior during the swim test could not explain the enhanced corticosterone after swimming in the CLP survivor mice.

3.4. Evidence of decreased ventral hippocampal activity in sepsis survivors

Brain regions previously implicated in affective behavioral changes after stress are strong candidate areas to mediate the behavioral changes seen in sepsis survivor mice. To explore which of these brain regions might be involved, we measured mRNA for the immediate early gene, cfos, after forced swim stress at 14 days, the time of increased anxiety-like behavior and HPA axis reactivity in sepsis survivors. In situ hybridization was used to compare cfos mRNA between sham and CLP survivor mice in the following areas known to be involved in affective behavior and HPA axis activity: the prefrontal cortex (infralimbic, prelimbic, and anterior cingulate areas), paraventricular nucleus of the hypothalamus, amygdala (central, medial, and basolateral amygdala) and hippocampus along the dorsal-ventral axis (CA1 and CA3 pyramidal cell layers and dentate granule cell layer from dorsal, intermediate, and ventral levels). Fig. 4 illustrates the anatomic levels chosen. Table 1 shows mean cfos mRNA optical density from all analyzed brain regions and the outcomes of statistical comparisons between sham and CLP groups. Optical density of cfos mRNA differed significantly between sham and CLP mice only in the ventral hippocampus, where CLP survivor mice showed decreased cfos mRNA in all three subregions (CA1, CA3, and dentate gyrus; images in Fig. 4F–G). This finding suggests that, in comparison to sham, sepsis survivor mice exhibit less activity in ventral hippocampal principal cells in response to a novel stressor.

3.5. Sepsis increases glucocorticoid receptor immunoreactivity in the ventral hippocampus

The hippocampus is a well-known target of glucocorticoids, and the glucocorticoid receptor (GR) is known to regulate innate avoidance behavior in mice. Changes in GR protein levels in the hippocampus could therefore mediate differences in ventral hippocampal function and increased avoidance behavior 14 days after CLP. We used immunohistochemistry to label GR in sections from the dorsal and ventral hippocampus in CLP survivor and sham mice, and compared the optical density of labeling (Fig. 5). GR immunoreactivity was primarily seen in CA1 pyramidal cells and dentate granule cells (Fig. 5A). There was no difference in the optical density of GR immunoreactivity in CLP survivor and sham mice in the dorsal hippocampus ($F(1,1) = 26) = 0.99$, $p = 0.33$) (Fig. 5B). In contrast, CLP survivor mice had increased GR immunoreactivity in the ventral hippocampus in both CA1 and dentate gyrus principal cell layers ($F(1,24) = 7.1$, $p = 0.014$) (Fig. 5B–F), suggesting a region-specific increase in GR expression in the ventral hippocampus after sepsis.

4. Discussion

In this study, we describe a specific behavioral and neuroendocrine syndrome in C57Bl/6 male sepsis survivor mice. These mice displayed

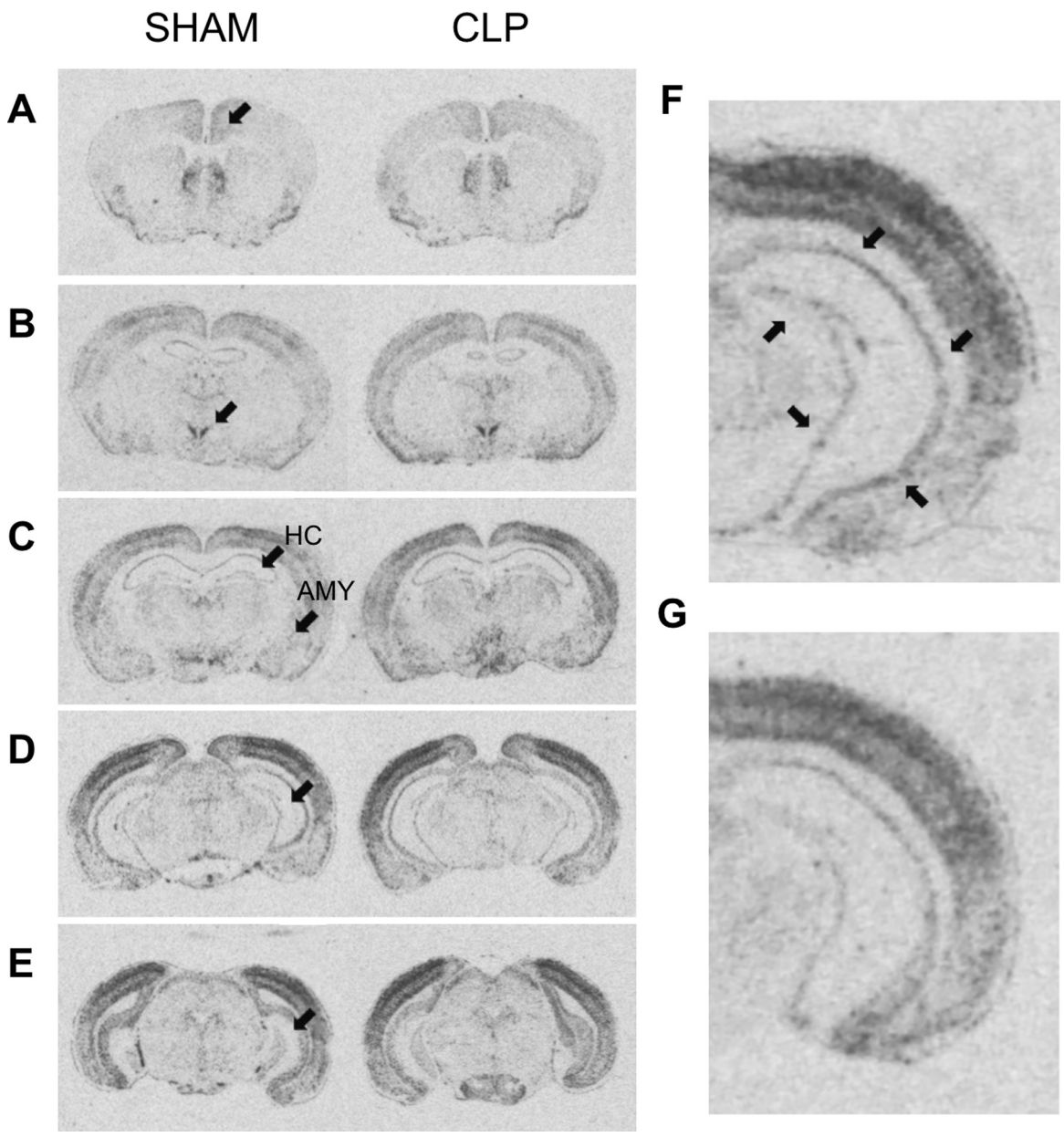

Fig. 4. In situ hybridization for cfos mRNA after swimming stress in sham and CLP mice. Sections containing prefrontal cortex (A), paraventricular nucleus of the hypothalamus (B), dorsal hippocampus (C, HC), amygdala (C, AMY), intermediate hippocampus (D), and ventral hippocampus (E) were analyzed as in the methods. The brain region of interest for each section are indicated by arrows in the sham sections. F-G, Higher magnification representative images of sham (F) and CLP (G) sections containing ventral hippocampus, with arrows to indicate the borders of ventral hippocampus on the sham section (F).

increased avoidance behavior at 14 days in the open field, increased behavioral despair at 50 days in the tail suspension test, and evidence of enhanced HPA axis activity. Sepsis survivors showed decreased cfos mRNA after a novel stressor specifically in the ventral hippocampus, suggesting decreased activation of this brain region compared with the sham condition. Increased hippocampal GR in sepsis survivors was also limited to the ventral hippocampus. To our knowledge, this is the first implication of the ventral hippocampus in the neurobiologic sequelae of acute stress, and we suggest that dysfunction in this brain region could underlie the survivors' phenotype.

The neuroendocrine phenotype in murine sepsis survivors is similar to the long-term consequences of other acute stressors in rats. For example, rats exposed to a novel stressor after a single acute stress show increased corticosterone (Armario et al., 2008). This is similar to our finding here of increased corticosterone after swimming in sepsis survivors, which indicates either an enhanced peak or slower decay. Previous work also showed increased hippocampal GR mRNA in the hippocampus of rats after single prolonged stressor (Liberzon et al., 1999), similar to our finding of increased ventral hippocampal GR immunoreactivity in murine sepsis survivors. These similarities in the long-term neuroendocrine consequences of sepsis (in mice) and other acute stressors (in rats) suggest some shared neurobiology, with the possibility of shared targets for prevention and treatment of stress-induced psychiatric disorders in humans.

In our study, long-term effects of CLP were present only in ventral, not dorsal, hippocampus. We suggest that the unique sensitivity of the ventral hippocampus to sepsis may explain the behavioral and neuroendocrine phenotype. A functional distinction along the dorsal-ventral axis of the human and rodent hippocampus has long been made, with dorsal and ventral aspects more involved in cognition and emotion, respectively (Fanselow and Dong, 2010; Kheirbek et al., 2013). Lesions to the ventral hippocampus consistently affect innate anxiety-

Table 1
Decreased ventral hippocampal cfos mRNA in CLP survivors compared with sham. Integrated optical density for cfos mRNA from the prefrontal cortex (PFC), infralimbic (IL), prelimbic (PL), and anterior cingulate areas (ACA) shows no difference between CLP and sham groups. There were no differences in integrated optical density between groups in the paraventricular nucleus of the hypothalamus (PVN), or in the amygdala including the central (CeA), medial (MeA), and basolateral (BLA) areas. There was no difference in optical density of cfos mRNA between groups in any subregion in the dorsal hippocampus, but a significant decrease in cfos mRNA in the ventral hippocampus. N = 6 for CLP, 10 for SHAM.

cfos mRNA optical density						
		Mean ± SD				
		SHAM	CLP	F	p	q
PFC	ILA	0.0115 ± 0.001	0.0122 ± 0.00259	F(1, 54) = 0.353	0.555	0.486
	PLA	0.00962 ± 0.000849	0.00954 ± 0.00196			
	ACA-v	0.0106 ± 0.00098	0.0122 ± 0.00182			
	ACA-d	0.00944 ± 0.000884	0.00957 ± 0.00133			
Hypothalamus	PVN	0.0503 ± 0.00721	0.0427 ± 0.00542	N/A	0.415	0.486
Amygdala	CeA	0.0048 ± 0.000757	0.00472 ± 0.00061	F(1, 42) = 0.475	0.494	0.486
	MeA	0.00863 ± 0.000777	0.00772 ± 0.00124			
	BLA	0.00854 ± 0.000998	0.00788 ± 0.00127			
Dorsal hippocampus	CA1	0.0783 ± 0.00902	0.07072 ± 0.008433	F(1, 42) = 0.402	0.529	0.486
	CA3	0.0549 ± 0.00548	0.0549 ± 0.00751			
	DG	0.0426 ± 0.00485	0.0387 ± 0.00592			
Intermediate hippocampus	CA1	0.0410 ± 0.00670	0.0299 ± 0.00215	F(1, 39) = 2.13	0.152	0.399
	CA3	0.0277 ± 0.00407	0.024 ± 0.00232			
	DG	0.0126 ± 0.00189	0.0101 ± 0.000904			
Ventral hippocampus	CA1	0.0520 ± 0.00733	0.0318 ± 0.00277	F(1, 39) = 8.69	0.0054	0.0284*
	CA3	0.0201 ± 0.00303	0.0113 ± 0.00216			
	DG	0.0101 ± 0.00199	0.00369 ± 0.00110			

like behavior (Bannerman et al., 2003; Herman et al., 1998; Jimenez et al., 2018; Kheirbek et al., 2013). This brain region is also involved in negative feedback to the HPA axis (Herman et al., 1998; Jankord and Herman, 2008). In our model, the volume and dendritic spine density in the ventral pyramidal cell layer was not changed in long-term CLP survivors, and there was no evidence of hippocampal cell death (Singer et al., 2016). Alterations in ventral hippocampal function after sepsis may therefore be due to functional changes resulting from long-term alterations in immune signaling (Singer et al., 2016), neurogenesis (Anderson et al., 2015), or endocrine signaling that need to be further explored.

Based on our results, we suggest two mechanisms by which altered

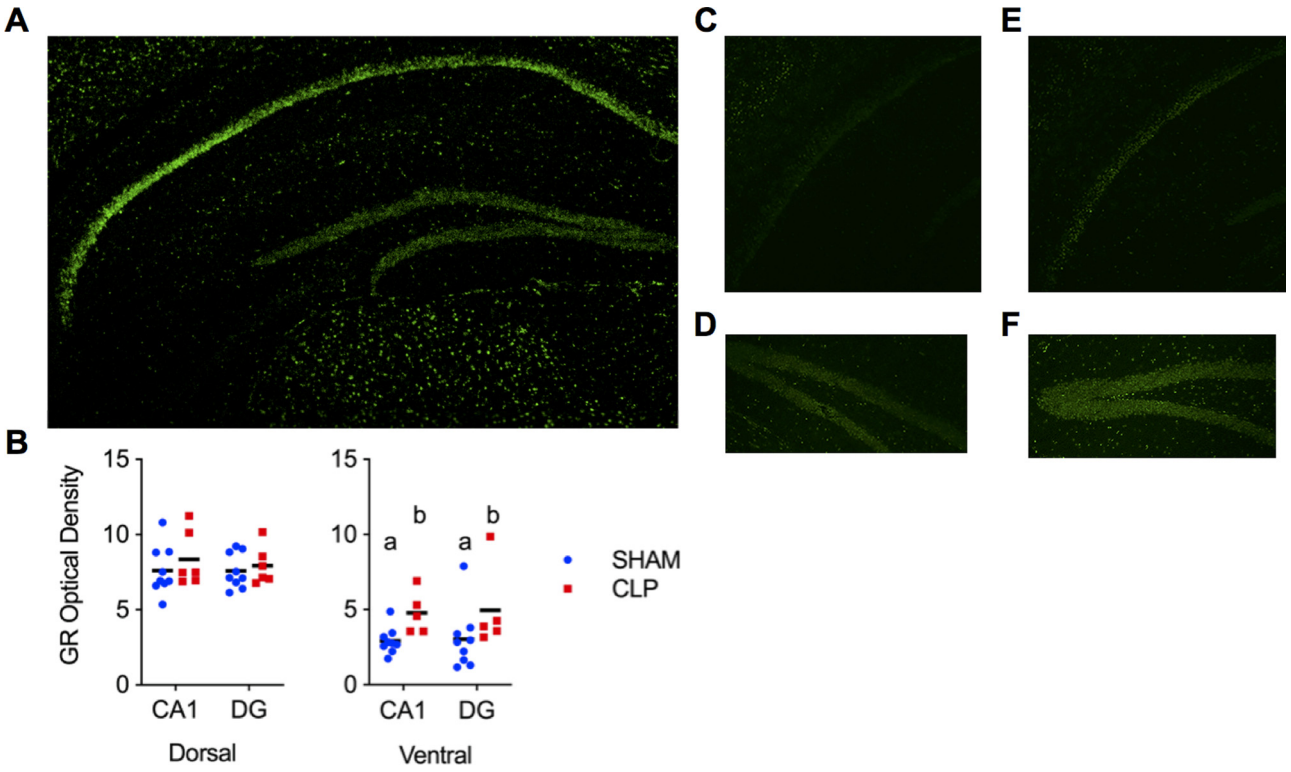

Fig. 5. Immunohistochemistry for GR 14 days after CLP. A, GR labeling is prominent in the CA1 pyramidal cell layer and dentate granule cell layer of the hippocampus. B, Optical density of GR labeling in the dorsal and ventral hippocampus shows a significant increase after CLP only in the ventral hippocampus (overall effect of group a vs b, $p < 0.05$) N = 9 SHAM, 6 CLP (dorsal) and 5 CLP (ventral). C–F, Representative images of GR immunolabeling in ventral CA1 of sham (C) and CLP (E), and ventral dentate gyrus of sham (D) and CLP (F).

neuroendocrine signaling could contribute to the behavioral phenotype in sepsis survivors. First, a decrease in ventral hippocampal principal cell activity, as suggested by the lower induction of cfos in sepsis survivors, could lead to decreased negative feedback to the HPA axis from this brain region. This decrease in negative feedback could be responsible for the increased corticosterone after stress seen in sepsis survivor mice. We further hypothesize that over time, persistently increased HPA axis activity with repeatedly elevated corticosterone levels in sepsis survivors may cause a progressive behavioral phenotype analogous to depression in humans. In future experiments, we could test the role of glucocorticoids in the depressive-like phenotype that develops in 50-day survivors through pharmacological or surgical interventions to decrease circulating corticosterone in CLP survivors.

Second, this HPA axis hyperactivity could lead to a compensatory upregulation of ventral hippocampal GR, as seen in sepsis survivor mice in this study. Whatever the mechanism for upregulation, increased ventral hippocampal GR may contribute to the increased avoidance behavior seen in sepsis survivor mice. Over- and under-expression of forebrain GR leads to increased and decreased avoidance behavior, respectively, suggesting that forebrain GR promotes avoidance or anxiety-like behavior. (Boyle et al., 2006; Wei et al., 2004) Studies using these models also suggest that GR promotes behavioral flexibility to pharmacologic or experience-dependent manipulation. (Boyle et al., 2006; Wei et al., 2004; 2012) Since increased caution and behavioral flexibility may be desirable in a hazardous environment, this increased hippocampal GR could be adaptive for the organism after an acutely stressful experience. Thus, sepsis may have lasting consequences on neuroendocrine signaling that may be adaptive in some situations, but likely contributes to negative affect in survivors. Future experiments could test the role of ventral hippocampal glucocorticoid receptors in the sepsis survivor phenotype through selective disruption of GR expression in this brain region.

Our study has several limitations. We only performed the tail suspension test at one time point, 50 days after surgery, so we do not know at what point this phenotype of behavioral despair emerges after CLP. Furthermore, the tail suspension test findings of increased immobility in sepsis survivors differed from those in the two-day forced swim test, which showed no difference in immobility between sham and CLP survivor mice. This could reflect differences between the tests, as previous work has shown differences in their sensitivity to various interventions (Bai et al., 2001). It could also relate to the difference between the two experiments, as the swimming mice had undergone several prior tail blood collections and thus, were different in their handling history compared to the mice undergoing tail suspension.

We chose to measure cfos mRNA to assess differences in neural activity patterns in different brain regions after swim stress. Immediate early genes such as cfos are transiently increased by cellular stimuli, and thus are used as a marker for neuronal activation during the hour prior to euthanasia (Cullinan et al., 1995). Cfos in particular shows a robust response to swimming in multiple brain areas (Cullinan et al., 1995), and different patterns of cfos mRNA induction have been associated with particular behavioral phenotypes in rodents (Jama et al., 2008; Kerman et al., 2011). However, other immediate early genes show varied activity patterns after swimming (Cullinan et al., 1995), and different cell types may show a preference for the induction of specific immediate early genes (Tronson et al., 2009). Thus, our experiments may not have captured all the differences in neural activity between sham and CLP survivors. Furthermore, since we only measured differences in cfos at the 14-day time point, we do not know whether these activity patterns persisted or differed at the 50-day time point. Finally, although we are able to capture global differences in activity using cfos, we do not know exactly how the activity patterns differ in specific ventral hippocampal cells during behavioral tasks. More sophisticated in vivo recording methods should be employed to better understand how ventral hippocampal function differs in sepsis survivor mice.

In our study, we only used male mice. Prior literature suggests an effect of female reproductive hormones on the inflammatory response to sepsis (Kennedy et al., 2014; Maggioli et al., 2016; Puder et al., 2001), which thus needs to be considered when incorporating females into CLP experiments. Corresponding experiments using females with consideration of reproductive hormone status will be important.

In summary, we present a murine post-sepsis syndrome involving negative affective behavior and HPA axis hyperactivity. We demonstrate novel changes to activity and GR expression specifically in the ventral hippocampus, and we suggest that dysfunction in this brain region underlies the post-sepsis syndrome. Important future directions include further defining the role of the ventral hippocampus in the behavioral and endocrine changes in sepsis survivors, understanding the unique sensitivity of this brain region to acute inflammatory illness, and exploring the translational relevance of these mechanisms to the human post-ICU syndrome.

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Declaration of Competing Interest

The authors have no conflicts of interest to disclose.

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