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Septic patients frequently develop cognitive impairment that persists beyond hospital discharge. The impact of sepsis on electrophysiological and molecular determinants of learning is underexplored. We observed that mouse survivors of sepsis or endotoxemia experienced loss of hippocampal long-term potentiation (LTP), a brain-derived neurotrophic factor (BDNF)-mediated process responsible for spatial memory formation. Memory impairment occurred despite preserved hippocampal BDNF content and could be reversed by stimulation of BDNF signaling, suggesting the presence of a local BDNF inhibitor. Sepsis is associated with degradation of the endothelial glycocalyx, releasing heparan sulfate fragments (of sufficient size and sulfation to bind BDNF) into the circulation. Heparan sulfate fragments penetrated the hippocampal blood-brain barrier during sepsis and inhibited BDNF-mediated LTP. Glycoarray approaches demonstrated that heparan sulfate's avidity for BDNF increased with sulfation at the 2-*O*-position of iduronic acid and *N*-position of glucosamine. Circulating heparan sulfate in endotoxemic mice and septic humans was enriched in 2-*O*- and *N*-sulfated disaccharides; furthermore, the presence of these sulfation patterns in the plasma of septic patients at intensive care unit (ICU) admission predicted persistent cognitive impairment 14 days after ICU discharge or at hospital discharge. Our findings indicate that circulating 2-*O*- and *N*-sulfated heparan sulfate fragments contribute to septic cognitive impairment.

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The authors have declared that no conflict of interest exists.

Abstract

Septic patients frequently develop cognitive impairment that persists beyond hospital discharge. The impact of sepsis on electrophysiological and molecular determinants of learning is underexplored. We observed that mouse survivors of sepsis or endotoxemia experienced loss of hippocampal long-term potentiation (LTP), a brain-derived neurotrophic factor (BDNF)-mediated process responsible for spatial memory formation. Memory impairment occurred despite preserved hippocampal BDNF content and could be reversed by stimulation of BDNF signaling, suggesting the presence of a local BDNF inhibitor. Sepsis is associated with degradation of the endothelial glycocalyx, releasing heparan sulfate fragments (of sufficient size and sulfation to bind BDNF) into the circulation. Heparan sulfate fragments penetrated the hippocampal blood-brain barrier during sepsis and inhibited BDNF-mediated LTP. Glycoarray approaches demonstrated that heparan sulfate's avidity for BDNF increased with sulfation at the 2-*O*-position of iduronic acid and *N*-position of glucosamine. Circulating heparan sulfate in endotoxemic mice and septic humans was enriched in 2-*O*- and *N*-sulfated disaccharides; furthermore, the presence of these sulfation patterns in the plasma of septic patients at intensive care unit (ICU) admission predicted persistent cognitive impairment 14 days after ICU discharge or at hospital discharge. Our findings indicate that circulating 2-*O*- and *N*-sulfated heparan sulfate fragments contribute to septic cognitive impairment.

Introduction

Septic patients frequently experience cognitive dysfunction that persists beyond hospital discharge, impairing survivors' quality of life and ability to return to work (1-3). This cognitive impairment has been largely attributed to brain injury occurring early in sepsis, arising from pathogenic processes including cerebral microvascular thrombosis, metabolic derangements, and interleukin-1β-dependent neuroinflammation (4-8). Less is known about the long-term functional effects of sepsis on the electrophysiological and molecular neuronal pathways responsible for learning.

Classic neurosurgical studies have identified the hippocampus as the anatomic center of memory. Learning requires synaptic plasticity between key hippocampal neuronal networks, including the strengthening of Cornu Ammonis Region 3 (CA3) to Cornu Ammonis Region 1 (CA1) neuronal connections through a process called long-term potentiation (LTP), the key molecular mechanism driving spatial memory (9). Hippocampal LTP is dependent upon the neuronal growth factor brain-derived neurotrophic factor (BDNF), as demonstrated by the induction of cognitive impairment by experimental BDNF sequestration (10). Interestingly, experimental and human sepsis has been associated with hippocampal pathology, including acute dysfunction of the hippocampal blood-brain barrier as well as later loss of hippocampal volumes in sepsis survivors (11-13).

There is increasing appreciation for the importance of the endothelial glycocalyx in sepsis pathophysiology. The glycocalyx is a ubiquitous endovascular layer enriched in heparan sulfate, a linear polysaccharide of repeating glucosamine-hexuronic (iduronic or glucuronic) acid

disaccharide units. These disaccharides may be sulfated at specific sites (including the 2-*O*-position of iduronic acid and/or the 6-*O*- or *N*-position of glucosamine), imparting a domain patterning of negative charge. During sepsis, the glycocalyx is fragmented, releasing heparan sulfate hexa- and octasaccharides into the bloodstream (14-16). These fragments have the capacity to interact with soluble proteins (such as growth factors) with remarkable specificity through sulfation-based electrostatic interactions, influencing a variety of homeostatic and/or pathologic signaling pathways (17). Shed fragments circulate for several days after sepsis onset and correlate with clinically-significant outcomes including acute kidney and lung injury (15, 18).

Given the importance of the growth factor BDNF to cognition, as well as the ability of circulating heparan sulfate fragments to influence growth factor signaling, we hypothesized that hippocampal penetration of circulating heparan sulfate fragments leads to sequestration of BDNF, impairing LTP and inducing septic cognitive impairment. In this report, we employed multimodal ex vivo, in vivo, and human studies to demonstrate that *N*- and 2-*O*- sulfated heparan sulfate fragments are released into the circulation during sepsis and inhibit BDNF-mediated hippocampal LTP, leading to cognitive dysfunction in both mice and humans.

Results and Discussion

To establish a model of septic cognitive dysfunction, we induced endotoxemia in 8 to 12 week-old male and female C57BL/6 mice through intraperitoneal injection of lipopolysaccharide (LPS, 10 μg/g body weight). Animals injected with LPS had a mortality of 18%, with 70% of deaths occurring within 3 days of LPS injection (**Supplementary Figure 1A**). Endotoxemic mice displayed sickness behavior for 3 days, followed by resumption of normal behavior and weight gain by 7 days (**Supplementary Figure 1B**). To determine if post-septic mice demonstrated memory deficits that persisted despite resumption of normal activity, we performed hippocampal-dependent neurobehavioral testing (contextual fear conditioning) on wild-type C57BL/6 mice 7 days after intraperitoneal LPS or saline (control). Contextual memory was apparent in saline-treated mice but was impaired in post-LPS mice (**Figure 1A)**. This post-LPS deficit in memory coincided with loss of hippocampal long-term potentiation (LTP), as measured using 300 μm-thick living hippocampal brain slices isolated from 8-12 week-old C57BL/6 mice 7 days after LPS (or saline, **Figure 1B**). We observed similar loss of LTP in mice 7 days after cecal ligation and puncture (CLP, or sham, **Figure 1C**), a widely used model of polymicrobial peritonitis-induced sepsis. Intriguingly, post-LPS hippocampi maintained BDNF content (**Figure 1D, Supplementary Figure 2**) as well as the ability to induce LTP in response to exogenous BDNF (100 ng/mL, **Figure 1E**). Treatment of endotoxemic mice with 7,8-dihydroxyflavone (7,8 DHF, 5 μg/g body weight administered daily via intraperitoneal injection), a well-tolerated selective agonist of the BDNF receptor tyrosine receptor kinase B (TrkB), prevented loss of memory at 7 days, as demonstrated by normalization of contextual fear conditioning (**Figure 1F**). Taken together, these findings indicate that post-endotoxemic/post-septic mice demonstrate impaired memory and loss of LTP, despite the presence of BDNF within the hippocampus and

persistence of hippocampal responsiveness to TrkB activation—suggesting induction of a competitive inhibitor of BDNF within the septic hippocampus.

Our group and others have shown that highly-sulfated heparan sulfate hexa- to octasaccharide fragments are shed into the plasma in animal (19) and human sepsis (14) as a consequence of endothelial glycocalyx degradation. We confirmed these findings using novel high-sensitivity mass spectrometry multiple reaction monitoring (MRM, (19)) analyses of plasma collected after intraperitoneal LPS in mice (**Figure 2A**) and in a separate cohort of human septic patients (Molecular Epidemiology of SepsiS in the Intensive Care Unit, MESSI, **Figure 2B**). Consistent with the known ability of ultralow molecular weight heparins to cross the blood-brain barrier in healthy mice (20), we observed that circulating heparan sulfate fragments also penetrate the blood-brain barrier, as intravenously-administered fluorescein-labeled heparan sulfate octasaccharides were observed in the hippocampus (**Figure 2C**) and cortex (**Supplementary Figure 3**) in both septic and non-septic mice. We accordingly observed an increase in hippocampal heparan sulfate content at the time of peak circulating heparan sulfate (1 day after LPS, **Figure 2D**). Interestingly, accumulation of hippocampal heparan sulfate persisted for 7 days after LPS, a timepoint characterized by impaired cognition (**Figure 1A**). Persistence of brain heparan sulfate has been recognized in other neurodegenerative disease states, such as Alzheimer's dementia (21-23). Hippocampal heparan sulfate content eventually normalized in mice 14 days after LPS (in comparison to mice 14 days after saline), coincident with improvement in cognition (**Supplementary Figure 4**).

We have previously demonstrated that circulating heparan sulfate oligosaccharides can influence growth factor signaling by electrostatically binding positively charged residues of growth factor ligands (19). To determine if hippocampal-penetrating heparan sulfate fragments interfere with BDNF, the neurotrophin responsible for hippocampal LTP, we isolated hippocampal slices from healthy (non-septic) 8 to 12 week-old C57BL/6 male and female mice and measured LTP in the presence or absence of highly-sulfated heparan sulfate (heparin) octasaccharides, approximating the known size and sulfation pattern of circulating heparan sulfate fragments after sepsis (14). Exposure of hippocampal slices to highly-sulfated octasaccharides inhibited LTP as compared to matched control slices isolated from the same mice (**Figure 2E**). Heparan sulfate-mediated inhibition of LTP could be reversed by the downstream activation of TrkB, as octasaccharides were unable to impede LTP when co-administered with 7,8 DHF (**Figure 2E**). This suggests the negative effect of heparan sulfate on hippocampal LTP was mediated by inhibition of BDNF/TrkB signaling rather than any nonspecific injurious effect of the glycosaminoglycan. These findings support that sepsis-induced circulating heparan sulfate fragments penetrate the septic hippocampus and inhibit BDNF-specific molecular mechanisms of learning.

Heparan sulfate disaccharides may be sulfated at the *N*- and 6-*O*- positions of glucosamine or the 2-*O*- position of iduronic acid (**Figure 3A**), imparting a domain patterning of negative charge that determines the ability of the heparan sulfate polysaccharide to selectively bind soluble ligands. We performed surface plasmon resonance (SPR), a technique that allows for quantification of glycosaminoglycan-protein interaction, to determine the sulfation sites that participate in heparan sulfate-BDNF binding. Heparan sulfate fragments bound BDNF in a length- and sulfation-dependent manner (**Supplementary Figure 5**). Since SPR does not inform

the precise sequence or pattern of sulfation necessary for BDNF binding, we employed a glycosaminoglycan microarray (glycoarray) comprised of 52 heparan oligosaccharides of varying sulfation patterns (24). This approach revealed that BDNF-avid oligosaccharides commonly contained disaccharides with both *N*- and 2-*O*- sulfation sequences (**Figure 3B, Supplementary Figure 6**). Intriguingly, circulating heparan sulfate fragments collected from post-LPS mice (**Figure 3C**) and septic humans (**Figure 3D, Supplementary Figure 7, Supplementary Table 1**) were similarly enriched in *N*- and 2-*O*- sulfation sequences on mass spectrometry MRM analysis. This enrichment in *N*- and *O*- sulfation was observed in the hippocampi of post-septic mice and persisted for 7 days (**Figure 3E, Supplementary Table 2**), indicating that mice maintained BDNF-avid heparan sulfate fragments within their hippocampi at timepoints coincident with post-septic cognitive impairment.

We performed additional glycoarray experiments to define the avidity of heparan sulfate oligosaccharides for pro-BDNF, a proenzyme incapable of promoting LTP (25). Pro-BDNF demonstrated no binding to any heparan sulfate oligosaccharide sequence (**Supplementary Figure 8A**), suggesting that the cleavage of pro-BDNF to active BDNF exposes a heparan sulfate binding site. Other known heparan sulfate binding proteins (e.g. antithrombin III, platelet factor 4) bound to heparan sulfate sequences distinct from BDNF. Similarly, the heparan sulfate 10e4 antibody (26) failed to bind BDNF-avid sequences (**Supplementary Figure 8B-D**). We leveraged this inability of the 10e4 antibody to bind BDNF-avid sequences to demonstrate that sequestration of non-BDNF-avid heparan sulfate sequences failed to prevent loss of LTP **(Supplementary Figure 9**), further supporting the sulfation sequence specificity of the observed BDNF inhibitory effect of heparan sulfate.

To determine the translational relevance of our findings, we measured heparan sulfate in plasma samples collected at day 0 (i.e. at ICU presentation) from septic patients enrolled in the Neurocognitive Impairment in Respiratory Failure and Shock (NIRFS) study of the MESSI cohort (**Table 1**) to determine if the presence of circulating *N*- and 2-*O*- sulfated heparan sulfate predicted cognitive impairment in sepsis. NIRFS patients with persistent cognitive impairment after sepsis (as quantified by a Montreal Cognitive Assessment (MoCA) score < 21 or a cognitive inability to perform the test at either hospital discharge or 14 days after ICU discharge) were those who had increased circulating *N*- and 2-*O*- sulfated heparan sulfate at the time of ICU admission (**Figure 3F, Supplementary Table 3**). Taken together, our findings demonstrate that the presence of circulating NS/2S-enriched, BDNF-avid heparan sulfate fragments at sepsis onset predicts cognitive impairment up to two weeks after ICU discharge.

In summary, our findings demonstrate that septic neurocognitive dysfunction is not only a consequence of inflammatory or ischemic brain injury, but also may arise from septic interference with biologic processes (i.e. hippocampal BDNF-TrkB signaling) necessary for memory and cognition. Detection of circulating *N*- and 2-*O*- sulfated-heparan sulfates may therefore allow identification of septic patients at high risk of prolonged cognitive impairment, enabling the future development (and personalized implementation) of sulfation sequence-targeted therapeutics to improve memory and other patient-centered outcomes in sepsis survivors.

Methods

Detailed methods are included in the Supplementary Methods section.

Statistics

Statistical analysis for animal experiment, SPR, glycoarray, and human subjects heparan sulfate fragment quantification data was performed using Prism (Graphpad, La Jolla, CA). Analysis of all other human subjects data was performed using Stata (College Station, TX). All replicates refer to biological replicates, performed on different days to avoid temporal batch effect. No statistical outliers were excluded. Data represent mean ± standard error of the mean or (for whiskers) based upon Tukey criteria. A p value of less than 0.05 was considered statistically significant for all analyses.

Study Approval

<u>Animal experiments</u>

All animal experiments were performed with approval from the University of Colorado Office of Laboratory Animal Resources Institutional Animal Care and Use Committee (IACUC). Experiments were performed in accordance with the IACUC Guidebook published by the National Institutes of Health as well as the ARRIVE guidelines (27). Male and female C57BL/6 mice between the ages of 8 and 12 weeks purchased from both Charles River Laboratories (Wilmington, MA) and Jackson Laboratories (Bar Harbor, ME) were used in all studies. At least 3 biologic replicates were performed for microscopy and glycosaminoglycan quantification results and at least 6 were performed for all other experiments.

Human subjects

Human samples and accompanying clinical data were collected from ICU patients enrolled in the NIRFS study, a sub study of the MESSI cohort, a single-center, prospective cohort of patients admitted to the ICU at the Hospital of the University of Pennsylvania. This study was approved by the Institutional Review Board of the University of Pennsylvania. Subjects or their available surrogates provided written consent.

Author Contributions

Study design was performed by JAH, BJA, SAM, JL, NJM, BJA, RJL, PSH, and EPS. Animal neuroelectrophysiology and behavioral experiments were performed by JAH, JEO, RD, and JAF. Microscopy was performed by JAH and YYang. Western blotting was performed by SAM and KO. Analytical glycobiology experiments were performed by JAH, GS, YYu, FZ, and XH. Patient enrollment and acquisition of data were performed by BJA and NJM. Data analysis and manuscript preparation were performed by JAH, BJA, JEO, RD, GS, FZ, JL, RJL, NJM, PSH, and EPS. EPS was responsible for overall coordination and oversight of the project.

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Figures

Figure 1. Cognitive impairment in mouse survivors of endotoxemia/sepsis is BDNF/TrkB-responsive. A. Memory impairment occurs in mice 7 days after intraperitoneal LPS (10 μg/g body weight), as demonstrated by loss of freezing behavior (representing fearful memory of a previous paw shock) after contextual fear conditioning (CFC). **B.** Living hippocampal slices isolated from mice 7 days after intraperitoneal LPS (as compared to saline) demonstrated impaired long-term potentiation (LTP), the brain-derived neurotrophic factor (BDNF)-dependent neuronal process responsible for spatial memory. **C.** Loss of LTP is similarly seen 7 days after cecal ligation and puncture (CLP), a model of polymicrobial peritonitis-induced sepsis. Loss of LTP occurs 7 days after LPS despite (**D.**) maintenance of hippocampal BDNF content and (**E.**) preserved responsiveness to excess (100 ng/ml) exogenous BDNF. **F.** Maintenance of BDNF-responsive molecular machinery of learning after sepsis is further demonstrated by reversal of memory deficits in post-LPS mice treated with daily 7,8 dihydroxyflavone (DHF, 5 μg/g IP), a direct agonist of the BDNF receptor tyrosine receptor kinase B (TrkB). For all analyses ** $p < 0.01$; * $p < 0.05$ by t-test. For LTP measurements, left panels represent mean/standard error of groups; right panels represent average change from baseline over final 10 minutes of measurement (each data point represents a unique biological replicate).

Figure 2. Sepsis-associated circulating heparan sulfate fragments penetrate the hippocampus and impede LTP. Mass spectrometry multiple reaction monitoring (LC-MS/MS MRM) analyses demonstrate that heparan sulfate is shed into the plasma of (**A.**) mice 24 hours after intraperitoneal LPS (10 μg/g body weight, compared to saline control) and (**B.**) human patients with sepsis (enrolled and followed longitudinally in the MESSI cohort; control samples

represent normal blood donors). **C.** Accordingly, there is an increase in heparan sulfate content within the hippocampus of LPS-injected mice that persists for 7 days after injection. **D.** Intravenous administration of fluorescein-labeled, highly-sulfated heparan sulfate (heparin) octasaccharides (250 μg) to mice 24 hours after intraperitoneal LPS (10 μg/g) or saline penetrated the hippocampal blood-brain barrier, as demonstrated using confocal microscopy of fresh isolated hippocampal slices. Scale bar 100 μm. **E.** Highly-sulfated heparan sulfate (heparin) octasaccharides (degree of polymerization 8, dp8) induce loss of LTP when perfused (2.5 μg/ml) over hippocampal slices isolated from healthy (non-septic mice). LTP is rescued by simultaneous perfusion with the TrkB agonist 7,8 DHF (250 nM). **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ by ANOVA with Tukey's correction for multiple comparisons (**A, C, E**) or Kruskal-Wallis with Dunn's test for multiple comparisons (**B, D**). TBS = Theta Burst Stimulation.

Figure 3. Importance of heparan disaccharide sulfation to BDNF binding and septic cognitive impairment. **A.** Heparan sulfate disaccharides may be sulfated at the *N*-("NS") and 6-*O*-("6S") positions of glucosamine and the 2-*O*-("2S") position of iduronic acid. **B.** Glycoarray analyses demonstrate that heparan sulfate oligosaccharides which bind to BDNF (n = 12) are enriched in NS and 2S compared to low-affinity (n = 40) oligosaccharides. 3S sulfation (found only in heparin) is not associated with binding. **C,D.** Circulating heparan sulfate fragments detected by mass spectrometry in plasma collected (**C.**) 24 h after intraperitoneal LPS (10 μg/g body weight) in mice or (**D.**) at the time of intensive care unit (ICU) admission in patients (n = 20) with sepsis are enriched in *N*-sulfation (including NS, NS2S, NS6S, TriS disaccharides) and 2-*O*- sulfation (NS2S, 2S, 2S6S, TriS). Control patients are normal blood donors (n =9). **E.**

Mouse hippocampi after sepsis were enriched in *N*- and 2-*O* sulfation at 7 days (n = 3 for all time points and groups)**. F.** Patients who demonstrated moderate/severe cognitive impairment (Montreal Cognitive Assessment score < 21 or cognitive inability to perform the test, n = 6) at time of hospital discharge (or day 14 after ICU discharge) were those who previously demonstrated (at time of ICU admission, day = 0) circulating heparan sulfate enriched in NS2S disaccharides or a combination of NS, NS2S, and NS6S disaccharides. Normal/mild impairment, n = 14 patients. Box line represents median; borders represent 25th and 75th percentiles. Whiskers represent upper and lower adjacent values (1.5 x interquartile range, per Tukey method). Outside values were included in analyses. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ by t-test for single comparisons (**B-D**), ANOVA with Tukey's correction for multiple comparisons (**E**), or Wilcoxon-Rank sum test (**F**).

Table 1. Characteristics of NIRFS Study Participants. * n = 17, 3 patients unable to complete the MoCA due to severe cognitive impairment.

Characteristic	
Age (years)	58 (41-64.5)
Male gender	12 (60%)
Caucasian race	13 (65%)
Years of education	14 (12-16)
Comorbid conditions	
Hypertension	6 (30%)
Diabetes	6 (30%)
Chronic kidney disease	5 (25%)
Congestive heart failure	4 (20%)
Cerebrovascular disease	1 (5%)
Cirrhosis	1 (5%)
Active malignancy	9 (45%)
Organ transplant	7 (35%)
Anxiety or Depression	5 (25%)
APACHE III score	100 (93.5 – 124)
Septic shock at presentation	13 (65%)
Vasopressor shock at presentation	12 (60%)
Any septic shock	15 (75%)
Acute respiratory failure at presentation	12 (60%)
Invasive ventilation at presentation	6 (30%)
Any invasive ventilation	15 (75%)
Acute respiratory distress syndrome	10 (50%)
Duration of mechanical ventilation (days)	3 (1-7)
ICU length of stay (days)	6.5 (4.5-14)
Montreal Cognitive Assessment Score (MoCA)*	24 (22-25)
Moderate/Severe Cognitive Impairment	6 (30%)
Trail Making Test Part B (seconds)	95.9 (66.1 – 139.8)

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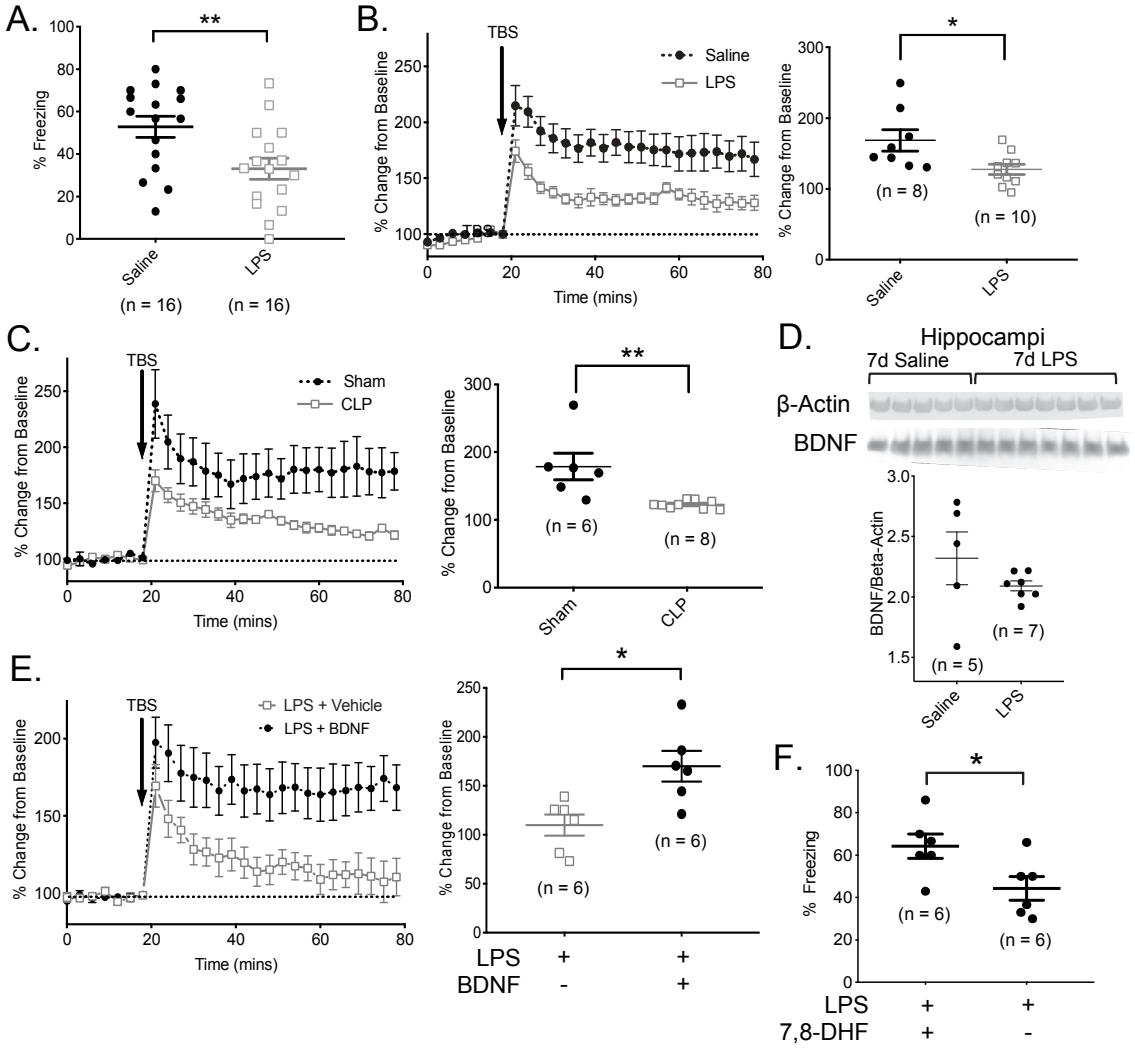

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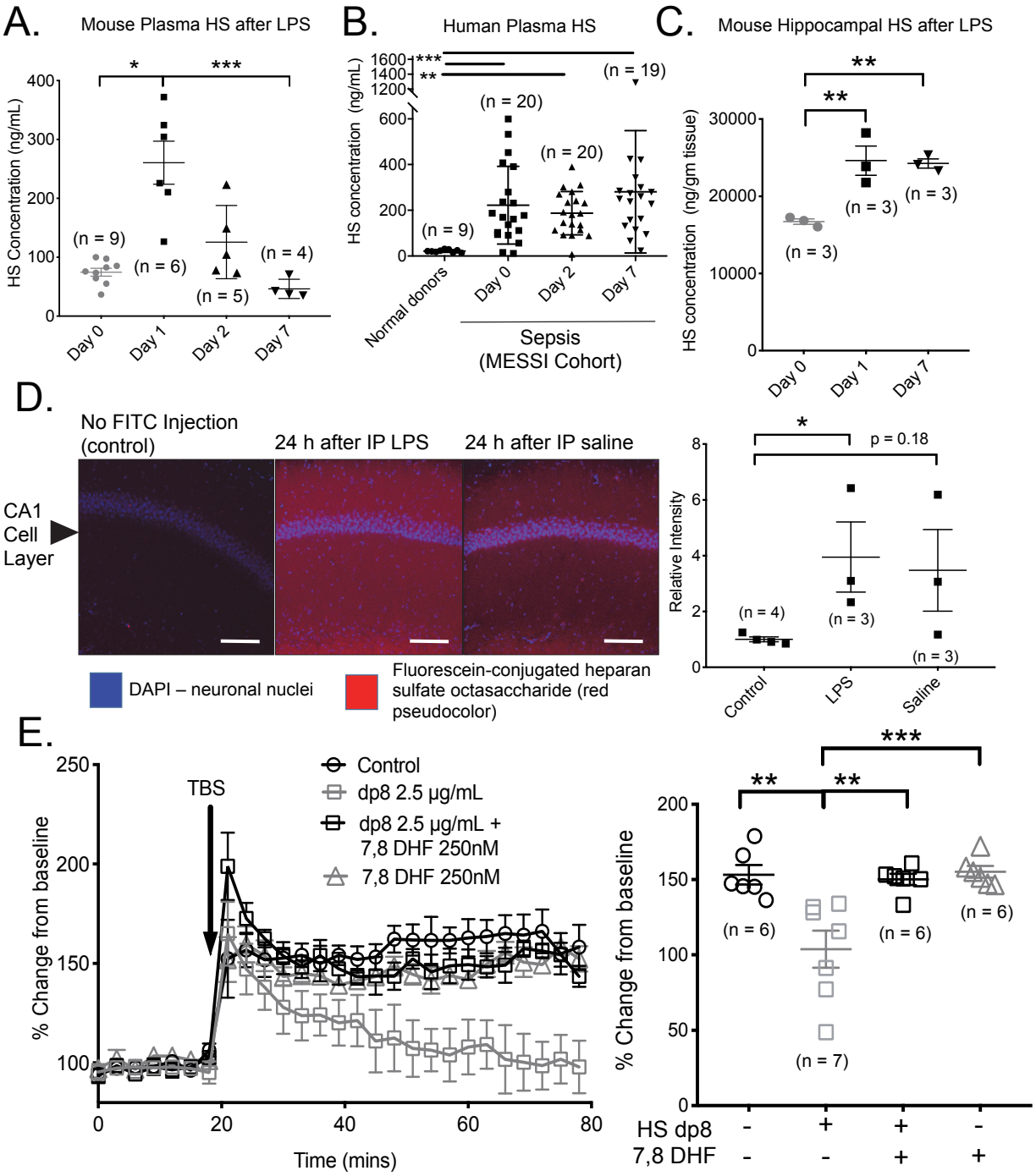

Figure 2. Sepsis-associated circulating heparan sulfate fragments penetrate the hippocampus and impede LTP. Mass spectrometry multiple reaction monitoring (LC-MS/MS MRM) analyses demonstrate that heparan sulfate is shed into the plasma of (**A**.) mice 24 hours after intraperitoneal LPS (10 μg/g body weight, compared to saline control) and (**B**.) human patients with sepsis (enrolled and followed longitudinally in the MESSI cohort; control samples represent normal blood donors). **C**. Accordingly, there is an increase in heparan sulfate content within the hippocampus of LPS-injected mice that persists for 7 days after injection. **D**. Intravenous administration of fluorescein-labeled, highly-sulfated heparan sulfate (heparin) octasaccharides (250 μg) to mice 24 hours after intraperitoneal LPS (10 μg/g) or saline penetrated the hippocampal blood-brain barrier, as demonstrated using confocal microscopy of fresh isolated hippocampal slices. Scale bar 100 μm. **E**. Highly-sulfated heparan sulfate (heparin) octasaccharides (degree of polymerization 8, dp8) induce loss of LTP when perfused (2.5 μg/ml) over hippocampal slices isolated from healthy (non-septic mice). LTP is rescued by simultaneous perfusion with the TrkB agonist 7,8 DHF (250 nM). **** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ by ANOVA with Tukey's correction for multiple comparisons (**A**, **C**, **E**) or Kruskal-Wallis with Dunn's test for multiple comparisons (**B**, **D**). TBS = Theta Burst Stimulation.

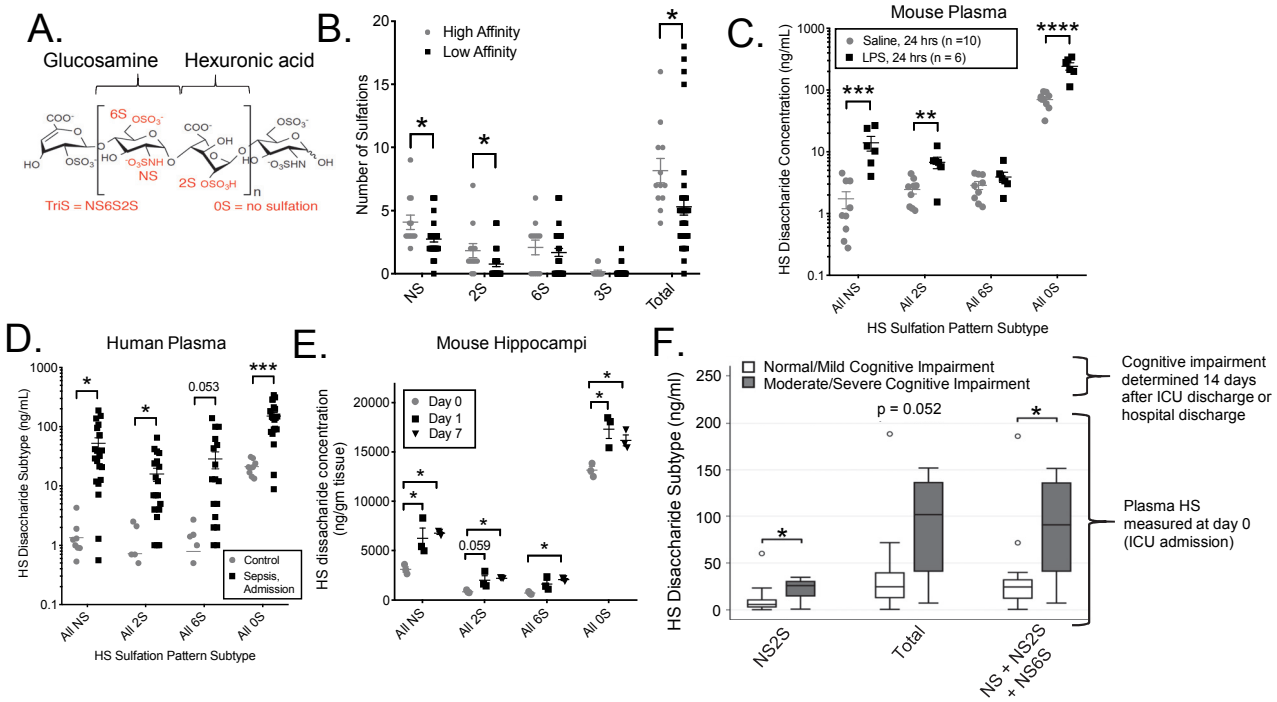

Figure 3. Importance of heparan disaccharide sulfation to BDNF binding and septic cognitive impairment. A. Heparan sulfate disaccharides may be sulfated at the N-("NS") and 6-O-("6S") positions of glucosamine and the 2-O-("2S") position of iduronic acid. **B**. Glycoarray analyses demonstrate that heparan sulfate oligosaccharides which bind to BDNF (n = 12) are enriched in NS and 2S compared to low-affinity (n = 40) oligosaccharides. 3S sulfation (found only in heparin) is not associated with binding. **C**,**D**. Circulating heparan sulfate fragments detected by mass spectrometry in plasma collected (**C**.) 24 h after intraperitoneal LPS (10 µg/g body weight) in mice or (**D**.) at the time of intensive care unit (ICU) admission in patients (n = 20) with sepsis are enriched in N-sulfation (including NS, NS2S, NS6S, TriS disaccharides) and 2-O- sulfation (NS2S, 2S, 2S6S, TriS). Control patients are normal blood donors (n =9). **E**. Mouse hippocampi after sepsis were enriched in N- and 2-O sulfation at 7 days (n = 3 for all time points and groups). **F**. Patients who demonstrated moderate/severe cognitive impairment (Montreal Cognitive Assessment score < 21 or cognitive inability to perform the test, n = 6) at time of hospital discharge (or day 14 after ICU discharge) were those who previously demonstrated (at time of ICU admission, day = 0) circulating heparan sulfate enriched in NS2S disaccharides or a combination of NS, NS2S, and NS6S disaccharides. Normal/mild impairment, n = 14 patients. Box line represents median; borders represent 25th and 75th percentiles. Whiskers represent upper and lower adjacent values (1.5 x interquartile range, per Tukey method). Outside values were included in analyses. **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ by t-test for single comparisons (**B-D**), ANOVA with Tukey's correction for multiple comparisons (**E**), or Wilcoxon-Rank sum test (**F**).