Lipopolysaccharide exposure in a rat sepsis model results in hippocampal amyloid- β plaque and phosphorylated tau deposition and corresponding behavioral deficits

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Abstract Sepsis is a severe systemic inflammatory response to infection associated with acute and chronic neurocognitive consequences, including an increased risk of later-life dementia. In a lipopolysaccharide-induced rat sepsis model, we have demonstrated neuroinflammation, cortical amyloid-beta plaque deposition, and increased whole brain levels of phosphorylated tau. Hippocampal abnormalities, particularly those of the dentate gyrus, are seen in Alzheimer's disease and age-related memory loss. The focus of this study was to

determine whether A β plaques and phosphorylated tau aggregates occur in the hippocampus as a consequence of lipopolysaccharide administration, and whether behavioral abnormalities related to the hippocampus, particularly the dentate gyrus, can be demonstrated. Male Sprague Dawley rats received an intraperitoneal injection of 10 mg/kg of lipopolysaccharide endotoxin. Control animals received a saline injection. Seven days post injection, A β plaques and phosphorylated tau in the hippocampus were quantified following immunostaining. Behavioral tests that have previously been shown to result in specific deficits in dentate gyrus-lesioned rats were administered. Lipopolysaccharide treatment results in the deposition of beta amyloid plaques and intracellular phosphorylated tau in the hippocampus, including the dorsal dentate gyrus. Lipopolysaccharide treatment resulted in behavioral deficits attributable to the dorsal dentate gyrus, including episodic-like memory function that primarily involves spatial, contextual, and temporal orientation and integration. Lipopolysaccharide administration results in hippocampal deposition of amyloid-beta plaques and intracellular phosphorylated tau and results in specific behavioral deficits attributable to the dorsal dentate gyrus. These findings, if persistent, could provide a basis for the higher rate of dementia in longitudinal studies of sepsis survivors.

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Introduction

Sepsis is a systemic inflammatory response to bacterial endotoxins that can result in a spectrum and severity of clinical conditions that can range from relatively mild to severe. These conditions can include shock, multi-organ dysfunction, and multi-organ failure. Sepsis is associated with both short- and long-term clinical neurocognitive problems (Lamar et al. 2011; Barichello et al. 2019). Acutely, patients with sepsis may suffer from delirium (Ebersoldt et al. 2007). Seventy percent of sepsis survivors have neurocognitive impairment when they are discharged from the hospital and 45% still suffer from neurological limitations at 1 year (Hopkins et al. 2004). In longitudinal studies, sepsis has been associated with an increased risk for cognitive impairment and dementia, although not specifically Alzheimer's disease (Chou et al. 2017; Iwashyna et al. 2010). This is particularly true in older patients.

We have previously characterized the neuropathological consequences of an experimental rat model of sepsis produced by a single high dose of systemically administered *E. coli* lipopolysaccharide endotoxin (LPS) (Wang et al. 2018). In addition to systemic findings associated with sepsis, this model results in secondary neuroinflammation as evidenced by an increase in cerebral cytokine levels as well as microglial proliferation. The LPS administration also results in increases in whole-brain content of soluble amyloid beta (A β) (1–42), a fibrillogenic amyloid species, and the subsequent accumulation of A β aggregates throughout the cortex which are morphologically and compositionally similar to diffuse (senile) plaques. There is also an increase in whole brain levels of phosphorylated tau in LPS-treated rats. There is no direct evidence that sepsis increases the risk of Alzheimer's disease. However, some features are common to sepsis-induced neuropathology and to Alzheimer's disease, including neuroinflammation, amyloid plaque deposition, and phosphorylated tau formation. These common features could potentially contribute to the long-term neuropathological consequences of sepsis.

Emerging evidence has indicated that altered neurogenesis in the adult hippocampus represents an early event in the course of AD as well as in age-related memory loss (Toda et al. 2019; Hollands et al. 2016). Hippocampal adult neurogenesis, particularly in the dorsal dentate gyrus (granule cell layer and subgranular zone, GCL/SGZ), is also vulnerable to LPS exposure (Fujioka and Akema 2010). Deficits in

episodic memory are among the earliest neurocognitive hallmarks of Alzheimer's disease and have been attributed to impairments in synaptic plasticity and adult neurogenesis of the hippocampus (Jahn 2013; Gallagher and Koh 2011; Saab et al. 2009; Bäckman et al. 2001). There is some evidence that episodic memory dysfunction, A β plaque deposition, and tauopathy may also accompany age-related episodic memory decline (Maass et al. 2018). Episodic-like memory in rodents, which involves both spatial, temporal, and contextual processing and the spontaneous exploration of novel environments, is dependent upon the dentate gyrus integrity (Vorhees and Williams 2018). Specific deficits in these functions in the rat have been reproducibly generated by stereotactic ablation of the dorsal dentate gyrus (Dees and Kesner 2013; Lee et al. 2005; Goodrich-Hunsaker et al. 2008; Kesner et al. 2015; Morris et al. 2013). Thus, animal behavioral models for dorsal dentate gyrus dysfunction have been well-validated.

We have previously reported hippocampal abnormalities on magnetic resonance imaging (MRI), in the LPS-induced rat sepsis model. These abnormalities include impaired vascularity, blood-brain barrier permeability, decreased brain metabolites, and free increased free radical formation (Towner et al. 2018). The focus of this study was to further interrogate the effect of LPS exposure on hippocampal pathology, specifically to determine whether A β plaques and phosphorylated tau aggregates occur in the hippocampus as a consequence of LPS administration, and whether behavioral abnormalities related to hippocampal function, particularly those of the dorsal dentate gyrus, can be demonstrated in this experimental sepsis model.

Methods

Overall study design

Male Sprague Dawley ($n = 9$ for each experiment) rats (250 g, 2 months old) received a single intraperitoneal injection of 10 mg/kg of lipopolysaccharide endotoxin (LPS). Control animals ($n = 9$ for each experiment) received a saline injection. A β plaques and phosphorylated tau in the hippocampus were quantified following immunostaining. Behavioral tests that have been shown to result in specific deficits in dentate gyrus-lesioned rats were administered.

Study site and ethics statement

Animal experiments were performed with the approval of and compliance with the policies of the University of Utah Institutional Animal Care and Use Committee (protocol no. 17-11012), which adhere to the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. All efforts were made to minimize suffering.

Animal husbandry

Two-month-old (250 g) male Sprague Dawley rats were purchased from Harlan Laboratories (Denver, CO). Rats were housed 2 per cage in HEPA-filtered clear plastic Theron #8 expanded rat cages (Theron Caging Systems, Inc., Hazelton, PA) with Paperchip bedding (Shepard Specialty Papers, Watertown, TN) under pathogen-free conditions within a room operating on a 12-h light/dark cycle (light onset at 7:00 am). Water and food were freely available. Animal welfare and body weight were assessed daily.

Inflammatory stimulus

Rats received a single intraperitoneal injection of 10 mg/kg of LPS (Sigma-Aldrich, St. Louis, MO). Control animals received an equal volume of intraperitoneal saline.

Brain preparation

Brains were immediately removed from euthanized animals 7 days post LPS or saline administration, flash-frozen in isobutene, embedded at -23°C , and stored at -80°C . Cryomicrotome sections at 20- μm slice thickness were obtained in the coronal plane at 20- μm thickness, mounted on glass slides, air-dried, and stored at -80°C .

A β plaque identification

A β (1–42) plaques were quantified by immunostaining of formic acid-treated coronal 20- μm cryomicrotome cortical brain sections through the region of the mid hippocampus according to a published protocol (Ly et al. 2011). Cryomicrotome sections were blocked with goat serum. For A β (1–42), mouse monoclonal antibody against rat A β (1–42)-specific antibody (AnaSpec Inc., Fremont, CA, cat no. AS-55922), the validation

and specificity of which has been reported (Levites et al. 2006). Visualization of antigen-antibody complexes was facilitated with the ABC Kit and VIP HRP substrate (Vector Lab, Burlingame, CA). The average number of plaques was recorded in each of 9 control and 9 LPS-treated rats from three $5\times$ microscopic fields in 3 adjacent brain slices.

Phosphorylate tau identification

Cryomicrotome sections mounted on glass slides at 20- μm thickness were fixed in 10% formalin with heated antigen retrieval using citrate buffer, pH 6.0 for 20 min. Sections were immunostained using phosphorylate tau (p-tau) (ser 262) rabbit polyclonal antibody (Santa Cruz Biotechnologies, Santa Cruz, CA, cat no. sc-32828) at a 1:1000 dilution at room temperature for a 1-h incubation. Validation and specificity of this antibody have been previously reported (Hoshi et al. 1996; Tashiro et al. 1997; Alonso et al. 2001). Visualization of antigen-antibody complexes was performed using the ABC Kit and VIP HRP substrate (Vector Lab, Burlingame, CA), or with the Alexa fluor 488 labeling kit (Invitrogen, Eugene, CA) according to kit instructions. For fluorescence microscopy, nuclei were counterstained with DAPI according to the ThermoFisher Scientific protocol. The average number of positive staining cells was quantified in 9 LPS-treated and 9 control rats. For each rat, two regions were examined: the dorsal dentate gyrus and the lateral CA1/CA2 region. For each region, the average number of positive cell bodies in 3 separate $40\times$ high-powered fields per region was recorded.

Behavioral testing

General experimental setup and apparatus The behavioral testing was performed in one of two apparatus which were constructed for the experiments. The floor of each apparatus measured 64 cm (L) $\times$ 64 cm (W), and each side 64 cm (W) $\times$ 40 cm (H). The apparatus sat 65 cm from the floor and rested on a circular 119-cm-diameter round platform that was covered with white non-transparent plastic. This plastic was drawn upon with dry-erase markers for reproducible cue (object) placement and then subsequently erased. The sides of the apparatus were glued together with the floor of the object to construct a cube with an open top. The apparatus were constructed of either clear or red Plexiglas.

Clear Plexiglas allowed rats to see extra-maze cues located outside the walls of the testing apparatus. These were visually distinct objects placed 30 cm outside of each wall while the apparatus was placed on the testing platform. The extra-maze cues consisted of shapes printed on paper measuring 12–17 cm (L) $\times$ 12–17 cm (W). Shapes were selected to be visually distinct from each other. The red Plexiglas apparatus was used to restrict the rats' field of vision while testing, because rats are unable to perceive the wavelengths of red light and the walls appear opaque black to the rat while allowing the experimenter to see through the walls. Intra-maze object cues measured 6 cm (L) $\times$ 6 cm (W) $\times$ 6 cm (H) $\pm$ 2 cm and were non-porous hard plastic models of cartoon animals. One heavy washer was glued to the bottom of each cue to prevent moving or tipping during testing. Preventing odor collection on cues was accomplished by disinfecting and deodorizing all cues and the testing box with HDQ Neutral disinfectant cleaner (Spartan Chemical Company, Maumee, OH) after each use. Multiple sets of identical objects were utilized to avoid cleaning difficulties between study and testing sessions during the experiments. A black (red Plexiglass) start box was used to introduce the rats to the test platform and house the rats during inter-session intervals (ISI) between study and test phases. The start box dimensions measured 24 cm (L) $\times$ 15 cm (W) $\times$ 17 cm (H) and had a guillotine style door for exiting. The circular testing platform supporting the training apparatus was surrounded by a black plastic curtain hung on scaffolding of the dimension 1.5 m (L) $\times$ 1.5 m (W) $\times$ 2.1 m (H) to allow the extra-maze cues to be controlled by the experimenter.

Sequence, handling, and habituation Male Sprague Dawley rats received a single intraperitoneal (IP) injection of 10 mg/kg of lipopolysaccharide endotoxin (LPS). Control rats received an equal volume of IP normal saline. Nine LPS-injected and 9 saline-injected (control) rats were used for each experiment. The rats were gently handled by the experimenter daily on days 3–6 post injection. Each rat was utilized for only one experiment of neurocognitive function. On the 7th-day post injection, rats underwent habituation. During this time, rats were placed in the training apparatus and allowed to explore for 15 min in the absence of object cues. The habituation phases allowed the rats to acclimate to new environmental conditions. Rats were habituated to the training apparatus relevant to the

experimental design. If two boxes were required for an experiment, habituation sessions to each box were performed one after another. The actual behavioral tests were begun on the 8th-day post IP injection.

Recording and analysis The basis of the behavioral tests administered relies upon differences in duration of exploration of objects that are familiar (previously encountered) or those that are novel (not previously encountered). Examples of exploratory behavior are pawing, biting, sniffing, and rearing within ≤ 3.0 cm of the object. The normal response is for a rat to show a shorter interval of exploration (less preference) for familiar objects than for objects or configurations that are novel. A video camera and monitor were suspended above the apparatus to allow observations and analysis. Timing of object exploration began with the subjects' investigation of the object and stopped when exploration was terminated. A stopwatch was used to record the exploration time for each object. The data for the test phase was calculated individually for each rat using the following equation for the discriminating ratio: (exploration of novel – exploration of familiar)/(exploration of novel + exploration of familiar). This discriminating ratio indicates for which object a rat displays a preference by an increased interval of exploration during the test phase. A positive ratio indicates that the novel cue or configuration was explored more (which is the normal response), and a negative ratio indicated that the familiar object or configuration was explored more. A ratio of zero means that both objects or configurations were explored equally. Differences in mean values of discriminating ratios between control and LPS-treated animals were compared by one-way ANOVA analysis of variance with the Dunnett type I error protection (against control tests). A statistically significant difference between groups was defined as $p < 0.05$. The experimenter was blinded to whether individual rats had previously received an IP injection of LPS or of saline.

Object recognition and object-context recognition The experimental procedure was adapted from a published method (Dees and Kesner 2013) which assessed the ability of the rats to perform an object recognition task with and without the added interference of spatial context produced by surrounding environmental objects. Initial handling and habituation were performed as described above. On the test day (the 8th day of recovery following IP injection of either LPS or saline), the rat was placed in a

box apparatus that was either constructed of clear Plexiglas, whereby 2 external spatial context cues could be seen or a red Plexiglas box (which is seen as opaque black to the rat), whereby external objects were not visible. Each rat was tested in both the black box (for the object recognition experiment) and the clear box (for the object-context recognition experiment) on sequential days, with the order reversed (counterbalanced) for half of the animals. Different objects were used for the first and second days. LPS-treated and control rats perform two 5-min exploratory sessions known as “study phases.” During these study sessions, the rats were placed in the start box within either the red or clear testing apparatus containing two internal unique object cues denoted as A and B, separated by a distance of 25 cm (Fig. 1a). The guillotine door on the start box was raised to allow the rat to exit. After the first study session, the rat was returned to the start box for a 3-min inter-session interval (ISI). During the final ISI, both objects were removed and replaced with identical replicas in the same locations. After the first ISI, the start box, housing the rat, was returned to the apparatus and the guillotine door was opened. The rat exited the start box for a second 5-min exploratory session (study phase 2). Upon conclusion of study phase 2, the rat was placed back in the start box for another 3-min ISI. During the ISI, one object was replaced with an identical replica and the other object cue was replaced with a novel object (cue C), maintaining a distance of 25 cm between the objects. The object that was substituted with a novel one was the object with the lower exploratory score during study phase 2. The rat was then placed in the apparatus as before and allowed to explore the objects for 5 min (test phase). During the test phase, the duration of exploration for each object (the familiar object and the novel object) was recorded individually. On day 9 of testing, the same behavioral tests were utilized as on day 8, with the exception that the rat was tested in the apparatus to which the rat was not previously exposed. If the red (black to the rat) box was used initially, the clear box was utilized on the following day, and vice versa. For the clear box (object-context recognition experiment), two unique and visually distinct objects were also placed 30 cm outside the box (D and E) but were not changed between the study and test phases (Fig. 1c).

Object-place recognition (spatial novelty detection) The behavior methods utilized were adapted from those previously reported (Lee et al. 2005). This test evaluates the ability of rats to identify an object as familiar despite

the object having been moved. Testing was performed in the clear Plexiglas apparatus in a similar manner to that described above. Two sequential 5-min study phases were conducted in the apparatus with the 2 internal cues, separated by 25 cm, as well as two external cues. A 3-min ISI separated the two study phases. After the second ISI, a 5-min test phase was performed. For the test phase, one of the internal objects was moved 45° relative to the other, retaining a distance of 25 cm between the objects (Fig. 2a). The external cues were not moved or changed and were 30 cm from the box. During the test phase, one of the two internal objects was moved in a counterbalanced fashion in the positive and negative direction for half of the rats.

Metric spatial processing recognition The experiments performed were adapted from those previously reported (Goodrich-Hunsaker et al. 2008), which were designed to test the effect of lesions of the dorsal dentate gyrus, CA1, and CA2 in identifying a spatial configuration change when the distance between two (familiar) objects was changed. The clear Plexiglas testing apparatus was utilized for testing, with two external cues that were not changed between study and test phases. Handling and habituation were performed as described above. Two study phases and a test phases were performed in sequence as above with two cues, the center of the box at either 18 cm or 32 cm apart (half the rats initially tested at 18 cm, and the others have at 32 cm between the objects). During the test phase, the objects were replaced with clean identical cues, but the distance between the objects was changed to either 18 cm (from 32 cm) or 32 cm (from 18 cm), relative to the original location during the study phases (Fig. 3a). Exploration of the cues was consolidated into one exploration score.

Object feature configuration recognition and object spatial feature configuration recognition The experiment was adapted from that previously reported (Kesner et al. 2015), which was designed to test the role of the dorsal dentate gyrus in object-place and complex object-place processing. The apparatus utilized for the experiment was made of red Plexiglas testing (seen as opaque black to the rat). After handling and habituation, rats were exposed to two sequential study phases with intervening IS intervals as described above, followed by a test phase. In the study phase of the object feature configuration recognition test, the rat was placed in the experimental apparatus containing two sets of

Fig. 1 Object recognition and object-context recognition test. The ability of LPS-treated and control rats to recognize a novel object was tested in the absence and presence of external contextual cues. The experimental arrangement (**a**) and results (**b**) are shown in the upper panel for the object recognition test, and (**c**) and (**d**) in the lower panel for the object-context recognition test. There was no significant difference ($p = 0.37$) between LPS and control animals

compound objects. Each set consisted of two objects, with the two objects touching each other, AB and CD, as shown in Fig. 4a. The two compound cue sets (AB and CD) were separated by a distance of 32 cm. On the

following day (9th-day post injection), a single repeat study phase of 5 min was performed with the same cues present as the previous day (AB and CD), with the exploration time of both configured cue sets recorded.

Fig. 2 Object-place recognition (spatial novelty detection) test. The experimental arrangement is shown on the left (**a**) and the results on the right (**b**). In this test, one of two familiar objects was moved 45° relative to the other object from its original location (the distance between the objects unchanged). The control rats

were nonetheless able to recognize that the object had moved to a novel location and reexplored it, while the LPS-treated rats were mildly impaired in this capacity, compared with controls, with a difference that was of borderline significance ($p = 0.05$)

Fig. 3 Metric spatial processing test. The experimental arrangement is shown on the left (**a**) and the results on the right (**b**). When the distance between two familiar objects was changed between the study and test phases, the LPS-treated rats were less able to

appreciate that the spatial distance between the objects had been changed and showed significantly less exploratory behavior toward the new spatial arrangement than the controls did ($p < 0.01$)

Following this third study phase, the rat was placed into the start box for a 3-min IS interval. During this time, the cues were replaced with familiar objects, but the configuration of one of the sets was changed, thus AB and

CA. To summarize, if the study phase cue configuration was initially AB and CD, the change in configuration during the test phase was AB and CA. All of the elements were familiar (previously encountered) but one of

Fig. 4 Object feature configuration test (upper panel) and object spatial feature configuration recognition test (lower panel). The left figures (**a**, **c**) show the experimental arrangement and the right figures (**b**, **d**) show the results. When there was no distance separating the 2 elements within 2 sets of paired objects (upper panel), there was no significant difference ($p = 0.41$) in the performance of LPS-treated rats when compared with that of control rats

in being able to recognize that the one of the sets of familiar elements had assumed a new arrangement. However, when a distance of 2 cm separated the sets of paired objects, the LPS-treated rats were less able to appreciate the new arrangement and demonstrated significantly less exploratory behavior of the novel configuration than the controls did ($p < 0.05$)

the sets was arranged in a novel configuration. The set of cues to reconfigure was established by which set of objects received a smaller exploration score during study phase 3. The object spatial feature configuration recognition test was essentially the same as object feature configuration experiment but with one difference. Rather than having the two elements within each set of compound objects touching each other, the cues in each set were separated by a distance of 2 cm (Fig. 4c).

Temporal object recognition The procedures were adapted from those previously reported (Morris et al. 2013) which confirmed that dorsal dentate gyrus integrity is required for temporal associative processing of spatial events. The clear Plexiglas testing apparatus was utilized. This allowed the rats to view extra-maze cues during the experiment. Rats were handled and habituated as above. In this experiment, the study phase was conducted over three consecutive days and is illustrated in Fig. 5a. On day 8 (following injection), the animal was placed in the testing apparatus and allowed to explore for 5 min with an object positioned in spot A. After a 3-min ISI interval, the rat was returned to the testing apparatus for another 5-min exploration study phase; however, during this study phase, a different object was positioned at spot B. On day 9 of testing, the same procedure was used as on day 8 of testing. However, different objects were used on this testing day and were placed in different locations than cues A and B presented on day 8. Specifically, these cues were placed at locations C and D. Presenting cues A and B on the same day and then C and D on the following day introduced a temporal association between the two objects presented on the same day. Day 10 consisted of a 1-min

cue trial where object A or C was presented alone. This was followed by a 3-min ISI interval. Next, the rat performed a 5-min preference test (for the greater exploration) between cues B and D. Exploratory time was collected individually for both cues during the test phase. A preference ratio was calculated individually for each rat. When the subject was exposed to cue A during the 1-min session, the preference ratio was calculated by the equation $(B - D)/(B + D)$. If cue C was presented in the 1-min session, the preference ratio was calculated by the equation $(D - B)/(D + B)$. These equations allowed for the assessment of preference (recognition that the object was less familiar and then resulted in increased exploration) for the cue that was temporally associated on the same day. Furthermore, if rats were presented with object A during the 1-min cue session, would they display a preference for cue B during the test phase between cues B and D? Positive ratios indicate the rat presented a preference for the cue that was temporally associated on the same day during the study phases. Negative ratios indicate the rat displayed a preference for the cue that was not temporally associated (i.e., cues were presented on different days). A ratio of zero indicates the rat showed no preference for either cue.

Results

Amyloid-beta plaques

LPS-treated rats demonstrated focal aggregates staining positive for A β in the hippocampus. Although there was a generalized increased background A β staining in the LPS-treated rats, there were also multiple focal A β

Fig. 5 Temporal object recognition test. Shown on the left (a) is the experimental sequence of events that extended over 3 days. On the right (b) are the results. The LPS-treated rats demonstrated a significant deficit in temporal object recognition when compared with control rats ($p < 0.0001$). In a cued-recall paradigm, the LPS-

treated rats were unable to temporally associate spatial events when presented in sequence on different days. Control rats, in comparison, had a significant exploratory preference for the spatial location previously paired with a specific cue, while LPS-treated rats did not show a preference

deposits which we have previously characterized in the cortex as similar morphologically and compositionally to diffuse A β plaques. As shown in Fig. 6, these plaques were most prominent in the dorsal dentate gyrus (in the expected location of the granule cell layer (GCL)/subgranular zone (SGZ) complex), the striatum lacunosum moleculare (SLM), and more diffusely in Ammon's horn, particularly in the lateral aspect of the CA1 and in CA2. The number of amyloid plaques was specifically quantified per 5 $\times$ field in the dorsal dentate gyrus and the SLM. In the dorsal dentate gyrus, the LPS-treated rats demonstrated an average of 21.40 plaques in the hippocampus per 5 $\times$ field (SE 2.67, 95% CI 15.25 to 27.57). For the SLM, the LPS rats demonstrated a mean of 28.92 A β plaques for 5 $\times$ field (SE 2.0, 95% CI 24.25 to 35.60). There were no A β plaques in the hippocampus of control rats, with a statistically significant difference between LPS-treated and control rats of $p < 0.0001$.

Phosphorylated tau

Prominent intracellular immunostaining for p-tau could be detected in many areas of the hippocampus of LPS-treated rats. Only faint (presumed nonspecific) staining was noted in the control rats. Specific quantification of intracellular staining was performed in the dorsal dentate gyrus, which includes the region of the granule cell layer (GCL) and subgranular zone (SGZ) and the lateral CA1/CA2 of the hippocampus. Shown in Fig. 7 are

representative p-tau immunostained 40 $\times$ fields from the lateral CA1/CA2 region of the hippocampus in LPS-treated and control rats. Within this region, the LPS-treated rats demonstrated 43.41 p-tau-positive cells per 40 $\times$ field (SE 3.07, 95% CI 27.27 to 43.06) while control rats demonstrated a mean of 6.96 p-tau-positive cells (SE 0.73, 95% CI 5.27 to 8.66), a difference that was statistically significant ($p < 0.0001$). For the dorsal dentate gyrus, LPS-treated rats demonstrated 29.50 p-tau-positive cells per 40 $\times$ field (SE 1.73, 95% CI 7.06 to 10.57), while control rats demonstrated a mean of 8.81 p-tau-positive cells per 40 $\times$ field (SE 0.76, 95% CI 7.06 to 10.57), a difference that was statistically significant ($p < 0.0001$). It should be noted that intracellular immunostaining staining for the control rats was faint. Although it was nonetheless quantified, it was thought possibly due to nonspecific binding of the antibody.

Behavioral studies

Following intraperitoneal injection of LPS, rats exhibit decreased mobility and decreased food intake for approximately 24 h, with significant improvement by 48 h. Thereafter, the LPS-treated rats are indistinguishable in outward behavior from control rats, including the degree of activity, feeding, self-grooming, and weight. There was no outward evidence of sickness at 1 week, when the behavioral tests were administered to the rats. Baseline habituation exploratory behavior was equal between LPS and control rats.

Fig. 6 A β plaque formation in the hippocampus of LPS-treated rats. Shown is a representative coronal slice through the mid right hippocampus. There were no A β plaques in the hippocampus of control rats. Although there was a generalized increased background A β staining in the LPS-treated rats, there were also multiple focal A β deposits which we have previously characterized in

the cortex as similar morphologically and compositionally to diffuse A β plaques. These plaques were most prominent in the dorsal dentate gyrus (closed arrowheads), the striatum lacunosum moleculare (SLM) (closed arrows), and in lateral aspect of the CA1 and in CA2 (open arrows)

Fig. 7 Intracellular p-tau accumulation in the hippocampus of LPS-treated rats. Shown are representative p-tau immunostained 40× fields from the lateral CA1/CA2 region of the hippocampus in control rats and LPS-treated rats. The control rats show only faint staining (left panel), thought to be nonspecific. The location of immunostaining in the LPS-treated rats is intracellular, suggested

by a circumferential rim of p-tau around central nuclei (center panel). A 40× view of a fluorescently immunostained region with application of deconvolution (right panel) shows a more 2-dimensional image of a p-tau staining mesh-like structure consistent with microtubule network morphology. Blue DAPI nuclear counterstaining was also performed

Object recognition and object-context recognition There was no significant difference ($p = 0.37$) in the ability of LPS-treated and control rats to discriminate between familiar and novel objects in the absence of external (context) cues (Fig. 3b). The mean discriminating ratio for the control rats was 0.13 (SE 0.11, 95% CI –0.11 to 0.37). The mean discriminating ratio for the LPS-treated rats was –0.05 (SE 0.17, 95% CI –0.43 to 0.32). As shown by Dees and Kesner (2013), this activity does not require dorsal dentate gyrus function. However, for object-context recognition, when there were external cues resulting in an environmental contextual component (which draws on dorsal dentate gyrus function), there was a significant difference in the degree of preferential exploratory behavior of the novel object displayed by the control rats when compared with that by the LPS-treated rats ($p < 0.05$). As shown in Fig. 3d, the control rats demonstrated increased exploratory behavior toward the novel object in a contextually enriched environment. The mean discriminating ratio for the control rats was 0.17 (SE 0.08, 95% CI –0.36 to 0.37). The LPS-treated rats, conversely, did not show a preference for the novel object when contextual cues were present. The mean discriminating ratio for the LPS-treated rats was –0.21 (SE 0.12, 95% CI 0.19 to 1.26). These results were similar to those reported by Dees and Kesner (2013) in comparing control rats with those in which dorsal dentate gyrus was chemically ablated by colchicine injection. Both groups displayed a preference for novel objects compared with that for familiar ones in the absence of external cues (object

recognition in a contextually absent environment). However, the dentate gyrus-lesioned rats, similar to the LPS-treated rats in the current experiment, displayed a decreased preference for novel cues when a contextual component was introduced (object-context recognition).

Object-place recognition (spatial novelty detection) In this study, one of two familiar cues was moved by 45°, although the distance between cues was maintained. Although the control rats demonstrated an increase in relative exploration of the moved cue, the LPS-treated rats were mildly impaired in recognizing that the object had been moved to a new location, showing a relative decrease in exploratory behavior for the object in the novel location (Fig. 4b). The difference between the LPS and control rats in this regard was borderline in statistical significance ($p = 0.05$). For this object-place discrimination (spatial novelty detection) test, control rats demonstrated a mean discriminating ratio of 0.43 (SE 0.10, 95% CI 0.21 to 0.64). For LPS-treated rats, the mean discriminating ratio was 0.04 (SE 0.16, 95% CI –0.32 to 0.40). This test has been previously utilized to demonstrate that rats with lesions in either the dorsal dentate gyrus or in CA3 demonstrate impairment in the ability to recognize a change in the placement of an object, suggesting that the dentate gyrus-CA3 network is essential for this function (Lee et al. 2005). CA1-lesioned animals were also reported to be mildly impaired in this capacity.

Metric spatial processing For this test, the same two objects remained paired between the study and test

phases, but the distance between them was changed for the test phase by moving both objects in or out relative to each other. The LPS-treated rats were less able than the controls to appreciate that the objects had been placed in a new spatial arrangement and showed significantly less exploratory behavior with the new spatial arrangement than did the controls ($p < 0.01$) (Fig. 5b). The control rats demonstrated a mean discriminating ratio of 0.03 (SE 0.12, 95% CI 0.30 to -0.24). LPS-treated rats demonstrated a mean discriminating ratio of -0.52 (SE 0.13, 95% CI -0.24 to -0.80). The neurocognitive deficit demonstrated by the LPS-treated rats is similar to that produced by stereotactic ablation of the dorsal dentate gyrus (Goodrich-Hunsaker et al. 2008).

Object feature configuration recognition When there was no distance separating the 2 elements within 2 sets of paired objects, there was no significant difference ($p = 0.41$) in the performance of LPS-treated rats when compared with that of control rats in being able to recognize that a novel experimental arrangement had been created by pairing one of the objects with a different, although familiar, cue (Fig. 6b). For control rats, the mean discriminating ratio for object feature configuration recognition was 0.23 (SE 0.06, 95% CI 0.37 to 0.10). For LPS-treated rats, the mean discriminating ratio was 0.12 (SE 0.06, 95% CI -0.01 to 0.25). These results were similar to those described, which demonstrated that the dorsal dentate gyrus is not necessary in object feature configuration recognition (Kesner et al. 2015).

Object spatial feature configuration recognition This experiment represented a modification of the above object feature configuration recognition test in which a distance of 2 cm separated the two individual objects in each pair. As shown in Fig. 6d, the LPS-treated rats displayed an inability to recognize that a novel rearrangement had been made with one of the pairs and showed a significantly reduced relative exploration of this pair, compared with the control rats ($p < 0.01$). The control rats demonstrated a mean discriminating ratio of 0.23 (SE 0.06, 95% CI 0.10 to 0.37). The mean discriminating ratio of the LPS-treated rats was 0.02 (SE 0.08, 95% CI -0.16 to 0.20). These results are similar to those previously reported (Kesner et al. 2015), which showed that dentate gyrus function is essential for detection of a change in pairing of objects when there is a spatial distance separating the paired objects.

Temporal object recognition The LPS-treated rats demonstrated a significant deficit in temporal object recognition when compared with control rats ($p < 0.0001$). The findings were similar to those previously reported by Morris et al. for rats with bilateral dorsal dentate gyrus ablation (Morris et al. 2013). In a cued-recall paradigm, the LPS-treated rats were unable to temporally associate spatial events when presented in sequence over 2 days. Control rats, in comparison, had a significant exploratory preference for the spatial location previously paired with a specific cue, while LPS-treated rats did not show a preference. As shown in Fig. 7b, control rats demonstrated a discriminating ratio of 0.26 (SE 0.05, 95% CI 0.15 to 0.37). The LPS-treated rats demonstrated a mean discriminating ratio of -0.50 (SE 0.05, 95% CI -0.612 to -0.38). The findings support that LPS-exposure results in deficits in temporal integration for proximal spatial events presented close in time in a pattern similar to the deficits produced with dorsal dentate gyrus ablation (Morris et al. 2013).

Discussion

The dentate gyrus of the hippocampus is capable of adult neurogenesis throughout life and contributes to the concept of hippocampal plasticity. Loss of adult hippocampal neurogenesis has been implicated both as an early event in the development of Alzheimer's disease and in age-related memory loss (Toda et al. 2019; Hollands et al. 2016). Lipopolysaccharide endotoxin (LPS), an important mediator of gram-negative sepsis, has been reported to suppress neurogenesis in the dentate gyrus, not by apoptosis, but by inhibiting neural precursor cells in the GCL/SGZ (Fujioka and Akema 2010). In longitudinal studies, sepsis has been associated with an increased risk of developing subsequent dementia and future cognitive impairment (Chou et al. 2017; Iwashyna et al. 2010). In older patients, gram-negative infection is the most common cause of sepsis (Rodrigues et al. 2017). When sepsis requires hospitalization for patients over 65 years of age, it subsequently results in a greater than threefold increase in cognitive decline compared with that in similar patients hospitalized for other reasons (Iwashyna et al. 2010). This raises that concern that sepsis may pose a subsequent particular risk to the brain health of older patients.

We have previously characterized the systemic and neuropathological findings of an LPS-induced rat sepsis

model (Wang et al. 2018; Towner et al. 2018; Rodrigues et al. 2017). This model results in secondary neuroinflammation and the accumulation of p-tau and cortical A β plaques that are morphologically and compositionally similar to diffuse (senile) plaques (Wang et al. 2018). This sepsis model also results in multiple MRI-based abnormalities in the brain, including those of the hippocampus, such as altered perfusion and blood-brain barrier disruption as well as hippocampal MRI spectroscopic findings of metabolic derangement and accumulation of free radicals (Towner et al. 2018).

In the current report, we further investigate the LPS-induced effects in the hippocampus in the rat sepsis model. We have documented that A β plaques and p-tau accumulate in the hippocampus as a result of LPS exposure. Specific subregions of the hippocampus have particularly prominent accumulation of A β plaques. These regions include the dorsal hippocampus, which includes the granule cell layer and subgranular zone (GCL/SGZ) and the striatum lacunosum moleculare (SLM). The lateral CA1/CA2 regions are also prominent in A β plaque deposition. Multifocal intracellular p-tau deposition was also identified and was specifically quantified in the dorsal dentate gyrus and the lateral CA1/CA2 regions. Advanced tauopathy is associated with neurofibrillary tangles and typical Alzheimer's A β plaques are defined as neuritic, which were not identified in our samples. However, the progression of tauopathy and the formation of neuritic plaques in Alzheimer's disease may take years to develop and these elements are also not specific for Alzheimer's disease. The findings of intracellular accumulation of p-tau and the deposition of A β plaques in vulnerable regions of the hippocampus 1 week following administration of a single high dose of LPS raises concern for the potential for progression to more advanced neuropathology.

The regions that demonstrate accumulation of p-tau and A β plaques following LPS exposure are among the first to show abnormalities in Alzheimer's disease and age-related cognitive impairment. The dorsal hippocampus plays a significant role in adult neurogenesis with neural stem cells in the SGZ that divide and migrate into the GCL, which is thought to exert regulatory control of adult neurogenesis of the SGZ. This complex contributes to the concept of hippocampal plasticity (Seri and Alvarez-Buylla 2002). The SLM contains perforant fibers connecting the entorhinal cortex (EC) with the hippocampus, particularly CA1, CA3, and the dentate gyrus. The EC-SLM connection pathway has been

implicated in the spread of tauopathy in early Alzheimer's disease and with tauopathy-related deficits in spatial memory and orientation in animal models (Maurin et al. 2014). The SLM is associated with tauopathy-related spatial memory and orientation abnormalities in Alzheimer's disease, mild cognitive impairment, and tauopathy in animal models (Liu et al. 2012; Clavaguera et al. 2009; Monacelli et al. 2003; Van Cauter et al. 2013; Delpolyi et al. 2007). The CA1 and CA3 regions of the hippocampus are among those that demonstrate the greatest magnitude of neuronal loss in Alzheimer's disease patients (Padurariu et al. 2012).

In the current study, we have demonstrated that specific neurocognitive deficits are apparent in the LPS-treated rats, when compared with those in controls. These deficits are associated with episodic-like memory activities that primarily involve spatial, contextual, and temporal orientation and integration. The behavioral abnormalities could not be attributed to acute sickness behavior resulting from LPS injection. At 1-week post LPS injection, LPS-treated rats exhibited no outward signs of sickness behavior and were indistinguishable from the control rats in normal activities, weight, and food and water intake. There was no difference in baseline exploratory activity during the habituation phases between LPS-treated and control rats. Furthermore, in the performance of tasks that did not require dentate gyrus function, there was no significant difference between LPS-treated and control rats. However, in tasks that require the integrity of the dorsal dentate gyrus, the LPS-treated rats were significantly impaired compared with controls.

There are a number of unanswered questions related to this study. First, the determinations were made at 1-week post LPS administration. Whether the animals eventually resolve with respect to A β plaque and intracellular p-tau deposition as well as behavioral deficits is unknown and will be critical to understanding the long-term neuropathological consequences of sepsis. Whether longitudinal inferences of human outcomes based on the rat sepsis model can be accurately made is also uncertain given the relatively short lifespan of rats compared with that of humans.

The rats utilized in this experiment were approximately 2 months of age. However, the sepsis model in these younger rats may nonetheless be informative of the neuropathology of aging. Sepsis, with its burden of accumulated oxidative damage, has been likened to an accelerated aging process (da Silva and Machado 2018, review).

Whether hippocampal damage and neurocognitive deficits resulting from LPS exposure would be more severe in older rats has not yet been established. Older individuals show a decrease in hippocampal neurogenesis and are more vulnerable to an overall worse from sepsis than younger patients (Seib and Martin-Villalba 2015; da Silva and Machado 2018, review).

The LPS-induced sepsis model is a severe systemic inflammatory insult. Whether lower levels of chronic or acute systemic inflammation may also have similar hippocampal effects is not known. There is supportive evidence that low-grade systemic inflammation does result in worsened functional disability in elderly patients with dementia (Cervellati et al. 2018). In addition, neurocognitive impairment associated with the aging process in primates has been associated with neuroinflammatory features seen in the current rat sepsis model, such as microglial activation (Shobin et al. 2017).

The neuropathological emphasis of this project was on the hippocampus. This does not exclude the possibility that other brain regions may also play a role in sepsis-induced neurocognitive compromise. Aging is known to be associated with myelin sheath defects, axonal loss, and a reduction in white matter volume (Robinson et al. 2018). We have previously reported cortical deposition of A β plaques in the LPS-induced rat sepsis model (Wang et al. 2018). Therefore, the neuropathological abnormalities resulting from aging as well as sepsis-induced neuropathology are likely multifactorial and may involve many regions of the brain.

The behavioral testing performed in this study focused on functions attributable to the dorsal dentate gyrus. Whether other hippocampal, prehippocampal, parahippocampal, or extrahippocampal subregion analysis would reveal additional specific neurocognitive deficits in the LPS-induced sepsis model is not yet known. For example, gait abnormalities have been described both in animals with whole-brain radiation as well as aging humans with cognitive decline (Ungvari et al. 2017; Beauchet et al. 2017). No obvious gross gait disturbances were observed in the LPS-treated rats. However, a full cataloging of neurocognitive deficits resulting from LPS exposure has not been performed.

It should be stressed that there was no claim being made to suggest that sepsis or LPS exposure causes Alzheimer's disease. Rather, the current study supports that some common neuropathological mechanisms of hippocampal vulnerability may be common to sepsis-

induced neurocognitive impairment and other dementias, such as Alzheimer's disease and age-related memory loss. Opportunities for mitigating the hippocampal damage done by LPS-induced sepsis have yet to be explored. An early and aggressive approach in treating gram-negative bacteria in the elderly may have far-reaching benefits, not only in the prevention of life-threatening sepsis but also in the prevention of accelerated cognitive compromise. Toward this goal, the current research is valuable in that it provides measurable consequences, both pathologically and behaviorally, of the effects of LPS on the hippocampus. This may provide a platform to evaluate the efficacy of therapies to mitigate the neuropathological consequences of sepsis.

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