

Nerve agent exposure and physiological stress alter brain microstructure and immune profiles after inflammatory challenge in a long-term animal model of Gulf War Illness

Detailed Data Collection Methods

Animals and Exposure Paradigm

Adult male Sprague Dawley rats (N=29; 250-300g) were purchased from Hilltop Lab Animals, Inc. (Scottsdale, PA, USA). Upon arrival, rats were single housed in a temperature ($21\pm 1^{\circ}\text{C}$) and humidity ($50\pm 15\%$) controlled room maintained under filtered positive-pressure ventilation on a 12-h light/dark cycle (lights on 0600 ET) in the CDC-Morgantown Animal Facility and allowed to acclimate for at least one week prior to the exposures. Rats were given ad libitum access to food (Teklad 2918 Global 18% rat chow; Envigo, Madison, WI, USA) and water and received daily health checks from animal husbandry personnel. Rats were exposed to CORT (200 mg/L in 0.6% EtOH) for 7 days followed by a single injection of DFP (1.5 mg/kg, i.p.) on Day 8, as previously described (Koo et al., 2018). Following this initiating exposure, the rats were re-exposed to CORT every other week for an additional 4 weeks and then challenged with the prototypical inflammagen, lipopolysaccharide (LPS; 0.25 mg/kg, s.c.) on Day 36. Rats were arbitrarily assigned to one of five exposure groups at the start of the study: Saline (N=5), CORT (N=5), CORT+DFP (N=7), CORT+LPS (N=5), CORT+DFP+LPS (N=7).

Tissue collection

Rats were euthanized at 6 hours post-LPS by decapitation for the evaluation of cytokine mRNA expression by qPCR. Immediately following euthanasia, whole brains were removed from the skull with the aid of blunt curved forceps. Frontal cortices and hippocampi were dissected free hand on a thermoelectric cold plate (Model TCP-2, Aldrich Chemical Co., Milwaukee, WI, USA) using a pair of fine curved forceps (Roboz, Washington, DC, USA). A small sample was removed from one lobe of the liver using dissecting scissors. Isolated brain and liver tissue samples were immediately placed on dry ice and stored at -80°C .

qRT-PCR

The total RNA from the frontal cortex, hippocampus, and liver were isolated using Trizol® reagent (Thermo Fisher Scientific, Waltham, MA, USA) and Phase-lock heavy gel (Eppendorf, AG Hamburg, Germany), and purified using RNeasy mini-spin columns (Qiagen, Valencia, CA, USA). Total RNA (1 ug) was reverse transcribed to cDNA using Superscript III and oligo (dT)12-18 primers (Thermo Fisher Scientific, Waltham, MA, USA) in a 20 µL reaction. Real-time PCR analysis of the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and of the proinflammatory mediators, TNFα, IL-6, CCL2, IL-1β, leukemia inhibitor factor (LIF), and oncostatin M (OSM), as well as the astrocyte marker glial fibrillary acidic protein (GFAP) was performed in an ABI7500 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA) in combination with TaqMan® chemistry as previously described. All PCR amplifications (40 cycles) were performed in a total volume of 50 µL, containing 1 µL cDNA, 2.5 uL of the specific Assay of Demand primer/probe mix (Thermo Fisher Scientific, Waltham, MA, USA), and 25 uL of Taqman® Universal master mix (Thermo Fisher Scientific, Waltham, MA, USA). Sequence detection software (version 1.7; Applied Biosystems/Thermo Fisher Scientific, Waltham, MA, USA) was used to determine threshold cycle (CT) values.