Chronic Pain State Mediates Development of Hippocampal and Renal Inflammatory Responses

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Introduction

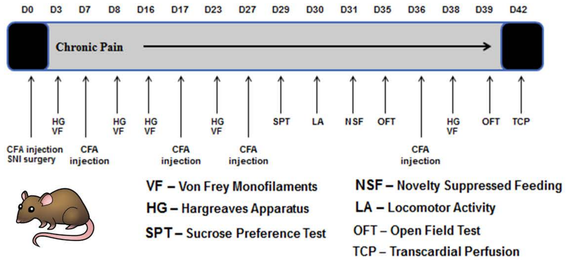
Chronic pain and related stress have been previously linked to the development of mood disorders and dysfunction of peripheral organs such as the kidney. While the underlying neurophysiological mechanisms remain elusive, here we examined the effects of chronic pain on activation of immune-inflammatory responses in the brain and kidney.

Biochemical analysis of the hippocampus, a limbic brain region that regulates mood and stress responses, was conducted in animals exposed to chronic inflammatory pain [multiple injections of Freund's adjuvant (CFA) into the hind paw]. Specifically, we assessed hippocampal expression levels of ionized calcium binding adaptor molecule 1 (IBA1) and NLRP3 inflammasome proteins, known markers of microglial activation and neuroinflammatory responses, respectively.

NLRP3 is also implicated in the pathogenesis of kidney diseases by regulation of renal inflammation and injury. Immunocytochemical analysis was conducted to assess the effects of chronic pain (CFA) on the expression of NGAL, IL-18, and NLRP3 protein in the renal glomeruli and tubules. Together, our studies are aimed to increase our mechanistical understanding of the bidirectional relationship between chronic pain and kidney dysfunction. Further understanding of this relationship could contribute to the identification of novel treatment strategies to diminish both mental health and renal physiological consequences of chronic pain.

Materials and Methods

Inflammatory and Neuropathic Pain Models
Adult male Sprague Dawley rats (Charles River, Wilmington, MA), age and weight matched (250-300 g), were exposed to either inflammatory (injection of Complete Freund's Adjuvant, CFA) for 42 days. Saline injections were used as controls.



Thermal Hyperalgesia – Rats were placed on a thermal analgesiometer (Hargreaves Apparatus), and both latency and duration of a hind paw withdrawal from the thermal source was measured in seconds.

Mechanical Allodynia – Rats were placed on an elevated mesh platform and plantar surface of their hind paws were stimulated with von Frey monofilaments (2.6-522mN) using the up-down method and a "response" was noted if paw withdrawal was evoked.

Sucrose Preference Test (SPT) – Rats were habituated to 1% sucrose solution for 48 h. Following a 4-6 h liquid deprivation, consumption of both water and sucrose solution were recorded during a 1 h test. Anhedonia was identified as reduction in sucrose intake when compared to controls.

Open Field Test (OFT) – Rats were placed inside an open field container for 10 min and their movement in the central vs. outer zone was recorded. Analysis was performed using AnyMaze software.

Western Blotting – The ipsilateral and contralateral hippocampi were dissected, followed by total protein and RNA extraction using PARIS kit (Ambion) with added phosphatase and protease inhibitors. Membranes were incubated with NLRP3, Casp-1, MKP-1 (Santa Cruz) or IL-1 β (Abcam) antibodies, while GAPDH protein was used as a loading control. Membranes were visualized via chemiluminescence and analyzed using ChemiDoc System (Bio-Rad).

Immunostaining and Imaging
Fixed kidney sections stained with NGAL antibody (Rabbit Polyclonal, Santa Cruz Biotechnology, Dallas, TX), NLRP3 antibody (Rabbit Monoclonal, Abcam, Cambridge, UK), or IL18 antibody (Chicken Polyclonal, Santa Cruz Biotechnology, Dallas, TX). Actin was visualized by phalloidin conjugated with Alexa Fluor488 (Life Technologies, Carlsbad, CA). Sections were imaged with a fluorescence microscope via 10x/0.25 objective (Zeiss, Jena, Germany).

Image Processing
A MATLAB algorithm was coded to semi-automatically measure the fluorescence intensity of glomeruli and tubules. The program iterates through a series of 10 images per sample and allows the user to select up to 10 glomeruli and tubules per image.

Results

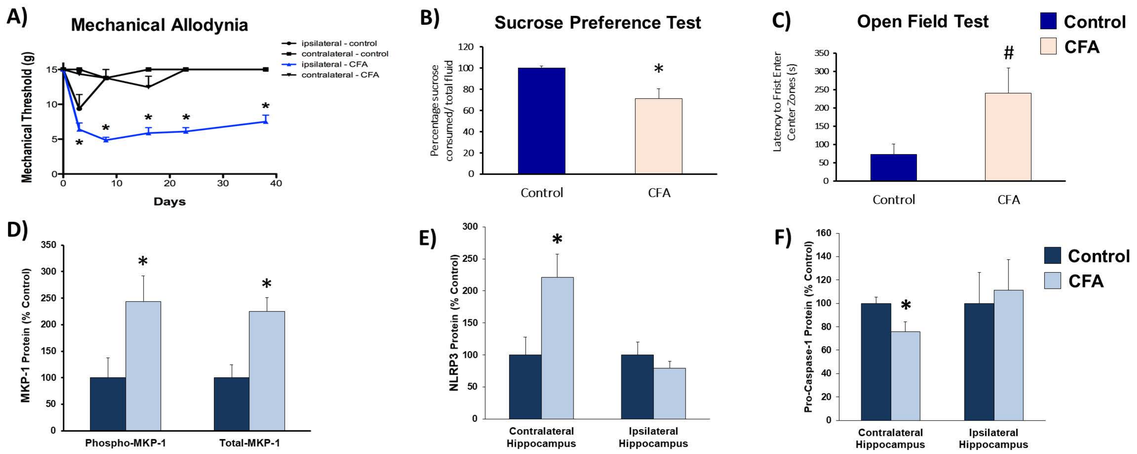


Figure 1. Pain and depressive-like behaviors and quantitative hippocampal protein analysis in CFA Animals. A) Mechanical sensitivity (allodynia) was assessed on days 3, 8, 16, 23, and 38 after CFA administration. Behavioral emotionality was assessed by measuring sucrose preference **(B)** and latency to enter the center zone in an open field test **(C)** Quantitative analysis of hippocampal MKP-1 **(D)**, NLRP3 **(E)**, and pro-caspase-1 **(F)** proteins. Data are expressed as mean % change or fold change over saline control group +/- S.E.M. (n=6); P < 0.05 compared to the control group.

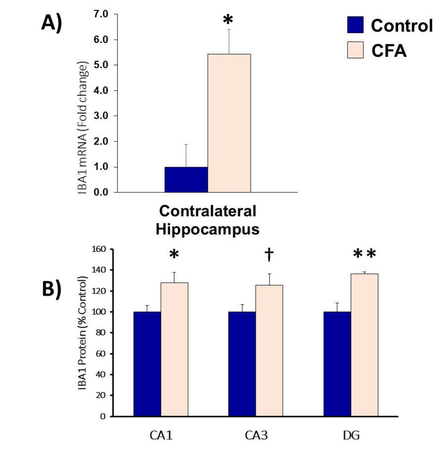


Figure 2. IBA1 mRNA and protein expression. A) qPCR was used to assess changes in IBA1 mRNA expression. Data is expressed as fold change. **B)** Immunohistochemical staining was used to assess changes in IBA1 protein levels throughout the hippocampus subregions (CA1, CA3, DG).

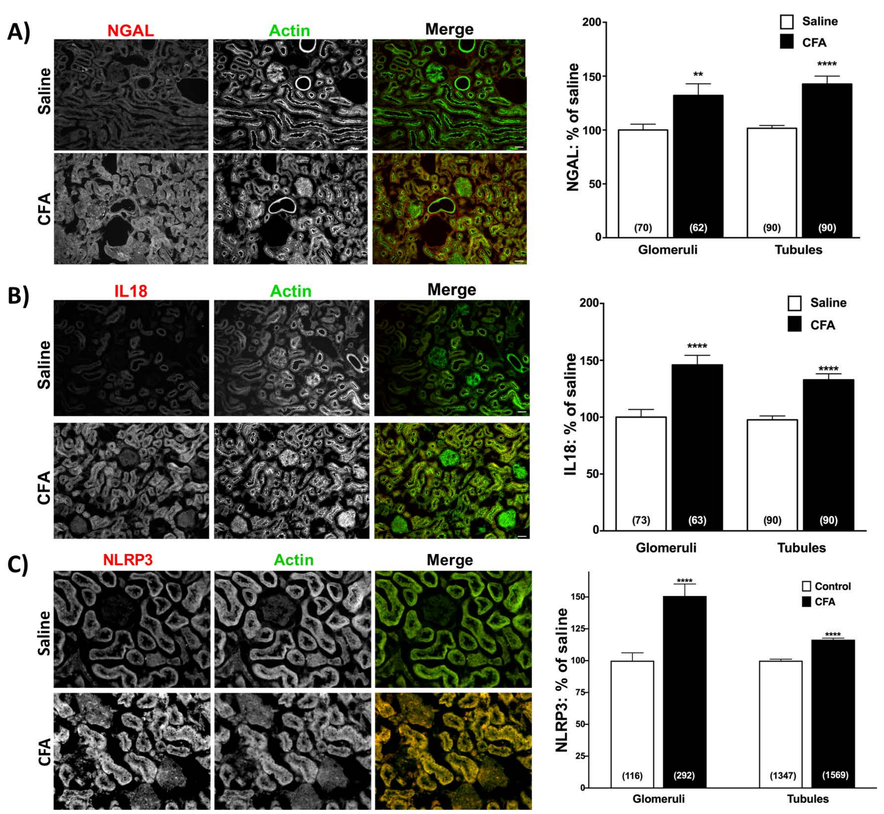
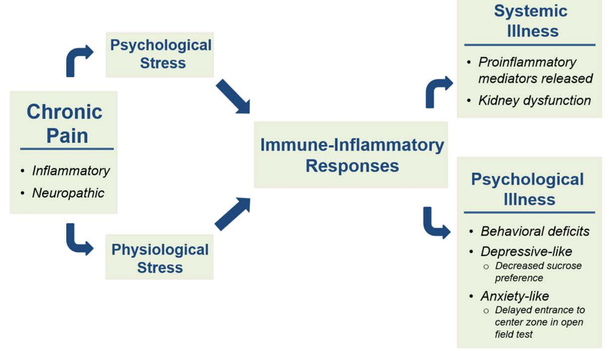


Figure 3. NGAL, IL-18, and NLRP3 protein expression in renal cortical slices in control or CFA treated rats. Representative image of perfusion-fixed kidney sections from rats treated with saline or CFA. Left image shows **A)** NGAL, **B)** IL-18, or **C)** NLRP3 immunostaining (red), middle shows actin visualized by Alexa Fluor488 phalloidin (green) and right shows merged composite of both protein and actin. Also shown are statistical analyses of NGAL, IL-18, and NLRP3. Signal in sham-operated rats defined as 100%. Bars and error bars represent the mean +/- standard errors from 3-independent experiments, respectively. Number of glomeruli and tubules given in parenthesis. **P<0.01, ****P<0.0001. Scale bar is 50 µm.



Summary/Conclusions

- Chronic CFA administration produced both pain (e.g., mechanical allodynia, hyperalgesia) and depressive-like behaviors (e.g., anhedonia)
- Chronic CFA administration evoked quantitative changes in MKP-1, NLRP3, and pro-caspase-1 hippocampal protein levels compared to control.
- Chronic CFA administration induced an increase in IBA1 mRNA expression as well as an increase in protein levels in the CA1 and CA3 regions of the hippocampus and the dentate gyrus.
- Chronic CFA administration significantly increased NLRP3 protein levels in glomeruli and tubules of the kidney. Increase in NLRP3 protein levels was associated with an increase in NGAL and IL-18 protein levels.
- Chronic pain-related stress induced depressive-like behaviors and renal inflammation and injury. Activation of NLRP3 inflammasome proteins is proposed as signaling mechanisms activated by chronic pain-related stress.

Acknowledgement

This work is supported by PhRMA Foundation Starter Grant (V.D.) and Iowa Osteopathic Education and Research (IOER) Funds (F.D. and V.D.)