Journal Club

Editor's Note: These short reviews of recent *JNeurosci* articles, written exclusively by students or postdoctoral fellows, summarize the important findings of the paper and provide additional insight and commentary. If the authors of the highlighted article have written a response to the Journal Club, the response can be found by viewing the Journal Club at www.jneurosci.org. For more information on the format, review process, and purpose of Journal Club articles, please see https://www.jneurosci.org/content/jneurosci-journal-club.

Microglia and Reactive Oxygen Species Are Required for Behavioral Susceptibility to Chronic Social Defeat Stress

Christopher A. Guevara,* Pamela Del Valle,* and Cynthia R. Mercedes

Graduate School of Biomedical Sciences, Nash Family Department of Neuroscience and Friedman Brain Institute, Icahn School of Medicine at Mount Sinai, New York, New York 10029

Review of Lehmann et al.

Psychosocial stress occurs in response to a potentially life-threatening interaction and leaves a lasting impact that can be detected and measured physiologically and psychologically. This type of stress is a major risk factor for the development of psychopathologies like depression and anxiety (Corcoran et al., 2002; Yang et al., 2015), making it a subject of intense interest. In recent years, strong correlative arguments have been made that suggest that at least some of the negative behavioral outcomes of psychosocial stress may lie downstream of immune responses, but causal evidence has been thin.

A prevalent animal model for studying the effects of psychosocial stress on the brain and body is chronic social defeat (CSD), a paradigm in which animals undergo daily bouts of physical subordination by a larger mouse for ~10 d (Iñiguez et al., 2016). After undergoing CSD, rodents display several social and anxiety-like behaviors that are similar to those observed in human depression and are reversed by treatment with antidepressants (Vialou et al., 2013).

CSD is also associated with an increase in inflammatory cytokines in the serum of rodents exhibiting depressive-like behaviors, and similar increases are seen in patients with treatment-resistant major depressive disorder (Hodes et al., 2014), suggesting that inflammatory markers may mediate the development of psychopathologies after stress. A possible avenue by which peripheral inflammation has been thought to induce inflammation in the CNS is through microglia, the primary immune responders of the brain and a unique myeloid cell population (Dionisio-Santos et al., 2019). Microglia can become activated by challenges like psychosocial stress, but the mechanisms by which microglia communicate during or after stressful events are not entirely understood (Galic et al., 2012).

As a result of infection, trauma, or CSD, activated microglia produce reactive oxygen species (ROS; Wolf et al., 2017). ROS can be beneficial to the CNS by damaging exogenous pathogens and playing important roles in the development of neural circuits (Zhang et al., 2016; Oswald et al., 2018), but when ROS are produced at high and sustained levels, they can damage cell membranes, proteins, and DNA of neighboring neurons (Spencer et al., 2016). Excessive production of ROS by microglia can also stimulate microglial production and secretion of proinflammatory substances, which can create a self-reinforcing cycle of microglial activation that negatively impacts neuronal health and viability (Spencer et al., 2016).

In addition to microglia, neurons also generate ROS after stressful events. A study showing that stress increases ROS production in neurons also showed that accompanying anxiety-like behaviors were reduced when ROS production was limited (Seo et al., 2012). Similarly, there is a positive correlation between anxiety-like behavior in mice and levels of ROS activity in neurons and glia (Rammal et al., 2008). Although these data suggest that microglia and neurons may respond similarly to stressful events, the dependency of ROS production, if any, on one cellular population versus another is not well understood.

To directly evaluate the contribution of microglia to social stress responses and ROS production, Lehmann et al. (2019) first depleted microglia in mice by administering PLX5622, an inhibitor that blocks access to a microglial survival factor, during and after CSD. Behavioral responses to the depletion of microglia were then monitored using two assays: an anxiety-like light/dark test measuring time spent in or crossing between brightly lit and dark compartments of a cage, and a two-chamber social interaction test measuring time spent near a perforated chamber housing a novel mouse versus one that was empty. Whereas CSD mice with intact

Received Sept. 9, 2019; revised Dec. 8, 2019; accepted Dec. 21, 2019.

This work was supported by T32 MH087004 (C.A.G., P.D.V.). We thank Dr. George Huntley, Dr. Deanna Benson, Dr. Swati Gupta, and Roxana Mesias for feedback on the paper.

The authors declare no competing financial interests.

*C.A.G. and P.D.V. contributed equally to this work.

Correspondence should be addressed to Christopher A. Guevara at christopher.guevara@icahn.mssm.edu or Pamela Del Valle at pamela.delvalle@icahn.mssm.edu.

https://doi.org/10.1523/JNEUROSCI.2175-19.2019

microglia showed anxiety-like behavior, mice depleted of microglia sustained baseline pre-stress behavior after CSD, presenting no anxiety-like behavior. These results suggest that microglial depletion protects mice from anxiety-like behavioral effects of stress, whereas microglia activation during CSD in microglia-intact mice may contribute to anxiety-like behavior.

To understand the role of ROS in responses to psychosocial stress, 2-OH-E fluorescence, a marker for ROS activity, was quantified in tissue sections after CSD. Microglia-depleted mice demonstrated a significant decrease in the intensity of 2-OH-E after CSD compared with CSD-exposed microglia-intact mice, which presented significantly elevated 2-OH-E intensity. To determine whether changes in behavior were due to elevated ROS during stress, an antioxidant, *N*-acetylcysteine (NAC), was injected intracranially. As expected, NAC-treated mice produced less 2-OH-E than controls. This intervention also reduced the social avoidance and anxiety-like behavioral effects of CSD. Specifically, microglia-intact mice injected with NAC showed more social interaction and more crosses between light and dark chambers after CSD compared with mice that were injected with vehicle.

Having shown that depleting microglia or reducing ROS protected against CSD-induced behavioral changes, Lehmann et al. (2019) asked whether microglia are the source of the damaging ROS. To do so, they allowed microglia to repopulate the brain after microglia-depleted mice underwent CSD. Surprisingly, this led to an increase in ROS, as measured by an increase in 2-OH-E intensity, and a reinstatement of CSD-induced anxiety-like behavior and social avoidance. Conversely, mice with continued microglia depletion showed consistently reduced 2-OH-E levels and a sustained protection from the anxiety-like behavior and social avoidance effects of CSD. These data support that microglia serve as the principal source of ROS and are necessary to mediate CSD-induced behaviors.

Considering that NAC-driven inhibition of ROS prevented anxiety-like and social avoidance behaviors, Lehmann et al. (2019) asked whether antioxidant administration during microglia repopulation would have a protective effect. The data show that NAC administration attenuated the behavioral effects of CSD in both microglia-intact and repopulated mice. Together, these findings suggest that microglia contribute to the ROS activity measured and drive social avoidance and anxiety-like behavioral effects of CSD stress.

The findings of Lehmann et al. (2019) contribute directly to a growing body of research probing the causal relationship between microglia, social avoidance, and anxiety (Yirmiya et al., 2015; McKim et al., 2018; Wohleb et al., 2018). Data from one study suggests that microglia are necessary to recruit circulating inflammatory monocytes to the brain, which they believe serve as the source for inflammatory cytokine-dependent modulation of behavioral responses to stress (McKim et al., 2018). Consistent with this interpretation, McKim et al. (2018) showed that administration of clonazepam (a benzodiazepine used to treat certain anxiety disorders) blocked microglial activation, monocyte recruitment, social avoidance, and anxiety-like behaviors in mice that underwent CSD. These findings fit well with previous research showing that antidepressants suppress microglial activation and inhibit proinflammatory cytokine production. They also support the hypothesis that microglia are key regulators of social avoidance and anxiety-like behaviors resulting from psychosocial stress (Yirmiya et al., 2015).

Although Lehmann et al. (2019) identified an increase in ROS production after CSD that was prevented by microglial depletion, a direct mechanism by which microglia influence ROS production remains to be defined. Studies by Ding et al. (2017) and Kawai et al. (2018) propose NADPH oxidase and voltage-gated proton channels as potential targets that may influence microglia-specific ROS production. However, it is not known whether these proteins contribute significantly to the behavioral readout observed in the study by Lehmann et al. (2019). Given that NAC administration fully protected against the behavioral effects of CSD, the contribution of NADPH oxidase and voltage-gated proton channels may be minor in this context. Future studies would need to manipulate NADPH oxidase and voltage-gated proton channels to better understand the direct link between ROS production, microglia, and the behavioral effects of psychosocial stress.

One of the most intriguing observations by Lehmann et al. (2019) was that even when CSD takes place when microglia are depleted, mice with repopulated microglia show increased ROS and exhibit behaviors resembling mice having intact microglia throughout CSD. If most microglia were depleted, newly proliferated microglia that produce ROS at levels similar to those in non-microglia-depleted mice must have had a "memory" of CSD. In other words, either the small population of microglia that survived depletion or neighboring cells exhibit lasting CSD-related effects. A variety of epigenetic modification targets have been shown to directly influence microglial memory (Desplats et al., 2019) and may explain the findings by Lehmann et al. (2019). For example, it has been shown that broadly inhibiting histone deacetylases (HDACs), enzymes that modulate DNA expression, decrease cytokine release, suggesting a decline in microglial activation (Suh et al., 2010). Relevant targets may also be contained within the microglia-specific transcriptional changes in mice that have undergone psychosocially stressful events (Lacal and Ventura, 2018). To further support that epigenetic modifications contribute to a microglial memory after challenging events, a study by Wendeln et al. (2018) found that microglia underwent epigenetic remodeling after inflammatory stimuli were peripherally applied in mice, and that this remodeling was impaired when HDAC 1 and 2 were knocked out in microglia. Localizing the sites and agents underlying microglial memory forms part of an exciting avenue of research that provides a continuing expansion of potential targets for therapeutic intervention.

Subsequent experiments could build on the findings by Lehmann et al. (2019) by identifying the microglial epigenetic signatures of animals that did not show social avoidance and anxiety-like behaviors as a result of CSD. In this regard, Lehmann et al. (2019) did not address the natural susceptibility and resiliency-to-stress spectrum of their animals before CSD, nor did they assay behavioral susceptibility or resiliency to CSD effects before microglia depletion. A previous study from this group showed that the gene expression profile of microglia in the brains of mice resistant to the social avoidance and anxiety-like behavioral effects of CSD was largely similar to home-cage, unstressed controls. Such expression profiles were heavily enriched in genes implicated in reducing the effects of stress (Lehmann et al., 2018). In contrast, stress-susceptible mice, which are socially avoidant and exhibit anxiety-like behavior after CSD, showed elevated levels of expression of microglial gene profiles implicated in inflammation, oxidative stress, and blood–brain barrier breakdown. These observations suggest a difference in microglial gene expression profiles between stress-resilient and susceptible animals, as well as demonstrate the importance of observing microglial ge-

netic signatures before microglial depletion and after microglial repopulation in relation to CSD.

The work by Lehmann et al. (2019) highlights the relevance of microglia and ROS in mediating behavioral responses to psychosocial stress. It also serves to underscore that such responses are a collaborative effort that crosses cell type and requires multiple molecular mechanisms that are nevertheless lasting and coordinated by a form of cellular memory.

References

Corcoran C, Mujica-Parodi L, Yale S, Leitman D, Malaspina D (2002) Could stress cause psychosis in individuals vulnerable to schizophrenia? CNS Spectr 7:33–38, 41–42.

Desplats P, Gutierrez AM, Antonelli MC, Frasch MG (2019) Microglial memory of early life stress and inflammation: susceptibility to neurodegeneration in adulthood. Neurosci Biobehav Rev. Advance online publication. Retrieved December 2, 2019. doi:10.1016/j.neubiorev.2019.10.013.

Ding X, Zhang M, Gu R, Xu G, Wu H (2017) Activated microglia induce the production of reactive oxygen species and promote apoptosis of cocultured retinal microvascular pericytes. Graefes Arch Clin Exp Ophthalmol 255:777–788.

Dionisio-Santos DA, Olschowka JA, O'Banion MK (2019) Exploiting microglial and peripheral immune cell crosstalk to treat Alzheimer's disease. J Neuroinflammation 16:74.

Galic MA, Riazi K, Pittman QJ (2012) Cytokines and brain excitability. Front Neuroendocrinol 33:116–125.

Hodes GE, Pfau ML, Leboeuf M, Golden SA, Christoffel DJ, Bregman D, Rebusi N, Heshmati M, Aleyasin H, Warren BL, Lebonté B, Horn S, Lapidus KA, Stelzhammer V, Wong EH, Bahn S, Krishnan V, Bolaños-Guzman CA, Murrough JW, Merad M, et al. (2014) Individual differences in the peripheral immune system promote resilience versus susceptibility to social stress. Proc Natl Acad Sci U S A 111:16136–16141.

Iñiguez SD, Aubry A, Riggs LM, Alipio JB, Zanca RM, Flores-Ramirez FJ, Hernandez MA, Nieto SJ, Musheyev D, Serrano PA (2016) Social defeat stress induces depression-like behavior and alters spine morphology in the hippocampus of adolescent male C57BL/6 mice. Neurobiol Stress 5:54–64.

Kawai T, Tatsumi S, Kihara S, Sakimura K, Okamura Y (2018) Mechanistic insight into the suppression of microglial ROS production by voltage-gated proton channels (VSOP/Hv1). Channels 12:1–8.

Lacal I, Ventura R (2018) Epigenetic inheritance: concepts, mechanisms and perspectives. Front Mol Neurosci 11:292.

Lehmann ML, Weigel TK, Cooper HA, Elkahloun AG, Kigar SL, Herkenham M (2018) Decoding microglia responses to psychosocial stress reveals blood–brain barrier breakdown that may drive stress susceptibility. Sci Rep 8:11240.

Lehmann ML, Weigel TK, Poffenberger CN, Herkenham M (2019) The behavioral sequelae of social defeat require microglia and are driven by oxidative stress in mice. J Neurosci 39:5594–5605.

McKim DB, Weber MD, Niraula A, Sawicki CM, Liu X, Jarrett BL, Ramirez-Chan K, Wang Y, Roeth RM, Sucaldito AD, Sobol CG, Quan N, Sheridan JF, Godbout JP (2018) Microglial recruitment of IL-1β-producing monocytes to brain endothelium causes stress-induced anxiety. Mol Psychiatry 23:1421–1431.

Oswald MCW, Garnham N, Sweeney ST, Landgraf M (2018) Regulation of neuronal development and function by ROS. FEBS Lett 592:679–691.

Rammal H, Bouayed J, Younos C, Soulimani R (2008) Evidence that oxidative stress is linked to anxiety-related behaviour in mice. Brain Behav Immun 22:1156–1159.

Seo JS, Park JY, Choi J, Kim TK, Shin JH, Lee JK, Han PL (2012) NADPH oxidase mediates depressive behavior induced by chronic stress in mice. J Neurosci 32:9690–9699.

Spencer NG, Schilling T, Miralles F, Eder C (2016) Mechanisms underlying interferon-γ-induced priming of microglial reactive oxygen species production. PLoS One 11:e0162497.

Suh HS, Choi S, Khattar P, Choi N, Lee SC (2010) Histone deacetylase inhibitors suppress the expression of inflammatory and innate immune response genes in human microglia and astrocytes. J Neuroimmune Pharmacol 5:521–532.

Vialou V, Feng J, Robison AJ, Nestler EJ (2013) Epigenetic mechanisms of depression and antidepressant action. Annu Rev Pharmacol Toxicol 53:59–87.

Wendeln AC, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, Blank T, Wagner J, Häsler LM, Wild K, Skodras A, Blank T, Staszewski O, Datta M, Centeno TP, Capece V, Islam MR, Kerimoglu C, Staufenbiel M, Schultze JL, et al. (2018) Innate immune memory in the brain shapes neurological disease hallmarks. Nature 556:332–338.

Wohleb ES, Terwilliger R, Duman CH, Duman RS (2018) Stress-induced neuronal colony stimulating factor 1 provokes microglia-mediated neuronal remodeling and depressive-like behavior. Biol Psychiatry 83:38–49.

Wolf SA, Boddeke HW, Kettenmann H (2017) Microglia in physiology and disease. Annu Rev Physiol 79:619–643.

Yang L, Zhao Y, Wang Y, Liu L, Zhang X, Li B, Cui R (2015) The effects of psychological stress on depression. Curr Neuropharmacol 13:494–504.

Yirmiya R, Rimmerman N, Reshef R (2015) Depression as a microglial disease. Trends Neurosci 38:637–658.

Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, Dong W (2016) ROS and ROS-mediated cellular signaling. Oxid Med Cell Longev 2016:4350965.