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P114. Impaired Microglial Pruning of Excitatory Synapses on Developing CRH-Expressing Hypothalamic Neurons Exacerbates Stress Responses Throughout Life

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Background: The developmental origins of stress-related mental illnesses are well-established, and early-life stress/adversity (ELA) is an important risk factor. We and others have found that early-life exposure to an impoverished environment and unpredictable maternal care (in a limited bedding and nesting [LBN] paradigm) provokes major alterations in cognitive and emotional function, including anhedonia, accompanied by aberrant connectivity between the hippocampal-limbic system and reward/pleasure-related regions. Within the hypothalamus, this early-life adversity causes an increase in the number of excitatory synapses onto corticotropin-releasing hormone (CRH)-expressing neurons in the paraventricular nucleus (PVN). Such synaptic changes suffice to induce large-scale and enduring epigenomic changes in the expression of neuronal genes, including Crh. However, the mechanisms by which early-life adversity modulates synapse development or persistence in developing brain circuits remain unknown. We hypothesize that microglia contribute to normal synapse reduction on CRH + neurons in the developing PVN, and that adverse early-life experiences interfere with this function, leading to lifelong stress vulnerabilities.

Methods: To interrogate microglial function, we employed dual-reporter transgenic mice with visible CRH + neurons and microglia and two-photon time-lapse imaging in acute slices of the PVN. We obtained these

hypothalamic slices from P8 male and female mice (n = 8-10/group) that were reared in LBN or control cages from P2 to P8. We then visualized live microglial process dynamics and their interactions with CRH neurons. In fixed tissue, we utilized 3D-reconstruction confocal microscopy and immuno-detection of pre- and post-synaptic markers to quantify in high-resolution the developmental trajectory of synapse density and engulfment by microglia in the PVN (n = 8-10/group). To probe whether microglial function is required for normal synapse development, we inhibited microglial phagocytosis with a targeted Mer tyrosine kinase inhibitor and assessed synapse number on CRH + neurons (n = 5-8/group). In a final mechanistic experiment, we capitalized on cell type-specific DREADD technology to express activating Gq-DREADDs in microglia and delivered CNO continuously via a subcutaneous slow-release pellet from P3 to P10 (n = 6-12/group). We then probed whether this exogenous activation of microglia prevented the effects of ELA on microglial function, synapse number on CRH + neurons, and lifelong stress responses. Statistical analyses included t-tests, or one-way or two-way ANOVA, as appropriate.

Results: Here we find that ELA increases the number and function of excitatory synapses onto stress-sensitive hypothalamic corticotropin-releasing hormone CRH + neurons (t[12.44] = 2.95, p = 0.01), and implicate disrupted synapse pruning by microglia as a key mechanism. Microglial process dynamics in live imaging, and engulfment of synaptic elements by microglia, were both attenuated in ELA mice (t[16] = 2.79, p = 0.01), associated with deficient signaling of the microglial phagocytic receptor Mer. Accordingly, selective chemogenetic activation of ELA microglia (with CX3CR1-Gq-DREADDs) increased microglial process dynamics and reduced excitatory synapse density to control levels (F[2,23.8] = 3.76, p = 0.04). In terms of functional outcomes, selective early-life microglial activation also mitigated the adrenal hypertrophy and prolonged stress responses in adult ELA mice (F[3,24] = 11.11, p < 0.0001).

Conclusions: These findings establish microglial actions during development as powerful contributors to experience-dependent sculpting of stress-related brain circuits. The manipulation of microglial function during development to prevent stress-related emotional disorders in adulthood may provide novel targets for therapeutics or preventative interventions in neuropsychiatric disorders.

Keywords: Microglia, CRH + Neurons, chemogenetics, 2-photon Techniques, Synaptic Pruning

Disclosure: Nothing to disclose.