

**Title:** Impact of Early Life Pain on Microglia Expression and Activation in the Periaqueductal Gray of Adult Male and Female Rats

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**Introduction:** The developing brain is extremely sensitive to exogenous signals, and recent studies suggest that adverse experiences during the perinatal period, including maternal stress, trauma, or infection, may profoundly alter developing neural circuits. We have previously shown that a single inflammatory insult on the day of birth significantly alters opioidergic neural circuits within the midbrain PAG, permanently changing subsequent responses to pain and stress. Microglia, the resident immune cells of the brain, are particularly vulnerable to early life perturbations, which may have profound consequences for future immune responses. However to date, the impact of early life pain on microglia expression and activation are unknown.

**Methods:** On the day of birth, male and female rat pups received an injection of the inflammatory agent carrageenan into the hindpaw or were handled. In adulthood (postnatal day 60-90), rats were implanted with a Thermicron iButton to monitor autonomic output, and 7 days later, received an injection of the endotoxin lipopolysaccharide (LPS) to induce an immune challenge. Tissue was collected six hours after LPS injection and stained for microglia using immunohistochemistry. Microglia were characterized as ameboid, activated or quiescent based on morphology.

**Results:** No differences microglial colonization in the PAG were observed for early life pain versus control rats; similarly, no sex differences were observed. PAG sections are currently being analyzed for sex and treatment effects in glial morphology. We predict that the rats that received an early life injury will have more glial activation than the rats who were handled, indicating a lower threshold for activation. We also predict a difference in male and female microglial activation, consistent with what we have observed in past studies.

**Discussion:** While early life injury does not affect the density or baseline phenotype of microglial cells in the PAG, it may result in a lower activation threshold in comparison to controls. These results would have significant implications for subsequent immune challenges, and may increase the risk for infants who experience early life pain (e.g. premature infants born into the NICU) for increased risk of developing autoimmune disorders.