

From Breath to Call: Neuromodulatory Role of IL-6 in Neonatal Brainstem function. Ultrasonic Vocalization in Neonates

Isaac Vargas, PhD., Emily Silva, David Villalobos, Jose Otero MD, PhD, Michele Alves MS, PhD.

Department of Cellular and Molecular Medicine, Herbert Wertheim College of Medicine, Florida International University, Miami, FL.

Background: Neonatal sepsis is the leading cause of morbidity and mortality in newborns. IL-6 is emerging as a cytokine with neuroprotective or detrimental effects and has been shown to regulate neurodevelopmental processes. Our previous work demonstrate that IL-6 plays a key role modulating neonatal immune activation and brainstem neuroinflammation, which disrupts breathing. The brainstem circuits controlling autonomic breathing overlap with those that generate vocalizations, transforming respiratory rhythms into patterned calls. Ultrasonic vocalizations (USV) are a key communicative behavior in mice that provide sensitive readout for respiratory-vocalization circuit integrity. Our study sought to determine how IL-6 loss alters neonatal brainstem circuitry, using USVs as an integrative measure of respiratory–vocalization network function.

Methods: *Il6^{tm1Kopf}/J* (IL6^{KO}) and IL6^{+/+} mice were utilized throughout postnatal day (PD) 5 to 7. At PD5 they were *i.p* administered with either saline or LPS. Following 3, 24, 48 and 72 hours USVs were recorded for 5 min in a soundproof chamber using a condenser microphone connected to Ultrasound Gate hardware. All analysis was performed using the VocalMat add on MATLAB and R Studio for the data analysis.

Results: To evaluate the neuromodulatory effects of IL6 in neonatal brainstem circuits, we first determined whether IL-6 ablation alters USVs. Baseline recordings revealed that IL6^{KO} pups exhibited a significant reduction in call number and mean bandwidth compared to IL6^{+/+} at PD7 and PD8. To further characterize these differences, we mapped syllables features using a diffusion map, which revealed genotype-dependent shifts in syllables centroid distances, highlighting the impact of IL6 loss on vocalization structure. Neonatal immune activation by LPS resulted in a significant reduction calls in acute phase (3h) in both IL6^{+/+} and IL6^{KO}. However, during the early-adaptive phase (24 h), this effect was reversed exclusively in IL-6^{+/+} mice, which displayed an increased number of calls that persisted throughout the adaptive response at 72 h. Consistent with these findings, syllables centroid distances demonstrated that LPS induced marked changes in call patterns in IL-6^{+/+} pups, whereas IL-6 ablation prevented these adaptive shifts at 24 h. At 48 h post-LPS, the vocalization repertoire in IL-6^{+/+} mice became more similar between LPS-treated and control groups, suggesting convergence of call patterns during adaptation. In contrast, IL-6^{KO} pups that received LPS maintained a distinct syllable repertoire throughout the adaptive phase. Analysis of established calls categories a showed positive association with flat and short calls in IL6^{KO} regardless of the immune activation with LPS.

Conclusions: Our findings highlight IL-6 as a key neuromodulator of neonatal breathing-vocalization circuits, shaping adaptative vocal behavior in response to immune activation.

Acknowledgements: This work was supported by: NIH/NHLBI R01HL132355 for JJO and FIU Start-up for JO and MA.