

Lipopolysaccharide-induced Ca²⁺ oscillations in neonatal rat *nucleus tractus solitarius* (NTS) *in vitro*

Melisa Custer, Noah Osman, Christopher G. Wilson*

Center for Perinatal Biology, Loma Linda University, Loma Linda, California, U.S.A.

39.23

Abstract

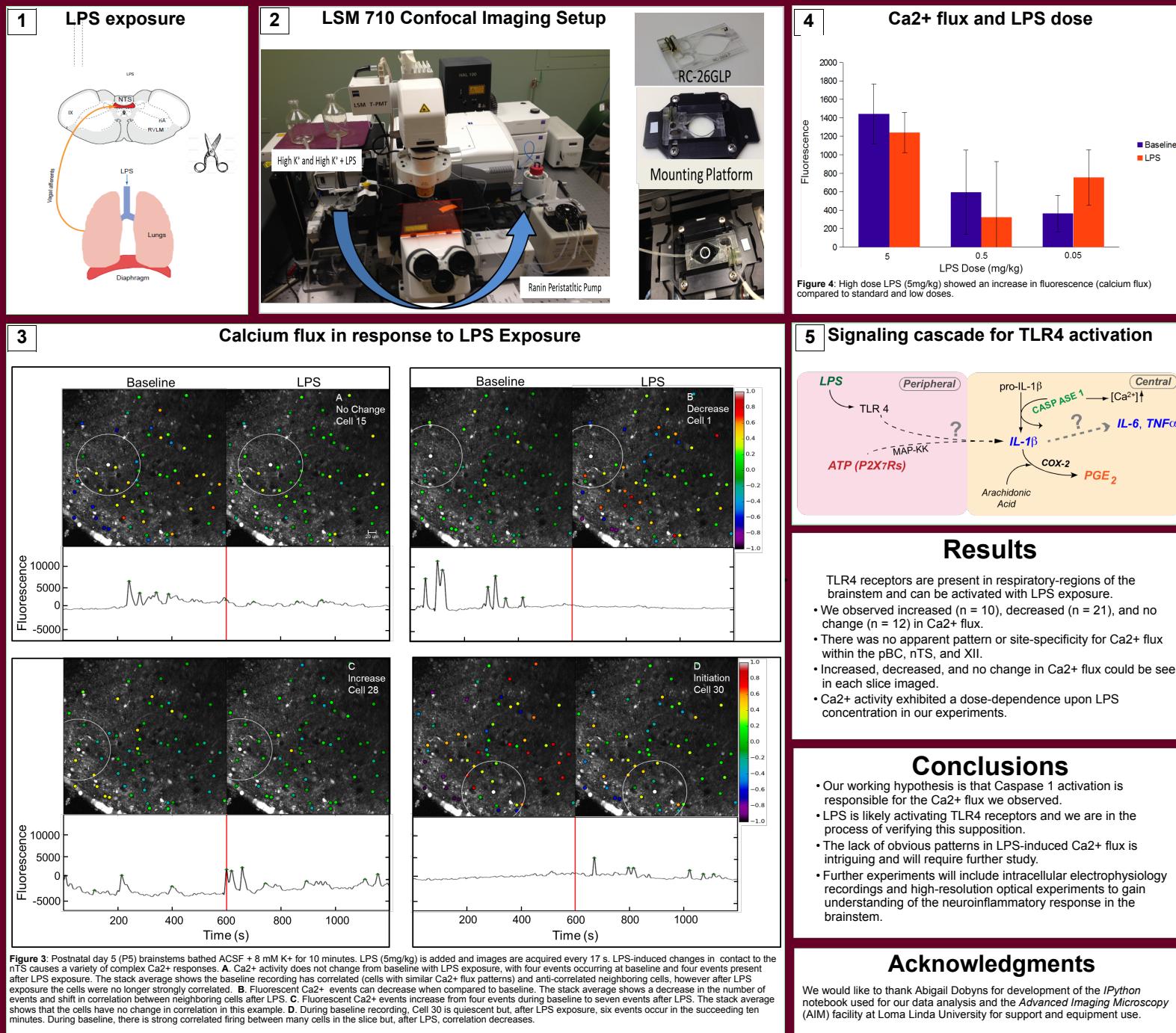
Lipopolysaccharide (LPS), or endotoxin, is commonly used to induce an inflammatory response in animal models. In premature infants, systemic infection (sepsis) causes tachypnea and an increase in the number of apneas over time. The mechanism by which sepsis alters breathing control centrally is unknown. Our laboratory has previously shown that LPS instilled into the airway of 10 day old rats causes changes in IL-1 β expression in brainstem regions associated with breathing control—including the rostral medulla, NTS, and the hypoglossal (XII) motor nucleus. We hypothesized that expression and release of cytokines in the brainstem alters neuronal activity in these areas which, in turn, alters breathing rhythm. To test this hypothesis, we performed experiments using confocal imaging to evaluate changes in calcium flux in neurons within the NTS before and after exposure to LPS (0.5 mg/kg). We cut acute organotypic slices (300 μ m) from brainstems of postnatal day 1 to 5 rat pups. These slices included the preBötzinger complex, XII motoneurons, and the NTS. Fluo-4 AM was used to label the slices, prepared in DMSO/Pluronic and incubated for 1 hour with 95% O₂/5% CO₂ bubbled in the chamber. Then the slices were transferred to a Zeiss LSM-710 NLO for imaging calcium transients. We found that NTS neurons showed an increase in response to LPS-containing perfusion. In 5 cells we saw calcium transients that showed changes in burst duration within 10 minutes after LPS infusion began. Baseline burst durations started at 0.024 (SD 0.03) per minute and increased within 10 minutes of LPS exposure to 0.564 (SD 0.16). The bursts declined after 30 minutes of exposure to 0.192 (SD 0.21) and continued to fall with bursts slowing to 0.012 (0.03) after 50 minutes of exposure. The changes in network activity we observed may be the underlying substrate for the changes in breathing pattern seen in sepsis. Changes in the NTS may gate afferent inputs carried via the vagus nerve to the CNS and gating of these afferent inputs may be critical to the breathing changes we have previously observed in our rat models.

Hypothesis

We hypothesized that LPS induced activation of TLR4 receptors in the brainstem alters neuronal activity in respiratory-related regions of the brainstem.

Methods

Sprague-Dawley rat pups (postnatal days 1 to 5) were anesthetized (n=5) with isoflurane and the brainstems removed. Two organotypic slices, one rostral, one caudal with each containing preBötzinger Complex (280–300 μ m) were cut on a VT-1000 Vibratome. Fluo-4 AM (prepared in DMSO/Pluronic) was used to label slices. Slices were incubated for 1 hour and bubbled with 95% O₂/5% CO₂. Slices were transferred to a Zeiss LSM-710 NLO confocal/multi-photon microscope for imaging calcium transients. 30 minute videos were taken. In the first 10 minutes (baseline), the tissue was bathed with artificial cerebrospinal fluid (ACSF) with 8–9 mM [K⁺]. The next 20 minutes LPS (0.5 mg/kg) was added to the ACSF. Image stacks were continuously acquired during the 30 min experimental period and then analyzed *post-hoc* with *IPython* notebooks specifically developed for imaging analysis. We quantified raw F intensity, flash duration, frequency of flashes, and evaluated correlated flash patterns.



Results

TLR4 receptors are present in respiratory-regions of the brainstem and can be activated with LPS exposure.

- We observed increased (n = 10), decreased (n = 21), and no change (n = 12) in Ca²⁺ flux.
- There was no apparent pattern or site-specificity for Ca²⁺ flux within the pBC, nTS, and XII.
- Increased, decreased, and no change in Ca²⁺ flux could be seen in each slice imaged.
- Ca²⁺ activity exhibited a dose-dependence upon LPS concentration in our experiments.

Conclusions

- Our working hypothesis is that Caspase 1 activation is responsible for the Ca²⁺ flux we observed.
- LPS is likely activating TLR4 receptors and we are in the process of verifying this supposition.
- The lack of obvious patterns in LPS-induced Ca²⁺ flux is intriguing and will require further study.
- Further experiments will include intracellular electrophysiology recordings and high-resolution optical experiments to gain understanding of the neuroinflammatory response in the brainstem.

Acknowledgments

We would like to thank Abigail Dobyns for development of the *IPython* notebook used for our data analysis and the *Advanced Imaging Microscopy* (AIM) facility at Loma Linda University for support and equipment use.