Mapping Changes in Uterine Activity During Late Gestation with Wide-area *in vivo* Calcium Imaging in Mice

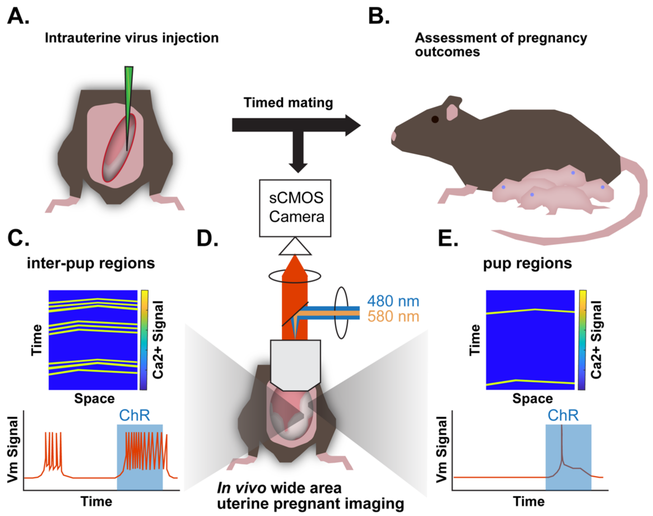
David J. Combs,[1,2], Sarah K. England,[3] Adam E. Cohen.[2,4]

[1]Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School. [2]Department of Chemistry and Chemical Biology, Harvard University. [3]Department of Obstetrics and Gynecology – Center for Reproductive Health Sciences, Washington University School of Medicine. [4]Department of Physics, Harvard University.

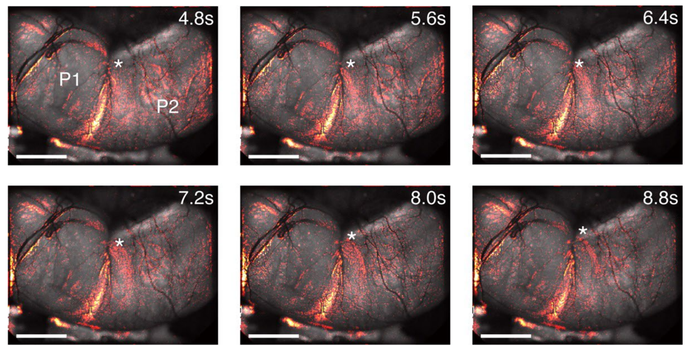
Background

- Uterine contractions support labor & delivery, conception and implantation.
- Despite knowledge of many molecular factors influencing uterine excitation, an understanding of how activity is coordinated at organ-scale is lacking.
- Traditional methods for studying excitation events in the uterus suffer from limited spatiotemporal resolution, mechanical interference, and/or dissected (*i.e.* damaged) preparations.

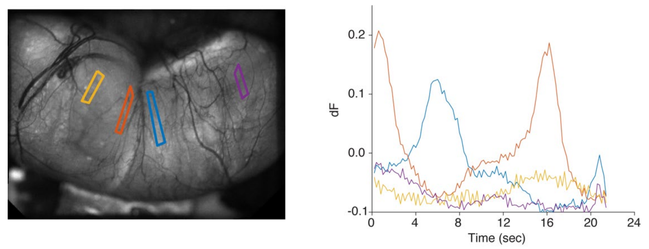
Construct expression, timed breeding, and imaging



GCaMP8m reveals whole-organ calcium activity patterns in live pregnant mice

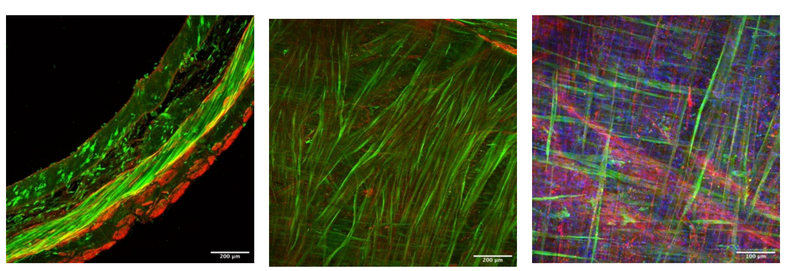


Time series of change in fluorescence (dF) overlaid on a grayscale image of the basal fluorescence signal. Asterisk marks advancing calcium wave. Field of view shows the most cervical (P1) and next most cervical (P2) implanted pups in the left uterine horn in a pregnant uterus at 18 dpc. Scale bar = 1 cm.



Regions of interest (left0 Intensity traces (right) showing the change in fluorescence (dF) at an area near the junction between two implantation sites (blue and orange) and areas overlying pup sites (yellow and purple).

Viral vectors injected prior to mating enable GCaMP8m expression in circular and longitudinal layers in late pregnancy



Left and Middle: confocal maximum intensity projection of axial ring (left) and tissue strip (middle) of uterus from late pregnant (dpc 18) mouse. 10X objective, 200 micron scale bars. Right: confocal maximum intensity projection of tissue strip from late pregnant (dpc 18) mouse. 20X objective, 200 micron scale bars. Green: anti-GFP-Alexa-488; Red: anti-SMA-Alexa-637; Blue DAPI.

Conclusions & Future Directions

- Developed approach for *in vivo* calcium imaging of uterine activity at organ scale in pregnant mice.
- Implantation sites appear to show more activity in late gestation than non-implantation sites.
- Future work will better define the contraction patterns of the pregnant uterus in late gestation and during labor

Acknowledgements: This work was supported by: BIRCWH K12 (K12HD051959), Harvard Anesthesia T32 (GM007592)), SOAP Young Investigator Award