Bolder BioPATH, Inc.

Boulder, Colorado. Est. 2003

Pre-Clinical Contract Research Organization

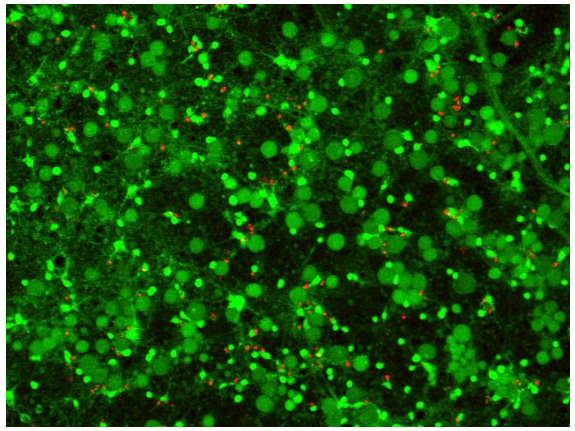
BolderBioPATH

Pre-Clinical Pharmacology & Pathology CRO

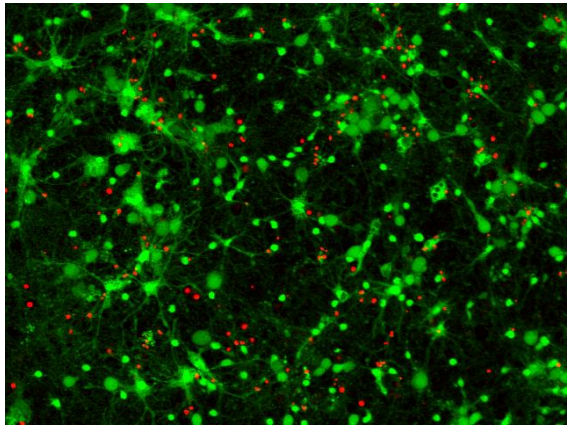
AGING/NEURODEGENERATION/INJURY

Glutamate Neurotoxicity

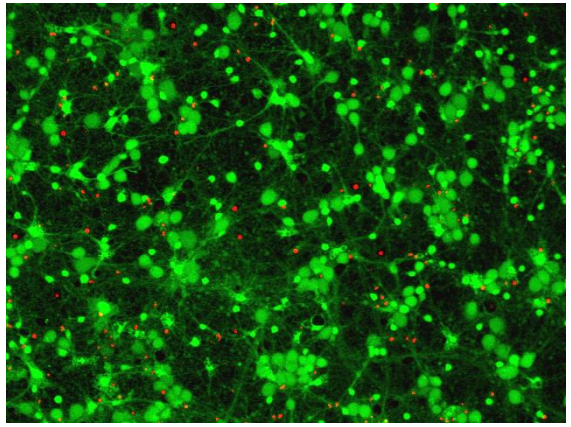
Control



Glutamate



10 µM treatment
+ Glutamate



Green = Live Cells (Calcein AM)

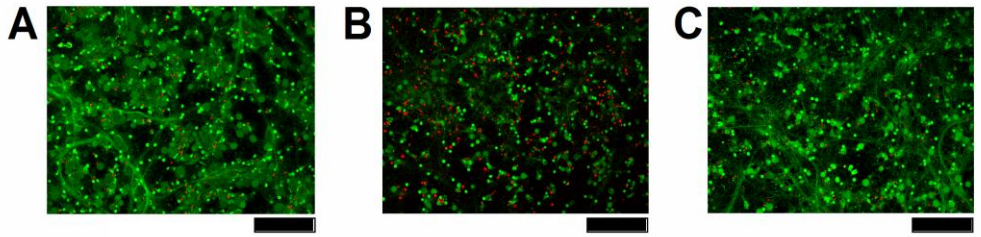
Red = Dead Cells (Ethidium Homodimer-1)

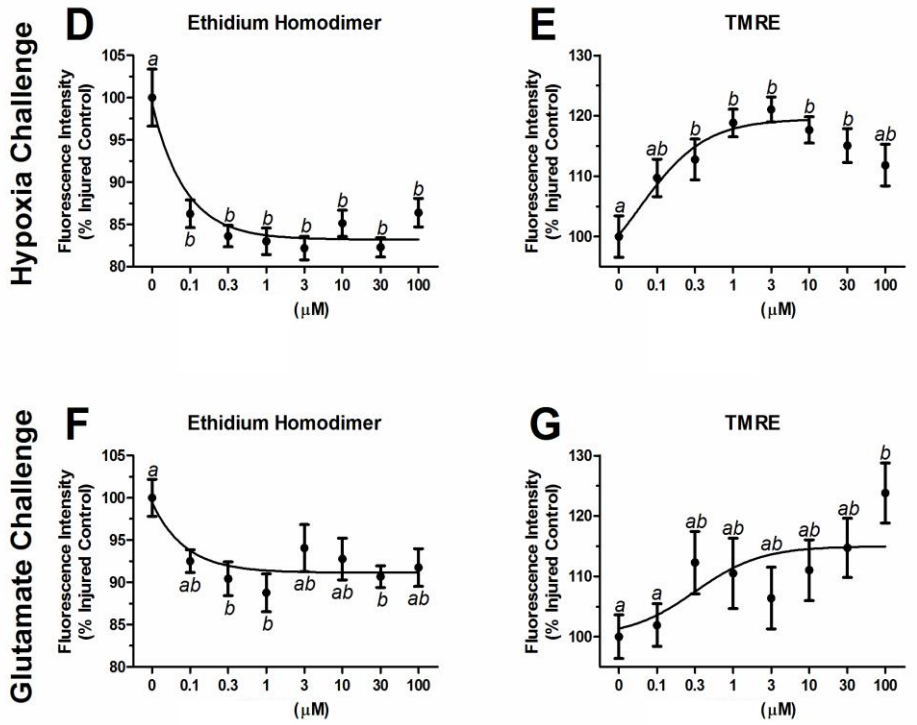
Primary hippocampal neurons 10 days *in vitro*
48hr pretreatment prior to 1hr glutamate exposure (50 µM)
Live dye and imaging 24hrs post-glutamate

Aβ, NMDA and LPS also available as insults

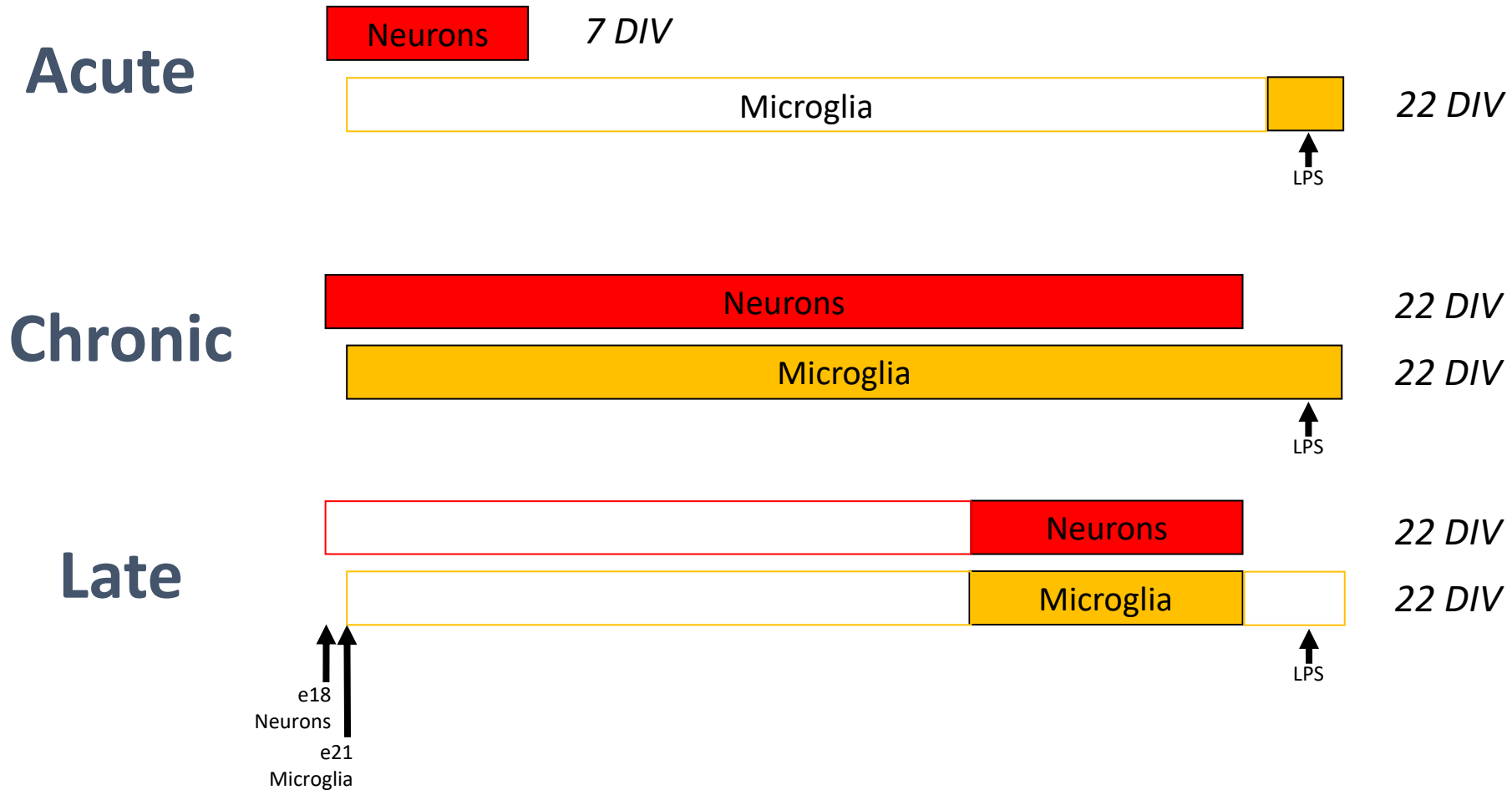
HYPOXIA AND NEUROTOXIN ENDPOINTS

Ethidium Homodimer, TMRE, Calcien AM, calcium imaging

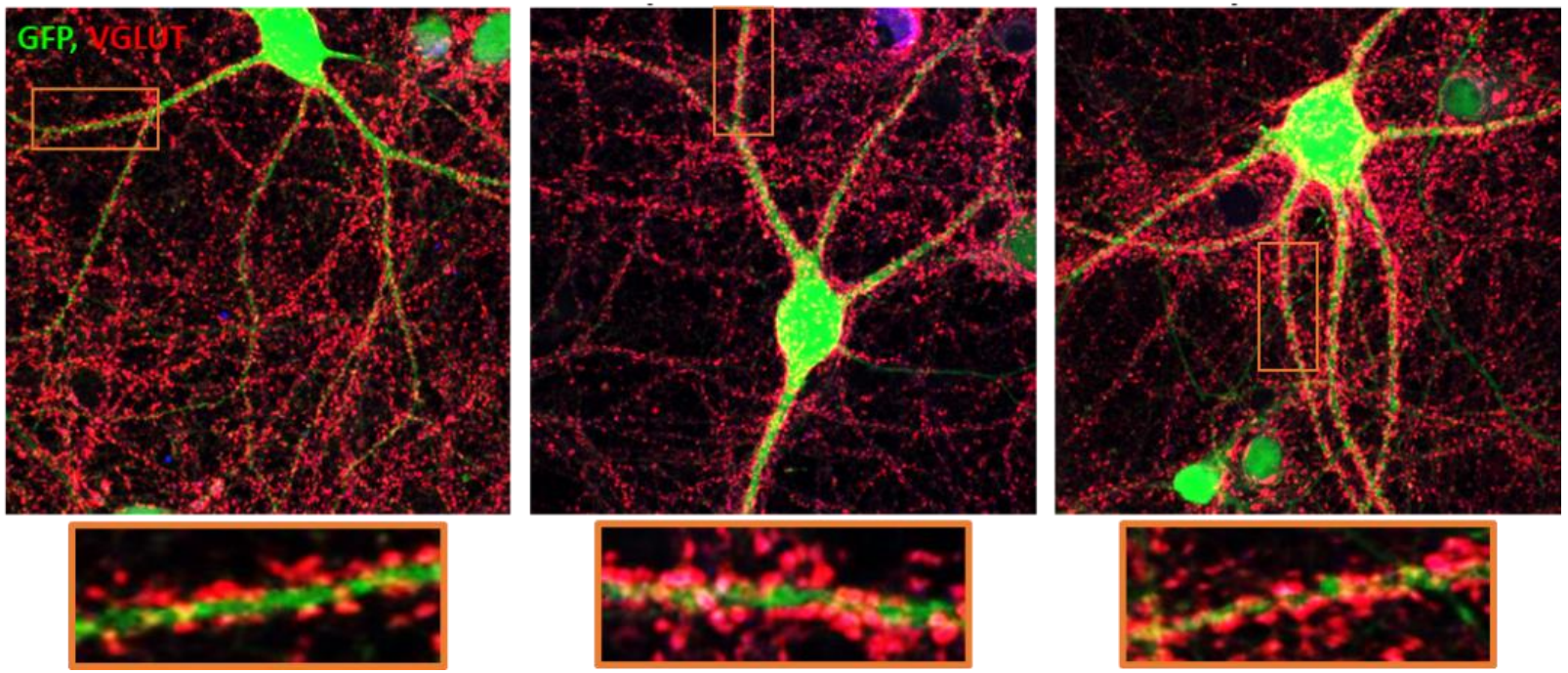




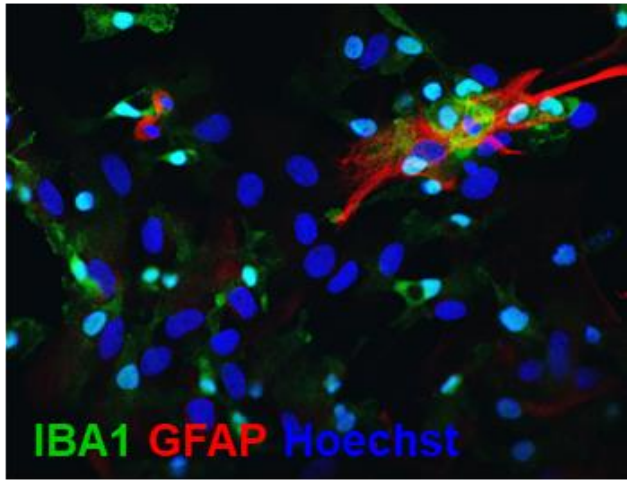
RANGE OF TREATMENT PERIODS

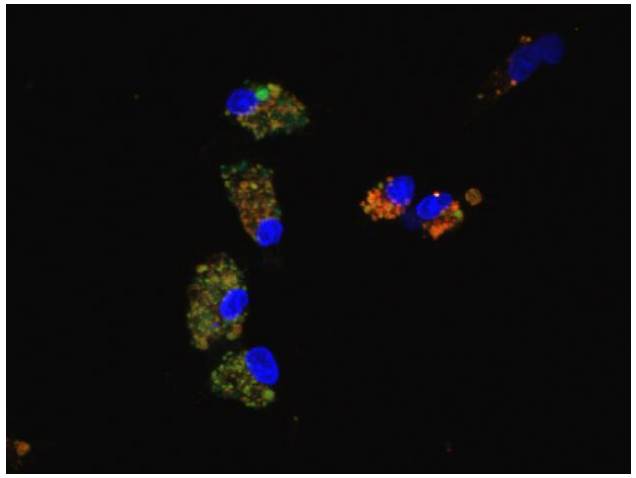


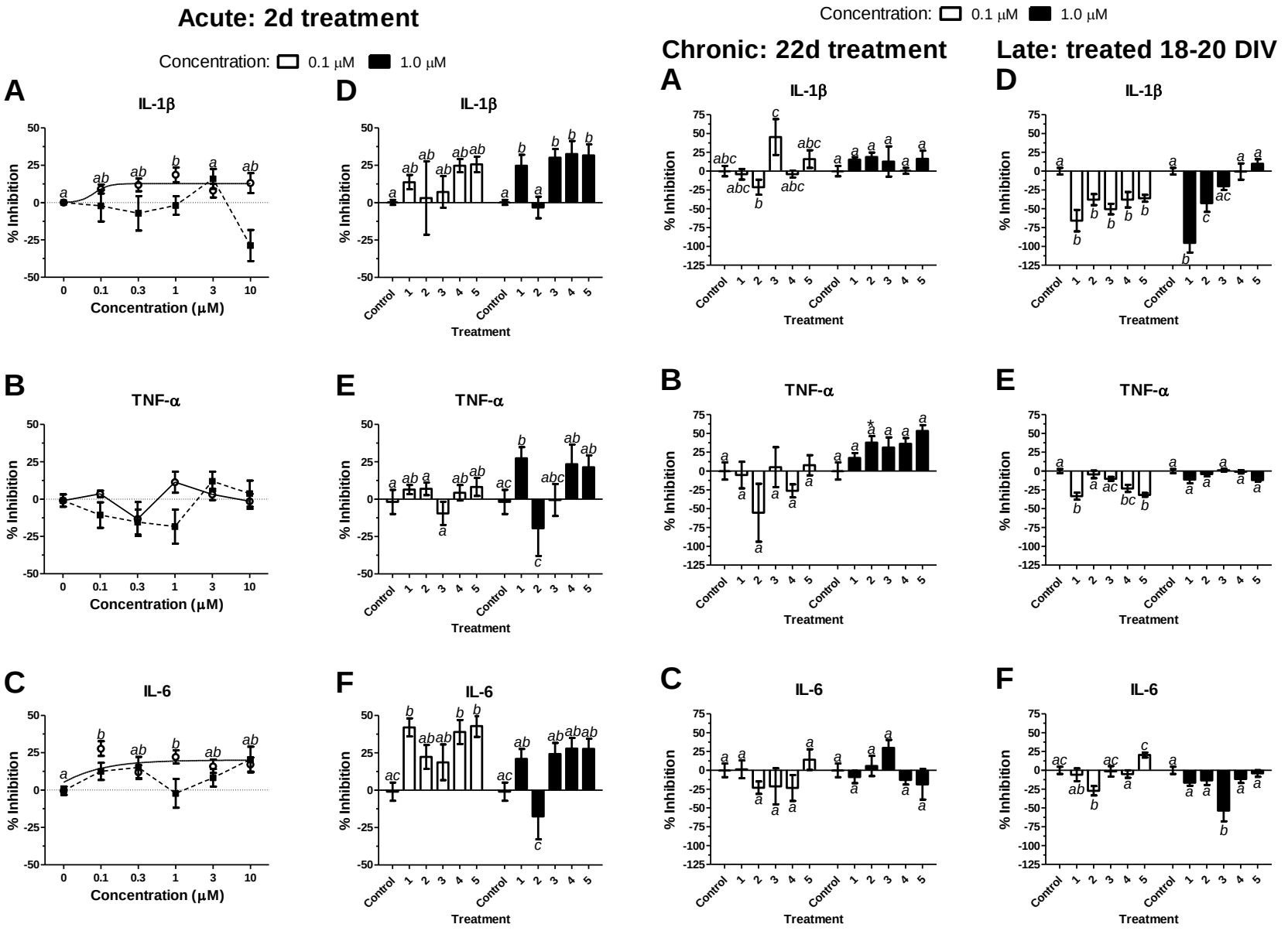
SYNAPTIC SIZE & DENSITY



MICROGLIAL ACTIVATION BY LPS

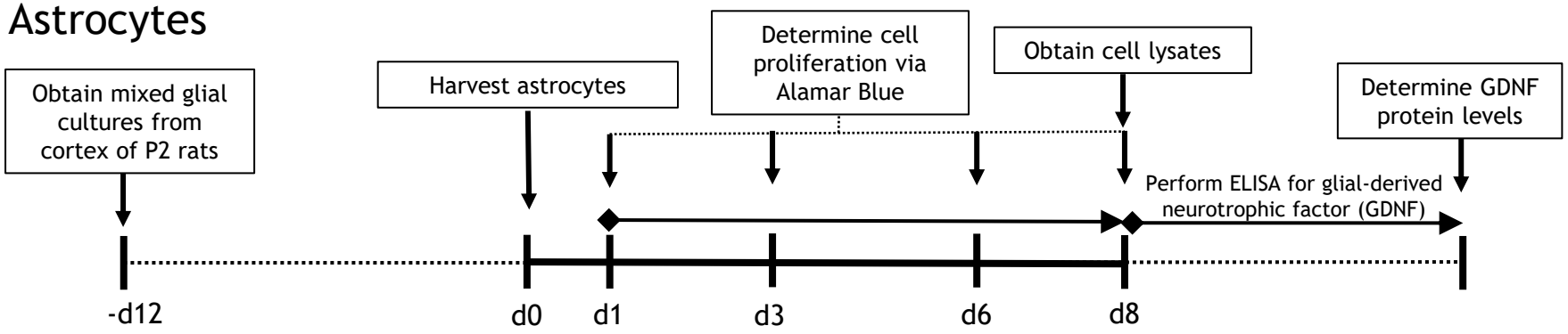


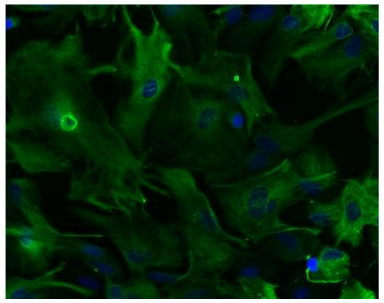




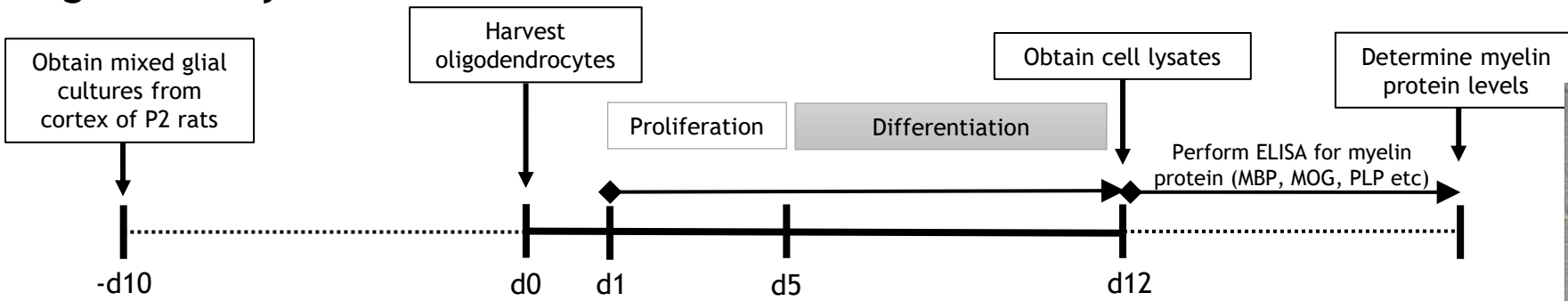
TIMELINES FOR ASTROCYTE & OLIGODENDROCTYES

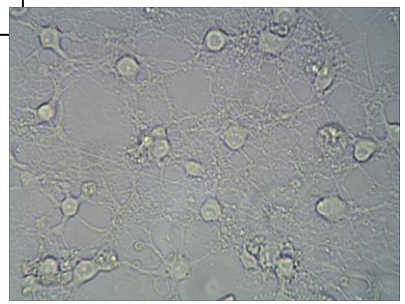
Astrocytes





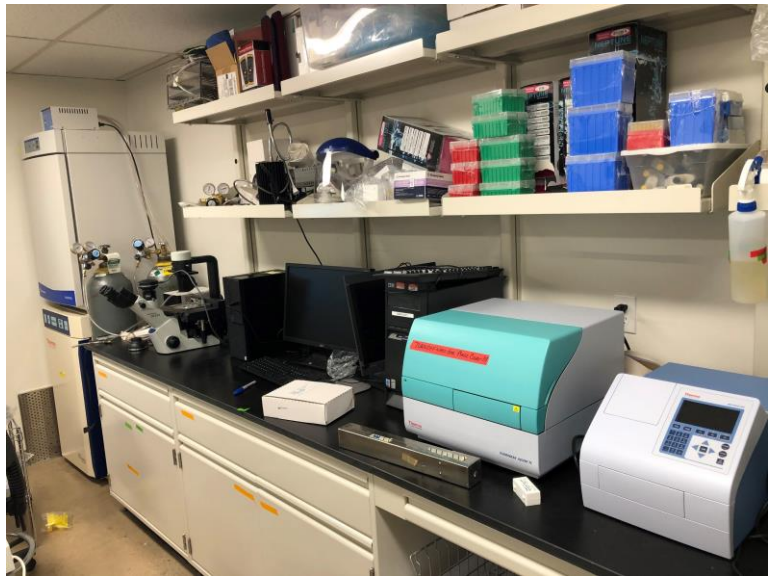
Oligodendrocytes





EQUIPMENT THAT MAKES IT HAPPEN

In vitro lab

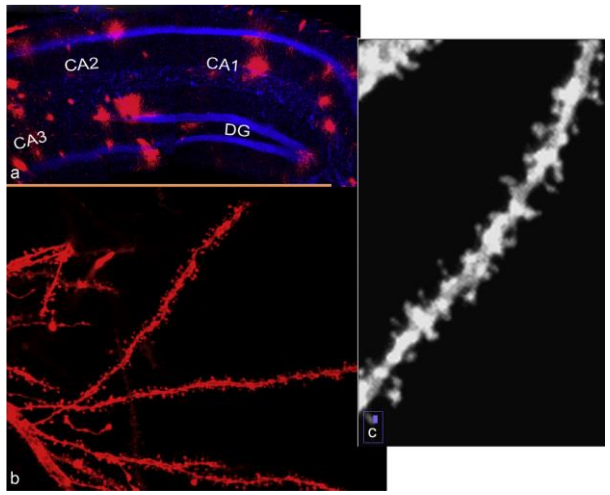


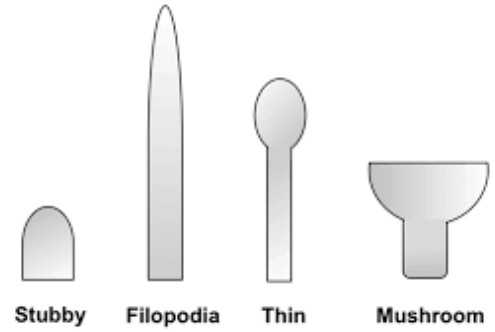
96-well fluorimeter, 96-well spectrophotometer (qRT-PCR not shown), 4 incubators

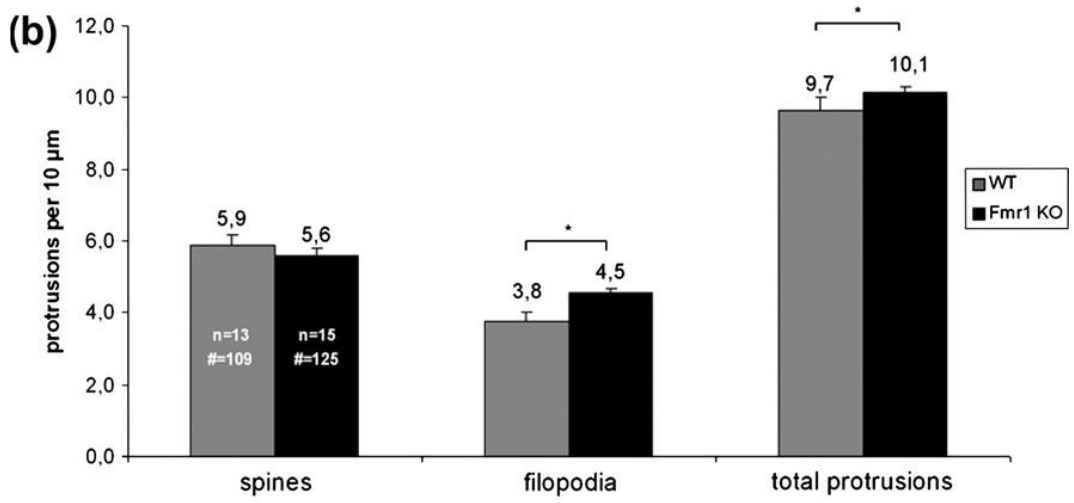
4-laser confocal microscope with automated stage, 2 e-phys channels and calcium imaging

EX VIVO SPINE MORPHOLOGY

- Spines are protrusions that are physical connection points between neurons. Spines can take on different forms: stubby, filopodia, thin and mushroom
- Intellectual disability, autism, Alzheimer's disease can lead to altered spine morphology as well as increase/decrease in number







Levenga J, et al, 2011

SKILL SET SUMMARY

- ***In vivo* capabilities in rats and mice (piglets and sheep available via 3rd-party collaboration)**
  - Surgery (e.g. OVX, ADX, s.c. & i.c. implants, stereotactic, i.v. catheterization, bone defect)
  - Video analysis of gait, exercise and male sexual performance
  - Monitoring of skin & body temperature (model of menopausal hot flash)
  - Open field (locomotor activity and anxiety)
  - Motor coordination (rotarod, etc.)
  - Elevated plus maze (anxiety-like), Forced swim test (depressive-like)
  - Morris Water Maze, Barnes Maze and fear conditioning(cognition)
  - Sociability
  - Sensory gating
  - Tissue harvesting for endpoint of choice
  - Fertility, EEG, Sleep, and Acute & Chronic inflammation in multiple systems available through in-house and 3rd-party collaborations

- ***In vitro* capabilities**
  - Human stem cells and brain organoids derived from them
  - Primary neurons, oligodendrocytes, astrocytes and microglia
  - Osteoblasts, retinal epithelial cells, cell lines and macrophages

- **Commonly used endpoints**
  - *In silico* and proteomic approaches to idea development & hypothesis generation
  - Multiplexed ELISA, immunohistochemistry, enzymology, electrophysiology
  - Confocal & electron microscopy (TEM and SEM)
  - Glutamate challenge, hypoxia challenge, oxidative stress, LPS stimulation
  - Biomarkers for viability, proliferation, growth, development/maturation and immune activation

- **Under development**
  - Integration with genetics and personalized medicine
  - Coordination and integration with other BBP models

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