

Oxycodone dependency & Neurodevelopment

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Asian Pacific Rim Development Program

Dr. Pendyala's Laboratory

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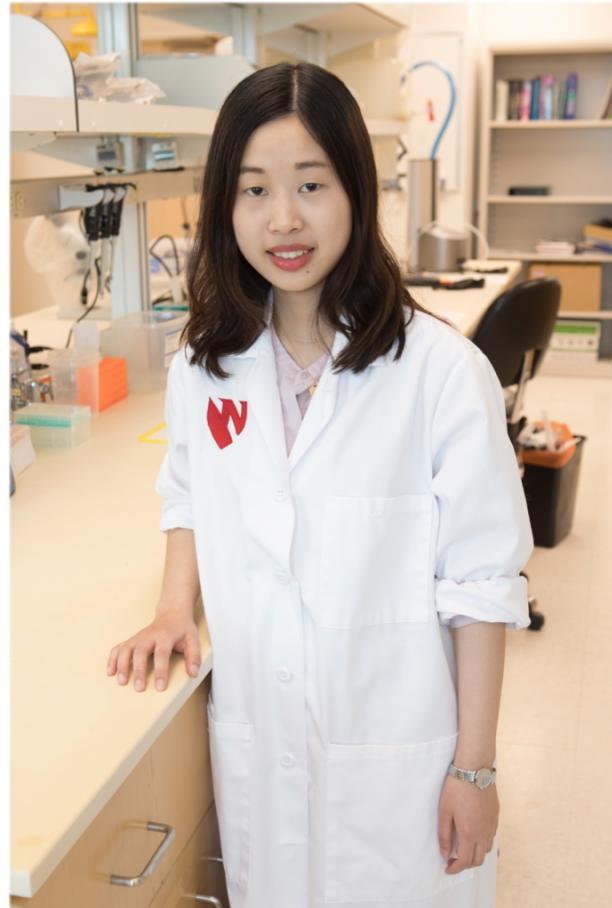


About me...

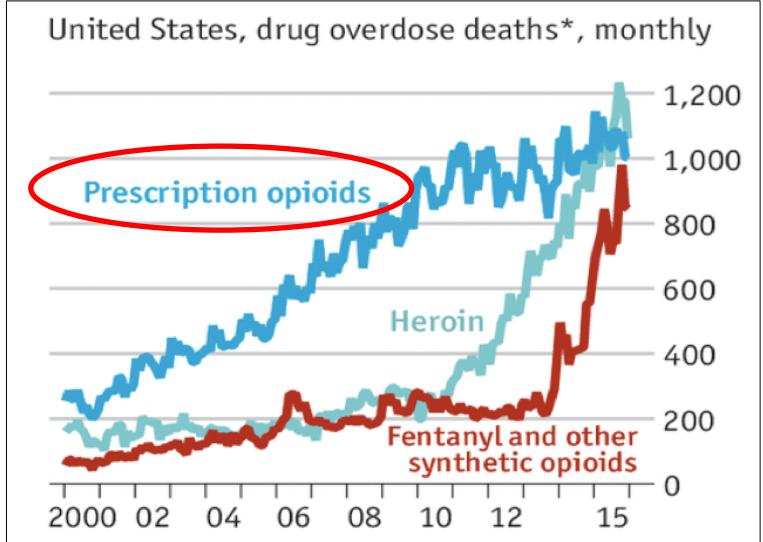
- 3rd year Medical Student
- Clinical interest in reproductive genetics (OB/GYN & pediatrics)
- Ambition to pursue a PhD in Neuroscience



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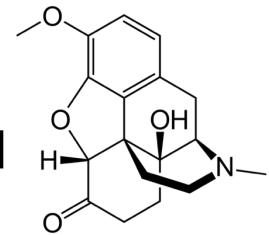
Prescription Opioids Crisis



Deaths from opioid overdose:

<http://scr.zacks.com/News/Press-Releases/Press-Release-Details/2017/Egalet-EGLT-The-Guardian-of-Misuse-and-Abuse/default.aspx>

- Oxycodone is a semi-synthetic prescription painkiller sold under brand names such as Percocet and OxyContin.
- 81% of global Oxycodone consumption occurs in the US.
- Oxycodone prescriptions will be their greatest ever in 2018.





Baby on Drugs image source:
<http://www.hookedsober.com/wp-content/uploads/2017/03/Rise-in-opioid.jpg>

Scientific Premise

Understanding behavioral, molecular and synaptic correlates with *in utero* and post-natal Oxycodone exposure

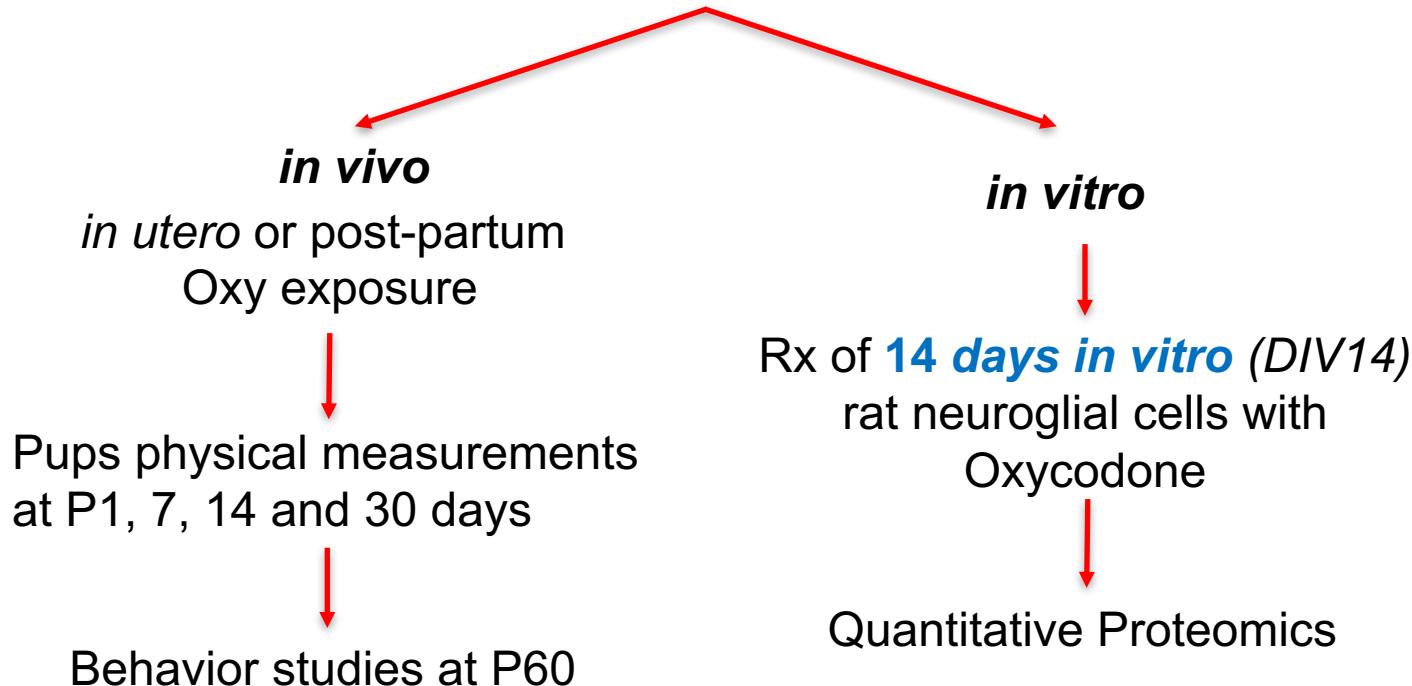
Hypothesis

In utero Oxy (IUO) exposed offspring display altered neurodevelopment associated with enhanced synaptic and behavioral deficits compared to post natal Oxy (PNO) offspring.



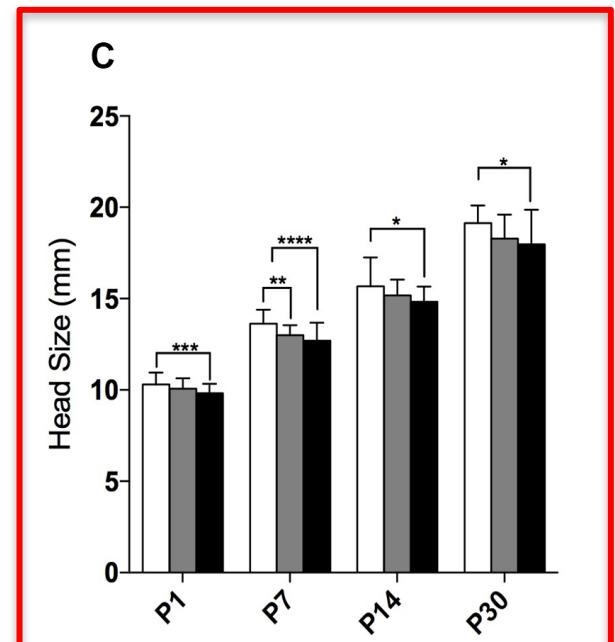
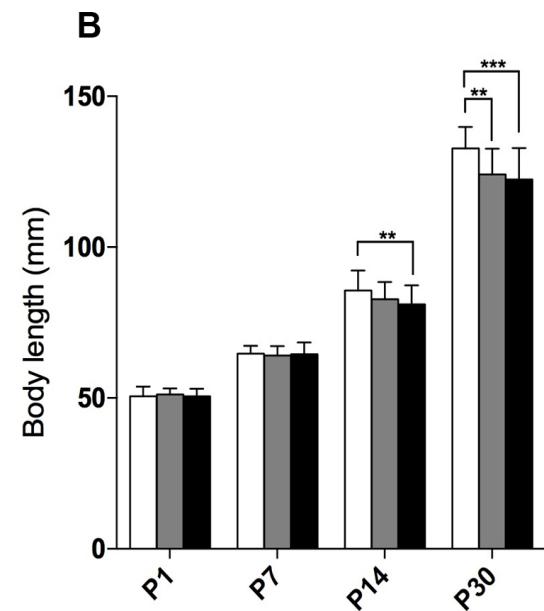
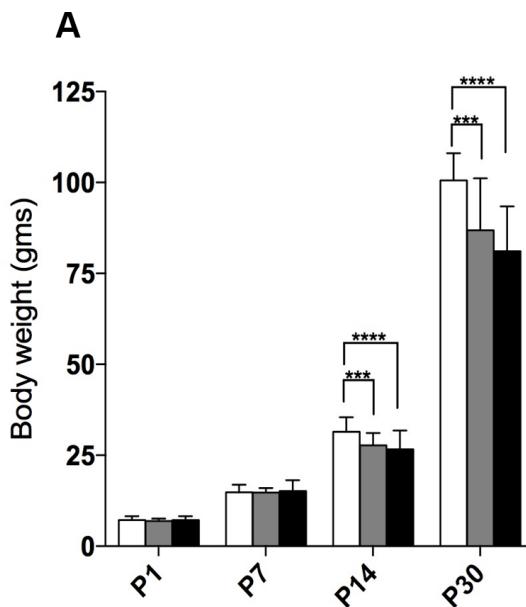
Methods

We used offspring at post natal day (P14) from 3 groups:
Saline, post-natal Oxy (PNO) and *in utero* Oxy (IUO) exposed pups
P14 represents a key stage of brain development – **synaptogenesis**



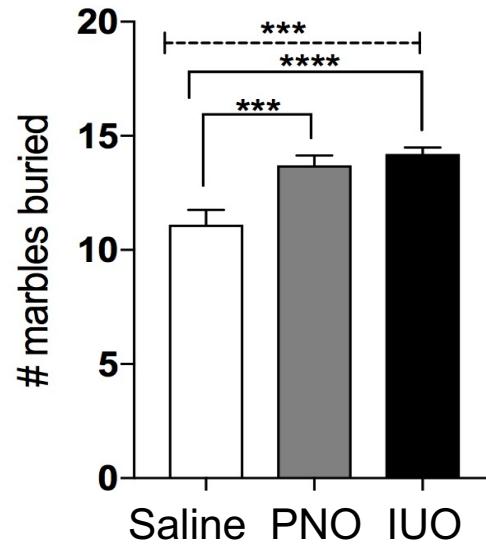
IUO offspring display altered brain development from birth

Saline
PNO
IUO



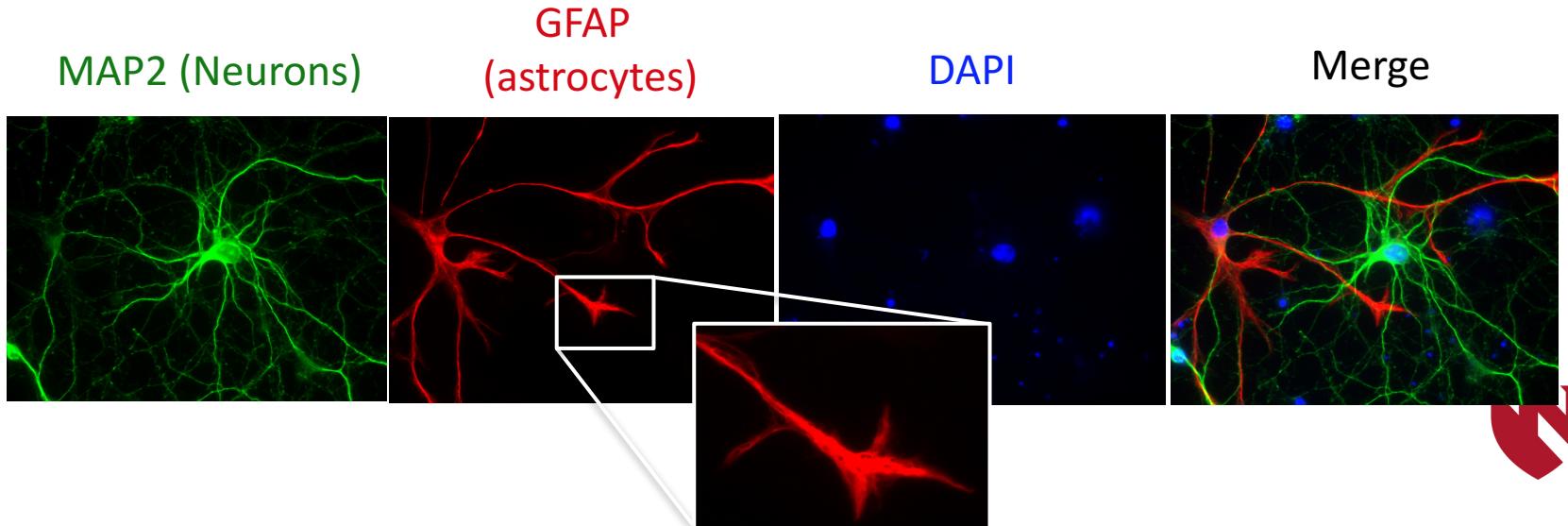
IUO offspring display alterations in behavior

P60 old offspring from the three groups were subjected to marble burying test which depicts anxiety or obsessive compulsive disorder (OCD) behavior.



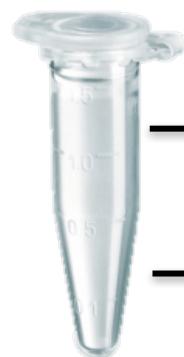
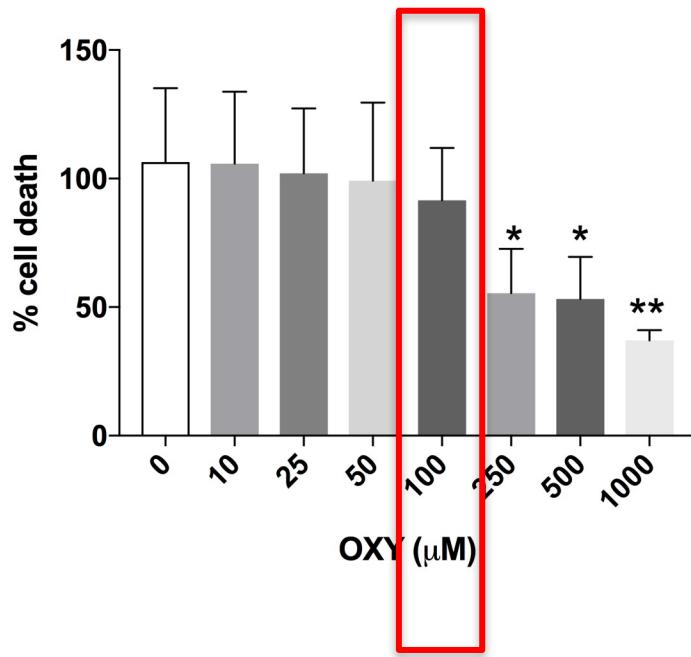
Understanding molecular mechanisms of Oxy exposure at the synapse *in vitro*

- Communication between neurons and glial cells key for proper brain function occurs at the **synapse**.
- Mixed neuro-glial cultures from E18 rat cortices were isolated and cultured for 14 days.



Oxy cytotoxicity assay

P14 mixed cultures were treated with **100 μ M Oxy** for **24h**.



- Supernatant → EV analysis
- Protein → Proteomics*

* Currently in progress



Mass Spec analysis of control samples

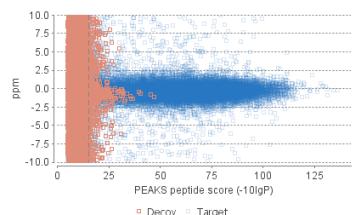
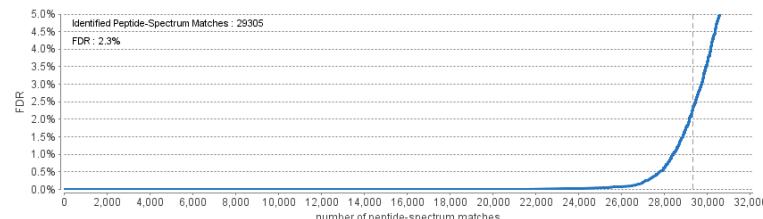
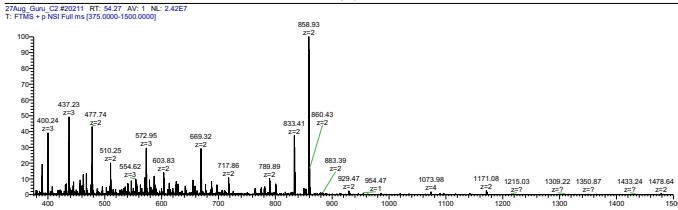
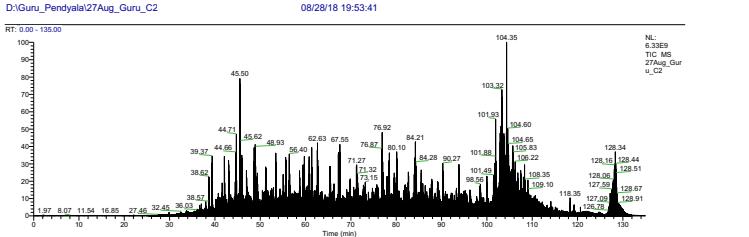


Table 1. Statistics of data.

of MS scans 7441

of MS/MS scans 57847

Table 2. Result filtration parameters.

Peptide -10lgP ≥ 15

Peptide Ascore ≥ 0

Protein -10lgP ≥ 20

Proteins unique peptides ≥ 0

De novo ALC Score $\geq 50\%$

Table 3. Statistics of filtered result.

Peptide-Spectrum Matches 29391

Peptide sequences 22455

Protein groups 2533

Proteins 2562

Proteins (#Unique Peptides) 1738 (>2); 313 (=2); 492 (=1);

FDR (Peptide-Spectrum Matches) 2.3%

FDR (Peptide Sequences) 2.9%

De Novo Only Spectra 16064

What next ?

In vivo

- Changes in synaptic architecture of P14 brains using Golgi-Cox staining
- Rx with anti-inflammatory drugs to reverse altered synaptic deficits

In vitro

- Quantitative Proteomics to identify key protein targets altered by Oxy treatment; elucidation of mechanisms
- RNA sequencing of cell supernatants to identify changes in key extracellular vesicle (EV) miRNA expression



Accomplishments

Learnt an array of techniques:

- Animal handling including i.p. injections, oral gavaging
- Brain areas dissection using brain matrix
- Biochemical isolation synaptosomes
- Western blot
- Mixed neuro-glial cultures, cell cytotoxicity assay
- Immunocytochemistry and microscopy



Acknowledgements

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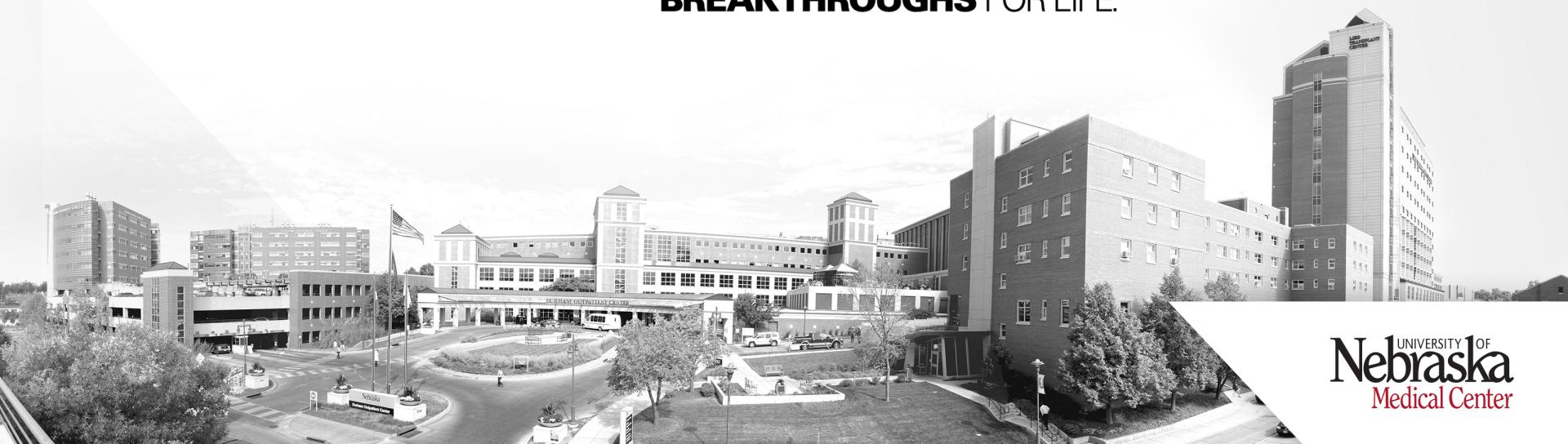
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