# Recent Studies on the genetics of NAS

Richard D. Egleton, PhD

OB/GYN grand rounds

- I have no conflicts associated with theses studies

- The studies were funded by NIH grants 2U54GM104942-02, 5P20GM103434, and 1P20GM121299

# Objectives

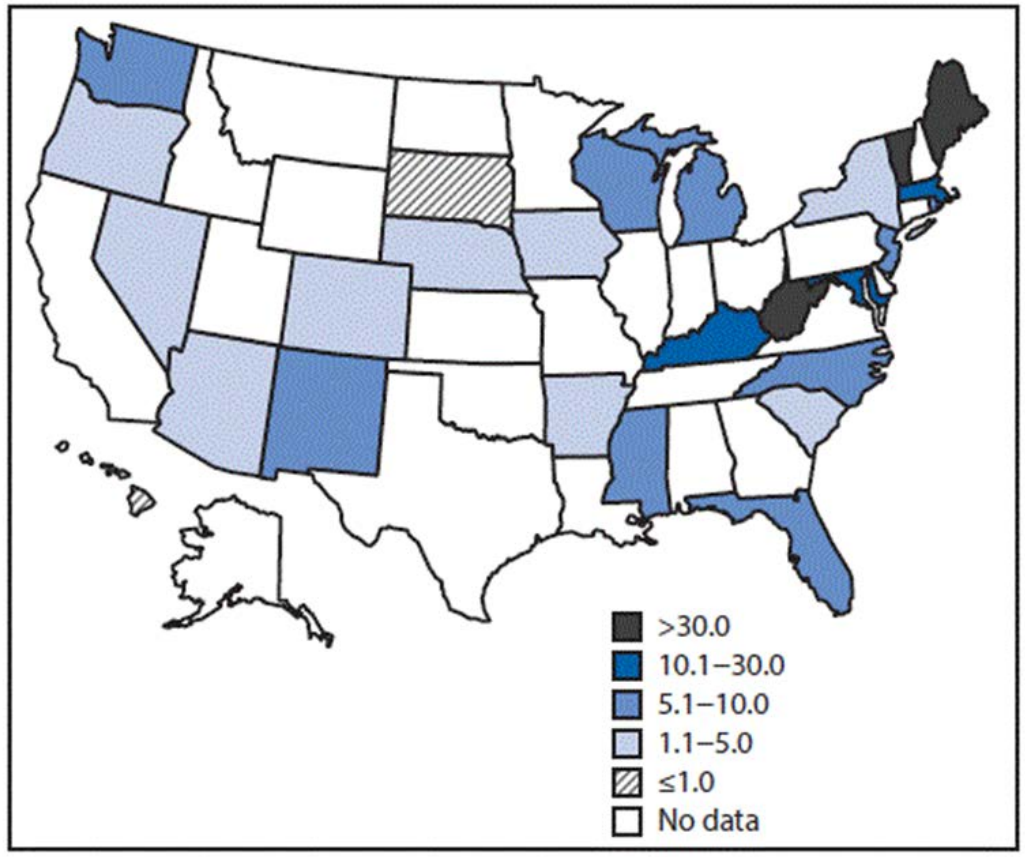
1. Discuss issues associated with NAS
2. Outline the importance of studying genetics for various aspects of OUD.
3. Describe recent genetic studies with the MARC program

# Neonatal Abstinence Syndrome (NAS)

- Passive exposure of the newborn occurs when a mother uses a neuroactive drug during her pregnancy
- When the infant is deprived of these substances through the birthing process, a withdrawal syndrome may develop
- Classic NAS consists of a wide variety of CNS signs of irritability, GI problems, autonomic signs of dysfunction, and respiratory  symptoms
- The hallmark of neonatal withdrawal is a striking disorder of movement, most aptly termed “jitteriness”
- Autonomic over-reactivity is typically exhibited by yawning, sneezing, mottling and fever
- Cerebral irritation results in an irritable and hypertonic infant

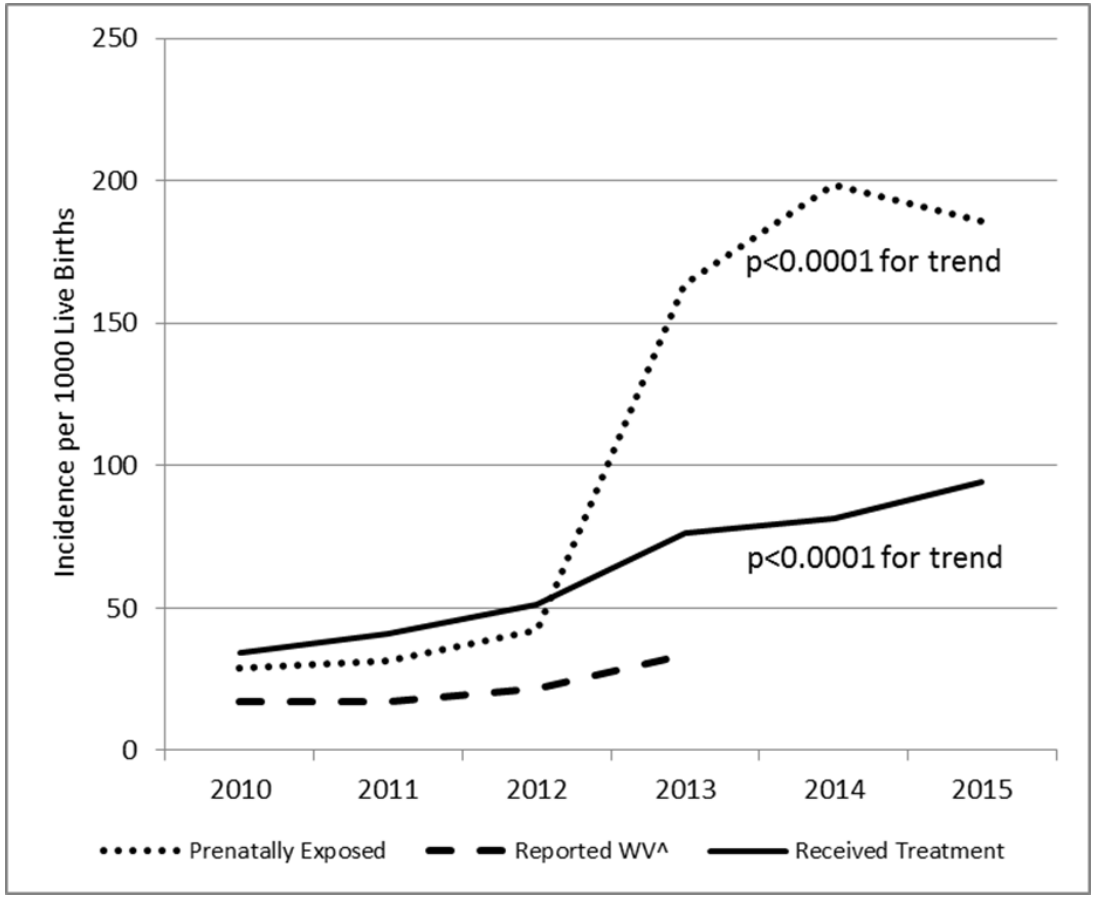
# NAS Prevalence

## NAS incidence rates 2012–2013




Ko *et al.*. Incidence of Neonatal Abstinence Syndrome — 28 States, 1999–2013. MMWR Morb Mortal Wkly Rep 2016;65:799–802.

## NAS Births per 1000 at CHH



# Neonates treated for NAS with Methadone at CHH

| Year | 2010 | 2011 | 2012 | 2013 | 2014 | 2015 |
|---|---|---|---|---|---|---|
| Live Births | 2436 | 2494 | 2641 | 2801 | 2755 | 2810 |
| Prenatal Exposures | 71 | 79 | 111 | 459 | 547 | 522 |
| NAS per 1000 | 29.1 | 31.7 | 42.0 | 163.9 | 198.5 | 185.8 |
| Pharmacologically Treated NAS | 83 | 102 | 135 | 214 | 224 | 265 |
| Treated NAS per 1000 | 34.1 | 40.9 | 51.1 | 76.4 | 81.3 | 94.3 |
| %multi-Substance | 46% | 47% | 58% | 54% | 52% | 53% |

# From a state perspective

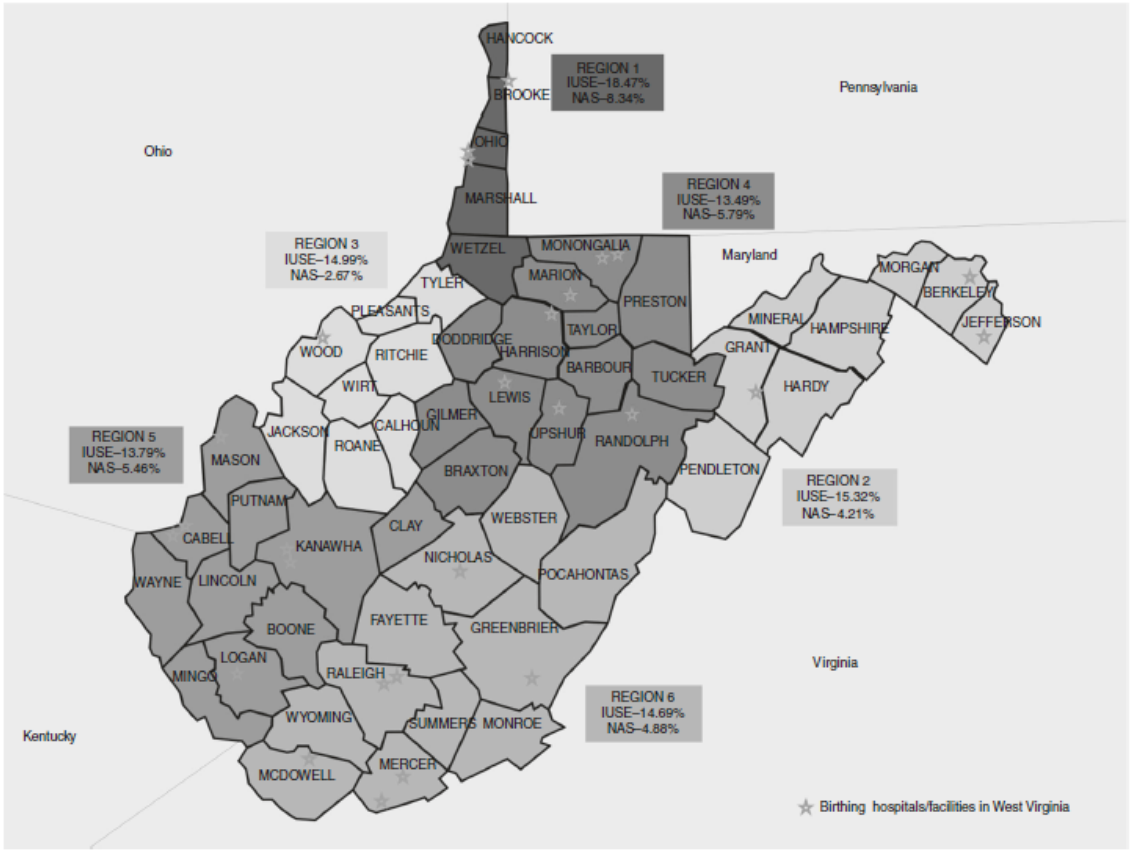
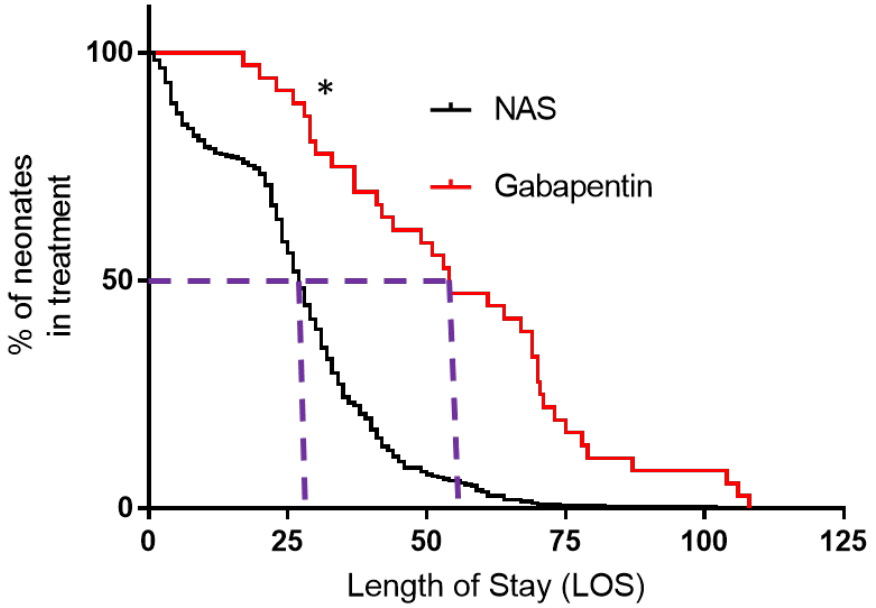



**Fig. 2** Project WATCH surveillance data on prevalence of intra-uterine substance exposure (IUSE) and neonatal abstinence syndrome (NAS) in West Virginia by SAMHSA sub-state regional classification system, $N = 20{,}002$

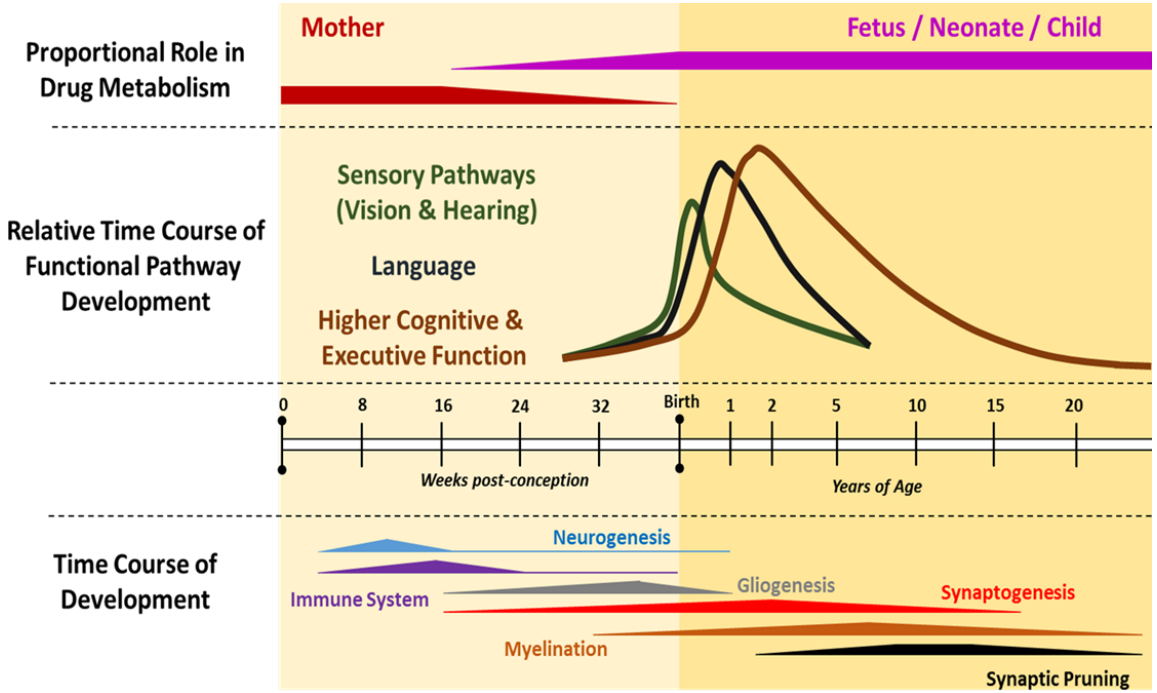
**Umer, *et al.* Pediatric Research (2019) 85:607–611**

# Drug combinations alter NAS profile




% of neonates still recievng medical treatment at CHH for NAS for years 2014-2015. Black line represents total NAS babies over this period, while the red is neonates that had exposure to gabapentin in utero. The curves are significantly different (p<.0001 Chi Square), with median LOS of 27 (NAS) and 54 (gabapentin) days.

# What are the potential long term consequences of inter uterine exposure



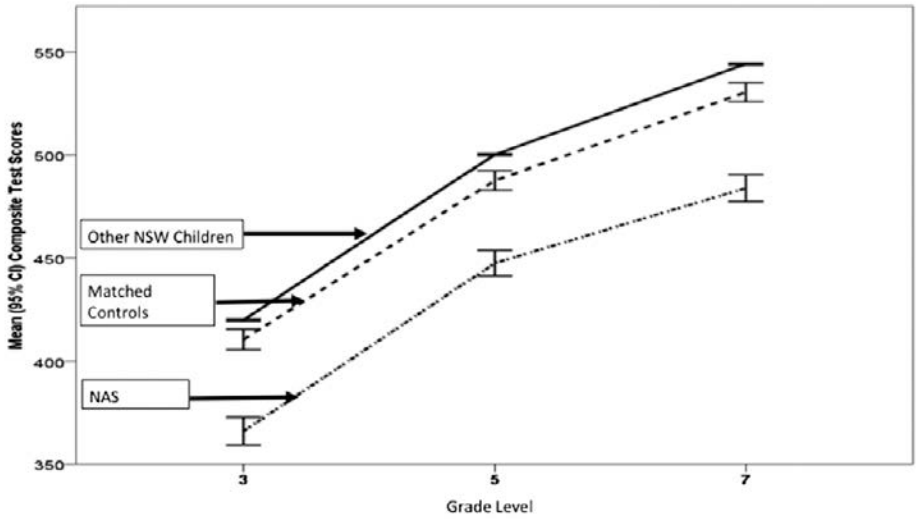




FIGURE 2
Composite NAPLAN test scores between children with NAS, control, and other NSW children.

**To cite:** Oei JL, Melhuish E, Uebel H, et al. Neonatal Abstinence Syndrome and High School Performance. *Pediatrics*. 2017;139(2):e20162651

# So what are we doing in Biomedical Sciences?

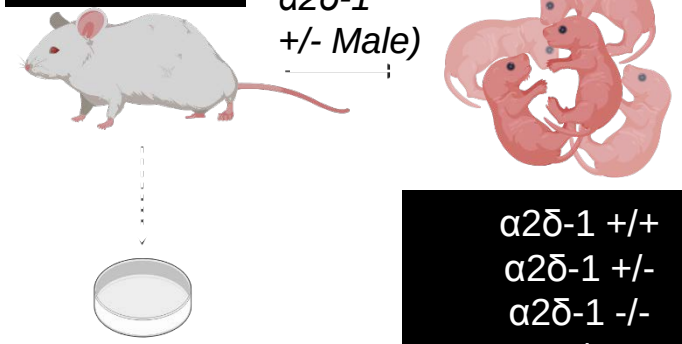
- Two primary research projects
  - Opioids + gabapentin in neuronal development
    - Animal model
  - Genetic markers

# How does prenatal drug exposure affect synaptic development *in vivo*?

Taylor Boggess

Pregnant Dam α2δ-1 +/-

*(Bred with α2δ-1 +/- Male)*



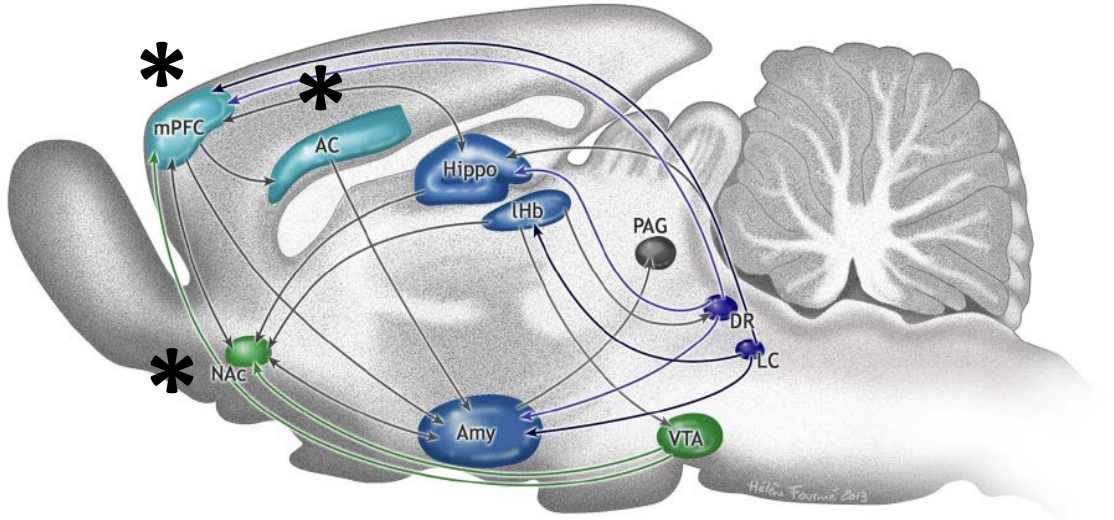
α2δ-1 +/+
α2δ-1 +/-
α2δ-1 -/-
M / F

Drug/Vehicle Condensed Milk Mixture (Daily from E7 to P11)

Drug treatments:
5mg/kg buprenorphine, 30mg/kg gabapentin, or both

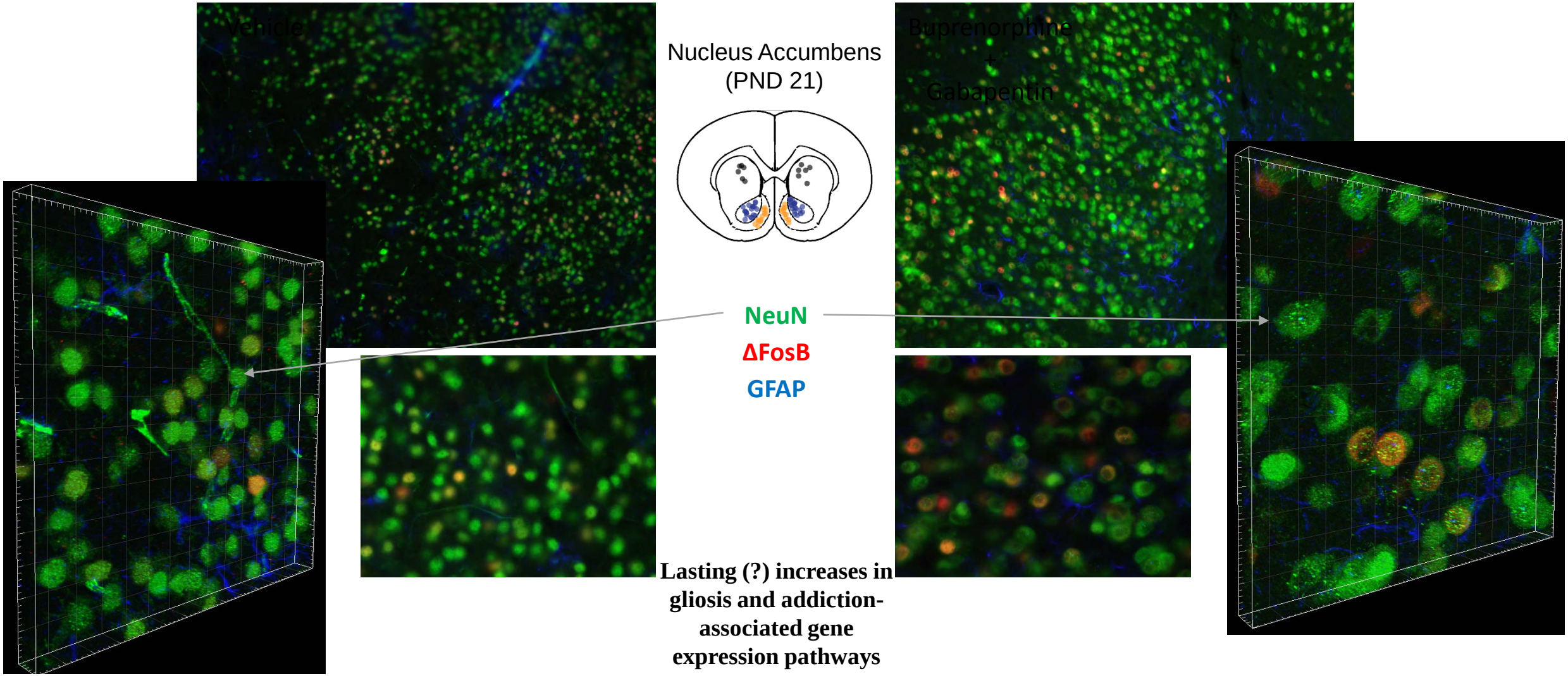
P21
Pups sacrificed, brains isolated and fixed in paraformaldehyde

Sectioning, immunohistochemical (IHC) staining, and confocal microscopy




Belzung, Turiault & Griebel, *Pharm Biochem Behav* 2014

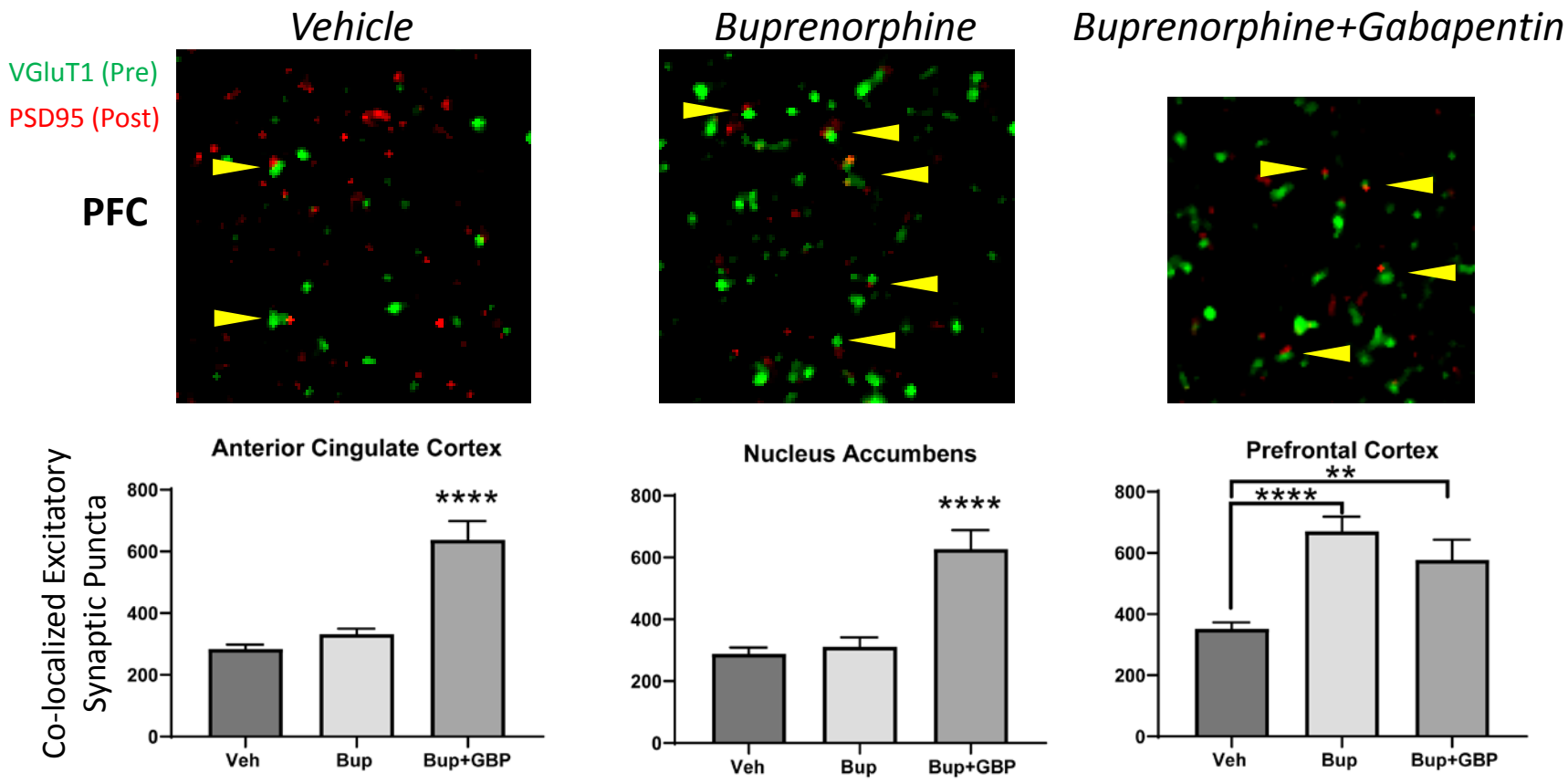
# Addiction-related changes in astrocytes and gene transcription



# How does prenatal drug exposure affect synaptic development *in vivo*?

PND 21 α2δ-1 +/- male and female mice

Taylor Boggess

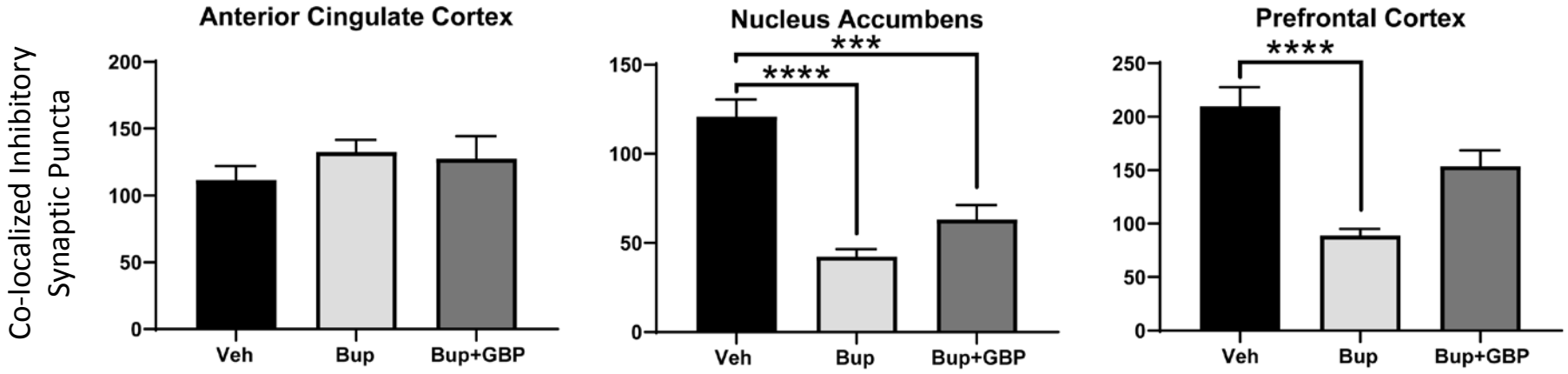



Prenatal drug exposure increases excitatory connectivity in reward-associated brain regions

# Are inhibitory synapses also impacted by drugs of abuse?

PND 21 α2δ-1 +/- male and female mice

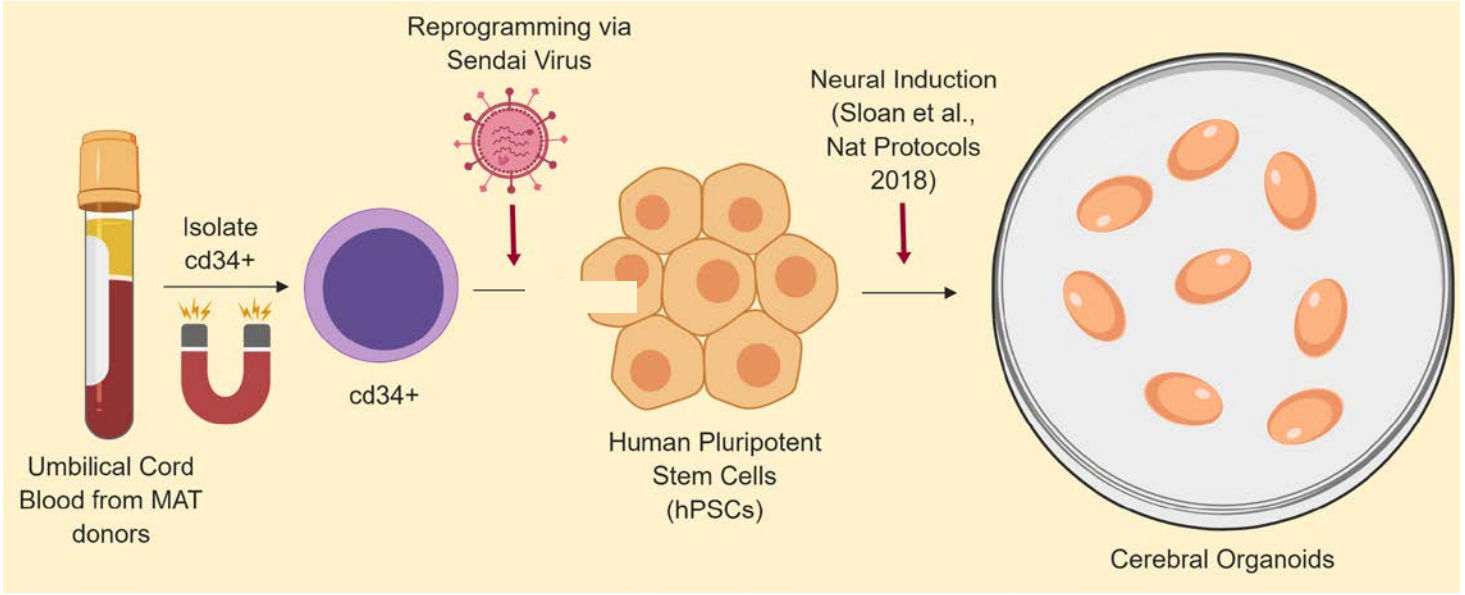
Taylor Boggess



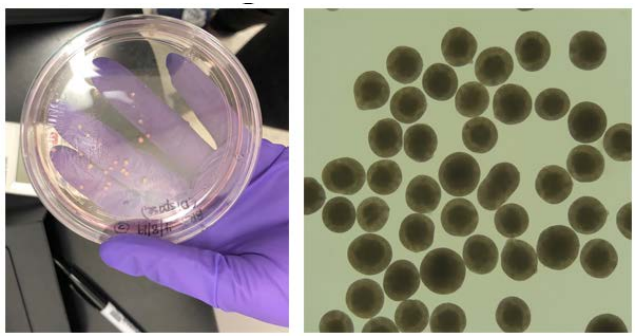

Decreased inhibitory connectivity in (some) reward-associated brain regions

*(overall hyperexcitability)*

# Making a Brain in a Dish
# CD34→hPSC→Organoid Workflow




David Chaffin / Mary Gibbs



Ania Mazur & Jesse Stevens

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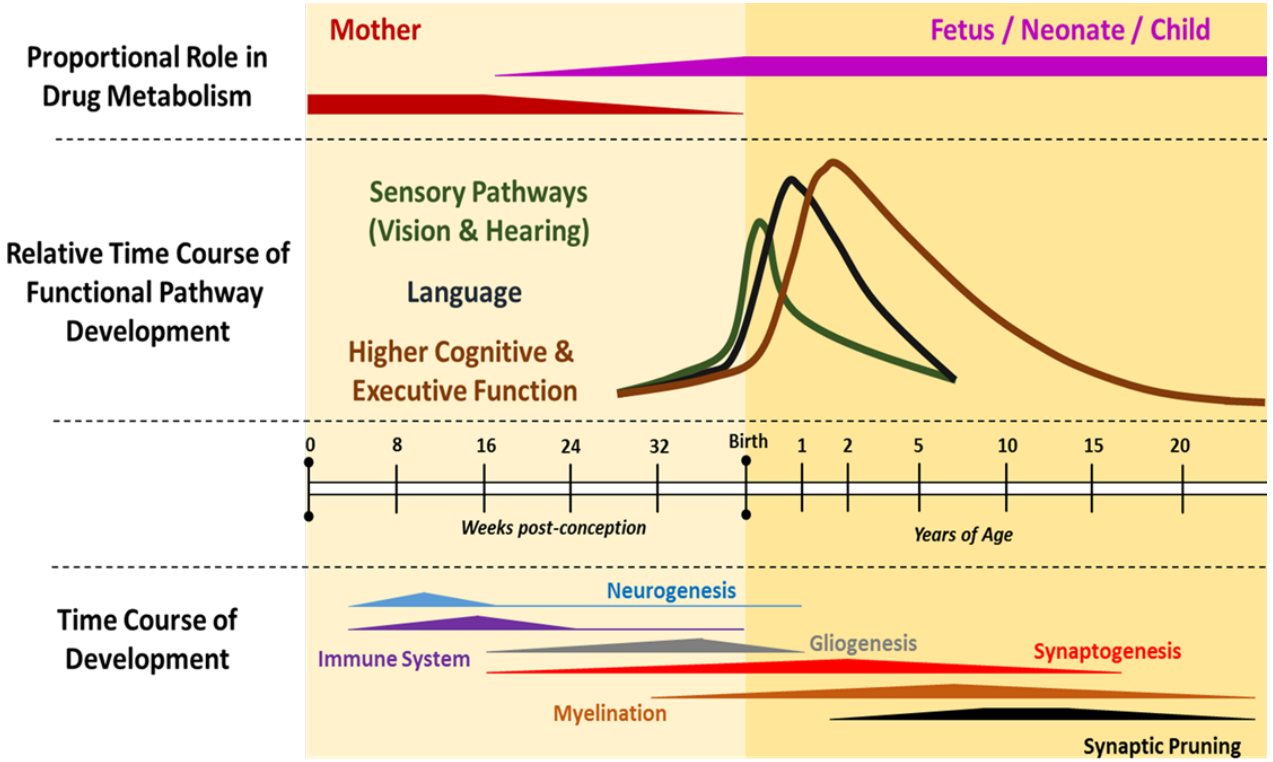
# Whole exome study

## Rationale:

- **Only ~50% of exposed infants have pharmacologically treatable NAS, but we cant predict which**
  - **Fetal development and pregnancy outcomes are regulated by a complex interplay between both mother and fetus.**
  - **Alterations in the expression patterns of genes in either the neonate or mother can have profound effects on development.**
  - **It is thus important to consider the maternal-neonate dyad as a unit as well as individually.**




## Hypothesis

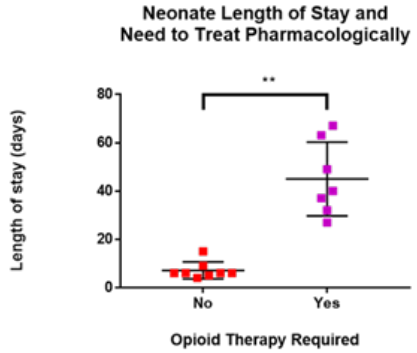
NAS intensity is in part due to variants in genes involved in maternal and neonate metabolism of opioids and in genes related to brain and placental  development.

# Study design

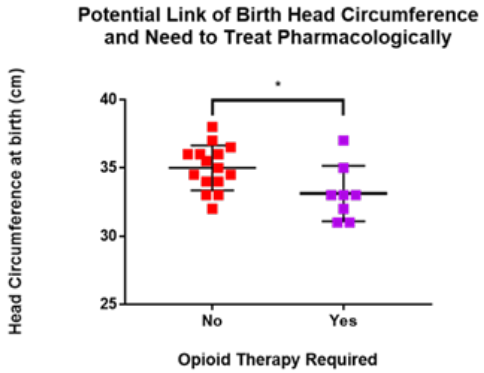
- Patients recruited at 36 weeks of pregnancy from WVU and Marshall MAT programs.
- DNA collected by cheek swab and isolated. Whole exomes sequenced by the WV-CTSI genomic core at 100x coverage using an Illumina HiSeq .
- Once the sequences were obtained the dyads' exomes were analyzed using a logistic regression model for needing or not needing treatment at each genomic locus. Maternal dominant model is being presented
- Network analysis of various aspects of OUD using STRING via Cytoscape

# Patients in Cohort

| | No treatment (+/- sem) | Neaded treatment (+/- sem) |
|---|---|---|
| Number of Dyads | 14 (61%) | 9 (39%) |
| Mother | | |
| Age years | 29.3 (0.76) | 29.3 (1.4) |
| BUP dose mg | 10.4 (1.03) | 12.9 (1.3) |
| # of previous treatment programs | 1 (0.3) | 1.6 (0.6) |
| Weeks of treatment | 23.4 (3.1) | 24.9 (3.4) |
| Neonate | | |
| Gestational Age weeks | 38.9 (0.4) | 38.1 (0.4) |
| Weight g | 3368 (155) | 2985 (179) |
| Length cm | 49.4 (1.0) | 47.9 (1.3) |




Comparison of length of stay for neonates that did or did not require treatment with an opioid to control their NAS. Scatter plot for each group with associated mean and SD. LOS was significantly longer in neonates that required opioid therapy (*p<0.01 Students t-test).




Comparison of head circumference at birth in neonates that did or did not require treatment with an opioid to control their NAS. Scatter plot for each group with associated mean and SD. Head circumference was significantly smaller in neonates that required opioid therapy (*p<0.05 Students t-test).

# No difference between our groups in SNPs for Opioid receptors or metabolizing enzymes!

**Table 4.** Percent of our preliminary patient population with indicated opioid receptor single-nucleotide polymorphisms

| Gene | dbSNP ID | % of patients | ExAc* or EUR^ variant allele freq. |
|---|---|---|---|
| *OPRM1* | rs146319173 | 6 | 0.1%* |
| | rs6912029 | 15 ★ | 3.7% |
| | rs34427887 | 25 | 6.4% |
| | rs1799971 | 20 ★ | 16.2% |
| | rs677830 | 24 ★ | 22.7% |
| | rs540825 | 14 ★ | 22.9% |
| *OPRK1* | rs16918931 | 18 | 7.7% |
| | rs113472418 | 18 | 7.7% |
| | rs12548098 | 29 | 14.5% |

* non-ancestral allele freq. from Broad Institute's Exome Aggregation Consortium (ExAC) database of 60,706 unrelated individuals [32];
^ non-ancestral allele freq. from 1000 Genomes' database of 1006 European individuals [33] (given that the WV Appalachian population under study is overwhelmingly of European/Caucasian descent).

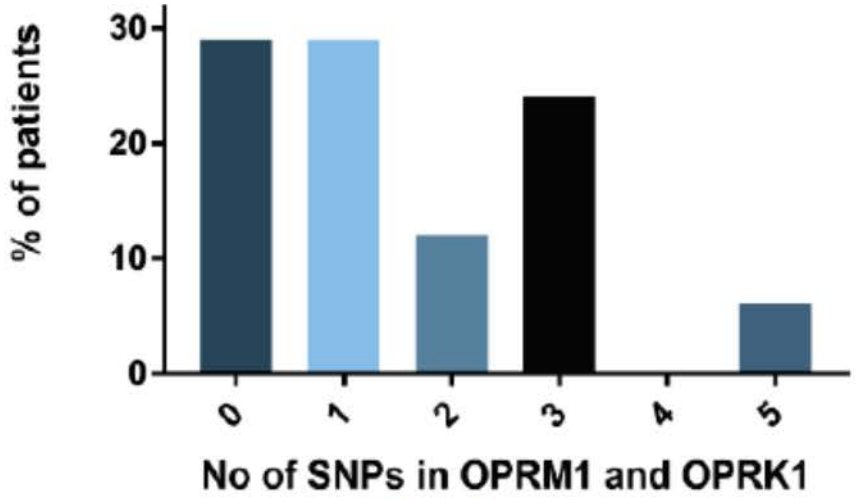



**Figure 5.**
Distribution of *OPRM1* & *OPRK1* SNPs in our pilot patient population. Over 70% have one or more SNPs in these opioid receptor genes.

★ SNPs linked to altered OPRM1 function associated with either pain response or addiction

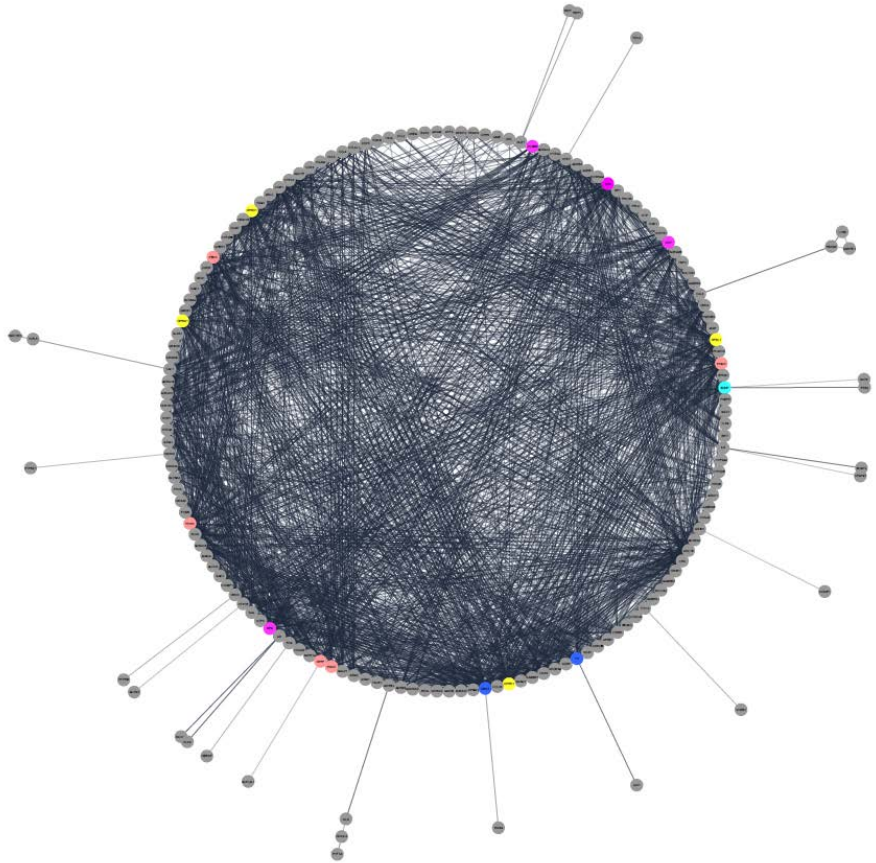
# Polymorphisms more common in mothers of treated or non-treated neonates

| Maternal treated neonates | | | | | | Maternal non-treated neonates | | |
|---|---|---|---|---|---|---|---|---|
| **Gene** | **rs#** | **OR** | **Gene** | **rs#** | **OR** | **Gene** | **rs#** | **OR** |
| OR4Q2 | 12587697<br>80024306<br>12586792 | 5.5<br>4.1<br>7.1 | FTCD | 61735841 | 5.8 | PITPNM3 | 3809835 | 0.05 |
| SCN1B | 67701503<br>67486287 | 10<br>10 | RNF215 | 5749088 | 2.5 | SLC2A14 | 36141788 | 0.07 |
| CNN2 | 2304260 | 7 | DSE | 10485183 | 2 | FANCA | 2239359 | 0.10 |
| APOL4 | 6000173<br>2227168 | 5.1<br>5.6 | PHIP | 9350797 | Full convergence | MAP2K3 | 56020453 | 0.17 |
| RNF212 | 60035268 | 4.1 | ANKDD1A | 34988193 | 10.3 | ATF7IP | 2231909 | 0.27 |
| PRSS55 | 61743179 | 15 | NPSR1 | 6972158 | 12.6 | ZNF773 | 67054060 | 0.40 |
| C5orf38 | 62333235 | 10 | NUP210 | 7628051 | 4.9 | ACE | 4363 | 0.27 |
| TANC1 | 4664277 | 7 | NIPSNAP3B | 3739741 | 12.6 | NLRP8 | 306496 | 0.04 |
| SLC26A8 | 743923 | 4.6 | | | | DCAF8L2 | 5926895 | 0.16 |

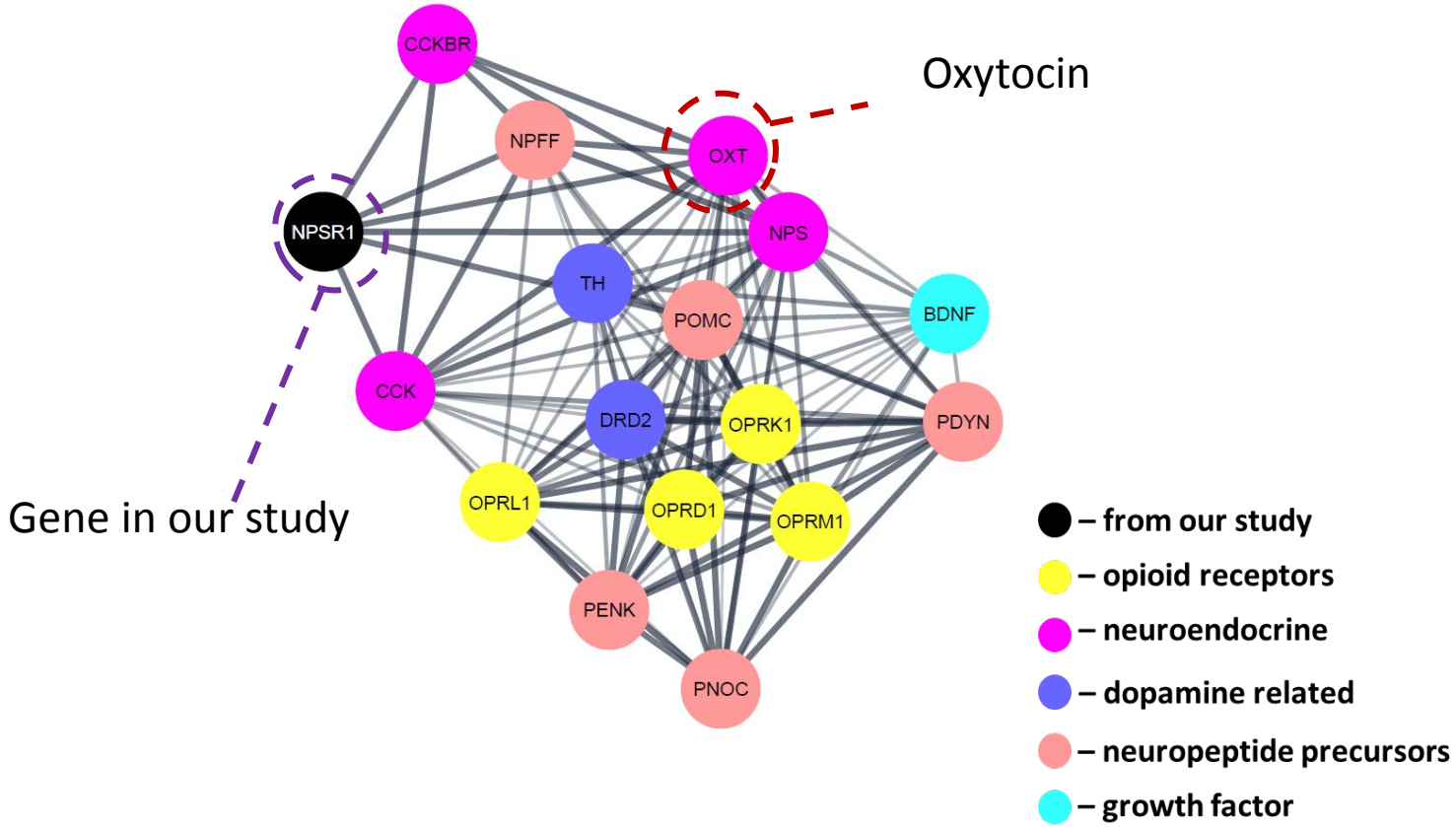
Total 21 SNPs in 17 genes more common in mothers of neonates that required opioid treatment for NOWS
Total of 9 SNPs in 9 genes more common in mothers of neonates that did not require opioid treatment for NOWS

# Gene networks involved in opioid abuse, dependence, and withdrawal

Total number of genes that have  been associated with abuse, dependence, or withdrawal (216) based on Cytoscape analysis of String disease networks.

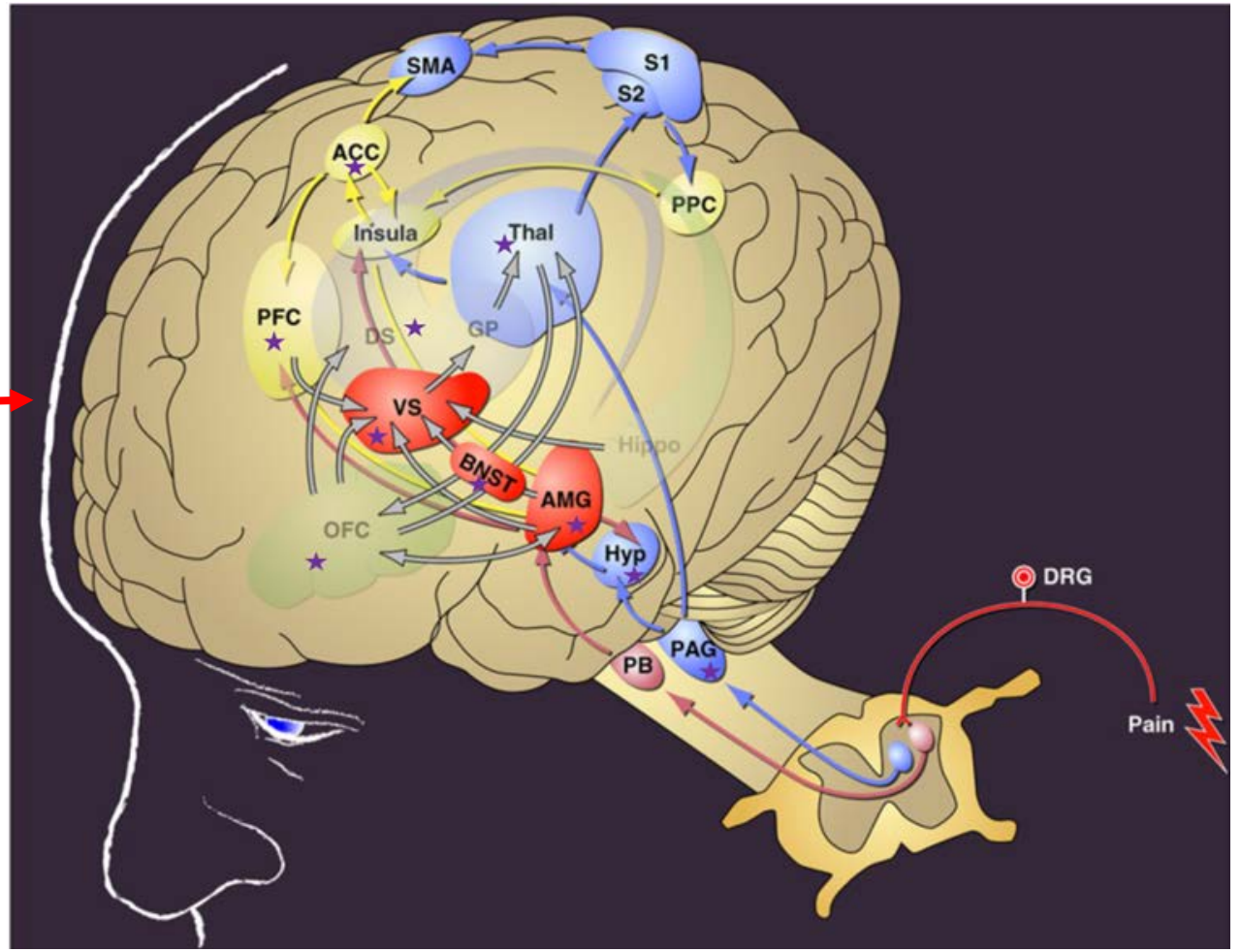


Associated genes based on merged network analysis. Sixteen genes found in all three String pathways. NPSR1 potential risk gene form our study interacts with this network



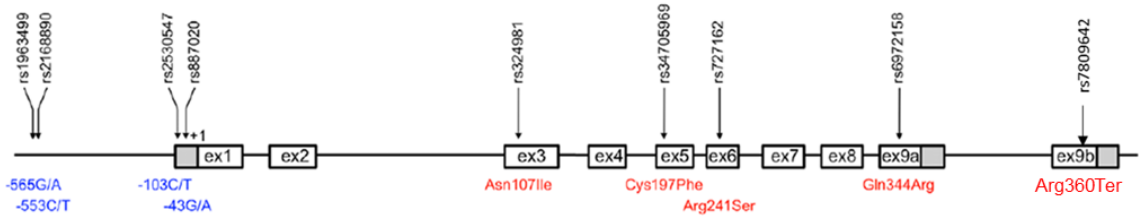
# Neuropeptide S receptor 1 (NPSR1)

- rs6972158 in our study OR of 12.6
- G-coupled receptor  for neuropeptide S
  - Regulator of neuroendocrine and autonomic function
  - Expressed in areas of brain associated with various aspects of OUD
  - Regulation of oxytocin
    - Important neuropeptide for pregnancy, lactation, and attachment
  - Polymorphisms have been linked to:
    - Anxiety and depression
    - GI motility
    - Pain
    - Addiction
    - Specifically rs6972158 – risk factor for somatization of trauma and anxiety



# Other NPSR1 polymorphisms

Polymorphisms seen on NPSR1 in our study population. Allelic rates for mothers and also the 1000 genome rate for the American population.




| rs# | change | AA | 1000 genome | Our Population (# Mothers)<br>Neonate no opioid treatment<br>Neonate needed opioid treatment |
|---|---|---|---|---|
| 2530547 | C-T | Non coding | 0.42 | 0.38 (4)<br>0.83 (3) |
| 324981 | A-T | N107I | 0.41 | 0.47 (14)<br>0.33 (9) |
| 34705969 | G-T | C197S | 0.02 | 0.03 (14)<br>0.00 (9) |
| 727162 | C-G | R241S | 0.35 | 0.29 (13)<br>0.22 (9) |
| 6972158 | A-G | Q344R | 0.23 | 0.27 (14)<br>0.56 (9) * |
| 7809642 | C-T | R360Ter | 0.1 | 0.12 (12)<br>0.44 (9) * |
| | C-A | R360R | 0.06 | 0.04 (12)<br>0.06 (9) |

* Indicates a significantly different allelic frequency when compared to 1000 genome data by Chi-Squared analysis

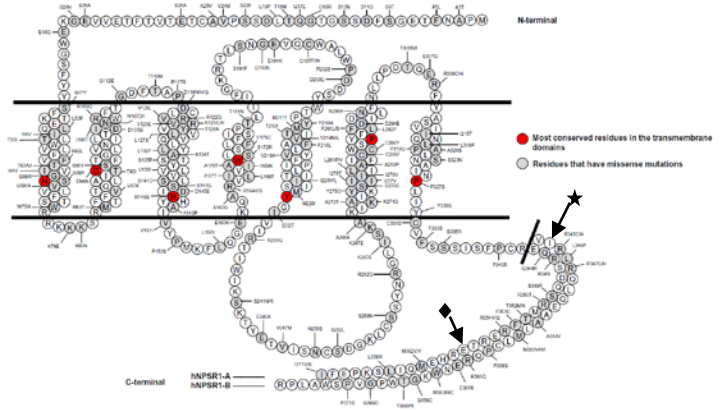
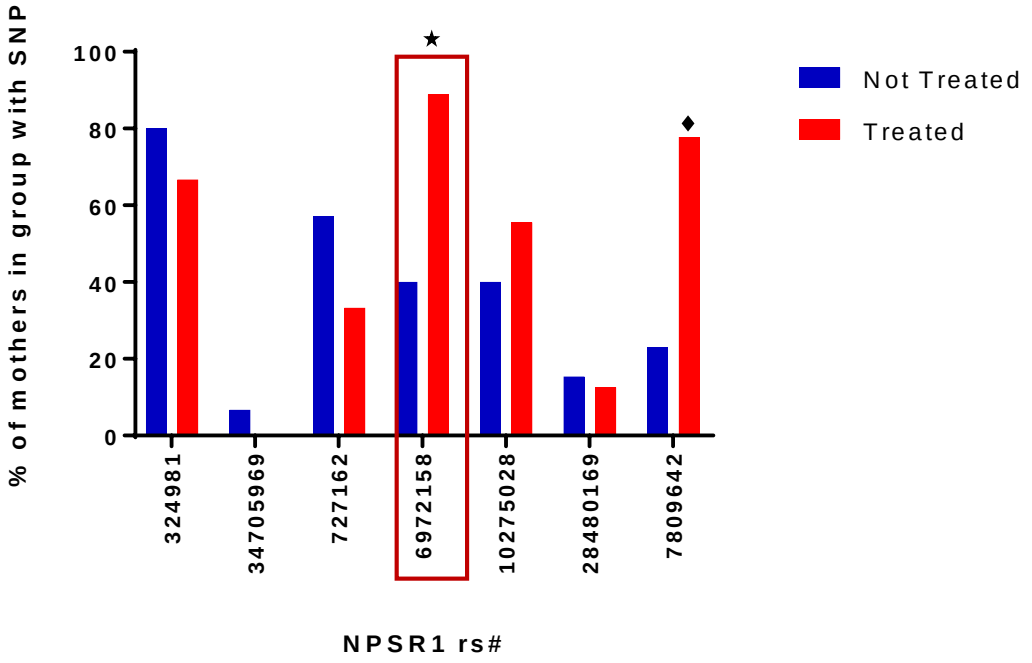



Fig. 1 Two-dimensional illustration of human NPSR1-A and NPSR1-B. The most conserved residues in the transmembrane domains are shaded in *red*. Residues that have naturally occurring mutations or polymorphisms identified by Exome Aggregation Consortium database are shaded in *gray*.

**Maternal expression of NPSR1 SNPs within our patient population**



# Why could maternal NPSR1 SNPs be important for long term effects of opioid exposure in utero?

Peptides 46 (2013) 6–12

Contents lists available at SciVerse ScienceDirect

Peptides

journal homepage: www.elsevier.com/locate/peptides

Morphine dependence is associated with changes in neuropeptide S receptor expression and function in rat brain

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12214 · The Journal of Neuroscience, December 13, 2017 · 37(50):12214–12225

Systems/Circuits

Neuropeptide S Activates Paraventricular Oxytocin Neurons to Induce Anxiolysis

Thomas Grund,[1] Stephanie Goyon,[2] Yuting Li,[3] Marina Eliava,[4] Haikun Liu,[3] Alexandre Charlet,[2,5] Valery Grinevich,[4]* and Inga D. Neumann[1]*

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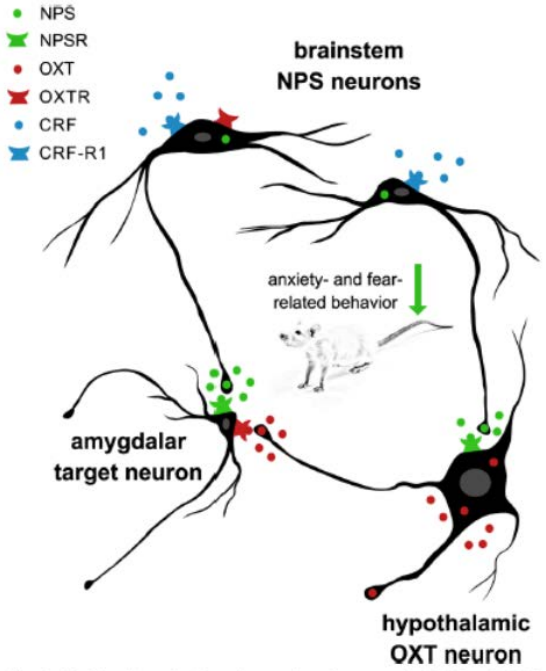


**Fig. 2** Working hypothesis on interactions between the NPS and OXT systems in the context of anxiety. Enhanced activity of brainstem NPS neurons evokes NPS release within the amygdala, e.g., during stressful events, in a CRH/CRHR1-dependent manner, thereby modulating amygdala responsiveness. Moreover, NPS activates OXT neurons within the hypothalamic PVN in an NPSR-dependent manner and consequently OXT release within the PVN and within limbic brain areas, such as the amygdala, from OXT projecting neurons. Altogether, these routes of NPS beneficially modulate fear- and anxiety-related behavior in rodent models

Stimulation of NPSR1 leads to a downstream increase in oxytocin, thus a reduction of NPSR1 activation will lead to a reduction in OXT levels

**Gund, cell tissue res (2019) 375: 123**

If you change oxytocin levels in the maternal brain, it will impact,maternal mental health and attachment, and  thus potentially impact neonate development.

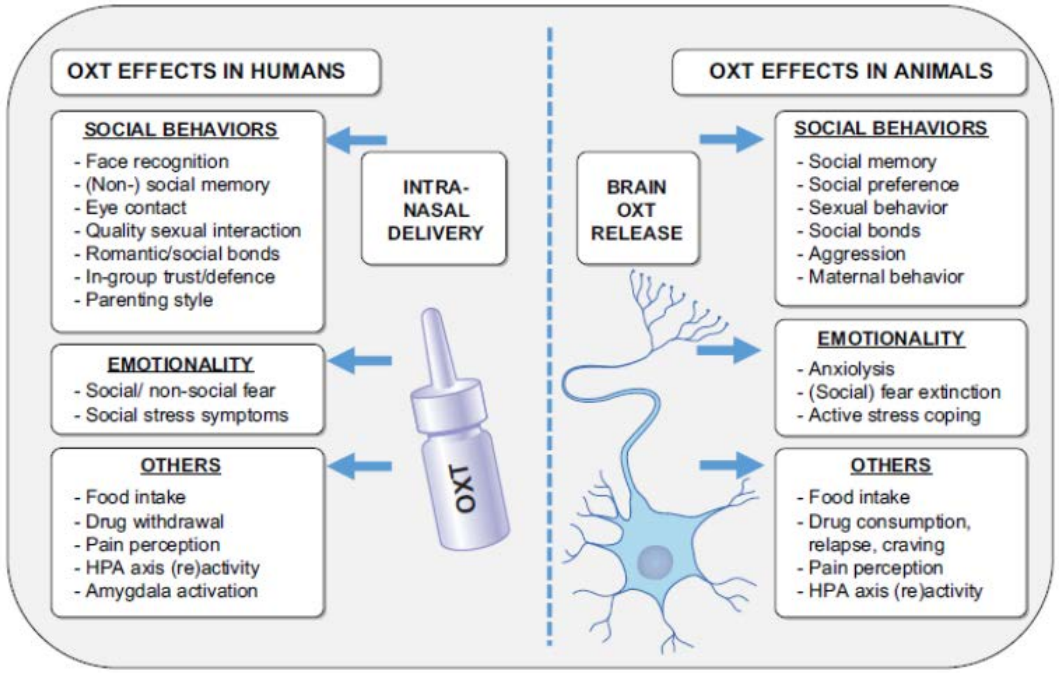


**FIGURE 10.** Summary of effects of synthetic or endogenous OXT on social behaviors, emotionality, and other functions reported in humans after intranasal delivery (*left*) and in animals (*right*).

# Summary and future studies

Summary:

- In a preliminary whole exome study we found:
  - 21 SNPs in 17 genes more common in mothers of neonates that required opioid treatment for NAS.
  - 9 SNPs in 9 genes more common in mothers of neonates that did not require opioid treatment for NAS.
- Network analysis using Cytoscape indicates that one of our candidate genes of interest NPSR1 involved in multiple aspects of OUD, thus a change in function could impact our population outcomes.
- Analysis of NPSR1 SNPs indicate that several SNPs with known links to behavioral health and addiction are differentially expressed in our cohort.

Future studies:

- Validate NPSR1 SNP profiles as a biomarker for NAS intensity, using a candidate gene approach.
- Investigate if NPSR1 SNPs lead to changes in maternal-neonate attachment due to oxytocin regulation.
- Animal modeling.

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