NIDA NATIONAL INSTITUTE ON DRUG ABUSE
The Science Of Drug Abuse & Addiction

Yale/NIDA Neuroproteomics Center

Summary and Future Directions

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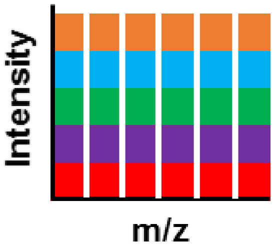
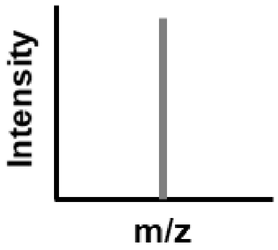
Some general themes and approaches

DIA and PRM

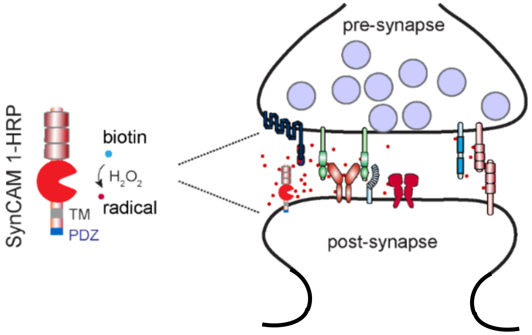
Isolate and fragment in consecutive mass/charge (m/z) windows



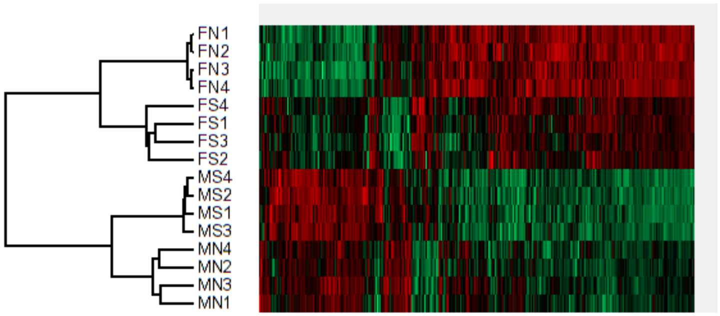
Isolate and fragment m/z of interest



Proximity Biotinylation

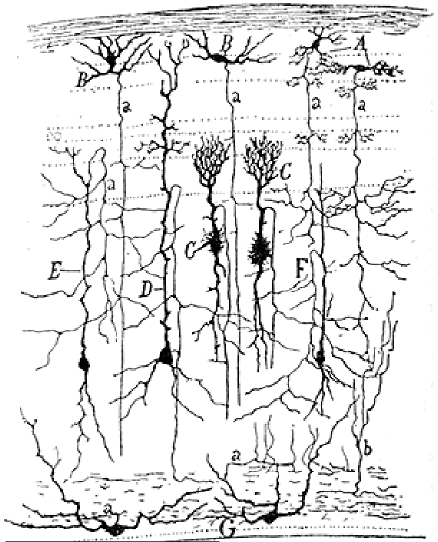


Sex Differences



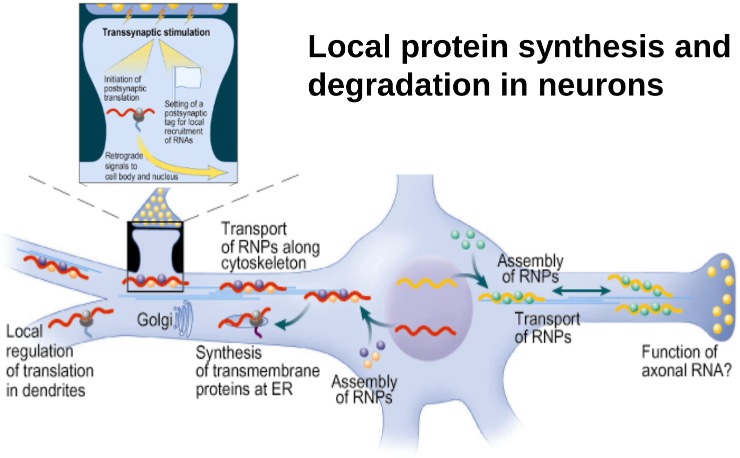
Some general themes and approaches

Diversity of neuronal subtypes



Ramón y Cajal

Local protein synthesis and degradation in neurons



Cell Type Specific Proteomics: Kitchen et al Nature Neurosci. 2014; Wilson & Nairn, Proteomes 2018.

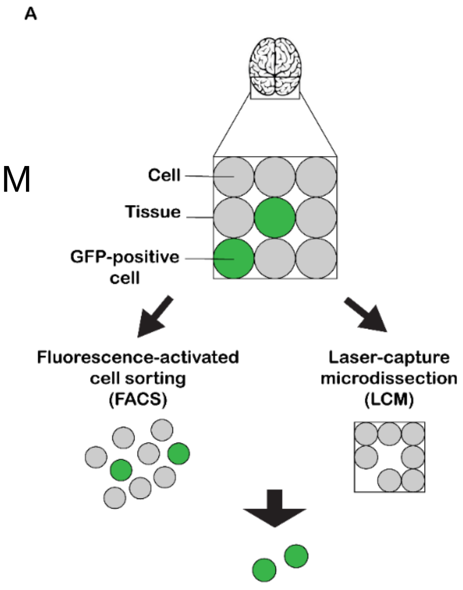
<u>Specific challenges for cell type-specific proteomic studies of the CNS</u>

- Huge amount of cell type variability with specific and distinct patterns of gene/protein expression and regulation
- Complex intermingling of neuronal sub-types
- Complex cell shapes and sub-compartments
- Cell separation or sub-cellular fractionation leads to very low amounts of proteins to analyze

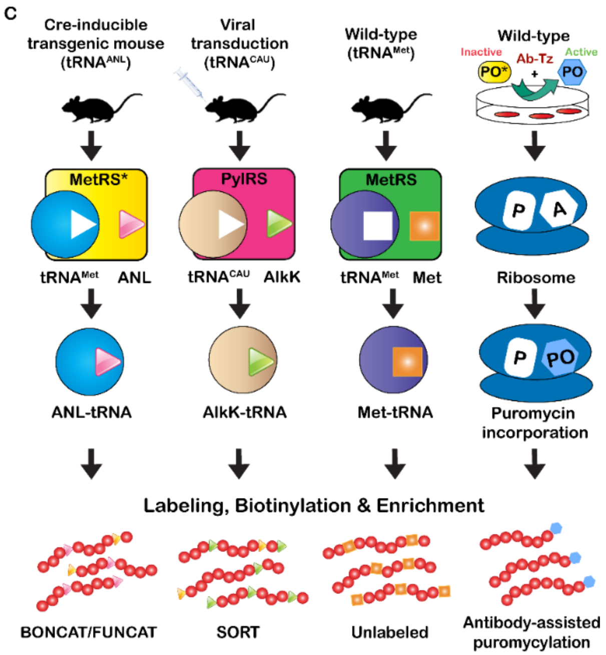
- **Methods lag single cell and single nuclei RNAseq**

Cell-Type-Specific neuronal proteomics

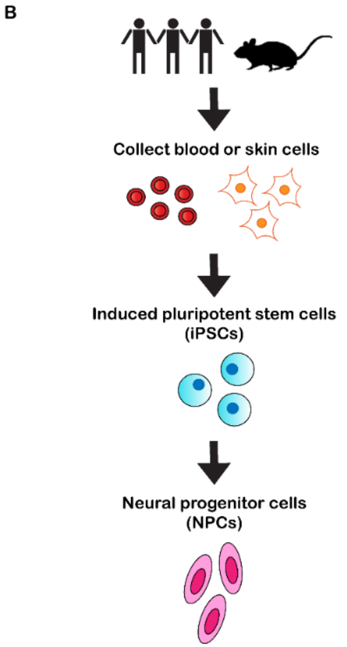
FACS and LCM



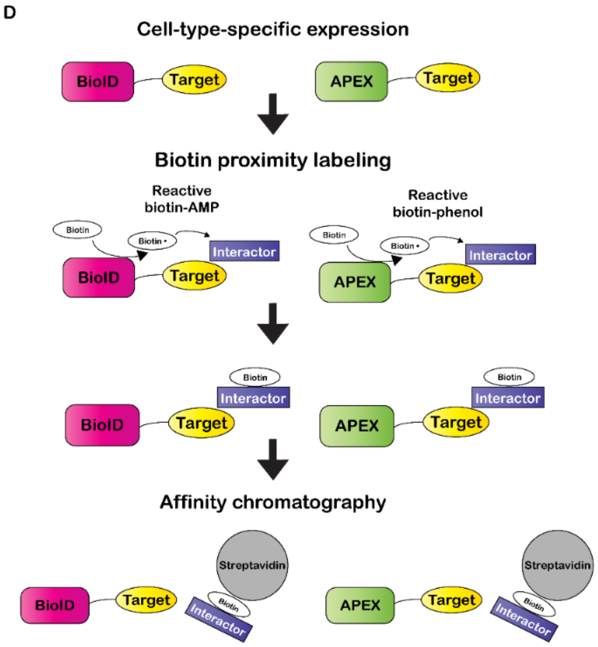
Non-Canonical Amino acid incorporation + Puromycin incorporation



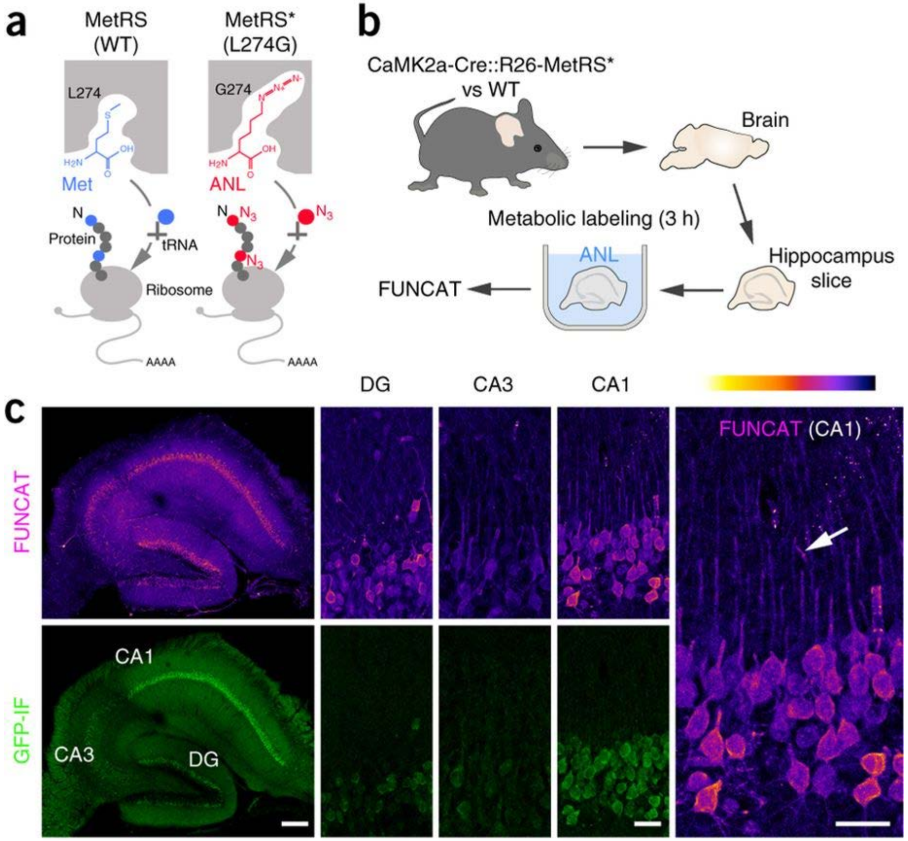
iPSCS/NPCs

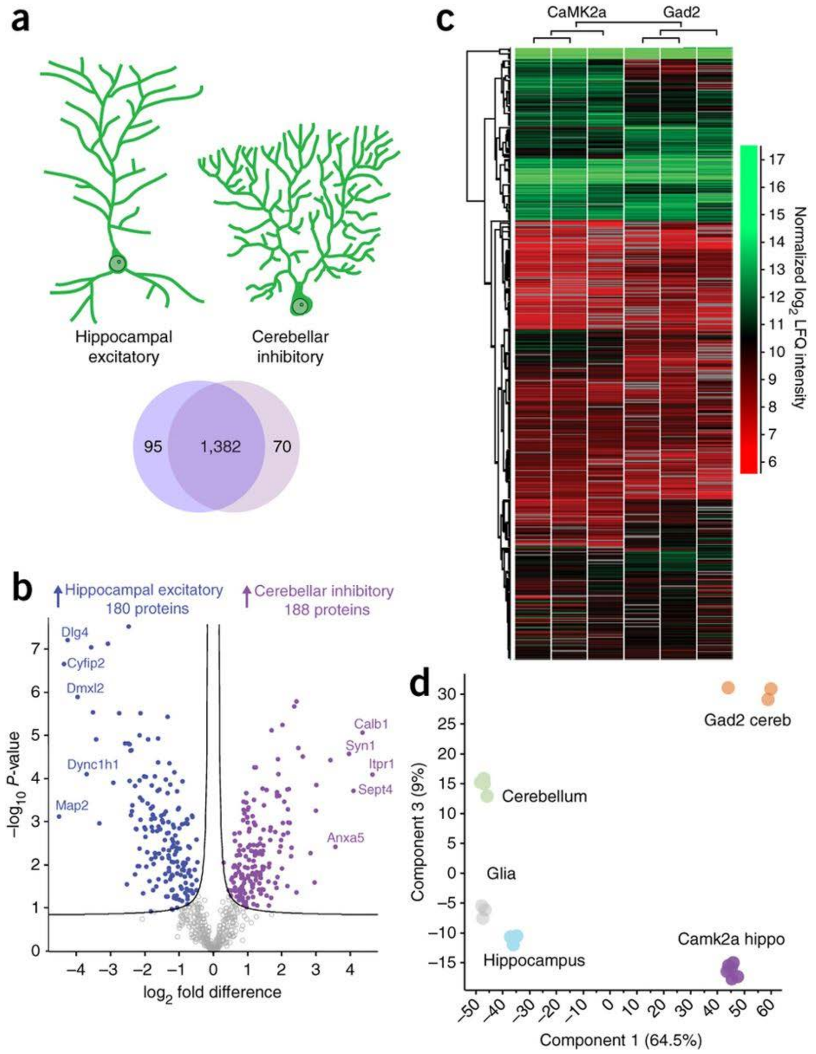


Proximity Biotinylation



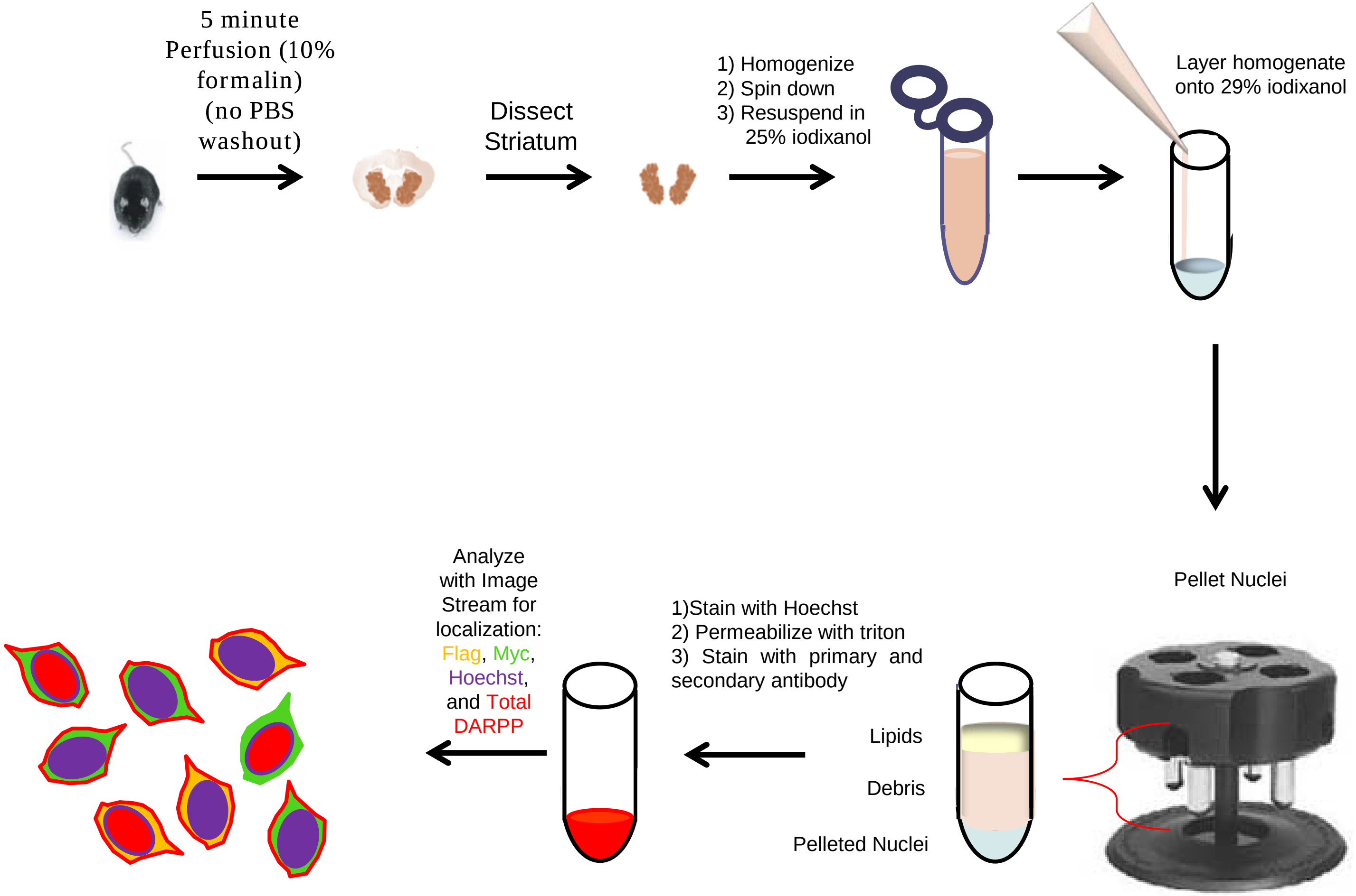
Cell-type-specific metabolic labeling of nascent proteomes *in vivo*





Alvarez-Castelao et al Nature Biotechnology 2017

D1 and D2 MSNs and FACS sorting



5 minute Perfusion (10% formalin) (no PBS washout)

Dissect Striatum

1) Homogenize
2) Spin down
3) Resuspend in 25% iodixanol

Layer homogenate onto 29% iodixanol

Pellet Nuclei

Lipids

Debris

Pelleted Nuclei

1)Stain with Hoechst
2) Permeabilize with triton
3) Stain with primary and secondary antibody

Analyze with Image Stream for localization: Flag, Myc, Hoechst, and Total DARPP

D1 and D2 MSNs and FACS sorting

Protein Threshold: 1.0% FDR
Min # Peptides: 2
Peptide Threshold: 95%

This view includes lower scoring matches and includes entire protein clusters.

Splicing factor 3A subunit 1 OS=Mus musculus GN=Sf3a1 PE=1 SV=1

Counting unit: Individual Proteins
Evaluate based on: Presence/Absence
1: D1
2: D2
3: No Category Specified

Accession	Protein Name
S12A5_MOUSE	Solute carrier family 12 ...
GTR3_MOUSE	Solute carrier family 2, f...
SORL_MOUSE	Sortilin-related receptor...
SAM50_MOUSE	Sorting and assembly m...
SPTN1_MOUSE	Spectrin alpha chain, no...
SPTB1_MOUSE	Spectrin beta chain, ery...
SPTB2_MOUSE	Spectrin beta chain, no...
STRBP_MOUSE	Spermatid perinuclear R...
DX39B_MOUSE	Spliceosome RNA helica...
SF3A1_MOUSE	Splicing factor 3A subun...

☑ Show significance threshold line
☑ Show zero fold change line

There are no GO terms in your experiment.

1268 Proteins at
18.0% Minimum
2 Min # Peptides
0.1% Decoy FDR
45577 Spectra at
95.0% Minimum
0.01% Decoy FDR

Some general themes and approaches

D1/D2 MSN FACS

- Further optimization for fixation and cell sorting in progress
- Minimize sample volumes and handling steps
- Combine with TMT multiplex approach or DIA/PRM

D1/D2 MSN Proximity Biotinylation

- Use available cell type-specific driver lines and viral constructs

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Thanks to participants, audience, YSM and NIDA