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## Neuropeptides: From the first metazoans to the synaptic credit assignment problem

Compelling phylogenomic evidence indicates that the last common ancestor of all animals with nervous systems lacked neurons and synapses, but coordinated sensory, motor and digestive cell types via secreted ligands homologous to today's neuropeptides and receptors homologous to today's neuropeptide-selective GPCRs. Peptidergic networks never went away. As pressures for speed and size drove the evolution of synaptic networks, peptidergic networks never stopped being essential to homeostasis, adaptation and memory storage by the synaptic newcomers.

**Genetic first light on peptidergic network.** Until the recent advent of molecular genetics, architectures of neuropeptide networks have been invisible to science. Peptidergic network graphs are determined not by structurally obvious synaptic contacts, but by much less obvious factors: relatively obscure sites of cellular secretions and receptor display, the biophysics of interstitial peptide diffusion and degradation, intricately intermingled but loosely stereotyped dendrite forms, and differential patterns of peptide and receptor gene expression.

**Positive Feedback from Paired Peptide Ligands and Receptors.** Single-cell transcriptomics now offers our first panoramic, if slightly foggy - views of peptidergic network graphs. Here we find on new evidence that certain neurons express genes in cognate pairs, where individual cells both ligand precursor protein and receptor for one and the same peptide. Well-established facts of GPCR signal transduction suggest that some of these pairings may predispose cells to explosive, regenerative signaling due to positive feedback from reception to secretion of the same peptide.

**Neuropeptide Signal Regeneration and Credit Assignment.** Regenerative neuropeptide signaling entails intriguing possibilities of intracellular and intercellular signal propagation, threshold non-linearities, and new presently dark places to look for solutions to the enigmatic synaptic credit assignment problem. Here we present transcriptomic evidence supporting a regenerative signaling hypothesis, outline possible experimental tests and hope to inspire ideas about new, energy-efficient alternatives to conventional back-prop for training artificial neural networks.

## Functional Annotation of Neuropeptide Precursor Protein (NPP) and Neuropeptide-Selective G-Protein-Coupled Receptor (NP-GPCR) Genes

1. 30 sets of cognate NPP / NP-GPCR pairs, categorized by primary signal transduction cascade and then sorted by mean mRNA-Seq counts detected per cell in mouse brainwide.

2. Right-most column lists useful aggregates summing counts for all NP-GPCRs cognate to each of the 30 listed NPP genes, and corresponding gene-like symbols.

Neuropeptide Product NPP Gene Mean Counts Cognate NP-GPCR Aggregate Primary Transduction Pathway

cholecystokinin Cck 8,972 Gq/G11 Phosphopase C Activation

gastrin-releasing peptide Grp 8,458 Gs Adenylyl Cyclase Activation

parathyroid hormone-like protein Pth 8,059 Gs Adenylyl Cyclase Activation

thyrotropin-releasing hormone Trh 5,900 Gs Adenylyl Cyclase Activation

vasoactive intestinal peptide Vip 0,972 Gs Adenylyl Cyclase Activation

neurokinin-1 neuropeptide Y Npy 0,956 Gs Adenylyl Cyclase Activation

pro-enkephalin Pro 0,108 Gs Adenylyl Cyclase Activation

calcitonin gene-related peptide CGRP 0,059 Gs Adenylyl Cyclase Activation

hypothalamic-releasing hormone Hrh 0,027 Gs Adenylyl Cyclase Activation

endothelin 1 Endt 0,016 Gs Adenylyl Cyclase Activation

urocortin 1 Ucr 0,015 Gs Adenylyl Cyclase Activation

adrenomedullin Adm 0,013 Gs Adenylyl Cyclase Activation

preproenkephalin Pnk 1,999 Gs Adenylyl Cyclase Activation

neurokinin-2 neuropeptide Y Npy 0,956 Gs Adenylyl Cyclase Activation

pro-enkephalin Pro 0,350 Gs Adenylyl Cyclase Activation

neuropeptide FF-amide Nppf 0,041 Gs Adenylyl Cyclase Activation

tachykinin 1 Tkr 0,210 Gs Adenylyl Cyclase Activation

arginine vasopressin Arg 0,128 Gs Adenylyl Cyclase Activation

pro-enkephalin Pro 0,111 Gs Adenylyl Cyclase Activation

galanin and GMAP Gal 0,029 Gs Adenylyl Cyclase Activation

... and its associated anatomic parcellation...

Region Symbol Region Label Neurons Sampled Number of Cells

VIS Primary Visual 14,000 10 - 120

VIS Visual Panel Association 3,538 10 - 50

AUD Auditory 1,433 10 - 32

SSt Primary Somatosensory 5,640 10 - 60

GSt Gustatory 1,596 10 - 50

Mot Motor 9,004 10 - 95

PLA Paralimbic 1,540 10 - 31

QHb Orbital 1,380 10 - 31

ACA Anterior Circular 5,056 10 - 61

TPE Temporal-Anterior-Posterior 1,580 10 - 31

ENT Entorhinal 1,577 10 - 30

PPB Parahippocampal 6,529 10 - 66

Hippocampal Formation 6,529 10 - 66

... and its associated cell-type taxonomy...

3 Classes 8 Neighborhoods 42 Subclasses 388 Clusters

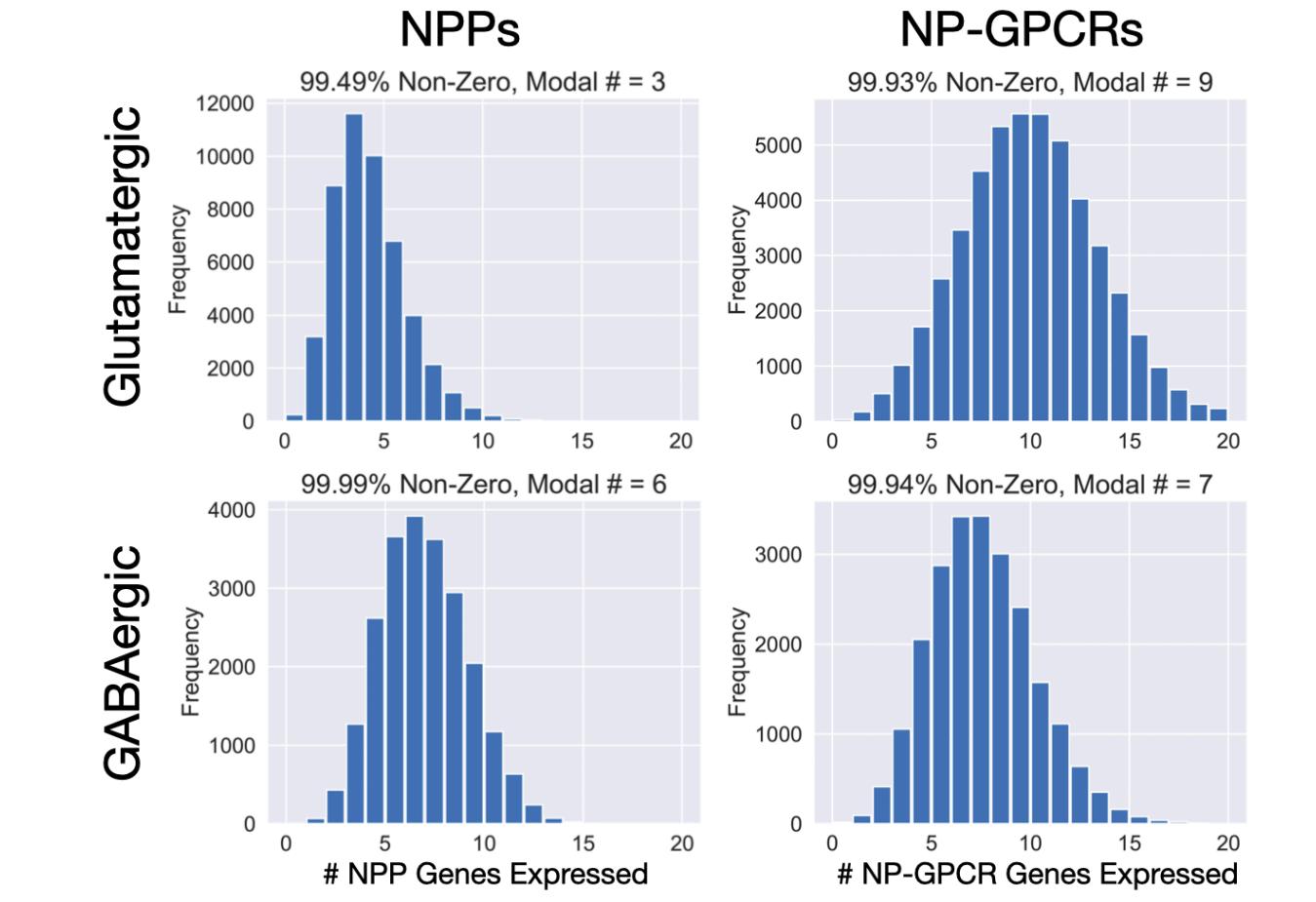
6 Neighborhoods 1 Subclass 7 Neighborhoods 34 Subclasses

2 Classes 7 Neighborhoods 34 Subclasses

Neurotaxony Badge Cell Count 2 Classes 7 Neighborhoods 34 Subclasses

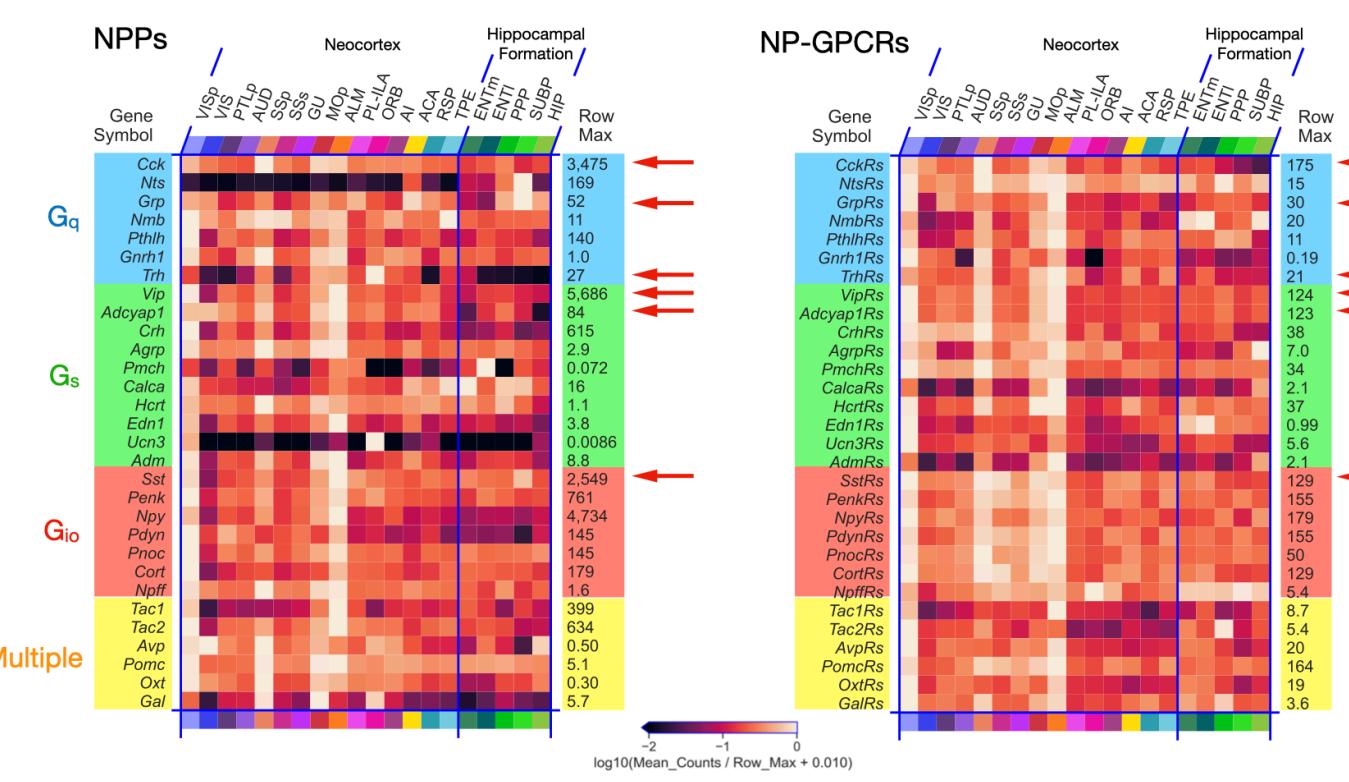
## All Neurons in Mouse Cortex Are (Very Probably) Peptidergic and Peptide Receptive

Frequency histograms show that almost all cortical neurons express multiple NPPs and multiple NP-GPCRs. Very few lack expression of either.

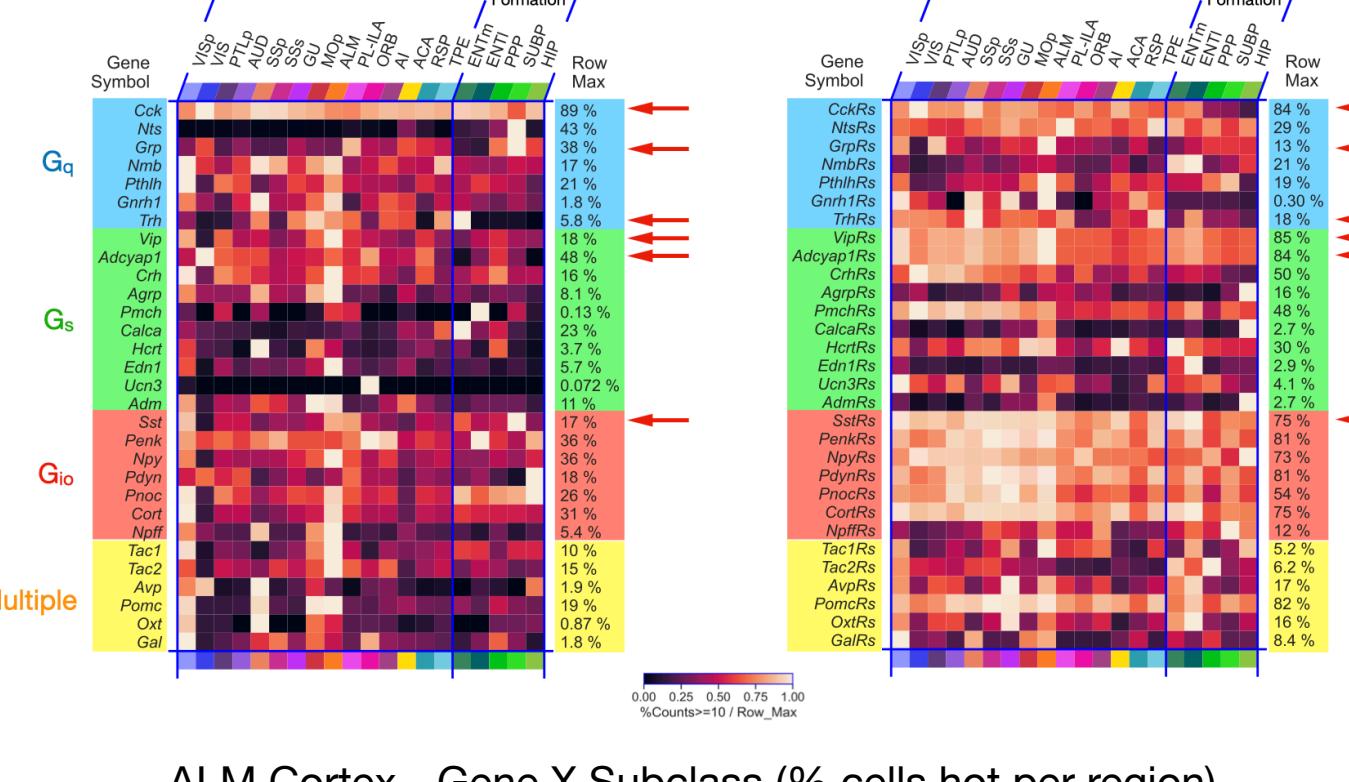


## Expression of 30 NPPs and Cognate NP-GPCRs in Mouse Isocortex and Hippocampal Formation (row normalized with red-arrow callouts to network graph examples)

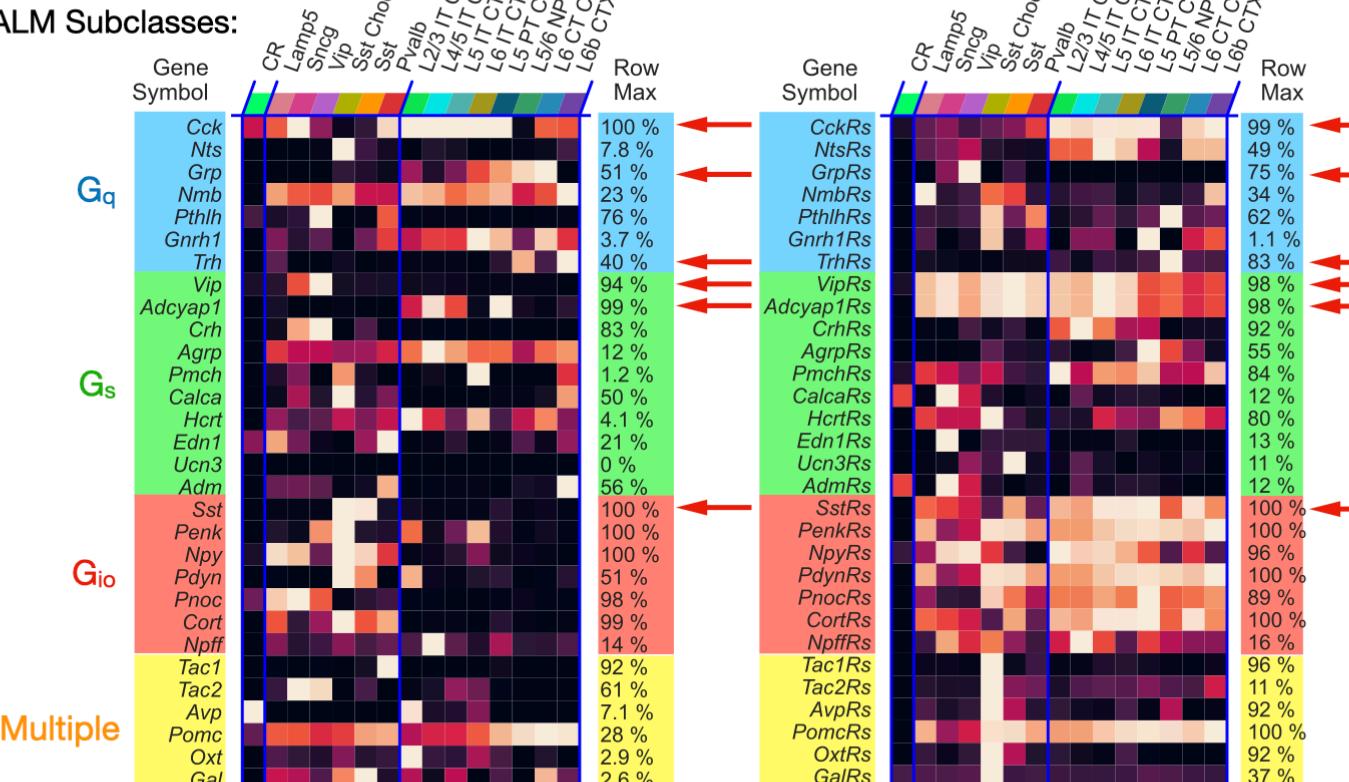
Gene X Region (mean counts per region)



Gene X Region (% cells hot per region)



ALM Cortex - Gene X Subclass (% cells hot per region)



## Transcriptomic Data and Taxonomy Resource

The present analysis is based on primary data documented by:

**A Taxonomy of Transcriptomic Cell Types Across the Isocortex and Hippocampal Formation (2021) Cell 184(12): p. 3222-3244.**

Yao, Z., van Velthoven, C. T., Nguyen, T. N., Gody, J., Sedenio-Cortes, A. E., Bafna, F., Bertagnoli, D., Casper, T., Chiang, M., Crichton, K., Ding, S. L., Fong, O., Garren, E., Glandon, A., Gouwens, N. W., Gray, J., Graylock, B., L., Hawrylycz, M. J., Hirschstein, D., Kroll, M., Lathia, K., Lee, C., Levi, B., McMullen, D., Mok, S., Pham, T., Ren, Q., Rimorin, C., Shapovalova, N., Sulc, J., Sunkin, S. M., Tie, M., Torkelson, A., Tung, H., Ward, K., Dee, N., Smith, K. A., Tasic, B., Zeng, H.

... and its associated anatomic parcellation...

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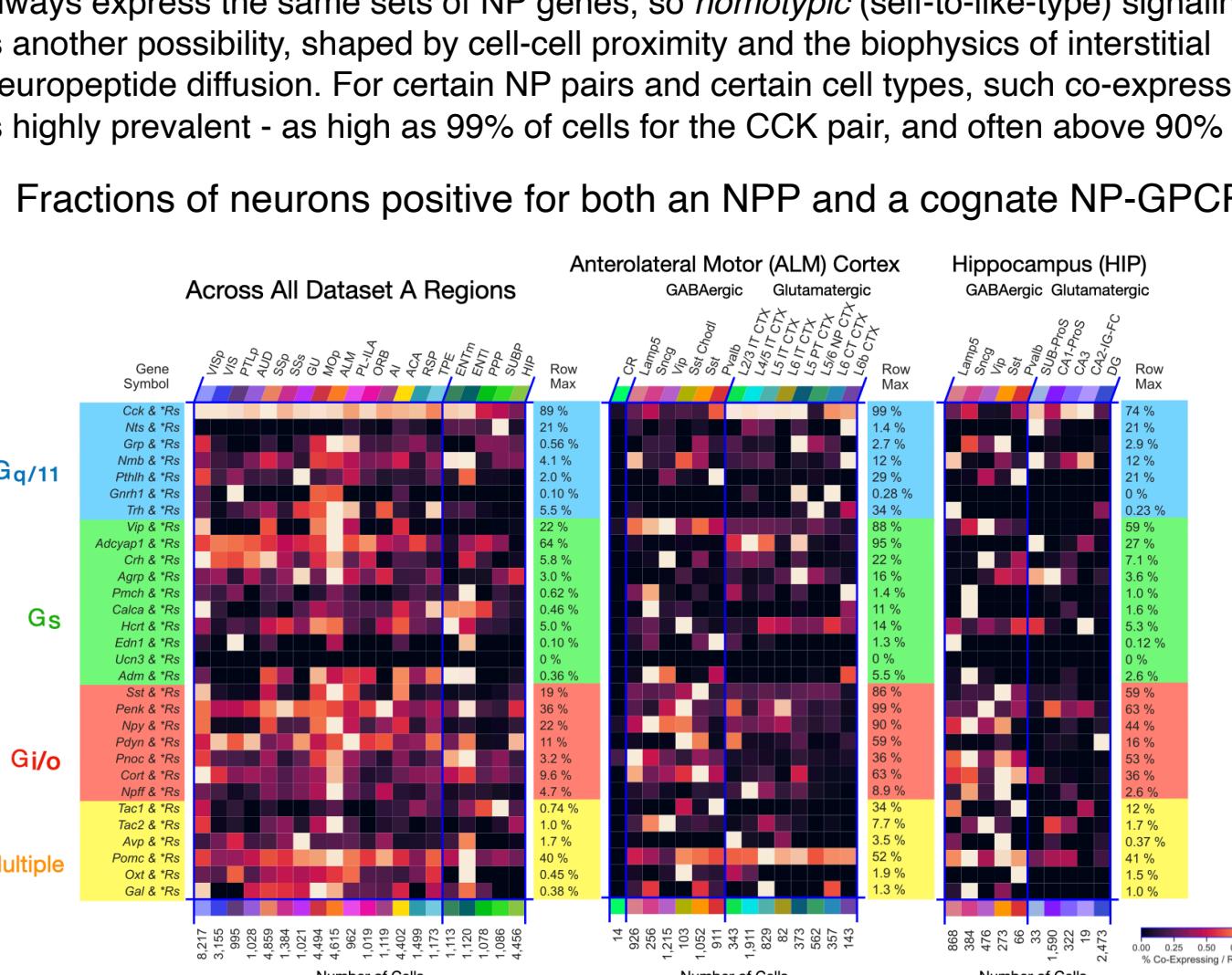
2 Classes 7 Neighborhoods 34 Subclasses

Neurotaxony Badge Cell Count 2 Classes 7 Neighborhoods 34 Subclasses

## Many individual neurons express both members of a cognate NPP / NP-GPCR pair

Expression of a given NPP and a cognate NP-GPCR in one single cell suggest most obviously *autocrine* (self-to-self) signaling. Cells of the same type, however, almost always express the same sets of NP genes, so *homotypic* (self-to-like-type) signaling is another possibility, shaped by cell-type proximity and the biophysics of interstitial neuropeptide diffusion. For certain NP pairs and certain cell types, such co-expression is highly prevalent - as high as 99% of cells for the CCK pair, and often above 90%

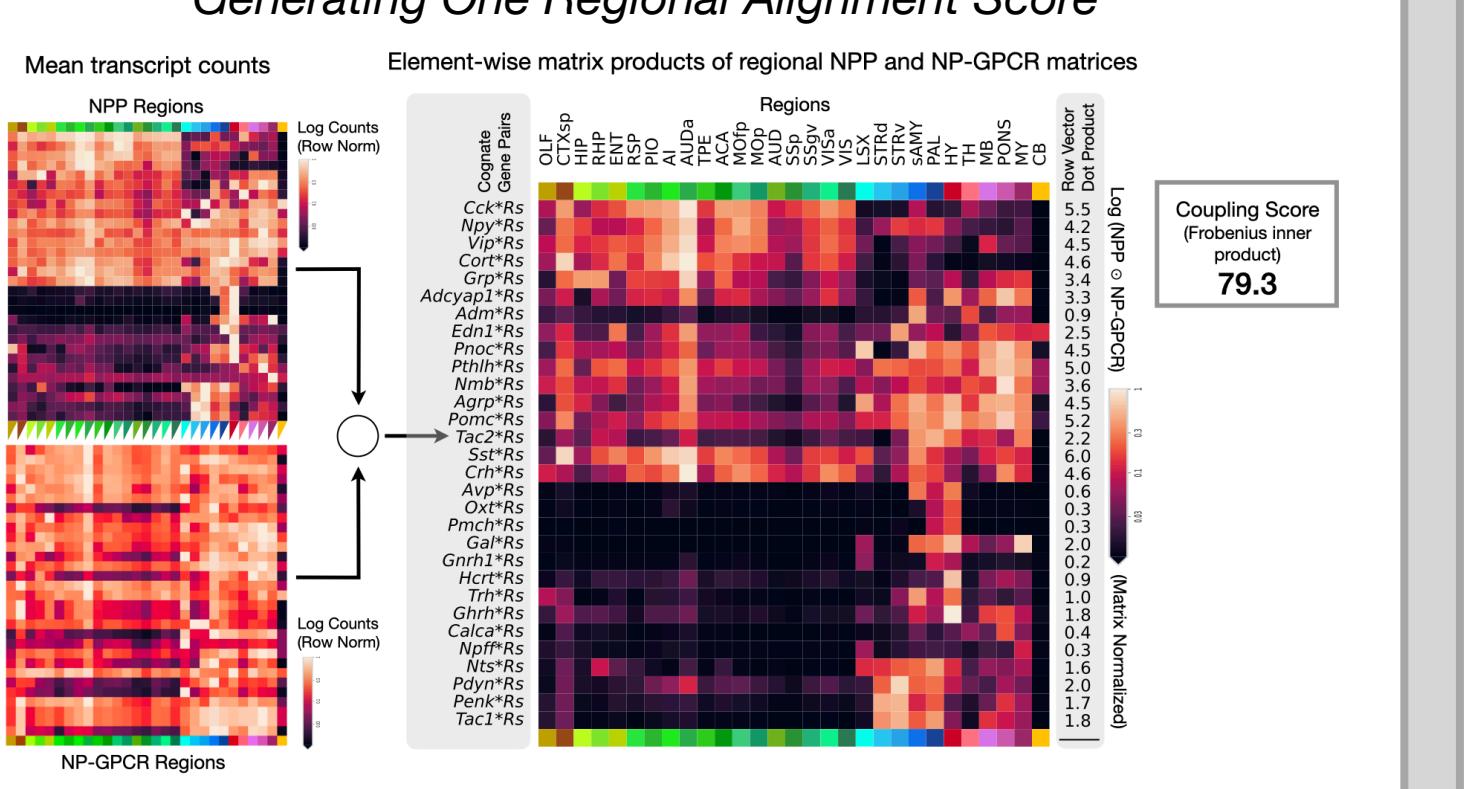
Fractions of neurons positive for both an NPP and a cognate NP-GPCR



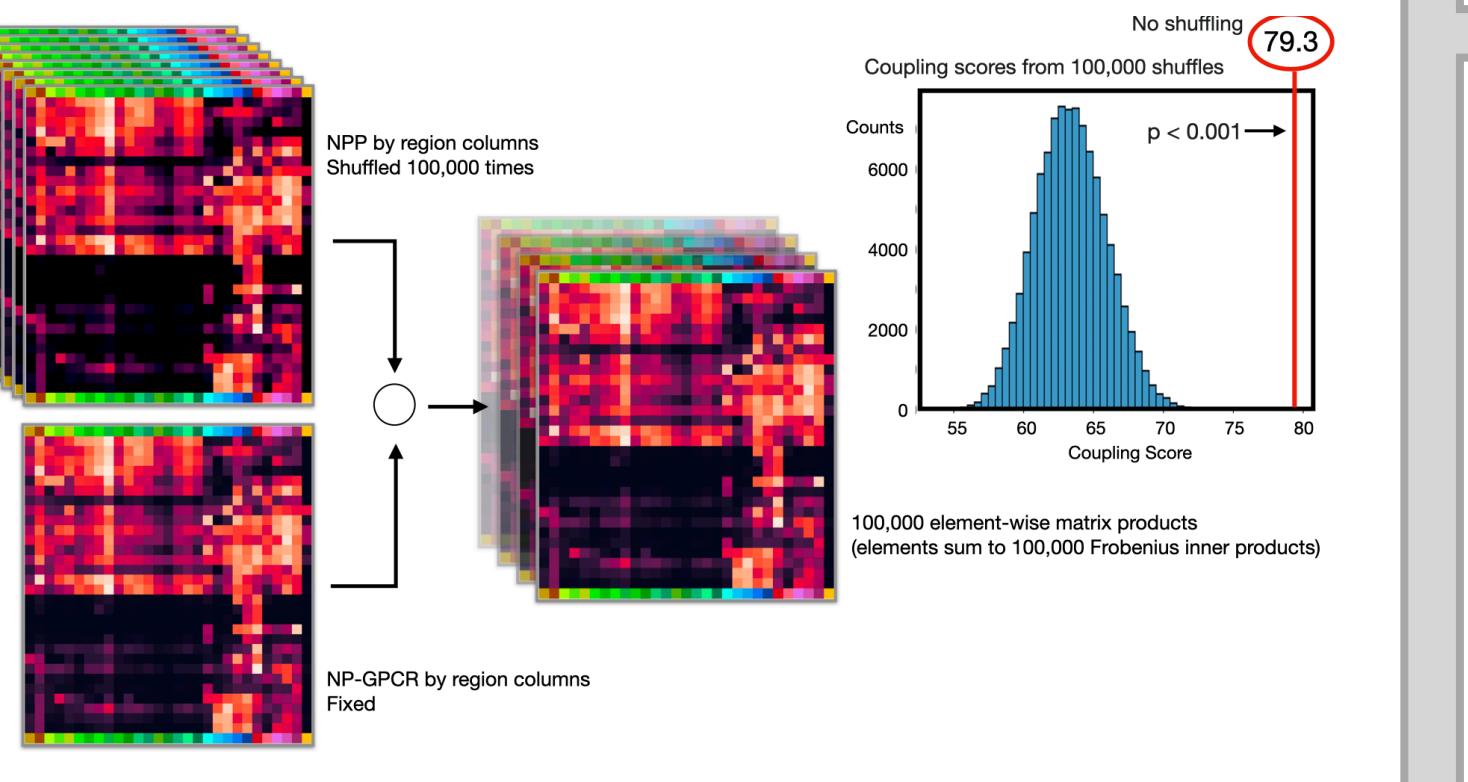
## Regional Alignment of Peptide and Receptor Expression Make a Case for Localized Signaling

"Alignment" here refers to region by region matching between NPP and NP-GPCR expression patterns, quantifiable as the Frobenius inner product (akin to a dot product) of gene by region NPP and NP-GPCR matrices. The observation that such product scores decline dramatically when alignment of regions is perturbed by random shuffles suggests that the natural alignment of expression patterns confers fitness for local signaling.

Generating One Regional Alignment Score



Shuffling Regional NPP Vectors Weakens Regional Alignment

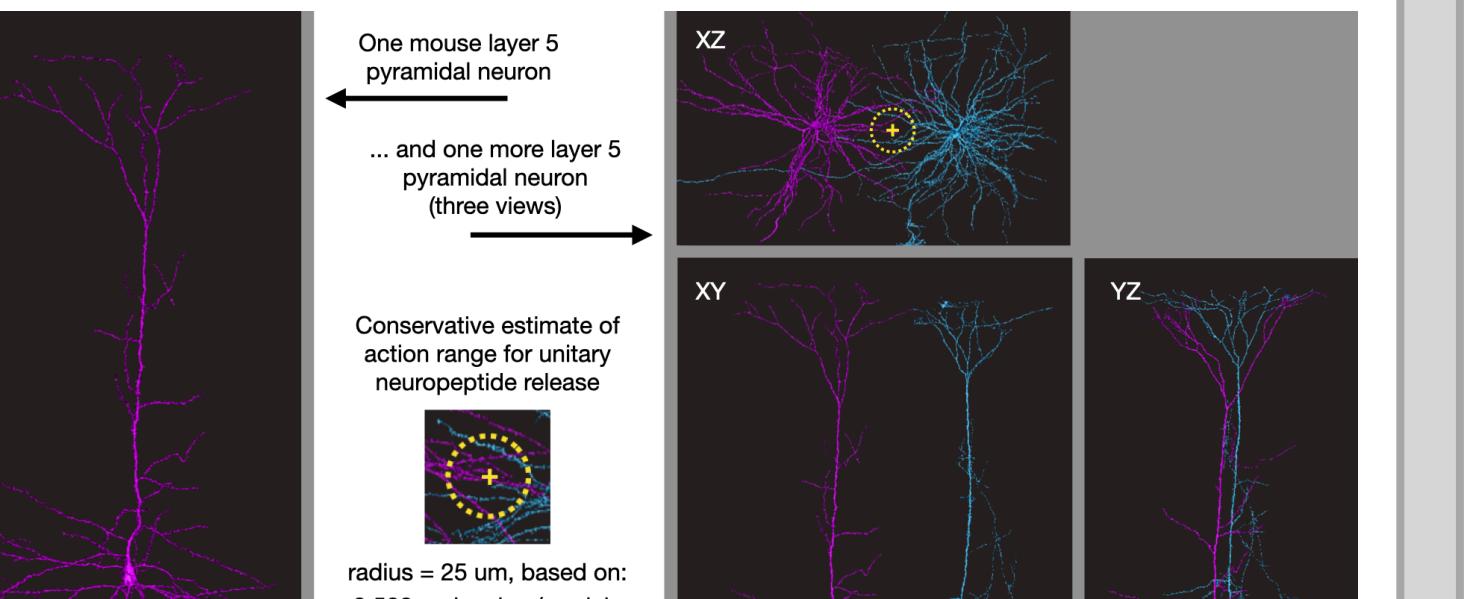


## Anatomic Constraints on Neuropeptide Signaling

Biophysics of interstitial diffusion guarantee that proximity of secretion site and cognate receptor will strongly constrain neuropeptide signaling strength. Unfortunately, only crude estimates of the key parameters have been possible up until now.

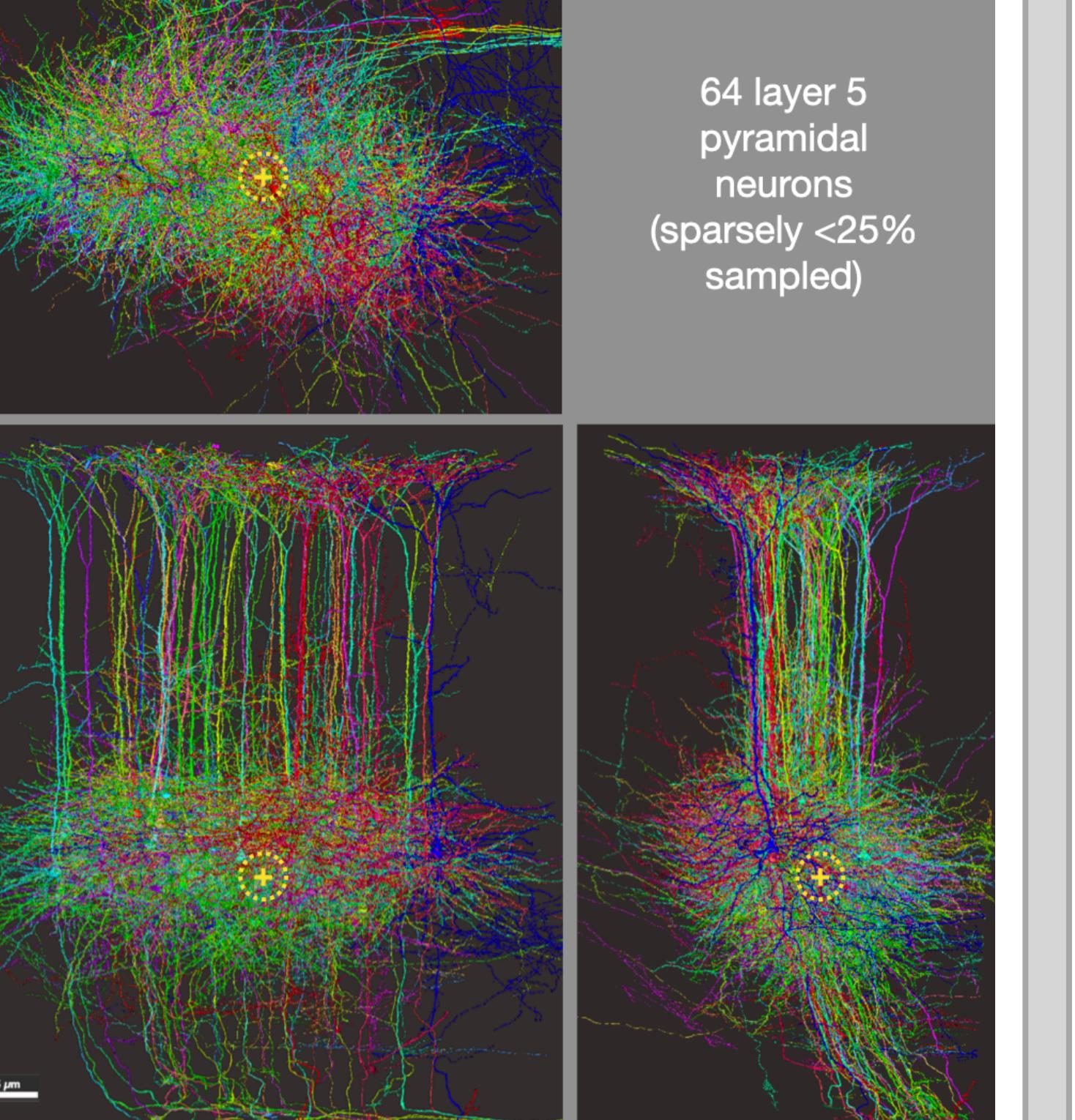
Since most neuropeptide release and receptor sites in cortex are likely dendritic, the intricate intermingling of cortical dendrites has loomed as one vexing area of uncertainty. The sparse labeling formerly necessary to capture cortical dendritic morphologies has hidden intermingling from our view. The dense anatomic visualizations newly available from computational volume electron microscopy (<https://www.microns-explorer.org/>) are now solving this problem.

The illustrations below are intended to evoke ways the new volume EM visualizations are likely to lead to dramatic improvements in our abilities to grapple the cell-type and proximity factors that will constrain coming models of local neuropeptide signaling.



radius = 25 um, based on: 2.50 micrometers diameter D = 3x10^-6 cm/sec volume fraction = 0.1 tortuosity = 1.6 GPCR EC50 = 0.5 nM

64 layer 5 pyramidal neurons (sparsely <25% sampled)



## Transcriptomic Prediction of Peptidergic Networks

Detection of cognate NPP and NP-GPCR messengers in one given limited brain region suggests a peptidergic signaling link between expressing cells within diffusion distance. Such an inference is strengthened by evidence that neuropeptides are often secreted from dendrites and cell bodies, which intermingle extensively within local brain regions. The graphic below exemplifies the construction and annotation of an adjacency matrix predicting a directed peptidergic network graph for one limited cortical region (ALM) for one NPP (Cck).

Predicting one neuropeptide network graph from NPP and NP-GPCR expression

Region: ALM

Subclass: GABAergic

Element-wise matrix products of regional NPP and NP-GPCR matrices

Regions: L2/3 IT CTX, L3 IT CTX, L5 IT CTX, L6 IT CTX, L7 IT CTX, L8 IT CTX, L9 IT CTX, L10 IT CTX, L11 IT CTX, L12 IT CTX, L13 IT CTX, L14 IT CTX, L15 IT CTX, L16 IT CTX, L17 IT CTX, L18 IT CTX, L19 IT CTX, L20 IT CTX, L21 IT CTX, L22 IT CTX, L23 IT CTX, L24 IT CTX, L25 IT CTX, L26 IT CTX, L27 IT CTX, L28 IT CTX, L29 IT CTX, L30 IT CTX, L31 IT CTX, L32 IT CTX, L33 IT CTX, L34 IT CTX, L35 IT CTX, L36 IT CTX, L37 IT CTX, L38 IT CTX, L39 IT CTX, L40 IT CTX, L41 IT CTX, L42 IT CTX, L43 IT CTX, L44 IT CTX, L45 IT CTX, L46 IT CTX, L47 IT CTX, L48 IT CTX, L49 IT CTX, L50 IT CTX, L51 IT CTX, L52 IT CTX, L53 IT CTX, L54 IT CTX, L55 IT CTX, L56 IT CTX, L57 IT CTX, L58 IT CTX, L59 IT CTX, L60 IT CTX, L61 IT CTX, L62 IT CTX, L63 IT CTX, L64 IT CTX, L65 IT CTX, L66 IT CTX, L67 IT CTX, L68 IT CTX, L69 IT CTX, L70 IT CTX, L71 IT CTX, L72 IT CTX, L73 IT CTX, L74 IT CTX, L75 IT CTX, L76 IT CTX, L77 IT CTX, L78 IT CTX, L79 IT CTX, L80 IT CTX, L81 IT CTX, L82 IT CTX, L83 IT CTX, L84 IT CTX, L85 IT CTX, L86 IT CTX, L87 IT CTX, L88 IT CTX, L89 IT CTX, L90 IT CTX, L91 IT CTX, L92 IT CTX, L93 IT CTX, L94 IT CTX, L95 IT CTX, L96 IT CTX, L97 IT CTX, L98 IT CTX, L99 IT CTX, L100 IT CTX, L101 IT CTX, L102 IT CTX