Transcriptomic Evidence for Homotypic Neuropeptide Signaling Architectures in Mouse Brain

148.01

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Toward Neuromodulatory Network Architectures

Neuromodulatory signaling by secreted ligands such as neuropeptides, dopamine, serotonin and many others is essential to all neuronal circuit function and plasticity. Such signaling is predominantly extrasynaptic, so neuromodulatory signaling architectures differ greatly from those defining synaptic networks. While synaptic networks are defined by axonal "wiring" and focal sites of contact, neuromodulatory networks reflect the spatial and cell-type specific patterning of differential ligand secretion and selective receptor display. Neuromodulatory networks are further shaped powerfully by the biophysics of interstitial ligand diffusion and the complex intracellular signal amplification cascades characteristic of neuromodulatory receptors.

The recent availability of brain-wide single-cell transcriptomic data and cell-type taxonomies now offers panoramic new perspectives on neuromodulatory architectures and very rich sources of physiologically testable hypotheses. Here we expose a few of these new vistas for a set of robustly expressed neuropeptide ligands and their cognate selective receptors.

Evidence for Homotypic Neuropeptide Networks

Architectures of neuropeptide (NP) signaling networks are particularly amenable to prediction from transcriptomic data and cell-type taxonomies since numerous distinct NP ligands and NP receptors are encoded by genes expressed in many different highly cell-type-specific patterns. Here we describe results from single-cell mRNA sequencing (scRNA-Seq) data from 4,116,323 single cells sampled from regions spanning the entire mouse brain. These results suggest that every neuron in most brain regions robustly expresses transcripts encoding one or more of 30 neuropeptide precursor proteins (NPPs) and one or more of 51 neuropeptide-selective G-Protein-Coupled Receptors (NP-GPCRs).

These scRNA-Seq results suggest that large fractions of individual cells of certain types in certain brain regions probably express *both* an NPP and a receptor selective for that same NP-GPCR, that is, a "cognate" receptor. Though such findings may immediately suggests "autocrine" (self-to-self) signaling, they may also be understood as evidence for "homotypic" (self-to-same-type) signaling. Here we outline possible homotypic signaling networks and suggest ways that they may be highly consequential for brain function and plasticity.

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 brainwide.*
2. *The right-most column lists artificial but useful aggregates of NP-GPCRs cognate to each of the 30 listed NPP genes, and corresponding gene-like symbols. These aggregates simply sum counts for all individual NP-GPCR genes cognate to a given NPP.*

Neuropeptide Product	NPP Gene	Mean Counts	Conjugate NP-GPCR Genes	NP-GPCR Aggregate	Primary Transduction Pathway
cholecystokinin	Cck	8.540	Cckbr Cckar Gpr173	Cckxrs	G_{q} + Phospholipase C Activation
neurotensin	Nts	5.348	Ntsr1 Ntsr2	Ntsrs	
gastrin-releasing peptide	Grp	3.156	Grpr	Grprs	
neuromedin U	Nmu	2.198	Nmur2	Nmurs	
parathyroid hormone like	Pth2	0.059	Ptr2r	Pth2rs	
gonadotropin-releasing	Gnrh1	0.056	Gnrr	Gnrhrs	
thyrotropin releasing	Trh	0.054	Trhr Trhr2	Trhrs	
vasoactive intestinal	Vip	0.972	Adcyap1r1 Vipr1 Vipr2	Viprs	G_{s} + Adenylate Cyclase Activation
corticotropin releasing	Crh	0.303	Crhr1 Crhr2	Crhrs	
corticotropin releasing	Crh	0.203	Crhr1 Crhr2	Crhrs	
agouti related	Agrp	0.138	Mc3r Mc4r	Agrprs	
cocaine and amphetamine	Cart	0.099	Gpr160	Cartprs	
calcitonin/calcitonin-related	Calcrl	0.059	Calcr	Calcrls	
hypocretin	Hcrt	0.027	Hcrtr1 Hcrtr2	Hcrtrs	
urotensin 2	Urn2	0.016	Oxtr	Urn2rs	
urotensin 2	Urn2	0.014	Oxtr	Urn2rs	
adrenomedullin	Adm	0.013	Calcr	Admrs	
somatostatin	Sst	2.706	Sstr1 Sstr2 Sstr3 Sstr4 Sstr5	Sstrs	G_{i} + Adenylate Cyclase Inhibition
prepronociceptin/ophanin	Pnoc	1.399	Oprl1 Oprk1 Oprl1 Oprm1	Pencrs	
prodynorphin	Pdyn	0.664	Oprk1 Oprl1 Oprd1 Oprm1	Pdyncrs	
prodynorphin	Pdyn	0.664	Oprk1 Oprl1 Oprd1 Oprm1	Pdyncrs	
preproenkephalin	Penk	0.355	Oprd1	Penkrs	
prolactin-releasing peptide	Prlh	0.061	Sstr1 Sstr2 Sstr3 Sstr4 Sstr5	Prlhrs	
neuropeptide FF amide	Npff	0.041	Npffr1 Npffr2	Npffrs	
tachykinin 1	Tac1	1.406	Tacr1	Tacrs	G_{q} + Phosphoinositide Hydrolysis
tachykinin 2	Tac2	0.210	Tacr2	Tacrs	
arginine vasopressin	Avp	0.128	Avpr1a Avpr1b Oxtr	Avprs	
pro-apolipoprotein A1	Apoa1	0.115	Mas1 Mas1l Gpr151 Gpr23 Gpr37	Apoars	
orexin	Hcrt	0.111	Hcrtr1 Hcrtr2	Hcrtrs	
galanin and GMAP	Gal	0.091	Galr1 Galr2	Galrs	

Data, Cell-Type Taxonomy and Code Resources

Isocortex & Hippocampal Formation

Dataset A: Plate-Based Single-Cell RNA-Seq (SMART-Seq v4)
Average 7,677 Genes Mapped per Neuron
71,518 Neurons Sampled from 20 Regions
(15 Isocortex, 5 Hippocampal Formation)
2 Classes, 36 Subclasses, 358 Clusters

Dataset B: Droplet-Based Single-Cell RNA-Seq (10x v2)
Average 2,768 Genes Mapped per Neuron
1,169,213 Neurons Sampled from 18 Regions
(15 Isocortex, 3 Hippocampal Formation)
2 Classes, 36 Subclasses, 364 Clusters

A Taxonomy of Transcriptomic Cell Types Across the Isocortex and Hippocampal Formation. (2021) bioRxiv 10.1101/2021.03.10.435991.

Yao, Z. van Velthoven, C. T., Nguyen, T. N., Goldy, J., Sedeno-Cortes, A. E., Baftizadeh, F., Bertagnolli, D., Casper, T., Chiang, M., Crichton, K., Dee, N., Diep, D., Gelfand, E., Hirokawa, K., Jeanne Kowalski, A., Kroll, M., Lathia, K., Lee, C., Lee, B., Mihalas, O., Mok, G., Pham, T., Rai, C., Ransford, O., Rimorin, C., Ruiz, A., Sanchez, R., Sunkin, S., Tan, P., Wa, N., Smallwood, A., Tung, H., Ward, M., Dee, N., Smith, K., A., Tasic, B., Zeng, H. (2021).

The two publications referenced above include links to the major resource data and taxonomy tables from which this poster's data figures are derived. The python scripts and minor tables used to generate these figures may be downloaded from a GitHub repository (https://github.com/profsjsmith/extrasynaptic_networks).

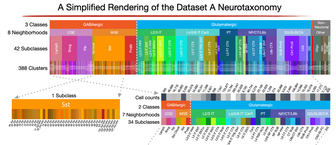
Why Three Different Datasets?

Key findings are first introduced here using dataset A to take advantage of its superlative sampling depth. We'll proceed then to exploit the scaling advantages of droplet method and progress from cortical areas only (dataset A) to brainwide sampling (dataset C). Comparing results from datasets B (droplet-based) to dataset A (plate-based) will offer a valuable perspective on how method choice impacts results.

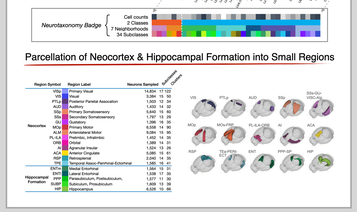
Dataset A: Neocortex & Hippocampal Formation, Cell-Type Taxonomy

Plate-Based Single-Cell RNA-Seq (SMART-Seq v4)
Average 7,677 Genes Mapped per Neuron
71,518 Neurons Partitioned into 2 Classes, 36 Subclasses, 358 Clusters

A Simplified Rendering of the Dataset A Neurotaxonomy



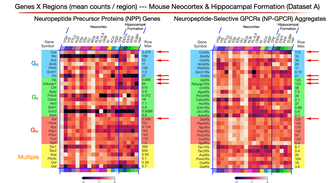
Parcellation of Neocortex & Hippocampal Formation into Small Regions



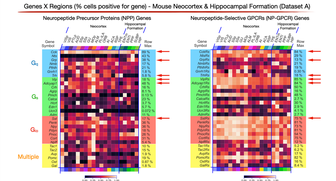
Dataset A: mRNA expression matrices for the top 30 NPPs and aggregates of their cognate NP-GPCRs in isocortex and hippocampal formation

Values in each heatmap row are normalized to row maximum, with that value numeric at right. Red arrows call out NPPs and NP-GPCR aggregates used below as illustrative examples.

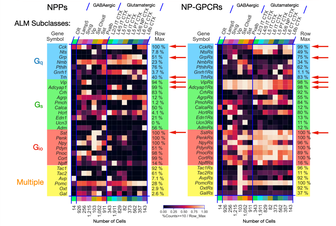
Genes X Regions (mean counts / region) --- Mouse Neocortex & Hippocampal Formation (Dataset A)



Genes X Regions (% cells positive for gene) - Mouse Neocortex & Hippocampal Formation (Dataset A)



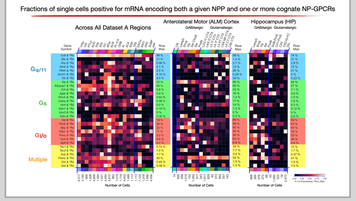
Genes X Subclasses (% cells positive for gene) --- ALM Cortex, by Subtype (Plate-Based)



Expression in single cells of conjugate NPP and NP-GPCR gene pairs

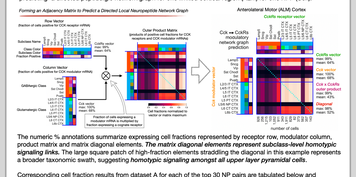
Expression of a given NPP and a cognate NP-GPCR in one single cell most obviously suggests *autocrine* (self-to-self) signaling. There is considerable evidence, however, that neuropeptides are often secreted from dendrites and cell bodies and that neurons of similar type tend to flock together and densely intermingle their processes. Thus, co-expression of NPP and cognate NP-GPCR also suggests the possibility of *homotypic* (self-to-like-type) signaling. Note from the heatmaps below that for certain gene pairs, certain regions and certain cell types, such co-expression is highly prevalent - as high as 99% of cells for CCK and cognates in certain glutamatergic subtypes, and often above 90%.

Fractions of single cells positive for mRNA encoding both a given NPP and one or more cognate NP-GPCRs



Prediction of Peptidergic Network Graphs from NPP / NP-GPCR Pair Expression

Detection of cognate NPP and NP-GPCR messenger RNAs 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).



The numeric % annotations summarize expressing cell fractions represented by receptor row, modulator column, product matrix and matrix diagonal elements. *The matrix diagonal elements represent subclass-level homotypic signaling links.* The large square patch of high-fraction elements straddling the diagonal in this example represents a broader taxonomic swath, suggesting *homotypic signaling amongst all upper layer pyramidal cells.*

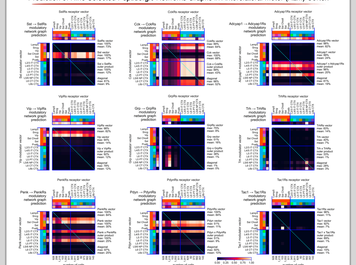
Corresponding cell fraction results from dataset A for each of the top 30 NP pairs are tabulated below and illustrated in more detail by the following adjacency matrices for three representative regions and the nine representative NP pairs highlighted in this table.

Dataset A: Cell Fraction Results from Network Graphs Predicted for Regions ALM and HIP

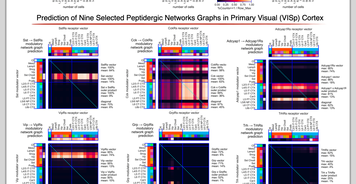
Anterolateral motor (ALM) cortex | Hippocampus (HIP)

(highlighted predictions illustrated below)

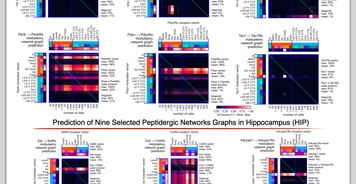
Prediction of Nine Selected Peptidergic Networks Graphs in Anterolateral Motor (ALM) Cortex



Prediction of Nine Selected Peptidergic Networks Graphs in Primary Visual (VISp) Cortex

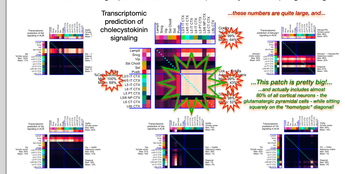


Prediction of Nine Selected Peptidergic Networks Graphs in Hippocampus (HIP)



Interlude

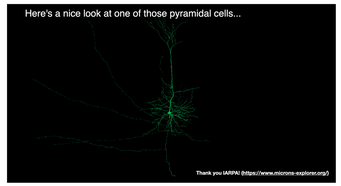
Is there something special about CCK (cholecystokinin) signaling?



So all those many pyramidal cells might be communicating with one another via activity-dependent CCK secretion and CCK-selective GPCR activation?

Are they close enough to "hear" their neighbors "speak"?

What might happen if they are?



Points to ponder:

(1) Although definite information is in somewhat short supply, a conservative guess would suggest that a cognate GPCR might be activated at a distance of 15 um or more from release of a dense-core vesicle.

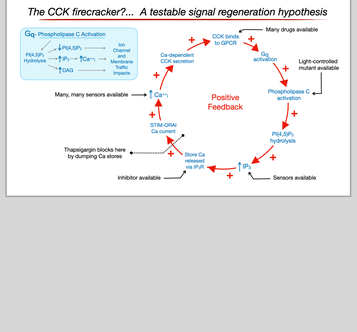
(2) There is evidence that CCK is secreted - perhaps primarily - from dendrites.

(3) It is likely that CCK-selective GPCRs are displayed on dendrites.

(4) Since the known CCK-selective GPCRs signal primarily via phospholipase C activation and intracellular calcium elevation, CCK might trigger a regenerative cycle of CCK-triggered CCK release (see the next and final slide...) that could produce signal amplification, intracellular and cell-to-cell signal propagation and lead to all sorts of interesting non-linearities.

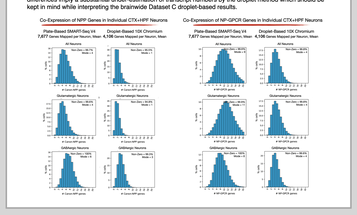
Just a thought, folks!

The CCK firecracker?... A testable signal regeneration hypothesis

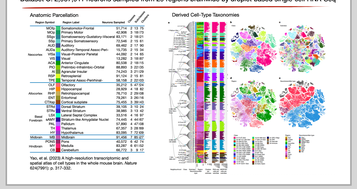


Comparing single-cell mRNA sampling by plate-based and droplet-based RNA-Seq methods

Results presented so far have mined Dataset A (~70,000 neurons), obtained using the very thorough, but very expensive, plate-based SMART-Seq v4 sampling method. Dataset B, where a population of neurons similar to that sampled by the plate method for Dataset A, was sampled by the droplet method provides for a direct methods comparison. In sampling neurons isolated from the same isocortical and hippocampal formation regions, the plate-based method yielded a modal number of 4-5 distinct NPP-encoding transcripts detected per neuron, with only 0-3% zeroes, while the droplet method yielded a modal number of 1-2, with 4.5% zeroes. The frequency histograms below illustrate results of this comparison in more detail. These striking sampling differences imply a substantial under-estimation of transcript numbers by the droplet method which should be kept in mind while interpreting the brainwide Dataset C droplet-based results.

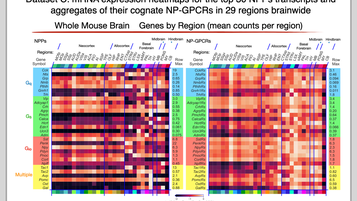


Dataset C: 2,957,911 neurons sampled from 29 regions brainwide by droplet-based single-cell RNA-Seq

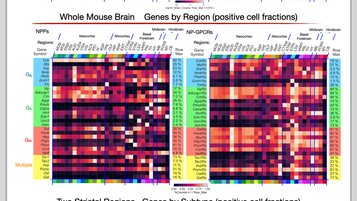


Dataset C: mRNA expression heatmaps for the top 30 NPPs transcripts and aggregates of their cognate NP-GPCRs in 29 regions brainwide

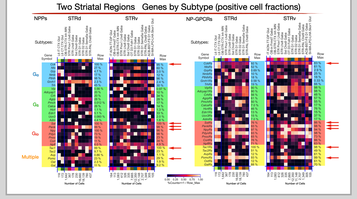
Whole Mouse Brain | Genes by Region (mean counts per region)



Whole Mouse Brain | Genes by Region (positive cell fractions)



Two Striatal Regions | Genes by Subtype (positive cell fractions)

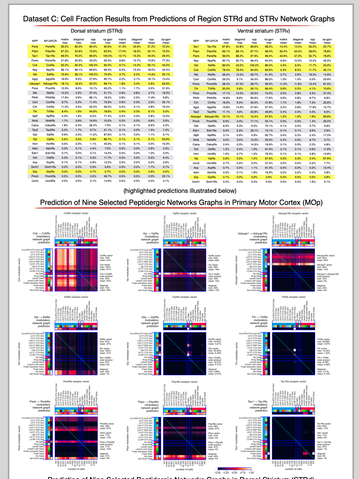


Dataset C: Cell Fraction Results from Predictions of Region STRd and STRv Network Graphs

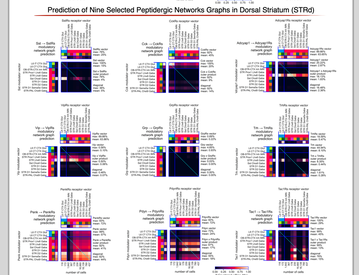
Dorsal striatum (STRd) | Ventral striatum (STRv)

(highlighted predictions illustrated below)

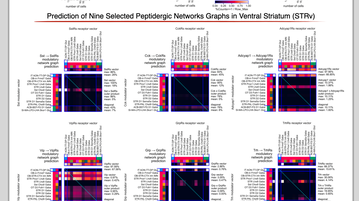
Prediction of Nine Selected Peptidergic Networks Graphs in Primary Motor Cortex (MOp)



Prediction of Nine Selected Peptidergic Networks Graphs in Dorsal Striatum (STRd)



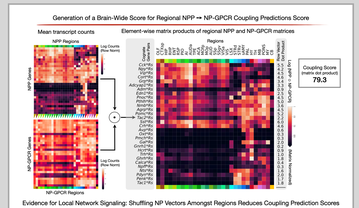
Prediction of Nine Selected Peptidergic Networks Graphs in Ventral Striatum (STRv)



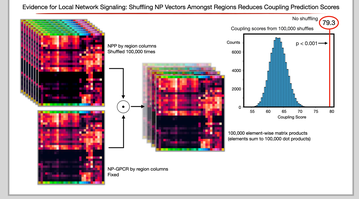
Dataset C: Cell Fraction Results for Predictions of Nine Selected Peptidergic Networks Graphs in Regions Primary Motor Cortex, Dorsal Striatum, Ventral Striatum

(highlighted predictions illustrated below)

Generation of a Brain-Wide Score for Regional NPP → NP-GPCR Coupling Predictions Score



Evidence for Local Network Signaling: Shuffling NP Vectors Amongst Regions Reduces Coupling Prediction Scores



Bibliography

Authors' antecedent neuropeptide publications

1. Smith, S.J. and M. von Zastrow, *A Molecular Landscape of Mouse Hippocampal Neuromodulation.* Frontiers in Neural Circuits, 2022. **16**.
2. Liu, Y.H., et al. *Biologically-plausible backpropagation through arbitrary timespans via local neuromodulators.* in *36th Conference on Neural Information Processing Systems (NeurIPS 2022).* 2022. New Orleans.
3. Smith, S.J., *Transcriptomic evidence for dense peptidergic networks within forebrains of four widely-divergent tetrapods.* Curr Opin Neurobiol, 2021. **71**: p. 100-109.
4. Liu, Y.H., et al., *Cell-type-specific neuromodulation guides synaptic credit assignment in a spiking neural network.* Proc Natl Acad Sci U S A, 2021. **118**(51).
5. Smith, S.J., et al., *New light on cortical neuropeptides and synaptic network plasticity.* Curr Opin Neurobiol, 2020. **63**: p. 176-188.
6. Smith, S.J., et al., *Single-cell transcriptomic evidence for dense intracortical neuropeptide networks.* Elife, 2019. **8**.

Some neuropeptide generalities

7. Sun, W., et al., *Spatial transcriptomics reveal neuron-astrocyte synergy in long-term memory.* Nature, 2024.
8. Yanez-Guerra, L.A., D. Thiel, and G. Jekely, *Premetazoan Origin of Neuropeptide Signaling.* Mol Biol Evol, 2022. **39**(4).
9. Kuo, C.S., et al., *Neuroendocrinology of the lung revealed by single-cell RNA sequencing.* Elife, 2022. **11**.
10. Casello, S.M., et al., *Neuropeptide System Regulation of Prefrontal Cortex Circuitry: Implications for Neuropsychiatric Disorders.* Front Neural Circuits, 2022. **16**: p. 796443.
11. Wan, K.Y. and G. Jekely, *Origins of eukaryotic excitability.* Philos Trans R Soc Lond B Biol Sci, 2021. **376**(1820): p. 20190758.
12. Melzer, S., et al., *Bombesin-like peptide recruits disinhibitory cortical circuits and enhances fear memories.* Cell, 2021. **180**(22): p. 5822-5834 e25.
13. Jekely, G., *The chemical brain hypothesis for the origin of nervous systems.* Philos Trans R Soc Lond B Biol Sci, 2021. **376**(1821): p. 20190761.
14. Arendt, D., *Elementary nervous systems.* Philos Trans R Soc Lond B Biol Sci, 2021. **376**(1821): p. 20200347.
15. Ren, J., et al., *Single-cell transcriptomes and whole-brain projections of serotonin neurons in the mouse dorsal and median raphe nuclei.* Elife, 2019. **8**.
16. Varoqueaux, F., et al., *High Cell Diversity and Complex Peptidergic Signaling Underlie Placozoan Behavior.* Curr Biol, 2018. **28**(21): p. 3495-3501 e2.
17. Jekely, G., et al., *The long and the short of it - a perspective on peptidergic regulation of circuits and behaviour.* J Exp Biol, 2018. **221**(Pt 3).
18. Banghart, M.R., X.J. He, and B.L. Sabatini, *A Caged Enkephalin Optimized for Simultaneously Probing Mu and Delta Opioid Receptors.* ACS Chem Neurosci, 2018. **9**(4): p. 684-690.
19. Jekely, G., *Global view of the evolution and diversity of metazoan neuropeptide signaling.* Proc Natl Acad Sci U S A, 2013. **110**(21): p. 8702-7.
20. Rice, M.E., *Seeing a Tree Within the Forest: Selective Detection and Function of Somatodendritic Cholecystokinin Release From Dopamine Neurons in the Ventral Tegmental Area.* Biological Psychiatry, 2023. **93**(2): p. 110-112.
21. Martinez Damonte, V., et al., *Somatodendritic Release of Cholecystokinin Potentiates GABAergic Synapses Onto Ventral Tegmental Area Dopamine Cells.* Biol Psychiatry, 2023. **93**(2): p. 197-208.
22. Tao, L., et al., *Parallel Processing of Two Mechanosensory Modalities by a Single Neuron in C. elegans.* Dev Cell, 2019. **51**(5): p. 617-631 e3.
23. Pitra, S., et al., *NMDA receptors potentiate activity-dependent dendritic release of neuropeptides from hypothalamic neurons.* J Physiol, 2019. **597**(6): p. 1735-1756.
24. Persson, C.M., et al., *Pool size estimations for dense-core vesicles in mammalian CNS neurons.* EMBO J, 2018. **37**(20).
25. Ludwig, M. and J. Stern, *Multiple signalling modalities mediated by dendritic exocytosis of oxytocin and vasopressin.* Philos Trans R Soc Lond B Biol Sci, 2015. **370**(1672).
26. Crosby, K.M., et al., *Postsynaptic Depolarization Enhances GABA Drive to Dorsomedial Hypothalamic Neurons through Somatodendritic Cholecystokinin Release.* J Neurosci, 2015. **35**(38): p. 13160-70.
27. Gillard, E.R., et al., *Vasopressin autoreceptors and nitric oxide-dependent glutamate release are required for somatodendritic vasopressin release from rat magnocellular neuroendocrine cells responding to osmotic stimuli.* Endocrinology, 2007. **148**(2): p. 479-89.
28. Ludwig, M. and G. Leng, *Dendritic peptide release and peptide-dependent behaviours.* Nat Rev Neurosci, 2006. **7**(2): p. 126-36.

Some Range of neuropeptide action references

29. Chini, B., M. Verhage, and V. Grinevich, *The Action Radius of Oxytocin Release in the Mammalian CNS: From Single Vesicles to Behavior.* Trends Pharmacol Sci, 2017. **38**(11): p. 982-991.
30. Banghart, M.R., et al., *Caged naloxone reveals opioid signaling deactivation kinetics.* Mol Pharmacol, 2013. **84**(5): p. 687-95.
31. Banghart, M.R. and B.L. Sabatini, *Photoactivatable neuropeptides for spatiotemporally precise delivery of opioids in neural tissue.* Neuron, 2012. **73**(2): p. 249-59.
32. Leng, G. and M. Ludwig, *Neurotransmitters and peptides: whispered secrets and public announcements.* J Physiol, 2008. **586**(23): p. 5625-32.

Some references on cholecystokinin impacts upon memory and cognition

33. Barde, S., et al., *Substance P, NPY, CCK and their receptors in five brain regions in major depressive disorder with transcriptomic analysis of locus coeruleus neurons.* European Neuropsychopharmacology, 2024. **78**: p. 54-63.
34. Asim, M., et al., *Cholecystokinin neurotransmission in the central nervous system: Insights into its role in health and disease.* BioFactors, 2024.
35. Zhang, Z., et al., *Cholecystokinin Signaling can Rescue Cognition and Synaptic Plasticity in the APP/PS1 Mouse Model of Alzheimer's Disease.* Mol Neurobiol, 2023.
36. Zhang, X., et al., *Cholecystokinin B receptor antagonists for the treatment of depression via blocking long-term potentiation in the basolateral amygdala.* Mol Psychiatry, 2023. **28**(8): p. 3459-3474.
37. Su, J., et al., *Entorhinohippocampal cholecystokinin modulates spatial learning by facilitating neuroplasticity of hippocampal CA3-CA1 synapses.* Cell Rep, 2023. **42**(12): p. 113467.
38. Lau, S.H., et al., *The potential role of the cholecystokinin system in declarative memory.* Neurochem Int, 2023. **162**: p. 105440.
39. Hawkins, R.D., et al., *Distribution, cellular localization, and colocalization of several peptide neurotransmitters in the central nervous system of Aplysia.* Learn Mem, 2023. **30**(5-6): p. 116-123.
40. Feldmeyer, D., *Structure and function of neocortical layer 6b.* Front Cell Neurosci, 2023. **17**: p. 1257803.
41. Nassel, D.R. and S.F. Wu, *Cholecystokinin/sulfakinin peptide signaling: conserved roles at the intersection between feeding, mating and aggression.* Cell Mol Life Sci, 2022. **79**(3): p. 188.
42. Ma, Y. and W.J. Giardino, *Neural circuit mechanisms of the cholecystokinin (CCK) neuropeptide system in addiction.* Addict Neurosci, 2022. **3**.
43. Tinoco, A.B., et al., *Ancient role of sulfakinin/cholecystokinin-type signalling in inhibitory regulation of feeding processes revealed in an echinoderm.* Elife, 2021. **10**.
44. Rehfeld, J.F., *Cholecystokinin and Panic Disorder: Reflections on the History and Some Unsolved Questions.* Molecules, 2021. **26**(18).
45. Rehfeld, J.F., *Cholecystokinin and the hormone concept.* Endocr Connect, 2021. **10**(3): p. R139-R150.
46. Chen, X., et al., *Cholecystokinin release triggered by NMDA receptors produces LTP and sound-sound associative memory.* Proc Natl Acad Sci U S A, 2019. **116**(13): p. 6397-6406.
47. Rehfeld, J.F., *Cholecystokinin-From Local Gut Hormone to Ubiquitous Messenger.* Front Endocrinol (Lausanne), 2017. **8**: p. 47.
48. Rehfeld, J.F., *Cholecystokinin expression in tumors: biogenetic and diagnostic implications.* Future Oncol, 2016. **12**(18): p. 2135-47.
49. Schoofs, L., et al., *A Conserved Dopamine-Cholecystokinin Signaling Pathway Shapes Context-Dependent Caenorhabditis elegans Behavior.* PLoS Genetics, 2014. **10**(8).
50. Oikonomou, E., M. Buchfelder, and E.F. Adams, *Cholecystokinin (CCK) and CCK receptor expression by human gliomas: Evidence for an autocrine/paracrine stimulatory loop.* Neuropeptides, 2008. **42**(3): p. 255-65.
51. Galloplin, T., et al., *Cortical sources of CRF, NKB, and CCK and their effects on pyramidal cells in the neocortex.* Cereb Cortex, 2006. **16**(10): p. 1440-52.
52. Dodd, J. and J.S. Kelly, *The actions of cholecystokinin and related peptides on pyramidal neurones of the mammalian hippocampus.* Brain Research, 1981. **205**(2): p. 337-350.
53. Beinfeld, M.C., D.K. Meyer, and M.J. Brownstein, *Cholecystokinin in the central nervous system.* Peptides, 1981. **2**: p. 77-79.

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