An Intriguing Puzzle of Cortical Cholecystokinin (CCK) Gene Expression

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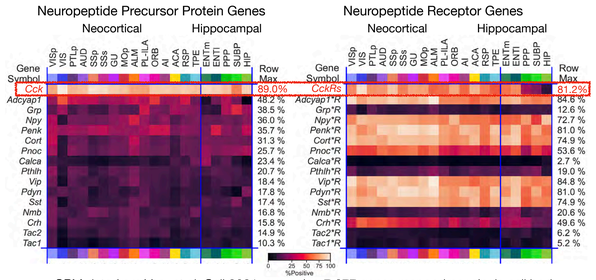
Transcriptomic Survey of Neuropeptide Precursor Protein (NPP) and Cognate Selective G Protein-Coupled Receptor (NP-GPCR) Gene Expression in Single Neuronal Somata Isolated from Mouse Cortex

mRNA from 16 NPP and 21 cognate NP-GPCR genes listed are abundant, *Cck* transcripts are the most abundant by far - top 0.1%, genome wide!

NPP Gene	Neuropeptide Product Name	Cognate NP-GPCR Genes		Aggregate Label	Primary Transduction Pathway
Cck	cholecystokinin	*Cckbr*		*CckRs*	Gq/11 family: Phospholipase C Activation ↓PIP2, ↑ IP3, ↑DAG,↑Ca2+
Grp	gastrin releasing peptide	*Grpr*		*GrpRs*	
Pthlh	parathyroid hormone-like peptide	*Pth1r*		*PthlhRs*	
Nmb	neuromedin B	*Nmbr*		*NmbRs*	
Adcyap1	adenylate cyclase activating peptide	*Adcyap1r1,Vipr1*		*Adcyap1Rs*	Gs family: Adenylyl Cyclase Activation ↑ Cyclic AMP
Vip	vasoactive intestinal polypeptide	*Adcyap1r1,Vipr1,Vipr2*		*VipRs*	
Crh	corticotropin releasing hormone	*Crhr1,Crhr2*		*CrhRs*	
Calca	calcitonin/calcitonin-related peptide	*Calcrl*		*CalcaRs*	
Penk	preproenkephalin	*Oprd1,Oprk1,Oprl1,Oprm1*		*PenkRs*	Gio family: Adenylyl Cyclase Inhibition ↓ Cyclic AMP
Npy	neuropeptide Y	*Npy1r,Npy2r,Npy5r*		*NpyRs*	
Cort	cortistatin	*Sstr1,Sstr2,Sstr3,Sstr4*		*CortRs*	
Pnoc	prepronociceptin	*Oprl1*		*PnocRs*	
Sst	somatostatin	*Sstr1,Sstr2,Sstr3,Sstr4*		*SstRs*	
Pdyn	prodynorphin	*Oprd1,Oprk1,Oprl1,Oprm1*		*PdynRs*	
Tac2	tachykinin 2	*Tacr2*	*Tacr3*	*Tac2Rs*	Multiple Pathways
Tac1	tachykinin 1	*Tacr2*	*Tacr1*	*Tac1Rs*	

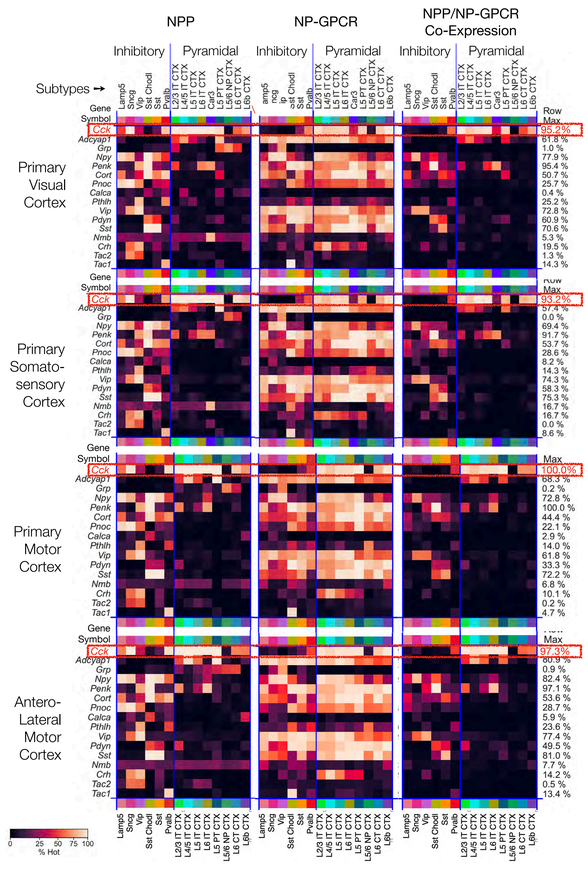
Potential targets for GPCR regulation include nearly every ion channel and receptor!

Deep mRNA Sequencing of 71,518 Somata from 20 Cortical Regions



CPM data from Yao, et al. Cell 2021, averaging 7,677 genes mapped per single cell body

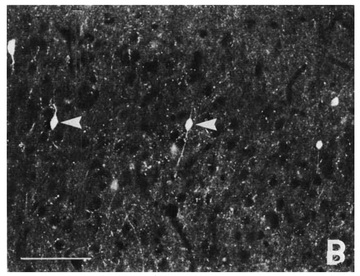
Regional Conservation of Expression Patterns Suggests Functional Importance,
Patterns of Single-Cell NPP/NP-GPCR Co-Expression Included!



What's So Puzzling?

1. *mRNA transcripts* encoding CCK peptides are extremely abundant in almost all cortical pyramidal cells, but most attempts at CCK *peptide* detection in these cells by immunohistochemistry have reported negative results.
2. This is all the more puzzling because *both* transcripts and peptides are readily detected in a small subset of inhibitory neurons, long classified accordingly as the "CCK+" subset.
3. Actions of CCK peptides released by CCK+ inhibitory neurons are well documented, but not so for CCK peptide release from pyramidal cells.
4. The widespread co-expression in single pyramidal cells of genes encoding both CCK peptides and receptors suggests paracrine/autocrine signaling, but we have found no reports of evidence for such.

CCK peptide immunofluorescence in rat cortex



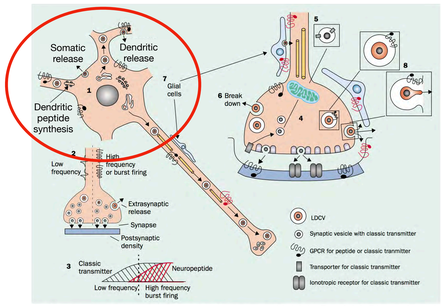
Morino, Herrera-Marschitz, Castel, Ungerstedt, Varro, Dockray & Hokfelt (1994)

Most of the dark voids seen here are unlabeled pyramidal cell bodies. The brightly labeled cells (arrows) are CCK+ inhibitory neurons.

Solutions?

1. Pyramidal cell CCK transcripts are actually useless vestiges of evolutionary history - never actually transcribed. *The very wide conservation and very high abundance of these metabolically costly molecules makes this seem unlikely.*
2. Unlike the CCK+ interneurons, pyramidal cells may simply secrete peptide fast enough to keep accumulation below readily detectible levels.
3. Translation of CCK transcripts may occur only upon demand, perhaps linked to depletion of a small, readily releasable pool of CCK vesicles.
4. Efficient intracellular transport of CCK-containing vesicles may disperse them rapidly into the pyramidal cell's large dendrites and/or long axons, making clear detection in somata problematic.
5. Differences between pyramidal and inhibitory neurons in vesicular CCK processing or storage may result in differential exposure of epitopes to antibody binding.
6. The much brighter CCK staining of CCK+ inhibitory neurons may have hijacked the pyramidal cells' share of experimental attention on CCK secretion biophysics.
7. The co-expression of transcripts encoding CCK and its receptors in single cells may have escaped experimental inquiry prior to the relatively recent advent of hints from single-cell mRNA-Seq data, such as those portrayed in the "NPP/NP-GPCR Co-Expression" column at left.

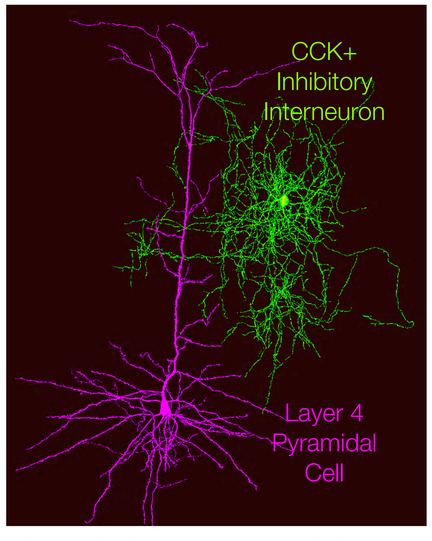
Some Canonical Neuropeptide Generalities to Consider



Hökfelt, Bartfai & Bloom (2003)

1. Neuropeptides are packaged into large, dense-cored vesicles (DCVs) and transported throughout neurons.
2. Neuropeptide secretion occurs via extra-synaptic DCV exocytosis at sites on somata, dendrites and axons that show no "active zone" specializations.
3. DCV exocytosis is triggered by intracellular [Ca2+] elevation resulting from surface Ca2+ entry and/or Ca2+ release from endoplasmic reticulum stores.
4. Neuropeptide receptors are displayed on dendritic, somatic and axonal surface membranes.

Reconstructions from MICrONS Project Volume Electron Microscopy Data



The elaborate arbors of these two cortical neuron types differ in that the pyramidal cell's arbor is almost entirely dendritic, while that of the CCK+ interneuron is mainly axonal. The pyramidal cell dendrite is much larger in diameter than the interneuron's axon. This difference could contribute to more rapid transport of DCVs from the pyramidal cell's soma.

What Makes this Puzzle Intriguing?

The transcriptomic results outlined here suggest that CCK might serve to couple activity extra-synaptically amongst the pyramidal packed densely into cortex, depending upon just how our CCK puzzle is resolved. Here we'll speculate based on one possible scenario that we see as likely and potentially important enough to merit some physiology:

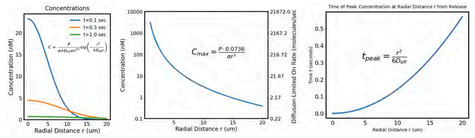
(1) We'll assume the puzzle outcome where CCK peptide is secreted by most or all pyramidal cells (as hinted by the transcriptomic data) in the canonical Ca-dependent fashion and that dendrites are a major site of secretion;

(2) It is already well established that cortical pyramidal cells are depolarized by CCK peptide via the Gq/11-family CCKBR receptor. We'll assume that this receptor is displayed on dendrites.

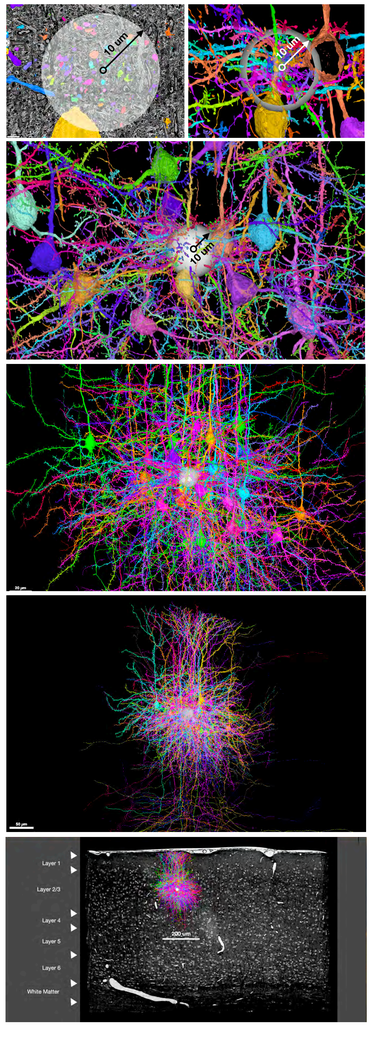
This scenario implies the prospect of direct bi-directional signaling between (almost) every pair of pyramidal cells within some constrained diffusion distance of each other. It also implies the prospect of autocrine (self-to-self) signaling. Two further question arise...

How many nearby neurons might be impacted directly by release of CCK from one DCV?

An estimate of 5000 CCK molecules per DCV, concentration profiles from Nicholson's (2000) model for brain interstitial diffusion, and a receptor capture radius of 3 nm yields an rough action radius estimate of ~10um.

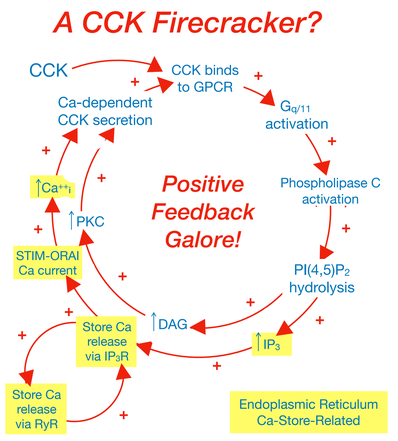


Projecting that 10um radius sphere into a MICrONS data volume as depicted below suggests that dendritic release of one DCV's contents could impact dendrites of ~50 neurons .



What might we expect autocrine CCK signaling to look like?

Well-documented abilities of Gq/11-family GPCRs to raise [Ca2+] and of elevated [Ca2+] to trigger CCK secretion raises the very intriguing prospect of regenerative signaling.



A diagram representing signal transduction steps in a positive feedback configuration that may come into play when a pyramidal neuron is exposed to an active CCK peptide. This diagram neglects other, restorative pathways that must also come into play to maintain the system within a critical operating zone.

Positive feedback loops bring distinctive abilities to regenerate signals in space and time, to create waves and oscillations, and to create sharp non-linearities. Such abilities adapt networks to complex computational roles.

Acknowledgements and Resources

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Stuart Thompson
Mark von Zastrow

A GitHub Repo
contains this a copy of this poster, a supporting bibliography, pointers to all data resource files, and all data graphics scripts

https://github.com/profsjsmith/Neuropeptides

Data Resources
Single-Cell RNA-Seq and Taxonomy: The Allen Institute for Brain Science
Volume Electron Microscopy: The MICrONs Consortium (Chiefs: Tolias, Reid, Seung)