Introduction to Neurotranscriptomics

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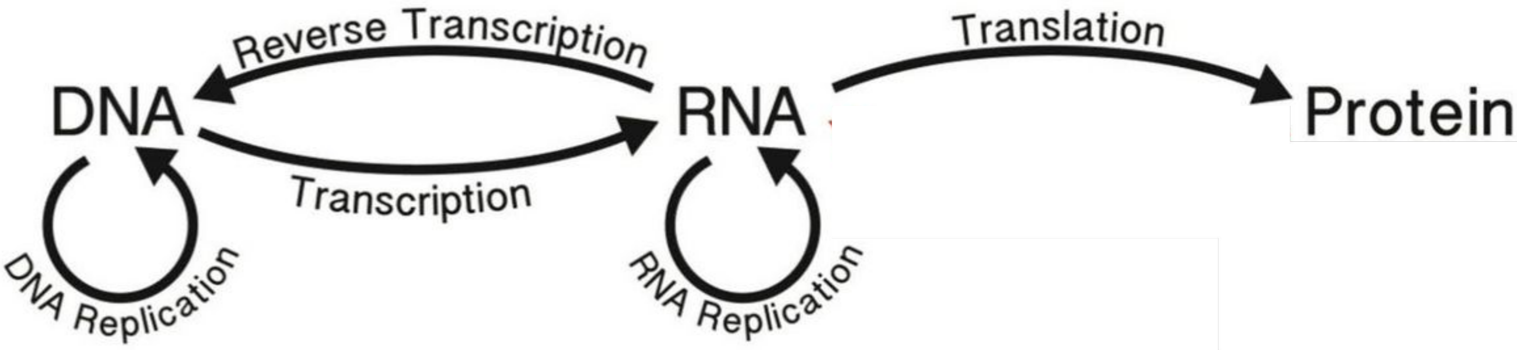
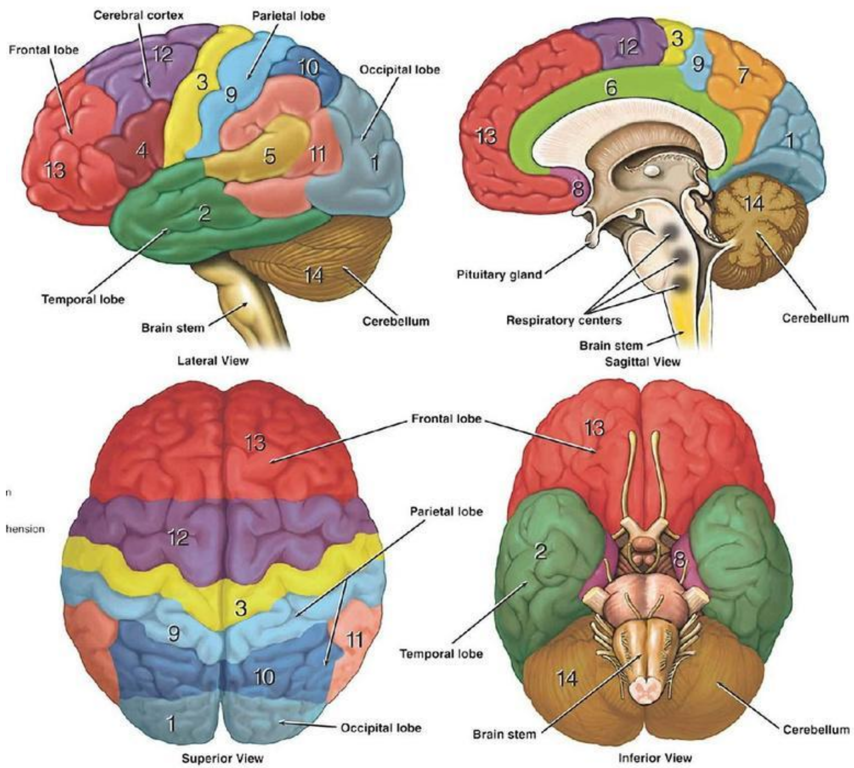
@babasaraky

Central Dogma of Biology

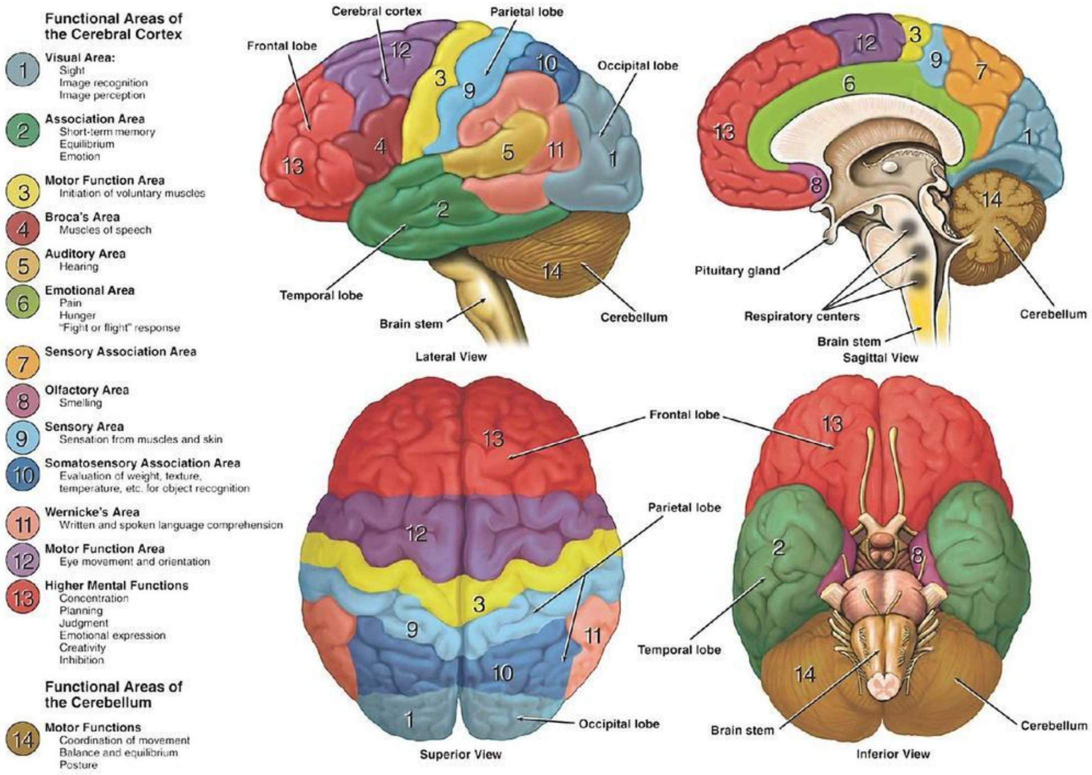


Why should you study neurotranscriptomics?

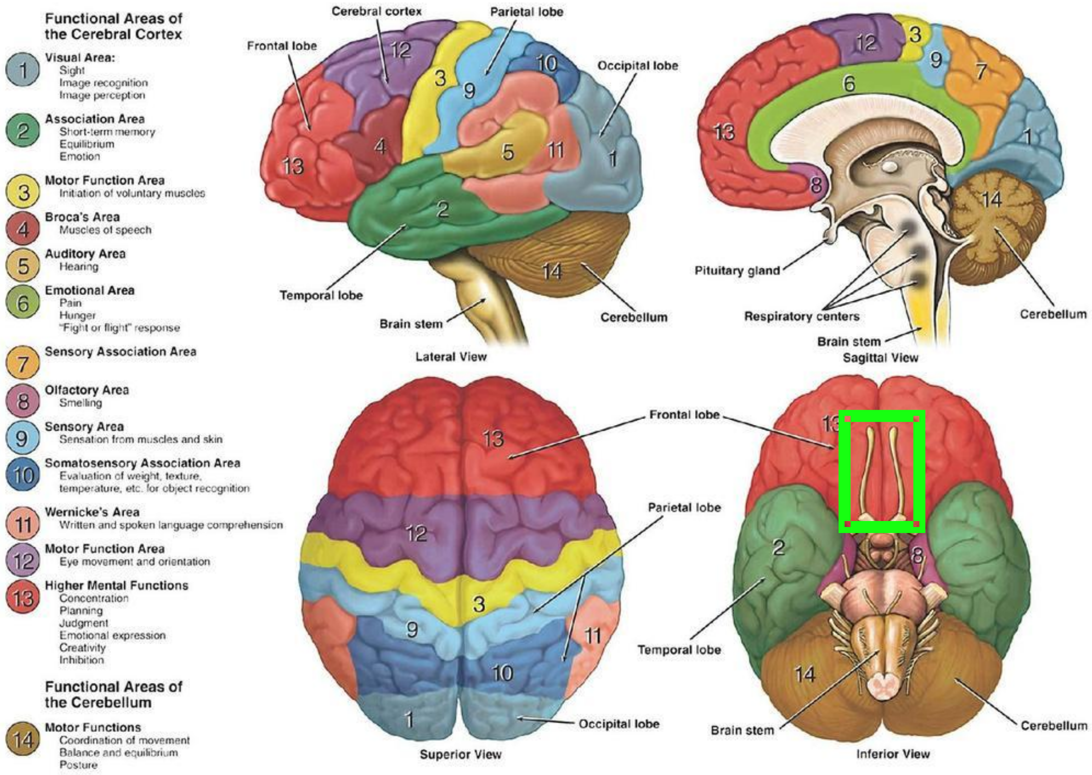
Complexities of the brain

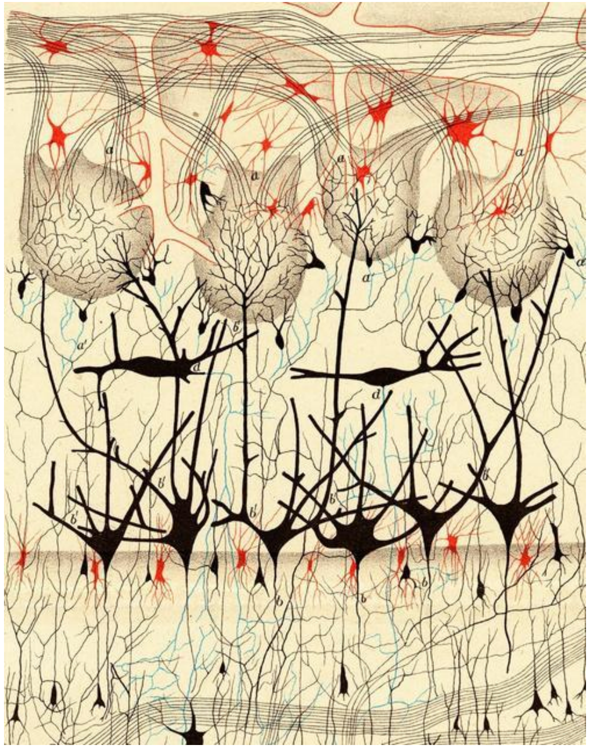


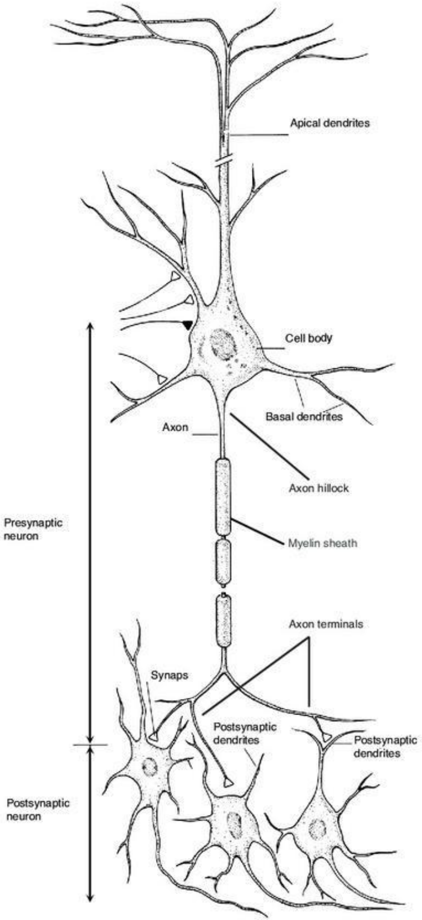
Complexities of the brain



Complexities of the brain

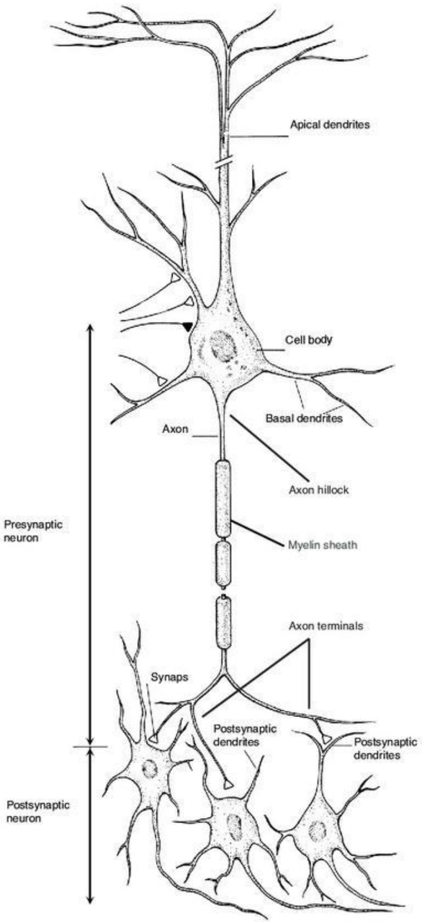


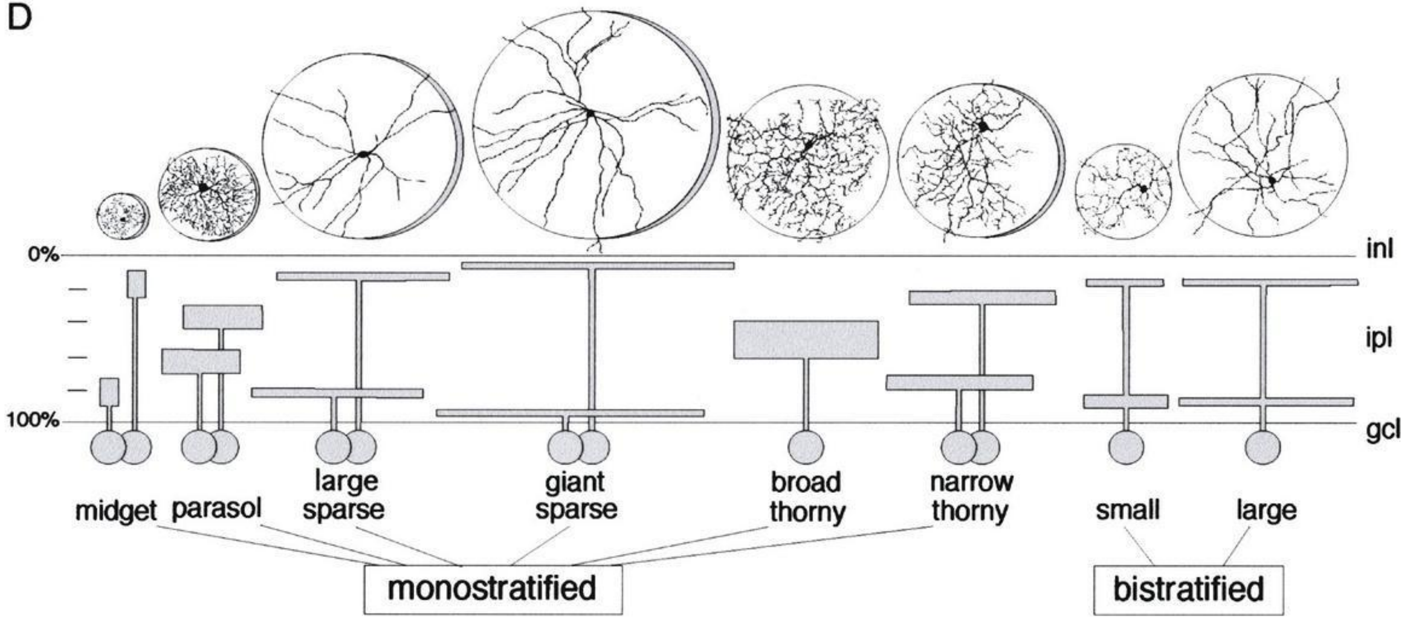




Dacey et al. Neuron. 2003

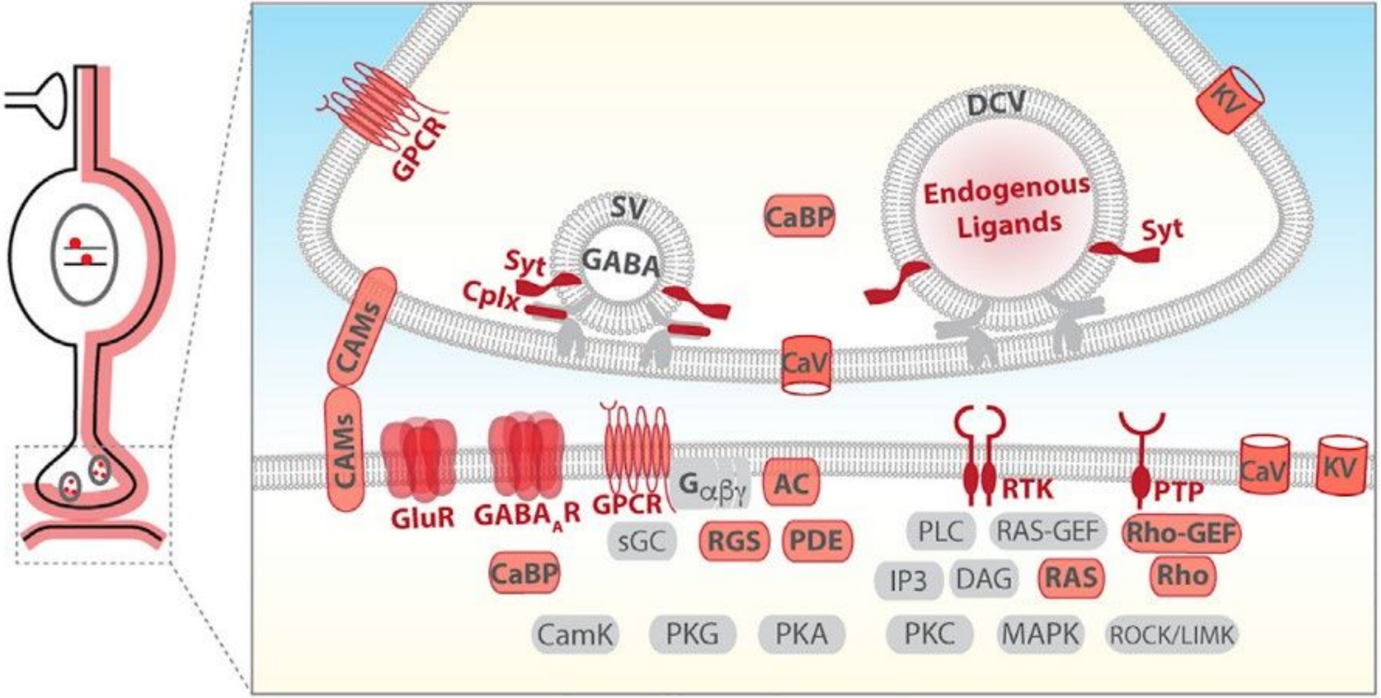
Structure determines function





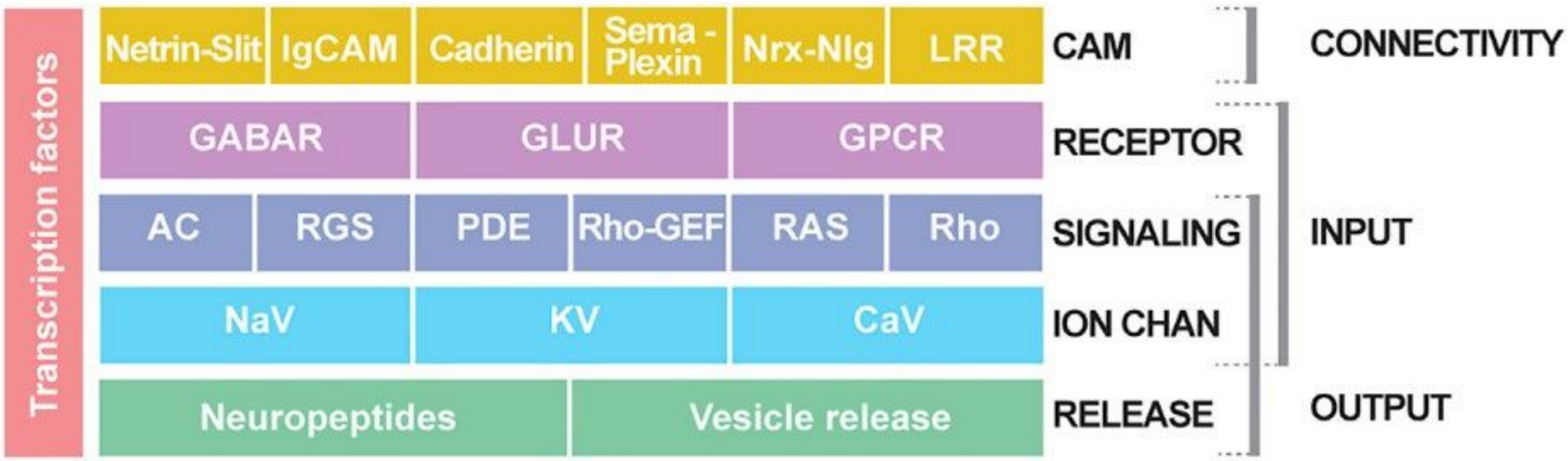
Dacey et al. Neuron. 2003

Neurons are also classified by receptors and transmitters



Paul et al. Cell. 2017

Gene expression underlies the diversity in neuronal structure, plasticity, and thus function



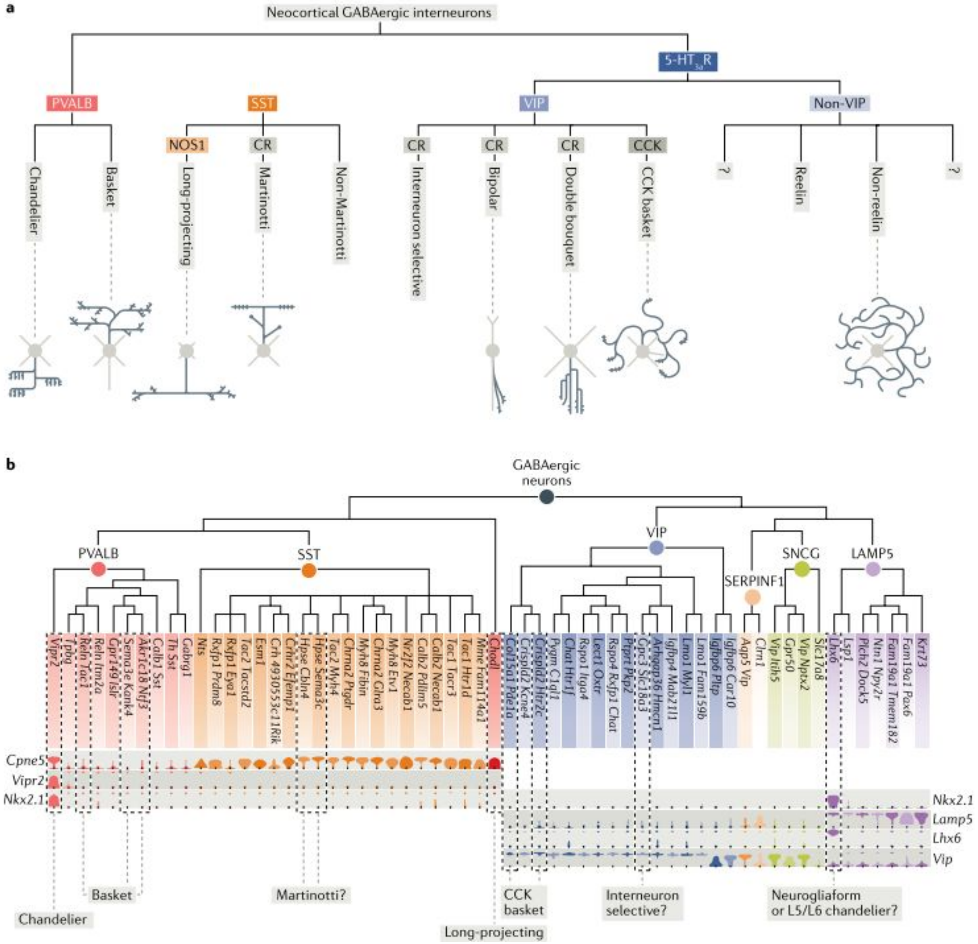
Paul et al. Cell. 2017

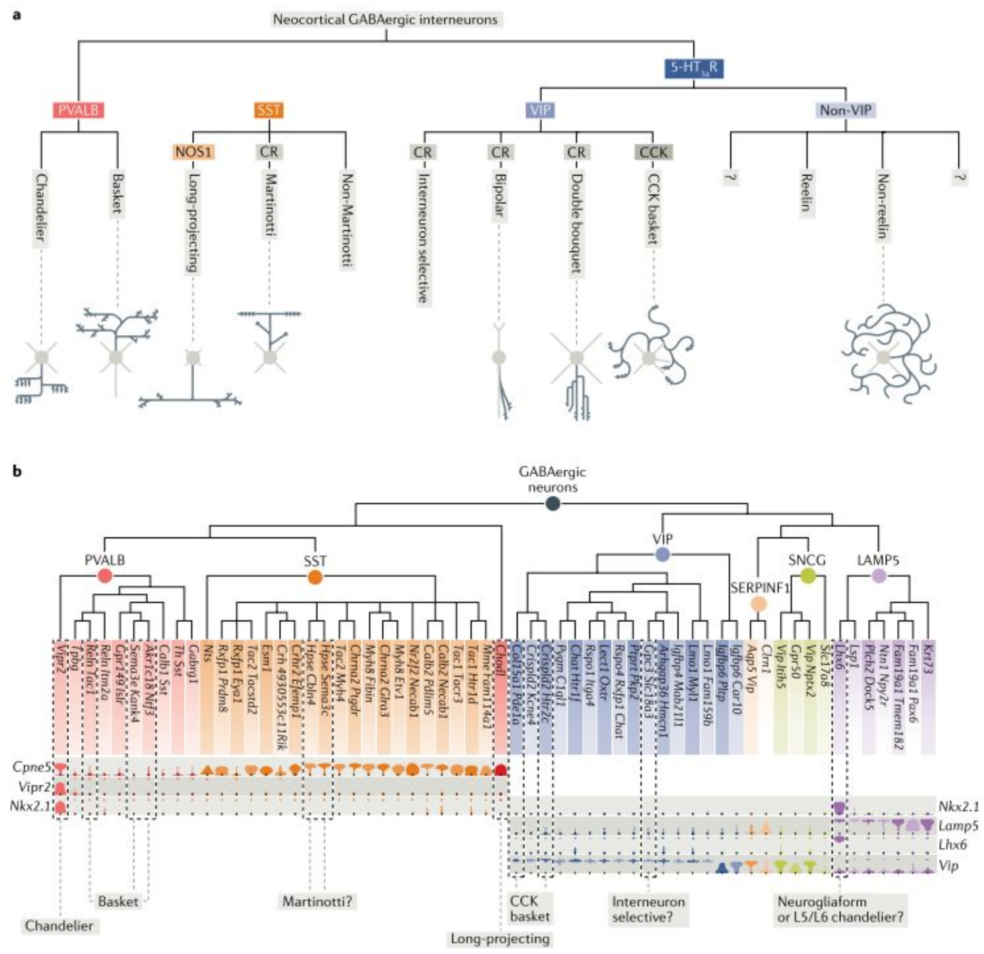
Why should you study neurotranscriptomics?

Why should you study neurotranscriptomics?

- Cell type classification: Identify biomarkers of cell types
- Development: Mature neurons are post mitotic, so they serve as a great study system
- Behavior, Neuronal Plasticity/response: Inform molecular studies, identify immediate early response genes
- Disease: identify genomic markers of brain disorders, find potential therapeutic strategies for neurological diseases

Transcriptomics can reveal subtypes of well characterized cells



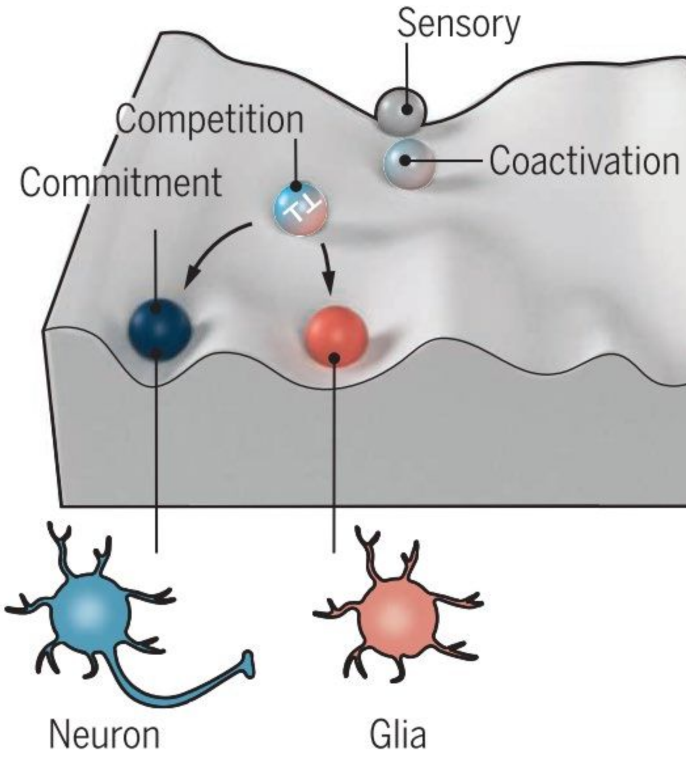


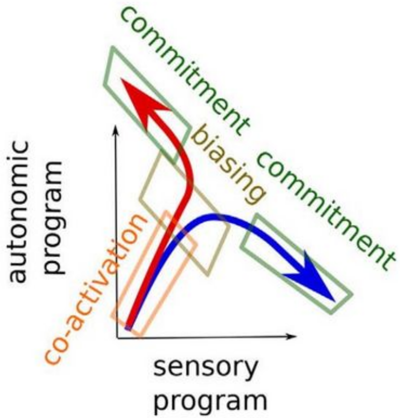
Transcriptomics can reveal subtypes of well characterized cells

Neuronal biomarker genes tend to be

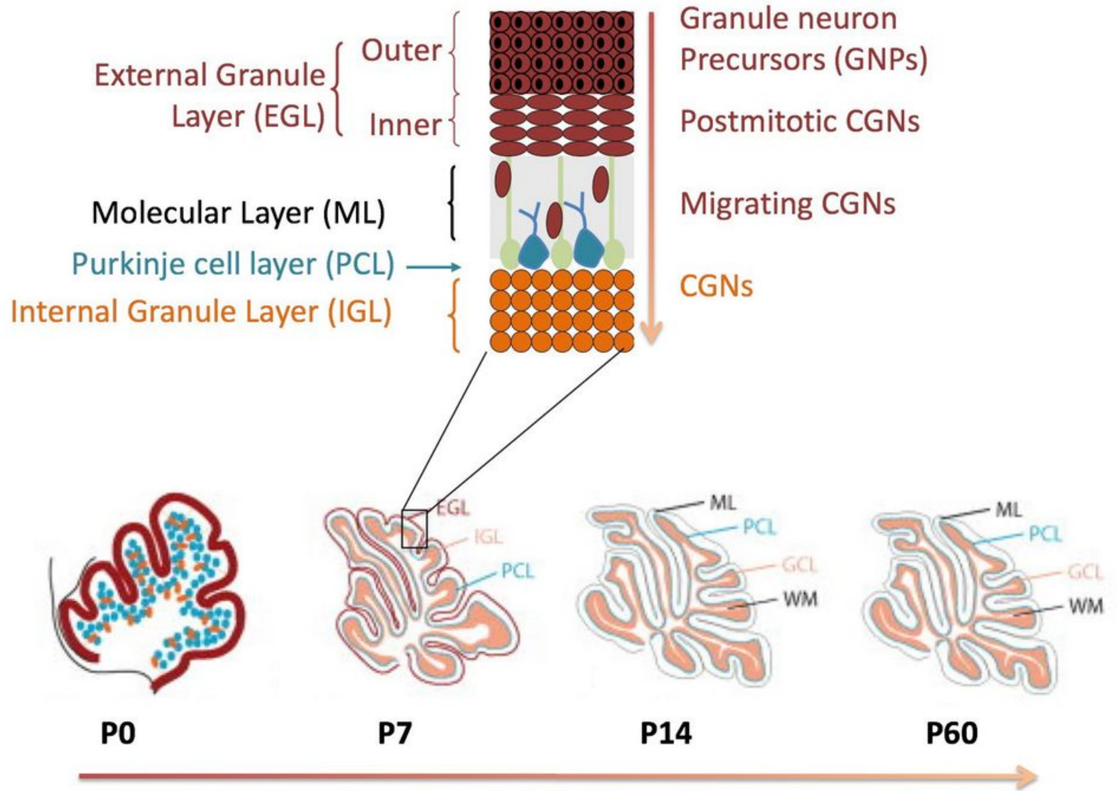
1. Cell adhesion molecules
2. Receptors for neurotransmitters
3. Vesicle release Ion
4. channel genes
5. Signaling
6. Transcription factors

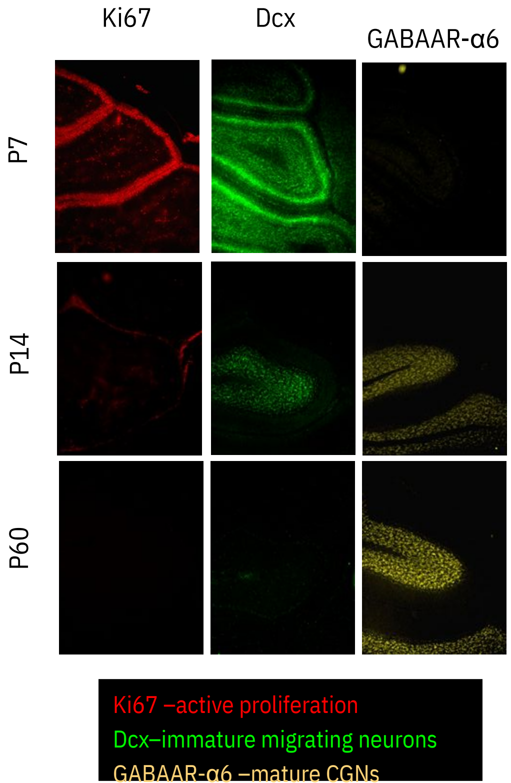
Gene expression determines cell fate





Study system for development





Frank et al. 2015. Nat Neurosci

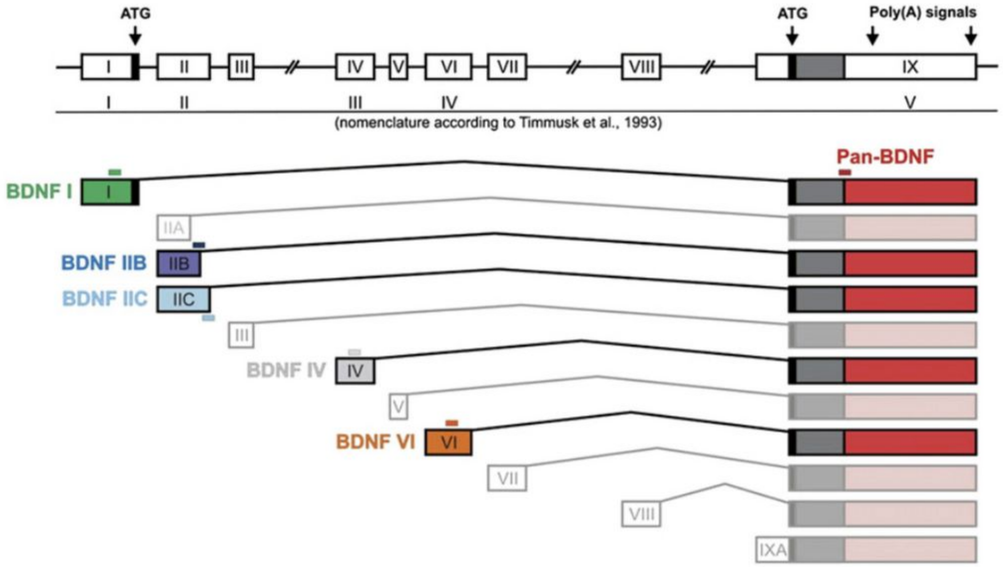
Response genes can explain behavior and plasticity

Table 2
A limited list of IEG mutant mice that exhibit abnormality in neuronal plasticity and cognitive functions.

Gene	Type of knockout	Phenotypes (impairments otherwise mentioned)	Reference
*Arc/arg*3.1	Conventional full knock-out (KO)	Hippocampal late-LTP/LTD; spatial and fear memory; taste aversion	Plath et al. (2006)
	GFP knock-in (KI) full KO	Orientation selectivity in visual cortex	Wang et al. (2006)
	GFP-KI full KO	Ocular-dominance plasticity in visual cortex	McCurry et al. (2010)
	Conventional full KO	Experience-dependent synaptic scaling in visual cortex	Gao et al. (2010b)
bdnf	Promoter IV-specific mutation KI	Inhibitory circuit development in neocortex	Hong et al. (2008)
	GFP-STOP KI in Exon IV	Aberrant spike-timing-dependent plasticity in prefrontal cortex	Sakata et al. (2009)
c-*fos*	CNS-specific KO	Hippocampal LTP; spatial and contextual fear memory	Fleischmann et al. (2003)
	D1R-expressing cell-specific KO	Cocaine-induced dendritic morphological and behavioral changes	Zhang et al. (2006)
*fos*B	Conventional full KO	Enhanced cocaine sensitivity	Hiroi et al. (1997)
*homer1*a/*Vesl*1s	IEG-subtype specific KO	Long-term fear memory formation; remote memory transition	Inoue et al. (2009)
Tissue plasminogen activator (t-PA)	Conventional full KO	Hippocampal late-LTP with GABA-transmission inhibition	Frey et al. (1996)
	Conventional full KO	Striatal LTD; hippocampal late-LTP; active avoidance task	Huang et al. (1996)
*zif*268 (*egr*1, *krox*24, *NGFI-A*)	LacZ-KI full KO	*In vivo* dentate gyrus late-LTP; spatial memory; taste aversion	Jones et al. (2001)
	LacZ-KI full KO	Reconsolidation of object recognition memory	Bozon et al. (2003)

**Plasticity is the ability for changes to brain function and structure to change given a stimulus

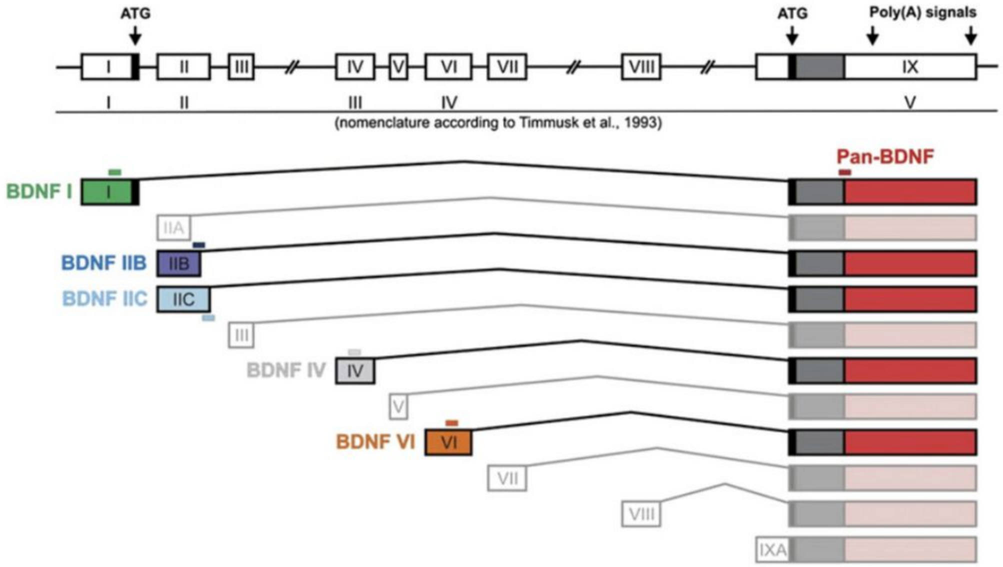
BDNF –a regulator of plasticity in the brain



Chiaruttiniet al. Mol Cell Neurosci. 2008

Pearson-Fuhrhopet al. Topics in Stoke Rehab. 2009

BDNF –a regulator of plasticity in the brain



- Immediate early gene
- Depolarize post synaptic neurons to elicit long-and short-term effects on
  - Ion channels
  - NMDA receptors
  - Neurotransmitters
  - Neuropeptide synthesis
  - Excitability
- Brain region specific effects

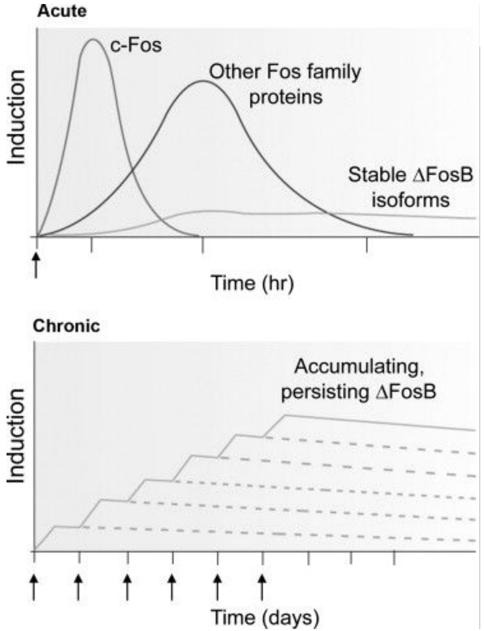
Chiaruttiniet al. Mol Cell Neurosci. 2008

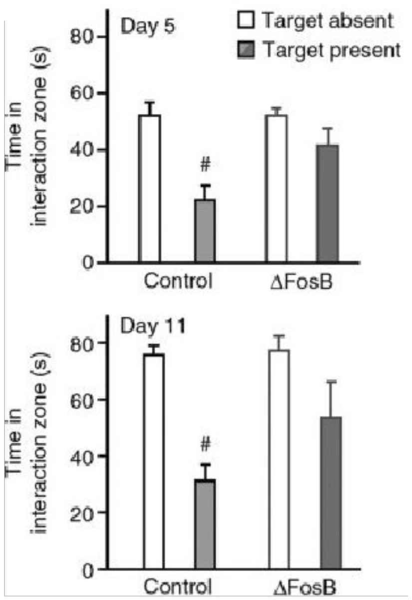
Pearson-Fuhrhopet al. Topics in Stoke Rehab. 2009

Gene mutations tend to underly the mechanisms of brain disorders

- Immediate early genes often responsible for learning, memory and development
- Memory deficits Schizophrenia, autism, bipolar disorder, obsessive-compulsive disorder, panic disorder, major depression, anxiety, PTSD

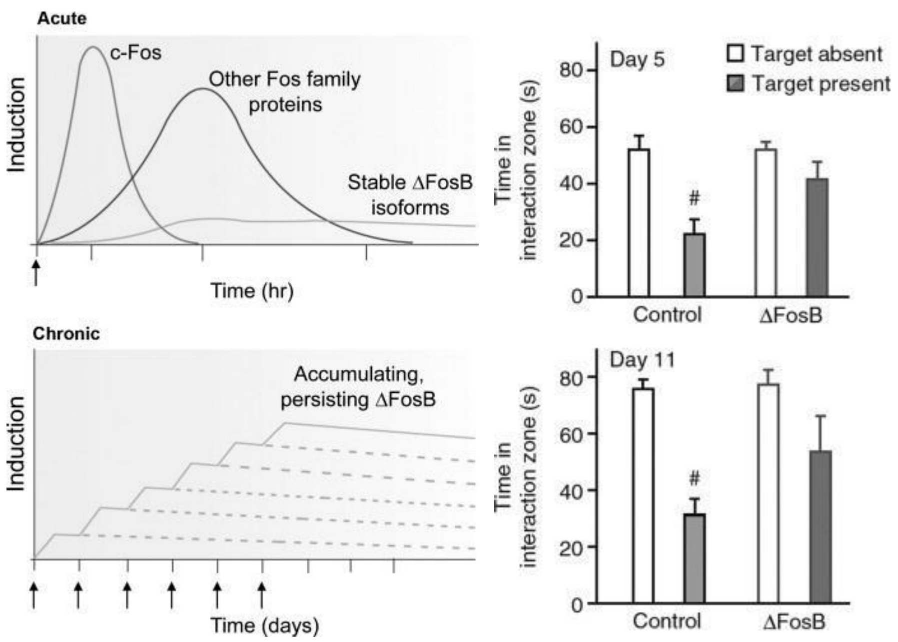
Examples IEGs and brain disorders

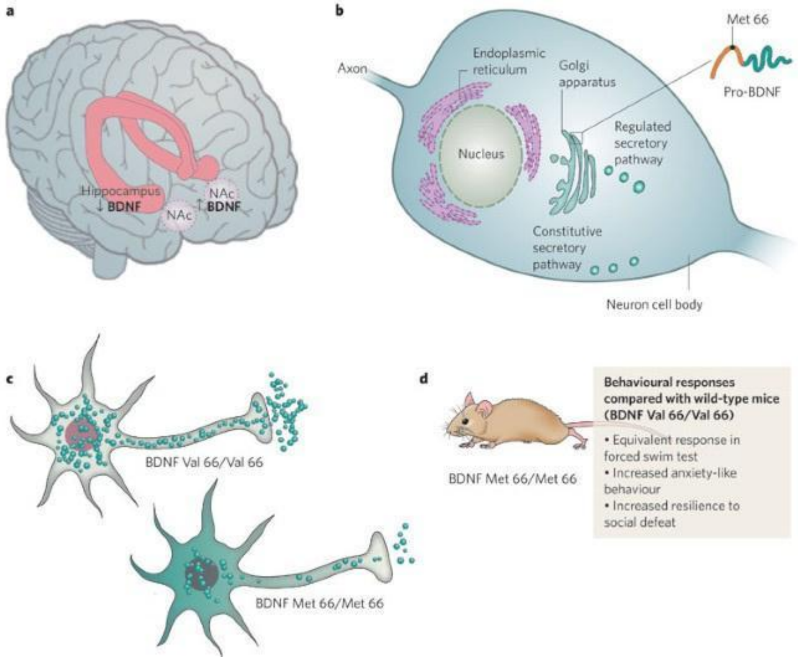




Nestler. Eur J Pharmacol. 2015
Krishnan & Nestler. Nature. 2008

Examples IEGs and brain disorders





Nestler. Eur J Pharmacol. 2015
Krishnan & Nestler. Nature. 2008

Brain Transcriptome Databases

Table 1. Highlighted brain transcriptome databases [a]

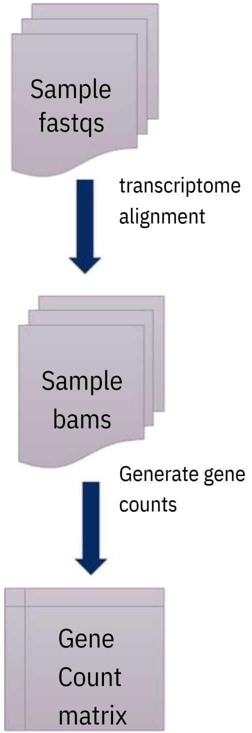
Analysis	Web Interface	Reference	Species	Age	Sample	Method	Isoform	Accession
Spatiotemporal	http://hbatlas.org	Johnson et al., 2009	Human	Lifespan	Multi, macrodissection	Microarray	–	GSE13344
		Kang et al., 2011						GSE25219
	http://hbatlas.org/mouseNCXtranscriptome	Fertuzinhos et al., 2014	Mouse	Postnatal	Ctx layer, microdissection	RNA-seq	–	SRP031888
	http://www.blueprintnhpatlas.org	Bakken et al., 2016	Macaque	Lifespan	Multi, macrodissection, and LMD	Microarray	–	At database
Spatial	http://human.brain-map.org	Hawrylycz et al., 2012	Human	Adult	Multi, macrodissection, and LMD	Microarray	–	At database
	http://genserv.anat.ox.ac.uk/layers	Belgard et al., 2011	Mouse	Adult	Ctx layer, microdissection	RNA-seq	+	GSE27243
	http://rakiclab.med.yale.edu/transcriptome	Ayoub et al., 2011	Mouse	Embryonic	Ctx embryonic layer, LMD	RNA-seq	+	GSE30765
	http://www.brainspan.org/lcm	Miller et al., 2014	Human	Midfetal	Multi, LMD	Microarray	–	At database
	https://www.gtexportal.org	GTEx Consortium, 2015	Human	Adult	Many tissues and cell lines	RNA-seq	+	At database
Temporal	http://braincloud.jhmi.edu	Colantuoni et al., 2011	Human	Lifespan	Prefrontal Ctx, macrodissection	Microarray	–	GSE30272
Cell type- specific	http://brainrnaseq.org	Zhang et al., 2014	Mouse	Adult	Ctx, genetic labeling, immunopanning	RNA-seq	+	GSE52564
		Zhang et al., 2016	Human	Fetal/adult	Ctx, Hp, immunopanning	RNA-seq	–	GSE73721
	http://genetics.wustl.edu/jdlab/csea-tool-2	Doyle et al., 2008	Mouse	Adult	Multi, genetic labeling, ribosome affinity purification	Microarray	–	GSE13379
		Xu et al., 2014						
	http://decon.fas.harvard.edu	Molyneaux et al., 2015	Mouse	Embryonic	Ctx, transcription factor FACS	RNA-seq	+	GSE63482
	http://hipposeq.janelia.org	Cembrowski et al., 2016	Mouse	Adult	Hp, genetic labeling, manual selection	RNA-seq	–	GSE74985
	http://neuroseq.janelia.org	Sugino et al., 2017	Mouse	Adult	Multi, genetic labeling, manual selection	RNA-seq	+	GSE79238
Single-cell	http://linnarssonlab.org/cortex	Zeisel et al., 2015	Mouse	Adult	Ctx, Fluidigm	RNA-seq	–	GSE60361
	http://genebrowser.unige.ch/science2016	Telley et al., 2016	Mouse	Embryonic	Ctx, ventricle dye, FACS, Fluidigm	RNA-seq	–	NA
	https://portals.broadinstitute.org/single_cell	Shekhar et al., 2016	Mouse	Adult	Retina, genetic labeling, Drop-seq	RNA-seq	–	GSE81905
	https://portals.broadinstitute.org/single_cell	Habib et al., 2016	Mouse	Adult	Hp, single nuclei, FACS, sNuc-seq	RNA-seq	–	GSE84371
	https://bit.ly/cortexSingleCell	Nowakowski et al., 2017	Human	Fetal	Ctx, ganglionic eminence, Fluidigm	RNA-seq	–	PRJNA295469
	http://gbmseq.org	Darmanis et al., 2017	Human	Adult	Ctx tumor, immunopanning, FACS	RNA-seq	–	GSE84465
Integrative	https://www.encodeproject.org	ENCODE Project Consortium, 2012	Many	Many	Many tissues and cell lines	Multiomics	+	Many
	http://celltypes.brain-map.org	Tasic et al., 2016	Mouse	Adult	Ctx, genetic labeling, FACS	RNA-seq	–	GSE71585

[a] Ctx, Cortex; Hp, hippocampus; multi, multiple brain regions. Isoform column indicates availability of isoform information via web interface.

Keil et al. J Neurosci. 2018

Transcriptomics pipeline/workflow

Preprocessing



Transcriptomics pipeline/workflow

Analyses

Preprocessing

Sample fastqs

↓ transcriptome alignment

Sample bams

↓ Generate gene counts

Gene Count matrix

Clustering

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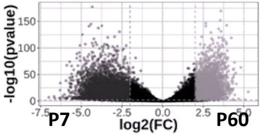
Differential Expression



DESeq2 limma Glimma edgeR

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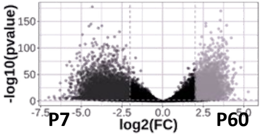

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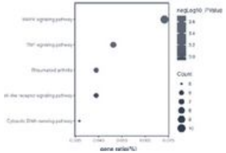
Functional Enrichment



GSEA Gene Set Enrichment Analysis

MetaCore

GREAT

KEGG Kyoto Encyclopedia of Genes and Genomes

reactome

DAVID Bioinformatics Database

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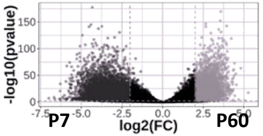

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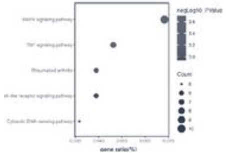
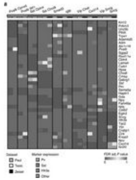


Coregulated Gene Expression



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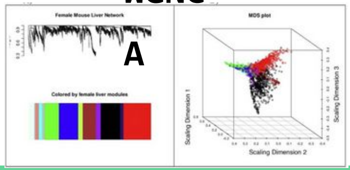

MetaNeighbo
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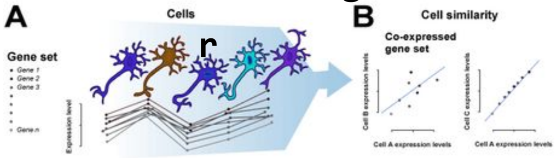
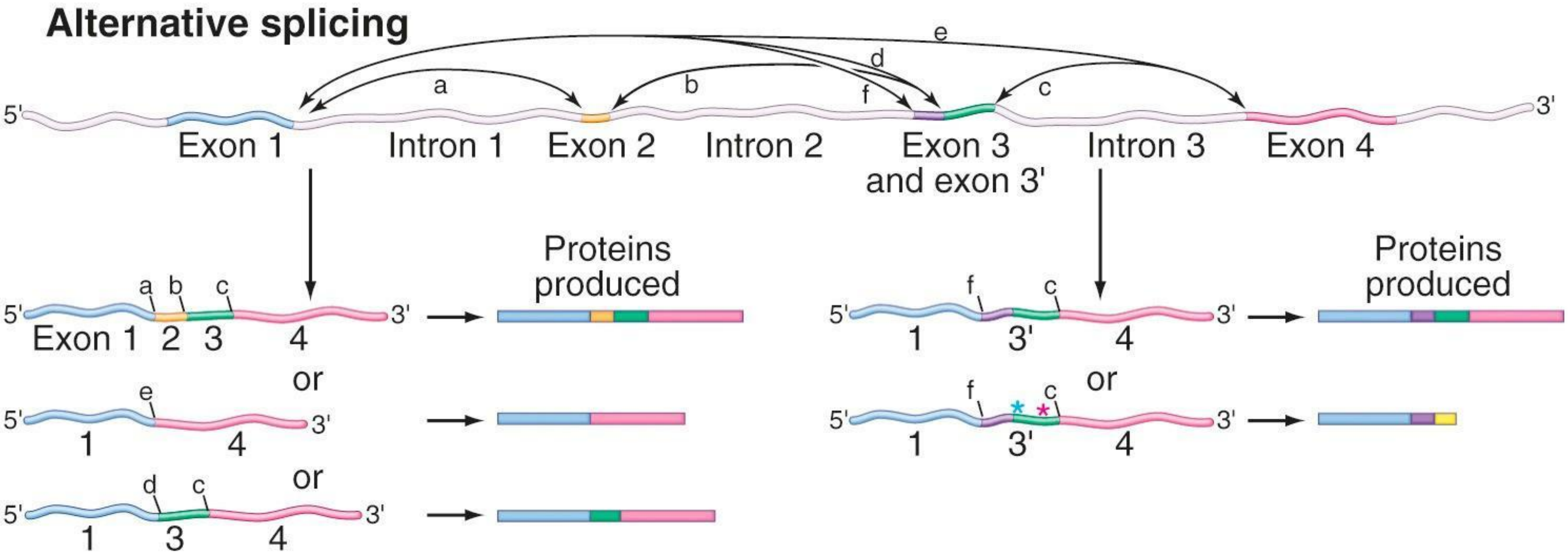


mRNA splicing



How is transcription captured?

•Extract RNA from cell •RNA preparation •Sequencing library preparation • Sequencing •Data capture •Data analysis

Questions?

Thank you for listening!