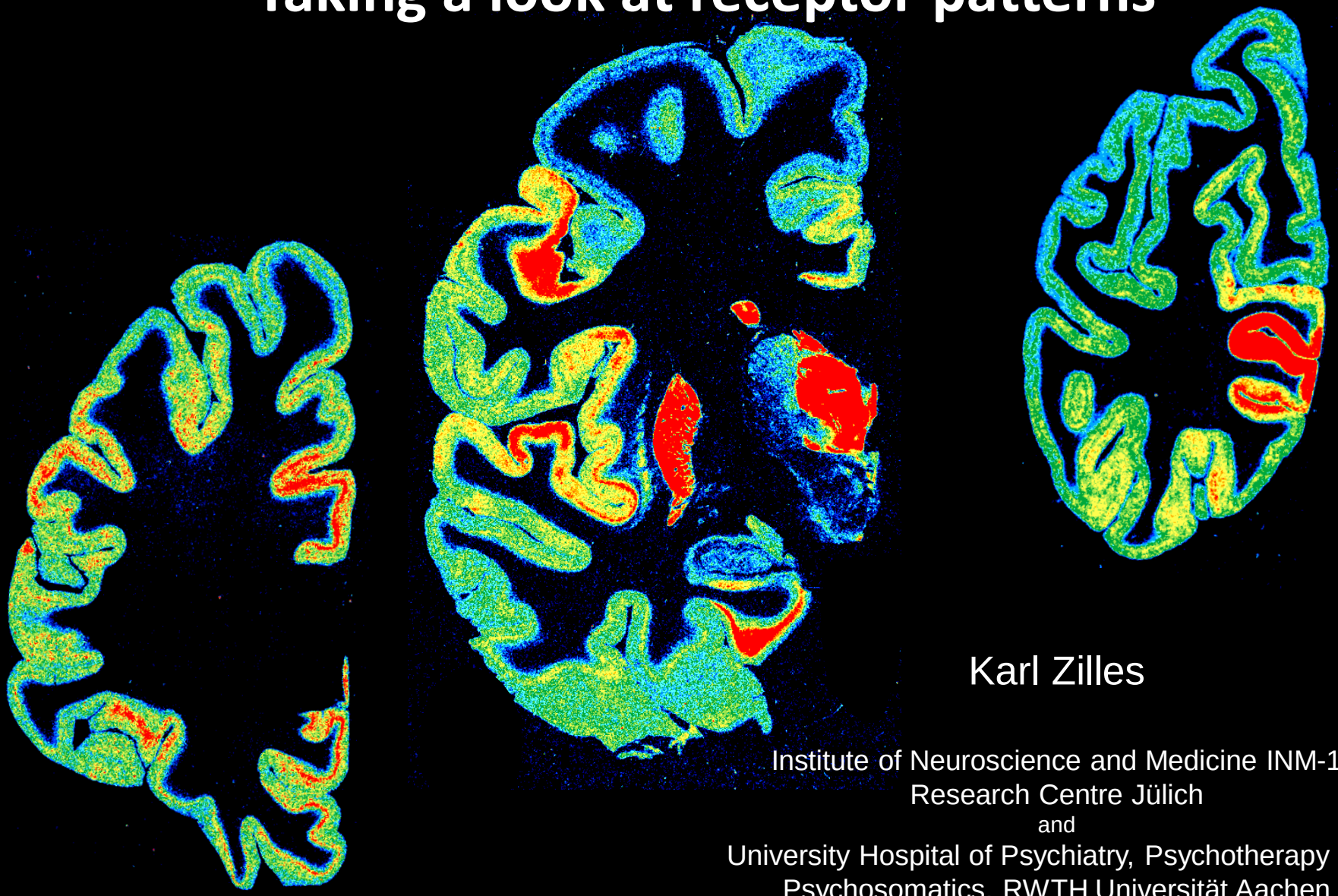
Anatomy in the resting state?
Taking a look at receptor patterns



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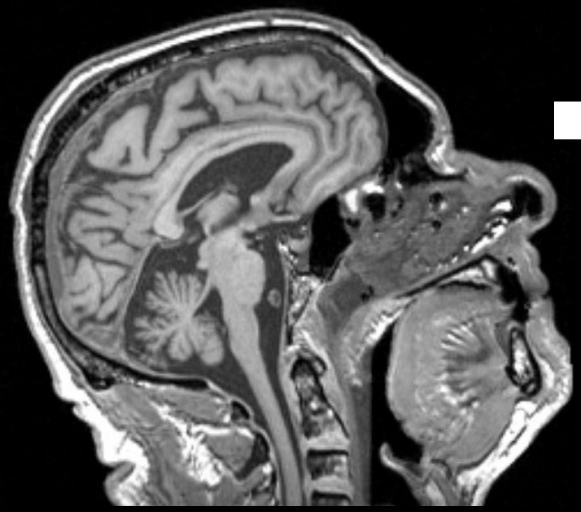
Regionally and functionally specific endowment of the brain with neurotransmitter receptors

1. How to visualize receptors at high spatial resolution throughout the entire brain

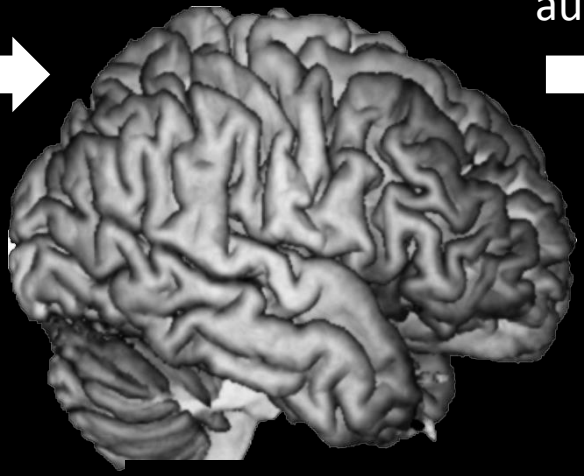
Zilles, K., Schleicher, A., Palomero-Gallagher, N., Amunts, K.:
Quantitative analysis of cyto- and receptorarchitecture of the human brain, pp. 573-602.
In: Brain Mapping: The Methods, 2nd edition (A.W. Toga and J.C. Mazziotta, eds.). Academic Press (2002)

Quantitative *in vitro* Receptor Autoradiography: Method (1)



in situ post mortem MRI

MRI

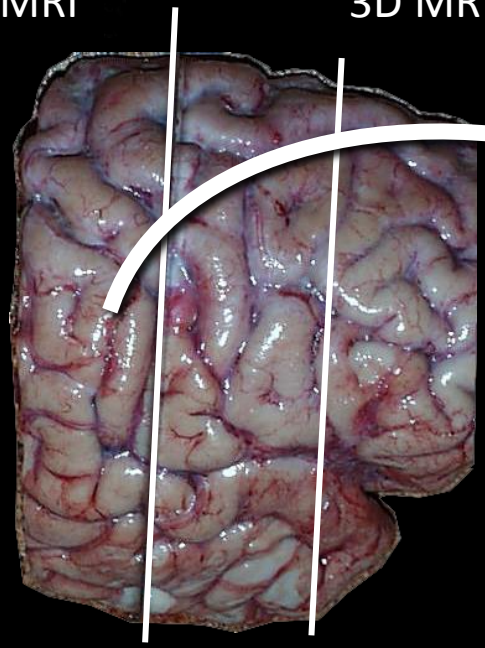


3D MR volume

autopsy

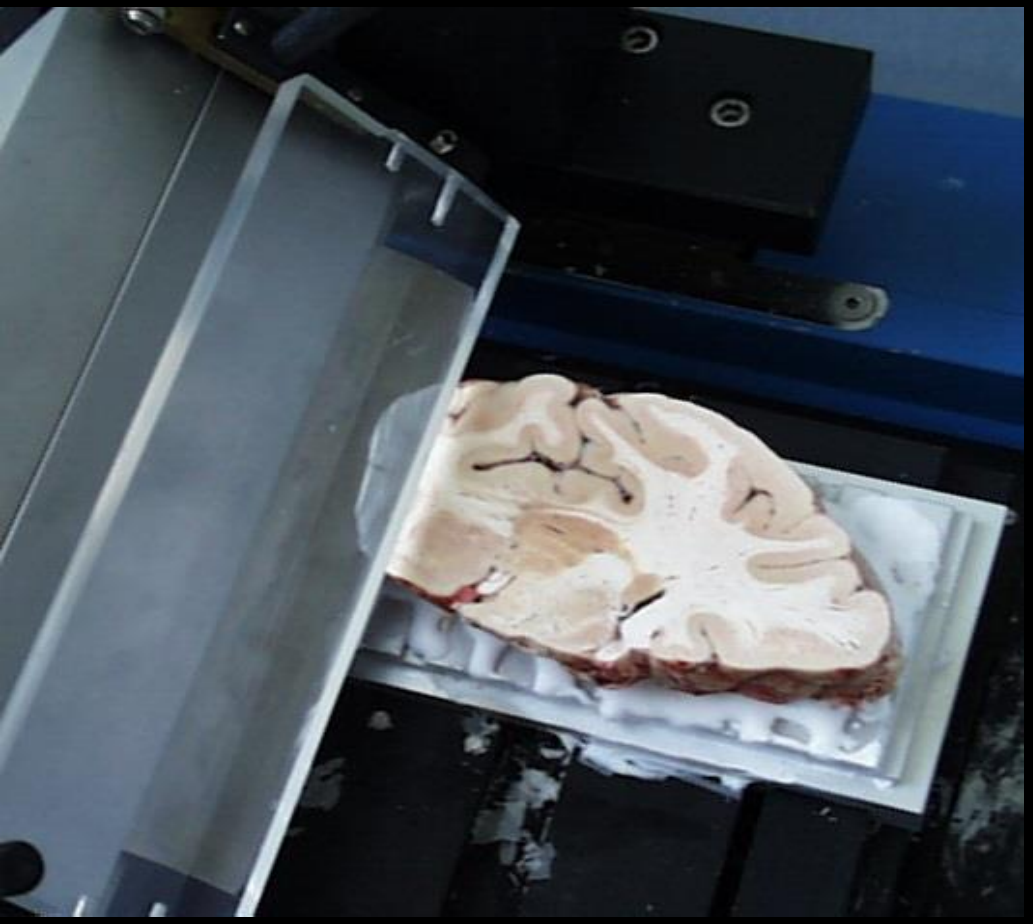
unfixed post mortem brain

dissection into slabs



mounting on the cryomicrotome chuck

Serial sections (20 µm) mounted onto glass slides

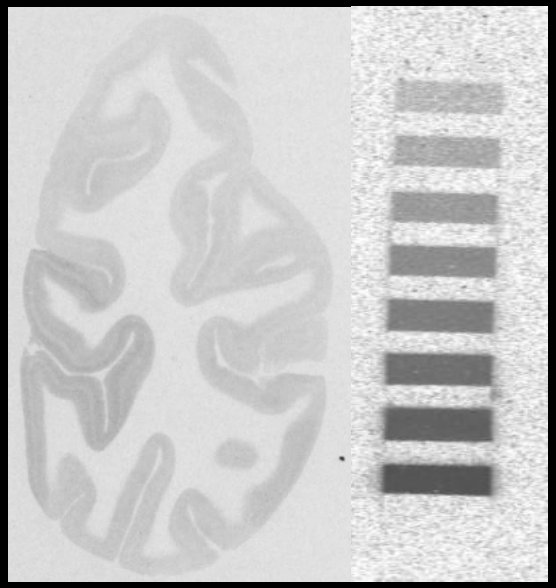


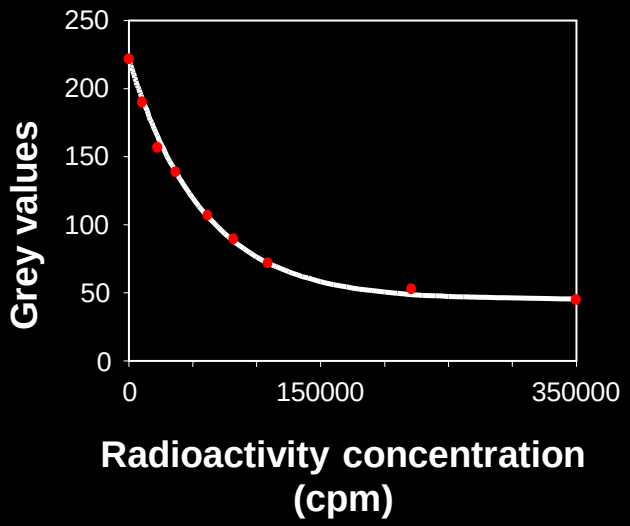
- Preincubation: re-hydrate sections. Remove endogenous substances
- Main incubation: buffer solution with [^{3}H]-ligands which specifically bind to a given receptor type
- Washing step: terminate binding procedure and eliminate surplus [^{3}H]-ligand and buffer salts
- Exposition of labeled sections and scales of known radioactivity concentrations to tritium-sensitive film
- Digitization of the films, measurement of receptor densities in fmol/mg protein, and color coding
- Histological staining of alternating sections for cell bodies and myelinated fibers

Examined receptor binding sites

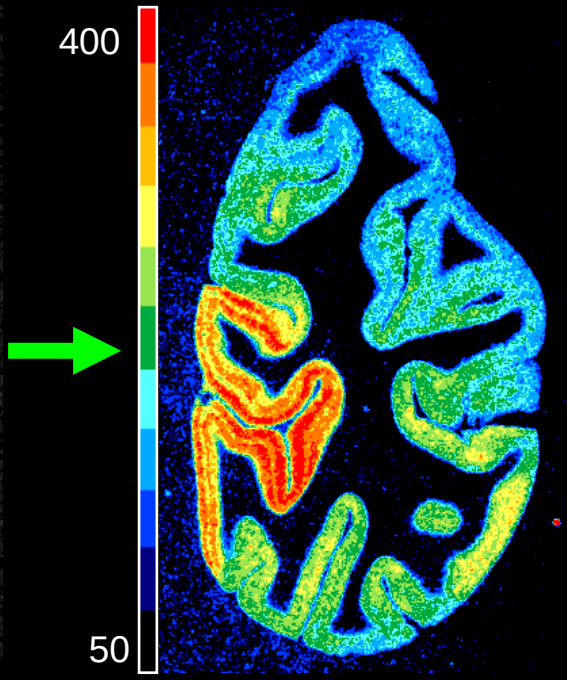
Neurotransmitter	Receptor	[^{3}H] Ligand
Glutamate	AMPA	AMPA
	Kainate	kainate
	NMDA	MK-801
GABA	$GABA_A$	muscimol,
	$GABA_B$	CGP 54626
	$GABA_ABZ$	Flumazenil
Acetylcholine	M_1	pirenzepine
	M_2	oxotremorine-M, AF-DX 384
	M_3	4-DAMP
	nicotinic $\alpha_4\beta_2$	epibatidine
Noradrenaline	α_1	prazosin
	α_2	UK 14,304 RX-821002
Serotonin	$5\text{-}HT_{1A}$	8-OH-DPAT
	$5\text{-}HT_2$	ketanserin
Dopamine	D_1	SCH 23390

Image processing





$$C = \frac{1}{S_a} \cdot \frac{K_D + L}{L} \quad \text{[fmol/mg protein]}$$



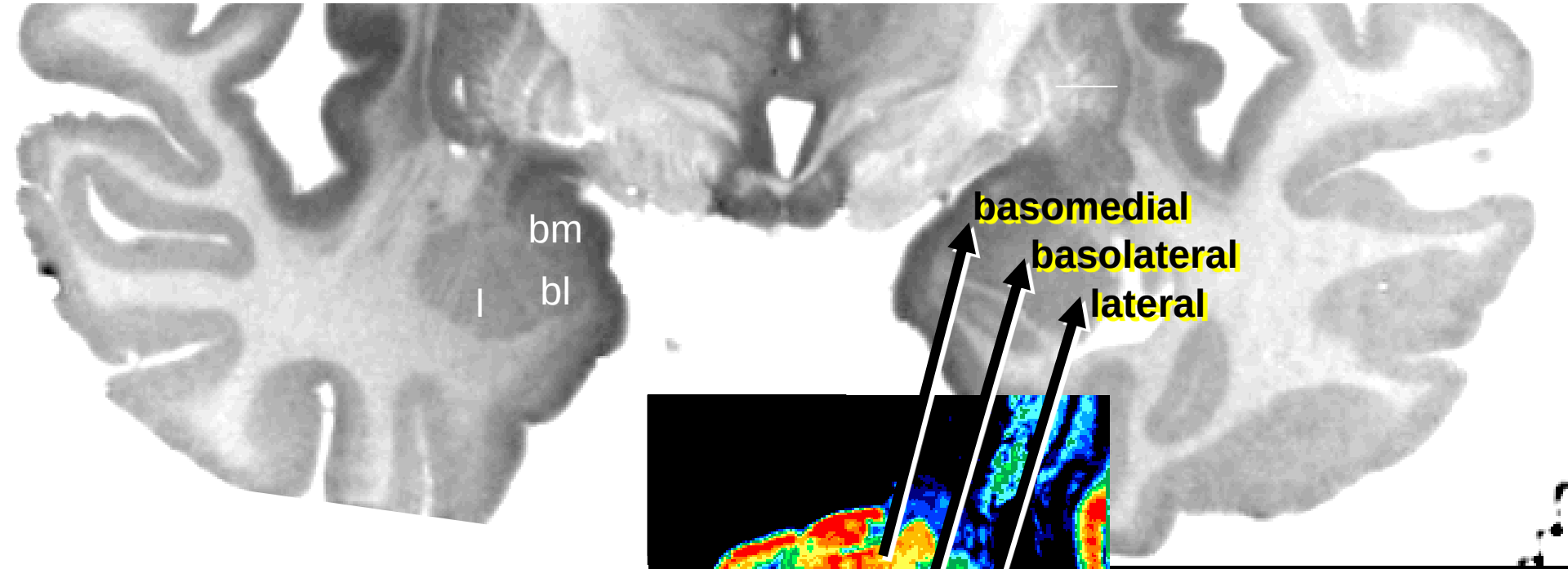
Linearised and color-coded autoradiograph

grey values or colors encode receptor densities

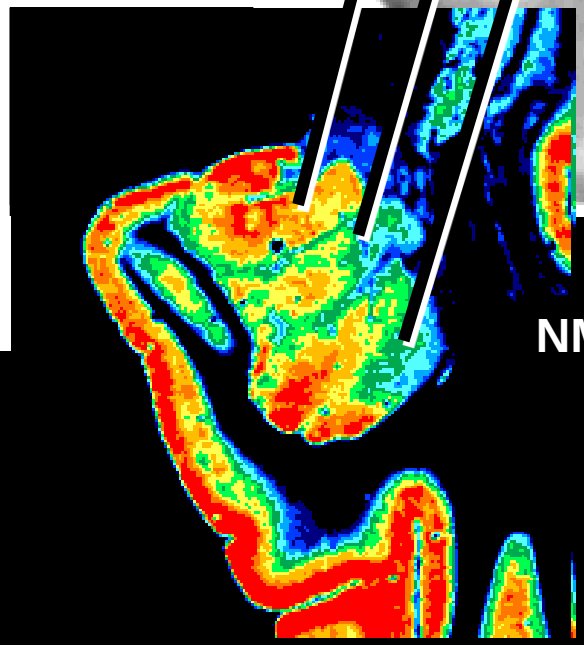
Regionally and functionally specific endowment of the brain with neurotransmitter receptors

2. Regional and laminar distribution of *single receptor types* in the cerebral cortex

The amygdala must be further subdivided based on it‘s distinct and heterogenous receptor distribution



Structural MRI at 4 Tesla:
spatial resolution 350 μm isotrop

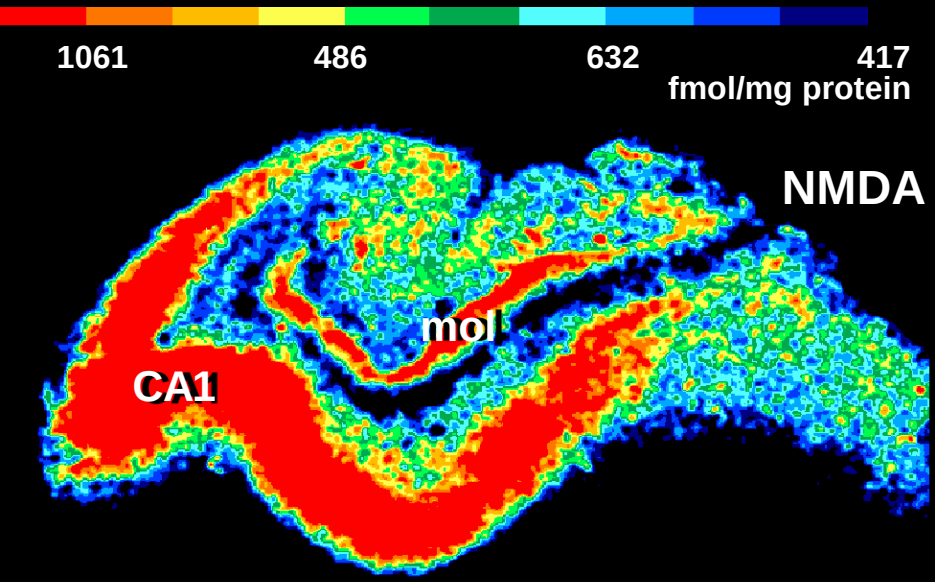


NMDA receptor

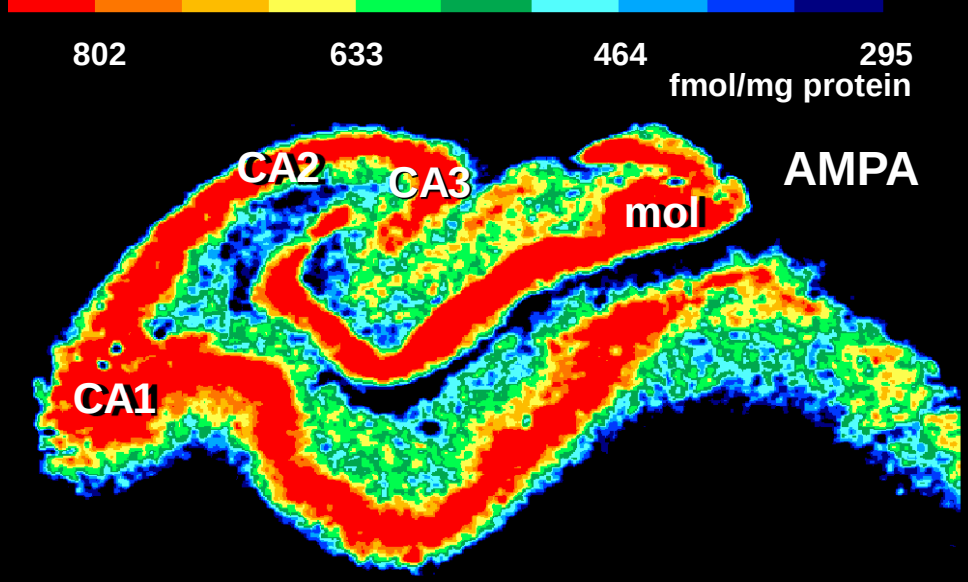
Receptor autoradiography

The termination fields of ***the trisynaptic pathway in the hippocampus*** show distinct regional and laminar expression patterns of different glutamatergic receptors

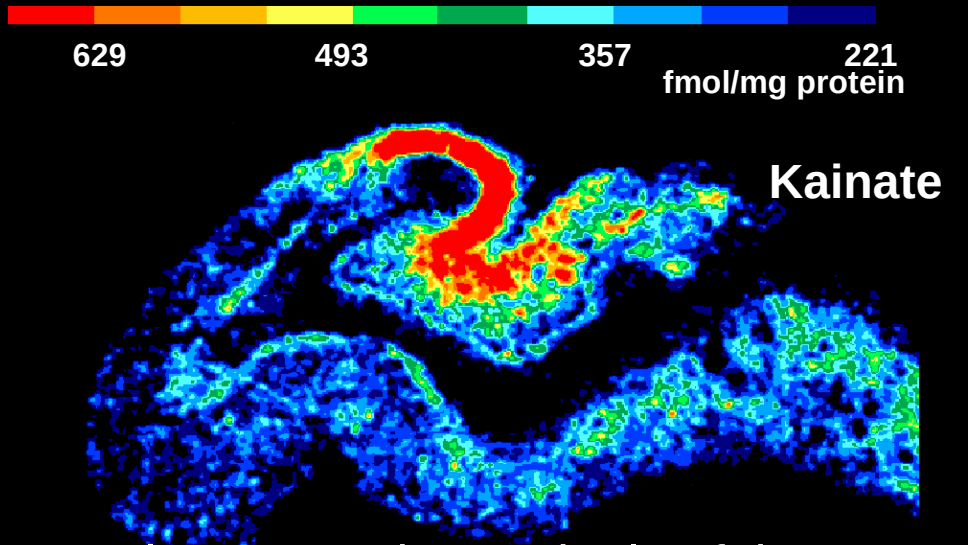
- ***Perforant path*** to CA and molecular layer of DG
- ***Mossy fibers*** from DG to CA3
- ***Schaffer collaterals*** from CA3 to CA1



Glutamatergic terminals of the ***perforant path*** and the ***Schaffer collaterals***



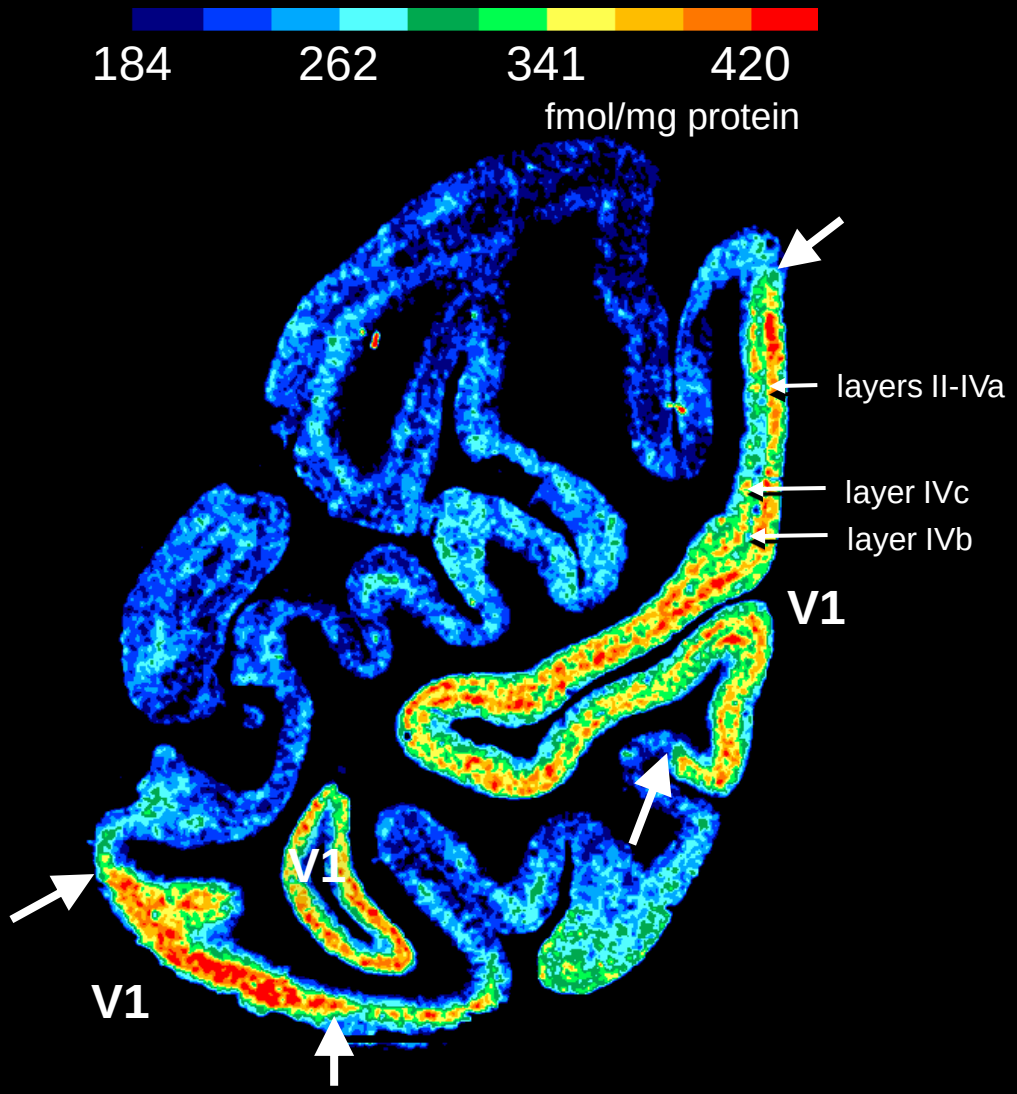
Glutamatergic terminals of the ***perforant path*** and the ***Schaffer collaterals***



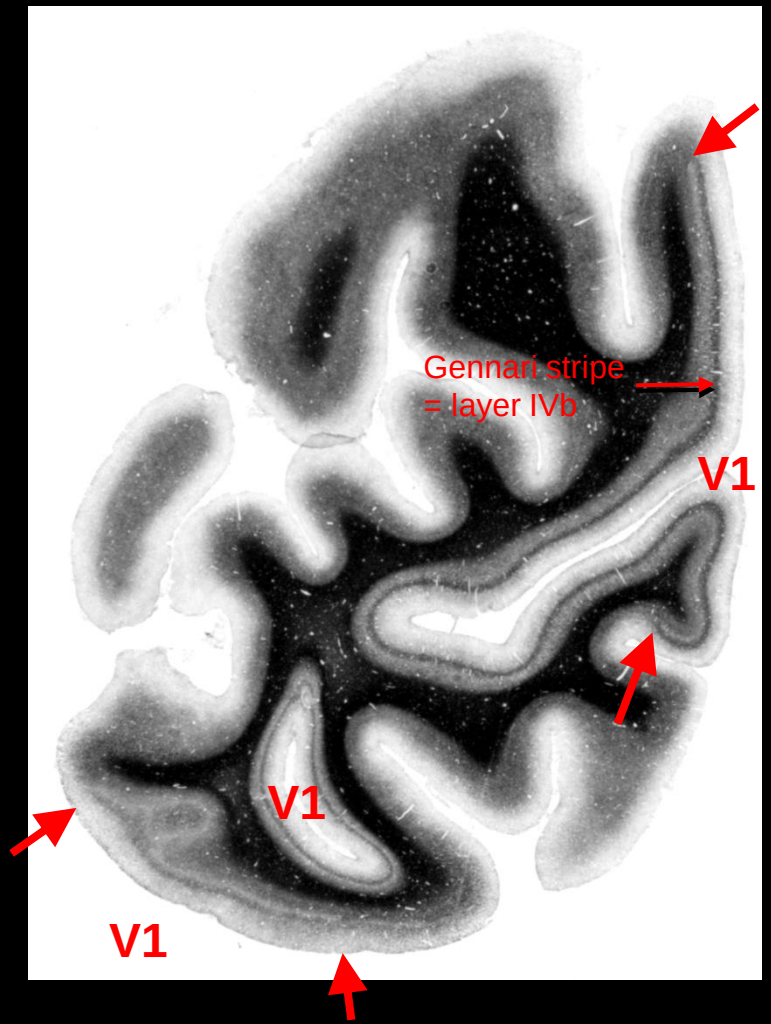
Glutamatergic terminals of the ***mossy fibers***

Cholinergic muscarinic M_2 receptor and primary sensory areas: Matching receptor- and myeloarchitecture

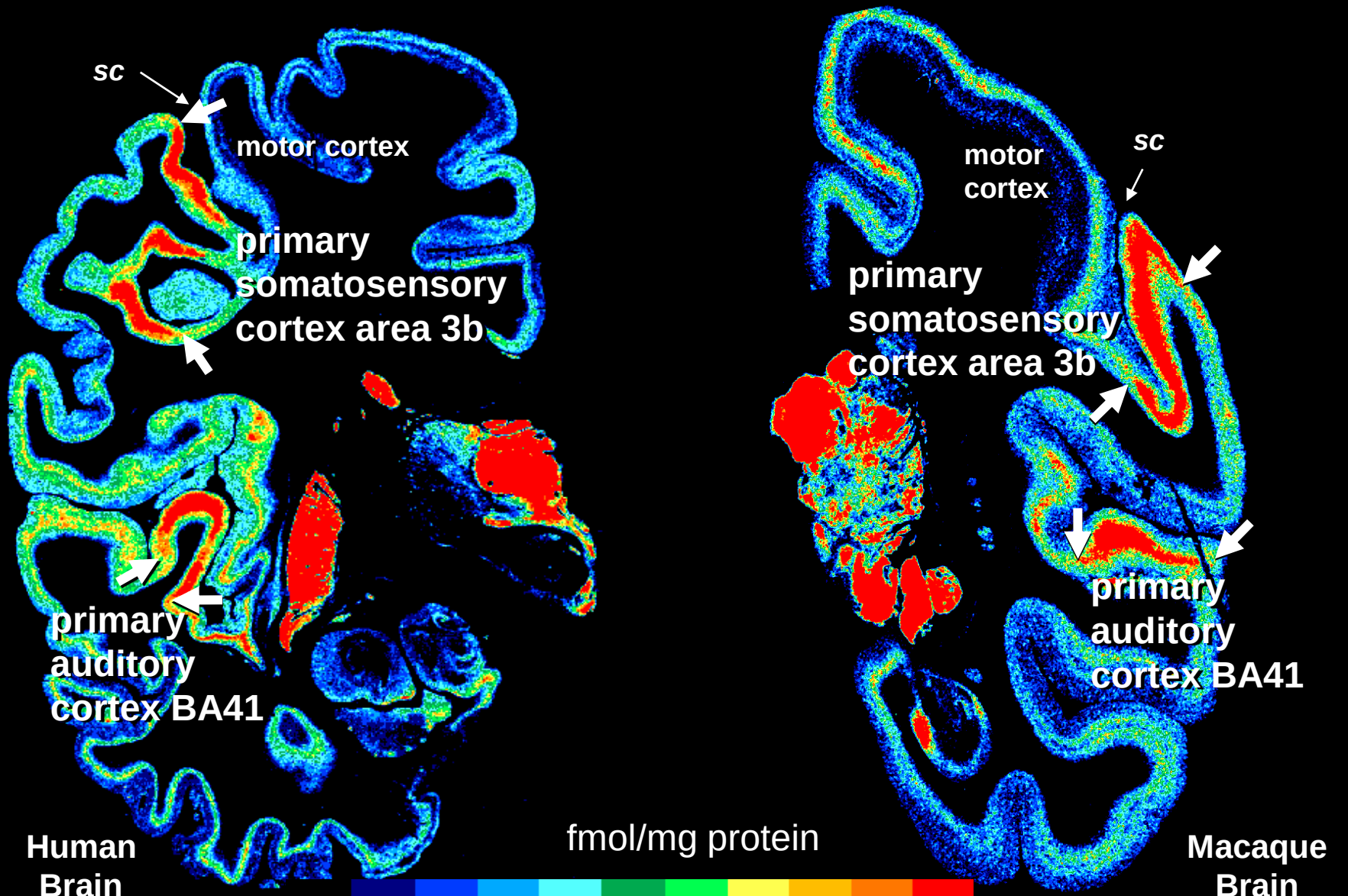
Receptor autoradiography



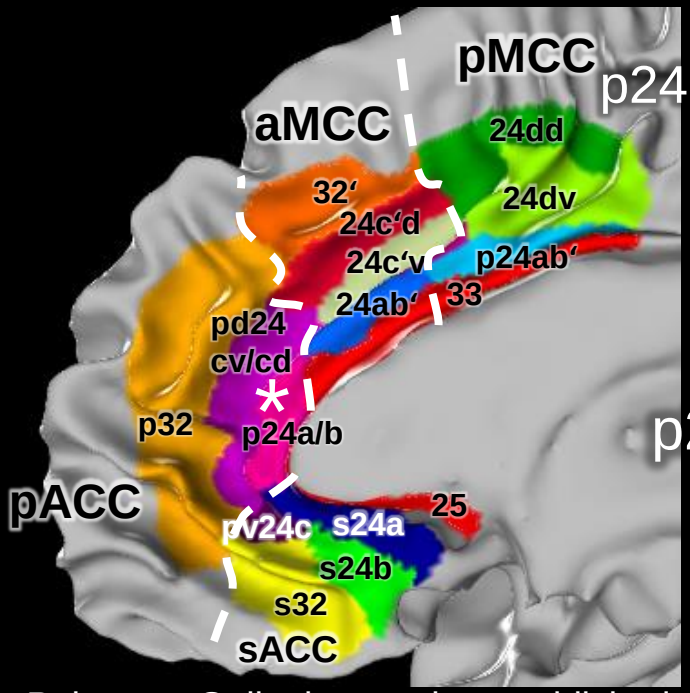
Myelin staining



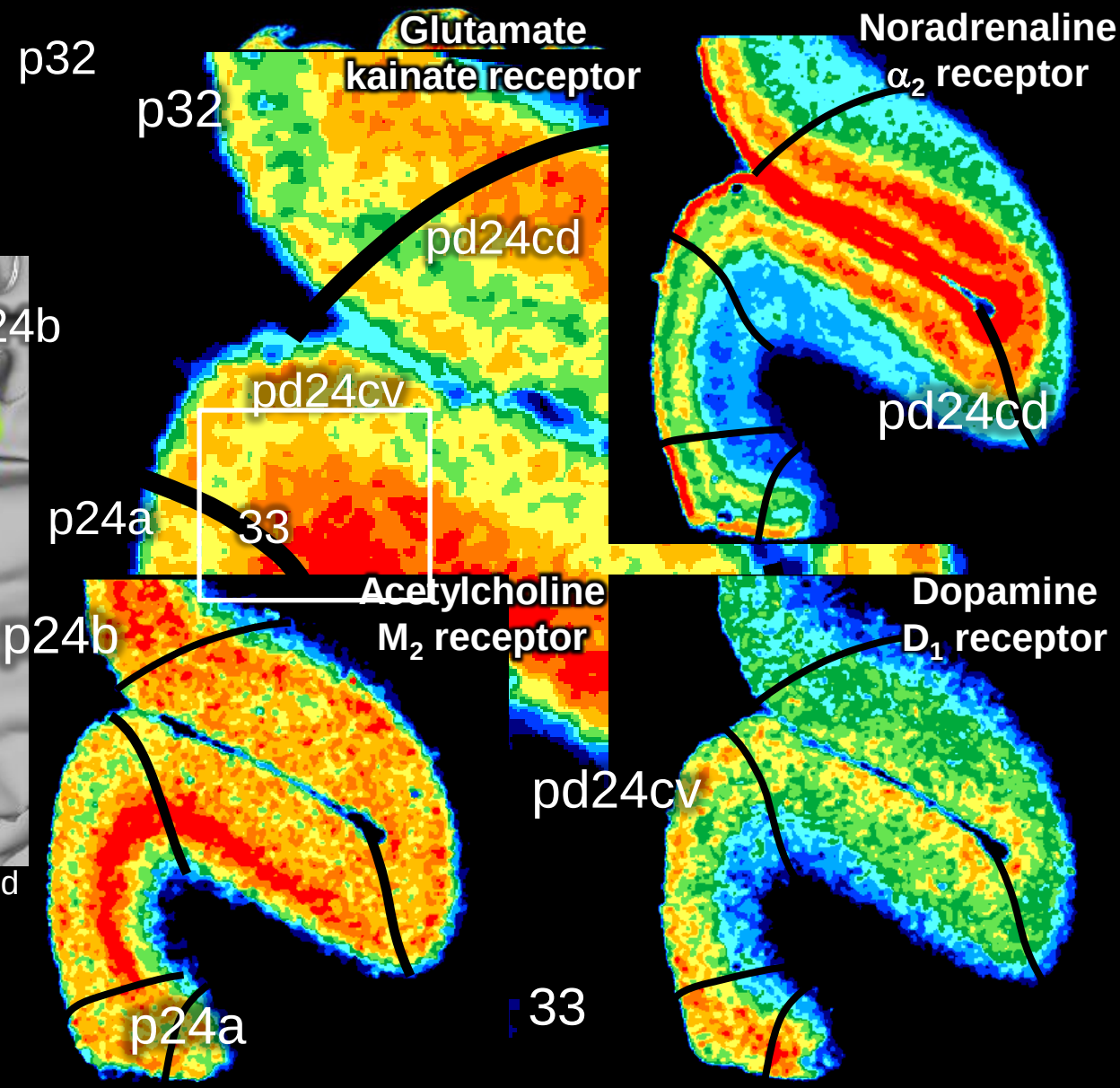
Cholinergic muscarinic M_2 receptor and primary sensory areas: Receptorarchitecture



Multiple receptors and anterior cingulate cortex



Palomero-Gallagher et al., unpublished



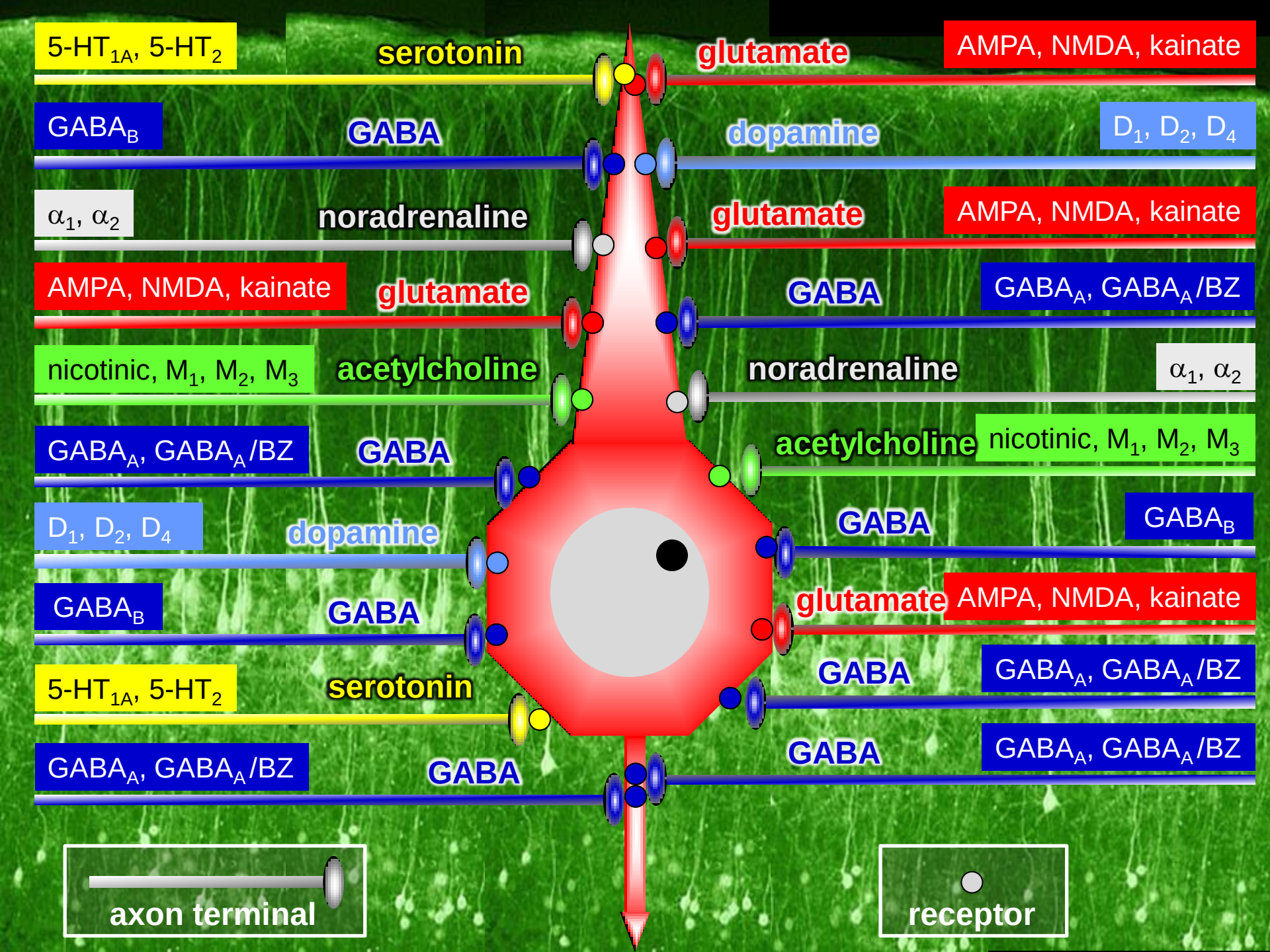
Palomero-Gallagher, Mohlberg, Zilles, Vogt (2008) *J Comp Neurol* 508: 906-926.

Palomero-Gallagher & Zilles (2009) In: Cingulate Neurobiology & Disease. Oxford University Press, pp. 31-63.

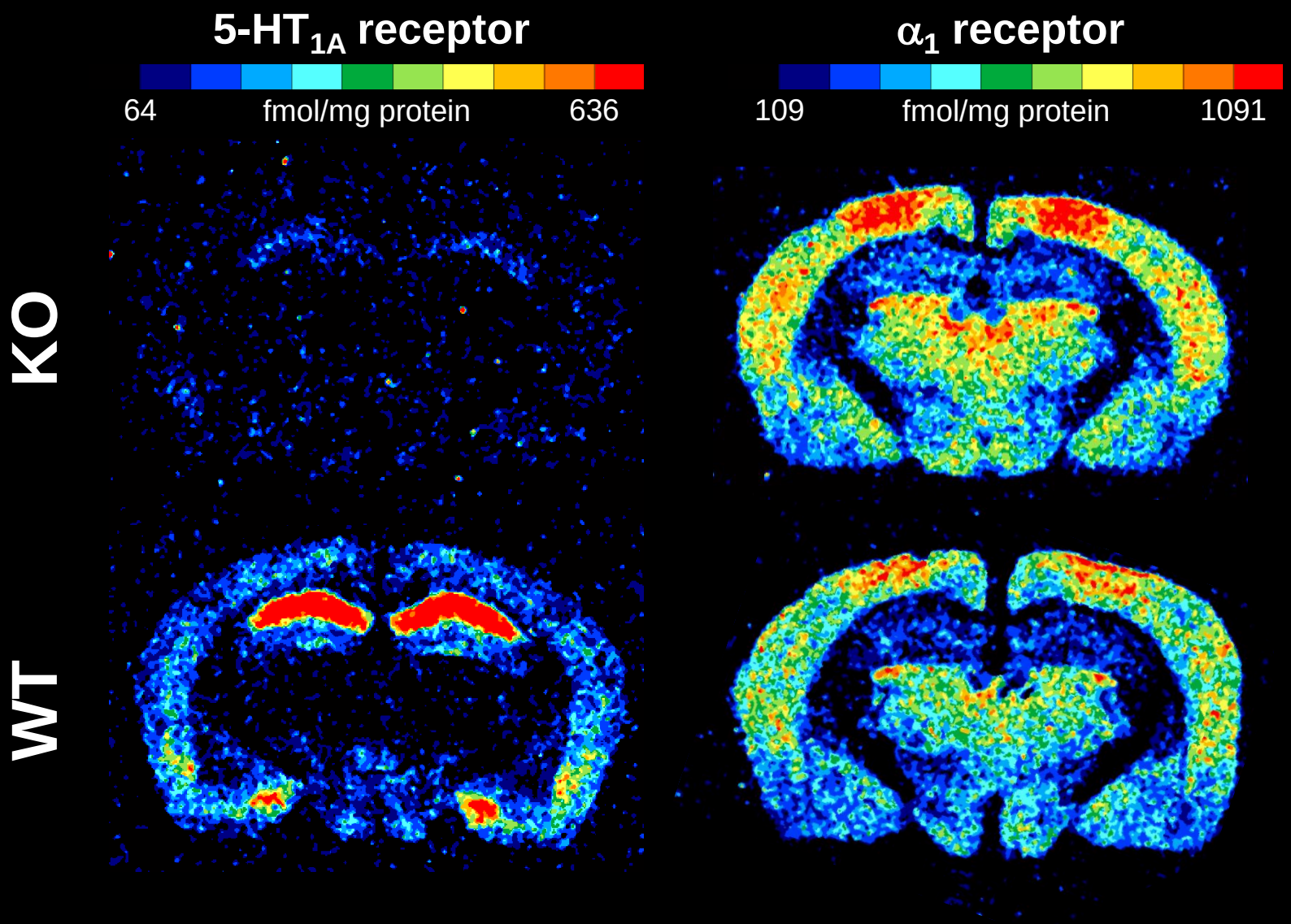
Regionally specific endowment of the brain with neurotransmitter receptors

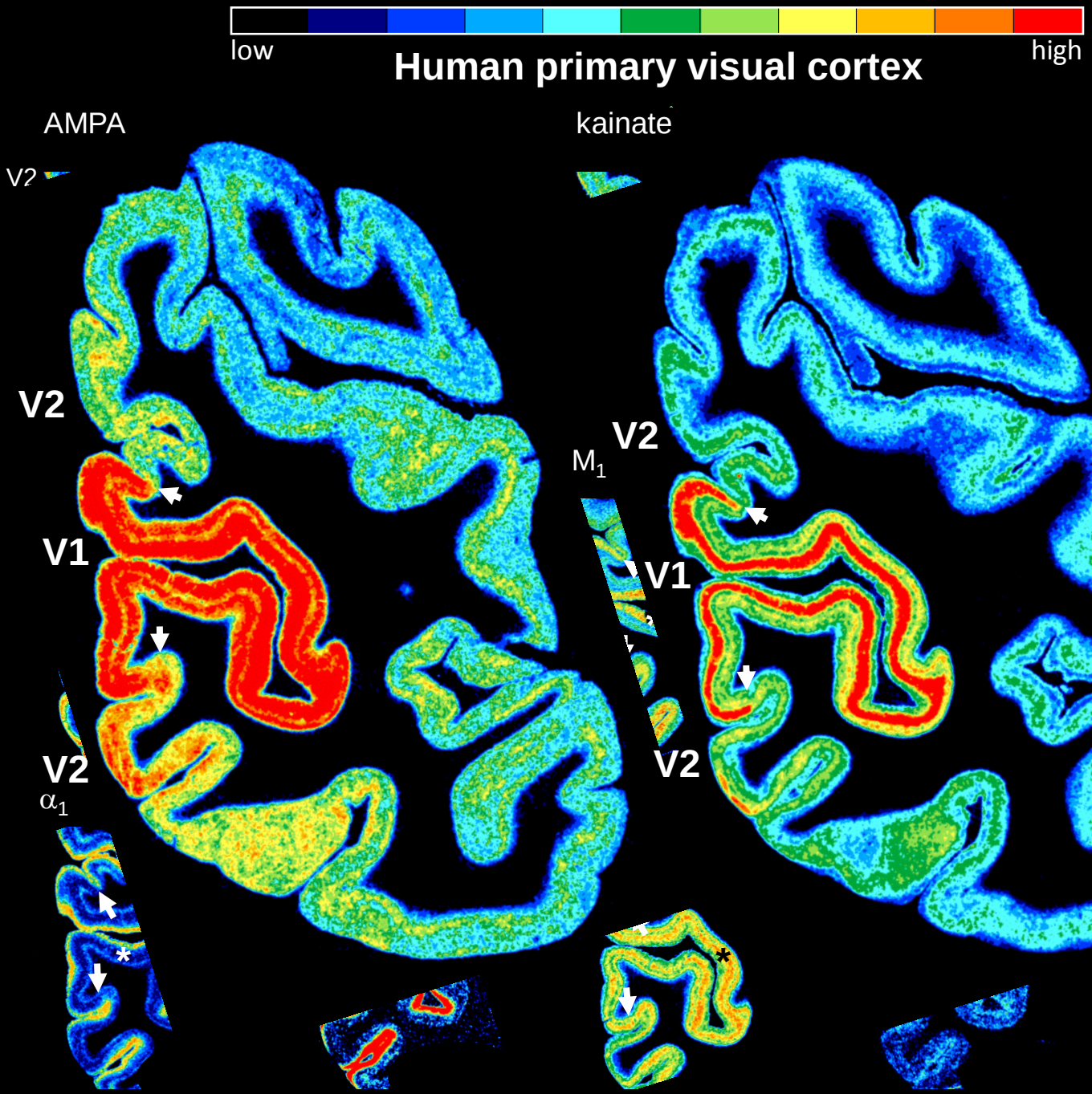
3. Regional and laminar distribution of multiple receptor types in the cerebral cortex

- RECEPTOR FINGERPRINTS -


5-HT1A, 5-HT2
serotonin
glutamate
AMPA, NMDA, kainate
GABAB
GABA
dopamine
D1, D2, D4
α1, α2
noradrenaline
glutamate
AMPA, NMDA, kainate
AMPA, NMDA, kainate
glutamate
GABA
GABAA, GABAA/BZ
nicotinic, M1, M2, M3
acetylcholine
noradrenaline
α1, α2
GABAA, GABAA/BZ
GABA
acetylcholine
nicotinic, M1, M2, M3
D1, D2, D4
dopamine
GABA
GABAB
GABAB
GABA
glutamate
AMPA, NMDA, kainate
5-HT1A, 5-HT2
serotonin
GABA
GABAA, GABAA/BZ
GABAA, GABAA/BZ
GABA
GABA
GABAA, GABAA/BZ
axon terminal
receptor

On the way to understand specificity and mechanisms behind receptor fingerprints: Conditional 5-HT_{1A} receptor knockout

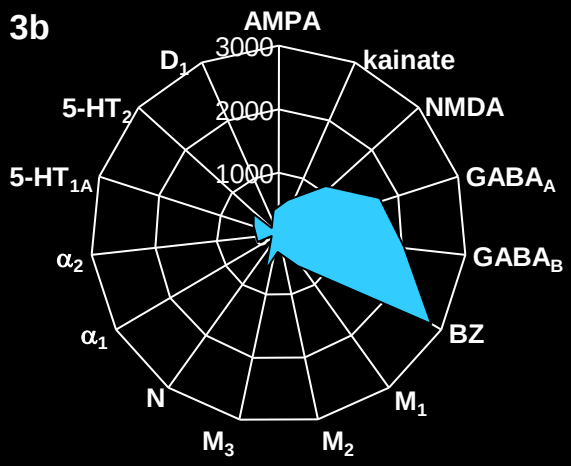




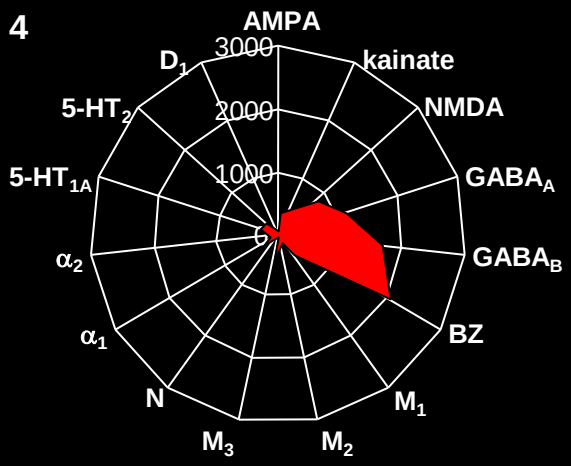
Zilles, Palomero Gallagher: Comparative analysis of receptor subtypes that identify primary cortical sensory areas. In (Kaas, Striedter, Krubitzer, Herculano-Houzel, Preuss, eds.) Evolution of the Nervous System. Elsevier, Oxford (2017)

Fingerprints: functional specificity

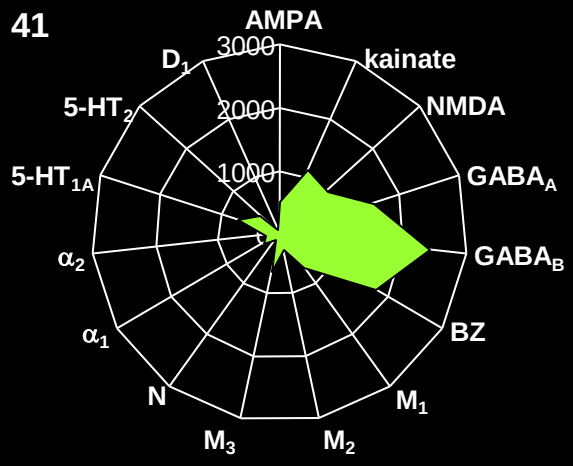
primary somatosensory



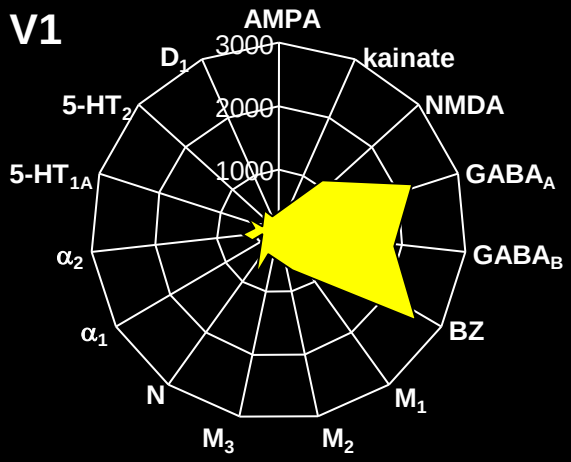
primary motor

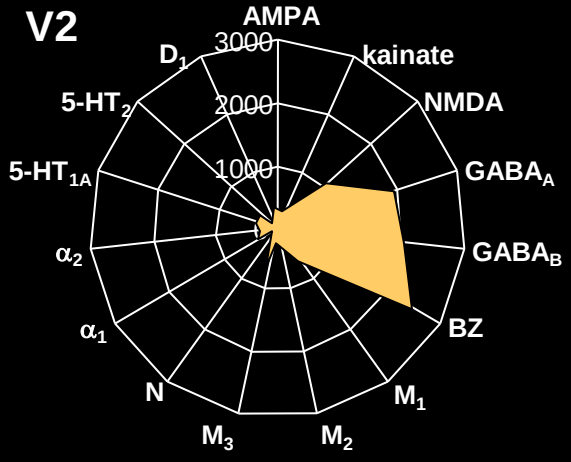


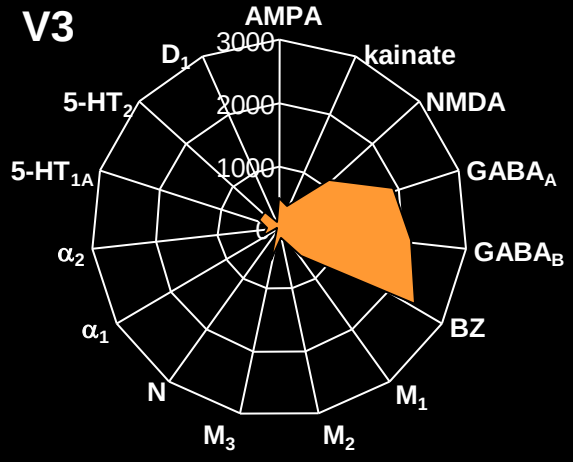
primary auditory



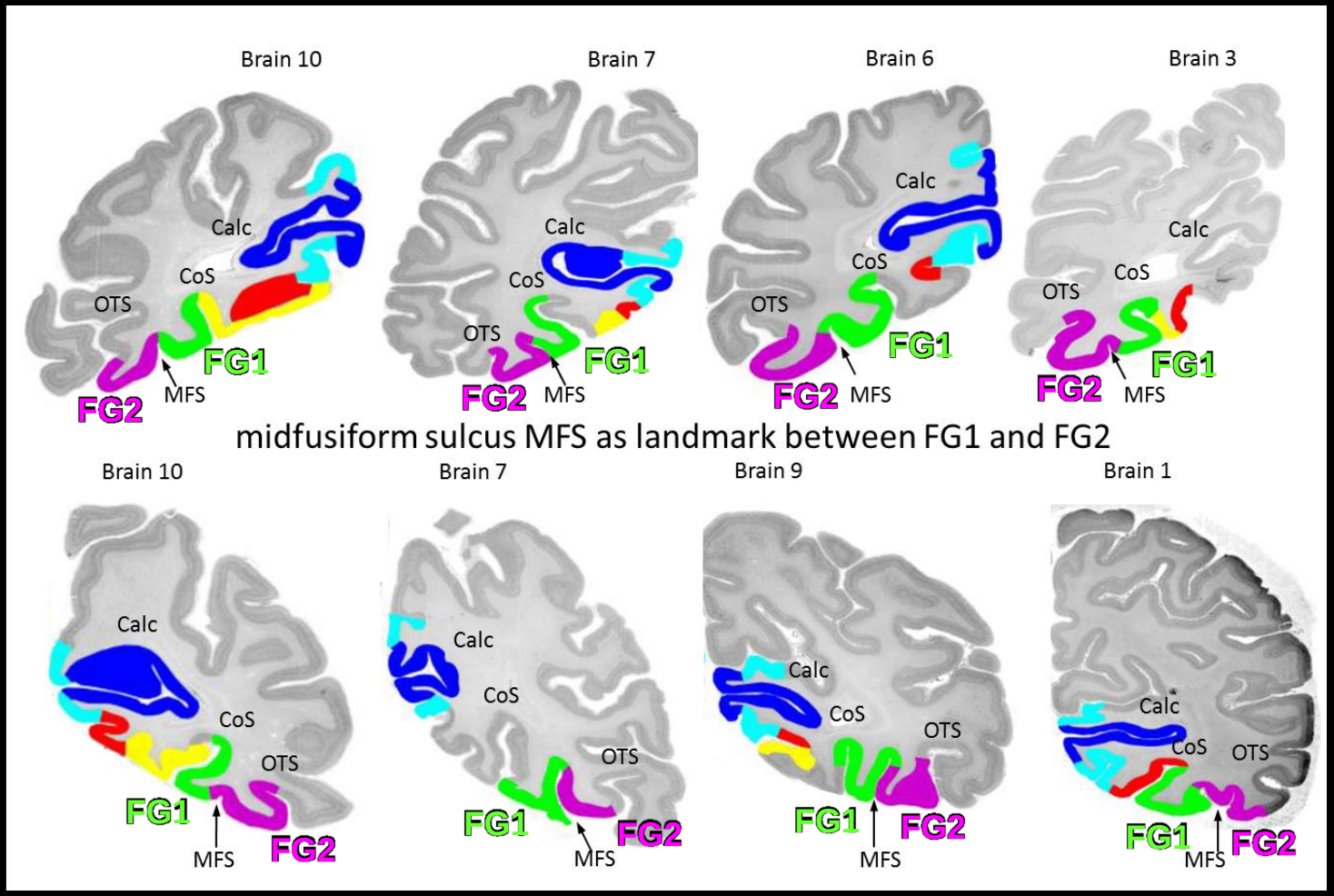
primary (V1), and higher (V2, V3) visual areas







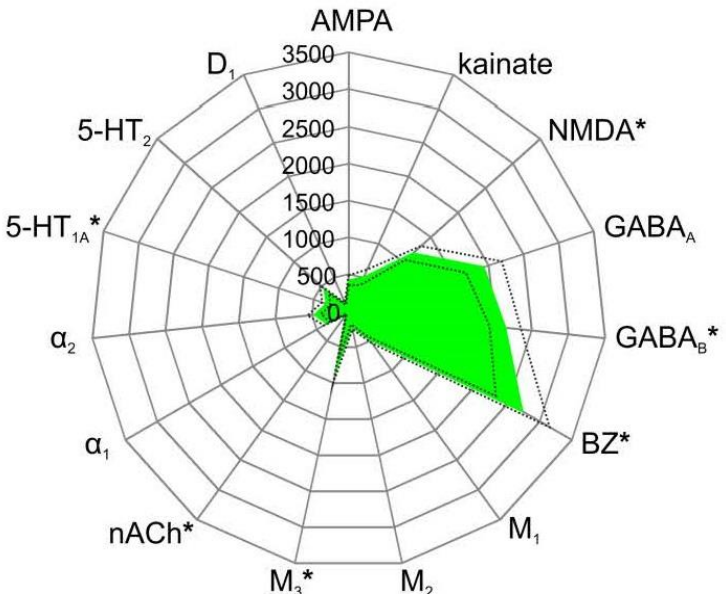
Ventral visual areas in human extrastriate cortex (FG2, FG2)



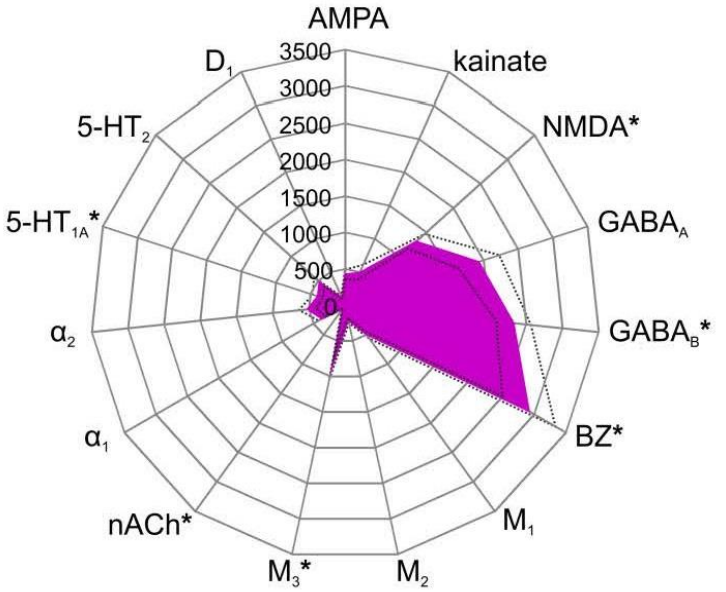
Weiner, K., Golarai, G., Caspers, J., Mohlberg, H., Zilles, K., Amunts, K., Grill-Spector, K.: The mid-fusiform sulcus: A landmark identifying both cytoarchitectonic and functional divisions of the human fusiform gyrus. *Neuroimage* 84: 453-465 (2014)

Gomez, J., Barnett, M.A., Natu, V.S., Mezer, A., Palomero-Gallagher, N., Weiner, K.S., Amunts, K., Zilles, K., Grill-Spector, K. (2016). Growth of tissue in human cortex is coupled with the development of face processing. ***Science*** 355: 68-71 (2017)

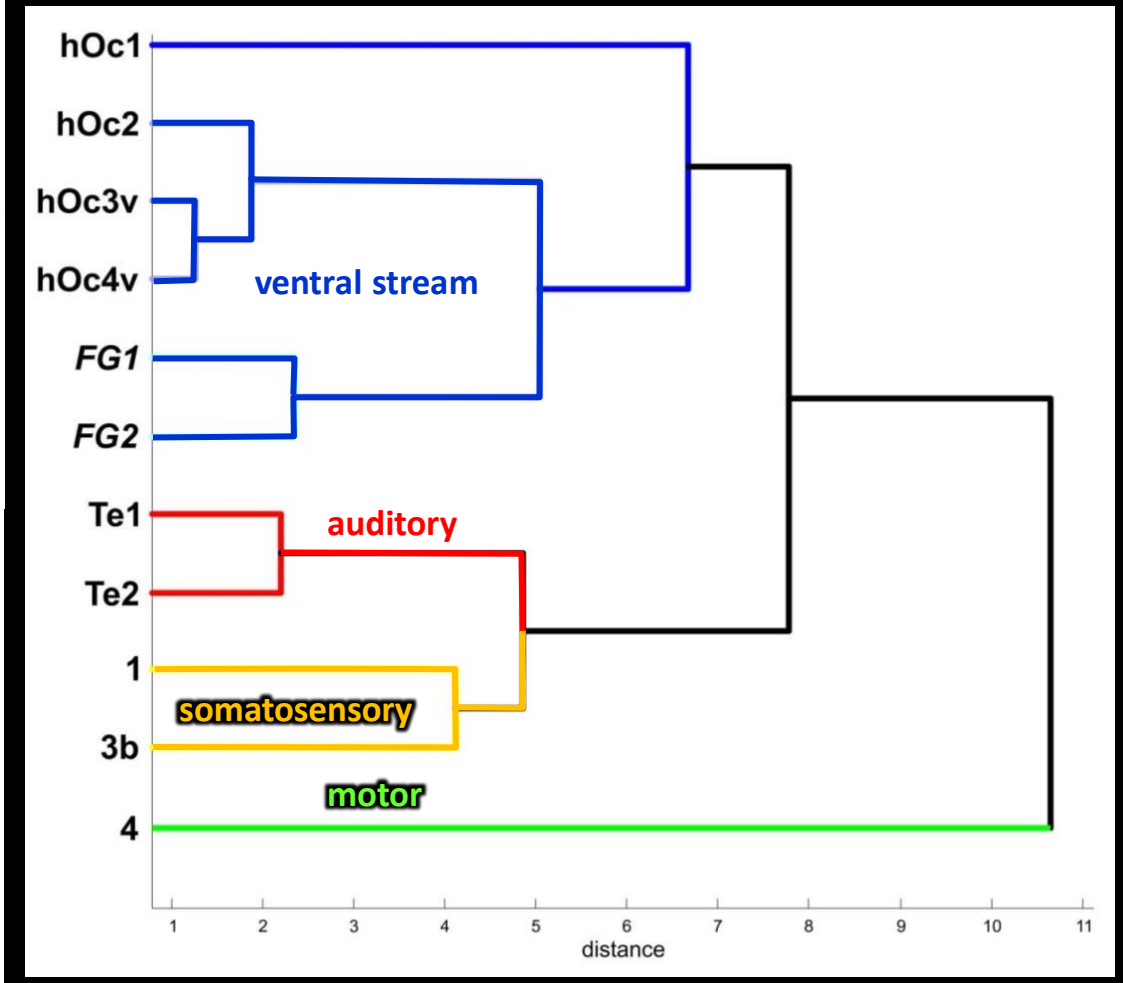
Receptor fingerprint of FG1



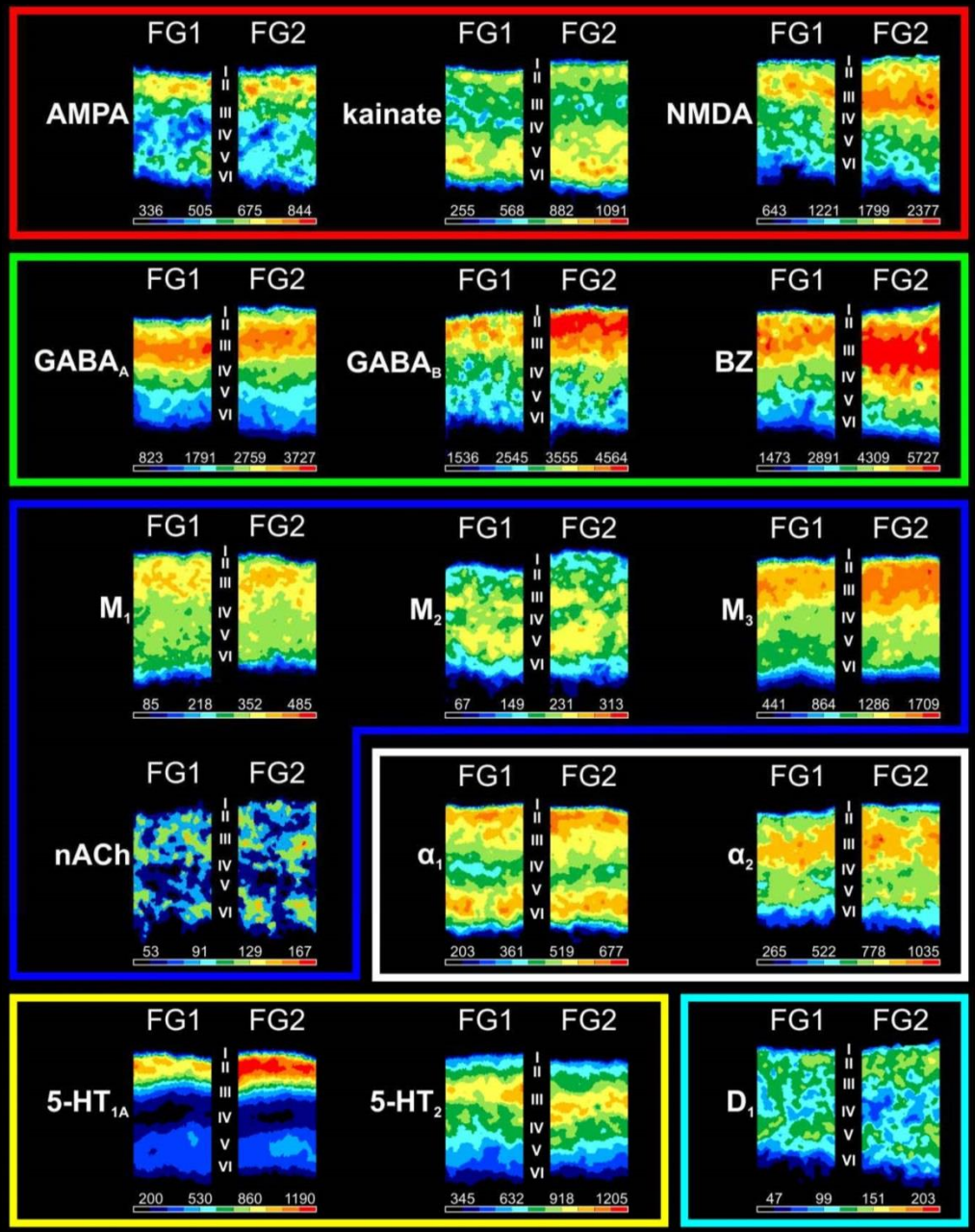
Receptor fingerprint of FG2

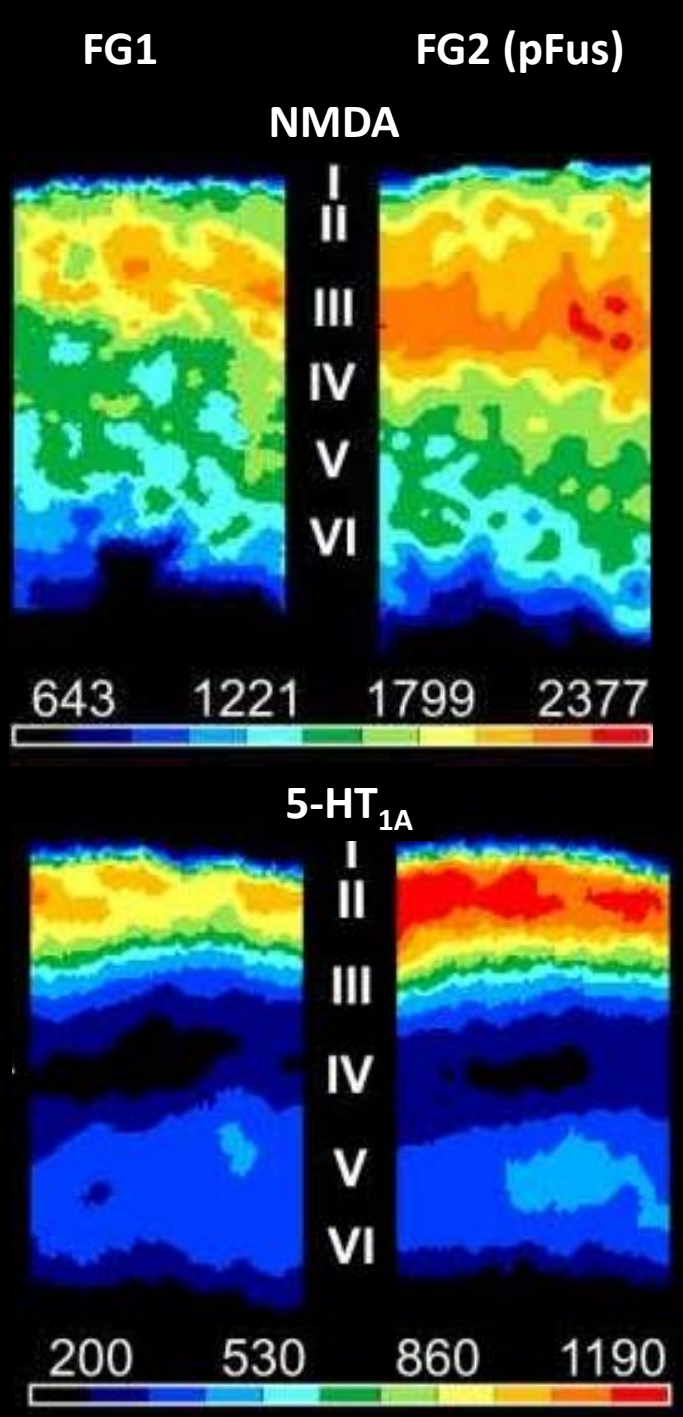


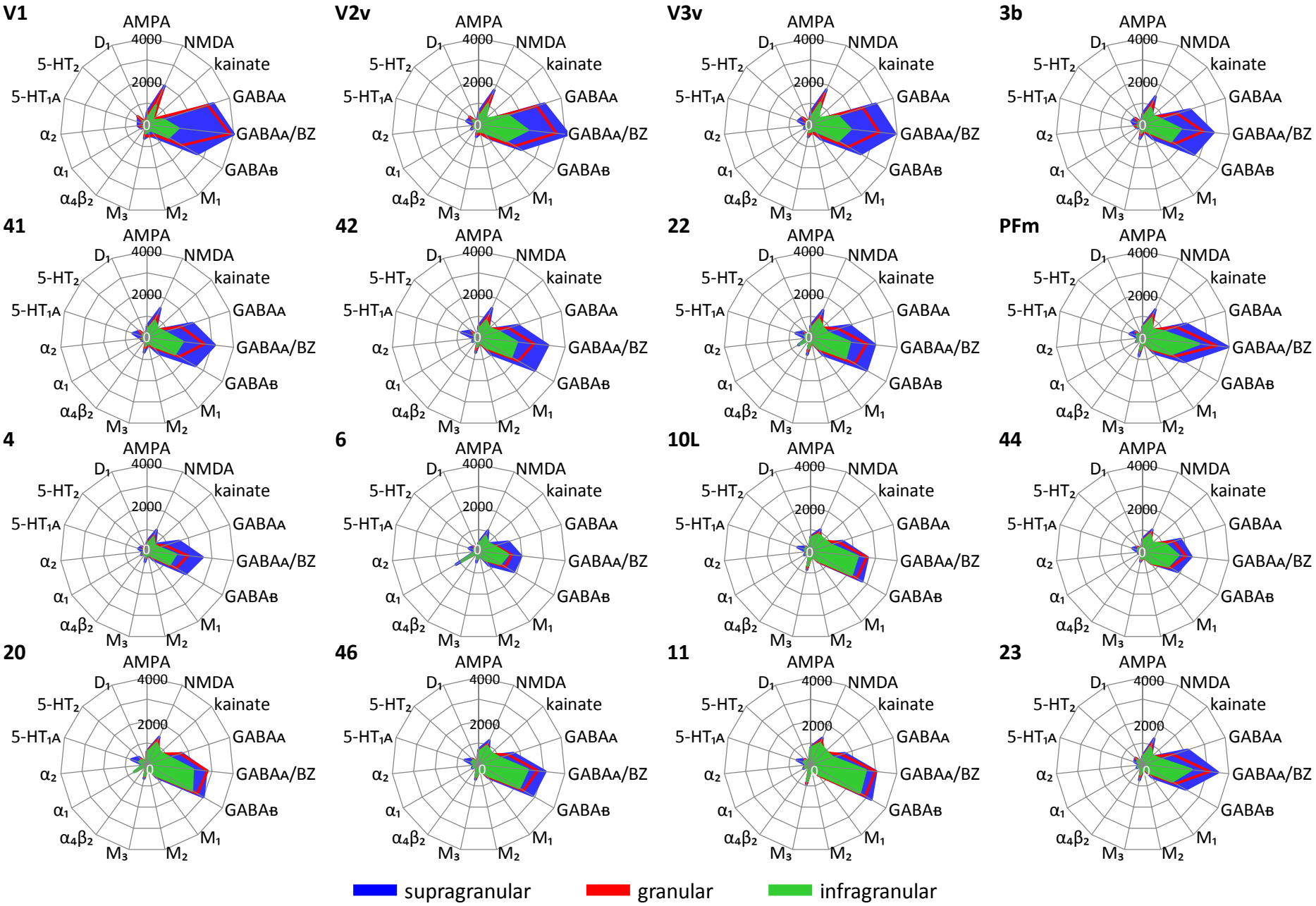
Hierarchical Cluster Analysis



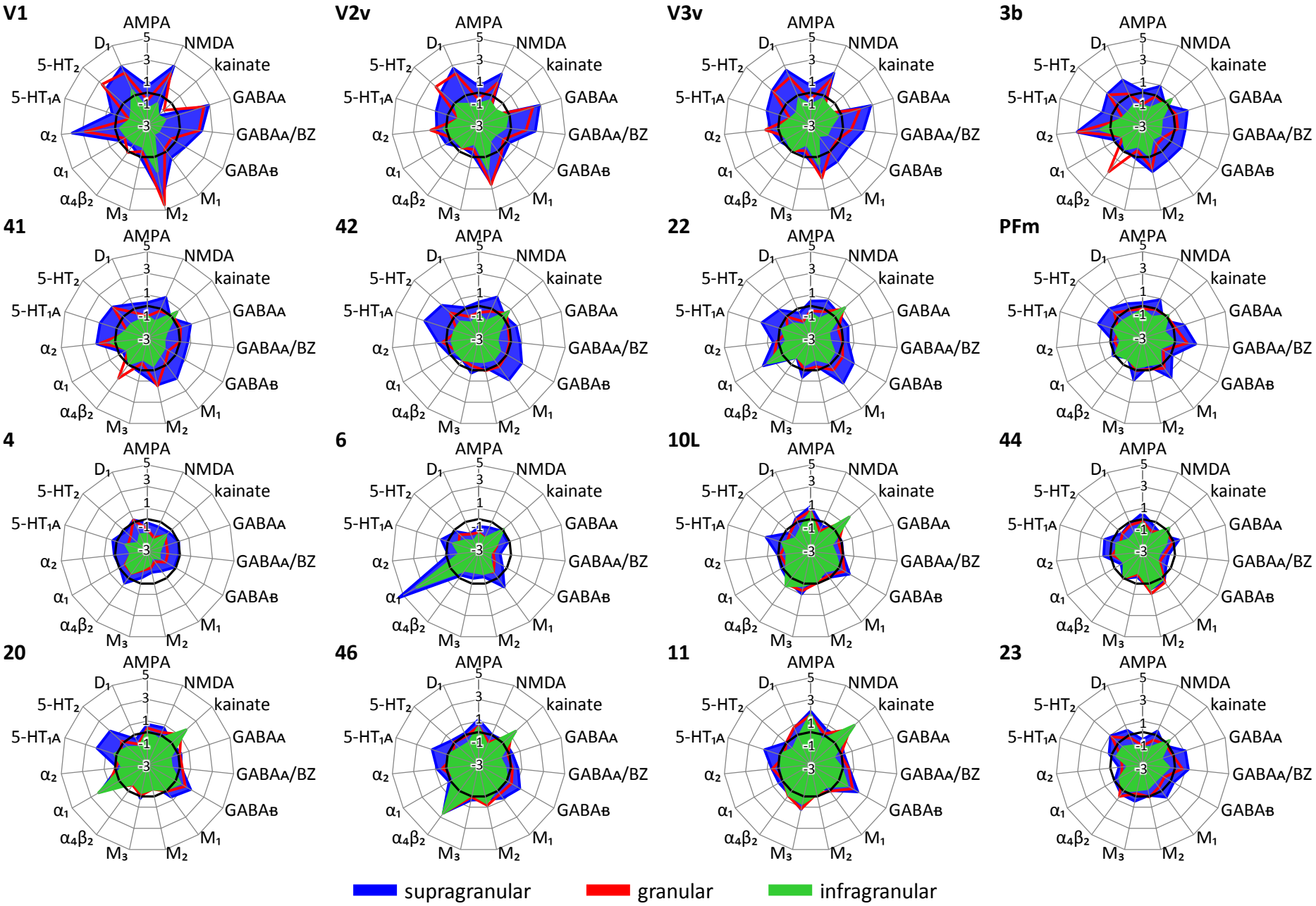
Caspers, J., Palomero-Gallagher, N., Caspers, S., Schleicher, A., Amunts, K., Zilles, K.: Receptor architecture of cytoarchitectonic visual areas FG1 and FG2 of the posterior fusiform gyrus. Brain Struct. Funct. 220: 205-220 (2015)


FG1
FG2
AMPA
I
II
III
IV
V
VI
336 505 675 844
kainate
255 568 882 1091
NMDA
643 1221 1799 2377
GABA$_A$
823 1791 2759 3727
GABA$_B$
1536 2545 3555 4564
BZ
1473 2891 4309 5727
M$_1$
85 218 352 485
M$_2$
67 149 231 313
M$_3$
441 864 1286 1709
nACh
53 91 129 167
α$_1$
203 361 519 677
α$_2$
265 522 778 1035
5-HT$_{1A}$
200 530 860 1190
5-HT$_2$
345 632 918 1205
D$_1$
47 99 151 203
glutamate
GABA
acetylcholine
adrenaline
serotonin
dopamine


FG1
FG2 (pFus)
NMDA
I
II
III
IV
V
VI
643 1221 1799 2377
5-HT$_{1A}$
200 530 860 1190



Zilles K, & Palomero-Gallagher N: Transmitter receptor fingerprints in regions and layers of the human cerebral cortex. Frontiers Neuroanat (submitted)



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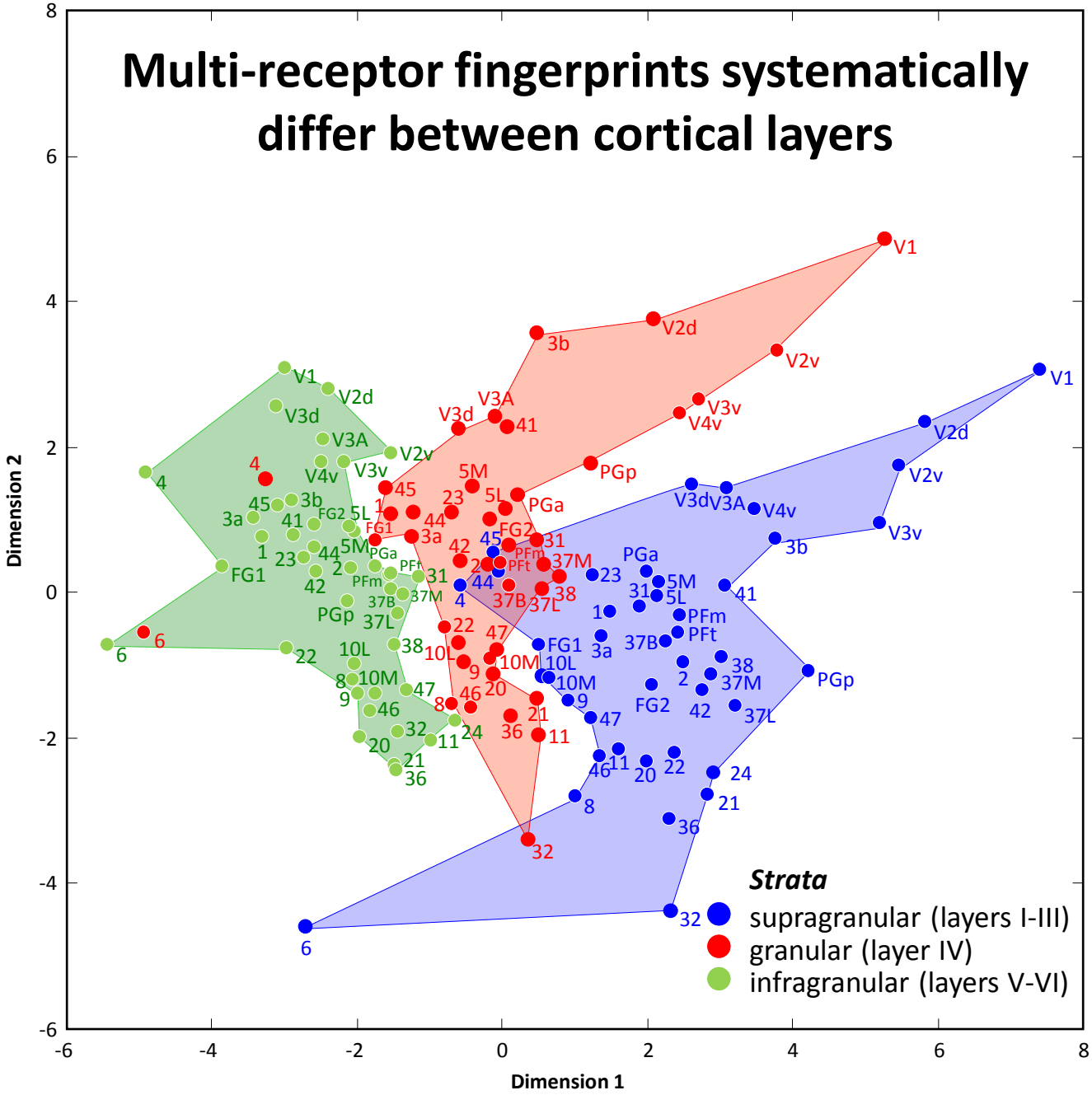
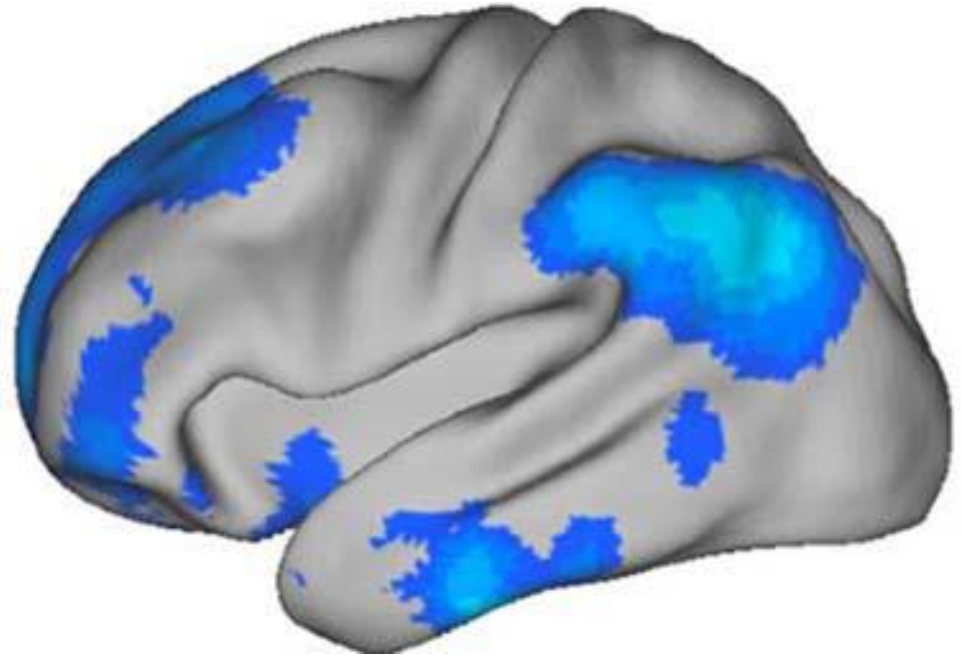
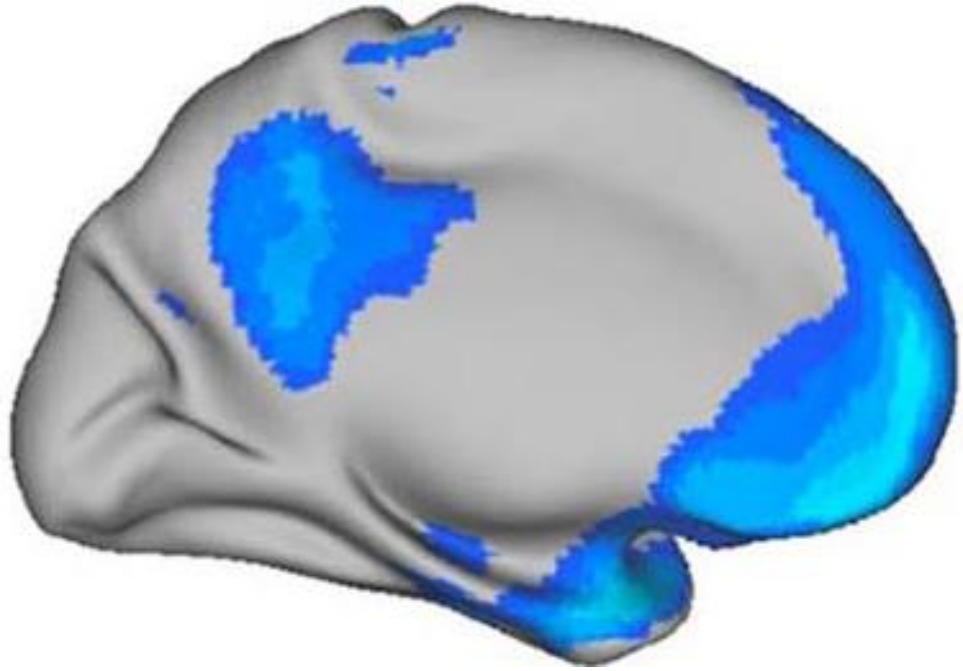
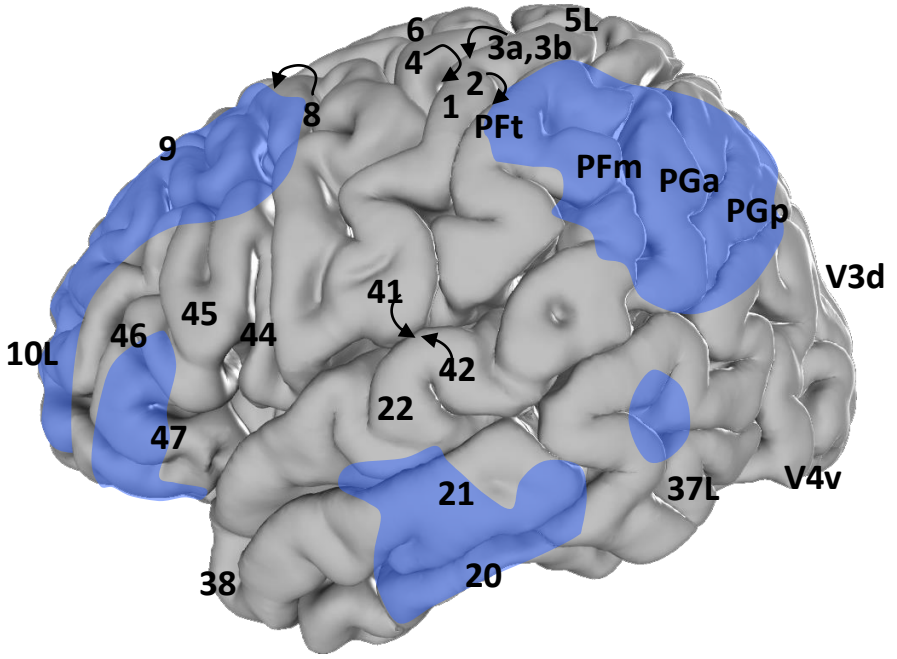


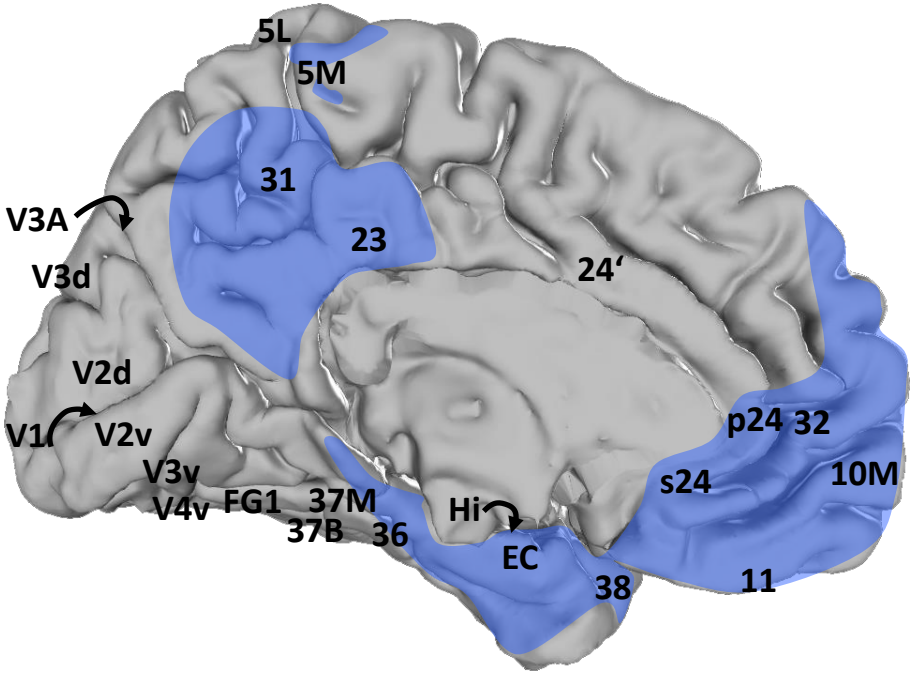
Zilles K, & Palomero-Gallagher N: Transmitter receptor fingerprints in regions and layers of the human cerebral cortex. Frontiers Neuroanat (submitted)

Receptors and the Default Mode Network





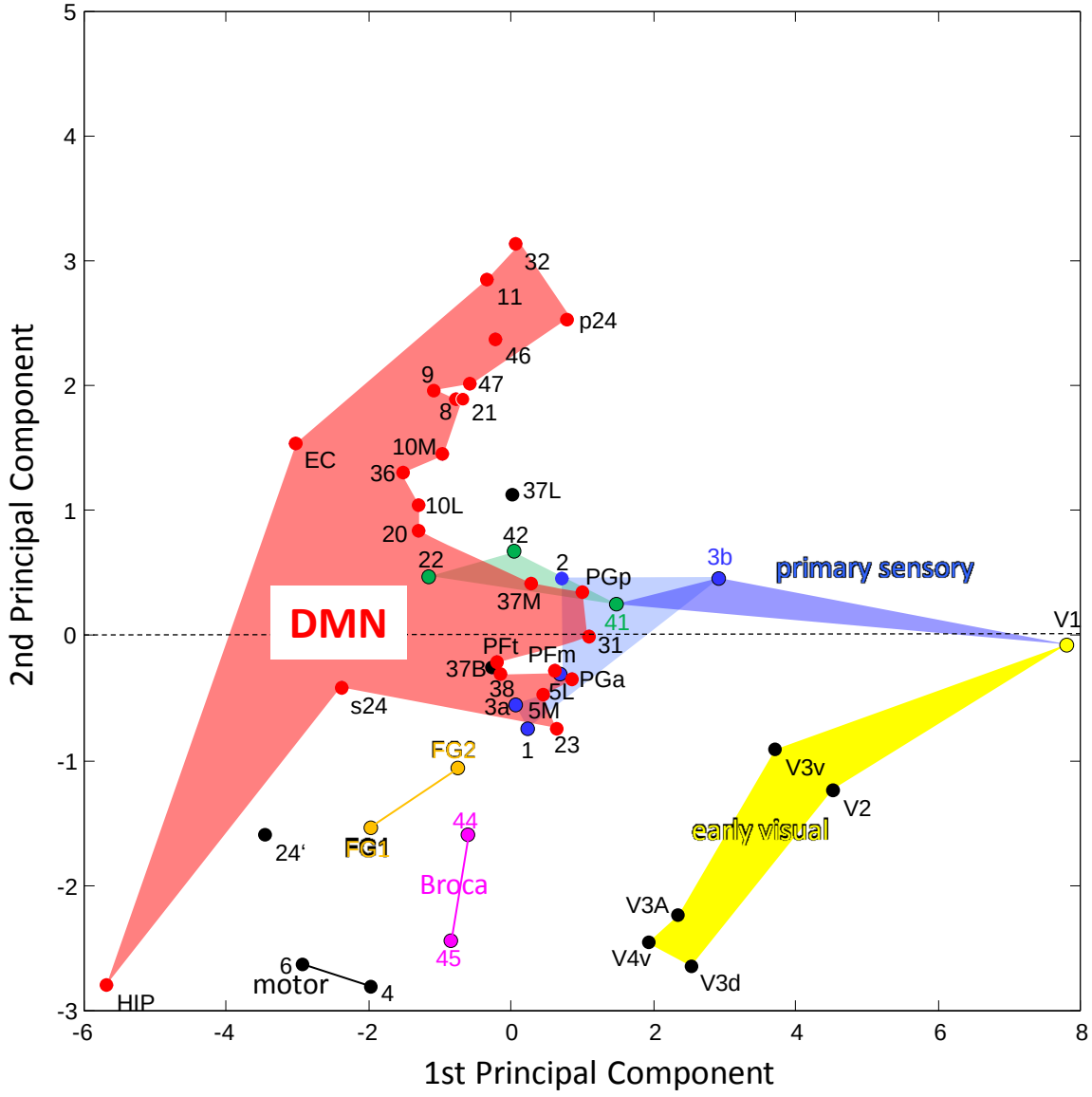




DMN areas defined by Buckner et al. (2008) *Ann NY Acad Sci* 1124: 1-38

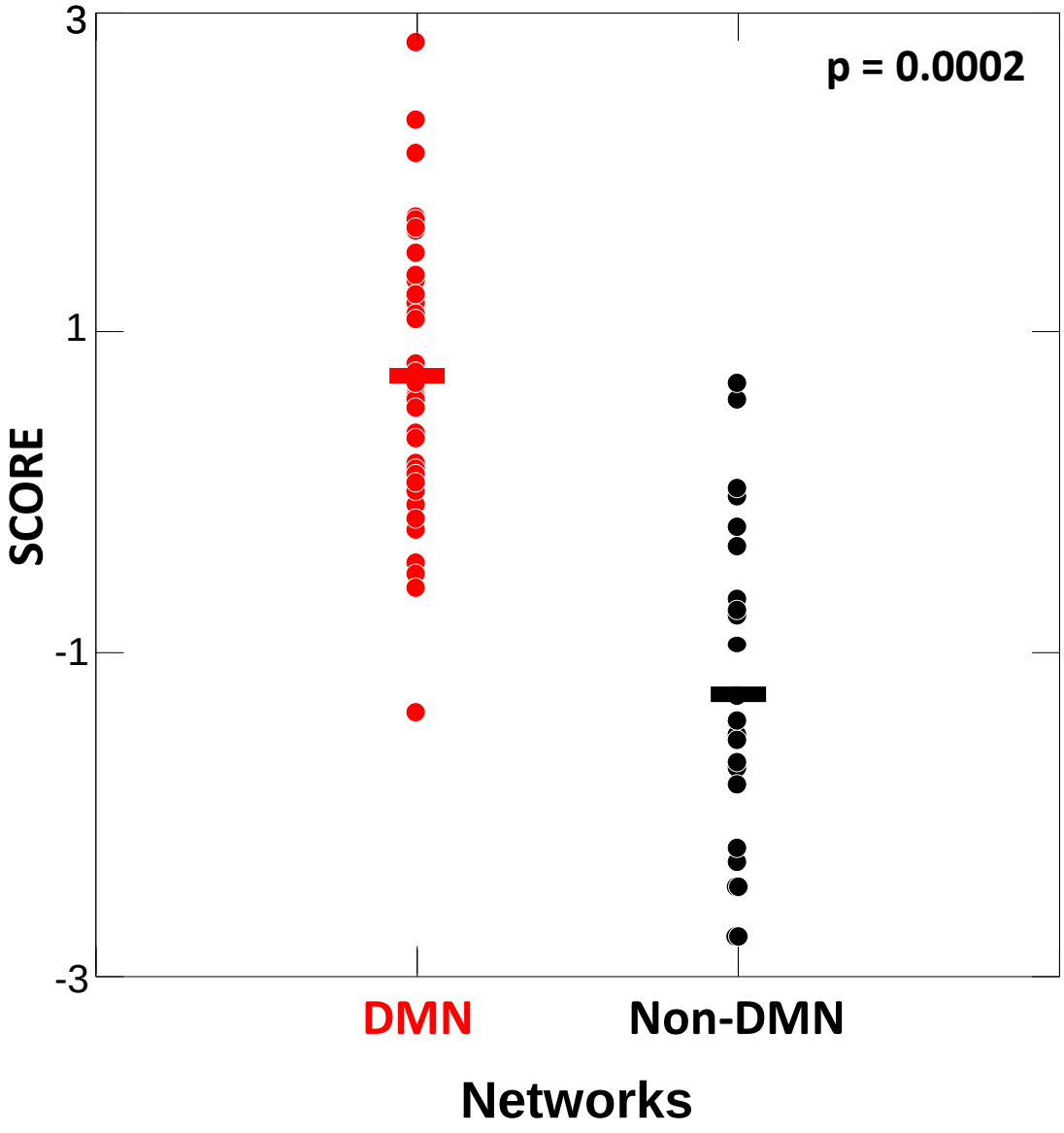
Cytoarchitectonic areas defined by Brodmann (1909) and JuBrain

Principal component analysis of receptor densities (averaged over all cortical layers) and default mode network DMN



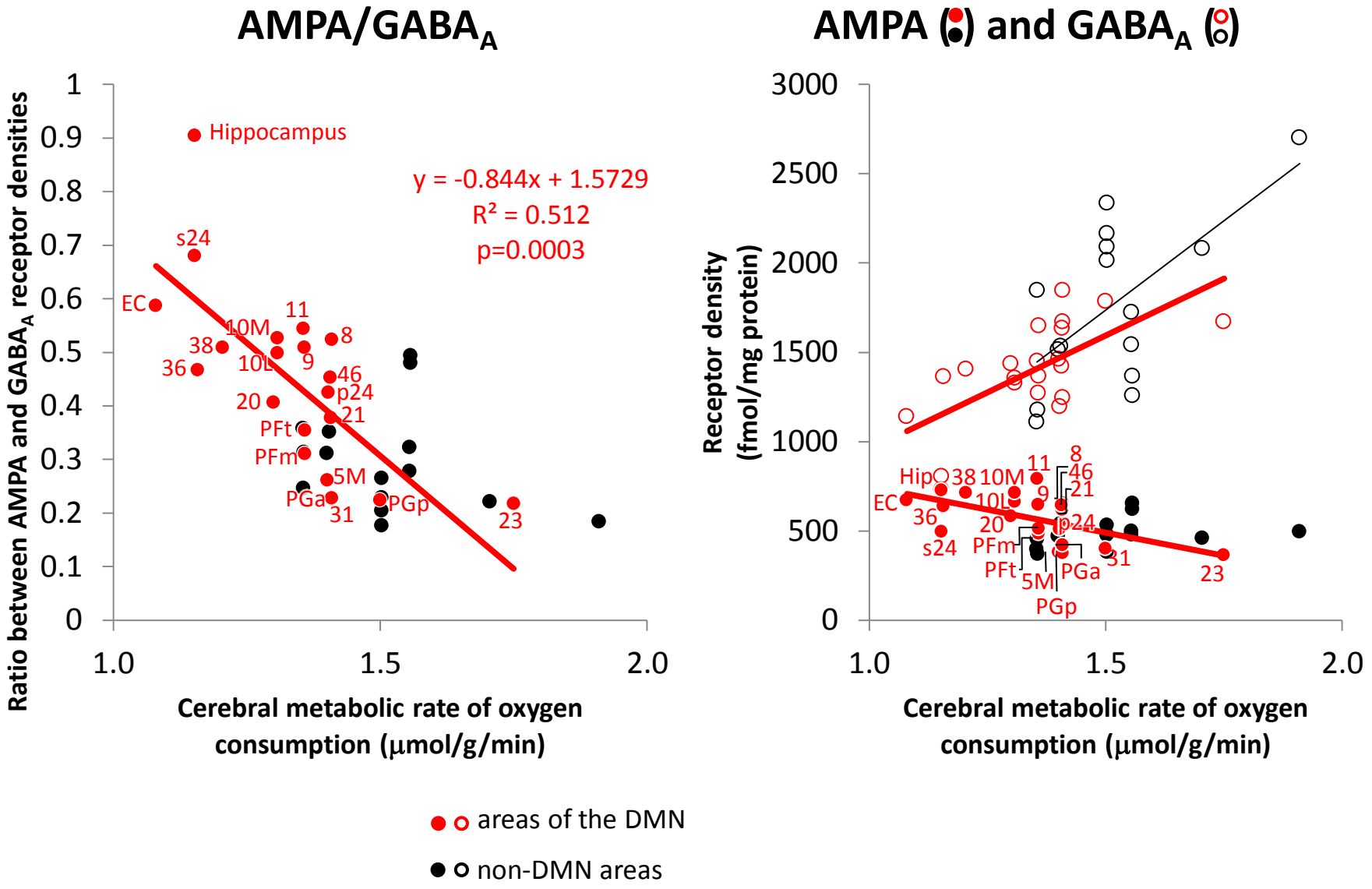
- posterior cingulate cortex (BA23)
- precuneus (BA31)
- anterior cingulate cortex (p24, s24)
- ventral medial prefrontal cortex (10m, BA32, BA11)
- dorsal medial prefrontal cortex (medial part of BA9)
- inferior parietal lobule (PGa, PGp, PFm, PFt)
- lateral temporal cortex (BA20, BA21)
- anterior temporal pole (BA38)
- hippocampus, entorhinal (EC), and parahippocampal (BA36) cortex

Discriminance analysis of the densities (averaged over all cortical layers) of all receptor types between default mode network DMN areas and Non-DMN areas



Contribution of each receptor type to the segregation between DMN and Non-DMN areas

Receptor	Standardized score
M_2	-1.6384
Kainate	-1.2543
α_2	1.2023
AMPA	1.1607
5-HT_2	1.1296
NMDA	-0.9699
M_3	0.7538
$GABA_B$	0.6951
D_1	-0.3707
$\alpha_4\beta_2$	0.3540
5-HT_{1A}	-0.2558
α_1	-0.2137
$GABA_A$/BZ	-0.0288
$GABA_A$	0.0235
M_1	-0.0221



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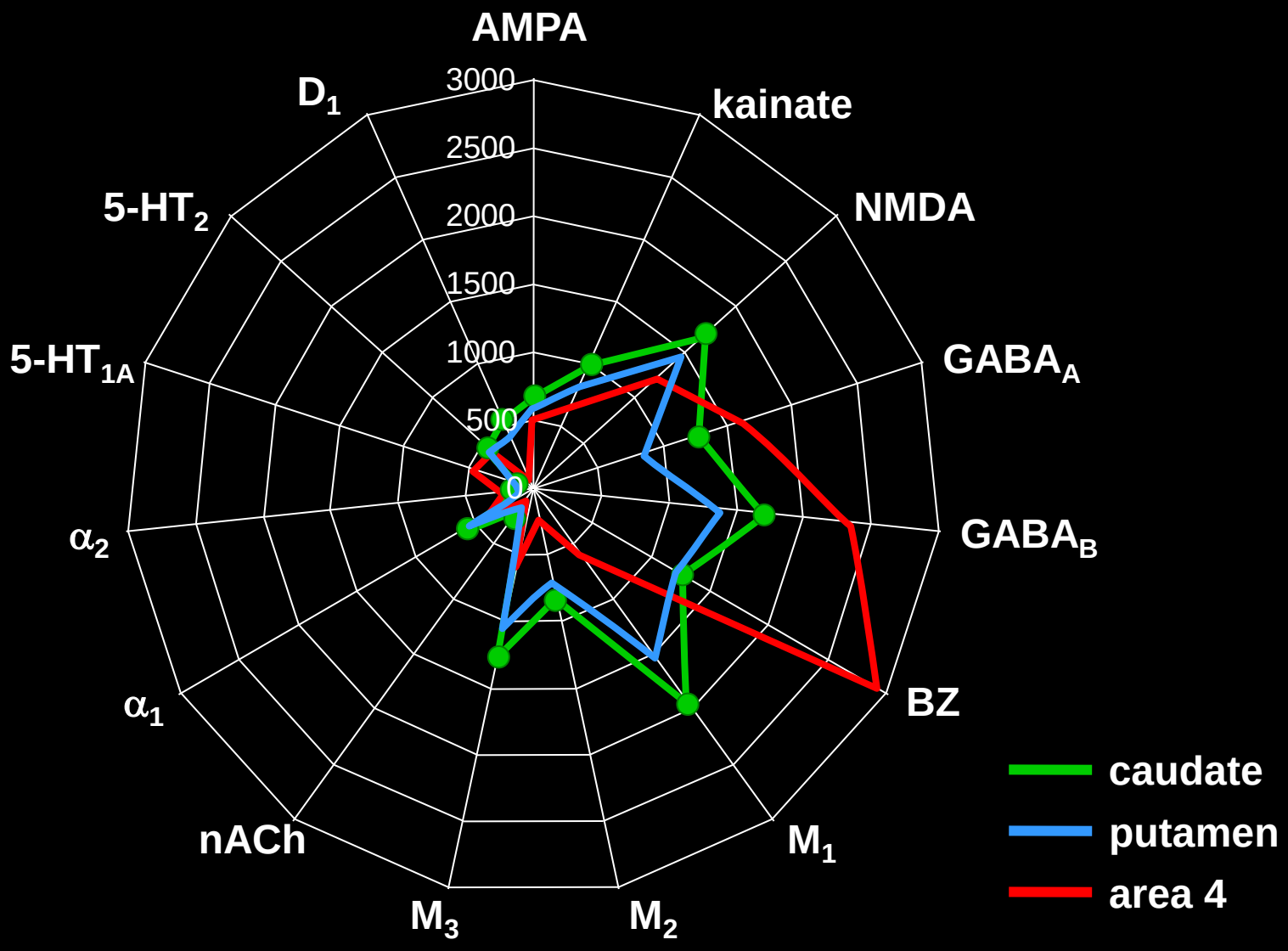
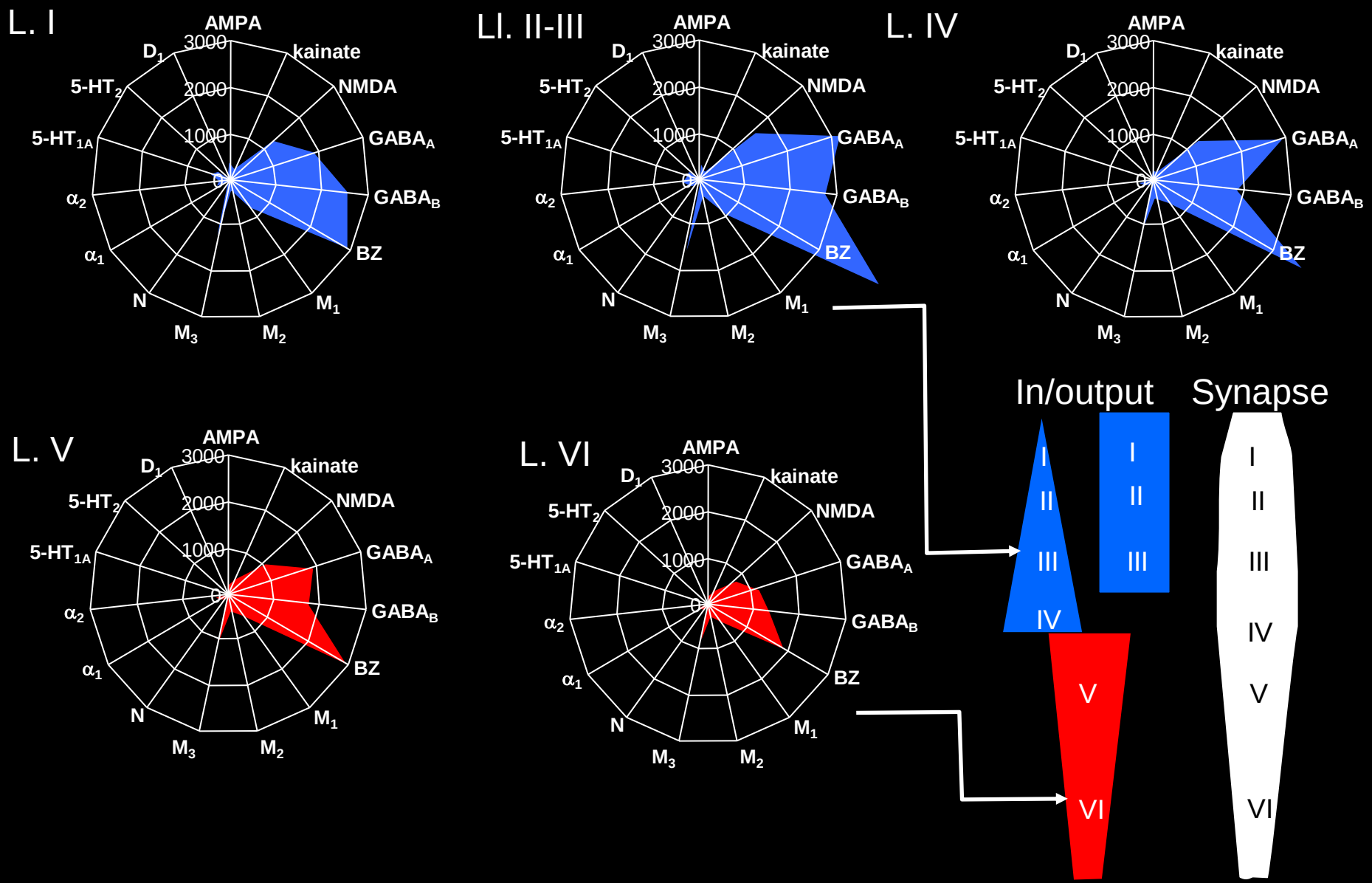
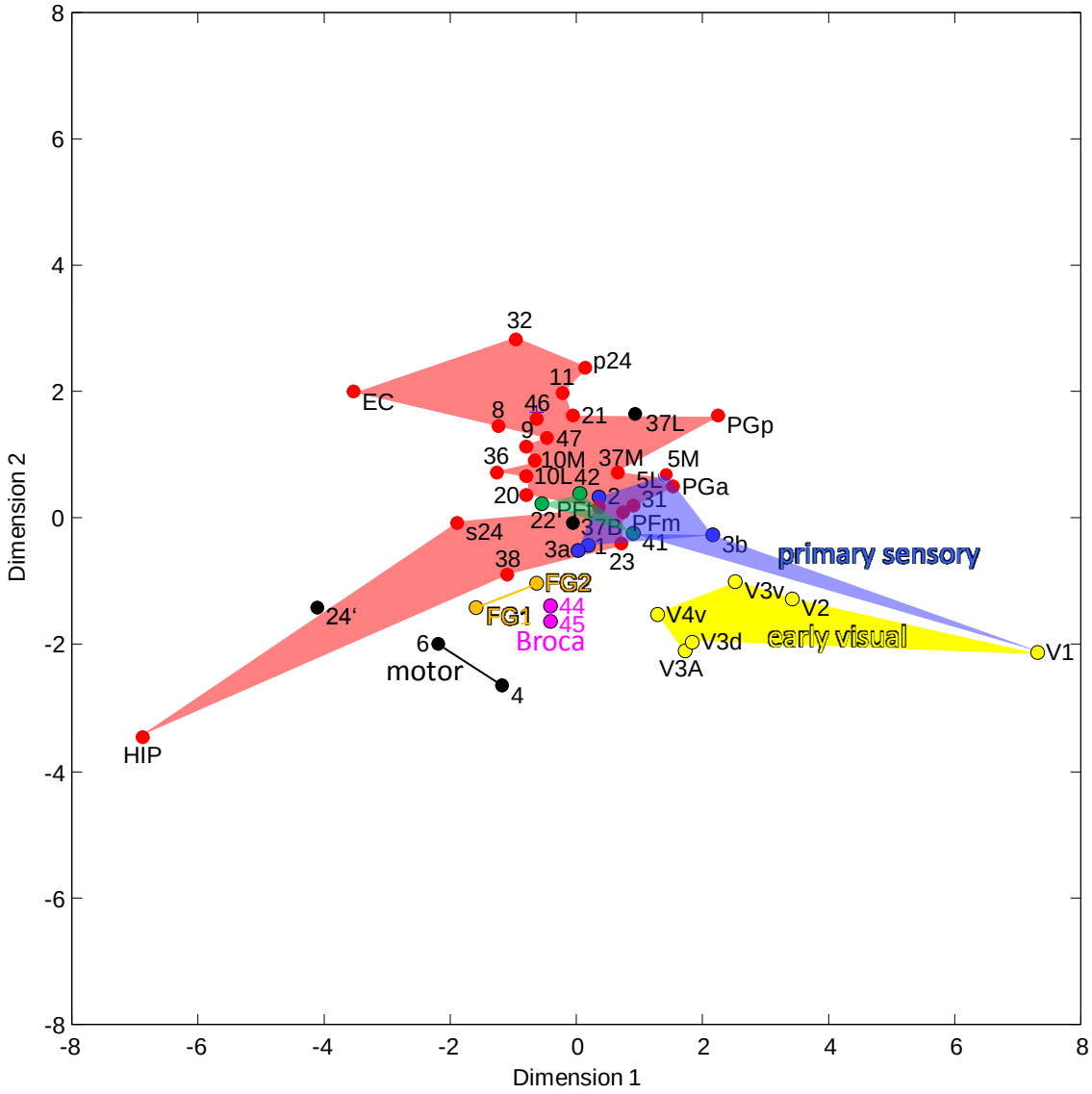
A receptor fingerprint is…



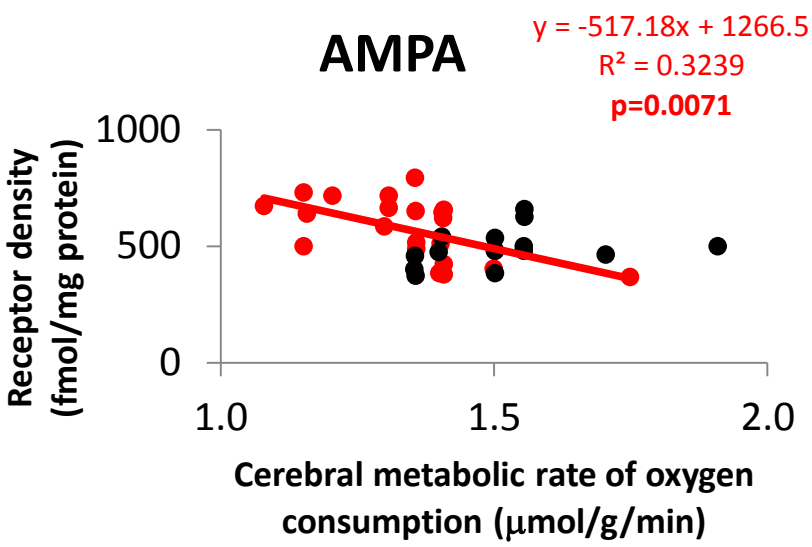
Receptor Fingerprints of the primary visual cortex: layer specificity

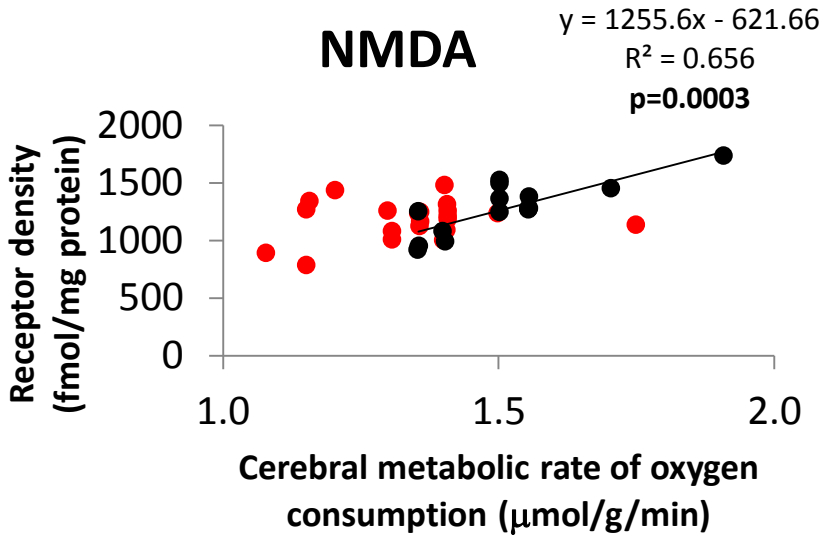


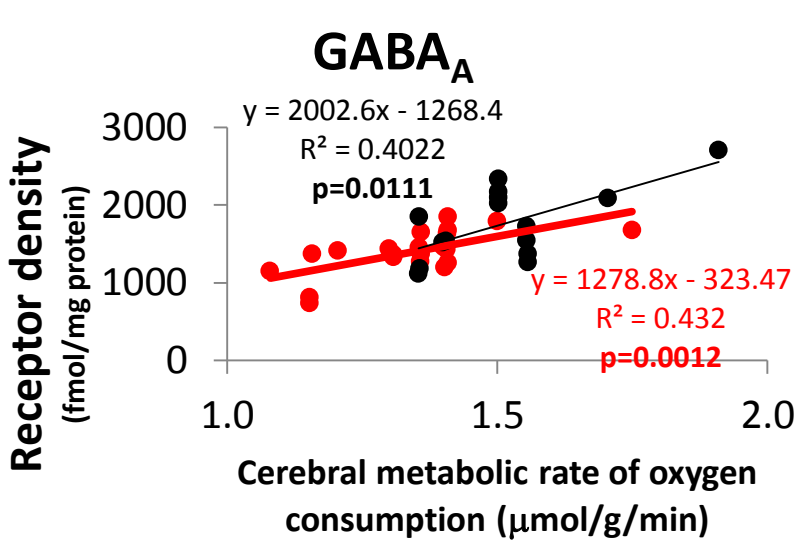
Multidimensional scaling analysis of receptor densities (averaged over all cortical layers) and default mode network DMN

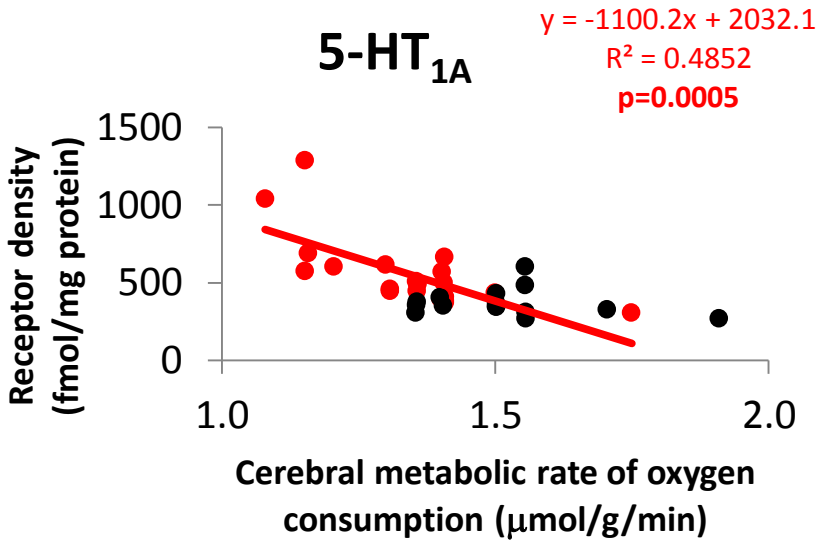


- posterior cingulate cortex (BA23)
- precuneus (BA31)
- anterior cingulate cortex (p24, s24)
- ventral medial prefrontal cortex (10m, BA32, BA11)
- dorsal medial prefrontal cortex (medial part of BA9)
- inferior parietal lobule (PGa, PGp, PFm, PFt)
- lateral temporal cortex (BA20, BA21)
- anterior temporal pole (BA38)
- hippocampus, entorhinal (EC), and parahippocampal (BA36) cortex









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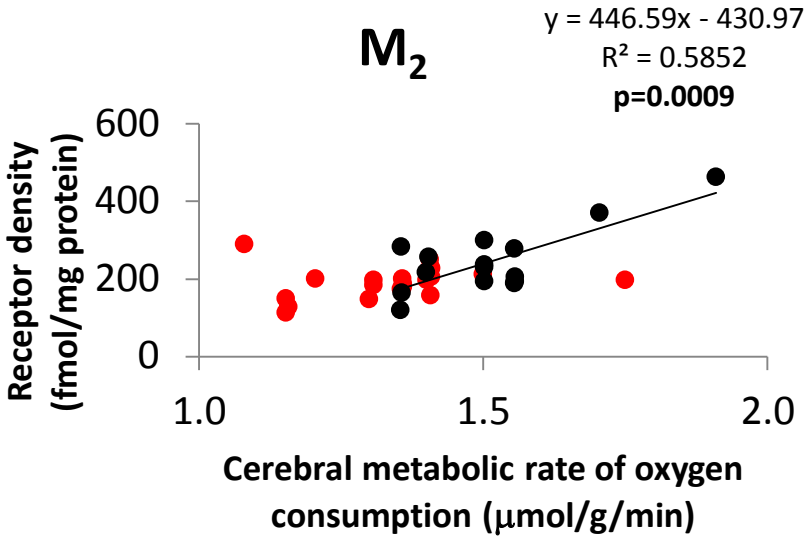

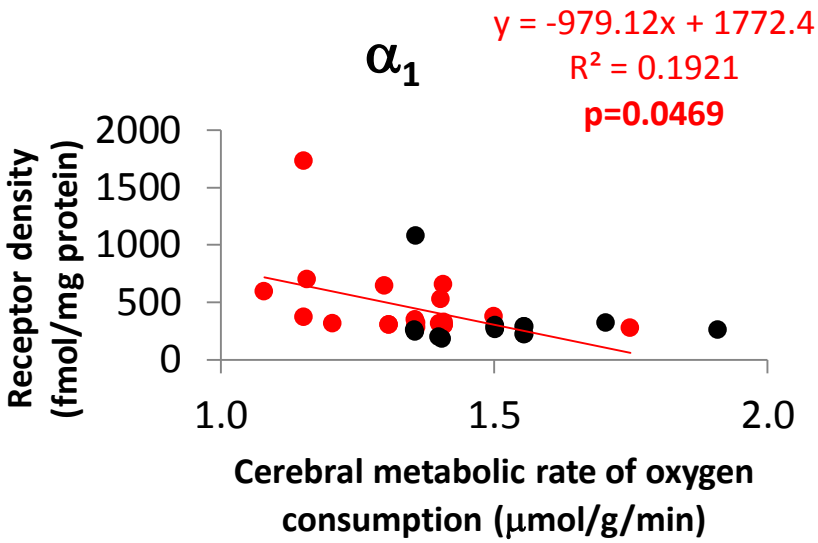

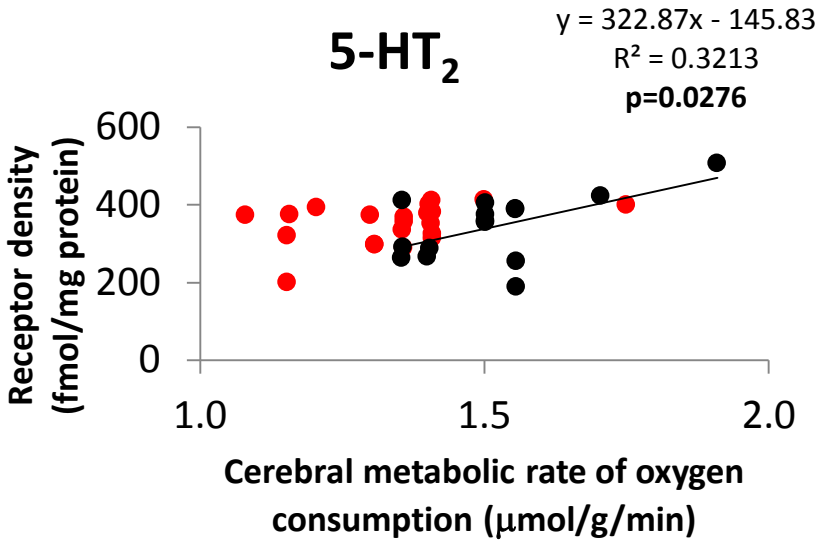

DMN: cerebral metabolic rate of oxygen consumption *decreases* with increasing AMPA, 5-HT_{1A} and α_1 receptor densities, but *increases* with increasing $GABA_A$ receptor densities

Non-DMN: cerebral metabolic rate of oxygen consumption *increases* with increasing NMDA, $GABA_A$, M_2 and 5-HT_2 receptor densities

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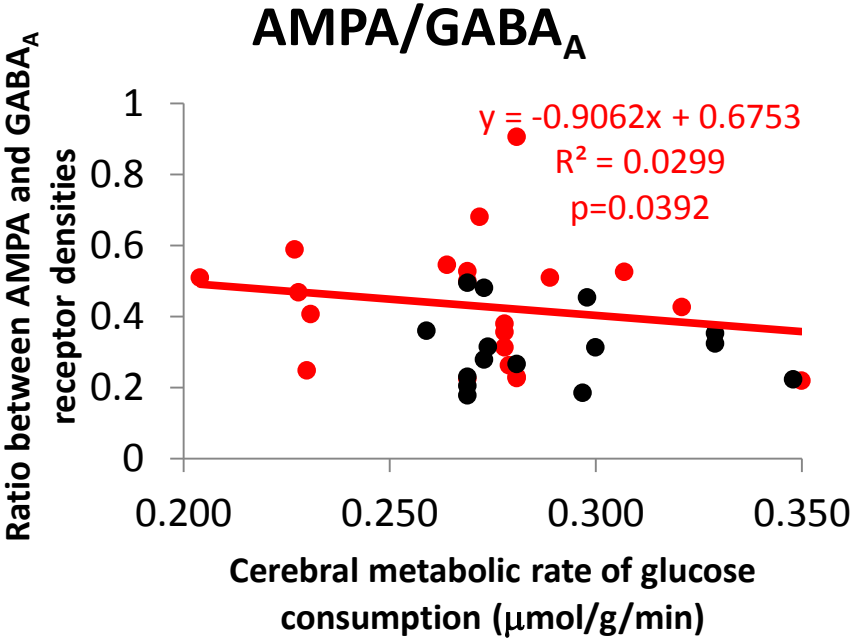

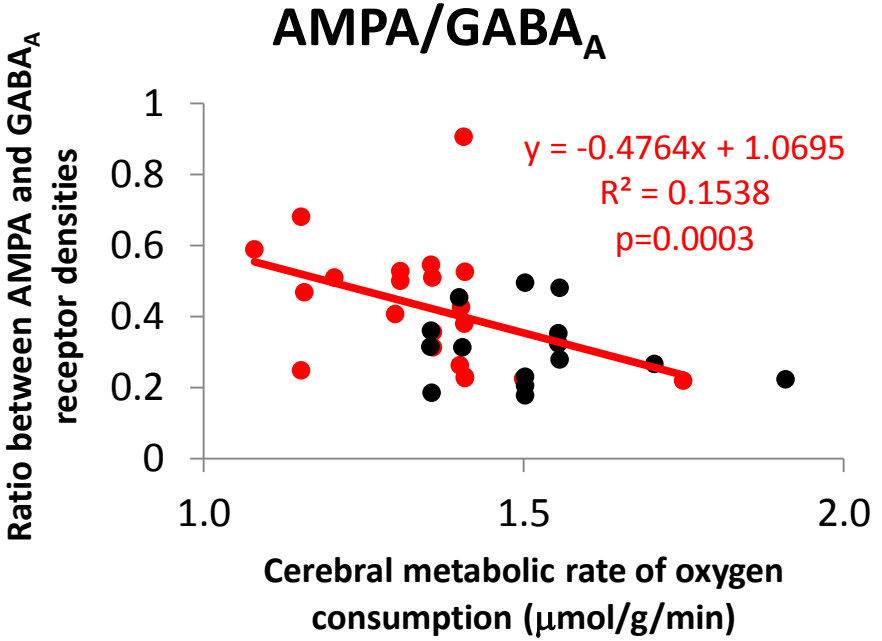

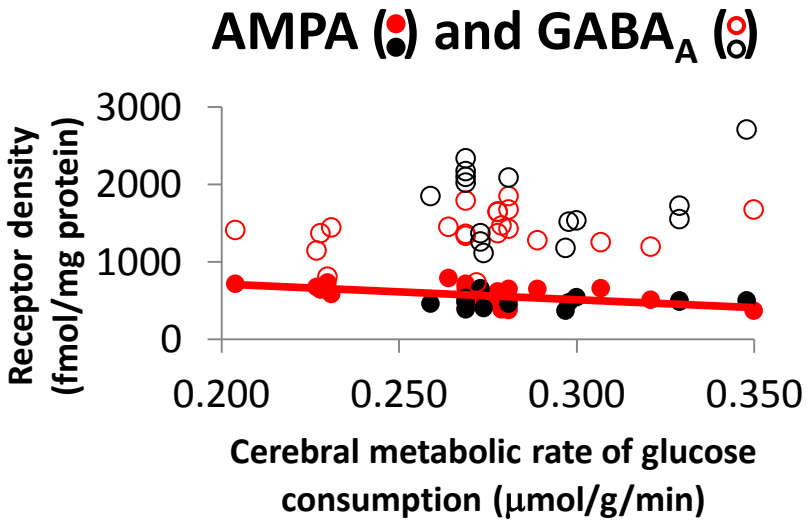

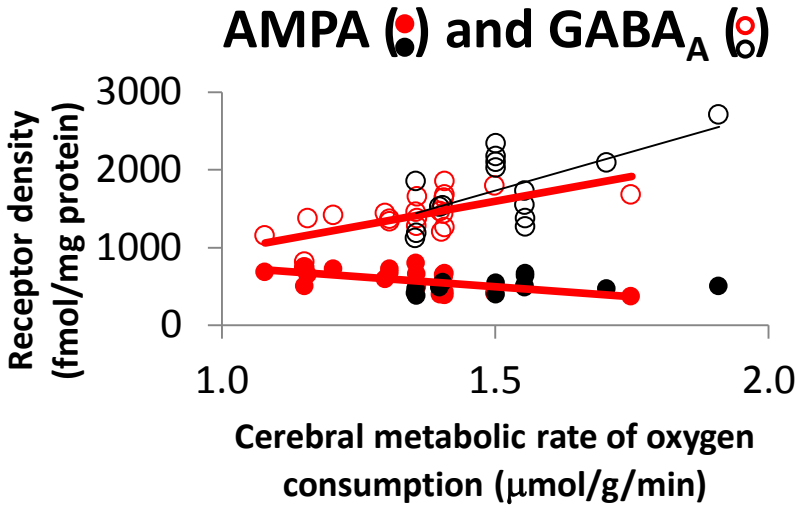

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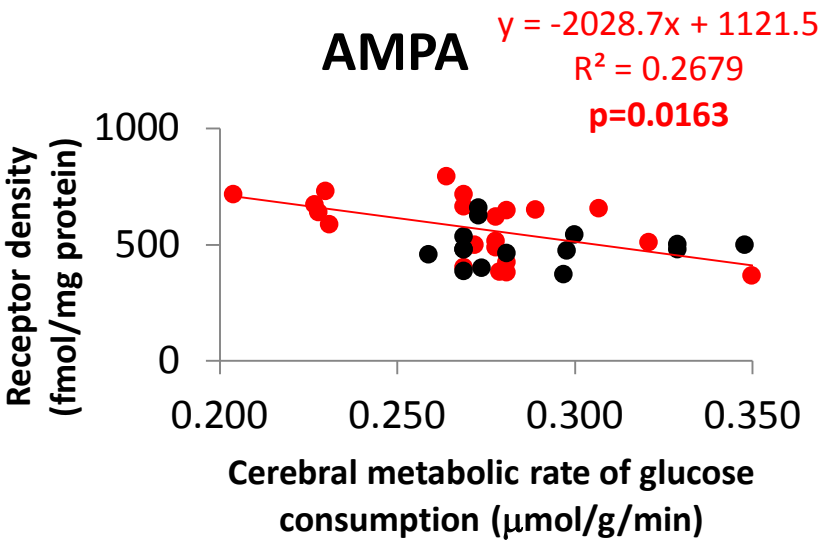

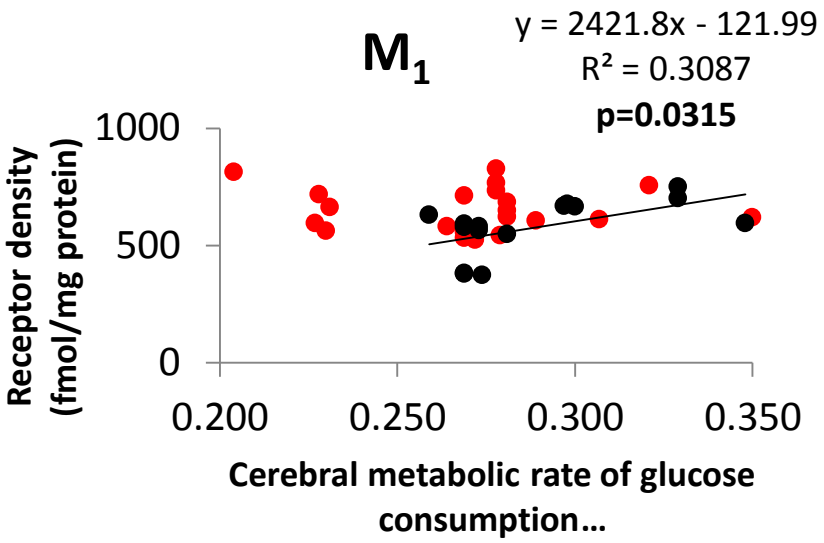

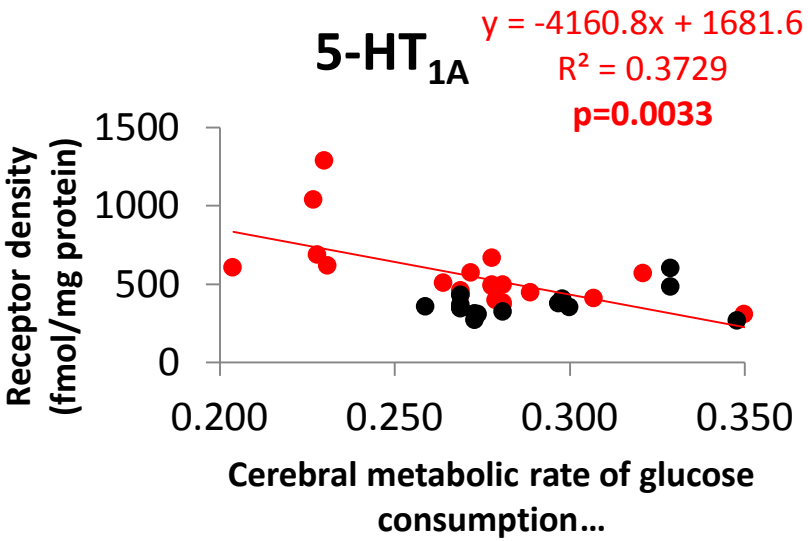

DMN: cerebral metabolic rate of glucose consumption *decreases* with increasing AMPA and 5-HT_{1A} receptor densities

Non-DMN: cerebral metabolic rate of glucose consumption *increases* with increasing AMPA and M_1 receptor densities

Metabolic data from: Hyder F, Herman P, Bailey CJ, Møller A, Globinsky R, Fulbright RK, Rothman DL, Gjedde A (2016) Uniform distributions of glucose oxidation and oxygen extraction in gray matter of normal human brain: No evidence of regional differences of aerobic glycolysis. *J Cerebral Blood Flow & Metabolism* 36(5): 903–916

Summary

High densities of receptors significantly correlate, or show a non-significant trend, with **low** cerebral metabolic rates of glucose or oxygen consumption in **DMN** areas. A notable exception is the positive correlation of the densities of inhibitory $GABA_A$ receptors with the cerebral metabolic rate of oxygen consumption.

High densities of receptors significantly correlate, or show a non-significant trend, with **high** cerebral metabolic rates of glucose or oxygen consumption in **non-DMN** areas (mainly primary and higher sensory areas, motor areas).