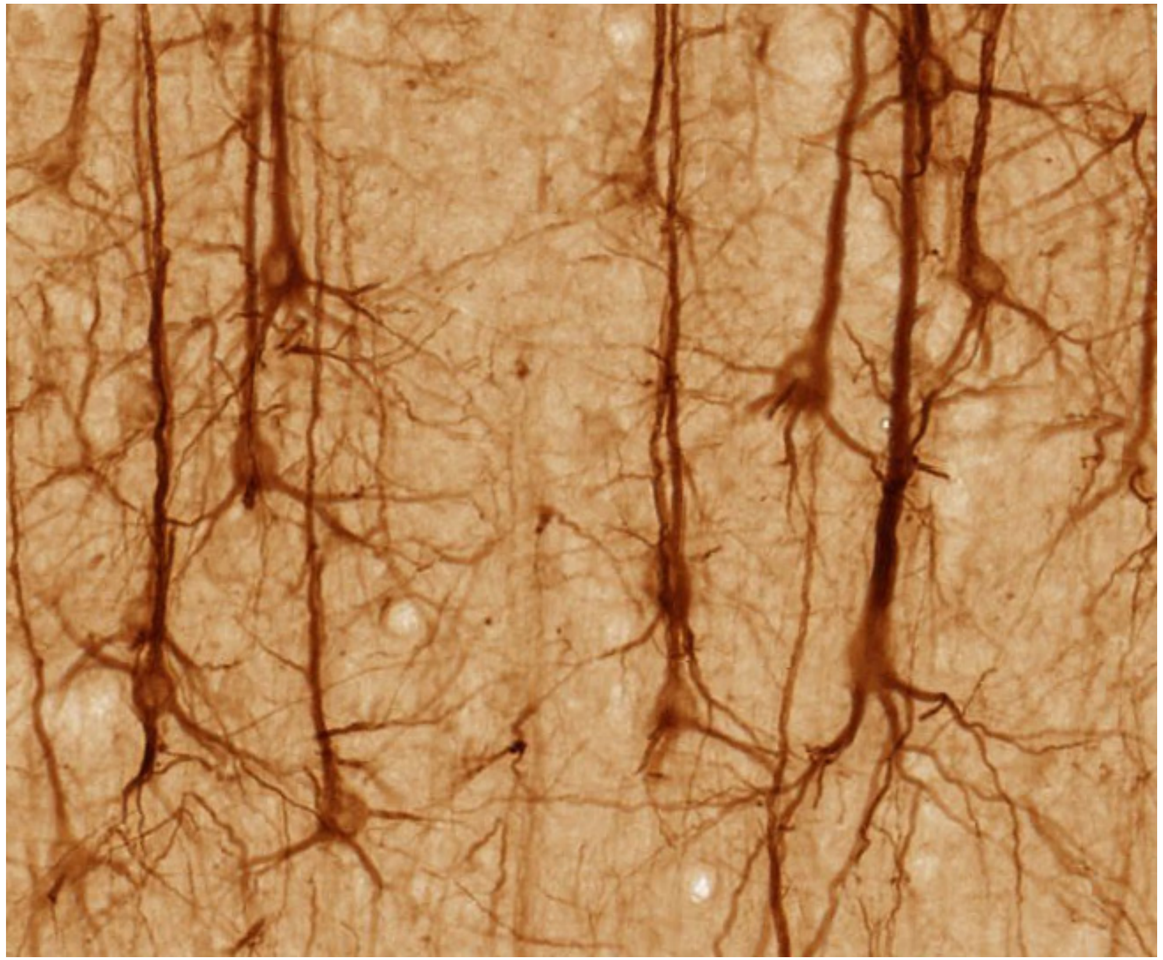
Functional and molecular neuroimaging *in vivo*

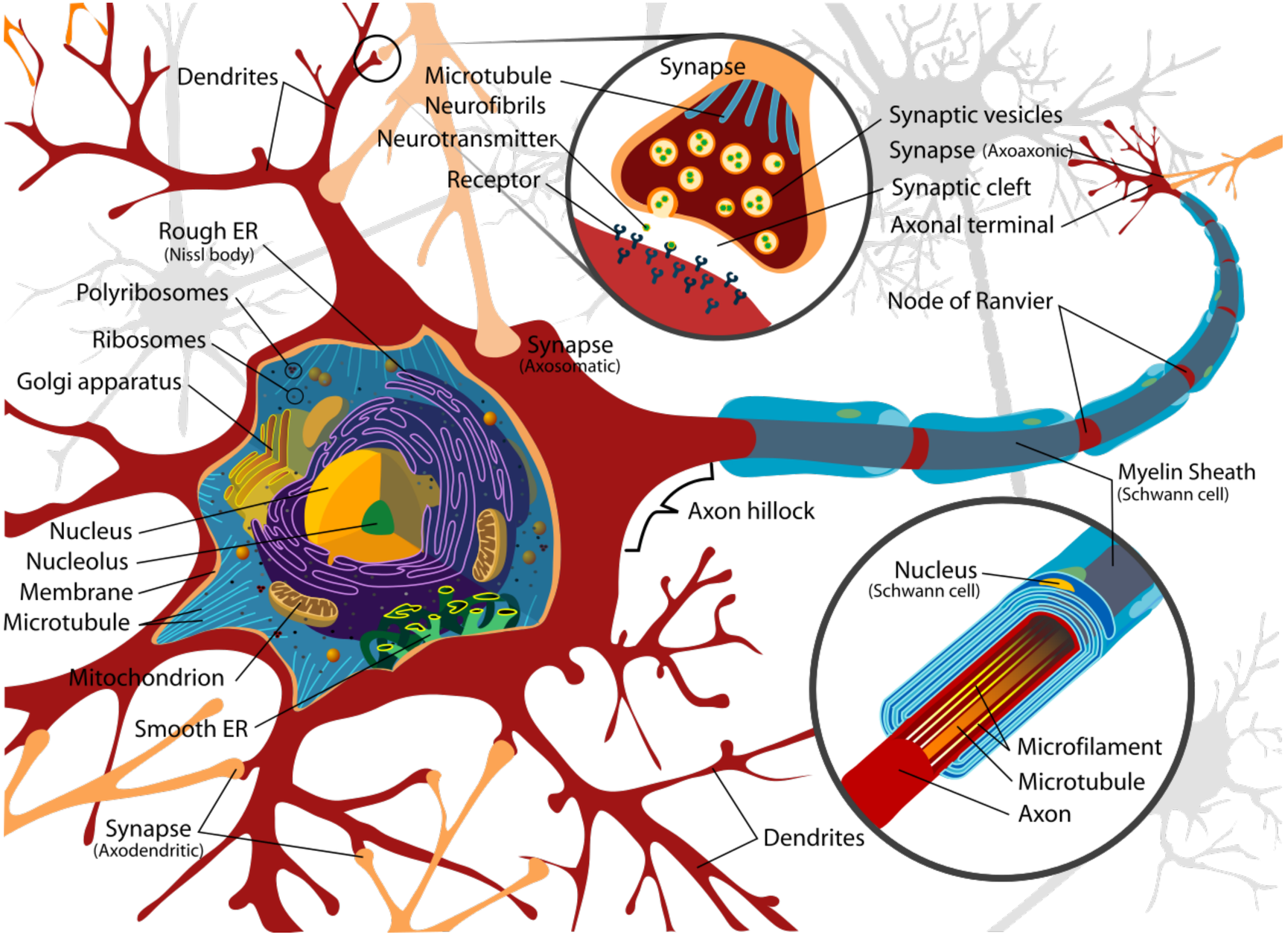
Lauri Nummenmaa
Aalto University and Turku PET Centre

Why do we need to understand brain function?

- To aid in clinical diagnosis
- To understand physiology of the CNS
- To develop drugs that target CNS
- To understand ‘higher’ mental functions such as emotions and memory



Neuroscientist’s three main problems


Dendrites
Microtubule
Neurofibrils
Neurotransmitter
Receptor
Synapse
Synaptic vesicles
Synapse (Axoaxonic)
Synaptic cleft
Axonal terminal
Rough ER
(Nissl body)
Polyribosomes
Ribosomes
Golgi apparatus
Synapse
(Axosomatic)
Node of Ranvier
Myelin Sheath
(Schwann cell)
Axon hillock
Nucleus
Nucleolus
Membrane
Microtubule
Mitochondrion
Smooth ER
Synapse
(Axodendritic)
Dendrites
Nucleus
(Schwann cell)
Microfilament
Microtubule
Axon

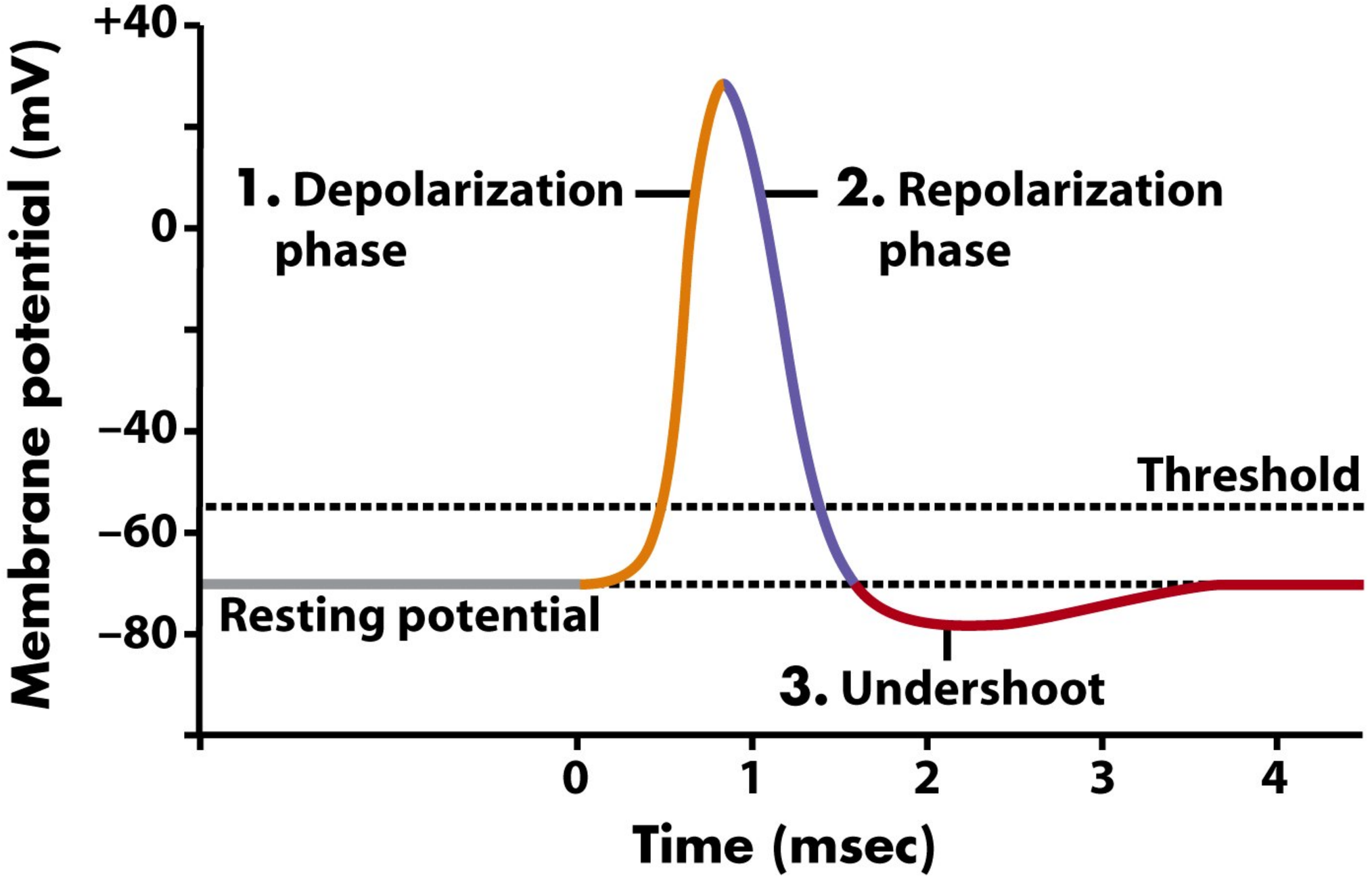
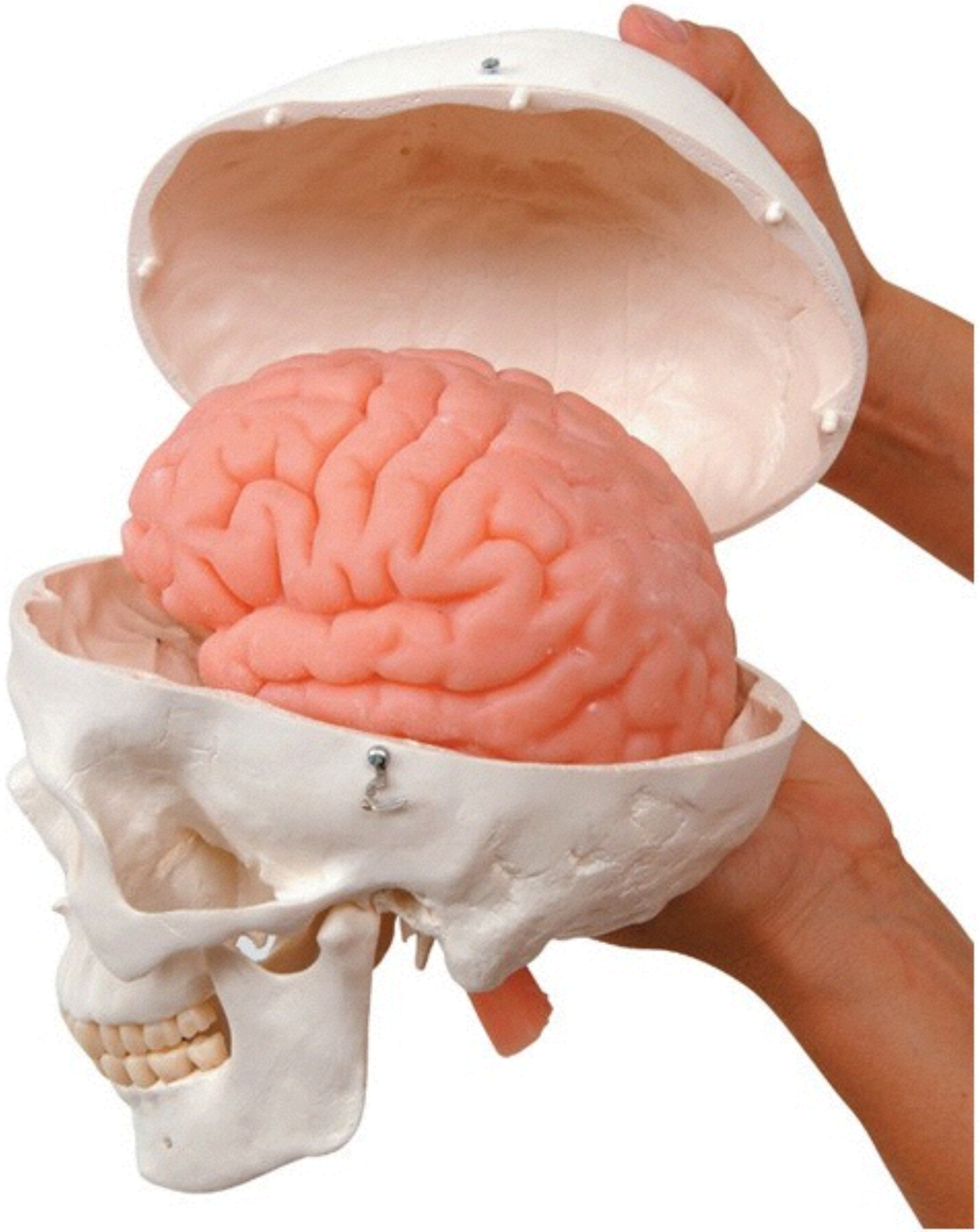
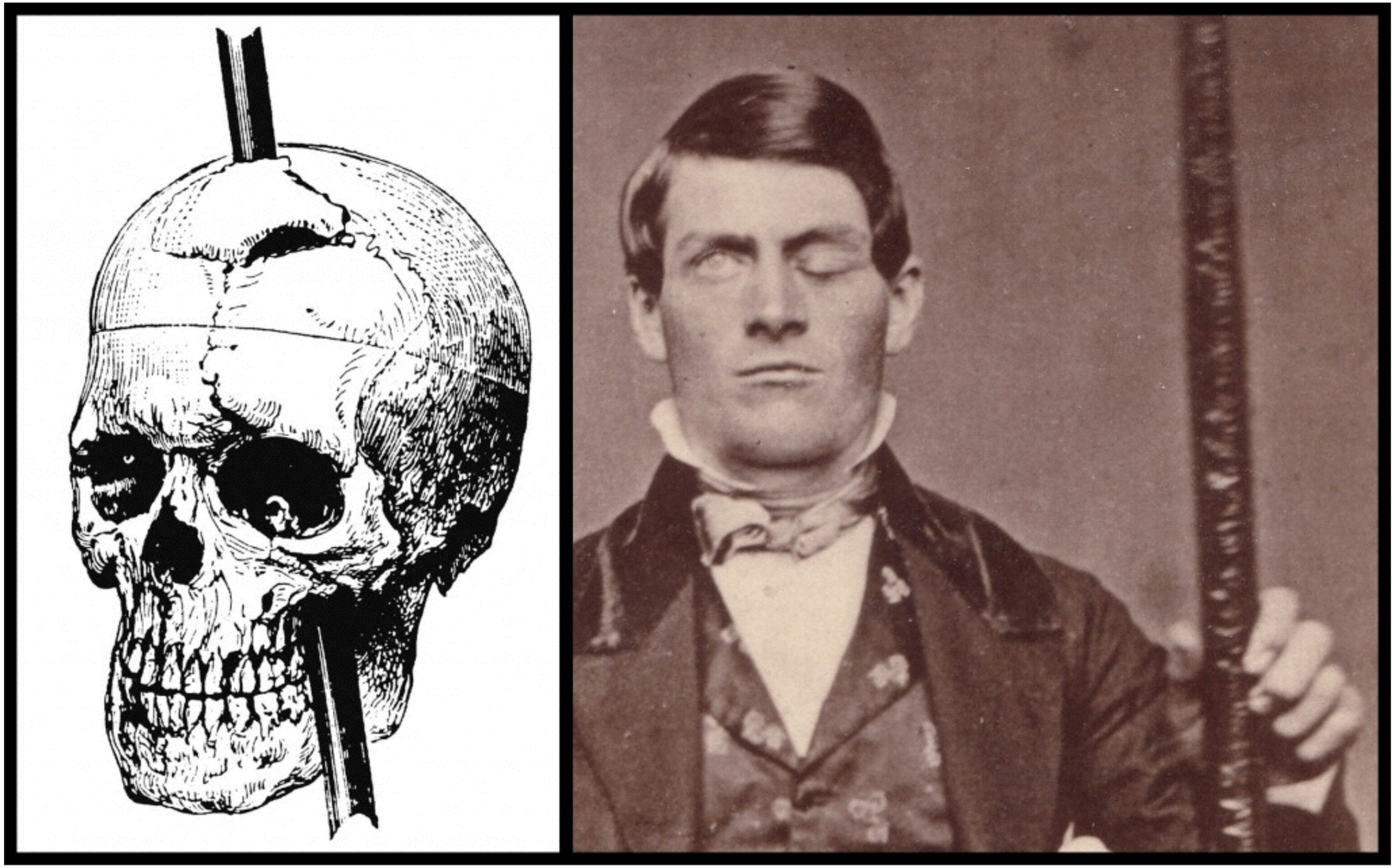
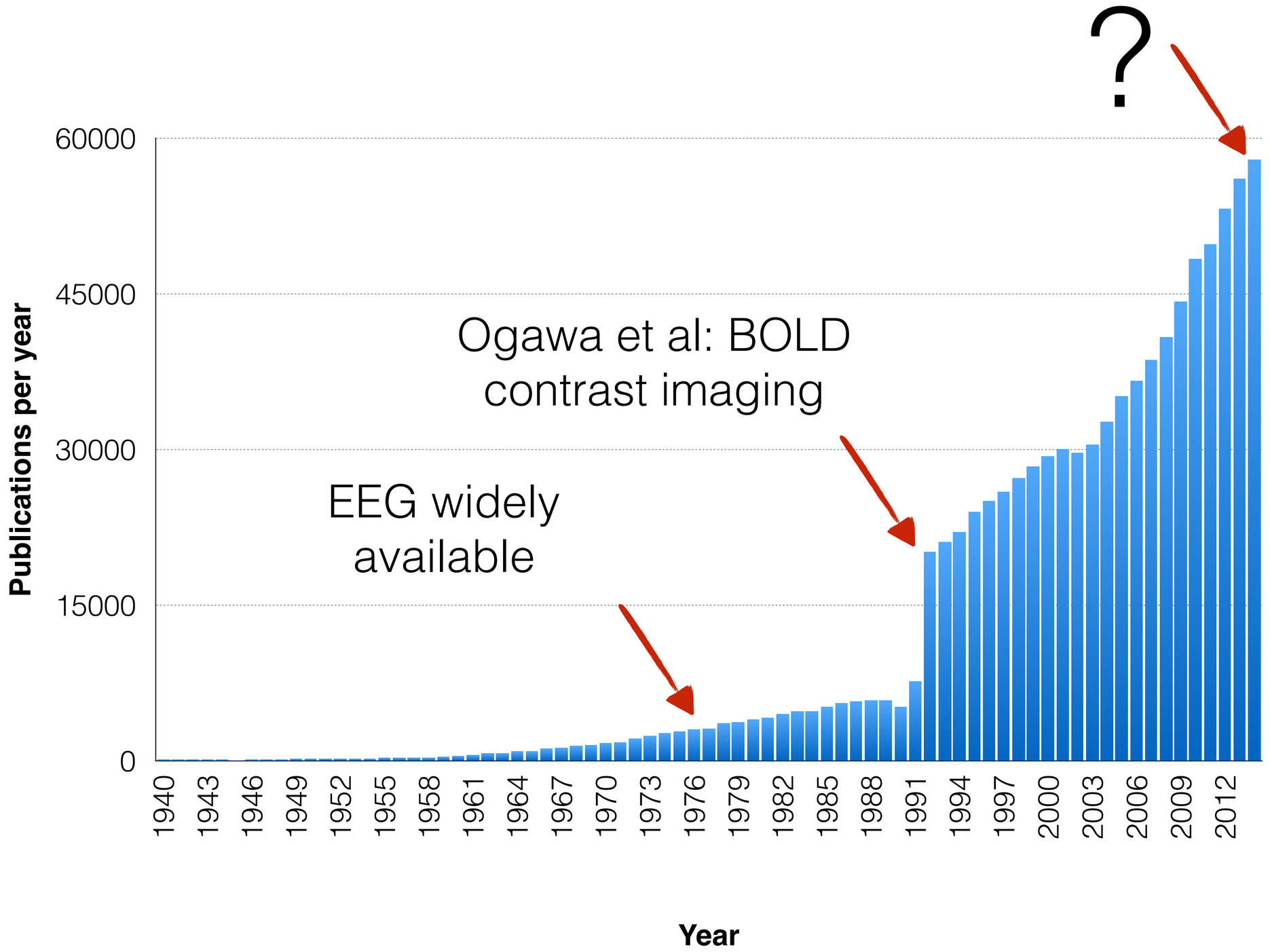


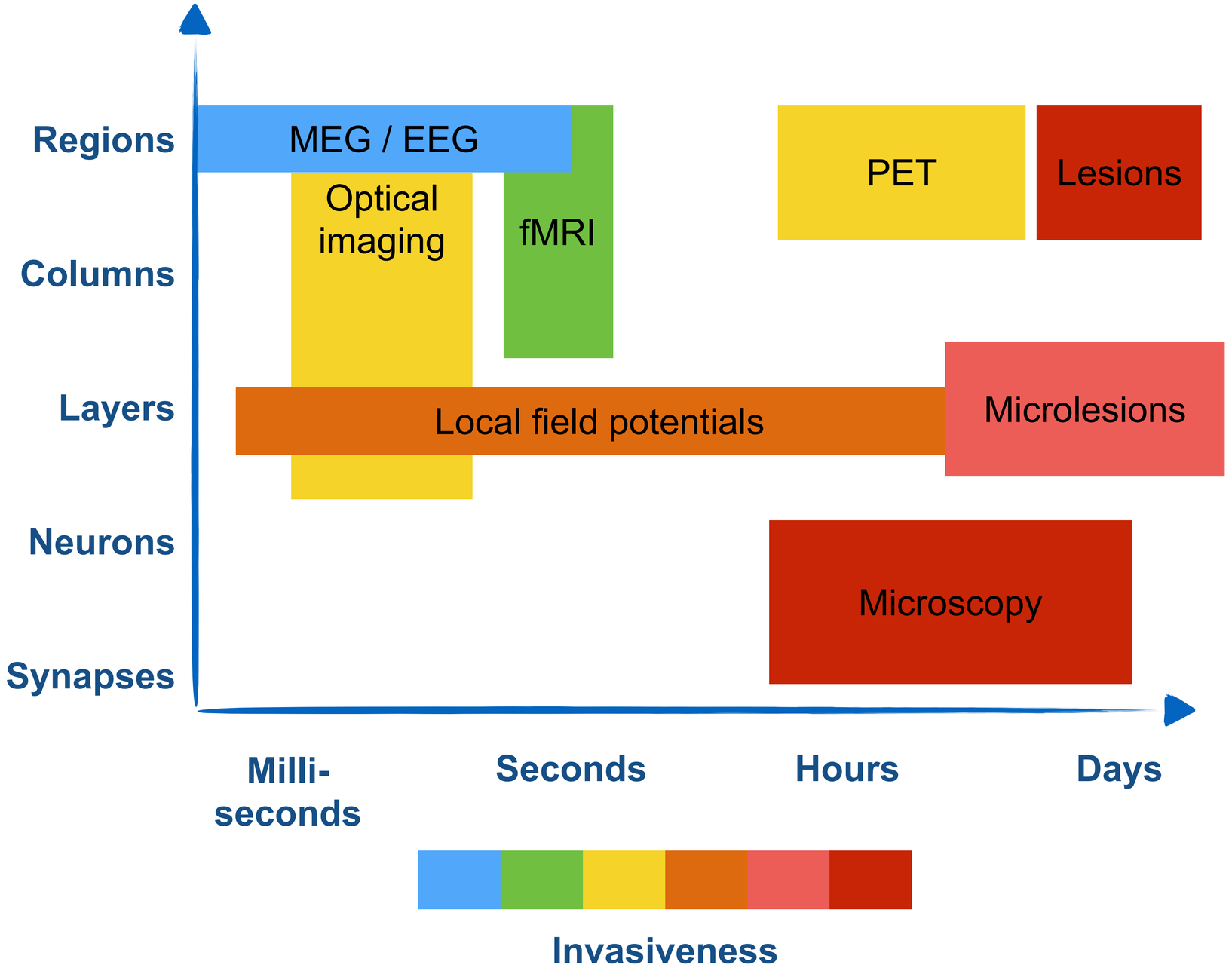
Figure 45-5 Biological Science, 2/e

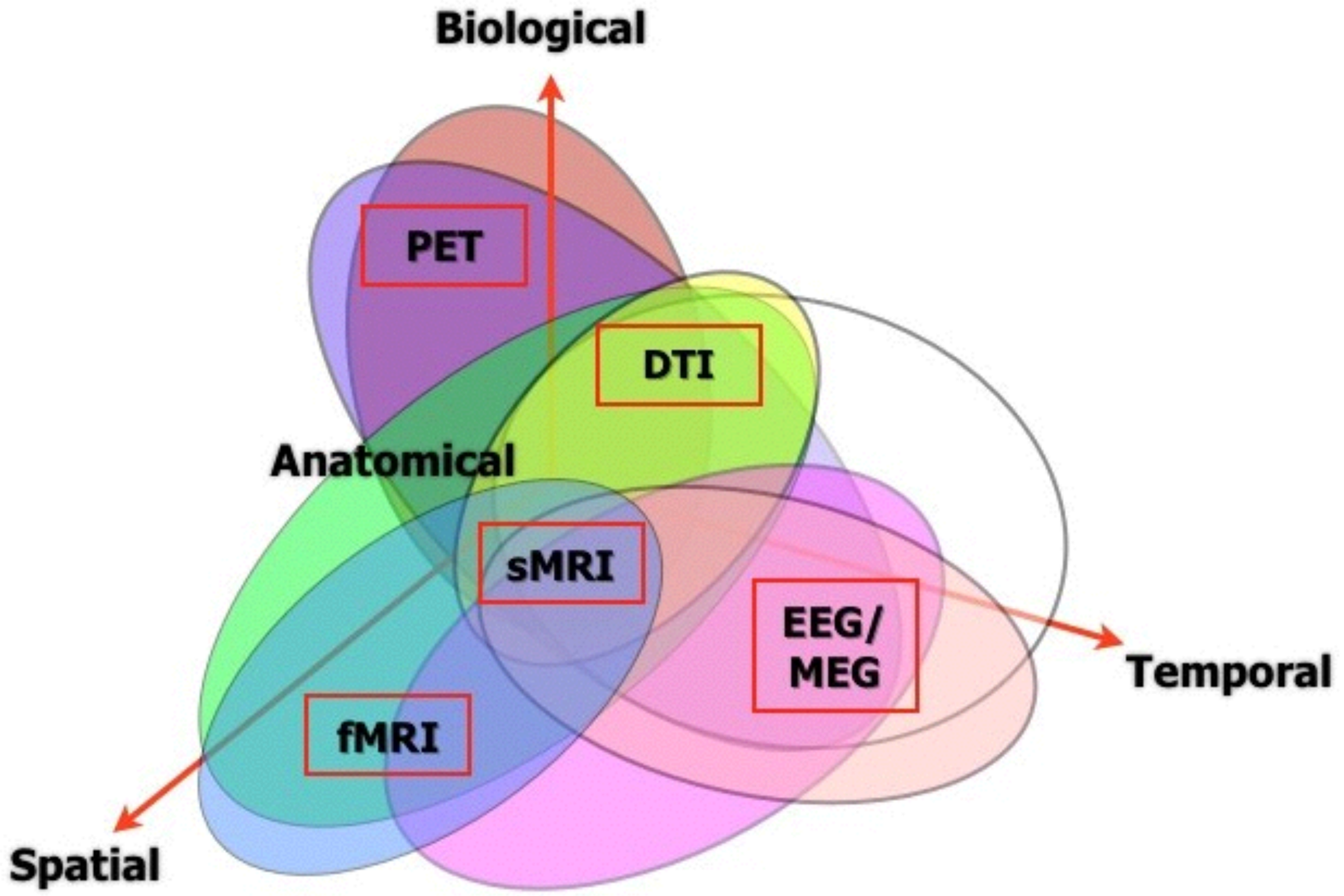


Traditional cognitive neuroscience




?
60000
45000
30000
15000
0
Publications per year
Ogawa et al: BOLD contrast imaging
EEG widely available
1940
1943
1946
1949
1952
1955
1958
1961
1964
1967
1970
1973
1976
1979
1982
1985
1988
1991
1994
1997
2000
2003
2006
2009
2012
Year

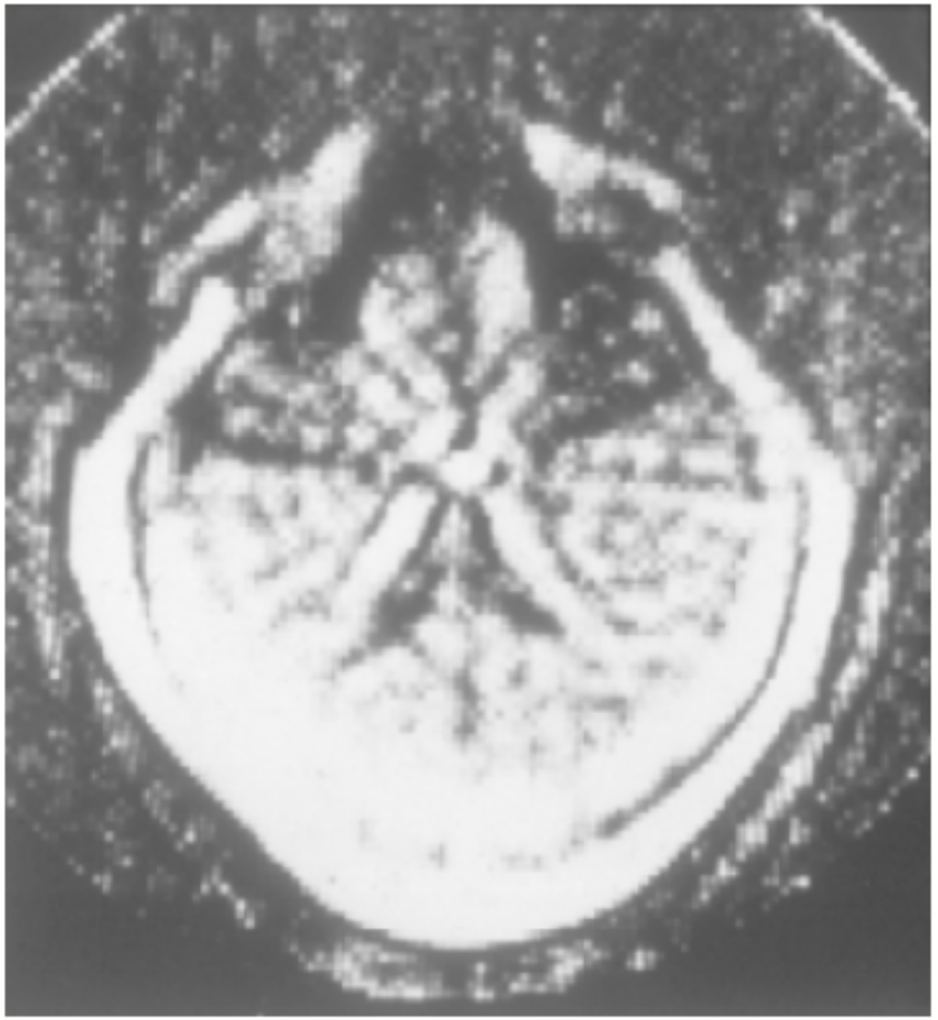

Regions
Columns
Layers
Neurons
Synapses
MEG / EEG
Optical imaging
fMRI
PET
Lesions
Local field potentials
Microlesions
Microscopy
Milli-seconds
Seconds
Hours
Days
Invasiveness


Biological
PET
DTI
Anatomical
sMRI
EEG/
MEG
Temporal
fMRI
Spatial

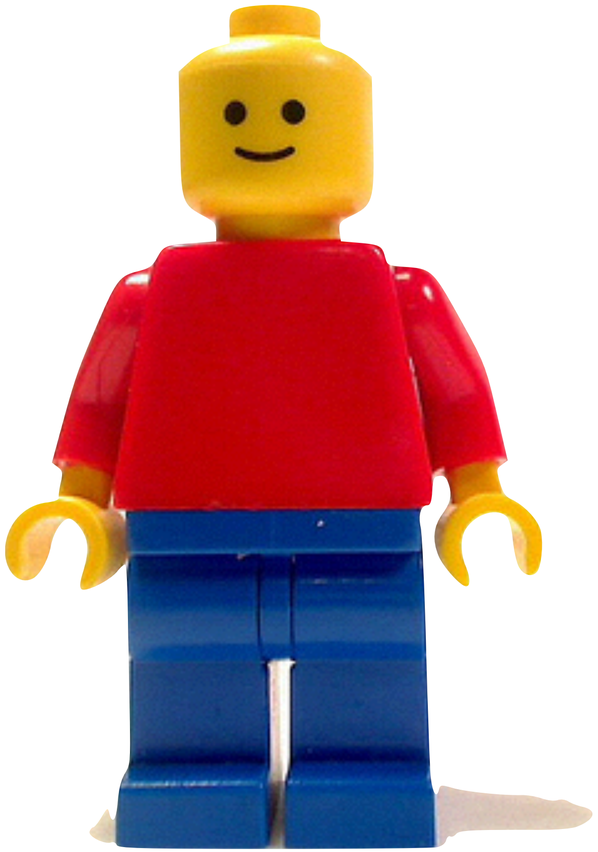
Part 1: Principles of magnetic resonance imaging

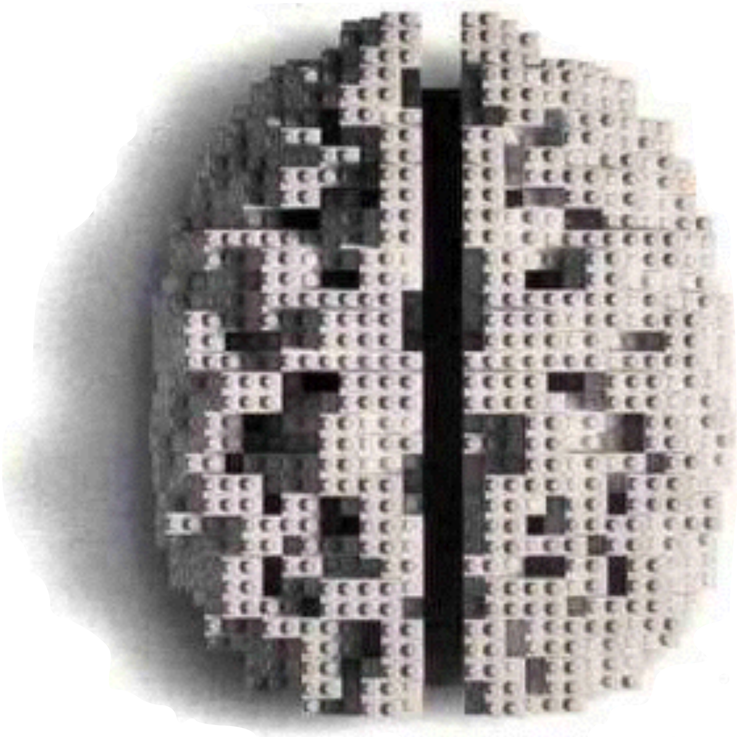
Magnetic resonance imaging

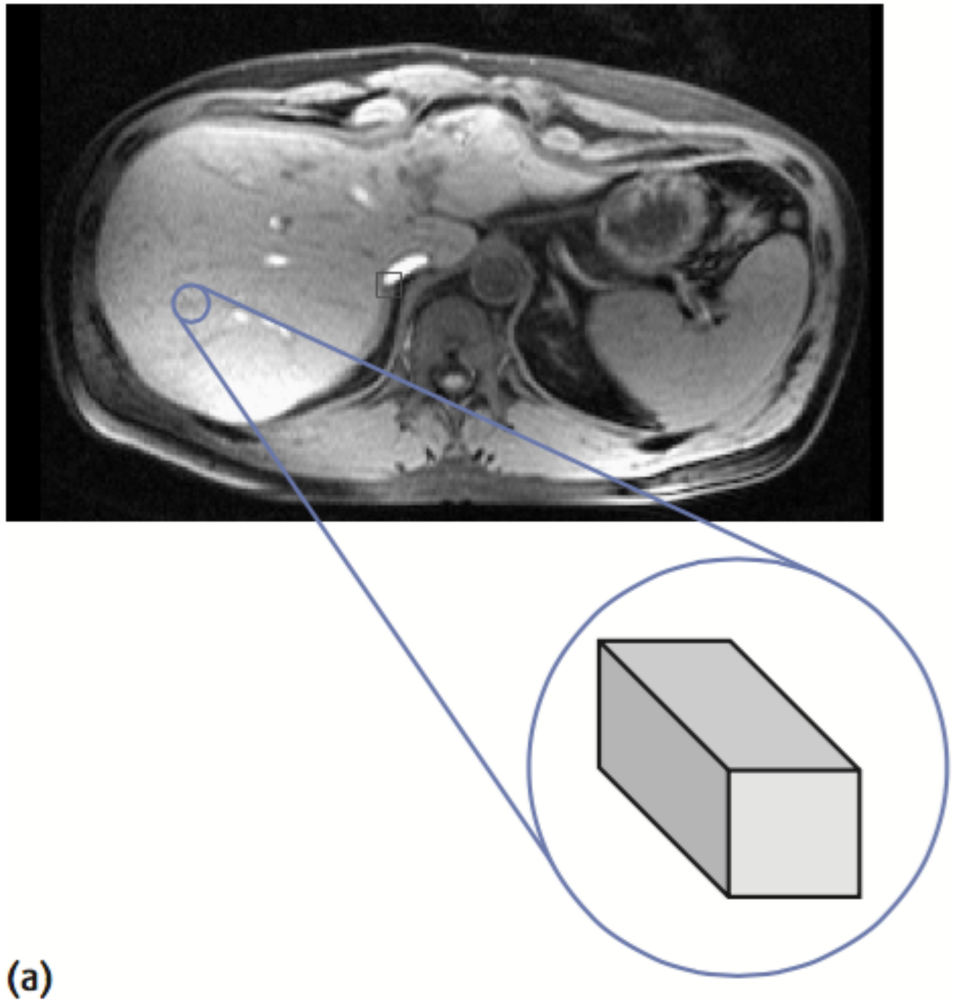
- Based on magnetic resonance of hydrogen nuclei omnipresent in human tissue
- By measuring RF signals emitted by hydrogen nuclei excited in a strong magnetic field we can study multiple tissue types in vivo
- By altering the excitation sequence we can highlight different tissues and their properties



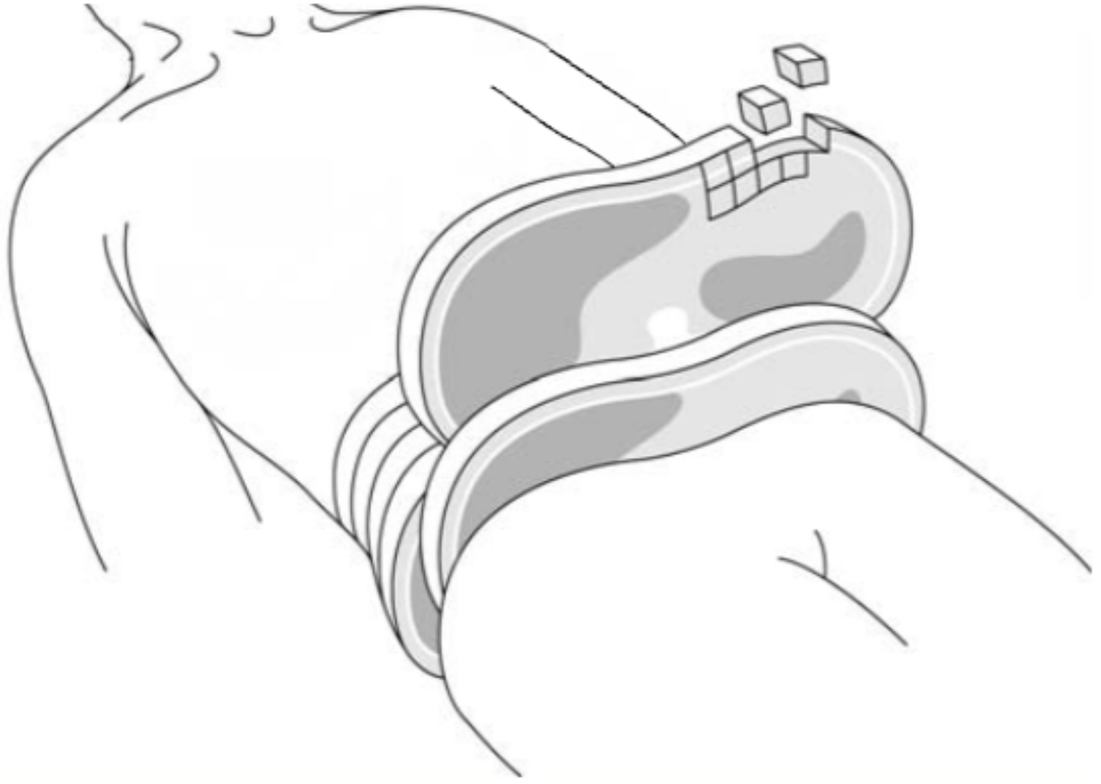
EMI central laboratories







(a)

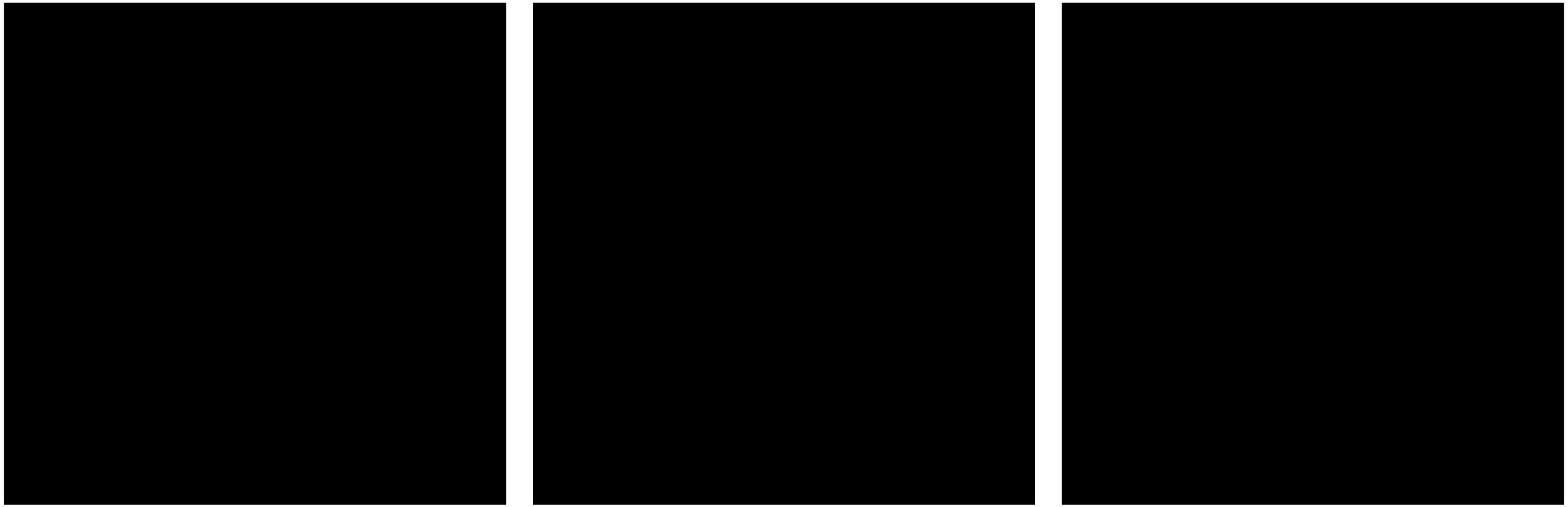


(b)

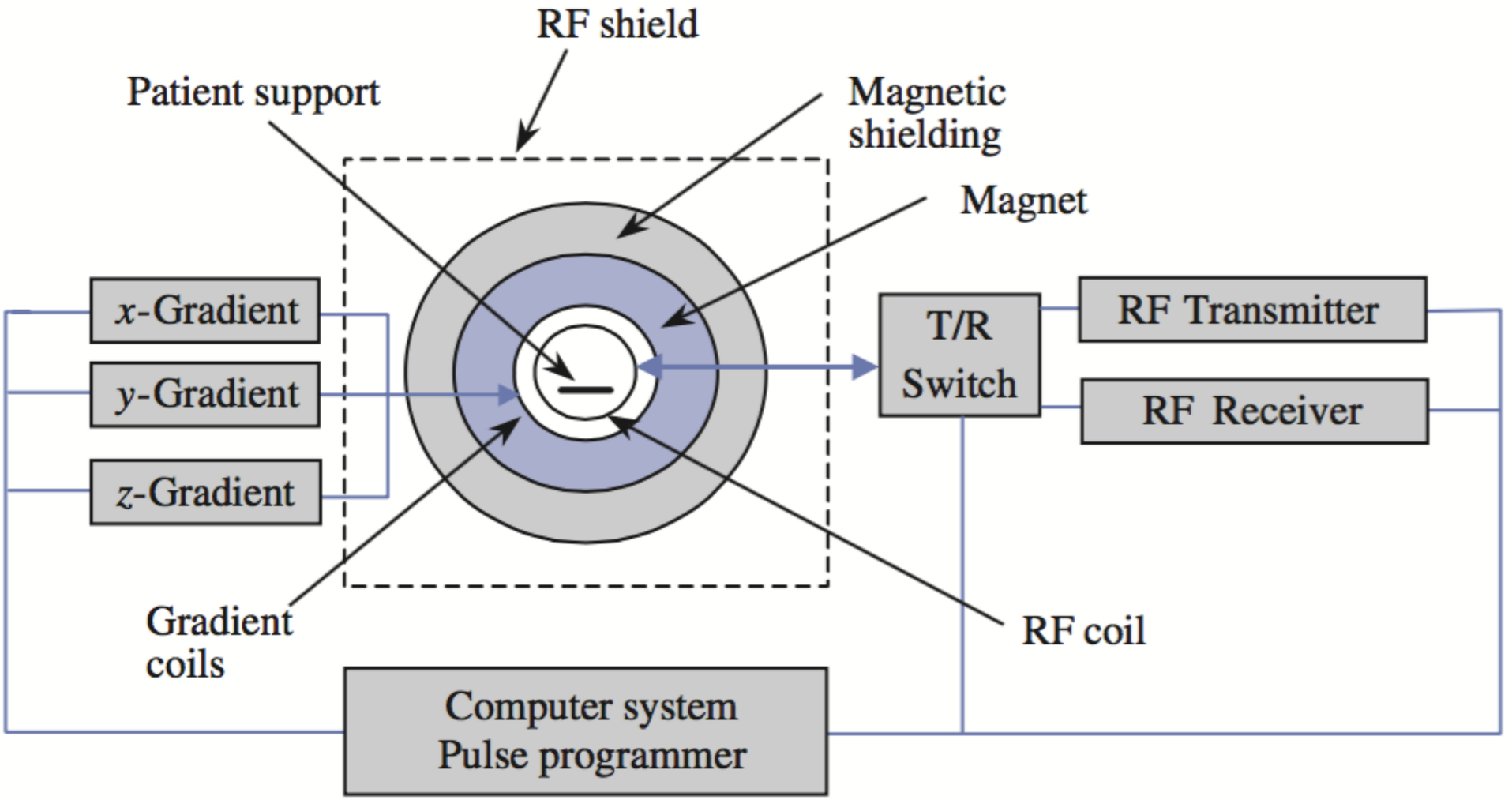
Axial

Coronal

Sagittal



0.5 mm isotropic voxels


RF shield
Patient support
Magnetic shielding
Magnet
x-Gradient
y-Gradient
z-Gradient
T/R Switch
RF Transmitter
RF Receiver
Gradient coils
RF coil
Computer system
Pulse programmer

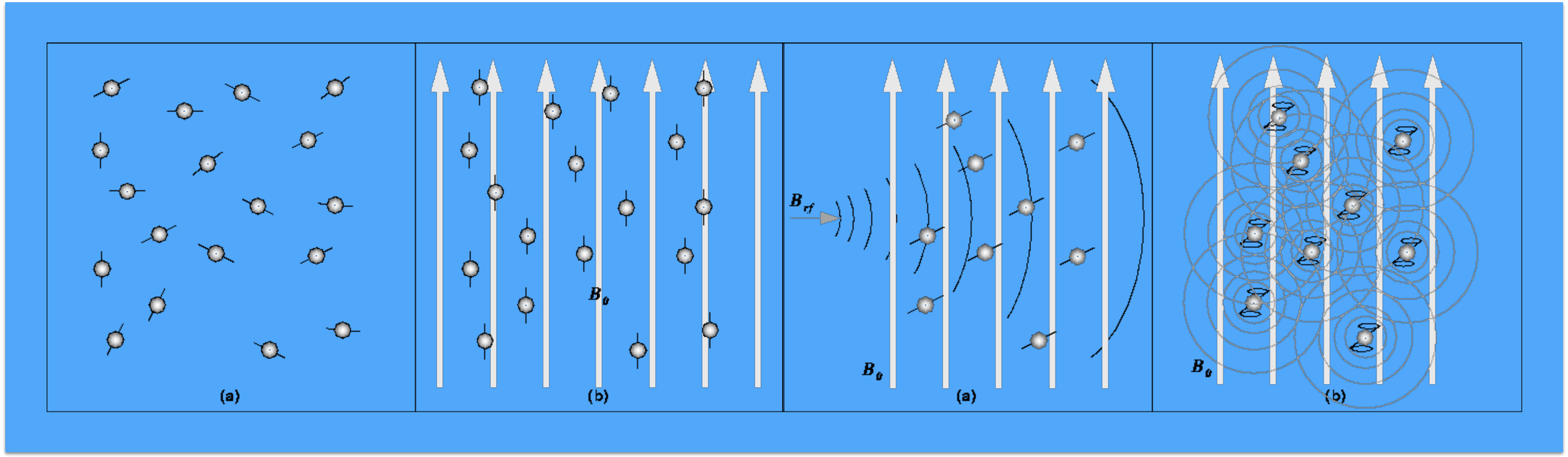
MRI in a nutshell

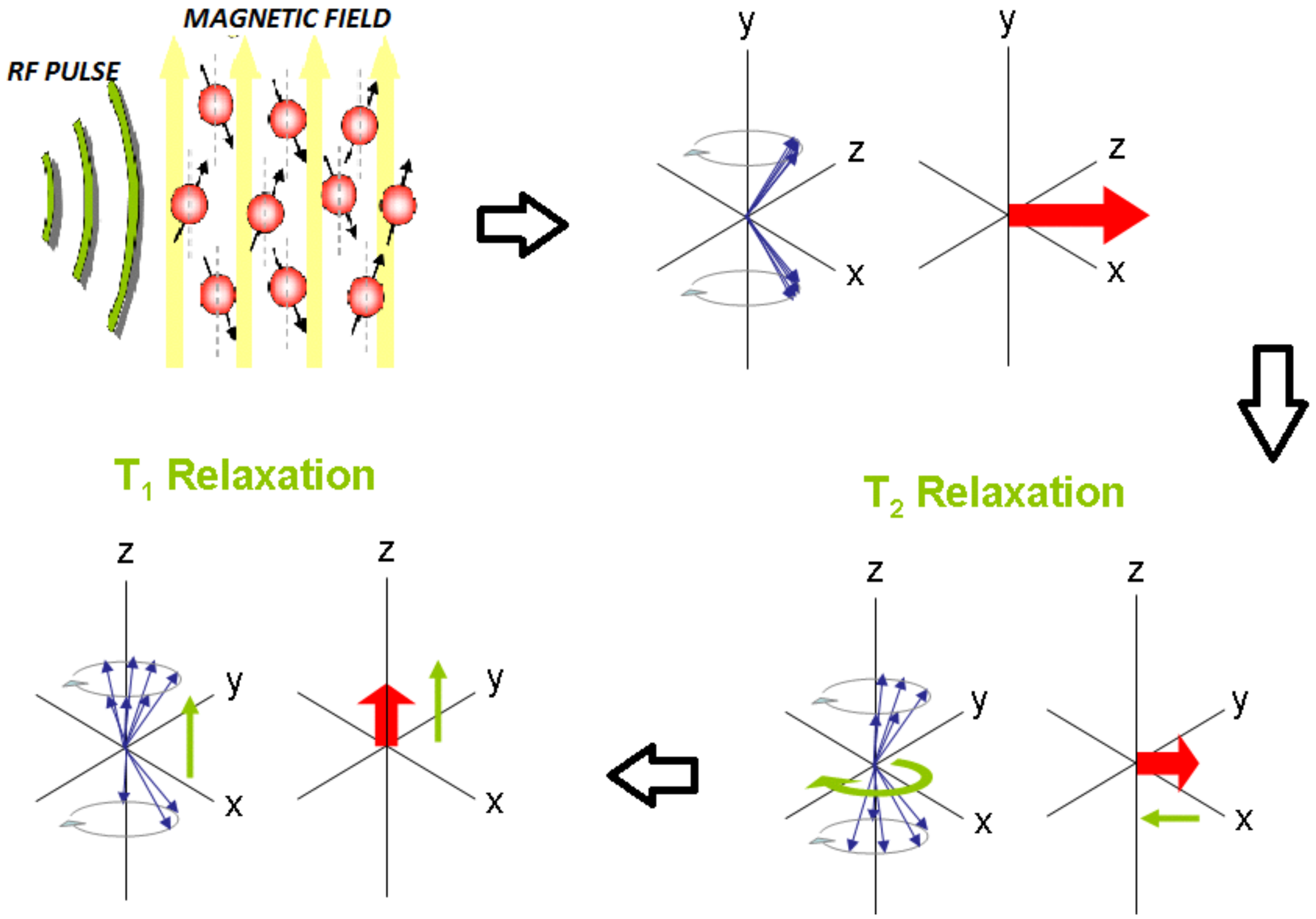
No magnetic field

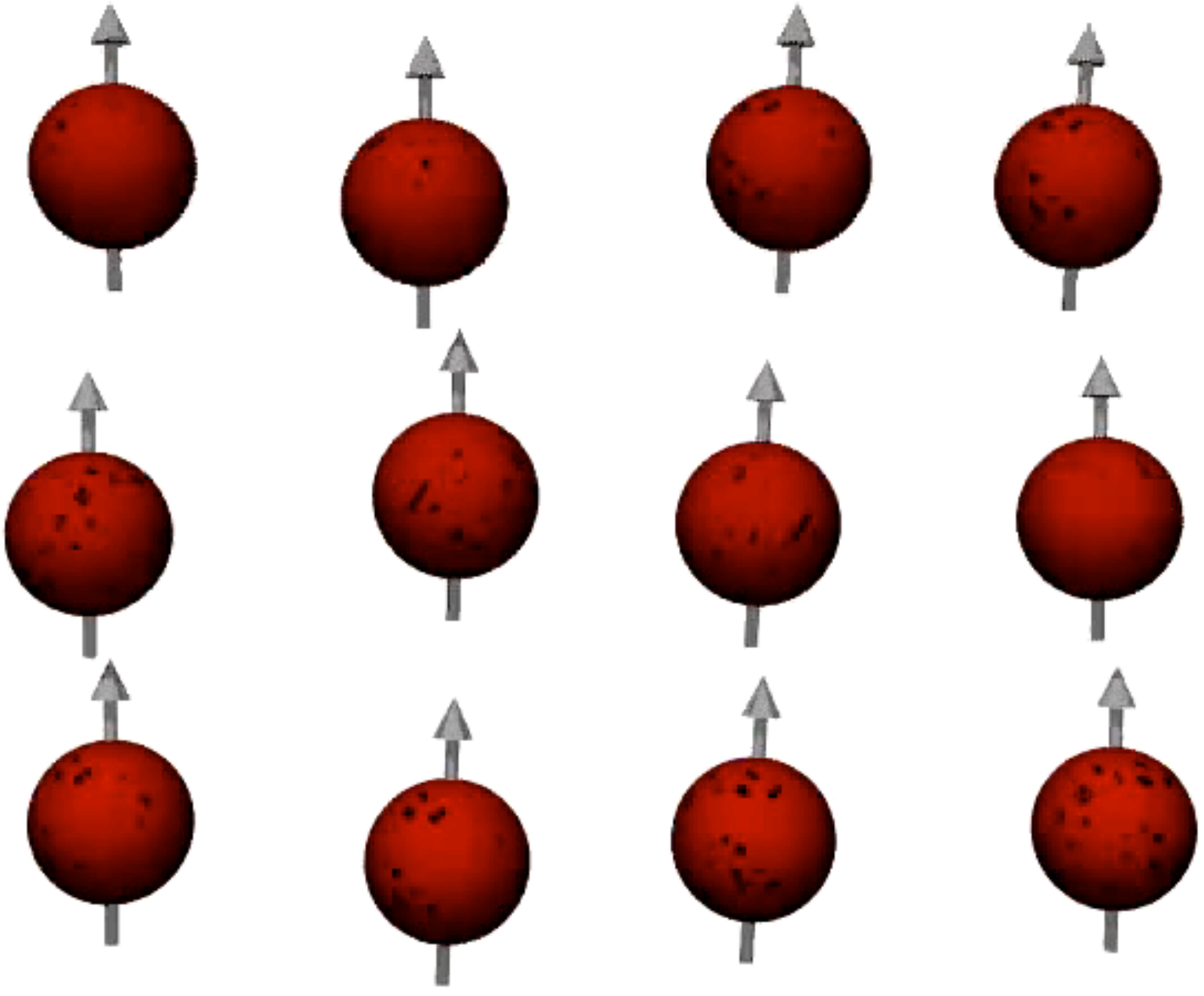
Strong magnetic field

RF pulse

Magnetic resonance



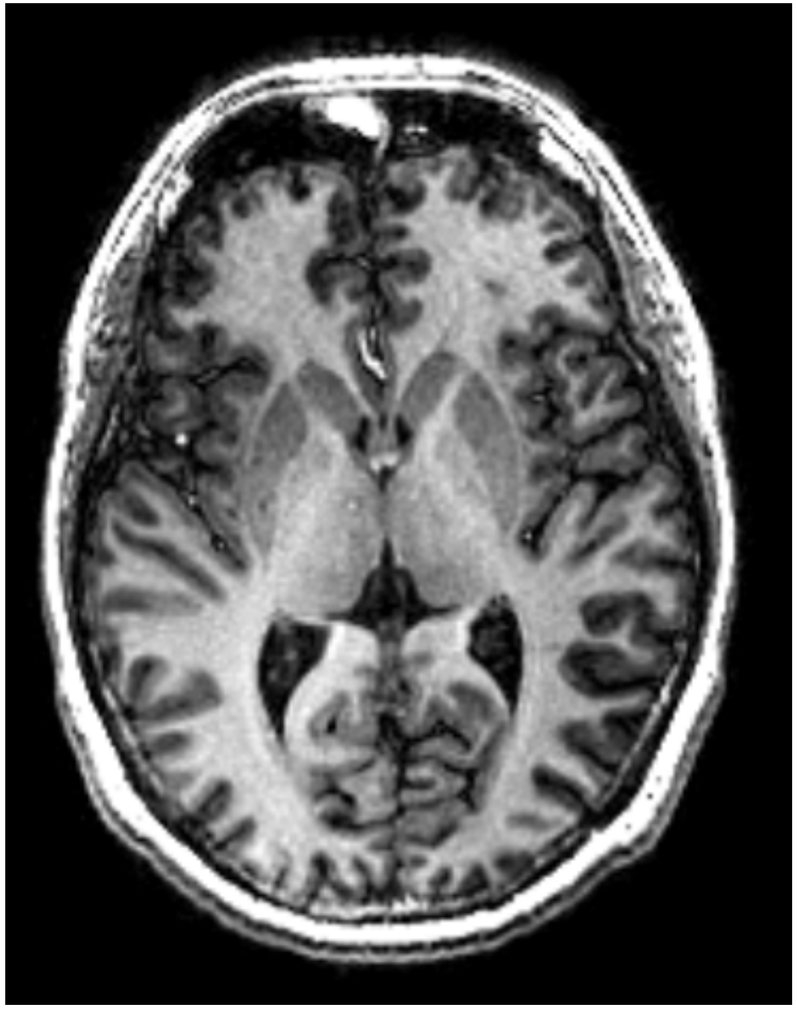

MAGNETIC FIELD
RF PULSE
y
z
x
y
z
x
T1 Relaxation
z
y
x
z
y
x
T2 Relaxation
z
y
x
z
y
x



Proton density, relaxation time and contrast

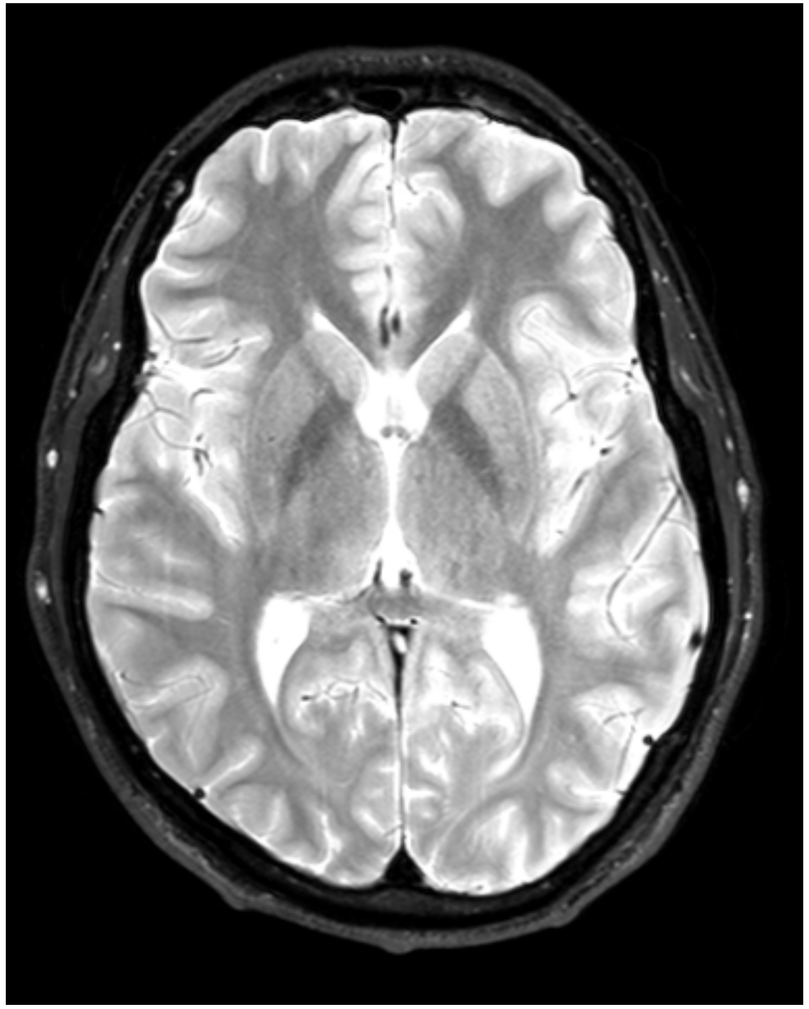
- Proton Density (PD) = number of hydrogen atoms in a volume
  - High in watery tissue such as CSF, low in bone
- Spin-lattice relaxation time (T1)
  - Long in fluids, medium in water-based, short in fat-based tissue
- Spin-spin relaxation time (T2); always shorter than T1 in tissue
  - Long in fluids, medium in water-based, short in fat-based tissue
- Image contrast determined (primarily) by PD, T1 and T2 and their weighting in the pulse sequence

T1-weighting



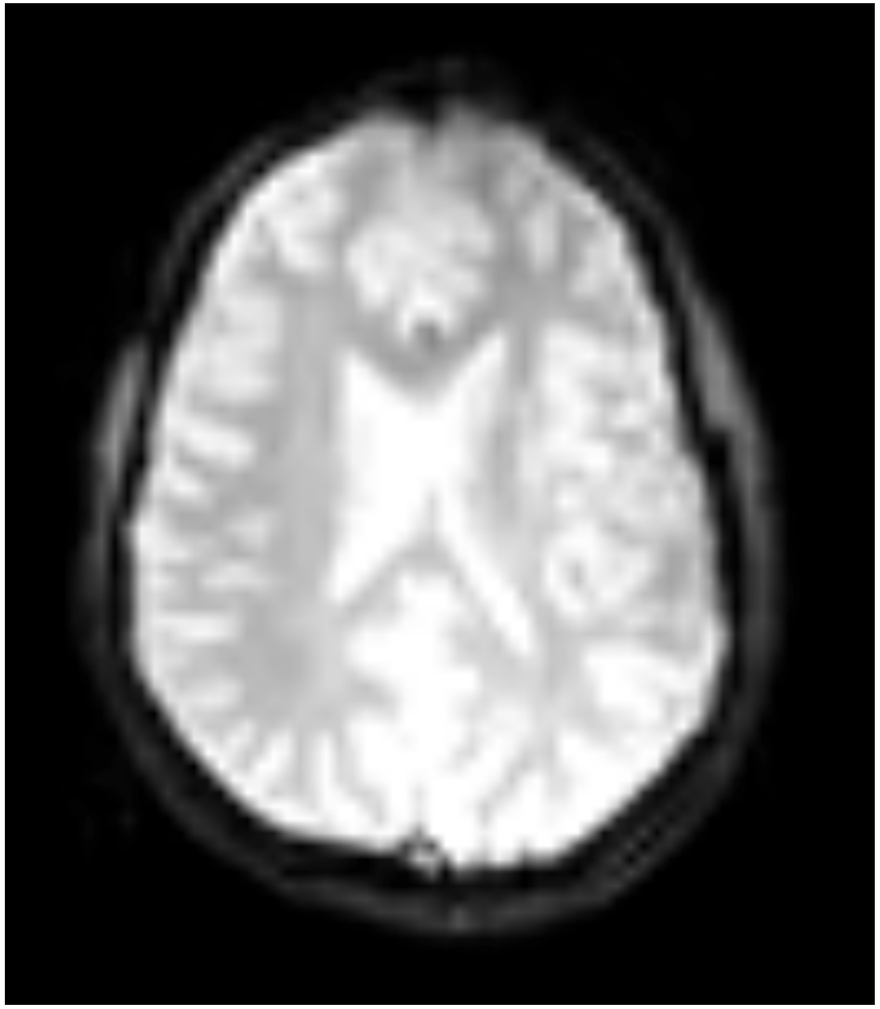
1 mm isotropic voxel

T2-weighting



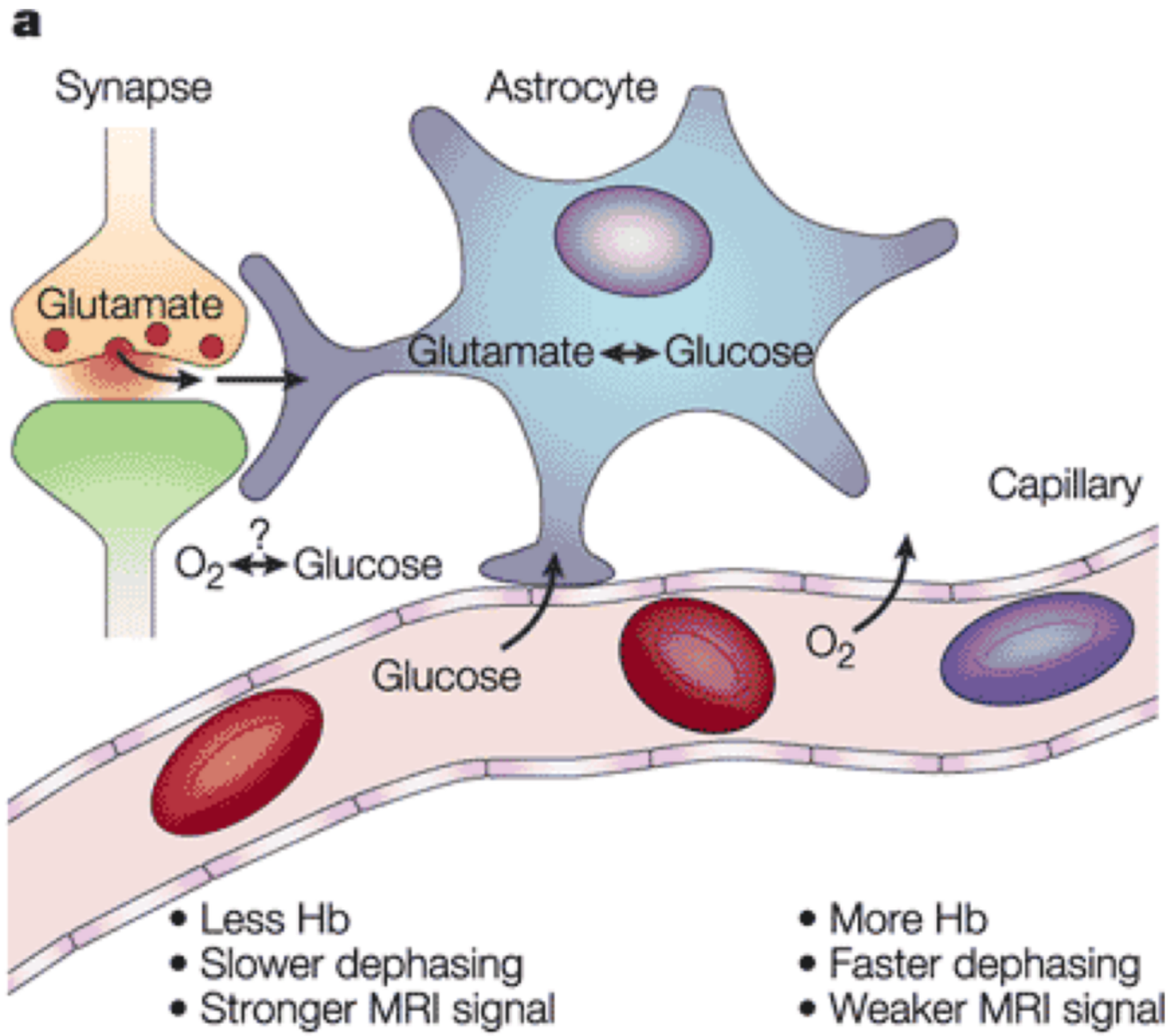
1 mm isotropic voxel

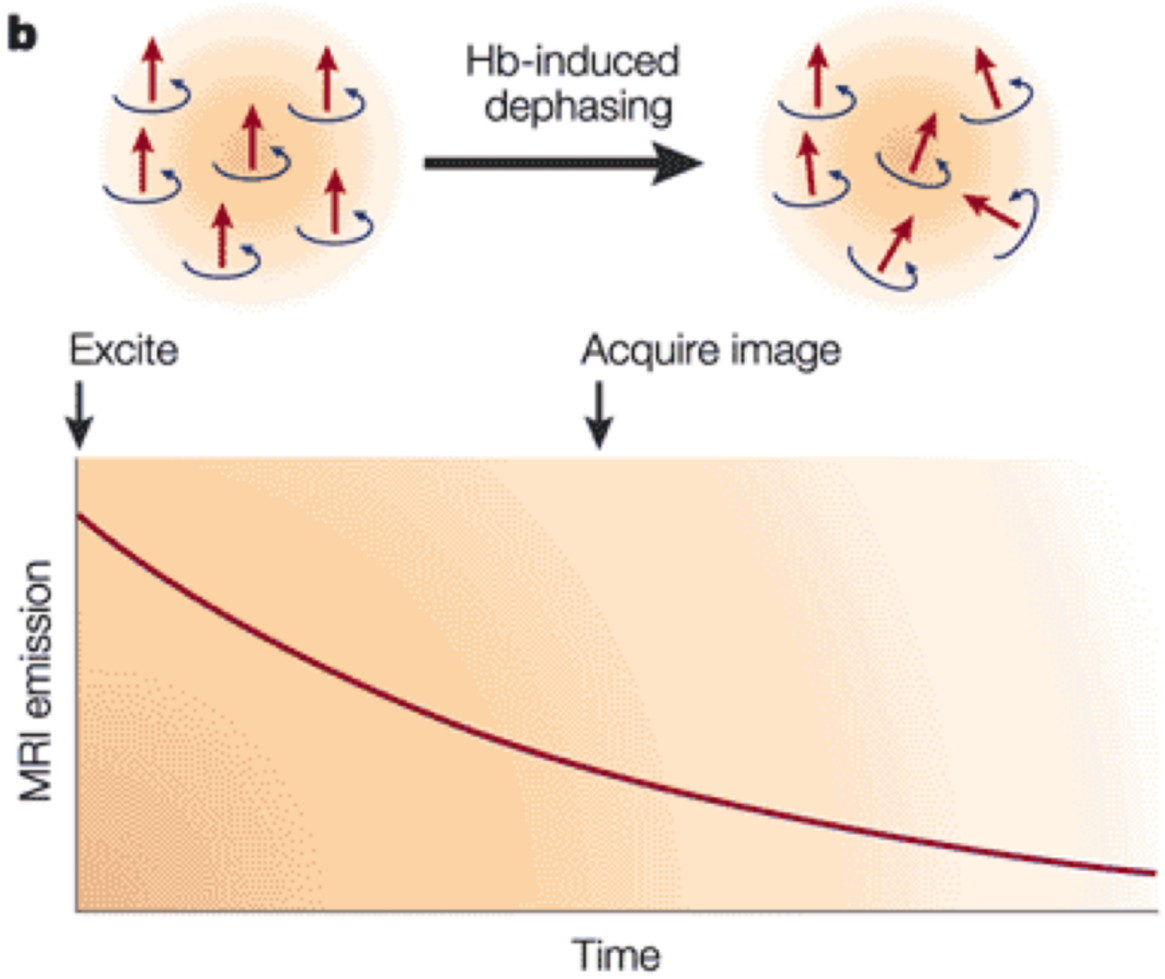
T2*-weighting (EPI)



3 mm isotropic voxel

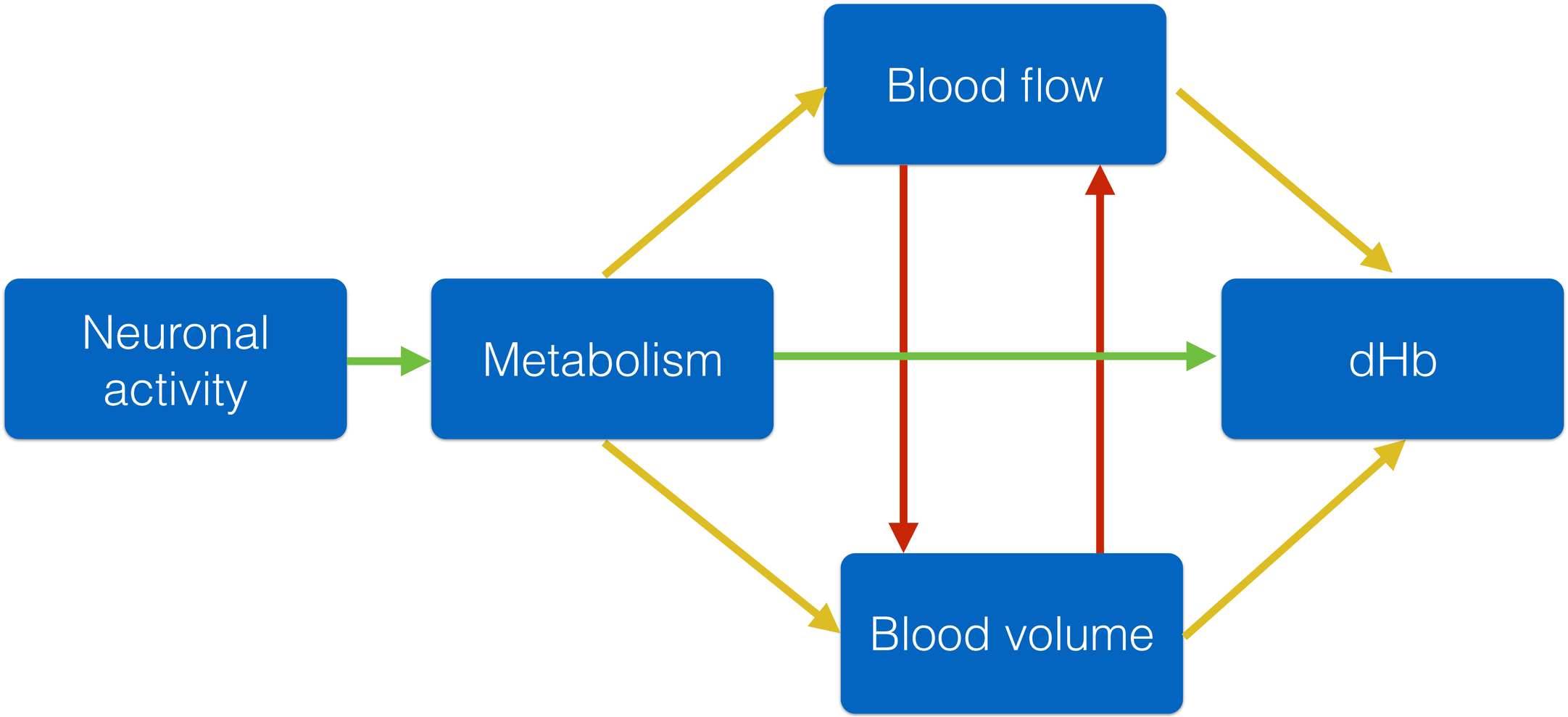
Blood oxygenation dependent (BOLD) contrast

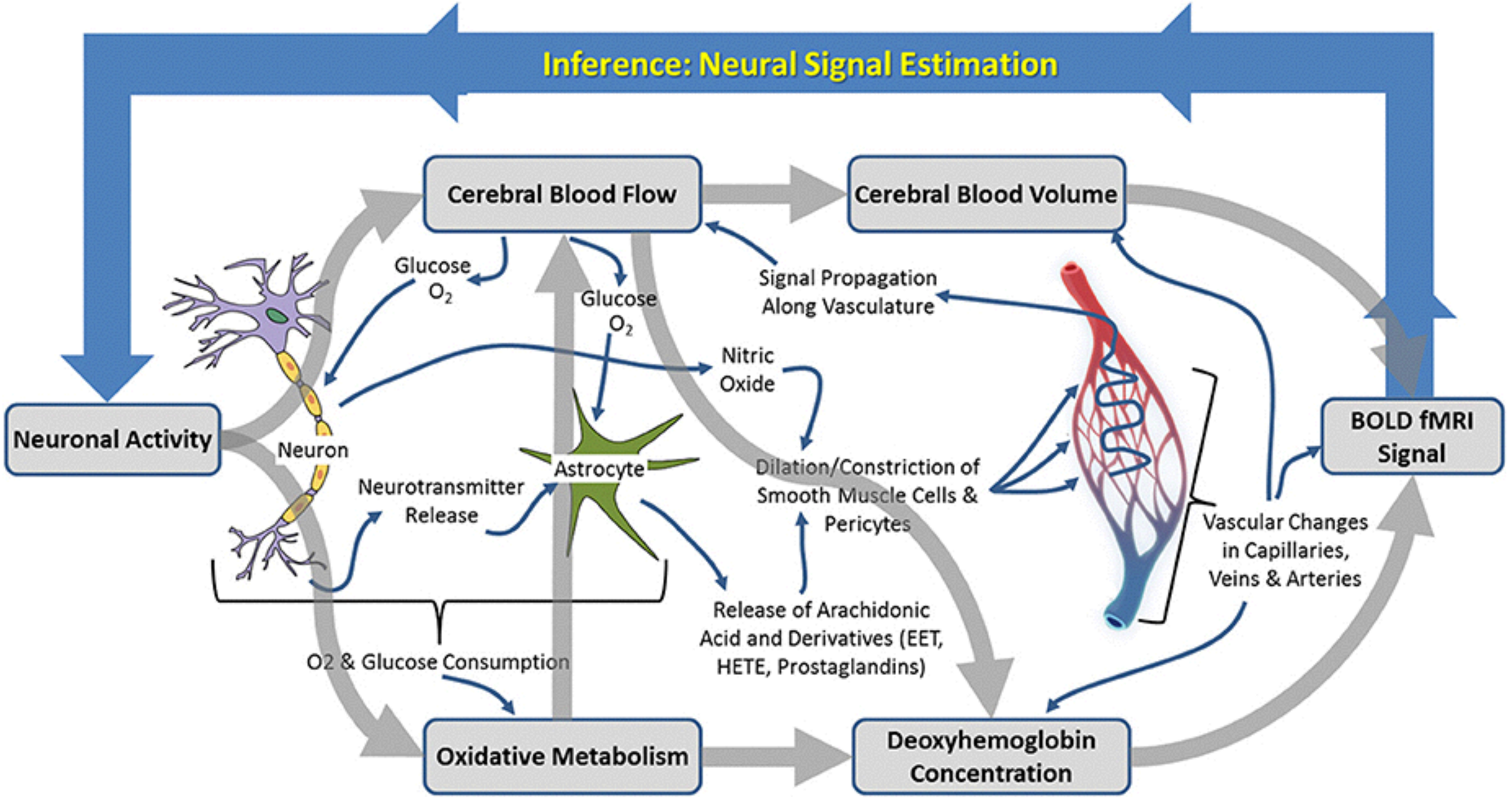




Heeger & Ress 2002 NRN

Pathways to BOLD contrast

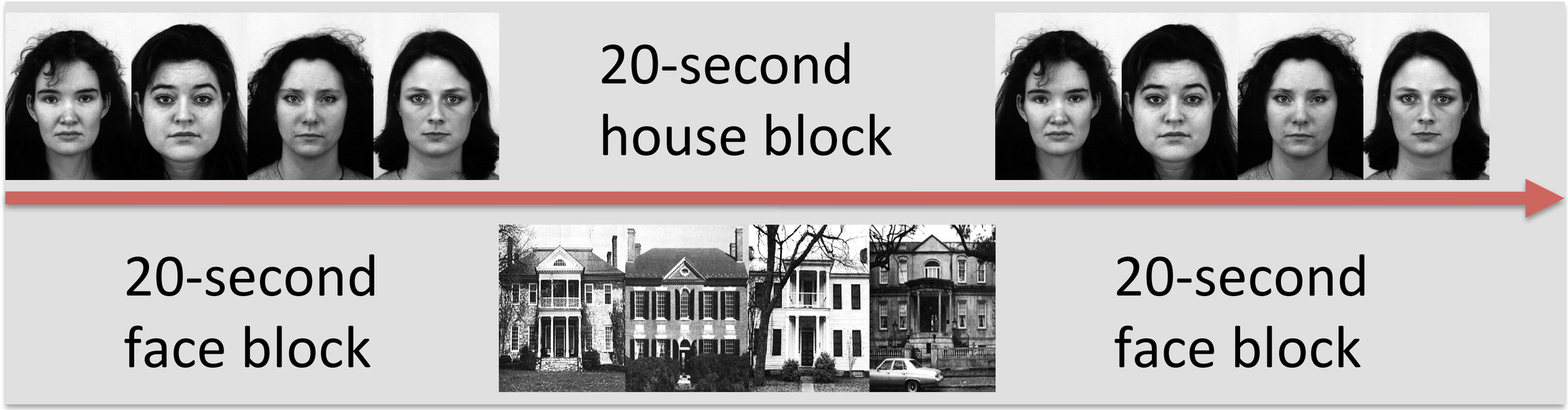




Martin (2014 Front Neurosci)

Part 2: Statistical analysis in fMRI

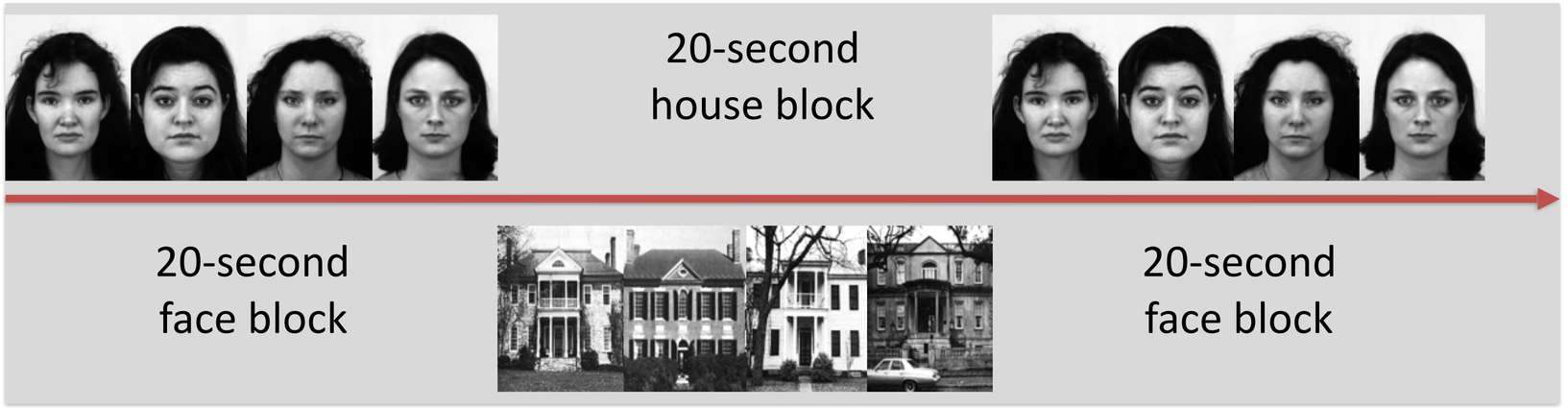
Cognitive subtraction and boxcar design

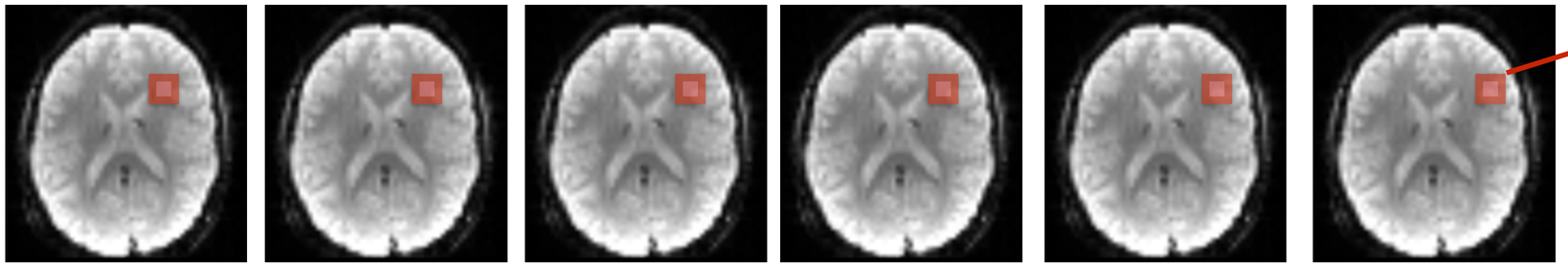


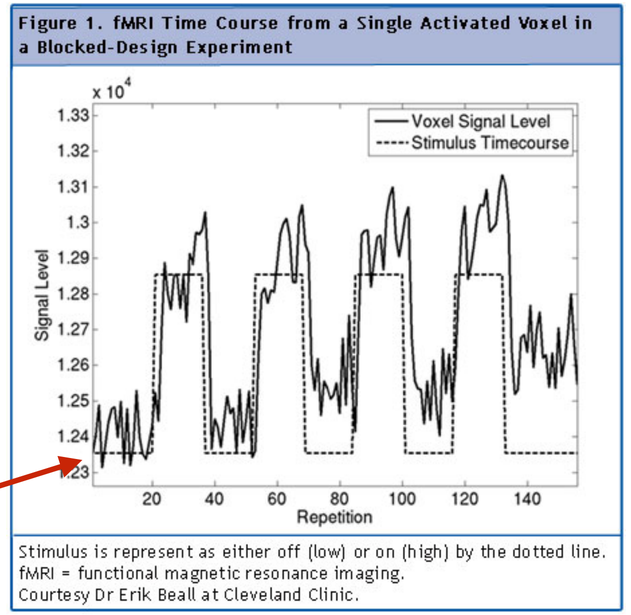
AIM Localize brain regions that are more sensitive to faces versus non-face objects

DESIGN Blocked experiment using cognitive subtraction assuming pure insertion

Cognitive subtraction and boxcar design

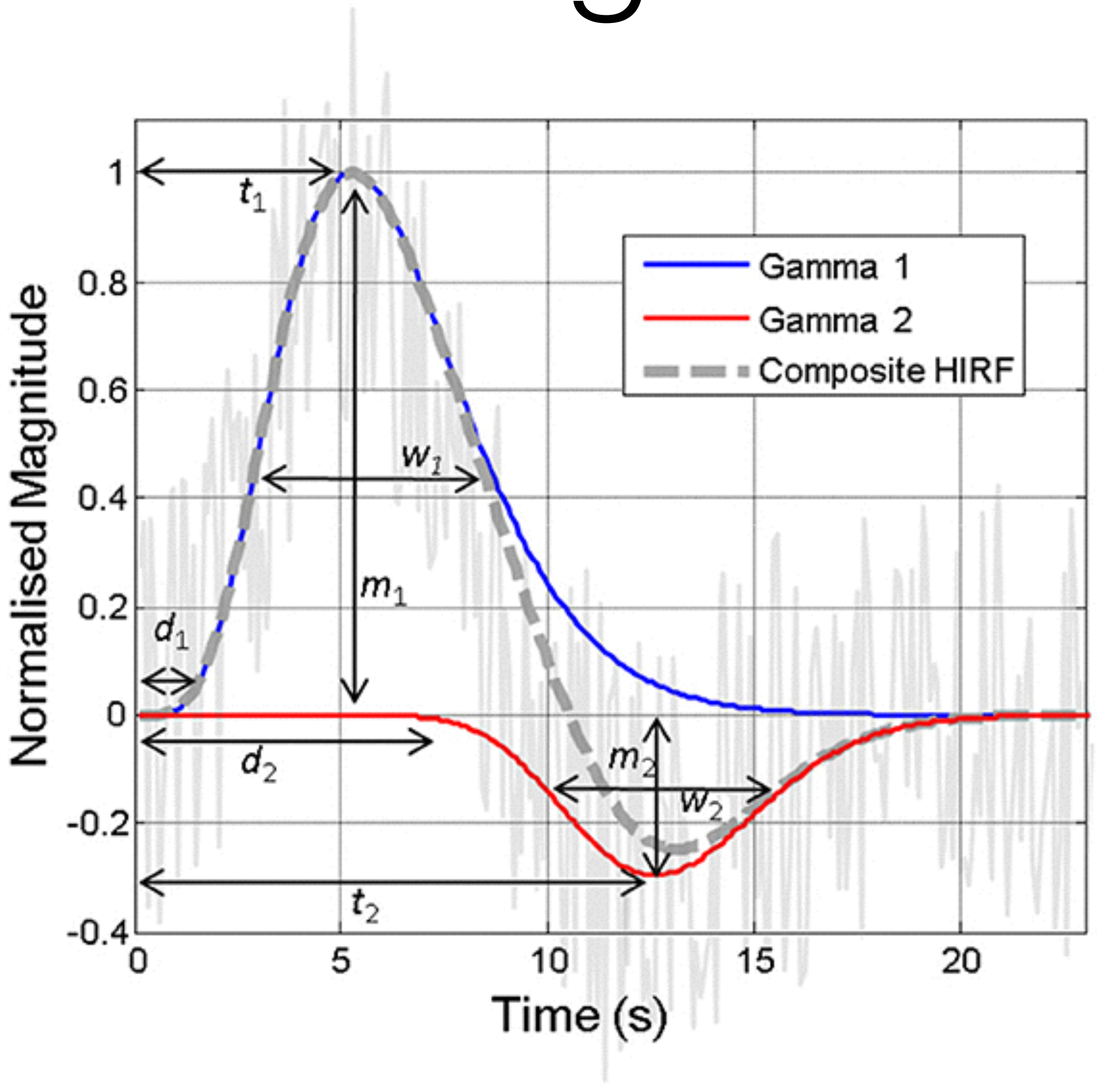






Acquiring one 3D functional volume takes about 1.5 seconds
We can distinguish events ~100ms apart, yet their actual timing can be resolved with about 2-s accuracy

Canonical double gamma HRF



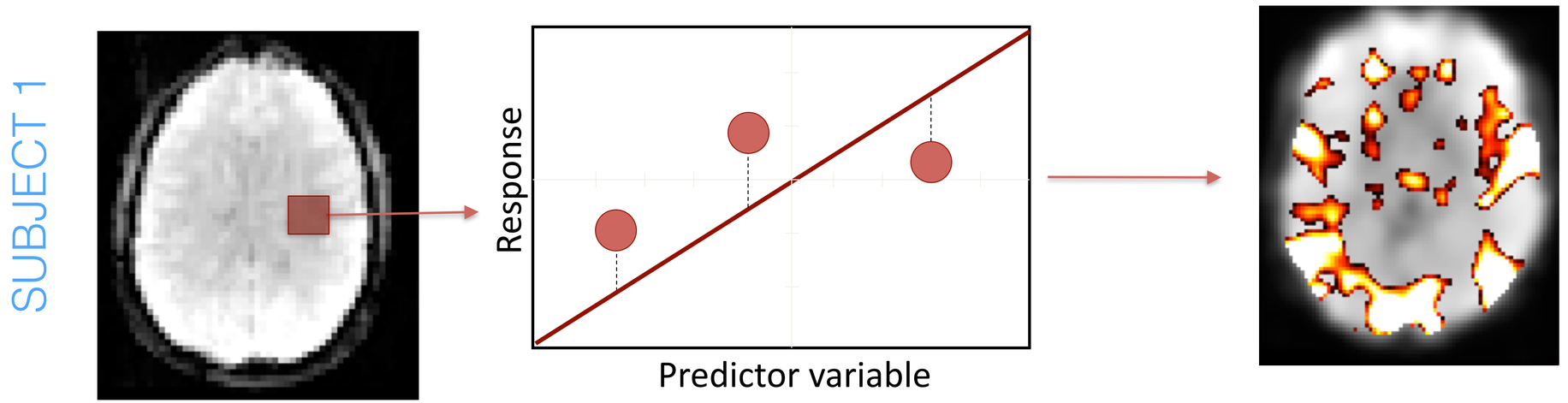
Martin (2014 Front Neurosci)

First-level model

EPI DATA

UNIVARIATE MODEL FOR EACH VOXEL

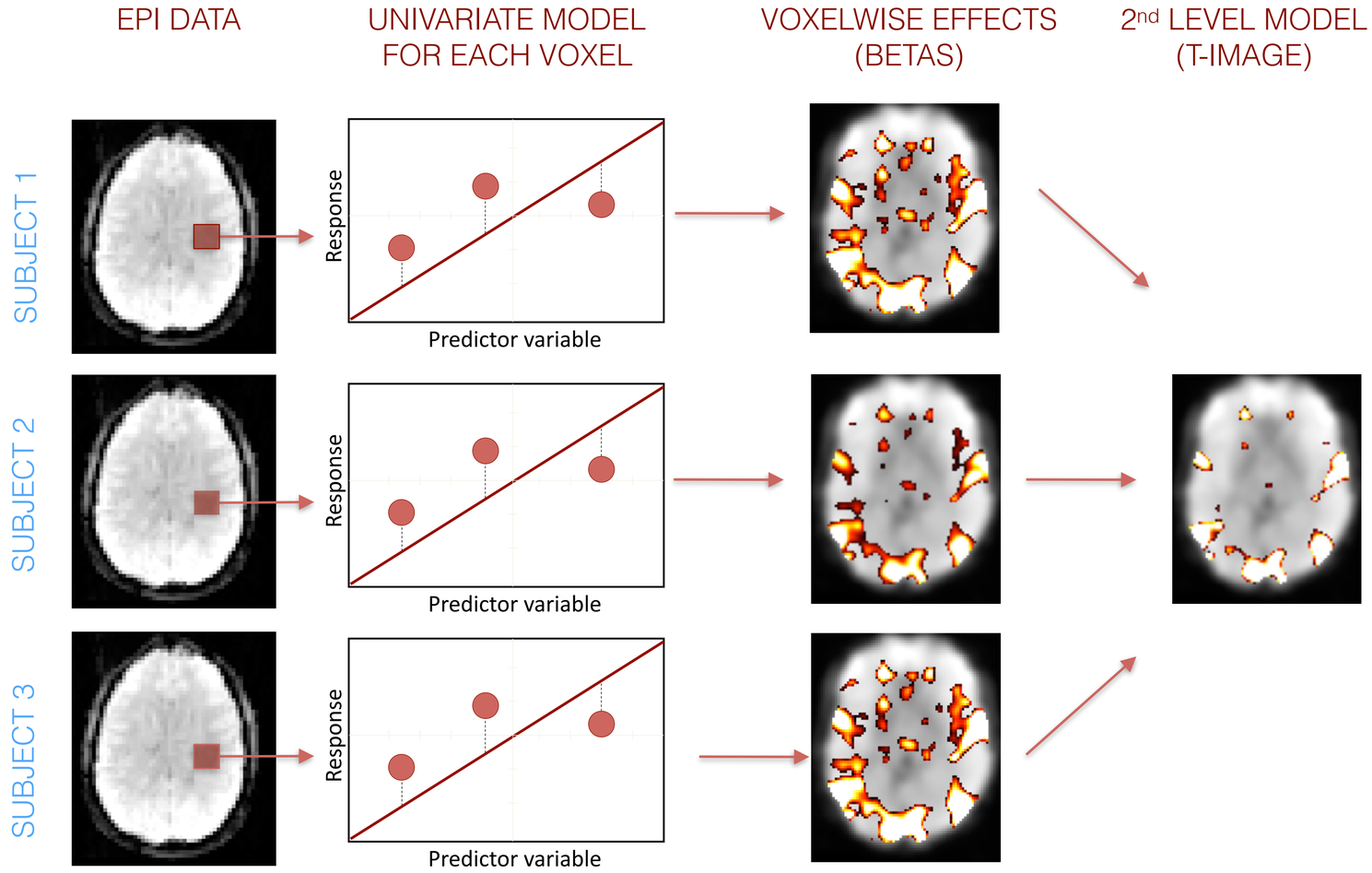
VOXELWISE EFFECTS (BETAS)



Estimates task-induced activation in single subject. However effects are not fixed across subjects. For population-level inference, need to account for between-subjects variance

STATISTICAL SIGNIFICANCE AT 1ST LEVEL = RELIABILITY OF EXPERIMENTAL MANIPULATION ON BRAIN ACTIVITY IN THIS PARTICULAR SUBJECT

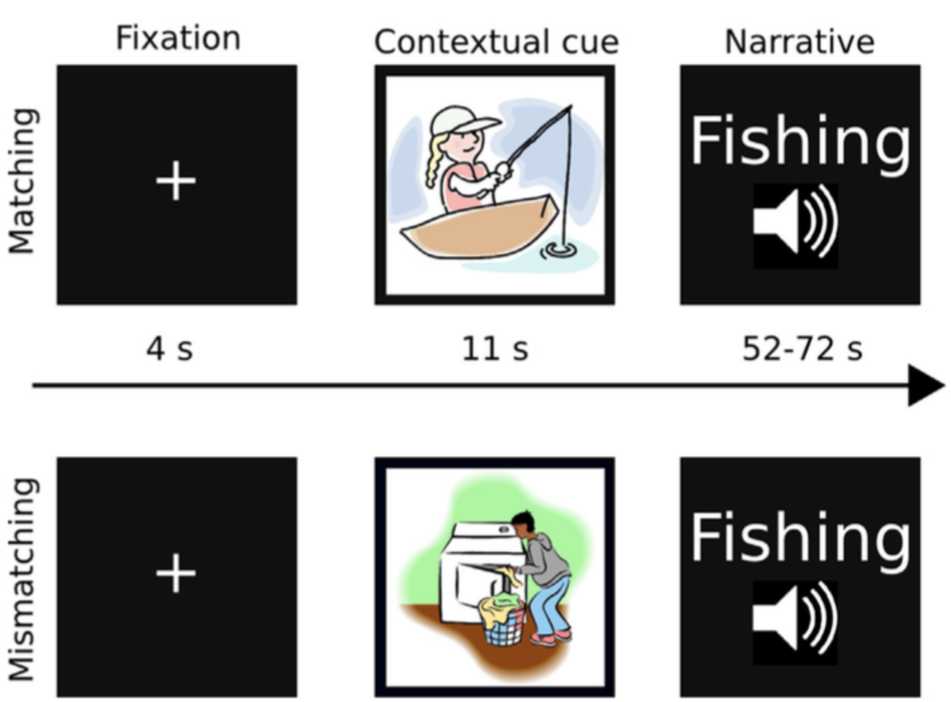
First and second level models

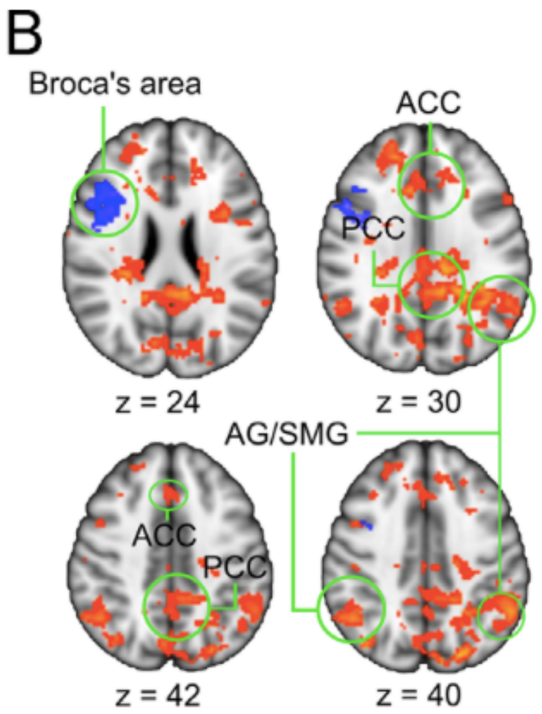


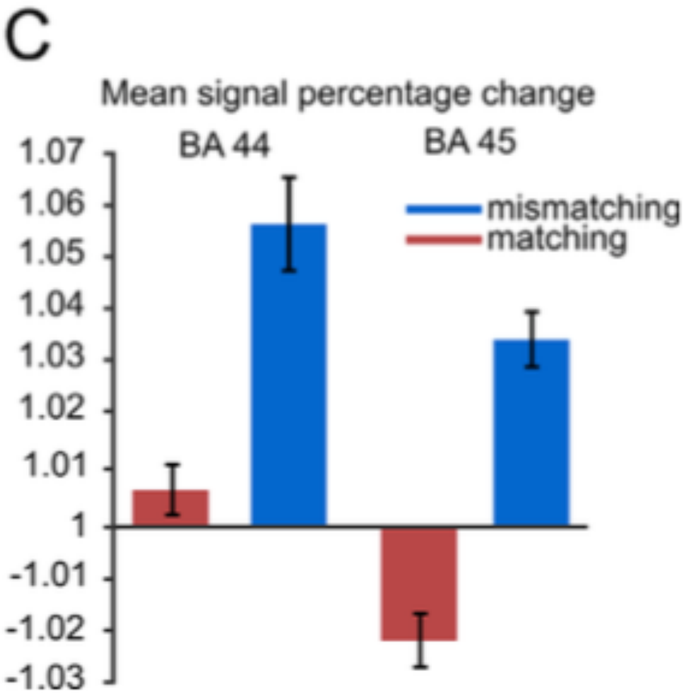
STATISTICAL SIGNIFICANCE AT 2ND LEVEL = RELIABILITY OF EXPERIMENTAL MANIPULATION ON BRAIN ACTIVITY ACROSS SUBJECTS

Part 3: Experimental designs for functional MRI

Boxcar design

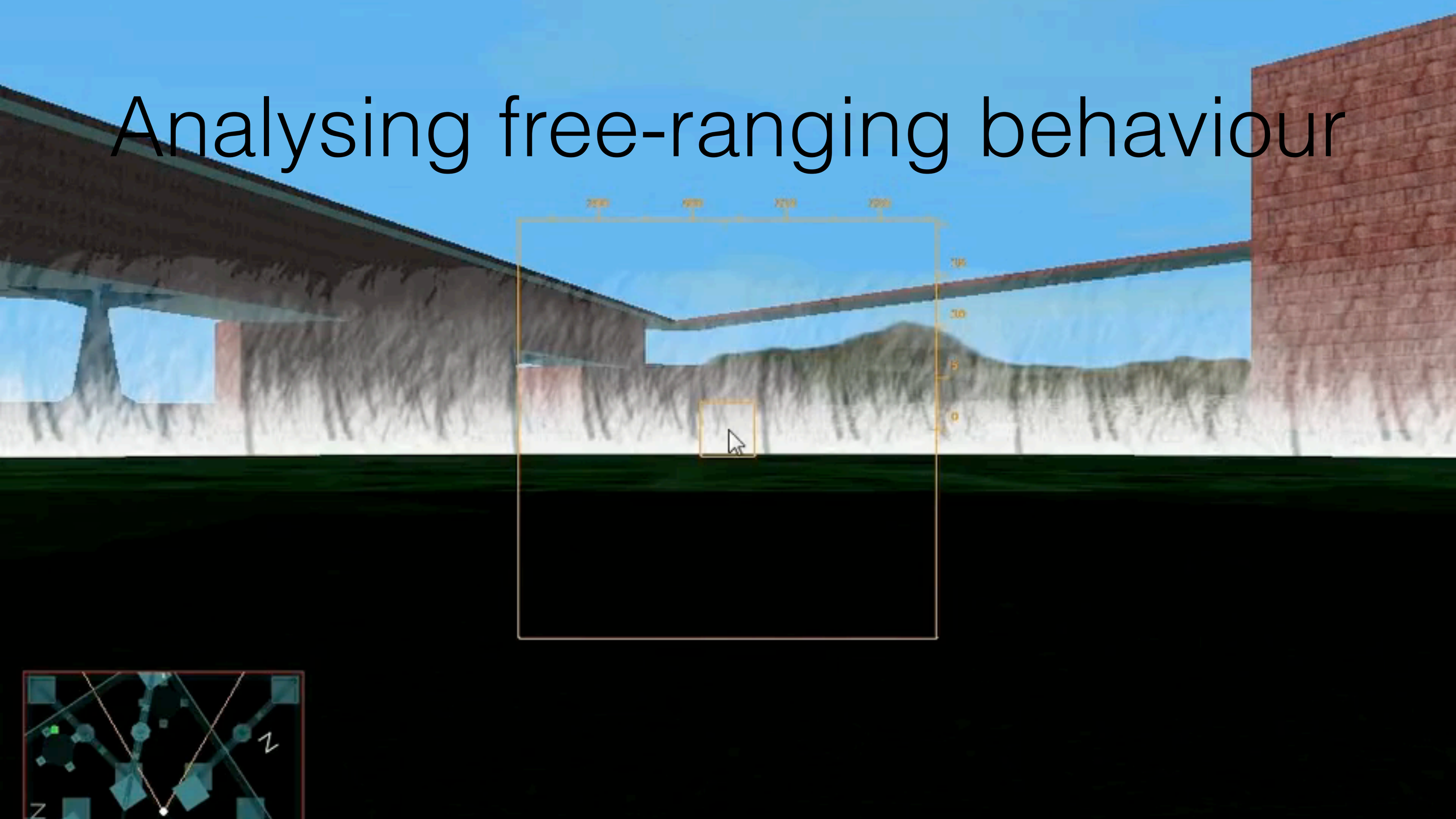




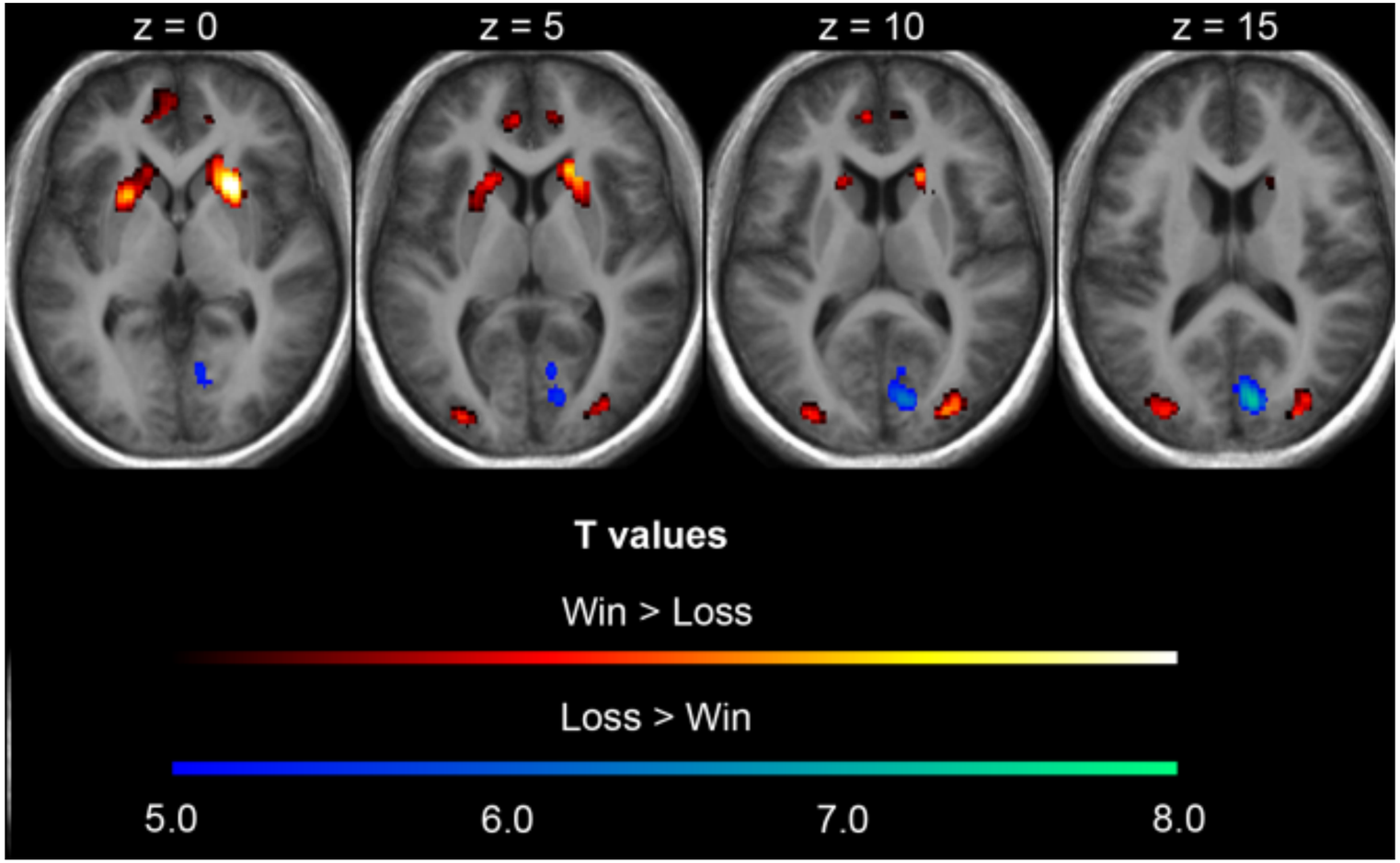


Smirnov, Glerean, Lahnakoski, …, & Nummenmaa (2014 Neurospychologia)

Analysing free-ranging behaviour

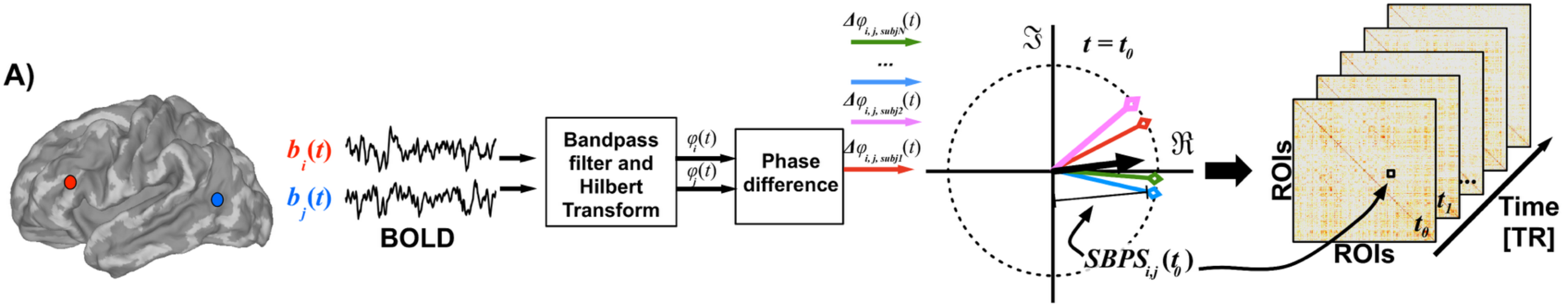


Winning activates the stratal reward circuit

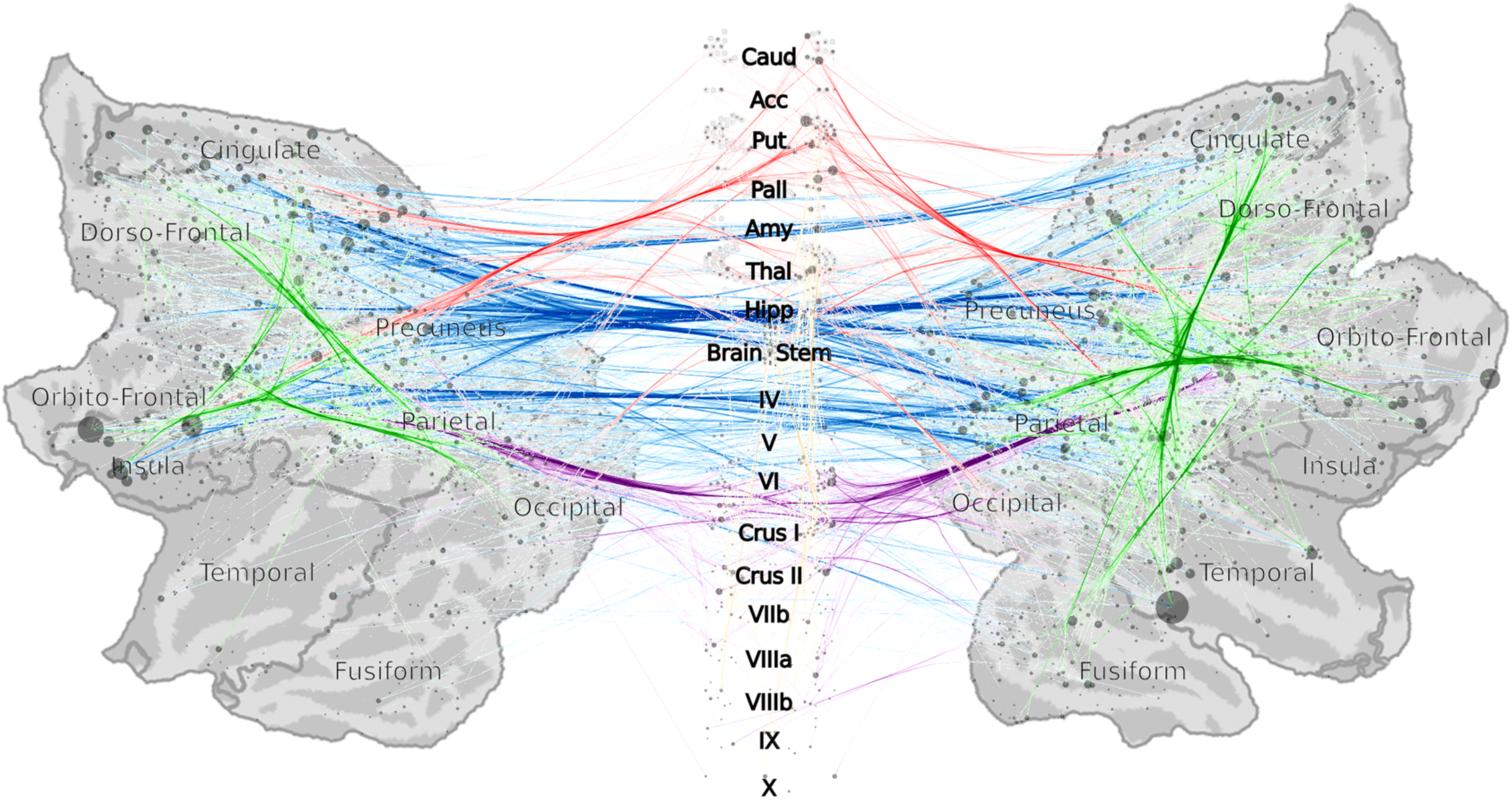


Kätsyri, Ravaja, Hari & Nummenmaa (2013 Cereb Cortex)

Connectivity analysis

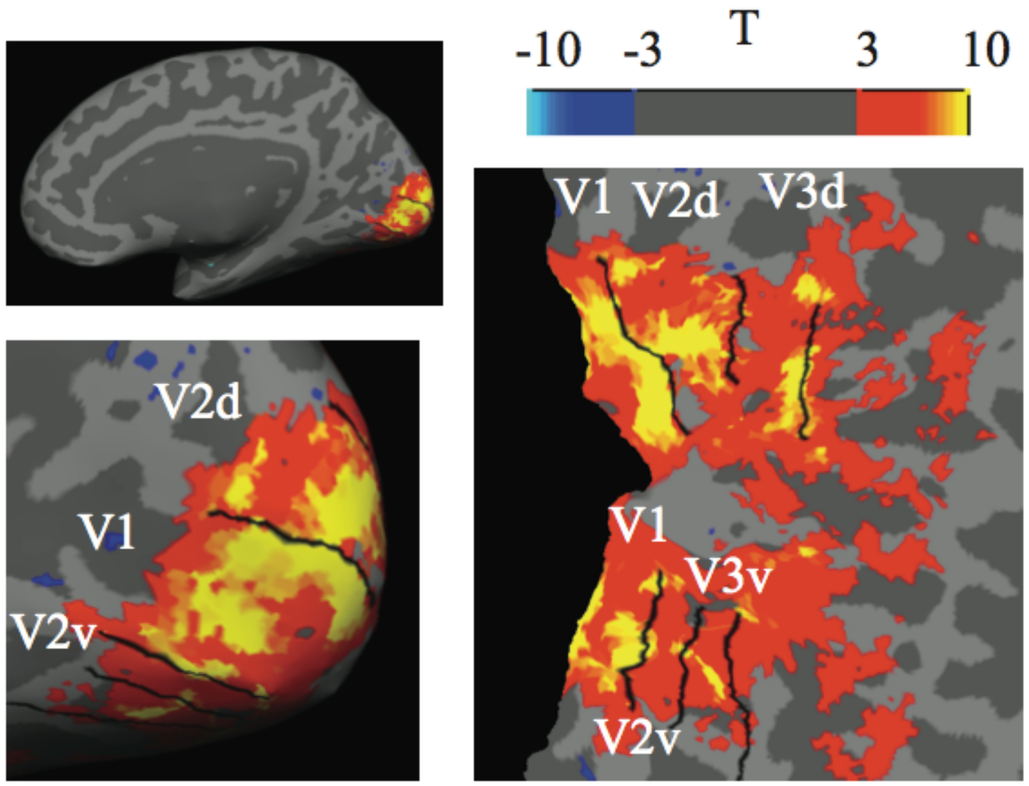


Nummenmaa et al (J Neurosci, 2015)

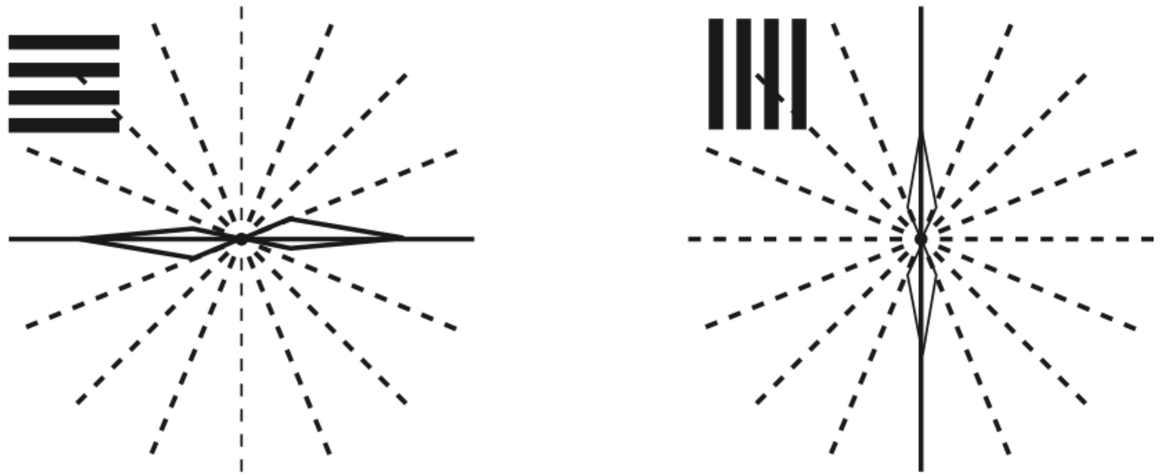


Nummenmaa et al (2014 Neuroimage)

Sub-voxel resolution with pattern recognition?



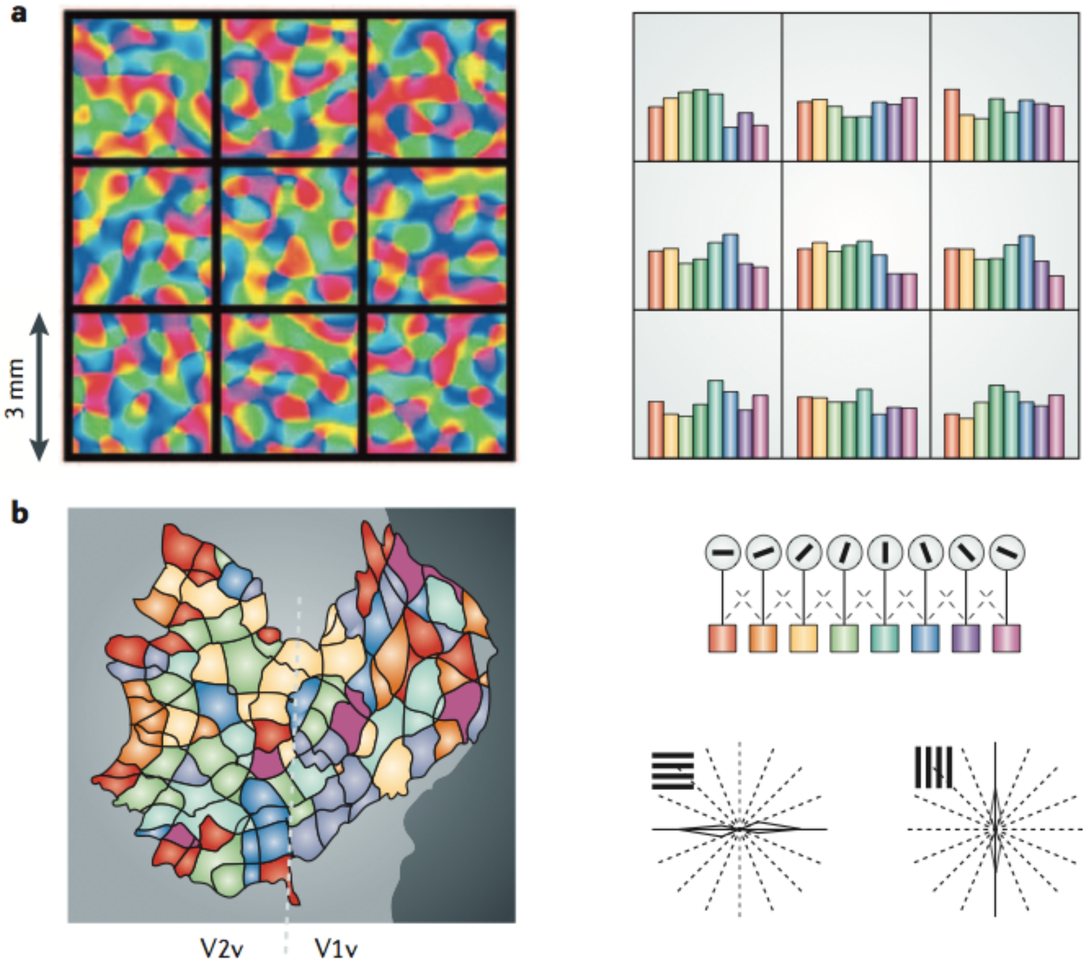
Sharifan, Nummenmaa & Vanni (unpublished work)



Rees & Haynes (2006 Nat Rev Neurosci)

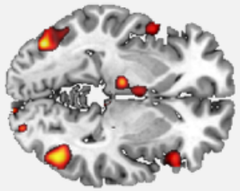
Sub-voxel resolution with pattern recognition?

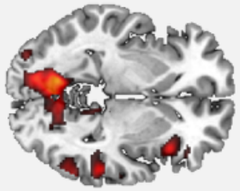
- Orientation preferences are systematically mapped in V1
- Regions with different orientation tuning separated by 0.5 mm and cannot thus be discriminated by conventional EPI imaging
- Due to slight irregularities in the orientation specific maps, different voxels contain uneven distribution of orientation-specific cells
- Differences are small, but when taken into account together they allow estimation of orientation selectivity

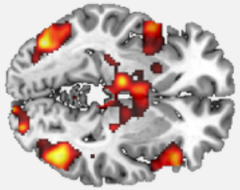


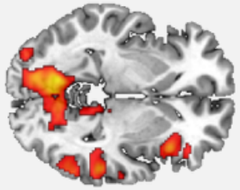
Rees & Haynes (2006 Nat Rev Neurosci)

Training data


Happy

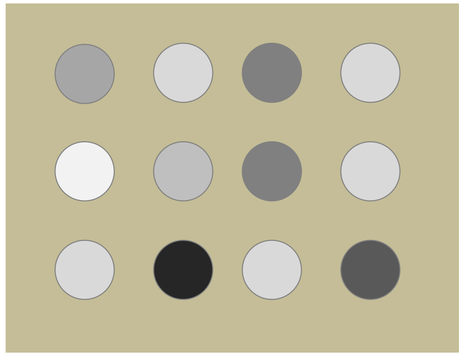

Fearful


Angry

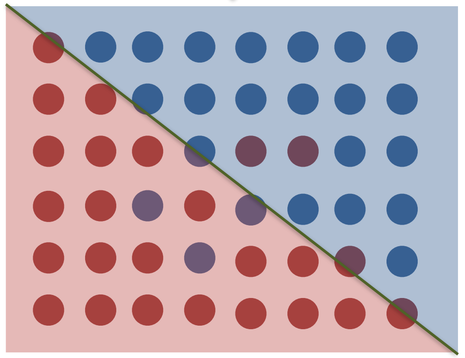

Sad

Classifier learns the association between emotions and brain states from the training data

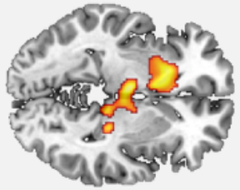
Feature selection

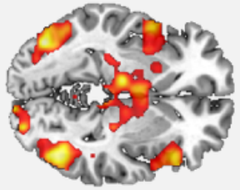


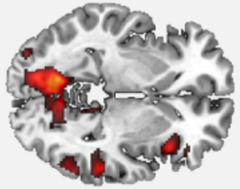
CLASSIFIER
learns to associate emotions with brain states

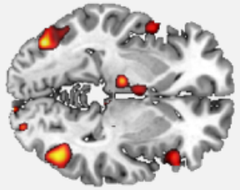


Test data


???


???


???


???

Classifier is tested with a novel dataset it has never seen before

Exp 1
Induction

Task begins

+

1 second

Movie

10 seconds

ITI

+

14 seconds

Exp 3
Both

Exp 2
Cued
Imagery

Task begins

+

1 second

Emotion word

Happy

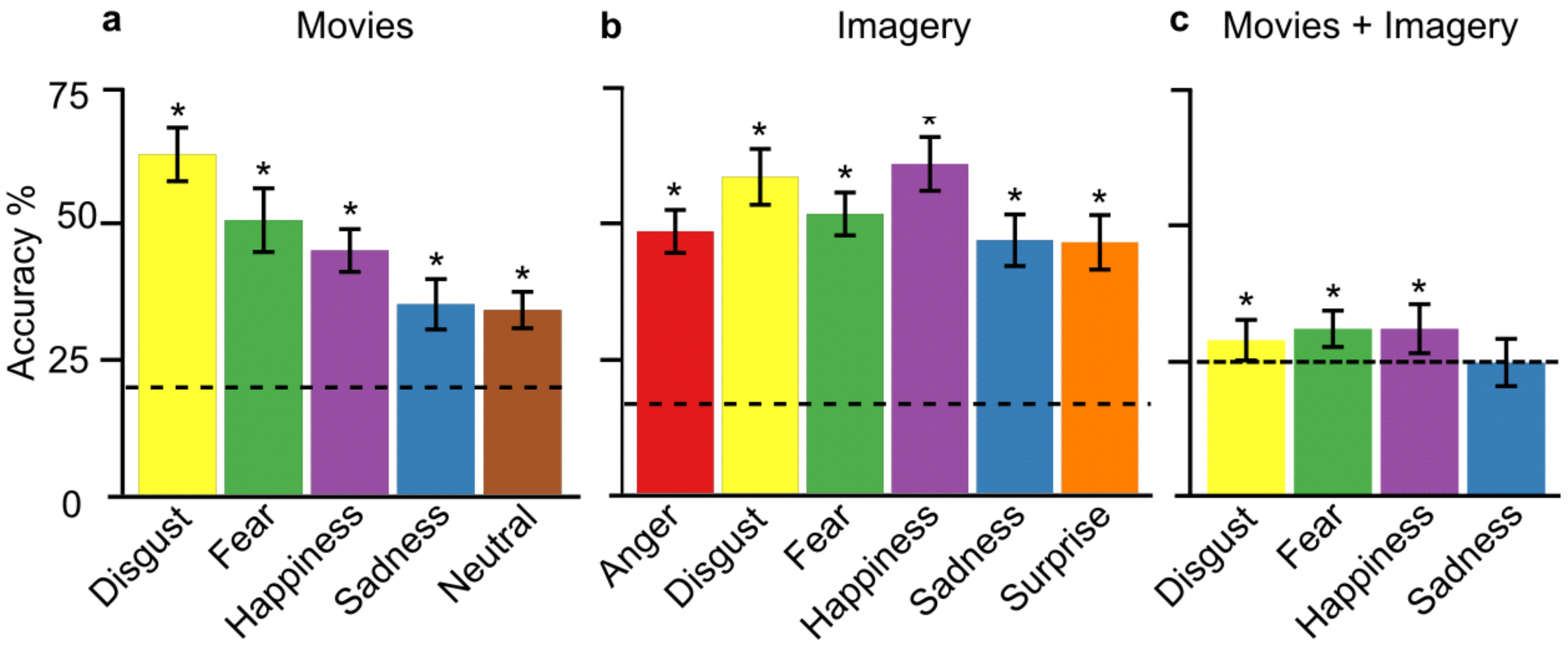
2 seconds

Imagery

+

14 seconds

Saarimäki et al (Cereb Cortex 2015)



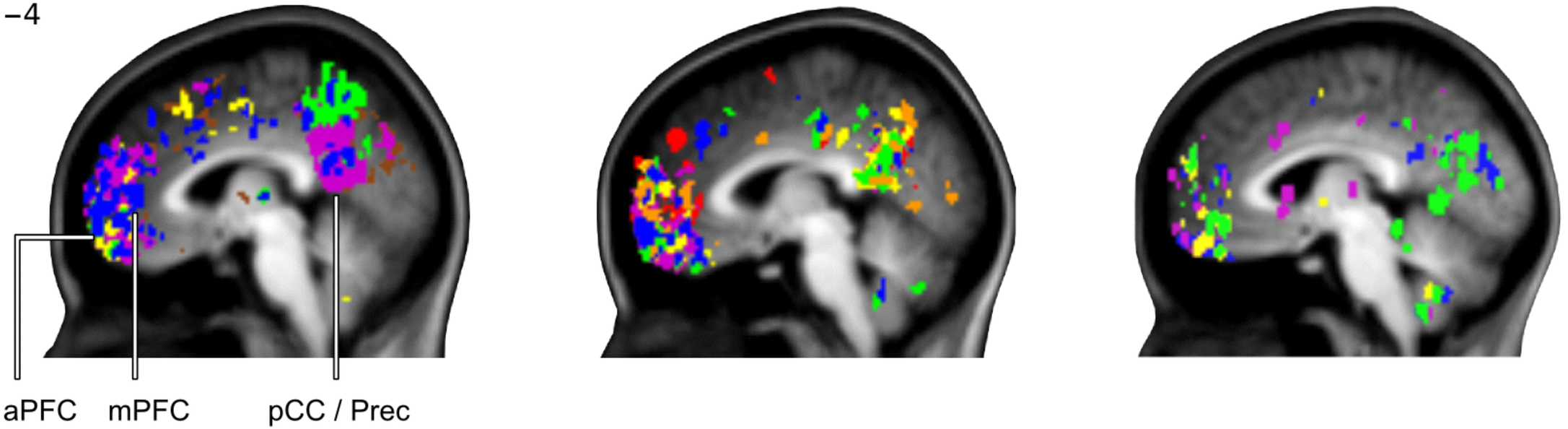
Saarimäki et al (Cereb Cortex 2015)

Movies

Imagery

Crossmodal

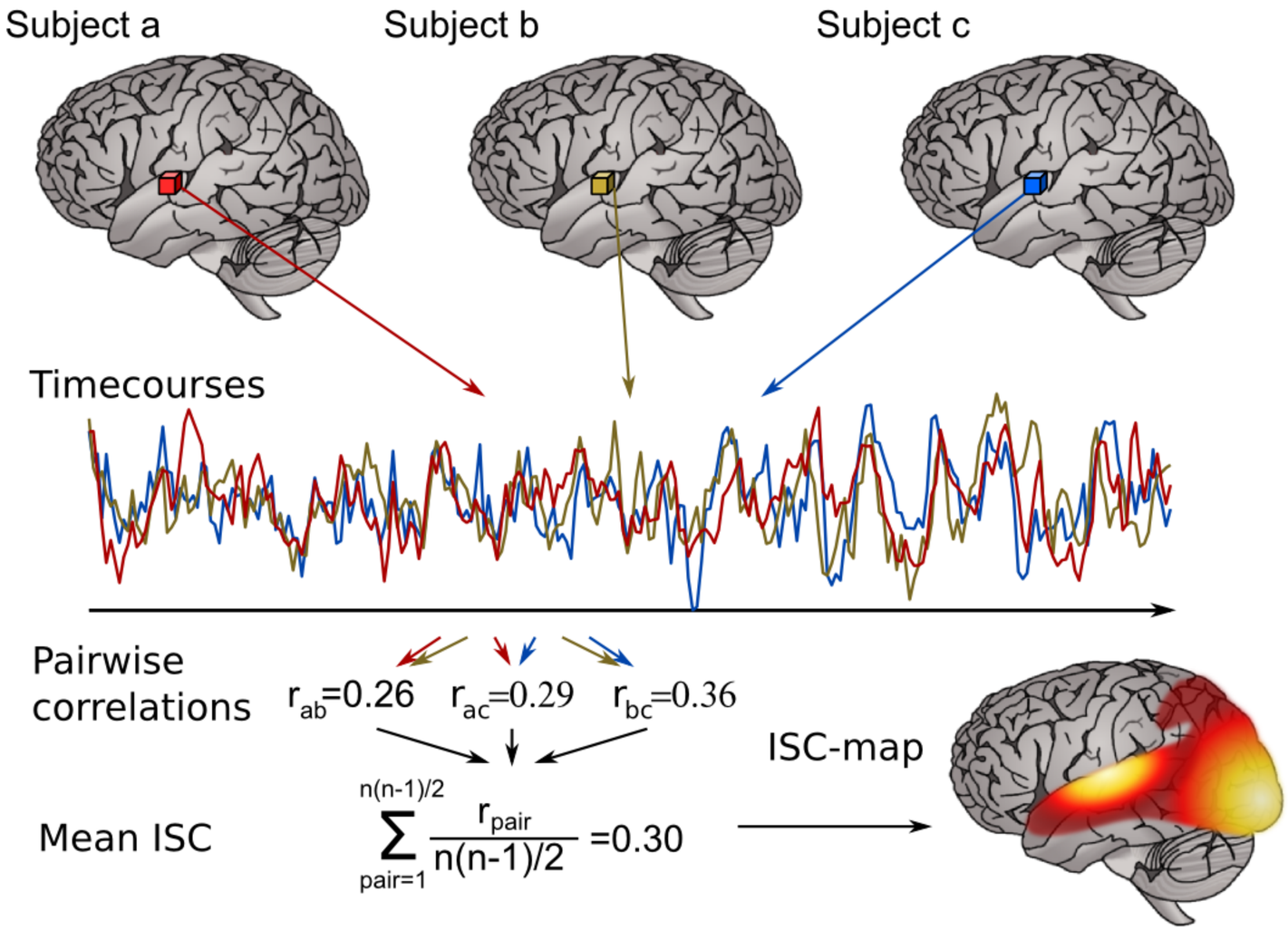
x = −4

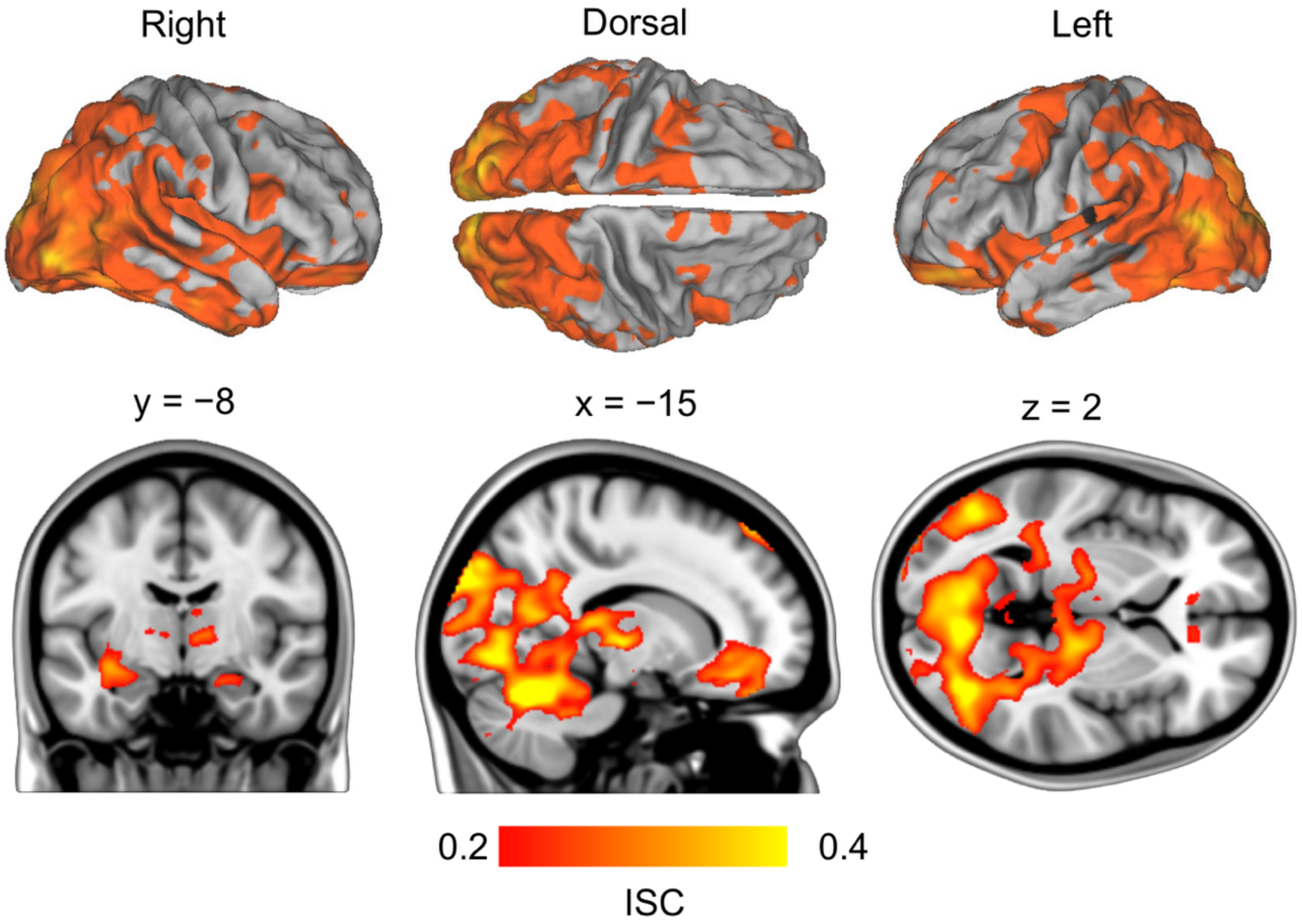


Anger Disgust Fear Happiness Neutral Sadness Surprise

Saarimäki et al (Cereb Cortex 2015)

Intersubject synchronisation





Nummenmaa et al (2012 Proc Natl Acad Sci U.S.A)

5 seconds

15 seconds

29 to 132 seconds

5 seconds

Fixation cross

Text describing the general context of the upcoming movie

Movie clip

Fixation cross
cross, next trial

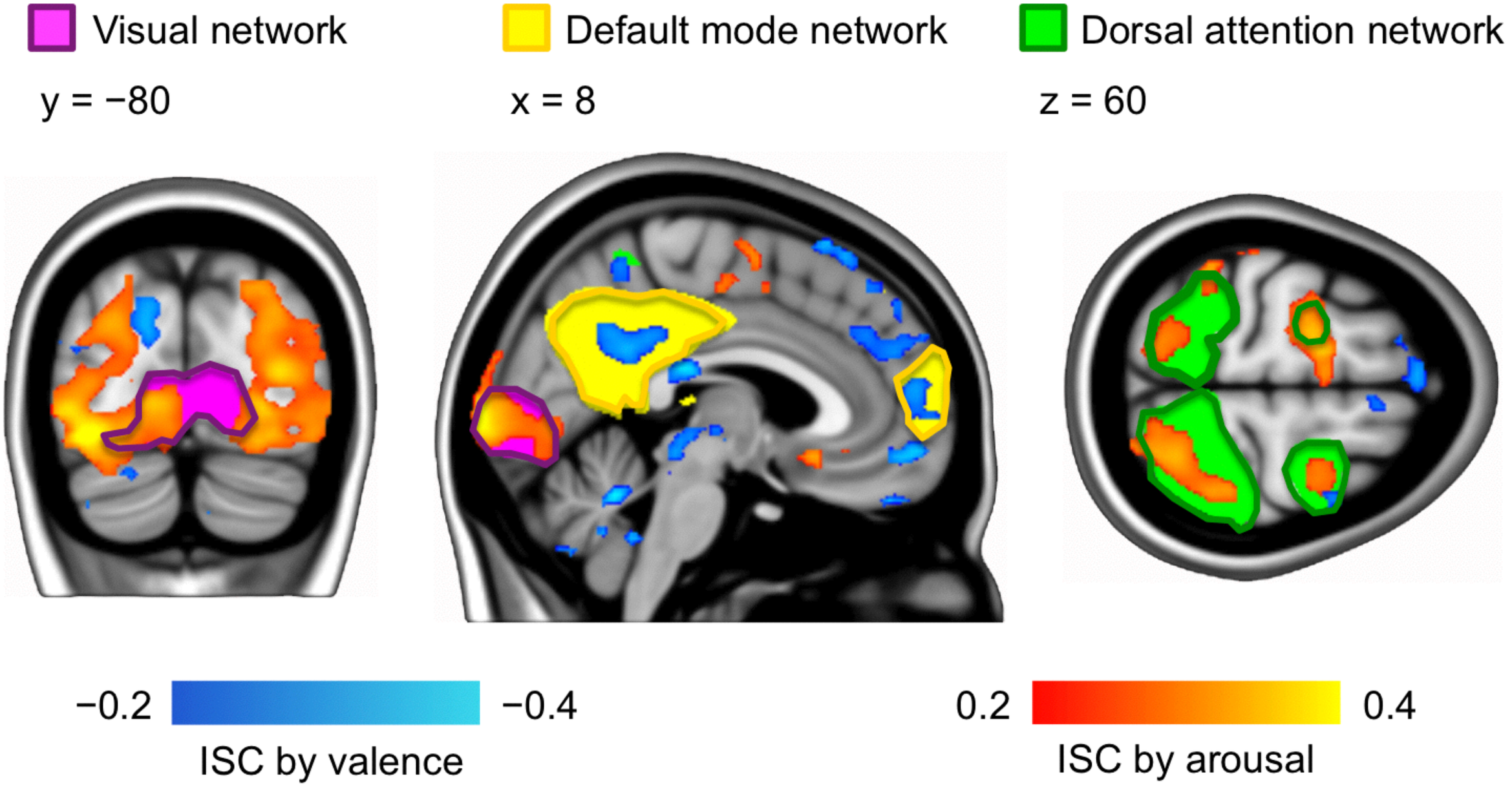
Nummenmaa et al (2012 Proc Natl Acad Sci USA)

Emotions make brains tick together

Francis Ford Coppola: *The Godfather*
Paramount Pictures (1972)

0.2 — 0.6
Intersubject synchronisation

Nummenmaa et al (2012 Proc Natl Acad Sci USA)

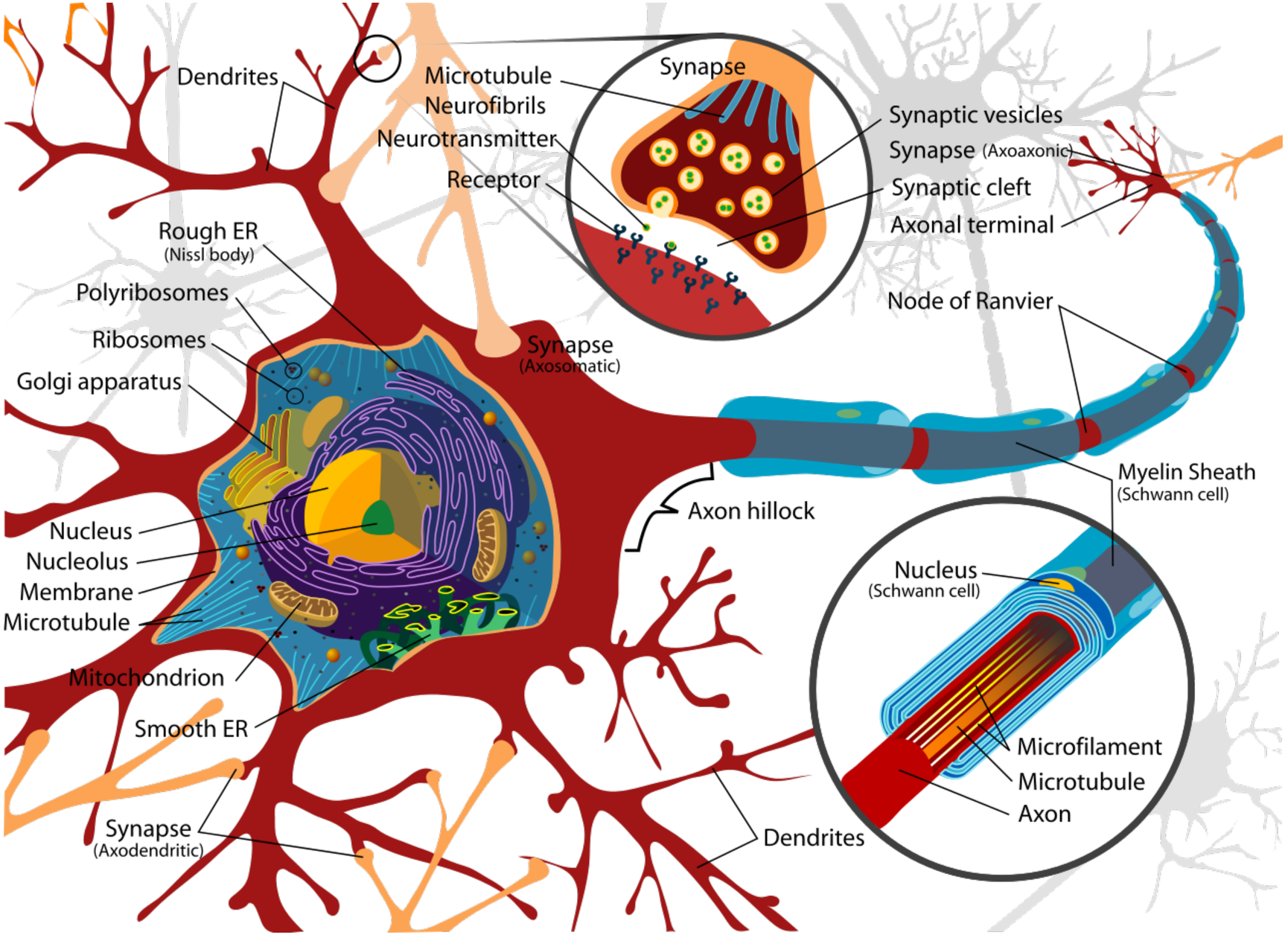


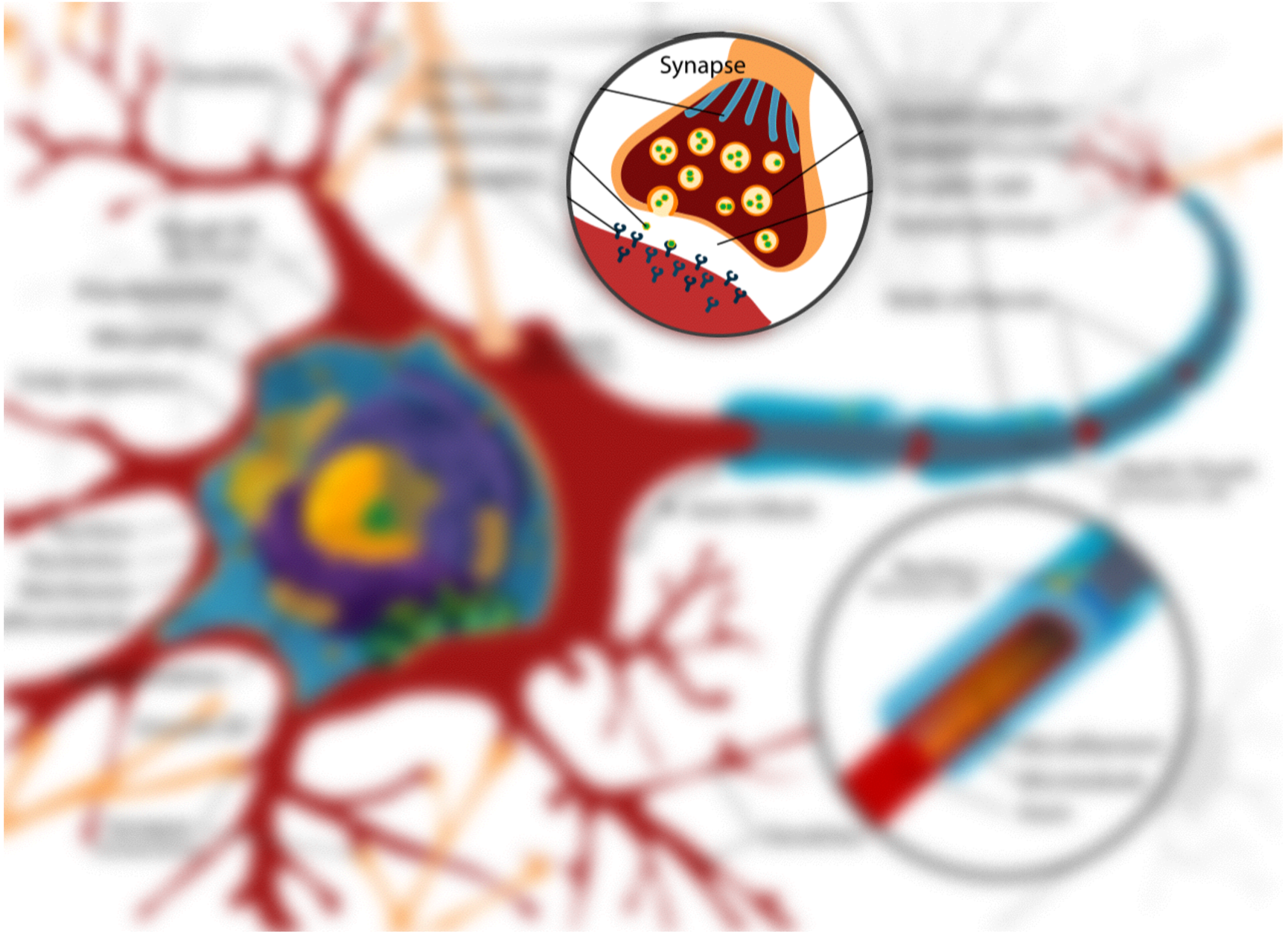
Nummenmaa et al (2012 Proc Natl Acad Sci USA)

Summary - MRI

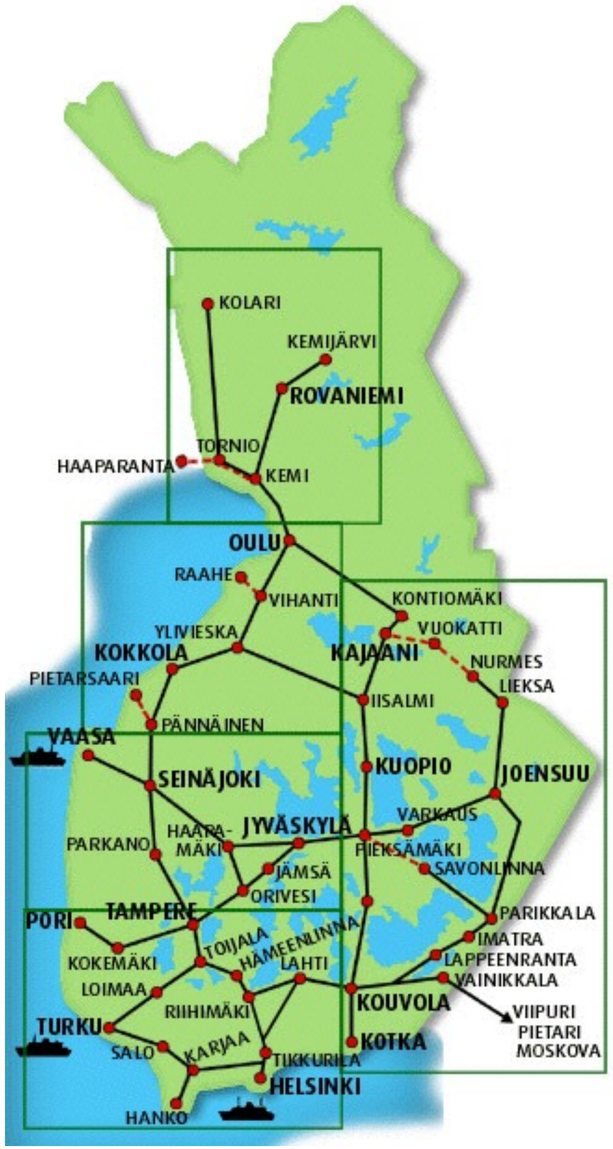
- Noninvasive method for imaging soft tissue
- Can be used for quantifying Hb / dHb ratio from blood
- Indirect measure of brain activity
- Full-volume, high bandwidth, high spatial resolution
- Slow, unspecific, does not measure neural activity directly

Part 4: Principles of Positron Emission Tomography

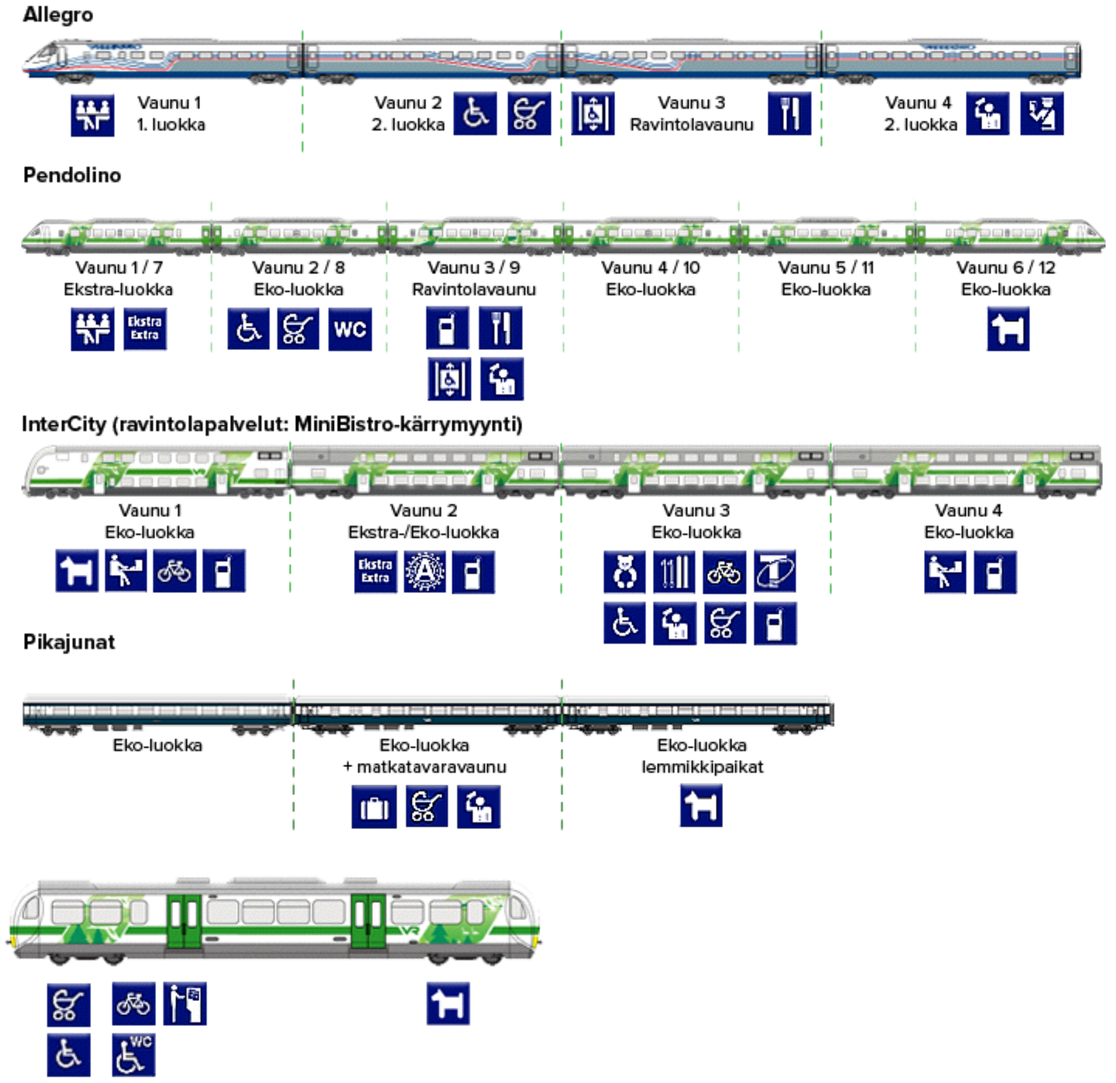

Dendrites
Microtubule
Neurofibrils
Neurotransmitter
Receptor
Synapse
Synaptic vesicles
Synapse (Axoaxonic)
Synaptic cleft
Axonal terminal
Rough ER
(Nissl body)
Polyribosomes
Ribosomes
Golgi apparatus
Synapse
(Axosomatic)
Node of Ranvier
Myelin Sheath
(Schwann cell)
Axon hillock
Nucleus
Nucleolus
Membrane
Microtubule
Mitochondrion
Smooth ER
Synapse
(Axodendritic)
Dendrites
Nucleus
(Schwann cell)
Microfilament
Microtubule
Axon


Synapse

Synaptic connections

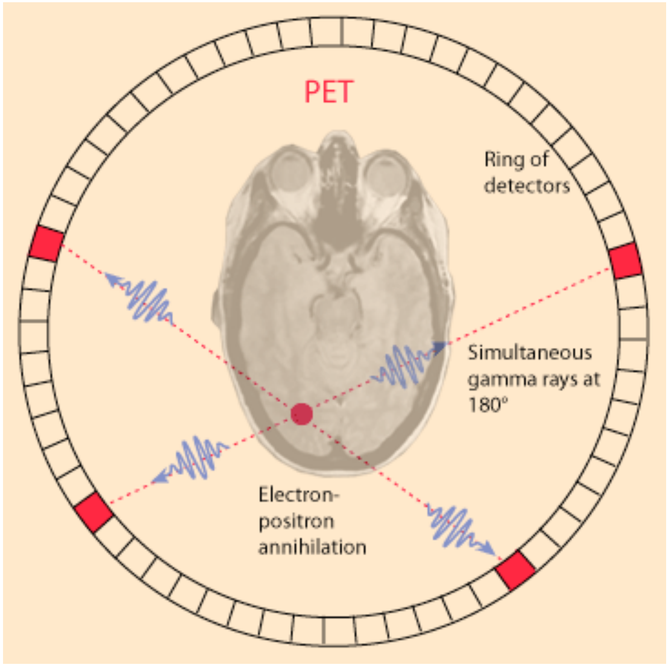


Neurotransmitters

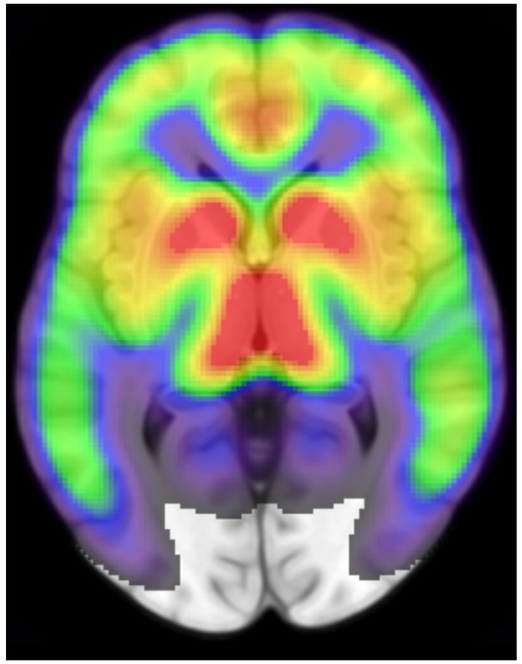


PET camera

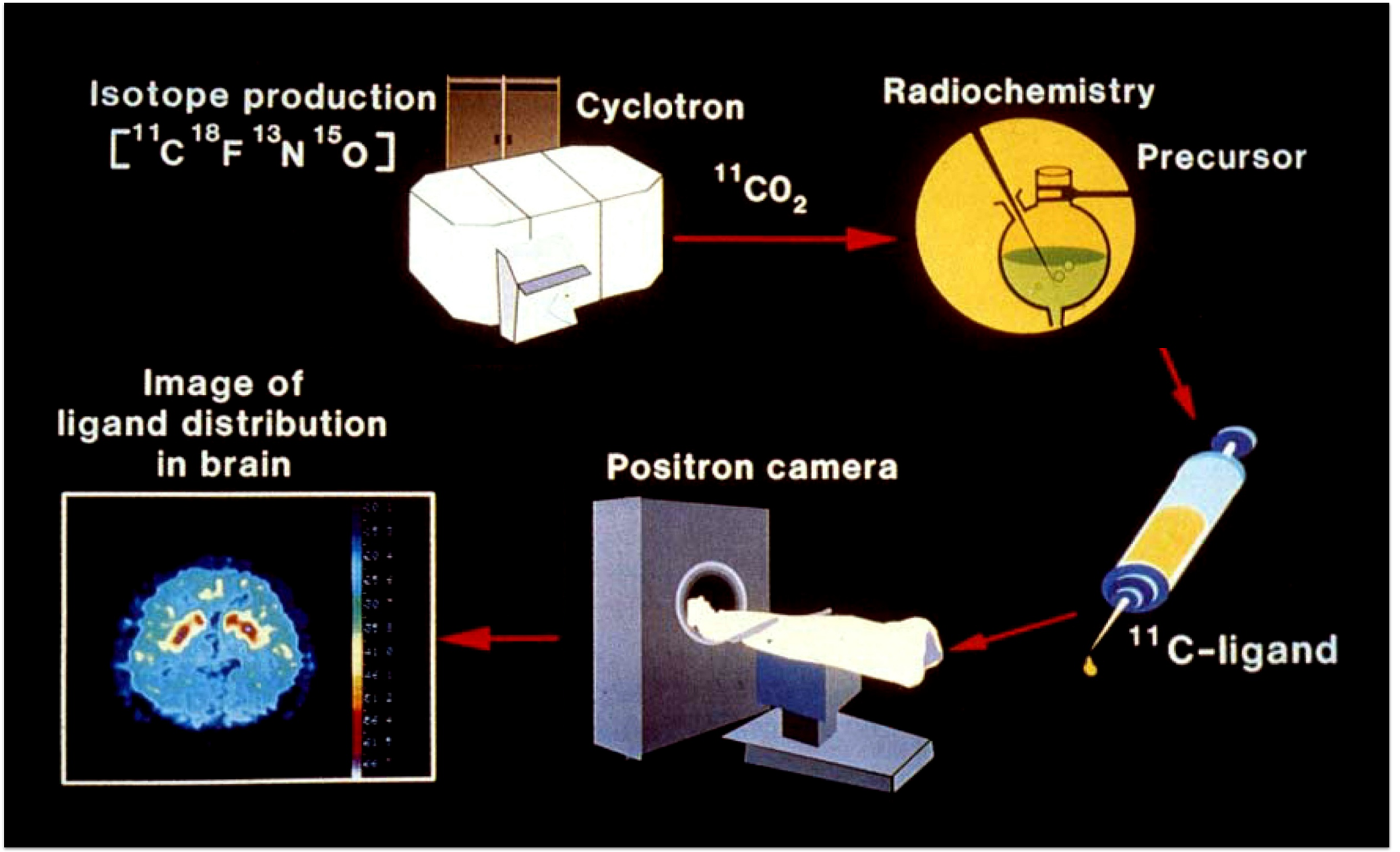
Coincedence detection



Reconstructed image



Positron Emission Tomography allows in vivo quantification of the distribution of specific chemical compounds. It can thus be used for studying specific neurotransmitter systems.


Isotope production
[^{11}C ^{18}F ^{13}N ^{15}O]
Cyclotron
$^{11}CO_2$
Radiochemistry
Precursor
^{11}C-ligand
Positron camera
Image of
ligand distribution
in brain

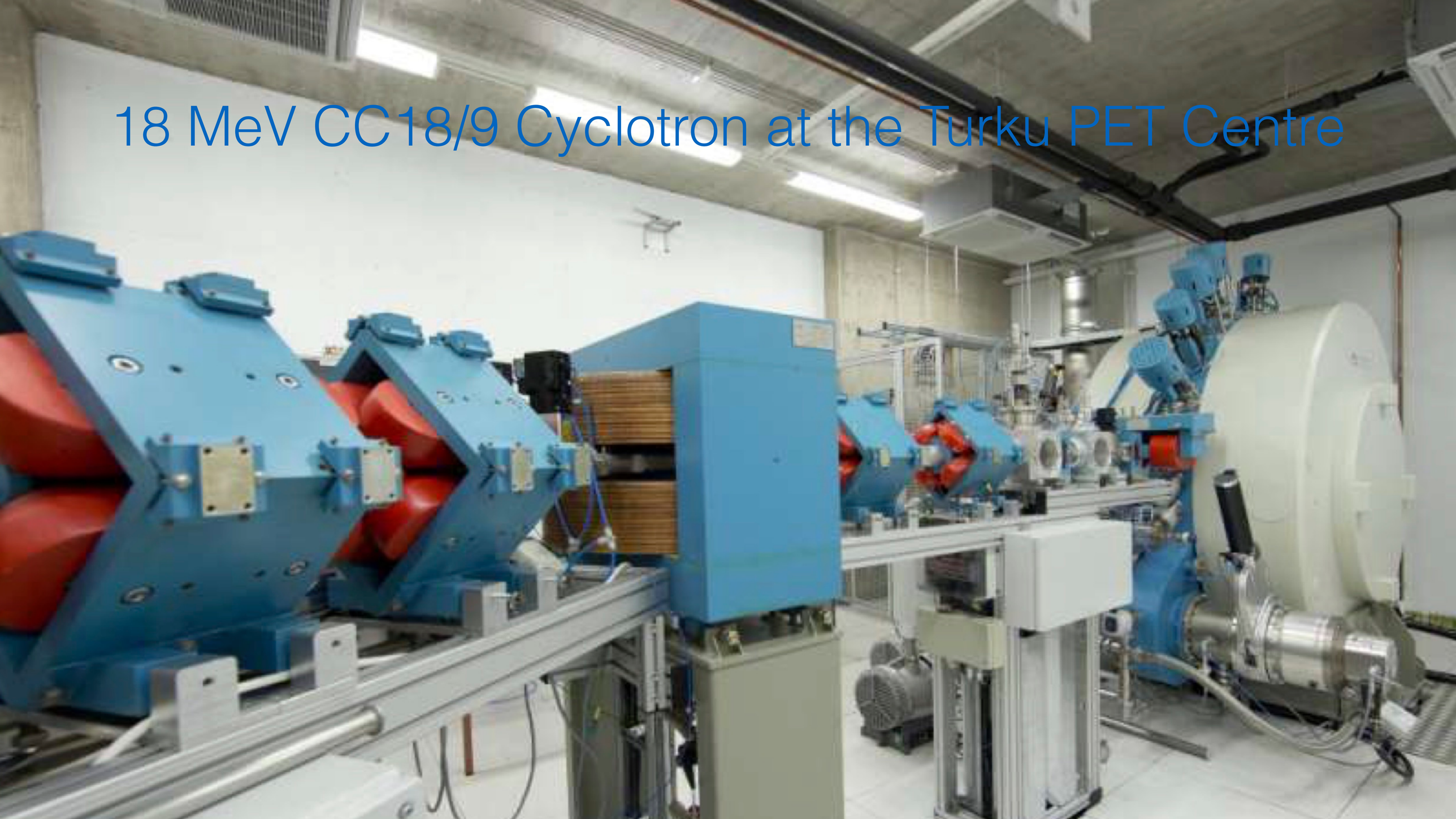
Radiochemistry

- Radioligands: Biologically active, unstable isotopes
- Decay via positron emission
- Short half-life required for sufficient SNR and reasonable scan duration
- Need to be synthesised close to PET camera
- Radiochemistry allows investigation of any biological circuit as long as it can be radiolabeled
- Radiochemistry is the “pulse sequence design for PET”

What makes a good radioligand?

- Optimal target density and ligand affinity: Density x Affinity ≈5
- High brain uptake
- Optimal lipophilicity (LogP=2.5–4); sufficiently high to cross blood-brain barrier but not too high to cause non-specific binding
- Not substrate for efflux transporters at BBB (e.g., P-gp)
- High pharmacological selectivity
- No brain-penetrant radiometabolites
- Quantifiable plasma protein binding
- Amenability to rapid labelling with high specific activity
- Fast enough kinetics to allow measurement in a few hours

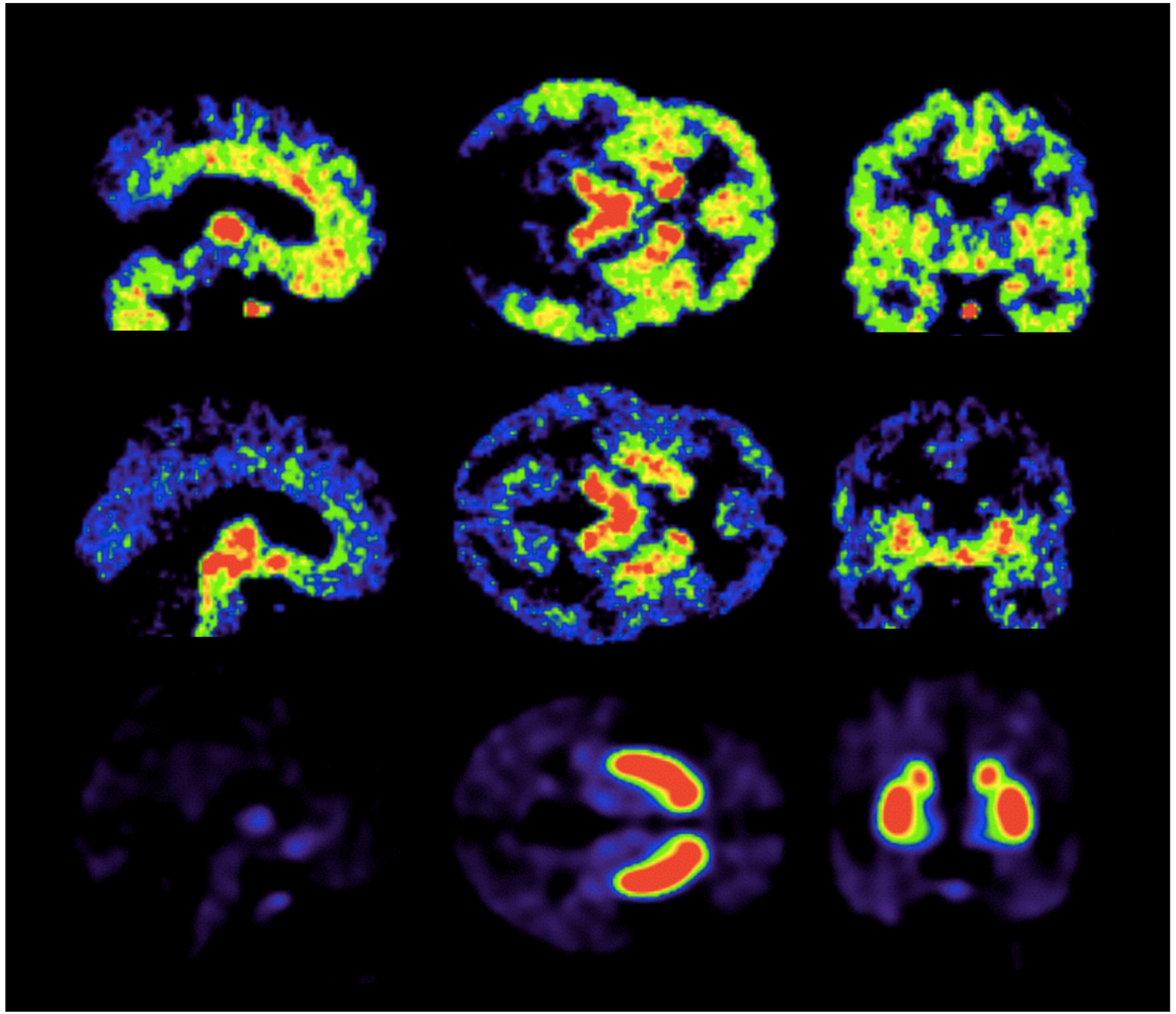
18 MeV CC18/9 Cyclotron at the Turku PET Centre



[11C] carfentanil
MOR tracer

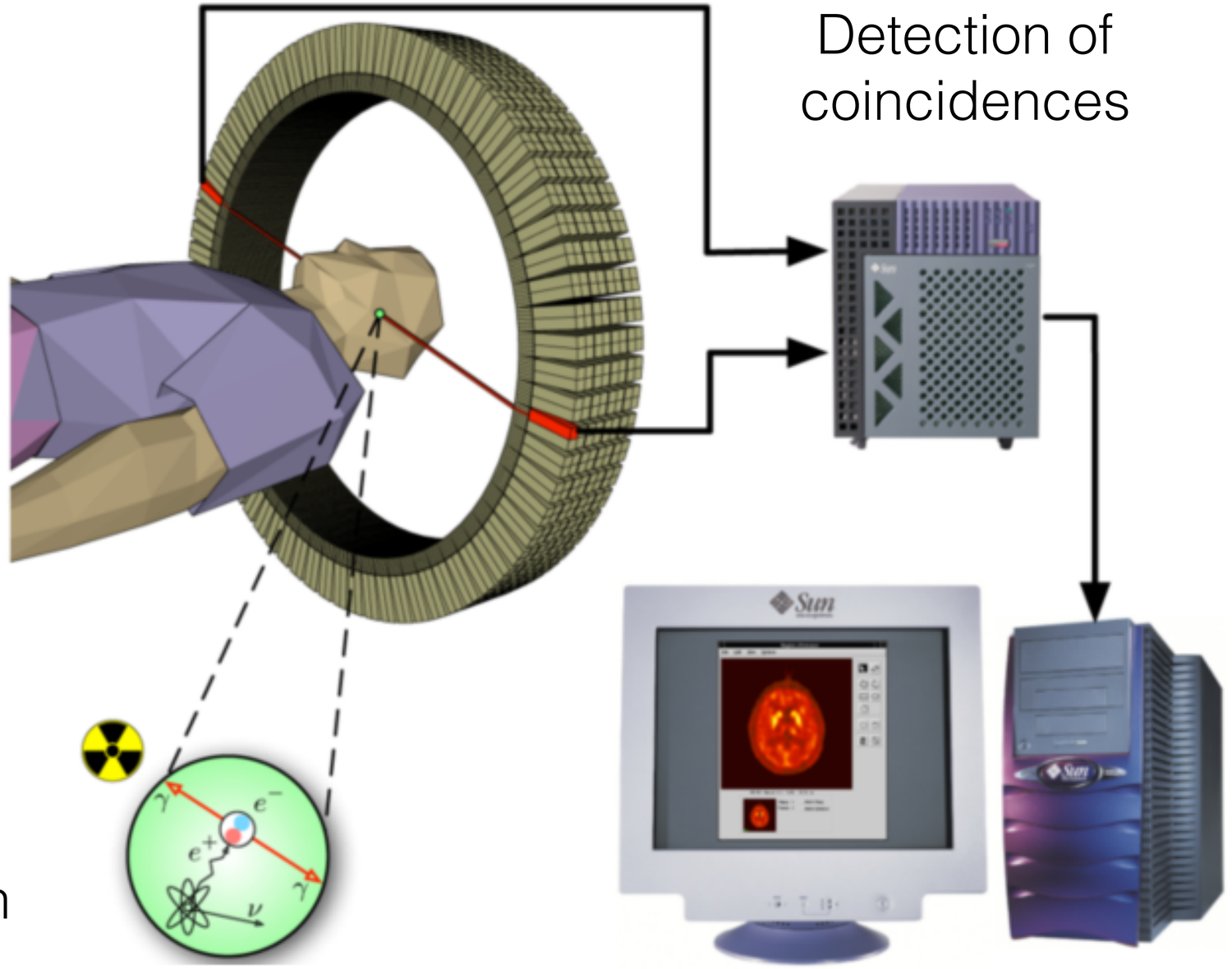
[11C] MADAM
SERT tracer

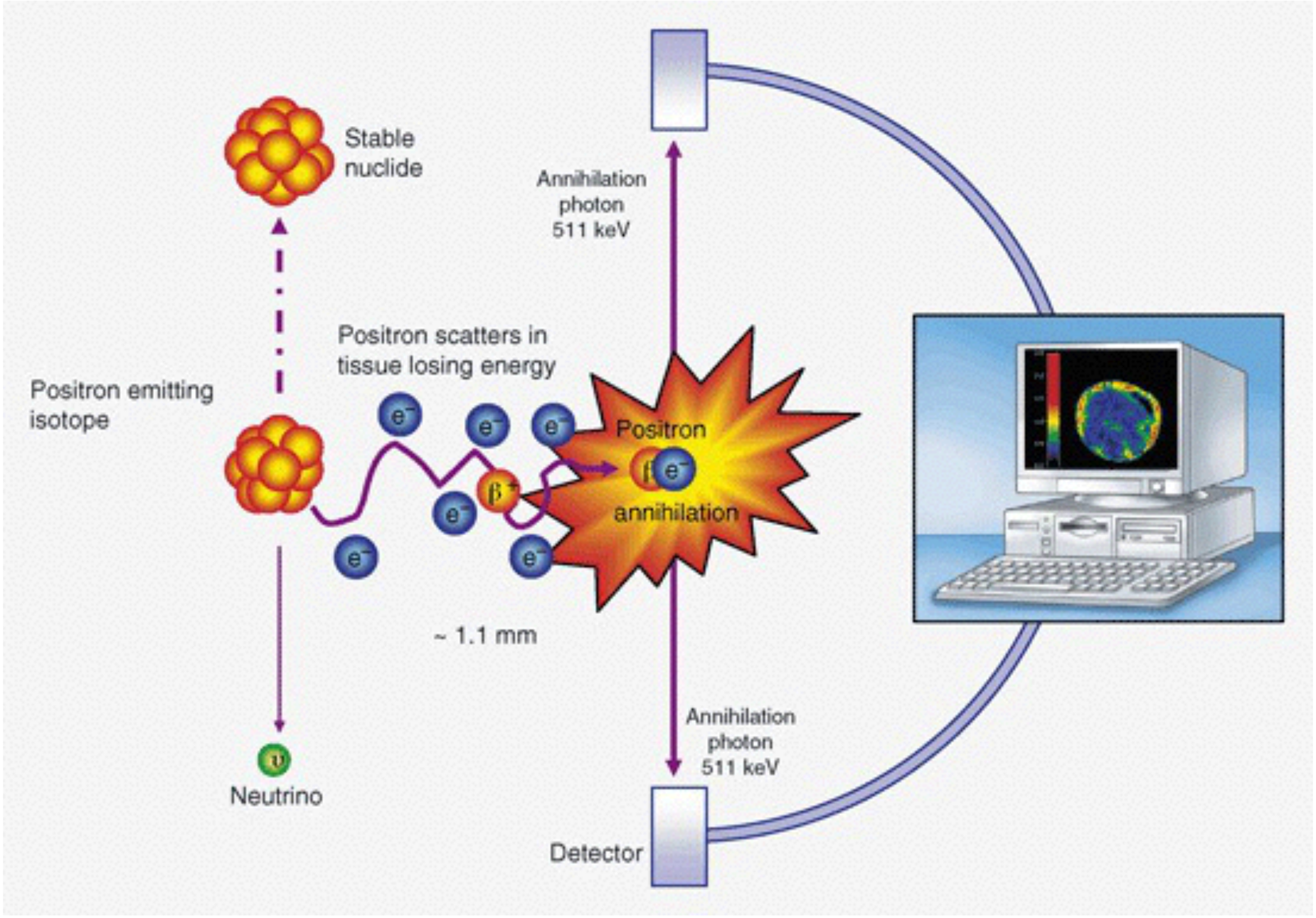
[11C] raclopride
D2R tracer



Some common PET tracers

Stable molecule	Isotope	Half-life	Typical tracer	Target molecule / system
Fluoride [F]	[18F]	118 min	[18F]FDG	glucose analogue
Carbon [C]	[11C]	20 min	[11C]carfentanil	MOR / KOR / DOR -receptor
Nitrogen [N]	[13N]	10 min	[13N] ammonia	perfusion
Oxygen [O]	[O15]	2 min	H2O15	perfusion

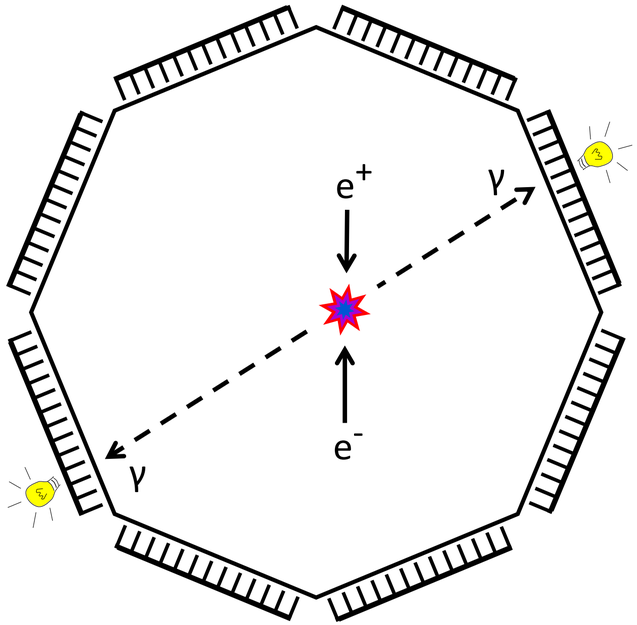

Detection of coincidences
Positron emission
Image reconstruction

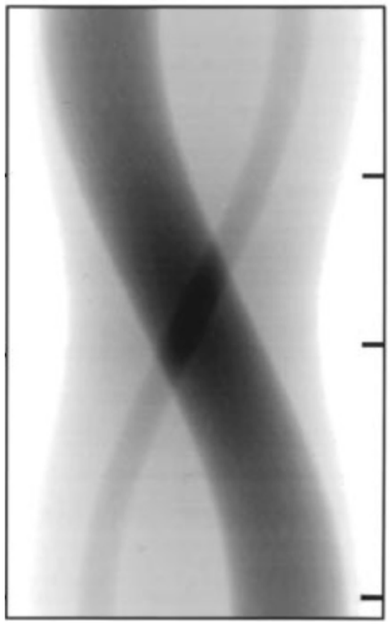

Stable nuclide
Annihilation photon 511 keV
Positron scatters in tissue losing energy
Positron emitting isotope
Positron
annihilation
~ 1.1 mm
Neutrino
Annihilation photon 511 keV
Detector

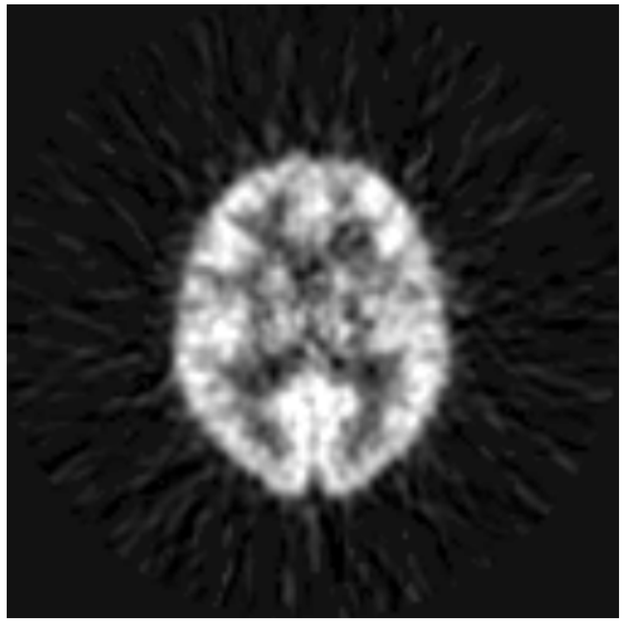
Coincidence

Sinogram

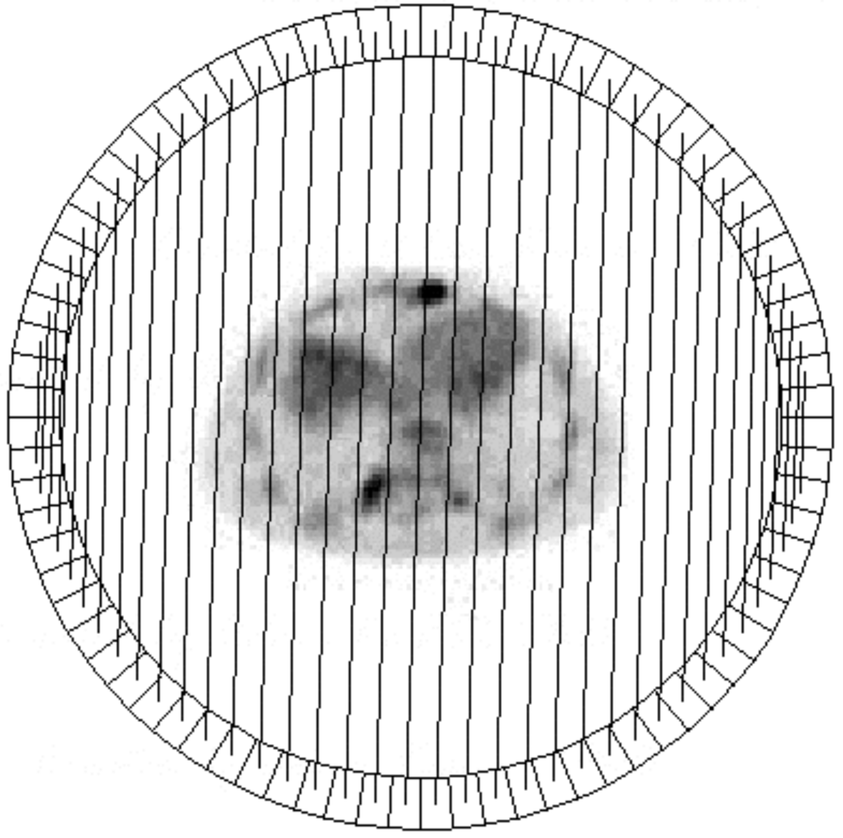
Reconstructed slice







Lines of response between PET detectors

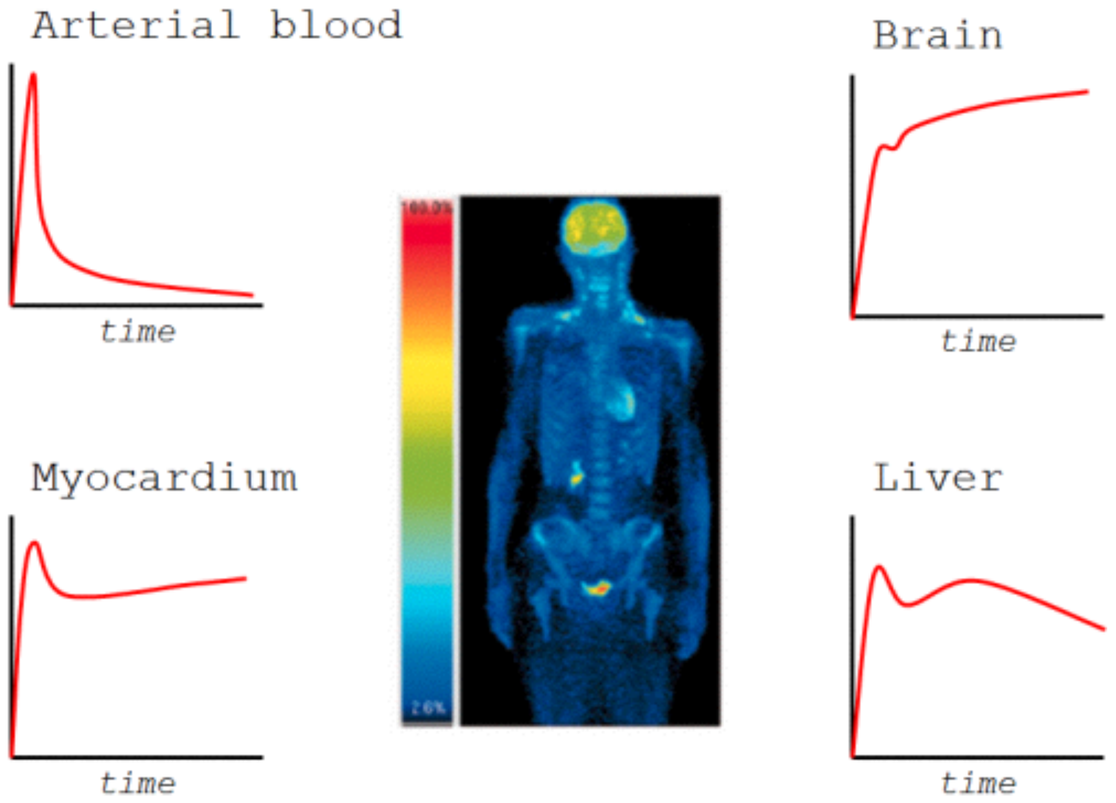


Angle: 0 °

Corresponding location in sinogram

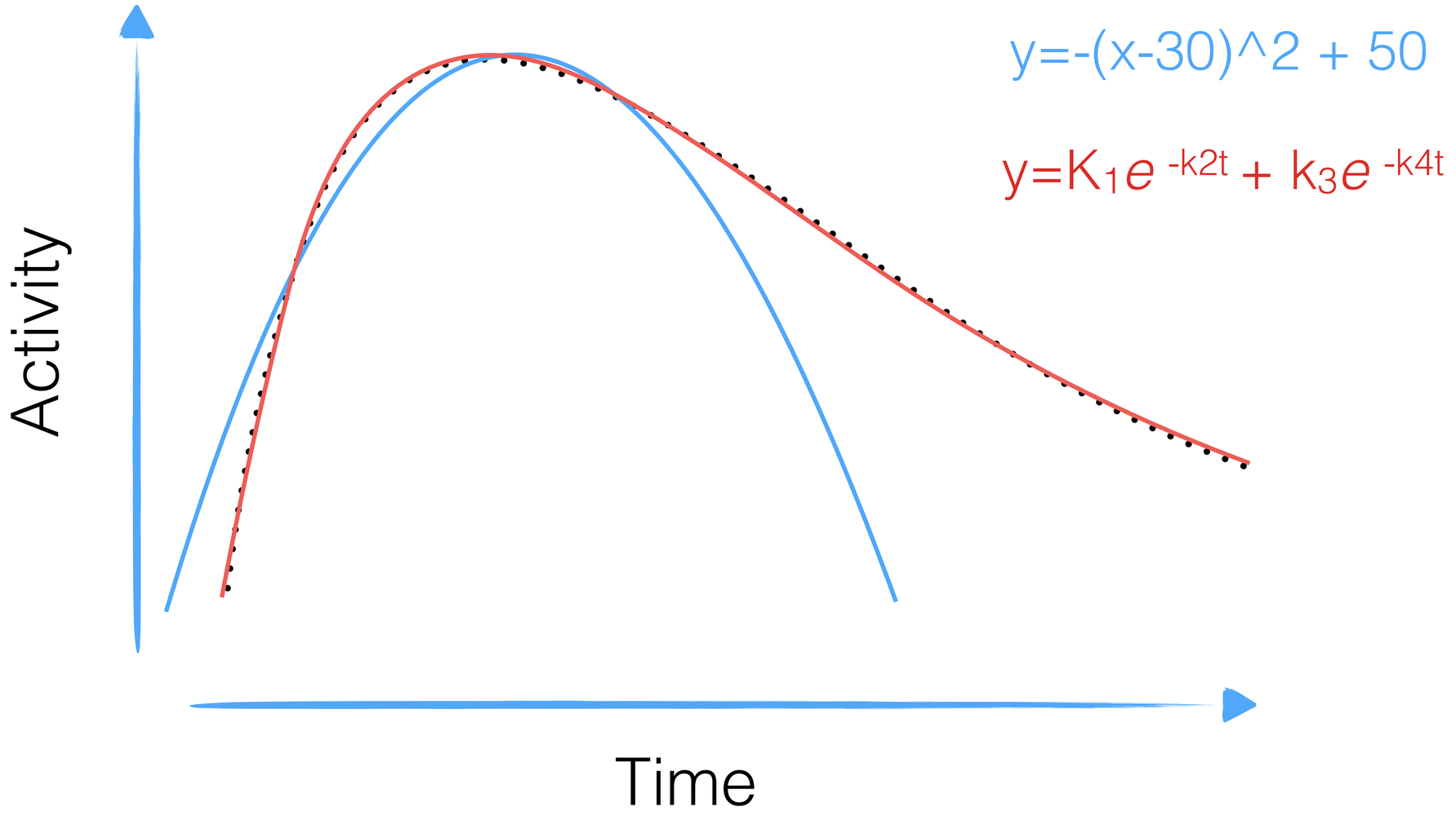
Modelling

- Modelling transforms radioactivity concentration into biologically relevant pharmacokinetic information
  - **No modeling** (‘raw’ radioactivity)
  - **Standardised uptake value** (SUV; control for injection and weight)
  - **Kinetic modeling** (arterial plasma as input)
  - **Reference tissue model** (reference tissue as input; not always possible as e.g. with H2O)

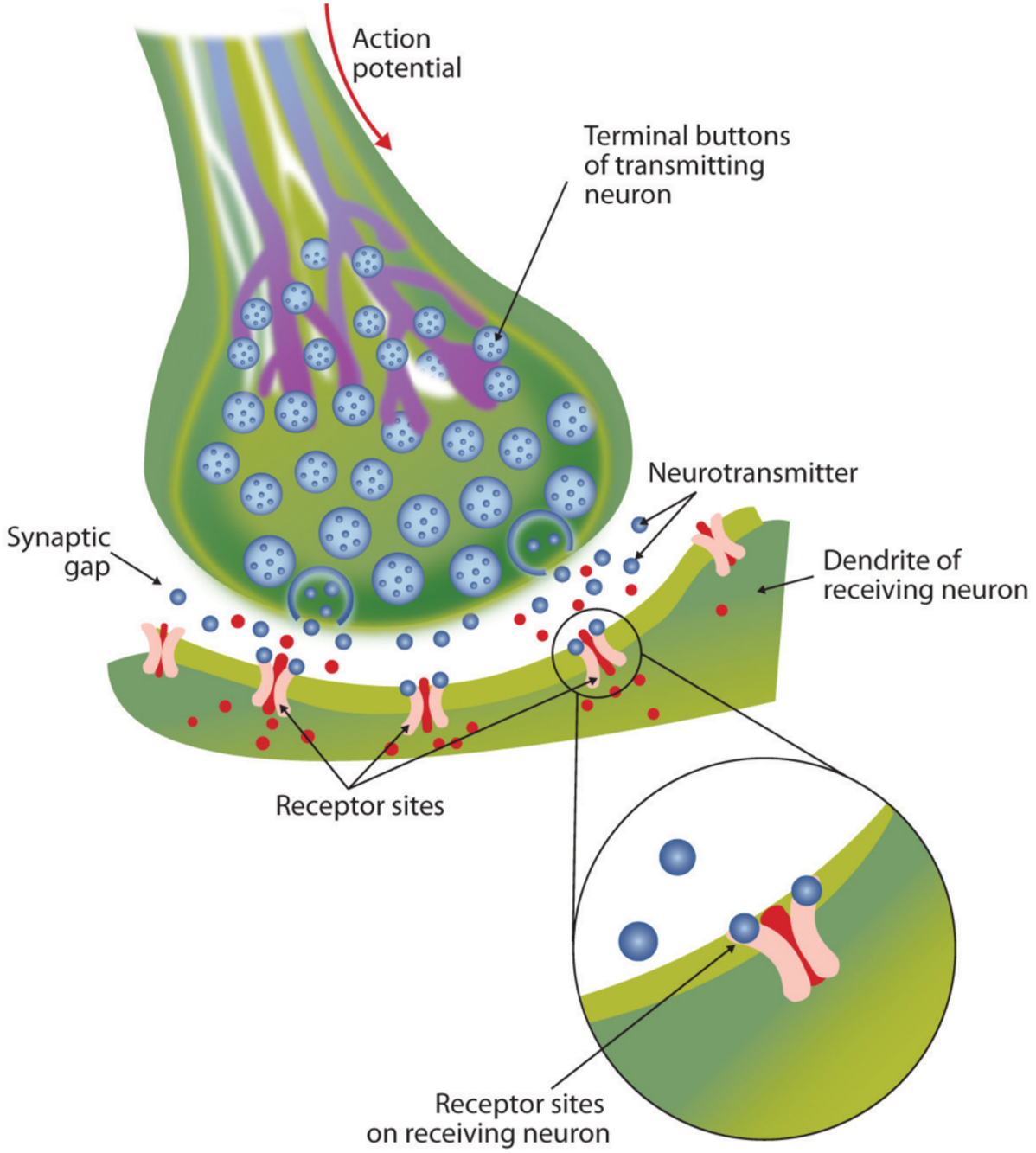


Oikonen (Turku PET Centre)

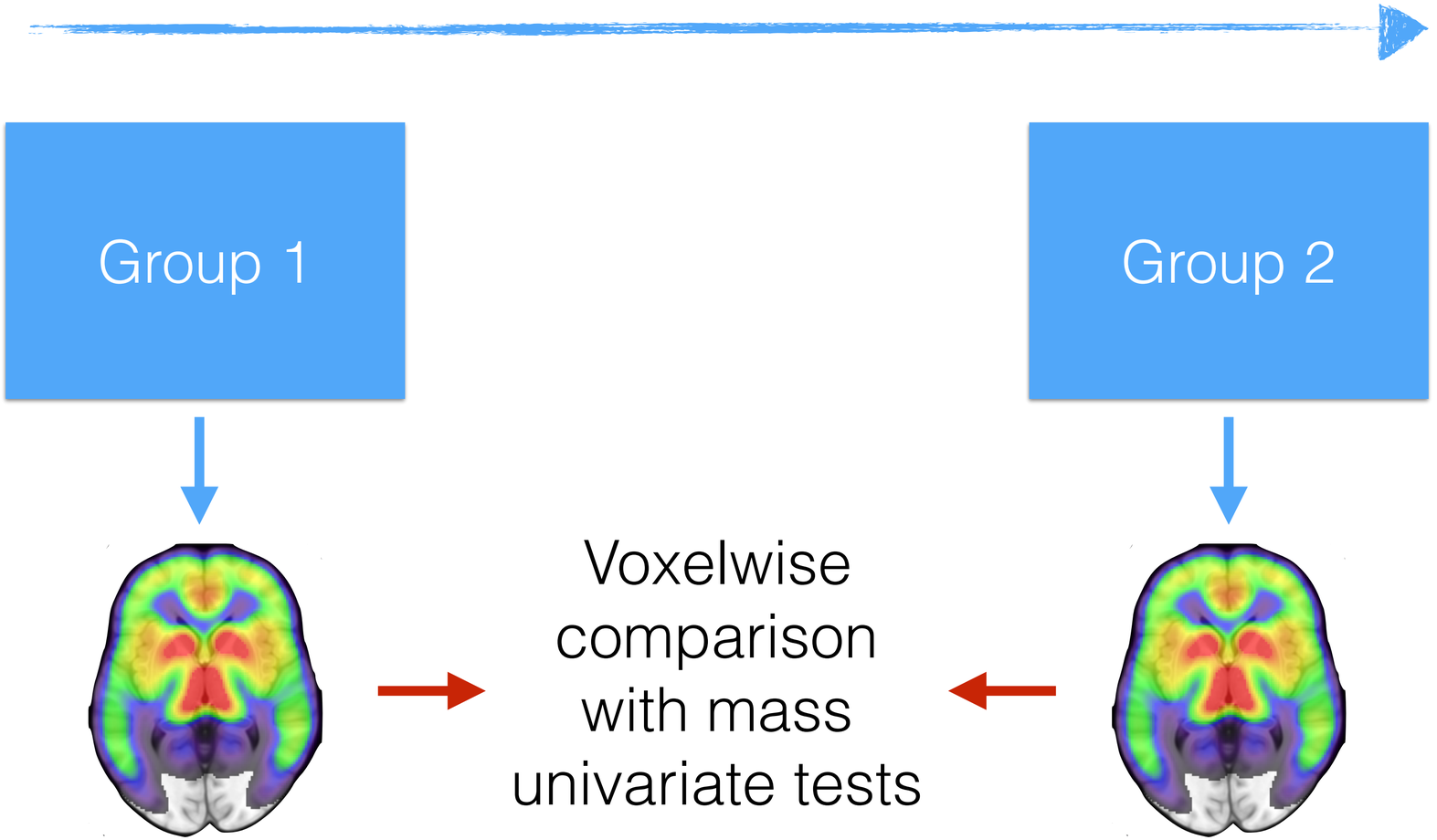
Fitting a function to the data

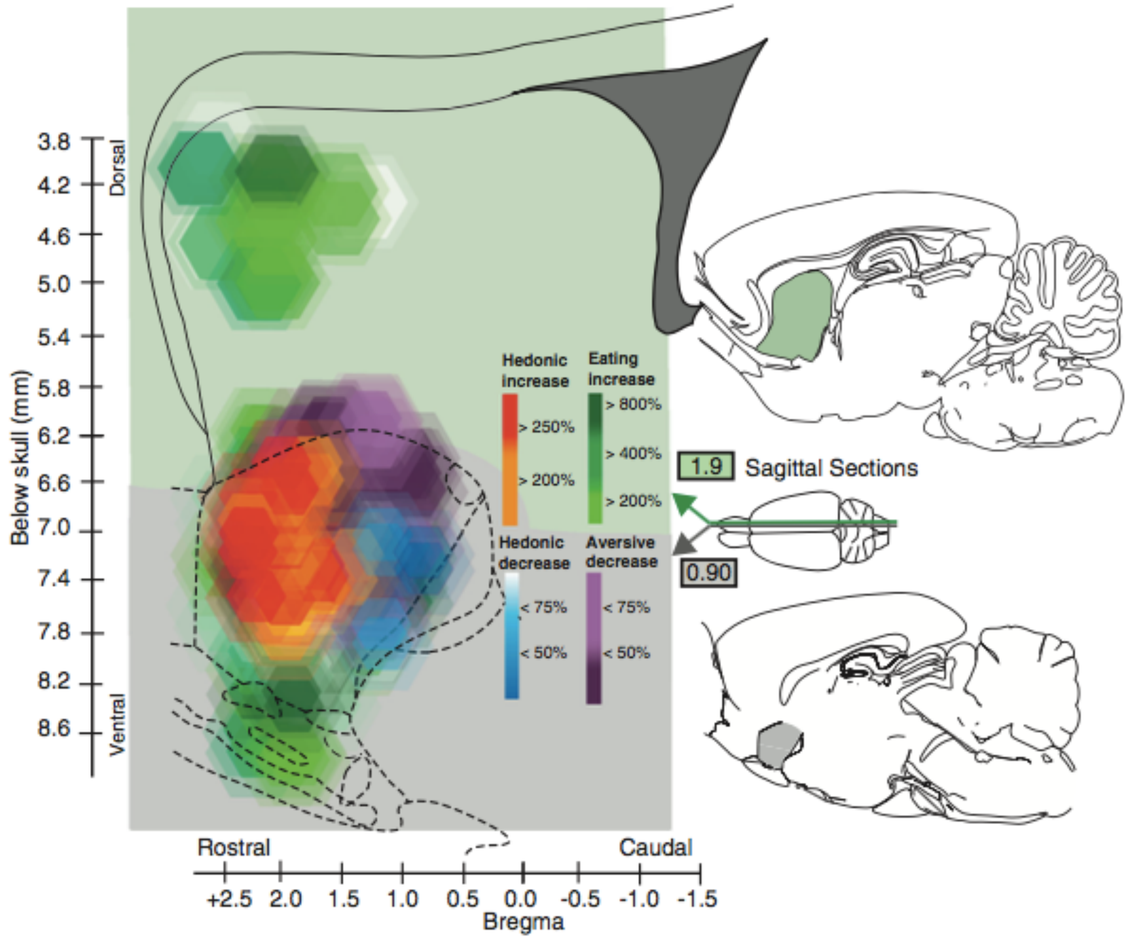


Part 5: Experimental designs for PET

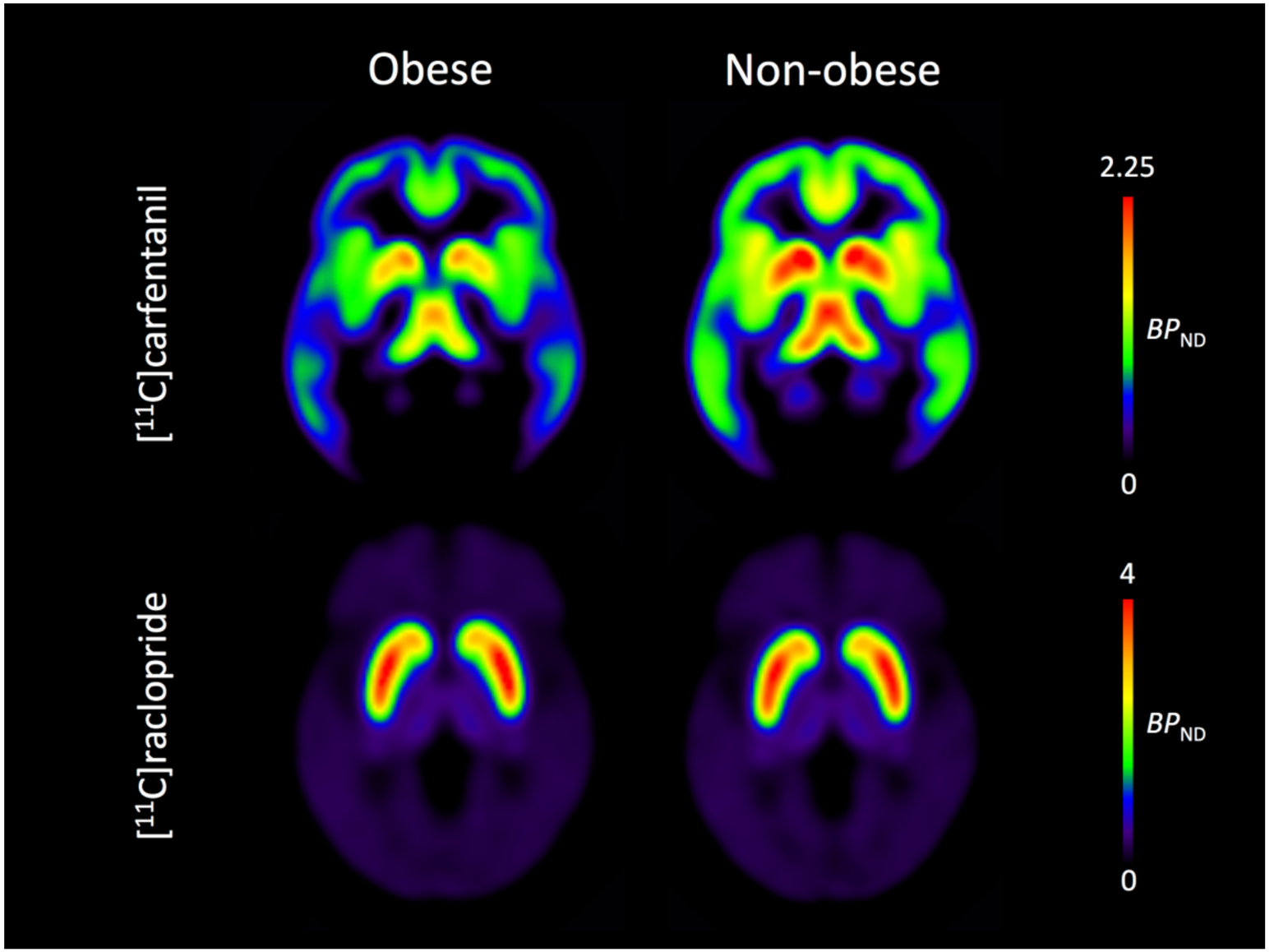

Action potential
Terminal buttons of transmitting neuron
Neurotransmitter
Synaptic gap
Dendrite of receiving neuron
Receptor sites
Receptor sites on receiving neuron

Between-groups design

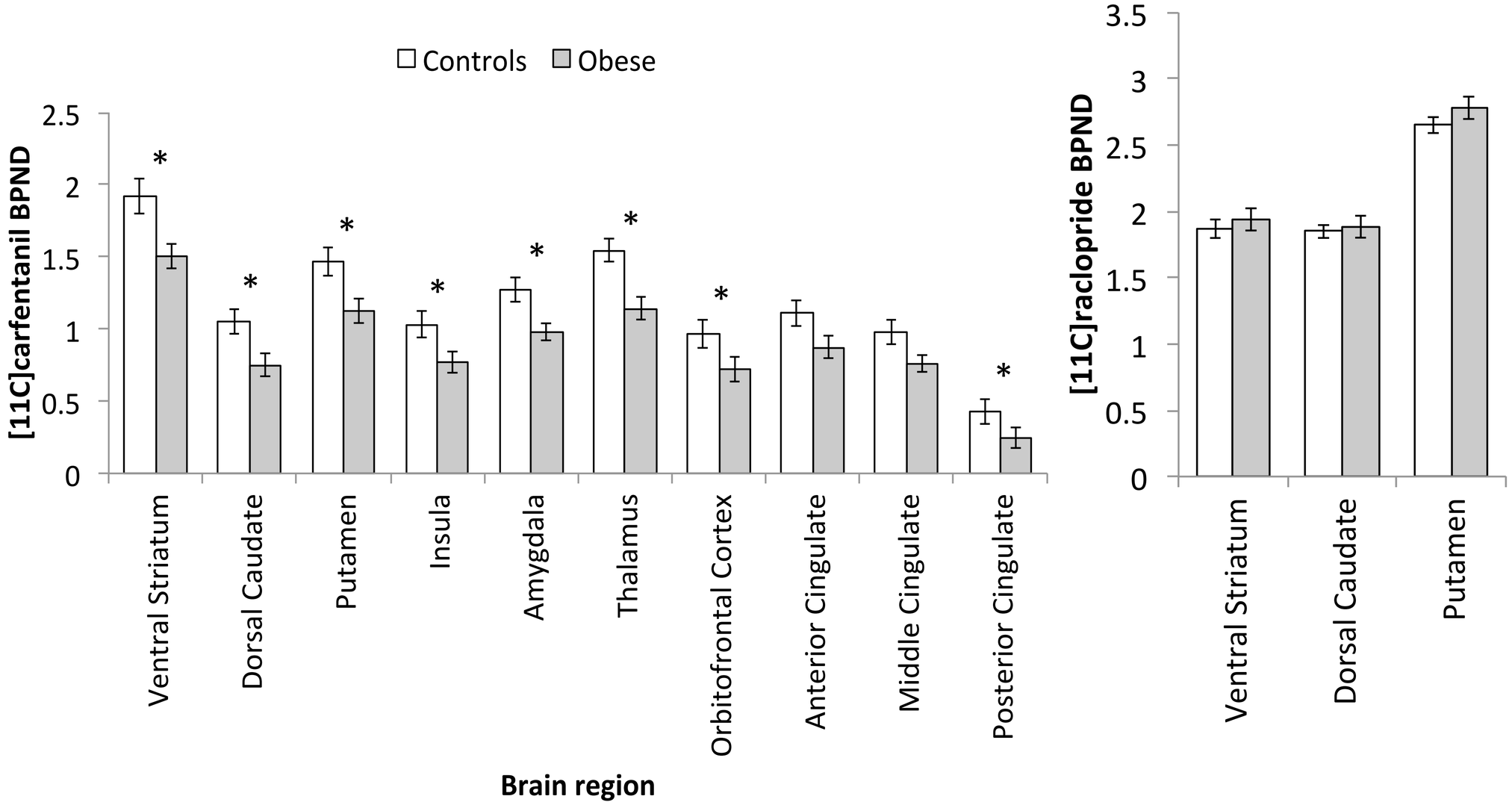




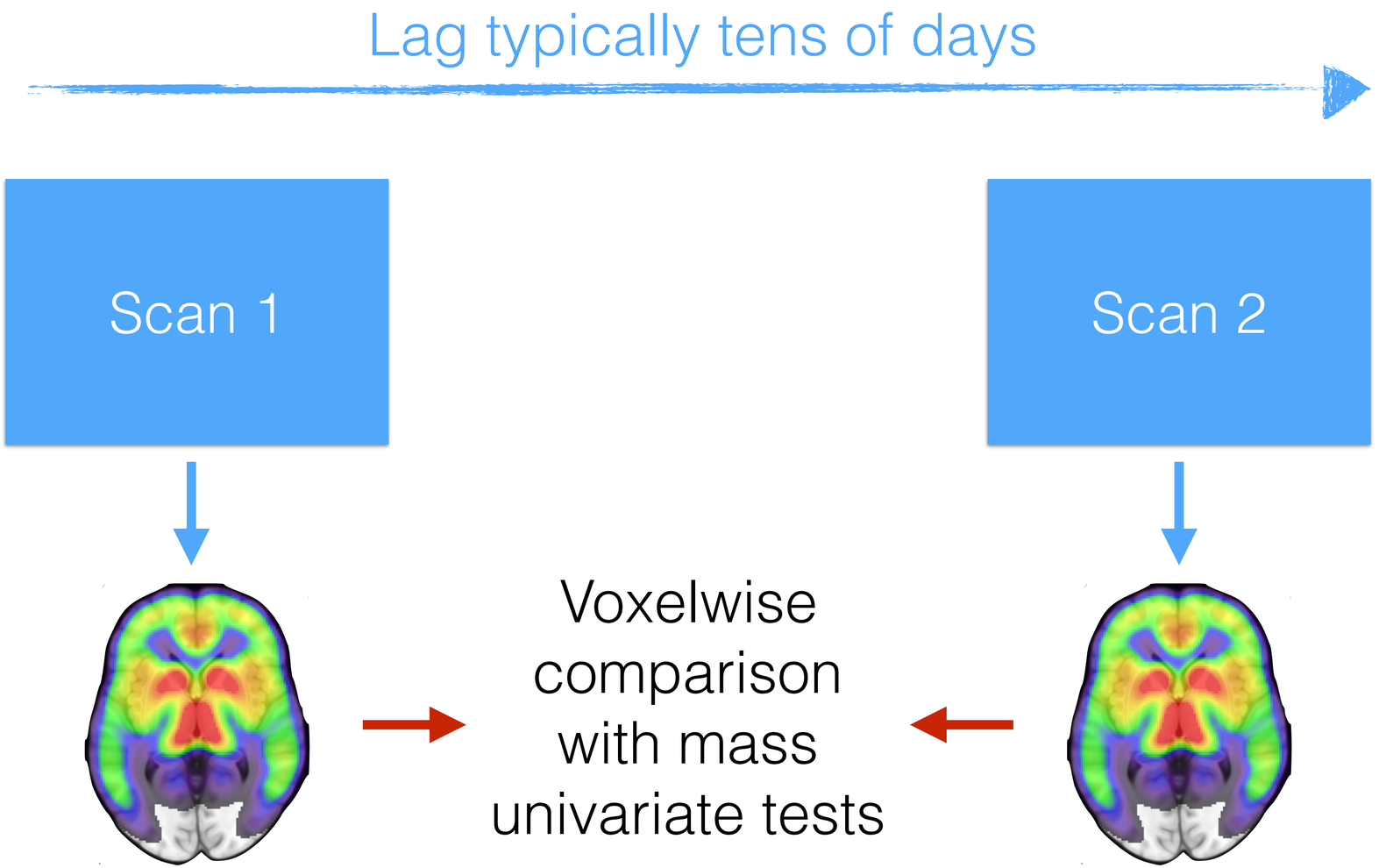
Berridge & Kringelbach (2013 CiN)

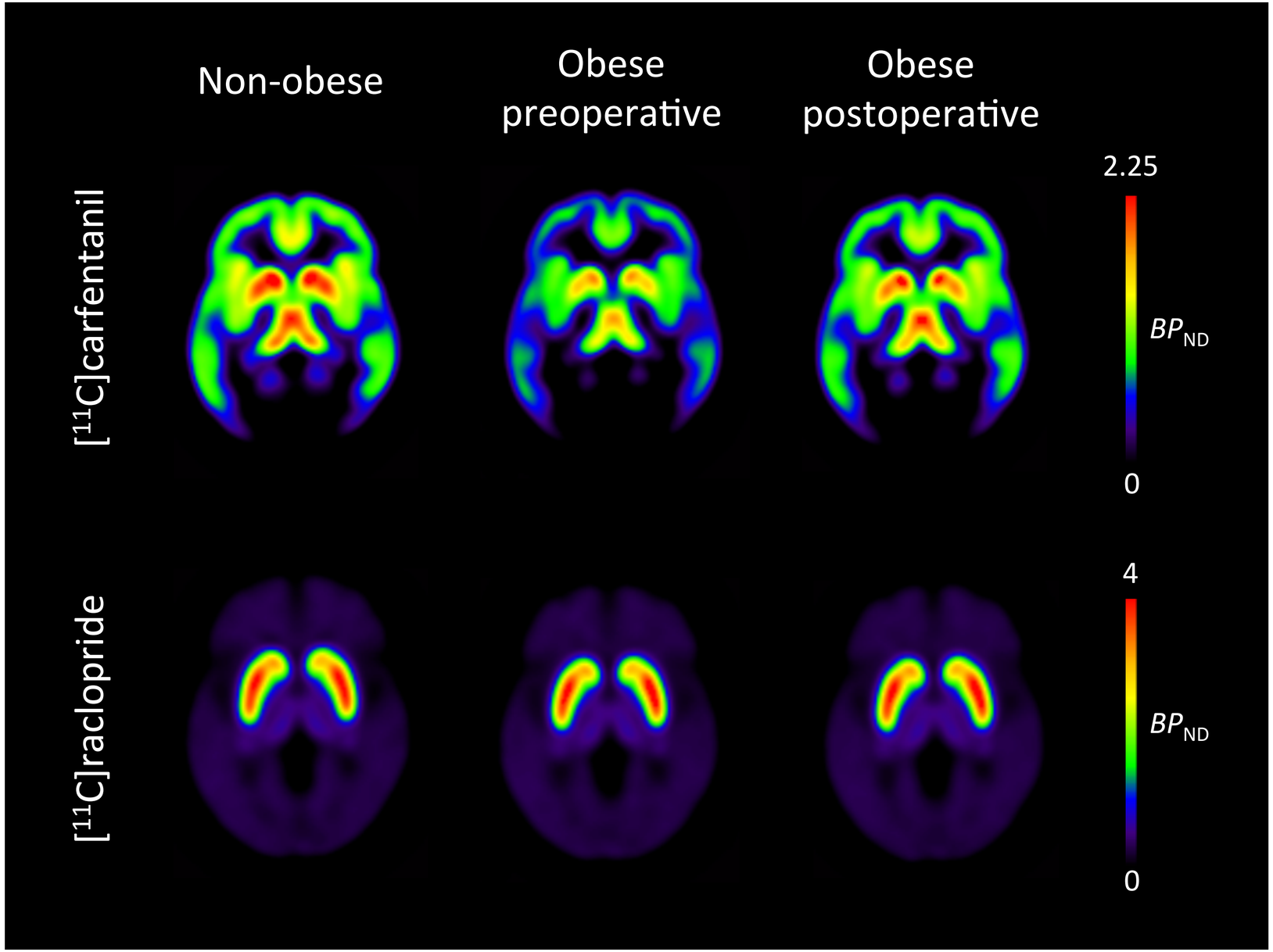


Karlsson et al (2015 J Neurosci)


Controls
Obese
[11C]carfentanil BPND
2.5
2
1.5
1
0.5
0
*
Ventral Striatum
Dorsal Caudate
Putamen
Insula
Amygdala
Thalamus
Orbitofrontal Cortex
Anterior Cingulate
Middle Cingulate
Posterior Cingulate
Brain region
[11C]raclopride BPND
3.5
3
2.5
2
1.5
1
0.5
0
Ventral Striatum
Dorsal Caudate
Putamen

Longitudinal design





Karlsson et al (2015 Mol Psych)

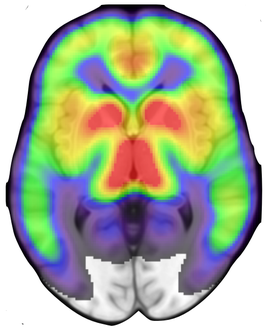
Functional PET (challenge paradigm)

Temporal resolution tens of minutes

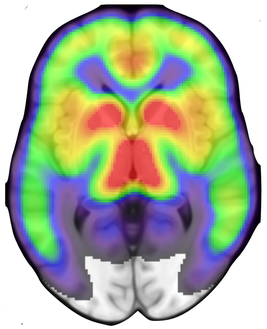
Task 1

Decay break

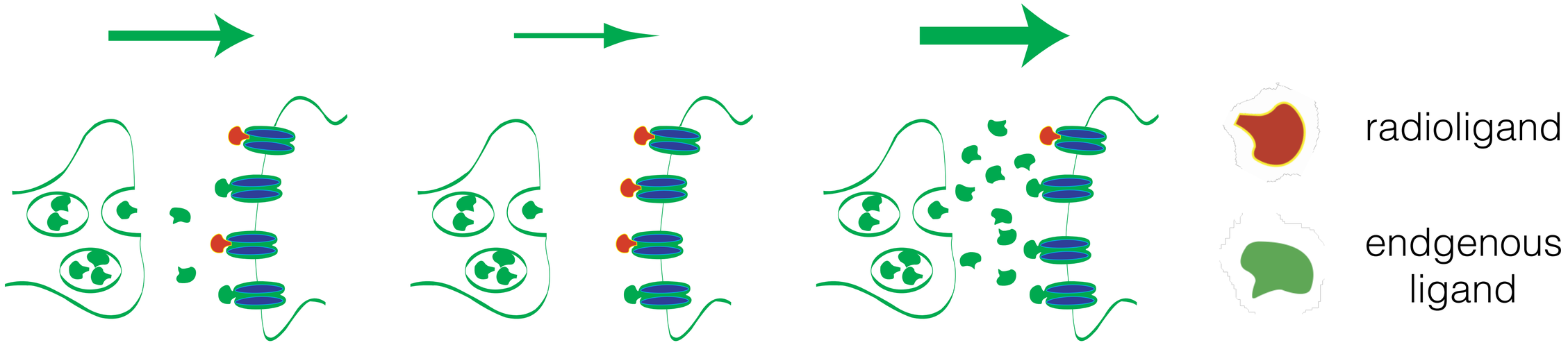
Task 2



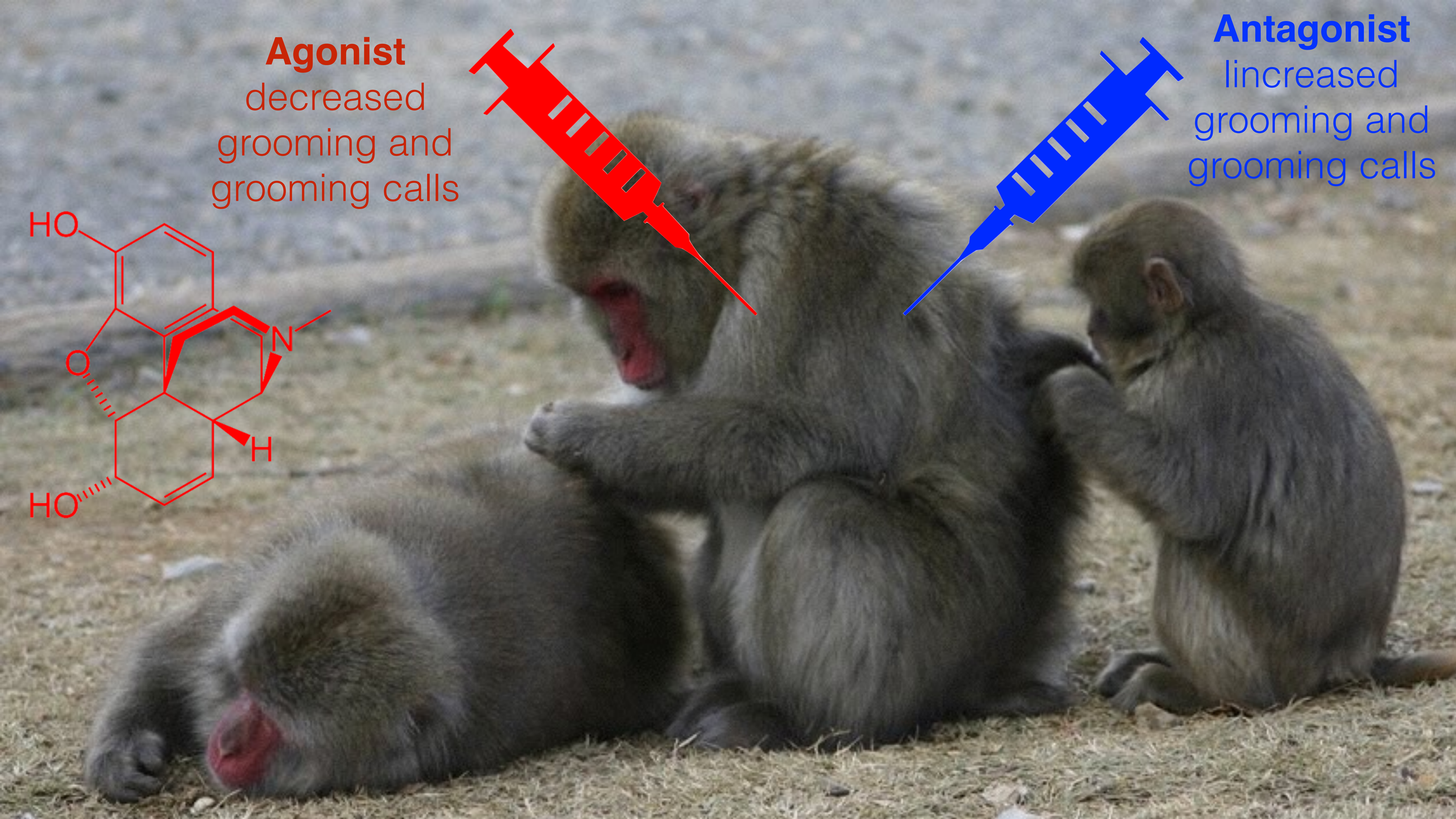
Statistical comparison (cognitive subtraction)

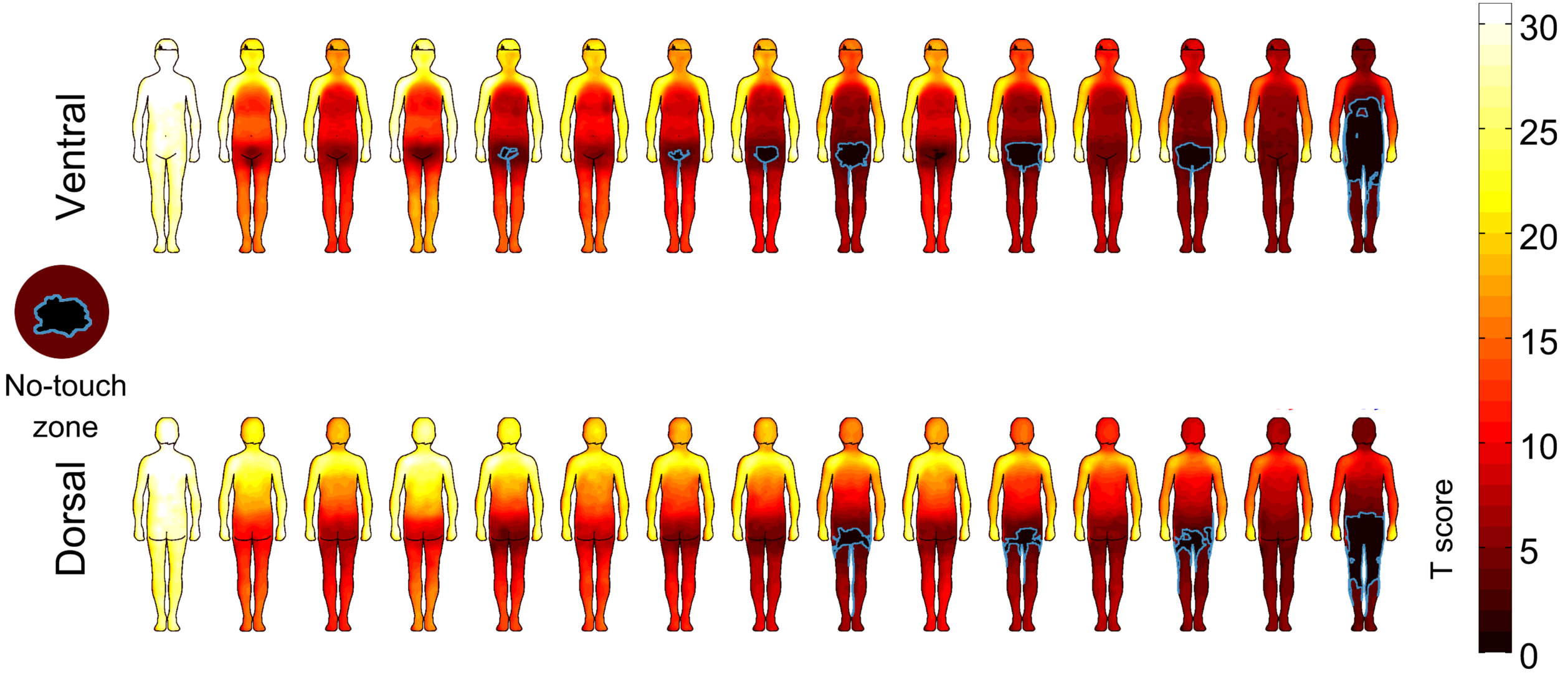


Challenge paradigm

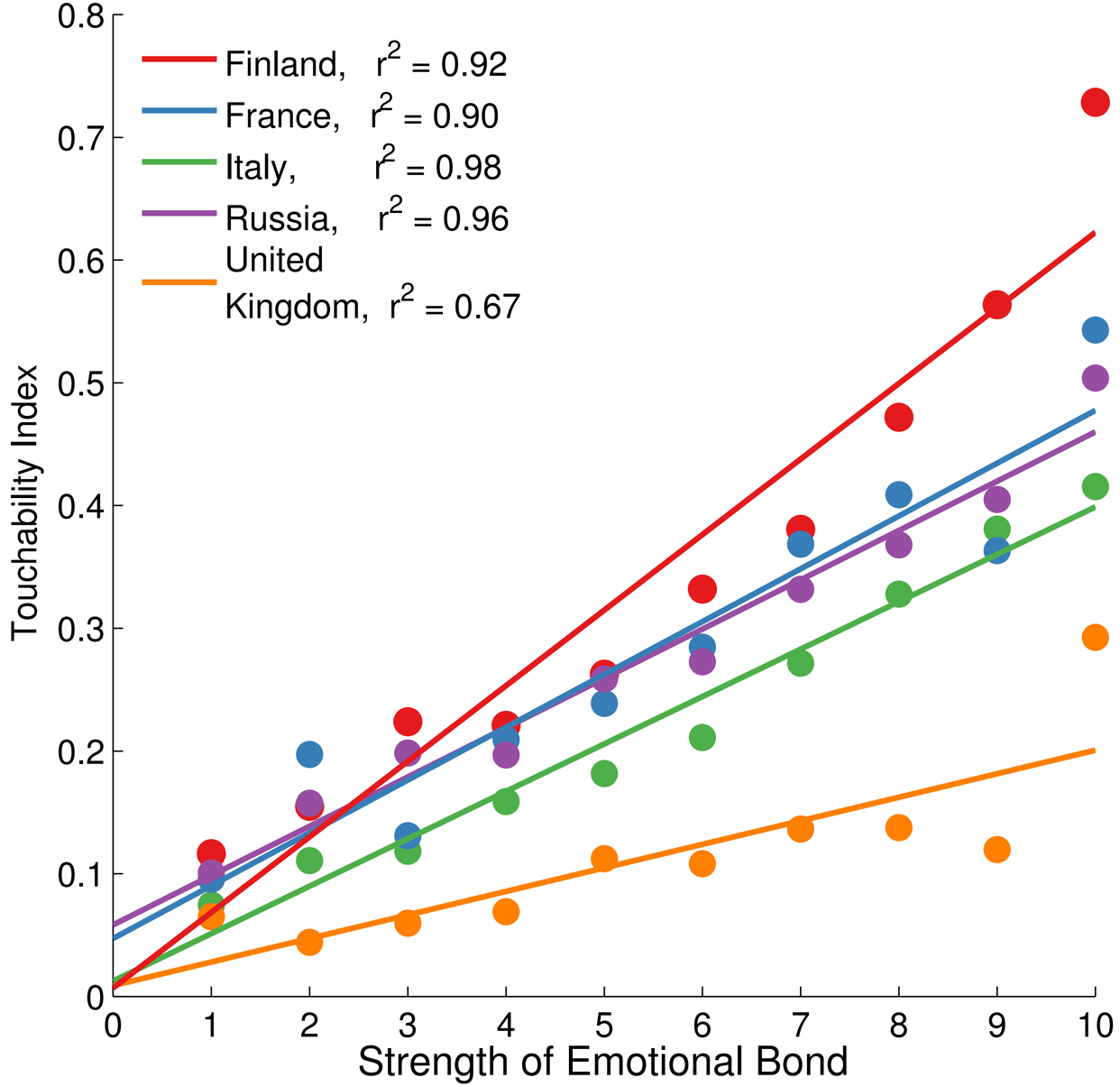


Zubieta (2001 Science)


Agonist
decreased
grooming and
grooming calls
HO
O
N
H
HO
Antagonist
lincreased
grooming and
grooming calls

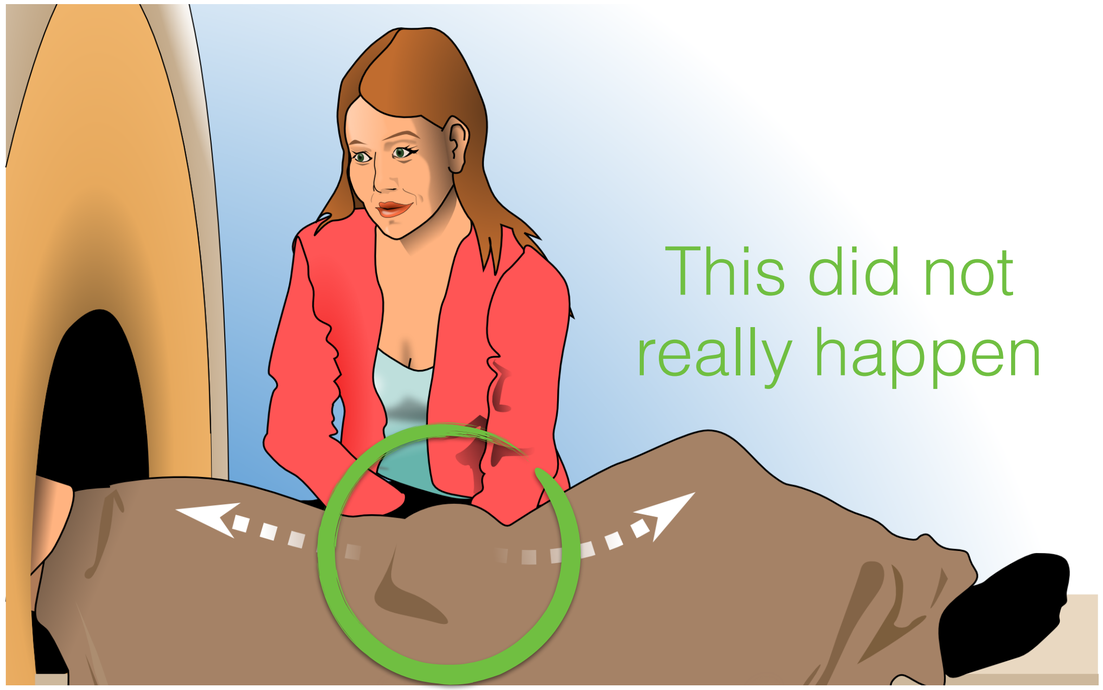


Suvilehto, Glerean, Dunbar Hari & Nummenmaa (2015; Proc Natl Acad Sci USA)

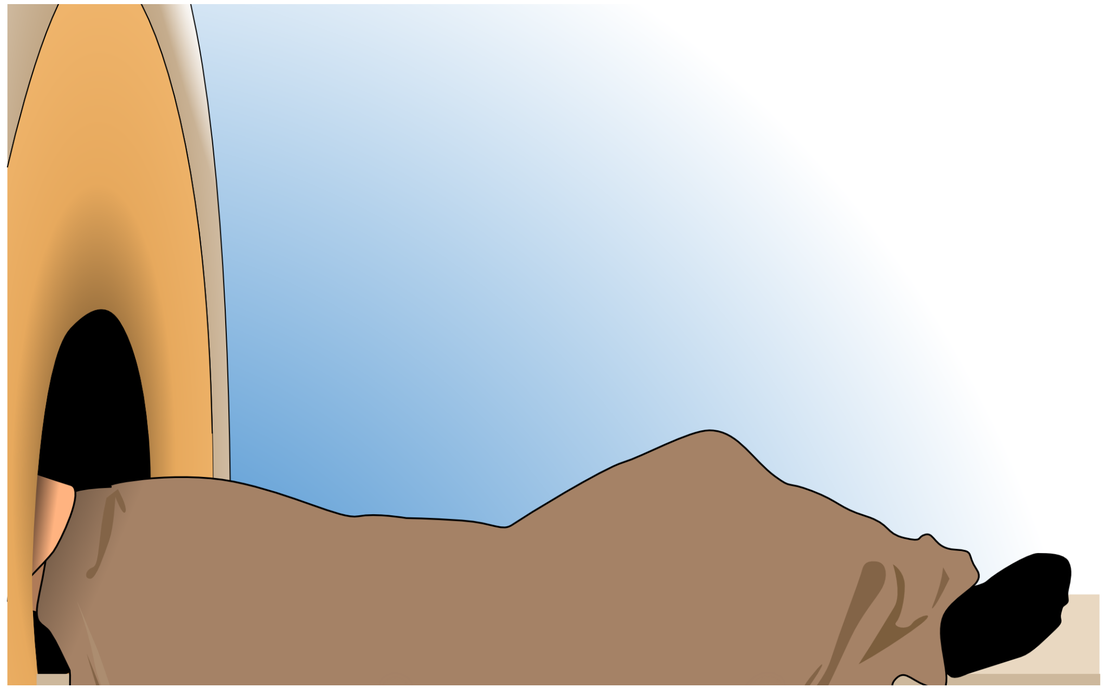


Suvilehto, Glerean, Dunbar Hari & Nummenmaa (2015; Proc Natl Acad Sci USA)

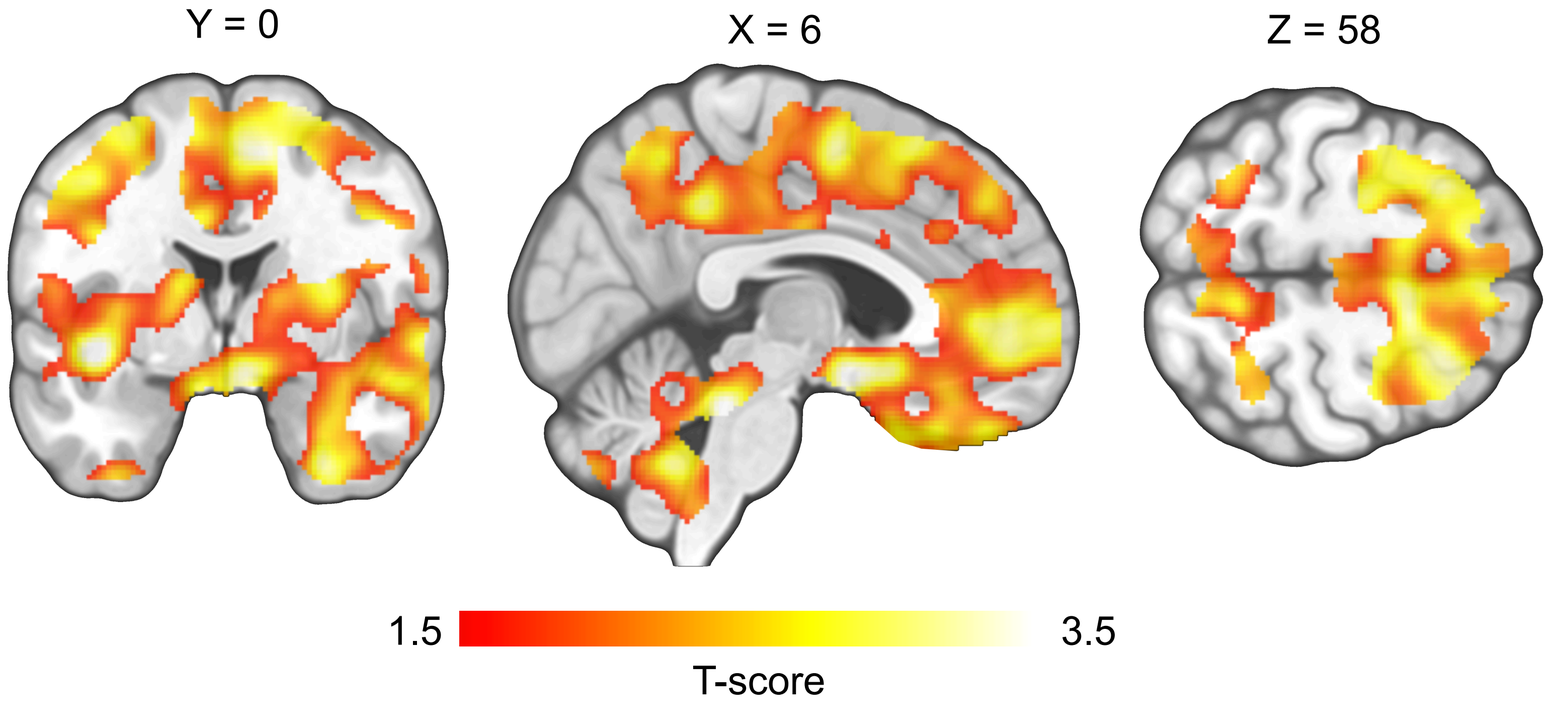
Social touch

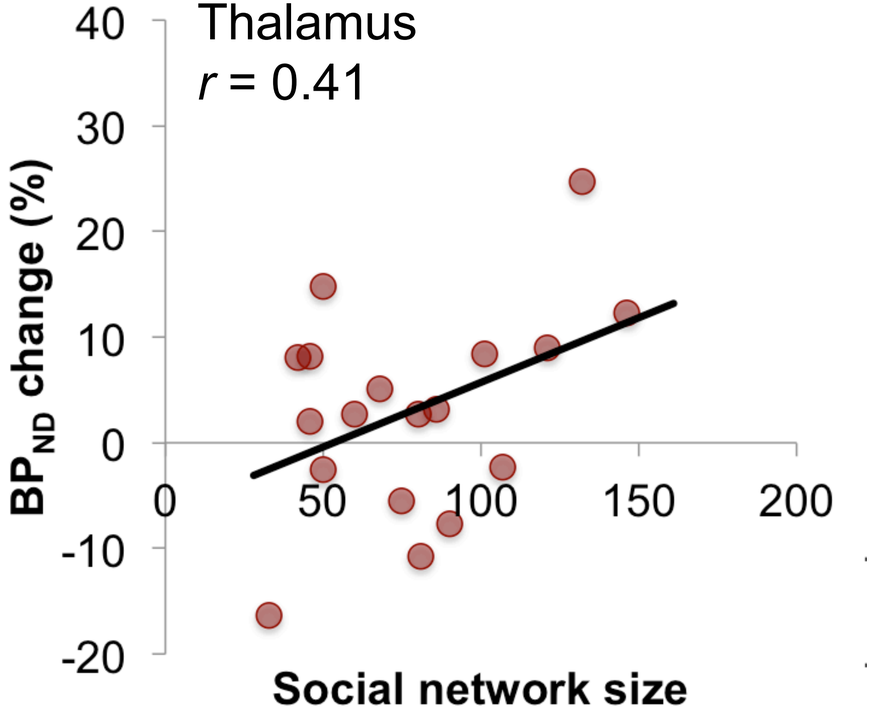


Baseline

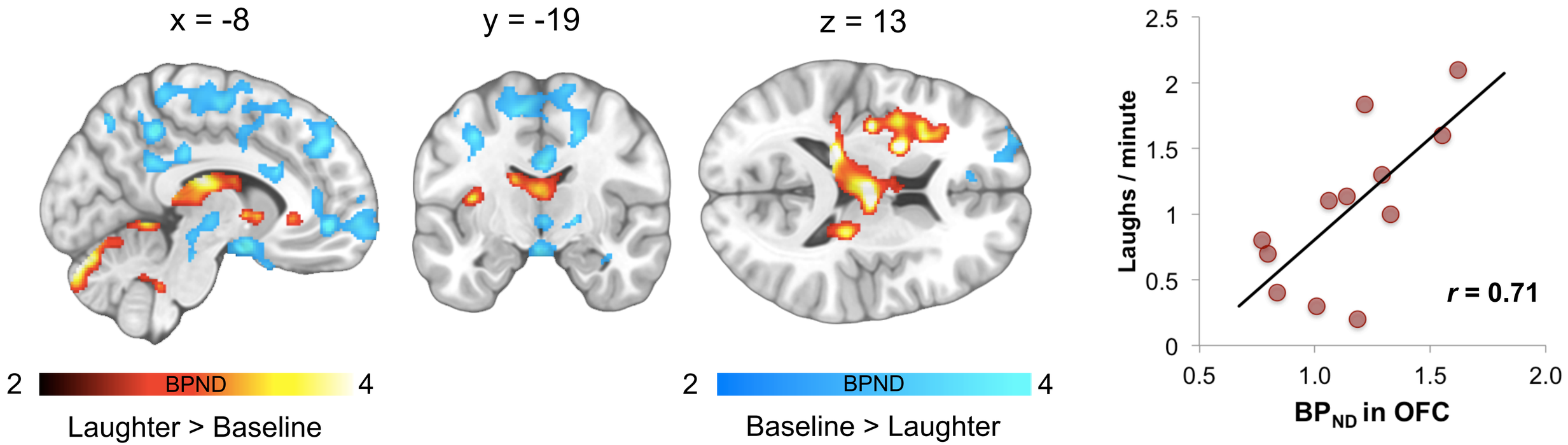


Nummenmaa et al (in revision; NeuroImage)





Nummenmaa et al (in revision; NeuroImage)

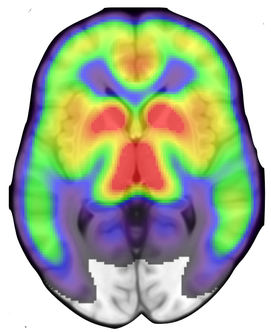


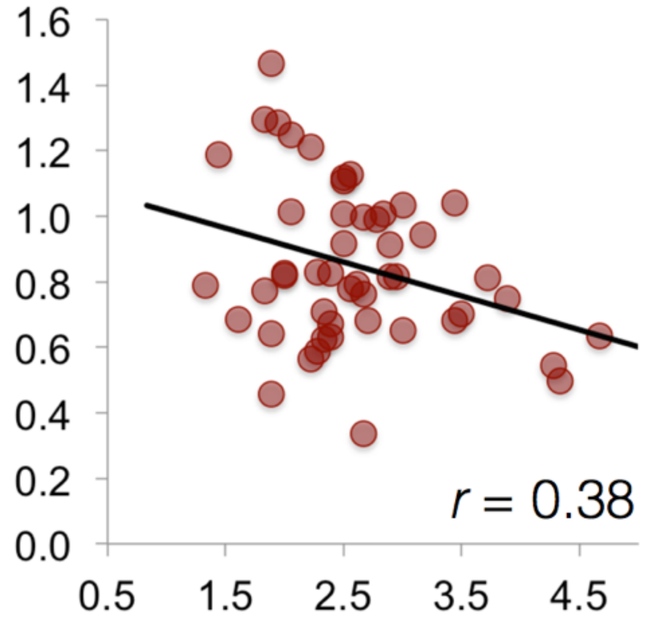
Manninen et al (in preparation)

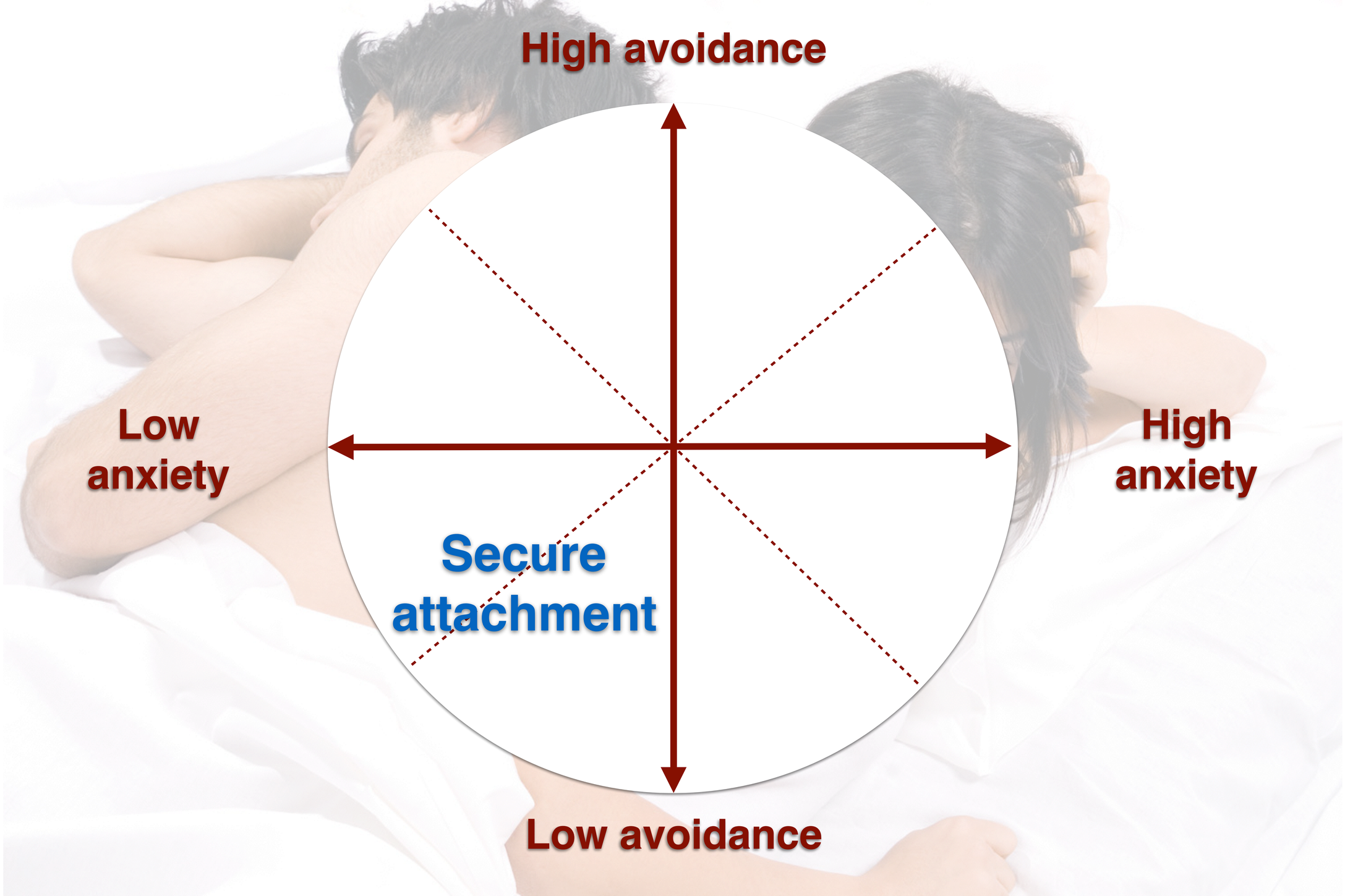
Correlational design

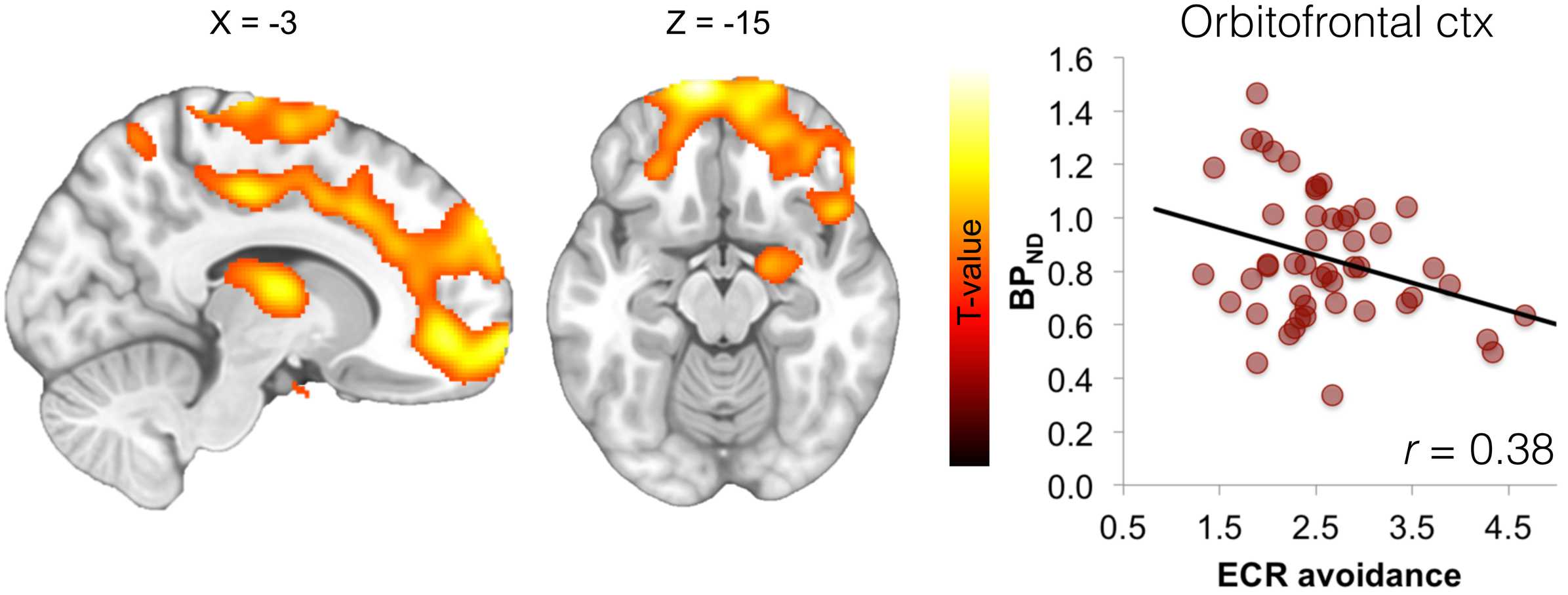
Baseline scan

Behaviour






High avoidance
Low anxiety
High anxiety
Secure attachment
Low avoidance



Nummenmaa et al (2015 Hum Brain Mapp;)

Summary - PET

- Based on radiolabeled tracers
- Allows quantification of any biological system as long as it can be radiolabeled
- Excellent chemical resolution
- Spatial resolution limited due to positron scattering
- Temporal resolution depends on tracer kinetics; typically from minutes to hours and often not relevant (no functional imaging)


"That's all Folks!"