Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

Consortium leader

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

***A projekt az Európai Unió támogatásával, az Európai Szociális Alap társfinanszírozásával valósul meg.

Biomedical imaging

(Orvosbiológiai képalkotás)

PHARMACOLOGICAL FMRI

(fMRI alkalmazása a gyógyszerkutatásban)

VIKTOR GÁL, ZOLTÁN VIDNYÁNSZKY

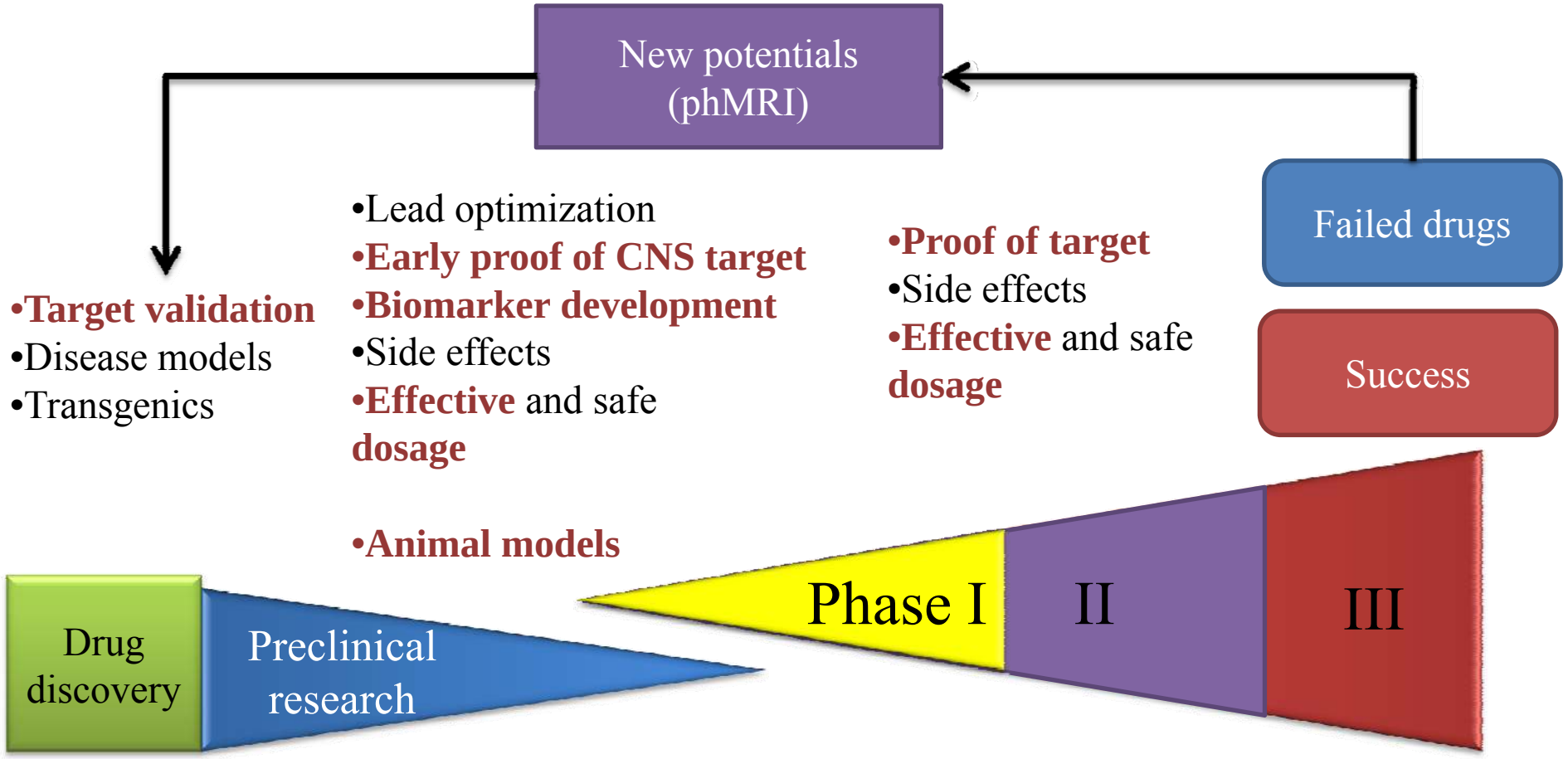
The clinical challenges in drug discovery

- Chronic diseases are increasing: Alzheimer's disease, psychiatric diseases, diabetes, atherosclerosis, arthritis, ...
  - Early onset
  - Slow progression
  - Poor prognosis
- Clinical trials extremely difficult and costly:
  - Long duration (> 3 years)
  - Many co-morbidities, huge group sizes (> 1'000 patients / arm)
  - Low chance of success (8% entering phase 1 will reach market)

Solution

- Search for early indicators (biomarkers)
  - stratify patient population
  - monitor therapy efficacy
- Imaging

Stages of CNS drug discovery, candidate role of phMRI



Role of Neuroimaging in drug discovery and development

Four interrelated categories:

- Neuroreceptor mapping
  - PET tracers
  - SPECT tracers
- Structural imaging to examine morphological changes and their consequences.
- Metabolic mapping
  - 18FDG
  - magnetic resonance spectroscopy
- Functional mapping (fMRI and FDG PET) to examine disease-drug interactions

Role of human and animal fMRI in drug discovery

fMRI is of most value at two distinct stages in the process of drug discovery:

- neuroscientific investigation of mechanisms of drug action
- providing quantitative markers of drug action, or endpoints, in candidate compounds for the clinic

fMRI also provides a means of comparing the potential mechanisms of drug action, at the systems level, between the animal models and humans, as the compound is transferred from animals to humans. This approach offers two benefits:

- the potential for verification of the similarity between the animal model and the human and hence the value of the animal model in future testing.
- the potential for reduction of animal use for investigating mechanisms of drug action and their replacement with comparatively small cohorts of human volunteers.

Applications of phMRI

- Measuring
  - Pharmaco-dynamic response
  - Pharmaco-kinetic characteristics
- Patient categorization
  - Stratification, subgroup definition
- Target identification:
  - proof of mechanism
- Early phase outcome study
- Alternative/surrogate marker of outcome

Advantages of phMRI

- **High information content**
  - novel information
  - faster than conventional analyses
- **Multi-modal**
  - from anatomy to function and molecular information
- **Non-invasive**
  - minimal interference with physiology
  - repeated assessments, intrinsic controls, chronic treatment studies
  - increased statistical power
  - reduced group sizes
- **Bridging the gap: translational research**
  - mouse to man
  - identical readouts in pre-clinical and clinical studies

Biomarkers

Definition: A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic response to a therapeutic intervention

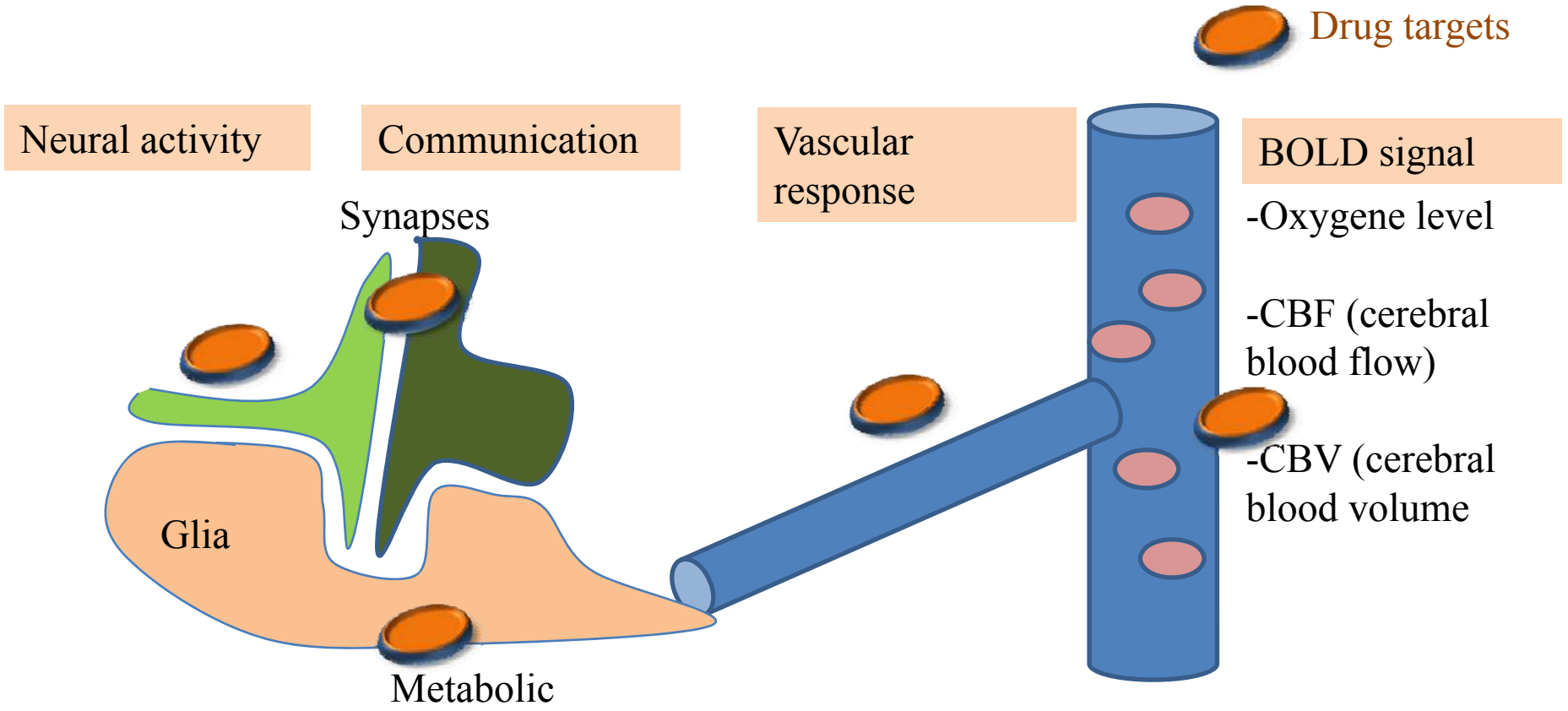
(Lesko & Atkinson, Annu Rev Pharmacol Toxicol 2001)

Biomarkers and the Pharmaceutical Industry

Imaging biomarkers enable:

- characterization of patient populations
- quantification of the extent to which new drugs reach intended targets,
- alter proposed pathophysiological mechanisms,
- achieve clinical outcomes as well as predict drug response.

- Is the drug affecting neuronal activity or just the haemodynamic response?
- FMRI for investigating regional neurovascular coupling mechanisms through pharmacological challenges



Modelling drug-induced responses

➢**BOLD/CBF changes in baseline activity**

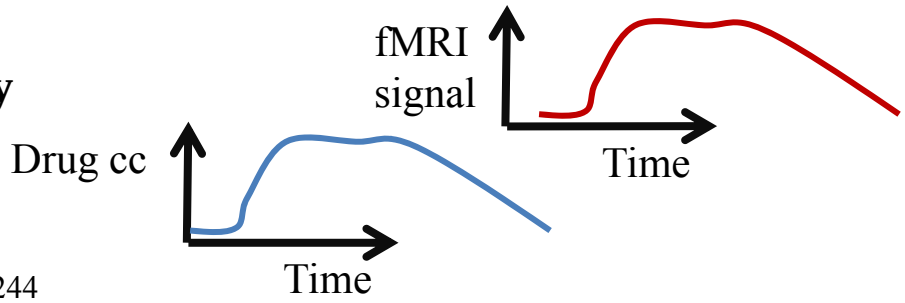
No repetitive specific sensory stimulus(Single evoked activity)

Cocaine: Breiter HC, et al, Neuron, 1997; 19:591-611

Nicotine: Bloom AS, et al, Human Brain Mapping, 1999; 8:235-244

Methamphetamine Völlm et al. *Neuropsychopharmacology* 2004, **29,** 1715–1722

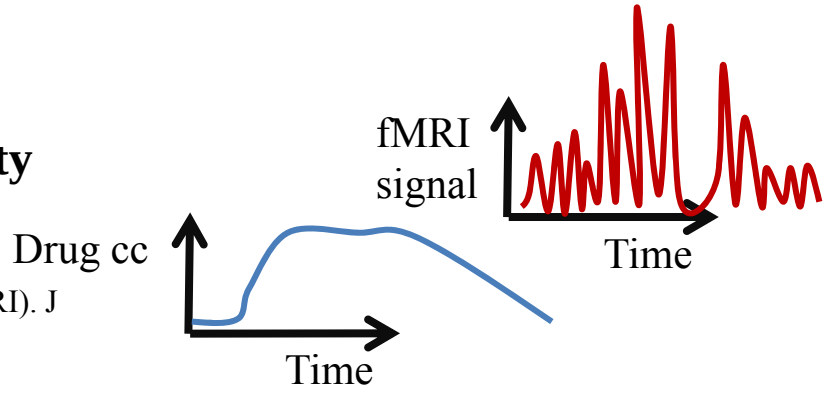
MDMA: Brevard et al. / Magnetic Resonance Imaging **24** (2006) 707– 714



➢**Modulation of stimulus induced activity**

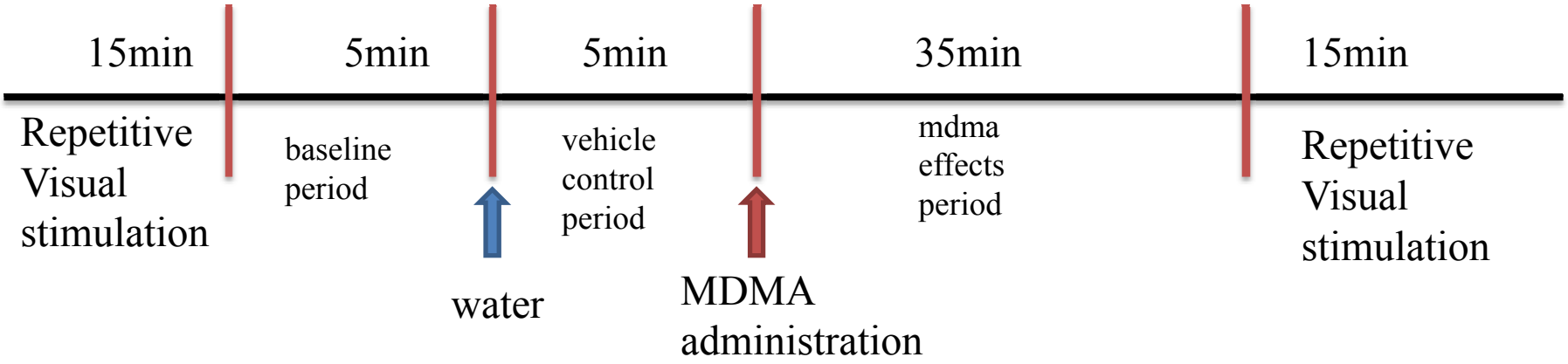
More treatment/disease specific

Remifentanil: Tracey I (2001). Prospects for human pharmacological functional magnetic resonance imaging (phMRI). J Clin Pharmacol 41: 21S–28S.



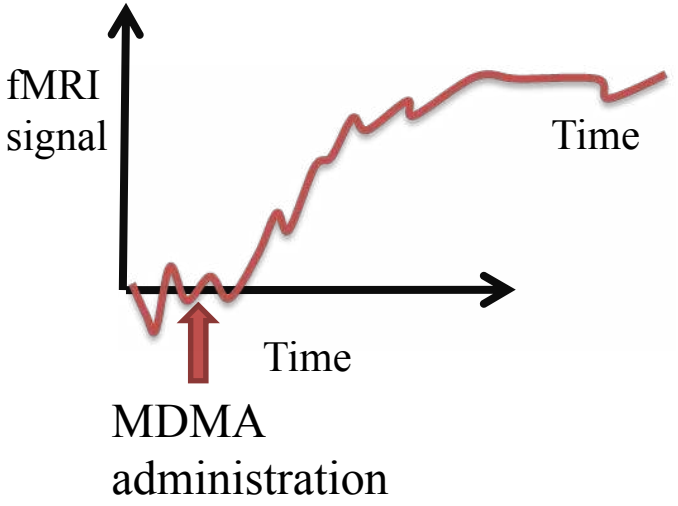
Drug-induced responses: example

Effects of MDMA (3,4-methylenedioxymethamphetamine) on monkey brain Brevard et al. / Magnetic Resonance Imaging 24 (2006) 707– 714



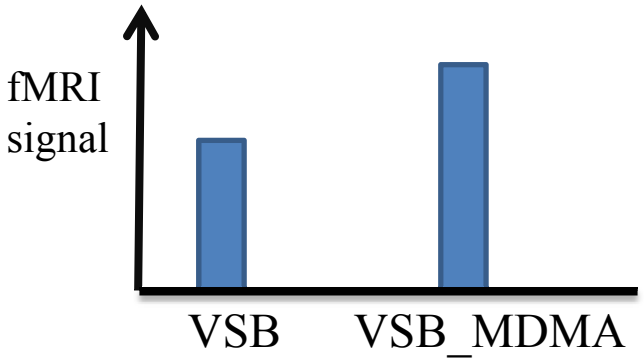
Drug-induced responses: example

BOLD changes in baseline activity
Raphe nucleus, hypothalamus, hippocampus, amygdala, striatal and visual areas followed the same tonic activation pattern



Modulation of stimulus induced activity
VSB: average amplitude of BOLD response to visual stimulation, before MDMA

VSB_MDMA: average amplitude of BOLD response to visual stimulation (after MDMA administration)

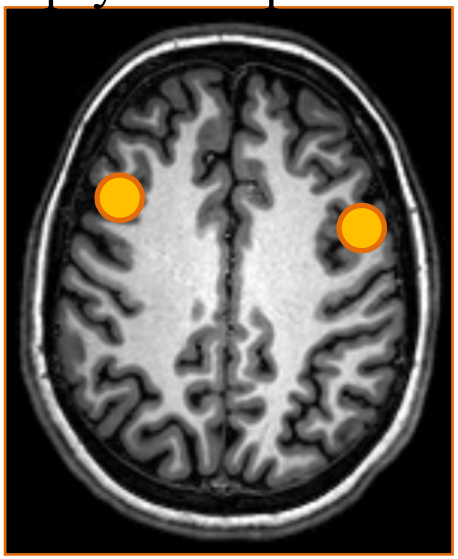


Classification of patients: defining subgroups in range disorders

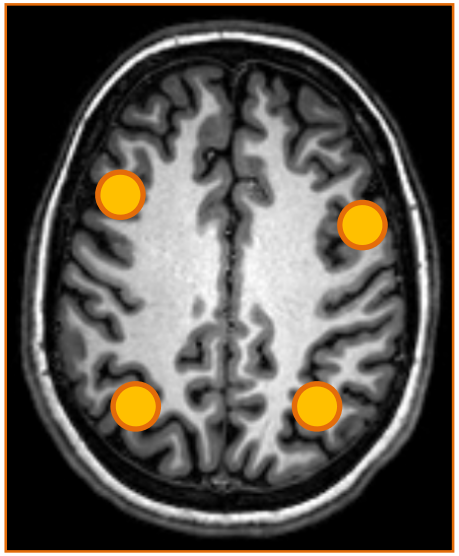
- Intermediate phenotype of schizophrenia : (Mac Donald et al am J Psychiatry, 2005)
- Expectancy AX Context Processing Task

Schizophrenia patients

Nonschizophrenia psychosis patients

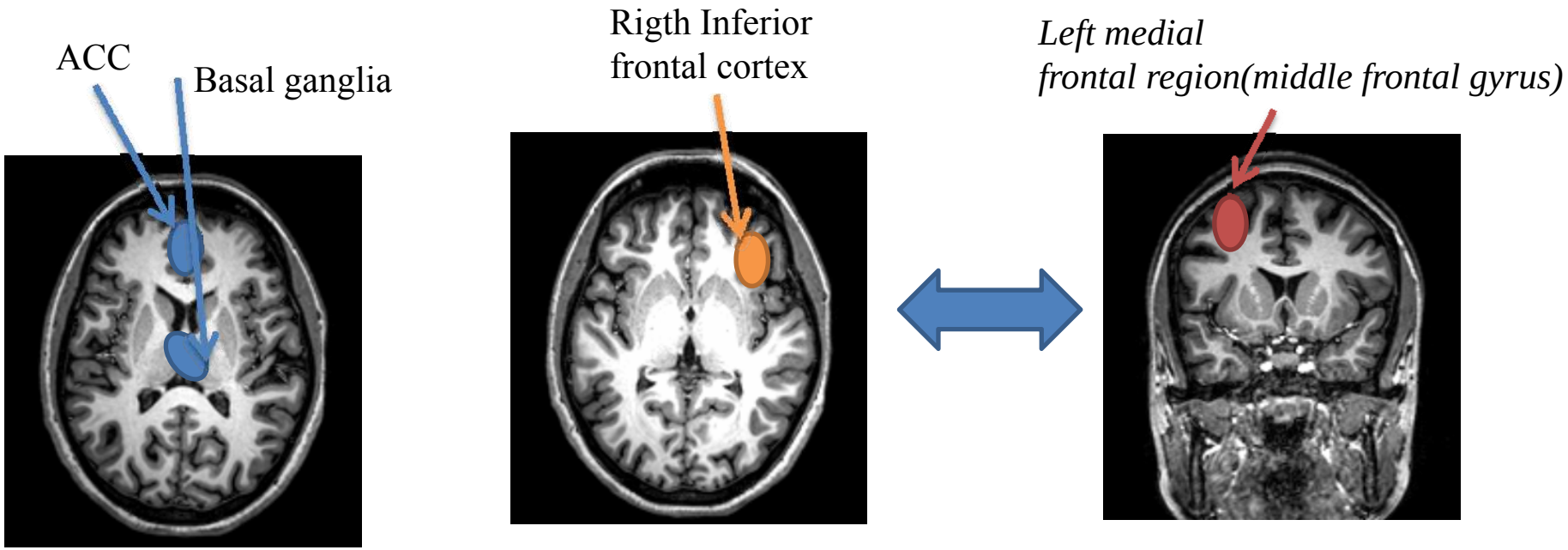


Healthy subjects



Early phase outcome measure: proof of concept (target)

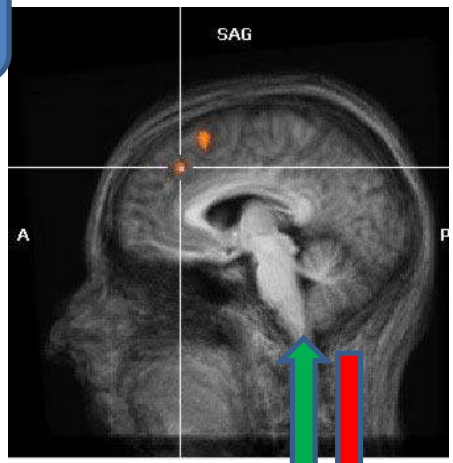
- Stroop task brain activation: basal ganglia, ACC, inferior frontal cortex (with right hemisphere dominance)
- Abnormal brain activation (left dominance over right hemisphere in the frontal cortex) in MS patients transiently normalizes after *rivastigmine* administration



Factors influencing pain experience

COGNITIVE STATE

CONTEXT



MOOD

Molecular and anatomic STRUCTURE

SPINAL CORD

NOCICEPTIVE modulation

Aδ, C fibers harmful input

Perceived pain intensity depends on:

Pain is highly subjective experience as illustrated by the definition given from the International Association for the Study of Pain (Merksey and Bogduk, 1994)

"*an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.*"

Neuropathic Pain: *caused by damage to or malfunction of the nervous system (no impending tissue damage in the background)*

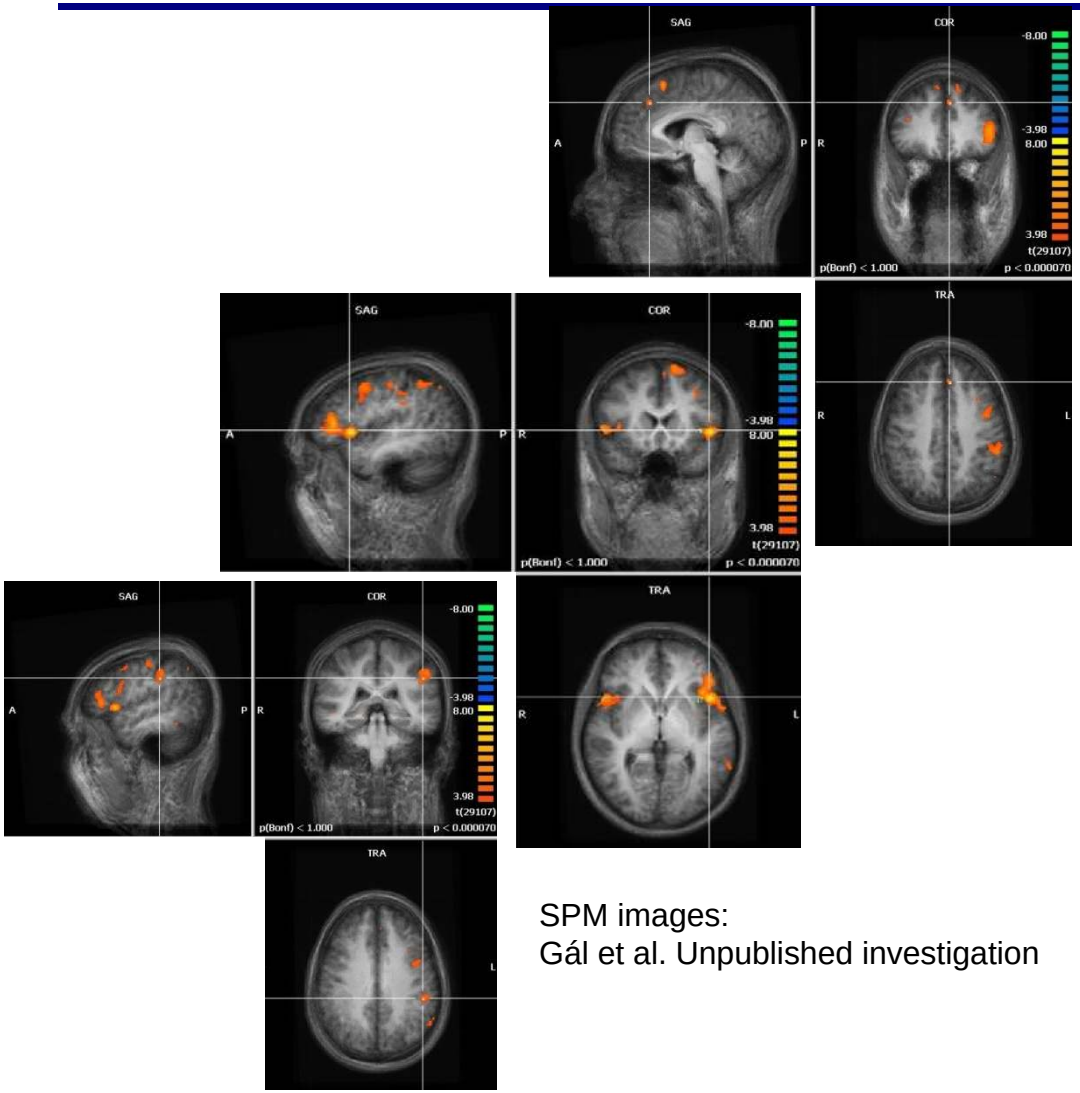
Chronic Pain: *pain that persists for more than three months*

- one of largest medical health problems in the developed world, affecting ~ 20% of the adult population, particularly women and the elderly *(Breivik et al., 2006).*
- to improve the ability to diagnose chronic pain and develop new treatments we need robust and objective ''readouts'' of the pain experience.

fMRI biomarker for chronic pain

should provide an opportunity to:

- assess and correlate pain signals at varying times in either pre-intervention or post-intervention settings.
- generate a unique brain processing "fingerprint" in response to a specific task or stimulus
- correlate behavioral pain scores with most important and relevant brain regions
- generate more specific and relevant definition of pain in early clinical studies (Phase I and II); smaller studies could assess most promising endpoints



SPM images:
Gál et al. Unpublished investigation

Pain matrix

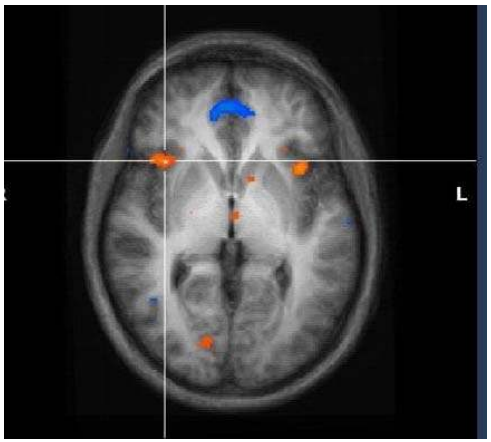
main components:

- Thalamus
- S1/S2
- Insula (several divisions)
- ACC (several divisions)
- Prefrontal

Modulation of the pain system via Remifentanil

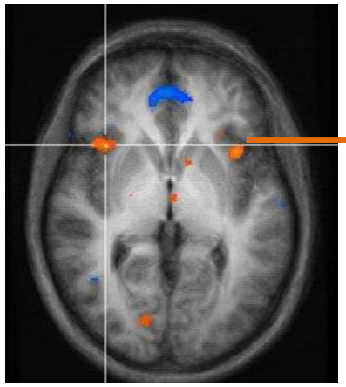
- Modulation of the pain system
- Subjective pain experience controlled by „objective” FMRI vs.
- Identifying regions associated with analgesia
- Novel therapeutic strategies
- FMRI dose-response relationship
  - Finding effective dosage
- Phasic thermal pain
- Remifentanil (peripheral and CNS pain killer) 0, 0.5, 1.0, 2.0 ng/ml
- computer controlled infusion

Tracey I (2001). Prospects for human pharmacological functional magnetic resonance imaging (phMRI). J Clin Pharmacol 41: 21S–28S.

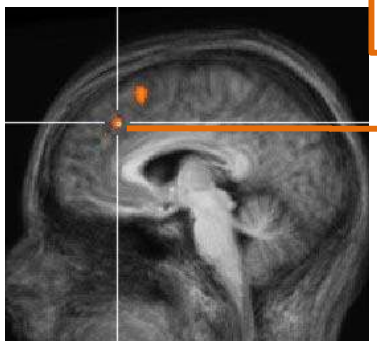


Remifentanil

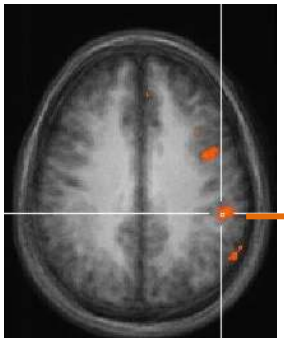
Dose dependent suppression of pain related activity within the pain matrix



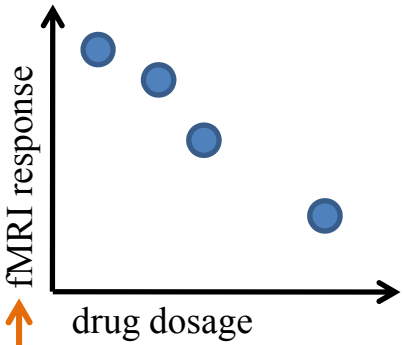
Insula

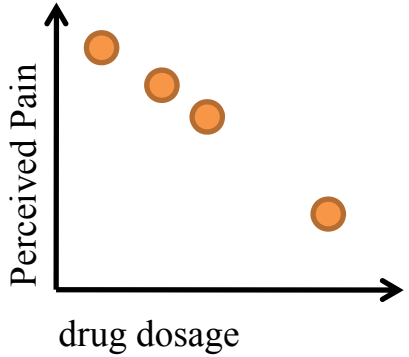


Anterior cingulate cortex



SII

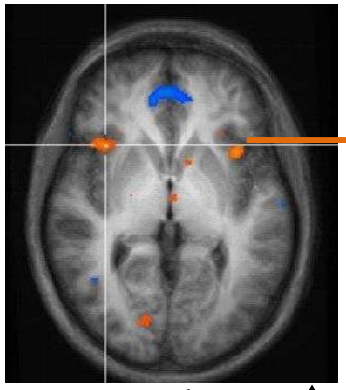




SPM images:
Gál et al. Unpublished investigation

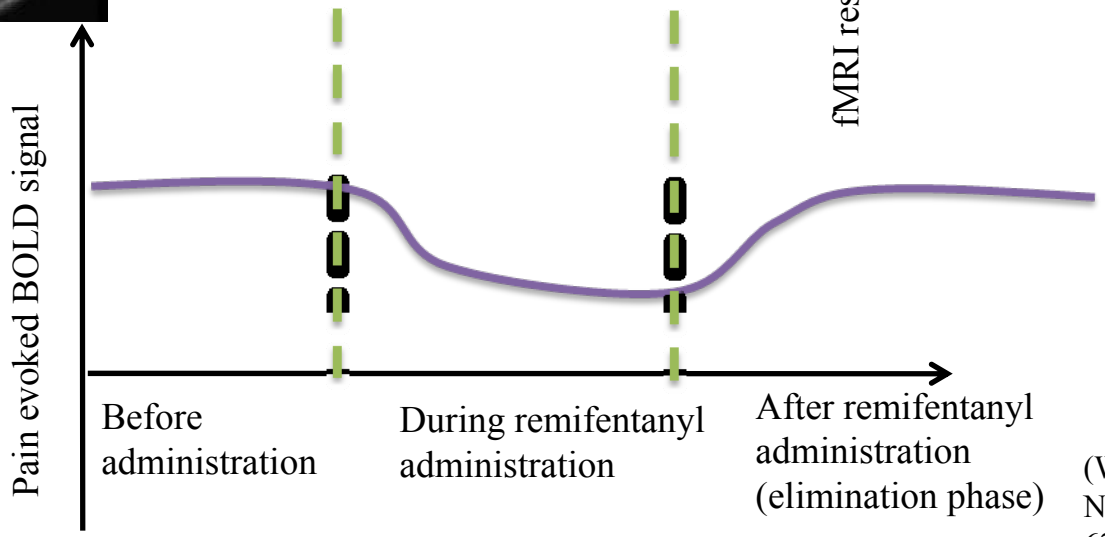
Dynamic modulation of pain matrix activity

SPM images: Gál et al. Unpublished investigation:



Right insula

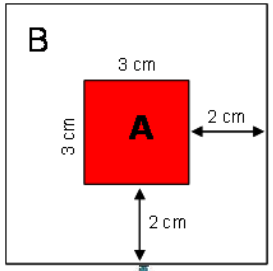
Insula



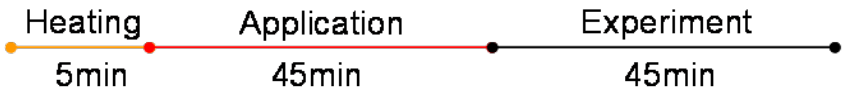
(Wise et al., 2003 Neuropsychopharmacology ***29***, 626-635.)

Chronic pain model: Central sensitisation by topical capsaicin treatment (Petersen and Rowbotham, 1999, Zambreanu et al. Pain 2005)

-Topical application of capsaicin, a vanilloid receptor agonist, which elicits ongoing discharge in C-nociceptors and induces an area of hyperalgesia.

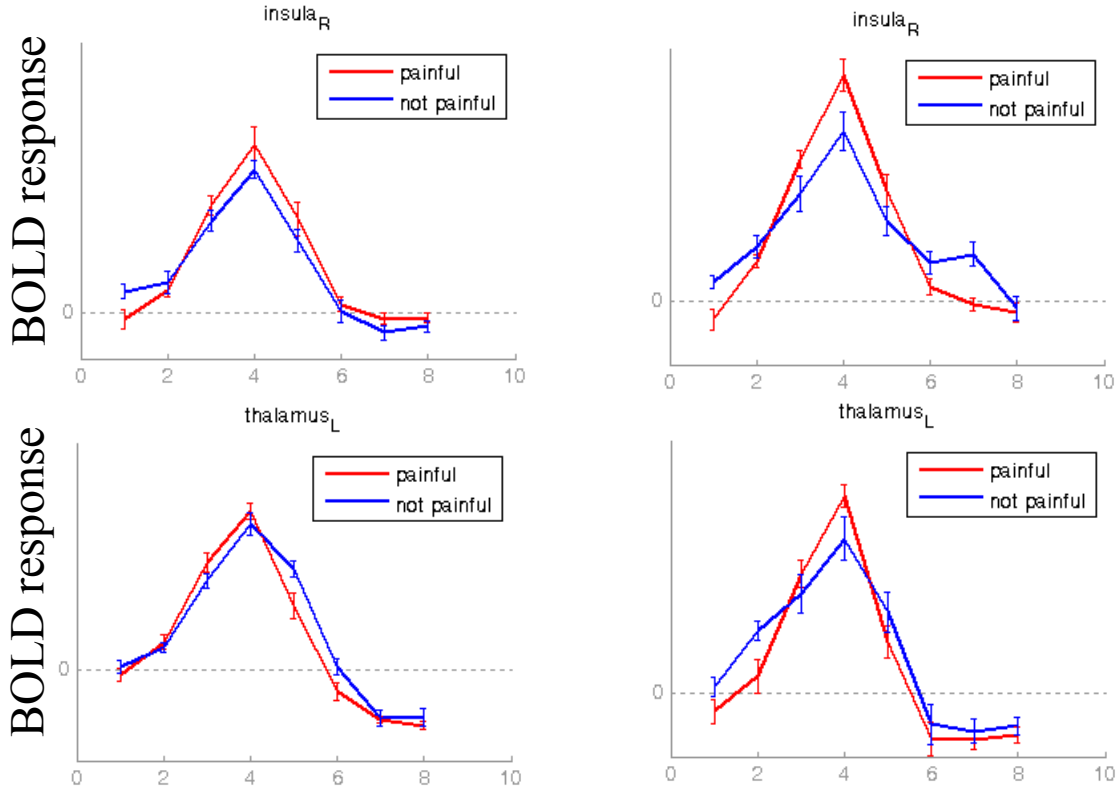


Computer controlled MR-compatible mechanical stimulus presentation equipment



Conditions	Capsaicin treatment	Treated area	Stimulation	Model of sensitization
1	Yes/ Hyperalgesia	A	A	Peripheral sensitization
2	No/ Control	-	A	Control
3	Yes/ Hyperalgesia	A	B	Central sensitization
4	No/ Control	-	B	Control

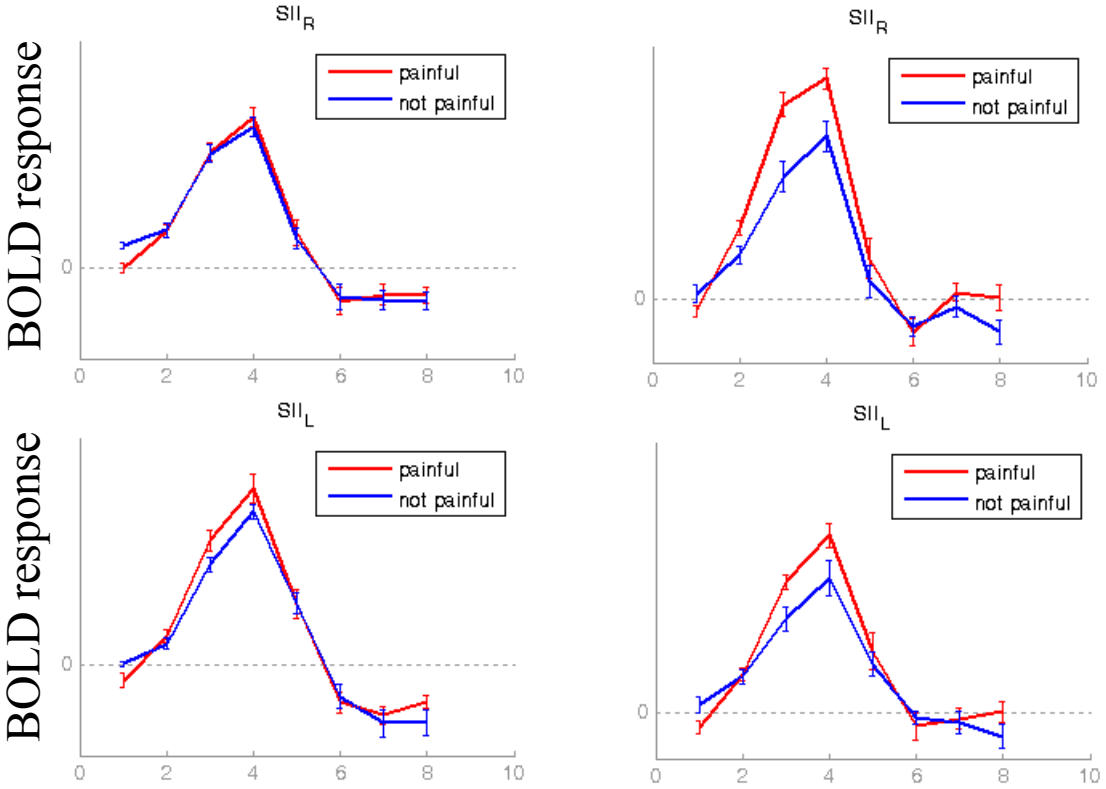
Central sensitisation by topical capsaicin treatment



Gál et al.
Unpublished
investigation

Effects of central sensitiszation: talamus, insula anterior, – BOLD responses in the different brain areas in the conditions of untreated (left column) and central sensitization (right column) when subjects categorized painful and non-painful stimuli

Central sensitisation by topical capsaicin treatment



Gál et al.
Unpublished
investigation

Effects of central sensitiszation: S2 cortex (left, right) – BOLD responses in the different brain areas in the conditions of untreated (left column) and central sensitization (right column) when subjects categorized painful and non-painful stimuli

Animal fMRI

Comparing to human fMRI:

- Larger number of samples
- Testing of potentially noxius/lethal/less known
  - Intervention
  - stimulation (e.g. intracranial microstimulation)
  - chemical agents
  - genetic manipulations
- Translation of small animal models to human models)
  - May validate other drug development methods

Bridging the gap: fMRI in translational studies

Biomarkers

Animal models

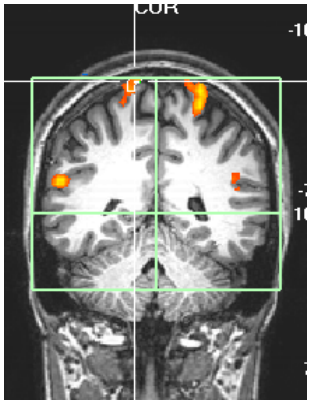
Preclinical imaging

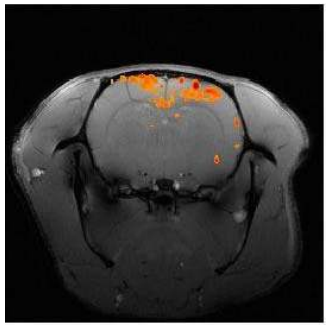
Drug discovery

Basic neuroscience

Clinical trials

Transgenic approach





Better match than behaviour?

Animal pharmacological MRI: issues

- **Is it predictive:**

Do we learn anything about drugs in the human situation?

- Major differences in receptors, circuits and function
- Experiments normally carried out on anesthetized animals

- **Is it cost and time efficient:**

How does it compare to conventional methods?

- **Is it relevant:**

What can we learn about new compounds?

- Difficulties at detecting tonic activation via BOLD methods
- Limited stimulus delivery and behavioral response

- **Is it ethical:**

May animals be "used" for research?

Ultra High field MRI

Typical strengths: 4.5T, 7T, 9.4 T

Bruker, Varian

- Pros:
  - high SNR
  - Higher chemical shift (also disadvantage)
  - 100μm or lower spatial resolution
  - T1 higher
  - Shorter imaging sessions (due to high SNR)
    - High susceptibility effects (even 15% signal change in BOLD), lower stimuli repetition required
    - Spin echo also gives BOLD contrast!
- Cons
  - High susceptibility effects
  - Poor field homogeneity
  - Variable signal loss
  - Higher TR for T1

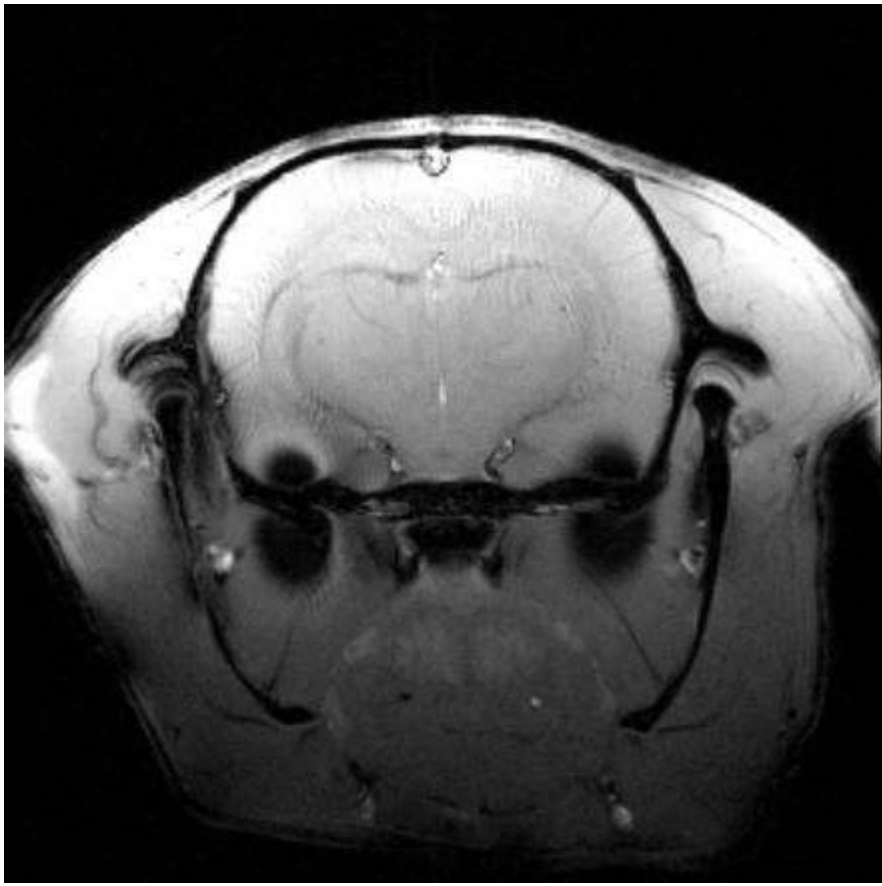
Resolution

- Spatial

Typical

  - 0.1x0.1x0.3mm , 4-6 slices, 5min
  - 0.015x0.015x0.3mm , 20 slices, 1 day
  - Cytoarchitecture can be visualized

- Time
  - Normally acquisition of a volume is not faster than at lower fields, but:
  - even single events (stimulus) can be detected by gradient or spin echo EPI
  - percent signal change can be 10 times higher than at 3T
  - Spectroscopy is accelerated substantially



Preparation

- Intubation
- Catheterization (through the tail vein)
- Placement of the monitors:
  - ECG heart rate
  - Respiration (piezo-electric transducer)
  - Rectal temperature probe
- Mechanical stabilization
  - acrylic stereotactic head holder (incisor bar and blunt earplugs,)
- Insertion of heating tube

Immobilization

Training

Mechanical restraining

Anesthesia:

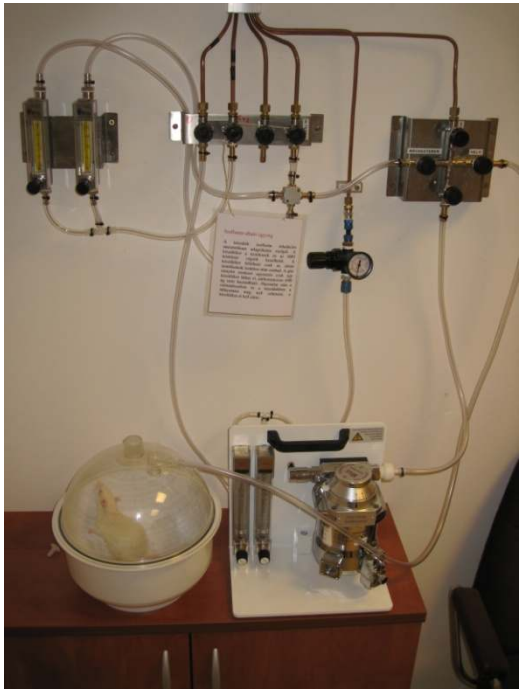
- α-chloralose
- Propofol
- Medetomidine
- Isoflurane

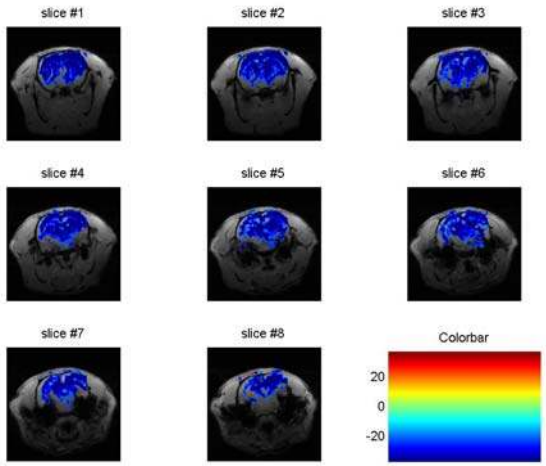
Paralysis

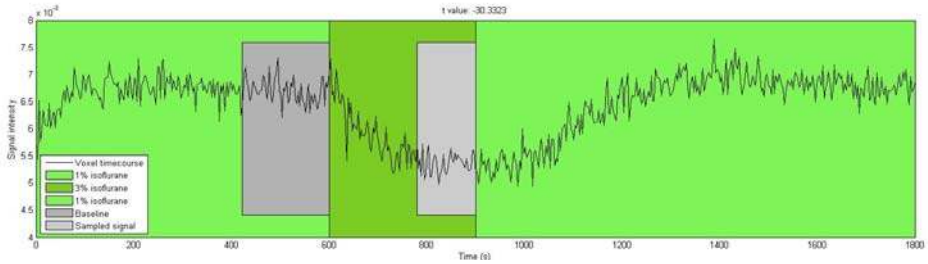
- mivacurium (curarization)
  - Enable awake, conscious experiments
  - Serious ethical issues

Direct effect of anesthesia on BOLD signal

Elevating isofluran concentration decreases baseline level

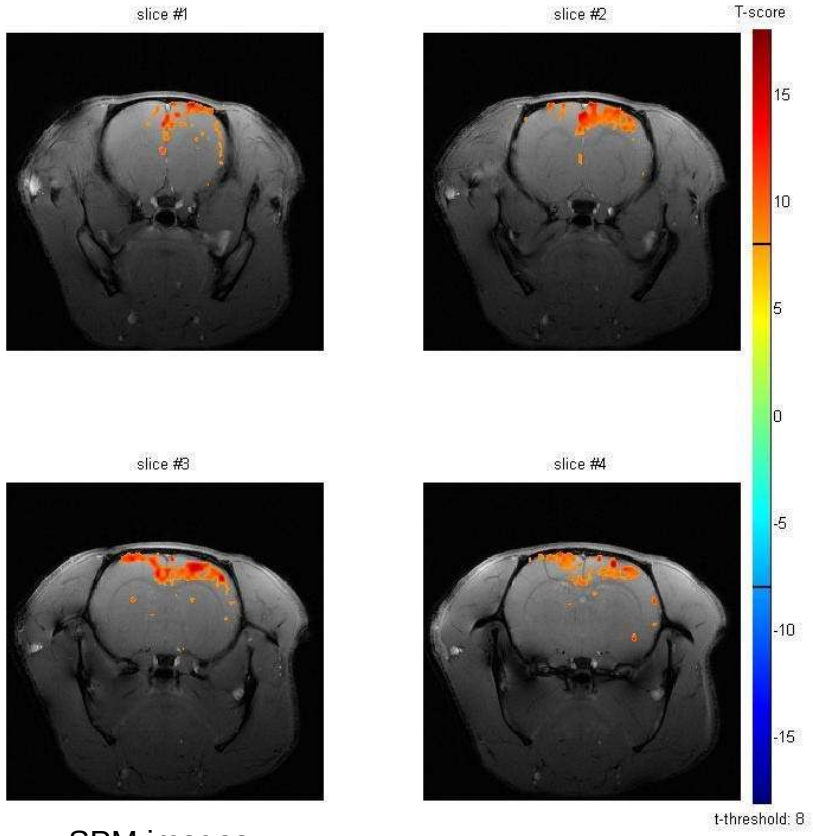






Paw stimulation (electrical)

- One of the most frequently used sensory stimulation in small animal fMRI
- Needle electrodes are inserted under the skin/fixed around fingers
- Basic research in somatosensory system
- Indirect effect of drugs, anesthesia on the sensory system (deprivation)
- Scanning parameter optimization



SPM images:
Gál et al. Unpublished investigation

Normalization of small animal brains

Why normalize?

- Multiple subject experiments
- To report anatomical localization of fMRI effects
- Coregister with other modalities (MRI, autoradiography)

How normalize/coregister?

- 3D digital atlases (Schweinhardt et al., 2003 Schwarz et al., Neuroimage 2006) derived from
  - •Rat: Paxinos and Watson, 2005
  - •Mouse Paxinos and Franklin, 2001;
- Automation of coregistration
  - •Tissue probability maps, brain templates coregistered with known atlases
  - •In-house brain templates
  - •Via finding anatomical landmarks

MRI Contrast agents

Animal MRI has the advantage to use potentially noxious contrast agents more freely than in human studies. Types of contrast materials used in clinical practice:

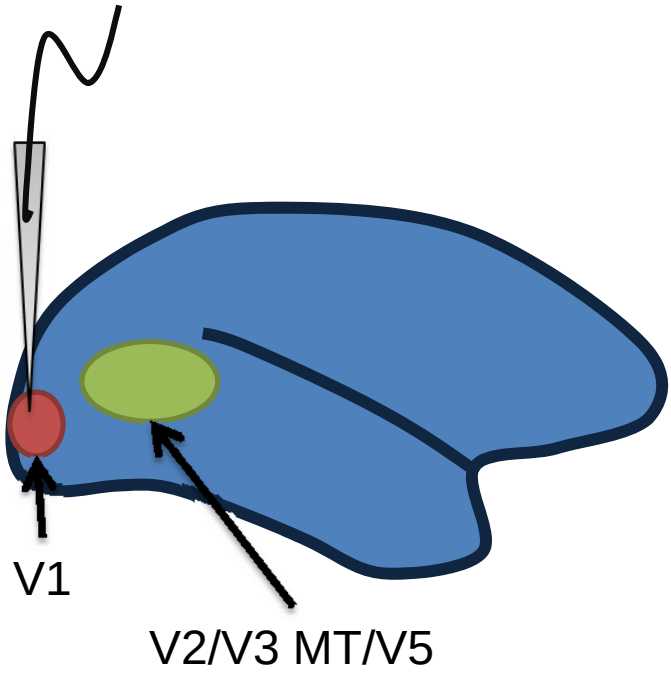
- Oral
- Intravascular
  - Gadolinium (and complexes): Paramagnetic
  - Manganese (and complexes): Paramagnetic
  - Iron oxide: Superparamagnetic
    - SPIO : Superparamagnetic Iron Oxide (SPIO) and UltraSPIO
    - Reduces T2 and T2*
    - Intravascular time: depending on particles size and coating
      - With long iv time they can be used as fMRI contrast agent (next slide)

Potential Functional MRI Contrast agents

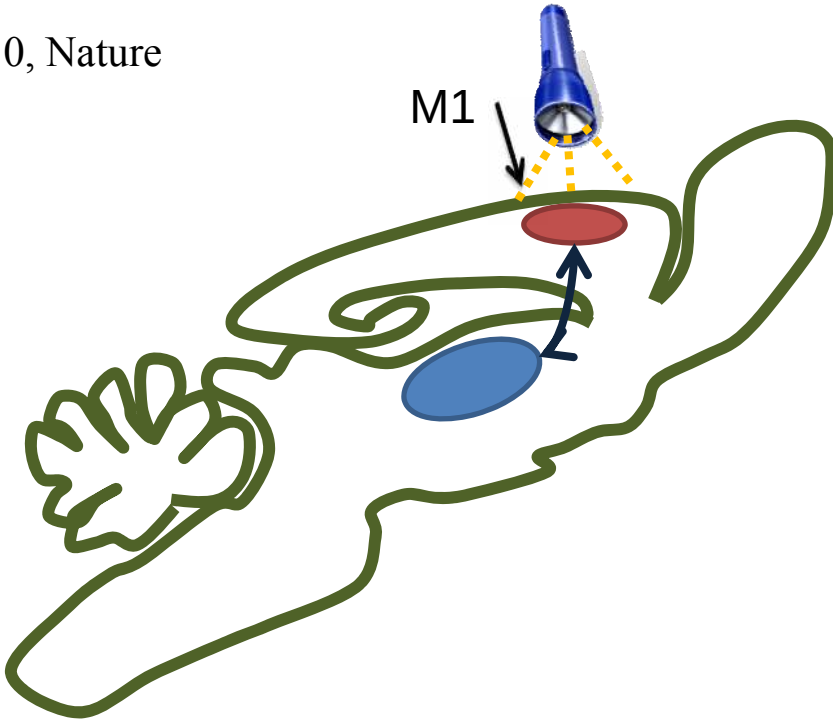
- Indicators of change in local blood flow
  - MION-47, USPIO with long blood half-life
- Indicators for Ca2+ and other metal ions
  - BAPTA-based Gd3+ complex
  - Mn2+ as Ca2+ mimetic
- pH indicators
  - Phosphonated Gd3+ complex, Endogenous amide protons
- Probes for metabolic activity
  - Exogenous hemoglobin
- Genetically controlled contrast agents
  - Ferritin
  - Transferrin (Tf)- conjugated SPIOs
  - Artificial lysine-rich protein

Electrical microstimulation and fMRI

- Local and remote connections of a specific (stimulated) site can be mapped
- Method can detect selective modulatory effects of pharmacological agents on specific connections
- Methodological challenge: MR compatible electrode, MR signal is contaminated by electrical stimulation
- Pioneering work of Logothetis Lab: monkey V1 microstimulation (Tolias et al 2005, Neuron)
  - Significant BOLD signal change in V1, and extrastriate visual areas



Optogenetic fMRI Lee et al 2010, Nature



- Electrical stimulation is not selective:
  - Afferents and efferents, passing axons
  - Inhibitory and excitatory neurons are also activated
- Injection of viral vector (AAV5-CaMKIIa::ChR2(H134R)-EYFP)
  - into primary motor cortex
  - expression of channelrhodopsin (ChR2)
  - only in Ca^{2+}/calmodulin-dependent protein kinase II (CaMKIIa)-expressing **principal cortical neurons**, (not in GABAergic or glial cells)
  - Activation/measurement 10 days after viral injection

➢Optical (laser diode) stimulation of motor cortex resulted in BOLD response shown in the site of stimulation and in relevant thalamic nuclei

Summary

Examples demonstrate promising capabilities of phMRI in:

- Measuring Pharmaco-dynamic response and pharmaco-kinetic characteristics
- Patient categorization, target identification:
- Early phase outcome or surrogate biomarker of outcome

via BOLD/CBF changes in baseline activity or modulation of stimulus induced activity

Animal fMRI broaden the potential of phMRI enabling:

- Larger number of samples
- Testing of potentially noxius/lethal/less known chemical agents (drugs), intervention, stimulation (e.g. intracranial microstimulation) and genetic manipulations
- Translation of small animal models to human models (bridging the gap)
  - May validate other drug development methods