Functional MRI in animal models: pharmacological and manganese-enhanced MRI

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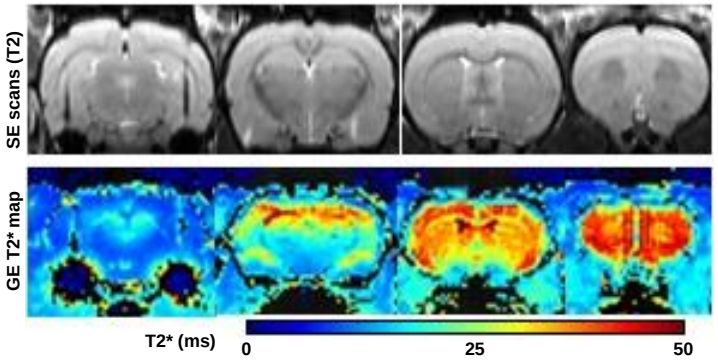


Outline

- **Why use fMRI in animal models?**
- **Two fMRI applications in rat models: pharmacological MRI (blood-flow based) and manganese-enhanced MRI**

Why animal models?

Combination of behavioural/cognitive testing with manipulation and analysis of brain function enable the discovery of causal brain-behaviour relations.

Why fMRI in animal models?

Disadvantages

- Costs (£ per scanner, maintenance)
- Lower temporal and spatial resolutions than other available techniques (e.g., electrophysiology, post-mortem histology)

Advantages

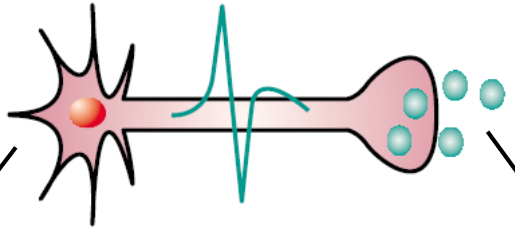
- Non-invasive (more or less), enabling longitudinal studies
- Time course of activity changes across whole brain within same animal
- Digital image acquisition enables efficient 3D visualisation and quantification of data
- 'Translational bridge': direct comparison with human MRI studies

Functional MRI: Two principal approaches

Based on blood flow

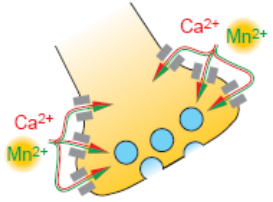
Based on molecular probes

Neuronal/synaptic activity



Haemodynamic response

Accumulation of molecular probe



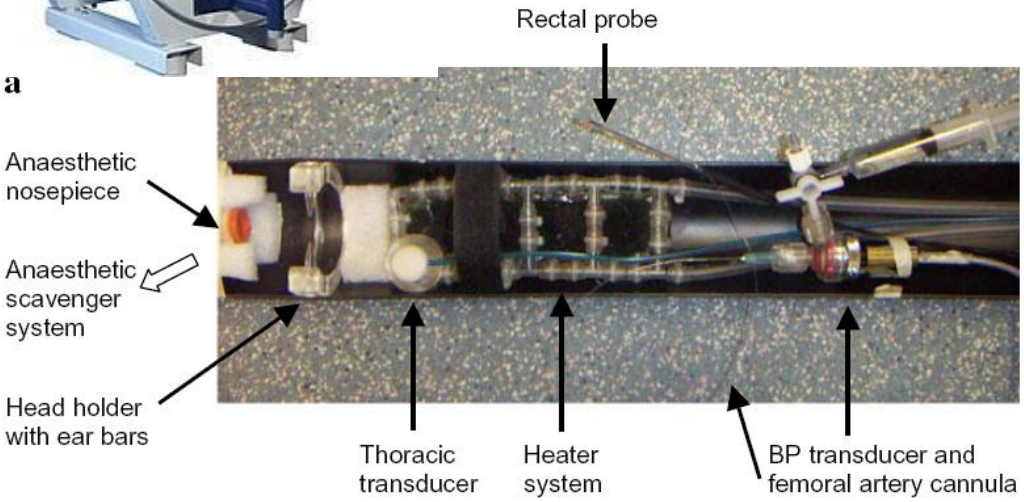
Examples:
BOLD MRI,
rCBV MRI

Detection by MRI

Example:
Manganese-enhanced MRI (MEMRI)
(for further examples, see: Jasanoff, 2007, *Curr. Opin. Neurobiol*)

Functional MRI in rats: general set-up and methodological considerations

7-T small-animal scanner



General methodological considerations

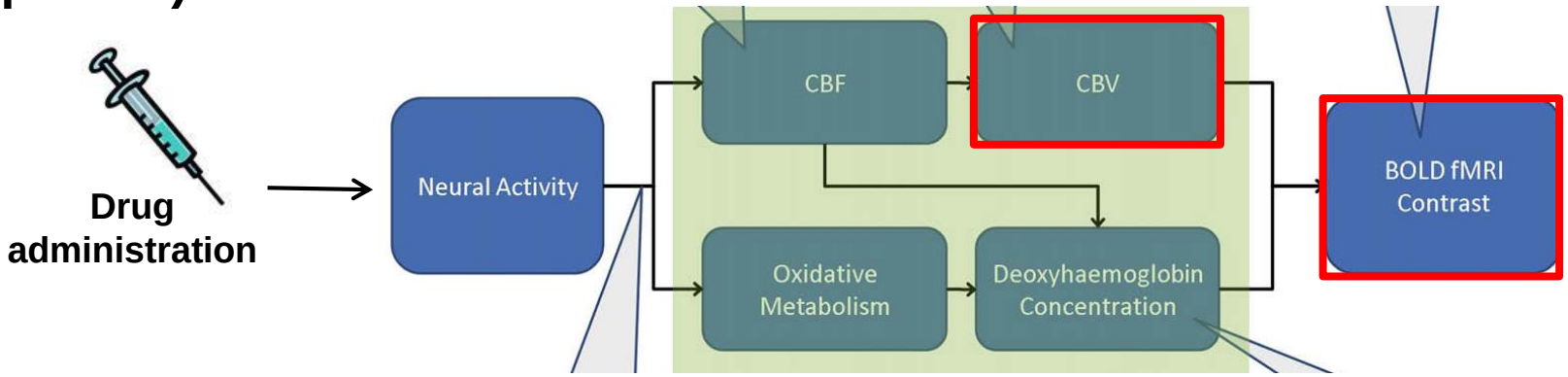
- **Brain size**
  - **-small brain requires high spatial resolution**
  - **-small brain facilitates use of high fields**
- **Anaesthesia**
- **Physiological control**
- **Appropriate imaging sequence**

Application of blood flow-based fMRI in rats: pharmacological MRI

- **Investigation of the time course of regional changes in brain function in response to pharmacological stimuli**

- **Potentially, a cross-species tool to identify mechanisms of drug action and to determine biomarkers of therapeutic drug action**

- **Typically, drug-induced changes in BOLD contrast or rCBV are measured in between-subjects design (- reduced statistical power!)**



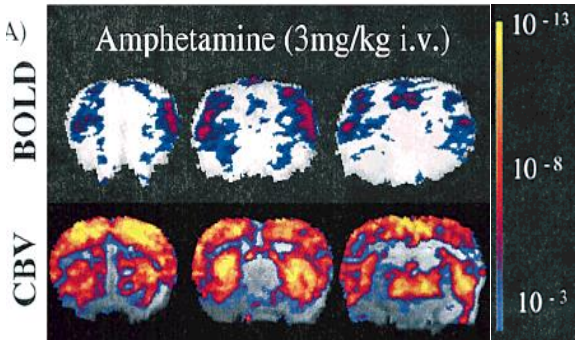
Comparison of BOLD and rCBV imaging

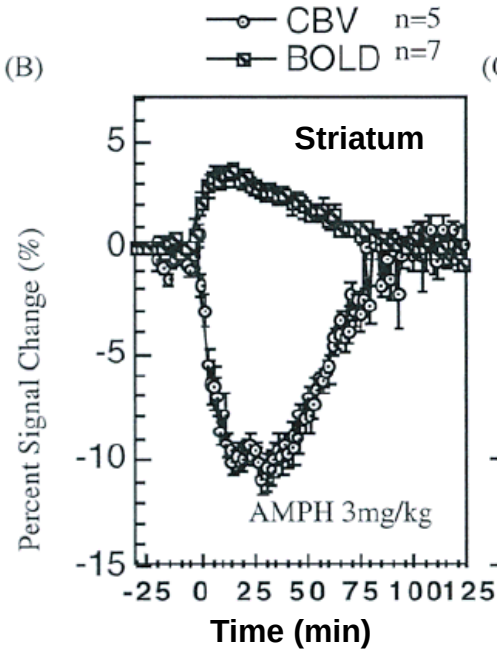
Table 2
Comparisons of BOLD and Superparamagnetic CBV Contrast Agent Imaging

BOLD	IRON	Advantage
Much lower CNR at common field strengths (up to at least 4.7T)	Much greater CNR than BOLD at common field strengths	IRON
Heavy resting CBV (vascular) weighting at common field strengths	Low resting CBV (vascular) weighting	IRON
No direct relationship to a physiologic parameter	Direct relation to a physiologically relevant parameter, rCBV	IRON
Endogenous contrast, no injections; possibly comparable to IRON contrast at high field strengths (7–14 T)	Requires injection of large amount of iron	BOLD
Possibility of measuring $CMRO_2$ indirectly	No longer able to measure BOLD (or $CMRO_2$)	BOLD

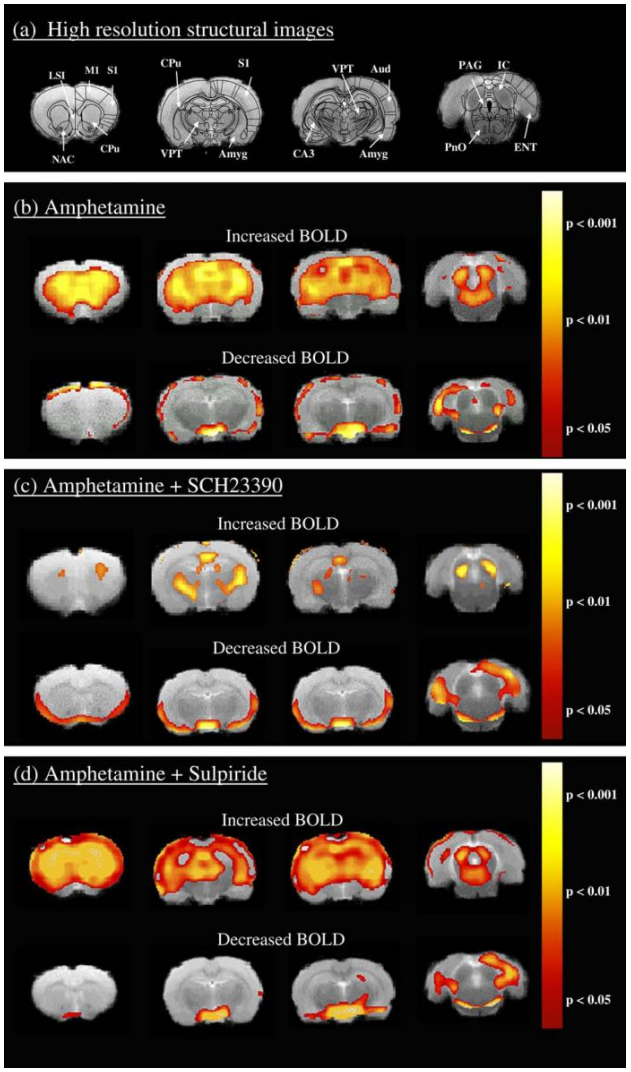
YC Chen, JB Mandeville, TV Ngyuen, A Talele, F Cavagna, BG Jenkins (2001) *J Magn Res Imaging* 14:517-524

Pharmacological MRI of amphetamine effects

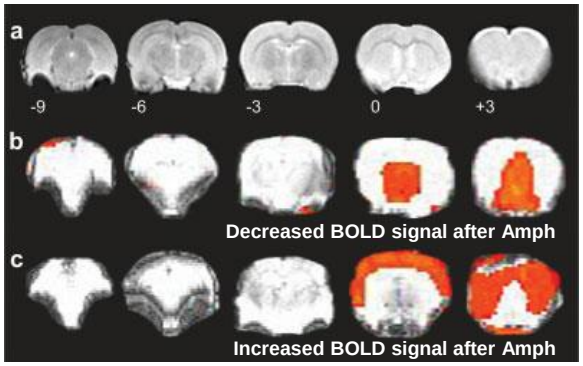




YC Chen, JB Mandeville, TV Ngyuen, A Talele, F Cavagna, BG Jenkins (2001) *J Magn Res Imag* 14:517-524



AL Dixon, M Prior, PM Morris, YB Shah, MH Joseph, AMJ Young (2005) *Neuropharmacology* 48:236-245



MA Preece, NR Sibson, JM Raley, A Blamire, P Styles, Sharp T (2007) *Synapse* 61:925-32

Problems

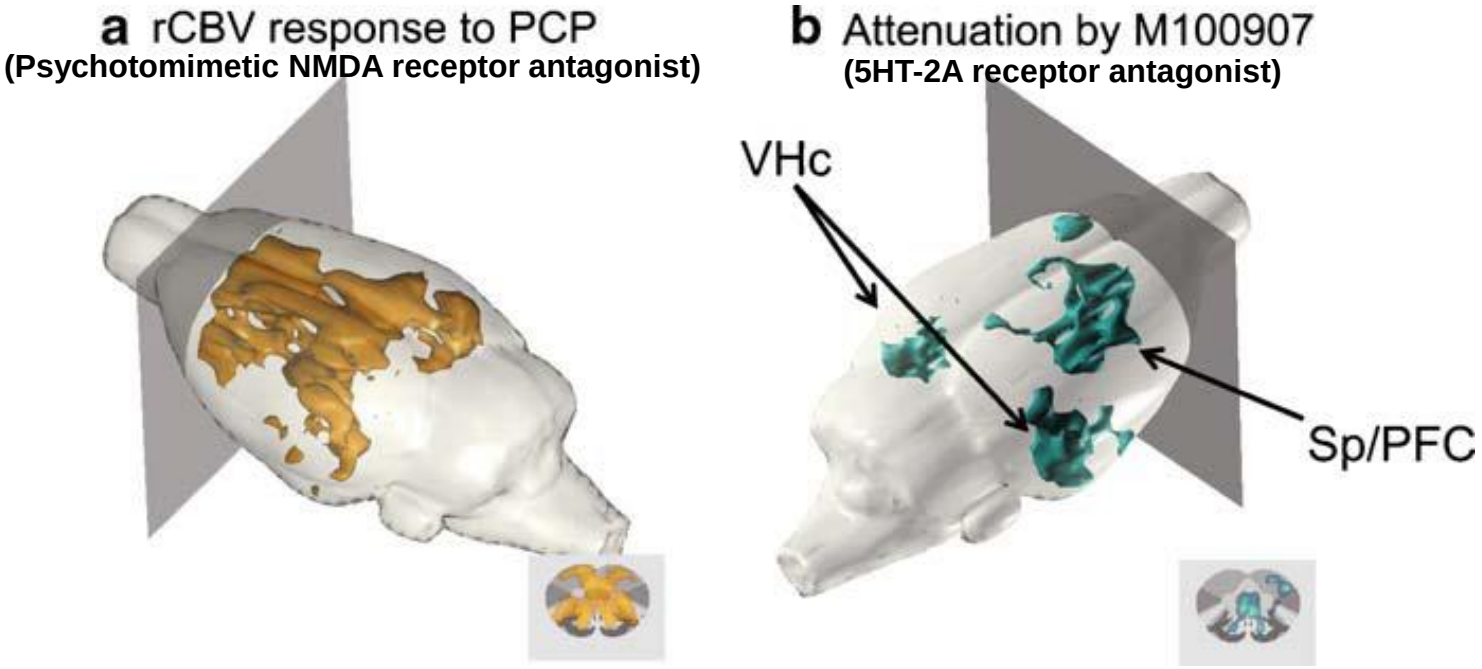
- Very high amphetamine dose!
- Do signal changes reflect specific neuronal activation?

Other applications of pharmacological MRI

- **Effects of other psychotomimetics (NMDA receptor antagonists) and antipsychotic drugs/drug candidates**

- **Effects of drugs modulating serotonin function (relevant to depression and to antipsychotic action)**

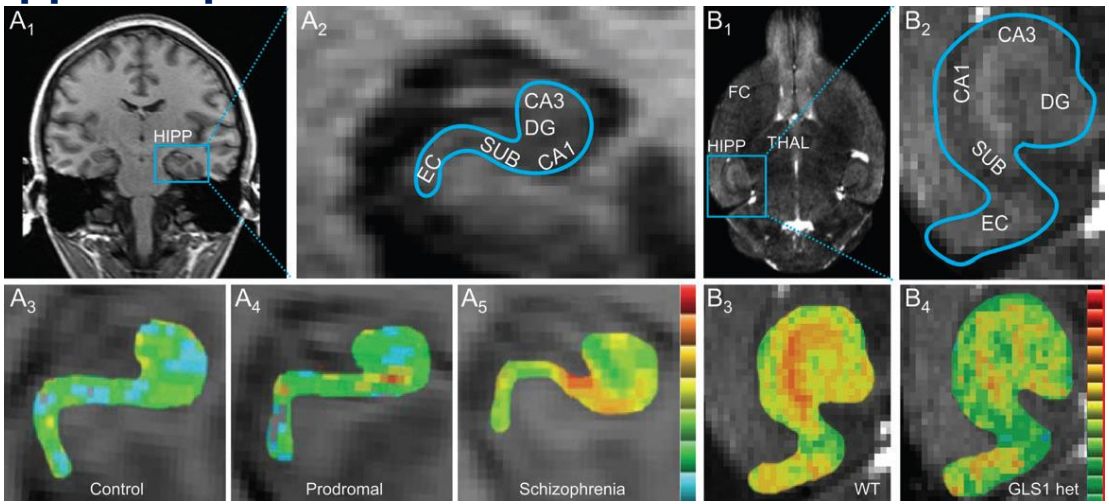
- **Etc.**



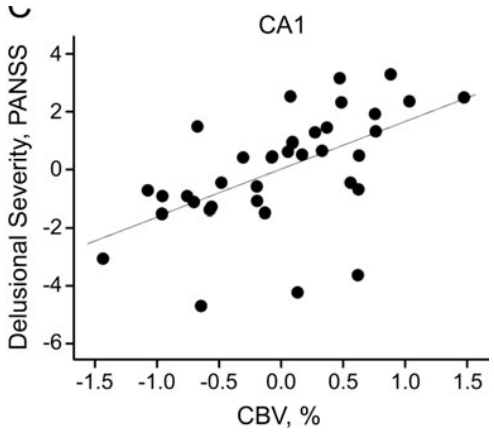
For overview, see: A Bifone, A Gozzi (2011) *Curr Top Behav Neurosci* 7: 323-357 (PDF file available from my webpage)

Coordinated translational studies in animal models and patients using fMRI

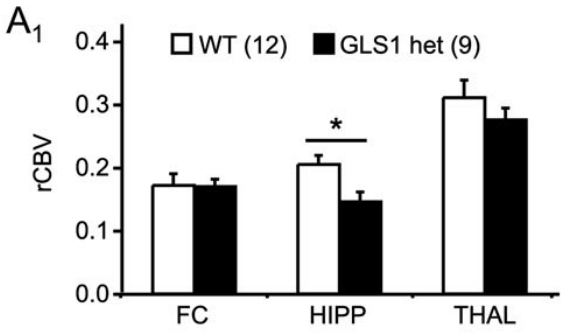
Hippocampal CBV measurements in mice and men

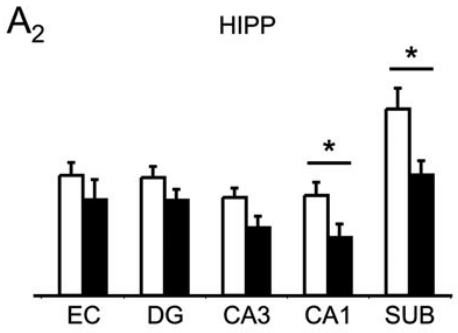


CA1 CBV correlates with psychotic symptoms



Strategies to reduce CA1 CBV can be studied in mice

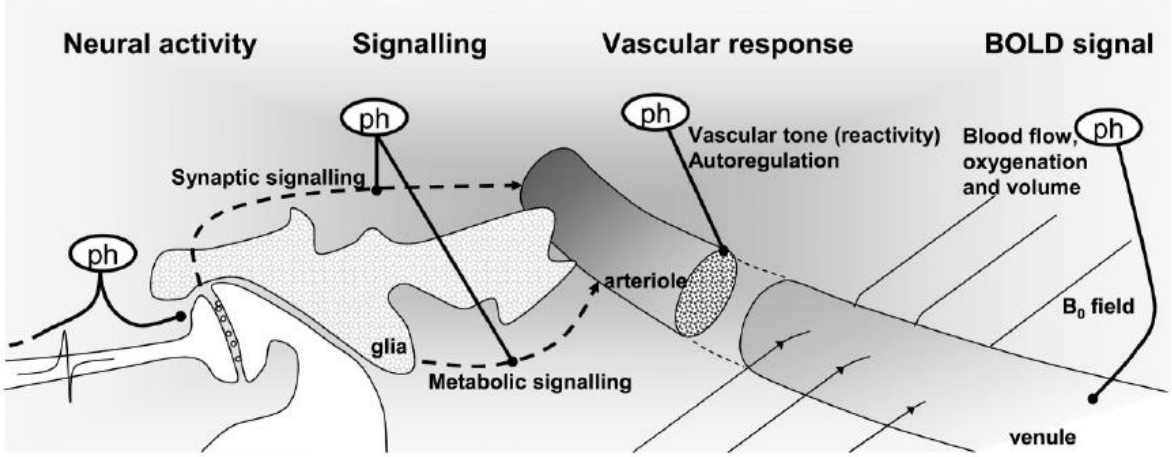




I Gaisler-Salomon, SA Schobel, SA Small, S Rayport (2009) *Schizophr Bull* 25:1037-1044

Problems of pharmacological MRI

- **Do the drug-induced MR signal changes reflect specific neuronal drug effects?**



RG Wise, I Tracey (2006) *J Magn Res Imaging* 23:862-876, Fig. 5

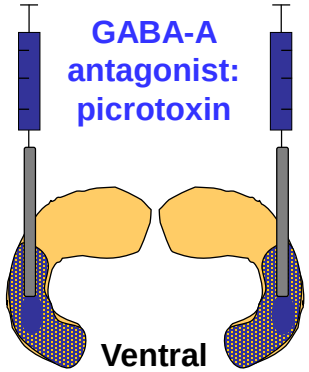
- **Key statistical problem: drug-induced response is a single temporally extended event**

-Difficult to distinguish from slow baseline drift

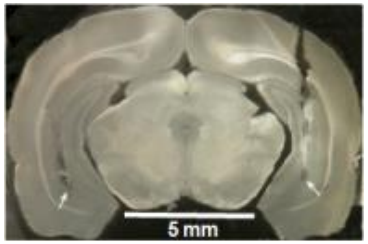
-Repeated-measures designs are impossible

Compare: A Pohlmann, H Barjat, LC Tilling, MF James (2007) *Conf Proc IEEE Eng Med Biol Soc:*3411-3416

Use of BOLD MRI to detect neural-network effects of brain site-specific pharmacological stimulation

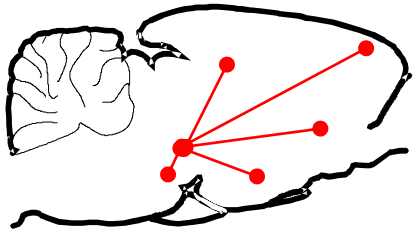


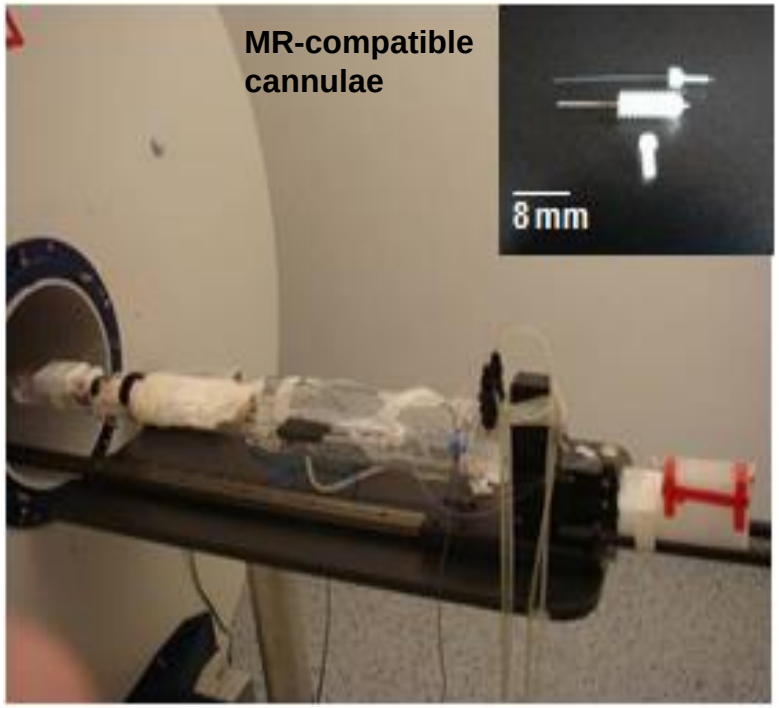
Ventral hippocampus



Compare **McGarrity et al., 2017, *Cereb Cortex***

Neural-network effects?

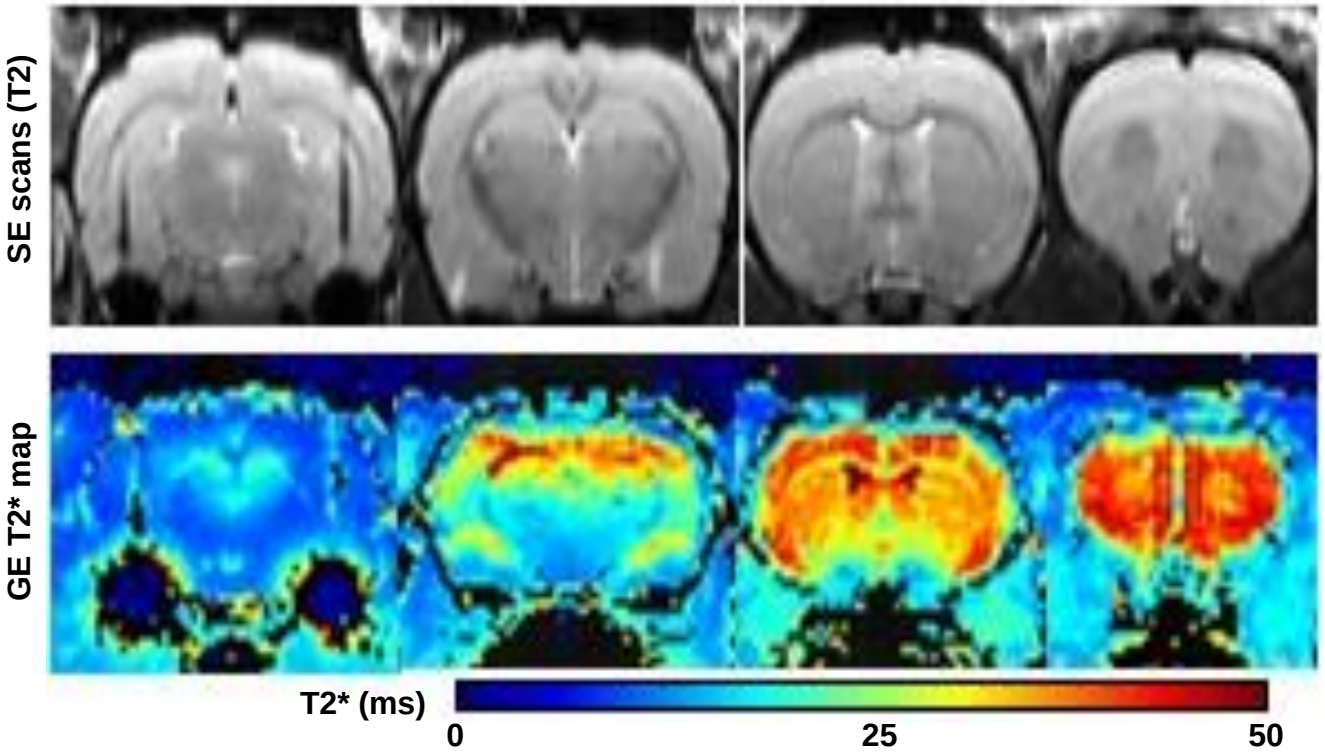




Key methodological challenges:

- **GE or SE imaging?**
- **Is imaging sequence sensitive to specific neuronal activations via synaptic pathways?**
- **Appropriate statistical analysis?**

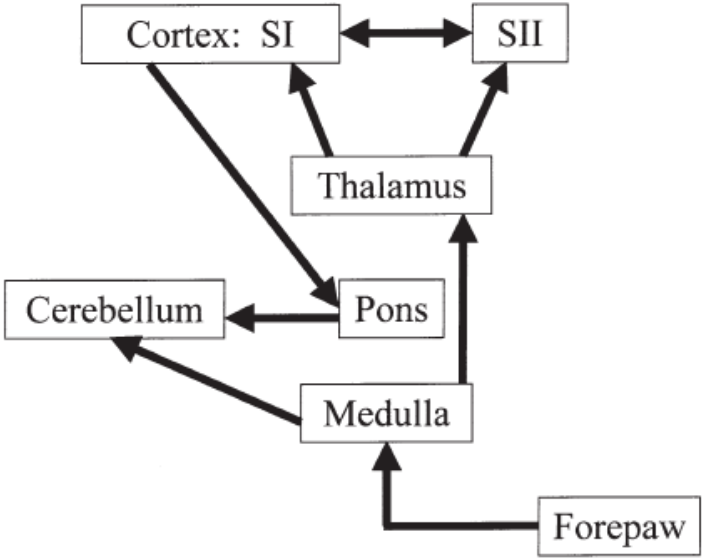
Whole brain imaging using SE and multiple-GE sequences



T Bast, M Prior, D Schluppeck, *unpublished data*

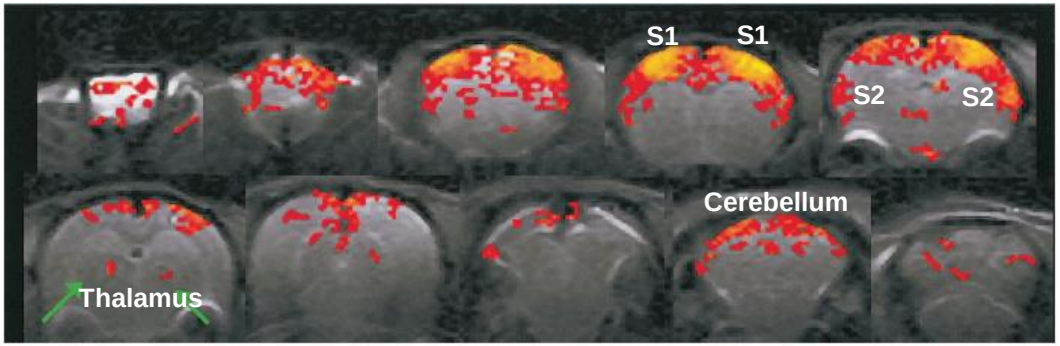
Forepaw stimulation paradigm as positive control: Regional brain activation via well-defined synaptic pathway

Somatosensory pathway

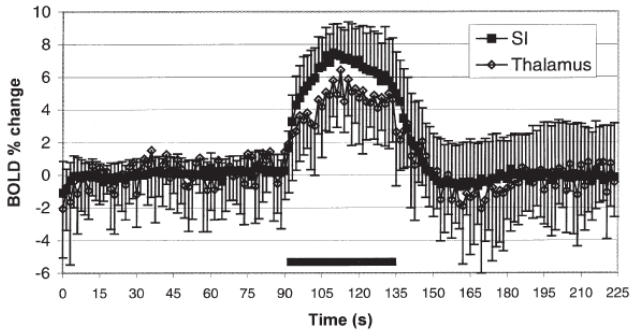


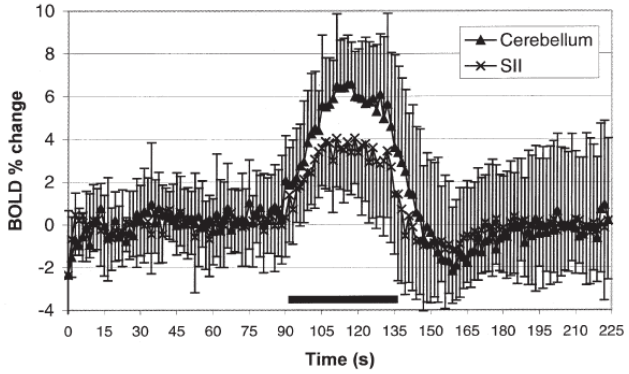
MRI (SE-EPI) of rat somatosensory pathway

Most anterior



Most posterior



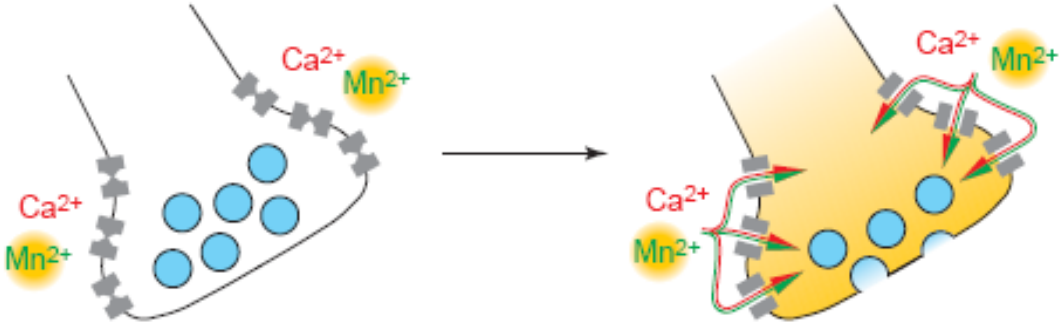


SD Keilholz, AC Silva, M Raman, H Merkle, AP Koretsky (2004) *MRI Medic* 52:89-99

Manganese-enhanced MRI (MEMRI)

- **Manganese (Mn^{2+}) acts as contrast agent, enhancing T1-dependent MR signal and facilitating high-resolution T1-weighted imaging**

- **Accumulation of Mn^{2+} in brain is activity-dependent**



- **Neuro-axonal Mn^{2+} uptake and transport: pathway tracing**

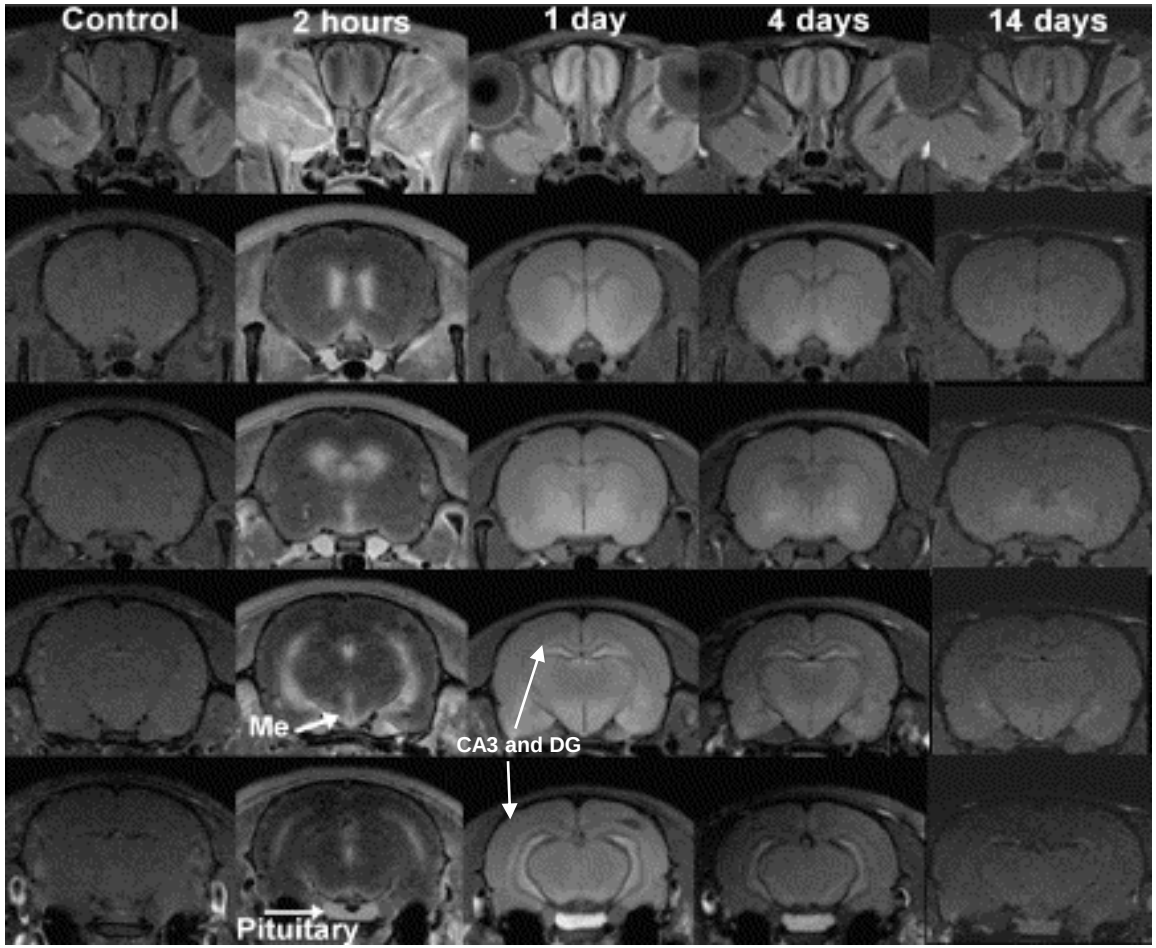
Uptake by voltage-gated Ca^{2+} channels → Sequestration in ER and packaging for transport → Axonal transport along microtubules → Release at synaptic cleft

- **Activity-dependent Mn^{2+} accumulation can be measured hours later using T1-weighted MRI**

MEMRI offers unique opportunities for high-resolution functional mapping: regional brain activation and activation of functional systems

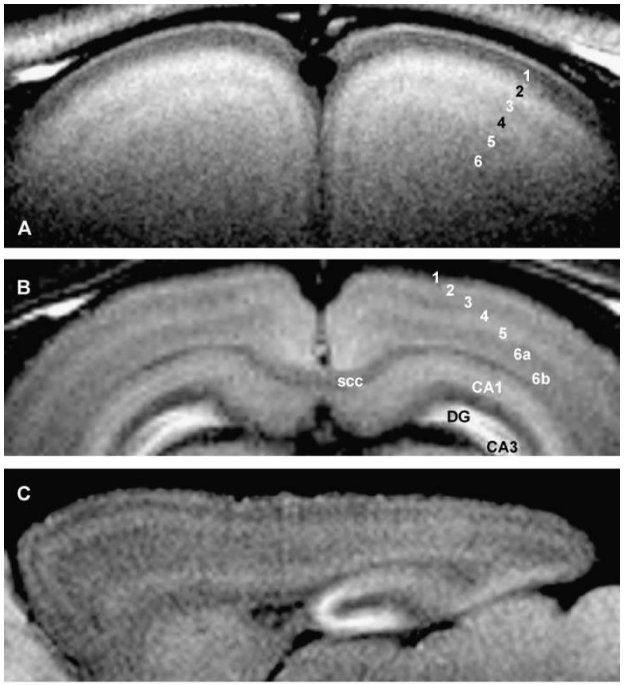
MEMRI of the rat brain

Time course of signal enhancement after systemic Mn^{2+} application*



*175 mg/kg (i.v.) $MnCl_2(X4H_2O)$

High anatomical resolution

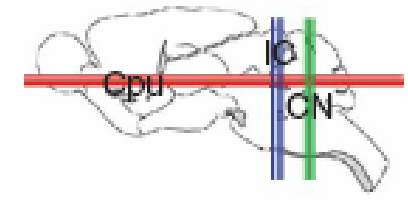


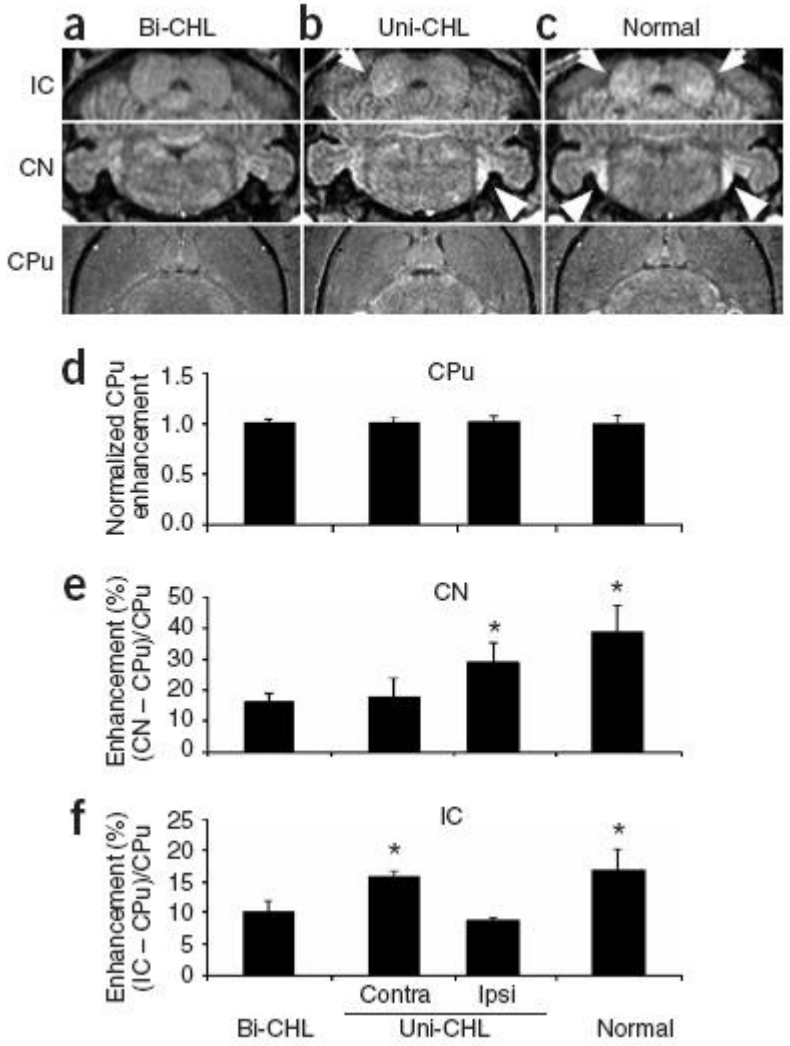
Resolution:
A: 50 X 50 X 750 um
B and C: 75 X 75 X 1000 um

I Aoki, Y-J Lin Wu, AC Silva, RM Lynch, AP Koretsky (2004) *Neuroimage* 22:1046-1059

MEMRI of auditory-evoked brain activity in mice

MEMRI after MnCl2 injection and 24h of broadband-noise exposure in normal mice and mice with conductive hearing loss



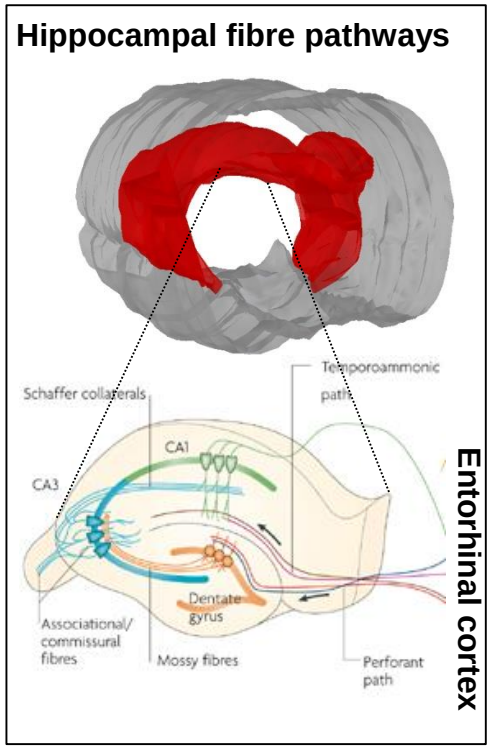


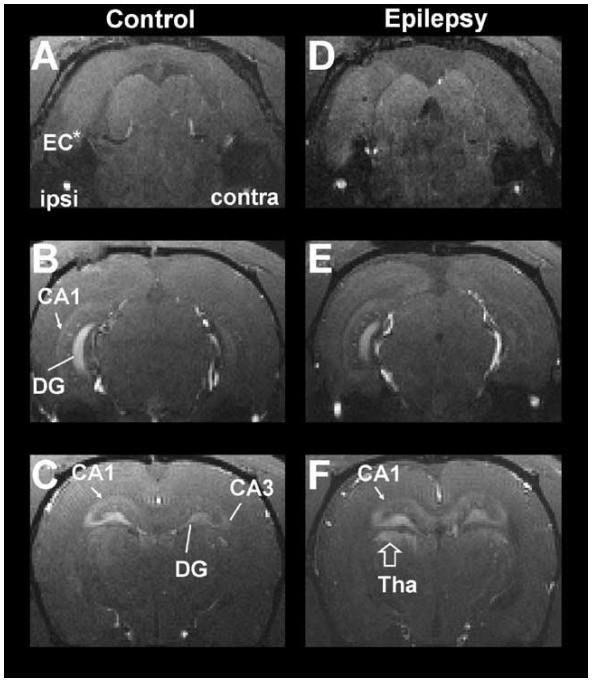
- **No signal enhancement in auditory cortex**
- **Signal enhancement was also detected in mice exposed to broadband noise as compared to quiet environment**
- **Tonotopic maps in IC could be detected**

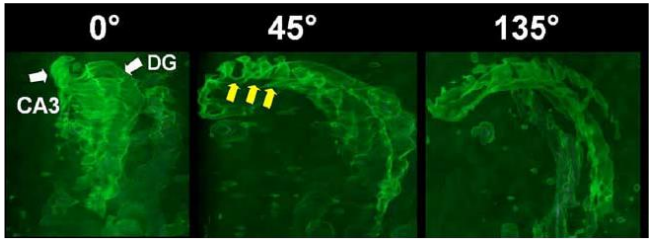
X Yu, YZ Wadghiri, DH Sanes, DH Turnbull (2005) *Nature Neurosci* 8:961-968

MEMRI of hippocampal pathway (mossy fibres) plasticity

Signal enhancement in mossy fibre pathway (DG-CA3) after Mn^{2+} injection into entorhinal cortex: effects of kainic acid-induced epilepsy







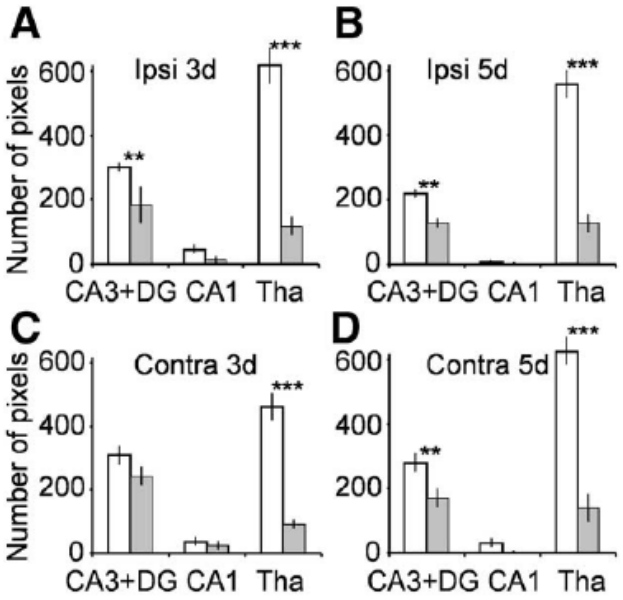
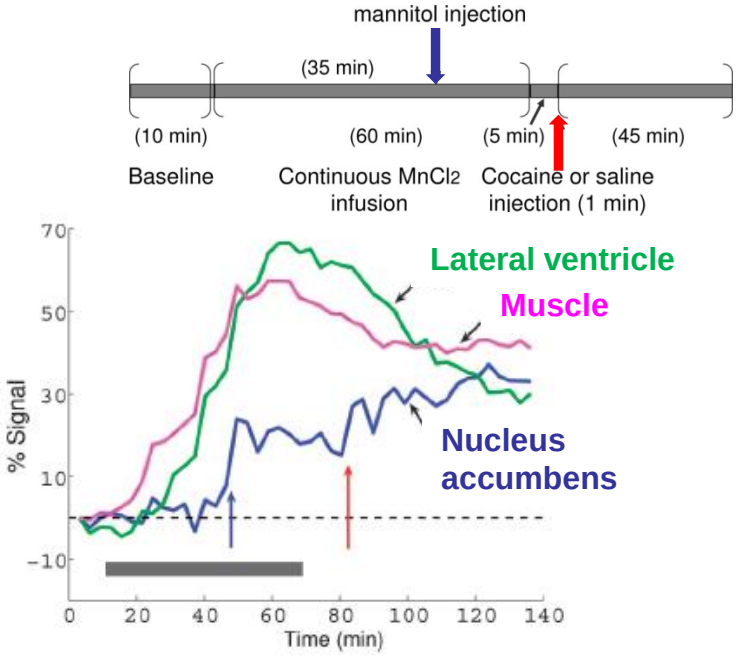


Fig. 3. Number of enhanced pixels in the dentate gyrus + CA3 subfield (DG + CA3), in the CA1, and in the dorsal thalamus 3 (A, C) and 5 days (B, D) after Mn injection in control rats (gray bar) and KA-treated rats (white bar). Data shown are for the sides ipsilateral (A, B) and contralateral (C, D) to the Mn injection site. Statistical significance was evaluated using Student's *t* test (**$P \leq 0.01$, ***$P < 0.001$). Values indicated are mean ± SEM.

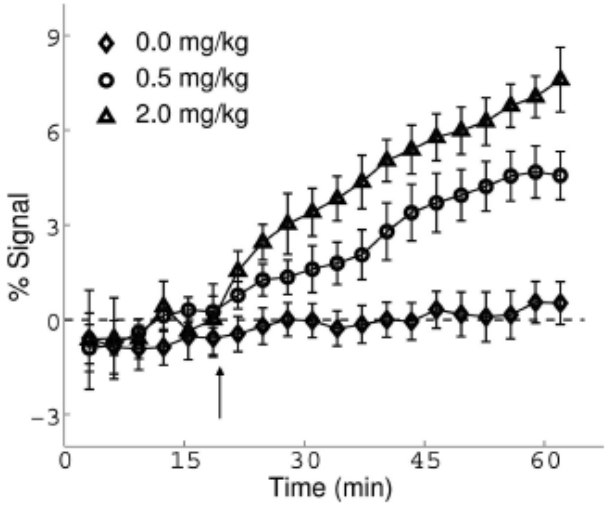
J Nairismagi, A Pitkanen, S Narkilahti, J Huttunen, RA Kauppinen, OHJ Grohn (2006) *Neuroimage* 30:130-135

MEMRI of cocaine-induced brain activation

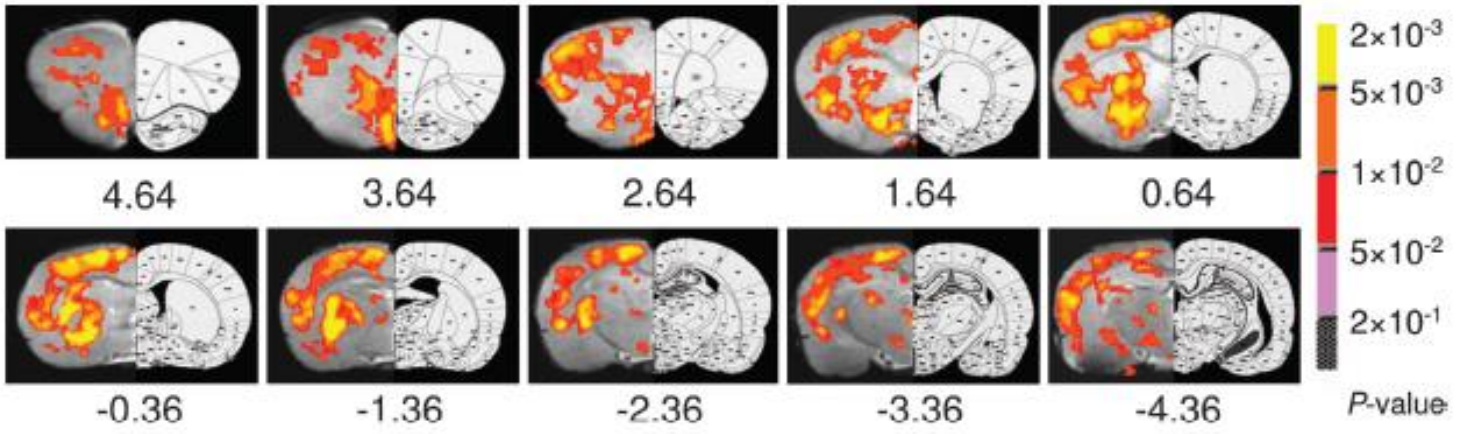
Experimental design: BBB disruption



Signal time course in nucleus accumbens



Brain-wide activation pattern resulting from acute cocaine



H Lu, Z-X Xi, L Gitajn, W Rea, Y Yang, EA Stein (2007) *Proc Nat Acad Sci USA* 104:2489-2494

MEMRI vs. blood-flow based fMRI

Advantages

- **Higher spatial resolution**
- **May reflect neuronal activation more directly (higher sensitivity; less confounded by vascular effects)**
- **Can be used to measure neural correlates of behaviour that occurred outside scanner**

Disadvantages

- **Lower temporal resolution**
- **Problems related to Mn^{2+} entrance into brain extracellular space (limited time window for functional mapping, requirement of BBB disruption)**
- **Toxicity!!!**
- **No directly comparable measure in humans (hampers 'translation')**

Toxicity of $MnCl_2$ at doses commonly used for MEMRI

Table 1. Toxicity data (LD_{50}) for $MnCl_2$

Species	Route	Dose
Rat	Oral	250 mg/kg
	Intraperitoneal	147 mg/kg
	Intravenous	92.6 mg/kg
	Intramuscular	700 mg/kg
Mouse	Oral	1031 mg/kg
	Intraperitoneal	121 mg/kg
	Intravenous	38 mg/kg

Source: MSDS for MnCl2 (125.84 g/mol; product nr. 244589, Sigma Aldrich, USA)

Table 2. Systemic doses of $MnCl_2$ used in current MEMRI experiments

Species	Route	Dose	Reference
Rat	Intravenous	54 mg/kg	Lin and Koretsky[1]
	Intravenous	60 mg/kg	Duong *et al.*[3]
	Intra-arterial	53 mg/kg	Aoki *et al.*[4]
	Intravenous	175 mg/kg	Aoki *et al.*[15]
Mouse	Nasal	65 mg/kg	Pautler *et al.*[7]
	Intravenous	175 mg/kg	Lee *et al.*[24]
	Intravenous	6.6 mg/kg	Hu *et al.*[5]
	Intraperitoneal	20 mg/kg	Watanabe *et al.*[17]

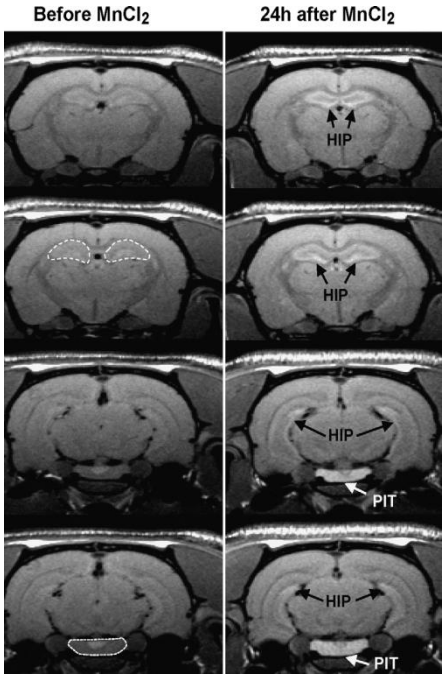
[Doses refer to $MnCl_2$ X $4H_2O$ (197.84 g/mol)]

". . . current MEMRI experiments are being performed at . . . doses . . . as shown in Table 2, with good results and few adverse effects reported. For example, we have been able to reliably administer up to 175 mg/kg intravenously in rats up to 250 g body weight and in mice up to 25 g body weight with only minor and temporary side effects that resolved slowly over 30–60 min after administration."

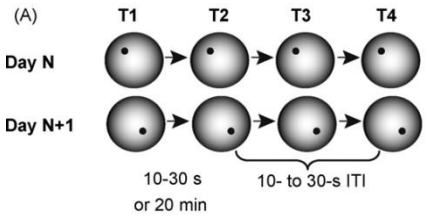
Silva et al, 2004, NMR Biomed

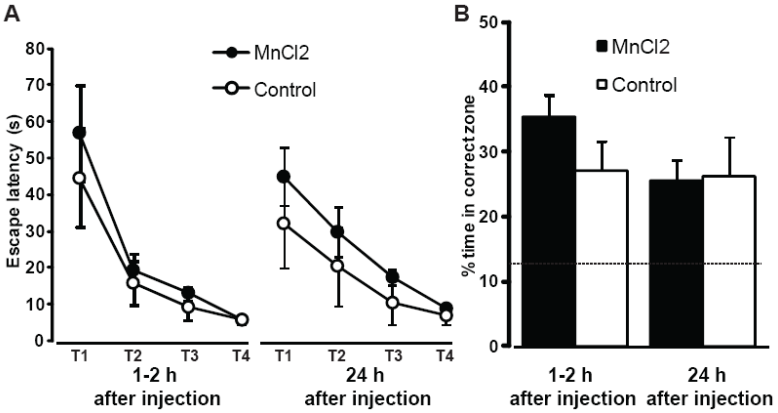
MEMRI of rat brain: Hippocampal signal enhancement without disruption of hippocampus-dependent behaviour

Hippocampal signal enhancement after 200 µmol/kg $MnCl_2$ (i.p.)

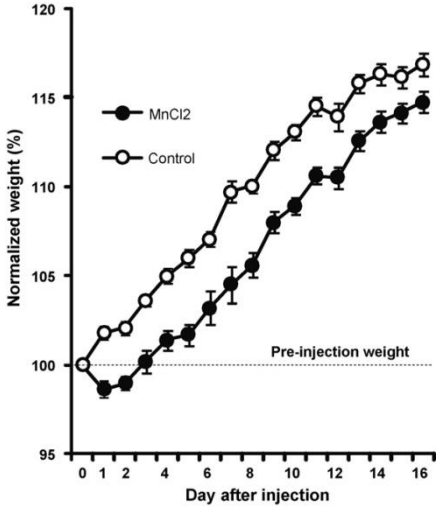


Intact hippocampus-dependent behaviour on rapid place learning test





Weight loss



Consistent with findings by others that Mn^{2+}-induced hippocampal signal enhancement can be obtained without disruption of hippocampal function (Eshenko et al., 2010, Neuroimage; Eshenko et al., 2011, Magn Res Imaging).

SJ Jackson, R Hussey, MA Jansen, GD Merrifield, I Marshall, A MacLullich, JLW Yau, T Bast (2011) *Behav Brain Res* 216:293-300

fMRI in animal models: summary and conclusions

- **Several fMRI approaches in animal models have been developed.**

- **These hold promise for efficient mapping of the time course of regional activation across the brain (especially drug-induced effects, but potentially also correlates of behaviour and exposure to sensory stimuli).**

- **Animal fMRI may act as translational bridge between animal model and human studies.**

- **However, potential of animal fMRI has yet to be realised: by and large, this approach has not yet produced new insights into brain function and dysfunction.**

Selected review papers

R Wise & I Tracey (2006) Role of fMRI in drug discovery. *J Magn Res Imaging* 23:862-876.

CA Steward, CA Marsden, MJW Prior, PG Morris, YB Shah (2005) Methodological considerations in rat brain BOLD contrast pharmacological MRI. *Psychopharmacology*180:687-704.

AC Silva, JH Lee, I Aoki, AP Koretsky (2004) Manganese-enhanced magnetic resonance imaging (MEMRI): methodological and practical considerations. *NMR Biomed* 17:532-543.

A Bifone, A Gozzi (2011) Functional and pharmacological MRI in understanding brain function at a systems level. *Curr Top Behav Neurosci* 7: 323-357(PDF file available from my webpage)

I Gaisler-Salomon, SA Schobel, SA Small, S Rayport (2009) How high-resolution basal-state functional imaging can guide the development of new pharmacotherapies for schizophrenia. *Schizophr Bull* 25:1037-1044

Single-Photon-Emission-Computerised Tomogrophy (SPECT) brain imaging

Injection into blood of radioactive tracer emitting single photons, e.g. ^{99m}Tc, ^{201}Tl

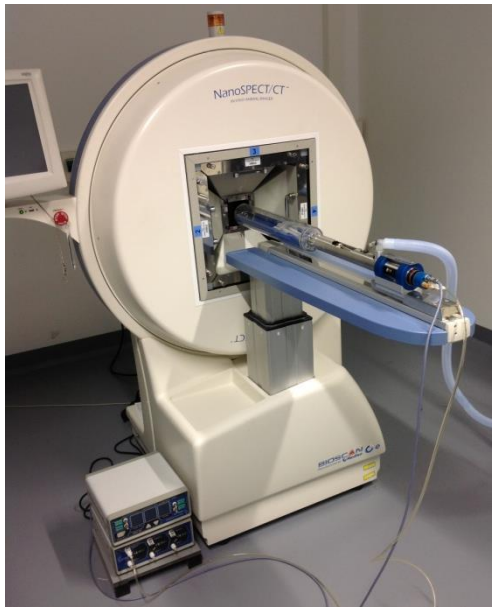
↓

Animal undergoes behavioural or pharmacological testing, during which tracer accumulates in brain depending on regional neural activity

↓

SPECT/CT imaging

Small animal SPECT/CT



Selected reading

SR Meikle, FJ Beekman, SE Rose (2006) Complementary molecular imaging technologies: high resolution SPECT, PET and MRI. *Drug Discov Today: Technologies* 3: 187-194.

FJ Beekman, F van der Have (2007) The pinhole: gateway to ultra-high resolution three-dimensional radionucleotide imaging. *Eur J Nucl Med Mol Imaging* 34:151-161.

Kolodziej, A., Lippert, M., Angenstein, F., Neubert, J., Pethe, A., Grosser, O. S., ... & Goldschmidt, J. (2014). SPECT-imaging of activity-dependent changes in regional cerebral blood flow induced by electrical and optogenetic self-stimulation in mice. NeuroImage, 103, 171-180.