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

Tobias Bast, School of Psychology, University of Nottingham
Tobias.Bast@nottingham.ac.uk
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

Detection by MRI

Examples: BOLD MRI, rCBV 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

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!)

Drug administration

[Diagram showing the relationship between drug administration, neural activity, CBF, CBV, oxidative metabolism, deoxyhaemoglobin concentration, and BOLD fMRI contrast]
Comparison of BOLD and rCBV imaging

Table 2
Comparisons of BOLD and Superparamagnetic CBV Contrast Agent Imaging

<table>
<thead>
<tr>
<th></th>
<th>BOLD</th>
<th>IRON</th>
<th>Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Much lower CNR at</td>
<td>Much greater CNR than BOLD at common field strengths</td>
<td></td>
<td>IRON</td>
</tr>
<tr>
<td>common field strengths (up to at least 4.7T)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heavy resting CBV (vascular) weighting at common field strengths</td>
<td>Low resting CBV (vascular) weighting</td>
<td></td>
<td>IRON</td>
</tr>
<tr>
<td>No direct relationship to a physiologic parameter</td>
<td>Direct relation to a physiologically relevant parameter, rCBV</td>
<td></td>
<td>IRON</td>
</tr>
<tr>
<td>Endogenous contrast, no injections; possibly comparable to IRON contrast at high field strengths (7–14 T)</td>
<td>Requires injection of large amount of iron</td>
<td></td>
<td>BOLD</td>
</tr>
<tr>
<td>Possibility of measuring CMRO₂ indirectly</td>
<td>No longer able to measure BOLD (or CMRO₂)</td>
<td></td>
<td>BOLD</td>
</tr>
</tbody>
</table>

Pharmacological MRI of amphetamine effects


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

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

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

GABA-A antagonist: picrotoxin

Ventral hippocampus

MR-compatible cannulae

Key methodological challenges:
- GE or SE imaging?
- Is imaging sequence sensitive to specific neuronal activations via synaptic pathways?
- Appropriate statistical analysis?

Compare McGarrity et al., 2017, Cereb Cortex
Whole brain imaging using SE and multiple-GE sequences

T2* (ms)

SE scans (T2)

GE T2* map

T2* (ms) 0 25 50

T Bast, M Prior, D Schluppeck, unpublished data
Forepaw stimulation paradigm as positive control: Regional brain activation via well-defined synaptic pathway

MRI (SE-EPI) of rat somatosensory pathway

Somatosensory pathway

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

![Diagram of Mn\(^{2+}\) uptake and transport]

- 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\(_2\)O)

High anatomical resolution

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

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

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 thalamic 3 (A, C) and 5 days (B, D) after Mn injection in control rats (grey 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.
MEMRI of cocaine-induced brain activation

Experimental design: BBB disruption

Signal time course in nucleus accumbens

Brain-wide activation pattern resulting from acute cocaine

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$_2$

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Oral</td>
<td>250mg/kg</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>147mg/kg</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>92.6mg/kg</td>
</tr>
<tr>
<td>Mouse</td>
<td>Intramuscular</td>
<td>700mg/kg</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>1031mg/kg</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>121mg/kg</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>38mg/kg</td>
</tr>
</tbody>
</table>

Source: MSDS for MnCl$_2$ (125.84 g/mol; product nr. 244589, Sigma Aldrich, USA)

Table 2. Systemic doses of MnCl$_2$ used in current MEMRI experiments

<table>
<thead>
<tr>
<th>Species</th>
<th>Route</th>
<th>Dose</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>Intravenous</td>
<td>54mg/kg</td>
<td>Lin and Koretsky$^1$</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>60mg/kg</td>
<td>Duong $et al.$$^3$</td>
</tr>
<tr>
<td></td>
<td>Intra-arterial</td>
<td>53mg/kg</td>
<td>Aoki $et al.$$^4$</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>175mg/kg</td>
<td>Aoki $et al.$$^{15}$</td>
</tr>
<tr>
<td>Mouse</td>
<td>Nasal</td>
<td>65mg/kg</td>
<td>Pautler $et al.$$^7$</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>175mg/kg</td>
<td>Lee $et al.$$^{24}$</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>6.6mg/kg</td>
<td>Hu $et al.$$^5$</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal</td>
<td>20mg/kg</td>
<td>Watanabe $et al.$$^{17}$</td>
</tr>
</tbody>
</table>

[Doses refer to MnCl$_2$ X 4H$_2$O (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).

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.


**Single-Photon-Emission-Computerised Tomography (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**

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**Selected reading**


SPECT CBF imaging to reveal brain activation changes caused by hippocampal disinhibition


**GABA-A antagonist:** picrotoxin

Ventral hippocampus

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

**Metabolic hippocampal overactivity**

Neural-network effects?

Distal activation in medial prefrontal and ventral striatal areas

Hippocampal metabolic overactivity has emerged as key SZ biomarker


D Vincenz, F Stober, I Heinemann, J Goldschmidt, T Bast, *unpublished data*