Mn-Based MRI contrast

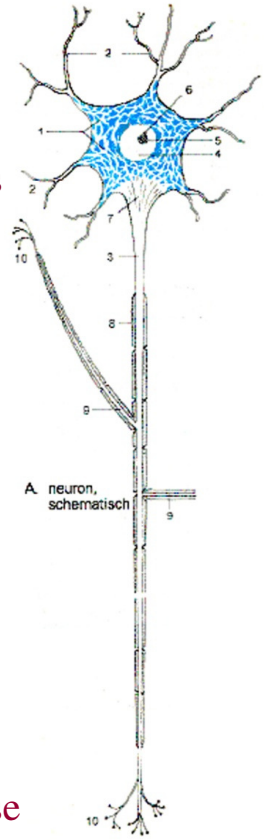
MEMRI neuro applications:

Bio Imaging Lab, University of Antwerp
Annemie van der Linden

Central nervous system

dentrites

neuron



axon

synapse

Manganese-enhanced MRI (**MEMRI**)

evolved in the late nineties when Alan Koretsky (NIH) and associates pioneered the use of **MEMRI** for brain activity measurements as well as neuronal tract tracing

Using MnCl2 > Mn(2+)

Manganese-enhanced MRI (**MEMRI**)

relies upon the following three main properties of **Mn(2+)**:

(1)it is a paramagnetic ion that shortens the T(1)

(2) it is a calcium (Ca(2+)) analog that can enter excitable cells, such as neurons and cardiac cells via voltage gated Ca(2+) channels

(3)once in the cells Mn(2+) can be transported along axons by microtubule dependent axonal transport and can also cross synapses trans synaptically to neighboring neurons

How to get Mn2+ ions in the brain?
Manganese administration routes

systemic injection of MnCl2:
intraperitoneal [i.p.]
subcutaneous [s.c.]
intravenous [i.v.]

Manganese administration routes
Systemic injections
Traversing the BBB?

- After a short systemic exposure, Mn2+ is cleared from the blood, in the range from minutes up to hours
- Increased influx into the brain > uptake mechanism at the level of the **choroid plexus and the ventricular ependyma**, rather than direct uptake through the BBB
- Identical results after **intraventricular injection**

Manganese administration routes
alternative administration routes Traversing the BBB?

Alternatively, one can reversibly break the BBB

- by applying an osmotic shock during the intravenous infusion of Mn2+

Mn2+ can also be delivered directly into a particular brain ROI

- Via olfactory epithelium and the eye (circumventing the BBB)
- or more invasive via **focal brain injections**

Chemical configuration
and cellular uptake of manganese

Mn2+ uptake involves transport systems:

- calcium channels
- Na/Ca exchanger
- active calcium uniporter
- Na/Mg antiporter
- divalent metal transporter DMT1 (also known as DCT1 and NRAMP2)
- carrier-mediated, identity of the Mn2+-carrier(s) is unknown

Mn2+ efflux across the BBB

- does not appear to occur through a carrier but rather by diffusion

Chemical configuration and cellular uptake of manganese neurons

In excitable cells such as neurons

- Mn2+ can be incorporated by **L-type voltage gated calcium channels**
- This was verified by utilizing Ca2+ channel blockers (diltiazem or verapamil) which prevents the uptake of Mn2+ into cells. This has been verified in brain as well as the heart
- Additional support comes from the accumulation of Mn2+ in specific brain areas that contain neuronal populations with high spontaneous activity

Subcellular distribution and axonal transport of manganese

- The largest subcellular concentration of manganese is found in the mitochondria, endoplasmatic reticulum and lysosomes
- Excitable tissue, experiencing frequent Ca2+ spikes, is likely to accumulate mitochondrial Mn2+

Subcellular distribution and axonal transport of manganese

- mammalian axons exhibit two major anterograde transport processes with a differential speed
  - **Slow axonal transport** 0.01– 0.33mm/h and transports mitochondria
  - **Fast axonal transport** 2–16mm/h. within vesicles > Mn(2+)
- Interestingly, although the speed of fast axonal transport is constant, the **amount of vesicles** that can be transported per unit of time can change **according to the neuronal activity**

Subcellular distribution and axonal transport of manganese

Transsynaptic transport mechanism

- Mn2+ released at the synaptic cleft within synaptic vesicles together with glutamate
- Mn2+ incorporated in the post-synaptic cells by ligand-gated Ca channels such as NMDA receptors.

Fluorescence quenching techniques has shown this

MEMRI

neuronal connectivity and activation

Universiteit Antwerpen

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Universiteit Antwerpen

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

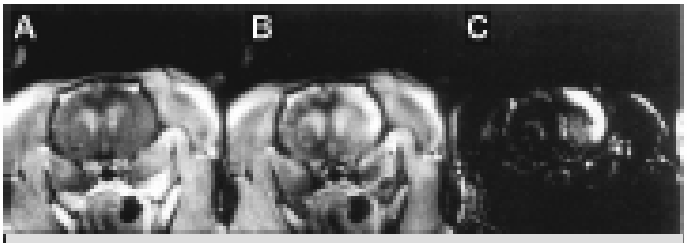
Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

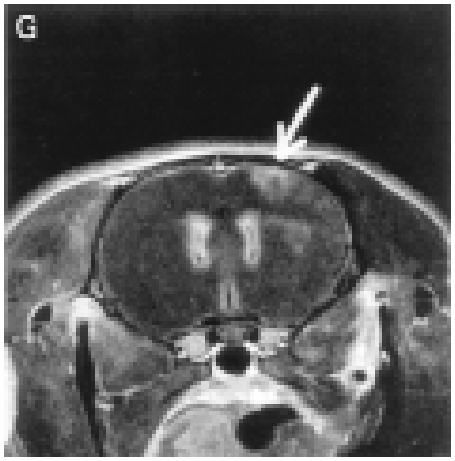
'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Activation- induced manganese dependent contrast **(AIM)MRI**

Lin and Koretsky, *MRM* 38, 378, **1997**



0.3 % halothane
3.6 µmol/min MnCl2 infusion iv
A: intact BBB
B: unilateral BBB disruption
C: difference image
G: Mn infusion 30 sec before electrical forepaw Stimulation, and BBB rupture (mannitol)



MEMRI
neuronal connectivity and activation

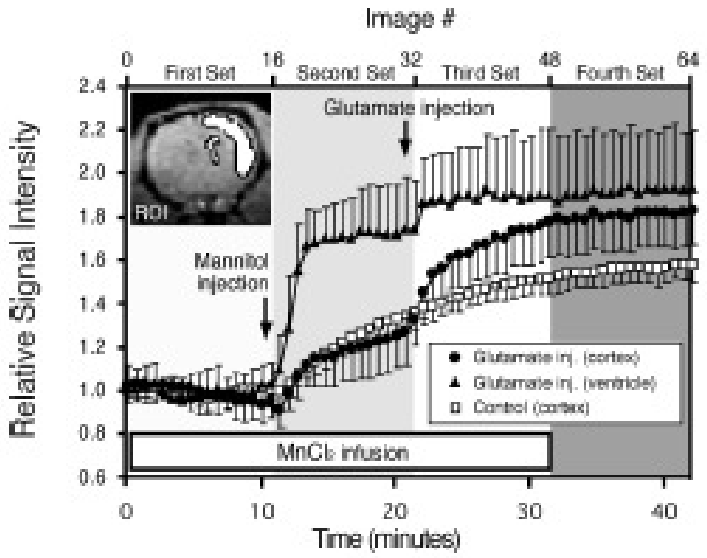
Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Dynamic activation induced manganese dependent contrast **(DAIM)MRI**
Aoki et al, MRM 48: 927, **2002**



MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

MEMRI tract tracing
Olfactory and visual pathways

For **tract tracing** one needs

- to target the area (inject $MnCl_2$)
  from which the axonal projections start (shaping the circuit of interest)
- thus circumventing the BBB
  1. Obvious for sensoric system (less invasive, remote from the brain)
     1. Target nostrils (olfactory epithelium) to study the olfactory circuit
     2. Target the retina to study the visual circuit
     3. Target the auditory nerves to study the auditory system
  2. Less obvious: injections in the brain targeting a nucleus of the circuit

ME MRI tract tracing

Watanabe et al, *MRM*, 46, 424, **2001**

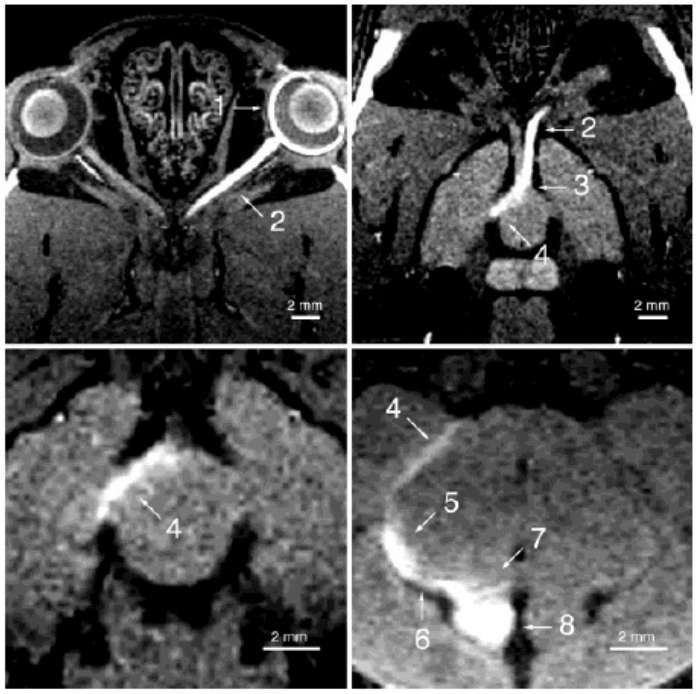
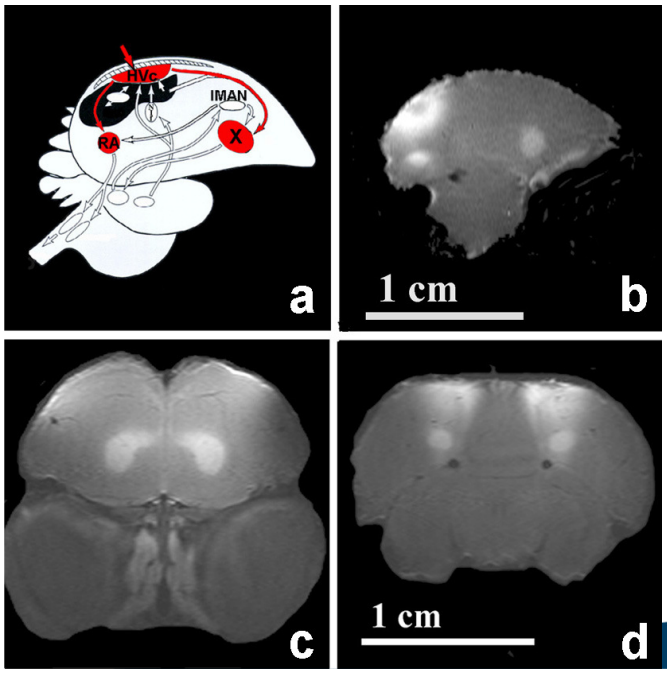


Fig. 1 (sections indicated in Fig. 1). Enhanced structures are: (1) left retina, (2) left optic nerve, (3) optic chiasm, (4) right optic tract, (5) right lateral geniculate nucleus, (6) right brachium of the superior colliculus, (7) right pretectal region, and (8) right superior colliculus.

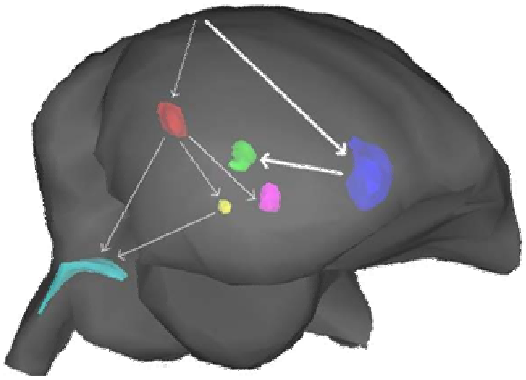
ME MRI tract tracing
other circuits: the song control system

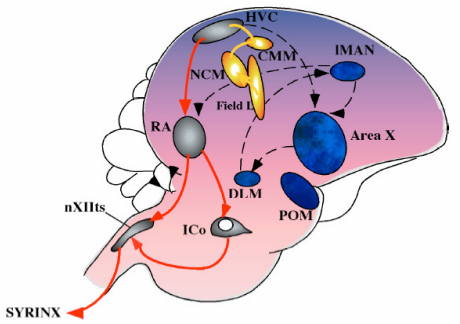
Van der Linden et al,
Neuroscience 112, 467, **2002**



Trans synaptic transport capacity of manganese

Tindemans et al *NMRB*, 19, 18, **2006**





MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Activity dependent ME MRI tract tracing

Pautler and Koretsky, *Neuroimage, 16, 441,* ***2002***

Activation of the main olfactory bulb with common odours

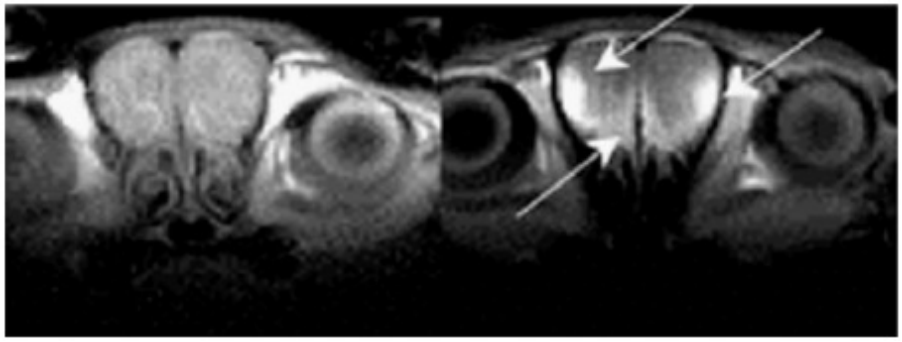


FIG. 3. MRI images of the olfactory bulb of a mouse exposed to Mn^{2+} only (left) and Mn^{2+} plus amyl acetate (right). MEMRI images of the mouse were obtained 1.5 h after exposure to aerosolized Mn^{2+} alone or in the presence of amyl acetate. The localized accumulation of Mn^{2+} is seen as positive contrast enhancement in the olfactory bulb.

Pautler and Koretsky, *Neuroimage*, 16, 441, **2002**

Activation of the accessory olfactory bulb with pheromones

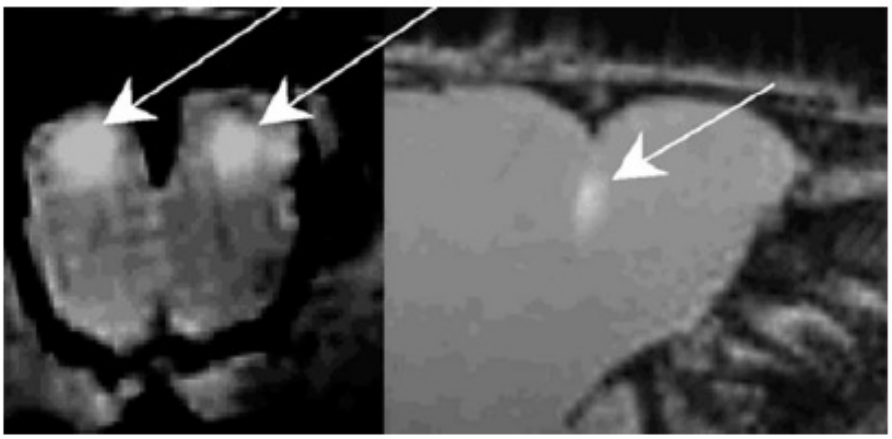


FIG. 2. Accessory olfactory bulb enhancement by MEMRI after exposure to pheromones and Mn^{2+}. Areas enhanced are readily detected as bright regions in axial (left) as well as sagittal (right) slices. The arrowheads point to the bilaterally symmetric accessory olfactory bulb, which appears as bright circles in the axial slice and is located caudal in the olfactory bulb and rostral to the main cortical areas in the saggital slice. Mice were exposed to pheromones (in the form of male mouse urine) as well as Mn^{2+} and enhancement in the accessory olfactory bulb always exhibited positive contrast enhancement ($n = 15$).

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

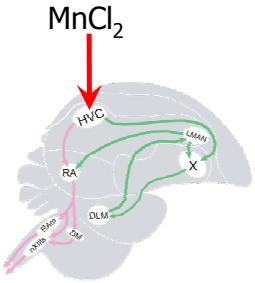
Dynamic Manganese Enhanced [**DME]MRI**
the song control system

- the dynamics of axonal manganese transport were monitored as manganese induced signal intensity (SI) enhancement in the projected areas
- Date are translated into a Hill plot (function describing a sigmoid curve)
- This so called **Dynamic Manganese Enhanced [DME]MRI** can then be used as a quantitative tool to monitor the activity of the projecting neurons in the injection area

Manganese Enhanced MRI

Pautler et al. (1998)

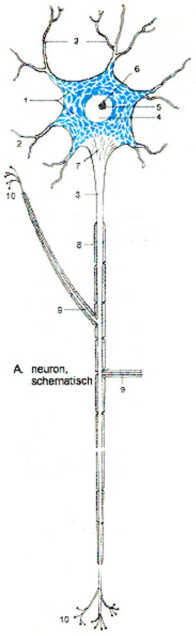
Mn^{2+} is transported along axons of a circuit



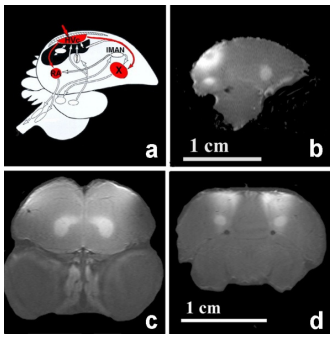
neuron

axon

synapse

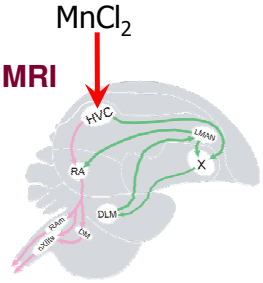


Mn^{2+} is a biological Ca^{2+} analogue
Mn2+ is paramagnetic



Universiteit Antwerpen

Dynamic Manganese Enhanced MRI : DME MRI

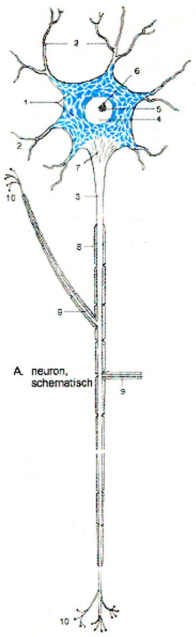


Mn^{2+} is transported along axons of a circuit

neuron

axon

synapse

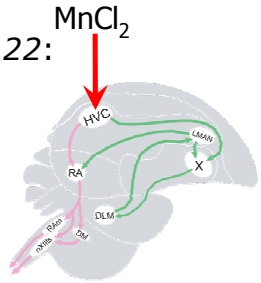


Mn^{2+} is a biological Ca^{2+} analogue

Uptake and transport activity dependent

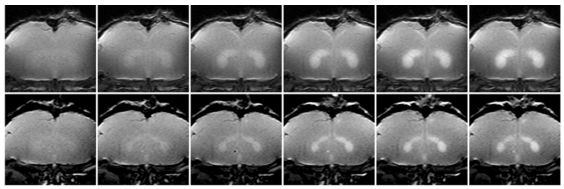
Universiteit Antwerpen

Van der Linden et al. *Neuroscience 122*: *467*, **2002**

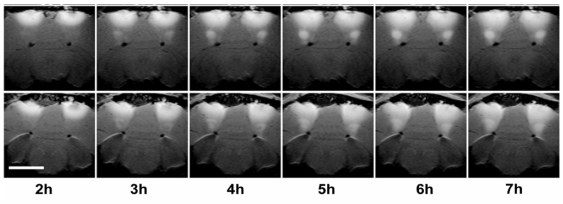


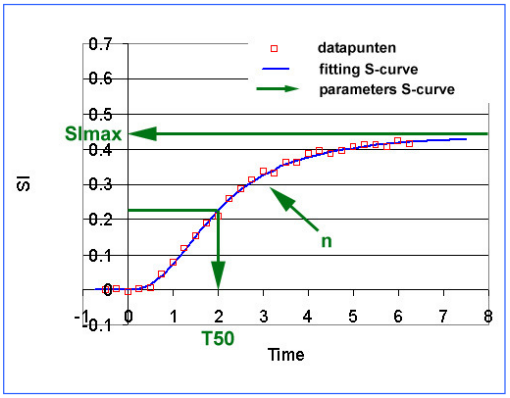
Accumulation of Mn^{2+} in Area X and RA

Area X



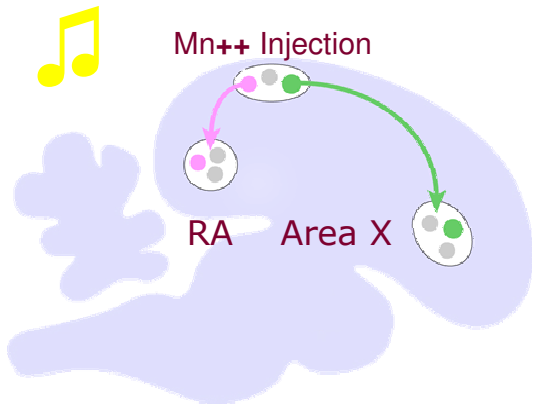
RA

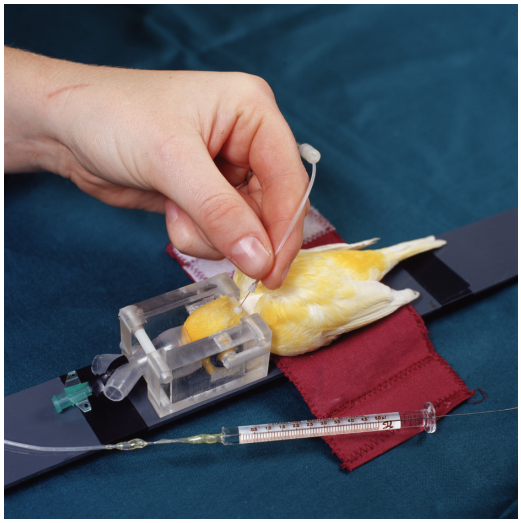




Study **repeatedly** the response of different neuronal populations in HVC to song using DME MRI

Tindemans et al. *Eur. J. Neurosc: 18:3352*, **2003**

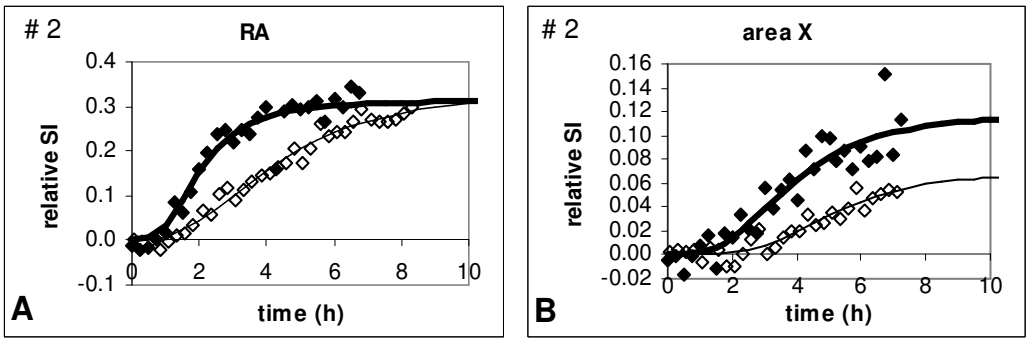




Follow Mn^{2+} uptake in RA and X

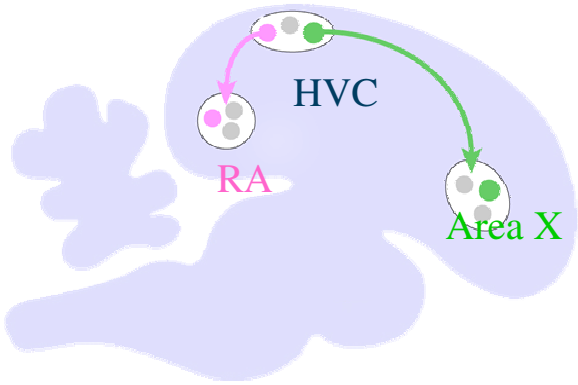
Study repeatedly the response of different neuronal populations in HVC to song using DME MRI

Tindemans et al. *Eur. J. Neurosc: 18:3352*, **2003**



Thick lines and full squares show the song stimulated results
n, SI_{max} provide a correlate for activity of that particular type of projecting neuron

Conclusion:
Different response of RA projecting and Area X projecting HVC neurons to song exposure



Differentiate the activity of neuronal populations from the same nucleus without having the resolution to do so..

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

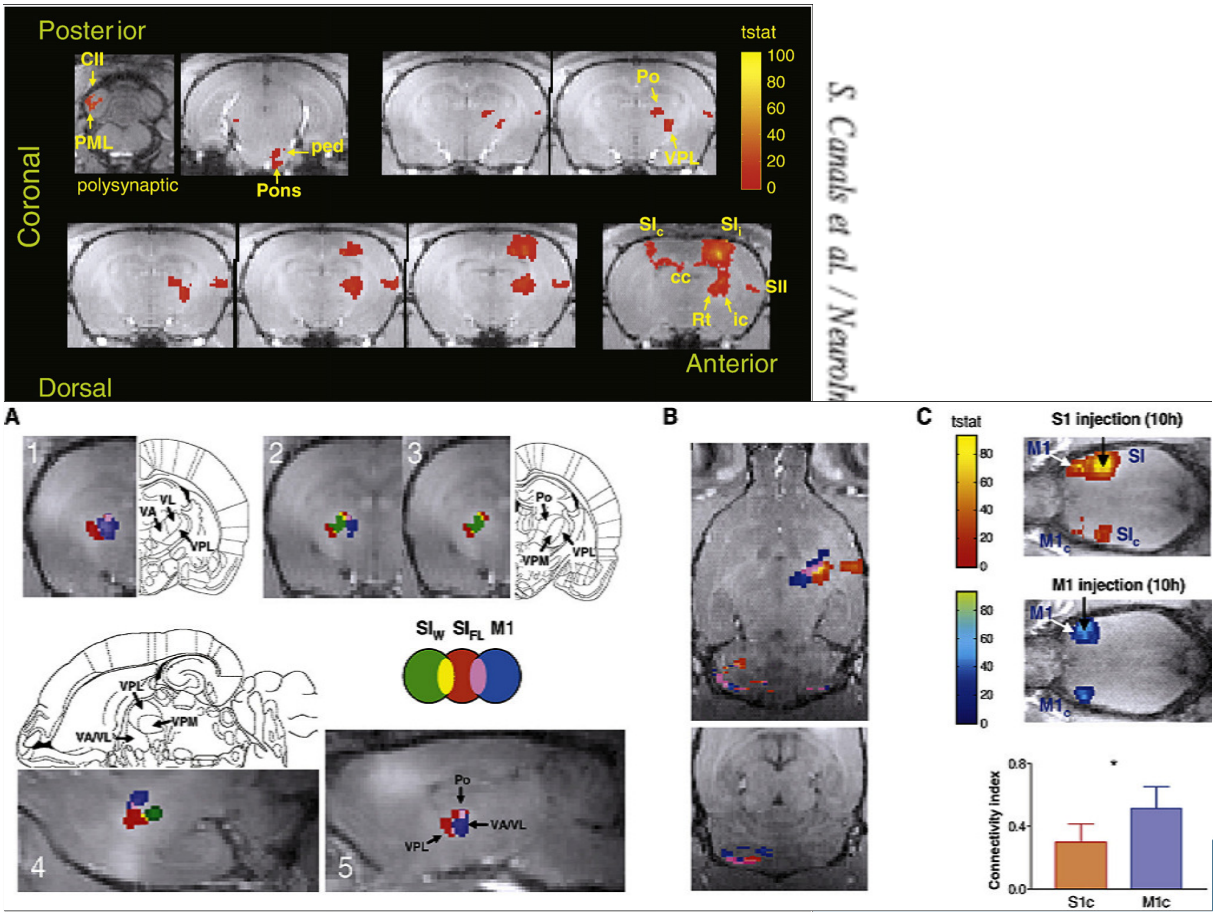
Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Neuronal connectivity
Connectivity Index **(CnI)**

Canals et al, *NeuroImage 40, 458,* **2008**

- continuously infusing very low concentrations of Mn2+into the target area using osmotic pumps coupled to chronically implanted brain cannulae.
- corticofugal somatosensory and motor pathways in individual animals.
- describe a **connectivity index (CnI)** based on Mn2+ transport



MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Remodelling of neuronal circuitries

NeuroImage 34 (2007) 1650 – 1657

Changes in neuronal connectivity after stroke in rats as studied by serial manganese-enhanced MRI

Jet P. van der Zijden,[a,*] Ona Wu,[a,b] Annette van der Toorn,[a] Tom P. Roeling,[c] Ronald L.A.W. Bleys,[c] and Rick M. Dijkhuizen[a]

[a]*Image Sciences Institute, University Medical Center Utrecht, Bolognalaan 50, 3584 CJ, Utrecht, The Netherlands*
[b]*Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital/Massachusetts Institute of Technology/Harvard Medical School, Charlestown, MA, USA*
[c]*Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands*

JOURNAL OF MAGNETIC RESONANCE IMAGING 28:863–870 (2007)

Original Research

Manganese-Enhanced MRI in a Rat Model of Parkinson's Disease

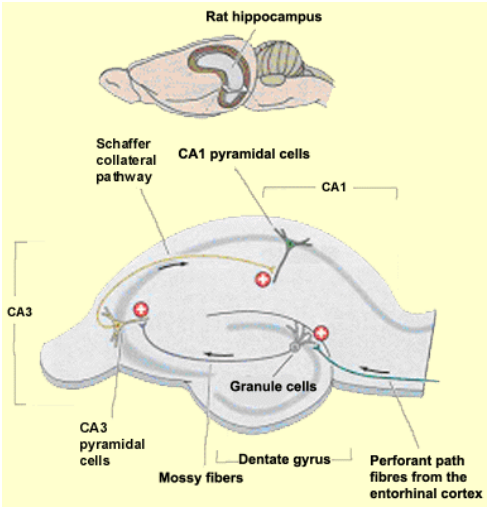
Galit Pelled, PhD,[1,2] Hagai Bergman, MD,[3] Tamir Ben-Hur, MD, PhD,[4] and Gadi Goelman, PhD[1*]

Remodelling of neuronal circuits

- as a consequence of hyperactivity during seizures
- Sprouting of granule cell axons or mossy fibers is one of the most consistent neuropathologic findings in the hippocampus of animals or humans with temporal lobe epilepsy, providing one of the most extensively characterized examples of activity-induced axonal plasticity in the brain
- Nairismagi, J., Pitkanen, A., Narkilahti, S., Huttunen, J., Kauppinen, R.A., and Grohn, O.H., Manganese-enhanced magnetic resonance imaging of mossy fiber plasticity in vivo, *Neuroimage*, 30, 130, 2006

intraperitoneal kainic acid injection
epilepsy model

MEMRI signal
in the dentate gyrus
and the CA3 subregion
of the
hippocampus



injection of MnCl2 into the entorhinal cortex
both in control and kainic acid injected rats

Nairismagi, *Neuroimage, 30, 130*, **2006**

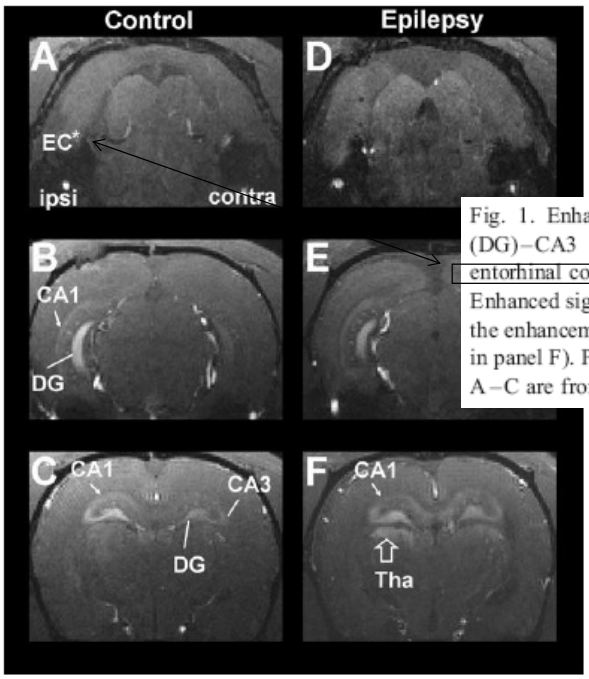
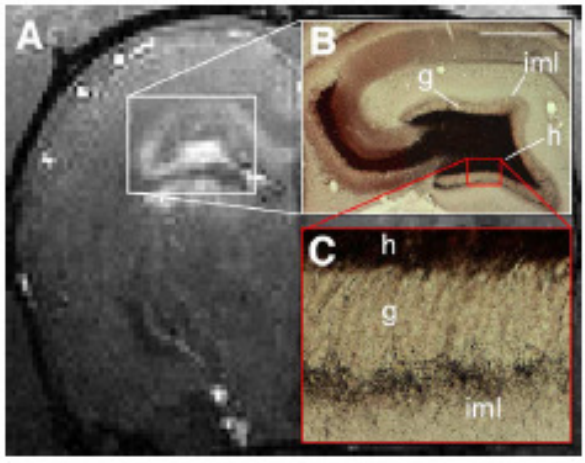


Fig. 1. Enhancement of the mossy fiber pathway in the dentate gyrus (DG)–CA3 region at day 3 after stereotactic $MnCl_2$ injection into the entorhinal cortex (EC). Asterisk indicates the location of the injection site. Enhanced signal is also present in the CA1 subfield (arrowhead). Note also the enhancement of the dorsal thalamus in the epileptic animal (open arrow in panel F). Panels are arranged from caudal (A, D) to rostral (C, F). Panels A–C are from a control rat and D–F from a KA-treated rat.

Nairismagi, Neuroimage, 30, 130, **2006**



A: ME MRI hyperintensity in the hippocampus of epileptic rat
B: corresponding silver staining (mossy fiber sprouting)
C: enlarged field of **inner molecular layer (iml)** showing sprouted mossy fibers
g: granule cell layer
h: hilus
1mm scale bar

Universiteit Antwerpen

www.elsevier.com/locate/ynimg
NeuroImage 30 (2006) 130–135

Manganese-enhanced magnetic resonance imaging of mossy fiber plasticity in vivo

Jaak Nairismägi,[a,b] Asla Pitkänen,[b,d] Susanna Narkilahti,[b] Joanna Huttunen,[c] Risto A. Kauppinen,[a,e] and Olli H.J. Gröhn[a,*]

[a]*Department of Biomedical NMR and National Bio-NMR Facility, Virtanen Institute for Molecular Sciences, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland*
[b]*Epilepsy Research Laboratory, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland*
[c]*Department of Cognitive Neurobiology, A. I. Virtanen Institute for Molecular Sciences, University of Kuopio, Finland*
[d]*Department of Neurology, Kuopio University Hospital, Kuopio, Finland*
[e]*School of Sport and Exercise Sciences, The University of Birmingham, Birmingham, United Kingdom*

www.elsevier.com/locate/ynimg
NeuroImage 40 (2008) 1718–1730

Manganese enhanced MRI detects mossy fiber sprouting rather than neurodegeneration, gliosis or seizure-activity in the epileptic rat hippocampus

Riikka J. Immonen,[a] Irina Kharatishvili,[b] Alejandra Sierra,[a] Christine Einula,[b] Asla Pitkänen,[b,c] and Olli H.J. Gröhn[a,*]

[a]*A.I. Virtanen Institute for Molecular Sciences, Biomedical NMR Research Group, Biomedical Imaging Unit, Department of Neurobiology, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland*
[b]*A.I. Virtanen Institute for Molecular Sciences, Epilepsy Research Group, Department of Neurobiology, University of Kuopio, PO Box 1627, FIN-70211 Kuopio, Finland*
[c]*Department of Neurology, Kuopio University Hospital, PO Box 1777, FIN-70211 Kuopio, Finland*

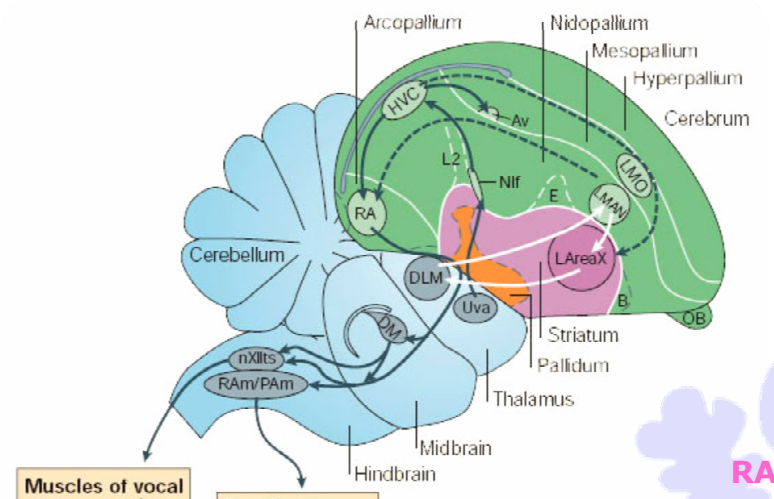
ALSO systemic MEMRI can reveal axonal sprouting

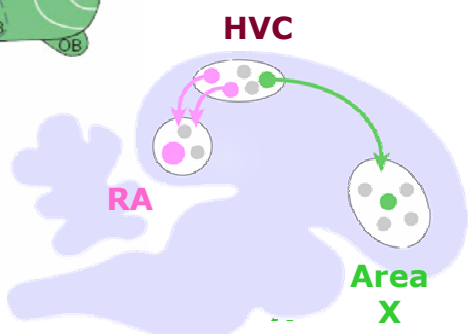
Universiteit Antwerpen

Remodelling of neuronal circuits

- Axonal plasticity
- MEMRI is a very useful method to examine network plasticity and regeneration in songbirds
- (review: **Van der Linden** et al, *NMRB,* 17, 602, **2004**)

Song control system



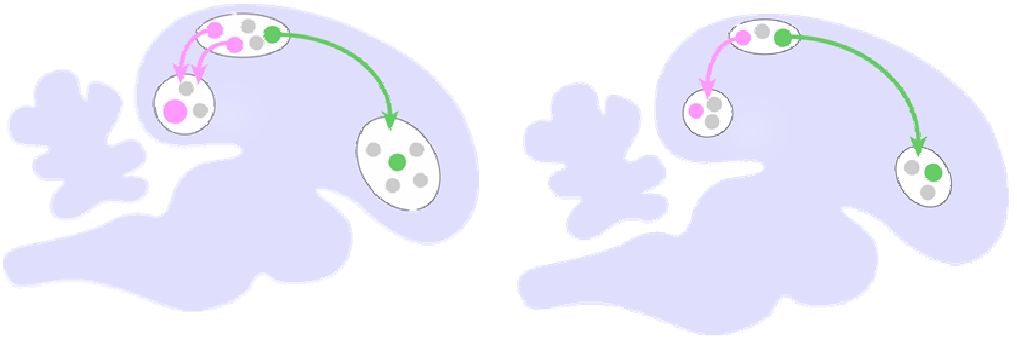


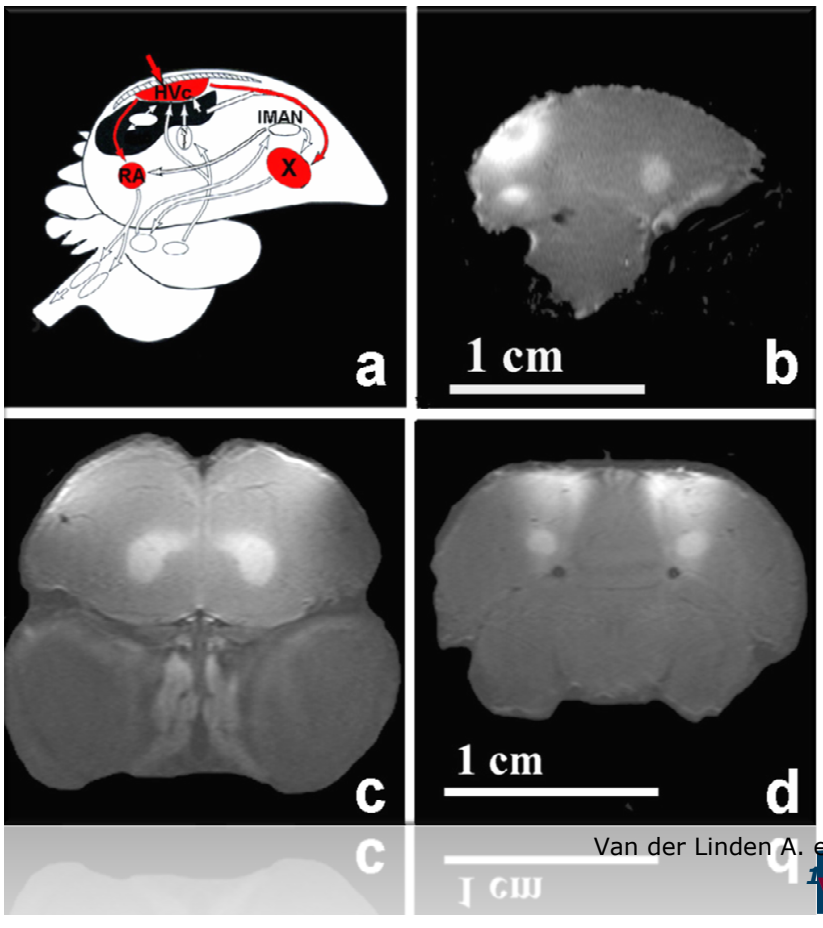
NEUROPLASTICITY in SCS

Delicate balance between adult neurogenesis and cell death, cell volume and cell density changes : volume changes in song control nuclei

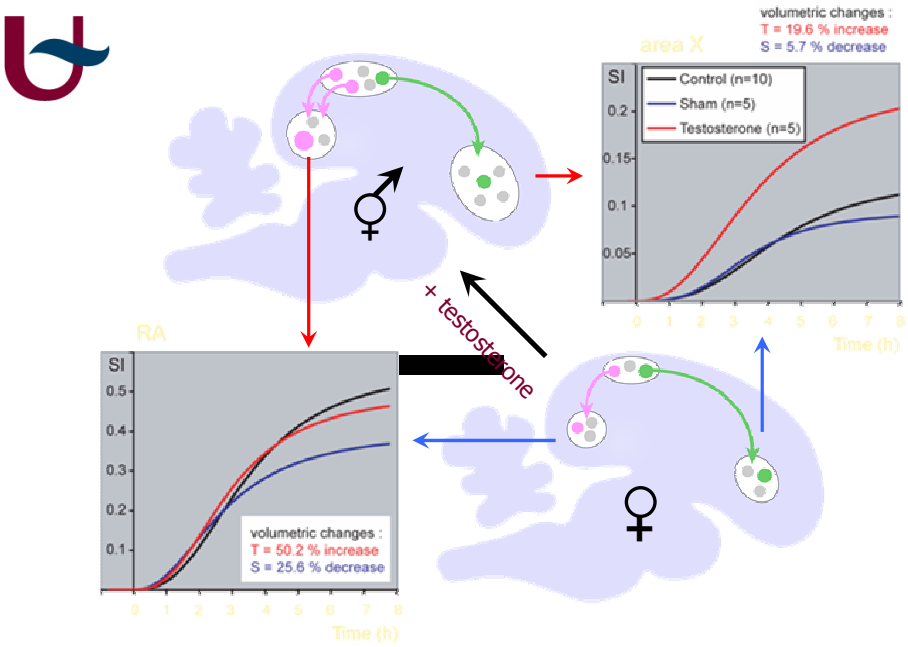
Creation of new axonal projections and dentrites: altered neuronal connectivity

Spring **Summer**





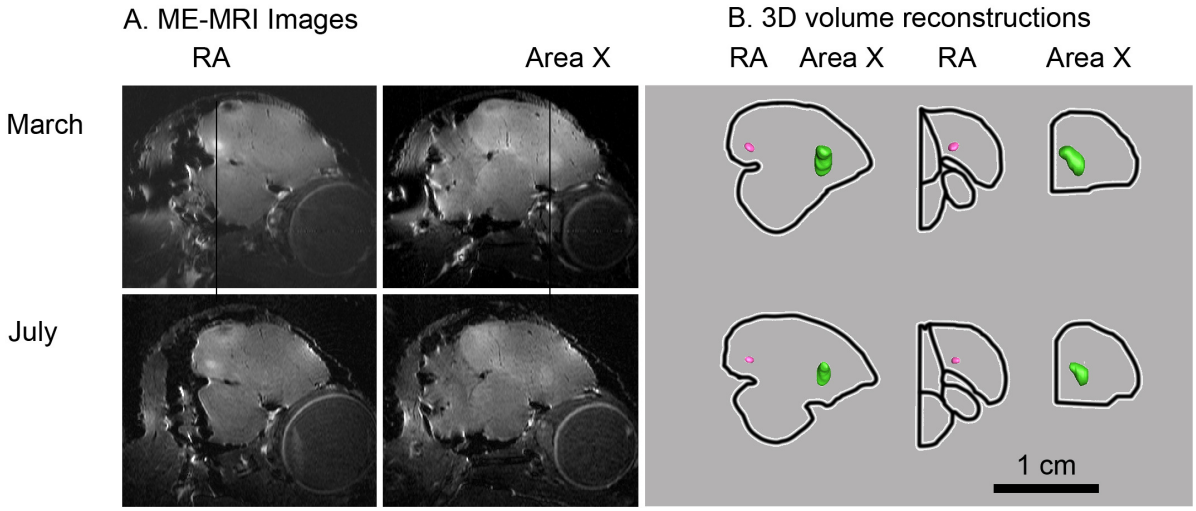
Van der Linden A. et al. (2002) ***Neuroscience, vol 1***



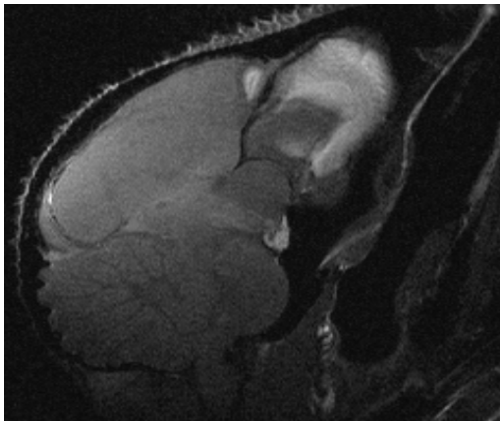
Female starlings implanted with Testosterone or placebo

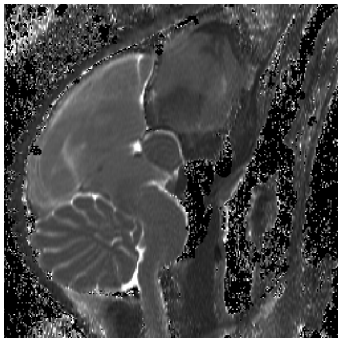
In vivo MRI of seasonal volumetric and functional plasticity of song control nuclei in relation to song output in female songbirds.

Van Meir V. et al., ***NeuroImage 2006, 31(3):981-992.***



Manganese uptake through the nostriles and transport to the OB (1 hour)
upon smelling milfoil or no particular smell
in different seasons





MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Manganese Transfer Index **(MTI)**

Magnetic Resonance in Medicine 60:169–175 (2008)

Assessing Transneuronal Dysfunction Utilizing Manganese-Enhanced MRI (MEMRI)

Faridis Serrano,[1] Mitchell Deshazer,[1] Karen D.B. Smith,[1] Jeyarama S. Ananta,[4] Lon J. Wilson,[4,5] and Robia G. Pautler[1–3*]

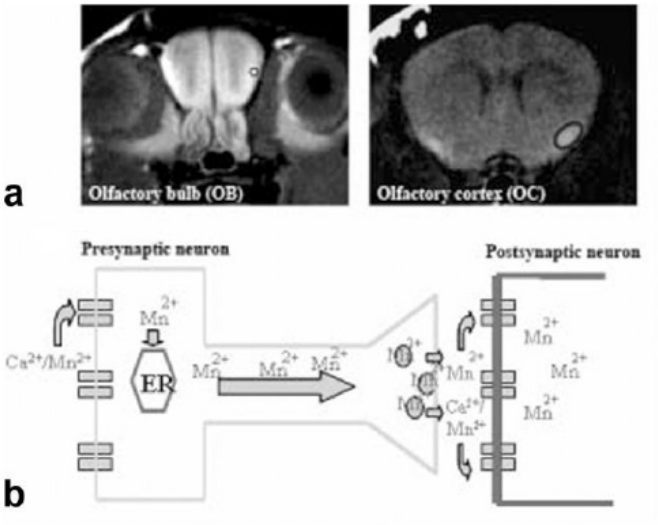
Transneuronal efficiency of manganese ion (Mn2) movement is quantified by the **manganese transfer index (MTI)** as a means to assess overall changes in neuronal function.

Tested with pharmacological agents (MTI decrease)
- Isoflurane: decreases synaptic vesicle release
- Memantine: decreases postsynaptic uptake of Ca2 and Mn2

Applied in knockout mice with neuronal dysfunction

Universiteit Antwerpen

Manganese Transfer Index MTI



$$\text{MTI} = \frac{\text{signal intensity (postsynaptic neuron, eg. OC)}}{\text{signal intensity (presynaptic neuron, eg. OB)}}$$

FIG. 1. **a:** The image demonstrates the region of interest (ROI) selected in the olfactory bulb (presynaptic) and olfactory cortex (postsynaptic). **b:** Cartoon explaining the concept of manganese transfer index (MTI). The MTI value assesses the transneuronal efficiency of Mn^{2+} ion and is defined as the ratio of the signal intensity in the postsynaptic neuron in relation to the signal intensity in the presynaptic neuron. MTI = signal intensity (postsynaptic neuron)/signal intensity (presynaptic neuron).

$MnCl_2$ intranasally via nasal lavage

Manganese Transfer Index **(MTI)**

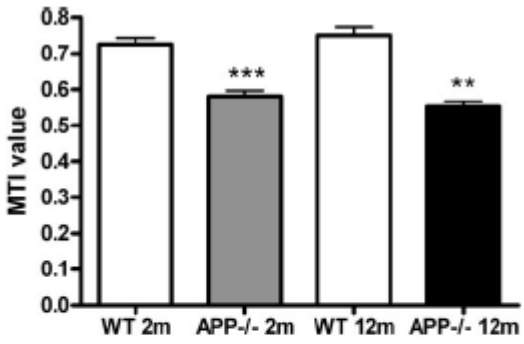


FIG. 4. Graph of the MTI value in age-matched 2 months wildtype (n = 7, white bar) and APP−/− mice (n = 6, black bar) and 12 months wildtype (n = 4, white bar) and age-matched APP−/− mice (n = 3, black bars). The data indicates that there is an age-independent decrease in the MTI value in the APP−/− mouse model, ***P = 0.0001; **P = 0.0016. Values represent an average in each group and their standard error (SEM).

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Axonal Transport Rates

Smith et al, NeuroImage 35, 1401 (2007)

Currently, there are NO OTHER methods available to measure in vivo axonal transport.

NeuroImage 35 (2007) 1401–1408

In vivo axonal transport rates decrease in a mouse model of Alzheimer's disease

Karen Dell Brown Smith,[a] Verena Kallhoff,[b] Hui Zheng,[b,c,e,f] and Robia G. Pautler[a,d,e,*]

[a]Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[b]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[c]Huffington Center on Aging, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[d]Department of Radiology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[e]Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA
[f]Department of Molecular and Cellular Biology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, USA

These data indicate that in vivo axonal transport rates decrease prior to plaque formation in the Tg2576 mouse model of AD.

Axonal Transport Rates

Smith et al, NeuroImage 35, 1401 (2007)

- The olfactory system of the mouse provides access to a well-defined white matter projection with minimal invasiveness to the animal.
- The olfactory system is targeted early in the time-course of AD making it an ideal target for monitoring disease progression
- a nasal lavage of MnCl2

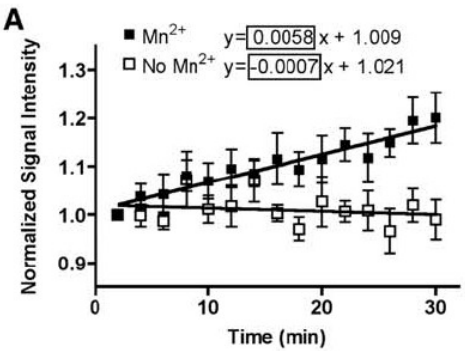
Axonal Transport Rates

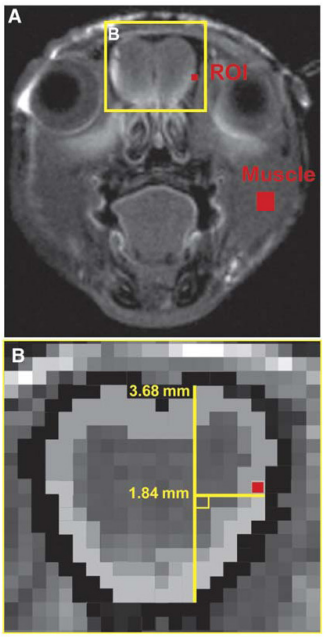
Smith et al, NeuroImage 35, 1401 (2007)

The differences between Mn2+ treated and control mice (no Mn2+) demonstrate the increased signal intensity acquired using MEMRI.

Data were quantified as a function of change in signal intensity (ΔSI) over time (min). Slope of line acquired through linear regression.

The slope is reflective of the rate of axonally transported Mn2+

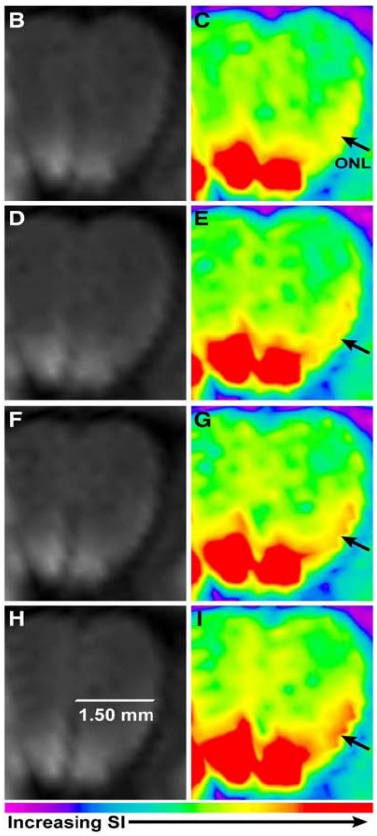




Universiteit Antwerpen

Axonal Transport Rates

Smith et al, NeuroImage 35, 1401 (2007)

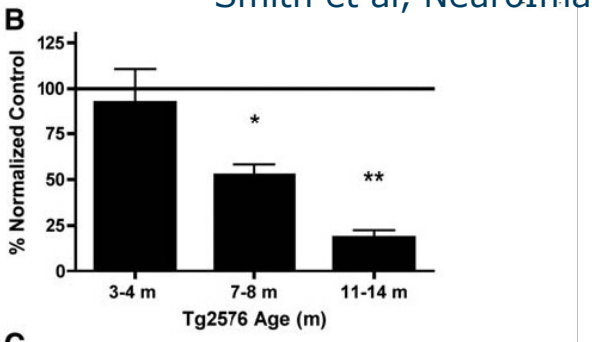


Demonstration of the movement of Mn2+ (red) through the olfactory bulb using sequential scans. (B, C) 2 min, (D, E) 12 min, (F, G) 22 min, (H, I) 32 min.

Arrow denotes region of interest located by finding the lengthwise midpoint of the olfactory bulb and extending That point out to the olfactory neuronal layer (ONL)

Axonal Transport Rates

Smith et al, NeuroImage 35, 1401 (2007)



C

WT	ΔSI/Time (min)	Tg2576	ΔSI/Time (min)	t-test
3-4 m	0.00797 ± 0.0019 N=4	3-4 m	0.00737 ± 0.0015 N=4	p>0.05
7-8 m	0.00676 ± 0.0013 N=6	7-8 m	0.00353 ± 0.0004 N=6	p<0.05 *
11-14 m	0.00975 ± 0.0023 N=4	11-14 m	0.00178 ± 0.0004 N=4	p<0.015 **

B. gradual and significant decrease with age in the axonal transport rate of the Tg2576 mutant as percent of control.

C. raw data for WT controls and the Tg2576 animals at the three different ages.

Axonal Transport Rates

Smith et al, NeuroImage 35, 1401 (2007)

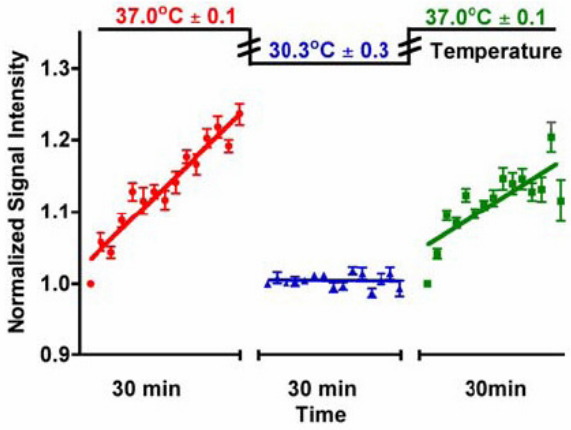


Fig. 3. Axonal transport is dependent upon body temperature. At 37.0 °C the SI increase in Mn^{2+} transport is ● 0.00679±0.001, n=10 vs. reduced temperature, 30.3 °C, ▲ −0.00131±0.002, n=10. It also shows that the transport rate recovers with a return to normal temperature (■ 0.00589±0.002, n=10). Difference in ΔSI/Time (min) between both 37 °C groups and the 30.3 °C group is significant (*) with a p-value of <0.01, df=29 (one-way ANOVA).

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

systemic injections of manganese: the least invasive approach

- It has been demonstrated in mice and rats that an intraperitoneal (i.p.), intravenous (i.v.) or subcutaneous (s.c.) injection of MnCl2 leads to unique MRI contrast revealing the **neuroarchitecture of the brain**

- Wadghiri, Y.Z., Blind, J.A., Duan, X., Moreno, C., Yu, X., Joyner, A.L., and Turnbull, D.H., Manganese-enhanced magnetic resonance imaging (MEMRI) of mouse brain development, *NMR Biomed.*, 17, 613, **2004**

Wadghiri et al NMRB, 17, 613, **2004**

MEMRI provides an efficient and powerful in vivo method
•for analyzing neonatal brain development
•in normal and genetically engineered mice

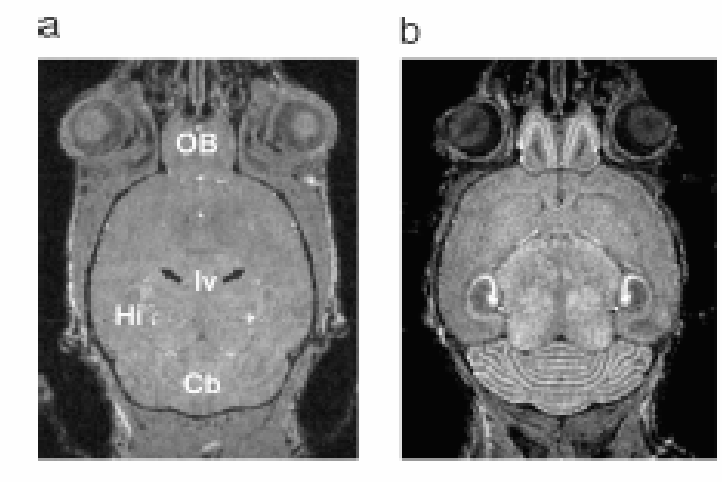
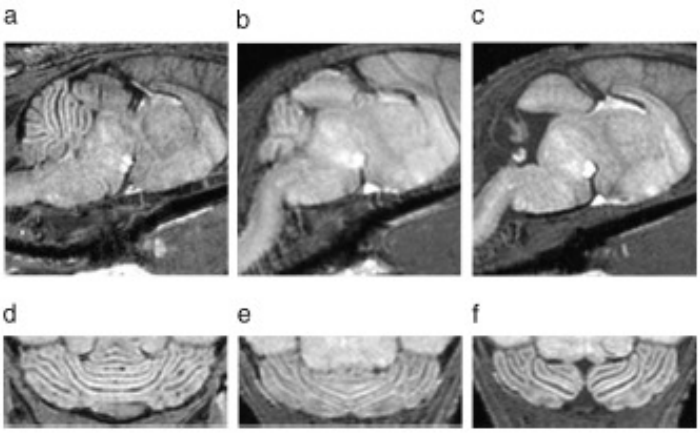
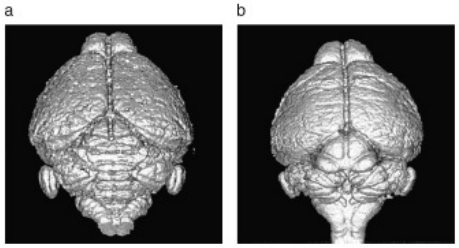


Figure 1. MEMRI enhancement is maximized 24 h after i.p. injection of $MnCl_2$. Horizontal T_1-weighted GE images before (a) and 24 h after (b) injection of $MnCl_2$ in an adult mouse brain show enhancement in olfactory bulb (OB), hippocampus (Hi) and cerebellum (Cb). Quantitative analysis

Wadghiri et al NMRB, 17, 613, **2004**





a: wild type
b: Gbx2-CKO mutant mouse

a-d: wild type
b-e: mild genotype Gbx2-CKO mutant mouse
c-f: Gbx2-CKO mutant mouse with severe deletion of vermis

systemic injections of manganese: the least invasive approach

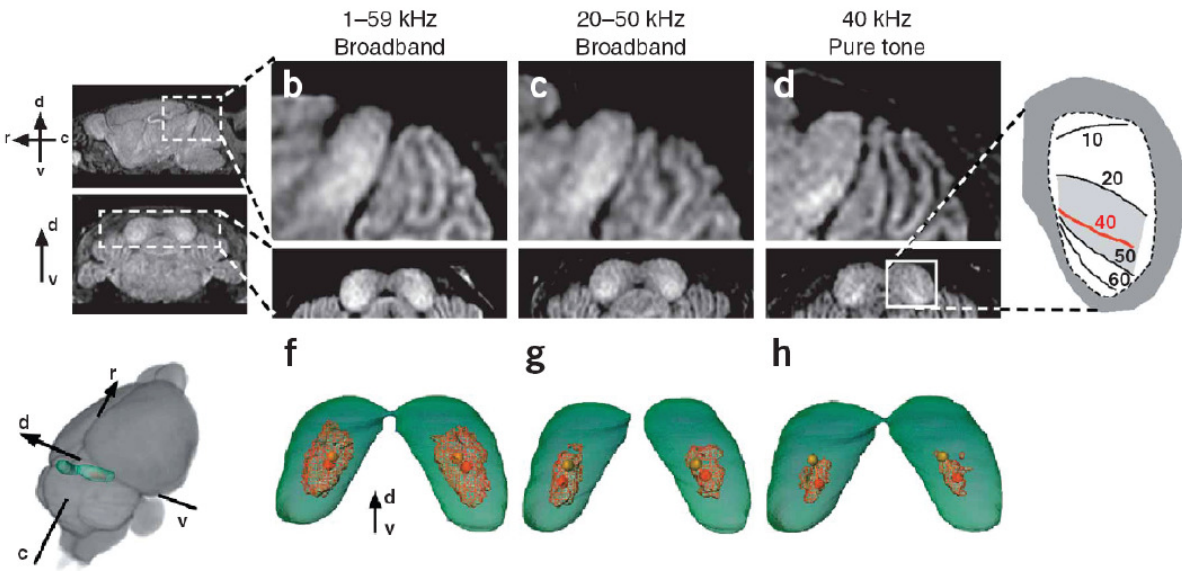
study neural substrate of awake behaviour

- Brain activation in ***awake*** **small animals can be monitored by performing MRI *after* the presumed activity has occurred preceded by a systemic injection of manganese**
- MEMRI becomes then quite homologue to histological discrimination of IEG expression (cfos) as it highlights areas with prior activity but probably harbours the same drawbacks in terms of specificity
- This method has been proven capable of providing a sensitive and effective method for mapping the mouse auditory brainstem
- Yu, X., Wadghiri, Y.Z., Sanes, D.H., and Turnbull, D.H., In vivo auditory brain mapping in mice with Mn-enhanced MRI, *Nat Neurosci*, 8, 961, 2005

Yu et al, *Nat Neurosci, 8, 961*, **2005**

- MEMRI for 100 micron resolution tonotopic mapping of the mouse inferior collilulus (IC)
- 21 days old mice whereby the IC showed obvious differences in mice exposed to defined stimuli
  - (b) After broadband (1-59 kHz) stimulation
  - (c) After high-frequency broadband (20-50 kHz) stimulation
  - (d) After 40 kHz pure-tone stimulation: enhancement was restricted to an isofrequency band in excellent agreement with electrophysiological maps
- **Intraperitoneal** administration of MnCl2 allowed longitudinal imaging starting even from early postnatal stages of mouse auditory brain development

Yu et al, Nat Neurosci, 8, 961, **2005**



An isofrequency band in excellent agreement with electrophysiological maps

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Study neural substrate of awake behavior with more invasive approach

Chen et al, *NeuroImage 37, 221* **(2007)**

ELSEVIER

www.elsevier.com/locate/ynimg
NeuroImage 37 (2007) 221–229

Imaging unconditioned fear response with manganese-enhanced MRI (MEMRI)

Wei Chen, Jeff Tenney, Praveen Kulkarni, and Jean A. King*

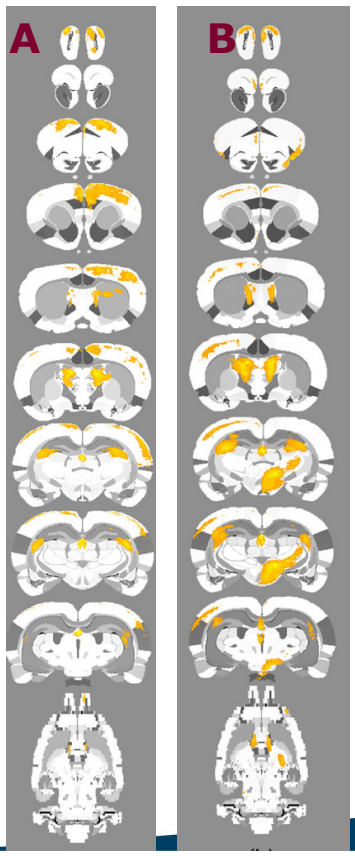
University of Massachusetts Medical School, Department of Psychiatry, Center for Comparative Neuroimaging, 55 Lake Avenue North, Worcester, MA 01655, USA

Study neural substrate of awake behavior with more invasive approach

Chen et al, *NeuroImage 37, 221* **(2007)**

- Animals trained to restraining in magnet
- rats catheterised in the femoral vein and the right common carotid artery (CCA)
- After surgery, animals were returned to home-cages awake for scent and Mannitol administration.
- Rats were infused in the femoral vein with 120 mM MnCl2 at a rate of ml/h for a total of 30 min in their home cage.
- after starting the infusion, a bolus of 20% D-mannitol was given into the right carotid artery at a concentration of 5 ml/kg via the prepared catheters.
- One minute after the mannitol injection, rats were exposed to either odorless air (control), lemon (novel/arousing) or TMT (fear-inducing stimulus) until the end of the 30 min infusion period
- After infusion awake restrained in MRI

Study neural substrate of awake behavior with more invasive approach



Chen et al,
NeuroImage 37, 221 **(2007)**

B. Fox (fear) smell activated the unconditional fear pathway: amygdala + hypothalamus

A. Lemon (novel) smell compared to fear-inducing odor demonstrated enhanced uptake in the cingulated and prefrontal cortices. In addition, as expected the hippocampus showed significantly enhanced manganese contrast after novelty exposure.

Neural substrate correlated with behaviour

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations**

Mn binding enzymes

Yang et al, *MRM 59, 1329* **(2006)**

Magnetic Resonance in Medicine 59:1329–1339 (2008)

Manganese-Enhanced MRI Detection of Neurodegeneration in Neonatal Hypoxic-Ischemic Cerebral Injury

Jian Yang,[1,2] Pek-Lan Khong,[3] Yanxin Wang,[3] Andrew Chi-Yuen Chu,[4] Shu-Leong Ho,[4] Pik-To Cheung,[5] and Ed X. Wu[1,2]*

Mn-enhanced MRI (MEMRI) for detecting neurodegenerative processes by monitoring enzymatic activities of Mn-superoxide dismutase (Mn-SOD) and glutamine synthetase (GS), which are Mn-binding enzymes against the oxidative stress and glutamate excitotoxicity in neurodegeneration

Mn binding enzymes
Yang et al, MRM 59, 1329 (2006)

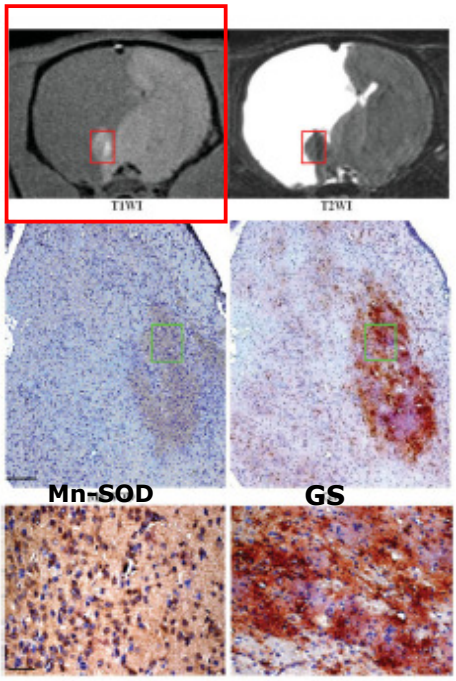


FIG. 8. Typical T_1WI and T_2WI at day 49 after H-I insult (late H-I phase) from an H-I rat in Group 1 (first row); corresponding Mn-SOD and GS staining at ×100 with scale bar = 100 μm (second row) and ×400 with scale bar = 50 μm (third row) in the ipsilateral basal ganglia area surrounding the cyst. Intensive Mn-SOD staining and strong GS staining spatially correlate with the hyperintensity in the T_1WI.

Mn-superoxide dismutase (Mn-SOD)
Glutamine Synthetase (GS)

Day 49 after Ischemic Insult

MEMRI
neuronal connectivity and activation

Activation-Induced Manganese-Dependent MRI (AIM)MRI
- **Dynamic AIM MRI: DAIM MRI**

Tract tracing with MEMRI
- **Tract tracing s.s.**
- **Activity dependent MEMRI**
- **Dynamic MEMRI: DMEMRI**
- **Neuronal Connectivity**
- **Remodelling of neuronal circuitries**
- **Manganese Transfer Index**
- **Axonal Transport Rates**

Study neural substrate of awake behavior
- **the least invasive approach**
- **the most invasive approach**

'Non Neuronal Activity' but 'Neuropathology Related' Mn uptake
- **Mn-binding enzymes**
- **Microglial activations and astrocytes**

- *Glial cells are non neuronal components of the CNS that interact closely with neurons and with each other*
- *There are 3 different types:*
  astrocytes, oligodendrocytes and microglial cells
- *They play an important role in neuroprotection, inflammation…*

Glial cells, particularly astrocytes represent a "sink" for brain manganese

- Contribute significantly to signal enhancements after manganese administration
  Unlike neurons, astrocytes have the ability to concentrate Mn2+ at levels 50-fold higher than the culture media
- Areas of high astrocyte density include the hypothalamus and hippocampus

  Areas with low astrocyte density include the cerebral cortex, neostriatum, midbrain, medulla oblongata, and cerebellum
- This could only partly explain the observed differential contrast enhancements in the brain after systemic injection

Microglial activations

Haapanen et al, *MRI 25, 1024* **(2007)**

ELSEVIER

Magnetic Resonance Imaging 25 (2007) 1024–1031

IMAGING

In vivo MRI reveals the dynamics of pathological changes in the brains of cathepsin D-deficient mice and correlates changes in manganese-enhanced MRI with microglial activation☆

Aleksi Haapanen[a], Usama Abo Ramadan[b], Taina Autti[c], Raimo Joensuu[d], Jaana Tyynelä[a,*]

[a]*Institute of Biomedicine/Biochemistry and Neuroscience Research Program, University of Helsinki, P.O. Box 63, FIN-00014 Helsinki, Finland*
[b]*Experimental MRI Laboratory, Department of Neurology, Helsinki University Central Hospital, FIN-00029 HUS, Helsinki, Finland*
[c]*Department of Radiology, Helsinki University Central Hospital, FIN-00029 HUS, Helsinki, Finland*
[d]*AstraZeneca R&D Mölndal, DECS-Imaging, S-43183 Mölndal, Sweden*

Haapanen et al, *MRI 25, 1024* **(2007)**

Neuropathologically, CTSD (Cathepsin D) deficient mice (CTSD_/_) are characterized by selective neuronal degeneration, gliosis and accumulation of autofluorescent proteinaceous storage material in neurons

MEMRI and histological stainings revealed that the hyperintense signal areas in MEMRI matched perfectly with areas of microglial activation in the brains of CTSD_/_ mice at the terminal disease stage

Universiteit Antwerpen

Haapanen et al, *MRI 25, 1024* **(2007)**

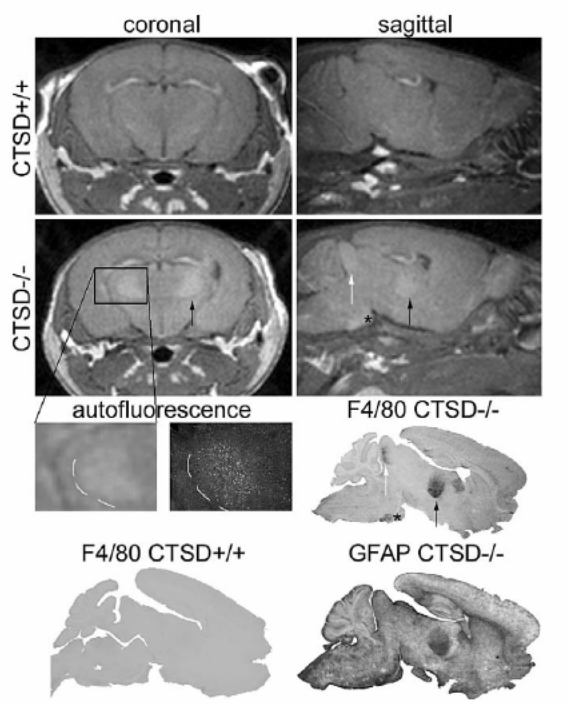


Fig. 2. Correlation between manganese-enhanced high-resolution T_1-weighted 3D images of CTSD−/− and CTSD+/+ mice (n=4) and glial activation at P25. Note the hyperintense areas in the thalamus (black arrow), inferior colliculus (white arrow) and olivary region (black asterisk) of the sagittal sections of CTSD−/− mice. Immunohistochemical staining of the corresponding brain slices using F4/80 antibody shows microglial activation in the same areas, while staining using GFAP antibody shows generalized astrocytosis in the brains of CTSD−/− mice. Higher magnification of the coronal MR image shows the thalamus in more detail, and the micrograph of the corresponding brain area, taken under ultraviolet light, shows the accumulation of autofluorescent storage material in the thalamus of CTSD−/− mice.

Accumulation of autofluorescent storage material

Immunohistochemical staining F4/80 for microglial activation

GFAP antibody for astrocytosis staining

Conclusion MEMRI in brain research

- the majority of reported MEMRI applications focuses on activity, connectivity and mapping of **somatosensory neuronal circuits**
- MEMRI harbours great potential for the study of **neuronal development, activity and plasticity** in different small animal models
- Only animal work: Manganese based contrast agents in the clinic (manganese dipyridoxal diphosphate (MnDPDP)) for liver imaging: Mn is **chelated** > loose all the advantages of the **ion** Mn2+ capacities for MRI.

Conclusion MEMRI in brain research

- ***in vivo* non invasive tool to link behaviour, performed in a non restricted environment and while awake, with its neuronal substrate**
- **behavioural phenotyping in neurodegenerative mice models > inserting MEMRI into protocols for phenotyping** the neural substrate of the observed modified behaviour

Literature

- book chapter :
  Molecular and Cellular MR Imaging, Edited by Michel M.J. Modo and Jeff W.M. Bulte, CRC Press. Chapter 20:
  Functional Cellular Imaging with Manganese by Vincent Van Meir and Annemie Van der Linden: p 369-392
- Book Chapter:
  Advances in Neurobiology
  Volume Title: Neural Metabolism In Vivo
  Editors: In-Young Choi, Ph.D. and Rolf Gruetter, Ph.D.
  Contrast agents, relaxation, in vivo calcium imaging by A. Van der Linden, V. Van Meir, D. Longo and S. Aime.
- Annemie.vanderlinden@ua.ac.be