

# Tracing neuronal pathways by MRI using WGA coated iron oxide nanoparticles

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## Introduction

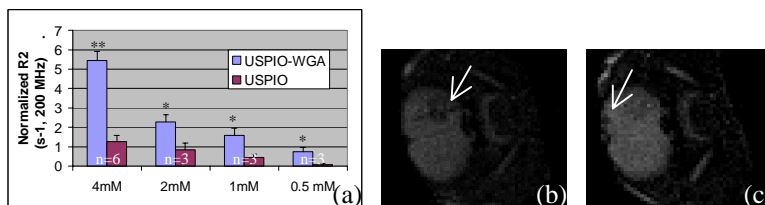
Neuronal tract tracing by Magnetic Resonance Imaging (MRI) is known to be possible with  $Mn^{2+}$  ions which are able to enter neurons through  $Ca^{2+}$  channels, and to be transported along the axon (1). An uptake of dextran coated iron oxide nanoparticles by brain neurons was shown (2). The lectin Wheat Germ Agglutinin (WGA) has a high affinity for axon terminals and is used in histology as a retrograde tracer of neuronal tracts (3, 4). Monocrystalline Iron Oxide Nanocompound was coupled to WGA (MION-WGA) and a neuronal uptake as well as slow axonal transport of this contrast agent were observed (5, 6). WGA-ferrite particles were also used to magnetically label rat fetal brain tissue before experimental brain transplantation (7). In this work, we study the *in vitro* internalization of WGA coupled ultra small particles of iron oxide (USPIO), the USPIO-g-WGA, by neurons and evaluate, in a preliminary experiment, the connectivity between two brain regions by MRI, using this tracer.

## Methods

USPIO-g-WGA were obtained by successive reactions of epichlorhydrin and WGA on the dextran coating. For *in vitro* experiments, neuron primary cultures were prepared from 18 days rat embryos brain. Briefly, embryos were removed from the ether sacrificed pregnant female. Their brain was extracted and placed in a calcium free buffer. Brain cells were mechanically separated by passing through sterile Pasteur pipettes of decreasing diameter. After 10 days of culture on 75 cm<sup>2</sup> poly-L-lysine coated plates in Neurobasal medium supplemented with B27, cells were scrapped and resuspended at 10<sup>7</sup> cells/ml in the wells of 96-wells tissue culture dishes for the incubation with a solution of contrast agent. After 30 min of incubation, cells were washed, transferred in PCR tubes, and finally resuspended in 100  $\mu$ l of 2% gelatin for MR imaging. For the *in vivo* protocol, a male NMRI mouse was anaesthetised with Nembutal and placed in a stereotaxic frame. The animal was injected with 1  $\mu$ l USPIO-g-WGA (9.73 mM Fe) in the right striatum, using a 20  $\mu$ m-tipped glass pipette. MRI was performed in a 4.7 T magnet with a Bruker Avance 200 spectrometer. T<sub>2</sub>-weighted MR images (*in vitro*: 2mm slice, TR=3000ms, TE=15ms, 24 echoes, NA=2, matrix = 256 x 256, FOV = 4cm, *in vivo*: 2mm slice, TR=2000ms, TE=30ms, 8 echoes, NA=2, matrix size = 128 x 128, FOV = 3.5cm) were acquired. PARAVISION software was used to measure T<sub>2</sub> values on the MR images.

## Results

R<sub>2</sub> measurements (Fig. 1a) performed on T<sub>2</sub>-weighted MR image of PCR tubes containing neuronal cells incubated with various concentrations of USPIO-g-WGA and USPIO (from 0.5 to 4 mM Fe) show a greater R<sub>2</sub> enhancement of cells incubated with USPIO-g-WGA. *In vivo*, the injection site of USPIO-g-WGA can be seen on the T<sub>2</sub>-weighted MR image acquired immediately after administration in the brain striatum (Fig. 1b). 5 days later, the initial dark spot has nearly disappeared and is now found in the brain cortex (Fig. 1c).



**Fig. 1:** R<sub>2</sub> measurements on cell tubes MR images at 200 MHz (a) (\*:p<0.05, \*\*:p<0.01), and T<sub>2</sub>-weighted MR images (echo: 60 ms) of a mouse brain acquired immediately after (b) and 5 days after (c) administration of USPIO-g-WGA (arrows indicate the injection site (b) and the site of migration of USPIO-g-WGA (c)).

## Discussion and conclusions

WGA has a high affinity for axon terminals and interacts with N-acetylglucosamine (3, 4). In this work, we have shown that our USPIO-g-WGA allows for a magnetic labeling of neuron primary cultures *in vitro*, and a subsequent T<sub>2</sub> effect detectable in MRI. WGA is also known to be transported along the axon of neurons. The first *in vivo* results show that USPIO-g-WGA can be transported along the cortico-striatal pathway, and confirms some findings of slow axonal transport (1-7 mm/day) reported after intraneural injection of MION-WGA in facial or sciatic nerve (5, 6).

## Acknowledgements

Many thanks to Mrs Marlène Genlain and to Dr Guy Cheron for their precious help. This work was financially supported by the PAI program of the Federal State of Belgium (phase V, P5/04).

## References

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