# Validation of multi-fibre probabilistic tractography of corticothalamic projections in the Göttingen minipig brain

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**Introduction**

The powerful neuron tract-tracing methods successfully used for studying connections in animal brains can evidently not be applied to humans due to their invasive nature. Instead diffusion MRI tractography may provide a method by which it is possible to study brain connectivity non-invasively, and the method may be pivotal for the understanding of brain regions, both in physiological and in pathological conditions. However, the accuracy of tractography has not been exhaustively validated against independent anatomical data. In the present study, tractography was assessed as a non-invasive method for studying connections in the porcine brain. Distribution patterns of corticothalamic projections were obtained using multifibre probabilistic tractography and compared with the corresponding projection patterns obtained using manganese-enhanced magnetic resonance imaging (Mn-MRI) and standard immunohistochemical visualization of a histological tracer.

**Method**

A baseline, high resolution 3D T1-weighted MPRAGE was obtained in five young Göttingen minipigs on a 3T Siemens Trio scanner, voxel size: 0.6x0.6x0.6 mm$^3$. The histological tracer, biotinylated dextram amine (BDA), and manganese (MnCl) were then stereotaxically injected into the prefrontal cortex (PFC) and uptake and transportation of Mn$^{2+}$ were visualized in vivo on day two using MRI. Following an additional 12 day survival period, the brains were perfusion fixated and the histological tracer was detected using immunohistochemical staining techniques.

High-resolution diffusion weighted images were obtained from the perfusion fixed brains on an experimental 4.7T Varian Inova scanner on which sequence parameters had been optimized to increase the sensitivity for crossing fibres (Alexander et al., 2002). The available post mortem data offered unique opportunities for optimizing scanning parameters, and well known physiological sources of data corruption such as motion were excluded. To achieve a full volume coverage the total number of slices was scanned in an interleaved fashion over two sessions (2x36 slices) and two repeats ensured a signal-to-noise ratio above 23. Final DTI parameters were: TE: 80.1 ms; TR: 5000 ms; matrix: 128x128; voxel size: 0.63x0.63x0.63 mm$^3$; delta: 16.1ms; DELTA: 58.4ms; b: 4090 s/mm$^2$; 61 directions; FOV: 80x80 mm. Tractography was performed using multifibre probalistic methods based on a mixture model of up to two fibre populations, which has advantages over single tensor representations, allowing more accurate definition of the routes and termini of connections (Parker and Alexander, 2003).

**Results**

Manganese tracing provided dense labelling of the main projection sites within a two day period. A good correlation was observed with the histological tracer when comparing the labelling of projections to the thalamic mediodorsal (MD) nucleus. In vivo tract tracing using manganese also revealed significant contralateral projections to the MD nucleus, high levels of corticocortical connectivity through the genu of the corpus callosum and indicated some of the segregated circuits that unite the frontal cortex, the thalamus and the basal ganglia. A good correspondence was observed when comparing the fibre pathways visualized using manganese with the results of probabilistic tracking (fig1). In accordance with the Mn-MRI labelled tracts, multi-fibre tractography demonstrated pathways from the PFC towards the internal capsule, the ventral striatum and the ventral pallidum. Further projections are then segregated into at least two major tracks. One bundle of fibres is routed through the rostral aspect of the thalamus. Another fibre bundle is directed toward the substantia nigra (SN) and midbrain tegmentum. However, due to the intra-voxel orientational heterogeneity in central gray structures a complete trajectory to the final projection site in the mediodorsal thalamic nucleus or the SN was not acquired.

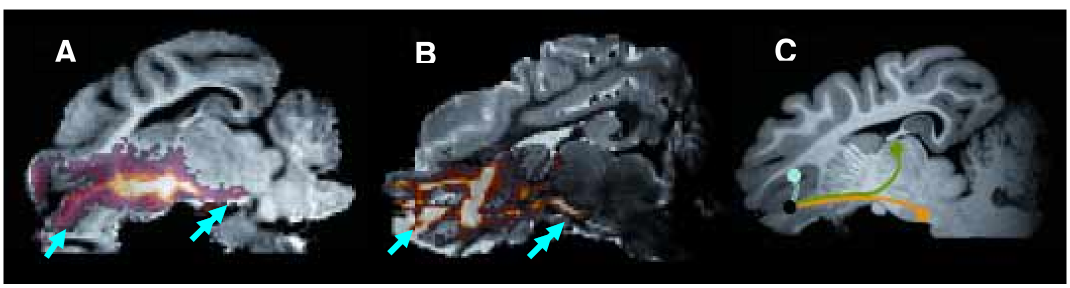


Fig.1: (A) Mn-MRI enhanced tract from medial PFC (arrow) towards basal ganglia (double arrow). (B) Probabilistic tract from seed point (arrow) medial PFC towards basal ganglia (double arrow). (C) Several circuits are revealed from both in-vivo Mn-MRI and in situ tractography; contralateral corticocortical connections (cyan), cortico-substantia nigra connections (yellow) and corticothalamic connections (green).

**Conclusion**

Using the porcine brain as a complex gyrated animal model, we demonstrate a high correspondence between traditional neuronal tract tracing techniques, manganese enhanced MRI and diffusion MRI tractography. Based on these results, tractography may be considered as a promising method for studying anatomical brain connectivity in vivo and non-invasively in the human brain. However, even though tractography is performed on an optimized in situ environment excluding predictable in vivo data corruption sources such as motion, physiological noise and geometric distortions, the use of tractography is limited when studying projections crossing basal grey matter structures with low anisotropy.

**References**

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