Title: Novel Gd-Based Neuroanatomical Tract-Tracers for Optical and Magnetic Resonance Imaging

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The advance of axonal tract-tracing has revolutionized neuroscience in the past three decades[1]. The elementary purpose of neuronal tract-tracing is to chart anatomical connections within the nervous system providing useful information on afferent and efferent connectivity in the brain. Biocytin is a classical neuroanatomical tract-tracer that is taken up by neurons and transported in both antero- and retrograde directions. Our aim was to develop non-toxic, efficient neuronal tracers that allow both, *in vivo* brain connectivity studies by means of MRI and postmortem-microscopic investigation in fixed tissue, in the same experimental animal.
We have designed five novel biocytin-based neuroanatomical tract-tracers (L_1-L_5). In newly modified-biocytin (L_1), propylamine is linked to amide of biocytin to make it *tert*-amide, which is stable to biotinidase degradation. The propylamine of $L_1$was used as a linker to link, FITC as a fluorescent moiety (L_2) or Gd-DOTA as MR detectable part (L_3). L_4 has an amide linkage between amine of GdDO3A-EA and carboxylate of biocytin while L_5 consists of a novel precursor based on serine containing Gd-DO3A. This precursor has an amine and a carboxylate group available for coupling of biotin and *l*-lysine.
In vivo histological experiments with L_1 demonstrated an increased molecular stability compared with biocytin and excellent neuronal tract-tracing capabilities. *In vitro* efficiency of cell internalization of L_2 into N18 neuroblastoma cells was demonstrated by fluorescence microscopy. *In vitro* MRI of L_3-L_5 with increasing concentrations of avidin were performed at 7T. The increase in R_2 for L_3-L_5 (300%-100% respectively) demonstrated strong binding of all tracers in the pocket of tetrameric avidin through biotin.
The above studies and preliminary results reveal a new strategy for neuroanatomical tract-tracing, which combines the powerful spatial resolution of the conventional microscopic techniques with the whole brain tri-dimensional coverage and *in vivo* applicability of MRI.

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