# Identification of Neural Stem Cells in Rat Brain Using Their Specific Magnetic Resonance Spectroscopy Signature

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**Abstract:**The objective of this study was to identify neural stem cells (NSC) in the living rodent brain based on their specific signature in microMRI (μMRI) spectroscopy. Using singular value decomposition (SVD) analysis to filter the spectra, we revealed NSC spectral peak from both the endogenous NSC, localized in the dentate gyrus, and exogenous NSC, implanted into the rat cortex. This study has significant consequences for the future utilization of the human MRI spectroscopy for the identification of NSC in a variety of neurological disorders.

**Introduction:**

Currently, there are no clinical, high-resolution imaging techniques that enable investigations of the survival, migration, fate, and function of unlabeled NSC and their progeny. Over the past few years, molecular and cellular MR imaging has emerged as a new field for visualization of macromolecules and cells in the living organisms, and has been attempted for identification and tracking of NSC.[1] Among several paramagnetic reagents, superparamagnetic iron oxide (SPIO) particles have been used in both molecular and cellular MR imaging.[2] Dual photosensitive and magnetic markers such as gadolinium rhodamine dextran (GRID) have also been used to monitor implanted stem cells *in vivo* and *ex vivo*.[3,4] However, exogenous markers become diluted as cells proliferate and differentiate, with resulting loss of contrast as the marker concentration diminishes. Moreover, exogenous markers cannot be used to identify endogenous populations of NSC. Our work has focused on a non-invasive method to identify both endogenous and exogenous NSC using brain MR spectroscopy. Using NMR spectroscopy, we have identified a NSC specific signal at a chemical shift of 1.28 ppm.[5] This peak is well resolved from the metabolite peaks such as N-acetyl compounds (2.02 ppm), Creatine (3.03 ppm), Choline (3.22 ppm), and Myoinositol (3.52 ppm), which are present in neural tissue. More importantly, this peak is not found in purified cultures of isolated neurons, astrocytes, and oligodendrocytes, respectively, distinguishing the 1.28 ppm metabolite as unique to NSC. Herein, we present our data on identification of NSC in the living rat brain, using μMRI spectroscopy.

**Methods:**

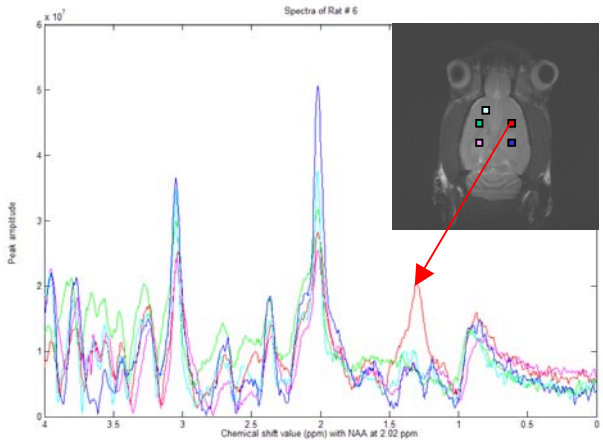
**NSC culture and transplantation:** Embryonic brains (day 12) of C57Bl6 mice were digested in 2mg/mL collagenase for 2 hrs at 37°C. Cells were filtered through a 40 μm filter three times and plated at 50,000 cells/ml on methylcellulose-coated 6-well plates, in the Neurocult Basal Media with 10% Proliferation supplement (Stem Cell Technologies). To enable proliferation of NSC, growth factors (EGF, FGF-2, 20 ng/mL) were added every two days, as described.[5] Neurospheres were collected after 14 days *in vitro* and trypsinized to single cells. They were resuspended in the PBS, pH 7.25, just before transplantation. Five million NSC were injected transcranially into the cortex of an adult Sprague-Dawley rat (stereotactic coordinates X:Y:Z=3:1:1.5mm from bregma) in 5 μl of PBS. As a control, the contralateral cortex was injected with 5 μl of PBS only. Rats were scanned within the 6 hrs following transplantation.

**Spectroscopy**: A 400 MHz Bruker Avance small animal MRI system (Bruker Biospin MRI, Inc.) was used to acquire spectra. Rats were anesthetized with an intraperitoneal injection of Nembutal (40 mg/kg) and allowed to breathe spontaneously. If necessary, anesthesia was maintained with isoflurane (1- 2 %). Body temperature and respirations were monitored by a small animal monitoring and gaiting system (SA Instruments, Inc.) Each rat was immobilized supine with its head resting on of a 3 cm diameter surface coil. $T_2$ weighted anatomical images were acquired to identify the hippocampus (site of endogenous NSC) and injection sites (exogenous NSC). The PRESS pulse sequence (TE/TR = 8/2000 ms) was used with six CHESS pulses for inner and outer volume solvent suppression. After shimming over the selected voxel, spectra were collected with a spectral width of 16 ppm, 2048 data points and up to 1024 averages over a maximum of 54 minutes. Voxel volumes of 2.5, 3 and 4 $mm^3$ were used to interrogate the injection sites.

**Analysis:** The data were processed by a method that is based on singular value decomposition (SVD). First, the modes of the water signal were estimated from the raw data followed by reconstruction of the water signal and its removal from the data. Afterwards, the periodogram of these data was used for calibration by using the position of the NAA peak (set at 2.02 ppm) as a standard. The SVD method was then repeatedly implemented on data from selected frequency bands to estimate the NAA, Cr, and NSC quantities.

**Results:**

**Figure 1**: Five spectra showing the metabolite signals from five locations in the rat brain. The color key is as follows: red – stem cell injection site, green – saline injection site, cyan – frontal cortex, magenta – left side hippocampus 2.5 $mm^3$ voxel, blue – right side hippocampus 5 $mm^3$ voxel. Using our μMRI spectroscopy protocol, we have reliably detected major metabolites in the brain tissue, such as NAA, Choline and Creatine (Fig.1). In addition, we can clearly detect the NSC peak within both




hippocampus, where NSC normally reside, and the injection site, where exogenous NSC are localized. The signal from NSC occurs between 1.27 and 1.30 ppm in spectra where the SNR is 30 or greater. Control cortical sites, where NSC do not reside, do not have the NSC peak. There is a clear difference in the peak amplitude when endogenous $0.2x10^6$ NSC are compared with exogenous $5x10^6$ NSC, demonstrating that our μMRI methodology can discern different quantities of NSC.

**Discussion:**

This is the first study that demonstrates our ability to visualize NSC in the living rat brain, using μMRI spectroscopy. We have been able to reliably and repetitively detect both endogenous and exogenous NSC (N=5). Further clarification of the specificity of the NSC peak as well as correlation of the peak amplitude to the number of endogenous and exogenous cells is currently in progress. This technique indicates the enormous possibility of tracking the survival, distribution and differentiation of NSC in the human brain.

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