FMRI and florescence imaging of neuronal networks and local anatomy of the regenerated sciatic nerve in rats

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Introduction:

Neurons in the peripheral nervous system, in contrast to neurons in the central nervous system have a unique ability to regenerate following nerve trauma. Discovering the special characteristics and the mechanisms underlying peripheral nerve regeneration, will contribute in developing new therapies for treating peripheral and central nerves trauma. Although many techniques are available today for evaluating the architectural changes that regenerated neurons exhibit (1,2), determining the regenerated neuron's functional properties *in vivo,* in a non-invasive manner, still remains a challenge.

We have applied the sciatic nerve crush model in rats, and implemented a multi-modal imaging approach to assess nerve regeneration that included: (i) functional MRI for assessing non-invasively the functional level of the regenerated sciatic nerve *in vivo* (ii) optical imaging *in vivo* for evaluating the rate of nerve regeneration, and (iii) histological methods in order to correlate the functional findings with changes the nerves undergo at the cellular level.

Methods:

Sciatic nerve crush: Sprague-Dawley Rats (70-90 g**,** 4 weeks old) were anesthetized with 2% Isoflurane, and the right sciatic nerve (which innervates more then 85% of the hindpaw) was exposed and was crushed with 0.8 mm tip forceps for 20 seconds (n=12). For optical imaging studies another group of rats (n=6) underwent the same procedure as described above. The crush location was marked with a fine suture. Two µl of the lipophilic membrane dye (DiO, 5%) was injected into the nerve bundle right above the crush site. The rats' skin was sutured and the rats were let to recover at least 3 days prior to MR or optical imaging. Rats were fed a special soy diet which has been shown previously to reduce post-operative pain, and were treated with pain analgesics for a week following the surgeries. In addition, rats were placed in an enriched environment for three hours, four times a week. After completing the MR imaging procedures, the sciatic nerve proximal to the crush location was removed for histology. Staining for myelin (LFB), axons (silver stain) and cell morphology (H&E) were performed on 10 µm coronal sciatic nerve sections.

Animal preparation for functional MRI: Rats were initially anesthetized with 2% Isoflurane during the surgical procedures. Rats were orally intubated and placed on a mechanical ventilator throughout the surgery of catheterizing the artery and the femoral vein, and the fMRI experiment. Two short stimulation electrodes were inserted in each hindpaw. After surgery, anesthesia was maintained with a constant α-chloralose infusion. Each animal was secured in a head holder with ear bars and a bite bar to prevent head motion and was strapped to a plastic cradle. End-tidal CO_2, rectal temperature, tidal pressure of ventilation, heart rate, and arterial blood pressure were continuously monitored during the experiment.

Image Acquisition: All images were acquired with an 11.7 T / 31 cm horizontal bore magnet (Magnex), interfaced to an AVANCE console (Bruker) and equipped with a 9-cm gradient set, capable of providing 64 G/cm with a rise time of 120 µs. A 2 cm diameter surface coil that was attached to a head holder was used to transmit and receive the MR signal. A single-shot, spin-echo EPI sequence with a 64 × 64 matrix was run with the following parameters: effective echo time (TE), 30 ms; repetition time (TR), 1.0-1.5 sec; bandwidth, 200 kHz; and a field of view, 2.64 × 2.64 cm. Brain coverage was obtained with 5, 2-mm thick slices, spaced 0.2 mm apart (4). 190 images were acquired for a total experiment time of 4 min (3).

Somatosensory Stimulation Paradigm: A World Precision Instruments stimulator supplied 2 mA, 300 µs pulses repeated at 3 Hz to both forepaws upon demand. The paradigm consists of 40 scans during rest and 10 scans during hindpaw stimulation.

Data Analysis: Analysis of the fMRI time series was performed using STIMULATE. A correlation coefficient was calculated from cross-correlation of the unfiltered time series with a boxcar waveform representing the stimulation period. The activation threshold was set at 0.20-0.4, and only groups that include at least four activated pixels were considered significant.

Optical imaging: Detection of the transport of the membrane dye within the regenerated nerve was executed using confocal fiber optic based fluorescence microscopy (Cell-Vizio, Mauna Kea Technologies) at different time points following the sciatic nerve crush. Rats were anesthetized with 2% Isoflurane, and a 1.8 mm diameter fiber optic with a spatial resolution of 2.5 µm and field of view of 240 µm x 170 µm was placed on the exposed sciatic nerve *in vivo*. The rate of the membrane dye transport within the regenerated nerve was evaluated with respect to the initial marked crush site.

Results and Discussion:

Histological staining for myelin, axons and cell morphology confirmed the degeneration process that the rats initially exhibit 3-7 days after the crush procedure, and the regeneration process that occurred in the rats at different time points (Figure 1d).

Due to the crush procedure, the membrane dye that was injected above this location could not diffuse distally towards the paw within the neurons' membrane. However, optical imaging of the crushed sciatic nerve at different time points following the crush procedure, demonstrated that the dye was transported distally at a rate of 1-1.5 mm/day, which agrees with previous studies (Figure 1c).

Rats that were imaged 3 to 7 days after the crush procedure exhibit normal somatosensory (SI) cortical BOLD activation in response to sensory stimulation of the healthy limb, whereas no fMRI signal was detected in response to sensory stimulation of the traumatized limb. FMRI preformed 15 to 30 days after the crush procedure demonstrated return of SI cortical activity in response to sensory stimulation of the traumatized nerve. However, the average number of pixels activating in response to the regenerated limb sensory stimulation was significantly lower (by 50%) compared to the number of pixels activating in response to stimulation of the healthy limb (Figure 1a,b).

In conclusion, in agreement with previous studies, two weeks after traumatizing the sciatic nerve, the rats exhibit close to full regeneration, as was detected by the optical imaging and the histological methods that were carried out. However, the fMRI preformed in different time points in the regeneration process demonstrated that although architectural changes might confirm the presence of neuronal regeneration, the regenerated neurons do not exhibit full functional recovery at these time points. These findings suggest that fMRI is sensitive enough to detect the functional characteristics of the regenerated neurons *in vivo* and offer complementary information to anatomical assessment.

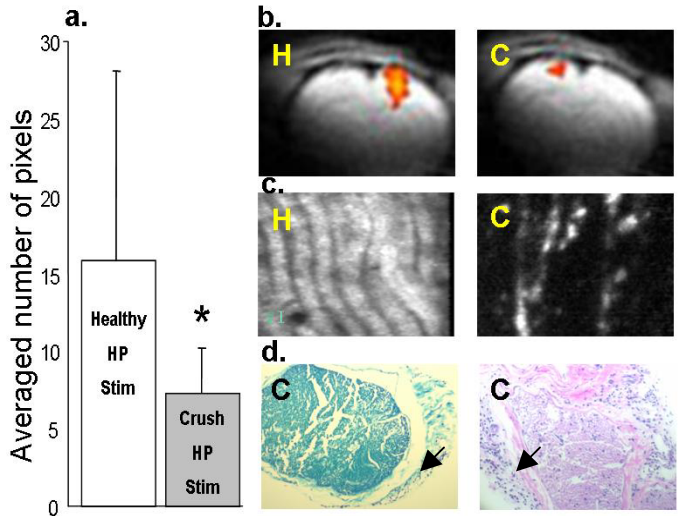

Figure 1. a. Averaged number of pixels (+ SD) activated above 0.2 cross-correlation threshold following healthy hindpaw (HP) and crushed HP for rats that were imaged 15-30 days following crushed procedure. P value (<0.05) was determined according to Two-Tail student T-test performed between groups.
b. Representative BOLD cross correlation (>0.2) activation maps from one representative rat overlaid on an EPI spin echo image are shown. Sensory stimulation of the healthy (H) hindpaw resulted with normal SI cortical activation, whereas sensory stimulation of the crushed HP (C, 15 days following crush) resulted with much smaller SI activation.
c. Optical fiber microscopy images showing the axons in the healthy (H) sciatic nerve, and the axonal growth (5 days following crush) in the crushed (C) nerve while it regenerates (FOV=240X170, 2.5 µm resolution).
d. Histology staining performed on coronal sciatic nerve distal to the crushed site 5 days (right) and 15 days (left) following crush. In both morphology (right) and myelin (left) staining, immune systems cells are present (black arrows), which indicate wallerian degeneration processes. Where morphology shows degeneration 5 days after crush, myelin shows close to full mylenation (regeneration) 15 days following crush.

References:

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