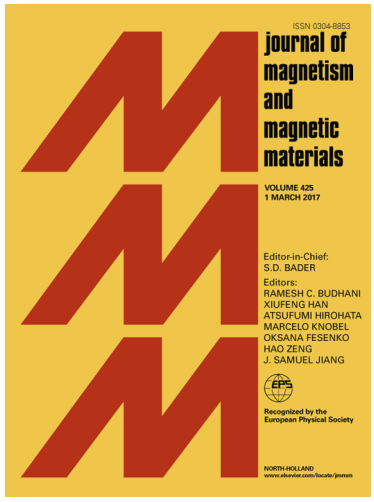

Magnetic drilling enhances intra-nasal transport of particles into rodent brain

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Abstract:

Getting drugs deep into the brain to treat cancers, neurological disease, and behavioral disorders is challenging. In this work, we tried to improve the efficiency of intra-nasal transport into the brain via the cribriform plate using magnetic particles. We and others have used magnetic particles for delivering heat, drugs, and genes. We performed experiments with mouse cadavers that received 250-nm-wide intra-nasal magnetic rods intra-nasally under different combinations of magnetic fields. We found that the application of helical dynamic gradients to the particles (i.e., both rotational and linear) improved transport from the nose into the brain, as compared to linear magnetic gradients alone. On histological examination, no tracks were observed to suggest significant damage to the brain during the transport process. We are currently building a system for testing with live animals, with eventual proposed application to humans.

Keywords:

Nasal delivery; Magnetic drug delivery; Magnetic rods; Brain.

Introduction:

In normal circumstances, the blood-brain barrier (BBB) provides natural protection to the central nervous system (CNS) against noxious substances found in the body's circulatory system. When the introduction of exogenous material into the brain is desired (e.g. for therapeutic applications), the BBB prevents 98% of small-molecules and an even greater percentage of large molecules from

reaching intended targets [1]. Current strategies for crossing the BBB involve molecular 'Trojan horse' delivery systems [2,3], disruption of the BBB through energy deposition (radio-frequency [4,5], ultrasound [6,7]). Drugs can be placed into the cerebrospinal system (e.g. via lumbar or ventricular injection) or directly into the brain parenchyma and thereby bypass the BBB, but such injections must be done expertly to avoid long-term neuronal damage [8]. An alternative strategy for delivering drugs to the brain is to administer them intra-nasally. The brain has natural projections into the nose, for example, olfactory nerves which penetrate pores in the cribriform plate of the ethmoid bone [1]. Intra-nasally-administered drugs have been used to relieve migraines [9,10] and improve memory [11,12]. Unfortunately, the efficiency of intra-nasal transport is too low for many useful drugs [13]. We and others have shown that magnetic particles can be pulled, pushed, and concentrated into tissues under the influence of magnetic gradients [14,15]. Such particles can be loaded with various payloads, including drugs [16] and nucleic acids [17], and can be heated for tumor treatment or vibrated for neuronal stimulation [18]. We wanted to see which configurations of magnetic gradients would be most successful in transporting magnetic particles intra-nasally into the brain.

Material and methods:

Experiments were conducted with one-day-old (pinky) mouse cadaver heads placed in glass tubes within the configuration shown in Fig. 1. The configuration contained a 0.8T NdFeB magnet whose purpose was to pull particles from the mouse's nose into its intra-cranial cavity. Two Helmholtz coil pairs were placed around the mouse head (one pair on the lateral sides of the mouse heads, and one pair on the anterior-posterior sides). The Helmholtz coils were activated parallel at a frequency of 200 Hz and coil currents of 1-4 Amperes (A), which generated the force of 15-60 mT on a magnetic dipole depending on the applied currents. Magnetic rods composed of Au-Fe-

Au-Fe-Au (Fig. 2) were mass-produced using template-guided synthesis methods as previously described [19–21]. The magnetic rods (250 nm wide and 2 microns long) contain two magnetic segments which have orthogonal easy axes of magnetization. As a result, the rods can be pulled or pushed along their long axis and rotated around their short axis at the same time, using a helical magnetic gradient [22]. The rods were dispersed in 500 microliters deionized water with sonication for two minutes. The concentration of iron in the solution was $7x10^{-5}$ mg/ml. Particle solution was injected with a syringe and 22-gauge needle into both nostrils (15 microliters per nostril) for control and test experiments as in Table 1. Each set of experiments were performed with three mouse cadavers. In the control experiment (A in Table 1), the permanent NdFeB magnet and Helmholtz coils were removed from the configuration. In experiment B of Table 1, the permanent NdFeB magnet was placed to pull the particles, and the Helmholtz coils were not activated. In experiments C-F, the permanent NdFeB magnet was placed near the head, and the Helmholtz coils were activated with increasing currents (1,2,3,4 A, respectively).

After 40 minutes, the mouse heads were removed from the magnetic apparatus, cut along the midline and the brains removed. The brains were stained with Prussian Blue (Sigma-Aldrich) for 15 minutes. The brains were then visualized directly with an optical microscope at 40x magnification and the particles were counted manually. To better demonstrate the anatomic location of the particles, after the initial set of experiments (A-F), an additional mouse was exposed to the parameters of test F (4 A Helmholtz coil, NdFeB) and the head immersed in 10% formaldehyde for two days, processed and sliced by cryotome and stained by hematoxylin and eosin stain (to show the brain anatomy) followed by Prussian blue solution (to stain the magnetic particles). The slices were tile-scanned at 20x using a Nikon eclipse optical microscope.

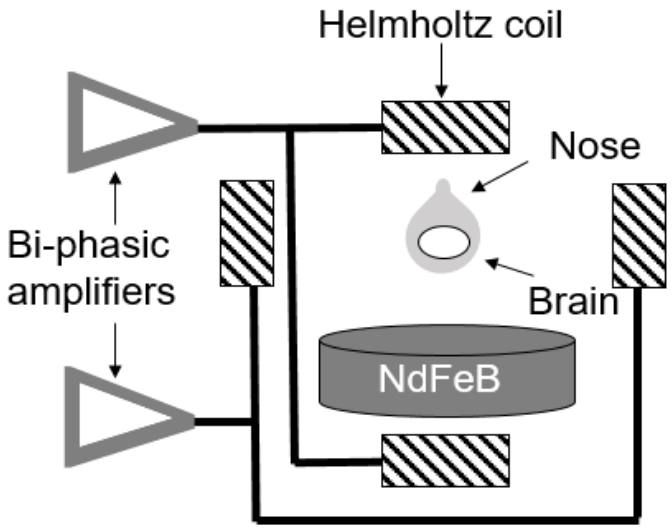

FIG. 1. Experimental set-up. Nd-Fe-B magnet applied linear magnetic gradient to pull particles from mouse nose into brain. Helmholtz coils applied rotational magnetic field in combination with linear gradient to drill particles through nose and cribriform plate into brain.

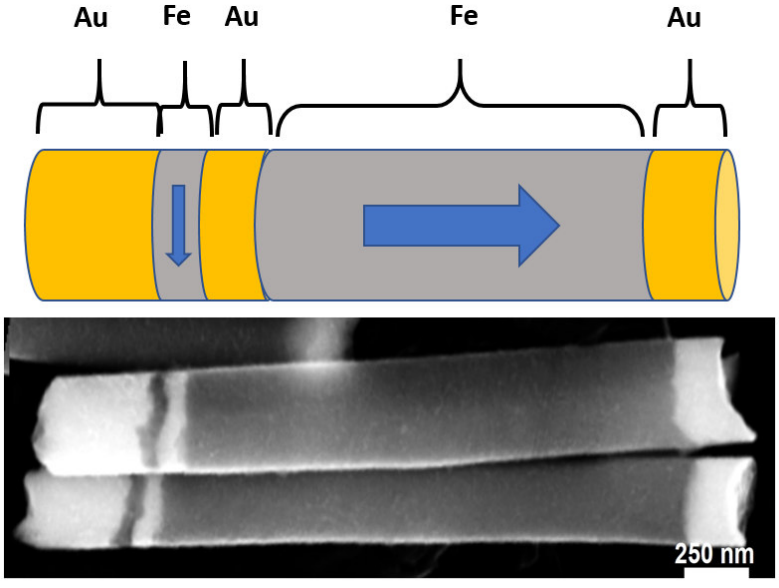

FIG. 2. Particle composition. Top: Arrows in schematic show the direction of magnetizations for each of the two iron segments of Au-Fe-Au-Fe-Au rods. Bottom: Scanning electron micrograph of rods.

Experiment	Linear magnetic gradient applied	Helmholtz rotation applied
A	no	no
B	yes	no
C	yes	1 A
D	yes	2 A
E	yes	3 A
F	yes	4 A

Table 1. Experimental conditions

Results

Figure 3 a-c show optical microscopy images of mouse brains. In the control case (no magnetic field applied), and in the cases where currents less than 4 A were applied to the Helmholtz coils, no particles were transported into to the brain (Fig. 3a). When particles were only pulled without rotation, a few particles (6+/-1 per square-mm (Fig. 4)) were transported to the brain (Fig. 3b). When currents of 4 A were applied (Fig. 3c), particle densities of 420+/-20 per square-mm field were observed in the brain (Fig. 4). Fig. 4 indicates that drilling improves transport into the brain 60 times as compared to linear gradient alone. Fig. 5 shows that 72% of particles were deposited in the olfactory bulb, and 28% in more distant sections of the brain. No tracks were observed in the brain to suggest damage during particle transit.

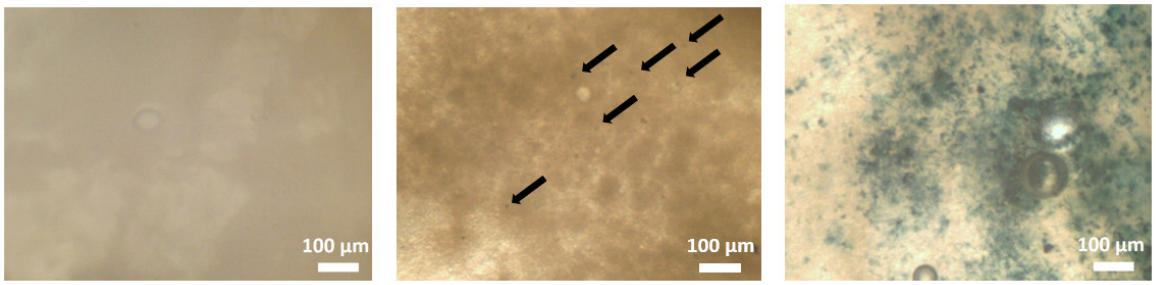

FIG. 3. Particles deposited in mouse brains. Left: Optical microscopy images of stained mice cadaver brains in control experiment, with no magnetic pulling or drilling, showing no particles. Middle: Arrows point to particles pulled into brain by NdFeB magnet without rotation. Right: Particles fill field of view in case where particles were pulled with NdFeB magnet and rotated with 4 Acurrents applied to Helmholtz coils.

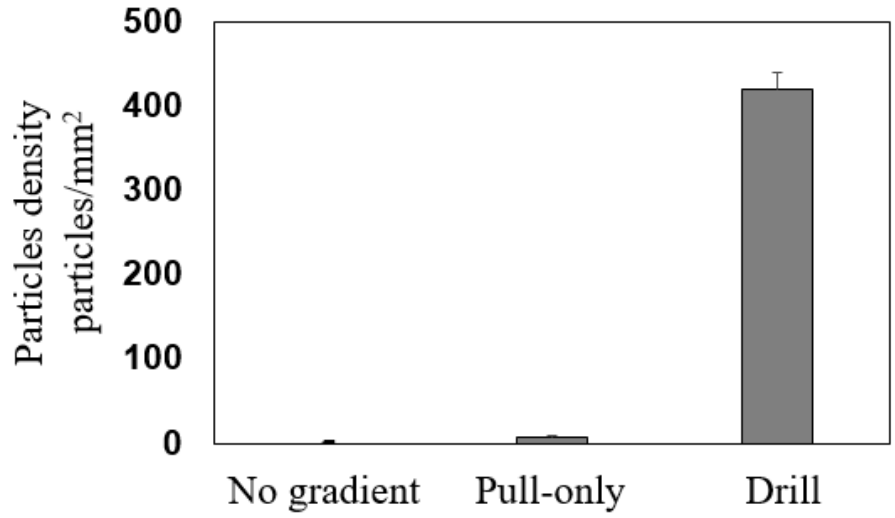

FIG. 4. Quantitative particle density measurement. Drilling improves transport into brain 60 times as compared to linear gradient alone.

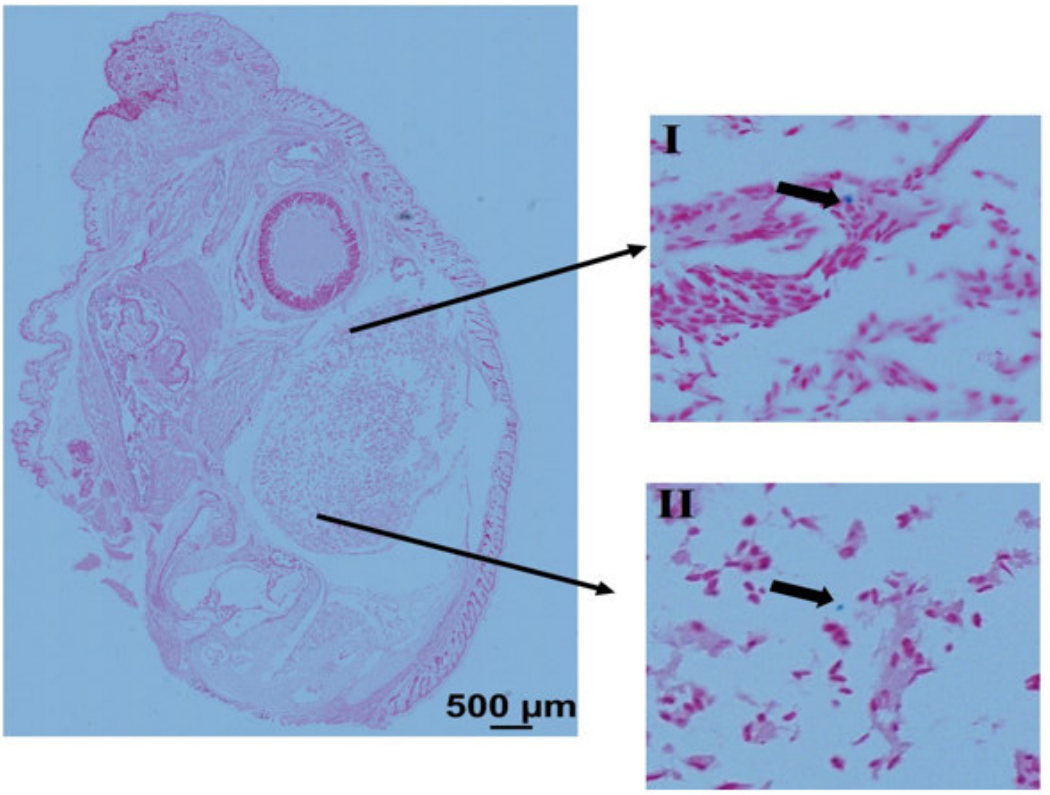

Fig. 5. Particles deposited in mouse brain with magnetic drilling. Arrows point to representative particle deposits in sections of olfactory bulb (inset I) and distal brain (inset II). No tracks are noted in brain.

Discussion

Our experiments demonstrated that application of a helical dynamic magnetic field resulted in enhanced intra-nasal transport into the cadaveric rodent brain (as compared to application of a linear gradient alone). This finding is consistent with our prior published results for very small (10 nm) pores in cartilage, where magnetic-induced vibration enhanced penetration by two orders of magnitude [23], presumably because of effective reduction in local viscosity [24]. No particle tracks were observed in the brain after drilling, suggesting that the particles traveled individually

without brain damage, consistent with earlier studies in brain slices [25]. The cribriform pore size in the one-day-old mice is considerably smaller than in humans (where pore size ranges from 1 to several millimeters in diameter) [26].

Conclusions

This work demonstrated that magnetic particles could be efficiently transported from the nose into the brain in a cadaveric rodent model using dynamic helical magnetic fields. As discussed above, intra-nasal delivery in humans can be difficult, and we therefore expect that magnetic particle drilling would eventually be helpful in human applications. We have previously shown that particles can be concentrated using remote pulsed magnetic fields [14], and that magnetic transport within brain slices does not affect neuronal function [27]. We are currently constructing platforms for MRI-guided image therapy and particle manipulation for experimental use, in which particles with various payloads would be administered intra-nasally and concentrated to specific locations in the brain while images of the particles in the tissue are provided [28]. The payloads could include drugs, genes, or the particles could impart heat or vibration as needed for tumor treatment or neurostimulation, respectively.

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- Intra-nasal magnetic delivery was performed to guide magnetic rods to the brain.
- Multi-segmented micro magnetic rods were used for magnetic delivery.
- Pulling and spinning magnetic fields were applied simultaneously.
- Particles were transported by translational and rotational motions.
- Pull and drill rods can greatly improve the transport of drugs to the brain.