Syllabus Outline:

Specialty area: Molecular Imaging & MEMRI

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Highlights:

- Overview of MEMRI
- Setting up for a successful MEMRI experiment: 1) How to perform a successful Nasal Lavages or deep brain injection of Mn2+; 2) How to set up a successful i.v. line in rodents for cardiac and muscle studies.
- Examples of studies that can be conducted using MEMRI in the brain, heart and muscle
- Validating brain, heart and muscle MEMRI studies with non-MRI methods.

TALK TITLE: "Molecular Imaging & MEMRI"

TARGET AUDIENCE : The target audience is basic researchers and clinicians interested in using MEMRI in preclinical models to understand physiological processes or as a drug testing platform.

OUTCOME/OBJECTIVES: Attendees of this presentation should understand some of the basic applications of MEMRI in brain, heart and muscle studies. Additionally, attendees should also learn some of the details and trip on how to perform nasal lavages in mice, set up i.v. lines in rodents for muscle and heart MEMRI studies and prepare solutions for MEMRI studies. Additionally, attendees will learn what types of studies can be conducted with MEMRI in brain, heart and muscle work. Last, some non-MRI validation approaches for MEMRI studies will be described.

PURPOSE: The purpose of this presentation is to give audience members more of a "how-to" description on how to set up MEMRI experiments.

METHODS: The methods used in this presentation include:

Nasal lavage of Manganese: Axonal transport rates can be measured in vivo utilizing a Manganese Enhanced MRI (MEMRI) protocol either through intranasal administration or through deep brain injection. For the nasal lavages, a concentrated solution of $MnCl_2$ (0.77 g/ml) is pipetted into the nasal cavity of the mouse at a total of 4 ml (2 ml/naris). Allow Mice to recover for 45 minutes on a heating pad, allowing the loading of Mn^{2+} into the olfactory receptor neurons located in the olfactory epithelium. The mice are then sedated with isoflurane and loaded into the magnet. The zero time point for imaging is 60 minutes post Mn^{2+} exposure. Spin lattice (T1)-weighted, spin-echo 2D data sets are then acquired of the mouse brain using a multi-slice/multi-echo 2D imaging protocol with the following parameters: matrix dimensions = 128x128; FOV= 3.0 cm x 3.0; slice thickness =1 mm; repetition time (TR)= 504.1 ms; echo time (TE) =8.2 ms; number of averages (NA) = 2; number of repetitions = 15; time per image = 2 min. Four axial slices should be selected with the first slice aligned with the leading edge of the olfactory bulb. In all studies, slice 2 of the 4 slices is a good target to assay for axonal transport in a region of interest (ROI) selected in the dorsal lateral portion of the olfactory bulb. Changes in the signal intensity of this ROI are measured and signal intensities are normalized to non-enhanced muscle outside of the brain. A least squares method is then used to determine the change in signal intensity over time, reflective of the rate of transport of Mn^{2+}.

IV Administration of Manganese: 50 mM $MnCl_2$ is dissolved in bivine (50 mM) and administered i.v. for 30 minutes at a rate of 0.2ml/hr.

Setting up Tail vein Lines in mice and rats:

A) Supplies needed include 29 gauge (rats) or a 30 gauge needle (mice); micro medical tubing with a 0.28 mm inner diameter x .64 mm outer diameter and a syringe pump (if needed); electronic coffee mug heater; glass beaker filled with warm water; saline; silk suture; scissors; hemostats; forceps; 1 ml syringe and 30 gauge needle; magnifying visor as needed.
B) To make your own catheter that will reach the animal in the magnet (if necessary), gently use hemostats to bend the needle off of an insulin syringe. Make sure that the needle isn't closed off. Using the hemostats, carefully insert the needle (blunt side into the tubing) into the tubing until it covers about 1/3 of the needle. Fill the 1 ml syringe with saline (make sure the 30 gauge needle is attached --- no bubbles!). Attach this to the other end of the tubing and gently fill the tubing with saline until a few drops come out of the needle on the opposite end of the tubing. Remove the 1 ml syringe and needle from the back of the tubing -- this is important as it will allow blood to more readily back flow into the tubing once the needle has successfully entered the vein.
C) To set up a tail vein line, anesthetize and maintain the animal using isoflurane anesthesia. Keep the warm water heated on the coffee mug heater. Make sure it is warm but not scalding. Next, use the beaker of water to soak the animals tail -- this will cause the veins to become more apparent, especially under anesthesia.
D) Cut off about 10 cm of silk suture and put it underneath the tail of the animal where you hope to insert the needle.
E) With the opening side up, carefully identify the tail vein either to the left or the right of the tail. Gently insert the needle in line with the vein (sometimes wearing a magnifying visor is very helpful in finding the veins, especially in pigmented mice). Make sure you don't insert the needle in too far, otherwise, the needle will poke through the entire vein. Once inserted, sometimes it is helpful to gently pull back on the needle. A needle successfully placed in the tail vein will result in a nice back flow of blood.
F) Once you see the back flow of blood, carefully use the suture to tie the needle down to the tail. Make sure the knots are made on the needle and not the tubing itself.
G) Attach the needle and syringe filled with saline to the back end of the tubing. If the needle has remained in the vein, the blood will clear and not leak around the site of needle entry. Additionally, if the tail turns a whitish color around where the needle is located, that is indicative that the needle is no longer in the vein. Last, if there is resistance when the saline is injected into the vein, this indicates that the needle is no longer in the vein.
H) Upon confirmation that the tail vein line is properly set up, the animal is now ready for infusion or for placement into the magnet for infusion of the agent.

RESULTS: Some of the results that will be described include using MEMRI to monitor disease progression as well as responses to therapeutics in brain, heart and muscle will be highlighted. Specifically, mouse models of Alzheimer's disease, cardiac inotropy and muscular dystrophy will be described.

CONCLUSION: At the conclusion of the session, the translational impact of the MEMRI work will be discussed. The focus will be primarily on drug screening work in pre-clinical models and why MEMRI is likely not suitable for the clinic. Last, potential complementary studies to MEMRI more appropriate for the clinic will be discussed.

REFERENCES: Suggested reading for MEMRI studies include the following references:

1. Manganese enhanced MRI (MEMRI): neurophysiological applications. Inoue T, Majid T, Pautler RG. Rev Neurosci. 2011;22(6):675-94. doi: 10.1515/RNS.2011.048. Epub 2011 Nov 18.
2. Manganese-enhanced MRI: an exceptional tool in translational neuroimaging.Silva AC, Bock NA. Schizophr Bull. 2008 Jul;34(4):595-604. doi: 10.1093/schbul/sbn056. Epub 2008 Jun
3. Biological applications of manganese-enhanced magnetic resonance imaging. Pautler RG. Methods Mol Med. 2006;124:365-86. Review.
4. Manganese-enhanced magnetic resonance imaging (MEMRI). Massaad CA, Pautler RG. Methods Mol Biol. 2011;711:145-74. doi: 10.1007/978-1-61737-992-5_7.
5. Manganese enhanced magnetic resonance imaging (MEMRI): a powerful new imaging method to study tinnitus. Cacace AT, Brozoski T, Berkowitz B, Bauer C, Odintsov B, Bergkvist M, Castracane J, Zhang J, Holt AG. Hear Res. 2014 May;311:49-62. doi: 10.1016/j.heares.2014.02.003. Epub 2014 Feb 26.
6. Applications of manganese-enhanced magnetic resonance imaging (MEMRI) to image brain plasticity in song birds. Van der Linden A, Van Meir V, Tindemans I, Verhoye M, Balthazart J. NMR Biomed. 2004 Dec;17(8):602-12. Review.
7. In vivo, trans-synaptic tract-tracing utilizing manganese-enhanced magnetic resonance imaging (MEMRI). Pautler RG. NMR Biomed. 2004 Dec;17(8):595-601. Review.
8. Applications of manganese-enhanced magnetic resonance imaging (MEMRI) to imaging of the heart. Wendland MF. NMR Biomed. 2004 Dec;17(8):581-94. Review.
9. Manganese-enhanced magnetic resonance imaging (MEMRI): methodological and practical considerations. Silva AC, Lee JH, Aoki I, Koretsky AP. NMR Biomed. 2004 Dec;17(8):532-43. Review.
10. Chem Senses. 2008 Jan;33(1):73-8. Epub 2007 Sep 27.Thallium transport and the evaluation of olfactory nerve connectivity between the nasal cavity and olfactory bulb. Kinoshita Y1, Shiga H, Washiyama K, Ogawa D, Amano R, Ito M, Tsukatani T, Furukawa M, Miwa T.