Published in final edited form as:

Rev Neurosci. 2011 ; 22(6): 675–694. doi:10.1515/RNS.2011.048.

Manganese enhanced MRI (MEMRI): neurophysiological applications

Taeko Inoue¹, Tabassum Majid², and Robia G. Pautler^{1,2,3,4,5,*}

¹Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA

²Translational Biology and Molecular Medicine (TBMM) Graduate Program, Baylor College of Medicine, Houston, TX, USA

³Department of Neuroscience, Baylor College of Medicine, Houston, TX, USA

⁴Department of Radiology, Baylor College of Medicine, Houston, TX, USA

⁵Huffington Center on Aging, Baylor College of Medicine, Houston, TX, USA

Abstract

Manganese ion (Mn^{2+}) is a calcium (Ca^{2+}) analog that can enter neurons and other excitable cells through voltage gated Ca^{2+} channels. Mn^{2+} is also a paramagnetic that shortens the spin-lattice relaxation time constant (T_1) of tissues where it has accumulated, resulting in positive contrast enhancement. Mn^{2+} was first investigated as a magnetic resonance imaging (MRI) contrast agent approximately 20 years ago to assess the toxicity of the metal in rats. In the late 1990s, Alan Koretsky and colleagues pioneered the use of manganese enhanced MRI (MEMRI) towards studying brain activity, tract tracing and enhancing anatomical detail. This review will describe the methodologies and applications of MEMRI in the following areas: monitoring brain activity in animal models, *in vivo* neuronal tract tracing and using MEMRI to assess *in vivo* axonal transport rates.

Keywords

axonal transport; imaging; manganese; MEMRI; mouse models; MRI; neuronal activity; neurophysiology

Introduction

Mn^{2+} is a trace element that is essential for many cellular processes (Santamaria, 2008). For example, Mn^{2+} is an essential cofactor for enzymes including mitochondrial superoxide dismutase (Miao and St Clair, 2009), pyruvate carboxylase (Zwingmann et al., 2004), arginase (Patchett et al., 1991) and glutamine synthetase (Wedler and Denman, 1984). Deficiencies in Mn^{2+} have been linked to bone malformations, skin lesions and also epileptic seizures (Strause et al., 1986; Friedman et al., 1987). In excess, however, Mn^{2+} is neurotoxic with Mn^{2+} occurring most notably in the striatum resulting in manganese poisoning, referred to as ‘manganism’ (Santamaria, 2008). Symptoms of manganism include Parkinson’s disease like symptoms, including tremors, bradykinesia, rigidity, hallucinations and memory loss (Mena et al., 1967; Barbeau, 1984; Santamaria, 2008).

Mn^{2+} has a similar ionic radius to that of Ca^{2+} and acts as a Ca^{2+} analog. Mn^{2+} can enter excitable cells through voltage gated Ca^{2+} channels and the Na^+/Ca^{2+} exchanger (Narita et al., 1990; Wadhvani et al., 1992; Mason et al., 1993; Kerper and Hinkle, 1997; Finley, 1998). Indeed, Mn^{2+} has long been used in the field of fluorescence microscopy to indirectly monitor Ca^{2+} influxes through fura-2 quenching (Merritt et al., 1989; Narita et al., 1990; Tas et al., 2008; Huang et al., 2009). An additional property of Mn^{2+} is that it is paramagnetic and functions as a positive magnetic resonance imaging (MRI) contrast agent (Mendonça-Dias et al., 1983; Geraldès et al., 1986; Cory et al., 1987; Fornasiero et al., 1987). The paramagnetic properties of Mn^{2+} cause a reduction in T_1 relaxation times of water resulting in positive contrast enhancement or a 'bright' signal in T_1 -weighted MRI images of tissues where Mn^{2+} has accumulated.

These combined properties of Mn^{2+} make this ion a very useful MRI contrast agent for anatomical and functional neuroimaging studies. Indeed, manganese enhanced MRI (MEMRI) has been increasingly used as a research tool over the past 10 years (Aoki et al., 2004b) with three main applications: 1) Monitoring neuronal activity as Mn^{2+} enters excited cells through Ca^{2+} channels; 2) *in vivo* neuronal tract tracing and 3) enhancement of anatomical detail in T_1 -weighted MRI images. In this review, we will discuss these first two topics and expand upon neuronal tract tracing and applications towards measuring *in vivo* axonal transport rates.

Activity induced manganese (AIM) MRI

One of the first developments of MEMRI was through the efforts of Lin and Koretsky to assess neuronal activity. The premise of the work was to use the calcium (Ca^{2+}) analog, manganese ion (Mn^{2+}), to monitor increases in Ca^{2+} influx with MRI in response to increased neuronal activity. This group demonstrated in the rat model that transiently breaking the blood-brain barrier (BBB) through hyperosmotic shock with mannitol and infusing a solution of manganese chloride ($MnCl_2$) resulted in nonspecific accumulation of Mn^{2+} ion throughout the brain (Lin and Koretsky, 1997). Lin and Koretsky then performed specific activation paradigms that included activation with glutamate applied intravenously. They observed an increase in the MRI signal intensity in the ventricles and also in the brain that was a result of the infused $MnCl_2$. Additionally, Lin and Koretsky demonstrated specific patterns of enhancement in T_1 -weighted MRI images in response to forepaw stimulation and light anesthesia. This groundbreaking work was the first demonstration that it is possible to directly assess neuronal activity with Mn^{2+} and monitor the output with MRI. Since this initial technical development, other groups have further developed and characterized AIM MRI. For example, Aoki et al. used a stroke model to characterize changes in the signal intensity in AIM MRI. Specifically, Aoki et al. furthered the electric forepaw stimulation and glutamate stimulation paradigms initially used by Lin and Koretsky, to show that AIM MRI can be used as a functional tool in disease models (Aoki et al., 2004a). A comparison of AIM, diffusion weighted MRI imaging (DWI), and T2W in Figure 1 demonstrates an application of AIM to assess functional alterations observed in a rat model of stroke. In this model of stroke, the failure of adequate ATP production leads to injured ATP-dependent sodium potassium pumps, leading to more open Ca^{2+} channels and thus increasing the amount and intensity of Mn^{2+} influx into the cell. In particular, panels 1 and 2 of Figure 2, show an increase of fos expression, in similar areas to the enhanced Mn^{2+} signal, as well as a verification of the same areas, using an overlap of an activation map, produced by blood oxygenation level detection (BOLD) functional MRI (fMRI). To validate AIM MRI as a functional tool, cerebral blood flow (CBF) was compared to the AIM results as well as c-fos expression to confirm that AIM was measuring Ca^{2+} influx indirectly (Aoki et al., 2004a).

Aoki and colleagues, built upon the AIM methodology further to include a dynamic component, dynamic AIM (DAIM). The DAIM MRI paradigm includes a series of four image datasets. In the first data set, MnCl_2 is infused and a baseline image is obtained before BBB disruption. In the second data-set, the BBB is broken with mannitol to assess nonspecific Mn^{2+} accumulation before functionally stimulating the brain. In the third dataset, the images are acquired during functional stimulation in order to precisely measure the Mn^{2+} in neurons as a result of an intentional stimulus. Finally, in the fourth dataset, once all stimulation has ended, the brain is imaged again to look at signal reduction without any stimuli present (Aoki et al., 2002).

There are several unique characteristics of the DAIM method. First, the DAIM strategy provides a more accurate picture of the signal obtained from anesthesia by differentiating signal intensity changes as a specific result from stimulation vs. nonspecific changes from light anesthesia. Second, a brain map can be obtained that can account for both background signal and nonspecific stimulation (Aoki et al., 2002).

In particular, this group worked with both glutamate stimulation and electrical forepaw stimulation to determine which stimulant and how much of it was needed to see a change in signal intensity over time with respect to the accumulation of Mn^{2+} . Figure 3 shows the paradigm that was used with both kinds of stimulation.

Aoki et al. compared both forms of stimulation using both AIM and DAIM. A comparison of time course and imaging between DAIM MRI and AIM MRI using glutamate administration and forepaw electrical stimulation in rats is shown in Figure 4. Overall, the group compared signal enhancement with both methods between the ventricles and the brain hemisphere. They observed that DAIM values were higher in the brain hemisphere as compared to the ventricles, whereas AIM was more consistent in signal enhancement within both areas. However, in the electrical forepaw stimulation paradigm, DAIM provided a more accurate map to the forelimb region of the somatosensory cortex, whereas AIM signal enhancement did not show a significant difference between the somatosensory cortex and other regions (Aoki et al., 2004a). This optimization of the DAIM method illustrates a model that can be used in future disease states for specific mapping techniques.

An additional application of AIM MRI was used with stimulation with kainic acid in rats to assess hippocampal lesions in a rat model of temporal lobe epilepsy. High resolution diffusion weighted imaging (DWI) and spin-spin weighted imaging (T_2 WI) were used to assess lesions as a result of the kainic acid administration. In particular, the group was interested in kainic acid neurotoxicity by evaluating signal enhancement after activation. The paradigm of stimulation is outlined in Figure 5. The scans were obtained over a period of 168 total hours after stimulation (Hsu et al., 2007).

In this study, the group coupled MRI with histopathology validation, as well as behavioral observations. For behavioral observations, those rats that were induced with kainic acid were less active than those who were sham treated. Upon utilizing AIM MRI, the group found that MR signal intensity increases in the CA3 region and then the CA1 region of the hippocampus. They observed that the kainic acid induced group had higher signal intensity in the CA3 region 1–2 h after injection. In order to investigate why the signal intensity changed over time, hematoxylin & eosin (H&E) staining was used across several time points to observe swelling of dendrites and shrinking of cell bodies in the CA3 region. In addition, 48 h after the kainic acid injection, there was severe loss of neurons, as a result of the swelling of the dendrites in this region. Thus, the group concluded that proliferation of astrocytes in the brain may be a possible explanation for the signal changes over time. These changes seen histopathologically over time matched up with the changes in signal intensity

over time. In addition, the group investigated the mechanism by which this swelling and change in signal intensity occurs by staining for GFAP, concluding that proliferation of astrocytes was the main reason for changes in signal intensity in the CA1 region of the hippocampus in particular. An illustration of these findings is shown in Figure 6. To validate these findings, the group used c-Fos staining within different conditions of anesthesia in order to confirm that kainic acid administration has an *in vivo* effect on hippocampal lesions in addition to signal intensity changes seen in the MRI analysis (Hsu et al., 2007). This was the first example of a study that applied AIM MRI techniques to changes in kainic acid induced hippocampal lesions, indicating that this technique may be applied to many different disease models.

The Turnbull group developed a parametric mapping method that was based on MEMRI methods to study activity patterns in the olfactory and somatosensory cortex of young rats and mice. They also used these methods to study sound evoked activity in the mouse auditory cortex and provided an elegant approach towards developing functional maps in response to auditory stimuli. Particularly, this group assessed the auditory midbrain of mice and used the voxel-wise methods also used in functional MRI (fMRI) studies. The 3D maps developed by this group showed the volume of the activity regions identified by MEMRI increased with stimulus amplitude (Yu et al., 2008). Specifically, the group developed a strategy for measuring signal intensity for each voxel defined within active inferior colliculus (IC) regions. Once these regions were established, they were used as ROIs for further analyses. The statistical mapping is shown in Figure 7.

Once this statistical map was established, MEMRI images were then obtained after three different decibel level tones from the IC. The group then continued to develop a more specific threshold for the *p*-value to increase the accuracy. They used a combination of threshold detection as well as Gaussian filtering to develop the map. This map displayed specificity by demonstrating that the 'volume of active IC region was directly proportional to the magnitude of the *p*-value threshold'. The algorithm for the construction of these maps is displayed in Figure 8.

Further mapping and statistical validation was completed to demonstrate that the peak changes in SI increased as the stimulus amplitude increased. Finally, ANOVA analysis was used to assess 40 kHz stimulation at three peak signal intensity points. This map allows AIM MRI to be used to analyze both spatial location of neuronal activity and magnitude evoked by sound stimuli with different energies (Yu et al., 2008). The final map and analysis obtained from this work is shown in Figure 9.

This statistical validation was able to confirm that the signal changes were not a result of changes in activity, but rather activation of specific neurons that were firing as a result of the sound amplitude changes. This application of AIM MRI allows for specific statistically accurate mapping that can then be compared to tonotopic and electrophysiological maps that have been constructed of various brain regions outside of the auditory cortex.

There are differences between AIM MRI auditory mapping and other tonotopic mapping strategies. The first of these is that a specific stimulus is used to activate multiple sets of neurons, whereas electrophysiological maps use individual neurons to generate a map. Thus, this method allows for investigations into supra-threshold activity patterns whereas it is much more difficult to get a bigger picture sense of what is going on in the auditory cortex than with other tonotopic methods.

Additional applications of AIM MRI include the work of Howles et al. who used AIM MRI to map responses to mechanical stimulation of the vibrissae pad in both right and left hemispheres of the brain. They were able to use ultrasound to open the BBB across the

whole mouse brain and thus were able to compare stimulated (one half) to unstimulated (other half) manganese enhanced activation of the mouse brain (Howles et al., 2010).

In addition, mapping studies from Fa et al. of rat brain activation have recently used AIM MRI after repetitive transcranial magnetic stimulation to measure brain activity with T_1 weighted scans. This group showed that AIM MRI can be used to measure proportional changes in signal intensity as a result of frequency stimulation in rats (Fa et al., 2011).

An additional example is the work of Gröschel et al. They have used AIM MRI and auditory mapping to evaluate a murine disease model of noise induced hearing loss and confirmed that the noise-induced hearing loss is Ca^{2+} dependent activity in the central auditory pathway. Using AIM MRI, the group found that mice had a significant threshold shift in hearing. This confirmed that AIM MRI can be used to show fundamental, calcium induced changes in the central auditory brainstem and pathway of mice. This application could lead to further disease models specifically with regards to the auditory maps that have now been studied (Gröschel et al., 2010).

Thus far, we have discussed the development of AIM MRI. The Koretsky group was the first to develop and characterize Mn^{2+} as a reliable and specific contrast agent and to develop the technique of AIM MRI. Upon further validation and statistical refinement, Aoki et al. continued to develop an extension of AIM MRI, i.e., DAIM MRI, which provides higher specificity and less contamination from background signals. The continued refinement of AIM MRI was then used to develop maps of the auditory cortex and also has been applied to models of stroke, hearing loss, and brain injury in rats and mice. Further application of AIM MRI may include other disease models as well as other areas of interest within the rodent brain in the future.

Manganese as an *in vivo*, trans-synaptic tract tracer

Historically, the field of neuronal tract tracing uses a wide variety of anterograde and retrograde tracers, such as biotinylated dextran (Rajakumar et al., 1993), horseradish peroxidase and fluorogold (Kristensson and Olsson, 1971; Tillet et al., 1993). The disadvantage of these tracers, however, is that they required the sacrifice of the animal in order to visualize tracts, and they do not allow for longitudinal studies on the same animal.

Manganese ion (Mn^{2+}) has long been known to enter neurons *via* voltage gated calcium (Ca^{2+}) channels (Mason et al., 1993; Kerper and Hinkle, 1997; Finley, 1998). After Mn^{2+} enters neurons, the ion is transported along axons *via* micro-tubule-based axonal transport to presynaptic termini (Sloot and Gramsbergen, 1994; Pautler et al., 1998; Takeda et al., 1998; Henriksson et al., 1999; Pautler and Koretsky, 2002). Once there, Mn^{2+} is released at the synapse and taken up by post-synaptic neurons (Sloot and Gramsbergen, 1994; Pautler et al., 1998; Sloot and Takeda et al., 1998; Henriksson et al., 1999; Pautler and Koretsky, 2002; Serrano et al., 2008). This property of Mn^{2+} , along with the fact that Mn^{2+} is a powerful paramagnetic ion, resulting in positive contrast enhancement in spin-lattice (T_1)-weighted MRI images, (Mendonça-Dias et al., 1983; Geraldès et al., 1986; Cory et al., 1987; Fornasiero et al., 1987) allows for MRI-detectable, *in vivo*, trans-synaptic tract tracing. Some of the major reports of the use of MEMRI tract tracing and its use within different pathways, and animal systems are described within this section.

Pautler, Silva and Koretsky were the first to describe the use of Mn^{2+} as an MRI-detectable tract tracer (Pautler et al., 1998). In this study, they used Mn^{2+} to trace two sensory pathways: the olfactory pathway and the visual pathway. In the olfactory work, a solution of $MnCl_2$ or $CaCl_2$ (control) was applied to the naris of mice and mice were imaged using T_1 -weighted MRI beginning at 24 h post-application. At 24 h post-application, there was

increased contrast seen within the olfactory bulbs of mice treated with MnCl_2 as shown in Figure 10, while mice given CaCl_2 showed no change in contrast over the same amount of time. Monitoring these mice for a period of up to 240 h after MnCl_2 application showed that the peak contrast enhancement occurred at approximately 29 h post-application, and that tissue returned to control signal levels within the olfactory bulb and primary olfactory cortex at approximately 240 h post-application (Pautler et al., 1998).

Importantly, they showed that over time, the signal intensity returned to normal levels indicating that the Mn^{2+} ion was removed from the brain. Furthermore, they demonstrated through administration of colchicine that the transport of Mn^{2+} was microtubule based.

To determine the cellular distribution of Mn^{2+} , they used subcellular fractionations on homogenized olfactory bulbs. They reported increases in Mn^{2+} within all subcellular fractions with the largest concentrations of Mn^{2+} observed within the microsomal fraction compared to untreated control animals (Pautler et al., 1998).

Next, they assessed tract tracing with MEMRI in the visual pathway. They performed intravitreal injections of MnCl_2 into the eyes of mice and imaged the mice with T_1 -weighted MRI. Signal enhancement in the optic nerve was seen as early as 2 h post-injection. Twenty-four hours post-injection, they showed that there was signal enhancement in the eye, the optic nerve all the way to the superior colliculus as shown in Figure 11. Using this data, they were able to approximate the transport of Mn^{2+} at 2 mm/h (Pautler et al., 1998).

The findings of this group demonstrated that Mn^{2+} can be used to trans-synaptically trace neuronal pathways *in vivo* and detect these tracings with MRI. They also alluded to the idea that MEMRI could be utilized to measure the rate of transport through these neuronal pathways (Pautler et al., 1998).

Following this first reported use of MEMRI in neuronal tract tracing within the olfactory and visual systems by the Koretsky group in 1998, Pautler and Jacobs published MEMRI tract tracing from the striatum and the amygdala within the mouse central nervous system (Pautler et al., 2003). They stereotactically introduced MnCl_2 into the murine striatum and the amygdala and used a 11.7T MRI to visualize the signal enhancement due to Mn^{2+} uptake and transport. Twenty-four hours post-injection, they visualized Mn^{2+} -based enhancement within the dorsal striatum to the dorsal prefrontal cortex, globus pallidus, stria terminalis, lateral septal nucleus and thalamus, as well as tract tracing to the substantia nigra and induncular nucleus. Forty-eight hours post-injection into the amygdala, direct tracing to the subiculum, entorhinal cortex, hippocampus and thalamus were observed as shown in Figure 12, with Mn^{2+} based enhancement observed within the hippocampus, fornix, septal nucleus and habenula. The presence of Mn^{2+} based enhancement within the prelimbic cortex (from striatum), as well as the stria terminalis (from amygdala) once again demonstrated the trans-synaptic activity of Mn^{2+} as was observed in the olfactory system. They also reported complete loss of Mn^{2+} based signal enhancement from all regions of the brain at 10 days post-injection indicating Mn^{2+} washout from central nervous system tissue over a period of several days with no signs of manganese-based toxicity within the injected animals. Not only did the Pautler and Jacobs group use MEMRI in the tracing of major neuronal tracts leading from the striatum and the amygdala, but they also showed delineation of small tracts leading from these brain regions and demonstrated the ability of Mn^{2+} to travel trans-synaptically across neuronal connections (Pautler et al., 2003).

Following the Koretsky group report of the first use of MEMRI in neuronal tract tracing, several groups published the use of MEMRI tract tracing in other animal systems. In 2002, Van der Linden et al. reported tract tracing within the song control nuclei (SNC) within song birds (Van der Linden et al., 2002). MnCl_2 was stereotactically introduced into the high

vocal center (HVC) of European starlings. These birds were then imaged 6 h post-injection with a T_1 -weighted MR protocol utilizing a 7T horizontal bore MR microscope. Van der Linden et al. were able to detect signal changes within the nucleus robustus archistriatalis (RA) and area X using MEMRI as described in Figure 13. These changes persisted up to 24 h post-injection within area X; however, the RA was no longer visible at this time point. After a period of 4 weeks or 3 months, two birds were re-injected with $MnCl_2$, with no signs of structural abnormalities, with both the RA and area X showing similar contrast enhancements as was seen previously. Using this technique, they measured the volumes of both the RA and area X in male and female starlings using a 3D MEMRI protocol. They verified that the volume of area X was significantly larger in males than in females, while there were no significant size differences in the RA between males and females. These findings were contrary to previous, histologically-based reports using Nissl staining and autoradiography, where area X was found to only be slightly larger in males than females and RA volumes measured by MEMRI were found to be significantly larger than those previously reported by histology (Van der Linden et al., 2002).

This group also assessed the kinetics of Mn^{2+} uptake within these two brain regions of the starlings. First of all, they reported that transport of Mn^{2+} was temperature dependent, and that the rate of signal intensity change could be described sigmoidally. Using the known distance between the injection site and area X and RA, they were able to approximate the rate of transport from the HVC to its respective targets, which they estimated to be approximately 2 – 6 mm/h which is in agreement with fast axonal transport rates. Second, they observed differential uptake of Mn^{2+} between male and female starlings within both area X and the RA. Their findings showed that MEMRI could be utilized in a system other than mice, namely within the SNC of European starlings (Van der Linden et al., 2002). Sentence should read: They alluded to the idea that MEMRI could also be used to measure the rate of transport through axons which was later reported by Smith et al. (Smith et al., 2007, described later within this review).

The use of MEMRI as a tract tracer was first reported in nonhuman primates by the Logothetis group in 2002 (Saleem et al., 2002). They injected $MnCl_2$ into the right putamen (pu) and the left caudate nucleus (cd) as indicated in Figure 14, both of which are known to have direct projections into the globus pallidus (GPi) and the substantia nigra (SN). They reported signal enhancement in the area surrounding the injection site (shown in Figure 14) in some of the cases, indicating some diffusion effects of Mn^{2+} through tissue. However, there was enhancement seen within the globus pallidus and substantia nigra regions, both of which have been previously verified to have direct projections from the striatal regions into which Mn^{2+} was injected. There was also enhancement observed within regions of the brain with indirect connection to the striatum, such as the ventral anterior and lateral nuclei, as well as the Habenular nucleus. These regions of the brain have direct connections through the GPi or SN, but not to the pu or cd. These findings further demonstrated that Mn^{2+} could not only be transported along neuronal tracts, but also could cross synapses to trace secondary connections (Saleem et al., 2002).

They also conducted studies on the validation of the specificity of Mn^{2+} as a neuronal tract tracer by injecting wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) into the same coordinates as $MnCl_2$ was injected. These verification studies showed that Mn^{2+} indeed traveled along neuronal tracts and the labeling with WGA-HRP coincided with the regions where enhancement was seen due to Mn^{2+} uptake (Saleem et al., 2002).

The Michaelis group delineated the hippocampal formation within the murine brain utilizing MEMRI (Watanabe et al., 2004). Using cannulae placed bilaterally within the posterior hippocampal formation, a solution of $MnCl_2$ was injected in the murine brain several days

post-insertion of the cannula. Using a 2.35 T magnet, they found that there was increased signal enhancement due to Mn^{2+} accumulation as early as 2 h post-injection within the injection site. At 6 h post-injection, there is clear enhancement of the fiber bundles within the hippocampal-septal pathway, with the greatest signal increase seen within the septal complex at the dorsal part of the lateral septal nucleus as shown in Figure 15. They also estimated that the speed of Mn^{2+} transport through this pathway was approximately 2–6 mm/h by assessing the known distances of this pathway and the rate at which the signal intensity due to Mn^{2+} accumulation occurred, although they did not discuss in detail how this measurement was made. The findings of this paper showed that there was increased SI observed within the hippocampal-septal pathway within 2 h post-injection, with optimal signal observed at 6 h post-injection. Not only did this report evaluate the morphological and functional properties of the hippocampal pathway, it alluded to the use of MEMRI as a tool to evaluate learning and memory, as well as the dependence of Mn^{2+} uptake on neuronal activity (Watanabe et al., 2004).

The Richmond paper published in 2008 addressed three Mn^{2+} and MEMRI-based topics: 1) $MnCl_2$ toxicity; 2) Mn^{2+} detection and 3) mapping of Mn^{2+} transport within rhesus and cynomolgus monkeys (Simmons et al., 2008). Using a 1.5 T magnet and either a high (800 mM) or a lower (120 mM) concentration $MnCl_2$ solution, they injected subjects three times over the course of a year (800 mM) or 4 months (120 mM) into the orbitofrontal cortex (OFC) and/or anterior cingulate cortex (ACC), and mapped the transport of Mn^{2+} . In the case of high-concentration $MnCl_2$ injections, they found tracing within the corticostriatal thalamic loop including white matter tract within the internal capsule found between the OFC and the ventral striatal regions. Histological examination of the brain tissue, however, revealed neuronal loss in the area directly surrounding the injection site. Despite neuron loss in the area exposed to the highest concentration of $MnCl_2$, functionally and morphologically intact neurons were found downstream of this area. Injections of the lower concentrations of $MnCl_2$ injections into the OFC resulted in similar signal enhancement within the corticostriatal thalamic loop as seen in the high-concentration animal. Signal was more prominent and localized to the lateral portion of area 13, with enhancement also found within area 11. There was transport of Mn^{2+} also seen to parts of the striatum and globus pallidus. In the case of injections into the ACC, signal enhancement was observed along corticocortical projections to the dorsolateral prefrontal and orbitofrontal cortices, as well as the dorsal caudate and putamen. Mn^{2+} based enhancement was even observed to cross the corpus callosum to the contralateral putamen. Histological staining showed that there was no neuronal loss seen at the injection site within the OFC, with a small area of neuronal loss at the ACC injection site. Behavioral assays performed on these monkeys showed that $MnCl_2$ injections did not result in any deficits or alterations in social or motor reactions, and electrophysiological recordings of healthy neurons isolated from these animals also showed that they retained their normal responses to stimulation. This work demonstrated that high-concentration $MnCl_2$ solutions yield excellent contrast as well, but result in neuronal cell loss within the injection site by histological examination. Lower concentrations of $MnCl_2$ solution can be utilized to trace tracts within the CNS of non-human primates, such as rhesus and cynomolgus monkeys with little to no toxicity, as well as deficits in animal behavior or deficits in electrophysiological properties of isolated neurons (Simmons et al., 2008).

Most of the early work in MEMRI utilized the tract tracing property to map connections within the CNS. The more recent work utilizes MEMRI tract tracing as a means to assess nerve function and integrity after regeneration of injured neuronal tracts. Matsuda et al. studies the regeneration of the sciatic nerve after crush or cut injury in rats (Matsuda et al., 2010). Sprague-Dawley rats were given a right sciatic nerve crush injury or a cut injury. The sciatic nerve function was assessed using injections of $MnCl_2$ solution into the distal part of

the sciatic nerve at 3 days, and 2, 4, and 12 weeks post-injury. They reported decreased Mn^{2+} -based signal enhancement in all time points post-injury compared to uninjured animals, with even the 12 week post-injury group showing significantly lower Mn^{2+} -based signal enhancement. Axonal tracing using fluorogold, and histomorphometrical examinations of the peroneal nerve showed Wallerian degeneration at 3 days and at 2 weeks post-injury. At 4 and 12 weeks post-injury, they found that both fiber number and fiber diameter was increased from what was found days after injury. Axonal tracing with the fluorogold showed full recovery at 12 weeks, contrary to axonal tracing with MEMRI. The results of their study indicate that regeneration of the damaged nerves can be assessed with MEMRI; however, the sensitivity of MEMRI is lower than that of tracing using fluorogold early post-injury (Matsuda et al., 2010).

Sandvig et al. also utilized MEMRI as a tract tracer to assess the integrity of regenerating fibers within mice, rats, frogs and fish (Sandvig et al., 2011). Lower vertebrates, such as frogs and fish have been known to spontaneously regenerate injured nerve fibers. They utilized MEMRI to compare the optic nerve integrity after crush injury (ONC) and/or lens injury (LI). After *intra vit* injection of Mn^{2+} , they show that transport of Mn^{2+} did not occur past the injury site to the distal portion of the optic nerve in all animals assessed. However, after monitoring animals for a period of up to 6 months signal enhancement was seen in the retinal projections in frogs (at 3 m) and in fish (at 6 m) indicating regeneration of the nerve after injury as shown in Figure 16. They also showed that the uptake rate of Mn^{2+} was much lower in fish and frogs compared to rats and mice by approximately a quarter to an eighth due to a heavy dependence on temperature. As a result, signal enhancement was not seen in the retinal projection until 48 h in frogs and 96 h in fish after *intra vit* injection of Mn^{2+} . They were able to demonstrate also, that MEMRI could be utilized for long-term monitoring and assessment of nerve regeneration in rodents as well as fish and frogs (Sandvig et al., 2011).

Prior to MEMRI, the field of tract tracing utilized invasive techniques that introduced tracers, such as fluorogold. They were limited in that they required the sacrifice of the animal in order to visualize these agents and also did not allow for longitudinal studies within the same animal. MEMRI has revolutionized the field in that is a relatively fast, noninvasive and *in vivo* imaging modality allowing for visualization of neuronal tracts to assess for connectivity and integrity of neuronal tracts of several different animal models. Since its first reported use in 1998, MEMRI as a neuronal tract tracer has been successfully utilized in a number of animal models, ranging from small rodents, such as mice and rat, songbirds, to nonhuman primates, such as macaques.

MEMRI axonal transport

As mentioned previously, Mn^{2+} , being a Ca^{2+} analog, is taken up by voltage-gated Ca^{2+} channels into neurons (Mason et al., 1993; Kerper and Hinkle, 1997; Finley, 1998). Once inside the cell, Mn^{2+} ions are taken into the endoplasmic reticulum where they are likely packaged into vesicles (Tillet et al., 1993; Slood and Gramsbergen, 1994; Pautler et al., 1998; Henriksson et al., 1999; Pautler and Koretsky, 2002). The ion is then shuttled along axons *via* microtubule-based transport where it can be released at the synapse to be taken up once again by post-synaptic neurons (Tillet et al., 1993; Pautler et al., 1998; Slood and Takeda et al., 1998; Henriksson et al., 1999; Pautler and Koretsky, 2002). This ability of Mn^{2+} to be taken up *via* voltage-gated Ca^{2+} channels has not only been exploited by the field of tract tracing, but also has been used to functionally assess the rate at which transport occurs through neurons. This axonal transport mechanism is crucial and necessary for the normal functioning of neurons and the entire system as a whole. Perturbations in axonal transport and its machinery have been associated with diseased states, such as Alzheimer's

disease, diabetes, as well as with normal aging (Smith et al., 2007; Cross et al., 2008). Reports of the use of MEMRI to assess perturbations in axonal transport rates in aging and several disease models are reviewed within this section.

Bearer et al. studied some of the characteristics of Mn^{2+} based transport (Bearer et al., 2007). Using two different murine models, they determined: 1) whether axonal transport of Mn^{2+} required electrical activity within the optic nerve and 2) furthering the initial work of Pautler and Koretsky, whether axonal transport of Mn^{2+} was mediated *via* a kinesin motor protein-based mechanism. First, to assess the effects of $MnCl_2$ on function of the optic nerve, they injected different volumes (ranging from 0.5 to 0.125 μ l), as well as concentrations of $MnCl_2$ (ranging from 50 to 200 mM) and measured visual evoked potentials (VEP) of injected mice. They found that even the smallest volumes and lowest concentrations (which was undetectable by their 11.7 T magnet) resulted in decreased VEP response compared to saline injected mice. For the rest of their studies, they injected mice with 0.25 μ l of 200 mM concentration of $MnCl_2$. They utilized two different mouse models to further characterize Mn^{2+} transport: the CBA (Rd1 $^{-/-}$) mouse that is visually blind due to lack of photoreceptor cells, as well as the kinesin light chain 1 (KLC $^{-/-}$) knockout mouse (Bearer et al., 2007).

CBA mice, despite having intact connections from the retina through the optic nerve, have no visual evoked potentials due to their lack of photoreceptors. They do, however, show Mn^{2+} based signal enhancement within the optic nerve after injections of $MnCl_2$ solution into the vitreous of the eye, indicating that transport of Mn^{2+} is not dependent on electrical activity. In fact, they showed that the rate of Mn^{2+} based signal enhancement was similar in both CBA mice and mice that were visually intact (C57 control mice). Mn^{2+} based enhancement, however, was not apparent in the midbrain of CBA mice, but was observed in the midbrain of C57 controls. They concluded that although electrical activity was not necessary for the transport of Mn^{2+} along axons, it was necessary for trans-synaptic movement of Mn^{2+} . Histological studies performed several months after injection of $MnCl_2$, showed that there were approximately 25% fewer axons within the optic nerve of both CBA and C57 control mice injected with $MnCl_2$ compared to the uninjected optic nerve. However, despite this report of fewer axons within the $MnCl_2$ injected optic nerve, they did not observe any signs of inflammation or infection. In fact, electron microscopy images showed that $MnCl_2$ injected optic nerves in both CBA and C57 control mice, showed normal morphology and ultrastructure indicating no signs of toxicity (Bearer et al., 2007).

Injection of $MnCl_2$ into the vitreous of the KLC1 $^{-/-}$ mice showed delayed transport of Mn^{2+} through the optic nerve as shown in Figure 17. There was little Mn^{2+} based enhancement to be seen within T_1 -weighted MRI images in the optic nerve up to 2.5 h post-injection. At 24 h, however, the enhancement within the optic nerve within KLC1 $^{-/-}$ was similar to that of WT control mice. This delayed signal enhancement indicates that the transport of Mn^{2+} is not solely dependent on a KLC1-based mechanism and that there are other motors involved in the movement of Mn^{2+} through axons (Bearer et al., 2007).

Bearer et al. demonstrated that Mn^{2+} injections into the eye can disrupt visual evoked potentials in response to light in a volume, as well as concentration-dependent manner. Electrical activity was not required for the axonal transport of Mn^{2+} , however, it is required for trans-synaptic movement. Finally, they demonstrated that Mn^{2+} transport is not solely dependent on KLC1 for its movement through axons (Bearer et al., 2007).

Smith and Pautler first reported MEMRI-based axonal transport measurements in 2007 in a mouse model of Alzheimer's disease, the Tg2576 mouse (Hsiao et al., 1996). The Tg2576 overexpresses human amyloid precursor protein (hAPP) containing the Swedish mutation.

This results in increased levels of amyloid beta (A β) at 6–7 months of age, and A β plaque formation by 10–12 months of age (Hsiao et al., 1996). As a result, animals from both pre-plaque and post-plaque were assessed during this study to determine how A β accumulation affects axonal transport.

Animals were intranasally lavaged with 4 μ l of 0.75 mg/ml MnCl₂ dissolved in sterile nanopure water while anesthetized on a cocktail of ketamine and xylazine. One hour post-lavage, animals were imaged using a 9.4 T Bruker Avance Biospec Spectrometer (Bruker BioSpin, Billerica, MA). T₁-weighted scans were obtained approximately every 2 min for 15 repetitions (Smith et al., 2007).

The signal intensity was then measured within an ROI selected with the dorsal olfactory neuronal layer of the olfactory bulb across all 15 repetitions (shown in Figure 18). The signal intensity was then plotted against time to show a rate of Mn²⁺ accumulation within that region which is reflective of the rate of axonal transport as demonstrated in Figure 19. By utilizing MEMRI, they were able to show that axonal transport rates were not only decreased in the older 11–14 month old animals after plaque formation, but also within younger 7–8 month Tg2576 prior to A β plaque formation compared to WT control mice (Smith et al., 2007).

Several key validation experiments were performed over the course of their study. First, they assessed the temperature dependence on axonal transport as assessed with MEMRI and found that the physiological temperature of 37°C was required for the normal transport of Mn²⁺ to occur. Next, they repeated the initial work by Pautler and Koretsky and treated mice with colchicine, a microtubule-destabilizing drug. Microtubules have previously been shown to be a key component in the transport of organelles and vesicles through cells. Their destabilization would result in the disruption of any Mn²⁺ transport if they were involved in the movement of Mn²⁺ loaded vesicles. Their results showed that Mn²⁺ transport was indeed disrupted with treatment of colchicine with the signal enhancement due to Mn²⁺ accumulation within the olfactory bulb being abolished. The results of their experiments verified that transport machinery, i.e., microtubules, is required to actively transport Mn²⁺ along axons and that axonal transport rates from the olfactory neuronal layer in the naris to the olfactory bulb is decreased in a mouse model of Alzheimer's disease compared to WT control mice before plaque formation and is exacerbated after plaque formation (Smith et al., 2007).

Work performed by Massaad and Pautler utilized the same mouse model of AD (Tg2576) to show that axonal transport was rescued with the overexpression of an endogenous mitochondrial antioxidant, superoxide dismutase 2 (SOD2) (Massaad et al., 2010). The Tg2576 mouse (Hsiao et al., 1996) was crossed with a transgenic mouse that overexpressed SOD2 (Ho et al., 1998) and the resulting progeny (WT, Tg2576, SOD2, and Tg2576/SOD2) were then assessed for alterations in axonal transport. Looking at three age groups in these animals, i.e., 4 month (pre-A β accumulation), 8 month, (post-A β accumulation, pre-plaque deposition) and 12–16 month (post-plaque deposition), and utilizing the same methodology as Smith et al., they reported that axonal transport rates were unperturbed in aged 12–16 month WT and SOD2 genotypes, but was decreased in the Tg2576 mice (shown in Figure 20). Most interestingly, however, was that the axonal transport rates in Tg2576 mice were rescued with the overexpression of SOD2 back to near WT levels (Massaad et al., 2010).

Aside from assessing axonal transport rates in the Tg2576 mouse model of Alzheimer's disease, Sharma and Pautler also assessed at how these rates were perturbed in a mouse model of diabetes mellitus type 1 or insulin dependent diabetes mellitus (IDDM). This model of DMT1 was induced by injecting WT C57 mice with streptozotocin (STZ) to

destroy the pancreatic beta cells that produce insulin. One week post-STZ inject, after all animals were verified to have become hyperglycemic, their axonal transport rates were measured using a nasal lavage of MnCl_2 solution and assessing the change in signal intensity within the olfactory bulb. STZ-injected mice were shown to have a decreased rate of change of Mn^{2+} based signal enhancement over time (approx. 75% that of control) compared to the vehicle-injected group. Interestingly, they showed that this deficit could be recovered if STZ injected animals were given insulin, or this deficit could be reproduced in nondiabetic mice if they were given glucose. These experiments were also repeated in SOD2 overexpressing mice given STZ. The deficits in axonal transport seen in WT, STZ-injected mice, were completely abolished in SOD2, STZ-injected mice, indicating that these deficits are potentially due to an oxidative stress induced mechanism. Histological assessment of the nasal cavity and epithelium with dihydroethidium, a superoxide specific indicator, showed elevated levels of superoxide in tissue of WT, STZ-injected mice, which were recovered down to WT levels in the SOD2, STZ-injected mice. Their results also indicated that there was an oxidative stress-related effect on axonal transport rates as assessed by MEMRI (Sharma et al.).

MEMRI has been used to not only assess neurodegenerative diseases and diabetes. The Minoshima group assessed age related decreases in axonal transport rates in Sprague-Dawley rats (Cross et al., 2008). Similar to previously reported work in mice, rats were intranasally lavaged with MnCl_2 and then imaged using a 1.5 T MR scanner over a period of up to 10 days. Assessing Mn^{2+} transport over this longer period of time allowed them to assess its movement from the nasal cavity, through the olfactory bulb, anterior olfactory nucleus and finally to the piriform cortex. After studying three age groups, young (4 months), mid-aged (9 – 13 months) and aged (24 – 25 months), they reported a significant age-related decline in transport rates of Mn^{2+} from the nasal cavity through the lateral olfactory tract in the aged rats compared to the young and mid-aged rats as shown in Figure 21. The average peak signal enhancement time was also shorter in young mice vs. mid-aged and aged rats in that the peak was reached at 11–12 h in young and 24 h in the mid- and aged rats. The results of this study showed that axonal transport is not only perturbed in a diseased state, but also there is an age-related decrease seen with aging (Cross et al., 2008).

Traditionally, the measurement of axonal transport rates were done using the introduction of large tracer molecules, which due to their size, may not give an accurate representation of transport within an *in vivo* system. Also, historical techniques required the sacrifice of the animal and excision of the neuron, which did not allow for longitudinal studies. The discovery of MEMRI and its application in measuring axonal transport rates has revolutionized this field in allowing for fast, non-invasive *in vivo* measurements, as well as longitudinal studies across several time points without sacrifice of the animal. As a result, MEMRI has proven to be an invaluable tool in the field neuroscience.

Conclusion

From the first report of MEMRI in 1998 in tracing neuronal connections within the central nervous system, to the present where it is being utilized to visualize axonal regeneration, MEMRI has revolutionized the neuroscience field. As summarized here, MEMRI allows for relatively fast, noninvasive, longitudinal and *in vivo* imaging in many different animal models ranging from small amphibians, such as frogs to non-human primates, such as rhesus monkeys. It can be administered by various means, intranasally *via* lavage (Pautler et al., 1998; Smith et al., 2007), optically *via* injections into the vitreous (Pautler et al., 1998; Sandvig et al., 2011), intracerebrally *via* stereotaxic injections (Van der Linden et al., 2002; Saleem et al., 2002; Pautler et al., 2003; Watanabe et al., 2004) etc. to allow for the visualization of different pathways within the nervous system.

Currently, however, one of the major hurdles in utilizing MEMRI is cellular toxicity. High exposure to Mn^{2+} has been shown to result in Parkinsonian-like symptoms in exposed individuals, such as miners, as well as animals used in early MEMRI studies (Santamaria, 2008). As a result, this has prevented extension of MEMRI into human use. More recently, however, several Mn^{2+} -based contrast agents have been developed. GE Healthcare developed Teslascan® for liver imaging (which has been discontinued since), while currently, Eagle Vision Pharmaceutical Corp (Exton, PA, USA) is testing another Mn^{2+} -based contrast agent called SeeMore™ for heart imaging. The limitations of MEMRI, however, can be addressed by increasing the sensitivity for detection of Mn^{2+} in a clinical setting. Also, as clinical scanners are at a lower magnetic field, allowing for better relaxivity, lower concentrations of Mn^{2+} can be utilized in humans than are used in animals imaged at higher magnetic fields, lowering the risk of Mn^{2+} -based toxicity.

Overall, MEMRI has proved to be an invaluable tool in the field of neuroscience allowing for *in vivo* studies on connectivity of neuronal circuits as well as studies that look neuronal activity and function within specific brain regions.

Acknowledgments

Funding for this manuscript was in part supported by NIH/NIA R01AG029977 (Pautler), the BCM Diabetes and Endocrinology Research Center, MRI Core, NIH/NIDDK P30DK079638 and the Huffington Center Training Grant, NIH/NIA T32AG000183.

References

- Aoki I, Tanaka C, Takegami T, Ebisu T, Umeda M, Fukunaga M, Fukuda K, Silva AC, Koretsky AP, Naruse S. Dynamic activity-induced manganese-dependent contrast magnetic resonance imaging (DAIM MRI). *Magn Reson Med*. 2002; 48:927–933. [PubMed: 12465100]
- Aoki I, Naruse S, Tanaka C. Manganese-enhanced magnetic resonance imaging (MEMRI) of brain activity and applications to early detection of brain ischemia. *NMR Biomed*. 2004a; 17:569–580. [PubMed: 15617055]
- Aoki I, Wu YJL, Silva AC, Lynch RM, Koretsky AP. In vivo detection of neuroarchitecture in the rodent brain using manganese-enhanced MRI. *NeuroImage*. 2004b; 22:1046–1059. [PubMed: 15219577]
- Barbeau A. Manganese and extrapyramidal disorders (a critical review and tribute to Dr. George C. Cotzias). *Neurotoxicology*. 1984; 5:13–35. [PubMed: 6538948]
- Bearer EL, Falzone TL, Zhang X, Biris O, Rasin A, Jacobs RE. Role of neuronal activity and kinesin on tract tracing by manganese-enhanced MRI (MEMRI). *NeuroImage*. 2007; 37:S37–S46. [PubMed: 17600729]
- Cory DA, Schwartzentruber DJ, Mock BH. Ingested manganese chloride as a contrast agent for magnetic resonance imaging. *Magn Reson Imaging*. 1987; 5:65–70. [PubMed: 3586874]
- Cross DJ, Flexman JA, Anzai Y, Maravilla KR, Minoshima S. Age-related decrease in axonal transport measured by MR imaging in vivo. *NeuroImage*. 2008; 39:915–926. [PubMed: 17980625]
- Fa Z, Zhang P, Wu W, Wang Z, Huang F, Yang L, Chang H, Xu R, Wen Z, Zhang J, et al. Functional mapping of rat brain activation following rTMS using activity-induced manganese-dependent contrast. *Neurol Res*. 2011; 33:563–571. [PubMed: 21708064]
- Finley JW. Manganese uptake and release by cultured human hepato-carcinoma (Hep-G2) cells. *Biol Trace Elem Res*. 1998; 64:101–118. [PubMed: 9845466]
- Fornasiero D, Bellen JC, Baker RJ, Chatterton BE. Paramagnetic complexes of manganese(II), iron(III), and gadolinium(III) as contrast agents for magnetic resonance imaging. The influence of stability constants on the biodistribution of radioactive aminopolycarboxylate complexes. *Invest Radiol*. 1987; 22:322–327. [PubMed: 3583652]

- Friedman BJ, Freeland-Graves JH, Bales CW, Behmardi F, Shorey-Kutschke RL, Willis RA, Crosby JB, Trickett PC, Houston SD. Manganese balance and clinical observations in young men fed a manganese-deficient diet. *J Nutr.* 1987; 117:133–143. [PubMed: 3819860]
- Geraldes CFGC, Sherry AD, Brown RD III, Koenig SH. Magnetic field dependence of solvent proton relaxation rates induced by Gd³⁺ and Mn²⁺ complexes of various polyaza macrocyclic ligands: implications for NMR imaging. *Magnet Reson Med.* 1986; 3:242–250.
- Gröschel M, Götze R, Ernst A, Basta D. Differential impact of temporary and permanent noise-induced hearing loss on neuronal cell density in the mouse central auditory pathway. *J Neurotrauma.* 2010; 27:1499–1507. [PubMed: 20504154]
- Henriksson J, Tallkvist J, Tjälve H. Transport of manganese via the olfactory pathway in rats: dosage dependency of the uptake and subcellular distribution of the metal in the olfactory epithelium and the brain. *Toxicol Appl Pharm.* 1999; 156:119–128.
- Ho YS, Vincent R, Dey MS, Slot JW, Crapo JD. Transgenic models for the study of lung antioxidant defense: enhanced manganese-containing superoxide dismutase activity gives partial protection to B6C3 hybrid mice exposed to hyperoxia. *Am J Respir Cell Mol Biol.* 1998; 18:538–547. [PubMed: 9533942]
- Howles GP, Bing KF, Qi Y, Rosenzweig SJ, Nightingale KR, Johnson GA. Contrast-enhanced in vivo magnetic resonance microscopy of the mouse brain enabled by noninvasive opening of the blood-brain barrier with ultrasound. *Magnet Resonan Med.* 2010; 64:995–1004.
- Hsiao K, Chapman P, Nilsen S, Eckman C, Harigaya Y, Younkin S, Yang F, Cole G. Correlative memory deficits, Aβ₄₂ elevation, and amyloid plaques in transgenic mice. *Science.* 1996; 274:99–102. [PubMed: 8810256]
- Hsu YH, Lee WT, Chang C. Multiparametric MRI evaluation of kainic acid-induced neuronal activation in rat hippocampus. *Brain.* 2007; 130:3124–3134. [PubMed: 17785345]
- Huang CC, Cheng HH, Lin KL, Cheng JS, Tsai JY, Liao WC, Fang YC, Jan CR. Tamoxifen-induced [Ca²⁺]_i rise and apoptosis in corneal epithelial cells. *Toxicology.* 2009; 255:58–64. [PubMed: 18992300]
- Kerper LE, Hinkle PM. Cellular uptake of lead is activated by depletion of intracellular calcium stores. *J Biol Chem.* 1997; 272:8346–8352. [PubMed: 9079658]
- Kristensson K, Olsson Y. Retrograde axonal transport of protein. *Brain Res.* 1971; 29:363–365. [PubMed: 4107258]
- Lin YJ, Koretsky AP. Manganese ion enhances T1-weighted MRI during brain activation: an approach to direct imaging of brain function. *Magn Reson Med.* 1997; 38:378–388. [PubMed: 9339438]
- London RE, Toney G, Gabel SA, Funk A. Magnetic resonance imaging studies of the brains of anesthetized rats treated with manganese chloride. *Brain Res Bull.* 1989; 23:229–235. [PubMed: 2819480]
- Mason MJ, Mayer B, Hymel LJ. Inhibition of Ca²⁺ transport pathways in thymic lymphocytes by econazole, miconazole and SKF 96365. *Am J Physiol.* 1993; 264:C654–C662. [PubMed: 8384787]
- Massaad CA, Amin SK, Hu L, Mei Y, Klann E, Pautler RG. Mitochondrial superoxide contributes to blood flow and axonal transport deficits in the Tg2576 mouse model of Alzheimer's disease. *PLoS ONE.* 2010; 5:e10561. [PubMed: 20479943]
- Matsuda K, Wang HX, Suo C, McCombe D, Horne MK, Morrison WA, Egan GF. Retrograde axonal tracing using manganese enhanced magnetic resonance imaging. *NeuroImage.* 2010; 50:366–374. [PubMed: 20074651]
- Mena I, Marin O, Fuenzalida S, Cotzias GC. Chronic manganese poisoning. Clinical picture and manganese turnover. *Neurology.* 1967; 17:128–136. [PubMed: 6066873]
- Mendonça-Dias MH, Gaggelli E, Lauterbur PC. Paramagnetic contrast agents in nuclear magnetic resonance medical imaging. *Semin Nucl Med.* 1983; 13:364–376. [PubMed: 6359418]
- Merritt JE, Jacob R, Hallam TJ. Use of manganese to discriminate between calcium influx and mobilization from internal stores in stimulated human neutrophils. *J Biol Chem.* 1989; 264:1522–1527. [PubMed: 2536366]
- Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. *Free Radic Biol Med.* 2009; 47:344–356. [PubMed: 19477268]

- Narita K, Kawasaki F, Kita H. Mn and Mg influxes through Ca channels of motor nerve terminals are prevented by verapamil in frogs. *Brain Res.* 1990; 510:289–295. [PubMed: 2158851]
- Patchett ML, Daniel RM, Morgan HW. Characterisation of arginase from the extreme thermophile- ‘*Bacillus caldovelox*’. *BBA-Protein Struct M.* 1991; 1077:291–298.
- Pautler RG, Koretsky AP. Tracing odor-induced activation in the olfactory bulbs of mice using manganese-enhanced magnetic resonance imaging. *NeuroImage.* 2002; 16:441–448. [PubMed: 12030829]
- Pautler RG, Silva AC, Koretsky AP. In vivo neuronal tract tracing using manganese-enhanced magnetic resonance imaging. *Magn Reson Med.* 1998; 40:740–748. [PubMed: 9797158]
- Pautler RG, Mongeau R, Jacobs RE. In vivo trans- synaptic tract tracing from the murine striatum and amygdala utilizing manganese enhanced MRI (MEMRI). *Magn Reson Med.* 2003; 50:33–39. [PubMed: 12815676]
- Rajakumar N, Elisevich K, Flumerfelt BA. Biotinylated dextran: a versatile anterograde and retrograde neuronal tracer. *Brain Res.* 1993; 607:47–53. [PubMed: 7683244]
- Saleem KS, Pauls JM, Augath M, Trinath T, Prause BA, Hashikawa T, Logothetis NK. Magnetic resonance imaging of neuronal connections in the macaque monkey. *Neuron.* 2002; 34:685–700. [PubMed: 12062017]
- Sandvig A, Sandvig I, Berry M, Olsen Ø, Pedersen TB, Brekken C, Thuen M. Axonal tracing of the normal and regenerating visual pathway of mouse, rat, frog and fish using manganese- enhanced MRI (MEMRI). *J Magn Reson Imaging.* 2011; 34:670–675.
- Santamaria AB. Manganese exposure, essentiality & toxicity. *Indian J Med Res.* 2008; 128:484–500. [PubMed: 19106442]
- Serrano F, Deshazer M, Smith KDB, Ananta JS, Wilson LJ, Pautler RG. Assessing transneuronal dysfunction utilizing manganese- enhanced MRI (MEMRI). *Magn Reson Med.* 2008; 60:169–175. [PubMed: 18581360]
- Sharma R, Buras E, Terashima T, Serrano F, Massaad CA, Hu L, Bitner B, Inoue T, Chan L, Pautler RG. Hyperglycemia induces oxidative stress and impairs axonal transport rates in mice. *PLoS One.* 2010; 5:e13463.10.1371/journal.pone.0013463 [PubMed: 20976160]
- Simmons JM, Saad ZS, Lizak MJ, Ortiz M, Koretsky AP, Richmond BJ. Mapping prefrontal circuits in vivo with manganese-enhanced magnetic resonance imaging in monkeys. *J Neurosci.* 2008; 28:7637–7647. [PubMed: 18650340]
- Sloot WN, Gramsbergen JBP. Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia. *Brain Res.* 1994; 657:124–132. [PubMed: 7820609]
- Smith KDB, Kallhoff V, Zheng H, Pautler RG. In vivo axonal transport rates decrease in a mouse model of Alzheimer’s disease. *Neuroimage.* 2007; 35:1401–1408. [PubMed: 17369054]
- Strause LG, Hegenauer J, Saltman P, Cone R, Resnick D. Effects of long-term dietary manganese and copper deficiency on rat skeleton. *J Nutr.* 1986; 116:135–141. [PubMed: 3944652]
- Takeda A, Devenyi A, Connor JR. Evidence for non- transferrin- mediated uptake and release of iron and manganese in glial cell cultures from hypotransferrinemic mice. *J Neurosci Res.* 1998; 51:454–462. [PubMed: 9514199]
- Tas PWL, Stössel C, Roewer N. Inhibition of the histamine-induced Ca²⁺ influx in primary human endothelial cells (HUVEC) by volatile anaesthetics. *Eur J Anaesthesiol.* 2008; 25:976–985. [PubMed: 18631421]
- Tillet Y, Batailler M, Thibault J. Neuronal projections to the medial preoptic area of the sheep, with special reference to monoaminergic afferents: immunohistochemical and retrograde tract tracing studies. *J Comp Neurol.* 1993; 330:195–220. [PubMed: 8491868]
- Van der Linden A, Verhoye M, Van Meir V, Tindemans I, Eens M, Absil P, Balthazart J. In vivo manganese-enhanced magnetic resonance imaging reveals connections and functional properties of the songbird vocal control system. *Neuroscience.* 2002; 112:467–474. [PubMed: 12044464]
- Wadhvani KC, Murphy VA, Smith QR, Rapoport SI. Saturable transport of manganese(II) across blood-nerve barrier of rat peripheral nerve. *Am J Physiol.* 1992; 262:R284–R288. [PubMed: 1539737]

- Watanabe T, Radulovic J, Spiess J, Natt O, Boretius S, Frahm J, Michaelis T. In vivo 3D MRI staining of the mouse hippocampal system using intracerebral injection of MnCl₂. *NeuroImage*. 2004; 22:860–867. [PubMed: 15193616]
- Wedler FC, Denman RB. Glutamine synthetase: the major Mn(II) enzyme in mammalian brain. *Curr Top Cell Regul*. 1984; 24:153–169. [PubMed: 6149889]
- Yu X, Zou J, Babb JS, Johnson G, Sanes DH, Turnbull DH. Statistical mapping of sound-evoked activity in the mouse auditory midbrain using Mn-enhanced MRI. *NeuroImage*. 2008; 39:223–230. [PubMed: 17919926]
- Zwingmann C, Leibfritz D, Hazell AS. Brain energy metabolism in a sub-acute rat model of manganese neurotoxicity: an ex vivo nuclear magnetic resonance study using [1–¹³C] glucose. *Neurotoxicology*. 2004; 25:573–587. [PubMed: 15183011]

Biographies

Taeko Inoue is a PhD candidate at Baylor College of Medicine in the Department of Molecular Physiology and Biophysics in Houston, Texas, USA. Working under the mentorship of Dr. Robia G. Pautler, her current research focuses on oxidative stress and how it plays a role in perturbing physiological mechanisms in Alzheimer's disease and several other neurodegenerative diseases. Originally from the country of Belize, Taeko previously received her BS and MS degrees at the University of Texas at Brownsville in the field of physics. Over the course of her studies, she has gained much experience in handling rodents as test subjects, biochemical and immunohistological techniques, as well as in utilizing magnetic resonance imaging to assess neuronal function, such as axonal transport rates, tract tracing, cerebral blood flow and, of course, anatomy within small rodents.

Tabassum Majid is a PhD student in the Translational Biology and Molecular Medicine program at Baylor College of Medicine in Houston, Texas, conducting research in Dr. Robia Pautler's laboratory that focuses on the role of oxidative stress in neurodegeneration. Originally from Ellicott City, MD, she earned her Bachelor of Science degree in a self-designed Interdisciplinary Studies major, focusing on neurodegenerative disorders of the aged at the University of Maryland, Baltimore County. While there, she gained experience teaching in an undergraduate classroom and participated in both basic and clinical research projects on Parkinson's disease, traumatic brain injury, and spinal cord injury.

Robia G. Pautler, PhD is an Associate Professor at Baylor College of Medicine in the Departments of Molecular Physiology and Biophysics, Neuroscience and Radiology with appointments in the Translational Biology and Molecular Medicine Graduate Program and the Huffington Center on Aging. Originally from Fort Collins, CO, Dr. Pautler earned her BS in Microbiology from Colorado State University. She then went on to pursue her graduate studies at Carnegie Mellon University where she earned her PhD in Biology/MRI under the mentorship of Dr. Alan Koretsky. She did her post-doctoral training at Caltech in Dr. Scott Fraser's laboratory after which she moved to Baylor College of Medicine for a faculty position.

Figure 1.

Comparison of AIM (A), diffusion weighted image (DWI) (B), T2-weighted (T2W) image (C), and apparent diffusion coefficient (ADC) map (D) on the same stroke model. The high density area which is localized within the lateral side of the caudate-putamen and lateral cortex on AIM was smaller than both DWI hyperintensity and decreased ADC. A slight decrease in signal intensity was observed on the T2W image. [Reproduced from Aoki et al. (2004a). Copyright © 2004 John Wiley & Sons, Inc.]

Figure 2.

Activation of the rat brain by the intra-carotid arterial injection of hypertonic NaCl. The top and bottom panels show Fos immunoreactivity in the cortex, lateral habenular nucleus, paraventricular hypothalamic nucleus and supraoptic nucleus. The middle panel shows T1-weighted images. Areas in which the signal intensity increased significantly ($p < 0.05$) are shown in color. The bottom panel shows spatial overlaps of activation maps from the same two rats shown in Figure 1. The first column shows the combined maps of BOLD (red), CBF (green), and the overlap between the two (yellow). The second column shows the combined maps of BOLD (red), T1-weighted AIM (green) and the overlap between the two (yellow). The third column shows the combined maps of CBF and T1-weighted AIM. [Reproduced from Aoki et al. (2004a). Copyright © 2004 John Wiley & Sons, Inc.]

Figure 3.

Typical DAIM time courses of relative signal intensities in the cortex and ventricle, obtained from the glutamate administration (solid symbols) and control (open squares) groups. The inset indicates typical regions-of-interest. The first set of images (1–16) was obtained before mannitol administration, but after the start of MnCl_2 infusion. The second set (17–32) was obtained before injection of glutamate after breaking the BBB with mannitol. The signal increases significantly in a nonspecific manner following opening of the BBB. The third set (33–48) was obtained after glutamate injection. The signal intensity in the cortex of the glutamate administration group (solid circles) increases substantially after glutamate injection, but not in the control group following saline administration (open squares). The fourth set (49–64) was obtained during rest after stopping the MnCl_2 infusion. [Reproduced from Aoki et al. (2002). Copyright © 2002 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 4.

Comparison between DAIM and AIM MRI. The left column shows (A) typical DAIM functional map and (B) AIM images from the glutamate administration group. The right column shows (C and D) corresponding maps for the forepaw electrical stimulation group. While the (A and C) DAIM maps only show the regions specifically activated by the stimulation paradigm, the (B) and (D) AIM images show contamination from nonspecific activation, such as ventricles (arrows). [Reproduced from Aoki et al. (2002). Copyright © 2002 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 5.

Timetable of MR scanning and drug administration. A series of high resolution DWI, T2WI, T1WI were repeatedly acquired before and at 0.1, 1.2, 2.3, 24, 48, 72, 120, and 168 h after KA administration. The arrow indicates the time of KA i.v. injection. Arrowheads indicate the times of two Gd-DTPA i.v. injections (1 h 45 min and 2 h 45 min post KA). The dashed line indicates the time of Mn²⁺ i.v. infusion. [Reproduced from Hsu et al. (2007). Copyright © 2007 Oxford University Press.]

Figure 6.

Representative high-resolution images and a corresponding histological section in normal control rats showing layer organization in the intact hippocampus and selected regions of interest (ROIs). (A) DWI showing distinct hippocampal layers *in vivo* (n=6). (B) T2WI did not allow for identification of hippocampal layers. (C) A magnified view of the hippocampus region in the white rectangle shown in (A) indicating six hippocampal layers, including the CA3 stratum oriens (subregion 1), CA3 pyramidal cell layer (subregion 2), CA 3 stratum radiatum (subregion 3), hippocampal fissure (subregion 4), CA1 stratum radiatum (subregion 5) and CA1 pyramidal cell layer (subregion 6). (D) Immunostaining of VGCC in brain section showing the layers in the hippocampus (n=5). The ROIs were shown as black circles. Arrows indicated hippocampal layers and the corresponding ROIs. The arrowhead indicates the control ROI in the secondary visual cortex. The scale bar represents 500 μm . [Reproduced from Hsu et al. (2007). Copyright © 2007 Oxford University Press.]

Figure 7.

Statistical mapping in the mouse IC from MEMRI images. 3D MEMRI images of the IC were segmented and analyzed *via* voxel-by-voxel statistical comparisons. t -Test analysis was performed, comparing the signal intensities (SI) in each voxel, between 3D images from two groups of mice: V_1 array of SI values for Group 1=stimulated, V_2 array for Group 2=quiet controls. For each voxel (red cube), the resulting p -value was assigned after 8-bit gray scale conversion. In this way, a 3D p -map was created to encode the statistically significant different IC regions between the two groups of mice. [Reproduced from Yu et al. (2008). Copyright © by Elsevier, Inc.]

Figure 8.

Gaussian filtering is a method for reducing the number of false positive errors. Multiple voxel-wise t -tests were made, comparing MEMRI images of mice maintained in a quiet environment and mice exposed to 40 kHz. The resulting statistical maps demonstrated difference between the two groups (quiet vs. 40 kHz=red) using a variable p -value threshold (p_{th}): $p_{th}=0.05$ (A–C), $p_{th}=0.02$ (D–F), and $p_{th}=0.01$ (G–I). Some scattered active voxels were observed in the original statistical maps, which are more likely to be the false positive voxels (A, D, G). A 3D Gaussian filter was employed to smooth the 3D p -map by convolving the data with a $3 \times 3 \times 3$ Gaussian kernel (B, E, H). A constrained smoothing function (Amira) was also employed for display purposes only, generating sub-voxel weights to naturally smooth the surface of the activity contour. In the example shown, this smoothing function has been applied to the filtered p -map data (C, F, I) to show the effect of the smoothing operation. [Reproduced from Yu et al. (2008). Copyright © by Elsevier, Inc.]

Figure 9.

MEMRI signal intensities were analyzed within the active IC regions defined by the 3D p -maps. Signal intensity (SI) differences (ΔSI) between sound stimulated and quiet control images were calculated in each voxel with p -value ≤ 0.05 . Color-coded 2D maps show ΔSI in coronal sections of the IC (A, B, C; color bar below). (D) The mean SI was significantly higher in 40 kHz stimulated mice than in quiet controls (65 dB vs. quiet, $p=0.00032$, 77 dB vs. quiet, $p=0.00090$, 89 dB vs. quiet, $p=0.00022$). (E) Scatter plot of the peak ΔSI vs. peak sound stimulus amplitude, showing the regression line (center) and 95% confidence bands (outer curves). [Reproduced from Yu et al. (2008). Copyright © by Elsevier, Inc.]

Figure 10.

(A) Three sagittal slices of a mouse treated with Mn^{2+} in the naris from a representative 3D T_1 -weighted MRI sequence. Note the highlighting of the olfactory bulb as well as the primary olfactory cortex leading from the bulbs. (B) Four axial slices from the same mouse treated with Mn^{2+} in the naris from a 3D T_1 -weighted MRI sequence. Note the highlighting of the outer layers of the olfactory bulbs where the olfactory glomeruli are located. In addition, the enhanced contrast continues caudally into the primary olfactory cortex. Due to the length of the scan, mice were sacrificed before 3D imaging. [Reproduced from Pautler et al. (1998). Copyright © 1998 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 11.

Sequential, oblique horizontal slices from a 3D T1-weighted MRI set of a mouse 24 h after Mn²⁺ was injected intravitreally into one eye. Note the enhanced contrast of the eye on the right, as well as the optic nerve, as compared with the control eye. In the sequence of slices from panels (A) to (F), the enhanced contrast crosses over to the contralateral side of the brain to the area of the superior colliculus. Due to the length of the scan, mice were sacrificed before 3D imaging. [Reproduced from Pautler et al. (1998). Copyright © 1998 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 12.

MEMRI images of mouse injections in the basolateral amygdala. All tracings due to Mn^{2+} accumulation within the brain are seen as hyperintense regions. From the site of injection in the amygdala (A), tracings to the entorhinal cortex (EC), subiculum (S), and the hippocampus (HC) are apparent. Starting ventrally (approx. 4.5 mm below the top of the brain) and moving dorsally in horizontal sections, in the top two panels, the aforementioned structures are clearly visible (left side of the figure). Homotopic connections to the hippocampus on the right side of the brain are also evident. In the lower left panel, projections to the fornix (F) from the hippocampus are visible. Fornix enhancements, the lateral septal nucleus (SN), and the habenulla (H) also exhibited positive contrast enhancement due to Mn^{2+} accumulation. In the lower right panel the final closure of the hippocampal formation (DHC) is visible. Note that all homotopic connections are of less signal intensity than connections on the side ipsilateral to injection (see Table 2). [Reproduced from Pautler et al. (2003). Copyright © 2003 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 13.

Sagittal (A, C), horizontal (D) and coronal (E, F) *in vivo* MEMRI of the male starling brain obtained 6 h after MnCl_2 injection into HVC. The injection area is indicated by the arrow in panel (B) and by the signal-enhanced areas on the corresponding sagittal MRI slices in panels (A) and (C). In the images, the brighter signal enhanced areas demonstrate the labeling by Mn^{2+} as is the case for the RA and area X. A schematic illustration of the song-control system redrawn based on a figure in Nottebohm (1991) is provided in (B). Panel (C) (a duplicate of panel A) illustrates the different planes of imaging for panels (D), (E) and (F). Image resolution in the coronal plane is $97\ \mu\text{m}$ (pixel size). Scale bar=10 mm. IMAN, Lateral magnocellular nucleus of the anterior neostriatum; DLM, dorsolateral nucleus of the medial thalamus; xNIIts, nucleus hypoglossus pars tracheosyringalis. [Reproduced from Van der Linden et al. (2002). Copyright © 2002 Elsevier Science Ltd.]

Figure 14.

Schematic of manganese chloride (MnCl_2) injection sites and the actual injection sites in the striatum of Case 2 (F97). (A) Schematic lateral views of the monkey brain show the injection sites in the rostral part of the right putamen (pu) and the head of the left caudate nucleus (cd). (B–D) Stereotaxic coordinates for the caudate and putamen injections with respect to the Horsley-Clark (Hc) stereotaxic coordinate system in a sagittal MR image (B) and dorsal view of the rendered brain [(C), 45 h post-injection] are shown. The horizontal and vertical dashed lines labeled Hc0 show the horizontal and frontal zero planes, respectively, in the Horsley-Clark system. The solid yellow lines indicate the coordinates of the centers of the caudate and putamen injection sites shown in (D). The red structures in (C) show the brain vasculature as determined by angiography. Blood vessels appear bright white (high signal) in the anatomical scans, as does the manganese (Mn^{2+}) signal (see D). To minimize the error in identifying tracer-containing regions, the signal originating from the vasculature was removed from the anatomical scans by subtracting the corresponding angiography scans. The green structures represent the extents of the Mn^{2+} signal at the sites of the left caudate (cd) and right putamen (pu) injections (spherical regions) and the subsequent path of the tracer in the basal ganglia. The injection coordinates for the caudate were anterior (cdA) 22.0 mm, lateral (cdL) 5 mm, and dorsal (cdD) 13.5 mm. The injection coordinates for the putamen were anterior (puA) 22.0 mm, lateral (puL) 12.5 mm and dorsal

(puD) 9 mm. In (D), the bright regions indicate the spatial extent of the Mn^{2+} signal at the caudate and putamen injection sites in coronal, horizontal, and sagittal MR images at 45 h post-injection. Both injections were well localized within the corresponding structures. The green arrow (sagittal image, bottom left) indicates the needle penetration tract for the putamen injection. The green arrowhead (sagittal image, bottom right) indicates axonal transport of Mn^{2+} from the caudate injection site. Major blood vessels are labeled with red arrows in the coronal and sagittal images. Fr0, Frankfurt zero plane; CA, carotid artery; MCA, middle cerebral artery; OA, ophthalmic artery; and PCA, posterior cerebral artery. [Reproduced from Saleem et al. (2002). Copyright © 2002 Elsevier Science Ltd.]

Figure 15.

The mouse hippocampal formation in (top) a histologic section and corresponding horizontal T1-weighted MR images (left) 2 h and (right) 6 h after intrahippocampal injection of (middle) 200 mM MnCl₂ (no. 7). Pronounced signal enhancements in the dentate gyrus (DG) and CA3 subfield are complemented by only mild signal increases in the CA1 subfield and subiculum (S). [Reproduced from Watanabe et al. (2004). Copyright © 2002 Elsevier Science Ltd.]

Figure 16.

Oblique 2D slices from the MRI 3D volumes obtained from animals in the frog (a, c, e, g) and fish (b, d, f, h) group: (A, B) Normal controls showing the Mn^{2+} -enhanced (a) frog and (b) fish ON 48 h and 96 h, respectively, after *ivit* + injection; (C, D) Mn^{2+} -enhanced ON of (b) frog and (c) fish 48 h and 96 h, respectively, after ONT and *ivit* MnCl_2 injection, with clear delineation of the lesion; (E, F) Mn^{2+} -enhanced regenerating ON of (e) frog at 1 mpl and (f) fish at 3 mpl; (G, H) Mn^{2+} -enhanced regenerating ON of (g) frog at 3 mpl and (h) fish at 6 mpl. [Reproduced from Sandvig et al. (2011). Copyright © 2003 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 17.

Time-lapse MRI of optic nerve enhancement after Mn^{2+} injection into the right eye of KLC +/+ (wildtype littermate) and KLC -/- mice. (A) Representative images from two time-lapse sequences, KLC+/+ (top) and KLC-/- mice, captured at 30 min, 1, 1.5 and 2.5 h after injection of 0.25 μ l of 200 mM Mn^{2+} into the right eye. Note that by 2.5 h after injection in the littermate, Mn^{2+} signal is present along the optic nerve, possibly reaching the chiasm. In contrast in the KLC-/- mouse, Mn^{2+} enhancement is only detected in a short segment at the beginning of the optic nerve. (B) At 24 h Mn^{2+} signal has progressed along the optic track in both genotypes, demonstrating that the optic tract is intact. (C) ROI intensity measurement (position indicated by circles in left panel) demonstrate that over the first 160 min after Mn^{2+} injection into the vitreous, intensity (the ratio of optic track vs. cheek muscle) increased in the optic tract by 150% in the wildtype KLL++ while no intensity increase was detected in the KLC-/- mouse. Error bars represent the sum of the standard deviations of the average voxel intensities in optic tract vs. cheek muscle ROIs. For KLC+/+ these were 10% and 6%, respectively and for KLC-/- 7% and 6%. [Reproduced from Bearer et al. (2007). Copyright © 2007 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 18.

(A) Successive visualization of the region of interest (ROI) tracks changes in Mn^{2+} enhanced signal intensity during the second hour post- Mn^{2+} lavage. Muscle was used to control for background effects. (B) This figure demonstrates how the ROI was determined using the length of the olfactory bulb and 90° angle to find the midpoint on the olfactory neuronal layer. Each pixel measures 0.23 mm. [Reproduced from Smith et al. (2007). Copyright © 2003 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 19.

The differences between Mn²⁺ treated and control mice (no Mn²⁺) clearly demonstrate the increased signal intensity acquired using MEMRI. Data were quantified as a function of the change in signal intensity (ΔSI) over time (min). Slope of line acquired through linear regression. *t*-Test of Mn²⁺ vs. no Mn²⁺ ^a*p*-Value<0.0001. ^a*p*-Value is significant. (Mn²⁺ 0.00587±0.001 ΔSI /time (min); n=8; No Mn²⁺: -0.00071±0.001 ΔSI /time (min); n=5). [Reproduced from Smith et al. (2007). Copyright © 2003 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]

Figure 20.

SOD-2 overexpression improves the axonal transport deficits displayed by Tg2576 mice.

(A) The graph represents the rates on axonal transport as measured *in vivo* by MEMRI in 12 to 16 months old WT, SOD-2, Tg2576 and Tg2576/SOD-2 mice. Significance was assessed by a one-way ANOVA with Dunnett's post-test for multiple comparisons. ***p*, 0.01.

[Reproduced from Massaad et al. (2010)].

Figure 21.

Mn^{2+} through the lateral olfactory tract is delayed with aging. Setting a statistical threshold of $Z=4.0$ (after Bonferroni's correction for comparison across multiple pixels), group-wise statistical maps of changes in MR intensity indicate significant peak enhancement in both anterior (blue arrow) and posterior (purple arrow) at the EARLY (11–12 h) time point. Mid-age group statistical maps indicate sub-threshold enhancement of both anterior and posterior VOI until the 24 h time point. In contrast, aged group peak z -value exceeded the threshold by 24 h in the anterior VOI, but was still sub-thresh-old in the posterior VOI. By 36 h post $MnCl_2$ administration, aged group posterior VOI reached statistical threshold. Statistical maps are shown in coronal slices superimposed onto a template MRI for localization purposes. Anterior tract=+4.6 mm, posterior tract=+0.2 mm from bregma landmark. [Reproduced from Cross et al. (2008) Copyright © 2003 John Wiley & Sons, Inc. Reprinted with permission of Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.]