Published in final edited form as:

Neuroimage. 2012 August 15; 62(2): 1009–1013. doi:10.1016/j.neuroimage.2012.01.008.

Using Manganese-Enhanced MRI to Understand BOLD

Afonso C. Silva, Ph.D.

Cerebral Microcirculation Unit, Laboratory of Functional and Molecular Imaging National Institute of Neurological Disorders and Stroke, National Institutes of Health Bethesda, MD 20892 USA

Abstract

The 1990s were designated “The Decade of the Brain” by U.S. Congress, perhaps in great anticipation of the impact that functional neuroimaging techniques would have on advancing our understanding of how the brain is functionally organized. While it is impossible to overestimate the impact of functional MRI in neuroscience, many aspects of the blood oxygenation level-dependent (BOLD) contrast remain poorly understood, in great part due to the complex relationship between neural activity and hemodynamic changes. To better understand such relationship, it is important to probe neural activity independently. Manganese-Enhanced MRI (MEMRI), when used to monitor neural activity, is a technique that uses the divalent manganese ion, Mn^{2+} , as a surrogate measure of calcium influx. A major advantage of using Mn^{2+} as a functional marker is that the contrast obtained is directly related to the accumulation of the ion in excitable cells in an activity dependent manner. As such, the contrast in MEMRI is more directly related to neural activity than hemodynamic-based fMRI techniques. In the present work, the early conceptualization of MEMRI is reviewed, and the comparative experiments that have helped provide a better understanding of the spatial specificity of BOLD signal changes in the cortex is discussed.

Keywords

animal models; blood-brain barrier; brain; cerebral blood flow; functional magnetic resonance imaging; manganese; neural activity

Introduction

Ever since the inception of BOLD fMRI in the early 1990s (Bandettini et al., 1992; Kwong et al., 1992; Ogawa and Lee, 1990; Ogawa et al., 1990a; Ogawa et al., 1990b; Ogawa et al., 1992), it became clear that a major research effort would be needed to understand the nature of the contrast per se, from its biophysical characteristics, to its complex dependence on changes in cerebral blood flow (CBF), cerebral blood volume (CBV) and metabolism (oxygen consumption), its spatial characteristics, and its temporal evolution. Manganese-enhanced MRI (MEMRI) (Lin and Koretsky, 1997; Pautler et al., 1998) was conceptualized as an attractive alternative to measure neural activity more directly, independently of the hemodynamic changes associated to changes in neural activity.

Address Correspondence to: Afonso C. Silva, Ph.D., Head, Cerebral Microcirculation Unit, NIH – NINDS, 10 Center Drive MSC 1065, Building 10 Room B1D106, Bethesda MD 20892-1065, USA, Tel: +1-301-402-9703, Fax: +1-301-480-2558, SilvaA@ninds.nih.gov.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

MEMRI is based on using the divalent manganese ion, Mn^{2+} , as a biological analogue of calcium, an essential second messenger that enters excitable cells in the central nervous system (CNS) via both ligand-gated and voltage-gated channels. MEMRI combines the efficiency of Mn^{2+} as a MRI relaxation agent (Fornasiero et al., 1987; Mendonca-Dias et al., 1983) with its rich biological properties. The efficiency as an MRI contrast agent comes from the fact that Mn^{2+} has 5 unpaired electrons in its 3d shells, which give rise to a strong magnetic moment and gives manganese high influence over the T_1 and T_2 relaxation times of surrounding water. Indeed, in his 1973 seminal paper, Paul Lauterbur used the paramagnetic properties of the $MnSO_4$ to decrease the longitudinal relaxation time of water as a means to create image contrast (Lauterbur, 1973). The rich biological properties of manganese turn the divalent ion into a particularly useful reporter of Ca^{2+} -dependent cellular processes in the CNS, the heart, the liver, and the pancreas. For example, in the CNS, Mn^{2+} can enter excitable cells via the principal routes of transport of Ca^{2+} and/or Mg^{2+} (Crossgrove and Zheng, 2004), such as the voltage-gated Ca^{2+} channels, the sodium (Na^+)/ Ca^{2+} exchanger and the Na^+ / Mg^{2+} antiporter, as well as the active Ca^{2+} uniporter in mitochondria (Takeda, 2003). Once inside the cells, Mn^{2+} can bind to proteins and nucleic acids with high affinity, often competing for binding sites with Ca^{2+} and/or Mg^{2+} . Inside neurons, Mn^{2+} is transported along axonal tracts in an anterograde manner (Sloot and Gramsbergen, 1994; Takeda et al., 1998), and is released into the synaptic cleft, upon reaching the pre-synaptic membrane, along with the neurotransmitter glutamate (Takeda et al., 1998), raising the possibility that Mn^{2+} may influence synaptic transmission in the brain (Takeda et al., 2002).

The combination of all the properties of manganese mentioned above confer to the metal a unique ability to influence MRI contrast in biological systems, especially to the major organs of the body, such as the brain, the heart, the liver and the pancreas. By exploring one or more of the features listed above, researchers have been able to utilize manganese as an MRI contrast agent according to three main application categories, reviewed extensively in (Bock and Silva, 2007; Hu et al., 2010; Koretsky and Silva, 2004; Lee and Koretsky, 2004; Pautler, 2006; Silva and Bock, 2008; Silva et al., 2004). First, manganese delivered systemically can be used to enhance contrast in the tissue and organ of interest. This class of applications has clearly benefited MRI of the brain, in which a unique, region-specific enhancement of its cytoarchitecture is obtained. Second, the movement and transport of Mn^{2+} along interconnected neuronal pathways of the CNS, including the brain and the spinal cord, confer to manganese the ability to act as an MRI-detectable neuronal tracer, which can be used to visualize functionally specific neuronal tracts. Third, because Mn^{2+} will enter excitable cells, it can be used as a marker of functional activity in the brain, the heart and the pancreas. A major advantage of using Mn^{2+} as a functional marker is that the contrast obtained is directly related to the accumulation of the ion in excitable cells in an activity dependent manner. Therefore, Activity-Induced Manganese-dependent (AIM)-MRI, as originally coined by Lin and Koretsky (Lin and Koretsky, 1997), should more directly reflect neural activity and should show better spatial localization than fMRI techniques based on detecting functional hemodynamic changes as surrogate markers of increased neural activity.

Activity-Induced Manganese-dependent (AIM)-MRI

Ever since he became a tenure-track faculty member at Carnegie Mellon University, in 1987, Alan Koretsky became interested in using MRI and MRS to measure brain function. His initial effort was in devising MRI techniques to measure CBF (for a detailed and precise account, see Alan's article in this commemorative issue), and in that context I joined the Koretsky group as a graduate student in 1992 to do my doctoral work on the recently invented arterial spin labeling (ASL) technique. At that time, the first papers on BOLD

fMRI were being published (Bandettini et al., 1992; Kwong et al., 1992; Ogawa and Lee, 1990; Ogawa et al., 1990a; Ogawa et al., 1990b; Ogawa et al., 1992), and we started following with great interest the use of hemodynamic-based techniques to measure brain function. Together with me, another graduate student, Yijun Lin, joined the Koretsky laboratory that same year. Yijun was strongly interested in learning to use MRI to measure brain function. However, she wanted to pursue a more direct pathway to measure neural activity that did not involve using the hemodynamic changes measured by BOLD and ASL. That's when Alan suggested using manganese. The properties of Mn^{2+} as a paramagnetic contrast agent with positive contrast were long known (Fornasiero et al., 1987; Lauterbur, 1973; Mendonca-Dias et al., 1983), and Alan knew from the literature that manganese behaved as a Ca^{2+} analogue in the heart (Hunter et al., 1980), the brain (Drapeau and Nachshen, 1984) and the neuromuscular junction (Kita et al., 1981; Meiri and Rahamimoff, 1972; Narita et al., 1990), entering cells via voltage-gated Ca^{2+} channels. In particular, Mn^{2+} was known to enter excitable cells during nerve action potentials through voltage-gated calcium channels, as entry was specifically blocked by the voltage-gated Ca^{2+} -channel blocker verapamil (Narita et al., 1990). Therefore, it became clear to Alan and to Yijun that there was great potential in using manganese as an MRI-marker of neural activity.

There was a lot to be done initially. Yijun chose to use an isotonic solution of MnCl_2 , injected systemically into the femoral vein, to optimize the rate of infusion and total dosage. Because of my engineering/physics background, I helped the project by building RF coils, programming pulse sequence and optimizing sequence parameters, and occasionally with rat surgery when the late Maryann Butowicz was not available. The initial results were quite informative. Yijun showed that the largest initial signal enhancement in the head were from the muscle and the cerebral ventricles, demonstrating that Mn^{2+} transport across the blood-brain barrier (BBB) was slow (Lin and Koretsky, 1997). There was no measurable cortical enhancement two-hours into the MnCl_2 infusion. To show that cortical enhancement would be obtained upon brain activation, Yijun decided to inject the animal with glutamate, the most common excitatory neurotransmitter in the mammalian CNS. Because glutamate does not cross the BBB, it was necessary to co-administer a hypertonic solution of mannitol. I had some experience in using mannitol to break the BBB in the rat (Silva et al., 1997b) and thus again I helped Yijun with the experiments. The results obtained (Lin and Koretsky, 1997) were so complete that it is important to review them here. Yijun started MRI acquisition and the infusion of MnCl_2 through the femoral vein. Fifty minutes after onset of the infusion, a hypertonic solution of mannitol was injected through the right common carotid artery to break the BBB. This did not cause significant changes in MRI signal intensity, demonstrating that a leaky BBB by itself does not cause manganese accumulation in the brain. Forty minutes after breaking the BBB, glutamate was injected through the same artery, causing a large signal enhancement on the right side of the brain. The cortex showed the largest signal enhancement, demonstrating that brain activation is necessary to cause entry of Mn^{2+} into the cells. To show that the signal enhancement was specific to glutamate-induced increases in neural activity, Yijun repeated the experiment using inhalation of 10% CO_2 in the breathing gas mixture in place of glutamate. CO_2 inhalation is a robust method to increase CBF without increasing neural activity, and it is the most common approach in calibrated fMRI (Davis et al., 1998). Inhalation of CO_2 in the presence of Mn^{2+} , after breaking the BBB, did not cause signal enhancement. However, when glutamate was given after CO_2 , a robust increase of more than 2-fold in signal intensity was obtained. Therefore, Yijun showed in a single experiment that infusion of MnCl_2 by itself does not cause parenchymal MEMRI signal enhancement, that breaking the BBB in the absence of a functional stimulus does not cause parenchymal MEMRI signal enhancement, and that the MEMRI signal changes obtained by stimulating the cortical neurons with glutamate are independent of CBF changes. Moreover, Yijun also showed that AIM-dependent MRI enhancement could be obtained by lowering the level of anesthesia, by performing

somatosensory stimulation inside the magnet, and even by injecting MnCl_2 outside the magnet while the animal was behaving in the cage (Lin and Koretsky, 1997). For all this work Yijun was awarded the 1997 Young Investigator's Rabi Award at the ISMRM meeting in Vancouver.

MEMRI of Neuronal Tracts

Another graduate student in the Koretsky lab, Robia Pautler, joined the Koretsky lab at Carnegie Mellon in 1994 and also became interested in working with manganese. Robia, however, realized that Mn^{2+} could be used as an MRI-detectable neuronal tract tracer, based on previous work showing transport of manganese in the olfactory system of the northern pike fish (Tjälve et al., 1995) and of the rat (Takeda et al., 1998; Tjälve et al., 1996). Using T_1 -weighted MRI, Robia showed that topical administration of a MnCl_2 solution to the naris as well as to the intravitreal eye space of mice would cause specific enhancement of contrast along the olfactory and visual pathways, respectively, showing for the first time that local injections of Mn^{2+} would be useful to trace functionally specific neuronal tracts with MEMRI (Pautler et al., 1998). She also showed that the observed enhancement along the respective olfactory and visual pathways were consistent with anterograde transport of manganese and the crossing of neuronal synapses (Pautler et al., 1998). In fact, Robia combined the activity-dependent properties with tract-tracing properties of Mn^{2+} to have the initial accumulation of Mn^{2+} dictated by local neural activity, and then to trace the subsequent neuronal pathways. This approach was recently used by Kai-Hsiang Chuang and colleagues to detect, in the olfactory bulbs of mice, distinct MEMRI maps of functional activity in the olfactory bulb in response to exposure of mice to different odorants such as octanal, acetophenone, or carvone (Chuang et al., 2009). Moreover, Kai-Hsiang showed that functional maps in the mitral cell layer were similar to those in the glomerular layer, and that analysis of specific Mn^{2+} signal peaks indicated that MEMRI maps can be resolved at the level of single glomeruli (Chuang et al., 2009).

Using MEMRI to Understand BOLD

In 1997, I moved to the Center for Magnetic Resonance Research (CMRR) at the University of Minnesota to do my postdoctoral training with Seong-Gi Kim. Having focused my doctoral work at Carnegie Mellon on the development of ASL techniques to measure CBF (Silva et al., 1997a; Silva et al., 1995, 1997b; Zhang et al., 1995), I wanted to extend its use to the measurement of functional CBF changes to focal brain stimulation. Coming from a basic science lab focused on rodent work, it was only natural to stick to animal models as the experimental platform. Fahmeed Hyder at Yale University in New Haven, USA, and Mathias Hoeft at the Max-Planck Institute for Neurological Research in Cologne, Germany, had developed a rodent model of somatosensory stimulation that became the best established and most utilized experimental model of focal brain activation (Hyder et al., 1994; Kerskens et al., 1996). Seong-Gi and I decided to implement the chloralose model for our own studies. A major concern at the time in the major laboratories across the globe was to characterize the spatial and temporal features of the hemodynamic response function (HRF). Two main hot issues in BOLD activation at the time were the influence of draining veins on both spatial and temporal evolution of the BOLD HRF (Chen et al., 1998; Gao et al., 1996; Menon et al., 1997; Oja et al., 1999) and the initial dip (Ernst and Hennig, 1994; Hu et al., 1997; Marota et al., 1999; Yacoub and Hu, 1999; Yacoub et al., 1999). Seong-Gi and I decided to implement an EPI-based ASL technique to obtain simultaneous BOLD and CBF measurements of the rat cortex during somatosensory stimulation (Silva et al., 2000; Silva et al., 1999). The main results were that, under well-controlled experimental conditions, the amplitude of BOLD and CBF changes over somatosensory cortex were proportionally related over a large range of CBF (Silva et al., 1999), and that the changes in CBF at the

onset of stimulation are fast and earlier than the BOLD changes (Silva et al., 2000). In addition, we were unable to detect the presence of an early negative BOLD response after the onset of stimulation, showing that in rat somatosensory cortex the CBF response is dynamically coupled to changes in oxygen consumption (Silva et al., 2000).

In 1998 Timothy Duong joined Seong-Gi Kim's laboratory as a post-doctoral fellow. Tim was also interested in learning fMRI, and the conditions were ripe for him to continue on the same line of work, studying the spatial characteristics of BOLD- and CBF-based fMRI. His first project in the lab was to compare AIM-MEMRI to the simultaneous BOLD and CBF measurements we had obtained with the EPI-ASL sequence. I was really excited about participating in these experiments, as they would be the first attempt to reproduce Yijen's results obtained a few years earlier (Lin and Koretsky, 1997). The BOLD/CBF sequence was already optimized, along with the somatosensory stimulation parameters to be used in the α -chloralose anesthetized rat (Silva et al., 1999).

Using a similar approach to the one employed by Yijen, Tim administered a solution of 120 mM MnCl_2 IV at a rate of 2 ml/hr (Duong et al., 2000). Ten minutes after onset of the MnCl_2 infusion, the BBB was broken with mannitol, and the rat forepaw was stimulated while quantitative T_1 maps were serially obtained with a 10 min temporal resolution. The resulting changes in T_1 due to the accumulation of Mn^{2+} in the primary somatosensory cortex were robust and well localized, with the highest accumulation of Mn^{2+} localized to cortical layer IV (Duong et al., 2000). There was also excellent co-registration of both BOLD- and CBF-based fMRI maps with the AIM-MEMRI maps, in demonstration that at high magnetic field strengths and high spatial resolution, hemodynamic-based fMRI techniques are spatially accurate (Duong et al., 2000).

Refinement of MEMRI Techniques to Measure Brain Function

Since the pioneering work of Lin and Koretsky (Lin and Koretsky, 1997) and the experiments of Duong and colleagues (Duong et al., 2000), brain activity in rodents has been successfully probed by many groups with MEMRI (Aoki et al., 2003; Aoki et al., 2002; Berkowitz et al., 2007; Brozoski et al., 2007; Fa et al., 2010; Fa et al., 2011; Lu et al., 2010; Lu et al., 2007; Weng et al., 2007). In these studies, MnCl_2 was infused into the bloodstream while the brain was stimulated pharmacologically or with a sensory stimulation paradigm. Local increased brain activity led to an increased Mn^{2+} influx and thus to increased contrast on T_1 -weighted MRI. While functional activation maps produced with MEMRI have the advantage of being based on calcium influx, they present a strong sensitivity to the anesthetic depth. Deep anesthesia significantly suppresses activity, making it difficult to detect signal changes. Light anesthesia, on the other hand, may induce stimulus unrelated, or spatially unspecific activation. In order to control for the anesthetic depth, a dynamic MEMRI technique was proposed to reduce non-specific signals associated with light anesthesia (Aoki et al., 2002). This technique relies on acquisition of a baseline "resting" activity signal during infusion of Mn^{2+} , but before presentation of the stimulus, to probe for quiescent rates of Mn^{2+} enhancement unrelated to the intended stimulus paradigm (Aoki et al., 2002).

Another disadvantage of MEMRI is that, in order to get sufficient accumulation of Mn^{2+} in the active regions of the cerebral cortex, it is necessary to open the BBB. This is because the cortex is located remotely from the major ventricular bodies in the brain, where transport of manganese is highest (Aoki et al., 2004; Bock et al., 2008). Disruption of the BBB is usually done with a highly concentrated ($\sim 25\%$) solution of D-mannitol, a hypertonic agent that causes temporary and reversible breakage (Neuwelt et al., 1979), or with an antibody that targets the endothelial barrier antigen (Lu et al., 2010). After BBB disruption and upon brain

stimulation, Mn^{2+} accumulates in active regions on a short time scale (minutes), but like in the case of systemic administration, once accumulated it does not leave the regions for several hours or days. The difference in Mn^{2+} influx and efflux rates offers the advantage of allowing Mn^{2+} to be delivered outside the MRI, while the animal is being stimulated or behaving in complex environments (Eschenko et al., 2010; Lin and Koretsky, 1997). A disadvantage, however, is that rapid changes in activity, and in particular, de-activation, cannot readily be followed with MEMRI.

While the BBB imposes a significant limitation to the delivery of Mn^{2+} to the cortex, regions of the brain situated adjacent to the main ventricles can be studied without the need to disrupt the BBB. This is the case of the hypothalamus, an endocrine organ in the CNS that is responsible, along with the Pit, for regulation of several aspects of the energy homeostasis system, such as appetite and maintenance of stable osmotic pressure in blood vessels and other extracellular compartments (Kuo et al., 2006). Indeed, Jimmy Bell's group in London has successfully studied hypothalamic function in the rodent brain using MEMRI without the need to disrupt the BBB (Chaudhri et al., 2006; Kuo et al., 2006; Kuo et al., 2007; So et al., 2007).

Another interesting way to avoid breakage of the BBB is to deliver manganese systemically and to perform the stimulation over many hours, in a way to guide the uptake of manganese to areas of the brain that are maintained active with the continued stimulation. This approach was successfully employed by Xin Yu, Dan Turnbull and colleagues to investigate the tonotopic organization of the mouse inferior colliculus (Yu et al., 2005), and by Eschenko and colleagues (Eschenko et al., 2010), who employed an osmotic pump to slowly release manganese over a few days, allowing mapping of the sensorimotor pathway in freely behaving rats (Eschenko et al., 2010). An advantage of such approach is that the cumulative dosage of manganese employed can be substantially reduced, minimizing toxicity concerns and potentially allowing for longitudinal studies to be carried out.

Conclusions

MEMRI allows measurement of brain function independently of hemodynamic changes and with unmatched sensitivity. As such, the contrast in MEMRI is more directly related to neural activity. MEMRI has helped with a better understanding of the spatial specificity of BOLD signal changes in the cortex (Duong et al., 2000). The accumulation is stable for long enough times to acquire high resolution images, enabling functional mapping of small brain areas such as the hypothalamic nuclei, individual glomeruli and single whisker barrels. However, there are many limitations to the functional information that can be obtained from MEMRI. First, the BBB imposes a significant limitation in the amount of manganese available at the extracellular space. While it has been possible to map neuronal activity with MEMRI without breaking the BBB (Yu et al., 2005), this approach requires an extension of the experimental time to many hours, and significant care needs to be employed to discern functionally specific activation from non-specific manganese uptake. In addition, even when the BBB is disrupted to facilitate access of manganese to the parenchyma, the disruption may not be uniform, leading to possibly incomplete maps to be obtained. Second, because of the long clearance time of Mn^{2+} , MEMRI techniques produce static activation maps that do not inform on temporal characteristics of functional brain activation. Therefore, MEMRI cannot be used to understand the temporal evolution of BOLD. Finally, because MEMRI contrast reflects accumulation of Mn^{2+} , it remains to be determined whether MEMRI can also report decreases in neural activity (i.e., deactivation).

Acknowledgments

I am most grateful to Alan P. Koretsky for critical reading of the manuscript. This research was supported by the Intramural Research Program of the NIH, NINDS.

References

- Aoki I, Ebisu T, Tanaka C, Katsuta K, Fujikawa A, Umeda M, Fukunaga M, Takegami T, Shapiro EM, Naruse S. Detection of the anoxic depolarization of focal ischemia using manganese-enhanced MRI. *Magn Reson Med*. 2003; 50:7–12. [PubMed: 12815672]
- 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, Wu YJ, Silva AC, Lynch RM, Koretsky AP. In vivo detection of neuroarchitecture in the rodent brain using manganese-enhanced MRI. *Neuroimage*. 2004; 22:1046–1059. [PubMed: 15219577]
- Bandettini PA, Wong EC, Hinks RS, Tikofsky RS, Hyde JS. Time course EPI of human brain function during task activation. *Magn Reson Med*. 1992; 25:390–397. [PubMed: 1614324]
- Berkowitz BA, Roberts R, Luan H, Bissig D, Bui BV, Gadianu M, Calkins DJ, Vingrys AJ. Manganese-enhanced MRI studies of alterations of intraretinal ion demand in models of ocular injury. *Invest Ophthalmol Vis Sci*. 2007; 48:3796–3804. [PubMed: 17652754]
- Bock NA, Paiva FF, Nascimento GC, Newman JD, Silva AC. Cerebrospinal fluid to brain transport of manganese in a non-human primate revealed by MRI. *Brain Res*. 2008; 1198:160–170. [PubMed: 18243167]
- Bock NA, Silva AC. Manganese-enhanced MRI of the brain. *Future Neurology*. 2007; 2:297–305.
- Brozoski TJ, Ciobanu L, Bauer CA. Central neural activity in rats with tinnitus evaluated with manganese-enhanced magnetic resonance imaging (MEMRI). *Hear Res*. 2007; 228:168–179. [PubMed: 17382501]
- Chaudhri OB, Parkinson JR, Kuo YT, Druce MR, Herlihy AH, Bell JD, Dhillon WS, Stanley SA, Ghatei MA, Bloom SR. Differential hypothalamic neuronal activation following peripheral injection of GLP-1 and oxyntomodulin in mice detected by manganese-enhanced magnetic resonance imaging. *Biochem Biophys Res Commun*. 2006; 350:298–306. [PubMed: 17007819]
- Chen W, Zhu XH, Kato T, Andersen P, Ugurbil K. Spatial and temporal differentiation of fMRI BOLD response in primary visual cortex of human brain during sustained visual stimulation. *Magn Reson Med*. 1998; 39:520–527. [PubMed: 9543413]
- Chuang KH, Lee JH, Silva AC, Belluscio L, Koretsky AP. Manganese enhanced MRI reveals functional circuitry in response to odorant stimuli. *Neuroimage*. 2009; 44:363–372. [PubMed: 18848997]
- Crossgrove J, Zheng W. Manganese toxicity upon overexposure. *NMR Biomed*. 2004; 17:544–553. [PubMed: 15617053]
- Davis TL, Kwong KK, Weisskoff RM, Rosen BR. Calibrated functional MRI: mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci U S A*. 1998; 95:1834–1839. [PubMed: 9465103]
- Drapeau P, Nachshen DA. Manganese fluxes and manganese-dependent neurotransmitter release in presynaptic nerve endings isolated from rat brain. *J Physiol*. 1984; 348:493–510. [PubMed: 6325673]
- Duong TQ, Silva AC, Lee SP, Kim SG. Functional MRI of calcium-dependent synaptic activity: cross correlation with CBF and BOLD measurements. *Magn Reson Med*. 2000; 43:383–392. [PubMed: 10725881]
- Ernst T, Hennig J. Observation of a fast response in functional MR. *Magn Reson Med*. 1994; 32:146–149. [PubMed: 8084231]
- Eschenko O, Canals S, Simanova I, Beyerlein M, Murayama Y, Logothetis NK. Mapping of functional brain activity in freely behaving rats during voluntary running using manganese-enhanced MRI: implication for longitudinal studies. *Neuroimage*. 2010; 49:2544–2555. [PubMed: 19896539]

- Fa Z, Zhang P, Huang F, Li P, Zhang R, Xu R, Wen Z, Jiang X. Activity-induced manganese-dependent functional MRI of the rat visual cortex following intranasal manganese chloride administration. *Neurosci Lett*. 2010; 481:110–114. [PubMed: 20600598]
- Fa Z, Zhang P, Wu W, Wang Z, Huang F, Yang L, Chang H, Xu R, Wen Z, Zhang J, Zeng Y, Jiang X. Functional mapping of rat brain activation following rTMS using activity-induced manganese-dependent contrast. *Neurol Res*. 2011; 33:563–571. [PubMed: 21708064]
- 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]
- Gao JH, Miller I, Lai S, Xiong J, Fox PT. Quantitative assessment of blood inflow effects in functional MRI signals. *Magn Reson Med*. 1996; 36:314–319. [PubMed: 8843386]
- Hu, TCC.; Waghorn, B.; Yanasak, N.; Silva, AC. Manganese-Enhanced Magnetic Resonance Imaging: Applications to Preclinical Research. In: McQueen, CA., editor. *Comprehensive Toxicology*. Oxford: Elsevier; 2010. p. 199-219.
- Hu X, Le TH, Ugurbil K. Evaluation of the early response in fMRI in individual subjects using short stimulus duration. *Magn Reson Med*. 1997; 37:877–884. [PubMed: 9178239]
- Hunter DR, Komai H, Haworth RA, Jackson MD, Berkoff HA. Comparison of Ca²⁺, Sr²⁺, and Mn²⁺ fluxes in mitochondria of the perfused rat heart. *Circ Res*. 1980; 47:721–727. [PubMed: 6774832]
- Hyder F, Behar KL, Martin MA, Blamire AM, Shulman RG. Dynamic magnetic resonance imaging of the rat brain during forepaw stimulation. *J Cereb Blood Flow Metab*. 1994; 14:649–655. [PubMed: 8014212]
- Kerskens CM, Hoehn-Berlage M, Schmitz B, Busch E, Bock C, Gyngell ML, Hossmann KA. Ultrafast perfusion-weighted MRI of functional brain activation in rats during forepaw stimulation: comparison with T2-weighted MRI. *NMR Biomed*. 1996; 9:20–23. [PubMed: 8842029]
- Kita H, Narita K, Van der Kloot W. Tetanic stimulation increases the frequency of miniature end-plate potentials at the frog neuromuscular junction in Mn²⁺-, CO₂+-, and Ni²⁺-saline solutions. *Brain Res*. 1981; 205:111–121. [PubMed: 6258705]
- Koretsky AP, Silva AC. Manganese-enhanced magnetic resonance imaging (MEMRI). *NMR Biomed*. 2004; 17:527–531. [PubMed: 15617051]
- Kuo YT, Herlihy AH, So PW, Bell JD. Manganese-enhanced magnetic resonance imaging (MEMRI) without compromise of the blood-brain barrier detects hypothalamic neuronal activity in vivo. *NMR Biomed*. 2006; 19:1028–1034. [PubMed: 16845705]
- Kuo YT, Parkinson JR, Chaudhri OB, Herlihy AH, So PW, Dhillon WS, Small CJ, Bloom SR, Bell JD. The temporal sequence of gut peptide CNS interactions tracked in vivo by magnetic resonance imaging. *J Neurosci*. 2007; 27:12341–12348. [PubMed: 17989298]
- Kwong KK, Belliveau JW, Chesler DA, Goldberg IE, Weisskoff RM, Poncelet BP, Kennedy DN, Hoppel BE, Cohen MS, Turner R, et al. Dynamic magnetic resonance imaging of human brain activity during primary sensory stimulation. *Proc Natl Acad Sci U S A*. 1992; 89:5675–5679. [PubMed: 1608978]
- Lauterbur PC. Image Formation by Induced Local Interactions: Examples Employing Nuclear Magnetic Resonance. *Nature*. 1973; 242:190–191.
- Lee JH, Koretsky AP. Manganese enhanced magnetic resonance imaging. *Curr Pharm Biotechnol*. 2004; 5:529–537. [PubMed: 15579042]
- 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]
- Lu H, Demny S, Zuo Y, Rea W, Wang L, Chefer SI, Vaupel DB, Yang Y, Stein EA. Temporary disruption of the rat blood-brain barrier with a monoclonal antibody: a novel method for dynamic manganese-enhanced MRI. *Neuroimage*. 2010; 50:7–14. [PubMed: 20026228]
- Lu H, Xi ZX, Gitajn L, Rea W, Yang Y, Stein EA. Cocaine-induced brain activation detected by dynamic manganese-enhanced magnetic resonance imaging (MEMRI). *Proc Natl Acad Sci U S A*. 2007; 104:2489–2494. [PubMed: 17287361]

- Marota JJ, Ayata C, Moskowitz MA, Weisskoff RM, Rosen BR, Mandeville JB. Investigation of the early response to rat forepaw stimulation. *Magn Reson Med*. 1999; 41:247–252. [PubMed: 10080270]
- Meiri U, Rahamimoff R. Neuromuscular transmission: inhibition by manganese ions. *Science*. 1972; 176:308–309. [PubMed: 5019787]
- Mendonca-Dias MH, Gaggelli E, Lauterbur PC. Paramagnetic contrast agents in nuclear magnetic resonance medical imaging. *Semin Nucl Med*. 1983; 13:364–376. [PubMed: 6359418]
- Menon RS, Ogawa S, Strupp JP, Ugurbil K. Ocular dominance in human V1 demonstrated by functional magnetic resonance imaging. *J Neurophysiol*. 1997; 77:2780–2787. [PubMed: 9163392]
- 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]
- Neuwelt EA, Frenkel EP, Diehl JT, Maravilla KR, Vu LH, Clark WK, Rapoport SI, Barnett PA, Hill SA, Lewis SE, Ehle AL, Beyer CW Jr, Moore RJ. Osmotic blood-brain barrier disruption: a new means of increasing chemotherapeutic agent delivery. *Trans Am Neurol Assoc*. 1979; 104:256–260. [PubMed: 121949]
- Ogawa S, Lee TM. Magnetic resonance imaging of blood vessels at high fields: in vivo and in vitro measurements and image simulation. *Magn Reson Med*. 1990; 16:9–18. [PubMed: 2255240]
- Ogawa S, Lee TM, Kay AR, Tank DW. Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc Natl Acad Sci U S A*. 1990a; 87:9868–9872. [PubMed: 2124706]
- Ogawa S, Lee TM, Nayak AS, Glynn P. Oxygenation-sensitive contrast in magnetic resonance image of rodent brain at high magnetic fields. *Magn Reson Med*. 1990b; 14:68–78. [PubMed: 2161986]
- Ogawa S, Tank DW, Menon R, Ellermann JM, Kim SG, Merkle H, Ugurbil K. Intrinsic signal changes accompanying sensory stimulation: functional brain mapping with magnetic resonance imaging. *Proc Natl Acad Sci U S A*. 1992; 89:5951–5955. [PubMed: 1631079]
- Oja JM, Gillen J, Kauppinen RA, Kraut M, van Zijl PC. Venous blood effects in spin-echo fMRI of human brain. *Magn Reson Med*. 1999; 42:617–626. [PubMed: 10502748]
- Pautler RG. Biological applications of manganese-enhanced magnetic resonance imaging. *Methods Mol Med*. 2006; 124:365–386. [PubMed: 16506430]
- 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]
- Silva AC, Bock NA. Manganese-enhanced MRI: an exceptional tool in translational neuroimaging. *Schizophr Bull*. 2008; 34:595–604. [PubMed: 18550591]
- Silva AC, Lee JH, Aoki I, Koretsky AP. Manganese-enhanced magnetic resonance imaging (MEMRI): methodological and practical considerations. *NMR Biomed*. 2004; 17:532–543. [PubMed: 15617052]
- Silva AC, Lee SP, Iadecola C, Kim SG. Early temporal characteristics of cerebral blood flow and deoxyhemoglobin changes during somatosensory stimulation. *J Cereb Blood Flow Metab*. 2000; 20:201–206. [PubMed: 10616809]
- Silva AC, Lee SP, Yang G, Iadecola C, Kim SG. Simultaneous blood oxygenation level-dependent and cerebral blood flow functional magnetic resonance imaging during forepaw stimulation in the rat. *J Cereb Blood Flow Metab*. 1999; 19:871–879. [PubMed: 10458594]
- Silva AC, Williams DS, Koretsky AP. Evidence for the exchange of arterial spin-labeled water with tissue water in rat brain from diffusion-sensitized measurements of perfusion. *Magn Reson Med*. 1997a; 38:232–237. [PubMed: 9256102]
- Silva AC, Zhang W, Williams DS, Koretsky AP. Multi-slice MRI of rat brain perfusion during amphetamine stimulation using arterial spin labeling. *Magn Reson Med*. 1995; 33:209–214. [PubMed: 7707911]
- Silva AC, Zhang W, Williams DS, Koretsky AP. Estimation of water extraction fractions in rat brain using magnetic resonance measurement of perfusion with arterial spin labeling. *Magn Reson Med*. 1997b; 37:58–68. [PubMed: 8978633]
- Sloot WN, Gramsbergen JB. Axonal transport of manganese and its relevance to selective neurotoxicity in the rat basal ganglia. *Brain Res*. 1994; 657:124–132. [PubMed: 7820609]

- So PW, Yu WS, Kuo YT, Wasserfall C, Goldstone AP, Bell JD, Frost G. Impact of resistant starch on body fat patterning and central appetite regulation. *PLoS One*. 2007; 2:e1309. [PubMed: 18074032]
- Takeda A. Manganese action in brain function. *Brain Res Brain Res Rev*. 2003; 41:79–87. [PubMed: 12505649]
- Takeda A, Kodama Y, Ishiwatari S, Okada S. Manganese transport in the neural circuit of rat CNS. *Brain Res Bull*. 1998; 45:149–152. [PubMed: 9443831]
- Takeda A, Sotogaku N, Oku N. Manganese influences the levels of neurotransmitters in synapses in rat brain. *Neuroscience*. 2002; 114:669–674. [PubMed: 12220568]
- Tjalve H, Henriksson J, Tallkvist J, Larsson BS, Lindquist NG. Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. *Pharmacol Toxicol*. 1996; 79:347–356. [PubMed: 9000264]
- Tjalve H, Mejare C, Borg-Neczak K. Uptake and transport of manganese in primary and secondary olfactory neurones in pike. *Pharmacol Toxicol*. 1995; 77:23–31. [PubMed: 8532608]
- Weng JC, Chen JH, Yang PF, Tseng WY. Functional mapping of rat barrel activation following whisker stimulation using activity-induced manganese-dependent contrast. *Neuroimage*. 2007; 36:1179–1188. [PubMed: 17537649]
- Yacoub E, Hu X. Detection of the early negative response in fMRI at 1.5 Tesla. *Magn Reson Med*. 1999; 41:1088–1092. [PubMed: 10371439]
- Yacoub E, Le TH, Ugurbil K, Hu X. Further evaluation of the initial negative response in functional magnetic resonance imaging. *Magn Reson Med*. 1999; 41:436–441. [PubMed: 10204863]
- Yu X, Wadghiri YZ, Sanes DH, Turnbull DH. In vivo auditory brain mapping in mice with Mn-enhanced MRI. *Nat Neurosci*. 2005; 8:961–968. [PubMed: 15924136]
- Zhang W, Silva AC, Williams DS, Koretsky AP. NMR measurement of perfusion using arterial spin labeling without saturation of macromolecular spins. *Magn Reson Med*. 1995; 33:370–376. [PubMed: 7760704]