# Contribution of the Extracellular Sodium Pool to the Brain's Triple Quantum Sodium MR Signal

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## ABSTRACT

The goal of this study is to estimate the contribution of the extracellular sodium pool to the triple quantum (TQ) sodium MR signal in the brain. The extracellular sodium MR signal is separated from the intracellular sodium MR signal in the *in vivo* rat brain using the thullium shift reagent, $TmDOTP^{5-}$, which does not cross the cell membrane.[1] In order to get the shift reagent (SR) across the blood brain barrier (BBB), hyperosmotic mannitol is used to temporarily break the BBB.[2] The magnitudes of the single quantum (SQ) and TQ signals from the rat's head are measured after the administration of $TmDOTP^{5-}$ (following bolus injection of mannitol to temporarily break the BBB). The results of this study indicate that within the SNR of the experiment there is very little contribution to the TQ signal from the sodium in the extracellular brain, vascular, and muscle spaces in the head.

## METHODS

Four Sprague-Dawley rats (~450 g) were prepared under isoflurane (2%), $N_2O$ (30%), and balance $O_2$, administered via endotracheal tube and volume controlled artificial respiration. The femoral artery and both common carotid arteries (CCA) were cannulated with PE-50 polyethylene catheters. The femoral artery cannula was used for the continuous monitoring of arterial blood pressure using a strain gauge transducer (to monitor the rat's status and state of anesthesia), during both the surgery and the MR scanning. During surgery rectal temperature was maintained at 37°C with a servo-controlled heating pad and a rectal thermistor, and during MR scanning, by a thermostatic cradle (with recirculating heated water). The CCA cannulas were used for the administration of mannitol and the SR (80 mM $TmDOTP^{5-}$). SQ and TQ sodium FID's were acquired on a 3T whole body MRI scanner (GE MS, Milwaukee, WI) using a custom-built, dual-tuned ($^1H/^{23}Na$), dual-quadrature, 8-pole, RF birdcage coil (5cm diameter). First, experiments were done to determine the time-course, optimal dose, and the more efficient route of administration of the mannitol. From these early experiments, bolus injection of 25% w/v mannitol into the CCA was determined to be more effective than infusion for breaking the BBB (n = 5).

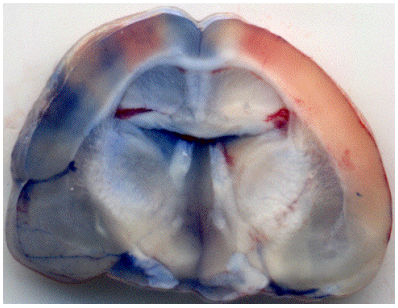

**Figure 1:** *A demonstration of the expected distribution of a shift reagent in the rat brain after breakage of the BBB. Trypan blue, a histology dye that does not cross the BBB, was injected directly into the right CCA after a bolus injection of 25% w/v mannitol into the right CCA.*

## RESULTS & CONCLUSIONS

Figure 1 demonstrates the effectiveness of mannitol administration (3.8ml, 25% w/v solution) for breaking the BBB. In Figure 1, a section from a harvested rat brain is presented where a mannitol bolus to the right CCA was followed by right CCA injection of trypan blue.[3] (Trypan blue is a stain that can not cross the intact BBB.) Figure 1 shows that the trypan blue (after mannitol) did cross the BBB on the right side of the brain leading to a cerebral-blood-flow-modulated blue staining of the brain parenchyma. Little staining is observed in the contralateral hemisphere (some staining occurs there due to circulation through the circle of Willis and late systemic redistribution of the dye). The staining demonstrated in Figure 1 was not observed with the administration of mannitol at the same concentration into the femoral vein (all body organs except the brain were stained in that case). Figure 1 also illustrates that a heterogeneous distribution of the SR is expected; therefore, the linewidth of the SR-shifted peak in the measured spectra will be affected by the ensuing spread of resonant frequencies in the SR-shifted extracellular space.

Figure 2 shows SR-aided spectra of a rat head after bolus injection of mannitol and then the SR (4.0ml of 80mM $TmDOTP^{5-}$) into both CCA's. The left spectrum is from a SQ sodium FID acquired with TE = 0.1ms, TR = 100ms, and 2410 averages, and the right spectrum is from a TQ sodium FID acquired with TE = 0.1ms, TR = 120ms, and 428 averages. Collected seven minutes after the SR injection, the SQ spectrum demonstrates the change in resonant frequency caused by the $TmDOTP^{5-}$. Two resonances are visible with a clear spread of resonant frequencies for the shifted peak (3-6 KHz frequency offset). The shifted resonance in this SQ spectrum corresponds to signals from the extracellular space in the brain as well as the vascular and muscle extracellular spaces in the head. The TQ spectrum has a peak at the center frequency, 0 KHz offset (i.e., the unshifted, intracellular sodium pool). However, there is no evidence of TQ signal from the extracellular sodium pool (i.e. at the 3-6 KHz frequency offset of the SR-shifted extracellular space).

In conclusion, these results indicate that within the SNR of the experiment there is very little contribution to the TQ signal from the sodium in the extracellular brain, vascular, and muscle spaces in the head. The findings from these experiments will have important implications for our ongoing efforts to understand the role of TQ sodium MRI for monitoring the intracellular sodium concentration in evolving focal brain ischemia.

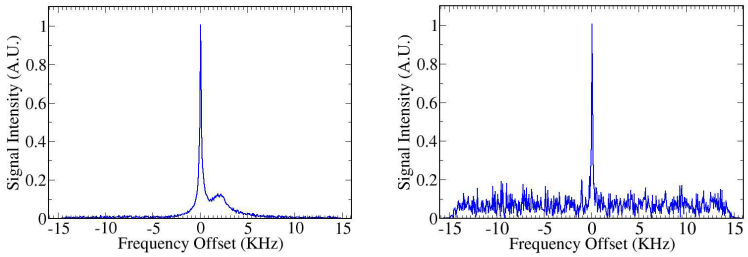


**Figure 2:** *SR-aided SQ (left) and SR-aided TQ (right) spectra of rat head after bolus injection of mannitol followed by injection of 80mM $TmDOTP^{5-}$. All boli were administered through catheters in the right and left CCA's. The SQ spectrum demonstrates the presence of a shifted resonance, which corresponds to signals from the extracellular space in the brain as well as the vascular and muscle extracellular spaces in the head. Little to zero contribution is observed in the TQ spectrum at the frequency of the SR-shifted extracellular sodium pool.*

## REFERENCES

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