Assessment of Neuronal Marker and Brain Iron in aphakia Mice Model for Parkinson's Disease Using Novel MRI Contrasts

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Introduction Iron facilitates the generation of free radicals which are thought to play a role in dopaminergic (DA) neuronal loss in PD.[(1),(2)] To evaluate the link between iron accumulation and neuronal loss, it is desirable to assess iron content and neuronal integrity in the SN of patients with PD simultaneously. *Aphakia* mice represent a highly specific mouse model of dopaminergic neuronal loss in SNc and are characterized by deficiency of the transcription factor Pitx3.[(3)] It has been shown that DA neurons are absent in SN of the Pitx3 deficient new-born mice, whereas this cell population is relatively intact in ventral tegmental area (VTA/10) and in the retrorubral field (RRF/A8)[(4)]. The most significant difference between the cell loss in *aphakia* mice and PD patients is that in the mice the absence of SN DA cells results from the developmental defects and not from a degenerative process as adults. Here, we employ $T_{1\rho}$ and $T_{2\rho}$ MRI methods recently developed in our laboratory [which are based on the relaxations during adiabatic pulses with different modulation functions] for the investigation of the specificity of $T_{1\rho}$ MRI as a measure of neuronal density and the $T_{2\rho}$ as a method for quantification of the iron loading in the brain using *aphakia* mice. In this work the correlation between $T_{1\rho}$ measurements and neuronal content and the $T_{2\rho}$ measurements with iron loading ***combined with the histological analysis and the atomic emission spectroscopy*** (for the iron concentration measurements in the brain) are conducted.

Methods MR imaging was performed at 9.4T MRI/MRS system. After the animals were positioned in the magnet, transverse multislice images were obtained with a rapid relaxation enhancement (RARE) sequence. For the relaxation measurements the TurboFLASH imaging readout [4 segments] was used. Images were measured using (0.078 mm)^2 in-plane resolution, FOV = (2 mm)^2, 256^2 matrix, and slice thickness = 0.3 mm. The $T_{1\rho}$ and $T_{2\rho}$ measurements were performed as described in prior work[(5),(6)] using variable numbers (m) of hyperbolic secant HS1 and HS4 adiabatic full passage (AFP) pulses. For $T_{2\rho}$ measurements (pulse sequences CP^{HS1} and CP^{HS4}, with hyperbolic secant HSn (n=1 or 4)[(5)] pulses in the AFP pulse train)[(6)] the AFP pulse train was placed after the coherence excitation by an adiabatic half passage (AHP) pulse and the magnetization was returned back to the longitudinal (Z') axis using another AHP pulse placed prior to the TurboFLASH imaging readout. For $T_{1\rho}$ measurements, the AFP pulse train was placed prior to the imaging readout (pulse sequences entitled $(HS1)_m$-90° and $(HS4)_m$-90° with the HS1 and HS4 pulses in the AFP pulse train, respectively)[(7)].

Results and Discussion

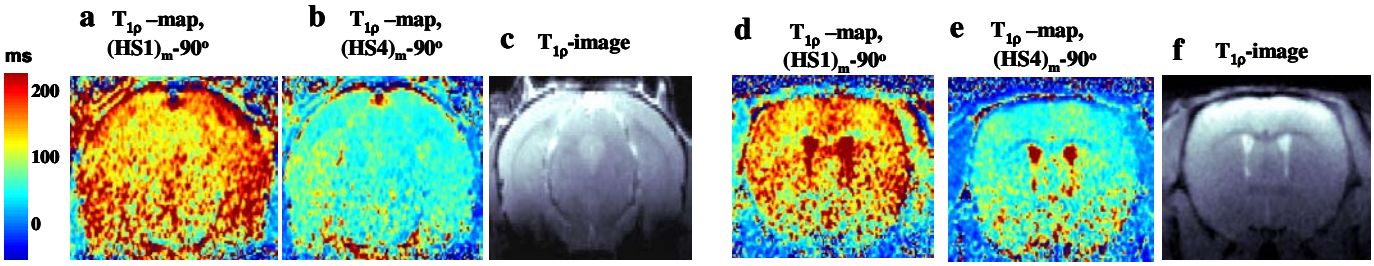

Figure 1. (a,b) Representative $T_{1\rho}$ maps obtained with the $(HS1)_m$-90° (a) and $(HS4)_m$-90° (b) pulse sequences in the area of SN of *aphakia* mice; (d,e) $T_{1\rho}$ maps obtained with the $(HS1)_m$-90° and (d) $(HS4)_m$-90° pulse sequences in the striatum. c),f) $T_{1\rho}$ images obtained at the TE= 51 ms.

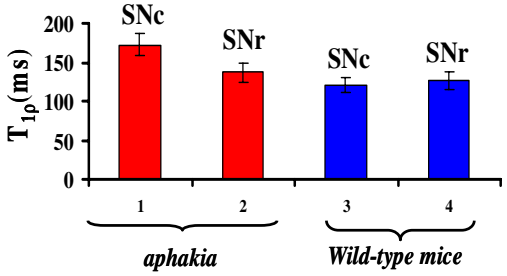

Figure 2. $T_{1\rho}$ time constants measured in the areas of SNc and SNr of aphakia and wild-type mice.

To investigate the correlation between $T_{1\rho}$ measurements and the neuronal density in the *aphakia* mice model, we performed the $T_{1\rho}$ measurements in the areas of SNc, SNr (substantia nigra pars reticular), striatum and cerebellum. The representative $T_{1\rho}$ maps are shown in the Fig. 1. It can be seen that the $T_{1\rho}$ measurements are AFP pulse modulation functions dependent. We also performed the same measurements on control mice from the same colony. Analysis was performed in a blinded manner. Our preliminary results from the scans of 7 aphakia mice and 6 controls show a statistically significant difference in the $T_{1\rho}$ in the area SNc between *aphakia* mice and the wild type mice measured with the $(HS1)_m90^o$ sequence. We have found in the SNc of *aphakia* mice $T_{1\rho}(HS1)_m90^o$ =172 ± 14 ms and in the SNc of the wild type mice $T_{1\rho}((HS1)_m90^o)$ = 121 ± 10 ms (significant difference between *aphakia* mice and controls, p<0.001, 2-tailed). The $T_{1\rho}((HS1)_m$-$90^o)$ of the wild type mice measured in the SNr were similar to that of SNc (SNr: $T_{1\rho}(HS1)_m90^o$ = 127 ± 11 ms). On the other hand, $T_{1\rho}$ in SNc and SNr (SNr: $T_{1\rho}((HS1)_m90^o)$= 137 ± 12 (ms)) in *aphakia* mice were different (Figure 2). In *aphakia* mice the deficiency of DA neurons occurs in SNc mainly, although there is also a decrease in the number of DA dendrites extending into the SNr and nerve fibers projecting to the striatum from the SNc.

Thus far iron content in the SNc of *aphakia* mice that are affected by the lack of DA neurons has not been assessed using atomic emission spectroscopy. Our preliminary results suggest, however, that $T_{1\rho}$ and $T_{2\rho}$ measurements can reflect the deficiency of DA neurons in the SNc and potentially iron accumulation, respectively, assessed by MRI. Histological analysis of neuronal density and atomic emission spectroscopy measurements of iron concentrations are underway in our laboratory.

References

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Acknowledgment This work was supported by BTRR - P41 RR008079, the Keck Foundation and the Mind Institute.