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Detection of neuronal loss using $T_{1\rho}$ MRI assessment of $^1\text{H}_2\text{O}$ spin dynamics in the aphakia mouse

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Abstract

The loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNc) is well characterized in Parkinson's disease (PD). Recent developments in magnetic resonance imaging (MRI) techniques have provided the opportunity to evaluate for changes in cellular density. Longitudinal relaxation measurements in the rotating frame ($T_{1\rho}$) provide a unique magnetic resonance imaging (MRI) contrast in vivo. Due to the specificity of $T_{1\rho}$ to water-protein interactions, the $T_{1\rho}$ MRI method has strong potential to be used as a non-invasive method for quantification of neuronal density in the brain. Recently introduced adiabatic $T_{1\rho}$ magnetic resonance imaging (MRI) mapping methods provide a tool to assess molecular motional regimes with high sensitivity due to utilization of an effective magnetic field sweep during adiabatic pulses. In this work, to investigate the sensitivity of $T_{1\rho}$ to alterations in neuronal density, adiabatic $T_{1\rho}$ MRI measurements were employed in vivo on itx3-homeobox gene-deficient aphakia mice in which the deficit of DA neurons in the SNc is well established. The theoretical analysis of $T_{1\rho}$ maps in the different areas of the brain of aphakia mouse suggested variation of the $^1\text{H}_2\text{O}$ rotational correlation times, τ_c . This suggests τ_c to be a sensitive indicator for neuronal loss during neurological disorders. The results manifest significant dependencies of the $T_{1\rho}$ relaxations on the cell densities in the SNc, suggesting $T_{1\rho}$ MRI method as a candidate for detection of neuronal loss in neurological disorders.

Keywords

$T_{1\rho}$ MRI; Substantia nigra pars compacta; Neuronal loss

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative disorder characterized by dopaminergic (DA) neuron degeneration in the substantia nigra pars compacta (SNc) (Kaur et al.,2004; Dexter

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et.al., 1991; Dexter et al., 1989; Damier et al., 1999; Berg et al., 2001; Michell et al., 2004). PD is an etiologically heterogeneous disorder and its underlying causes can be discriminated at the endophenotypic level (as defined by imaging studies); PD cases with the high-risk genetic profile have aberrant nigrostriatal pathway trajectories but PD cases with the low-risk genetic profile do not. Presently there is no method of detecting Parkinson's disease (PD) before the onset of symptoms, a fact that may hamper the development of preventative therapies. To date, the exact causes of SNc neuronal loss in PD remain poorly understood (Kaur et al., 2004).

Conventionally, MRI contrast originates from differences in longitudinal and transverse relaxation of the tissue $^1\text{H}_2\text{O}$ in the absence of radiofrequency (RF) irradiation, as described by the time constants, T_1 and T_2 , respectively. It is well understood that T_2 -weighted MRI is sensitive to exchange and diffusion of the nuclear spins between different magnetic sites in the tissues. Because of the presence of ferritin in the SNc, and ferritin's accompanying ferromagnetic properties with its induced magnetic field inhomogeneities, the MRI signal has enhanced sensitivity to water diffusion in the tissue of the SNc (Gossuin et al., 2000; Haacke et al., 2005; Jensen et al., 2001).

Unlike T_2 -weighted MRI relaxation methods, T_1 weighted MRI reflects water-protein interactions. The reason is that T_1 relaxation in vivo originates from dipolar interactions predominantly, and to some extent isochronous exchange (e.g., exchange between spins with identical chemical shifts). A field dependent inversion recovery (FDIR) T_1 MRI method promises to provide a biological marker to map out neuronal cell density in Parkinson's disease, although further studies demonstrating the technique's specificity to dopaminergic cell loss are necessary (Raff et al., 2006).

Conventionally, in vivo rotating frame $T_{1\rho}$ studies have employed pulse sequences utilizing a traditional "continuous wave" (CW) RF "spin lock," that is, with a constant irradiation frequency (ω_{RF}). Recently we developed novel MRI techniques for human studies that can be extremely helpful in providing new insights into the molecular processes characterizing neurological diseases such as PD. These methods utilize the so-called "adiabatic" pulses (based on the sweep of the radiofrequency (RF) during the pulses) and exploit a novel MRI contrast based on the rotating frame longitudinal and transverse relaxations, $T_{1\rho}$ and $T_{2\rho}$ respectively, during the adiabatic pulses. (Michaeli et al., 2004; Michaeli et al, 2006). They provide the capability to enhance the sensitivity to molecular dynamics, which are generally not accessible by conventional T_1 and T_2 MRI methods. This is due to the fact that the MR relaxations during adiabatic pulses can be "tuned" to the relevant motional regimes in heterogeneous tissue (e.g., to different characteristic time-scales of the dynamic processes). This provides a valuable tool to assess molecular motion in-vivo and to generate a unique tissue contrast in MRI (Michaeli et al., 2008). Moreover, we have used two different RF modulation functions to affect the adiabatic pulses. During adiabatic pulses the relaxation rate constant $R_{1\rho}(t) (\equiv 1/T_{1\rho})$ is time-dependent as a result of modulating the pulse functions, $\omega_1(t)$ and $\omega_{\text{RF}}(t)$. [Here, $\omega_1(t)$ and $\omega_{\text{RF}}(t)$ each have units of rad/s.] Detailed descriptions of hyperbolic secant family (HSn) adiabatic pulse modulation functions are given in (Michaeli et al., 2004; Michaeli et al, 2006). Briefly, HSn pulses are stretched versions of the hyperbolic secant pulse (Silver et al., 1984), and n denotes the stretching factor. According to this description, the original hyperbolic secant pulse is referred to as the HS1 pulse. As n becomes larger, the HSn pulse amplitude-modulation (AM) function becomes flatter. Thus, the time evolution of magnetization during an HSn pulse can change significantly with a change of n .

In specific relation to human patients with PD, we previously hypothesized that $T_{1\rho}$ may be sensitive to cellular loss, while $T_{2\rho}$ should be sensitive to iron content, since the signal in this case is affected by the diffusion in the local field gradients or exchange between magnetic sites with different chemical shifts (Michaeli et al., 2007). We anticipated that $T_{1\rho}$ and $T_{2\rho}$ MRI

techniques may provide greater ability to detect changes in neuronal loss and iron content in PD patients as compared to traditional T_1 and T_2 MRI methods. We therefore recently implemented these techniques on a 4 T magnet and embarked on a study of PD and age-matched control subjects, aiming to assess iron deposits and neuronal integrity in the SNc. First, $T_{2\rho}$ MRI, which is expected to be reflective of iron-related mechanisms, demonstrated a statistically significant difference between the PD and control group, while routine T_2 MRI did not. Second, $T_{1\rho}$ measurements, which we hypothesized to reflect neuronal density, revealed results consistent with our hypothesis of detecting neuronal loss in the SNc in PD. These preliminary results encourage using sub-millimeter resolution $T_{1\rho}$ and $T_{2\rho}$ MRI methods as quantitative non-invasive tools to assess Parkinson's disease-related pathology in vivo (Michaeli et al., 2007). With regards to $T_{1\rho}$ the capacity to non-invasively detect neural cell loss in discrete brain regions would provide a powerful tool for the study of numerous neurodegenerative conditions. Unfortunately, however, the multiple pathological changes occurring in the Parkinson's diseased brain prevented our previous study from definitively concluding that $T_{1\rho}$ is truly sensitive to neural cell loss, as other factors ranging from inflammation and iron accumulation to intracellular protein misfolding may have influenced the results. We therefore set out to specifically test the hypothesis that $T_{1\rho}$ can indeed detect selective neural cell loss.

Aphakia mice represent a highly specific model of neuronal loss in SNc and are characterized by deficiency of the transcription factor Pitx3 (Munckhof et al., 2003). It has been shown that the loss of Pitx3 in aphakia mice results in the embryonic loss of A9 DA neurons in the SNc, while A10 DA neurons in the ventral tegmental area (VTA) and A8 DA neurons in the retrorubral field (RRF) are relatively preserved (Nunes et al., 2003). Subsequently, aphakia mice have a greater than 90% decrease in dorsal striatal DA levels and a significant reduction in spontaneous locomotor activity. Thus, the distribution of DA cell loss in this model correlates with that observed in human PD. Moreover, cell loss occurs via a cell-intrinsic mechanism during development, allowing specific investigation of imaging techniques to specific cell loss without confounding factors related to inflammation and intracellular pathology, that are present in popular toxin-induced animal models of Parkinson's disease.

In this work we performed $T_{1\rho}$ measurements using different modulation functions of adiabatic pulses and applied rigorous theory for the analysis of the $T_{1\rho}$ measurements in the SNc, SNr, VTA, striatum, hippocampus and cerebellum. For the interpretation of NMR data the dynamic processes were modeled with an equilibrium two-site-exchange (2SX) system approximation (Michaeli et al., 2006). We have applied the theoretical formalisms for simultaneous rotating frame dipolar relaxation and 2SX equilibrium maintained during the adiabatic pulses. The theoretical interpretation of the experimental results enabled us to extract the fundamental parameters for tissue water dynamics, e.g., the rotational correlation times of $^1\text{H}_2\text{O}$ in different locations and the populations of the exchanging sites. These were found to be significantly different between aphakia mice versus control mice in the area of SNc, suggesting capability of the $T_{1\rho}$ method to assess neuronal cell degeneration.

METHODS

Animals

Adult homozygote aphakia mice were used for experiments, with heterozygote mice serving as controls. Founder animals were obtained from Jackson, and subsequently bred under SPF conditions in the animal facility at the University of Minnesota. Adult male mice were used to avoid potential effects of hormonal fluctuations. Studies were performed in accordance with guidelines provided by the Institutional Animal Care and Use committee at the University of Minnesota.

Tissue Processing and Immunohistochemistry

Animals were deeply anesthetized using a ketamine/Xylazine cocktail. Cardiac perfusion was then performed with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were postfixed in paraformaldehyde for 24 hours and then transferred to a 30% sucrose solution until they sank. Floating sections of 50 μ m were obtained using a freezing microtome and stored in an anti-freeze solution. Floating sections were blocked in PBS containing 5% normal donkey serum and 0.3% triton X for 2 hours. Primary antibodies, (Rb $\times$ TH or Sheep $\times$ TH, Pel-Freez) were diluted at 1:100 in block solution and incubated with sections overnight at 4 degrees C. Secondary antibody (Rabbit or Sheep Alexa 488 or 555, Invitrogen) was diluted at 1:500 in block solution. DAPI was used as a nuclear counterstain. Floating sections were mounted onto Fisher Superfrost plus slides and dried. Slides were dehydrated in increasing concentrations of ethanol followed by Xylene and coverslipped using DPX mounting media. Images were captured using a fluorescent microscope (Nikon Eclipse E600) equipped with a Spot- Insight B/W camera and Spot Advanced 3.4.5 software for windows (Diagnostic Instruments, Inc), then processed using Adobe Photoshop.

MRI protocol

MR imaging was performed with an MRI system using a Varian Unity INOVA console (Varian Associates, CA, USA) interfaced to a 31 cm bore 9.4T magnet (Oxford Magnet Technology, Oxford, UK). Shimming was performed with a fully adiabatic version of FASTMAP (Fast Automatic Shimming Technique by Mapping Along Projections) (Gruetter and Tkac, 2000). Mice were anesthetized with 1.75% isoflurane. Body temperature was monitored with a rectal probe and maintained at 37 $^{\circ}$ C with the aid of circulating water blank. Pulse rate and blood O₂ saturation levels were monitored.

After the animal was positioned in the magnet, transverse multislice images were obtained with a rapid relaxation enhancement (RARE) sequence (repetition time (TR) = 4s, echo train length = 8, echo spacing = 15 ms, TE = 60 ms, seven slices, two averages). For the relaxation measurements, the TurboFLASH imaging readout [4 segments] was used (Haase, 1990). Images were measured using (0.076 mm)² in-plane resolution, FOV = (20 cm)², 256² matrix, and slice thickness = 0.3 mm. The T_{1 ρ} measurements were performed as described in prior work (Michaeli et al., 2006) using variable numbers (m) of HS_n (n=1 and 4) AFP pulses of the HS_n family (Silver et al., 1984). The phases of the adiabatic full passage (AFP) pulses were prescribed according to MLEV-4 (Levitt et al., 1982). For T_{1 ρ} measurements, the AFP pulse train was placed prior to the TurboFLASH imaging readout. T_{1 ρ} measurements of one brain slice required ~10 minutes for each relaxation measurement, and the total scan time (including shimming, RF calibrations, scout imaging, and slice positioning) was approximately 75 minutes per animal.

Every AFP pulse had a bandwidth of 3.2 kHz and pulse length T_p set to 3 ms. TR = 4.5 s was used. The optimum RF power levels were determined using the region-of-interest (ROI) signals. Four images were collected with m = 4, 8, 12, 16, where m is number of AFP pulses in the pulse train. The peak power of AFP pulses $\omega_1^{\max}/(2\pi) \approx 3$ kHz was used. Relaxation T_{1 ρ} maps were generated from pixel-by-pixel analysis. All MR signal intensities (SI) measured with the T_{1 ρ} technique exhibited mono-exponential decay indicating a fast exchange condition. Even though with the shortest and longest pulse-train durations there is a possibility to detect intermediate/slow exchange contribution that could not be assessed due to limited data available. Thus, for the interpretation of NMR data the dynamic processes were modeled with an equilibrium 2SX system approximation, comprising of two water populations coupled by an equilibrium exchange as follows: 'A' is the water population associated with macromolecules, and 'B' is the free water population. These two sites have relaxation times T_{1 ρ ,A} and T_{1 ρ ,B} that maintain equilibrium during RF pulse. At the two exchanging sites, the

relaxation rate constants are modulated since $\omega_{\text{eff}}(t)$ is time-dependent. Thus, the sensitivity to different correlation times changes during the RF pulse. The formalism of the 2SX used in this work is described in Appendix and is valid in fast, intermediate and slow exchange regimes.

The relaxation time constant values were obtained by fitting the individual pixel signal intensity decays to monoexponential decay functions. ROIs in SNc, SNr, VTA, hippocampus, striatum, and cerebellum were obtained by carefully outlining anatomical regions in blinded manner, with the assistance of a neuroanatomy atlas. Furthermore, each image used for the $T_{1\rho}$ maps was carefully compared with the RARE images to avoid improper slice positioning. After the ROI was determined on the anatomical image on each hemisphere, the ROI was copied to the $T_{1\rho}$ maps for the calculation of the relaxation time constants at the left and right sides of the SN. The statistical analyses were performed on the mean values of the relaxation time constants obtained from the ROIs. $T_{1\rho}$ maps were generated using the MATLAB software package (MATLAB 6.1, Mathworks, MA) using monoexponential decay function. The theoretical analysis of the experimental results were conducted using Mathematica 5.2 software package.

To investigate the relation between $T_{1\rho}$ measurements and the neuronal density in the aphakia mice, we performed the $T_{1\rho}$ measurements in the areas of SNc, SNr, VTA, striatum, hippocampus, and cerebellum. We also performed the same measurements on control mice from the same colony. Consistent with previous reports, loss of DA neurons was noted in the SNc of aphakia mice, with decreased DA neuronal fiber density in the dorsal striatum, compared to control mice (Figure 1).

While it is known that there is neuronal deficiency in aphakia mice in subregions of the SN it may be inexact to separate the SN into pars compacta and pars reticulata (SNc and SNr, respectively). Presently there is much skepticism as to the ability of MRI to segment the SN into the SNc and SNr [discussion during 1st International Neuroimaging meeting on Parkinson's disease, Innsbruck, Austria Feb 2–3, 2007]. The problem arises from the fact that not a T_1 -weighted and not T_2 -weighted MRI provide sufficient contrast for the accurate determination of the ROIs, making the segmentation inaccurate. Following that, we identified the ROIs combining MR images with the histology and anatomical atlas, as illustrated in Figure 2. This procedure was developed was successfully applied for the investigation of the specificity of functional MRI signal in cat (Harel et al, 2006).

A mixed effects repeated measures analysis in which the $T_{1\rho}$ fits are treated as random effects were performed. The statistical model $y_{ijk} = \mu_i + \alpha_{j(i)} + \varepsilon_{ijk}$ was fit where $i=1,2$ denotes control or aphakia, j denotes mouse identification 1 to 9, nested within group i , and $k=1,2$ denotes the side of the brain. The $\alpha_{j(i)} \sim N(0, \sigma_a^2)$ are normally distributed mouse-specific $T_{1\rho}$ values, repeated within a treatment group, independent of the two repeated measures within the mouse $\varepsilon_{ijk} \sim N(0, \sigma^2)$, one measurement from each side of the brain. For HS1 pulse sequence, this approach yields p-values of 0.000, 0.032, 0.001, 0.131, 0.037, and 0.679 from two-sided tests of no group differences $H_0: \mu_1 = \mu_2$, for SNc, SNr, dorsomedial to SNc, hippocampus, striatum, and cerebellum respectively. These values are slightly larger than those obtained treating $T_{1\rho}$ fits as independent observations within a treatment group via the t-test. However, the same conclusions are reached concerning statistical significance.

RESULTS

Our results from the scans of 9 aphakia mice and 9 control mice show a statistically significant difference in the $T_{1\rho}$ in the area SNc between aphakia mice and the control mice measured with the (HS1)_m90° pulse sequence. We have found in the SNc of aphakia mice $T_{1\rho}(\text{HS1})_{m90^\circ} = 165 \pm 4.2$ ms and in the SNc of the control mice $T_{1\rho}(\text{HS1})_{m90^\circ} = 134 \pm 2.3$ ms (Table 1, MEAN $\pm$ SE). On the other hand, the $T_{1\rho}(\text{HS1})_{m-90^\circ}$ of the wild type mice

measured in the SNr were similar to that of SNc (Table 1). In aphakia mice the deficiency of DA neurons occurs primarily in the SNc. 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.

The analysis of the $T_{1\rho}$ maps were performed in a blinded manner. For the interpretation of NMR data the dynamic processes were modeled with an equilibrium two-site exchange (2SX) approximation, comprising two water populations coupled by an equilibrium exchange: the water population associated with macromolecules (A), and the free water population (B). For the details of the theory employed see Appendix. The representative $T_{1\rho}$ maps from the aphakia mouse and from control mouse are shown in Figures 3. It can be seen that the $T_{1\rho}$ measurements are AFP pulse modulation function dependent. A significant dependence of the relaxation time constants on the adiabatic pulse modulation functions is shown in this figure. The difference $[^{HS1}T'_{1\rho B}] - [^{HS4}T'_{1\rho B}]$ maps are shown in Figures 3 c,f for control and aphakia mice; they exhibit specificity of the adiabatic contrast to water spin dynamics in different tissue locations.

The $T_{1\rho}$ measurements in the different areas of mouse brain are summarized in Figure 4a and Table 1, and in Figure 4b the calculated values of the rotational correlation times in the SNc and striatum are summarized (see also Table 3). The calculations were performed as described in detail in Michaeli et al. (Michaeli et al., 2006) and in Appendix. For the simulations the intrinsic populations of the free water pool associated with the macromolecules $P_A=0.2$ and the free water pool $P_B=0.8$ was used (Michaeli et al., 2006, Michaeli et al., 2008). From our theoretical analysis the correlation times of water population associated with macromolecules (site A) $\tau_{cA}=8 \times 10^{-9}$ s were obtained, which is consistent with the human brain study (Michaeli et al., 2006). A significant difference between $T_{1\rho}$ values measured with the HS1 AFP pulses in the SNc was obtained in aphakia mice versus control mice ($T_{1\rho}$ (SNc)/ $T_{1\rho}$ (SNr) = 1.16, Table 1). In the same table we present the results of the $T_{1\rho}$ measurements in striatum, cerebellum and the VTA areas.

DISCUSSION

It can be seen from Figure 4 and Table 1 that smaller differences between aphakia and control mice were also observed in the VTA and striatum, consistent with the presence of decreased dopaminergic neurons and projections in the aphakia mice, however, no difference was observed in the cerebellum, which contains no dopaminergic neurons or projections. Table 2 reveals the similarity in the measurements between the aphakia and control mice as measured with the HS4 AFP pulses. This observation is in line with our previous finding, where we have demonstrated that the exchange condition is greatly modulated during the courses of AFP pulses, and is AFP pulse modulation functions dependent (Michaeli et al., 2006). An important result of the study presented is the alterations of the rotational correlation times between different areas of the brain of aphakia mice, as summarized in the Table 3. It can be seen that the correlation times of the free tumbling spin population (associated with the free water) are significantly faster in the SNc of the aphakia mouse as compared to the control mouse. This result is consistent with a decrease in the cell density in the SNc of the aphakia mouse as compared to controls (as represented by the histological slice in Figure 1) and suggest that $T_{1\rho}$ can reflect the deficiency of DA neurons in the SNc assessed by MRI.

In this work, the 2SX model was used to extract information from different water pools during neural degeneration. It should be realized that such models created to describe water dynamics in tissue are often oversimplified and thus, caution should be exercised when interpreting the results in terms of true water pools. In fact, a multi-site exchange pool models should be used to account for different relaxation processes in-vivo. However, such a models lead to a large number of the independent parameters for the data fitting, which makes implication of such a models problematic.

Interestingly, our study demonstrate that hippocampal cell loss as quantified using the $T_{1\rho}$ appears comparable to that observed in the SNc (Tables 1 and 2). Clearly, this is a noteworthy finding, and specific histologic investigation is required. Dopamine projections to adult neurogenic regions have been suggested to play an important role in regulation of neurogenesis. The relative paucity of such SNc-derived fibers in the aphakia model provides a very interesting tool to address this question. Given that quantification of hippocampal neurogenesis or cell density has not previously been performed on aphakia mice, and given the potential importance of this finding to the understanding of regulation of adult neurogenesis, as well as hippocampal development, this is a question that should be addressed in more detail in a separate manuscript.

Our $T_{1\rho}$ measurements exhibited similarity in the cerebellum between aphakia and control mice. Because DA neuron projections extend in multiple regions within the forebrain, but not in cerebellum, this similarity strongly confirms our finding on the sensitivity of $T_{1\rho}$ measurements to cellular density, including neuronal projections. In this work, we demonstrate that the adiabatic $T_{1\rho}$ technique detects the loss of the DA neurons in the SNc due to the significant difference in neuronal density between aphakia and control mice. We do not test here whether or not $T_{1\rho}$ relaxation measurements may similarly detect loss of other neuronal subtypes or glia. However, given that $T_{1\rho}$ is sensitive to $^1\text{H}_2\text{O}$ spin dynamics, we speculate that this technique would likewise be sensitive to the loss of other neuronal and non-neuronal cell types. As such, this technique may have applications in multiple conditions involving cell loss, including Huntington's disease, cerebellar ataxia and amyotrophic lateral sclerosis. The MRI method proposed here can provide quantitative tools in assessing the neural degeneration.

Our present findings are in line with our previous observations in humans, where an increase of the $T_{1\rho}$ time constants were measured in the SNc of subjects with PD as compared with controls. What was unclear after that study, however, was exactly what physiological variable in the Parkinsonian brains accounted for the increase in $T_{1\rho}$ time constant measurements. Potential factors that may impact $T_{1\rho}$ time constants could include inflammation, iron deposition, cell loss, microglial accumulation, and protein malformation. In this study, we intentionally used a simple model in which the only difference between experimental and control animals was the number of cells in a specific brain region, with loss of concomitant projections. We applied here theoretical model to estimate fundamental MR parameters in the different areas of the aphakia mice brain. The analysis show that the exchange rate constants in SNc of the aphakia mice are higher than in the wild-type mice and the correlation times are slower in controls (Table 3). In the current study, as an explanation for the changes in the $T_{1\rho}$ we observe we would not suggest that the gliosis is the mechanism of the increase in the $T_{1\rho}$ time constants in SN of aphakia mice. This would be possible if the model involved an injury, however, in this case, the cells fail to survive development via a cell-intrinsic mechanism. As such, the cells would have undergone apoptosis and not have induced a gliotic reaction. It is thought that the taking up the space - possible extra extracellular space, or possibly surrounding structures move closer to fill in the space. The agreement between the present results and those obtained in previous human subjects strongly suggests that this technique provides a sensitive measure of neuronal loss both in animal models and in neurodegenerative disease. Our findings lay the framework for future application of the adiabatic $T_{1\rho}$ technique for noninvasive longitudinal studies of neuronal loss in neurodegenerative disease.

CONCLUSION

The novel adiabatic $T_{1\rho}$ MRI relaxation method utilized here for the measurement of neuronal deficiency may provide unique information on the pathogenesis of neurological disorders, in particular PD. We demonstrated that the $T_{1\rho}$ relaxation measurements provide direct information about the fundamental dynamic parameters of the water spins in different areas of the brain. It had been shown that the change in density of neurons and their projections is

reflected in the tumbling of the water spins. This suggests that $T_{1\rho}$ measurements can potentially be used to quantify the neuronal loss in the SNc of PD patients, as well as likely other neurodegenerative conditions. The $T_{1\rho}$ measurements provide unique information as compared to conventional T_1 MRI due to the sweep of the effective magnetic field during the adiabatic pulses used for the measurements. Further studies will be warranted to confirm the applicability of our technique to additional neurodegenerative disease models, however we expect that this technique will prove capable of detecting cell loss in any given brain region of interest. In conclusion, adiabatic $T_{1\rho}$ contrast holds great potential for non-invasive detection of neural cell loss.

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APPENDIX

The MRI data for adiabatic $T_{1\rho}$ were collected with a AFP pulses of the hyperbolic secant family of pulses (HSn), using conditions and parameters which achieved magnetization inversion (i.e., adiabatic full passage), and the amplitude and frequency were modulated in the adiabatic condition. (Garwood M and DelaBarre L, 2001). Under this condition, the magnetization follows the trajectory of the effective magnetic field vector, B_{eff} . The effective field is expressed in units of rad/s, and as such, is given by $\omega_{\text{eff}} = \gamma B_{\text{eff}}$. Here γ is the gyromagnetic ratio. The amplitude and orientation of ω_{eff} are time dependent and these in turn are functions of the amplitude- and frequency modulations used for the pulse. One way to alter the modulations is to change the stretching factor, n . The amplitude modulation (AM) function of the HSn pulse becomes flatter as the factor n increases:

$$\omega_1(\tau) = \omega_1^{\text{max}} \text{sech}(\beta \tau^n), \quad (1)$$

where ω_1^{max} is the maximum pulse amplitude in rad/s and β is a constant. When $n = 1$, the pulse is the original hyperbolic secant pulse (Silver et al., 1984), whereas when $n > 1$, a stretched version of the pulse is produced (Tannus and Garwood, 1996). In the experiments performed here, all HSn pulse parameters were held constant except for the stretching factor ($n = 1$ or 4). With these adiabatic pulses, the rotating frame relaxation time constants (i.e., adiabatic $T_{1\rho}$) were measured using a preparation module consisting of consecutive identical HSn pulses, with no delays between pulses. During an adiabatic pulse the orientation of the effective frequency vector $\omega_{\text{eff}}(t)$ changes, as described by the angle $\alpha(t)$ between $\omega_{\text{eff}}(t)$ and the z' axis,

$$\alpha(t) = \tan^{-1} \left(\frac{\omega_1(t)}{\omega_0 - \omega_{RF}(t)} \right), \quad (2)$$

where $\omega_1(t)$ is the amplitude modulation function of the RF pulse, $\omega_{RF}(t)$ is the time-dependent frequency of the RF pulse, and ω_0 is the Larmor precession frequency. The time-dependent magnitude of the effective frequency vector is given by:

$$\omega_{eff}(t) = \sqrt{\omega_1^2(t) + (\omega_0 - \omega_{RF}(t))^2}. \quad (3)$$

Here, we consider the effect of an isochronous exchange ($\delta\omega = 0$ but between spins at sites A and B having different relaxation rate constants in the absence of exchange) maintaining equilibrium during the RF irradiation. The well-known equations describing the instantaneous apparent relaxation rate constants and populations of the sites A and B undergoing 2SX can be written for the time-dependent $R_{1\rho}(t)$ ($\equiv 1/T_{1\rho}(t)$) as (Woessner 1961; Woessner and Zimmerman, 1963; Lee and Springer, 2003):

$$R'_{1\rho A}(t) = \frac{1}{2} \left\{ R_{1\rho A}(t) + R_{1\rho B}(t) + k_A + k_B + \left[(R_{1\rho A}(t) - R_{1\rho B}(t) + k_A - k_B)^2 - 4k_A k_B \right]^{1/2} \right\} \quad (4)$$

$$R'_{1\rho B}(t) = \frac{1}{2} \left\{ R_{1\rho A}(t) + R_{1\rho B}(t) + k_A + k_B - \left[(R_{1\rho A}(t) - R_{1\rho B}(t) + k_A - k_B)^2 - 4k_A k_B \right]^{1/2} \right\} \quad (5)$$

$$P'_B(t) = \frac{1}{(R'_{1\rho B}(t) - R'_{1\rho A}(t))} (P_A R_{1\rho A}(t) + P_B R_{1\rho B}(t) - R'_{1\rho A}(t)); \quad P'_A(t) = 1 - P'_B(t) \quad (6)$$

Here, $R_{1\rho A}(t)$ and $R_{1\rho B}(t)$ are the intrinsic longitudinal rotating-frame relaxation rate constants of sites A and B, P_A and P_B are the intrinsic equilibrium populations (mole fractions) of spins in magnetic sites A and B, respectively, and k_A and k_B are the unidirectional rate constants for site egress (i.e., τ_A^{-1} and τ_B^{-1} , respectively). The relaxation rate constants $R_{1\rho A,B}(t)$ represent relaxation from mechanisms other than the equilibrium exchange, such as dipolar interactions or cross-relaxations. Thus, the fixed physiological parameters of an isothermal tissue system are P_A , k_A , P_B , and k_B . These are dependent of each other through the McConnell relationship: $P_A k_A = P_B k_B$ (McConnell, 1958). The measure of equilibrium kinetics is the rate constant: $k = k_A + k_B$. Equations (4)–(6) suggest that, in the presence of isochronous 2SX, the apparent rotating frame relaxation rate constant and population of the site A [$R'_{1\rho}(t)$ and $P'_A(t)$, respectively] can depend on the intrinsic parameters of site B [$R_{1\rho,B}(t)$ and P_B], and vice versa.

The intrinsic relaxation rate constant at sites A or B with no exchange is given by:

$$\overline{R_{1\rho(A,B)}} = \frac{1}{T_p} \int_0^{T_p} R_{1\rho(A,B)}(t) dt, \quad (7)$$

where T_p is the length of individual pulses. Here, the contributions from dipolar fluctuations at sites A or B during the course of AFP pulses can be calculated from (Michaeli et al., 2006; Blicharski, 1972):

$$R_{1\rho,dd}(t) = \frac{1}{10k_{dd}} \left(\frac{3\sin^2\alpha(t)\cos^2\alpha(t)}{1+\omega_{eff}^2(t)\tau_c^2} + \frac{3\sin^4\alpha(t)}{1+4\omega_{eff}^2(t)\tau_c^2} + \frac{2+3\sin^2\alpha(t)}{1+\omega_0^2\tau_c^2} + \frac{8-6\sin^2\alpha(t)}{1+4\omega_0^2\tau_c^2} \right) \quad (8)$$

where

$$\frac{1}{k_{dd}} = 2I(I+1)\hbar^2\gamma^4r^{-6}\tau_c, \quad (9)$$

Here τ_c is the correlation time for sites A and B ($\tau_{c,A}$ and $\tau_{c,B}$, respectively), r is the internuclear distance (1.58 Å for water), I and γ are the equivalent spin and gyromagnetic ratio of the nucleus, respectively, and $\hbar$ is Planck's constant. (Blicharski, 1972; Michaeli et al., 2008). Finally, the apparent populations at sites A and B are described by Eq. 10:

$$\overline{P_{A,B}} = \frac{1}{T_p} \int_0^{T_p} P'_{A,B}(t) dt \quad (10)$$

The SI decay curves were analyzed using bi-exponential decay function:

$$\frac{SI}{SI_0} = \overline{P_A} e^{-\overline{R_A}t} + \overline{P_B} e^{-\overline{R_B}t} \quad (11)$$

The theoretical description allows extract rotational correlation times τ_{cA} and τ_{cB} and exchange rate constants between two exchanging sites A and B.

The isolated identical spin pair 2SX model has only six independent parameters. Thus, to investigate exchange, we have simulated the SI decay of the tissue water measured with the (HS1)_m-90° and (HS4)_m-90° pulse sequences, using Eqs. (1)–(11), with the parameters listed in Table 3. The fundamental parameters are $\tau_{cA,B}$, $r_{A,B}$, $P_{A,B}$, and $k_{A,B}$. For the set of parameters in Table 3, the simultaneous analysis of two independent measurements of the SI decay curves with the HS1 and HS4 pulse modulations is necessary (Michaeli et al., 2006).

Figure 1.

The differences in the SNc and striatum of the aphakia and control mice obtained from histological analysis are shown. The neuronal density in the SNc and striatum were determined by counting the density of tyrosine hydroxylase (TH) neurons in aphakia mice and control mice after the MRI scans using histology for TH immunohistochemistry.

Figure 2.

Co-registration of MR image with the corresponding histological slice. a) High resolution $T_{1\rho}$ -weighted image; b) superimposed ROIs from the anatomical atlas and the MR image. c) Superimposed histological slice with the atlas and the MR image.

Figure 3.

$T_{1\rho}$ maps obtained in the slice containing SNc from one aphakia mouse using a) HS1 AFP pulses and b) HS4 AFP pulses. (c) difference map (a)-(b); $T_{1\rho}$ maps obtained in the slice containing SNc from one control mouse using d) HS1 AFP pulses and e) HS4 AFP pulses; (f) difference map (d)-(e).

Figure 4.

a) $T_{1\rho}$ time constants measured in different areas of aphakia (blue bars) and control (purple bars) mice using HS1 AFP pulses. b) Theoretical calculations of the rotational correlation times in SNc and striatum.

Table 1
Averaged calculated $T_{1\rho}$ (in ms, MEAN $\pm$ SEM) time constants measured using HS1 AFP pulses in *aphakia* and wild-type mice brain at 9.4 T [9 *aphakia* mice and 9 controls]

^a T _{1ρ} (HS1) (ms)								
SNc	SNr	SNc/SNr	VTA	Hippocampus	SNc/Hipp	Striatum	Cerebellum	SNc/Cerebellum
<i>Aphakia mice</i>								
<i>h</i> , <i>b</i> 165 ± 4.2	<i>b</i> 142 ± 5	1.16	<i>l</i> 152.8 ± 3.4	160.9 ± 4.4	1.03	<i>k</i> 155.9 ± 3	<i>d</i> 153 ± 3	1.08
<u>Control</u>								
<i>h</i> , <i>c</i> 134 ± 2.3	<i>c</i> 128 ± 3	1.05	<i>l</i> 136.2 ± 2.3	150.8 ± 3	0.89	<i>k</i> 141 ± 4.4	<i>d</i> 151.58 ± 4.7	0.88

^a Significant difference between $T_{1\rho}$ (HS1) and $T_{1\rho}$ (HS4) ($p < 0.001$, two-tailed)
^b Significant difference between SNc and SNr in *aphakia* mice ($p < 0.001$, two-tailed)
^c No significant difference between SNc and SNr in wild-type mice ($p = 0.055$, two-tailed)
^d No significant difference in cerebellum between *aphakia* and wild-type mice ($p = 0.72$, two-tailed)
^h Significant difference between SNc in *aphakia* mice and controls ($p = 0.001$, two-tailed)
^k No significant difference in striatum between *aphakia* and wild-type mice ($p = 0.177$, two-tailed)
^l Significant difference between VTA in *aphakia* mice and controls ($p = 0.002$, two-tailed)

Table 2
Averaged calculated T_{1ρ} (in ms, MEAN±SEM) time constants measured using HS4 AFP pulses in *aphakia* and wild-type mice brain at 9.4 T

^a T _{1ρ} (HS4) (ms)							
SNC	SNr	SNC/SNr	VTA	Hippocampus	SNC/Hipp	Striatum	Cerebellum
<i>Aphakia mice</i>							
<i>m</i> ₁ 86 ± 3	<i>e</i> 84 ± 3.7	1.02	77.8 ± 3.7	86.2 ± 1	1	<i>n</i> 90.5 ± 2	<i>g</i> 91.6 ± 2
<i>Control</i>							
<i>m</i> ₁ 95.6 ± 2.3	<i>f</i> 94.9 ± 1.3	1	91.6 ± 1.5	93 ± 1	1.027	<i>n</i> 82.3 ± 2	<i>g</i> 95.1 ± 1.6

^eNo significant difference between SNC and SNr in *aphakia* mice (p=0.8, two-tailed)
^fNo significant difference in cerebellum between SNC and SNr in wild-type mice (p=0.52, two-tailed)
^gNo significant difference in cerebellum between *aphakia* and wild-type mice (p=0.96, two-tailed)
^mNo significant difference in SNC between *aphakia* and wild-type mice (p=0.18, two-tailed)
ⁿNo significant difference in SNC between *aphakia* and wild-type mice (p=0.29, two-tailed)

Table 3

Simulation Parameter Values

Aphakia		
Area	$\tau_{cB}(s)$	$k_A(s^{-1})$
SNc	7.6×10^{-11}	40
SNr	9.5×10^{-11}	40
VTA	8.5×10^{-11}	40
Hippo		
campus	8×10^{-11}	40
Striatum	8.3×10^{-11}	40
Cerebellum	8.4×10^{-11}	40
Control		
SNc	13.5×10^{-11}	20
SNr	10.4×10^{-11}	40
VTA	9.4×10^{-11}	40
Hippo		
campus	8.7×10^{-11}	40
Striatum	9.4×10^{-11}	40
Cerebellum	8.5×10^{-11}	40

$I = 1/2$; $\hbar = 1.05459 \times 10^{-27}$ erg s; $\gamma = 26,75 \times 10^3$ rad/(s G); $\omega_I^{\max}/(2\pi) = 2.5$ kHz