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Why Does MTR Change With Neuronal Depolarization?

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T_1 and T_2 relaxation, and magnetization transfer (MT) of the rat brain were measured during experimentally induced spreading depression (SD). All measured MR parameters changed during SD: T_1 relaxation increased by approximately 13%, whereas the T_2 increase was substantially larger (88%). MT results showed an MT ratio (MTR) decrease of 9%. The lack of change in the MT exchange rate indicated that the MT processes between water and macromolecular protons are not affected by neuronal depolarization. The observed decrease in MTR was only caused by changes in T_1 and T_2 relaxation. Magn Reson Med 47: 472–475, 2002. © 2002 Wiley-Liss, Inc.

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The magnetization transfer ratio (MTR) has been reported (1,2) to increase during brain activation. The mechanism of this MTR change is still unknown. MTR is a phenomenological measure that is influenced by the MT exchange between macromolecular and water protons as well as by the direct effect of the radiofrequency (RF) saturation (3). There are, therefore, numerous possible mechanisms that may increase MTR during activation, such as increase in blood flow and oxygenation or changes on the macromolecular level due to neuronal polarization. The goal of this study was to measure the MT and relaxation of brain tissue during a controlled experiment of spreading depression (SD). SD is a phenomenon in which the neuronal tissue undergoes a near total depolarization, accompanied by a massive depression and rapid redistribution of ions (4,5). In the experimental setting, SD can be triggered by an application of high-intensity mechanical, chemical, or electrical stimulation to the cortical surface. Once triggered, neuronal depolarization propagates outward from the site of initiation, and entire depolarization of the hemisphere can be achieved (4,6). Recovery occurs within approximately 20 min after induced depression. SD, therefore, is an effective experimental method to probe physical processes accompanying neuronal depolarization. In this study we measured the T_1 and T_2 relaxation and MT of the rat brain prior to, during, and after SD in order to evaluate the mechanisms of MTR changes due to neuronal depolarization.

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EXPERIMENTAL METHODS

Male Wistar rats weighing 350–400 g (Charles River Laboratory, Montreal, PQ) were used. To minimize tissue swelling (specifically cortex), dexamethozone was administered by an intramuscular injection 2–4 h prior to the experiment. Following surgical anesthesia (sodium pentobarbital; 50 mg/kg), an incision (approximately 3 cm) was made along the mid-line and the skull was exposed by pulling back the muscle and the skin using a surgical clamp. One burr hole was made over one hemisphere of the cortex and the dura matter was removed. SD was achieved by chemically stimulating the cortical surface. KCl (20% by weight) was delivered to the cortical surface using a 10-gauge polyethylene tube positioned over the opening (see Fig. 1). The tubing was secured to the skull using a fast-drying adhesive and foam tape. The other end of the tube was connected to a Hamilton syringe, allowing for accurate release of KCl in steps of 0.05 mL to a total dose of 0.3 mL. Initial injection of the sodium pentobarbital was administered through I.P. injection followed by subsequent I.M. injections. The level of anesthesia was estimated by monitoring motor reflexes (hind-leg pinch) and the respiration rate. In addition, the core temperature of the animal was monitored using a rectal probe. While the rat was in the magnet, bore temperature was maintained at approximately 37°C using airflow control. To assess SD, a steady potential was measured (7) using a wire electrode made of Teflon-insulated stainless steel (approximately 75-μm diameter with a 50-μm exposed tip) inserted approximately 500 μm into the cortex. The electrode was disconnected from the measurement device following a confirmation of the SD, and the MR measurements were started.

A receive, single-loop RF coil (7 mm in diameter) was placed directly above the exposed brain hemisphere (Fig. 1) and secured using MR-compatible tape. The rat head was then placed inside an RF transmit saddle coil (30 mm in diameter) with the rat brain in its geometrical center. The rat was then placed inside the magnet with the tubing outside, allowing for KCl and anesthetic delivery without changing the experimental setup. The use of separate transmit and receive coils provided a uniform B_1 field (saddle transmit coil) necessary to perform accurate MT measurements, while measuring only the affected portion of the brain (small receive coil). The transmit and receive coils were actively decoupled to minimize their interference.

MR measurements were performed at 1.5 T using a superconducting, horizontal bore magnet (Nalorac, Martinez, CA). Pulse sequences were generated by a programmable console (SMIS, Surrey, England) configured to provide rectangular RF pulses as an input to an RF amplifier (model 3205; American Microwave Technology, Brea, CA). The duration of a π pulse was approximately 400 μs, and the

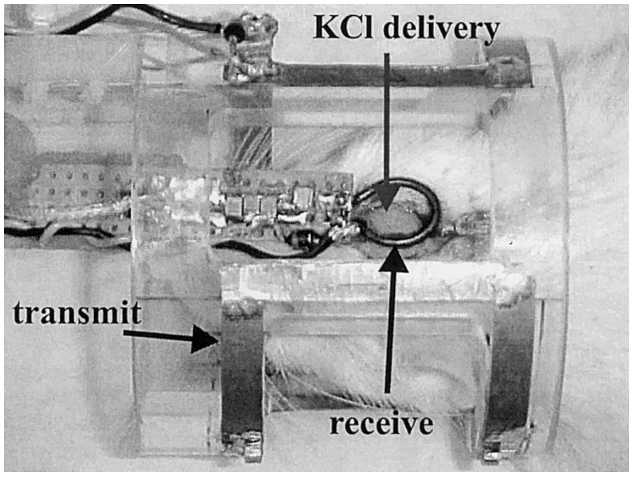

FIG. 1. Experimental setup. SD is induced by surface KCl delivery. MR experiments are performed using actively decoupled transmit and receive coils.

signal-to-noise ratio (SNR) was typically 1000 after a single $\pi/2$ excitation.

The nonimaging MR measurements consisted of the following:

1. T_1 relaxation time data were acquired using an inversion recovery (IR) sequence (8) with 12 TI values logarithmically spaced from 5 to 32 000 ms, with 10 s between each acquisition and the next inversion pulse, and two averages.
2. T_2 relaxation time data were acquired using a Carr-Purcell-Meiboom-Gill (CPMG) sequence (8,9) with TE/TR = 1/10 000 ms, 2000 even echoes sampled, and eight averages.
3. MT-weighted data were measured using a continuous wave (cw) saturation pulse of 7-s duration. For the standard MTR evaluation, the RF saturation amplitude, $\omega_1/2\pi$, was 330 Hz, and the offset frequency of the saturation, Δ, was 4 kHz. To quantitatively evaluate MT data (10) four RF saturation amplitudes ($\omega_1/2\pi$ = 85, 170, 330, and 580 Hz) and 12 off-resonance frequencies Δ (ranging from 0.1 to 100 kHz) were applied. The TR was 10 s, the number of averages was two, and the acquisition phase was cycled.

Immediately after placing the rat inside the magnet a series of baseline measurements consisting of T_1, T_2, and MTR were taken. Each measurement was repeated five times to assess the reproducibility of the MR data for the same brain over a period of 15 min.

Baseline MR measurements were followed by KCl administration up to 24 min, after which the T_2 measurements were repeated every 3 min; the results of these experiments were monitored. The KCl administration was repeated every 40 min, allowing for complete depolarization recovery. This sequence of events was repeated for T_1 and MTR measurements. The sequence of parameter measurements was randomly altered (five or six times) to avoid cumulative errors that might occur. Moreover, before and after each experimental session, T_2 decay was measured using a CPMG sequence to ensure continuity of sample signal characteristics. No changes in the T_2 decay curves were observed during these sessions.

Finally, for more detailed MT measurements after each KCl administration, the MT signal was measured for a single RF pulse amplitude, $\omega_1/2\pi$, and 12 offset frequencies, Δ; KCl was readministered and the measurement for another RF pulse amplitude was performed.

The experiments were performed for three rats with KCl injection. As a control, the same procedure was repeated for three rats with saline administration instead of KCl.

ANALYSIS

T_1 data was analyzed assuming monoexponential behavior. All T_2 decay data were analyzed using a non-negative least-squares (NNLS) algorithm (11,12) resulting in a fitted T_2 spectrum. As a single-parameter summary of these T_2 spectra, an average T_2 relaxation time, $\langle T_2 \rangle$, was calculated as the arithmetic mean of the T_2 spectrum (13).

The MTR was evaluated using the following equation:

$$MTR = \frac{M_0 - M_{SAT}}{M_0} \quad [1]$$

where M_0 and M_{SAT} denote signal amplitude measured without and with the RF saturation pulse respectively.

Quantitative MT data were fitted to a "two-pool" model (Fig. 2) quantifying the exchange between an unrestricted liquid pool (A) and a semisolid macromolecular pool (B) of restricted mobility (10). Given that the RF saturation rates in both pools, R_{rfA} and R_{rfB}, are dependent on RF irradiation amplitude and frequency off-resonance, the model estimates: R, the rate of exchange of longitudinal magnetization from pool B to A; the dimensionless parameters $1/R_A T2_A$, and RM_0^B/R_A, where R_A is the rate of longitudinal relaxation in pool A, and M_0^B is the fraction of magnetization that resides in pool B. Additionally, to investigate the effects of transverse cross-relaxation between liquid and semisolid pools on MTR, the experimental data was also fitted to the MT model (14) that includes these terms.

RESULTS

The release of KCl onto the cortical surface triggered an SD, as indicated by the rapid negative deflection of the extracellular steady potential. Following the large initial charge, a slow recovery to the baseline occurred over a period of 20–30 min.

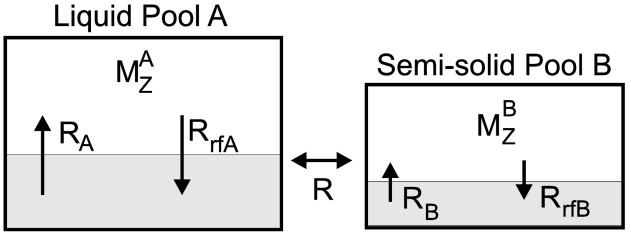

FIG. 2. Two-pool model of MT.

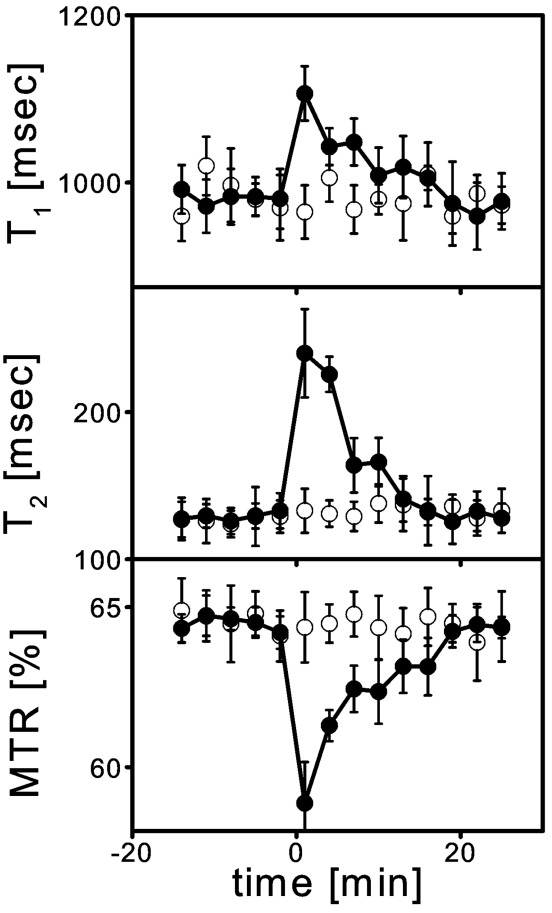

FIG. 3. T_1 and T_2 relaxation times and MTR as a function of time. Time $t = 0$ indicates KCl delivery. MTR measured for RF saturation pulse amplitude $\omega_1/2\pi = 330$ Hz and offset frequency $\Delta = 4$ kHz. Solid points = KCl; open circles = saline administration. Error bars represent biological deviations for three measured rat brains.

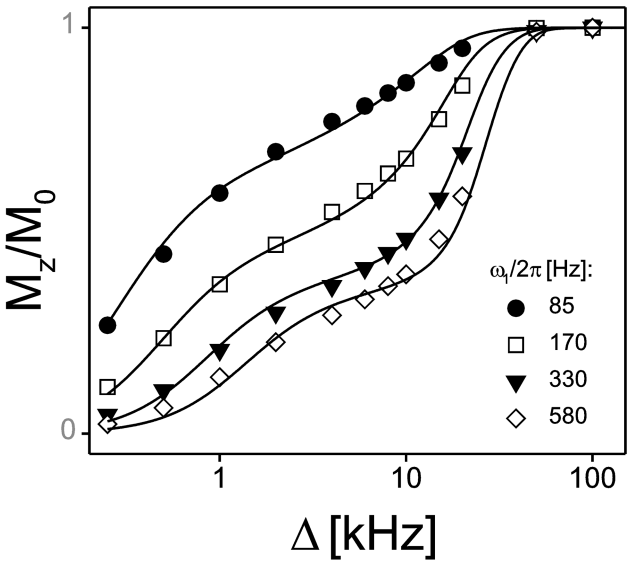

FIG. 4. MT data (data points) as a function of offset frequency, Δ, for four saturation pulse amplitudes $\omega_1/2\pi$. Solid lines represent fitted model.

The repeated baseline experiments in the same rat resulted in the T_1 measurement reproducibility of 5%, T_2 of 7%, and MTR of 0.2%. The repeated KCl administration resulted in changes in all measured MR parameters. The MR measurements with control saline administration showed no changes in the measured MR parameters.

Figure 3 shows longitudinal (T_1) and transverse (T_2) relaxation times and MTR as a function of time. Time $t = 0$ indicates KCl delivery. The error bars represent averages for three measured rat brains, and were obviously larger than the errors from the repeated baseline experiments in the same rate. Instant increase of both T_1 and T_2 relaxation times is followed by a slow return to normal, after approximately 20 min. T_1 increases after KCl delivery by approximately 13% (from 980 ± 70 to 1107 ± 33 ms), whereas the maximum T_2 change is substantially larger at 88% (from 128 ± 18 msec to 240 ± 45 ms). MTR decreases after KCl delivery from 0.65 ± 0.01 to 0.59 ± 0.02 (approximately a 9% change).

Figure 4 shows the results of the more detailed quantitative MT experiments for normal brain. The data points represent normalized signal as a function of RF pulse amplitude $\omega_1/2\pi$ and offset frequency Δ. Solid lines represent MT-fitted model curves using a super-Lorentzian absorption lineshape for the semisolid pool (15) that accurately describes the experimental data (average residual deviation of less than 2%). Table 1 shows the results of the two-pool analysis for normal and depolarized brain. The errors in the parameter estimates represent standard deviations for three measured brains and were slightly larger than the statistical errors of the fitting procedure. The exchange rate, R, for depolarized brain is similar to that of the normal brain. The MT model parameter RM_{0B}/R_A slightly decreases with depolarization while $1/R_AT2_A$ significantly decreases. The T_2 relaxation time of the semisolid pool B, $T2_B$ remains unchanged.

DISCUSSION

The release of KCl onto the cortical surface triggers an SD, as indicated by a rapid negative deflection of the extracellular steady potential (7). All measured MR parameters change during experimentally induced SD. T_1 relaxation time increases by approximately 13% with depolarization. Increase in the transverse relaxation time, T_2, is substantially larger (88%). MT results show an MTR decrease (9%). No change in the MT exchange rate, R, indicates that the MT exchange process between water and macromolecular protons is *not* affected by neuronal depolarization. Changes in the two-pool model parameters RM_{0B}/R_A and $1/R_AT2_A$ are consistent with increased T_1 and T_2 relaxation during SD. Incorporation of a transverse relaxation

Table 1
Estimated Two-Pool MT Parameters for Control and SD Brain

	R [sec^{-1}]	RM_0^B/R_A	$1/(R_AT2_A)$	$T2_B$ [μsec]
Normal	29 ± 3	2.1 ± 0.2	17.4 ± 0.6	7.6 ± 0.2
Depolarized	30 ± 4	1.7 ± 0.3	8.5 ± 0.8	7.5 ± 0.3

Errors represent biological deviations for three measured brains.

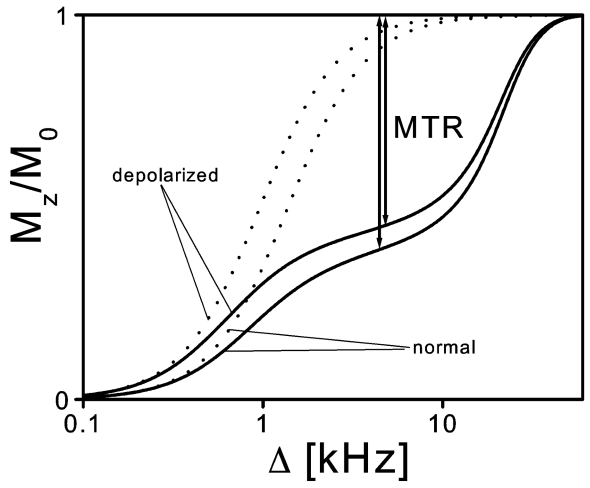

FIG. 5. MT curves (solid lines) and direct effect (dashed) for normal and depolarized brain. MTR is indicated by arrows.

term (14) did not change the fitted values of the exchange rate R, nor did it improve the quality of the fit significantly. However, it did decrease the value of parameter $1/R_A T2_A$ by approximately 30%, as would be expected.

MTR is a phenomenological measurement that has been shown to depend on the amount of MT and the direct saturation of free water by the RF pulse (3). Figure 5 shows the MT curves and direct effect for normal and depolarized brain as calculated using fitted parameters of the two-pool model analysis (Table 1). The direct effect curve in the case of depolarization is shifted towards smaller offset frequencies, Δ, which is mainly caused by the increase in T_2 relaxation. The offset frequency at the half maximum saturation $\Delta_{1/2}$ is given by (10): $\Delta_{1/2} = \omega_1/2\pi(T_1/T_2)^{1/2}$, and decreases by approximately 400 Hz during depolarization. Consequently, the MTR (as indicated by arrows in Fig. 5) decreases. However, the true MT effect, as defined as the maximum difference between the direct effect and the MT curve (16), remains unchanged, although the offset frequency at which the maximum MT effect occurs decreases.

In contrast, MTR has been reported to increase during activation, which has been attributed to increased blood flow and oxygenation (1,2). In the case of SD these variables remain constant; however, MTR decreases due to T_2 changes related to neuronal depolarization.

CONCLUSIONS

SD results in changes in the MR properties of the brain. Most dominant is an almost twofold increase in the T_2 relaxation, with a much smaller increase in T_1. The mechanism for these relaxation changes is not known. The small decrease in MTR with chronic depolarization is secondary to the changes in relaxation, and does not represent any change in MT exchange to macromolecules.

Whether the observed MTR changes reported for acute neuronal activation are also secondary to relaxation changes remains a question for further study.

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