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White Matter Glucose Metabolism during Intracortical Electrostimulation: A Quantitative [^{18}F]Fluorodeoxyglucose Autoradiography Study in the Rat

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[^{18}F]Fluorodeoxyglucose (FDG) autoradiography was used to analyze the effects of intracortical electrostimulation on local cerebral metabolic rate for glucose (LCMRglu). The hindleg area in rat brains was electrically stimulated with different frequencies (0, 0.1, 0.25, 0.5, 0.75, 1 stimulus trains per second, two animals per condition). The major result was a strong positive correlation between stimulation frequency and LCMRglu in the callosal fibers originating in the stimulated cortical area. At the highest stimulation frequency callosal LCMRglu was 50.01 μmol/min/100 g compared to 27.87 μmol/min/100 g at baseline. LCMRglu in gray and white matter control areas was stable across conditions. Direct injection of FDG in the stimulated cortex failed to produce increased callosal uptake, excluding the possibility that FDG uptake in the corpus callosum is related to axonal diffusion. Although several previous autoradiographic studies have demonstrated alterations in LCMRglu in white matter, correlations between neural activity and LCMRglu have never been explicitly addressed. Changes in white matter metabolism most likely reflect changes of electrical fiber activity and thus the presented results bear important implications for brain imaging studies.

Key Words: **white matter glucose metabolism; autoradiography; [^{18}F]Fluorodeoxyglucose; positron emission tomography; neural connectivity.**

INTRODUCTION

White matter represents the brain's information highway. The major unit of information transfer is the action potential whose production and propagation along the axons requires energy (Aiello and Bach-y-Rita, 2000). It is therefore reasonable to assume that increased rates of information transfer are associated with higher energy demand. Thus monitoring the energy supply of white matter may yield important information on the traffic on the highway, and with it on cerebral communication. This is especially interesting since the brain's energy consumption, e.g., glucose metabolism, can be evaluated noninvasively in humans using positron emission tomography (PET). Due to the tight coupling of local cerebral metabolic rate for glucose (LCMRglu) and regional cerebral blood flow (rCBF) monitoring the latter with [^{15}O]H_2O PET or fMRI may yield similar information. Despite these interesting aspects only few studies have explicitly addressed LCMRglu or rCBF in white matter. In their seminal [^{14}C]deoxyglucose (DG) study, Sokoloff and colleagues (1977) found an increase in LCMRglu of about 30 % in the corpus callosum of concious compared to anesthetized animals. Several other [^{14}C]DG studies found changes in white matter metabolism induced by e.g. electrical stimulation (Sharp and Evans, 1982) or drug administration (Duncan *et al.*, 1998). In human functional imaging, to our knowledge only one study investigated metabolic changes in white matter. Using [^{18}F] fluorodeoxyglucose (FDG) and PET, Karbe *et al.* (1998) analyzed glucose metabolism in bilateral cortical speech areas and in the connecting fibers of the corpus callosum.

Here we report the results of an [^{18}F]FDG autoradiography experiment in rats which investigated LCMRglu in electrically stimulated cortical areas as well as in the connecting fibers of the corpus callosum. The aim of the present study was to explore whether white matter energy consumption shows similar dependencies on neural activity as gray matter. Such a finding would have important implications for functional neuroimaging.

METHODS

Animals

Fifteen adult male Sprague–Dawley rats weighing 280–380 g were studied. Twelve hours before the experiment food was withheld with free access to water.

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The experiments were approved by the local veterinary authorities.

Experimental Procedure

Surgery was performed under halothane anesthesia and involved the placement of an arterio-venous shunt from the right femoral artery to the right femoral vein, tracheotomy for mechanical ventilation and craniotomy for the placement of a tungsten stimulation microelectrode in the right hindleg area, (1 mm posterior and 2 mm lateral to Bregma at 1.5 mm depth (Neafsey *et al.*, 1986). The stimulation position was adjusted to avoid large superficial blood vessels. Only one electrode penetration was performed per animal. The actual experiment was then performed under α-chloralose anesthesia according to the protocol described by Bonvento *et al.* (1994). The shunt was used for the continuous monitoring of arterial blood pressure, the collection of blood samples for the determination of plasma glucose, PaO_2, $PaCO_2$, and pH, the injection of the FDG at the beginning and pentobarbital at the end of the experiment and the continuous measurement of whole blood ^{18}F-activity. For the latter purpose the shunt was run through a coincidence probe that stored the data at 1-s intervals. The on-line arterial sampling procedure is described in detail elsewhere (Weber *et al.*, 2002). The electrical stimulation parameters (monopolar cathodal pulses, current 120 μA, duration 0.25 ms, trains of 30 pulses at 330 Hz, at train frequencies of 0, 0.1, 0.25, 0.5, 0.75 1 Hz, 2 experiments at each frequency) were kept constant during the entire experiment and produced movements of the contralateral hindleg. After penetration of the dura, halothane anesthesia was discontinued. To minimize the effects of spreading cortical depression, the experiment was started 30 minutes after the penetration of the dura (Lacombe *et al.*, 1992). The stimulation was started immediately before 130–190 MBq [^{18}F]FDG were injected over 35 seconds using an injection pump. The animals were sacrificed 45 min later using intravenous pentobarbital. The brain was quickly removed and frozen in chilled isopentane. For high-resolution autoradiographic images selected 10 μm slices were directly thaw-mounted (Duncan, 1992) on film (Kodak Biomax MR). For the delineation of anatomical structures some slices were stained with methylgreen-pyronin. For quantification brain slices (10-μm thickness, slice distance 200 μm) were placed on a phosphor imaging screen together with ^{14}C standards (calibrated for 10 μm tissue equivalents) for 240 min. Tritium sensitive screens (Fuji TR2025) were employed as their uncoated, thin sensitive layer yields higher resolution ^{18}F autoradiographies than ordinary screens. The data were then scanned in (Fuji BAS 1800 II, pixel size 50 μm) and converted to kBq/mg.

Quantification of Cerebral Glucose Metabolism

The procedure followed the [^{14}C]deoxyglucose method described by Sokoloff *et al.* (1977). It is based on a two-tissue compartment model. The exchange of ligand between the compartments is defined by the rate constants K1–k3. The operational equation used in this work takes into account changing plasma glucose levels during the experiment (Savaki *et al.*, 1980). It requires the knowledge of the cerebral FDG concentration at a specific time-point after injection (45 min) and the time-course of the FDG concentration in arterial plasma (input curve). The latter was obtained by multiplying the whole-blood values from the coincidence probe with a conversion function which had previously been established in three animals (Weber *et al.*, 2002). Values for K1–k3 used were 0.30, 0.40, 0.068 for gray matter (Ackermann and Lear, 1989) and 0.13, 0.22, 0.03 [min-1] for white matter. A lumped constant of 0.58 was chosen. The calculation was implemented in the software PMOD (Mikolajczyk *et al.*, 1998), which yielded values for glucose consumption in each pixel. Regions of interest (ROIs) were then defined over the stimulated cortex, the homotopic area contralaterally and the connecting corpus callosum. Additional ROIs were chosen over nonactivated parietal cortex and corpus callosum. The mean glucose consumption of all pixels in each ROI in five slices was calculated.

Direct Cortical Injection of [^{18}F]FDG

To exclude potential axonal diffusion of ^{18}F-activity [^{18}F]FDG was directly injected into the cortex. In three additional animals a pulled glass pipette filled with [^{18}F]FDG was inserted instead of the tungsten microelectrode. A thin silver wire ran through the pipette to deliver the electrical stimulation through the pipette. 0.5 μl [^{18}F]FDG (12 MBq/ml, concentration adjusted to produce similar cortical uptake as intravenous administration) was injected over 2 min using a microsyringe. The stimulation was performed at 1 stimulus train per second with the stimulation parameters described above.

RESULTS

Arterial blood pressure ranged from 110 to 130 mmHg and remained stable during the experiment in all animals. The values for $PaCO_2$, PO_2, oxygen saturation and pH were in the physiological range. Hematocrite values of all animals were in a narrow range (0.44–0.48). Representative autoradiographs (film exposure) from experiments with different stimulation frequencies are demonstrated in Fig. 1. Already visual inspection reveals an increase of FDG uptake with increasing stimulation frequency in the cortical stimulation site, the homotopic contralateral area and the

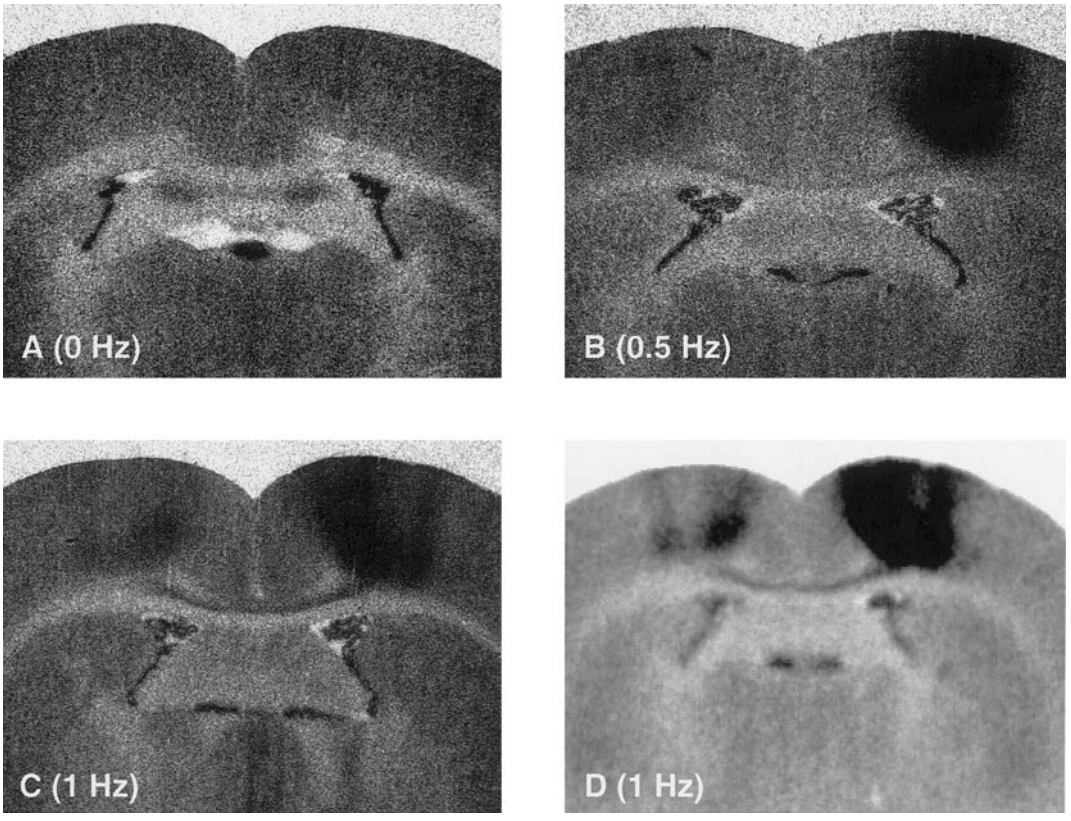

FIG. 1. Coronal [^{18}F]fluorodeoxyglucose (FDG) autoradiographs 45 min following injection of FDG. (A–C, thaw-mounted sections on film; D, phosphor imaging screen). The stimulation frequency was 0 in A, 0.5 in B, and 1 train per second in C and D. Visual inspection indicates increased glucose utilization with increased stimulation frequency in the stimulated hindleg cortex, the contralateral homotopic region and in the connecting fibers of the corpus callosum. Image resolution is highest on film. However, a serious disadvantage of film is demonstrated by the reduced contrast of stimulated area and surrounding cortex compared to the images from the phosphor imaging screen. This is due to saturation effects. Due to its large linear dynamic range the phosphor imaging screen is more suitable for quantification.

corpus callosum. The relationship of glucose metabolism and stimulation frequency is demonstrated in Fig. 2. In the corpus callosum adjacent to the activated fibers as well as in the cortical control region glucose metabolism was stable and the values show little variation among the animals (25.94 ± 2.28 and 48.31 ± 4.47 μmol/min/100 g, mean ± SD for white matter and cortex, respectively). In all other examined areas there was a clear increase of LCMRglu with stimulation frequency. In the corpus callosum the relationship of LCMRglu and stimulation frequency was linear and LCMRglu increased from 27.87 at baseline to 50.01 μmol/min/100 g at 1 Hz (mean of the two experiments). In the cortical stimulation area and the homotopic contralateral region LCMRglu increased from 47.98 to 288.56 and from 47.64 to 123.45 μmol/min/100 g respectively. In the stimulated area the incremental increase seems to taper off with the stimulation frequency.

In contrast, no evidence of ^{18}F-activity was found in the corpus callosum or any other structure outside the stimulation area following microinjections of FDG directly into the stimulation site prior to the beginning of stimulation (data not shown).

DISCUSSION

In the present study we provide evidence for a close relationship between glucose utilization and stimulation frequency in gray and white matter. Intracortical stimulation of the rat hindleg area produced increased local cerebral metabolic rates for glucose (LCMRglu) in cortical regions of the ipsilateral and contralateral hemisphere. However, the most important and new finding is the linear positive correlation between stimulation frequency and LCMRglu in the corpus callosum. This callosal activation was restricted to fibers connecting the stimulation site with the contralateral homotopic area. The pathways of the rat motor cortex have been studied extensively in several [^{14}C]deoxyglucose (DG) experiments (Sharp and Evans, 1982; Sharp, 1984; Sharp *et al.*, 1988).

Although it has been known since the introduction of the DG method (Sokoloff *et al.*, 1977) that the level of

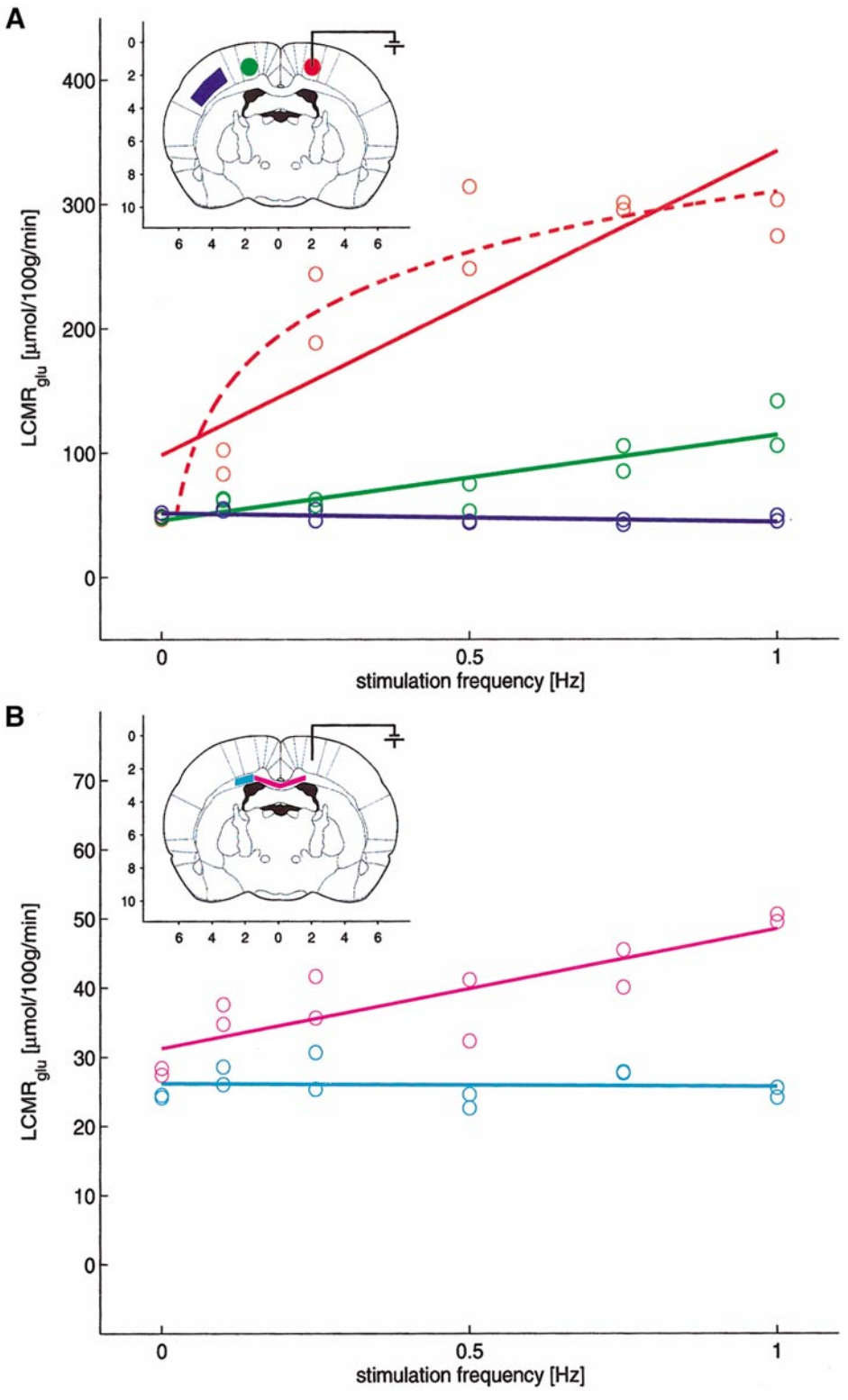

FIG. 2. Relationship between glucose metabolism and stimulation frequency. Increasing the stimulation frequency results in increased local cerebral metabolic rate for glucose (LCMRglu) in cortex (A) and in the corpus callosum (B), while control areas remain stable. Insets indicate localization of ROIs: red, stimulated hindleg cortex; green, contralateral homotopic cortex; blue, parietal cortex (control area); magenta, connecting fibers in the corpus callosum; cyan, nonactivated corpus callosum (control area). X- and Y-axis of insets represent the distance in mm from Bregma. Regression parameters (LCMRglu and stimulation frequency SF). Stimulated area log-fit: LCMRglu = 137.19 * log10(SF) + 294.4216, $F(1,10) = 57.52$, $P < 0.0001$, linear fit LCMRglu = 244.34 SF + 98.20, $F(1,10) = 28.86$, $P = 0.0003$; contralateral homotopic area: LCMRglu = 68.41 SF + 45.77, $F(1,10) = 35.33$, $P = 0.0001$; nonactivated parietal cortex: LCMRglu = −7.22 SF + 51.45, $F(1,10) = 5.61$, $P = 0.04$); activated corpus callosum: LCMRglu = 17.29 SF + 31.20, $F(1,10) = 27.05$, $P = 0.0004$; nonactivated corpus callosum: LCMRglu = −0.46 SF + 26.14, $F(1,10) = 0.0549$, $P = 0.82$.

anesthesia alters LCMRglu in white matter, only very few studies applying either autoradiographic or computed tomographic methods have analyzed white matter metabolism in detail. In accordance with the present study, Sharp *et al.* (1988) found an increased LCMRglu in the corpus callosum of the awake rat. However, they did not vary the stimulation strength and hence could not elaborate a stimulus-response relationship in white matter.

Increased LCMRglu was occasionally found in other white matter areas such as the internal capsule. However, the increase was not consistently found in all animals and was not as well correlated to stimulation frequency as in the corpus callosum. This might be attributed to the relatively long experimental period in which the threshold of motor responses varied considerably. At times the hindleg movements even ceased in the course of the experiment. To investigate activity-dependent LCMRglu in the corticospinal tract shorter experimental protocols would probably be more appropriate. It is known that central fiber tracts utilize glucose. Studies of the optic tract showed that axonal conduction and survival is severed after prolonged glucose withdrawal (Fern *et al.,* 1998). The energy demand in brain tissue is believed to be mainly related to the maintenance of ionic gradients by active pumps (Mata *et al.,* 1980; Erecinska and Dagani, 1990). In the case of myelinated axons, there is an increased density of sodium/potassium pumps at the nodes of Ranvier, whereas in unmyelinated fibers the energy demand is probably generally higher and more equally distributed along the axon (Aiello and Bach-y-Rita, 2000). The proportion of myelinated and unmyelinated axons in the corpus callosum is dependent on the studied species and above all on the developmental state. It appears that in adult rat brains at least half of the callosal axons remain unmyelinated (Innocenti, 1986).

One could raise the question whether the increased FDG uptake in the corpus callosum does indeed reflect increased local glucose metabolism or whether it could be caused by diffusion of FDG or metabolites along the axon. To test this possibility FDG was directly injected in the cortical stimulation area. The lack of callosal ^{18}F-activity in these experiments clearly speaks against axonal diffusion. Whether glucose is directly taken up by axons or whether energy supply is mediated by glial cells as proposed in gray matter (Magistretti and Pellerin, 1999) remains to be shown.

Of the overwhelming number of neuroimaging studies only one PET study (Karbe *et al.,* 1998) reported and interpreted changes in LCMRglu in white matter. The authors engaged subjects in a word repetition paradigm and reported significant inverse correlations between LCMRglu in the midbody and isthmus of the corpus callosum and the activated bilateral cortical areas relevant for speech processing. They argued that for hemispherically asymmetric information processing net callosal transfer is decreased by collateral inhibition.

The lack of reported white matter signal change might be explained by the relatively small alterations in the metabolic rate as compared to gray matter. Future studies will show whether the present results can be replicated when applying more subtle physiological, e.g., sensory stimulation. Another point of discussion is a possible effect of the applied anesthesia. It has been shown previously that α-chloralose can increase the cortical excitability. However, it seems very unlikely that the observed changes in white matter metabolism can be attributed to the anesthesia only, since similar callosal activity has been found in the awake rat (Sharp *et al.,* 1988).

Not surprisingly, strong correlations with stimulation frequency were also found in the cortex surrounding the stimulation electrode and in the contralateral homotopic area. As expected the highest increase of LCMRglu was found in the stimulation area. The improved fit of the logarithmic regression indicates a saturation at the higher stimulation frequencies (Fig. 2A). A question concerns the reliability of the absolute LCMRglu values in the stimulated area. The presented values were calculated with common rate constants and a common lumped constant for all gray matter. However, tissue alterations (e.g., due to electrode penetration and stimulation) might potentially lead to changes in the rate constants or the lumped contant. The here reported sixfold increase of LCMRglu in the stimulated area at the highest frequency is considerably larger in comparison to earlier studies. Although stimulating the cortex at higher frequencies and currents, Sharp *et al.* (1988) reported only a twofold increase in the stimulated area. This discrepancy might partly be explained by our use of the phosphor imaging system whose superb dynamic range allows a more accurate quantification. Quantification of film blackening as used by most groups is difficult at high intensities due to saturation effects, even if appropriate methods for linearization are applied. No change of glucose consumption was found in the control areas and LCMRglu was in accordance with the values reported in a recent study applying the same anesthesia (Nakao *et al.,* 2001). The small variation of LCMRglu among all animals in these areas demonstrates the reliability of the used methods.

In conclusion this study demonstrates strong positive correlations between cortical stimulation frequency and glucose metabolism in white and gray matter. The results regarding white matter bear important implications for neuroimaging studies. Normalization of data with white matter regions in semi-quantitative imaging studies is problematic unless the stability of white matter values across experimental conditions is proven. Furthermore, since changes of LCMRglu and potentially of regional cerebral blood flow in white mat-

ter can be attributed to changes of electrical fiber activity they contain potentially valuable information. Such changes should be considered when interpreting the findings of neuroimaging studies. In combination with anatomical data and recently developed methods for noninvasive fiber tracking (Conturo *et al.*, 1999) such results may provide direct information on the connectivity between activated cortical areas and could support and supplement correlational approaches (Horwitz *et al.*, 1992; McIntosh and Gonzalez Lima, 1994; Buchel and Friston, 1997).

However, whether direct monitoring of white matter metabolism is useful for the study of connectivity depends on the still open question whether white matter changes are detectable under physiological conditions, particularly when applying noninvasive imaging methods.

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