Published in final edited form as:

Neurobiol Dis. 2015 January ; 73: 269–274. doi:10.1016/j.nbd.2014.10.009.

In Vivo Magnetic Resonance Studies Reveal Neuroanatomical and Neurochemical Abnormalities in the Serine Racemase Knockout Mouse Model of Schizophrenia

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Abstract

BACKGROUND—Decreased availability of the *N*-methyl-D-aspartate receptor (NMDAR) co-agonist D-serine is thought to promote NMDAR hypofunction and contribute to the pathophysiology of schizophrenia, including neuroanatomical abnormalities, such as cortical atrophy and ventricular enlargement, and neurochemical abnormalities, such as aberrant glutamate and γ -aminobutyric acid (GABA) signaling. It is thought that these abnormalities directly relate to the negative symptoms and cognitive impairments that are hallmarks of the disorder. Because of the genetic complexity of schizophrenia, animal models of the disorder are extremely valuable for the study of genetically predisposing factors. Our laboratory developed a transgenic mouse model lacking serine racemase (SR), the synthetic enzyme of D-serine, polymorphisms of which are associated with schizophrenia. Null mutants (SR^{-/-}) exhibit NMDAR hypofunction and cognitive impairments. We used 9.4 Tesla magnetic resonance imaging (MRI) and proton spectroscopy (MRS) to compare *in vivo* brain structure and neurochemistry in wildtype (WT) and SR^{-/-} mice.

METHODS—Mice were anesthetized with isoflurane for MRI and MRS scans.

RESULTS—Compared to WT controls, SR^{-/-} mice exhibited 23% larger ventricular volumes ($p < 0.05$). Additionally, in a medial frontal cortex voxel (15 μ l), SR^{-/-} mice exhibited significantly higher glutamate/water (12%, $t = 1.83$, $p < 0.05$) and GABA/water (72%, $t = 4.10$, $p < 0.001$) ratios.

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Financial Disclosures

In the last two years, JTC served as a consultant for Abbvie Laboratories and En Vivo. In addition, a patent owned by Massachusetts General Hospital for the use of D-serine to treat serious mental illness could yield royalties. In the last two years, MJK received funding from PhotoThera, Inc., Michael J. Fox Foundation for Parkinson's Research, and Air Products and Chemicals, Inc. MDP, DM, JEJ, TEG, and GTK have no financial or potential conflict of interest disclosures.

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CONCLUSIONS—Collectively, these data demonstrate *in vivo* neuroanatomical and neurochemical abnormalities in the SR^{−/−} mouse comparable to those previously reported in humans with schizophrenia.

Keywords

γ-aminobutyric acid; glutamate; magnetic resonance imaging; magnetic resonance spectroscopy; schizophrenia; serine racemase knockout mouse; ventricular volume

Introduction

Schizophrenia is a devastating psychiatric disorder that affects 1% of the population worldwide (1). The disease manifests in several different endophenotypes through a combination of heterogeneous symptom domains including, positive symptoms (e.g., hallucinations, delusions, and thought disorder), negative symptoms (e.g., blunted affect and poverty of speech), and cognitive impairments (e.g., deficits in working memory and attention). Most current therapies do little to treat the negative symptoms and cognitive impairments, which are often reported as the most disabling by individuals with schizophrenia (2).

A prominent hypothesis proposes that activity of the *N*-methyl-D-aspartate receptor (NMDAR) is decreased in individuals with schizophrenia relative to healthy controls (2; 3; 4; 5). NMDAR hypofunction may be due, in part, to decreased availability of D-serine, an NMDAR co-agonist, given that genes encoding serine racemase (SR; the synthetic enzyme for D-serine), D-amino acid oxidase (DAAO; the degradation enzyme for D-serine), and the DAAO activator G72 are risk genes for schizophrenia (6; 7; 8; 9) and that expression of SR (10; 11) and DAAO (12; 13) is altered in individuals with schizophrenia. Evidence suggests that one of the consequences of NMDAR hypofunction is down-regulation of the fast-firing, parvalbumin-positive γ-aminobutyric acid (GABA) interneurons, resulting in disinhibition of pyramidal neurons (14; 15) and a hyperglutamatergic state (16; 17). Indeed, unmedicated (i.e., antipsychotic naïve or off antipsychotic medications for at least 2 weeks) humans with schizophrenia exhibit increases in glutamate levels (18; 19; 20), as well as increases in GABA levels (18; 21; 22). Furthermore, schizophrenia is characterized by neuroanatomical abnormalities, including cortical atrophy accompanied by ventricular enlargement (23; 24; 25). It is thought that these neurochemical (16; 26; 27; 28; 29) and neuroanatomical (30; 31; 32) aberrations directly correlate with symptom severity of the disorder.

Given the genetic complexity of schizophrenia, animal models play a crucial role in the investigation of the cellular and molecular underpinnings of the disorder. To that end, our laboratory developed a transgenic mouse line with a constitutive deletion of exon 1 of the SR gene, which encodes the catalytic domain of the enzyme (33). Null mutants (SR^{−/−}) from this line exhibit NMDAR hypofunction and cognitive impairments, thus serving as a model of the schizophrenia endophenotype mediated by decreased D-serine availability (33).

Previously, our laboratory demonstrated decreased dendritic spine density and complexity in SR^{−/−} mice *ex vivo*, effects that appear to result from deficits in molecular pathways mediating neuroplasticity (34; 35). The current study assessed brain structure and

neurochemistry in SR^{-/-} mice *in vivo* using ultra-high magnetic field (9.4 Tesla) magnetic resonance imaging (MRI) and ultra-short echo time proton magnetic resonance spectroscopy (MRS). We aimed to establish whether neuroanatomical and neurochemical abnormalities similar to those evident in schizophrenia also occur in SR^{-/-} mice. Specifically, we hypothesized that SR^{-/-} mice would exhibit increased ventricular volumes, as well as increased frontal cortex glutamate and GABA levels relative to WT mice.

Methods and Materials

SR^{-/-} mice

SR^{-/-} (n=12) and WT (n=12) mice were generated as previously described (33). Heterozygous (SR^{+/-}) sires and dams were bred to produce WT and SR^{-/-} offspring. Adult male mice (3 months old) were used. Animals were housed in groups of four (two WT and two SR^{-/-}) in polycarbonate cages and maintained on a 12:12 h light/dark cycle in a temperature- (22 °C) and humidity-controlled vivarium. Food and water were available *ad libitum*. Prior to imaging, animals were divided into scan pairs (one WT and one SR^{-/-}) and transported from the vivarium to the imaging facility. Scan order was counterbalanced. All animal procedures were approved by the McLean Hospital Institutional Animal Care and Use Committee.

In vivo neuroimaging

MRI images and MRS spectra were acquired *in vivo* from anesthetized (2% isoflurane) WT and SR^{-/-} mice using a 9.4 Tesla (Varian Inc., Direct Drive) horizontal-bore scanner equipped with a 60 mm ID, 100 G/cm, imaging gradient. Physiological parameters, including rectal temperature and respiration rate, were monitored and maintained throughout all scans.

MRI

A 2D fast-spin-echo sequence was used to acquire highly T₂-weighted coronal slices. Imaging parameters were: TR=3000 ms, TE=55 ms, in plane FOV 16 mm × 16 mm, in-plane acquisition matrix 256 × 256, 36 slices, 0.5 mm thickness (no gap), 32 averages (35 min total acquisition time). Masks delineating ventricular volumes were manually overlaid on MRI images in a blind manner with respect to genotype. Ventricular volumes were estimated from the masks using standard FSL command line tools (FSL, FMRIB, <http://fsl.fmrib.ox.ac.uk/fsl/>). Inter-rater reliability was assessed using four randomly chosen subjects (two WT mice and two SR^{-/-} mice). Volume tracing was highly correlated (Pearson's r=0.99) between experimenters.

MRS

Three multi-slice images were acquired orthogonally along sagittal, axial, and coronal planes to guide voxel placement. The voxel was positioned in the medial frontal cortex (see Figure 1). MRS spectra were acquired with a custom-made volume coil using an ultra-short echo-time STEAM (36) sequence with TR=4 s and TE=3 ms, mixing time (TM)=14 ms, 4096 complex points, 5000 Hz acquisition bandwidth, 1 ms 90° excitation pulse. Free-induction decays (FID) were acquired in groups of 128 averages (512 averages total, 34 min

total acquisition time). Water suppression was carried out using the VAPOR (37) scheme with a 30 ms sinc pulse (200 Hz water suppression bandwidth). Four averages (one phase cycle) were acquired for each unsuppressed water spectrum. These were acquired after shimming and before water suppressed spectrum acquisition, with exactly the same parameters as the suppressed spectrum acquisition but with water suppression amplitude set to zero. This ensures that the water suppression gradients were the same for subsequent eddy-current and phasing (Klose) correction. Nominal voxel size was $2.0 \text{ mm} \times 3.0 \text{ mm} \times 2.5 \text{ mm}$ (15 μl). Voxel shimming was carried out using FASTMAP (38), resulting in unsuppressed water linewidths of 10-13 Hz. Spectra were visually inspected and apodized with a 4 Hz exponential filter. The Klose correction, implemented in custom-written Matlab code (Mathworks, Inc, vR2012a), was used to correct the phase and any residual eddy currents using the phase of the unsuppressed water peak (39). FID blocks were added together using the NAA peak at 2 ppm to synchronize elimination of any residual frequency drift. Subsequently, spectra were automatically fitted with the LCModel (40) using a simulated basis created with GAVA (41). All steps were visually inspected against inadvertent errors. Metabolites with Cramér-Rao Lower Bound values exceeding 30% were considered unreliable and were excluded from analyses.

Statistical analysis

Two-sample t tests with equal and unequal variances were conducted using Stata software (v12, StataCorp, College Station, TX). Equality of variances was formally tested using the Levene-Brown-Forsythe robust test (42; 43) and indicated that equal and unequal variance tests were appropriate for glutamate and GABA, respectively. As we advanced *a priori* directional hypotheses for group differences in ventricular volumes, glutamate levels, and GABA levels, we conducted one-sided t tests to analyze for group differences in these measures.

One WT subject was excluded from the volumetric analysis and one WT subject was excluded from the MR spectral analyses due to their data values being outside of two standard deviations from their respective group means. In addition, acquisition of MRS data failed in one SR^{-/-} subject and one SR^{-/-} subject was excluded from the MR spectral analyses due to a 38% Cramér-Rao Lower Bound value.

Results

A qualitative difference in ventricle size between SR^{-/-} and WT mice was observed upon visual inspection of structural MRI images (see Figure 2A for representative SR^{-/-} and WT images). Indeed, volumetric analysis revealed significantly larger (23%) ventricular volumes in SR^{-/-} mice compared to WT controls ($p < 0.05$; see Figure 2B). A typical proton spectrum with LCModel fits of glutamate and GABA demonstrated good resolution of proton metabolites, including glutamate and GABA (see Figure 3A). There were no group differences in LCModel reported full width half maximum (WT: $\text{FWHM} = 0.0509 \pm 0.018$ ppm, SR^{-/-}: $\text{FWHM} = 0.0508 \pm 0.020$ ppm) or signal to noise ratio (WT: 10.0 ± 1.7 , SR^{-/-}: 11.6 ± 2.4) values or in the unsuppressed water peak (see Figure 3B). Therefore, all MRS statistical analyses were conducted using metabolite ratios with the unsuppressed water peak

as the denominator. Metabolites of interest were glutamate and GABA, for which an increase in SR^{-/-} mice was hypothesized, as well as choline, creatine, glutamine, lactate, myoinositol, *N*-acetylaspartate (NAA), and *N*-acetylaspartylglutamate (NAAG). We observed significantly higher glutamate/water (12% increase; one-tailed $t=1.83$, $p=0.0413$; see Figure 3C) ratios in SR^{-/-} mice compared to WT controls. We also observed significantly higher GABA/water (72% increase; one-tailed $t=4.10$, $p=0.0005$; see Figure 3D) ratios in SR^{-/-} mice compared to WT controls. When we excluded GABA values with CRLBs between 20-30% (5 WT mice and 1 SR^{-/-} mouse), the GABA elevation in SR^{-/-} mice was preserved ($t=4.10$, $p=0.0009$). There were no group differences in other metabolites (see Table 1).

Discussion

Using *in vivo* MRI and MRS, we identified neuroanatomical and neurochemical abnormalities in SR^{-/-} mice. Specifically, we observed ventricular enlargement in SR^{-/-} mice relative to WT controls, which not only complements our previous work showing cortical atrophy in SR^{-/-} mice *ex vivo* (34; 35), but also parallels findings in human studies documenting ventricular enlargements in individuals with schizophrenia (23; 24; 25). Furthermore, we observed elevated frontal cortex glutamate levels in SR^{-/-} mice, which also corresponds with findings in unmedicated humans with schizophrenia (18; 19; 20). Our glutamate finding is consistent with the theory that chronic NMDAR hypofunction in schizophrenia results in a compensatory hyperglutamatergic state (16; 17). In addition, we observed elevations in frontal cortex GABA levels in SR^{-/-} mice that correspond with GABA elevations reported in unmedicated (18; 21; 22) humans with schizophrenia. It is important to note that studies of humans with schizophrenia currently being treated with antipsychotic medications have yielded conflicting findings for both glutamate (18; 44; 45) and GABA (44; 46; 47; 48; 49). These discrepancies may be due to variability introduced as a result of factors related to medication and treatment (e.g., disease phase, disease length, response to treatment), not the pathophysiology of the disease. Furthermore, factors such as species differences, variations in the degree of NMDAR hypofunction among patients with schizophrenia, and the genetic specificity of the SR^{-/-} model versus polygenetic contributions to schizophrenia (50) must be considered when interpreting these findings. We conclude that SR^{-/-} mice appear to adequately model some, but not all, aspects of human schizophrenia and, thus, may be useful for studying the etiology of brain abnormalities associated with the disorder, as well as the efficacy of putative treatments.

A caveat with regard to the GABA elevations that we observed in SR^{-/-} mice is that the anesthetic gas that was used, isoflurane, has been shown to increase GABA concentration in the mouse brain as measured via MRS (51). It seems unlikely that such an effect could fully account for the group difference in GABA concentration that we observed since all mice were exposed to the same isoflurane concentration over the same time course. In addition, diffusion of intracellular metabolites, in general, has been shown to be augmented by isoflurane, as assessed by MRS (52), suggesting that acute effects of isoflurane on metabolite concentrations are likely due to increased molecular mobility. However, we cannot rule out that SR^{-/-} mice are particularly sensitive to the effects of isoflurane on GABA concentration, an alternative explanation that must be further explored. In addition, it

is possible that the elevated intracellular GABA levels observed with MRS reflect a reduction in GABA turnover, as evidence suggests that one of the consequences of NMDAR hypofunction is down-regulation of the fast-firing, parvalbumin-positive GABA interneurons, resulting in disinhibition of pyramidal neurons (14; 15; 16). Furthermore, the increase in GABA observed in the current study could be due to compensatory upregulation in non-fast-firing GABA neurons as an endogenous attempt to overcome the aforementioned down-regulation of fast-firing GABA interneurons.

Future studies are needed to investigate whether the frontal cortex neurochemical abnormalities reported here are present in other areas of the brain. Also, it will be important to confirm whether white matter abnormalities reported in individuals with schizophrenia (53) are present in the SR^{-/-} mouse model *in vivo*. The use of the SR^{-/-} mouse model also will allow for the investigation of behavioral correlates of schizophrenia in conjunction with ultra-high magnetic field MRI techniques. An area of particular interest is co-morbid schizophrenia and substance abuse, as substance abuse disorders are three to four times more prevalent in individuals with schizophrenia compared to the general population (54) and both gray and white matter abnormalities indicative of schizophrenia are compounded in patients who suffer from co-morbid alcohol dependence (55).

Given the heterogeneous symptom domains of schizophrenia, as well as the contribution of complex genetic and epigenetic factors to the presentation of the disease, it is essential that animal models are developed so that neural substrates and novel therapeutic targets can be identified. This study demonstrates *in vivo* neuroanatomical and neurochemical abnormalities in the SR^{-/-} mouse comparable to those reported in some individuals with schizophrenia, suggesting that the SR^{-/-} mouse models a specific endophenotype of the disease. These findings further validate the SR^{-/-} mouse model itself and strengthen the veracity of the NMDAR hypofunction hypothesis.

Acknowledgments

The authors thank Dr. Darrick Balu for his technical and analytical assistance, Dr. Amy Janes for her helpful analytical discussions, and Dr. Scott Lukas for his logistical assistance and helpful analytical discussions. This research was supported, in part, by NIH grants T32 DA015036, R01 MH51290, and S10 RR019356, by an internal grant from the O'Keefe family (O'Keefe Family Junior Investigator Award for Excellence in Imaging Research), and by the Counter-Drug Technology Assessment Center (CTAC), an office within the Office of National Drug Control Policy (ONDCP), via Contract Number DBK39-03-C-0075 awarded by the Army Contracting Agency. The content of the information does not necessarily reflect the position or the policy of the Government and no official endorsement should be inferred.

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Highlights

- Serine racemase knockout (SR^{-/-}) mice model phenotypes found in schizophrenia (Scz)
- We obtained the first in vivo MRI and MRS scans of SR^{-/-} mice
- SR^{-/-} mice had enlarged ventricles and increased frontal cortex glutamate and GABA
- SR^{-/-} mice exhibit some MRI and MRS abnormalities observed in Scz
- Imaging of SR^{-/-} mice may lead to new diagnostic methods and treatments for Scz

Figure 1.
T₂-weighted sagittal (left panel) and coronal (right panel) sections illustrating the placement of the 2.0 mm × 3.0 mm × 2.5 mm (15 μ l) frontal cortex voxel used for MRS analyses.

Figure 2.

A. T₂-weighted coronal slice images from representative WT (left panel) and SR^{-/-} (right panel) mice. SR^{-/-} images display enlarged ventricles compared to WT images. **B.** Ventricular volume (% WT) of WT (squares) and SR^{-/-} (circles) mice. SR^{-/-} mice exhibit greater ventricular volumes. — denotes sample means and * denotes statistical significance (p<0.05).

Figure 3.

MRS findings in WT (squares) and SR^{-/-} (circles) mice. **A.** Representative MR spectrum from a WT mouse illustrating metabolite peaks. **B.** Water concentration (% WT). There were no group differences. **C.** Glutamate/water ratios (% WT). SR^{-/-} mice exhibit increased (12%) glutamate levels. **D.** GABA/water ratios (% WT). SR^{-/-} mice exhibit increased (72%) GABA levels. — denotes sample means, * denotes statistical significance ($p < 0.05$), and # denotes statistical significance ($p < 0.01$).

Table 1

Summary statistics for all metabolites of interest.

	30% SD		
	WT	SR ^{-/-}	
GABA/H₂O	67.34 ± 11.83 (8)	115.78 ±	38.30 (12)
Glu/H₂O	521.98 ± 67.16 (11)	584.54 ±	91.52 (11)
Cho/H₂O	15.65 ± 1.57 (4)	10.90 ±	3.99 (6)
Cre/H₂O	18.12 ± 2.85 (9)	16.34 ±	1.77 (9)
Gln/H₂O	13.48 ± 1.78 (7)	12.22 ±	2.86 (10)
Lac/H₂O	14.91 ± 2.02 (8)	15.87 ±	3.23 (5)
Ins/H₂O	20.15 ± 2.52 (11)	18.96 ±	4.45 (11)
NAA/H₂O	19.25 ± 1.15 (11)	18.88 ±	2.09 (12)
NAAG/H₂O	20.07 ± 3.07 (5)	17.99 ±	2.17 (3)

Mean ± SD (N)