GLUTATHIONE AS A PREDICTOR OF NEUROPSYCHOLOGICAL IMPAIRMENT IN PATIENTS WITH RELAPSING REMITTING, SECONDARY PROGRESSIVE, AND PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS

By

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Abstract

Multiple sclerosis (MS) has long been characterized as an inflammatory disease of the central nervous system (CNS); however, recent research has suggested that neurodegenerative processes such as oxidative stress may be the primary force driving disease progression and associated neuropsychological impairment in this population. Recent work by our research group identified GSH, an important cerebral antioxidant, as a marker of oxidative stress-mediated neurodegeneration in patients with secondary progressive (SP) MS. However, the present study featured the first comparison of cerebral GSH concentrations among patients with RR, PP, and SP subtypes of MS and healthy controls. The primary aims of this study were to examine differences in GSH concentrations among subtypes of MS and to investigate whether reductions in GSH concentrations occurred in conjunction with neuropsychological impairments in processing speed, memory, and executive function. Results indicated that relative to RR patients, progressive (PP and SP) patients exhibited the largest reductions in GSH concentrations, with no significant differences between PP and SP patients. A similar pattern of outcomes was observed on the neuropsychological measures, with reductions in GSH being accompanied by a worsening of impairment in processing speed and a broadening of impairment to include deficits in learning and memory. These results support the hypothesis that even in the absence of inflammatory processes underlying acute clinical exacerbations, diffuse oxidative stress signals an ongoing neurodegenerative process that likely contributes to disease progression and cognitive decline over the course of the disease.

Keywords: multiple sclerosis, oxidative stress, glutathione, GSH, cognitive dysfunction, magnetic resonance spectroscopy, neuropsychological assessment

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Glutathione as a Predictor of Neuropsychological Impairment in Patients with Relapsing Remitting, Secondary Progressive, and Primary Progressive MS

Multiple sclerosis (MS) is a chronic, progressive disease characterized by neuroinflammation and neurodegeneration of the central nervous system (CNS). While the clinical profiles of patients with MS can be quite varied, upwards of 70% of patients report some level of neuropsychological dysfunction during the course of the disease (Kurtzke et al., 1972; Peyser, Rao, LaRocca, & Kaplan, 1990; Rao, Leo, Bernardin, & Unverzagt, 1991), with slowed speed of information processing cited as one of the most pervasive cognitive deficits observed in this population (Archibald & Fisk, 2000; Bergendal, Fredrikson, & Almkvist, 2007; Bodling, Denney, & Lynch, 2008, 2009, 2012; De Sonneville et al., 2002; DeLuca, Chelune, Tulsky, Lengenfelder, & Chiaravalloti, 2004; Demaree, DeLuca, Gaudino, & Diamond, 1999; Denney, Gallagher, & Lynch, 2011; Denney & Lynch, 2009; Kail, 1997, 1998; Kujala, Portin, Revonsuo, & Ruutiainen, 1994; Lengenfelder et al., 2006; Lynch, Dickerson, & Denney, 2010; Macniven et al., 2008; Rao, St. Aubin-Faubert, & Leo, 1989; Reicker, Tombaugh, Walker, & Freedman, 2007; Schulz, Kopp, Kunkel, & Faiss, 2006). While these deficits have long been established within the literature, the relationship between neurological impairment and neuropsychological dysfunction has been tenuous, and many of the most common physical or neurological indicators of disease progression (e.g., enhancing lesions in the brain) are often only mildly correlated with cognitive dysfunction (Filippi, M., et al., 1994; Lynch, Parmenter, & Denney, 2005; Ryan, Lee, Clark, & Campbell, 1996; Zivadinov et al., 2001). Fortunately, over the past decade, improvements in both magnetic resonance (MR) and neuropsychological assessment techniques have afforded increasingly sensitive methods for capturing some of the hallmark neurological and neuropsychological deficits in this population. One of these emerging techniques is

magnetic resonance spectroscopy (MRS), a non-invasive, *in vivo* imaging technique that measures cerebral metabolite concentrations. Studies evaluating the neurochemical profiles of patients with neurological disorders have become increasingly common in several literatures, including Alzheimer's and aging (e.g., Loos, Achten, & Santens, 2010; Parnetti et al., 1997; Valenzuela & Sachdev, 2001) and provide some advantages over using traditional magnetic resonance imaging (MRI; e.g., T_1- and T_2-weighted images). Recent work by our MS research group has used MRS techniques to measure one particular molecule of interest, glutathione (GSH). A recent pilot study demonstrated that GSH may provide a reliable marker of disease progression in patients with the secondary progressive (SP) subtype of MS (Choi, Lee, Denney, & Lynch, 2010). Yet, to date, no studies have explored the degree to which GSH correlates with other common features of MS, particularly neuropsychological impairment. Moreover, it is unclear how MS might impact GSH levels in relapsing remitting (RR) and primary progressive (PP) subtypes. The present study will be the first to examine the relationship between neuropsychological impairment and GSH levels in patients with RR, PP, and SP subtypes of MS. The following sections will review inflammatory and neurodegenerative processes hallmark to MS, introduce the concept of oxidative stress as it relates to neurodegeneration in each subtype of MS, explore the role of GSH as a marker of oxidative stress-mediated neurodegeneration, and review the current understanding of the relationship between neuropsychological impairment and neurobiological markers of disease progression.

Background

MS has long been conceptualized, and likewise treated, as predominantly a neuroinflammatory, demyelinating disease of the CNS. However, important distinctions arise between MS subtypes that are relevant when considering the relative contributions of

inflammatory and neurodegenerative processes to MS pathology. MS has most commonly been divided into three subtypes: RR, PP, and SP (Lublin & Reingold, 1996). While the conceptual descriptions of these categories are fairly distinct, in practice, classifying patients as to subtype can be challenging.

RR patients vary considerably in presentation, with some patients presenting with frequent, debilitating clinical exacerbations, while others having relatively few relapses; however, the general course is characterized by a series of relapses, or periods of exacerbated symptoms most commonly attributed to the development of a new lesion in the CNS. Because enhancing lesions are believed to be caused by acute inflammation of myelinated neuronal axons (Fassbender et al., 1998; Lin & Blumhardt, 2001; van Walderveen et al., 1999**)**, and because the RR subtype is characterized by the development of enhancing lesions, most of the support for viewing MS as an inflammatory disease centers on this subtype. However, in RR patients, rates of relapses and recovery following relapses are highly variable. While functional recovery follows most relapses, the speed and degree of recovery varies from patient to patient. In addition to the inflammatory processes occurring at this stage of the disease, neurodegenerative processes may also contribute to the diverse symptomatology. Indeed, axonal loss, and grey and white matter atrophy have been observed in RR patients, although to a lesser extent than more progressive subtypes (Simon et al., 1999; Wegner & Stadelmann, 2009). For RR patients, it is unclear whether these neurodegenerative features are occurring secondary to or independent of inflammation.

For many RR patients, the number of new enhancing MS lesions tends to decrease over time, and patients begin to experience a gradual worsening of symptoms that cannot be explained by the presence of enhancing lesions. At this stage of progressive worsening, patients are

unlikely to have as many distinct relapses and are less likely to recover from those relapses that occur. For many patients, the typical disease course evolves over time from the RR subtype to the secondary progressive (SP) subtype. Researchers believe that this shift in symptomatology marks a clear distinction between inflammatory and neurodegenerative processes in MS (Pittock & Lucchinetti, 2007; Trapp & Nave, 2008). In fact, recent studies have demonstrated that neurodegeneration can occur in the absence of inflammation and is likely to occur in patients with progressive forms of the disease (Pirko, Lucchinetti, Sriram, & Bakshi, 2007; Simon et al., 1999; Trapp & Nave, 2008). However, proponents of the inflammation theory have also observed long-lasting enhancing lesions among SP patients and have continued to view inflammation as a continuous process underlying disease progression in this progressive subtype.

In contrast to SP patients, PP patients experience gradual deterioration from the onset of the disease, with no relapses or periods of functional recovery. Although physical symptom presentation for PP patients is very similar to, and sometimes indistinguishable from SP subtype, the onset of symptoms constitutes a clear clinical difference between these patients. Due to the gradual worsening of symptoms observed at the onset of the disease, researchers suggest that the PP subtype may provide evidence for neurodegeneration. While the relative contributions of inflammation and neurodegeneration are currently under debate, mounting evidence suggests that neurodegeneration may have a much more prominent role in all subtypes of MS than previously thought (Pirko et al., 2007; Simon et al., 1999; Wegner & Stadelmann, 2009).

Oxidative stress and glutathione. A multitude of factors likely contribute to neurodegeneration in MS, including neurochemical disturbances such as glutamate excitotoxicity and oxidative stress (Bains, & Shaw, 1998; Coyle & Puttfarcken, 1993; Smythies, 1999). Oxidative stress is defined as the imbalance between the accumulation of reactive oxygen

species in the brain and the brain's capacity to detoxify and prevent subsequent neural damage (Ramalingam & Kim, 2012; Coyle & Puttfarcken, 1993). An increase in cerebral free radicals leads to oxidative damage and can have a significant impact on cognitive function (Beal, 1995; Harman, 1992).

Despite the brain's high susceptibility to oxidative stress, the brain also contains numerous antioxidants to scavenge reactive oxygen species and maintain metabolic stability. One such antioxidant is GSH, a tripeptide enzymatic substrate composed of L-glutamate, L-cysteine, and glycine. GSH is a major endogenous antioxidant produced by the cells (Meister & Anderson, 1983; Pompella, Visvikis, Paolicchi, De Tata, & Casini, 2003). It plays a crucial role in maintaining other important antioxidants such as vitamins C and E and is used in biochemical reactions such as DNA synthesis and repair, protein synthesis, amino acid transport, and enzyme activation (Meister, 1983). GSH is pervasive throughout mammalian cells and has been implicated in metabolic disturbances observed in other neurodegenerative disorders (Bains & Shaw, 1997; Coyle & Puttfarcken, 1993).

In the presence of oxidative stress, GSH combines with reactive oxygen species to reduce the neurodegenerative impact of free radicals on cells. As GSH reacts with free radicals, it becomes oxidized, resulting in lower concentrations of GSH (Cooper & Kristal, 1997). A reduction in GSH generally signifies an increase in oxidative stress, and, potentially, an increase in oxidative-stress-related neurodegeneration (Heales, Davies, Bates, & Clark, 1995). Thus, as opposed to using magnetic resonance imaging (MRI) techniques (e.g., T_1- and T_2-weighted images) that are more sensitive to the inflammatory features of MS (e.g., gadolinium-enhancing lesions), measuring GSH levels in the brain may provide a reliable method for quantifying neurodegenerative features of the disease.

While quantifying GSH can be quite challenging, investigators in our MS research group at the University of Kansas Medical Center have developed techniques for isolating GSH resonance signals from other cerebral metabolites (Choi, 2003a; 2003b; 2004). There are generally two ways to express GSH levels: as a ratio to creatine (Cr) levels, or as an absolute quantity. Using the former procedure assumes that Cr levels remain comparable between MS patients and controls, while the latter does not require this assumption. Researchers have debated whether GSH and other brain metabolites of interest should be quantified as ratios to Cr or as absolute quantities. While there is no gold standard for this measure, a recent review maintained that, for most studies, differences in Cr between MS patients and controls have been minimal and that expressing GSH levels as ratios to Cr is an acceptable practice (Caramanos, Narayanan, & Arnold, 2005).

Oxidative stress and MS subtypes. Because oxidative stress may provide a marker of neurodegeneration, it is useful to consider the theories and implications of oxidative stress within different subtypes of MS. There are several theories for how inflammatory and neurodegenerative processes interact to produce the neurological and neuropsychological disability observed in MS (Schulz, Lindenau, Seyfried, & Dichgans, 2000). The first, and historically most common theory stipulates that MS begins as an inflammatory disease; however, as the disease progresses and lesion load increases, oxidative stress becomes more prevalent, resulting in subsequent axonal neurodegeneration. Presumably, in this scenario, oxidative stress may not be observed until several years after disease onset and would typically be concomitant with progressive subtypes. An alternative theory states that oxidative stress-induced neurodegeneration occurs early on in the disease, as a separate process from inflammation. Several investigators have supported this hypothesis, noting widespread metabolic abnormalities

in normal appearing white matter (NAWM) that occur early and, importantly, are evident even during clinical remission (i.e., an absence of inflammation; Kirov et al., 2009; Inglese et al., 2003). This scenario could account for the highly heterogeneous disease course observed in RR patients, where oxidative stress contributes to many of the deficits observed in RR patients and is not necessarily related to enhancing lesion load. In this case, oxidative stress would likely be present, to some extent, early in the disease course and could potentially predict subsequent grey and white matter atrophy. A third theory, and one that has been vigorously debated within the literature, states that MS begins as a neurodegenerative disease, and that oxidative stress triggers the immune response responsible for inflammation and demyelination. In this scenario, oxidative stress would precede the development of enhancing lesions, and might even serve as a reliable predictor of inflammatory disease processes (Cooper & Kristal, 1997; Heales et al., 1999).

Given the emerging support for GSH as a marker of oxidative stress-mediated neurodegeneration, measuring GSH *in vivo* using MRS may provide a novel way to characterize neurodegeneration in all three subtypes of MS. If neurodegeneration is the predominant process underlying MS, as some emerging research has suggested, changes in GSH could be observed in all subtypes of MS, with lower levels of GSH being associating with greater neurodegeneration (Choi et al., 2010). In this case, GSH measures would probably not be highly correlated with neuroinflammatory markers (e.g., gadolinium-enhancing lesions in traditional MRI scans); however, GSH would be correlated with other more firmly established neuroimaging measures of neurodegeneration (e.g., atrophy), disability status, and neuropsychological performance.

Neuropsychological impairment in MS. In addition to neurochemical and neuroanatomical indicators of MS disease progression, neuropsychological measures have also

become central areas of focus in the MS literature. Several prominent literature reviews have cited a host of cognitive symptoms associated with MS (e.g., Brissart et al, 2012; Chiaravalloti & DeLuca, 2008; Jongen, Ter Horst, & Brands, 2012; Julian, 2011; Langdon, 2011), including problems with attention, information processing speed, executive functioning, and memory. To date, deficits in processing speed have been observed using several types of assessment tools, including reaction time (RT), rapid serial processing (RSP), and planning time measures. While most researchers agree that processing speed is the most common domain impacted by MS, some investigators have proposed that slowed processing speed may account for apparent deficits in other domains (Denney & Lynch, 2009; Hughes, Denney, & Lynch, 2011). This overlap becomes most evident in patients with significantly diminished processing speed, where apparent deficits in working memory or executive functioning emerge because patients are unable to process information at a rate necessary to effectively encode the information or process it at a higher level (Denney, Hughes, Owens, & Lynch, 2012). While researchers may disagree over the relative primacies of domain-specific deficits (e.g., processing speed versus memory), there is strong evidence that essential verbal skills and general intelligence are rarely affected by MS. These important distinctions noticeably separate MS-related cognitive impairments from those of other neurological disorders, including Alzheimer's and other dementias, where one finds pervasive deficits in language, memory, and reasoning.

In addition to identifying and describing these deficits across MS patients in general, research over the past decade has sought to more specifically differentiate subtypes of MS with regard to neuropsychological impairment. However, because distinguishing MS subtypes can be challenging even at the neurological level, only a handful of studies have attempted to directly compare cognitive deficits between subtypes. Huijbregts et al. (2006) demonstrated that while

patients with progressive (PP and SP) subtypes performed worse than controls on all tasks of the Brief Repeatable Battery of Neuropsychological Tests, RR patients only performed worse on speeded tasks that required higher-order processes (e.g., Word List Generation). A similar distinction was noted between SP and PP patients, where both groups exhibited poor performance relative to controls, but SP patients performed worse than PP patients on those same higher-order processing tasks. Potagas et al. demonstrated a similar finding, where RR patients or patients with clinically isolated syndrome (CIS) generally exhibited fewer deficits in verbal learning and memory relative to more progressive subtypes. De Sonneville also demonstrated significant deficits in attention and processing speed that were more severe in progressive patients relative to RR patients, with no significant differences emerging between PP and SP patients. These results suggest a general worsening of cognitive impairment in conjunction with disease progression, particularly within the domain of processing speed.

In addition to cognitive deficits, emotional dysfunction has also been observed in patients with MS. Although the mechanism underlying depression in MS is not entirely understood, reviews have cited neurotransmitter dysfunction as a result of neuronal damage as a primary biological pathway (Feinstein, 2011; Vattakatuchery, Rickards, & Cavanna, 2011). Furthermore, because MS is a chronic illness, research on coping has identified depression as a potential risk factor in patients with poor coping skills (Goretti, Portaccio, Zipoli, Razzolini, & Amato, 2010). Regardless of the mechanism, depression, stress, and fatigue are frequently reported among patients with MS. Therefore, assessing these areas of emotional functioning is critical for understanding whether patients' neuropsychological dysfunction stems predominantly from depressive features or directly from impairments in the aforementioned cognitive domains.

Numerous studies have investigated relationships between cognitive deficits and neurobiological changes associated with MS. However, to date, most of these studies have employed broad neuropsychological screeners with poor sensitivity to MS-specific deficits and neuroimaging measures that focus on neuroinflammatory processes. While these screeners are generally fast and easy to administer, they may not detect some of the more subtle deficits common to patients with MS (e.g., decreased processing speed). Similarly, neuroimaging techniques that neglect neurodegenerative processes may be missing a key event driving disease progression. These results suggest the need for more targeted neuropsychological assessment and the use of neuroimaging techniques that capture neurodegenerative processes.

The Present Study

By and large, the majority of MS neuroimaging studies have focused on structural differences observed between MS patients and healthy controls, with an emphasis on inflammatory processes. Recently, MRS techniques for measuring cerebral metabolic processes have become more common in the literature, and several studies have cited abnormalities in metabolites such as myo-inositol (mI), glutamate (Glu), choline (Cho), creatine (Cr), and n-acetyl-aspartate (NAA) in patients with MS (e.g., De Stefano & Filippi, 2007; Kirov et al., 2009; Aboul-Enein, Krssak, Hofterger, Prayer, & Kristoferitsch, 2010). However, little is known about GSH and the correlations between GSH and performance on neuropsychological measures. Therefore, the present study was the first to investigate the relationship between oxidative stress, as measured by GSH levels, and neuropsychological impairment in RR, PP, and SP MS patients. Moreover, this investigation employed a battery of computerized neuropsychological evaluations specifically designed to assess MS-related deficits in processing speed, verbal and visual memory, and executive function. In collaboration with investigators at the Hoglund Brain

Imaging Center at the University of Kansas Medical Center, *in vivo* GSH levels were quantified and examined in relation to performance on each cognitive measure.

The present study was the first to critically assess differences between healthy controls and MS patients (RR, PP, and SP subtypes) with regard to *in vivo* GSH levels and selected neuropsychological measures of processing speed, memory, and executive function. The primary aims of this study were the following: (a) to evaluate GSH concentrations among patient groups and healthy controls in order to investigate the role of neurodegeneration in disease progression; (b) to examine whether neuropsychological measures displayed a similar pattern of outcomes; and (c) to examine correlations between GSH levels and neuropsychological measures. We propose the following hypotheses: (a) that GSH concentrations will be lower for patients relative to controls, with concentrations being lowest in progressive patients; (b) that reductions in GSH concentrations will be accompanied by cognitive impairments, particularly by slowed information processing speed; and (c) that GSH levels will be at least modestly correlated with neuropsychological outcomes and disease-related variables (e.g., disability status).

Method

Participants

The original sample comprised a total of 15 RR (11 females, 4 males), 15 PP (5 females, 10 males), and 16 SP (12 females, 4 males) patients with clinically definite MS and 19 healthy individuals (10 females, 9 males) of comparable demographics. MS patients ranged in age between 18 and 65, with disease durations ranging between 1 and 33 years. Disability ratings based on the Expanded Disability Status Scale (EDSS; Kurtzke, 1983) ranged from 1 to 8.5. The healthy controls ranged in age between 24 and 65. Of the 65 total participants, movement artifact prevented the acquisition of GSH metabolite concentration in 19 individuals, yielding a final sample of 12 RR (10 females, 2 males), 13 PP (5 females, 8 males), and 11 SP (8 females, 3 males) MS patients and 10 (8 females, 2 males) healthy controls. For these remaining participants, MS patients ranged in age between 18 and 63, with disease durations ranging between 1 and 33 years. Disability ratings ranged from 1 to 8.5. The healthy controls ranged in age between 24 and 65.

All patients were under the care of the same neurologist (Sharon G. Lynch) at the University of Kansas Medical Center, and had been diagnosed with MS for at least one year prior to recruitment (in order to ensure accurate diagnosis). Consistent with prior studies in our laboratory, exclusionary criteria for patients were as follows: presence of any neurological disorder other than MS; history of drug or alcohol abuse, premorbid psychological disorder (e.g., depression), mental retardation, or head injury; visual acuity greater than 20/50 (corrected) or impaired color vision; symptomatic involvement of the hands; MS relapse within the past 30 days; or cognitive impairment of sufficient severity to interfere with comprehension of testing instructions. Additionally, because this study required participants to undergo an MRI,

additional exclusionary criteria were as follows: presence of cardiac or pacemaker, cardiac defibrillator, heart valve replacement, aneurysm/vascular clips, stints/filters/coils, kidney/liver transplant, kidney disease or diabetes, BB/foreign body/gunshot wound, neurostimulation device, permanent hearing aid, cochlear implant, medication skin patch, vascular IV access, hydrocephalus/spinal shunt, Harrington rods, eyelid spring/wire, permanent prosthetic device, dentures/partials, motion disorder, permanent body piercing(s), permanent eyeliner, bladder stimulation device, insulin pump, implantable device, or history of metal in the eyes. For female patients, pregnancy or presence of magnetic intrauterine device constituted additional exclusionary criteria. The same exclusionary criteria applied for healthy controls.

Measures

Demographic, self-report, and disease-related measures. The following information was collected from each participant on a demographic information form: name, date of birth, age, years of education, occupation, marital status, race/ethnicity, and handedness. Additionally, MS subtype, number of years since their initial diagnosis (disease duration), and EDSS rating was collected for MS patients. The demographic information form was followed by self-report questionnaires, which included the Beck Depression Inventory – Fast Screen (BDI-FS; Beck, 1996; Benedict, Fishman, McClellan, Bakshi, & Weinstock-Guttman, 2003), the Fatigue Severity Scale (FSS; Krupp, LaRocca, Muir-Nash, & Steinberg, 1989), and the Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). Each MS patient additionally completed the Multiple Sclerosis Neuropsychological Questionnaire – Patient Form (MSNQ; Benedict, et al., 2004), a measure of subjective cognitive impairment.

Expanded Disability Status Scale. The EDSS provided a measure of each MS patient's current level of disability. Functional status in each of eight categories (e.g., cerebral, brainstem,

cerebellar) was rated and combined to form a total EDSS score. Scores on this measure range from "0" to "10," with "0" being "normal neurological exam" and "10" being "death due to MS." Only MS patients were evaluated for EDSS.

Beck Depression Inventory–Fast Screen. The BDI-FS measured participants' perceived severity of depressive symptoms occurring over the past two weeks. Items on this scale corresponded to the psychological and non-somatic criteria for diagnosing major Depressive Disorders in the *Diagnostic and Statistical Manual of Mental Health Disorders – Fourth Edition* (DSM-IV; American Psychological Association, 2000), and exclude somatic criteria (e.g., psychomotor slowing) that may be more closely related to MS. The BDI-FS consists of seven items, each rated on a scale of "0" to "3," with "0" indicating no symptoms and "3" indicating severe symptoms. The total score on this measure is the sum of all items. Scores range from 0 to 21, with "0 to 3" being minimal symptoms, "4 to 6" being mild symptoms, "7 to 9" being moderate symptoms, and "10 to 21" being severe symptoms.

Fatigue Severity Scale. The FSS assessed participants' self-report of fatigue over the past seven days. The scale consists of nine items (e.g., "Exercise brought on my fatigue), each rated on a scale of "1" to "7," with "1" being "strongly disagree" and "7" being "strongly agree." The total score on this measure is an average of the nine items, with higher scores indicating greater fatigue.

Perceived Stress Scale. The PSS assessed the frequency with which participants have experienced daily stressful situations over the past month. The scale consists of 10 items, each rated on a scale of "0" to "4", with "0" being "never" and "4" being "very often." The total score on this measure is the sum of all items, including four reverse-scored items, with higher scores indicating greater perceived stress.

Multiple Sclerosis Neuropsychological Questionnaire – Patient Form. The MSNQ measured the frequency with which MS patents believe they have experienced cognitive weaknesses or impairment over the past three months. The scale consists of 15 items, each rated on a scale of "0" to "4," with "0" being "never" and "4" being "very frequent." The total score is the sum of all items, with higher scores indicating more frequent cognitive problems.

Neuropsychological assessments. Following completion of the initial demographic and self-report measures, participants completed a fixed battery of five computerized neuropsychological tests: The Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964), the Tower of London (TOL; Krikorian, Bartok, Gay, 1994; Shallice, 1982), the Stroop Test (Stroop, 1935), the Symbol Digits Modalities Test (SDMT; Smith, 1982), and the Brief Visuospatial Memory Test - Revised (BVMT; Benedict, Schretlen, Groninger, Dobranski, & Sphritz, 1996). These five tests yielded a total of 13 scores, each of which fell into one of three cognitive domains: processing speed, memory, or executive function. Processing speed measures assessed reaction time (RT), rapid serial processing (RSP), and planning time (PT). Memory measures assessed verbal and visual recall and recognition. Executive function measures assessed planning and attentional control.

Rey Auditory Verbal Learning Test. This is a computerized version of the RAVLT designed to assess verbal memory. On this test, participants were asked to listen to a list of aurally presented words that the examiner read from the computer screen. The first list, List A, comprised fifteen unrelated words. After each presentation, the participant was asked to verbally recall as many words from the list as he or she could recall. After the third trial, the examiner read a second list, List B, of 15 "distractor" words and the participant was asked to verbally recall as many of those words as possible. The participant was then asked to immediately recall

as many words as possible from List A. A delayed visual recognition trial of 50 words was performed 20 to 30 minutes after the recall, with each word presented individually on the screen. In this trial, the participant was instructed to press the "1" key if the word had been on the original word list (List A) and to press the "3" key if the word was not on the original list. Four scores were generated from this test: the total number of words recalled during the first three learning trials (Rey-A), the total number of words recalled during the immediate recall task (Rey-IR), the total number of correct delayed recognition responses (Rey-DR), and the response latencies (in seconds) for each delayed recognition item (Rey-DRRT). In terms of cognitive domain classifications, the Rey-DRRT provided an RT measure of processing speed and the Rey-A, Rey-IR, and Rey-DR provided measures of verbal learning and memory. While lower scores on the Rey-DRRT indicated faster speed, higher scores on the remaining measures indicated better learning and memory performance.

Tower of London. This computerized version of the TOL was designed to assess executive function (i.e., planning and strategic problem solving). In the bottom portion of the screen, three colored disks were arranged on three pegs (starting position). The upper portion of the screen displayed a model of the disks in a different arrangement (ending position). The participant was instructed to move the blocks in the lower portion of the screen to match the arrangement displayed in the upper portion of the screen, and to do so as quickly as possible and using a predetermined number of moves. The test consisted of 16 items ranging from 2-move to 5-move problems. Each problem was scored as one point for correct responses and zero points for incorrect responses. Two scores were generated from this test. The total sum of all item scores (TOL-Score) provided a measure of executive function (planning), and average planning

time (TOL-PT) provided a measure of processing speed (planning time). While greater TOL-Score scores indicated better performance, lower TOL-PT scores indicated faster planning.

Stroop Test. This computerized test was adapted from the original Stroop Color-Word Interference Test (Golden, 1978). It consisted of three 60-second trials. In the first trial, participants were asked to read a series of color words (e.g., "RED") printed in black letters. In the second trial, participants were asked to name the colors used to print a row of four X's. In the third trial, participants were asked to name the colors used to print an incongruent color word (e.g., BLUE printed in red letters). Participants responded verbally to items and pressed the space bar as quickly as possible to advance to the next item. The computer timed each trial and recorded the number of items completed in each trial. Four scores were generated from this test: the number of items completed in the first trial (Stroop-W), the number of items completed in the second trial (Stroop-C), a combined core for the first two trials (Stroop-WC), and relative interference (i.e., attentional control) score (Stroop-RI; Denney & Lynch, 2009). The Stroop-W, Stroop-C, and Stroop-WC provided RSP measures of processing speed and the Stroop-RI provided a measure of executive function. While higher scores on the trials indicated better performance, lower scores on the Stroop-RI indicated better attentional control.

Symbol Digit Modalities Test (SDMT). This computerized test was adapted from the paper-based SDMT (Smith, 1982). The test consisted of a single 90-second trial. During this test, a reference key was located at the top of the computer screen displaying nine nondescript geometric symbols with their corresponding digits (i.e., 1-9). Stimulus items consisted of symbols presented individually in the center of the computer screen. Participants were asked to state the number associated with the stimulus, and press the space bar to display the next item. The computer timed the trial and recorded the number of responses. Additionally, an Incidental

Learning (IL) trial was added to our computerized version of the SDMT in order to evaluate participants' acquisition of the associations between symbols and digits during the course of the preceding trial. Participants were shown a stimulus item without the reference key and were asked to say "yes" and press the "1" key if they saw the item on the previous reference key, and "no" and press the "3" key if they did not. If they responded "yes," a list of numbers 1 through 9 appeared on the screen and participants were asked to identify the number that went with the symbol. If they responded "no," the next stimulus item appeared on the screen. Two scores were derived from this measure: the total number of responses on the initial 90-second trial (SDMT) and the total number of correct number of responses on the IL trial (SDMT-IL). In terms of cognitive domain classification the SDMT provided an RSP measure of processing speed and the SDMT-IL provided a measure of visual memory. As in the case with the RAVLT, higher SDMT scores indicated better performance, while lower SDMT-IL scores indicated faster response times.

Brief Visuospatial Memory Test. This computerized test was adapted from the paper-based BVMT-R (Benedict, 1997). The test consisted of three visuospatial memory trials, where the participant was shown a 2x3 grid of six geometric shapes that appeared on the computer screen for 10 seconds. The participant was then asked to identify the six stimulus items from an array of 18 geometric shapes and to identify the position on the grid where each stimulus had been displayed. This trial was repeated two additional times. Participants were awarded two points for each correctly placed item and one point for each incorrectly placed item. The total sum of all item scores provided a measure of visual memory (BVMT), with higher scores indicating better performance.

Neuroimaging procedures. All MR scans were performed using a 3T scanner (Skyra, Siemans, Erlangen, Germany) at the Hoglund Brain Imaging Center located at the University of Kansas Medical Center. Participants were asked to lie supine in the MR scanner for the duration of all scans. Data acquisition consisted of a series of MRS scans, followed by a series of MRI scans. However, for the purpose of this study, we will only focus on the MRS scans.

MRS data acquisition. Participants were positioned for GSH chemical shift imaging (CSI) using a FLASH sequence of three-plane scout images such that the volume of interest (VOI), a 6 x 6 x 3-cm axial slab covering just above the corpus callosum, was placed in the iso-center of the magnet (Figure 1). MRS scans used a custom-made helmet coil designed to target frontal and parietal regions. Preliminary localized automated shimming was performed using the Massachusetts General Hospital shimming package to ensure homogeneity across the CSI slice. B_0 and B_1 mapping were performed to correct for signal variations caused by frequency shifts on GSH signals or any field inhomogeneities of the RF coil. GSH signals were measured using an MQ/SQ CSI sequence (TE/TR = 115/1500ms, FOV = 20cm, matrix = 12 x 12, slice thickness = 3cm), and an axial slice including frontal and parietal regions was imaged.

Concentrations of GSH were calculated using a gold standard internal reference method **(**Choi, Lee, Merkle, & Shen, 2004; 2006), the GSH-to-creatine (Cr) ratio. The phase and frequency of GSH signals in each voxel of CSI were corrected based on those of Cr signals in the corresponding voxels using a custom-written program and IDL software (RSI, Boulder, CO**)**. Frontal and parietal GSH concentrations were determined by overlaying the GSH data to anatomical MRI scans. In total, three GSH measures were obtained for the present study: a 2x4 voxel matrix corresponding to the frontal region, a 2x4 voxel matrix corresponding to the parietal region, and a 4x4 voxel matrix comprising the combined frontoparietal region (Figure 1).

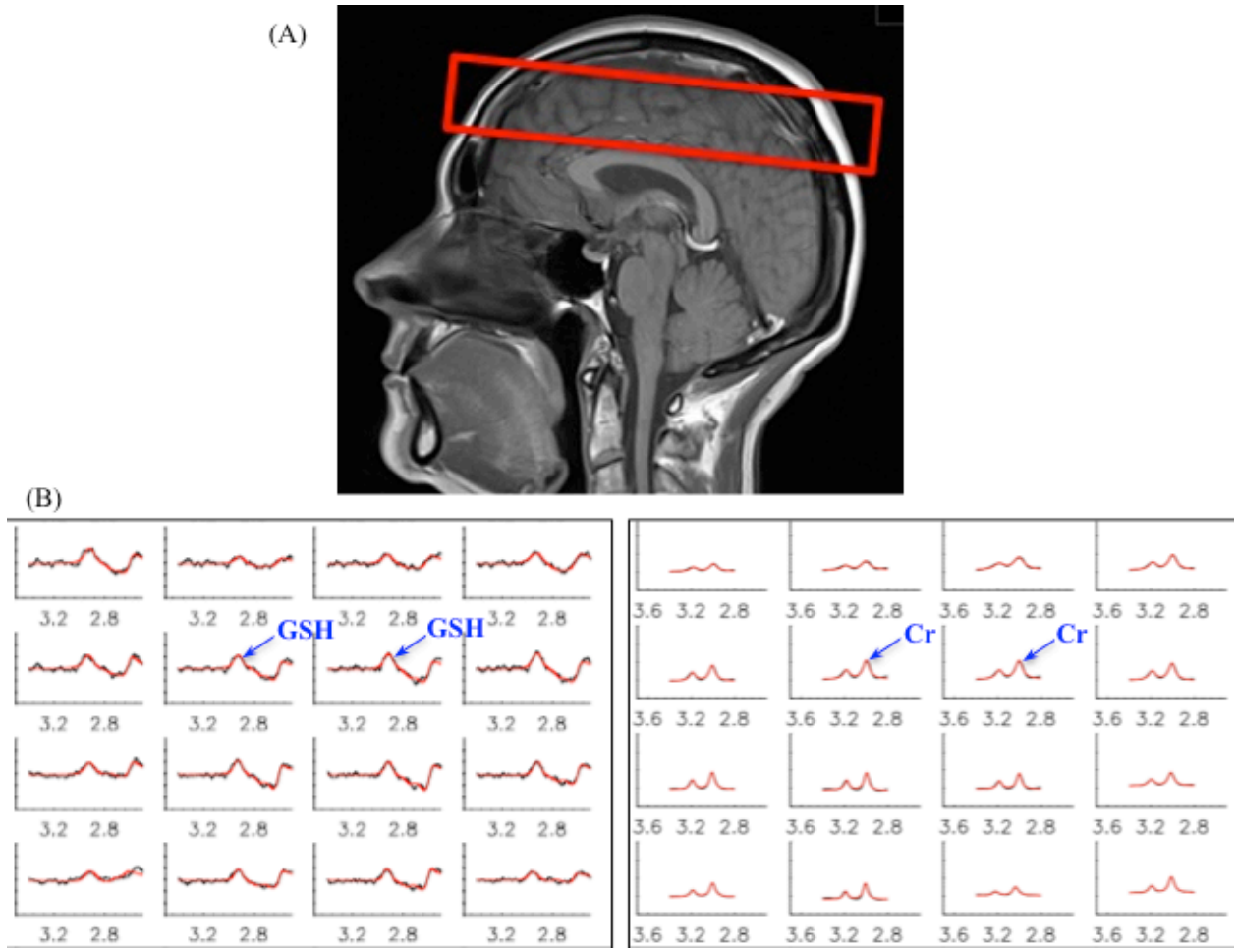

Figure 1. (A) Outline of glutathione (GSH) chemical shift imaging (CSI) slice, the volume of interest (VOI), overlaid on a T_1-weighted sagittal scout image of the brain. (B) Partial views of 4x4 frontoparietal voxel matrices for *in vivo* GSH CSI (left) and simultaneously measured creatine (Cr) CSI (right).

Procedures

This study was approved by the Human Subjects Committee at the University of Kansas Medical Center. MS patients were recruited during the course of their regularly scheduled appointments at the MS Clinic. After obtaining written consent and having the participant fill out an MRI safety form, a research assistant scheduled a time for the patient to return for their study appointment. Control participants were recruited through friends and family members of patients, research personnel, and employees at the University of Kansa Medical Center. Age and gender of control subjects was monitored in order to obtain approximate equivalency on these variables. Study appointments began with the administration of the demographic and self-report questionnaires, followed by the computerized neuropsychological assessments and finally the series of MR scans. In total, study procedures lasted approximately three hours.

Analyses

Preliminary Analyses. A preliminary omnibus analysis was performed to assess group differences (controls, RR, PP, SP) on demographic characteristics (gender, years of education, age), and self-report measures (BDI-FS, FSS, PSS). Variables that resulted in significant *F* statistics were further evaluated with a series of planned comparisons using Helmert contrasts. For the first contrast, controls were compared with all MS subtypes combined. The second contrast compared RR and combined progressive subtypes (PP and SP). Finally, the third contrast evaluated differences between PP and SP subtypes. The justification for these contrasts lies in evidence garnered from previous studies that (a) MS patients, regardless of subtype, often differ from healthy controls on these variables, (b) that these differences are subtler in RR patients relative to progressive subtypes, and (c) that PP and SP patients typically exhibit similar levels of these variables (De Sonneville et al., 2002). Demographic and self-report variables that

yielded significant group differences were explored as potential covariates for subsequent GSH and neuropsychological analyses.

In addition to demographic and self-report variables, disease-related variables (MSNQ, EDSS, disease duration) were also assessed for group differences using omnibus tests, followed by Helmert comparisons for significant variables. However, because these variables did not apply to controls, only the latter two Helmert contrasts (RR vs. progressive subtypes: PP vs. SP) were employed.

GSH and Neuropsychological Analyses. MRS data acquisition yielded GSH concentrations for three regions of interest: frontal, parietal, and combined frontoparietal. Neuropsychological assessment yielded 13 scores organized into three MS-relevant cognitive domains: processing speed, memory, and executive functioning. As in the case with demographic and self-report variables, group differences (controls, RR, PP, SP) for GSH concentrations and neuropsychological scores were evaluated using omnibus tests followed by Helmert contrasts. Additionally, Hedges' *g* effect sizes for each contrast were examined in order to identify the neuropsychological measures and GSH regions that yielded the largest group differences.

Correlational Analyses. With only patients being considered, associations between GSH concentrations and neuropsychological measures were calculated using Pearson product-moment correlations. Additionally, correlations between GSH concentrations and disease related variables were explored using Pearson product-moment correlations for MSNQ and disease duration and Spearman rank-order correlations for EDSS.

Results

Initial Group Differences

Means and standard deviations for all groups on continuous demographic, self-report, and disease-related variables are presented in Table 1. Groups did not differ with respect to gender (X^2 (3, N = 65) = 7.33, p = .06, Cramer's V = .34); however, an omnibus multivariate analysis of variance (MANOVA) revealed statistically significant group differences for age (F(3, 61) = 6.54, p < .001, η^2 = .24), years of education (F(3, 61) = 3.18, p < .05, η^2 = .14), depression (BDI-FS: F(3, 61) = 6.93, p < .001, η^2 = .25), fatigue (FSS: F(3, 61) = 14.51, p < .001, η^2 = .42), and perceived stress (PSS: F(3, 61) = 5.27, p < .01, η^2 = .21). Additionally, the three patient groups differed on disability status (EDSS: F(2, 43) = 44.25, p < .001, η^2 = .67) and disease duration (F(2, 43) = 4.95, p < .05, η^2 = .19), but not subjective cognitive impairment (MSNQ: F(2, 43) = 1.55, p = .23, η^2 = .07). Results of Helmert contrasts for significant initial variables are presented in Table 2. MS patients as a whole reported significantly greater levels of depression, fatigue, and stress relative to controls. Furthermore, patients with progressive subtypes were older, reported greater levels of depression and fatigue, and had greater disability ratings and longer disease durations than RR patients. Education comprised the only difference between PP and SP patients, with PP patients obtaining fewer years of education than SP patients.

Because of these initial group differences, age, education, BDI-FS, FSS, and PSS were examined as potential covariates using separate multivariate analyses of covariance (MANCOVAs). For GSH measures, none of these initial variables comprised significant covariates (all ps > .05). For neuropsychological test performance, fatigue and stress did not emerge as significant covariates (all ps > .05). Age was a significant covariate for performance on the Stroop-RI; education was a significant covariate for performance on eight

neuropsychological measures: Rey-DRRT, Stroop-W, Stroop-C, Stroop-WC, SDMT, Rey-A, Rey-IR, and Rey-DR; and BDI-FS emerged as a significant covariate for Stroop-C and SDMT performance. However, when these covariates were omitted and the analyses repeated, group differences on neuropsychological measures were nearly identical. Therefore, these variables were not included as covariates in subsequent analyses.

Table 1

Means and Standard Deviations of Demographic, Self-Report, and Disease-Related Variables

	CNTL (*N*=19)		RR (*N*=15)		PP (*N*=15)		SP (*N*=16)	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Age	47.79	11.73	39.67	9.54	54.13	7.59	51.56	8.36
Education (yrs)	15.53	1.90	15.07	1.58	14.07	1.58	15.94	1.98
BDI-FS	0.84	1.77	1.93	2.02	4.07	3.73	4.38	2.87
FSS	1.82	0.72	3.24	1.76	4.19	1.70	4.82	1.47
PSS	9.58	4.75	13.80	7.76	15.27	8.36	19.25	8.10
MSNQ	---	---	18.33	9.83	25.73	13.78	23.69	11.81
EDSS	---	---	1.87	0.69	5.83	1.16	5.63	1.78
Duration (yrs)	---	---	8.13	4.84	10.67	7.04	15.06	6.54

Table 2

Helmert Contrasts for Significant Demographic, Self-Report, and Disease-Related Variables

	Contrast Estimates		
	CNTL vs All MS	RR vs (PP+SP)	PP vs SP
Age	-.67	-13.18***	2.57
Education (yrs)	0.50	0.07	-1.87**
BDI-FS	-2.62***	-2.29**	-0.31
FSS	-2.27***	-1.26**	-0.63
PSS	-6.53**	-3.46	-3.98
EDSS	---	-3.86***	0.21
Duration (yrs)	---	-4.73*	-4.40

*$p < .05$. **$p < .01$. ***$p < .001$

GSH Concentrations

Mean GSH levels (frontal, parietal, and frontoparietal) for each group are depicted in Figure 2. Groups differences were observed for all three regions of interest (frontal: ($F(3, 42) = 4.54$, $p < .01$, $\eta^2 = .25$); parietal: ($F(3, 42) = 2.99$, $p < .05$, $\eta^2 = .18$); frontoparietal: ($F(3, 42) = 4.68$, $p < .01$, $\eta^2 = .25$). Helmert contrasts and Hedges *g* effect sizes for each region are presented in Table 3. For all three regions, GSH concentrations were significantly lower for MS patients than controls. Additionally, frontal and frontoparietal GSH levels were lower for progressive patients than for RR patients. No other significant group differences were observed. Hedges' *g* effect sizes for significant contrasts ranged from .81 to .98. While all three regions of interest were effective at differentiating controls and MS patients, only frontal and frontoparietal GSH concentrations were effective at distinguishing RR from progressive patients.

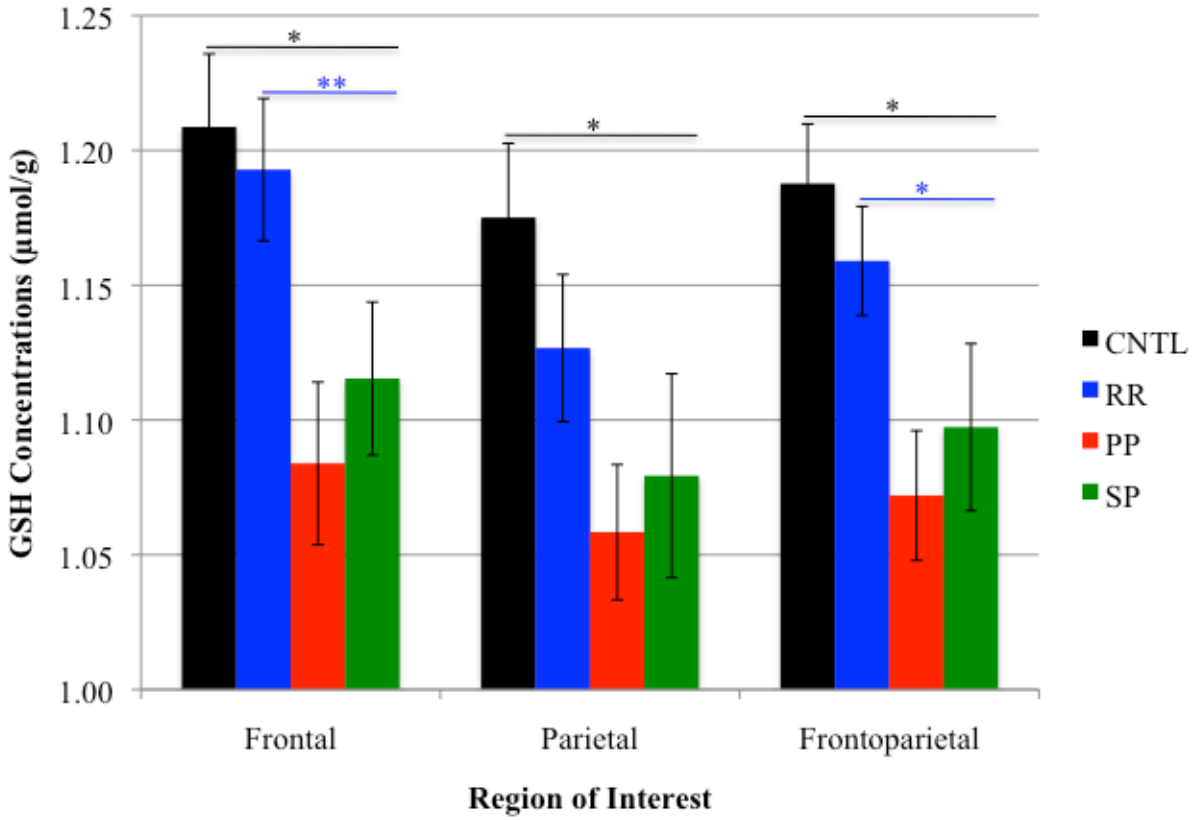

Figure 2. Comparison of GSH levels (means ± SEM) for Controls (n = 10), RR patients (n = 12), PP patients (n = 13), and SP patients (n = 11). *$p < .05$. **$p < .01$.

Table 3

Helmert Contrasts and Hedges' g for Significant GSH Measures

	CNTL vs All MS		RR vs (PP+SP)		PP vs SP	
	Contrast	*g*	Contrast	*g*	Contrast	*g*
Frontal	0.08*	.81	0.09**	.98	-0.03	-1.09
Parietal	0.09*	.91	0.06	.58	-0.02	-.64
Frontoparietal	0.08*	.96	0.07*	.91	-0.03	-.92

*$p < .05$. **$p < .01$. ***$p < .001$

Neuropsychological Outcomes

Table 4 presents means and standard deviations for all groups on neuropsychological measures included in this study. Groups did not differ significantly on any executive function scores (TOL-Score: ($F(3, 61) = 2.51$, $p = .07$, $\eta^2 = .11$); TOL-PT ($F(3, 61) = 1.34$, $p > .05$, $\eta^2 = .27$); Stroop-RI ($F(3, 61) = 0.50$, $p = .68$, $\eta^2 = .02$). However, group differences were significant for all other measures (all ps $< .05$), with eta-squared effect sizes ranging from .12 to .51. Helmert contrasts and Hedges *g* effect sizes for significant neuropsychological outcomes are presented in Table 5.

With regard to processing speed, MS patients as a whole performed more slowly than controls on all measures except the Stroop-W, and progressive patients performed more slowly than RR patients on all measures. There were no differences between PP and SP subtypes. Hedges' *g* effect sizes for significant contrasts ranged from .63 to 2.06. Effect sizes for the single RT measure (Rey-DRRT) and the four RSP measures were approximately equivalent in terms of distinguishing patients from controls and PP from SP patients; however, all four RSP measures were more effective than the RT measure for distinguishing RR from progressive patients.

For measures of verbal and visual memory, Hedges' *g* effect sizes for significant contrasts ranged from .52 to 1.50. Although visual tasks were generally more effective than verbal tasks for distinguishing patients and controls, verbal tasks were more effective for distinguishing RR and progressive subtypes. As in the case with processing speed measures, there were no differences between PP and SP subtypes.

Table 4

Means and Standard Deviations of Neuropsychological Variables

	CNTL (*N*=19)		RR (*N*=15)		PP (*N*=15)		SP (*N*=16)	
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*
Processing Speed								
Rey-DRRT	1.75	0.76	1.81	0.50	2.67	0.88	3.06	2.20
Stroop-W	76.21	17.17	80.47	14.64	58.53	22.01	60.69	22.21
Stroop-C	64.47	9.61	67.13	10.23	47.80	14.89	49.06	17.09
Stroop-WC	140.68	24.60	147.60	21.18	106.33	36.17	109.75	37.84
SDMT	61.79	11.82	61.33	9.59	36.53	10.17	39.31	15.13
Memory								
Verbal								
Rey-A	27.00	5.71	29.00	6.29	20.80	5.45	22.56	6.26
Rey-IR	8.16	3.08	8.60	3.29	5.27	2.52	5.69	3.01
Rey-DR	44.26	3.87	44.53	4.05	41.20	3.84	41.25	4.49
Visual								
SDMT-IL	40.95	2.42	37.27	4.06	36.60	3.09	36.00	2.76
BVMT	28.11	7.20	26.07	7.15	21.60	7.87	20.19	7.09
Executive Function								
TOL-Score	11.68	1.67	12.47	2.13	11.27	2.12	10.44	2.50
TOL-PT	9.75	4.10	11.98	4.61	12.39	4.37	12.43	5.51
Stroop-RI	27.19	10.94	26.95	9.96	30.72	12.37	29.77	8.79

Table 5

Helmert Contrasts and Hedges' g for Significant Neuropsychological Measures

	CNTL vs All MS		RR vs (PP+SP)		PP vs SP	
	Contrast	*g*	Contrast	*g*	Contrast	*g*
Processing Speed						
Rey-DRRT	-0.77*	-.66	-1.05**	-.85	-0.39	-.23
Stroop-W	9.65	.50	20.86***	1.12	-2.15	-.10
Stroop-C	9.81**	.73	18.70***	1.40	-1.26	-.08
Stroop-WC	19.46*	.63	39.56***	1.32	-3.42	-.09
SDMT	16.06***	1.14	23.41***	2.06	-2.78	-.22
Memory						
Verbal						
Rey-A	2.88	.46	7.32***	1.20	-1.76	-.30
Rey-IR	1.64*	.52	3.12**	1.03	-0.42	-.15
Rey-DR	1.94	.48	3.31*	.81	-0.05	-.01
Visual						
SDMT-IL	4.33***	1.50	0.97	.28	0.60	.20
BVMT	5.49**	.75	5.17*	.72	1.41	.19

*$p < .05$. **$p < .01$. ***$p < .001$

Correlations

Correlations between GSH concentrations and neuropsychological measures ranged from .07 to .52, from 0 to .43, and from .05 to .54 for frontal, parietal, and frontoparietal areas, respectively (Table 6). Frontal GSH levels were positively correlated with performance on all RSP processing speed and verbal memory measures, with correlations for these measures ranging from .36 to .52. Parietal GSH concentrations were positively correlated with the SDMT (r = .43), but not with any other scores. Frontoparietal GSH levels were positively associated with performance on the Stroop-C, Stroop-WC, SDMT, Rey-A, and Rey-IR. No significant correlations emerged for executive function measures. For all three regions, RSP measures resulted in larger correlations than RT measures, and correlations for verbal memory tasks were larger than those for visual memory tasks.

Correlations between GSH concentrations and disease-related characteristics ranged from .06 to .36 (Table 6). Frontal GSH concentrations were moderately correlated with MSNQ and frontoparietal concentrations were moderately correlated with EDSS. There were no significant correlations between GSH concentrations and disease duration.

Table 6

Correlations between GSH, Neuropsychological Measures, and Disease-Related Variables

	GSH Regions of Interest		
	Frontal	Parietal	Frontoparietal
Neuropsychological Measures			
Processing Speed			
Rey-DRRT	-.22	-.29	-.29
TOL-PT	-.27	-.24	-.29
Stroop-W	.37*	.20	.32
Stroop-C	.36*	.26	.35*
Stroop-WC	.38*	.23	.35*
SDMT	.52***	.43**	.54***
Memory			
Verbal			
Rey-A	.44**	.31	.43**
Rey-IR	.47**	.31	.44**
Rey-DR	.44**	.10	.30
Visual			
SDMT-IL	.25	.10	.19
BVMT	.20	.05	.14
Executive Function			
TOL-Score	.10	.12	.12
Stroop-RI	.07	.00	.05
Disease-Related Characteristics			
MSNQ	-.34*	-.20	-.32
EDSS†	-.29	-.30	-.36*
Disease Duration	-.23	.13	-.06

†Spearman rank-order correlations. *$p < .05$. **$p < .01$. ***$p < .001$

Discussion

The present study featured the first comparison of cerebral GSH concentrations among patients with RR, PP, and SP subtypes of MS and healthy controls. Consistent with our pilot study (Choi et al., 2011), frontal and frontoparietal GSH levels were lower for patients relative to controls. Additionally, relative to controls and RR patients, progressive (PP and SP) patients exhibited the largest reductions in GSH concentrations, with no significant differences between PP and SP patients. These results support the idea that even in the absence of acute clinical exacerbations (i.e., relapses), diffuse metabolic changes in GSH concentrations signal an ongoing neurodegenerative process that likely contributes to disease progression.

While the present study focused on GSH as one specific marker of diffuse neurodegeneration in MS, it is useful to consider whether other metabolites have exhibited similar patterns of outcomes among MS subtypes. Several studies have investigated N-acetylaspartate (NAA), a ubiquitous molecule that is found exclusively in neurons and serves as a marker of diffuse axonal injury (Kirov et al., 2009; Aboule-Enein et al., 2010). Aboule-Enein and colleagues (2010) noted group differences in NAA that were very similar to those observed for GSH in the present study. Specifically, SP patients exhibited the largest reductions in diffuse NAA concentrations relative to RR patients and controls. The striking similarity between NAA and GSH profiles among MS patients, particularly with regard to progressive subtypes, provides further support for neurodegeneration as a major force driving disease progression beyond the relapsing episodes of multifocal inflammation in brain tissues that seems to characterize RR patients.

In addition to examining group differences in GSH concentrations, the present study also investigated whether these differences occurred in conjunction with neuropsychological

impairments. The pattern of group differences on neuropsychological measures paralleled those for GSH concentrations, where progressive patients exhibited the most severe deficits, and no significant differences emerged between SP and PP patients. Additionally, GSH concentrations significantly correlated with select RSP measures of information processing speed and with additional measures of verbal memory, suggesting that oxidative stress as reflected by reductions in GSH concentrations may be driving these forms of cognitive impairment commonly observed in MS.

Differences in neuropsychological performance are perhaps best discussed in terms of a *worsening* and *broadening* of impairments (De Sonneville et al., 2002). These concepts are useful in conceptualizing the nature of cognitive impairment commonly observed over the course of MS. With regard to *worsening*, deficits in information processing speed, particularly on RSP measures, are typically observed early in the disease course and *worsen* with disease progression (De Sonneville et al., 2002; Parmenter, Shucard, Benedict, & Shucard, 2006). The emergence of processing speed deficits is generally attributed to the diffuse nature of MS pathology, where widespread axonal damage reduces the redundancy in connectivity that seems to characterize most tracts within the CNS, thereby detracting from the speed and efficiency of neuronal transmission (Kail, 1997; 1998). In the present study, effect sizes for progressive patients relative to RR patients were quite large, providing at least suggestive evidence of this worsening. With regard to *broadening*, the limited spectrum of deficits observed early in the disease course expands to include impairments in other cognitive domains as the disease progresses (Amato, Ponziani, Siracusa, & Sorbi, 2001). The emergence of these additional deficits is generally believed to be a consequence of more extensive neuronal injury, where redundancies in neurological tracts can no longer forestall the emergence of additional deficits in cognitive

operations (Bjartmar, Wujek, & Trapp, 2003). The present findings supported evidence for broadening, with the addition of two verbal memory deficits (Rey-A and Rey-DR) in progressive patients.

It is important to note that no differences were found between groups on any of the executive function measures; thus, the concepts of "worsening" and "broadening" did not appear to apply to this cognitive domain. This finding highlights one of the major inconsistencies within the MS literature. Whereas some investigators have cited evidence for executive function deficiencies (e.g., Beatty, Hames, Blanco, Paul, & Wilbanks, 1995; Heaton et al., 1985; Nilsson, Rorsman, Larson, Norrving, & Sandberg-Wollheim, 2008), others, including our own research group (Drew, Tippett, Starkey, & Isler, 2008; Helekar et al., 2010; Denney, Lynch, & Parmenter, 2008; Denney, Lynch, Parmenter, & Horne, 2004; Denney et al., 2011), have consistently failed to find these differences. A meta-analysis by Zakzanis (2000) attempted to resolve this issue, suggesting that while executive deficits are present in MS, they do not typically emerge until later stages of the disease. Even so, this conclusion conflicts with studies that have demonstrated executive dysfunction quite early in the disease process (Schulz, Kopp, Kunkel, & Faiss, 2006). Several investigators (Denney et al., 2011; DeLuca, Chelune, Tulsky, Lengenfelder, & Chiaravalloti, 2004) have suggested that impairments in executive function observed in MS patients may be better explained as an artifact of more basic deficits in processing speed, where executive function deficits emerged only when assessments were confounded with speed. For example, while Denney and Lynch (2009) found differences in the naming of print colors on incongruent Stroop stimuli, a measure of executive function (attentional control) that is confounded with speed, no group differences remained after statistically controlling for speed. This hypothesis was also supported in a study using the TOL (Owens, Denney, and Lynch,

2012), where patients performed worse than controls when the TOL was administered under explicit time constraints, but not under untimed conditions. Because the present study used an untimed version of the TOL, the lack of group differences observed on this measure, as well as on the Stroop-RI, supported the notion that executive dysfunction is not a hallmark deficit among MS patients at any stage of the disease.

While significant differences emerged between RR and progressive subtypes, no significant differences were observed between PP and SP patients. Therefore, it is possible that these two subtypes may be pathologically indistinguishable in terms of neurodegeneration, resulting in comparable GSH concentrations and neuropsychological test performance. Alternatively, given the relatively small sample sizes of 15 and 16 for PP and SP patients, respectively, it is possible that the present study lacked sufficient statistical power to detect differences in either GSH concentrations or neuropsychological test performance. Some investigators have suggested that SP patients may have more severe neuropsychological deficits than PP patients as a function of typically longer disease durations; however, this distinction is rather tenuous given that the measurement of disease duration generally begins when patients exhibit distinct episodes involving inflammatory processes and for PP patients, such episodes may be clinically "silent" and therefore undiagnosed.

In addition to small sample sizes, several limitations deserve mention. First, all of the patients who participated in this study were required to have cognitive abilities sufficient for understanding the instructions for neuropsychological assessments. Approximately 10 patients were screened as ineligible on the basis of prohibitive cognitive impairment during the recruitment phase of the study. Therefore, we cannot discount the possibility that executive dysfunction might have been evident in these excluded patients, and that these deficits would

have been correlated with GSH concentrations. Second, additional metabolic markers (e.g., NAA) and structural measures (e.g., global atrophy; lesion burden) of neurodegeneration were not included in the present study. Therefore, we cannot establish the relationship between GSH reductions and these other more firmly established markers of neurodegeneration in this sample. Finally, it is not entirely clear whether GSH concentrations provide a dynamic marker of active, ongoing neurodegeneration, or a more static marker of the total neurodegeneration accumulated over time (Choi et al., 2011). An analogous comparison might be made for the difference between daily blood glucose measurements and glycated hemoglobin (A1c) testing in patients with diabetes mellitus, where daily measurements provide a better dynamic marker of blood glucose changes and A1c levels provide a better summative marker of blood glucose control. While the former would be more useful for detecting the initiation of neurodegenerative processes, the latter would be more relevant in examining neurodegenerative changes over time.

In light of these limitations, future work in this area would benefit from including information concerning other brain metabolites and structural MRI scans. Additionally, a longitudinal study, with particular focus on GSH levels in RR patients who over time develop SP MS versus those who never undergo this conversion could provide valuable insight into whether GSH constitutes a dynamic or static marker of neurodegeneration as well as the predictive value of this marker in terms of future changes in clinical status. Comparative GSH concentrations in these groups could further clarify the role of oxidative stress-mediated neurodegeneration and the neurological and neuropsychological sequelae of this process.

In conclusion, the present study examined the role of oxidative stress-mediated neurodegeneration among patients with RR, PP, and SP MS and healthy controls. Reductions in diffuse cerebral GSH concentrations were accompanied by a worsening of impairment in

processing speed and a broadening of impairment to include deficits in learning and memory, and importantly, these impairments were more pronounced in PP and SP patients than in RR patients. As such, the present findings suggest that oxidative stress-mediated neurodegeneration comprises an event driving disease progression and related neuropsychological impairments in the later stages of MS.

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