FUNCTIONAL BRAIN IMAGING, COGNITIVE DYSFUNCTION AND ANTI-NR2 ANTIBODIES IN PATIENTS WITH NON-NEUROPSYCHIATRIC SYSTEMIC LUPUS ERYTHEMATOSUS

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NATIONAL UNIVERSITY OF SINGAPORE

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DECLARATION

I hereby declare that this thesis is my original work or work to which I substantially contributed and it has been written by me in its entirety. I have duly acknowledged all the sources of information which have been used in the thesis.

This thesis has also not been submitted for any degree in any university previously.

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ABSTRACT

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease which potentially affects major organs during the disease course. Neuropsychiatric SLE (NPSLE) is one of the major manifestations of SLE which carries unfavourable impact on the quality of life, vocational outcome and survival of patients with SLE. Cognitive dysfunction, which affects executive function, attention, learning and memory, is the commonest neuropsychiatric manifestation of SLE. Yet, cognitive impairment in lupus patients is often clinically unapparent and indeed, its functional neuro-pathogenesis remains unclear. Therefore, we attempted to approximate this knowledge gap by a series of studies reported in this thesis which can hopefully prompt further focussed research on the pathogenesis and clinical classification of cognitive dysfunction in SLE.

In the first study, patients with new-onset SLE without neuropsychiatric symptoms and their matched healthy counterparts went through the modified computerized Wisconsin Card Sorting Test (WCST) while they were undergoing functional magnetic resonance imaging (fMRI) of the brain concomitantly. Although the SLE patients and healthy controls had comparable performance on the WCST, SLE patients demonstrated inferior strategic planning skills which resulted in compensatory recruitment of additional cortical regions to compensate for their inferiority in strategic planning. Surprisingly, their inefficient strategic planning skills and the subsequent compensatory recruitment of cortical regions which boosted their cognitive function for error detection and conflict monitoring persisted even after control of the disease. These findings prompted the second important mechanistic study.

While the activity of a few anatomical brain regions were found to be altered in lupus patients, further studies on potential functional neural circuits which mediate cognitive impairment in SLE would be pathologically relevant. In the second study, by applying the same fMRI scan and WCST paradigms as the first study to patients with new-onset lupus and healthy controls, the cortico-basal ganglia-thalamic-cortical circuit and amygdala-hippocampus coupling, which were involved in response inhibition and active forgetting-learning dynamics respectively, were found to be compromised in lupus patients when the demand for working memory and learning reached the maximum during the WCST. An increase in the activity of the contralateral cerebellar-frontal connection was found to compensate for the compromised cortico-basal ganglia-thalamic-cortical circuit in the lupus patients in order to maintain their comparable WCST performance as their healthy counterparts. These findings confirmed that functional neural circuits were involved in mediating subclinical cognitive impairment in SLE and prompted us to perform the third study to investigate whether these dysfunctional circuits were associated with putative autoantibodies associated with cognitive dysfunction in SLE.

In the third study, while SLE patients were found to have significantly higher levels of serum anti-NR2 antibodies than their healthy counterparts, no significant association was found between the levels of the antibodies and lupus disease activity, the frequency of NPSLE as well as the abnormal fMRI signals of the hippocampus and amygdala in the lupus patients. The results suggest that serum anti-NR2 antibodies alone is unlikely an optimal marker to detect subclinical impairment of learning and memory in SLE.

Word count: 500

Keywords: fMRI, WCST, cognitive dysfunction, anti-NR2, SLE

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LIST OF ABBREVIATIONS

1st NF	first negative feedback
1st PF	first positive feedback
2nd NF	subsequent negative feedback
2nd PF	subsequent positive feedback
AC	anterior commissures
aCL	anti-cardiolipin
ACR	American College of Rheumatology
AD	Alzheimer's disease
ANA	anti-nuclear antibodies
ANAM	Automated Neuropsychological Assessment Metrics
anti-dsDNA	anti-double-strand DNA
anti-PL	anti-phospholipid
APC	antigen-presenting cells
APTT	Activated partial thromboplastin time
BA	Brodmann area
BAFF	B cell activating factor

BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
BOLD	blood-oxygen-level-dependent
CBF	cerebral blood flow
CD	cluster of differentiation
CD40L	CD40 ligand
Cho	Choline
CNS	central nervous system
Cr	Creatine
CSF	cerebrospinal fluid
DC	dendritic cells
dHb	deoxygenated hemoglobin
DLPFC	dorsolateral prefrontal cortex
DMN	default mode network
DRVVT	Dilute Russell Venom Viper test
EEG	electroencephalography
ELISA	Enzyme-linked immunosorbent assay

FE	feedback evaluation
fMRI	functional Magnetic Resonance Imaging
GP	globus pallidus
GWAS	genome-wide association studies
HRQoL	health-related quality of life
IF	intermediate neurofilament
IFN	interferon
IFS	inferior frontal sulcus
IL	interleukin
INA	intermediate neurofilament alpha-internexin
IQ	intelligence quotient
ITGAM	integrin alpha M
KCT	Kaolin clotting time
LAC	lupus anti-coagulant
mDCs	myeloid DCs
MHC	major histocompatibility complex
MMP	matrix metalloproteinase

MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
MTI	magnetization transfer imaging
MTR	magnetization transfer ratio
NAA	N-acetyl aspartate
NMDAR	N-methyl-D-aspartate receptor
NPSLE	Neuropsychiatric SLE
NSAIDs	nonsteroidal anti-inflammatory drugs
NUH	National University Hospital
OD_{450}	optical density at 450 nm
Hb	oxygenated hemoglobin
PBS	phosphate-buffered saline
PC	posterior commissures
pDCs	plasmacytoid DCs
PET	positron emission tomography
PFC	prefrontal cortex
PNS	peripheral nervous system

R4A mab	R4A monoclonal antibody
ROI	region of interest
RS	response selection
SD	standard deviation
SDI	Systemic Lupus International Collaborating Clinics / American College of Rheumatology damage index
SLE	systemic lupus erythematosus
SLEDAI	Systemic Lupus Erythematosus disease activity index
SLICC	Systemic Lupus International Collaborating Clinics
SNP	single nucleotide polymorphism
SPECT	single-photon-emission computed tomography
STAT4	signal transducer and activator of transcription 4
Th	T helper
TIMP	tissue inhibitor of metalloproteinases
TLR	toll-like receptor
TNF α	tumor necrosis factor alpha
TNFSF4	tumor necrosis factor superfamily member 4
TRAIL	TNF-related apoptosis-inducing ligand

TSA	Trichostatin A
WCST	Wisconsin Card Sorting Test
WAIS	Wechsler Adult Intelligence Scale
Zn	Zinc

PUBLICATIONS

Data presented in the studies in this thesis have been reported in the literature listed below, which have been published, in press or under review in international peer-reviewed journals.

*Mak, A., *Ren, T., Fu, E.H., Cheak, A.A., and Ho, R.C. (2012). A prospective functional MRI study for executive function in patients with systemic lupus erythematosus without neuropsychiatric symptoms. *Seminars in arthritis and rheumatism 41*, 849-858.

*Ren, T., Ho, R.C., and *Mak, A. (2012). Dysfunctional cortico-basal ganglia-thalamic circuit and altered hippocampal-amygdala activity on cognitive set-shifting in non-neuropsychiatric SLE. *Arthritis and rheumatism 12*, 4048-4059.

*Ren, T., Tay, S.H., Chua, Z., Ho, R.C., and *Mak, A. (2012). Serum anti-NR2 alone is not an ideal marker of subclinical working memory and learning deficits assessed based on abnormal real-time functional brain signals in systemic lupus erythematosus. (*under review*).

* denotes equal contribution

CHAPTER 1

INTRODUCTION

1.1 Systemic lupus erythematosus – an overview

1.1.1 Clinical classification of the disease

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease with predilection for women during their reproductive age. SLE potentially affects any organ systems during the disease course. Amongst various organ systems, the dermatological, musculoskeletal, haematological, renal and neuropsychiatric systems are commonly involved (Tsokos, 2011). Besides specific organ involvement, non-specific symptoms such as fatigue, weight loss, fever, anxiety and mild cognitive impairment are often the main presenting symptoms of lupus and they pose diagnostic challenge especially at the early stage of the disease (Lahita, 2011). Indeed, patients with SLE are often suspected to have other conditions such as rheumatoid arthritis (RA), fever of unknown origin and fibromyalgia before seeing a specialist or an experienced clinician (Blumenthal, 2002; Harvey et al., 1954; Hoffman, 1978). Furthermore, the mean interval between onset of symptoms and diagnosis can lapse as long as 5 years, during which the prognosis could be adversely affected (Lahita, 2011).

Perhaps due to the heterogeneity and complexity of SLE, the formal diagnostic criteria were not established until the American College of Rheumatology (ACR) defined the 11 criteria for the classification of SLE (Tan et al., 1982). This "1982 criteria" were subsequently revised on the 10th criterion of immunologic disorder in 1997 by deleting positive LE cell preparation and adding abnormal serum level of IgG or IgM anti-cardiolipin antibodies and positivity of lupus anticoagulant (Hochberg, 1997) (see Table

1.1). The presence of four or more criteria out of the 11 is required for formal classification for SLE. These criteria were demonstrated to have the sensitivity and specificity of as high as 96% (Gleicher, 1992). At the time when this thesis was being prepared, a new set of classification criteria for SLE has been validated and released by the Systemic Lupus International Collaborating Clinics (SLICC) (Petri et al., 2012). The SLICC classification criteria for SLE consist of 11 clinical and 6 immunological criteria. Patients must fulfill at least 4 criteria, including at least one clinical criterion and at least one immunological criterion or patients have biopsy-proven lupus nephritis with the simultaneous presence of antinuclear antibodies (ANA) or anti-double-strand DNA (anti-dsDNA) antibodies before they fulfill the criteria (Petri et al., 2012). The SLICC classification criteria demonstrate significantly reduced misclassification and improved sensitivity in the derivation and the validation sets when compared to the 1997 ACR criteria (Petri et al., 2012). Given that lupus criteria might be acquired and accrued over a period of time, the Boston weighted criteria for the classification of SLE which include patients who meet less than 4 of the 11 ACR criteria identify 7% more lupus patients when compared with the 1997 ACR criteria (Costenbader et al., 2002).

Table 1.1 The 1997 American College of Rheumatology Criteria for the classification of SLE

Criterion	Definition
1. Malar Rash	Fixed erythema, flat or raised, over the malar eminences, tending to spare the nasolabial folds
2. Discoid rash	Erythematous raised patches with adherent keratotic scaling and follicular plugging; atrophic scarring may occur in older lesions

3. Photosensitivity	Skin rash as a result of unusual reaction to sunlight, by patient history or physician observation
4. Oral ulcers	Oral or nasopharyngeal ulceration, usually painless, observed by physician
5. Arthritis	Nonerosive arthritis involving 2 or more peripheral joints, characterized by tenderness, swelling, or effusion
6. Serositis	i. Pleuritis-convincing history of pleuritic pain or rub heard by a physician or evidence of pleural effusion
	ii. Pericarditis-documented by ECG or rub or evidence of pericardial effusion
7. Renal disorder	i. Persistent proteinuria greater than 0.5 grams per day or greater than 3+ if quantitation not performed
	ii. Cellular casts-may be red cell, hemoglobin, granular, tubular, or mixed
8. Neurologic disorder	i. Seizures-in the absence of offending drugs or known metabolic derangements; e.g., uremia, ketoacidosis, or electrolyte imbalance
	ii. Psychosis-in the absence of offending drugs or known metabolic derangements, e.g., uremia, ketoacidosis, or electrolyte imbalance
9. Hematologic disorder	i. Hemolytic anemia-with reticulocytosis
	ii. Leukopenia-less than 4,000/mm^3 total on 2 or more occasions

	iii. Lymphopenia-less than 1,500/mm^3 on 2 or more occasions
	iv. Thrombocytopenia-less than 100,000/mm^3 in the absence of offending drugs
	i. Anti-DNA: antibody to native DNA in abnormal titer
	ii. Anti-Sm: presence of antibody to Sm nuclear antigen
	iii. Positive finding of antiphospholipid antibodies on:
10. Immunologic disorder	a) an abnormal serum level of IgG or IgM anticardiolipin antibodies,
	b) a positive test result for lupus anticoagulant using a standard method, or
	c) a false-positive test result for at least 6 months confirmed by *Treponema pallidum* immobilization or fluorescent treponemal antibody absorption test
11. Antinuclear antibody	An abnormal titer of antinuclear antibody by immunofluorescence or an equivalent assay at any point in time and in the absence of drugs known to be associated with "drug-induced lupus" syndrome

Source: Hochberg, 1997; Tan et al., 1982

1.1.2 Pathophysiology of SLE

The etiology of SLE is not fully known. A number of factors such as genetics, epigenetics, female hormone, gender, immune dysregulation and environmental triggers have been hypothesized and tested, for an aim to explain the mechanisms of the

breakdown of immune tolerance and aberrant autoimmune responses in patients with SLE (Crispin et al., 2010; Tsokos, 2011).

Genetics & Epigenetics

The concordance rates of SLE observed in monozygotic twins (24-56%) and dizygotic twins (2-5%) imply that genetic predisposition plays a crucial role in the development of SLE (Deapen et al., 1992; Moser et al., 2009). While defects in a single gene contribute to around 1-2% of the cases of SLE, the strongest single-gene defect which confers a high risk for SLE is the homozygous deficiency of C1q and C4 (Moser et al., 2009; Sestak et al., 2011). Lack of C1q or C4 has been suggested to be associated with compromised elimination of apoptotic materials and autoreactive B cells due to defective phagocytosis which leads to the enhanced stimulation of alloreactive T cells and subsequent interferon (IFN) γ production by the myeloid dendritic cells (DC) (Castellano et al., 2007).

In most cases, however, the higher risk of development of SLE is conferred by the cumulative effect of a number of SLE susceptibility genes and/or mutations rather than single gene defects. Early studies using candidate gene association analysis identified a number of at-risk genes for SLE including those encoding the major histocompatibility complex (MHC) class II (DR2 and DR3), tumor necrosis factor alpha (TNF α) and the early complement components (Sestak et al., 2011). With the recent advent of high-throughput genotyping technology, several genome-wide association studies (GWAS) identify a number of chromosome loci and genes associated with the increased susceptibility of SLE (Graham et al., 2008; Han et al., 2009; Harley et al., 2008; Hom et

al., 2008; Kozyrev et al., 2008; Yang et al., 2010). Functional studies have revealed the mechanisms by which some of these chromosome loci or genes are linked to the predisposition of SLE. For example, integrin alpha M (*ITGAM*), also known as CD11b or complement receptor 3, is one of the important components involved in complement cleavage and clearance of immune complex. A single nucleotide polymorphism (SNP) in the third exon of *ITGAM* (rs1153679) was shown to increase nephritis and discoid in lupus patients (Kim-Howard et al., 2010). The signal transducer and activator of transcription 4 (*STAT4*) transmits signals from receptors for IFN and interleukin (IL) and contributes to autoimmune responses. Located in the third intron of *STAT4*, rs7574865 has consistently been found to correlate with younger age of SLE onset, increased frequency of nephritis and the presence of anti-dsDNA antibodies (Kawasaki et al., 2008; Palomino-Morales et al., 2008; Sigurdsson et al., 2008). Involved in immune signal transduction, tumor necrosis factor superfamily member 4 (TNFSF4) is mainly expressed on the antigen-presenting cells (APC). Increased expression of TNFSF4 influences the susceptibility of SLE via either extended interaction between T cells and APCs or altering the consequence of T cell activation (Cunninghame Graham et al., 2008). A tagging SNP (rs2205960) which is located upstream of *TNFSF4* correlates with increased expression of TNFSF4 and increases the susceptibility of SLE (Chang et al., 2009; Cunninghame Graham et al., 2008; Han et al., 2009). In addition to gene polymorphisms, copy number variations of certain genes, such as *C4* and *toll-like receptor (TLR) 7*, have been linked to the increased risk for the development of SLE (Blanchong et al., 2001; Kelley et al., 2007).

Besides the impact of genetics on the pathogenesis of SLE, epigenetic regulation of gene expression via DNA methylation and histone acetylation has been shown to be involved in the development of SLE. Hydralazine and procainamide, which inhibit DNA methylation, are capable of inducing lupus-like manifestations in healthy individuals (Ballestar et al., 2006). For instance, demethylation of *cluster of differentiation (CD)11a* and *CD70* was shown to confer the susceptibility of SLE (Ballestar et al., 2006). In lupus T helper (Th) cells, the histone deacetylase inhibitor trichostatin A (TSA) has been found to restore the default expression of CD40 ligand (CD40L), resulting in the rebalance of IL-10 and IFN γ production (Mishra et al., 2001).

Female hormones & Gender

SLE has a predilection for women of child-bearing age. The female-to-male ratio is approximately 9 to 1 in adult SLE patients although the gender gap approximates in pediatric and late-onset SLE patients. Pregnancy and hormonal replacement therapy may increase the chance of disease flare (Buyon et al., 2005; Petri, 1997; Ruiz-Irastorza et al., 1996). These observations suggest that female hormones are likely to be involved in the pathogenesis of SLE. It has been postulated that the high estrogen to androgen ratio in females confers a higher risk for SLE through the inhibition of Th1 responses and upregulation of CD40L in T cells which favors the Th2 responses, leading to enhanced B cell responsiveness and autoantibody production (Mok & Lau, 2003). Of note, the X chromosome appears to contribute to the increased susceptibility of SLE independent of the physiological effects of female hormones. In genetically modified rodent models with the expression of XX, XO, or XXY, the presence of two X chromosomes confers elevated severity of SLE (Smith-Bouvier et al., 2008). This observation further supports

the role of CD40 in the pathogenesis of SLE because the CD40 gene is located on the chromosome X (Fuleihan et al., 1993).

Immune dysregulation

In SLE, T cells activation is aberrant, which is partially secondary to the alteration in T cell receptor signaling. CD3 complex is engaged in a fashion that leads to enhanced early signaling responses and consequent T cell activation (Crispin et al., 2008). The aggregation of lipid rafts on the cell surface membrane further amplifies the enhancement of T cell activation in patients with SLE (Li et al., 2007). Furthermore, cytokine physiology of lupus patients is dysregulated. For instance, IL-2, which is central to T cell activation and proliferation, is deficient in lupus T cells (Crispin et al., 2008). Insufficient production of IL-2 leads to defective activity of cytotoxic T cells, suppression of activation-induced cell death and prolongation of the survival of autoreactive T cells in lupus patients (Crispin et al., 2010). As the chief member of the IL-17 family of cytokines, IL-17 demonstrates proinflammatory properties and functions on various types of immunocytes in that it is capable of inducing the production of proinflammatory cytokines and chemokines, resulting in recruitment of monocytes and neutrophils to the vicinity of inflammation (Korn et al., 2009). IL-17 is primarily produced by activated T cells and critically involved in immune response against various bacteria and fungi (Korn et al., 2009). Patients with SLE demonstrated elevated levels of serum IL-17 and the levels of serum IL-17 has been demonstrated to correlate with lupus activity (Doreau et al., 2009).

Low number and hyperactivity are predominant abnormalities observed in lupus B cells (Crispin et al., 2010; Scheinberg & Cathcart, 1974), although lymphopenia is not restricted to lupus B lymphocytes since reduced number of circulating T cells has also been observed in patients with SLE (Scheinberg & Cathcart, 1974; Schulze-Koops, 2004). The number of naïve B cells is reduced in a disease activity-dependent manner, whereas the number of plasma cells increases in the peripheral blood of SLE patients (Odendahl et al., 2000). In addition, autoreactive B cells can escape immune surveillance while passing through various immune tolerance checkpoints without being deleted (Kumar et al., 2006), and this phenomenon may result in over-production of autoantibodies in patients with SLE (Crispin et al., 2010; Yurasov et al., 2006). Furthermore, the number of DNA-binding B cells which correlate with disease activity, increases regardless of antigen exposure (Jacobi et al., 2009).

DCs play a critical role in the development of immune responses against infection and malignancies but the dysfunction of DCs may lead to amplification of immune responses in autoimmune conditions such as SLE (Crispin et al., 2010). The presence of IFN α, CD40L, free nucleosomes and autoantibody-DNA complex in lupus sera is able to induce differentiation and activation of normal DCs (Blanco et al., 2001; Decker et al., 2005; Means et al., 2005). Quantitative and functional abnormalities of circulating plasmacytoid DCs (pDCs) and myeloid DCs (mDCs) have been revealed in patients with SLE (Ding et al., 2006; Gerl et al., 2010; Jin et al., 2010; Jin et al., 2008). Increased number of circulating pDCs was found in lupus patients (Jin et al., 2008). Lupus pDCs were demonstrated to reduce the differentiation of regulatory T cells and lead to altered cytokine profile as indicated by the persistently elevated production of IL-10 in SLE

patients (Jin et al., 2010). Furthermore, pDCs stimulate the production of type I IFN (Crispin et al., 2010), and upon exposure to IFN α, IFN-inducible genes including the *Fas*, *TNF-related apoptosis-inducing ligand (TRAIL)* and *B cell activating factor (BAFF)*, which are significantly involved in lupus pathogenesis (de Veer et al., 2001), were found to be up-regulated in patients with SLE (Feng et al., 2006). On the other hand, the number of circulating mDCs was found to be reduced in lupus patients (Jin et al., 2008). Lupus mDCs are characterized by accelerated differentiation, maturation and increased secretion of proinflammatory cytokines which are reflected by the increased expression of DC differentiation marker (CD1a), maturation markers (CD86 and CD80) and IL-8 respectively (Ding et al., 2006). Since lupus mDCs are capable of enhancing the proliferation and activation of allogeneic T cells in a more vigorous fashion than those of healthy individuals, such phenotypic abnormalities of mDCs might be functionally associated with the pathogenesis of SLE (Ding et al., 2006).

Environmental triggers

Smoking and ultraviolet light exposure were identified to be risk factors for the development of SLE (Furukawa et al., 1990; Simard et al., 2009). In addition, a number of organic and inorganic chemical agents such as aromatic amines (Reidenberg, 1981), trichloroethylene (Kilburn & Warshaw, 1992), insecticide (Beer et al., 1994), silicone (Press et al., 1992), gold (Robinson et al., 1986; Silverberg et al., 1970), mercury (Robinson et al., 1986) and cadmium (Ohsawa et al., 1988) were also suggested to be potential triggers of SLE. Epigenetic alterations observed in SLE might be attributed to medications and other potential environmental factors associated with lupus (Ballestar et al., 2006; Crispin et al., 2010). The possible role of viral infection in the development of

SLE has long been advocated (James & Robertson, 2012). Significantly higher seropositivity (Tsokos et al., 1983) and increased viral load of the Epstein-Barr virus (EBV) have been found in both pediatric and adult SLE patients (Kang et al., 2004). It has been suggested that molecular mimicry between viral proteins and certain self-antigens enables specific immune responses to cross-react with self-antigens (Crispin et al., 2010; James & Robertson, 2012). For example, antibodies against Epstein-Barr nuclear antigen can cross-react with a few highly specific lupus-associated autoantigens such as Sm B/B', and Sm D1(James & Robertson, 2012; Poole et al., 2006).

In summary, the complex interplay between genetics, epigenetics, female hormones, immune regulation and environment may pose impact on immune tolerance in a sequential or simultaneous manner, leading to the production of autoreactive lymphocytes, autoantibodies, immune complexes, and subsequent proinflammatory cytokines (see Figure 1.1). These cells and mediators initiate and perpetuate inflammation and potentially lead to damage of various organs and systems.

Figure 1.1 Overview of the pathogenesis of SLE
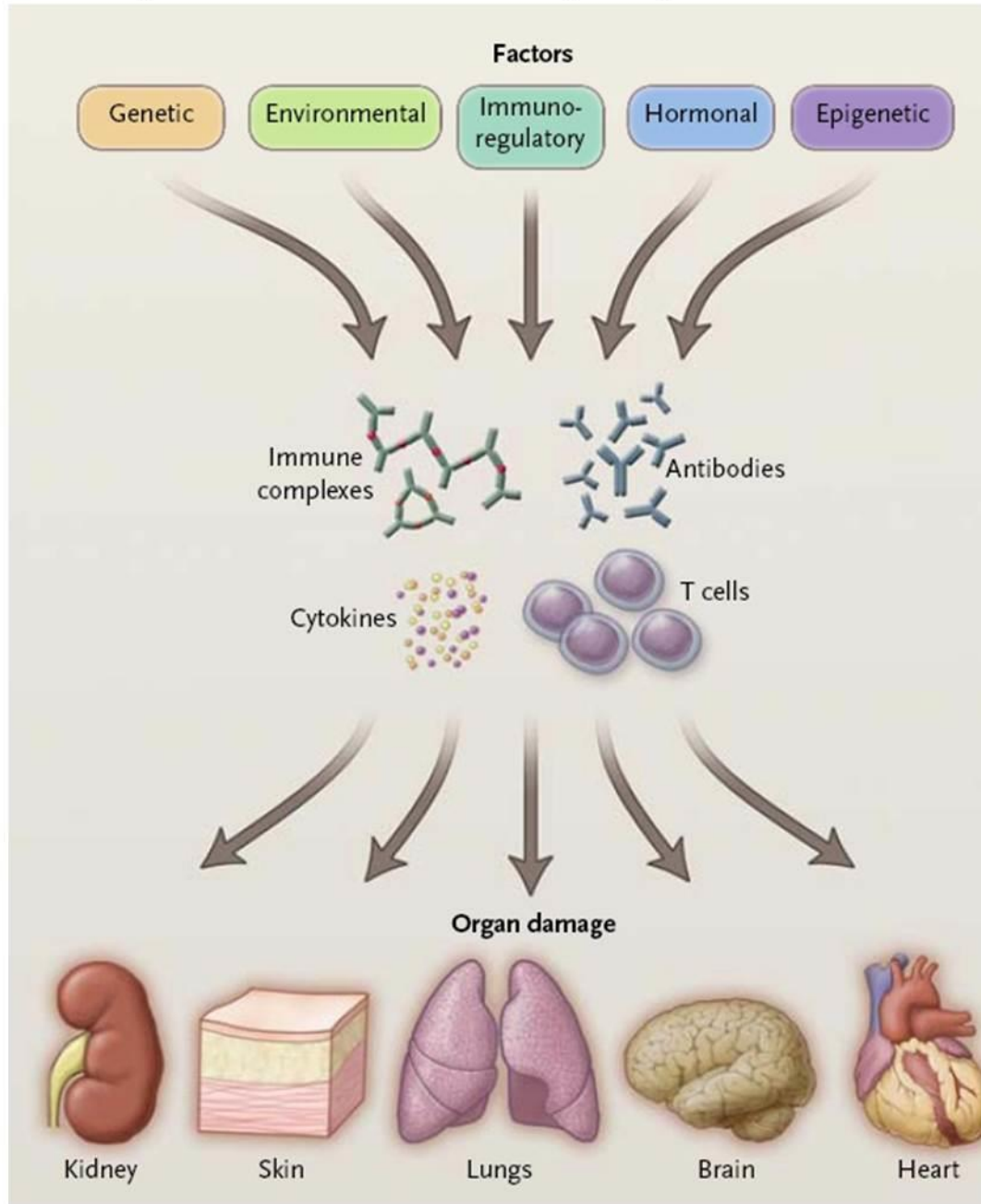
Reproduced with permission from Tsokos, 2011. Copyright Massachusetts Medical Society.

1.1.3 Management of SLE – brief overview

Due to the broad array of symptoms and signs and organ involvement in patients with SLE, the choice of treatment strategy must be tailored based on individual clinical characteristics such as organs and systems involved, disease severity and damage and baseline organ function. Currently, nonsteroidal anti-inflammatory drugs (NSAIDs), antimalarial agents, glucocorticoids and various immunosuppressants such as cyclophosphamide, azathioprine, cyclosporine and mycophenolate mofetil are commonly prescribed to treat SLE patients (Tsokos, 2011). NSAIDs have been proved to be useful in lupus patients with mild disease such as arthralgia and mild serositis. In patients with more active disease, such as proliferative glomerulonephritis, neuropsychiatric and severe hematological manifestations, potent immunosuppressants such as cyclophosphamide, mycophenolate and cyclosporine in combination with glucocorticoids remain the mainstay of treatment for SLE (Ballinger, 2012). Recently, biologics such as rituximab and belimumab have been increasingly used in the treatment of SLE, particularly if patients fail to respond to conventional immunosuppressive agents. While further discussion on lupus management is beyond the scope of this thesis, it must be stressed that clinicians taking care of lupus patients should be vigilant of the development of organ damage of their patients because SLE *per se* and its treatment can lead to chronic damage accrual, such as osteoporosis, diabetes mellitus and cardiovascular disease (Demas et al., 2010).

1.2 Neuropsychiatric SLE

1.2.1 Epidemiology and clinical classification

Disturbance of the central (CNS) and peripheral nervous systems (PNS) has been identified in patients with SLE (Lahita, 2011). Many patients develop some forms of neuropsychiatric SLE (NPSLE) of varying severity and the presence of NPSLE correlates with duration of the disease (Aranow et al., 2010). Epidemiologic studies showed that the prevalence of neuropsychiatric events among patients with SLE ranged from 37 to 95% (Ainiala et al., 2001; Brey et al., 2002; Hanly et al., 2004; Sanna et al., 2003b; Sibbitt et al., 2002). Moreover, NPSLE has been identified as one of the two major factors which slows the improvement of survival in patients with SLE in the past 50 years (Mak et al., 2012). However, the complexity and the relatively restricted accessibility to the nervous system render characterization and attribution of CNS involvement in SLE difficult. The criteria for classification of NPSLE classification had not been established until the ACR Ad Hoc committee designated 19 different neuropsychiatric manifestations attributable to SLE in 1999 (Anonymous, 1999). These ACR case definitions of NPSLE consist of 12 CNS and 7 PNS manifestations (see Table 1.2). For each of these presentations, a summary of non-SLE causes and guidelines for ascertainment by laboratory tests and imaging techniques are provided to help determine the cause of neuropsychiatric events in patients with SLE.

Although the ACR case definitions have improved the classification of NPSLE in clinical studies, their applications in clinical practice remains challenging. It is important to be aware that none of the defined syndromes are unique to SLE. Around two-thirds of neuropsychiatric events observed in lupus patients are attributable to non-SLE causes (Aranow et al., 2010). Effective application of the ACR criteria for NPSLE depends on the correct attribution of neuropsychiatric symptoms. Three particular conditions

including CNS infections, thrombotic thrombocytopenic purpura and posterior reversible encephalopathy syndrome require attention and exclusion where appropriate because these conditions may mimic CNS disease in patients with SLE (Aranow et al., 2010). Given the low specificity of ACR case definitions of NPSLE (Ainiala et al., 2001), classification based on anatomical location of neurological injury (Sanchez-Guerrero et al., 2008) and categorization into diffuse and focal diseases (Hanly, 2007) have been proposed. However, the clinical applicability of these alternative classification criteria requires further studies.

Table 1.2 ACR case definitions of NPSLE

Central nervous system	Peripheral nervous system
Aseptic meningitis	Guillain–Barré syndrome
Cerebrovascular disease	Autonomic neuropathy
Demyelinating syndrome	Mononeuropathy
Headache	Myasthenia gravis
Movement disorder	Cranial neuropathy
Myelopathy	Plexopathy
Seizure disorders	Polyneuropathy
Acute confusional state	
Anxiety disorder	
Cognitive dysfunction	
Mood disorder	
Psychosis	

Source: ACR, 1999

1.2.2 Common manifestations of NPSLE

Although the reported frequency of overall neuropsychiatric events using the ACR case definitions varies (37-95%), similar frequency of neuropsychiatric events have been identified in different SLE cohorts, including cognitive dysfunction (55-80%), headache (20-72%), mood disorders (10-57%), seizure (6-51%), cerebrovascular disease (5-18%) and anxiety (4-24%) (Ainiala et al., 2001; Bertsias & Boumpas, 2010; Brey et al., 2002; Hanly et al., 2004; Sanna et al., 2003b; Sibbitt et al., 2002). Of note, nearly half of all the 19 neuropsychiatric syndromes including cranial neuropathy, mononeuropathy, aseptic meningitis, movement disorders, demyelinating syndrome, Guillain–Barré syndrome, autonomic disorder, myasthenia gravis and plexopathy account for a frequency of less than 1% of patients with SLE (Bertsias & Boumpas, 2010).

Headache

The association between SLE and headache is debatable. Although headache (including migraine) is prevalent in SLE, a meta-analysis of epidemiological studies failed to demonstrate an increased frequency of headache in lupus patients in comparison with healthy subjects. Likewise, it was unable to demonstrate which subtypes of headache are more specific for SLE (Mitsikostas et al., 2004). Aspetic meningitis has been identified to cause headache in patients with SLE (Kanekura et al., 1993; Lancman et al., 1989). Anti-PL antibodies have been revealed to be associated with chronic headache in patients with SLE (Sanna et al., 2003a), but the association between anti-PL antibodies and migraine in patients with SLE remains inconclusive (Sanna et al., 2003a). It has been suggested that headache might be a common component of active lupus and it

likely results from non-SLE factors. With careful history taking and physical examination, lupus patients with headache might not require investigation more than that required for non-SLE individuals with headache (Bertsias & Boumpas, 2010; Lahita, 2011).

Mood disorder & Anxiety

Mood and anxiety disorders are common symptoms in SLE (Bachen et al., 2009; Mak et al., 2011; Miguel et al., 1994; Utset et al., 1994). Mood and anxiety disorders in lupus patients have been demonstrated to be associated with psychological and social factors but not with disease activity of lupus (Shortall et al., 1995). Cytokines, especially INF γ, have been revealed to be related to anxiety in lupus patients (Figueiredo-Braga et al., 2009). Since the features of mood disorder and anxiety observed in SLE patients do not differ from that in non-SLE conditions, it requires no additional assessment which is necessary for mood disorder and anxiety in non-SLE individuals.

Seizures

In patients with SLE, the majority of seizures occur as generalized seizures and only a minority of them is related to focal neurological events (Lahita, 2011). Seizures usually occur in lupus patients during active disease and only a minority of cases lupus-related seizures persist as a chronic disorder (Lahita, 2011). The presence of stroke and anti-PL antibodies is associated with seizures (Mikdashi & Handwerger, 2004; Sanna et al., 2003b). Although electroencephalography (EEG) and brain magnetic resonance imaging (MRI) are the main investigations of choice to evaluate seizures in lupus patients (Bertsias & Boumpas, 2010), another potential cause of seizures such as drugs, metabolic disturbance, renal dysfunction, CNS infection and reversible posterior encephalopathy

are required to be excluded by appropriate investigation if necessary (Beleza, 2012). Further discussion of these investigations will be out of the scope of this thesis.

Cerebrovascular disease

Patients with SLE have been shown to have an increased risk for cerebrovascular events when compared to the age- and sex-matched general population (Esdaile et al., 2001; Ward, 1999). Cerebrovascular accidents are broadly classified into ischemic and hemorrhagic stroke. More than 80% of cerebrovascular accidents in SLE patients are ischemic in nature, while the frequencies of multifocal disease, intracerebral hemorrhage and subarachnoid hemorrhage are between 3 and 12% (Bertsias & Boumpas, 2010). The management of cerebrovascular disease in SLE does not differ significantly from that of the non-SLE populations. An exception is that if lupus patients who suffer from stroke are positive for anti-PL and/or lupus anti-coagulant (LAC) antibodies for more than once at least 3 months apart, anticoagulation may be more beneficial than antiplatelet agents alone in reducing the probability of recurrent stroke (Furie et al., 2011).

1.3 Cognitive dysfunction in SLE

1.3.1 Overview

Definition

Cognition is the sum of intellectual functions including reception of external stimuli, information processing, learning, storage and expression that result in thought, which can be categorized into various domains including attention, memory, learning, reasoning, executive function, language, visuoperception, sensory-motor, judgment and

insight (Harrison & Ravdin, 2002; Kozora et al., 2008b). Individuals who have disturbance of any of these domains can be said to have cognitive dysfunction. Dysfunction in attention, information processing, learning and memory, visuospatial activities, executive function, abstract thinking and psychomotor speed are commonly described in the patients with SLE (Kozora et al., 2008b). The scale of cognitive dysfunction may be restricted to a certain cognitive domains or extended to a number of domains, depending on the properties of the pathogenic insults (Harrison & Ravdin, 2002).

Epidemiology

The awareness of the high prevalence of cognitive dysfunction in SLE has been well established since the first study was reported by Carbotte and colleagues in the mid-1980s (Carbotte et al., 1986). In their study, 62 female patients with SLE underwent a comprehensive test battery for assessment of cognitive function, which comprised the Wechsler Adult Intelligence Scale (WAIS), Wechsler Memory Scale, Consonant Trigrams, Rey Auditory-Verbal Learning Test, Rey-Osterrieth Complex Figure Drawing, Token Test, Trail Making Test, Stroop Color-Word Interference Test, Design Fluency Test, Benton Controlled Word Association Test, Animal Naming Test, Finger Tapping Test and Handedness Questionnaire (Carbotte et al., 1986). Sixty-six percent of the 62 patients showed cognitive dysfunction, which was significantly higher than that in patients with rheumatoid arthritis (17%) and healthy individuals (14%) (Carbotte et al., 1986). Subsequent epidemiological studies in SLE have found that cognitive dysfunction is the commonest NPSLE manifestation, which ranges from 55% to 80% (Ainiala et al., 2001; Bertsias & Boumpas, 2010; Brey et al., 2002; Hanly et al., 2004; Sanna et al.,

2003b; Sibbitt et al., 2002). In addition, subclinical cognitive dysfunction was shown to occur between 11 and 54% of lupus patients in a comprehensive review of 14 cross-sectional studies (Denburg & Denburg, 2003). Subclinical cognitive dysfunction was revealed in lupus patients without clinically overt neuropsychiatric symptoms who demonstrated poorer performance in cognitive tasks assessing attention, verbal memory and logical reasoning than healthy individuals (Kozora et al., 2008a; Kozora et al., 1996; Monastero et al., 2001).

Impact of cognitive dysfunction of SLE on employment and quality of life

Given the high prevalence of clinical and subclinical cognitive dysfunction in lupus patients, the impact of cognitive dysfunction on employment, functional outcome, and health-related quality of life (HRQoL) in patients with SLE has been extensively evaluated in a number of cross-sectional and cohort studies. HRQoL refers to specific aspects of quality of life that relates to an individual's physical and psychosocial well-being which includes the components of physical functioning, social functioning, role functioning, mental health and general health. Cognitive dysfunction has been identified as one of the major contributors to poor HRQoL in both pediatric and adult lupus patients (Kiani & Petri, 2010; Williams et al., 2011) and lupus patients were consistently shown to have significantly lower HRQoL scores when compared with age- and gender-matched healthy individuals (Sweet et al., 2004). Lupus patients, who demonstrate cognitive dysfunction particularly in the form of memory deficit, are more likely to report being incapable of working (Panopalis et al., 2007; Utset et al., 2006). In addition, memory impairment is correlated with the likelihood of unemployment in these patients (Panopalis et al., 2007). Furthermore, self-reported memory impairment and anxiety and

depressive symptoms, which are highly prevalent in SLE patients with the reported frequencies of around 51 and 74% respectively, have been identified to be two of the major factors associated with work disabilities (Mok et al., 2008).

Lupus patients with cognitive dysfunction often pose challenges to their attending physicians regarding diagnosis and management. Cognitive dysfunction is not specific to SLE. A number of non-SLE causes of cognitive dysfunction have been identified to be present in patients with SLE, such as cerebral ischemia and hemorrhage, hypertension, endocrinopathies, fever, antidepressants, anxiety, depression, metabolic disturbances, pain, fatigue and sleep disturbance (Hanly & Harrison, 2005). These factors must be addressed where appropriate and excluded before attributing cognitive dysfunction to SLE.

1.3.2 Potential pathogenic mechanisms of cognitive dysfunction

<u>*1.3.2.1 Autoantibodies*</u>

<u>*ANA*</u>

Findings from human studies regarding the relationship between the presence of ANA in the serum, cerebrospinal fluid (CSF) and occasionally neural tissue of patients with SLE have provided circumstantial evidence of how ANA is involved in the pathogenesis of neuropsychiatric syndromes in SLE (Bluestein et al., 1981; Hanly, 2005; Weiner et al., 2000a; Zvaifler & Bluestein, 1982). However, the association between ANA and cognitive dysfunction in SLE remains unclear (Denburg et al., 1987; Hanly et al., 1993; Papero et al., 1990). Direct involvement of ANA in cognitive dysfunction has

been revealed in animal studies, in which intracranial injection of ANA induced memory impairment, seizures and neuropathological alterations (Hanly, 2005).

Anti-NR2 antibodies

Anti-NR2 antibodies recognize the specific N-methyl-D-aspartate receptor (NMDAR) which consist of the NR2a and NR2b subunits and are most densely populated in the hippocampus and amygdala (Li & Tsien, 2009). Significantly higher levels of anti-NR2 antibodies have been found in the CSF of lupus patients with NPSLE compared to SLE patients without NPSLE and healthy individuals (Lauvsnes & Omdal, 2012). Serum levels of anti-NR2 antibodies were also found to be higher in patients with NPSLE than those in SLE patients without neuropsychiatric syndromes and healthy controls in a number of studies, but results were inconsistent (Lauvsnes & Omdal, 2012). More information about anti-NR2 antibodies will be discussed in the subsequent section of this thesis.

Anti-PL antibodies

Anti-PL antibodies predominantly direct against anionic phospholipids and phospholipid-binding proteins, and are associated with focal neuropsychiatric syndromes, such as cerebrovascular accidents and focal seizures (Love & Santoro, 1990; Sanna et al., 2003b). So far, anti-PL antibodies, in particular, anti-cardiolipin (aCL) antibody and LAC, have been extensively studied in patients with NPSLE (Colasanti et al., 2009). Pathogenic aCL antibody was able to cause cognitive dysfunction in rodent models (Sun et al., 1992; Ziporen et al., 2004). In patients with SLE, the association between aCL antibody and poor psychomotor speed, conceptual reasoning and executive function has

been demonstrated (Hanly et al., 1999; Lai & Lan, 2000; Menon et al., 1999). The effect of aCL antibody in cognitive dysfunction is possibly via thrombosis of intracranial vasculatures (Lahita, 2011) or microvasculopathy characterized by endothelial proliferation and fibrinoid necrosis (Hanly et al., 1992b). In addition, lupus patients who were positive for LAC yielded significantly higher risk for cognitive dysfunction when compared with those who were negative for LAC (Denburg et al., 1997). The association was suggested to be mediated via micro-thrombotic abnormalities or vasculopathy (Colasanti et al., 2009).

Anti-intermediate neurofilament alpha-internexin antibodies

Intermediate neurofilament alpha-internexin (INA) forms the type IV intermediate neurofilament (IF) throughout the development of CNS and PNS and has only been found in the CNS of the adulthood in murine models (Fliegner et al., 1990; Lee & Cleveland, 1994; Yuan et al., 2006). Murine models immunized by INA of the human sequence were demonstrated to have lupus-like cognitive dysfunction and hippocampal neuronal death (Lu et al., 2010). Furthermore, in patients with NPSLE, INA has been identified as a potential autoantigen associated with neuropsychiatric symptoms (Kimura et al., 2008). High titers of anti-INA antibodies have been found in both sera and CSF of around 50% of 67 lupus patients with NPSLE in a recent study (Lu et al., 2010). Moreover, the levels of both CSF and serum anti-INA antibodies were associated with cognitive dysfunction in these patients assessed by the mini-mental state examination (Lu et al., 2010).

1.3.2.2 Inflammatory mediators

Cytokines

Cytokines function as inflammatory mediators. Early studies identified the associations between a number of pro-inflammatory cytokines in the CSF including IL-2, IL-6, IL-8, IL-10 and INF α and neuropsychiatric manifestations in patients with SLE (Gilad et al., 1997; Hanly, 2007; Shiozawa et al., 1992). Serum C-reactive protein, a non-specific marker of inflammation, was demonstrated to correlate with poor performance in neuropsychological test that assessed executive function in patients with SLE (Shucard et al., 2007). Serum IL-6 which has been shown to be elevated in patients with active lupus, was demonstrated to be associated with learning impairment in lupus patients even after adjusting for depression and corticosteroid use (Kozora et al., 2001). It has been suggested that the association between IL-6 and cognitive dysfunction in patients with SLE might be mediated via neuronal and astrocytic degradation in the brain parenchyma (Fragoso-Loyo et al., 2007).

Matrix metalloproteinases

Matrix metalloproteinases (MMP), which belong to the family of endoperoxidases responsible for degrading extracellular matrix components (Birkedal-Hansen et al., 1993), are important inflammatory mediators of NPSLE. MMP-9 critically involves in the disruption of the blood-brain barrier (BBB), whereas the tissue inhibitor of metalloproteinases (TIMP) 1 plays a key role in stabilization of the BBB (Nagase et al., 2006). Serum level of MMP-9 is elevated in patients with Guillain–Barré syndrome and is correlated with disease severity of lupus (Creange et al., 1999). Serum and CSF of MMP-9 levels were shown to be elevated in lupus patients with cognitive dysfunction

when compared with those without NPSLE (Ainiala et al., 2004; Trysberg et al., 2004). The association between cognitive dysfunction and increased levels of MMP-9 was suggested to be related to CNS damage induced by neuronal and astrocytic degradation, cerebral micro-vasculopathy, cerebral ischemia and disruption of the BBB (Ainiala et al., 2004; Kozora et al., 2008b; Trysberg et al., 2004).

1.3.2.3 Neuropeptides

The role of vasopressin on cognition has been studied in rodent models and humans (Bennett et al., 1997; Zink & Meyer-Lindenberg, 2012). Animal studies demonstrated an association between vasopressin and attention and memory (de Wied & van Ree, 1982; Insel et al., 1999; Strupp & Levitsky, 1985). Murine models with deletion of a single nucleotide in the second exon of the vasopressin gene demonstrated deficits of attention and working memory (Colombo et al., 1992; Insel et al., 1999), although the mechanisms underlying these observations remain unknown. In humans, vasopressin has been suggested to affect emotional processing by modulating the activity and connectivity of a neural circuit connecting the medial prefrontal cortex and the amygdala (Zink et al., 2010). Increased hypothalamic expression of vasopressin was demonstrated in the MRL/*lpr* mouse model (Sakic et al., 1999) , which is an acceptable animal models for NPSLE (Gulinello & Putterman, 2011). Serum levels of vasopressin have been demonstrated to be increased in lupus patients with cognitive dysfunction but the mechanism underlying this observation remains unknown (Lapteva et al., 2006b).

1.3.2.4 Genetics

The genetic association with neuropsychiatric manifestation in patients with SLE has been investigated in a number of studies (Johansson et al., 2005; May et al., 2002; Nath et al., 2002; Oroszi et al., 2006; Pullmann et al., 2004; Rood et al., 1999). Polymorphisms of the apolipoprotein E, IL-10, TNF α and systemic lupus erythematosus susceptibility 3 genes have been identified to be associated with overall neuropsychiatric events in SLE patients (May et al., 2002; Nath et al., 2002; Pullmann et al., 2004; Rood et al., 1999), but their association with specific aspects of NPSLE remains unknown. The Met66 allele of Val66Met polymorphism located within the brain-derived neurotrophic factor (BDNF) gene has been found to be associated with better psychomotor and motor function in patients with SLE, suggesting a protective effect of BDNF Met66 allele on specific cognitive domain in these patients (Oroszi et al., 2006). Moreover, cognitive dysfunction has been shown to be related to the AA and TT genotype of the Xbal A/G polymorphism in the first intron of the estrogen receptor alpha gene in patients with SLE (Johansson et al., 2005). Despite that the aforementioned findings support the potential impact of genetic association on cognitive dysfunction in SLE patients, neither have the identified gene polymorphisms in SLE patients been evaluated for their relationship with cognitive function in the general or non-SLE population, nor have these results been replicated in independent studies (Kozora et al., 2008b). Thus the role of genetic factors in lupus-related cognitive dysfunction still needs to be cautiously interpreted (Kozora et al., 2008b).

Taken together, interactions of a number of factors including autoantibodies, inflammation mediators, neuropeptides and gene polymorphisms lead to insufficient elimination of antigen-autoantibody complexes, abnormal production of pro-

inflammatory cytokines and the disturbance of the integrity of the BBB. These abnormalities may subsequently contribute to the pathogenesis of cognitive dysfunction in patients with SLE (Kozora et al., 2008b) (see Figure 1.2).

Figure 1.2 Diagram summarizing the potential contributors to cognitive dysfunction in SLE.
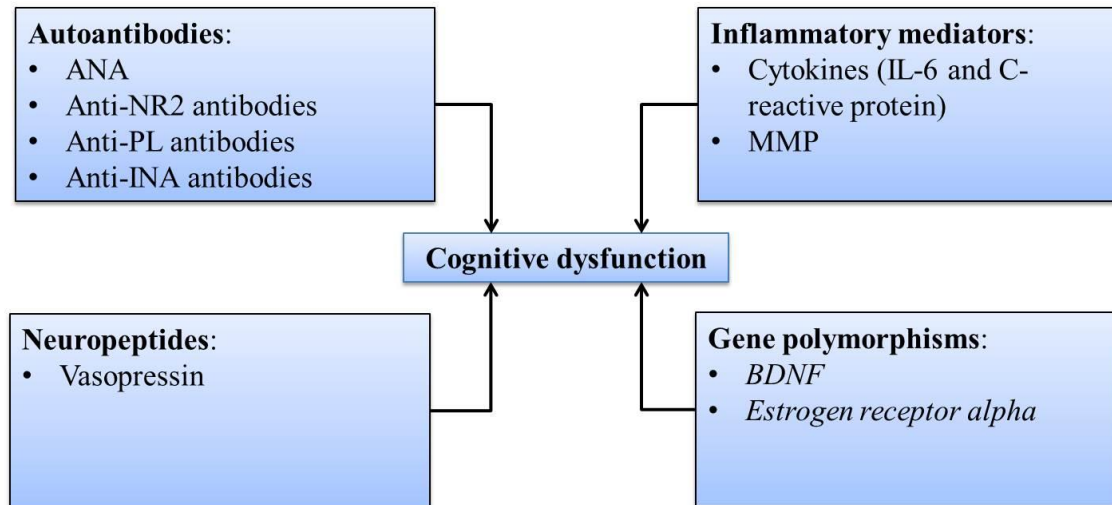
Abbreviation: SLE, systemic lupus erythematosus; ANA, anti-nuclear antibodies; anti-PL, anti-phospholipid; anti-INA, anti-intermediate neurofilament alpha-internexin; IL-6, interleukin-6; MMP, matrix metalloproteinase; BDNF, brain-derived neurotrophic factor

1.3.3 Neuropsychiatric assessment of cognitive dysfunction in patients with SLE

The ACR proposed a one-hour battery of comprehensive neuropsychological tests to aid clinicians and researchers to evaluate cognitive function in patients with SLE (see Table 1.3). This battery encompasses the evaluation of simple attention, complex attention, memory, visual-spatial processing, language, reasoning and/or problem solving, psychomotor speed and executive function (Anonymous, 1999). Disturbance of at least one of the eight domains is required to satisfy the ACR case definition for cognitive dysfunction (Anonymous, 1999), while the requirement for the involvement of three or more domains has been suggested to be defined as cognitive dysfunction by another

study (Ainiala et al., 2001). The reliability and validity of the ACR neuropsychological battery have been tested and the threshold of cognitive dysfunction have been empirically determined in lupus patients in comparison with healthy individuals (Kozora et al., 2004). Despite the comprehensiveness of these tests, the clinical applicability of ACR case definition is restricted by multiple factors. For instance, these tests require specialized training of administers and are prone to practising effects, not mentioning that it takes at least 1 hour to be completed (Lahita, 2011). Thus far, this battery has been employed in a few studies (Harrison et al., 2005; Jung et al., 2012; Kozora et al., 2007; Kozora et al., 2006), but it has yet to gain universal recognition. In addition, this battery cannot be utilized for clinical decision-making although it benefits population-based studies by offering standardization of the definition of cognitive dysfunction (Hanly & Fisk, 2011).

Table 1.3 ACR one-hour neuropsychological battery for SLE

North American adult reading test (to estimate premorbid IQ)
Digit symbol substitution test
Trail making test (Parts A and B)
Stroop color and word test
California verbal learning test
Rey-Osterrieth complex figure test (with delayed recall)
WAIS III Letter-number sequencing
Controlled oral word association test (FAS)
Animal naming
Finger tapping

Adapted from *ACR, 1999*

On the contrary, computer-based neuropsychological tests offer the advantages of being rapid and efficient in screening for cognitive dysfunction without special training of administers (Lahita, 2011). The Automated Neuropsychological Assessment Metrics (ANAM), consisting of a modified version of standard neuropsychological tests which evaluate basic information processing speed, attention, learning and memory and executive function (Bleiberg et al., 2000; Kane et al., 2007; Reeves et al., 2007), has been used in a number of studies addressing cognitive dysfunction in both pediatric and adult lupus patients (Antonchak et al., 2011; Benedict et al., 2008; Brey et al., 2002; Brunner et al., 2007; Hanly et al., 2010; Kane et al., 2007; McLaurin et al., 2005; Petri et al., 2008; Roebuck-Spencer et al., 2006). Furthermore, the ANAM is less dependent on the proficiency of English and reading ability as compared to the ACR proposed neuropsychological test battery. The ANAM has been demonstrated to possess strong agreements with the performance on the ACR proposed neuropsychological battery, especially the trail making test, stroop color and word test, and the digital symbol test (Roebuck-Spencer et al., 2006). However, practising effect has been suggested to confound the interpretation of ANAM (Bleiberg et al., 2004) and the ANAM may lack sufficient sensitivity to detect certain aspects of cognitive dysfunction such as impairment of memory, language and visuospatial function (Kozora et al., 2008b).

Apart from the comprehensive battery tests, neuropsychological tests which assess individual cognitive skill including the N-Back for working memory, verb generation task for language, continuous performance task for attention, and the Wisconsin Card Sorting Test (WCST) for executive function, have been utilized to study cognitive dysfunction in patients with SLE (Cavaco et al., 2012; Dahl et al., 2006;

Fitzgibbon et al., 2008; Shucard et al., 2011). Combination of these tests with neuroimaging techniques such as functional magnetic resonance imaging (fMRI) may help to elucidate the pathogenic mechanisms of the impairment of specific cognitive domain, regardless of whether the cognitive impairment is clinically overt or unapparent.

1.3.4 Neuroimaging studies of cognitive dysfunction in patients with SLE

Various neuroimaging tools have been used to study a number of aspects of cognitive dysfunction in patients with SLE by assessing the anatomical structure and function of the brain. Structural neuroimaging, such as MRI and magnetization transfer imaging (MTI), as well as functional neuroimaging techniques including positron emission tomography (PET), single-photon-emission computed tomography (SPECT), magnetic resonance spectroscopy (MRS) and fMRI are most frequently employed in studies of SLE patients (Kozora et al., 2008b).

The mechanism of the MRI technique is based on the fact that water is abundant in the tissue of human body (Novelline & Squire, 1997). By utilizing the physical property of water molecules which contain hydrogen nuclei or protons, a powerful magnetic field is deployed to align the magnetization of the protons and a radio frequency field is used to alter the magnetization (Novelline & Squire, 1997). Under this circumstance, the protons generate a rotating magnetic field recorded by an MRI scanner and the images of the scanned region are constructed based on the relative difference in the water content of different tissues (Novelline & Squire, 1997). Conventional MRI is able to identify periventricular white matter hyperintensities, infarcts, hemorrhages, cerebral atrophy and small focal lesions in the brain (Abreu et al., 2005; Kozora et al.,

1998; Sailer et al., 1997; West et al., 1995). However, neither the number of white matter hyperintensities nor the number and size of white matter lesions were found to be correlated with cognitive dysfunction in SLE (Abreu et al., 2005; Kozora et al., 1998).

MTI is an MRI-related technique which quantifies the capacity that the proteins in myelin have to exchange magnetization with the surrounding water molecules (Gochberg & Gore, 2007). Magnetization transfer ratio (MTR) can be calculated from the MTI images and has been proven to be a sensitive marker for pathological changes in the grey matter and "disease burden" in the normal-appearing white matter in a number of neurological disorders such as schizophrenia, dementia and multiple sclerosis (MS) (Bosma et al., 2000; Dousset et al., 1997; Filippi & Rovaris, 2000; Gupta et al., 1999; Inglese et al., 2001; Symms et al., 2004). Moreover, MTR decreases with increasing histopathological changes in these neurological conditions (Symms et al., 2004). MTI studies in patients with SLE have identified brain atrophy (Bosma et al., 2000) and selective grey matter damage (Steens et al., 2004). In particular, association between low MTR peak height value of the brain parenchyma and cognitive dysfunction has been demonstrated in NPSLE patients, suggesting that cognitive dysfunction might be related to the histopathological changes of the brain parenchyma in these patients (Emmer et al., 2008).

PET is a nuclear imaging technique that involves fluorodeoxyglucose, fluoro-L-dihydroxyphenylalanine, 16β-fluoro-5α-dyhidrotestosterone or 3-fluoro-3-deoxythymidine which measures the metabolic activity of tissues reflected by regional glucose uptake (Miele et al., 2008; Young et al., 1999). Patients with SLE yield abnormal brain metabolism, especially at the parieto-occipital regions (Csepany et al., 1997; Kao et

al., 1999a; Kao et al., 1999b; Komatsu et al., 1999; Otte et al., 1997; Sailer et al., 1997; Weiner et al., 2000b). Associations between attention impairment and hypometabolism of the prefrontal, inferior parietal and cingulate areas were demonstrated in SLE patients with mood disorders (Komatsu et al., 1999).

SPECT is another nuclear imaging technique which examines regional cerebral blood flow (CBF) following injection of a radioisotope into the bloodstream (Devous, 2005). SPECT studies on patients with SLE revealed reduced regional CBF to the temporal, parieto-occipital and parietal regions of the brain (Driver et al., 2008; Handa et al., 2003; Kao et al., 1999a; Kao et al., 1999b; Waterloo et al., 2001). In particular, reduction of perfusion in the watershed areas of both frontal lobes has been demonstrated to be correlated with the severity of cognitive dysfunction in the SLE patients (Driver et al., 2008). In addition, significant association has also been found between cognitive dysfunction and reduced regional CBF to superior parietal lobe and superior frontal lobe in patients with SLE (Waterloo et al., 2001).

MRS provides a noninvasive and quantitative regional measurement of different brain metabolites such as N-acetyl aspartate (NAA), choline (Cho), creatine (Cr) and lactate and has been widely used in evaluating CNS diseases (Gujar et al., 2005). Metabolic abnormalities can be visualized on MRS prior to the detection of structural lesions in SLE patients (Sibbitt & Sibbitt, 1993). Higher Ch:Cr ratio in the dorsolateral prefrontal regions and in the white matter of the frontal lobes have been found in SLE patients with cognitive dysfunction than those without (Filley et al., 2009; Kozora et al., 2005). High Ch:Cr ratio is related to inflammatory process, demyelination and gliosis (Ross & Michaelis, 1994), and is associated with the impairment of executive function in

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patients infected by the human immunodeficiency virus (Chang et al., 2002). Thus, the association between elevated Ch:Cr ratio and cognitive dysfunction in SLE is postulated to be mediated via early myelin injury and subsequent neuronal death due to inflammation in the white matter (Kozora et al., 2008b).

fMRI has been used to assess cognitive dysfunction of SLE patients in a number of studies . Further information on fMRI will be discussed in subsequent sections.

1.3.5 Management of cognitive dysfunction in SLE

Identification followed by treatment of any underlying causes of cognitive dysfunction is the initial management strategy for cognitive dysfunction in patients with SLE (Hanly & Harrison, 2005) (see Figure 1.3). The treatment rationale is based on clinical experience and relevant data on other similar diseases because there is a paucity of data on pharmacologic therapy for cognitive dysfunction in SLE (Kozora et al., 2008a). So far, only one double-blind, placebo-controlled trial has been carried out to evaluate the effects of corticosteroids on cognition in lupus patients with mild cognitive dysfunction (Denburg et al., 1994). These SLE patients were treated with prednisone 0.5 mg/kg per day for 21 days. Overall drug benefit for improvement of cognition was demonstrated in 5 out of 8 SLE patients who completed the trial and the beneficial effects persisted when these SLE patients continued to take glucocorticoids (Denburg et al., 1994). However, it remains uncertain whether the improvement in cognition could sustain if the glucocorticoids were tapered or when the treatment was withdrawn (Kozora et al., 2008b).

In addition to glucocorticoids, the use of aspirin was shown to be associated with better cognitive performance in SLE patients positive for anti-PL antibodies but negative

for vascular thrombosis in an observational study (McLaurin et al., 2005). Future clinical data is required to support the use of antiplatelet and/or anticoagulant therapy for cognitive dysfunction in this subset of SLE patients (Kozora et al., 2008b).

Cognitive rehabilitation programs have been attempted in stroke, dementia, traumatic brain injury and MS patients with cognitive dysfunction for retraining certain aspects of their impaired cognitive skills (Kozora et al., 2008b). SLE patients with self-perceived cognitive dysfunction demonstrated improved self-efficacy, memory function and ability to perform daily activities after psycho-educational intervention (Harrison et al., 2005).

Figure 1.3 Algorithm for management of SLE patients with cognitive dysfunction.
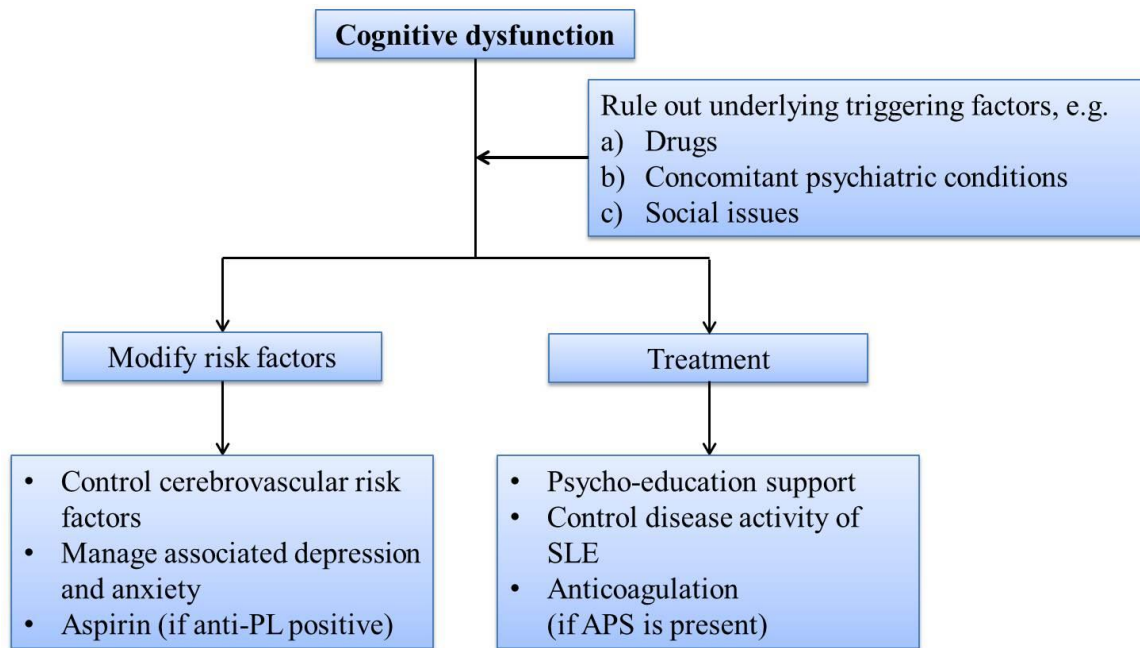
Abbreviation: anti-PL, antiphospholipid antibodies; APS, antiphospholipid syndrome
Source: Modified from Bertsias and Boumpas, 2010.

1.4 Executive function

1.4.1 Assessment of executive function

Executive function is conceptualized as a summary of cognitive functions including planning, working memory, attention, problem solving, verbal reasoning, response inhibition, mental flexibility and the initiation and monitoring of action (Chan et al., 2008), of which the majority is carried out by the prefrontal cortex (Koechlin & Summerfield, 2007; Miller, 2000; Wood & Grafman, 2003). Disturbance of normal executive function has been observed in a number of neurological, psychological and psychiatric disorders such as stroke (Zinn et al., 2007), Parkinson's disease (Weintraub et al., 2005), attention deficit hyperactivity disorder (Marchetta et al., 2008), autism spectrum disorder (Hill, 2004), depression (Fossati et al., 2002) and schizophrenia (Kerns et al., 2008). Currently, several measurements including the clock drawing test, stroop task, WCST, Tower of Hanoi, category test, random number generation and Delis-Kaplan executive function system are available to evaluate executive function. Amongst all, the WCST is most widely adopted (Nyhus & Barcelo, 2009). The WCST is primarily administered and scored in both the standard (Grant & Berg, 1948) and shortened versions (Heaton. et al., 1993). The conventional standard version of the paper-based WCST comprises 4 reference cards and 128 response cards (Heaton. et al., 1993). Participants are required to match the response card to one of the 4 reference cards based on one of the three prevailing rules (color, number or shape) which can be identified by trial, error and feedback from administrator. Once the identity of the correct rule is known, participants are required to maintain this sorting principle for the next ten consecutive trials until rule shift is indicated by an incorrect match. There is no pre-

determined time restriction on the conventional WCST and sorting continues until all cards are sorted or six correct sorting criteria are reached. The number of categories completed, perseverative errors and non-perseverative errors are commonly used to evaluate test performance (Nyhus & Barcelo, 2009). In addition, the WCST has been modified to be computer-based and applied in a number of functional imaging studies to investigate the component processes of executive function (Buchsbaum et al., 2005).

1.4.2 The temporally dynamic components of executive function

Executive function is postulated as a single unit by which the attention resources are allocated to support ongoing cognitive processes (Berman et al., 1986; Weinberger et al., 1986). This theory had been kept unchanged until evidence has emerged recently to support the notion that executive function consists of distinct yet interacting cognitive processes carried out by different cortical regions to achieve a common goal (Buchsbaum et al., 2005; Sylvester et al., 2003). Through creative modifications of the original WCST, neuroimaging studies have been attempting to dissect the presumed mental subcomponents of executive function and the brain involvement underlying these cognitive processes (Buchsbaum et al., 2005; Graham et al., 2009). The WCST rule shift has been found to be associated with activation at the posterior section of the inferior frontal sulcus (IFS) which facilitates the cognitive demand for response inhibition (Konishi et al., 1999a; Konishi et al., 1999b), whereas updating behavior in WCST is associated with left-lateralized activation at the IFS (Konishi et al., 2002). A modified paradigm was designed to dissect the WCST into four experimental stages (receiving negative feedback, matching after negative feedback, receiving positive feedback and matching after positive feedback) and two control stages (control matching and control

feedback) (Monchi et al., 2001). It has been assumed in this modified test that the brain activities upon receiving negative feedback are related to rule shift, while the cortical involvement following positive feedback is associated with updating the cognitive set in memory or maintenance of the established rule identity (Monchi et al., 2001). Different prefrontal regions have been demonstrated to be involved in executive function in a stage-specific manner. However, this strategy is compromised by the presence of ambiguous cards, in which the participants might receive positive feedback without identifying the underlying rule, resulting in contamination of updating behavior with rule searching. For instance, when One Red Triangle is to be sorted, the One Red Star reference card is unable to confirm whether the sorting rule is "color" or "number" even if the associated feedback is positive. This disadvantage is avoided by excluding the ambiguous cards and setting rational interval between card selection and feedback to facilitate the identification of associated cognitive processes (Graham et al., 2009). It was found that both caudates play a role in generating response without knowing the identity of the new rule, whereas the hippocampi serve the update and maintenance components of the established rule. Striatum-hippocampal interaction mediated via the medial cortical areas are found across the entire course of cognitive set-shifting, which casts critical insights into the temporally dynamic components of executive function (Graham et al., 2009).

1.4.3 Executive function in SLE

Executive function has been evaluated by formal neuropsychological tests and the WCST in both pediatric and adult patients with SLE (Conti et al., 2012; Hanly et al., 1992a; Loukkola et al., 2003; Monastero et al., 2001; Waterloo et al., 2002; Wyckoff et

al., 1995). Adult lupus patients with active disease demonstrated poorer performance on the WCST as reflected by the lower overall scores and more perseverative errors when compared to healthy individuals (Conti et al., 2012; Loukkola et al., 2003). Disturbance of visual memory was revealed in pediatric lupus patients assessed by the Wechsler Memory Scale (Wyckoff et al., 1995).

The pathogenesis of the executive function deficit in SLE patients remains elusive. Serum aCL antibodies were suggested to be associated with reduction of executive skills in SLE patients (Conti et al., 2012; Hanly et al., 1999), possibly mediated by modulating neuronal function (Conti et al., 2012). Since functional neuroimaging can potentially unravel the basic neuropathophysiology of cognitive dysfunction, it prompts further research which employs fMRI to study cognitive function in patients with SLE.

1.5 fMRI of the brain

1.5.1 The basic principle of fMRI and blood-oxygen-level-dependent signal

fMRI is a neuroimaging technique which has been developed recently to detect brain activities by measuring the relative changes in blood oxygenation and flow that occurs in response to neuronal activities (Huettel et al., 2004). Brain activation commonly presents as increased neural firing, axonal spiking, elevated synaptic transmission and active transport of calcium and potassium ions back and forth across the membranes of neurons (Frahm et al., 2004). All these processes are ATP-dependent. Since brain does not store glucose, it primarily relies on the oxidation of glucose from cerebral blood supply as the energy source. Thus, increase in blood flow conveys more glucose along with oxygenated hemoglobin (Hb) to regions where neuronal activity is

high. The amount of oxygen influx usually exceeds that of the oxygen consumed during glucose oxidation, resulting in a net decrease of deoxygenated hemoglobin (dHb) in the microvasculature around the active neurons (see Figure 1.4). Oxygenated Hb is diamagnetic with no unpaired electrons and magnetic movement, whereas dHb is paramagnetic with unpaired electrons and is 20% more susceptible to the magnetic field when compared to oxygenated Hb (Huettel et al., 2004). The different magnetic susceptibility of dHb results in dephasing of the magnetic resonance signals and darkening of the image in the voxels containing blood vessels with dHb in T2-weighted imaging (Ogawa & Lee, 1990; Ogawa et al., 1990b; Thulborn et al., 1982), while oxygenated Hb does not cause dephasing during the same imaging process (Ogawa et al., 1990a). Using dHb as an endogenous contrast, different magnetic properties of oxygenated Hb and dHb lead to differences of the magnetic resonance signals of the blood determined by the degree of oxygenation under the magnetic field. Since the degree of blood oxygenation is correlated with the level of neural activities, these differences can be visualized as the blood-oxygen-level-dependent (BOLD) signals detected by fMRI scanning which reflects regional brain activities (Ogawa et al., 1990a; Ogawa et al., 1990b). Although fMRI does not measure neural activities directly, it has been proved, using optogenetic tool, that neuron activation elicits increased BOLD signals (Lee et al., 2010), which gives critical credit to use fMRI to record brain activation.

Figure 1.4 Blood oxygenation changes when neurons shift from resting to activation.
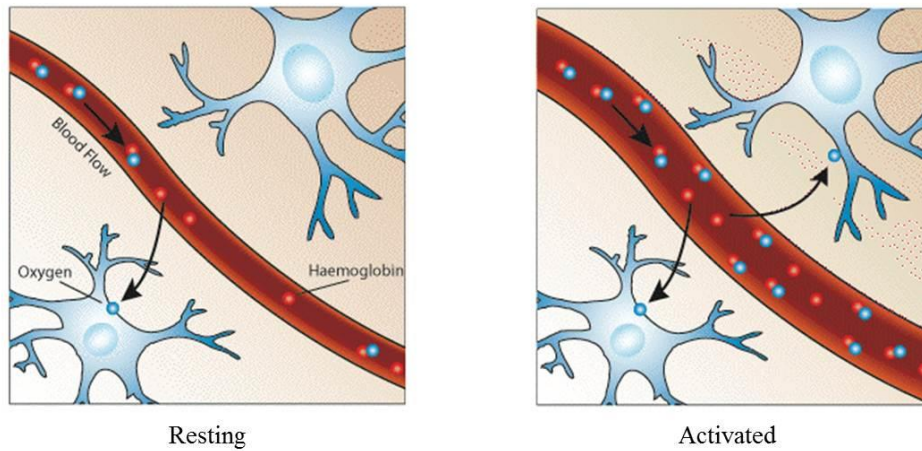
Resting   Activated

1.5.2 fMRI studies of neuropsychiatric disorders

So far, fMRI has been widely applied to study the neural basis and pathogenesis of mental and behavioral changes associated with a number of neuropsychiatric disorders. For instance, in patients with Alzheimer's disease (AD), compromised explicit memory encoding in the mesial temporal lobe and fusiform regions was revealed (Golby et al., 2005). In combination with memory test and genetic markers for AD, fMRI data were shown to be able to predict memory decline in patients with AD (Bookheimer et al., 2000). Resting-state fMRI assessing functional connectivity reveals uncoupling of the "hate circuit" involving the superior frontal gyrus, insula and putamen in patients with depression (Tao et al., 2011). As for schizophrenia, fMRI findings demonstrated that abnormal fronto–temporal lobe connections might be associated with cognitive dysfunction (Mitchell et al., 2001).

Based on the abnormal BOLD signals revealed in patients with neurological or neuropsychiatric disorders, fMRI has been suggested to hold promise for the early

detection and differential diagnosis, predicting future change in clinical status, and as a marker of alterations in brain physiology related to neurotherapeutic agents (Dickerson, 2007). The greatest potential of fMRI likely lies in the study of very early and preclinical stages of progressive neurological diseases at the point of subtle neuronal dysfunction, prior to overt anatomic pathology (Dickerson, 2007).

1.5.3 fMRI studies on SLE

At the time of writing this thesis, five fMRI studies have been published which addressed motor function and cognitive function in both pediatric and adult patients with SLE, in which two of them were conducted in SLE patients without neuropsychiatric syndromes. Using the resting-state fMRI procedure, altered brain activities in the cerebellum, posterior cingulate gyrus and the adjacent precuneus which are included in the default mood network were found in lupus patients without neuropsychiatric manifestations (Lin et al., 2011). Moreover, the fMRI signal changes in the cerebellum were positively correlated with lupus disease activity measured by the Systemic Lupus Erythematosus disease activity index (SLEDAI) (Lin et al., 2011). When SLE patients without NPSLE were stratified by disease duration and administered two functional paradigms which evaluated their working memory and emotional response, patients of short disease duration ($\leq$ 2 years) demonstrated significantly better performance in working memory task when compared with those of longer disease duration ($\geq$ 10 years) (Mackay et al., 2011). As for the functional brain signals, patients of short disease duration demonstrated significantly increased BOLD signals in the somatomotor, cingulate and prefrontal cortices and the Brodmann area (BA) 40 which belong to the working memory paradigm as well as in the amygdala and superior parietal area which

correspond to the emotional response paradigm when compared to those of longer disease duration (Mackay et al., 2011). No correlation was found between brain activation in the aforementioned regions and the SLEDAI and the SLICC damage index (Mackay et al., 2011).

In lupus patients with NPSLE, motor system and working memory were examined in two fMRI studies. Fourteen right-handed NPSLE patients and 14 matched healthy subjects underwent fMRI scan while they were performing a simple motor task in which participants were required to move the last four fingers of the right hand in a repetitive flexion-extension manner (Rocca et al., 2006). NPSLE patients showed significantly increased activation in the contralateral primary sensorimotor cortex, putamen and dentate nucleus which were included in classical sensorimotor regions, as well as cortical regions of the frontal and parietal lobes and visual system which were not frequently involved in motor function. These findings suggest that reorganization of cortical function seems to help maintain the normal motor function in NPSLE patients via increased activation of cortical regions normally subserving motor tasks and recruitment of extra pathways supporting more complex tasks (Rocca et al., 2006). A similar manner of cortical functional reorganization was demonstrated in another fMRI study which assessed working memory in patients with NPSLE (Fitzgibbon et al., 2008). While the performance of patients with NPSLE was the same as that of their healthy counterparts in the N-Back test, significantly increased brain activations in the supplementary motor area and both posterior inferior parietal lobules which subserve working memory were observed in patients with NPSLE (Fitzgibbon et al., 2008).

The fifth fMRI study involved ten childhood-onset SLE patients, of whom six showed cognitive dysfunction assessed by formal neuropsychological tests (DiFrancesco et al., 2007). Three paradigms including the verb generation task, continuous performance task and N-Back task were administered in these patients to assess their language, attention and working memory respectively. Compared to healthy controls, SLE patients failed to exhibit activation in the Wernicke's area which is involved in the comprehension of spoken and written language (Holland et al., 2001), suggesting that childhood-onset SLE patients might have language deficit due to abnormal neural activities in the Wernicke's area. During the continuous performance task, SLE patients demonstrated increased activation in the fusiform gyrus and visual association cortex, in which the activation patterns were similar to that associated with abnormal attention (Strakowski et al., 2004). Thus, the impairment of attention commonly observed in childhood-onset SLE was likely related to the abnormalities in fusiform gyrus and visual related cortex (DiFrancesco et al., 2007). When working memory was tested by the N-Back task, SLE patients demonstrated activation in the anterior dorsolateral prefrontal and the visual association cortices which are involved in working memory (DiFrancesco et al., 2007), implying that the same cognitive demand might pose greater challenge to the SLE patients and require more cortical activities than healthy subjects to perform the same task (Kozora et al., 2008b).

Taken together the results of all the 5 fMRI studies in lupus patients to date, alterations of neural circuits underlying resting state, working memory and emotional response may occur in lupus patients even without clinically overt NPSLE. Recruitment

of additional cortical pathways is required for lupus patients to maintain comparable motor function and working memory as those of healthy individuals.

1.6 NMDAR and the anti-NR2 antibodies

1.6.1 Function of the NMDAR

NMDAR is the receptor for the neurotransmitter glutamate and it serves as the predominant molecular device for controlling synaptic plasticity and memory function (see Figure 1.5) (Li & Tsien, 2009). As one of the major excitatory neurotransmitters, glutamate is present in most neurons in the brain. Stored in vesicles within the presynaptic neurons, glutamate is released with precise control to convey sensory information, respond to motor commands and mediate thought and memory that form cognition and emotion (Aranow et al., 2010). The NMDAR is a heterotetramer comprising four subunits: two NR1 subunits which have a binding site for glycine, and two of any four NR2A, NR2B, NR2C and NR2D subunits (Kutsuwada et al., 1992). NMDARs with the NR2A and NR2B subunits are mostly expressed in the hippocampus and the amygdala in the mammalian central nervous system (Ozawa et al., 1998) and are associated with learning and memory mediated by the hippocampus, and with emotional responses mediated by the amygdala (Aranow et al., 2010). In the hippocampus and amygdala, the NMDARs serve as voltage-gated calcium channels. Upon electrical stimulation to the neurons, glycine and glutamate bind to NR1 and NR2 of the NMDARs respectively to initiate the removal of magnesium ions which block the ion channel pore and the influx of calcium into the cells (Coan & Collingridge, 1985; Li & Tsien, 2009). Calcium influx depends on the duration of the "open state" of the ion channel. Excessive

calcium influx into the neurons leads to mitochondrion stress, activation of caspase cascades and eventual excitotoxic neuronal death (Laube et al., 1997; Lipton & Rosenberg, 1994). Interruptions of NMDAR by autoantibodies and antagonists against NMDAR have been suggested to be involved in autoimmune encephalitis, AD, seizures and schizophrenia (Chen & Lipton, 2006; Dalmau et al., 2007; Long et al., 2006). Moreover, NMDAR antagonists such as dizocilpine, phencyclidine and ketamine are able to cause drowsiness, halluncination, seizures and even coma (Ellison, 1995).

Figure 1.5 NMDAR and synaptic plasticity
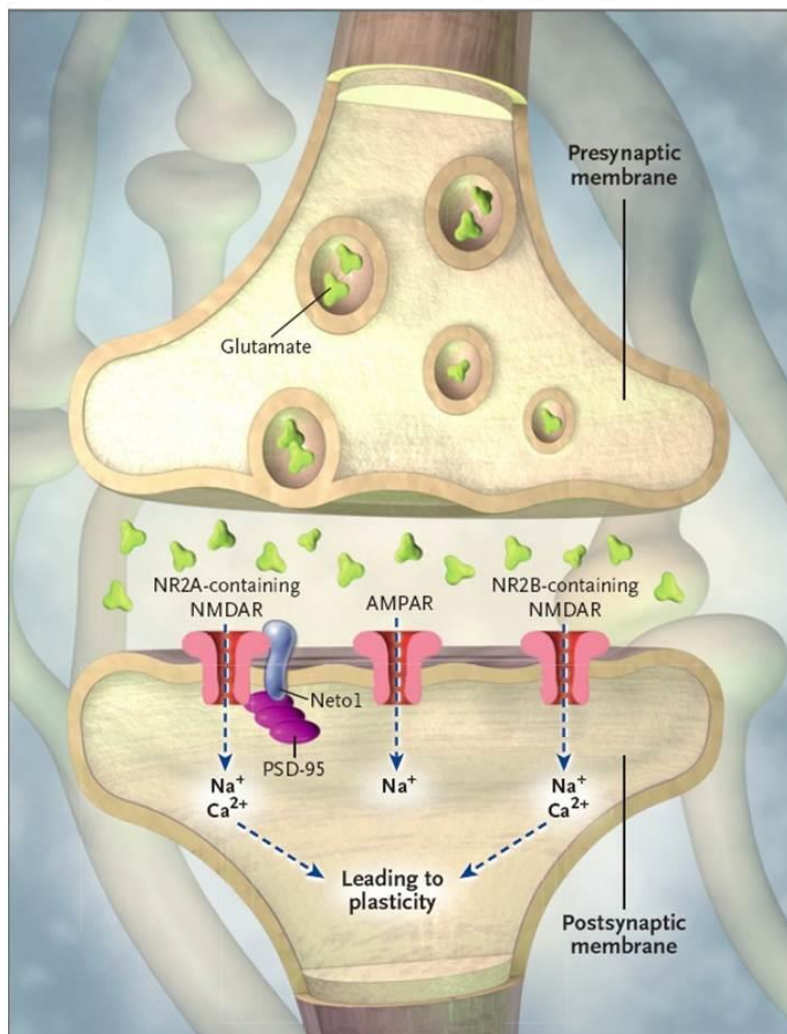
Reproduced with permission from Li and Tsien, 2009. Copyright Massachusetts Medical Society.

1.6.2 Nature of anti-NR2 antibodies

The genes encoding a subset of IgG isotype anti-dsDNA antibodies extracted from lupus patients and spontaneous lupus mouse models demonstrate somatic mutations, which carry the signature characteristics of T cell-dependent, germinal center maturing B cell response induced by protein antigens (Aranow et al., 2010; Paul et al., 1990). R4A is a monoclonal murine anti-dsDNA antibody which deposits in the glomeruli and causes nephritis (Gaynor et al., 1997). R4A is capable of binding to the DWEYS consensus sequence which is present in the extracellular, ligand-binding domain of both rodent and human NR2A and NR2B subunits of the NMDAR (DeGiorgio et al., 2001). Enzyme-linked immunosorbent assay (ELISA) and Western blot analysis showed that R4A could bind to NR2A and NR2B and was able to immunoprecipitate these two subunits from rodent brain lysate (DeGiorgio et al., 2001). Upon binding to the NMDAR, R4A functions to enhance NMDAR activation by prolonging the "open state" of the NMDAR associated ion channels which leads to higher calcium influx and triggers excitotoxic neuronal death (Aranow et al., 2010). Antibodies purified from the sera and CSF of lupus patients with progressive cognitive dysfunction using the same DWEYS consensus sequence in affinity chromatography lead to exitotoxic effects on hippocampal neurons as R4A does, suggesting that cognitive dysfunction might be attributed to the presence of anti-NR2 antibodies in the CSF (DeGiorgio et al., 2001).

1.6.3 Effects of anti-NR2 antibodies on cognition in murine models

It has been demonstrated in murine models that when anti-NR2 antibodies were directly injected into the mouse brain, they bound to the hippocampal neurons and caused

hippocampus-dependent memory impairment (Aranow et al., 2010; DeGiorgio et al., 2001), whereas anti-NR2 antibodies in the peripheral circulation caused neither brain pathology nor abnormal memory and learning in mice with an intact BBB (Kowal et al., 2004). However, when the integrity of BBB is compromised by lipopolysaccharide (LPS), the anti-NR2 antibodies in the circulation can gain access to the brain and preferably bind to the neurons in the hippocampi, leading to hippocampal neuronal death and persistent memory impairment (Kowal et al., 2006). These findings suggest that cognitive dysfunction mediated via anti-NR2 antibodies depend on the integrity of the BBB (Aranow et al., 2010; Diamond & Volpe, 2012). Furthermore, it has been demonstrated in rodent models that anti-NR2 antibodies affected the relevant neurons in a dose-dependent manner. Low concentration of anti-NR2 antibodies enhance excitatory postsynaptic potentials and cause transient cognitive dysfunction and emotional disturbance. High concentration of anti-NR2 antibodies can lead to neuronal death and irreversible cognitive dysfunction (Faust et al., 2010). Taken together, the findings in the murine model suggest that the anti-NR2 antibodies can affect cognitive function and emotional response upon their direct access to the brain, and the magnitude of these effects depend on the integrity of the BBB and the concentration of the antibodies in the CSF (Aranow et al., 2010; Diamond & Volpe, 2012).

1.6.4 The role of anti-NR2 antibodies in SLE

At the time of preparation of this thesis, 15 studies addressing the pathophysiology and potential clinical utility of anti-NR2 antibodies in human SLE were published (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Gono et al., 2011; Hanly et al., 2006; Hanly et al., 2008; Hanly et al., 2011; Hanly et al., 2012; Harrison et al., 2006;

Husebye et al., 2005; Kozora et al., 2010; Lapteva et al., 2006a; Omdal et al., 2005; Petri et al., 2010; Steup-Beekman et al., 2007; Yoshio et al., 2006). While these studies reveal significantly higher anti-NR2 antibodies in 10-35% patients with SLE when compared to those of the healthy individuals, the relationship between anti-NR2 antibodies and NPSLE remains inconclusive (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Gono et al., 2011; Hanly et al., 2006; Hanly et al., 2008; Hanly et al., 2011; Hanly et al., 2012; Harrison et al., 2006; Husebye et al., 2005; Kozora et al., 2010; Lapteva et al., 2006a; Omdal et al., 2005; Petri et al., 2010; Steup-Beekman et al., 2007; Yoshio et al., 2006). Three studies investigating CSF derived from SLE patients consistently demonstrated the association between anti-NR2 antibodies and seizures, acute confusional state, psychosis, severe refractory headache and cerebrovascular illnesses, and the levels of anti-NR2 antibodies in the CSF were correlated with disease severity of SLE (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Yoshio et al., 2006). However, results of studies addressing the potential correlation between serum anti-NR2 and NPSLE have been inconsistent. While the results from three studies identified significant associations between serum anti-NR2 antibodies and cognitive impairment, depression and overall frequency of NPSLE (Gono et al., 2011; Lapteva et al., 2006a; Omdal et al., 2005), this association was refuted by a higher number of studies (Fragoso-Loyo et al., 2008; Hanly et al., 2006; Hanly et al., 2008; Hanly et al., 2011; Hanly et al., 2012; Harrison et al., 2006; Husebye et al., 2005; Kozora et al., 2010; Petri et al., 2010; Steup-Beekman et al., 2007).

Thus, it has been advocated that when compared to CSF anti-NR2 antibodies, serum anti-NR2 antibodies alone may not be a sensitive marker to detect neuropsychiatric syndromes in patients with SLE (Lauvsnes & Omdal, 2012). Since

fMRI appears to be capable of detecting brain activities associated with cognitive dysfunction in a more sensitive manner (DiFrancesco et al., 2007; Fitzgibbon et al., 2008), it is likely to be an attractive strategy to use fMRI signals as surrogates to gauge the potential utility of serum anti-NR2 antibodies in order to detect cognitive dysfunction in patients with SLE, particularly if cognitive impairment is clinically unapparent. In addition, further studies addressing the clinical applicability of the combination of serum anti-NR2 antibodies and non-invasive assessment of the integrity of BBB to predict cognitive dysfunction in lupus patients may be another attractive research direction considering the role of BBB in the development of cognitive dysfunction and emotional disturbance associated with anti-NR2 antibodies in murine models (Diamond & Volpe, 2012).

1.7 Research questions and objectives of the thesis

1.7.1 Objectives

1) To assess the executive function in new-onset SLE patient and explore the potential changes in regional brain activation signals before and after achieving adequate disease control with the use of event-related fMRI.

2) To explore the potential neural circuits involved in subclinical cognitive dysfunction in new-onset SLE patients without NPSLE by analyzing the cognitive set-shifting processes with the use of event-related fMRI.

3) To explore the potential relationship between serum anti-NR2 antibodies and the BOLD signals of fMRI at the hippocampi and amygdala in SLE patients without clinically overt neuropsychiatric symptoms.

1.7.2 Hypothesis

1) New-onset SLE patients have different regional brain activation patterns associated with executive function and such activation patterns change over time with adequate disease control.

2) Specific neural circuits are involved in subclinical cognitive dysfunction in new-onset SLE patients without clinically overt neuropsychiatric symptoms.

3) Serum anti-NR2 antibodies are higher in SLE patients than healthy controls and the antibody level correlates with the BOLD signals of fMRI at the hippocampi and amygdala in SLE patients without clinically overt neuropsychiatric symptoms.

CHAPTER 2

PATIENTS AND METHODS

2.1 Recruitment of patients

All patients in the studies reported in this thesis were recruited from the lupus clinic of the Division of Rheumatology, Department of Medicine, National University Hospital (NUH), Singapore between March 2008 and June 2012. All participating patients fulfilled the ACR classification criteria for SLE (Hochberg, 1997). All studies were approved by the National Healthcare Group Domain-Specific Review Board in Singapore. Written informed consent was obtained from the patients and healthy controls before recruitment.

Study 3.1 Part A and Part B were conducted in the same 14 newly-diagnosed SLE patients. Seventeen newly-diagnosed patients with SLE were initially identified for the fMRI study, of whom one was ineligible as she presented with lupus psychosis and another two declined the invitation to participate due to work commitment. These 14 newly-diagnosed SLE patients were representative of the Singapore population in terms of the age of disease diagnosis (Jakes et al., 2012). Study 3.2 involved 12 out of the 14 SLE patients from Study 3.1 as well as 52 SLE patients from other study cohorts recruited from the same medical center.

2.2 Clinical evaluation and data collection

Various demographic and clinical data were collected from clinical interview and medical record review depending on the objectives of individual study. Demographic

information included age, gender, ethnicity and education level. Clinical data consisted of clinical manifestation of SLE at the time of disease onset, disease duration of SLE, serological information, disease activity and disease damage score and details of pharmacological management. Disease activity was assessed at study entry and every clinic visit by the same attending physician. Disease damage was assessed annually and at the time of repeat fMRI scans by the same attending physician.

2.3 Assessment of disease activity

Disease activity of SLE was evaluated by the SLEDAI (Bombardier et al., 1992) (see Table 2.1). The validated index consists of 24 items with individual definition for each. Presenting items are scored and summed based on the predetermined weights for each item. Higher weights are designated to more life-threatening item. A cut-off score of 3 or 4 is suggested to indicate active disease activity (Yee et al., 2011). Higher SLEDAI scores are shown to be associated with poor outcome in patients with SLE including death and tissue injury (Ibanez et al., 2011). The SLEDAI has been demonstrated to be a reliable tool for assessing lupus activity even when it is used by less experienced clinicians (Petri et al., 1992) and the reliability of the tool is not subject to geographic or cultural effects (Gladman et al., 1992, 1994). In addition, the SLEDAI has been revealed to be sufficiently sensitive to detect changes of disease activity over time (Gladman et al., 1994) and is therefore useful in prospective and longitudinal studies.

Table 2.1 The Systemic Lupus Erythematosus Disease Activity Index

Weight	Descriptor	Definition
8	Seizures	Recent onset. Exclude metabolic, infectious or drug cause
8	Psychosis	Altered ability to function in normal activity due to severe disturbance in the perception of reality. Include hallucination, incoherence, marked loose associations, impoverished though content, marked illogical thinking, bizarre, disorganized, or catatonic behaviour. Exclude uraemia or drug causes
8	Organic brain syndrome	Altered mental function with impaired orientation, memory or other intelligent function, with rapid onset fluctuating clinical features. Include clouding of consciousness with reduced capacity to focus, and inability to sustain attention to environment, plus at least two of the following: perceptual disturbance, incoherent speech, insomnia or daytime drowsiness, or increased or decreased psychomotor activity. Exclude metabolic, infectious or drug causes
8	Visual disturbance	Retinal changes of SLE. Include cytoid bodies, retinal haemorrhages, serious exudates or haemorrhages in

choroids, or optic neuritis. Exclude hypertension, infection or drug causes

8	Cranial nerve disorder	New onset of sensory or motor neuropathy involving cranial nerves
8	Lupus headache	Severe persistent headache; may be migrainous, but must be non-responsive to narcotic analgesia
8	CVA	New onset of cerebrovascular accident(s). Exclude arteriosclerosis
8	Vasculitis	Ulceration, gangrene, tender finger nodules, periungual, infarction, splinter haemorrhages, or biopsy or angiogram proof of vasculitis
4	Arthritis	More than 2 joints with pain and signs of inflammation (i.e. tenderness, swelling, or effusion)
4	Myositis	Proximal muscle aching/weakness, associated with elevated creatine phosphokinase/aldolase or electromyogram changes or a biopsy showing myositis
4	Urinary casts	Heme-granular or red blood cell casts
4	Haematuria	>5 red blood cells/high power field. Exclude stone, infection or other cause

4	Proteinuria	>0.5gm /24 hours. New onset or recent increase if more than 0.5gm/24 hours
4	Pyuria	>5 white blood cells/high power field. Exclude infection
2	New rash	New onset or recurrence of inflammatory type rash
2	Alopecia	New onset of recurrence of abnormal, patchy or diffuse hair loss
2	Mucosal ulcers	New onset of recurrence of oral or nasal ulcerations
2	Pleurisy	Pleuritic chest pain with pleural rub or effusion, or pleural thickening
2	Pericarditis	Pericardial pain with at least 1 of the following: rub, effusion, or electrocardiogram confirmation
2	Low complement	Decrease in CH50, C3, or C4 below the lower limit of normal for testing laboratory
2	Increased DNA binding	> 25% binding by Farr assay or above normal range for testing laboratory
1	Fever	> 38 ℃. Exclude infectious cause
1	Thrombocytopenia	< 100,000 platelets/mm^3

| 1 | Leukopenia | $< 3,000$ white blood cells/mm^3. Exclude drug causes |

2.4 Assessment of disease damage

SLE-related damage was assessed by the Systemic Lupus International Collaborating Clinics / American College of Rheumatology damage index (SDI), which is the standard tool accepted by the ACR to evaluate damage in SLE (Gladman et al., 1997). The reproducibility and reliability of SDI have been demonstrated in various international centers. Irreversible organ damage is scored by 41 items included in the SDI from the time of SLE onset. Damage item is individually defined and designated with a glossary of terms and the corresponding index. Damage which has been present for more than 6 months will be scored regardless whether it is caused by lupus, its treatment or concurrent diseases. Damage in the same organ system caused by repeat events is scored at most twice provided that a minimum of 6 months have lapsed between two consecutive events. The same lesion can be only scored once. Damage and the corresponding range has been defined for 12 organ systems, namely ocular (0-2), neuropsychiatric (0-6), renal (0-3), pulmonary (0-5), cardiovascular (0-6), peripheral vascular (0-5), gastrointestinal (0-5), musculoskeletal (0-6), skin (0-3), premature gonadal failure (0-1), diabetes mellitus (0-1) and malignancies (0-2).

2.5 Laboratory evaluation

2.5.1 Serological tests

The serological tests were performed in the standard laboratories of the NUH. Indirect immunofluorescence was used to detect the presence of ANA. ELISA would be performed subsequently if the indirect immunofluorescence for ANA was positive. Anti-dsDNA antibody was assessed by a standard ELISA procedure (Bio-Rad, CA, USA). Three phospholipid-dependent coagulation tests including activated partial thromboplastin time (APTT), kaolin clotting time (KCT) and dilute Russell Venom Viper test (DRVVT) were used to screen for LAC. In both the KCT and DRVVT systems, the presence of an inhibitor was determined by mixing equal amount of normal plasma and lupus plasma. LAC positivity was confirmed via platelet neutralization experiments in the APTT and DRVVT tests. Anti-β2 glycoprotein I antibodies (both IgG and IgM) and aCL were assayed using standard ELISA procedures (Quanta Lite, CA, USA).

2.5.2 Evaluation of brain activity by event-related fMRI

2.5.2.1 Evaluation of executive function and event-related fMRI

All SLE patients and healthy controls involved in the fMRI studies were instructed to perform the computer-based modified WCST (see Figure 2.1). Prior to the test, all participants underwent training to ensure satisfactory comprehension of the sorting criteria. During RS, four cards appeared along the top of a blue screen as reference and kept unchanged as the test ensued. On each trial, a candidate card which appeared at the central bottom of the blue screen was to be matched with one of the four reference cards based on one of the three rules (color, number, shape) randomly generated by the program (see Figure 2.2). Participants were given 4 seconds to respond, after which a bar would appear under the selected reference card, or else the words "too

late" would appear on the blue screen which signified trial termination. After the 4 seconds' selection time, fixation display of a white cross would appear at the center of the blue screen. After another 5 seconds, feedback would appear as "Right" or "Wrong" on the screen, indicating correct or incorrect card selection respectively. The feedback stimuli would appear for 500 milliseconds during which FE was allowed, then the display would change to fixation until the inception of the next trial.

Figure 2.1 Performing computer-based WCST in the fMRI scanning room

Figure 2.2 The modified Wisconsin Card Sorting Test

RS after 2nd PF: Maintain "Color" rule   RS after 1st NF: Propose "Number" rule   RS after 2nd NF: Select "Shape" as new rule   RS after 1st PF: Select using "Shape" rule

Wrong   Wrong   Right   Right

1st NF: Shift to another rule   2nd NF: Generate a new rule   1st PF: Update "Shape" as new rule   2nd PF: Maintain "Shape" rule

Course of cognitive set-shifting
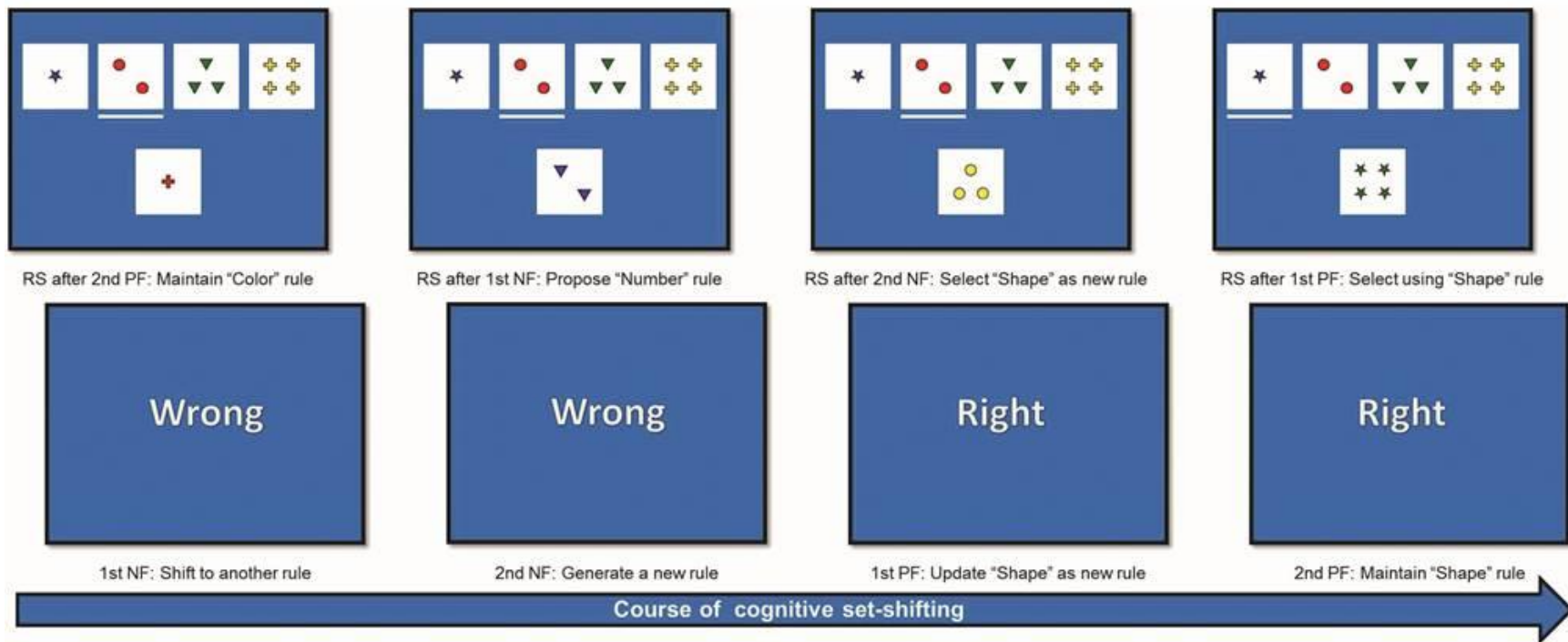
The identified rule kept unchanged randomly for 3 to 5 successive correct feedbacks until another rule was randomly generated. Appearance of first negative feedback (1st NF) after successive correct feedbacks signaled as a "shift" event for subjects to abandon previously prepotent rule and search for a new one. Subsequent negative feedback (2nd NF), if applicable, would serve as a "generate" event to identify the correct rule. The first positive feedback (1st PF) in a series of correct feedbacks was regarded as an "update" event to register the newly confirmed cognitive set in memory while subsequent positive feedback (2nd PF) served as a "maintain" event to keep the prevailing rule until a signal was received to shift to another rule.

Each scanning session contained 5 runs and each run lasted for 8 minutes. During the performance of the WCST and concomitant imaging, the sequence of the "shift", "generate", "update" and "maintain" events was kept constant throughout the course of the WCST while the predefined time interval of the imaging sequence was independent of the WCST event sequence.

2.5.2.2 Image acquisition

Functional imaging was performed on a Siemens Symphony 1.5T MRI scanner (Siemens, Erlangen, Germany) sited at the Functional Imaging Center, level 1, NUH. A blipped gradient-echo echoplanar imaging sequence was applied for functional imaging: time repetition = 3000 ms, flip angle = 90 °, field of view = 192 $\times$ 192 mm, pixel matrix = 64 $\times$ 64. Each run contained 156 whole brain acquisitions in a plane parallel to the line between the anterior and posterior commissures on the sagittal scout images: 32 oblique axial slices, 3-mm thick, 0.3-mm gap between slices, descending interleaved slice

acquisition. T1-weighted anatomical reference images were acquired by a magnetization prepared rapid acquisition gradient echo sequence: pixel matrix = 256 $\times$ 256, field of view = 206 $\times$ 206 mm, 80 slices, 2mm thick in the coronal plane.

2.5.2.3 Image processing

Image processing and analyses were performed using the software Brain Voyager QX (Version 2.1, Brain Innovation, Netherlands). Preprocessing steps, including slice scan time correction, motion correction, spatial smoothing (8-mm full width half-maximum) and linear trend removal, were performed prior to statistical mapping of brain activation during the fMRI paradigm. Subsequently, functional images were registered to the magnetization prepared rapid acquisition gradient echo images, followed by transformation of the realigned images into Talairach space. The Talairach transformation was achieved by first identifying the anterior commissures (AC), the posterior commissures (PC) and AC-PC plane in the anatomic images of each subject. Other fiduciary markers, including anterior point, posterior point, superior point, inferior point, right point and left point, were defined subsequently to generate transformation of anatomic images to a standard brain coordinate system.

2.5.3 Measurement of anti-NR2 antibody

Amidated and acetylated peptide of the sequence SVSYDDWDYSLEARV was synthesized (GenScript, NJ, USA) and used as a substrate of our home-made indirect ELISA for measuring the serum anti-NR2 antibodies (DeGiorgio et al., 2001; Husebye et al., 2005). The peptide has been demonstrated to have a purity of at least 95% assessed by high-performance liquid chromatography and it contains the pentapeptide consensus

sequence DWDYS which is the epitope recognized by the anti-NR2 antibodies (DeGiorgio et al., 2001; Gono et al., 2011). Maxisorp 96-well microplates (NUNC, NY, USA) were coated with 0.5 μg of the synthesized peptide in 100 μl phosphate-buffered saline (PBS) per well and kept overnight at 4 ℃. Plates were blocked with 3% fetal calf serum (Gibco, CA, USA) in PBS for 1 hour at 37 ℃ and subsequently incubated with serum samples at the dilutions of 1:500 and 1:1000 in PBS for 2 hours at 37 ℃. The microplates were then washed with PBS-Tween and incubated with horseradish peroxidase-conjugated goat anti-human IgG (Abcam, Cambridge, UK) for 1 hour at 37 ℃, followed by another wash with PBS-Tween. One hundred microliters (μl) of tetramethyl benzidine (BD Biosciences, CA, USA) were subsequently added to each well. The reaction was terminated 10 minutes later by adding 2 N H_2SO_4. Optical density at 450 nm (OD_{450}) was measured by a Multiskan FC ELISA Microplate Reader (Thermo Scientific, MA, USA). Since sample dilution at 1:500 worked most optimally for our immunoassays, the OD_{450} of serum samples at this dilution was reported. R4A monoclonal antibody (R4A mAb) was kindly provided by Dr Betty Diamond (The Feinstein Institute for Medical Research, Manhasset, NY, USA). The R4A mAb was serially diluted to serve as calibration for each plate (see Figure 2.3).

Figure 2.3 Standard curve for anti-NR2 ELISA
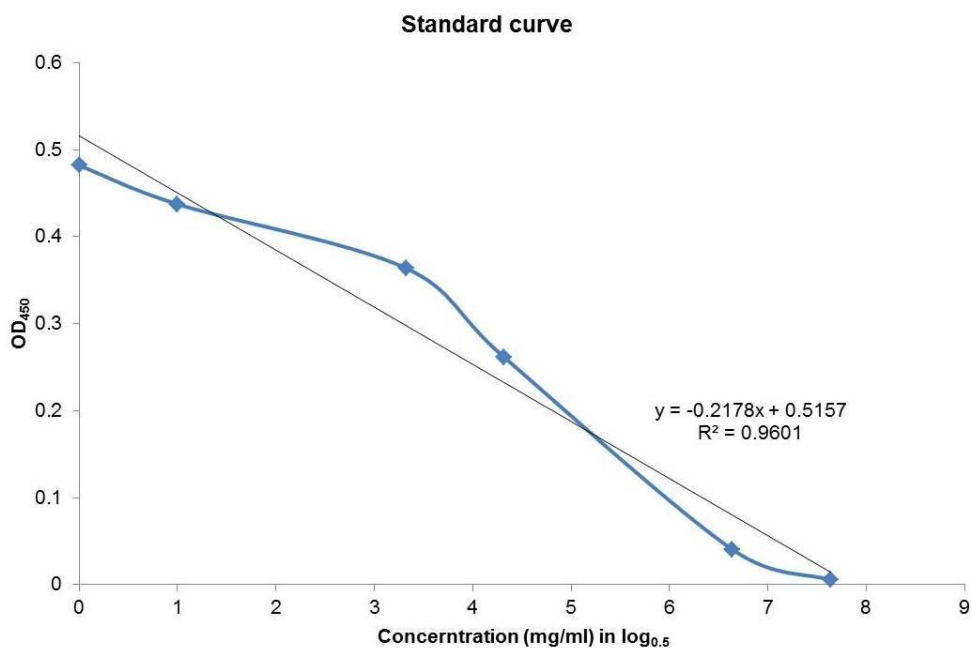
2.6 Statistical analysis

All statistical analyses were calculated by the SPSS program (version 18 or version 20). Values in this thesis were reported as mean $\pm$ standard deviation (SD) unless otherwise specified.

2.6.1 Comparison of continuous and categorical data

Comparison of continuous variables between two groups was carried out by Students't-test for independent samples when the data followed a normal distribution or if equal variance could be assumed, otherwise the Mann-Whitney U test would be used instead. Kurtosis and skewness were calculated to evaluate the normality of the data, of which the acceptable range for normal distribution was suggested to be between -1 to +1 (Chan, 2003). The Levene's test was used to test for equality of variance.

Comparison of categorical variables between two groups was executed by the χ^2 test. The Fisher's exact test would be performed instead when the frequency of occurrence was low.

2.6.2 Correlation analysis

Bivariate correlation was used to analyze the relationship between the serum levels of anti-NR2 antibodies and clinical characteristics of patients with SLE.

Statistical significance

A two-tailed P value < 0.05 was defined as statistical significance for all statistical tests unless otherwise specified.

CHAPTER 3

RESULTS

Study 3.1-Part A

A prospective functional MRI study for executive function in patients with systemic lupus erythematosus without neuropsychiatric symptoms

INTRODUCTION

Cognitive dysfunction, which primarily affects attention, reasoning, executive function, verbal memory, visual-spatial information processing and psychomotor speed (Bertsias et al., 2010; Hanly et al., 2005), has been recognized as one of the commonest NPSLE manifestations which may impose substantial physical, psychological and socioeconomic burden to patients and the community (Lau & Mak, 2009; Tam et al., 2008). Cognitive dysfunction is often clinically subtle and difficult to be recognized and despite that a standard battery of neuropsychological assessments for identifying cognitive dysfunction in SLE patients is currently available (Anonymous, 1999) and studies involving anatomical MRI and MRS have revealed the presence of structural cerebral white and gray matter abnormalities in patients with lupus (Ainiala et al., 2005; Lapteva et al., 2006a; Luyendijk et al., 2011), these assessments are not able to address the potential mechanism cognitive dysfunction in SLE.

fMRI has grown into the workhorse of brain imaging to study brain activation patterns associated with psychological tasks which probe specific aspects of cognition. While fMRI offers a non-invasive platform to evaluate abnormal brain activities in a

number of neurological conditions such as cerebrovascular disease, migraine and multiple sclerosis (Calautti & Baron, 2003; Filippi & Rocca, 2004; Rocca et al., 2003), its application to elucidate the neuropathological mechanism underlying cognitive dysfunction in SLE patients is rare. Two recent cross-sectional studies demonstrated increased brain activation in certain cortical regions associated with cognitive tasks assessing working memory, attention and language in certain cortical regions in SLE patients when compared with healthy controls (DiFrancesco et al., 2007; Fitzgibbon et al., 2008). These observations contribute to our preliminary understanding that significant brain activation with compensatory recruitment of additional cortical regions is required for SLE patients to maintain a comparable neuropsychiatric performance as healthy individuals.

Given that cognitive dysfunction can persist and even deteriorate over time in SLE patients (Cassano et al., 2007; Mok et al., 2006), it is imperative to study the functional brain pathology of cognitive dysfunction both prevalently and longitudinally in patients with new onset SLE, with an aim to elucidate the regions of abnormal brain activation and their changes over time. We therefore undertook this longitudinal study using event-related BOLD fMRI to assess executive function in new onset SLE patients and explore the changes in brain activation signals over time after adequate disease control with immunosuppressive therapy.

PATIENTS AND METHODS

Participants

Patients with newly diagnosed SLE based on the ACR revised classification criteria (Hochberg, 1997) were consecutively recruited from the Lupus Clinic, National University Hospital, Singapore. Healthy controls who were working in the the National University of Singapore were recruited and matched with SLE patients for age, sex, education level and intelligence quotient (IQ). Subjects were excluded if they were left-handed, if they were not proficient in English, if they had a history of neurological disorder, if they had clinically significant anxiety and/or depressive symptoms, as rated and defined by the HADS with a score ≥ 8 respectively (Mak et al., 2011; Tam et al., 2008), or if they had a concurrent or past history of psychiatric disorder or were using psychotropic medications. IQ was tested using the Wechsler Abbreviated Scale of Intelligence which consists of the assessment of verbal IQ, performance IQ and the full IQ by a certified clinical psychologist. Written informed consent was obtained before subject recruitment.

Wisconsin Card Sorting Test

Please refer to section 2.5.2.1

The fMRI protocol and imaging processing

Please refer to sections 2.5.2.2 and 2.5.2.3

Statistical analysis of BOLD signals

Beta-score maps were computed in the Talairach coordinate system by a random-effects general linear model. This model was constructed with separate regressors relative to a fixation baseline for RS and FE. Each hemodynamic response function convolved

each regressor, peaking at 6 seconds after onset of card stimuli or feedback evaluation, respectively. Jittering of the fixation intervals between FE and the subsequent RS assisted in event deconvolution (Graham et al., 2009). Composite beta-score maps were constructed for each event for SLE patients and healthy controls to identify significant group-activation regions. The different activation patterns between patients and healthy controls and within patients were assessed for RS and FE events separately and statistically displayed in respective beta-score maps. Application of cluster threshold to activation patterns for each map assured significance of difference.

Interpretation of activation signals

Although the traditional neural efficiency hypothesis suggests that individuals who perform better in cognitive tasks exhibit less task-relevant cortical activities demonstrated by functional imaging (Gray et al., 2003), more recent studies have provided evidence which supports that brain activation patterns are dependent on the complexity of the neuropsychological tests. For example, higher BOLD activation signals during RS and lower activation during FE were demonstrated in individuals with average IQ when compared with those with higher IQ during the performance of the WCST. These observations suggested that individuals with average IQ had poorer strategic planning during the FE stage, which required compensatory recruitment of additional cortical regions for average-IQ individuals to maintain a comparable executive performance as those with higher IQ. Thus, we hypothesize that during the WCST, decreased brain activation during FE suggests poor strategic planning skills and prompts recruitment of extra cortical regions and neural pathways during RS to compensate for the deficient strategic planning skills in order to maintain executive responses.

Clinical and serological disease activity of SLE was assessed using the SLEDAI and peripheral blood was obtained from patients on the day of fMRI scan. Serum was extracted by blood centrifugation and subsequently assayed for C3, C4 and anti-dsDNA levels by the standard laboratory of the hospital.

All patients were invited to undergo the second event-related fMRI scans if they demonstrated inactive disease activity indicated by SLEDAI $\leqslant$ 4 points (Yee et al., 2011) and a minimum of 6 months had elapsed from the date of the first scan, coupled with a repeat of SLEDAI assessment and blood tests on the day of the second scans.

RESULTS

Demographic, clinical and results of WCST performance of new-onset SLE patients and healthy controls

Fourteen patients with new-onset SLE and 14 matched healthy controls were recruited for the study. Table 3.1 lists the information on the demographics, clinical manifestation and disease activity index of individual lupus patients involved in this study. Twelve females were included in each group. The SLE patients and controls had comparable demographic characteristics in terms of mean age (39.38 $\pm$ 13.9 vs 34.07 $\pm$ 14.4, year, P = 0.33), duration of education (14.43 $\pm$ 3.4 vs 13.43 $\pm$ 3.4, year, P = 0.45) verbal IQ (96.23 $\pm$ 15.6 vs 100.43 $\pm$ 11.0, P = 0.42), performance IQ (101.50 $\pm$ 19.8 vs 104.86 $\pm$ 11.6, P = 0.59) and full IQ (99.31 $\pm$ 17.1 vs 103.14 $\pm$ 11.2, P = 0.49).

Table 3.1 Demographics, clinical manifestation and disease activity of SLE patients

No.	Age, Years	Sex	Education	Clinical manifestation of SLE	SLEDAI
1	47	F	Primary	Joint pain, Raynaud's and rashes	8
2	30	F	Undergraduate	Joint pain and nephritis	14
3	67	M	Secondary	Joint pain	12
4	28	F	Doctorate	Joint pain and rashes	24
5	54	F	Undergraduate	Joint pain and rashes	2
6	49	F	Secondary	Nephritis	8
7	34	F	Secondary	Nephritis, joint pain and rashes	8
8	30	F	Undergraduate	Joint pain and rashes	10
9	61	F	Undergraduate	Joint pain and rashes	8
10	26	F	Undergraduate	Joint pain and rashes	8
11	22	F	Master	Joint pain and pericarditis	12
12	33	F	Doctorate	Rashes and nephritis	0
13	34	M	Diploma	Rashes and nephritis	13
14	37	F	Undergraduate	Nephritis and cutaneous vasculitis	12

Abbreviations: SLE, systemic lupus erythematosus; F, female; M, male; SLEDAI, systemic lupus erythematosus disease activity index.

The majority of the SLE patients had active disease on recruitment indicated by SLE markers including low serum C3, C4 and high anti-dsDNA levels with mean $\pm$ SD (range) as 70.46 $\pm$ 25.0 (28.00-103.00) mg/dL [normal range (NR): 85-185], 15.42 $\pm$ 10.9 (3.00-38.00) mg/dL (NR: 10-50) and 132.50 $\pm$ 111.3 (1.00-250.00) IU (NR < 20) respectively. The mean $\pm$ SD (range) SLEDAI was 9.93 $\pm$ 5.7 (0-24.00). Both groups performed comparably in the WCST regarding the number of rules identified (19.54 $\pm$ 3.5

vs. 19.50 $\pm$ 3.0; P = 0.50), error per rule (3.04 $\pm$ 2.5 vs. 2.51 $\pm$ 0.7, P = 0.38), and reaction time on recruitment (1679.01 $\pm$ 411.3 ms vs. 1080.51 $\pm$ 228.8 ms, P = 0.11).

Differences in brain activation signals between SLE patients and healthy controls

Feedback evaluation

During 2nd NF, 1st PF and 2nd PF, healthy controls demonstrated significantly increased brain activation in a number of cortical regions including the medial frontal gyri and precentral gyri (BA6), the insular (BA13) and the anterior cingulate gyri (BA24/32) when compared with the new-onset SLE patients. The precentral gyri and medial frontal gyri are located within the premotor and supplementary motor cortices respectively, which subserve planning of complex and coordinated motor movements, decision making, computation and reasoning. The anterior cingulate gyri are involved in response inhibition, error detection and conflict monitoring and processing. Insular is suggested to be associated with integration of sensory information, self-awareness and cognitive functioning.

On the contrary, during the first NF when the rule shift was signaled, significantly elevated activation was revealed in the left middle, left superior, and right medial frontal gyri (BA10/11) and the right parahippocampal gyrus (BA34) in SLE patients when compared to healthy controls. These frontal gyri within BA10/11 have been suggested to be involved in cognitive branching (Walsh et al., 2009), during which previously running task is maintained in a pending state for subsequent retrieval and execution upon completion of the ongoing one. The right parahippocampal gyrus is mainly responsible for memory encoding and retrieval.

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Response Selection

During RS after 2nd NF, SLE patients demonstrated significantly enhanced activation in the right superior frontal gyrus (BA9) and both caudate bodies. Superior frontal gyrus of BA9 is located within dorsolateral prefrontal cortex which supports attention and working memory. The caudate body functions in event anticipation and formulation of response strategy for the ongoing trials.

Of note, the right precentral gyrus (BA4), postcentral gyri (BA3) and both superior parietal lobes (BA5/7) were significantly less activated during the rest of the RS events in the SLE group when compared with those of healthy controls. These cortical regions within BA3, 4 and 5/7 are primarily involved in processing of sensory information, motor execution and spatial orientation for goal-oriented behavior respectively.

A detailed summary of the signal differences during RS and FE events between SLE patients and healthy controls is detailed in Table 3.2.

Table 3.2 Regional differences in brain activation between patients with new-onset SLE and matched healthy controls

	Brain region	Talairach			Hemisphere	BA
		x	y	z		
RS after 1st NF						
SLE < Control	1 Postcentral gyrus	38	-27	48	R	3
	2 Precentral gyrus	47	-17	39	R	4
	3 Superior parietal lobule	20	-44	56	R	5
RS after 2nd NF						
SLE > Control	4 Superior frontal gyrus	8	52	22	R	9

	5 Caudate body	-10	8	16	L	−
	6 Caudate body	10	8	16	R	−
RS after 1st PF						
SLE < Control	7 Superior parietal lobule	-29	-53	59	L	7
	8 Postcentral gyrus	-30	-31	67	L	3
1st NF						
SLE > Control	9 Superior frontal gyrus	-23	60	10	L	10
	10 Middle frontal gyrus	-25	44	-11	L	11
	11 Medial frontal gyrus	4	50	0	R	10
	12 Parahippocampal gyrus	14	-6	-18	R	34
2nd NF						
SLE < Control	13 Precentral gyrus	-50	-5	33	L	6
	14 Precentral gyrus	50	-5	34	R	6
	15 Insula	-43	-4	11	L	13
	16 Insula	41	-6	16	R	13
1st PF						
SLE < Control	17 Anterior cingulate gyrus	6	7	43	R	32
2nd PF						
SLE < Control	18 Anterior cingulate gyrus	-5	-3	47	L	24
	19 Anterior cingulate gyrus	6	-3	46	R	24
	20 Medial frontal gyrus	-4	-4	53	L	6
	21 Medial frontal gyrus	5	-4	53	R	6

Abbreviations: BA, Brodmann area; R, right; L, left; RS, response selection; FE, feedback evaluation; NF, negative feedback; PF, positive feedback

Prospective data on clinical disease activity and WCST performance in SLE patients

WCST performance

The WCST performance during the follow-up fMRI scan did not differ significantly from that during the first fMRI scan in patients with SLE regarding the number of rules identified (19.36 ± 5.8 vs 19.54 ± 3.5, P = 0.906), error per rule (3.04 ± 2.5 vs 2.2 ± 0.4, P = 0.095) and reaction time (1679.01 ± 411.3 vs 1580.4 ± 347.4, P = 0.17).

Clinical and serological disease activity

After a mean $\pm$ SD (range) of 504.92 ± 267.0 (196- 1088) days of treatment, disease activities in the SLE patients were adequately controlled as indicated by both the clinical and serological features. The mean SLEDAI decreased significantly (2.64 ± 1.4 vs 9.93 ± 5.7, P < 0.001), whereas that of serum anti-dsDNA level tended to improve after disease control (83.42 ± 93.0 vs 132.5 ± 111.3, IU, P = 0.064). Serum C3 (89.08 ± 17.8 vs 70.46 ± 25.0, mg/dL, P = 0.009) and C4 (20.67 ± 8.8 vs 15.42 ± 10.9, mg/dL, P = 0.020) levels increased significantly, whereas daily prednisolone was significantly tapered (3.82 ± 3.5 vs 15.32 ± 18.4, mg/d, P = 0.023).

Differences in brain activation signals in SLE patients during the first fMRI scan and the follow-up fMRI scan

Feedback evaluation

All patients who had undergone the first fMRI scans underwent the follow-up scans after sufficient disease control. During the follow-up fMRI scan, reduced cortical activation in a number of brain regions during FE were persistently demonstrated in patients with SLE when compared to the first fMRI scan. During 1st NF, reduced activation was revealed in the left middle frontal gyrus (BA6), right cuneus (BA17) and both fusiform gyri (BA37) which functions in motor planning, pattern recognition of object and color processing and word recognition respectively, while no significant difference was noted during 2nd NF. During 1st PF, the right medial and superior frontal gyri and left precentral gyrus (BA6) and the right anterior cingulate gyrus (BA32) were demonstrated to be less activated. These regions within BA6 and BA32 are suggested to be associated with motor planning and error detection and conflict monitoring respectively. At the stage of 2nd PF, the left posterior cingulate gyrus (BA23), superior parietal lobule (BA5) and middle frontal gyrus (BA9) were revealed to have reduced neuronal activities, which subserve error detection and conflict monitoring, spatial orientation and response inhibition and attention sustaining.

Response selection

After adequate disease control, SLE patients distinctively showed activation in both anterior cingulate gyri (BA32) during RS after 1st NF. Notably, significantly reduced brain activation was demonstrated during all four RS events in a number of cortical regions including the right superior and inferior parietal lobes (BA7, 40), left insula and lingual gyri, right fusiform gyrus, and both inferior and right middle frontal gyri (BA13/18/19/9), and right medial frontal gyrus (BA8). The aforementioned cortical regions located within BA7, 40, BA13/18/19/9 and BA 8 are suggested to serve the

function of spatial orientation, mid-level processing of visual information and governance of eye movement respectively.

The BOLD signal differences between SLE patients at first fMRI scan and the follow-up fMRI scan is detailed in Table 3.3.

Table 3.3 Regional differences in brain activation in SLE patients between the first fMRI scan and the follow-up fMRI scan

	Brain region	Talairach			Hemisphere	BA
		x	y	z		
RS after 1st NF						
Follow-up > First	1 Anterior cingulate	-3	39	-6	L	32
	2 Anterior cingulate	3	38	-5	R	32
Follow-up < First	3 Inferior frontal gyrus	-48	5	30	L	9
	4 Middle frontal gyrus	46	13	34	R	9
RS after 2nd NF						
Follow-up < First	5 Inferior frontal gyrus	45	4	33	R	9
	6 Superior parietal lobule	21	-63	49	R	7
	7 Insula	-37	-21	7	L	13
	8 Fusiform gyrus	34	-72	-11	R	19
	9 Lingual gyrus	-21	-73	-8	L	18
RS after 1st PF						
Follow-up < First	10 Inferior parietal lobule	36	-46	39	R	40
RS after 2nd PF						
Follow-up < First	11 Medial frontal gyrus	7	27	41	R	8
1st NF						
Follow-up < First	12 Middle frontal gyrus	-16	-9	58	L	6

	13 Fusiform gyrus	-36	-57	-12	L	37
	14 Fusiform gyrus	25	-48	-9	R	37
	15 Cuneus	13	-81	9	R	17
1st PF						
Follow-up < First	16 Anterior cingulate	4	21	34	R	32
	17 Medial frontal gyrus	9	10	53	R	6
	18 Superior frontal gyrus	8	8	55	R	6
	19 Precentral gyrus	-32	-7	50	L	6
2nd PF						
Follow-up < First	20 Posterior cingulate gyrus	-3	-11	33	L	23
	21 Superior parietal lobule	-13	-41	56	L	5
	22 Middle frontal gyrus	-45	7	34	L	9

Abbreviations: BA, Brodmann area; R, right; L, left; RS, response selection; FE, feedback evaluation; NF, negative feedback; PF, positive feedback

DISCUSSION

The present prospective event-related fMRI study found that patients with new-onset SLE demonstrated inferior planning strategy which required compensation on response execution during the assessment of executive function when compared with healthy controls, even after achieving sufficient disease control.

Less efficient strategic planning skills regarding planning of complex and coordinated motor movement, decision-making, computation and reasoning (BA6), error detection, conflict monitoring and processing (BA24/32), and integration of sensory information (BA13) (Craig, 2009; Phan et al., 2002) were revealed in new-onset SLE patients when compared with healthy individuals. Although our sample of SLE patients possessed better planning skills regarding cognitive branching (BA10/11) and memory encoding and retrieval (BA34) during FE as compared with the healthy controls, their overall inferior planning strategy resulted in compensatory recruitment of additional cortical regions that boosted attention, working memory (BA9), and event anticipation and strategy formulation , sensory information processing (BA3), motor execution (BA4), and spatial orientation (BA5/7) in order to perform comparably as healthy controls.

After sufficient disease control, SLE patients continued to demonstrate poorer strategic planning skills regarding motor planning (BA6), response inhibition and sustaining of attention (BA9), error detection and conflict evaluation (BA23/32), pattern recognition of object (BA17), spatial orientation (BA5), and color information processing and word recognition (BA37) when compared to the time when their disease was active. These observations suggest that sufficient clinical and serological disease control of SLE

may not be able to improve the relevant strategic planning skill. Instead, recruitment of extra cortical areas was required to enhance response inhibition, error detection, and conflicting processing and monitoring (BA32) to maintain their executive functioning, despite the fact that they relied substantially less on spatial orientation (BA7/40), visual processing (BA9/13/18/19) and governance of eye movement (BA8) during RS. Although reduced dependence on spatial orientation and visual processing may be secondary to the beneficial effects of the clinical improvement of SLE, familiarization with the spatial and screen arrangement of the WCST task during their first scans may theoretically confound the interpretation.

BOLD signal changes are sensitive to even very minute alterations of cerebral perfusion. As a systemic inflammatory condition, SLE may cause a global change in vascular reactivity and lower rate of cerebral blood flow (Giovacchini et al., 2010; Yoshida et al., 2007). It can be argued that the diffuse deactivation of BOLD signals documented in this study were due to poorer global cerebral perfusion. However, this theory cannot explain the observations that BOLD signals were selectively suppressed in a confined number of cortical regions as shown in this study. Thus, other factors such as potential pathology in certain critical connections between brain networks may contribute (DiFrancesco et al., 2007). In view of this, we carried out a mechanistic study to explore the potential malfunction of brain networks and connections and the results will be presented in Part B of this study.

This study was limited by a small sample size. However, sample size of this magnitude has consistently been demonstrated sufficient sensitivity in detecting statistical significance in BOLD signals even in NPSLE na ïve lupus patients (DiFrancesco et al.,

2007; Fitzgibbon et al., 2008; Rocca et al., 2006). Second, the WCST was performed twice in our SLE patients and it can be argued that patients' learning effect of WCST might confound the interpretation. However, it has been suggested that such learning effect is minimal even if the WCST is reapplied within 1 month. In addition, temporal stability has been shown in most variables probed with the WCST (Ingram et al., 1999). Nevertheless, if learning effect was operant in our patients, the performance during the follow-up scan would have likely improved. Third, the WCST does not cover the assessment of other aspects of cognition such as language and psychomotor speed. Finally, patients with clinically overt neuropsychiatric lupus or cognitive impairment were obviously not included because they might not be able to follow and perform sophisticated neuropsychological test.

In summary, our results demonstrated that subclinical cognitive dysfunction persisted and worsened in patients with SLE despite sufficient disease control. This prompts future targeted research on potentially specific autoantibodies, cytokines, or neurotransmitter which direct against the involved brain regions. Moreover, emerging neuroscience studies focused more on neural circuit-level interaction between multiple cortical regions and cognition (Kapogiannis & Mattson, 2011; Ray & Strafella, 2012; Wilbrecht & Shohamy, 2010), potential neural circuits that might underlie the subclinical cognitive dysfunction in SLE patients would be explored in the Part B of this study.

Study 3.1-Part B

Dysfunctional cortico-basal ganglia-thalamic circuit and altered hippocampal-amygdala activity on cognitive set-shifting in non-neuropsychiatric systemic lupus erythematosus

INTRODUCTION

In the first part of Study 3.1 we have identified characteristic BOLD fMRI signal changes in a number of cortical brain regions in lupus patients without clinically overt neuropsychiatric manifestations. Evidence has recently suggested that cognitive dysfunction is likely related to abnormal sequential brain activities involving specific neural circuits. For instance, disturbance of executive function has been demonstrated to be associated with dysfunctional classic and extended executive circuits in schizophrenics (Eisenberg & Berman, 2010). The fronto-parietal and fronto-striatial-thalamic disconnections have been revealed to be associated with cognitive dysfunction in patients with mild cognitive impairment (Liang et al., 2011). Moreover, disturbance of the default mode network has been found in lupus patients without neuropsychiatric manifestations (Lin et al., 2011). Thus, it prompts to map the functional abnormalities occurring in potential neural circuits which mediate cognitive dysfunction in SLE patients by analyzing their sequential brain activation while they are going through various sequential stages of cognitive function test. We therefore undertook this fMRI study using the computerized modified WCST to explore the brain activities across all stages of cognitive set-shifting processes in patients with new-onset SLE without clinically overt neuropsychiatric symptoms.

PATIENTS & METHODS

Participants

Please refer to *Participants* of Study 3.1-Part A on Page 66-67

Wisconsin Card Sorting Test

Please refer to section 2.5.2.1

Image acquisition and imaging processing

Please refer to sections 2.5.2.2 and 2.5.2.3

Statistical analysis of BOLD signal

Beta-score maps were computed in the Talairach space using a random effects general linear model. This model was constructed with separate regressors relative to a fixation baseline, convolved with a canonical hemodynamic response function peaking at 6 seconds after onset of card stimuli or feedback, for RS and FE. Jittering of the fixation intervals between FE and subsequent RS facilitated in event deconvolution (Graham et al., 2009). Region of interest (ROI) was computed by analyzing the effect contrasts of the two categorical task factors, class (positive, negative) and order (first, subsequent) for both RS and FE. Significant clusters and peak voxels that survived the false-discovery rate (a statistical method to correct multiple comparisons) $q < 0.05$ were reported (Genovese et al., 2002). The cluster threshold was calculated by Cluster-level Statistical Threshold Estimator in the Brain Voyager QX software package.

RESULTS

Participants and WCST performance

We recruited 14 new-onset SLE patients and 14 matched healthy controls and they did not differ in age, sex, years of education and IQ. As far as the performance on the WCST is concerned, SLE patients and controls performed comparably in terms of the number of rules identified, the number of error per rule and the reaction time. The demographic information, clinical data and the WCST performance of the study participants have been presented in Part A of this study.

Signals on fMRI during response selection after negative feedback versus response selection after positive feedback

When rule shift was signaled, brain regions that demonstrated greater activation in RS after 1st NF when compared with RS after 1st PF (RS after 1st NF>RS after 1st PF) implied involvement in response inhibition of the previously prepotent rule and the need to search for a new rule. In healthy controls, significantly activated BOLD signals were found in both middle frontal gyri, left medial frontal gyrus, left inferior parietal lobule, right angular gyrus, right middle occipital gyrus, right middle temporal gyrus, left fusiform gyrus, right claustrum, right globus pallidus (GP) and both thalami. The GP and thalami, being the critical components of the cortico-basal ganglia thalamic-cortical circuit, were involved in response inhibition and change of behavioral set (Haber & McFarland, 2001; Stevens et al., 2007).

Intriguingly, besides brain activation in various regions within the frontal and parietal lobes, our lupus patients demonstrated activation at the declive of the cerebellar

vermis while signals in the right GP and thalami were concomitantly absent. This striking finding implies the cortico-basal ganglia-thalamic-cortical circuit was dysfunctional in patients with SLE when response to prevailing rule was inhibited. On the other hand, these patients elicited activated BOLD signals in the right parahippocampal gyrus and left posterior cingulate in the condition contrast of RS after 1st NF < RS after 1st PF, which was absent in the healthy controls, implying that SLE patients required additional activities in these two regions to boost reconfiguration of response strategy for adapting to a new rule. Brain activation profile of healthy controls and SLE during RS after 1st NF versus after 1st PF is shown in Table 3.4 and Figure 3.1.

Table 3.4 Region of interest during RS in healthy controls and patients with SLE

	Brain Region	Talairach			Hemisphere	BA
		x	y	z		
Healthy controls						
RS after 1st NF > RS after 1st PF	1 Middle frontal gyrus	41	25	30	R	9
(Response inhibition)	2 Middle frontal gyrus	-34	50	6	L	10
	3 Medial frontal gyrus	-1	19	45	L	8
	4 Angular gyrus	32	-56	39	R	39
	5 Inferior parietal lobule	-37	-56	42	L	40
	6 Middle occipital gyrus	30	-83	-3	R	18
	7 Middle temporal gyrus	50	-32	0	R	21
	8 Fusiform gyrus	-37	-56	-9	L	37
	9 Globus Pallidus	14	-2	3	R	—
	10 Thalamus	12	-7	9	R	—
	11 Thalamus	-10	-8	9	L	—
	12 Claustrum	29	19	0	R	—

RS after 1st NF < RS after 1st PF	No significant voxels					
RS after 2nd NF vs. RS after 2nd PF	No significant voxels					

Patients with SLE

RS after 1st NF > RS after 1st PF	13 Superior frontal gyrus	-31	55	15	L	10
(Response inhibition)	14 Middle frontal gyrus	32	10	51	R	6
	15 Middle frontal gyrus	29	49	3	R	10
	16 Middle frontal gyrus	-49	25	27	L	46
	17 Superior parietal lobule	-31	-68	51	L	7
	18 Inferior parietal lobule	44	-56	45	R	40
	19 Inferior parietal lobule	-37	-50	42	L	40
	20 Inferior parietal lobule	-55	-47	48	L	40
	21 Thalamus	-16	-8	6	L	—
	22 Claustrum	29	22	3	R	—
	23 Declive	35	-62	-21	R	—
	24 Declive	5	-74	-18	R	—
RS after 1st NF < RS after 1st PF	25 Posterior cingulate	-7	-47	21	L	30
(Reconfiguration of response strategy)	26 Parahippocampal gyrus	23	-14	-15	R	28
RS after 2nd NF vs. RS after 2nd PF	No significant voxels					

Abbreviations: ROI, Region of Interest; RS, response selection; FE, feedback evaluation; SLE, systemic lupus erythematosus; BA, Brodmann Area; NF, negative feedback; PF, positive feedback; R, right; L, left; vs., versus.

Figure 3.1 Composite general linear model of the BOLD activation pattern seen on the fMRI in healthy controls and patients with new-onset SLE during the condition contrast of RS after 1st NF > RS after 1st PF . Images are numbered according to the brain region numbering in Table 3.4.
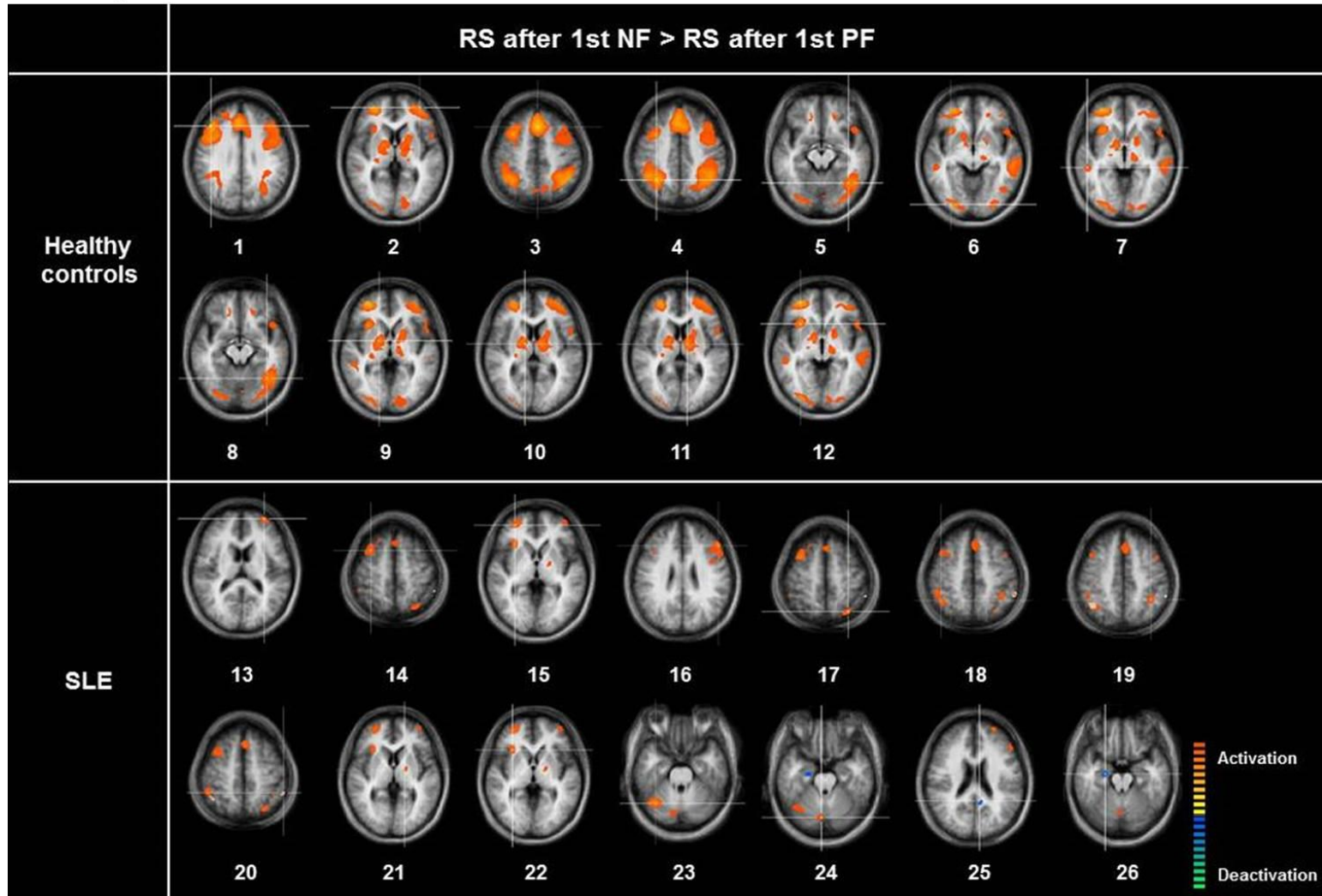
Brain regions that showed greater activation in 2nd NF in comparison to 2nd PF (2nd NF > 2nd PF) reflected involvement in generating identity of a new rule instead of keeping the prevailing one. In healthy controls, both middle frontal gyri, left medial frontal gyrus, precentral gyrus and both inferior parietal lobules were activated in this condition. In lupus patients, the right superior frontal gyrus, bilateral middle frontal gyri, right inferior parietal lobule, left superior parietal lobule, left cingulate gyrus and the right claustrum were activated instead. By contrast, brain regions that were activated in 2nd PF in comparison with 2nd NF (2nd PF > 2nd NF) signified their contribution to maintain the established rule rather than the generation of the identity of a new rule. In this condition, healthy controls had their right precentral gyrus, right precuneus, left inferior parietal lobule, left middle temporal gyrus and both hippocampi, left amygdala, right posterior cingulate, left anterior cingulate and left cingulate gyrus (limbic system) involved. Whereas in the lupus patients, limbic involvement in rule maintenance was demonstrated only in the right hippocampus and left anterior cingulate. Regions of brain activation in healthy controls and SLE for 2nd NF versus 2nd PF are described in Table 3.5 and Figure 3.2.

**Table 3.5 Region of interest during FE in healthy controls and patients with SLE**

	Brain Region	Talairach			Hemisphere	BA
		x	y	z		
Healthy controls						
2nd NF > 2nd PF	1 Precentral gyrus	-43	1	30	L	6
(Generation of new rule identity)	2 Middle frontal gyrus	29	49	3	R	10
	3 Middle frontal gyrus	-37	50	3	L	10
	4 Medial frontal gyrus	-1	19	45	L	8
	5 Inferior parietal lobule	35	-56	42	R	7
	6 Inferior parietal lobule	-43	-44	42	L	40
2nd NF < 2nd PF	7 Precentral gyrus	32	-29	57	R	4
(Maintenance of established rule)	8 Anterior cingulate	-7	43	-6	L	32
	9 Posterior cingulate	5	-50	18	R	30
	10 Cingulate gyrus	-10	-20	39	L	24
	11 Precuneus	14	-44	51	R	7
	12 Inferior parietal lobule	-49	-26	27	L	40
	13 Postcentral gyrus	56	-23	21	R	40
	14 Middle temporal gyrus	-52	-2	-9	L	21
	15 Middle temporal gyrus	-46	-59	9	L	39
	16 Hippocampus	29	-29	-9	R	—
	17 Hippocampus	-27	-29	-9	L	—
	18 Amygdala	-19	-11	-13	L	—
1st NF vs. 1st PF	No significant voxels					
1st NF vs. 2nd NF	No significant voxels					
1st PF vs. 2nd PF	No significant voxels					

Patients with SLE

2nd NF > 2nd PF	19 Superior frontal gyrus	29	49	12	R	10
(Generation of new rule identity)	20 Middle frontal gyrus	41	34	30	R	9
	21 Middle frontal gyrus	-31	58	6	L	10
	22 Middle frontal gyrus	-46	25	24	L	46
	23 Cingulate gyrus	-4	28	33	L	32
	24 Superior parietal gyrus	-37	-59	54	L	7
	25 Inferior parietal gyrus	41	-53	45	R	40
	26 Claustrum	29	19	0	R	—
2nd NF < 2nd PF	27 Precentral gyrus	50	-2	6	R	6
(Maintenance of established rule)	28 Paracentral lobule	-19	-38	54	L	5
	29 Anterior cingulate	-7	31	-9	L	32
	30 Hippocampus	29	-26	-12	R	—
1st NF vs. 1st PF	No significant voxels					
1st NF vs. 2nd NF	No significant voxels					
1st PF vs. 2nd PF	No significant voxels					

Abbreviations: ROI, Region of Interest; RS, response selection; FE, feedback evaluation; SLE, systemic lupus erythematosus; BA, Brodmann Area; NF, negative feedback; PF, positive feedback; R, right; L, left; vs., versus.

Figure 3.2 Composite general linear model of the BOLD activation pattern seen on the fMRI in healthy controls and patients with new-onset SLE during the condition contrast of 2nd NF > 2nd PF. Images are numbered according to the brain region numbering in Table 3.5.
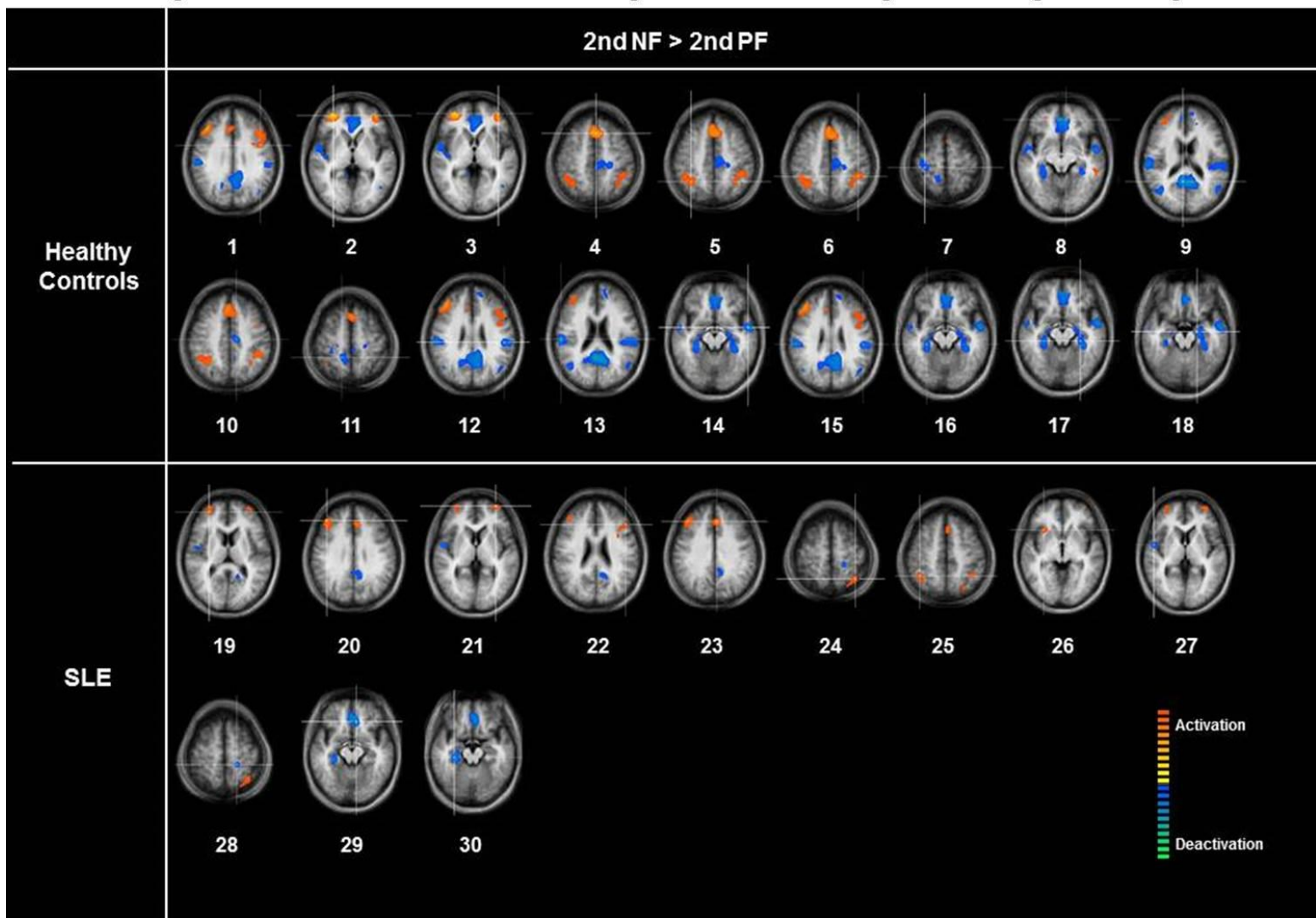
DISCUSSION

In this study, we hitherto found that the cortico-basal ganglia-thalamic-cortical circuit, which is involved in response inhibition, was dysfunctional in lupus patients even if they did not manifest clinically overt neuropsychiatric symptoms. To compensate for the dysfunction of the circuit, the contralateral cerebellar and frontal areas were activated in lupus patients. Additionally, lupus patients showed altered activities at the amygdala and hippocampus while they attempted to maintain prepotent rules during cognitive set-shifting.

While healthy controls experienced the maximum demand for withholding previous prepotent rule and response inhibition throughout the cognitive set-shifting processes, they showed increased brain activations at the right middle frontal gyrus, right GP and both thalami in a synchronous pattern across the cognitive set-shifting processes (Figure 3.3A). The dorsolateral prefrontal cortex (DLPFC) is involved in working memory, in the switching of cognitive sets and in inhibitory control of the prepotent response (Garavan et al., 1999; Haber & McFarland, 2001). Since the right middle frontal gyrus belongs to the DLPFC, it implies that it participates in conditions which require functional working memory and switching of cognitive sets. Furthermore, it was previously proposed that response suppression required the functional integrity of the prefrontal cortex, basal ganglia and thalami. Functionally, these three regions form the cortico-basal ganglia-thalamic-cortical circuit that was believed to coordinate the function of the cortical regions involved (Alexander et al., 1986; Garavan et al., 1999; Li et al., 2006; Logan & Cowan, 1984; Stevens et al., 2007).

The indirect pathway that connected these regions has been identified, in which the cortex, striatum, external segment of GP, subthalamic nucleus, internal segment of GP, thalami and cortex were involved sequentially to implement response suppression and change of behavioral set (Alexander et al., 1990). However, brain activation at the right GP and right thalamus in patients with SLE were not observed when the cognitive demand for inhibitory response control was dominating, implying that information flow from the striatum to the thalamus was compromised which resulted in the deficit of response control in SLE patients under this circumstance. Although the left thalamus was still activated in the lupus patients, the magnitude of the activation signal was lower than that of the healthy controls in the same region (Figure 3.3B).

In addition, a number of lesion studies have shown that GP is one of the important neural components which mediate executive cognitive function (Haaxma et al., 1993; Strub, 1989). For example, patients with advanced Parkinson's disease were unable to shift attention after bilateral pallidotomy (Scott et al., 2002). Likewise, disturbance of the metabolism in the thalamus was demonstrated in children with Sturge-Weber syndrome (Alkonyi et al., 2010), of which cognitive dysfunction is one of the commonest symptoms. Therefore, the absence of right GP and right thalamic activity in our lupus patients suggests that they had impaired response inhibition and reduced capability of behavioral change secondary to cortico-basal ganglia-thalamic-cortical circuit dysfunction.

Of note, the absence of brain activation at the right GP and right thalamus aforementioned was coupled with an increase in brain activity at the declive of the right cerebellar vermis in lupus patients. A few anatomical, physiological and neuroimaging studies demonstrated that cerebellar damage led to impairment of executive function,

spatial cognition, language and personality change (Schmahmann, 2010). On the other hand, over-reliance on cerebellar activities was demonstrated in patients with schizophrenia, alcoholism and cocaine abuse during evaluation of working memory, verbal working memory and executive control, respectively (Desmond et al., 2003; Hester & Garavan, 2004; Meyer-Lindenberg et al., 2001; Schlosser et al., 2003). In our study, the increased activity in the right cerebellum was coupled with activation of the left prefrontal cortex (left superior frontal gyrus and middle frontal gyrus) in lupus patients. Such activation pattern is reminiscent of increased brain activation in the left frontal-right cerebellar circuit in alcoholics during verbal memory test (Desmond et al., 2003), which is in keeping with the observations that cerebellar activation often occurs in conjunction with contralateral frontal lobe activation (Hester & Garavan, 2004). In our study, analysis of brain activities at the declive of the cerebellar vermis and the left middle and superior frontal gyrus revealed rather similar activation pattern during the course of cognitive set-shifting (Figure 3.3C), further implying the conjugated role of the right cerebellum and left frontal regions during different stages of cognitive set-shifting.

Neural activities of the cerebellum, coupled with brain activation at the contralateral frontal areas, have been suggested to compensate for articulatory and inhibitory controls in alcoholics and cocaine abusers respectively in order to maintain normal cognitive function (Desmond et al., 2003; Hester & Garavan, 2004). Taken together with another recent finding of the cerebellum-basal ganglia interconnections in an anatomical study performed in primates (Bostan & Strick, 2010), we postulated that in patients with SLE, the increased right cerebellar and its coupled left prefrontal activities may serve to compensate for the dysfunction of inhibitory control secondary to the

compromised cortico-basal ganglia-thalamic-cortical circuit in order to maintain the performance of the set-shifting tasks. Concurring with the findings of other functional imaging studies, the compensatory activations that aided the dysfunctional cortico-basal ganglia-thalamic-cortical circuit which we found in our study clearly illustrated the adaptability and plasticity of the brain to recruit neural networks to serve those function which has been damaged. For instance, in order to maintain performance levels, generalized brain activations were demonstrated in subjects with age-related degeneration in motor performance of the hands (Ward & Frackowiak, 2003). Relevant to SLE, a few recent studies demonstrated generalized brain activations in lupus patients when they were performing neuropsychological tasks (DiFrancesco et al., 2007; Fitzgibbon et al., 2008; Mackay et al., 2011). Importantly, compensatory BOLD signal intensity decreased in lupus patients with disease duration of longer than 10 years, suggesting that potential compensatory activations may decrease when irreversible neuronal damage has set in (Mackay et al., 2011).

Figure 3.3 Brain signal changes across the cognitive set-shifting process. A, Cortico–basal ganglia loop activity in healthy control subjects. B, Left thalamus activity in healthy controls and patients with new-onset SLE. C, Cerebellar–contralateral frontal activity in SLE patients. The Talairach x, y, and z coordinates for the right declive of the cerebellar vermis are shown in the key to C to distinguish the two graphs. Numbered images at the bottom correspond to the numbered β score graphs at the top.
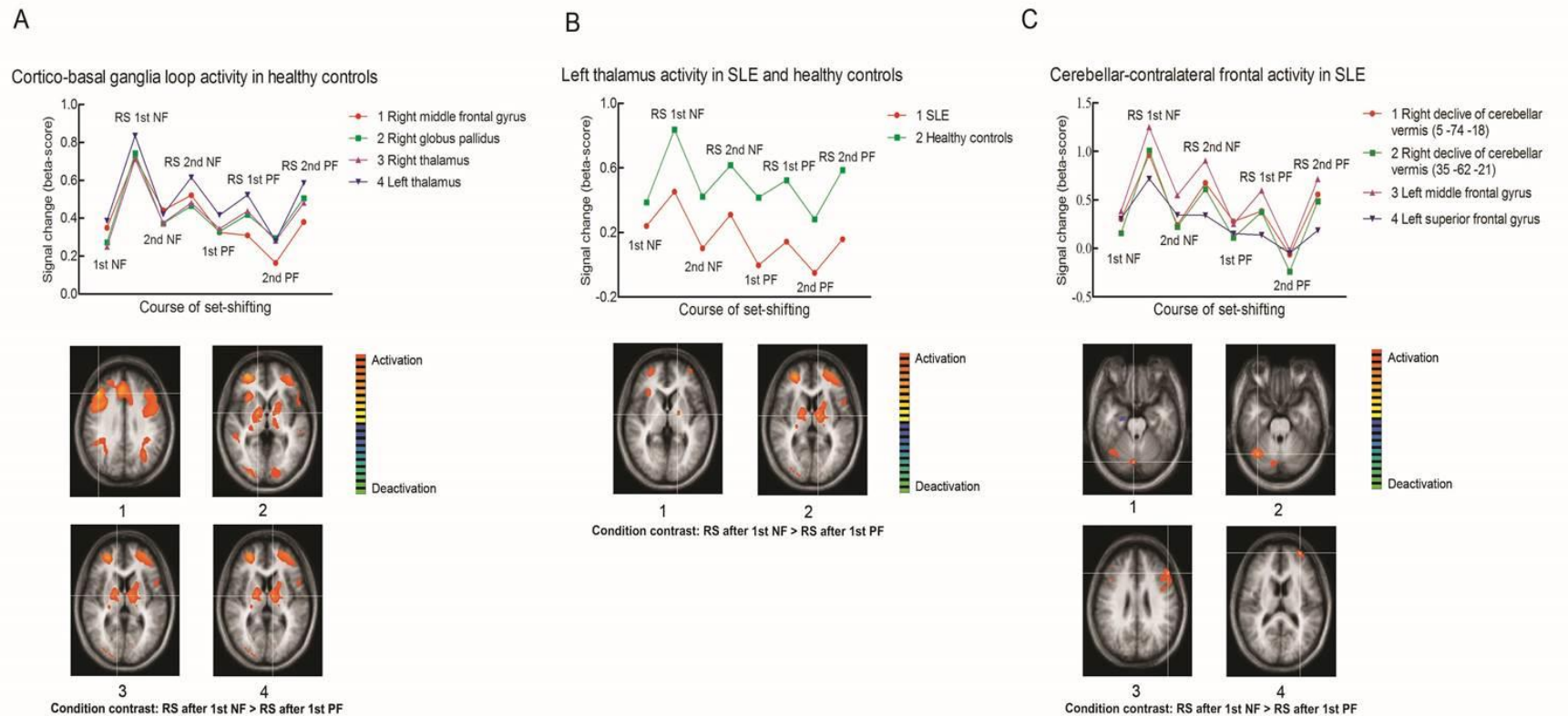
When study participants were posed to a challenge to adapt to a new rule during cognitive-set shifting, SLE patients activated their right parahippocampal gyrus and left posterior cingulate in order to reconfigure their response strategy and to adapt to a new rule, while amongst the healthy controls, no significant brain activities in these two regions were observed. The altered activities of the posterior cingulate and parahippocampus were consistent with two recently published studies in SLE patients without NPSLE and in patients with schizophrenia (Garrity et al., 2007; Lin et al., 2011), which suggested the attenuation of intrinsic DMN, episodic memory problem and related cognitive deficits. The DMN is regarded as a resting state of brain function and involved in planning of future events and on-going information processing.

Brain regions where involvement of new rule generation dominates that of the maintenance of an established rule were revealed during the condition contrast 2nd NF > 2nd PF. In healthy controls, both hippocampi were deactivated in this contrast, indicating their involvement in maintaining an identified rule. Most brain regions that contributed more to rule generation than maintenance in SLE patients and healthy subjects overlapped, suggesting that brain activities underlying this cognitive process remained intact in lupus patients. Further analysis revealed that activities of both hippocampi initially decreased during the "generate" event when the identity of the new rule was unknown. Subsequently during the "update" and "maintain" events while the new rule was identified and maintained in memory, both hippocampi were activated. This finding was consistent with the putative role of the hippocampus in set-shifting in which decreased hippocampal activity at the generation event would facilitate active forgetting of the previous rule and shift to a new cognitive set, while the subsequent increased
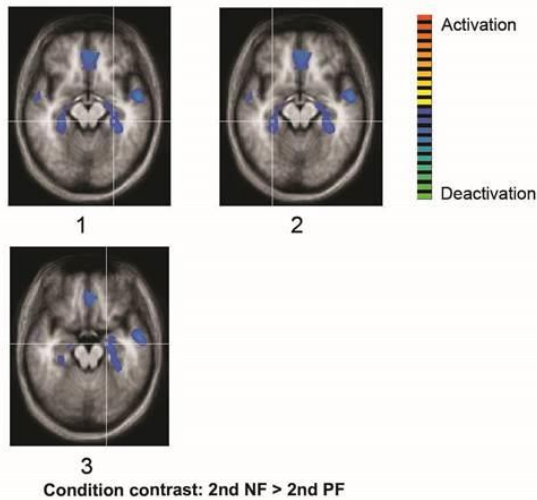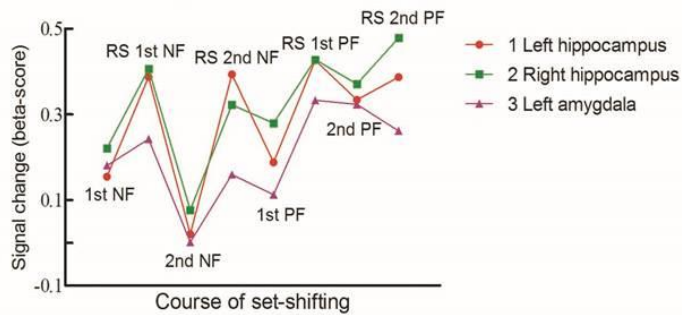
hippocampal activity at the update and maintenance events would enhance learning of the new rule to guide ensuing trials (Graham et al., 2009). Interestingly, the left amygdala appeared to follow a synchronous activity pattern as both hippocampi across all stages of set-shifting (Figure 3.4A).

The brain activation underlying FE has been proposed as a result of the response to reward or punishment of the feedback stimulus (Monchi et al., 2001). Hippocampal activities appear to be reinforced and maintained by the amygdala through generation of rewards (Joseph, 1996), suggesting the coupled role of amygdala and hippocampus in set-shifting processes observed in our study. However, the involvement of the left hippocampus and left amygdala in rule maintenance was absent in our SLE patients, implying a compromise of the active forgetting-learning dynamics in set-shifting despite that the right hippocampus was still required to maintain the established rule in a pattern similar to that in healthy controls (Figure 3.4B). As in patients with SLE, altered amygdala and hippocampal activities within DMN were demonstrated in patients with depression and Alzheimer's disease (Sheline et al., 2009; Zhou et al., 2010). These findings may signify a potential common pathological basis of the disrupted forgetting-learning dynamics which involves the amygdala and hippocampi in patients with SLE, depression and dementia.

Figure 3.4 Brain signal changes in the hippocampus and amygdala across the cognitive set-shifting process. A, Hippocampal and amygdala activity in healthy control subjects. B, Right hippocampal activity in healthy controls and patients with new-onset SLE. Numbered images at the bottom correspond to the numbered β score graphs at the top
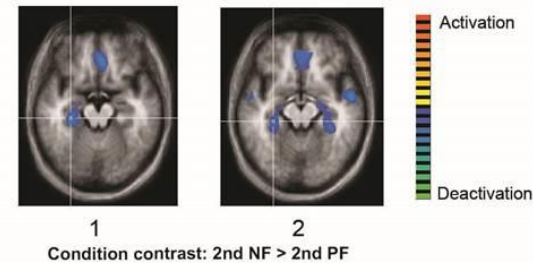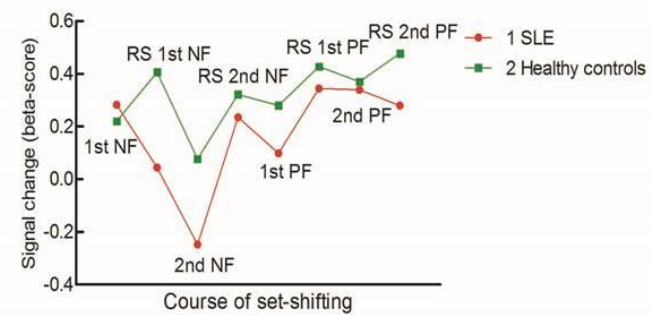
While further mechanistic evaluation is required to explain the BOLD signal changes especially those involved in hippocampus-amygdala coupling and the absence of BOLD signals in the hippocampus and amygdala in our patients with SLE, the well-investigated anti-ribosomal P and anti-N-methyl-D-aspartate receptor (NMDAR) antibodies may be potentially contributory. A clinical study in the 1980s demonstrated that the serum titre of anti-ribosomal P antibodies was selectively raised in lupus patients with active psychosis although not all subsequent studies could replicate the associations between the antibodies and psychosis and depression in patients with SLE (Bonfa et al., 1987). Nevertheless, induction of autoimmune depression in C3H/HeJ mice by intracerebroventricular injection of affinity-purified anti-ribosomal P antibodies revealed that the antibodies bound specifically to the pyramidal cell layer and dentate gyrus of the hippocampus and other areas of the limbic system which could be significantly counteracted by anti-idiotypic antibodies to anti-ribosomal P antibodies (Katzav et al., 2007). The NMDAR are mainly expressed in the neurons of the hippocampi and amygdala. Under physiologic conditions, the neurotransmitter glutamate activates the receptors and mediates learning and memory by manipulating synaptic plasticity (Diamond, 2010). In animal models, antagonising the NR2 subunit of the NMDAR (anti-NR2 antibody) led to impaired memory and learning (Diamond, 2010). Because anti-NR2 is present in the serum and cerebrospinal fluid in NPSLE patients (Diamond, 2010), a potential pathogenic role of anti-NR2 antibody in cognitive dysfunction is advocated (Aranow et al., 2010). Indeed, anti-NR2 purified from lupus patients was able to induce cognitive impairment, memory deficit and hippocampal neurotoxicity in BALB/c mice (Kowal et al., 2006; Lapteva et al., 2006b). Recently, it has been shown that anti-NR2

antibody exerted its cytotoxic effect of the neurons by increasing intracellular calcium through inhibition of the binding capacity of zinc (Zn) in the Zn-binding site of the NMDAR which resulted in reduced cell viability (Gono et al., 2011). The potential relationship between serum anti-NR2 antibodies and the fMRI BOLD signals at the hippocampi and amygdala in these lupus patients will be explored in the subsequent study reported in this thesis.

This study has a few limitations. First, the sample size is relatively small. However, sample size of this magnitude has consistently been demonstrating sufficient sensitivity of BOLD signals to achieve statistical significance (Graham et al., 2009; Monchi et al., 2001). Second, patients with SLE were given prednisolone treatment at the time of the fMRI scan, and the possible effects of medication on BOLD signals may confound our interpretation. Since the majority of patients underwent fMRI scan within 2 months of the diagnosis of SLE with the average (±SD) exposure of initial immunosuppressive therapy of 36.86 ± 35.5 days (range: 8-143 days) prior to the scan, the effect of treatment on BOLD signals should be minimal.

Taken together, our findings demonstrated that patients with SLE, even without clinically overt neuropsychiatric symptoms, had abnormal sequential brain activities involving the basal ganglia, hippocampi and amygdala, which indicated a compromised cortico-basal ganglia-thalamic-cortical circuit and hippocampus-amygdala coupling in comparison with healthy subjects. These results translate into potential compromise in response inhibition and the active forgetting-learning dynamics in lupus patients. Moreover, patients with SLE demonstrated over-reliance on the cerebellar-contralateral

frontal conjunction activities to compensate for the dysfunction of the compromised cortico-basal ganglia-thalamo-cortical circuit.

Study 3.2

Serum anti-NR2 alone is not an ideal marker of subclinical working memory and learning deficits assessed based on abnormal functional brain signals in patients with systemic lupus erythematosus

INTRODUCTION

In Study 3.1, we have identified a number of cortical regions, networks and connections that are associated with subclinical neuropsychiatric symptoms in SLE patients. However, as an autoimmune disease, the immune mechanism underlying the abnormal neural activities in these cortical regions and networks of patients with SLE remains unclear. Autoantibodies, being a hallmark of SLE, have been extensively studied for an aim to explain the mechanism of cognitive dysfunction in patients with SLE but the results have been inconclusive (Colasanti et al., 2009; Hanly et al., 2008; Hanly et al., 2011; McLaurin et al., 2005; Seaman et al., 1995). Anti-NR2 antibodies which direct against the NR2A and NR2B subunits of the NMDARs have been more consistently demonstrated to be capable of disturbing learning and memory, in both animal models and human studies (DeGiorgio et al., 2001). While a number of studies revealed significantly higher level of anti-NR2 antibodies in the CSF of lupus patients with neuropsychiatric symptoms (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Yoshio et al., 2006), the association between serum anti-NR2 antibodies and cognitive impairment remained inconsistent (Lauvsnes & Omdal, 2012). Furthermore, the potential use of serum anti-NR2 antibodies to detect subclinical impairment of learning and working memory indicated by abnormal BOLD fMRI signals is not thoroughly investigated.

In Part B of Study 3.1, we have found that when lupus patients were challenged with a high cognitive demand for learning and working memory, there was a complete absence of signals in the amygdala and left hippocampus, in contrast to their healthy counterparts whose signals in these brain regions were unaffected. Since the NMDARs are most densely populated in the hippocampi and amygdala and are involved in mediating learning and working memory, the discordant activities in the hippocampus and amygdala in lupus patients we found on fMRI are postulated to be related to the anti-NR2 antibodies. Additionally, because our preceding Study 3.1 provided objective evidence of brain involvement in subclinical cognitive impairment, the fMRI signals are able to offer a reasonably sensitive reference to examine if serum anti-NR2 antibodies are clinically useful to detect the subclinical cognitive impairment in patients with SLE. Hence, we undertook this study which carries two aims. First, we aimed to confirm if the serum level of anti-NR2 antibodies in patients with SLE is higher than that of healthy individuals and whether it correlates with clinical and serological disease activity of SLE. Second, we attempted to explore the potential clinical applicability of serum anti-NR2 antibodies in detecting subclinical neuropsychiatric manifestation of SLE by studying the relationship between serum anti-NR2 antibodies and the blood-oxygen-level-dependent (BOLD) signals of fMRI at the hippocampi and amygdala in lupus patients without clinically overt neuropsychiatric symptoms.

PATIENTS AND METHODS

Recruitment of study participants and clinical assessment of SLE

Patients who fulfilled the American College of Rheumatology classification criteria for SLE (Hochberg, 1997) were recruited between March 2008 and June 2012 consecutively from the Lupus Clinic of the National University Hospital, Singapore. Healthy subjects matched with lupus patients for age (±4 years) and sex were recruited during the same study period for comparison. The demographic data of the patients and healthy subjects were obtained during clinical interview and medical record review. Disease activity of the patients with SLE was measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) (Yee et al., 2011). Peripheral blood samples were collected via venipuncture of the antecubital veins and the sera were assayed for complement (C3, C4) and anti-dsDNA levels in the standard laboratory of the hospital. Written informed consent was obtained from the subjects recruited for this study and the study was approved by the local ethics committee.

Measurement of anti-NR2 antibody

Please refer to section 2.5.3

fMRI study

During the recruitment period of this study, 14 new onset lupus patients and 14 matched healthy controls were recruited in the preceding Study 3.1-Part B which investigated sequential brain activation during cognitive set-shifting. These 14 patients with new onset SLE had no history of neurological disorder and/or psychiatric disorder, clinically significant anxiety and/or depression and use of psychotropic medications. In addition, they were negative for antiphospholipid antibodies and lupus anticoagulants. The WCST paradigm, imaging acquisition, imaging processing and statistical analysis of

BOLD signal of this study are the same as those listed in the Method section of Study 3.1-Part B.

RESULTS

Demographic and clinical features of patients with SLE and healthy controls

We recruited 64 patients with SLE and 67 healthy controls. The age and gender proportion were comparable between the lupus and healthy control groups with matching. Amongst the lupus patients, the mean $\pm$ SD serum C3, C4 and anti-dsDNA levels were 77.86 $\pm$ 31.0 mg/dl, 17.05 $\pm$ 11.5 mg/dl and 101.46 $\pm$ 85.9 IU respectively. The mean $\pm$ SD SLEDAI was 6.70 $\pm$ 5.7. Among the 64 SLE patients, 6 had history of neuropsychiatric features in which 4 had psychosis, 1 had seizures and 1 had cerebrovascular disease. Also, amongst the 64 lupus patients recruited, 12 of them without a history of NPSLE who participated and were analyzed in the fMRI study had no significant difference in terms of age, gender and the serum levels of C3, C4 and anti-dsDNA and the SLEDAI when compared with those of the rest of the 52 patients with SLE. Table 3.6 summarizes the demographic and clinical information of patients with SLE and healthy subjects. Table 3.7 depicts the comparison of demographic information and clinical features between those patients who had and those did not have fMRI scan performed.

Table 3.6 Demographic and clinical features of study participants

	SLE (n = 64) Mean ± SD (range) [normal range] Number (%)	Control (n = 67)	*P* value
Age, years	40.02 ± 13.8	41.12 ± 13.2	0.585
Female, n	56 (87.5)	58 (86.6)	0.874
C3, mg/dl	77.86 ± 31.0 (16.00 - 201.00) [85 - 185]	—	—
C4, mg/dl	17.05 ± 11.5 (2.00 - 48.00) [10 - 50]	—	—
Anti-dsDNA, IU	101.46 ± 85.9 (2.00 - 250.00) [< 20]	—	—
SLEDAI	6.70 ± 5.7 (0 - 26)	—	—
Disease duration, months	41.84 ± 61.6	—	—
Prednisolone, mg/day	13.47 ± 15.0	—	—
Rash	28 (43.8)	—	—
Discoid rash	3 (4.7)	—	—
Photosensitivity	14 (21.9)	—	—
Oral ulcers	11 (17.2)	—	—
Arthritis	35 (54.7)	—	—
Serositis	5 (7.8)	—	—

Renal disorder	15 (23.4)	—	—
Neurologic disorder*	6 (9.4)	—	—
Haematological disorder	31 (48.4)	—	—
Immunological disorder	57 (89.1)	—	—
ANA positivity	63 (98.4)	—	—

Abbreviation: SLE, systemic lupus erythematosus; SD, standard deviation; anti-dsDNA, anti-double strand DNA; SLEDAI, Systemic Lupus Erythematosus Disease Activity index; ANA, anti-nuclear antibodies.
* Psychosis in four, seizure in one and cerebrovascular disease in one patients respectively

Table 3.7 Comparison of demographic and clinical features and anti-NR2 antibodies between SLE patients with and without fMRI scan

|	SLE with fMRI scan (n = 12)	SLE without fMRI scan (n = 52)	*P* value
	Mean $\pm$SD (range) [normal range] Number (%)		
Age, years	40.25 $\pm$14.3	39.96 $\pm$13.9	0.918
Female, n	10 (83.3)	46 (88.5)	0.631
C3, mg/dl	67.17 $\pm$25.5 (28.00-117.00) [85 - 185]	80.12 $\pm$31.6 (16.00-201.00) [85 - 185]	0.132
C4, mg/dl	12.92 $\pm$9.0 (3.00-34.00) [10 - 50]	17.92 $\pm$11.4 (2.00-48.00) [10 - 50]	0.146
Anti-dsDNA, IU	123.58 $\pm$104.4 (2.00-250.00) [< 20]	96.77 $\pm$80.6 (3.00-250.00) [< 20]	0.535
High anti-NR2	0 (0)	6 (11.5)	0.216
SLEDAI	9.00 $\pm$7.0 (4-25)	6.17 $\pm$5.3 (0-26)	0.221
Prednisolone, mg/day	15.00 $\pm$16.1	13.12 $\pm$14.9	0.396

Abbreviation: SLE, systemic lupus erythematosus; fMRI, functional magnetic resonance imaging; SD, standard deviation; anti-dsDNA, anti-double strand DNA antibodies; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index

Assessment of anti-NR2 antibodies in patients with SLE and healthy controls

SLE patients demonstrated significantly higher levels of serum anti-NR2 antibodies than that of the healthy controls (OD_{450} value: 0.147 $\pm$0.095 vs. 0.106 $\pm$0.080, P = 0.001) (see Figure 3.5). The proportion of patients with high anti-NR2 levels amongst the 12 lupus patients who participated in the fMRI study did not significantly differ from that of the rest 52 patients without undergoing fMRI (see Table 3.8). Additionally, the OD_{450} of anti-NR2 antibodies of these 12 patients ranged from 0.031 to 0.256, implying

that none of these 12 lupus patients who underwent the fMRI study had high anti-NR2 level. When analyzed based on the high/low anti-NR2 cut-off value which is 0.266, six of the 64 (9.4%) of lupus patients were categorized into the high anti-NR2 group but none of them had neuropsychiatric manifestation (see the footnote of Figure 3.5). Conversely, the OD_{450} of anti-NR2 antibodies in 6 lupus patients with history of neurological and/or psychiatric disorders ranged from 0.0765 to 0.265, indicating their serum anti-NR2 antibody levels were low.

Figure 3.5 Measurement of serum anti-NR2 antibodies in SLE patients and healthy controls
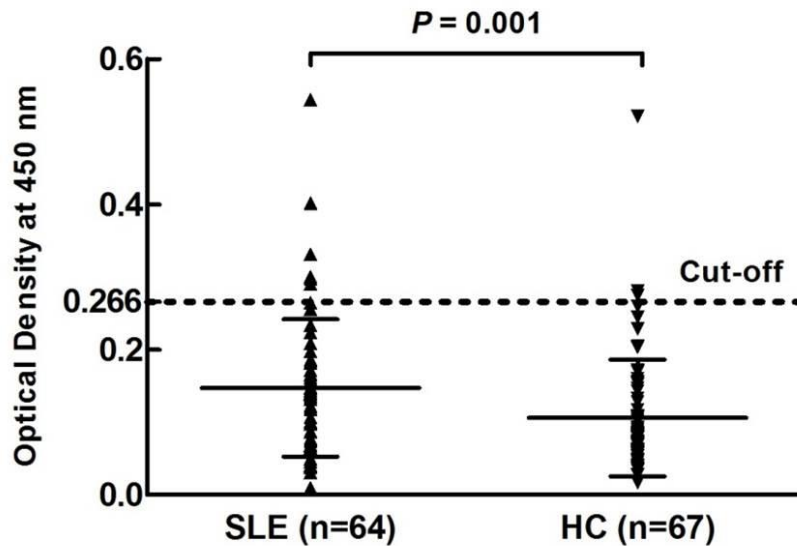
Footnote: Amongst the 6 out of the 64 SLE patients with high anti-NR2 level: 1 had discoid rash, 1 had photosensitivity, 1 had oral ulcers, 5 had arthritis, 1 had serositis, 2 had nephritis and 2 had haematological manifestations. All of the 6 high anti-NR2 patients were positive for ANA but none of them had NPSLE.

The high and low anti-NR2 groups in the SLE patients did not significantly differ in terms of mean age, gender proportion, serum C3 and anti-dsDNA levels and the SLEDAI, although the serum level of C4 was higher in the high anti-NR2 group (P = 0.003). No significant correlation was found between the serum anti-NR2 antibodies and serum anti-dsDNA antibodies, C3 and C4, and the SLEDAI when serum anti-NR2 antibody titer was assessed as a continuous variable. The comparison of clinical features between patients of the high and low serum anti-NR2 groups is shown in Table 3.8.

Table 3.8 Comparison of clinical characteristics between high and low anti-NR2 group and correlation between anti-NR2 antibodies and clinical parameters in SLE patients

	SLE (n = 64) Mean ±SD (range) [normal range] Number (%)		*P* value
	High anti-NR2 (n = 6)	Low anti-NR2 (n = 58)	
Age, years	46.67 ±17.4	39.33 ±13.4	0.336
Female	6 (100)	50 (86.2)	0.597
C3, mg/dl	94.17 ±13.6 (82.00-115.00) [85 - 185]	76.14 ±31.9 (16.00-201.00) [85 - 185]	0.073
C4, mg/dl	30.33 ±10.2 (21.00-48.00) [10 - 50]	15.57 ±5.7 (2.00-48.00) [10 - 50]	0.003
Anti-dsDNA, IU	44.83 ±33.8 (8.00-101.00) [< 20]	107.42 ±87.7 (2.00-250.00) [< 20]	0.175
SLEDAI	3.67 ±3.9 (0-10)	7.02 ±5.8 (0-26)	0.147
Disease duration, months	98.33 ±141.0	36.00 ±45.6	0.282

| Prednisolone, mg/day	5.83 ± 2.64	14.26 ± 15.5	0.464
Anti-NR2 versus	Spearman's correlation		
C3	- 0.107		0.406
C4	0.077		0.557
Anti-dsDNA	- 0.005		0.969
SLEDAI	- 0.054		0.676
Prednisolone	-0.062		0.625
Beta score (R Hippocampus)	0.294		0.354

Abbreviation: SLE, systemic lupus erythematosus; SD, standard deviation; anti-dsDNA, anti-double strand DNA; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; R, right.

WCST performance, abnormal neuronal activities at the hippocampus and amygdala and their association with anti-NR2 antibodies

The 12 SLE patients and 14 healthy controls recruited for the Study 3.1 showed no significant differences in the number of rules identified (17.83 vs. 19.50, p = 0.462), number of error per shift (3.23 vs. 2.51, p = 0.820) and reaction time (1726.04 vs. 1808.51, p = 0.705) regarding the performance of WCST. However, consistent with our findings in Part B of Study 3.1, only the right hippocampus was involved in the specific cognitive stage when learning and memory were most demanded during the WCST in the 12 SLE patients who involved in the fMRI study (see Figure 3.6), whereas both hippocampi and the left amygdala were involved during the same cognitive stage in the healthy counterparts. These findings signified that the impairment of working memory and learning was subclinical in our SLE patients. However, no significant relationship

was found between the level of serum anti-NR2 antibodies and the beta score of neuronal

activities at the right hippocampus in these 12 patients (Spearman's ρ=0.294, P = 0.354).

Figure 3.6 Discordant hippocampal and amygdala activities in 12 non-NPSLE patients

Cortical areas involved in learning are highlighted in blue
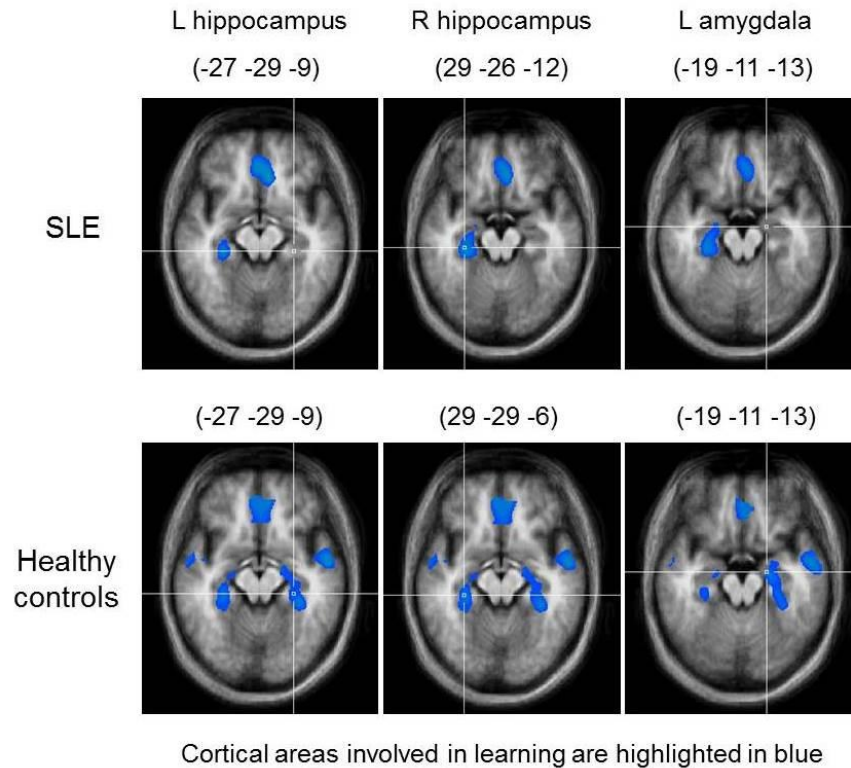
DISCUSSION

While it was found that the serum levels of anti-NR2 antibodies in patients with

SLE were significantly higher than that of the age- and gender-matched healthy controls,

we found no significant correlation between serum anti-NR2 antibodies and both clinical

and serological markers of lupus disease activity. In addition, patients with clinically

overt neuropsychiatric manifestation did not have high anti-NR2 antibody levels.

Although no significant correlation was found between serum C4 and anti-NR2

antibodies, serum C4 level was higher in the high anti-NR2 group than that of the low

anti-NR2 group. Mechanistically, unlike lupus nephritis where glomerular immune complex deposition and complement-dependent cytotoxicity are the main pathophysiological processes (Nangaku & Couser, 2005), anti-NR2 antibodies do not induce neuronal injury via immune complex formation because their Fab^1_2 fragments *per se* are capable of inducing neuronal death without the need for the Fc portion of the antibodies to activate the complement cascade (Diamond & Volpe, 2012). These findings strengthen the observations that the presence of anti-NR2 antibodies was unlikely related to the disease activity of SLE and immune-complex mediated neuronal injury (Diamond & Volpe, 2012; Gono et al., 2011; Kozora et al., 2010; Omdal et al., 2005).

When anti-NR2 antibodies from the sera or CSF of patients with SLE were introduced into mice brains, these mice demonstrated neuronal death in the hippocampus or amygdala and subsequently memory deficit and emotion disturbance caused by anti-NR2 antibodies (DeGiorgio et al., 2001; Huerta et al., 2006; Kowal et al., 2004). Since the NMDARs are highly expressed in the hippocampi and amygdala and are critical in learning and processing memory, it prompted us to examine whether serum anti-NR2 antibody levels were altered in lupus patients who demonstrated subclinical impairment in memory and learning on fMRI. The 12 lupus patients without NPSLE did not have high serum level of anti-NR2 antibodies and no significant correlation was found between their serum anti-NR2 antibodies and the magnitude of function brain activation at the right hippocampus on fMRI. Although studies addressing the relationship between serum anti-NR2 antibodies and neuropsychiatric lupus manifestations revealed inconsistent results (Gono et al., 2011; Hanly et al., 2006; Hanly et al., 2008; Hanly et al., 2011; Hanly et al., 2012; Harrison et al., 2006; Husebye et al., 2005; Kozora et al., 2010;

Lapteva et al., 2006a; Omdal et al., 2005; Petri et al., 2010; Steup-Beekman et al., 2007), all as-yet published studies consistently demonstrated a significant association between anti-NR2 antibodies in the CSF and NPSLE (Arinuma et al., 2008; Fragoso-Loyo et al., 2008; Yoshio et al., 2006). These data suggest that anti-NR2 antibodies in the CSF rather than those in the sera are more reliable in detecting neuropsychiatric syndromes in lupus patients.

In murine models, mice with intact BBB showed no detectable brain pathology and they performed normally in behavioral and cognitive tasks even if the anti-NR2 antibodies were present in the peripheral circulation (Wang et al., 2003). However, neuronal death at the hippocampi or amygdala and the resultant impairment of hippocampus-dependent memory and amygdala-related fear-conditioning were observed when anti-NR2 antibodies from lupus patients were introduced into the mice after a breach of the BBB by using lipopolysaccharide or epinephrine (Huerta et al., 2006; Kowal et al., 2006; Kowal et al., 2004). Given the evidence that the level of anti-NR2 antibodies in the CSF is positively correlated with that in the serum of lupus patients and the BBB of lupus patients is potentially leaking as a result of active disease and glucocorticoid treatment (Appenzeller et al., 2005; Yoshio et al., 2006), the serum anti-NR2 antibodies of our lupus patients might have penetrated the BBB and disturbed the neural mechanisms of working memory and learning as shown by the abnormal fMRI signals despite the potentially low level of the antibodies in the CSF. This phenomenon is not surprising because anti-NR2 antibodies at a very low concentration in the CSF (10 μg/ml) could induce transient alterations of synaptic potentials (Faust et al., 2010). Thus, the integrity of BBB appears to be a crucial factor in the pathogenesis of NPSLE.

Importantly, anti-NR2 antibodies preferentially bind to NMDARs during their activated state whereby the cognitive demand for learning and memory is high (Faust et al., 2010). In our 12 patients who underwent fMRI, neuroplasticity alterations of the hippocampi and amygdala were demonstrated while the cognitive demand for learning and memory reached the maximum during the WCST, which was also the moment when the NMDARs reached the activated state and invited binding with anti-NR2.

This study has a few limitations. First, fMRI scan was not performed in all of our lupus patients due to funding constraint and patients' reluctance owing to commitment of the long scanning time. Nevertheless, the 12 lupus patients who underwent fMRI had comparable demographic and clinical characteristics when compared to the rest of the SLE patients of this study. Second, because our aim is to test the clinical applicability of serum anti-NR2 for detecting subclinical NPSLE, CSF was not obtained in our patients for analysis. Thus, correlation between CSF and serum anti-NR2 levels could not be determined in our study. Third, as described previously in the conclusions of Study 3.1, medications such as glucocorticoids might affect BOLD fMRI signals despite the fact that it was practically challenging to match for disease activity and glucocorticoid doses in different groups.

In summary, while we found significantly higher serum levels of anti-NR2 antibodies in lupus patients in comparison with age- and sex-matched healthy controls, the presence of serum anti-NR2 antibodies in patients with SLE was unrelated to the clinical and serological disease activity of SLE. In addition, serum anti-NR2 antibodies are not associated with subclinical neuronal disturbance at the hippocampus and amygdala assessed based on fMRI. Taken together with the results of other studies

(Fragoso-Loyo et al., 2008; Hanly et al., 2006; Hanly et al., 2008; Hanly et al., 2011; Hanly et al., 2012; Harrison et al., 2006; Husebye et al., 2005; Kozora et al., 2010; Petri et al., 2010; Steup-Beekman et al., 2007), we conclude that serum anti-NR2 level alone is not an optimal biomarker to detect subclinical neuropsychiatric SLE. Since obtaining CSF in lupus patients without neuropsychiatric manifestations is hindered by practical and ethical issues, further studies assessing the clinical applicability of the combination of serum anti-NR2 level and non-invasive assessment of the integrity of BBB opens an attractive research direction which aims to detect and monitor subclinical neuropsychiatric manifestations in patients with SLE.

CHAPTER 4

CONCLUSIONS

4.1 Summary of the aims of this thesis

Although cognitive dysfunction has been identified as one of the most prevalent neuropsychiatric manifestations of SLE (Lahita, 2011) which carries a negative impact on the health-related quality of life and vocational as well as societal aspects in patients with SLE (Kiani & Petri, 2010; Panopalis et al., 2007; Williams et al., 2011), the underlying neuropathophysiology remains elusive. The aims of this thesis are to unravel the anatomical and functional brain involvement in subclinical cognitive dysfunction by employing BOLD fMRI and explore the potential role of serum anti-NR2 antibodies in subclinical cognitive dysfunction in patients with SLE. The rationale of using fMRI signals as surrogates to gauge subclinical cognitive dysfunction is because this modality of investigation has been consistently shown to be a sensitive tool to detect subclinical brain involvement, both anatomically and functionally. It is hoped that through the series of studies reported in this thesis, researchers and clinicians can further understand the underlying pathophysiology of cognitive dysfunction in SLE so that more focused and pragmatic research can be planned and implemented in order to target certain anatomical regions and functional pathways of the brain in SLE.

4.2 Summary of the results

It was shown in Part A of the first study that while patients with new-onset SLE demonstrated comparable WCST performance as their matched healthy counterparts,

deficits in executive function was revealed in these lupus patients as indicated by their inferior strategic planning skills revealed by the BOLD fMRI signals. As a result, additional cortical regions which are involved in the execution of goal-directed tasks were recruited in these lupus patients in order to compensate for their inferior strategic planning skills. Surprisingly, their inefficient strategic planning skills and the subsequent compensatory mechanism which boosted error detection and conflict processing persisted even after adequate control of their lupus disease activity. These observations may be able to explain why cognitive function may continue to decline even after adequate control of lupus disease activity.

With an aim to map potential dysfunctional neural pathways which mediate subclinical cognitive dysfunction in patients with SLE, the brain activities across different stages of cognitive set-shifting processes during the performance of the WCST in the new-onset SLE patients were explored by fMRI in Part B of the first study. During the condition when the cognitive demand for withholding previous prepotent rule and response inhibition was dominating, the cortico-basal ganglia-thalamic-cortical circuit was demonstrated to be involved in healthy individuals. On the contrary, the right GP and right thalamus within this circuit were dysfunctional in these lupus patients even if they had no clinically overt neuropsychiatric symptoms. To compensate for this compromised neural circuit, the contralateral cerebellum frontal connection was recruited in order to maintain comparable WCST performance as healthy individuals. During the condition when the subjects were actively forgetting the prevailing rule and attempting to maintain the identified rule, the healthy controls showed decreased activation at both the hippocampi and left amygdala. However, the involvement of the left hippocampus and

left amygdala in the same condition was absent in lupus patients, implying that the active forgetting-learning dynamics in lupus patients was disrupted. As for the potential implication in clinical practice, our findings suggest that physicians taking care of lupus patients may need to be aware of their potential reluctance to change their existing knowledge and adapt to new ones, such as in situations where pharmacological treatment and lifestyle need to be modified for optimizing disease control and preventing organ damage.

In the second study, we found that the serum levels of anti-NR2 antibodies were significantly higher in patients with SLE than that of the healthy individuals. No correlation was revealed between serum anti-NR2 antibodies and clinical and serological lupus disease activity. Furthermore, no significant association was demonstrated between serum anti-NR2 antibodies and subclinical neuronal disturbance at the hippocampus and amygdala in SLE patients without clinically overt neuropsychiatric symptoms as revealed by the BOLD fMRI signals. These findings appear to suggest that serum anti-NR2 level alone is not an optimal marker for subclinical neuropsychiatric manifestation of SLE.

4.3 Ethical consideration

All these three studies have obtained approval from the institutional ethics committee before they were carried out. Ethical issues that were commonly raised in biomedical studies, especially in studies involving fMRI, were taken into account in our studies.

Firstly, our fMRI studies were all conducted in adults with clinically normal cognitive function. Other individuals involved in Study 3.2 were adults as well. Thus, all

the studies presented here did not involve any vulnerable personnel, such as pregnant women or children. Secondly, written informed consent was sought from all participants in our studies. Thirdly, the fMRI scans were carried out using standard procedure in the Functional Imaging Center in NUH by certificated technicians. Lastly, the confidentiality and privacy of all study participants were carefully secured and all the results would be exclusively used in the research settings.

4.4 Future work

The dysfunctional cortico-basal ganglia-thalamic-cortical circuit and abnormal hippocampal-amygdala activities have been hitherto demonstrated in our new-onset non-neuropsychiatric SLE patients. Evaluation of aforementioned neural circuits in the SLE patients after sufficient disease control might be helpful to give further insight into the pathogenesis of cognitive dysfunction in SLE patients. Furthermore, it might be helpful to identify the cortical regions and neural circuits of which the abnormal activities are capable of predicting cognitive dysfunction in SLE patients by prospectively following up the SLE patients involved in the fMRI study, assessing their cognitive function and stratifying them based on their cognitive function condition.

Search for novel and specific mediators and antibodies against these neural pathways using rodent models would further enrich our knowledge of biological factors related to lupus cognitive impairment. In addition, our findings suggest that the serum titre of anti-NR2 antibodies alone is not a sensitive marker to detect subclinical cognitive dysfunction in patients with SLE although other studies have shown that its CSF equivalent is associated with neuropsychiatric manifestations in lupus patients. Given the

important role of the integrity of BBB in the pathogenesis of lupus cognitive dysfunction induced by anti-NR2 antibodies, further studies addressing the clinical applicability of the combination of serum anti-NR2 level and non-invasive assessment of the integrity of BBB is a relevant strategy to detect and monitor the subclinical neuropsychiatric symptoms in patients with SLE.

4.5 Overall summary of this thesis

In summary, patients with new-onset SLE without clinically overt neuropsychiatric symptoms demonstrated inferior strategic planning skills which required compensatory recruitment of additional cortical regions during the execution of goal-directed task in order to maintain normal executive function at the time of diagnosis and after sufficient disease control. Additionally, SLE patients demonstrated dysfunctional neural circuits and connections that contribute to response inhibition and active forgetting-learning dynamics during cognitive set-shifting. Furthermore, SLE patients were demonstrated to have significantly higher level of serum anti-NR2 antibodies although the level of the antibodies did not correlate with clinical neuropsychiatric symptoms and were not associated with abnormal neural activities in the hippocampus and amygdala as demonstrated by the BOLD fMRI signals in those regions. These findings collectively suggest that anti-NR2 alone was not an optimal marker for clinical and subclinical neuropsychiatric manifestations in patients with SLE.

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APPENDIX 1

Permission to use Figure 1.1

Ren Tao

From:	gtsokos@bidmc.harvard.edu
Sent:	Thursday, 6 September, 2012 4:35 PM
To:	Tao Ren
Subject:	Re: Inquiry to use one figure from your NEJM paper in my PhD thesis

There is no problem with me
Check also with the journal
Gct

On Sep 5, 2012, at 10:42 PM, "Tao Ren" <g0900145@nus.edu.sg> wrote:

> Dear Dr Tsokos,
>
> I am a PhD student at Yong Loo Lin School of medicine at National University of Singapore. I am preparing my PhD thesis which focuses on functional brain imaging of cognitive dysfunction and evaluation of anti-NR2 antibodies in patients with SLE. May I ask for permission to use the Figure 1 Overview of the pathogenesis of systemic lupus erythematosus from Systemic lupus erythematosus, N Engl J Med. 2011 Dec 1;365(22):2110-21 in the introduction of my PhD thesis? Thanks in advance!
>
> Best regards,
>
> Ren Tao
>
> **Ren Tao (Mr)** :: PhD student:: Department of Medicine, Yong Loo Ling School of Medicine :: National University of Singapore:: www.nus.edu.sg (W) :: *Company Registration No: 200604346E*
> Important: This email is confidential and may be privileged. If you are not the intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

APPENDIX 2

Permission to use Figure 1.4

Ren Tao

From:	Stuart Clare <stuart.clare@ndcn.ox.ac.uk>
Sent:	Monday, 17 September, 2012 5:27 PM
To:	Ren Tao
Subject:	Re: Inquiry for permission to use one figure from your PhD thesis in my PhD thesis

Dear Mr Tao,
Thanks for asking permission to use the figures. I am happy to grant you this permission.
Best wishes,
Stuart Clare

On 9 Sep 2012, at 10:35, Ren Tao wrote:

Dear Dr Clare,
I am a PhD student at Yong Loo Lin School of medicine at National University of Singapore. I am preparing my PhD thesis which focuses on functional brain imaging of cognitive dysfunction and evaluation of anti-NR2 antibodies in patients with SLE. May I ask for permission to use the Figure 3.17 Upon activation, oxygen is extracted by the cells, thereby increasing the level of deoxyhaemoglobin in the blood. This is compensated for by an increase in blood flow in the vicinity of the active cells, leading to a net increase in oxyhaemoglobin from the third chapter of your thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy October 1997 in the introduction part of my PhD thesis? I get the access to the full text of your PhD thesis at http://users.fmrib.ox.ac.uk/~stuart/thesis/. Thanks in advance!

Best regards,

Ren Tao

Ren Tao (Mr) :: PhD student:: Department of Medicine, Yong Loo Ling School of Medicine :: National University of Singapore:: www.nus.edu.sg (W) :: *Company Registration No: 200604346E*
Important: This email is confidential and may be privileged. If you are not the intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

Please use my new email address: stuart.clare@ndcn.ox.ac.uk

--
Stuart Clare
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APPENDIX 3

Permission to use Figure 1.5

Ren Tao

From:	Tsien, Joe <JTSIEN@georgiahealth.edu>
Sent:	Thursday, 6 September, 2012 8:40 PM
To:	Tao Ren
Subject:	RE: Inquiry to use one figure from your NEJM paper

Dear Mr. Tao:

Yes, you are very welcome to use it. Good luck with your defense.

Best,

Joe
Joe Z. Tsien
Co-Director, Brain and Behavior Discovery Institute
Georgia Research Alliance Eminent Scholar in Cognitive and Systems Neurobiology
Professor of Neurology, Medical College of Georgia
Georgia Health Sciences University
Augusta, GA 30912
Telephone: 706-721-3760
Fax: 706-721-3829
Email: jtsien@gerogiahealth.edu

From: Tao Ren [mailto:g0900145@nus.edu.sg]
Sent: Wednesday, September 05, 2012 10:23 PM
To: Tsien, Joe
Subject: Inquiry to use one figure from your NEJM paper

Dear Dr Tsien,

I am a PhD student at Yong Loo Lin School of medicine at National University of Singapore. I am preparing my PhD thesis which focuses on functional brain imaging of cognitive dysfunction and evaluation of anti-NR2 antibodies in patients with SLE. May I ask for permission to use the Figure 1 Neuron Showing Glutamate Receptors and Synaptic Plasticity from Memory and the NMDA Receptors, N Engl J Med 2009; 361:302-303 in the introduction of my PhD thesis? Thanks in advance!

Best regards,

Ren Tao

Ren Tao (Mr) :: PhD student:: Department of Medicine, Yong Loo Ling School of Medicine :: National University of Singapore:: www.nus.edu.sg (W) :: *Company Registration No: 200604346E*
Important: This email is confidential and may be privileged. If you are not the intended recipient, please delete it and notify us immediately; you should not copy or use it for any purpose, nor disclose its contents to any other person. Thank you.

APPENDIX 4

Hospital Anxiety and Depression Scale

Patients are asked to choose one response from the four given for each interview. They should give an immediate response and be dissuaded from thinking too long about their answers. The questions relating to anxiety are marked "A", and to depression "D". The score for each answer is given in the right column. Instruct the patient to answer how it currently describes their feelings.

A	**I feel tense or 'wound up':**	
	Most of the time	3
	A lot of the time	2
	From time to time, occasionally	1
	Not at all	0

D	**I still enjoy the things I used to enjoy:**	
	Definitely as much	0
	Not quite so much	1
	Only a little	2
	Hardly at all	3

A	**I get a sort of frightened feeling as if something awful is about to happen:**	
	Very definitely and quite badly	3

	Yes, but not too badly	2
	A little, but it doesn't worry me	1
	Not at all	0

D	**I can laugh and see the funny side of things:**	
	As much as I always could	0
	Not quite so much now	1
	Definitely not so much now	2
	Not at all	3

A	**Worrying thoughts go through my mind:**	
	A great deal of the time	3
	A lot of the time	2
	From time to time, but not too often	1
	Only occasionally	0

D	**I feel cheerful:**	
	Not at all	3
	Not often	2
	Sometimes	1
	Most of the time	0

A	**I can sit at ease and feel relaxed:**	
	Definitely	0

	Usually	1
	Not Often	2
	Not at all	3

D	**I feel as if I am slowed down:**	
	Nearly all the time	3
	Very often	2
	Sometimes	1
	Not at all	0

A	**I get a sort of frightened feeling like 'butterflies' in the stomach:**	
	Not at all	0
	Occasionally	1
	Quite Often	2
	Very Often	3

D	**I have lost interest in my appearance:**	
	Definitely	3
	I don't take as much care as should	2
	I may not take quite as much care	1
	I take just as much care as ever	0

A	**I feel restless as I have to be on the move:**	
	Very much indeed	3
	Quite a lot	2
	Not very much	1
	Not at all	0

D	**I look forward with enjoyment to things:**	
	As much as I ever did	0
	Rather less than I used to	1
	Definitely less than I used to	2
	Hardly at all	3

A	**I get sudden feelings of panic:**	
	Very often indeed	3
	Quite often	2
	Not very often	1
	Not at all	0

D	**I can enjoy a good book or radio or TV program:**	
	Often	0
	Sometimes	1
	Not often	2
	Very seldom	3

Scoring (add the As = Anxiety. Add the Ds = Depression).

The norms below will give you an idea of the level of anxiety and depression.

0-7 = Normal

8-10 = Borderline abnormal

11-21 = Abnormal

Adopted from:

Zigmond A.S. and Snaith R.P. (1983). The hospital anxiety and depression scale. Acta Psychiatrica Scandinavica *67*, 361-370.

APPENDIX 5

Wechsler Abbreviated Scale of Intelligence Record Form

APPENDIX 6

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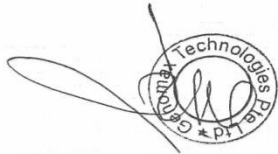
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