Neuronal NR4A1 and complement coordinate synaptic stripping by microglia in lupus

Han Xiaojuan

Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School

<https://orcid.org/0000-0002-2455-0424>

Xu Tianshu

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Fang Qijun

Nanjing Drum Tower Hospital, the Affiliated Hospital of Nanjing University Medical School

Dandan Wang

the Affiliated Drum Tower Hospital of Nanjing University Medical School

Genhong Yao

Nanjing University

Gang Hu

Nanjing Medical University

lingyun Sun (✉ lingyunsun@nju.edu.cn)

Department of Rheumatology and Immunology, Affiliated Drum Tower Hospital of Nanjing University Medical School

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Abstract

Up to 75% of systemic lupus erythematosus (SLE) patients experience neuropsychiatric (NP) symptoms, called neuropsychiatric SLE (NPSLE), yet the underlying mechanisms remain elusive. Here we showed that complement-coordinated elimination of synapses participated in NPSLE in MRL/lpr mice, a lupus-prone murine model. We demonstrated that lupus mice developed increased anxiety-like behaviors and persistent phagocytic microglia reactivation before overt peripheral lupus pathology. In lupus brain, C1q was increased and localized at synaptic terminals, causing the apposition of phagocytic microglia, ensuing synaptic loss, and neurological disease. We further determined that neuronal *Nr4a1* signaling was essential for attracting C1q synaptic deposition and then apposition of phagocytic microglia, resulting in synaptic loss and neurological disease. Minocycline-deactivated microglia, antibody-blocked C1q, or neuronal *Nr4a1* restored protected lupus mice from synapse loss and NP manifestations. Our findings revealed an active role of neurons in coordinating microglia-mediated synaptic loss and highlight neuronal *Nr4a1* and C1q as critical components amenable to pharmacological intervention in NPSLE.

Introduction

Systemic lupus erythematosus (SLE) is an incurable autoimmune disease characterized by abnormal immune responses that attack normal tissues such as kidney, skin, lungs, and brain. Neuropsychiatric (NP) manifestations occur in 40–90% of patients¹, with symptoms ranging from anxiety, depression, and cognitive impairment to psychosis, which are collectively referred to as neuropsychiatric lupus (NPSLE) or central nervous system (CNS) lupus, and remain a major cause of mortality^{2,3} in the SLE population. In many cases, certain autoantibodies, such as anti-NMDAR antibodies (antibodies that bind both DNA and the N-methyl-d-aspartate receptor)^{4,5} or anti-phospholipid antibodies^{6,7}, have been reported as mediators of NPSLE. However, only when a breach occurs in blood–brain barrier (BBB) integrity, mostly due to secondary vasculitis or neuroinflammation, can these antibodies traverse the BBB, enter the hippocampus and other parts of brain⁴, and cause excitotoxic neuronal death⁸. However, BBB compromise does not occur in early SLE cases, and even when barrier integrity is broken, some individuals do not exhibit brain pathology. Moreover, many studies have indicated that the presence of these antibodies in serum is not associated with active diffuse NPSLE^{9,10}, and removal of B cells and antibodies by chimeric models could not alleviate brain damage in lupus mice¹¹, suggesting that these autoantibodies may not be the primary mediators and are not directly related to brain disease in lupus.

Notably, NPSLE is typically present at lupus diagnosis or within the first year in patients^{12,13}, and emotional abnormalities are even detected before gross serological pathology in mice^{14,15}, suggesting that primary CNS factors rather than peripheral autoimmunity contributors are involved. However, the etiology and underlying mechanisms of the early brain injury remain largely undiscovered.

Here we investigate the hypothesis that NP symptoms in SLE can be caused by increased elimination of synaptic terminals. Elimination of synapses has been observed in Alzheimer's disease (AD)¹⁶ and during

aging¹⁷ and is central to the neurodegenerative process. The engulfment of dendritic processes by activated microglia¹⁸, the resident macrophages of the CNS, represents one potential mechanism for this phenomenon. Microglia-mediated synapse pruning is implicated in normal brain development^{19,20} and might be reactivated in the diseased brain²¹. In CNS inflammation, activated microglia can engulf synaptic terminals through complement C4-dependent²² or C3 activation mechanisms²³. More recent evidence has indicated that classical complement cascades are involved or even required in this synapse spine process, especially highlighting C1q's involvement. Genetic or pharmacologic blocking of complement pathway synaptic stripping can delay, prevent, or alleviate multiple age- or inflammation-related disorders^{24,25}.

Despite this mounting evidence that C1q assists microglial synapse stripping^{19,26}, the major source of C1q is still not clear, nor how it cooperates with microglia to orchestrate synaptic pruning. In addition to microglial phagocytosis, neuron-autonomous disruption of synaptic structure and/or activity may contribute to synapse changes in many conditions. For example, hyperexcitatory electrical activity resulted in a decreased number of dendritic spines in neurons in diseased brain²⁷, and overexpression of the major postsynaptic density (PSD) scaffolding protein PSD-95 enhances electrophysiological properties and increases spine size and/or density²⁸. However, no association between neuronal intrinsic disruption and microglia-mediated synaptic loss has been reported. It has been proposed that neurons can actively release paracrine signals to modulate phagocytic activity²⁹. One may thus hypothesize that the neuronal-driven complement coordinates microglia to execute synaptic degeneration, which remains to be tested experimentally. Moreover, the cellular and molecular foundations of neuronal intrinsic disruption in these contexts remain unknown and require further study.

Here, by conducting unbiased quantitative transcriptome analysis of hippocampal fractions in a widely used lupus-prone (MRL/MpJ-Fas^{lpr}) murine model, we identified significant alterations in a variety of pathways in lupus brain before the appearance of overt systemic lesions. Pathway analysis combined with molecular experiments highlighted reactivated microglia-mediated phagocytosis as a pathological mechanism of dendritic spine loss accounting for NPSLE. Strikingly, the extracellular protein complement C1q was markedly increased and majorly localized to synapses in MRL/lpr mice, which was also correlated with NP pathology. We also presented evidence that the increased C1q in the brain was primarily sourced from the circulation rather than secreted by resident nerve cells. We further highlighted that neuronal intrinsic *Nr4a1* defects and dysregulation of the actin cytoskeleton induced the engulfment of synapses. Furthermore, restoring neuronal *Nr4a1* expression preserved synapse removal by microglia and rescued synapse density in MRL/lpr mice. These findings offer novel grounds for therapeutic intervention in SLE-driven neuropsychosis and related disorders.

Results

MRL/lpr mice develop behavioral deficits and microglial reactivation before the appearance of overt peripheral SLE lesions.

To investigate the CNS disease process in lupus, we first monitored brain and systemic disease development between wildtype (WT) control (MRL/mpj) and lupus-prone MRL/MpJ-Fas^{lpr} (hereafter named MRL/lpr) mice (which have an insertion of a retrotransposon in the gene for Fas (CD95) resulting in defective apoptosis of lymphocytes and massive lymphoproliferation³⁰), the best established spontaneous model of SLE and extensively used in lupus-related NP studies³¹ because NP manifestations appear early in these mice³⁰.

Consistent with previous reports¹⁴, we found that serum anti-dsDNA antibody (Ab, the typical serologic indicator of lupus) was evident by 8 weeks in MRL/lpr female mice and continued to increase over time, with lupus nephritis onset at 18-20 weeks (Supplementary Fig. 1a). At 6 weeks of age, anti-dsDNA Ab titers were similar in MRL/lpr and congenic WT mice (Supplementary Fig. 1b), suggesting that no SLE serologic lesion is present in MRL/lpr mice at 6 weeks of age and that the disease onset time is approximately 8 weeks of age, as reported¹⁴.

To evaluate neuropathology in lupus, we then carried out a battery of behavioral tests on 16-week-old WT and MRL/lpr mice, the time point when mice began developing mild lupus nephritis (evidenced by increased proteinuria, although the difference was not statistically significant, (Supplementary Fig. 1a). We found that the lupus mice showed anxiety-like phenotypes in the open field test (OFT), a widely used indicator of anxiety-like behavior by evaluating the tendency of mice to remain close to the walls and avoid open spaces (central zone)³², and elevated plus maze (EPM) test, which is based on the animals' natural fear of heights and open spaces, as an additional measurement of anxiety-like behavior (Fig. 1b,c,d and Fig. 1e,f,g), cognitive defects indicated by performance in the novelty Y maze (Supplementary Fig. 1c,d), and increased depression-like phenotypes in the tail suspension test (TST) and forced swim test (FST) (Supplementary Fig. 1e,f). No motor or coordination defects were found in MRL/lpr mice (Supplementary Fig. 1g,h,i). **These findings suggest that MRL/lpr mice develop CNS disease and are suitable for NPSLE studies.**

In most patients, CNS lupus typically presents at an early phase during SLE^{2,12}, suggesting that NP manifestations may be driven primarily by brain-intrinsic factors rather than as a complication of systemic-autoimmune-activation. To determine whether the brain injury is a preexisting systemic pathology, we further examined the changed behavioral phenotypes observed in 16-week-old lupus mice as early as 6 weeks of age when mice had not yet developed peripheral pathology (Supplementary Fig. 1a,b). As shown in the OFT, 6-week-old MRL/lpr mice were less inclined to explore the central area of the OFT chamber than the peripheral zone (Fig. 1b,c), and likewise, the distance in the center (%) was significantly decreased compared with age-paired control mice (Fig. 1b,d). In the EPM test, MRL/lpr mice showed decreased entries (Fig. 1e,f) and time (Fig. 1e,g) in the open arms of the EPM compared with WT

animals. MRL/lpr mice also showed an increased immobility time in the TST but not FST at 6 weeks of age (Supplementary Fig. 1e,f) and no change in cognitive performance in the novelty Y maze (Supplementary Fig. 1c,d). **These results indicate that the MRL/lpr mice develop distinct anxiety- and depression- like behaviors that predate peripheral lesions at 6 weeks of age, in contrast to the WT control strain.**

Furthermore, a two-way repeated measures (RM) analysis of variance (ANOVA) (age by genotype) analyzing the distance in center revealed a main effect of genotype ($F(1,43) = 12.28$; $P = 0.0011$, $P < 0.01$) and no main effect of age ($F(1, 43) = 2.026$; $P = 0.1618$, $P > 0.05$) in the OFT (Fig. 1d). Two-way RM ANOVA analyzing head in the open arm revealed a major effect of genotype ($F(1,43) = 20.05$; $P < 0.0001$, $P < 0.01$) and a reduced effect of age ($F(1,43) = 3.895$; $P = 0.0549$, $P > 0.05$) in the EPM test (Fig. 1f). **Together, these results suggest that anxiety- and depression- like behaviors are major NP changes that occur early in lupus mice, and anxiety-like behaviors are more sensitive. We used this phenomenon as the indicator of NP manifestation in subsequent studies.**

Increased anti-NMDAR antibody-mediated excitotoxicity can induce neuronal apoptosis in mice with severe lupus lesions^{27,33}. TUNEL and Nissl's staining did not detect ongoing apoptosis before 12 weeks of age (Supplementary Fig. 2a) or loss of Nissl's⁺ neurons within the circuitry of the prefrontal cortex and hippocampus that mediate emotion and learning in MRL/lpr mice (Supplementary Fig. 2b). These findings suggest that apoptotic neuronal death is unlikely the cause of early behavioral abnormalities in SLE. Although peripheral inflammatory cells have also been reported as participants³⁴, notably, the MRL/lpr strain showed no cellular infiltration in the brain (Supplementary Fig. 2c), suggesting that rather than infiltrating immune cells, CNS-resident nonneuronal cells affect the NPSLE process at this early stage.

Microglia, resident macrophages in brain, respond to local inflammation or CNS damage by becoming reactive, increasing phagocytic activity and inflammatory cytokine production³⁵. To determine whether microglial activation participates in NPSLE development, we assessed the active state of microglia via cell morphology and density in MRL/lpr mouse brains during the early (6-8 weeks) and active (16-20 weeks) stages of SLE. As noted, in addition to increased IBA-1⁺ cell numbers (Fig. 1i), reactive microglia in MRL/lpr displayed ameboid morphology, as evidenced by increased cellular soma and decreased branching complexity of cytoplasmic processes (Fig. 1h and Supplementary Fig. 1m,o). Additionally, the coimmunostaining of IBA-1 with CD68, a lysosomal-localized indicator of microglial phagocytic activity³⁶, confirmed the reactivation of microglia (Fig. 1i and Supplementary Fig. 1m). We found significantly higher frequencies of reactive microglia in the cortex and hippocampus in MRL/lpr mice at 6 weeks compared with WT mice, which were further exacerbated at 16 weeks (Fig. 1h,i,j and Supplementary Fig. 1n), with similar microglial density in the cerebellum and midbrain (Fig. 1j)

Moreover, GFAP⁺ astrocytes within the hippocampus displayed no change across strains (Supplementary Fig. 2d), indicating microglia as major players. We also noticed an increase in IBA-1⁺ intensity in the prefrontal cortex of pristane-induced lupus mice (Supplementary Fig. 1p), as well as increased anxiety-

like performance in the OFT, as assessed by distance in the center (Supplementary Fig. 1q). These results were similar to those observed in MRL/lpr lupus-prone mice. Furthermore, relative to the observed rapid and progressive increase in IBA-1⁺ microglia intensity in the hippocampus beginning from the 6th postnatal week in MRL-lpr mice compared with age-paired WT mice (Fig. 1k,l), complement C3 deposition in the kidney of MRL-lpr mice was significantly increased at 16 weeks compared with age-paired WT mice (Fig. 1k,m).

Thus, the above data together indicate that NP abnormalities occur in the early stage of SLE, prior to peripheral pathology, and are closely related with the specific activation of microglia in brain.

Transcriptional profile reveals molecular changes in the hippocampus of MRL/lpr mice.

To determine the changes in the brain as reactive microglia and NP manifestations develop, we performed whole-transcriptome gene expression analysis of the hippocampus from MRL/lpr and WT mice at 6 weeks, when reactive microglia were detected (Fig. 1a, schematic).

The transcriptome microarray enabled the detection of the differential expression of 1,908 transcripts (cutoff of 1.5-fold change; $P < 0.05$; Fig. 2a and Supplementary Fig. 3a) and further filtering of 1,019 transcripts (873 upregulated, 146 downregulated in MRL/lpr; adjusted $P < 0.05$).

In addition, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis revealed signatures of cytokine-cytokine receptor interaction, antigen processing and presentation, activation of innate immune responses, and microglial proteins involved in ligand sensing and protein digestion, as well as of the complement and coagulation cascades. In detail, MRL/lpr mice showed enrichment of immune-activated pathways, including classes of microglial-mediated phagocytosis (KEGG terms 'Phagosome' and 'Protein digestion and absorption', Fig. 2b and Supplementary Fig. 3b) and the classical complement pathway (KEGG term 'the complement and coagulation cascades', Fig. 2c). In the above category, we identified genes associated with microglial-mediated phagocytosis (*Cx3cr1*, which encodes CX3CR1; *Fcgr2b*, which encodes FCGR2B; *Thbs1*, which encodes Thrombospondin1; *Itga2*, which encodes Integrin $\alpha 2$; *Tlr2*, which encodes TLR2; and *Tap1*, which encodes TAP1) and the classical complement pathway (*C1qa*, which encodes C1QA; *C6*, which encodes C6; *C1ra*, which encodes C1R; and *Cfp*, which encodes properdin), which were validated using quantitative PCR (qPCR) (Fig. 2d,e and Supplementary Fig. 3d). The microglial reactive phagocytosis was further confirmed by a dramatic induction of the immunofluorescence signal for CD68 in IBA-1⁺ cells in MRL/lpr hippocampus (Fig. 1i and Supplementary Fig. 1m). Although complement components contribute to peripheral tissue damage in SLE³⁷, their expression and function within the brain during lupus have not been well investigated. In murine CNS development and many disorders, C1q and C3 are reported to increase and are required for synaptic pruning³⁸. Immunoblotting confirmed the upregulation of C1q but not C3 protein in both the cortex and hippocampus of MRL/lpr mice (Fig. 2f and Supplementary Fig. 3e). Conversely, MRL/lpr mice expressed lower percentages of genes that regulate neuroactive ligand-receptor interaction, the calcium signaling

pathway, long-term depression, axon guidance, and synapse maintenance (Supplementary Fig. 3c and Supplementary information, Table S1), suggesting that synaptic dysfunction may also be involved.

Collectively, these data suggest a link between NP manifestations and increased microglial phagocytosis, as well as complement machinery, in the brain during SLE progression.

The aforementioned specific increase in reactive microglia in lupus mouse brains together with RNA-seq indicated elevation of phagocytic genes encouraged us to study whether microglial phagocytic activation is required for the NPSLE process. For this purpose, we treated MRL/lpr mice with minocycline, a BBB-permeable phagocytic activity inhibitor that can suppress microglial activation initiated at 5 weeks of age (shortly before reactive microglia were detected) (Supplementary Fig. 4a). Then, behavioral and biological analyses were performed at week 8. As expected, minocycline treatment significantly reduced both the IBA1⁺ microglial number and suppressed its phagocytic activation, as evidenced by reduced IBA1⁺/CD68⁺ fluorescence intensity in the hippocampus (Supplementary Fig. 4b,c), without affecting GFAP⁺ astrocytes and NeuN⁺ neurons (Supplementary Fig. 4d,e) and C1q intensity (Supplementary Fig. 4f). Notably, minocycline ameliorated the anxiety-like behaviors of MRL/lpr mice in both the OFT (Supplementary Fig. 4g,h) and the EPM test (Supplementary Fig. 4i,j).

To test whether the observed effect of minocycline was due to possible antimicrobial effects rather than suppressed phagocyte activation, we similarly treated a group of MRL/lpr mice with the broad-spectrum antibiotic amoxicillin-clavulanate⁴⁰ (Supplementary Fig. 5a). However, amoxicillin-clavulanate treatment did not protect lupus mice from brain disease (Supplementary Fig. 5b,c,d). **Collectively, these results suggest that increased phagocytosis reactivation of brain microglia is a key mediator of anxiety-like behaviors in MRL/lpr mice.**

Synapse loss due to microglial engulfment accounts for early behavioral defects in MRL/lpr mice

Neuron loss can contribute to emotion and cognitive dysfunction⁴. However, MRL/lpr mice displayed no differences in neuron numbers throughout the hippocampus and prefrontal cortices compared with WT controls at 8 weeks of age (Supplementary Fig. 2b). Sequence analysis detected alterations in synaptic activity-related genes (Supplementary Fig. 3c), which encouraged us to speculate that synaptic defects might be involved, reflecting an earlier subtle change that predates neuronal apoptosis. For confirmation, we quantified synaptic terminals and found that the numbers of synaptic puncta within CA3 (mossy fiber terminals) were decreased at 6 weeks in MRL/lpr mice compared with WT controls (Fig. 3a,b). Respectively, the decrease was traced to a 50% reduction in the number of postsynaptic terminals, with a lower change in the number of presynaptic terminals (Fig. 3a,b and Supplementary Fig. 6a). In diseased MRL/lpr mice, synapse loss occurred predominantly at excitatory (VGLUT1⁺) presynaptic terminals (Fig. 3c,d), but not at inhibitory presynaptic (VGAT1⁺) and postsynaptic (Gephyrin⁺) terminals (Supplementary Fig. 6d,e). Nevertheless, the densities of NeuN (Supplementary Fig. 6b) and phosphorylated neurofilament heavy chain (Supplementary Fig. 6c) within CA3 remained unchanged, indicating that both neurons and axons were preserved, rendering neuro-axonal degeneration an unlikely explanation for the observed loss

of synaptic input. We further quantified excitatory synaptic element numbers in the dentate gyrus (DG) granule neurons of Golgi-stained tissue. As observed, dendritic spine numbers were reduced in MRL/lpr mice (Fig. 3h,i), consistent with the immunostaining results.

Synapse pruning by microglia is involved in brain development³⁸ and neurodegenerative processes^{26,41}. We wondered if reactive microglia mediated synapse elimination via a similar process in our model. As noted, we found abundant IBA-1⁺ phagocytes in close apposition to or even enwrapping neurons in diseased MRL/lpr brains, whereas such phagocytic behavior was relatively rare in the controls (Fig. 3e). Moreover, the juxtaposition of microglia and neurons was associated with a loss of presynaptic SYP⁺ terminals in MRL/lpr mice but not WT controls (Fig. 3f) in the same cohort of mice. Furthermore, confocal microscopy showed reductions in SYP⁺ (Fig. 3g), especially excitatory VGLUT⁺ terminals (Fig. 3c), consistent with the Golgi staining results (Fig. 3h,i). Ultrastructure analysis also revealed displacement of synapses encapsulated by phagocytes (Fig. 3j and Supplementary information Fig. 6f). This evidence collectively indicated active roles of these “phagocytes” in synapse elimination.

To investigate the functional correlates of disturbed synaptic terminals, we performed electrophysiological analysis in acute slices of the hippocampus from MRL/lpr and WT mice. The input/output recordings revealed a significant reduction in basal synaptic activity (Supplementary Fig. 6g) in the lupus group. Long-term potentiation (LTP) indicated that excitatory postsynaptic potentials (EPSPs) were also significantly reduced in MRL/lpr slices compared with WT (Supplementary Fig. 6h), indicating that compromised excitatory synaptic transmission occurred in lupus brain, consistent with the reduced excitatory presynaptic and postsynaptic terminals in the hippocampus.

Together, these results suggest that microglial phagocytosis is a key mediator of the synaptic loss involved in early NP abnormalities in MRL/lpr mice.

Increased complement C1q accumulate at synapses in MRL/lpr mice.

The translome analyses combined with immunoblotting revealed a much higher abundance of C1q protein in lupus hippocampus. During the NPSLE process, the C1q signal was already prominent in the hippocampus at 6 weeks (Fig. 2f), especially in the DG and CA1/3 regions, in MRL/lpr but not WT mice (Supplementary Fig. 7a). However, unlike microglial activation, there was no significant progressive increase in C1q burden from 6 to 16 weeks of age (Supplementary Fig. 7b). A costaining experiment showed that increased C1q mostly collocated with neurons (Fig. 4b) and only slightly with microglia (Fig. 4a) in MRL/lpr hippocampus. In the periphery, classical complement C1q could function as a tag, labeling damaged cells for chemotactic phagocytes⁴². We thus hypothesized that elevated brain C1q might act as a marker to tag synapses for microglial pruning. Notably, further costaining of C1q and a synaptic marker (synapsin) revealed a significant proportion of C1q immunoreactive puncta located at synapses (Fig. 4c) in lupus brain, as evidenced by a much higher percentage of C1q-labeled synapses in the hippocampal CA3 of MRL/lpr brains ($23.6\% \pm 3.9\%$) than WT brains ($8.2\% \pm 0.9\%$) at 6 weeks of age (Fig. 4d).

We also performed Western blotting of C1q in purified PSD fractions versus total lysates in a different cohort of mice at 6 weeks of age and confirmed that C1q was greatly increased (although with interanimal variability) in the PSDs of MRL/lpr hippocampi and barely detectable in the WT PSDs (Fig. 4e,f). These biochemical data suggest that C1q accumulates at synapses in the MRL/lpr brain at or even before overt neurodegeneration has taken place. Importantly, antibodies against synaptophysin⁺ (SYP⁺, corresponding to presynaptic terminals) and PSD-95 coimmunoprecipitated C1q from lupus mouse hippocampal lysates at a higher level with PSD-95 (Fig. 4g), suggesting that C1q is predominantly associated with excitatory synapses, especially in the postsynaptic PSD-95 complex. This finding is consistent with observations that the decreased synapses in the MRL/lpr brain were primary excitatory ones (Fig. 3b,c). Consistent with the increased accumulation of C1q in the PSD of MRL/lpr mice, there was a strong positive correlation between the amount of C1q and the decreased center movement in the OFT (Supplementary Fig. 7c).

Although activated microglia have been reported to be a major source of C1q in inflammatory brains³⁸, the inhibition of microglial reactivation by minocycline did not reverse the C1q burden in MRL/lpr mice (Supplementary information, Fig.S4f, 7d), although it reduced the increase in C1q mRNA level in the hippocampus (Supplementary Fig. 7e), indicating that microglia may not be the major source of increased brain C1q protein, rendering the source of C1q a question in the lupus model.

C1q, as a highly abundant serum protein normally produced by many tissues and normally found in serum, is predominantly located in the periphery. During the embryonic period, aging, and neurodegenerative diseases (NDDs), increased brain C1q can be generated by brain-resident cells, such as activated microglia and insulted neurons. However, in autoimmunity conditions, many peripheral activated inflammatory cells can also generate abundant C1q. By immunoblotting C1q in brain, serum, and peripheral tissues of WT and MRL/lpr mice, we found that C1q levels were much higher in the serum and kidney than in the brain (Supplementary Fig. 7f). To further clarify whether increased brain C1q might source from serum, both WT and MRL/lpr mice were intravenously injected with a purified C1q (1 µg/ml). The injected C1q signal in the brain was detected within three hours, as expected due to some permeability of the BBB to C1q, despite intact BBB integrity in MRL/lpr mice at 6 weeks (Supplementary Fig. 7g,h). For additional confirmation, we used a parabiosis mouse model, which has been used to examine circulatory factors that are transferred from one animal to another (Supplementary Fig. 7i)⁴³, to show that connecting WT mice with MRL/lpr or WT littermates changed the C1q amount in the circulation of WT recipients 2 weeks after surgical connection. As expected, the hippocampal homogenate from WT mice in parabiosis with MRL/lpr mice exhibited increased C1q, whereas that from mice in parabiosis with WT controls did not (Supplementary Fig. 7j). Taken together, although the RNA-seq array show that increased C1q transcription in MRL/lpr hippocampus may be due to activated microglia or unfit neurons, however, the observed significant increase in C1q protein in the MRL/lpr mouse brain may originate at least partially from serum.

Although increased brain C1q levels have recently been reported in murine models of viral encephalitis²⁵, frontotemporal dementia³⁶, and Alzheimer's disease²⁴, they have rarely been reported in lupus. For confirmation, we further observed the PSD level of C1q in the pristane-induced SLE mice (PBS treatment as a control) at 4 months after induction when the mice developed behavioral symptoms without significant renal pathology. The average level of C1q in the PSDs of pristane-treated mice was significantly elevated compared with the controls, although it was lower than in the PSDs of MRL/lpr mice (Fig. 4h). Thus, at least in these mouse models, increased synaptic located C1q seems to function as a common pathologic molecule involved in brain damage during lupus development.

C1q tagging at synapses contribute to microglial synaptic pruning-mediated dendritic spine loss, and C1q-blocking approaches protect synaptic pruning and mitigate anxiety-like behaviors in MRL/lpr mice

Macrophage-mediated phagocytosis in the periphery often requires antibody and complement deposition; however, no differences in the amount of endogenous mouse IgG coating with VGLUT-1- or PSD95-positive staining in CA3 synaptic terminals in 6-week-old MRL/lpr mice were observed compared to the controls (Supplementary Fig. 7k,l). Notably, in addition to increased PSD accumulation, C1q also colocalized within cells with a neuronal morphology and with MAP2-positive neurites (Fig. 5a) in the hippocampus, confirming the idea that C1q rather than IgG may instruct phagocytes to engulf synapses in the early stage of NPSLE. Moreover, C1q protein was more frequently detected in synaptophysin⁺ (SYP⁺) perisomatic synaptic boutons surrounded by or adjacent to IBA-1⁺ cellular processes in MRL/lpr mice, in conjunction with a reactivated IBA-1⁺ morphology and reduced SYP⁺ presynaptic terminals (Fig. 5b).

To further examine the relation between C1q, synaptic loss, and microglial activation, we constructed a series of *in vitro* studies. First, we observed that exogenous C1q colocalized with PSD-95 in neuronal cultures derived from E17 MRL/lpr mice (Fig. 5c). Although C1q has been reported to be involved in the onset of many NDDs and as a classical complement signal, C1q also functions as an initiator to mediate cell death⁴⁴. However, directly adding C1q to neuronal cultures failed to disturb neuron survival, dendrites, and synapses, even at high concentrations (Supplementary Fig. 8a), suggesting that deposited C1q did not directly affect the integrity and survival of neurons in culture.

A previous study demonstrated that C1q inhibits the proinflammatory effects of HMGB1 on monocytes⁴⁵. To test whether C1q affected microglial activation, we incubated cultures of primary microglia isolated from WT neonatal mouse brains with or without C1q and assessed the downstream cytokine production, activation markers, and cell morphology. Cultures were performed in serum-free medium to avoid C1q contamination in the serum and to permit accurate control of the concentration of C1q. As anticipated, unlike LPS (a TLR agonist, classic microglia activator) and IFN α (a reported microglia activator in lupus mice), C1q did not alter the transcription of IFN-inducible genes or NF- κ B-dependent proinflammatory cytokines at the tested doses (0-50 μ g/mL C1q) (Supplementary Fig. 8c) and failed to change the phagocytic activity compared to IFN α and LPS (Supplementary Fig. 8d,e). These observations suggested

that C1q alone did not affect the active state of microglia in culture. **Thus, both increased C1q and reactivated microglia may be simultaneously required for synaptic elimination.**

For confirmation, we next conducted a microglia-neuron coculture system in which primary MRL/lpr mouse hippocampal neurons were cultured. One week later, they were cocultured with latent and LPS prime-induced reactive microglia (3:1, neuron: microglia ratio) for another 3 days (Fig. 5d). Indeed, dendrites of neurons cultured with both exogenous C1q and primed (reactive) but not latent microglia displayed reduced synapse density, as measured by PSD-95 clusters (Fig. 5e,f). Furthermore, when primed microglia were cocultured in a transwell insert, the nondirect-contactable coculture failed to induce C1q-microglia-axis-mediated synapse loss (Supplementary Fig. 8b,f), though the neurite length was slightly shortened. These findings highlighted the activation and close microglial-neuron interactions rather than soluble neurotoxic factors as key factors in microglia's orchestration of synaptic removal.

Given these findings, we hypothesized that the C1q-dependent guidance of reactive microglia to engulf synapses accounted for the synapse loss in NPSLE. Accordingly, we questioned whether inhibition of C1q could prevent synapse removal by microglia. To test this hypothesis, we used a C1q-blocking antibody, which potently blocked C1q binding to cultured neurons. We first tested whether the anti-C1q antibody could decrease microglial synapse engulfment *in vitro*. C1q-blocking antibody or isotype control was added concurrently with microglia to the neuron culture (Fig. 5d), and we found that C1q-blocking antibodies, but not the isotype control, prevented the loss of PSD-95 puncta along microglia-proximal dendrites (Fig. 5e,f).

To determine whether C1q-blocking antibodies could prevent synapse engulfment by microglia and rescue synapse loss *in vivo*, we injected C1q-blocking or isotype control antibodies into the bilateral hippocampus of 5-week-old MRL/lpr mice and analyzed the animals 3 weeks later. Remarkably, we measured a slight but significant rescue of synapsin puncta density in the CA3 region of MRL/lpr mice at 3 weeks after injection of C1q-blocking antibody relative to control IgG by both transmission electron microscopy (TEM) (Fig. 5g,h) and confocal imaging (Fig. 5i). C1q blocking also alleviated the anxiety-like behaviors of MRL/lpr mice (Fig. 5j,k) without affecting IBA-1⁺ microglia or CD68⁺ phagocyte intensity in the hippocampus (Supplementary Fig. 8g,h).

The neuronal Nr4a1 defect is an endogenous signal that is critical for the synaptic location of C1q in MRL-lpr mice.

We next investigated the molecular mechanism that guides C1q for synapse tagging. Complement C3 cleaved by C1q into the activated cleavage product C3d has been reported to be involved in phagocyte recruitment in the CNS^{23,25}, and numerous mechanisms have been reported to participate in C1q spinal guidance, including HMGB1 (high mobility group box 1)⁴⁶, small GTPase-regulating proteins²⁴, and altered neuronal action potential⁴⁷.

To investigate this phenomenon, we first compared C3 levels. Unexpectedly, no significant changes were found in either total C3 protein or cleavage products in lupus mice at 6 weeks of age or when NPSLE

developed (Supplementary Fig. 9a), indicating some other underlying mechanisms. The hippocampus microarray revealed a cluster of reduced genes in the “*Nr4a1* related signal transduction” pathway, but not others (Fig. 6a). *Nr4a1* belongs to a family of three immediate-early genes that encode three orphan nuclear receptors (*Nr4a1*, *Nr4a2*, and *Nr4a3*)⁴⁸. In the CNS, *Nr4a1/2/3* expression is controlled by NMDARs, CREB, and MEF2⁴⁸, which are key regulators of synaptic function. We confirmed the decrease in *Nr4a1* in the prefrontal cortex and hippocampus of 6-week-old MRL/lpr mice compared with WT littermates (Fig. 6b) as well as in both derived primary neurons (Fig. 6c) by q-PCR.

A protein-protein interaction analysis revealed that NR4A1, MEF2D, and TMOD3 act in a highly interconnected network, regulating the cellular response to endogenous stimuli, which in turn controls protein localization (Supplementary Fig. 9b), supporting the idea that *Nr4a1* may be related to C1q’s synaptic location. Moreover, *Nr4a1* expression is also maintained by synaptic activation^{48,49}, and *Nr4a1* loss-of-function causes deficits in L-LTP and long-term memory formation^{50,51} as well as dendritic spine loss⁴⁹. Thus, we reasoned that reduced *Nr4a1* might be an endogenous signal that reflects weakened neuronal activity and functions as a bridge connecting C1q to defective synapses, as C1q can recognize nonrobust synapses directly linked by local apoptotic-like processes in the synaptic compartment⁵². We confirmed this phenomenon by incubating neuronal cultures with AP5 (to inactivate NMDARs specifically), which resulted in decreased *Nr4a1* expression in a time- and dose-dependent manner (Supplementary Fig. 9c). Then, to examine how depletion of the NR4A1 proteins affects dendritic spines, we further used LV-shRNA-mediated knockdown in cultured hippocampal neurons (Supplementary Fig. 9d) and found that exogenous C1q bound to dendrites (colocalized with PSD-95⁺ dendrites) of neurons preincubated with sh*Nr4a1* (Fig. 6d) as well as neurons treated with AP5 but not untreated controls (Supplementary Fig. 9e). Moreover, neurons incubated with sh*Nr4a1* showed a 38–46% decrease in dendritic spine density compared with the control, with no effect on dendritic spine density, when cocultured with both primed microglia and exogenous C1q (Fig. 6e,f). **Therefore, this study presents a model in which reduced neuronal *Nr4a1* activity, an intrinsic signal, serves as a bridge to actively direct C1q and then microglial recruitment to conduct spine pruning.**

NR4A1 controls synapse density partially by disturbing the actin cytoskeleton, and dysregulation of the postsynaptic actin network contributes to the loss of dendritic spines in Tau-P301S mice²⁴. Thus, we hypothesized that *Nr4a1* knockdown could encourage the recognition of dysregulation in the synaptic cytoskeleton by C1q and investigated whether the pharmacological stabilization of filamentous actin (F-actin) would stabilize and prevent dendritic spine elimination. Neurons that had been treated with sh*Nr4a1* for 3 days *in vitro* (DIV 7+3) were incubated with fresh shRNAs and the F-actin-stabilizing agent phalloidin for another 2 days. Phalloidin had no effect on dendritic spines in control cultures (data not shown) but partially restored the spine density in neurons treated with shRNAs targeting *Nr4a1* (Fig. 6e,f). However, simple disassembly of F-actin seemed insufficient to explain the effect of low *Nr4a1* expression because the actin-stabilizing drug phalloidin did not achieve absolute restoration of spines. Indeed, many other mechanisms, either regulated by *Nra41* (e.g., BDNF, NMDAR activity) or not regulated by

Nr4a1 (astrocyte-derived molecules) have been reported to be involved in microglial synaptic loss regulation⁵³.

To probe the state of synaptic actin polymerization *in vivo*, we stained F-actin and the postsynaptic marker PSD-95 in the hippocampal CA3 region in WT and MRL/lpr mice. The fluorescence intensity of the F-actin signal that colocalized with PSD-95 clusters was reduced by 36% in MRL/lpr versus WT mice (Fig. 6g,h).

Together, these results highlight that neuronal *Nr4a1* is decreased in MRL/lpr mice, which can lead to dysregulation of the synaptic actin cytoskeleton and then guide C1q and microglia, synergistically contributing to the dendritic spine elimination observed in NPSLE.

To determine whether restoring neuronal NR4A1 expression could prevent synapse engulfment by microglia and rescue synapse loss *in vivo*, we injected an *Nr4a1* overexpression lentivirus or vector control into the bilateral hippocampus of 5-week-old MRL/lpr mice and analyzed the animals 3 weeks later (Fig. 7a). The injected *Nr4a1*-GFP lentivirus was expressed in neurons throughout the hippocampus (Supplementary Fig. 10a). The *Nr4a1* lentivirus resulted in a higher protein level than the control injection, confirming the specificity and effectiveness of the *Nr4a1* lentivirus (Supplementary Fig. 10b). In brains injected with LV-*Nr4a1*, the total C1q staining intensity was not affected, but neuron-located C1q was reduced in the CA1 region (Supplementary Fig. 10c,d,e), and a significant reduction was observed in PSD-95 or synapsin puncta within microglial lysosomes compared with LV-*Ctl* (Fig. 7b,c), suggesting that neuronal *Nr4a1* transcription could blunt C1q tagging and consequently synapse engulfment in MRL/lpr brains.

To investigate the role of NRA41 in synaptic plasticity, the input/output curves and LTP were examined in hippocampal slices. Rescued NRA41 expression partially restored the abnormal basal synaptic activity measured by the I/O amplitude in MRL/lpr mice (Fig. 7d). Compared with the control, LV-*Nr4a1*-treated mice showed enhanced induction of LTP in Schaffer collateral-CA1, although the maintenance (later population spike LTP) was not fully restored at 3 weeks after *Nr4a1* construct injection (Fig. 7e). Restored NR4A1 expression also improved the OFT performance of MRL/lpr mice (Fig. 7f,g), though the increase in center movement distance of LV-*Nr4a1*-injected mice did not reach a significant level compared with the control (Fig. 7h). In summary, our results demonstrate that neuronal NR4A1 rescue can reduce synapse removal by the C1q- microglial axis and lead to a recovery of synapse density and circuit function *in vivo* (Fig. 8).

Discussion

Our study of CNS lupus establishes that the brain is not merely a target of corrupted systemic immunity but assumes an intrinsic active role in the early stage of the disease process even before overt systematic pathology. In the lupus-prone MRL/lpr mice, weakened neurons underwent morphological and functional changes as intermediaries between complement C1q and phagocytes. Specifically, defective neurons

orchestrated their own synaptic stripping via activated microglia guided by C1q, producing an NR4A1-C1q-microglia regulatory axis.

The nervous system is one of the major organs affected, and NPSLE is a major source of morbidity in the SLE population, with mortality second only to that of lupus nephritis, affecting more than 50% of SLE patients¹². Apart from previous considerations that NP symptoms are passive damage attributable to secondary causes such as vasculitis or brain parenchyma injury^{3,54}, recent evidence has indicated that NPSLE develops early, even before the diagnosis of SLE, and along unique pathogenetic pathways compared with other SLE manifestations¹². Moreover, restoring systemic immunity early in the disease (e.g., B cell and/or autoantibody deficiency) does not prevent NP disease in many murine SLE models¹¹. Thus, the mechanistic basis of primary NPSLE manifestations is not yet fully understood. For these reasons, we focused on brain-intrinsic pathology and the molecular mechanisms that contribute to early NP symptoms. First, via a series of combinatorial behavioral analyses, we demonstrated that anxiety-like behaviors appeared earliest, representing the most specific phenotype before obvious lupus serology. Then, the hippocampus microarray combined with stereology analysis showed that while the core architecture of the brain and total neuron numbers appeared largely intact, multiple molecular changes occurred in the hippocampus. In detail, we found that the lupus brain is enriched in a specific set of genes associated with microglial phagocytosis and the classical complement pathway, but with defects in genes related to synaptic function.

Neuronal apoptosis occurs in NAb-induced NPSLE mice and patients with severe SLE in response to anti-double-stranded DNA antibodies cross-reacting with neuronal NMDA receptors^{5,55}. This antibody exposure could mediate the immediate excitotoxic death of binding neurons⁸. However, the neuronal numbers throughout the hippocampus and cortices of control and lupus mice displayed no differences. Thus, other milder, more insidious lesions may be responsible for the early brain disease. Synapse loss is a hallmark central to many neurodegenerative processes, such as aging, AD, and PD^{16,17,24}, although the underlying molecular mechanism is poorly understood. The unbiased identification of alterations in genes involved in synaptic function, the quantified reductions in synaptic terminals within hippocampal CA3, and the damaged neuronal circuit observed by electrophysiology provide compelling evidence that synapse loss rather than neuron death accounted for the early-onset NP abnormalities in lupus mice.

Microglia-mediated synapse pruning plays a pivotal role in ensuring correct synaptic connectivity during normal brain development^{38,56} but also contributes to synapse pathology in CNS disorders when the developmental synapse elimination process is reactivated^{23,36}. Indeed, hippocampus microarray analysis identified genes associated with microglial phagocytosis. This activation was further confirmed by increased microglial numbers, altered phagocytic morphology, and increased lysosomal-localized phagocytic markers. The increased synaptic material engulfed by microglia in lupus was also visualized by electron microscopy (EM) and light microscopy, and this engulfment could be restored by microglial deletion/phagocytosis inhibition, rendering microglial engulfment accountable for synapse loss. There are at least three possible mechanisms for microglial activation in this model: exposure to damage-

associated molecular patterns from weak neurons; binding of periphery-derived soluble, BBB-permeable inflammatory factors to specific receptors or TLR4, such as the reported type I IFN; and combined engagement of activating Fc receptors by DNRAb-immune complexes in later stages. In addition to brain-resident microglia, blood-derived monocytes can enter the brain via the compromised BBB and engulfed synapses and may contribute to the immunopathogenesis of viral de´ ja` vu disease⁵⁷, but this phenomenon was not directly addressed in our study.

The next question concerns why and how activated microglia target synapses, which remains unclear. Antibody and complement deposition are often required for macrophage-mediated phagocytosis in the periphery. However, substantial antibody deposition was not detected early in the lupus brain, suggesting that it may not be the initiating factor. Lines of evidence suggest that C1q, the initiator of the classical complement pathway, is key for synapse removal by phagocytes in inflammatory brains. Accordingly, increased C1q was detected in lupus brains from early disease stages. However, we did not find a significant progressive increase as the disease progressed; instead, C1q was more highly enriched in purified PSD fractions than in bulk tissue. Combined with the localization of C1q at excitatory synapses adjacent to microglia by light microscopy, the close correlation between C1q increase and anxiety behaviors supports a model of lupus in which synapses are tagged by C1q, which then guides microglia to perform pruning, resulting in synapse loss. We further confirmed the critical contributory role of C1q to neuronal damage by microglia *in vitro*; in the absence of C1q, neurons remain intact even when cocultured with primed active microglia. Microglial synaptic engulfment was also blunted by a C1q-blocking antibody *in vivo*. Thus, both *in vivo* and *in vitro* data suggested that a C1q-microglia axis targeting tagged neuronal dendrites for destruction leads to NP manifestations in lupus.

C1q, a part of the innate immune system, is abundant in serum and many peripheral tissues, in which secretion is increased to facilitate the clearance of damaged substances by phagocytes in autoimmune conditions³⁷. Thus, it is important to determine the source of the C1q that accumulated in the lupus brain. Although both neurons and microglia have been shown to secrete C1q^{35,58}, with microglia being the major contributor in neuroinflammatory conditions, little direct evidence has been presented to clarify the question, especially under pathological autoimmunity. We detected an increased C1q mRNA level in the lupus hippocampus, representing intracerebral transcription; however, microglial deletion did not significantly reduce the C1q load and synaptic deposition, though it restored the increased mRNA level in brain. Moreover, the protein levels displayed a much higher increase in mismatch with transcriptional upregulation, and the amount of C1q in peripheral tissue was significantly much higher than in brain, suggesting a possibility that peripheral C1q may be the major source of CNS deposited C1q in lupus mice. Both intravenously injected exogenous C1q protein and intravenously delivered homologous C1q can be detected in recipient brains, further confirming the serum as the major source.

We then asked whether synapses are selectively tagged by C1q and, if so, how C1q recognizes the tagged ones and whether there is a link between changes in the neuronal composition and synaptic C1q binding. Evidence suggests that C3, the downstream complement cascade of C1q, participates in synapse removal by phagocytes under neuroinflammatory conditions^{23,59}. Neither C3 total protein nor its active

cleavage product levels were significantly changed in brain. Thus, C3 may not be essential for disease precipitation or synaptic removal in the NPSLE setting. A recent study reported that tau-driven proteomic changes in PSDs could provide a signal for C1q recruitment²⁴, highlighting the concept that, in addition to phagocyte pruning, neuronal-autonomous disruption of synaptic composition and/or structure may participate in synapse loss. In principle, during development, synaptic intrinsic activity determines microglia-modulated pruning such that active synapses are preserved and fewer active synapses are removed⁴⁷, implying that the complement can “detect” morphofunctional changes at the synapse. In these contexts, many “detectable” signals have been deduced. For example, DNRAb excitotoxic surviving neurons can secrete HMGB1 to bind C1q directly⁵⁵. Many other signals, such as weakened neurotrophs, local reduced synaptic activity⁴⁹, and an abnormal spinal skeleton^{24,49}, are also possible.

Based on these findings, by mining sequencing data, we found a specific reduction in a set of activity-dependent genes regulating the actin cytoskeleton in the lupus hippocampus, the NMDAR/NR4A1/CREB transcriptional mechanisms. Neurons reduced in either activity or NR4A1 were confirmed to be preferentially recognized by C1q and then engulfed by microglia in neuron-microglia coculture. Lupus mice with restored NR4A1 expression in neurons were largely protected from synapse loss and microcircuit dysfunction^{51,60}. Thus, our data reveal a novel mechanism that operates when microglia prune synapses. Specifically, weak potential resulted in reduced NR4A1 and disrupted synaptic skeletal homeostasis, collectively functioning as a detectable signal for C1q tagging and promoting the juxtaposition of microglia to neurons, which is necessary for synaptic engulfment and displacement. However, neuronal NR4A1 expression can be determined by activity⁴⁹ and, in turn, can regulate many circuit-functional genes, triggering the transection of the actin cytoskeleton, synaptic loss, and thus disruption of neuronal connectivity^{49,51}; accordingly, whether weakened neuronal activity and reduced NR4A1 represent staggered sequences in the disease process or can occur independently of each other requires further clarification, as do the sequences between synaptic phagocytosis and displacement.

This study suggests that the early neuropathology seen in lupus-prone mice is characterized by a new homeostasis initiated by an inherent microglial phagocytosis and C1q-dependent dendritic loss. Moreover, our studies reveal that peripheral C1q may be a major source of elevated intracerebral deposition, especially in the early stages of autoimmune encephalopathy, in contrast to C1q generated *in situ* by brain cells in aging or aging-related NDDs. Thus, in this context, a multitarget therapy combining both systemic and brain focus may be a more aggressive strategy than focusing on either one alone. Given that C1q is a secreted protein mainly sourced from the periphery that enters the brain, where neurons play an active role in attracting C1q tag and microglia engulf synapses more avidly in the MRL/lpr brain, our study also points to a critical system-brain, microglia-neuron interaction in the synapse loss involved in NPSLE neuropathy.

Our studies also highlight the active role of neurons in disease participation, as alterations of NMDA receptor-mediated activity could contribute to altered synapse homeostasis. Several genes in this pathway are differentially expressed in the lupus brain, and it is remarkable that restoring neuronal

NR4A1 expression preserved synapse *in vitro* and *in vivo* at 3 weeks post injection. This rescue of synapse number suggests an ongoing dynamic turnover of synapses in the lupus brain that can be rebalanced in a favorable direction by manipulating neuronal activity-related genes and properties. Despite the amelioration of synaptic density, neuronal NR4A1 manipulation did not improve LTP. As revealed in the microarray analysis, however, the multiple other changes in the hippocampus might not be fixed by neuronal enhancement, which is also consistent with the concept that SLE is a multifactorial heterogeneous disease.

Further studies are needed to identify the pathways/factors by which neuronal damage interacts with systemic immune activation. Although astrocytes did not exhibit increased activation or colocalization with synaptic terminals, we cannot completely rule out their contribution to this process, as their functions of nerve support and neurotrophs differ from those of microglia^{61,62}. In NPSLE, increased intracerebral C1q burden co-clustered with reactive microglia, as crucial events in neuronal stripping, offering opportunities for pharmacological interventions. While NPSLE remains a heterogeneous disease with many symptoms and probably many causes, our findings suggest that early diagnosis and multiple brain-target interventions may be beneficial. BBB-permeable small molecule drugs or drugs approved by the United States Food and Drug Administration (FDA) for other purposes may be attractive candidates for exploratory clinical trials.

Materials And Methods

Mice

The MRL/MpJ-Fas^{lpr} (MRL/lpr; stock# 006825) and MRL/MpJ mice (MRL/mpj; stock # 000486) were used and genotyped according to the protocols provided by The Jackson Laboratory. For the pristane-induced SLE model, healthy C57BL/6J mice (female, 6-8 weeks old) were obtained from the Model Animal Research Center of Nanjing University (Nanjing, China)). None of the mice used in this study were involved in any previous test or drug treatment and were randomly assigned to groups at the beginning of each experiment. All animals were housed in groups of four to five mice per cage in the animal facility under standard laboratory conditions (12:12-h light cycle with free access to food and water), except where individually housed after surgery. We used a minimum of n = 3-6 mice per group (except n = 10-15 for the behavioral analyses) for most analyses, as this size provided 80% power to observe differences in effect size of at least 2 between groups. The exact sample size (n) for each experiment can be found in each Figure legend. All experiments were conducted in accordance with the NIH guidelines for animal research and were approved by the Animal Welfare Committee of the Affiliated Drum Tower Hospital of Nanjing University Medical School.

Primary mouse neurons and microglia

Mouse neuronal cultures were prepared from the hippocampi of mouse embryos on embryonic day 13 (E13) or E14 and plated on poly-D-lysine coated coverslips at a density of 80,000/24-well dish and cultured in Neurobasal medium (Invitrogen, 21103-049). Then, 50% of the medium was exchanged with fresh medium every 3 days. For primary microglial cultures, postnatal (P1-P2) pups were decapitated, the forebrain was triturated with a 5-ml serological pipette, and the homogenate was spun at 1000 x g for 5 min. The supernatant was discarded, and the pellet was resuspended with a 10-ml pipette and filtered through a 75-mm filter. Forebrains were cultured per 75-cm² flask in 15 ml of DMEM + 10% fetal bovine serum (FBS, Gemini Bio). After 24 h, the flasks were rinsed with Hank's Balanced Salt Solution (HBSS, Thermo Fisher Scientific), and new medium was added. Cultures were grown for an additional 10-14 days before microglia were shaken off the astrocyte feeder layer on a rocking platform for 2 h, pelleted, and resuspended in culture medium and plated. Primary cells from male and female pups were used.

Microglia-neuron cocultures and quantification for synaptic density

Microglia and cortical neuron cocultures were established when the neurons reached 7 DIV using a slightly modified protocol²⁴. Primary microglia were recovered by shaking the flasks and harvested as described above. The microglia were resuspended and added to neurons (or cultured in a Transwell insert) at a 1:3 (microglia: neuron) ratio. Cocultures were allowed to continue for 3 days before fixation with 4% PFA or 1 day before harvest. To quantify the synaptic density around microglia in cocultures, the presence of microglia, neurons and synapse was determined by immunofluorescent staining using antibodies against Iba-1 (Wako, 019-19741), MAP2 (Abcam, ab11267), and PSD95 (Abcam, ab2723), respectively. For each coculture coverslip, 10-15 microglial cells were chosen at random for imaging. During capture, microglial cells were centered, and images were acquired via Zeiss LSM710 confocal microscopy (Carl Zeiss Jena, Germany) with a 63× objective. Subsequent images were analyzed using the listed ModSholl Analysis Plugin Macro. The PSD95⁺; C1q⁺ puncta were quantified manually by surveying the extracellular periphery of the cocultures.

Pharmacological interventions

To inhibit phagocyte activation, minocycline (a tetracycline derivative that has been extensively studied to blunt neuroinflammation and phagocyte activation) was induced. Minocycline injections were performed as described⁵⁷. Briefly, mice received a once-daily intraperitoneal (i.p.) injection of minocycline (50 mg/kg) or PBS (control). To investigate the potential impact of the antibiotic, mice were randomized to receive daily oral administration of the broad-spectrum antibiotic amoxicillin-clavulanate (amoxicillin trihydrate: potassium clavulanate (4:1), 50 mg/ml in the drinking water) or mock (non-supplemented drinking water).

Stereotaxic antibody and *Nr4a1* lentivirus microinjection

The blocking anti-C1q antibody was produced using the variable domain sequences previously described⁵⁷, with isotype mouse IgG2b as the control. Five-week-old female mice were anesthetized with

3% isoflurane and placed on a stereotaxic apparatus (Stoelting) for surgery. All injections were performed with a 10- μ l syringe (Hamilton Company) with a pulled glass pipette tip glued to the end of needle and a syringe pump (Stoelting Quintessential stereotaxic injector). The injection coordinate was 1.25 mm anterior, $\pm$ 1.82 mm lateral, and 1.75 mm ventral to the bregma. One microliter of anti-C1q or isotype control antibody was injected at a rate of 0.5 μ l/min. For *Nr4a1* lentivirus microinjection, mice were randomly assigned and microinjected bilaterally with either the control or *Nr4a1*-GFP lentivirus (1 μ l of 1×10^9 viral genomes/ μ l, Hanbio, Shanghai, China) into the hippocampus using the above coordinates.

Tissue collection and sample preparation

Mice were anesthetized with 3% isoflurane and then transcardially perfused with ice-cold PBS. One hemisphere was dissected for isolation of the prefrontal cortex, hippocampus, cerebellum, and midbrain. A fraction of samples was used for RNA extraction, whereas other samples were homogenized in RIPA buffer (Pierce) containing phosphatase and protease inhibitors (Invitrogen), centrifuged for 15 min at $15,000 \times g$ and the supernatant was used for Western blotting. For RNA-seq, a fraction of the hippocampus samples was used.

Brain immunohistochemical and immunofluorescence staining

Mice were deeply anesthetized and transcardially perfused with PBS, followed by the addition of 4% paraformaldehyde (PFA) in PBS for fixation, post-fixed in 4% PFA overnight, and then cryoprotected in 20% sucrose. Alternatively, the right hemi-brain was drop-fixed for 48 h at 4°C in 4% PFA for immunohistochemistry, and the left forebrain was frozen and later homogenized. Brains were embedded in OTC and sectioned at 30 μ m using a freezing microtome. Sections were preserved in cryoprotectant (50% glycerol and 50% PBS) and stored at -20°C. For H&E staining, brains were embedded in paraffin and sectioned at 15 μ m. For fluorescent immunostaining, free-floating sections were rinsed in PBS, followed by PBS with 0.3% Triton X-100 (PBST), blocked with blocking buffer (5% goat serum, 5% bovine serum albumin in PBST), and incubated overnight with primary antibodies. Secondary antibodies were incubated for 1-2 h at room temperature, extensively washed in PBS, and mounted using ProLong Diamond medium (Invitrogen). For immunohistochemical bright field staining, endogenous peroxidases were neutralized (PBS/3% H_2O_2) and nonspecific binding was blocked. Then, sections were stained with primary and secondary antibodies and visualized by 3'-diaminobenzidine (DAB) immunostaining. The used primary and then secondary antibodies as listed in Supplementary information, Table S3. For neuron quantification, sections were stained with Nissl stain (0.05% thionine/0.08 M acetate buffer, pH 4.5). For apoptosis quantification, sections were stained with a TUNEL Kit (KGA7062, keygen). For synapse quantification, mouse brains were prepared according to the manufacturer's instructions, and sections were stained with an FD Rapid GolgiStain Kit (FD Neurotechnologies) as previously described¹⁷.

Tissue imaging and analysis

For total immunofluorescence analysis, sections were scanned via Zeiss LSM710 confocal microscopy (Carl Zeiss Co., Germany). Color thresholds in RGB space were used to identify the positive staining. The

mean fluorescence intensities of regions of interest (ROIs) were measured with Fiji software (ImageJ). For microglial engulfment analysis, sections were imaged using a Zeiss LSM 710 confocal microscope, reaching 32 z-stacks at 0.34- μ m steps with a 63x oil objective. Six to eight microglia within the hippocampal CA1 or CA3 region were analyzed per mouse. DAB-stained samples were imaged using a confocal microscope (Axiovert LSM510, Carl Zeiss Co., Germany), and the immunostaining signals were quantitatively analyzed using the Optical Fractionator method with Stereo Investigator software (Stereo Investigator software; Microbrightfield) as previously described⁶³. Golgi-stained samples were imaged via confocal microscopy (FV3000 Microscope, Olympus Co., Japan).

Tissue preparation for TEM

For TEM, methods were adopted as previously reported²¹. In brief, mice were perfused with a 2% PFA/2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4), and coronal sections (150 μ m) including hippocampus were cut using a vibratome and further fixed overnight. Then, tissues were processed using 2% osmium tetroxide and 4% uranyl acetate, dehydrated, and embedded in Eponate 12 resin (Ted Pella Inc., Redding, CA). Ultra-thin sections were cut at a thickness of 1 μ m, collected on copper grids, and imaged with FEI Tecnai G2 Spirit Bio TWIN TEM using FEI software. Fifteen distinct regions of the hippocampus were imaged per animal. Images were used to analyze the number of synapses with blinding to the genotype. A synapse was defined as an electron-dense postsynaptic density area juxtaposed to a presynaptic terminal filled with synaptic vesicles. Microglia were identified on the basis of their characteristic nuclear morphology, a single-profile endoplasmic reticulum, and electron-dark shading of the cytoplasm.

Electrophysiological recordings in hippocampal slices

Coronal brain slices containing the hippocampal formation were prepared as previously described⁶⁴. Animals were anesthetized with isoflurane, and brains were rapidly extracted from the skull and then transferred into ice-cold slicing artificial cerebrospinal fluid (ACSF) solution (mM:125 NaCl, 2.5 KCl, 1.25, NaH_2PO_4 , 25 NaHCO_3 , 10 D-glucose, 2.0 CaCl_2 , 1.5 MgCl_2 , saturated with 95% O_2 /5% CO_2), where hippocampus was isolated from cortices. Transversal slices (380 μ m thickness) were prepared with a Vibratome VT1200S (Leica, Germany) and recovered in incubation ACSF (same formula used for slicing ACSF) at 30°C for 30 min, followed by incubation at RT for an additional 1 h and transfer to a "submerged" type recording chamber and continuously perfusion with ACSF recording (mM:125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4 , 25 NaHCO_3 , 10 D-glucose, 2.0 CaCl_2 , 1.0 MgCl_2 , saturated with 95% O_2 /5% CO_2) with a peristaltic pump (BT100-2J, LongerPump, China) at 7 ml/min, $30.0 \pm 0.1^\circ\text{C}$. Recordings were acquired with an Axopatch 700B amplifier and Digidata 1440A (Molecular Devices, USA). pClamp10.7 software was used for the recording acquisition. For field excitatory postsynaptic potential (fEPSP) recordings, a recording pipette was placed in the middle of the stratum radiatum in CA1, 75-150 μ m deep in the tissue. fEPSPs were evoked by activating Shaffer collaterals with a glass pipette (1~2 M Ω) placed in the middle of the stratum radiatum 200-400 μ m away from the recording pipette. Input-output curves were generated by a series of stimuli in 0.01-mA steps. The amplitude of the fEPSPs was set at 35~40% of the maximal

responses for paired-pulse facilitation (PPF) recording. Paired stimuli (25, 50, 75, 100, and 200 ms intervals) were delivered, and the ratio of the amplitude and/or slope of the second fEPSP over the first one was calculated. For LTP recording, slices were stimulated with single test pulses every 30 s to elicit a stable baseline response for at least 30 min, and then LTP was induced by theta-burst stimulation (TBS, two trains of 10 bursts (5 Hz) were delivered with 20-s intervals; each burst consisted of four pulses of 100 Hz). Following TBS, the stimulus frequency was returned to 30 s for 60 min. Fifty to sixty minutes after stimulation corresponds to the early phase of LTP. All recordings were performed in a blinded manner.

Synaptosome fractionation

Synaptosomes were isolated using a modified version of the protocol outlined⁶⁵. Briefly, tissue was immersed in 10 volumes of HEPES-buffered sucrose (0.32 M, 5 mM HEPES, pH 7.4) and homogenized using a motor driven glass-Teflon homogenizer. The resulting homogenate was spun at 1200 x g to separate the nuclear fraction. A further spin at 15,000 x g was carried out to generate crude synaptosomes. These were then layered onto a discontinuous sucrose gradient and spun at 150,000 x g for 2 h. Purified synaptosomes were subsequently extracted and spun down.

RNA sequencing

A total amount of 3 µg of RNA per sample was used as input material for the RNA sample preparations, with mRNA purified from total RNA using poly-T oligo-attached magnetic beads. Sequencing libraries were generated using the NEBNext® Ultra™ RNA Library Prep Kit for Illumina® (NEB, USA) following the manufacturer's recommendations. Sequencing reads were aligned to the mouse reference genome mm10 (GRCm38.90) using STAR aligner (v2.5.1b) guided by the mouse GENCODE gene model release v15. HTSeq v0.6.0 was used to count the read numbers mapped to each gene. Then, the FPKM of each gene was calculated based on the length of the gene and the read count mapped to this gene. Raw count data were normalized by the voom function in the R limma package, and differential expression analysis was then performed using the DESeq2 R package (1.10.1). Differentially expressed genes (DEGs) were defined as having at least a 1.5-fold change in expression and adjusted $P < 0.05$ in comparisons of different genotypes.

Western blotting

Equal amounts of protein (30 µg) were separated by electrophoresis in precast 8–12% Bis-Tris Gels (Bio-Rad) and transferred to pre-wetted polyvinylidene difluoride membranes. The membranes were hybridized with the primary and then secondary antibodies. The membranes were hybridized with the primary and then secondary antibodies as listed in Supplementary Table S3. Then, ECL (Pierce®) was used to reveal the immunoreactive proteins, images were acquired using an Image Quant LAS 4000 mini (Uppsala, Sweden) luminescent image analyzer, and protein bands were quantified using ImageJ software (NIH). Protein levels were determined by normalizing to the level of ACTB and are presented relative to the control.

Mouse behavioral testing

All behavioral analyses were performed in the Animal Core Facility of Nanjing Medical University. For all behavioral tests, mice were transferred to the test room and equilibrated for at least 3 h prior to the tests, which were carried out between 9:00 and 18:00 h. Behavior was monitored through a video camera positioned in front or on top of the testing apparatuses and was recorded and later analyzed with a video tracking system (Top Scan software; Top Scan Software & Instruments, USA) by a blinded, experienced researcher. A panel of behavioral tests was used for phenotypic characterization of MRL/lpr as previously described¹⁵. These included the tail suspension test (TST) and the FST to assess depression-like behavior, the novelty Y maze test to assess memory, the OFT combined with the EPM test to examine anxiety-like behavior, and the OFT combined with the rotarod test to examine locomotion. Detailed materials and methods of these tests are provided in the Supplementary Information.

Parabiosis model

The mice to be joined in parabiosis were anesthetized and shaved along the opposite lateral flanks as reported in established protocols. The excess hair was wiped off with an alcohol prep pad. After further disinfection with betadine solution and 70% alcohol, identical incisions were created on the corresponding lateral aspects from the olecranon to the knee joint of each mouse. The olecranon and knee joints were each attached by a single 4-0 silk suture and tie, and the dorsal and ventral skins were sewed together with continuous 5-0 Vicryl suture. The mice were then kept on heating pads and continuously monitored until full recovery. Buprenorphine was used for analgesic treatment by subcutaneous injection every 8–12 h for 48 h after the operation.

Blood–brain-barrier leakage assay

Blood–brain-barrier leakage was assayed using established protocols⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶⁶. Mice were injected intravenously with 10-kDa FITC-dextran (2 mg per 20 g mouse). After 4 h of circulation, mice were euthanized, and brains were fixed in 4% PFA. Coronal brain sections (30 μ m) were stained with anti-CD31 (1:500, Abcam) to mark blood vessels and were imaged by confocal microscopy. Three fields of view from two brain sections per animal were quantified using ImageJ. Leakage was measured as a decrease in colocalization of FITC-dextran with CD31.

Assessment of lupus

Mice were monitored for the development of proteinuria and autoantibody titers during the course of the experiment. Urinary protein excretion was measured by a Bradford protein detection kit (KGA801-804, Keygen Biotech). Serum IgG anti-double stranded (ds) DNA antibody titers were measured by ELISA (FUJIFILM, Cat # 631-02699) according to the manufacturer's instructions, and serum samples were diluted 1:250 in buffer solution before being added to the plates for incubation.

Statistical analysis

For all statistical analyses, GraphPad Prism 6 Software was used. All replicate numbers (number of mice analyzed, unless otherwise indicated) and statistical tests are indicated in the legends. When possible, all analyses were performed with blinding to the genotype and/or treatment group. Error bars represent SEM in all figures. Analyses used include one-way ANOVA, two-way ANOVA, or the Student's t-test. For ANOVA analyses, Tukey's post hoc tests were used. Correlation was measured using Pearson's correlation coefficient, and the interactive effect was analyzed using multivariate linear regression. Significance is reported at $P < 0.05$.

Data Availability Statement

The RNA sequencing data that support the findings of this study have been deposited at the NCBI GEO data repository under accession number GSE154288. Source data underlying Figures¹⁻⁷ and Supplementary Figures¹⁻¹⁰ are available as a Source Data file. A detailed description of all statistical analyses performed for Figures¹⁻⁷ and Supplementary Figures¹⁻¹⁰, including a brief description for each Figure, sample size, statistical tests performed, P values, post hoc test, and post hoc test P values are provided as a Source Data file. Other data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declarations

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Author Contributions: X.H. designed and performed most of the experiments, analyzed and interpreted the data, and wrote the manuscript. Q.F., D.W., G.Y., and T.X. performed experiments and analyzed the data. G.H. assisted in experimental design. L.S. conceived the whole study, designed experiments, and wrote the manuscript.

Declaration of interests

The authors declare no competing interests.

Data Resources

The data that support the findings of this study are available from the corresponding authors upon request. The accession number for the RNA-seq data reported in this paper is GEO: GSE154288 (<https://www.ncbi.nlm.nih.gov/geo/info/linking.html>).

Additional information

Supplemental information includes eleven figures and three tables and can be found online at <https://>

Contact and materials availability

Further information and requests for resources and reagents should be directed to the Lead Contact, L.S. (lingyunsun@nju.edu.cn), or the first author, X.H. (hxj-719@163.com).

Figures

Figure 1

MRL/lpr mice develop anxiety-like phenotypes and reactive microglia before overt peripheral lesions (a) Schematic of the experiment to study CNS lupus development in SLE mice. (b-g) Behavior performance in Open field test (OFT, b,c,d) and Elevated plus maze (EPM, e,f,g) test of 6-week-old and 16-week-old female MRL/lpr mice (red) and matched littermate controls (black). $n = 11-12$ mice per group, Student's unpaired t-test. (h) Representative IBA-1 immunofluorescence images of hippocampus sections. Scale bars, 80 μm

and 10 μm in magnification. (i) Immunostaining of CD68, a microglial/macrophage lysosomal activation marker, positive IBA-1+ cells in MRL-lpr mice (n = 3 mice per group). Scale bars, 20 μm . Arrows indicate reactive microglia. (j) Quantitation of reactive microglia in frontal cortex (Cor), hippocampus (Hip), cerebellum (Cere) and midbrain (Mid) sections. n = 3-4 mice per group. (k-m) Representative image of IBA-1 in CA3 and complement C3 in kidneys from WT and MRL/lpr mice at indicated times, scale bar, 80 μm . Dot plots depict the average number of IBA-1+ cells per mm² (l) and renal pathological score (m) quantified in control (black) and MRL/lpr mice (red). n = 5 mice per group. Representative experiment of three independent experiments. Data are mean $\pm$ SEM. * P < 0.05, ** P < 0.01, *** P < 0.001, ns not significant, Student's unpaired t-test. See also Supplementary Fig. 1, 2.

Figure 2

Transcriptional profile reveals increased phagocytosis and complement signal activation in MRL/lpr mouse brain (a) Workflow of RNA-seq data analysis of hippocampus from MRL/lpr and WT control mice with heat map of differentially expressed genes. Bar chart displays 1,908 variable genes, which are part of the corresponding KEGG terms and have higher expression in WT- or MRL/lpr- hippocampus (bottom). KEGG, Kyoto Encyclopedia of Genes and Genomes. (b,c) Heat maps show relative expression of significantly altered genes (see methods) generated from hippocampal microarray of WT vs MRL/lpr mice at 6-week-old; each column represents individual mice. (d,e) Validation of select genes and pathways in a unique set of mice by qPCR. n = 5-6 mice per group. (f) Validation by immunoblotting of C3 and C1q in WT (n = 4) and MRL/lpr (n = 5) mice at 6-week-old. Data are mean $\pm$ SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns, not significant by one-way ANOVA with Tukey's post hoc test in (d,e). See also Supplementary Fig. 3-5.

Figure 3

Synapse loss due to microglia engulfment accounts for early NP manifestation in MRL/lpr mice. (a,b) Immunostaining and quantification of colocalized presynaptic and postsynaptic puncta using the markers synaptophysin (red) and PSD-95 (green), respectively, in 6-week-old WT and MRL/lpr brain sections. Data are the means of 2 staining experiments. Scale bar, 40 μm . $n = 6$ mice per group, * $P < 0.05$, ** $P < 0.01$, unpaired Student's t-test. (c,d) Immunostaining and quantification of staining area for

glutamatergic marker, VGLUT1, in 8-week-old WT or MRL/lpr mice treated with or without minocycline. n = 3, Scale bar, 20 μ m. (e) Confocal immunofluorescence images for presynaptic terminals (synaptophysin, SYP) and IBA-1 for phagocytes stained in the pyramidal layer of CA1 in brain sections. (f,g) Representative images and quantification of synaptophysin (SYP) bouton density in brain sections. Scale bar, 10 μ m, n = 20 neurons per mouse, n = 3 mice per group in (g). (h,i) Representative images and quantification of Golgi-stained dendritic spines from DG granule neurons in WT mice and MRL/lpr mice with or without minocycline treatment. n = 3 mice per group. Scale bars, 5 μ m. (j) Electron micrographs of microglia (MG; brown in the inset) surrounding synaptic elements (white arrow; green in the inset, up) and containing degraded components (red arrow; purple in the inset, right). Presynaptic terminals are indicated by asterisks. Scale bars, 500 nm. Data are mean $\pm$ SEM. * P < 0.05, *** P < 0.001, ns, not significant, by one-way ANOVA with Tukey's post hoc test in (d,h,g). See also Supplementary Fig. 6.

Figure 4

Increased C1q accumulates at synapses in MRL/lpr hippocampus (a,b) Representative images of C1q stained with IBA-1/NeuN in the dentate gyrus (DG) of 6-week-old WT and MRL/lpr mice. Scale bars as indicated. (c,d) Representative images and quantification of C1q (green) stained with synapsin (red)

puncta in the dentate gyrus (DG) of WT and MRL/lpr mice. Colocalized synapsin and C1q puncta are indicated by circles. Scale bar, 5 μ m. d, Graph shows percent of total synapsin clusters that colocalized with C1q. At least 120 synapses per mouse were analyzed. n = 5 mice per group. (e,f) Representative immunoblots and quantitation of C1q in PSD fractions and total lysates from WT and MRL/lpr mice. (g) Co-immunoprecipitation analysis of C1q with synaptophysin or PSD-95 antibodies from WT or MRL/lpr hippocampal lysates. (h) Representative immunoblots of C1q in PSD fractions from 4-month post-pristane-treated, normal control and 8-week-old MRL/lpr mouse hippocampus. Data are mean $\pm$ SEM. **P < 0.01, ***P < 0.001, two-tailed Student's t-test in (d), one-way ANOVA with Tukey's post hoc test in (f). Data are the mean $\pm$ SEM. **P < 0.01, ***P < 0.001, two-tailed Student's t-test in (d), one-way ANOVA with Tukey's post hoc test in (f). See also Supplementary Fig. 7.

Figure 5

Tag of C1q at synapses contributes to microglia synaptic pruning-mediated spine loss (a) Immunostaining for C1q with the neuronal marker MAP2 in WT and MRL/lpr mice at 6-week-old ($n = 3$). Scale bar, 40 μ m. (b) Immunostaining showing the colocalized synaptophysin, C1q, and IBA-1 puncta (with magnification insets, arrowheads depict colocalization) in WT and MRL/lpr mice. Scale bar, 20 μ m. Data are mean $\pm$ SEM. $n = 5$, ** $P < 0.01$, two-tailed Student's t-test. (c) MRL/lpr mouse-derived

hippocampi neurons were cultured alone or with exogenous C1q and stained for PSD-95 (green), labeled C1q (red), and DAPI (blue). Scale bar, 20 μ m. (d) Diagrams showing microglia-neuron (or Transwell) cocultures and Sholl analyses to quantify synapses around microglia. (e,f) Representative images of dendritic segments (e) and quantification of dendritic PSD-95 puncta density (f) in neuron-microglia cocultures, incubated with exogenous C1q, treated with anti-C1q antibodies or isotype control. Primary neurons of MRL/lpr mice were cocultured with latent or primed microglia and stained for IBA-1 (purple), PSD-95 (red), and MAP2 (green). Scale bar, 20 μ m. Data are pooled from three to five independent experiments (f). * $P < 0.05$, ** $P < 0.01$, ns, no significance by one-way ANOVA with Tukey's multiple comparisons test. (g,h,i) Representative histology images (TEM) and quantitation of synapse density in hippocampus sections. Scale bars, 500 nm. (n = 3 mice per group). (i) Density of synapsin puncta in CA1 region from control IgG or anti-C1q-injected MRL/lpr mice. Each dot represents the average value for one mouse. * $P < 0.05$, *** $P < 0.001$, one-way ANOVA with Tukey's post hoc test. (j,k) Behavioral performance changes of MRL/lpr mice post-antibody injection (n = 8 mice per group). * $P < 0.05$, two-tailed Student's t-test. All data are mean $\pm$ SEM. See also Supplementary Fig. 8.

Figure 6

Neuronal Nr4a1 deficiency is an endogenous signal that is critical for the synaptic location of C1q in MRL-lpr mice (a) Heat maps show the relative expression of significantly altered genes (involved in Nr4a signaling) generated from the hippocampal microarray of WT vs MRL/lpr mice at 6 weeks of age. (b,c) Representative PCR and quantification of Nr4a1 mRNA level in brain lysate from WT and MRL/lpr ($n = 5$) mice and in primary cultured hippocampal neurons. The relative expression was normalized to the

average of WT controls. (d) Representative images of dendritic segments and quantification of C1q colocalization after treatment of hippocampal neurons with the indicated shRNA for 3 days. Scale bar, 20 μm . At least 12 neurons per culture from two independent cultures were used for the analysis. (e,f) Dendritic segments of neurons after treatment with control or Nr4a1-specific shRNA and incubation with phalloidin or vehicle (e). Scale bar, 20 μm . The histogram shows the spine density along dendrites after treatment with shRNAs plus or minus phalloidin, as indicated (f). At least 11 neurons per culture from two independent neuronal cultures were used for the analysis. (g,h) Representative images of F-actin and PSD-95 in the hippocampal CA3 region. Quantitation of the intensity of F-actin signal that overlaps with PSD-95 puncta. Scale bar, 5 μm . Each dot represents the average for one mouse. Data shown are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns, non-significant by one-way ANOVA with Tukey's post hoc test in (b,f), unpaired t-test in (c,d,h). See also Supplementary Fig. 9.

Figure 7

Rescuing neuronal Nr4a1 in lupus mice protects synaptic pruning, restores hippocampus microcircuit function, and mitigates anxiety-like behaviors (a) Timeline of the experimental procedure in the lupus-prone mouse model. (b,c) (b) Representative confocal stacks of CD68+ lysosomes (red) and engulfed PSD-95 (green) puncta in the CA1 region of MRL-lpr mice injected with control or shNr4a1 lentivirus. Scale bar, 20 μ m. (c) Quantitation of the relative number of engulfed PSD-95 and synapsin puncta in CD68+ lysosomes normalized to LV-Ctl-injected mice. n = 4 mice/group, 30 cells/group. (d,e) Input-output relationship measuring basal synaptic function in LV-Ctl and LV-Nr4a1 injected MRL/lpr mice. Long-term potentiation (LTP) was induced by theta-burst stimulation over 60 min to evaluate synaptic

plasticity; the LTP magnitude averaged from the first and last 10 min of recordings represents the induction and maintenance of LTP, with the average from the first 10 min shown in the right histogram ($n = 8-10$ slices from three mice per group), $*P < 0.05$, $**P < 0.01$, two-way ANOVA, Bonferroni's test in (d), paired, two-tailed Student's t -test in (e). (f,g,h) Performance changes in OFT of MRL/lpr mice injection with LV-Ctl and LV-Nr4a1 ($n = 8$ mice per group). $*P < 0.05$, two-tailed Student's t -test in (g,h). Data are the mean $\pm$ SEM. See also Supplementary Fig. 10.

Figure 8

Graphical Abstract: Schematic of the neuron–complement–microglia-axis-coordinated synaptic loss in CNS lupus. In brief, our data inform a model in which neurons act as intermediaries between complement and microglia, synergistically driving their own synaptic loss, and subsequently regulate neuropsychiatric behaviors.

Supplementary Files

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