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ORIGINAL ARTICLE

Effects of prolonged treatment with memantine in the MRL model of central nervous system lupus

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Abstract

Neuropsychiatric manifestations and brain atrophy of unknown etiology are common and severe complications of systemic lupus erythematosus (SLE). An autoantibody that binds to *N*-methyl-D-aspartate (NMDA) receptor NR2 has been proposed as a key factor in the etiology of central nervous system (CNS) SLE. This hypothesis was supported by evidence suggesting memantine (MEM), an uncompetitive NMDA receptor antagonist, prevents behavioral dysfunction and brain pathology in healthy mice immunized with a peptide similar to an epitope on the NR2 receptor. Given that SLE is a chronic condition, we examined the effects of MEM in MRL/lpr mice, which develop behavioral deficits alongside SLE-like disease. A broad behavioral battery and 7-Tesla magnetic resonance imaging (MRI) were used to examine whether prolonged treatment with MEM (~25 mg/kg bodyweight in drinking water) prevents CNS involvement in this spontaneous model of SLE. Although MEM increased novel object exploration in MRL/lpr mice, it did not show other beneficial, substrain-specific effects. Conversely, MEM was detrimental to spontaneous activity in control $MRL^{+/+}$ mice and had a negative effect on body mass gain. Similarly, MRI showed comparable increases in the volume of periventricular structures in MEM-treated groups. We conclude that sustained exposure to MEM affects body growth, brain morphology and behavior primarily by pharmacological, and not autoimmunity-dependent, mechanisms. Substrain-specific improvement in exploratory behavior of MEM-treated MRL/lpr mice might indicate that the NMDA system is merely a constituent of a complex pathogenic cascade. However, it was evident that chronic administration of MEM is unable to completely prevent the development of a CNS SLE-like syndrome. (Clin Exp Neuroimmunol doi: 10.1111/j.1759-1961.2012.00032.x, September 2012)

Introduction

Systemic lupus erythematosus (SLE) is a severe autoimmune disease that primarily affects skin, kidneys and joints. In many SLE patients, however, neuropsychiatric manifestations and brain atrophy also occur at different phases of disease development.[1,2] The lack of insight into pathogenic mechanisms has necessitated the development of animal models, which show significant validity and usefulness in studying central nervous system (CNS) involvement.[3]

Two classes of SLE animal models have been established over the past decades. Inbred strains of NZB, NZB/W, BXSB and MRL mice spontaneously develop a systemic autoimmune disease, which steadily progresses over their lifespan. Conversely, "induced models" of SLE develop an acute autoimmune response to a systemically administered autoantigen.[4] More recently, immunization of healthy BALB/c mice with a pentapeptide (DWEYS) was shown to generate serum anti-DNA antibodies, which cross-react with a *N*-methyl-D-aspartate (NMDA) receptor in the brain.[5] Autoantibody binding resulted

in neurodegeneration and broad deficits in behavior, including altered emotional reactivity[6] and memory.[7] The pathogenicity of anti-NMDA antibodies was proposed to be mediated by enhanced postsynaptic transmission and excitotoxicity.[8] Consistent with this notion, both behavioral deficits and the demise of central neurons in an induced model of CNS SLE were prevented by the non-competitive NMDA receptor antagonist, memantine (MEM).[6,7] This effect appears to be the result of stabilized mitochondrial permeability,[8] and the result of the inhibition of autoantibody binding to the NMDA receptor.[7] However, inconsistencies among recent clinical reports and the fact that anti-NMDA receptor antibodies are detected in merely ~35% of SLE patients bring into question whether a dysfunctional NMDA system fully accounts for CNS manifestations in SLE.[9,10]

The "spontaneous" MRL model has been used for more than two decades,[11–14] and has been proven to be instrumental in documenting *bona fide* neurodegeneration of central neurons and cytotoxicity of cerebrospinal fluid (CSF) in SLE-like disease.[15,16] In particular, MRL/MpJ-Fas^{lpr}/J (MRL/lpr) and MRL/MpJ (MRL$^{+/+}$) mice spontaneously develop lupus-like manifestations (e.g. high serum levels of autoantibodies, skin lesions, lymph node and spleen enlargement, renal inflammation), but differ in their onset. Although MRL/lpr mice show high serum levels of autoantibodies and pro-inflammatory cytokines within the first 2 months of life, congenic MRL$^{+/+}$ mice develop similar symptoms much later.[17] Alterations in exploration, spatial learning and emotional reactivity represent key features of the "autoimmunity-associated behavioral syndrome" (AABS) in the MRL/lpr substrain.[18] Impaired performance in several paradigms have suggested that altered emotional reactivity and spatial learning are consequences of an accelerated form of SLE-like disease.[12,13] Furthermore, in comparison with the MRL$^{+/+}$ substrain, MRL/lpr mice show blunted responsiveness to palatable stimuli, impaired spontaneous and exploratory activity, and increased anxiety-related behavior.[20–24] Behavioral changes in lupus-prone mice are accompanied by infiltration of mononuclear cells into the choroid plexus and meninges, neuronal loss in limbic and cortical areas, as well as retarded brain growth and ventricular enlargement.[15,25–29] Similar to anti-NMDA antibodies in the peptide-induced model of CNS SLE,[6,7] brain-reactive antibodies of the immunoglobulin G class seem to account for CSF cytoxicity towards mature and immature neurons *in vitro*.[16,30,31]

The blood–brain barrier (BBB) is breached in diseased MRL/lpr mice, as evidenced by an upregulation of cell adhesion molecules in periventricular regions, widespread perivascular leakage,[26] and infiltration of immunocytes into the choroid plexus and multiple regions of brain parenchyma.[28,32,33] One might assume that "spontaneous" and "induced" models of CNS SLE (where the BBB is breached chemically) share comparable neuropathogenic mechanisms when autoantibodies cross the BBB. This notion is supported by elevated levels of anti-NMDA antibodies in both serum[34] and CSF of diseased MRL/lpr mice (D. Ma, B. Diamond, R. Klein, H. Zhao, M. Kapadia and B. Sakic, in preparation). If the NMDA hypothesis of CNS SLE is indeed true and MEM can be used as a therapeutic modality,[7] then prolonged treatment should have beneficial effects in the "spontaneous" CNS SLE model too. Taken together, the present study examines whether prolonged administration of MEM prevents the constellation of behavioral deficits and brain atrophy in the spontaneous MRL/lpr model.[18,35,36]

Methods

Mice

To avoid the potential confounding effects of estrus cycling on behavioral performance, male mice, which develop a comparable disease to MRL/lpr females,[17] were used. A total of 24 males from MRL/lpr and MRL$^{+/+}$ substrains were purchased from The Jackson Laboratory (Bar Harbor, ME, USA) at 4 weeks-of-age. Animals were matched for bodyweight and assigned into four groups ($n = 12$ mice/group) according to substrain and treatment. Mice were habituated over 5 days and singly-housed under standard laboratory conditions (light 08.00–20.00 hours, room temperature ~22°C, humidity ~62%, regular rodent chow and tap water *ad libitum*, bedding changed every 3–4 days). Two MRL/lpr mice died prematurely, thus reducing the sample size to $n = 46$. Bodyweight was recorded on a weekly basis and wet spleen weight (an index of autoimmune status) was measured on an analytical scale at death. All protocols were carried out in accordance with the rules and regulations of the Canadian Council of Animal Care, and approved by the local Animal Research Ethics Board.

Drug administration

To avoid confounding behavioral effects of injection-induced stress, memantine-hydochloride (MEM,

lot M9292; Sigma-Aldrich, St Louis, MO, USA) was dissolved in tap water and mice were allowed to drink it *ad libitum* from leak-proof bottles (Ancare, Bellmore, NY, USA). Based on body size and daily fluid intake (6–8 mL), a single mouse ingested between 20 and 30 mg/kg bodyweight daily, which was previously shown to fall within the therapeutic dose range in a mouse model of Alzheimer's disease.[37] The other half of the male mice were provided with drinking tap water (vehicle). Treatment started at 5 weeks-of-age, and persisted over 9 weeks: MEM was given 5 weeks before behavioral testing, was continued throughout the testing period (10–14 weeks-of-age) and terminated 2 days before death. The rationale for such a design was based on previous findings showing CNS involvement begins at approximately 8 weeks-of-age[26,38] and antedates systemic manifestations evident approximately 4 months after birth.[17]

Behavioral testing

A single test from our behavioral battery was given nightly to cohorts from each group in the order described below.

Sucrose preference test

Impaired preference for palatable stimulation is proposed to model anhedonia, the second core symptom of depression.[39] Indeed, in the MRL model, this paradigm shows a deficit in central reward circuits, and not changes in peripheral sensory input.[24] The 60-min sucrose preference test was carried out in the evening hours, as described earlier.[23] To determine the dose-response in a linear manner, 1–8% solutions were provided to mice and the consumption of sucrose mass was calculated for each concentration.

Spontaneous nocturnal activity

As described earlier,[12] spontaneous nocturnal activity was assessed from 18.00 to 08.00 hours by measuring distance and time traversed in computerized activity boxes (VersaMax; AccuScan Instruments, Columbus, OH, USA).

Open eld/novel object test

The novel object test was used to assess anxiety-like behavior and exploratory drive in a conflict (approach–avoidance) setting.[20] Each mouse was gently placed in a corner of a square table (160 × 160 cm, elevated ~50 cm) with a blue, steel cylinder (height = 12 cm) in its center. The test lasted 30 min (carried out daily from 18.00 to 22.00 hours) and behavior was videotaped with an overhead hard drive-based video camera. The table was cleaned with a mild solution of glass cleaner between trials. EthoVision XT 5 tracking software (Noldus Information Technology, Leesburg, VA, USA) was used to measure moving distance, moving time, "thigmotaxis" or time spent along the perimeter (thigmotaxic zone was defined as a 16-cm wide band along table edges). Time spent exploring the cylinder was assessed using a three-point tracking feature, with snout as the reference point during object sniffing, climbing or biting.

Climbing test

Spontaneous climbing is a behavioral pattern proposed to be controlled by the dopamine system.[40,41] Furthermore, several lines of evidence suggest aberrant dopaminergic neurotransmission in autoimmune MRL/lpr mice.[42–45] We further examined this notion and the effects of MEM by using a brief climbing test. Mice were placed in a rectangular box (height = 28 cm, weight = 26 cm, depth = 9 cm) made of wire-mesh and videotaped for 10 min. Duration and frequency of climbing, rearing and grooming were scored using the Observer XT software package (Noldus Information Technology).

Step-down test

Mouse readiness to escape from an elevated platform placed in an unfamiliar, brightly-lit and spacious environment is proposed to reflect an anxious response, which differs in MRL substrains.[20] Each mouse was gently placed on a wire-mesh covering a rectangular glass box. The time to step-down onto a black surface with all four paws was recorded in a 5-min trial. Step-down latency was assessed from video recordings using a stopwatch.

Rotarod test

Muscle strength and acquisition of sensorimotor coordination were assessed using the rotarod test (EZRod version 1.20; Accuscan Instruments). Three daily trials were carried out over 2 days, with the latency and speed at fall recorded under the following parameters: duration of trial 5 min, maximal speed 20 r.p.m. and time to maximal speed 15 s.

Beam walking

Being sensitive to motor cortex damage,[46] walking on a narrow beam is often used to test psychomotor coordination in rodents. "Shaping protocol" and other details were reported previously.[47] In the pres-

ent study, a single test was recorded and traversing time was analyzed with Observer XT scoring software (Noldus Information Technology).

Forced swim test

Increased floating in a no-escape situation is proposed to reflect depressive-like behavior.[48] In the present study, each mouse was gently lowered into a circular swimming pool (diameter 183 cm) along the inner side of the wall. Floating time during the 10-min test was measured by EthoVisionXT software (Noldus Information Technology) using swimming velocity <2.5 cm/s as the criterion for floating.

Morris water maze

Using the same aforementioned swimming pool, we measured spatial learning and memory formation, known to be affected in MRL/lpr mice.[12,19] Mice were trained in four, 2-min cue trials (day 1), with the platform above the water surface and a blue cylinder placed on the top. On day 2, the platform was hidden in the North West quadrant and four acquisition trials were carried out daily over 4 days. To examine whether a spatial learning strategy was used, a 2-min probe trial was carried out on day 6. "Cognitive flexibility" was measured in four reversal trials. All behaviors were measured with EthoVisionXT software (Noldus Information Technology). Latency to find the platform, distance traversed and swimming speed were recorded. The time spent in the NW quadrant was measured in the probe trial.

Tissue collection and MRI analysis

At death, bodyweight and wet spleen weight were recorded on an analytical scale. Tissue preparation and MRI recording with a multichannel 7.0-T MRI scanner (Varian, Palo Alto, CA, USA) were carried out as described in detail elsewhere.[35] The custom alignment procedure[49] was used to compute the volume of 62 structures in each of the specimens based on a 3-D anatomical MRI atlas of the mouse brain.[50] Two-way analysis of variance was carried out using the software package R (http://www.r-project.org/) for each anatomical structure with substrain, treatment and substrain : treatment interaction as factors. For comparison, a three-way ANCOVA was also carried out, which included bodyweight (covariate), substrain (factor), treatment (factor) and all interactions between the three. Each *F*-value obtained in the analysis was corrected for multiple comparisons across the 62 structures using the false discovery rate method.[51]

In addition to the analysis of anatomical structure volumes, whole brain maps of local volume differences were created by applying the aforementioned statistical procedures on a point-by-point basis throughout the brain.[52,53] This procedure allowed for the direct 3-D visualization of brain regions affected by each factor. For this analysis, the transformation data was smoothed with a 0.5-mm Gaussian kernel and the significance threshold established based on a 5% false discovery rate.

Statistical analysis

The specimen weight and behavioral results were analyzed by analysis of variance (ANCOVA) with substrain and treatment as between-group factors, and age, sucrose concentration and time as within-group factors. Student's *t*-test was used in *post-hoc* analysis. Pearson's and Spearman's correlations were used to assess associations between variables. Graphs show mean values ± SEM and significant differences of $P \leq 0.05$, $P < 0.01$ and $P < 0.001$ are indicated by *, **, and ***, respectively. All computations were carried out using the SPSS 16 statistical package (SPSS, Chicago, IL, USA).

Results

Although MRL/lpr mice were heavier before MEM treatment commenced (substrain: $F_{1,43} = 10.309$, $P = 0.003$), they were lighter than $MRL^{+/+}$ mice at the end of the study (substrain: $F_{1,42} = 16.538$, $P < 0.001$; Fig. 1a). This effect was largely accounted for by prolonged exposure to MEM in both experimental and control groups (treatment: $F_{1,42} = 9.518$, $P = 0.004$). As expected, splenomegaly (a peripheral marker of disease severity) was confirmed in MRL/lpr mice (substrain: $F_{1,42} = 88.528$, $P < 0.001$; Fig. 1b). Despite a trend for reduced spleen weight in drug-treated groups (treatment: $F_{1,42} = 3.248$, $P = 0.079$), an immunosuppressive effect of MEM seems unlikely given its association with overall growth impairment, as shown by a significant correlation between spleen size and body mass within the MRL/lpr group ($r_{20} = 0.582$, $P = 0.004$).

Blunted responsiveness to sucrose in MRL/lpr mice was confirmed by a lower slope of the regression line and raw concentration-intake data analysis (substrain × concentration: $F_{3,132} = 7.995$, $P < 0.001$, Fig. 2a). However, chronic MEM treatment increased performance of both substrains (treatment: $F_{1,44} = 5.941$, $P = 0.019$), suggesting a pharmacological, but not an immunomodulatory effect. Conversely, spontaneous

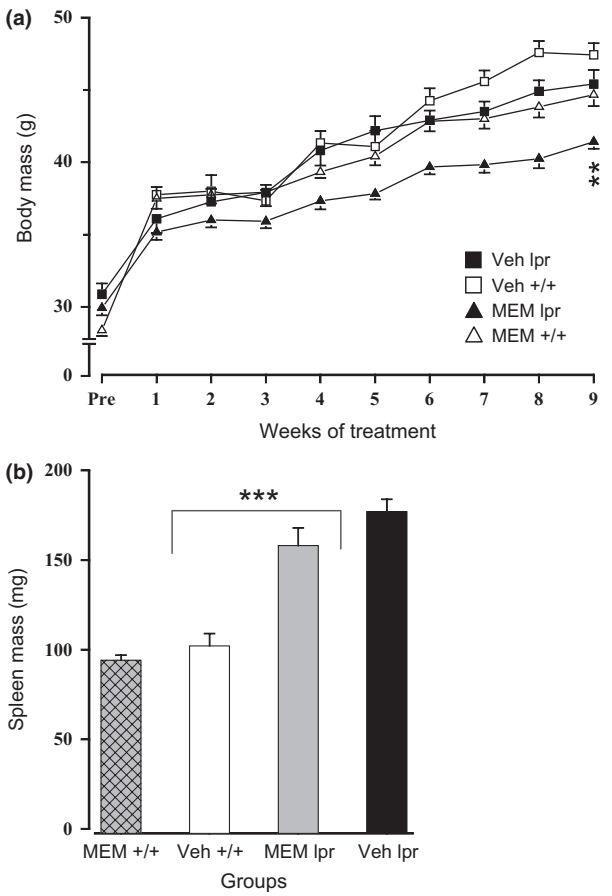

Figure 1 (a) Detrimental effects of sustained exposure to memantine (MEM) on growth, as evidenced by retarded body mass gain in MEM-treated groups. (b) Autoimmune status in MRL/lpr groups was confirmed by splenomegaly. MEM-treated mice showed a trend for decreased spleen mass, but this effect significantly correlated with the overall impairment in body growth ($P = 0.004$). Mean values ± SEM. $^{**}P < 0.01$; $^{**}P < 0.001$. Veh, vehicle.

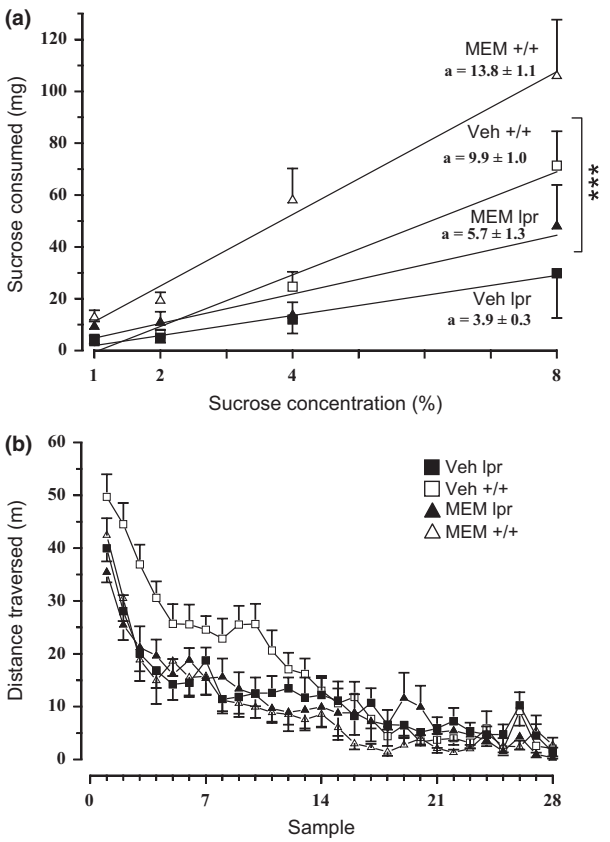

Figure 2 (a) Chronic treatment with memantine (MEM) improved responsiveness to sucrose in both MRL substrains. Although beneficial, this pharmacological effect is clearly independent of immunological status. (b) As expected, dissimilar spontaneous activity levels were confirmed by comparing vehicle (Veh)-treated MRL/lpr and $MRL^{+/+}$ groups. However, MEM significantly affected performance in the $MRL^{+/+}$ group, as evidenced by impaired novelty-induced hyperactivity, and shorter distances traversed and movement time (data not shown) during the night phase. Mean values ± SEM. $^{***}P < 0.001$.

activity in the $MRL^{+/+}$ control group was significantly reduced by MEM, as evidenced by a shorter distance traversed (substrain × treatment: $F_{1,43} = 6.113$, $P = 0.004$, Fig. 2b) and reduced movement time (substrain × treatment: $F_{1,43} = 4.34$, $P = 0.043$; data not shown). More interestingly, the novel object test showed an effect that could be immunomodulatory in nature. Namely, without affecting the performance of the control group, MEM treatment increased MRL/lpr exploration of a novel object (substrain × treatment: $F_{1,43} = 4.138$, $P = 0.048$, Fig. 3a). Other measures, such as moving distance, moving time and thigmotaxis, were not affected. Taken together, the results from the novel object test suggest exploratory drive and/or olfaction (rather than anxiety-related behaviours) were altered by sustained NMDA receptor blockade in autoimmune MRL/lpr mice. In the wire-mesh box, MRL/lpr climbed less frequently than $MRL^{+/+}$ mice (substrain: $F_{1,43} = 15.624$, $P < 0.001$; data not shown). However, the time they spent climbing the wall was not reduced by MEM, in contrast to MEM-treated $MRL^{+/+}$ controls (substrain by treatment: $F_{1,43} = 4.402$, $P = 0.042$; Fig. 3b). No significant group differences were detected with respect to rearing and grooming frequency or duration. In the step-down test, MEM treatment failed to reduce longer step-down latency in the MRL/lpr group (substrain: $F_{1,42} = 6.385$, $P = 0.012$; Fig. 4a).

As shown in Fig 4b, MRL/lpr mice showed no deficits in muscle strength or motor coordination when tested in the rotarod test. Conversely, their performance was better than control mice when fall latency (substrain: $F_{1,42} = 24.154$, $P < 0.001$) or speed at fall were considered (substrain: $F_{1,42} =$

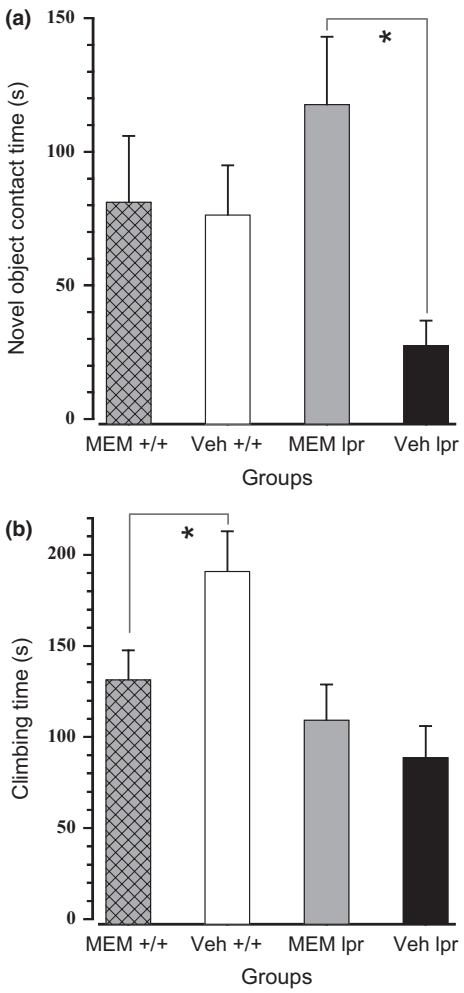

Figure 3 (a) After prolonged treatment with memantine (MEM), MRL/lpr mice spent significantly more time exploring the novel object. Considering this effect was substrain-specific, the results suggest MEM might be capable of inhibiting unknown immunopathogenic circuit(s) in autoimmune mice. (b) Conversely, sustained exposure to MEM decreased climbing time exclusively in $MRL^{+/+}$ controls, which (when untreated) spent more time climbing the mesh wall in comparison with diseased MRL/lpr mice. Mean values ± SEM. $*P \leq 0.05$. Veh, vehicle.

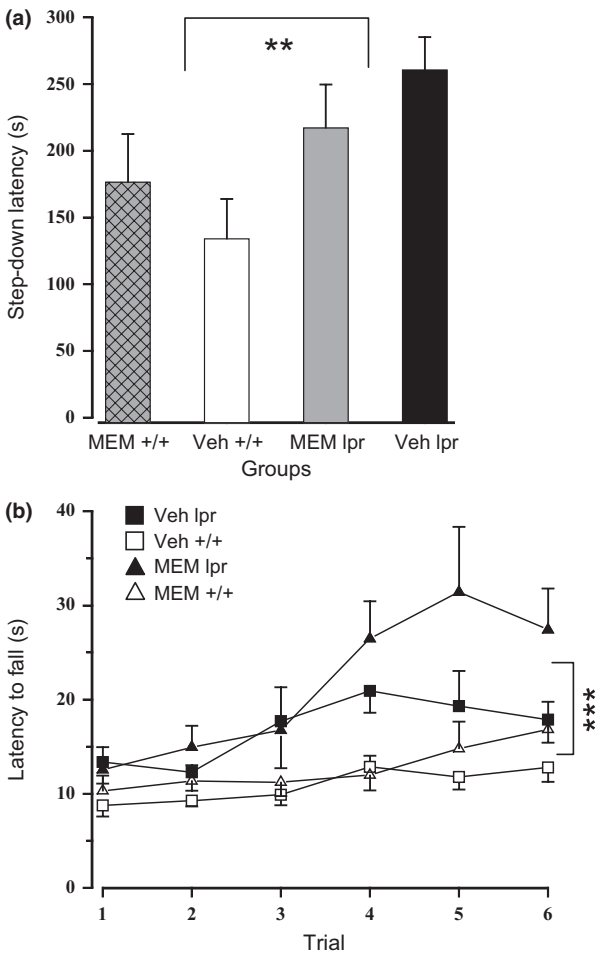

Figure 4 (a) Increased step-down latency in MRL/lpr mice was not affected by prolonged administration of memantine (MEM). (b) Performance of MRL/lpr mice in the rotarod test was consistently better than in control mice. However, significant negative correlations between body mass and fall speed showed that smaller animals (in this case MRL/lpr mice) were generally better performers on the rotarod test than heavy mice. Nevertheless, these results demonstrated that the diseased MRL/lpr group does not show deficits in movement coordination and muscle strength. More interestingly, sustained MEM administration improved sensorimotor learning over the testing period in both groups. Mean values ± SEM. $**P < 0.01$; $***P < 0.001$. Veh, vehicle.

19.341, $P < 0.001$). Furthermore, MEM treatment increased fall latency in both groups (treatment: $F_{1,42} = 4.672$, $P = 0.036$). However, significant negative correlations between body mass and fall speed (even within the group of untreated mice; falling speed $rho_{20} = –0.585$, $P = 0.004$) suggested that smaller mice were generally better performers on the rotarod test than heavy mice. In the beam walking test, MEM did not reduce longer traversing time in the MRL/lpr group (substrain: $F_{1,42} = 3.993$, P = 0.05; Fig. 5a). Similarly, it was ineffective in reducing immobility of MRL/lpr mice exposed to the forced swim test (substrain: $F_{1,42} = 5.329$, $P = 0.026$; Fig. 5b). In the Morris water maze, MEM failed to reduce longer latencies of MRL/lpr mice to locate the platform in cue and reversal trials (substrain: $F_{1,42} = 15.306$, $P < 0.001$; Fig. 6a). In contrast, treatment increased latencies in both substrains when tested in cue trials (treatment: $F_{1,43} = 8.168$, $P = 0.042$; Fig. 6b) and the probe trial (treatment: $F_{1,42} = 6.916$, $P = 0.012$; 7–10 s on average, data not shown). As shown earlier,[12] during "reversal learn-

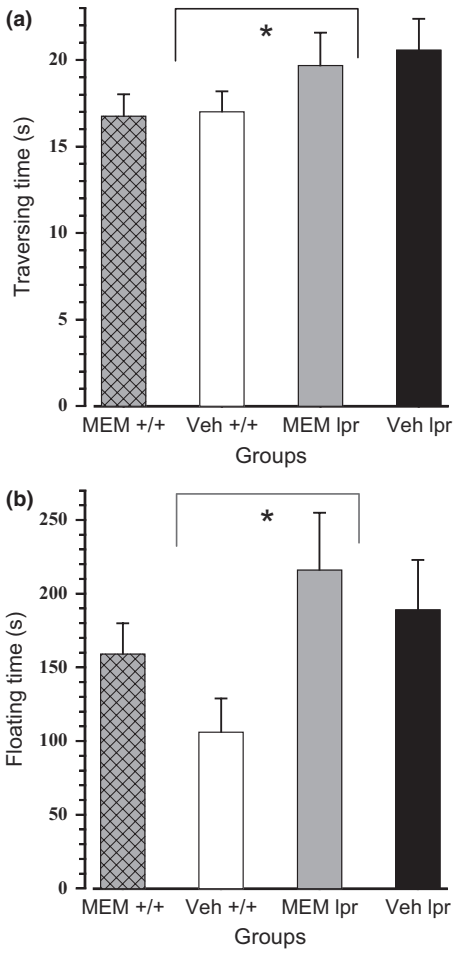

Figure 5 (a) In the beam-walking test, sustained memantine (MEM) treatment was not effective in reducing longer traversing time in the MRL/lpr substrain. (b) Similarly, it was completely ineffective in reducing increased immobility of MRL/lpr mice in the forced swim test. Mean values ± SEM. *$P \leq 0.05$. Veh, vehicle

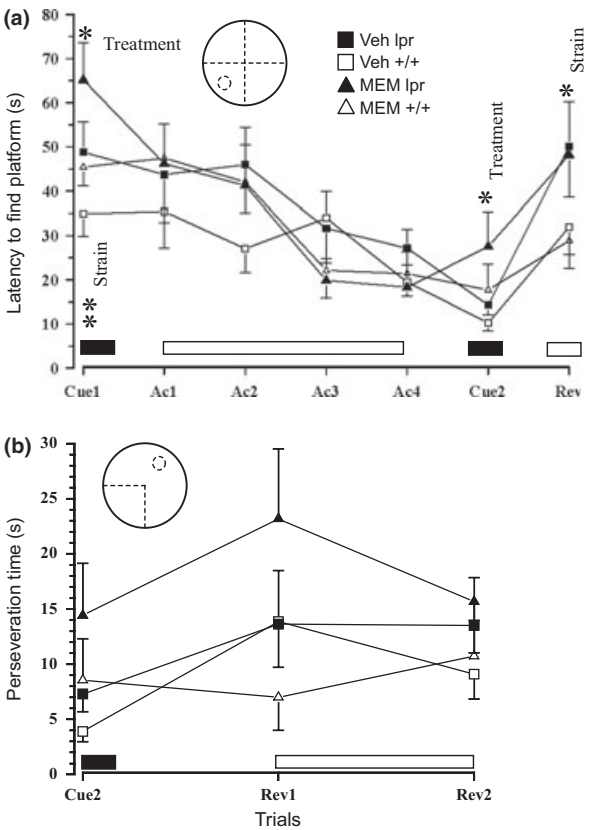

Figure 6 (a) Prolonged drug treatment of MRL/lpr mice failed to reduce increased latencies to locate the platform in cue and reversal trials. Conversely, memantine (MEM) comparably increased the latency in cue trials and the time spent in the southwest quadrant during the probe trial (data not shown). (b) During "reversal learning", longer search time in the MRL/lpr group was associated with increased perseveration of the previously learned response and could not be abolished with MEM treatment. Solid and open blocks indicate when the escape platform was either visible or invisible, respectively. Mean values ± SEM. *$P \leq 0.05$; **$P < 0.01$. Ac, acquisition trials; Cue, cue trials; Rev, reversal trials; Veh, vehicle.

ing", longer search time in the MRL/lpr group was associated with increased perseveration of a previously learned response (substrain: $F_{1,42} = 6.783$, $P = 0.013$; Fig. 6b).

Two-way analysis of variance with substrain and treatment as factors reproduced previously reported substrain effects on regional anatomical volumes.[35] Brain volume was found to be highly related to bodyweight at death, such that a significant proportion of interindividual variation could be explained. Including bodyweight as the first factor in a three-way ANCOVA with substrain and treatment, and all cross-terms as factors, bodyweight was found to be significant in all brain regions (false discovery rate [FDR] <5%). In addition, the effect of treatment after accounting for bodyweight and substrain was significant in 53 out of 62 regions. Table 1 shows the volume of anatomical structures identified in the atlas for each substrain and treatment group. Also shown is the effect of treatment for each region, computed using the ANCOVA model and accounting for bodyweight. The effect of treatment is a relatively uniform increase in volume across much of the brain that did not differ between the substrains.

No evidence supporting a differential effect of treatment between the two substrains was found. Regressing bodyweight against substrain and treatment showed a significant effect of treatment that did not differ by substrain, such that MEM-treated mice were 2.3 g lighter on average ($P < 0.01$). Recognizing that the association among brain vol-

Table 1 Brain region volumes for each substrain and treatment group

Region	Untreated MRL$^{+/+}$ mean ± SD (mm^3)	Untreated MRL/lpr mean ± sd (mm^3)	Treated MRL$^{+/+}$ mean ± SD (mm^3)	Treated MRL/lpr mean ± SD (mm^3)	Treatment effect (%)	Treatment effect significance
Dentate gyrus of hippocampus	4.39 ± 0.17	4.12 ± 0.18	4.57 ± 0.25	4.12 ± 0.16	5.8	0.0073
Cerebellar peduncle inferior	0.99 ± 0.03	0.93 ± 0.04	1.01 ± 0.05	0.94 ± 0.03	2.6	0.0089
Cerebral aqueduct	1.56 ± 0.05	1.46 ± 0.07	1.62 ± 0.09	1.46 ± 0.06	4.2	0.0089
Cerebral peduncle	2.92 ± 0.12	2.77 ± 0.13	3.01 ± 0.15	2.76 ± 0.11	5.0	0.0089
Colliculus inferior	5.26 ± 0.23	4.86 ± 0.32	5.38 ± 0.26	4.89 ± 0.21	4.6	0.0089
Colliculus superior	9.32 ± 0.40	8.40 ± 0.47	9.59 ± 0.56	8.52 ± 0.40	4.8	0.0089
Corpus callosum	15.90 ± 0.82	14.95 ± 0.66	16.47 ± 0.78	14.77 ± 0.65	5.5	0.0089
Habenular commissure	0.81 ± 0.03	0.77 ± 0.03	0.83 ± 0.04	0.76 ± 0.03	4.2	0.0089
Hippocampus	19.80 ± 0.88	18.60 ± 0.77	20.43 ± 1.12	18.52 ± 0.84	5.3	0.0089
Internal capsule	3.08 ± 0.13	2.90 ± 0.12	3.18 ± 0.18	2.89 ± 0.11	4.9	0.0089
Mammillary bodies	1.31 ± 0.04	1.24 ± 0.05	1.35 ± 0.06	1.24 ± 0.05	3.9	0.0089
Midbrain	22.80 ± 1.05	20.87 ± 1.35	23.41 ± 1.40	20.99 ± 0.89	4.4	0.0089
Olfactory bulbs	19.15 ± 0.64	18.06 ± 0.48	19.70 ± 0.98	18.37 ± 0.58	3.3	0.0089
Periaqueductal grey	9.46 ± 0.37	8.68 ± 0.44	9.77 ± 0.53	8.72 ± 0.38	4.4	0.0089
Posterior commissure	1.15 ± 0.05	1.08 ± 0.04	1.18 ± 0.06	1.08 ± 0.04	4.5	0.0089
Stratum granulosum of hippocampus	2.42 ± 0.10	2.29 ± 0.07	2.51 ± 0.12	2.26 ± 0.09	5.4	0.0089
Stria medullaris	1.15 ± 0.04	1.09 ± 0.04	1.19 ± 0.07	1.08 ± 0.04	4.8	0.0089
Stria terminalis	1.41 ± 0.06	1.33 ± 0.05	1.45 ± 0.08	1.32 ± 0.05	4.9	0.0089
Thalamus	16.87 ± 0.83	15.75 ± 0.60	17.59 ± 1.11	15.78 ± 0.60	5.8	0.0089
Fourth ventricle	1.07 ± 0.04	1.01 ± 0.04	1.10 ± 0.05	1.01 ± 0.04	3.6	0.0096
Arbor vita of cerebellum	7.91 ± 0.35	7.50 ± 0.39	8.08 ± 0.47	7.64 ± 0.39	3.4	0.01
Cerebellar peduncle superior	1.00 ± 0.05	0.93 ± 0.06	1.03 ± 0.06	0.94 ± 0.04	3.8	0.01
Cuneate nucleus	1.19 ± 0.04	1.13 ± 0.04	1.22 ± 0.06	1.12 ± 0.04	3.3	0.01
Facial nerve cranial nerve 7	1.08 ± 0.04	1.02 ± 0.04	1.11 ± 0.05	1.02 ± 0.04	3.9	0.01
Fasciculus retroflexus	1.64 ± 0.06	1.56 ± 0.06	1.68 ± 0.08	1.55 ± 0.06	3.9	0.01
Fimbria	3.68 ± 0.18	3.49 ± 0.13	3.81 ± 0.23	3.43 ± 0.17	5.7	0.01
Fornix	1.39 ± 0.06	1.32 ± 0.05	1.43 ± 0.07	1.31 ± 0.05	4.1	0.01
Cerebellar peduncle middle	1.76 ± 0.06	1.68 ± 0.05	1.81 ± 0.09	1.67 ± 0.07	4.2	0.011
Mammilothalamic tract	0.72 ± 0.03	0.68 ± 0.03	0.74 ± 0.04	0.68 ± 0.03	4.1	0.011
Pons	29.69 ± 1.18	27.43 ± 1.27	30.34 ± 1.38	27.71 ± 0.95	3.3	0.011
Pre para subiculum	2.47 ± 0.12	2.33 ± 0.16	2.50 ± 0.12	2.31 ± 0.09	3.8	0.011
Optic tract	1.31 ± 0.06	1.24 ± 0.05	1.35 ± 0.07	1.24 ± 0.05	4.3	0.012
Third ventricle	2.06 ± 0.08	1.95 ± 0.07	2.13 ± 0.11	1.94 ± 0.09	4.8	0.013
Anterior commissure pars posterior	1.60 ± 0.06	1.51 ± 0.05	1.64 ± 0.08	1.50 ± 0.06	3.8	0.013
Anterior commissure pars anterior	1.67 ± 0.07	1.55 ± 0.07	1.70 ± 0.08	1.55 ± 0.05	3.3	0.015
Interpedunclar nucleus	1.26 ± 0.05	1.19 ± 0.05	1.29 ± 0.06	1.19 ± 0.05	3.4	0.015
Ventral tegmental decussation	1.04 ± 0.04	0.98 ± 0.04	1.06 ± 0.05	0.97 ± 0.04	3.7	0.016
Bed nucleus of stria terminalis	1.94 ± 0.08	1.82 ± 0.07	1.98 ± 0.11	1.81 ± 0.07	3.7	0.017
Lateral olfactory tract	1.75 ± 0.06	1.65 ± 0.04	1.79 ± 0.08	1.64 ± 0.06	3.2	0.018
Subependymale zone rhinocele	0.60 ± 0.02	0.57 ± 0.02	0.61 ± 0.03	0.56 ± 0.02	3.5	0.018
Globus pallidus	3.61 ± 0.16	3.40 ± 0.15	3.69 ± 0.21	3.40 ± 0.13	3.5	0.019
Fundus of striatum	1.36 ± 0.06	1.30 ± 0.05	1.40 ± 0.07	1.28 ± 0.05	4.1	0.02
Cerebellar cortex	47.96 ± 1.83	46.68 ± 2.39	49.03 ± 2.46	46.40 ± 2.12	3.5	0.025
Medial septum	2.60 ± 0.11	2.47 ± 0.08	2.67 ± 0.15	2.45 ± 0.11	3.7	0.025
Hypothalamus	8.84 ± 0.45	8.39 ± 0.40	9.14 ± 0.51	8.44 ± 0.45	4.3	0.027

Table 1 (continued)

Region	Untreated $MRL^{+/+}$ mean ± SD (mm^3)	Untreated MRL/lpr mean ± sd (mm^3)	Treated $MRL^{+/+}$ mean ± SD (mm^3)	Treated MRL/lpr mean ± SD (mm^3)	Treatment effect (%)	Treatment effect significance
Pontine nucleus	2.03 ± 0.07	1.88 ± 0.05	2.08 ± 0.11	1.86 ± 0.09	3.8	0.027
Inferior olivary complex	0.91 ± 0.04	0.86 ± 0.04	0.93 ± 0.05	0.85 ± 0.03	3.5	0.034
Medial lemniscus medial longitudinal fasciculus	2.59 ± 0.12	2.39 ± 0.09	2.65 ± 0.13	2.40 ± 0.10	3.2	0.036
Superior olivary complex	1.29 ± 0.06	1.21 ± 0.06	1.31 ± 0.05	1.20 ± 0.04	2.4	0.038
Medulla	55.80 ± 2.18	51.39 ± 2.09	56.74 ± 2.57	51.75 ± 2.15	2.2	0.044
Striatum	23.16 ± 1.14	21.63 ± 0.92	23.78 ± 1.32	21.32 ± 1.31	3.9	0.049
Basal forebrain	6.83 ± 0.28	6.48 ± 0.23	6.97 ± 0.35	6.44 ± 0.24	2.8	0.049
Nucleus accumbens	4.69 ± 0.23	4.42 ± 0.17	4.81 ± 0.24	4.39 ± 0.19	3.2	0.049
Cerebral cortex frontal lobe	42.50 ± 2.29	40.42 ± 1.35	43.94 ± 2.17	39.55 ± 2.43	5.5	0.051
Corticospinal tract pyramids	2.33 ± 0.13	2.15 ± 0.08	2.38 ± 0.12	2.15 ± 0.10	3.4	0.051
Cerebral cortex parieto temporal lobe	70.18 ± 3.76	67.87 ± 2.63	71.80 ± 3.31	66.93 ± 3.44	3.7	0.07
Lateral septum	3.50 ± 0.17	3.30 ± 0.12	3.59 ± 0.24	3.22 ± 0.20	4.3	0.07
Cerebral cortex entorhinal cortex	8.89 ± 0.29	8.61 ± 0.30	9.04 ± 0.45	8.51 ± 0.34	2.6	0.088
Lateral ventricle	3.05 ± 0.17	2.96 ± 0.13	3.15 ± 0.20	2.82 ± 0.19	6.0	0.1
Olfactory tubercle	3.82 ± 0.20	3.66 ± 0.12	3.90 ± 0.20	3.61 ± 0.14	1.5	0.25
Amygdala	15.05 ± 0.65	14.54 ± 0.74	15.31 ± 0.79	14.01 ± 0.70	2.3	0.41
Cerebral cortex occipital lobe	5.83 ± 0.17	5.56 ± 0.26	5.85 ± 0.23	5.34 ± 0.26	1.9	0.77

Values are presented as mean ± standard deviation. The effect of treatment (estimated from the ANCOVA model) is expressed as a percentage of $MRL^{+/+}$ volume for that structure. Also shown is the significance of the treatment expressed as a false discovery rate to account for the multiple comparisons. Regions are sorted from most to least significant treatment effect.

ume, bodyweight and treatment could lead to a false association between treatment and brain volume, we examined the spatial pattern of volume change associated with these factors. The results of applying the same, three-way ANCOVA procedure at every point in the brain are shown in Fig. 7. For each factor, the subset of statistically significant points at a FDR of 5% are shown in color. The color scale shows the effect size on volume. As no interaction between substrain and treatment survived correction for multiple comparisons, the treatment effect was averaged for the two substrains. As seen in Fig. 7, treatment led to a pattern of brain volume increases that was different from that of substrain and bodyweight. The areas enlarged by MEM treatment were mainly periventricular. Bodyweight was associated with a pattern of increase that was largely uniform throughout the brain, although larger increases were observed in the temporal lobe.

Discussion

We previously established that the two MRL substrains differ in responsiveness to neurotransmitter modulators, such as quinpirole, amphetamine, sertraline and risperidone.[42,44,54] In the present study, we observed discrepancies in responsiveness to MEM when nocturnal activity and climbing behavior were considered. Although these findings might indicate dissimilar activity of the NMDA receptor system, the fact that MEM also binds with similar potency to other receptors[55,56] suggests detrimental effects in control mice and lack of responsiveness in MRL/lpr mice could also be mediated by other neurotransmitters. Such a mechanism would be particularly relevant to the dopaminergic system, where MEM might act as a receptor inhibitor and blocker of endocrine function.[57]

Based on the neuroprotective effects of MEM in a peptide-induced model of CNS SLE,[6,7] and clinical studies showing a relationship between circulating anti-NR2 antibodies and neuropsychiatric manifestations,[58,59] we expected that prolonged administration of MEM would prevent or attenuate the constellation of behavioral deficits and brain atrophy in the spontaneous MRL/lpr model of CNS SLE. However, the present results do not support the hypothesis that autoimmunity-associated behavioral dysfunction and brain pathology are mediated exclusively by changes to the NMDA system. Namely, except for the increase in novel object exploration, prolonged

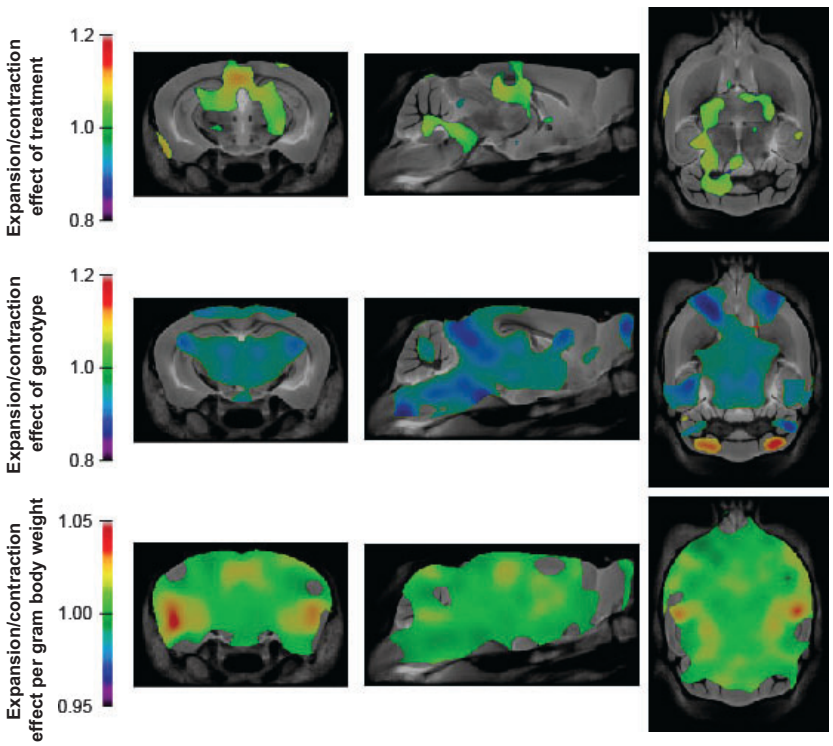

Figure 7 Coronal, sagittal and transverse sections are shown with a color overlay corresponding to the effect of treatment, substrain or bodyweight on local brain volume. The first row shows the relative difference of brain volume associated with treatment after accounting for bodyweight and substrain. The second row shows the relative size of MRL/lpr mice compared with $MRL^{+/+}$ after accounting for bodyweight. The third row shows the effect of bodyweight expressed in units of fractional volume increase per gram. Regions that are colored were significant at the false discovery rate of 5%.

exposure to MEM did not result in other beneficial effects in diseased MRL/lpr mice. More frequently, chronic MEM treatment produced comparable behavioral effects in both MRL substrains, as well as enlargement of brain volume. One might hypothesize that lack of more restorative effects represents the consequence of insufficient MEM dosage. However, significant effects on brain structure and function after the 9-week treatment are inconsistent with this possibility. Indeed, a more viable explanation is that SLE-like disease and CNS involvement in MRL/lpr mice are more severe and complex than modeled in the pentapeptide-immunized mice.[5] In other words, the NMDA system seems to act as one of multiple targets that account for the constellation of behavioral abnormalities in SLE patients and lupus-prone mice. Recent clinical reports are consistent with this possibility. In particular, levels of serum anti-NR2 antibodies are found to be associated with depressive mood, but not with cognitive dysfunction in CNS SLE patients.[60] Without the intention to anthropomorphize the current results, one might assume that the capacity of MEM to increase novel object exploration in MRL/lpr mice and the inability to prevent their "cognitive inflexibility" are in accordance with the aforementioned clinical findings. Another clinical study found that anti-NR2 antibodies are detected in the sera of 35% of SLE patients, but also failed to associate their presence with cognitive dysfunction.[61] Furthermore, Kozora et al.[62] failed to identify any significant relationships between serum anti-NR2 antibodies and global cognitive/memory indices, or with depression. The current lack of broad support for the anti-NMDA hypothesis and generalized behavioral dysfunction is further evidenced by a recent clinical trial in which prolonged MEM treatment largely failed to improve general cognitive function, with the exception of controlled oral word association.[63] Similarly, other clinical studies could not confirm the proposed relationship between serum anti-DNA and anti-NR2 receptor antibodies,[64,65] or the importance of serum anti-NR2 antibodies in the induction of CNS SLE.[66]

It was documented by our group that, when challenged with stimulants of dopamine release, MRL/lpr mice fail to increase sucrose intake[44] and behaviorally respond as control $MRL^{+/+}$ mice.[43,54] Therefore, the observed increase in sucrose intake ("anti-anhedonic" effect) in mice treated with MEM deserves particular attention. As aforementioned, MEM might affect the dopamine receptor system in a region- and cell-specific manner.[67–69] Therefore, direct stimulation of post-synaptic $D2^{high}$ receptors[57] in structures, such as nucleus accumbens, might be more effective than stimulation of dopaminergic

presynaptic neurons.[44] Whichever mechanism underlies MEM-induced increase in sucrose intake, it is clear that it does not depend on NMDA receptor blockade during lupus-like disease. Along the same line, despite significant negative correlations between body mass and rotarod performance, reduced bodyweight and improved rotarod performance in MEM-treated mice seem in concordance with reported effects of MEM on ingestive behavior[70,71] and sensorimotor capacity.[72]

Substantial within-group variability in brain volume was associated with bodyweight and was large enough to mask some of the morphological differences between substrains, as well as treatment-induced differences. Although incorporating bodyweight as a covariate allowed for the assessment of main effects, the observation that MEM reduces bodyweight complicates the interpretation of these results. A reduction in bodyweight caused by MEM treatment leads to a brain volume that is larger than expected. After accounting for bodyweight, we also observed a pattern of morphological change associated with MEM that was different from that associated with bodyweight alone. We interpret this as the direct effect of MEM on brain morphology that we were underpowered to detect without including bodyweight as a covariate.

Taken together, the obtained results suggest that effects of sustained exposure to MEM are largely pharmacological in nature, showing little restorative effect on behavior and brain morphology of autoimmune MRL/lpr mice. If the NMDA receptors were chronically blocked, then improved exploratory behavior in the MEM-treated MRL/lpr group suggests that the NMDA system is but one of multiple pathogenic circuits. Given the poor benefit-to-risk ratio, the present study represents the first line of experimental evidence that does not support chronic administration of MEM in the treatment of CNS SLE.

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