Mouse model of anti-NMDA receptor post–herpes simplex encephalitis

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Abstract

Objective
To develop an endogenous rodent model of postinfectious anti-NMDA receptor (NMDAR) encephalitis.

Methods
Six mice were inoculated intranasally with herpes simplex virus (HSV) 1 and subsequently treated with acyclovir for 2 weeks. Serum was collected at 3, 6, and 8 weeks postinoculation and tested for NMDAR antibodies through a cell-based assay. Eight weeks postinoculation, mice were killed and their brains were sectioned and immunostained with antibodies to postsynaptic density (PSD)-95 and NMDARs. Colocalization of hippocampal PSD-95 and NMDAR clusters, representing postsynaptic membrane NMDARs, was quantified via confocal imaging. Hippocampi were additionally analyzed for NMDAR and PSD-95 protein using Western blot analysis.

Results
Four of 6 mice (67%) developed serum antibodies to NMDARs: 1 at 3 weeks, 1 at 6 weeks, and 2 at 8 weeks postinoculation. As compared to inoculated mice that did not develop NMDAR antibodies, immunofluorescence staining revealed decreased hippocampal postsynaptic membrane NMDARs in mice with serum antibodies at 8 weeks postinoculation. Western blot analysis showed that mice that had NMDAR antibodies at 8 weeks had decreased total NMDAR but not PSD-95 protein in hippocampal extracts ($p < 0.05$).

Conclusions
Mice inoculated intranasally with HSV-1 developed serum NMDAR antibodies. These antibodies were associated with reduced hippocampal NMDARs, as has been shown in previous models where antibodies from patients with anti-NMDAR encephalitis were infused into mice, paving the way for future studies into the pathophysiology of autoimmune encephalitides.

Glossary

ACV = acyclovir; HSE = HSV encephalitis; HSV = herpes simplex virus; IgG = immunoglobulin G; NMDAR = NMDA receptor; PSD = postsynaptic density.

Herpes simplex virus (HSV)-1 is neurotropic[1] and highly destructive when it infects the brain. Despite treatment, HSV encephalitis (HSE) has a mortality of 10%–30%. Many patients with HSE are left with devastating sequelae, including short-term memory deficits, behavioral changes, and seizures. It was long thought that HSE recurs in a subset of patients despite treatment and known that some children develop post-HSE choreoathetosis.[2] However, repeat CSF analysis in these patients was negative for HSV. Surprisingly, retrospective analyses[3,4] detected NMDA receptor (NMDAR) antibodies in patients' sera and/or CSF, suggesting that the new neurologic symptoms actually represented autoimmune encephalitis. A subsequent prospective study has shown that 27% of patients with HSE develop autoimmune encephalitis, often associated with NMDAR antibodies.[5]

Based on these observations, the current study was undertaken to develop an endogenous rodent model of post-HSE NMDAR encephalitis to allow for detailed studies into the pathogenesis of autoimmune encephalitides. In the model described below, mice were infected with HSV-1 and subsequently treated with acyclovir (ACV), mirroring the clinical course of patients with HSE.

Methods

Please refer to the online supplemental methods (e-methods, links.lww.com/NXI/A92) for detailed descriptions of procedural details.

Standard protocol approvals

All animal procedures were approved by our Institutional Animal Care and Use Committee. Collection and banking of CSF from patients with autoimmune encephalitis was approved by our institutional review board.

Inoculation with HSV-1 and treatment with ACV

Six female BALB/c mice were intranasally inoculated with 0.5 (mouse 2) or 1×10^6 (mice 1 and 3–6) plaque-forming units of HSV-1 (strain 17 syn$^+$)[6] and treated with ACV (AuroMedics, 20 mg/kg/dose twice daily) via intraperitoneal injections for 2 weeks. Two age- and sex-matched control mice were inoculated with vehicle solution.

Serum collection and immunofluorescent cell-based assay for NMDAR Ab detection

Retro-orbital bleeds were performed preinoculation and at 3, 6, and 8 weeks post-HSV inoculation, and the presence of NMDAR antibodies in mouse sera was determined using a GluN1-NMDAR–transfected HEK293 cell–based assay as previously reported.[7]

Immunofluorescence and confocal microscopy

At 8 weeks, mice were killed, brains were harvested, and 7-μm-thick sections were stained for NMDARs and postsynaptic density (PSD)-95, similarly to previously described.[8] Spot detection and three-dimensional colocalization of NMDARs and PSD-95 were performed to quantify postsynaptic membrane NMDAR clusters.

Immunoblot analyses

Hippocampi were dissected and homogenized from thawed brain halves, nuclei were removed, and 10 μg protein (confirmed by bicinchoninic acid assay) of each sample was immunoblotted for NMDARs, PSD-95, and actin, similarly to previously described.[8]

Statistics

Results are expressed as mean with standard error of the mean error bars. NMDAR and PSD-95 colocalized spots, as well as hippocampal NMDAR and PSD-95 protein normalized to actin (loading control), were compared with one-way analysis of variance (ANOVA). Further pooled (NMDAR antibody–negative vs positive samples) were also compared with a Mann-Whitney U test. $p < 0.05$ was considered statistically significant.

Data availability

Data will be shared upon request from any qualified investigator.

Results

Mice inoculated intranasally with HSV-1 develop antibodies to NMDARs

Four of 6 mice (67%) inoculated intranasally with HSV-1 developed NMDAR antibodies in their sera, as demonstrated by a GluN1-transfected HEK293 cell–based assay (figure 1, A, B): HSE mouse 4 transiently at 3 weeks, HSE mouse 3 transiently at 6 weeks, and HSE mice 5 and 6 at 8 weeks postinoculation. No mice had NMDAR antibodies preinoculation, and serum antibodies were not detected in HSE mice 1 and 2 at any time point. HSE mouse 6 had immunoglobulin G (IgG) to an unknown antigen at 6 weeks (figure 1, B, arrows) and developed additional IgG to NMDARs at 8 weeks (figure 1, A, B).

Mice with antibodies to NMDARs have decreased hippocampal postsynaptic membrane NMDARs

Immunostaining for NMDARs (figure 2A) and colocalization with PSD-95 (figure 2C) revealed a decrease in hippocampal postsynaptic membrane NMDAR clusters in HSE mice 4–6

Figure 1 Mice inoculated intranasally with HSV-1 develop antibodies to NMDARs

HSE mouse	Preinoculation	Week		
		3	6	8
1	–	–	–	–
2	–	–	–	–
3	–	–	+	–
4	–	+	–	–
5	–	–	–	+
6	–	–	#	# +
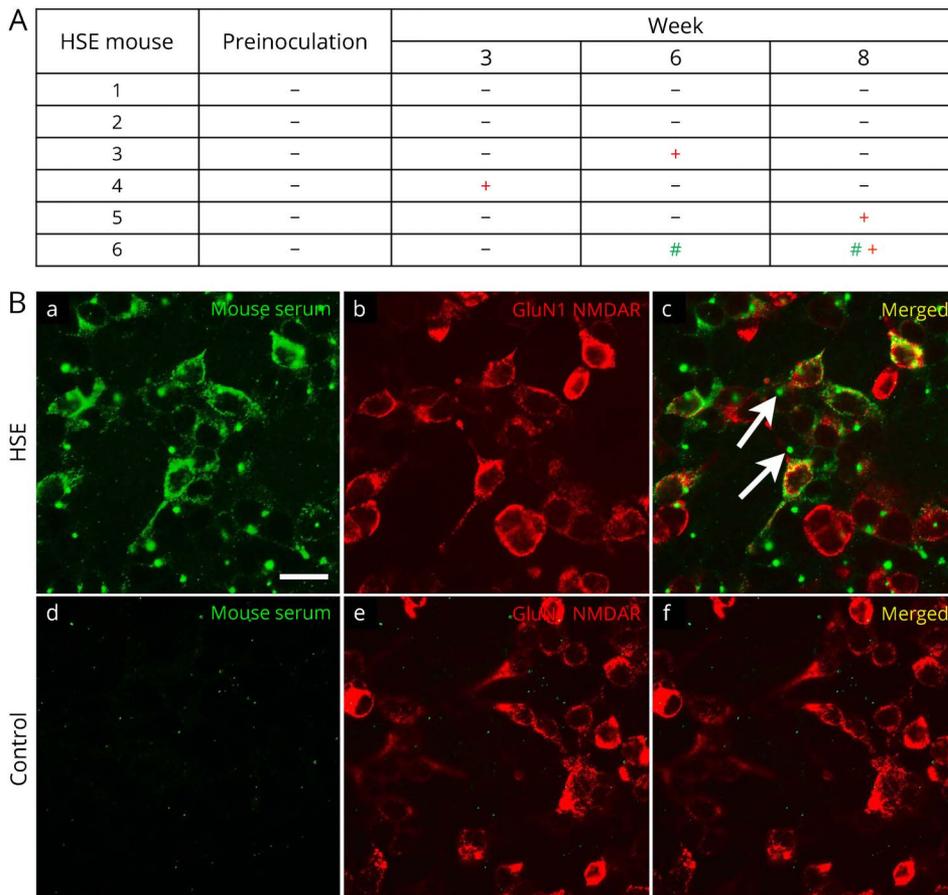
(A) NMDAR (GluN1 subunit)-expressing HEK293 cells incubated with mouse serum and probed with anti-mouse immunoglobulin G to evaluate for serum NMDAR antibodies (Abs) in HSE mice preinoculation and at 3, 6, and 8 weeks postinoculation with HSV-1. +: NMDAR Abs in serum; –: no NMDAR Abs in serum; #: antibody to unknown antigen in serum. (B) Representative images of GluN1-NMDAR–transfected HEK293 cell–based assay incubated with 8-week sample from HSE mouse 6 serum (a–c) or control serum (d–f) probed with anti-mouse immunoglobulin G (IgG; a, d) or commercial anti-NMDAR (GluN1) probed with anti-rabbit IgG (b and e). Merged images (c and f) show that whereas HSE mouse 6 developed serum antibodies that bind to NMDARs (as evident by colocalization with the commercial NMDAR Ab, c, yellow cell surface labeling), no NMDAR Abs are seen in control serum (f). Note the additional IgG Ab toward an unknown target (a, c; green dots, two of which are highlighted by arrows). Scale bar (a) = 10 μm.

compared with control mice and HSE mice 1–3. A significant difference was found between HSE mice 1 and 2 and HSE mice 5 and 6 (231 ± 23 vs 117 ± 12, respectively, $p = 0.03$) (figure 2B.a), as well as a trend toward a decrease between control mice and HSE mice 5 and 6 (204 ± 4 vs 117 ± 12, respectively, $p = 0.07$). When data were pooled based on serum NMDAR antibody status (control mice and HSE mice 1 and 2 vs HSE mice 3–6, figure 2B.b), a trend toward decreased hippocampal postsynaptic membrane NMDAR clusters was found (217 ± 12 vs 155 ± 25, $p = 0.20$).

### Mice with antibodies to NMDARs have decreased hippocampal NMDAR protein

Immunoblot analysis (figure 3A) revealed that HSE mice 5 and 6 had a significant decrease in total extranuclear hippocampal NMDAR but not PSD-95 protein compared with control mice and HSE mice 1 and 2 (figure 3B) (63.2 ± 9.3 vs 100.0 ± 8.3, $p = 0.0498$, and 101.3 ± 2.7, $p = 0.045$, respectively, normalized to mean of control as 100%). When data were pooled based on serum NMDAR antibody status (control mice and HSE mice 1 and 2 vs HSE mice 3–6), a similar decrease was found for hippocampal NMDAR (100.6 ± 3.6 vs 68.9 ± 5.0, $p = 0.029$) but not PSD-95 protein levels (figure 3C).

Discussion

In this mouse model of anti-NMDAR post-HSE encephalitis, we showed that two-thirds of mice inoculated intranasally with HSV-1 and treated with ACV developed serum NMDAR antibodies using the same cell-based assay that is used to detect NMDAR antibodies in human samples. One mouse (no. 6) developed antibodies to an unknown target in addition to NMDAR antibodies, further mirroring what has been reported in some patients after HSE.[5,9] Although NMDAR antibodies were not directly demonstrated to be binding to the mouse hippocampi in these studies, a decreased number of hippocampal postsynaptic NMDAR clusters and decreased hippocampal NMDAR protein were found in mice that had serum NMDAR antibodies at the time of sacrifice. The decrease in hippocampal NMDARs suggests that these mouse antibodies are similar to patient-derived NMDAR antibodies.[8] Effects on hippocampal NMDARs were less pronounced for mice that transiently developed NMDAR antibodies, suggesting that their effects may have been short lived.

The previously published animal model of NMDAR encephalitis[8,10] relies on adoptive transfer, infusing human NMDAR antibodies into mouse brains. Mice in that model have

Figure 2 Mice with antibodies to NMDARs have decreased hippocampal postsynaptic membrane clusters of NMDARs
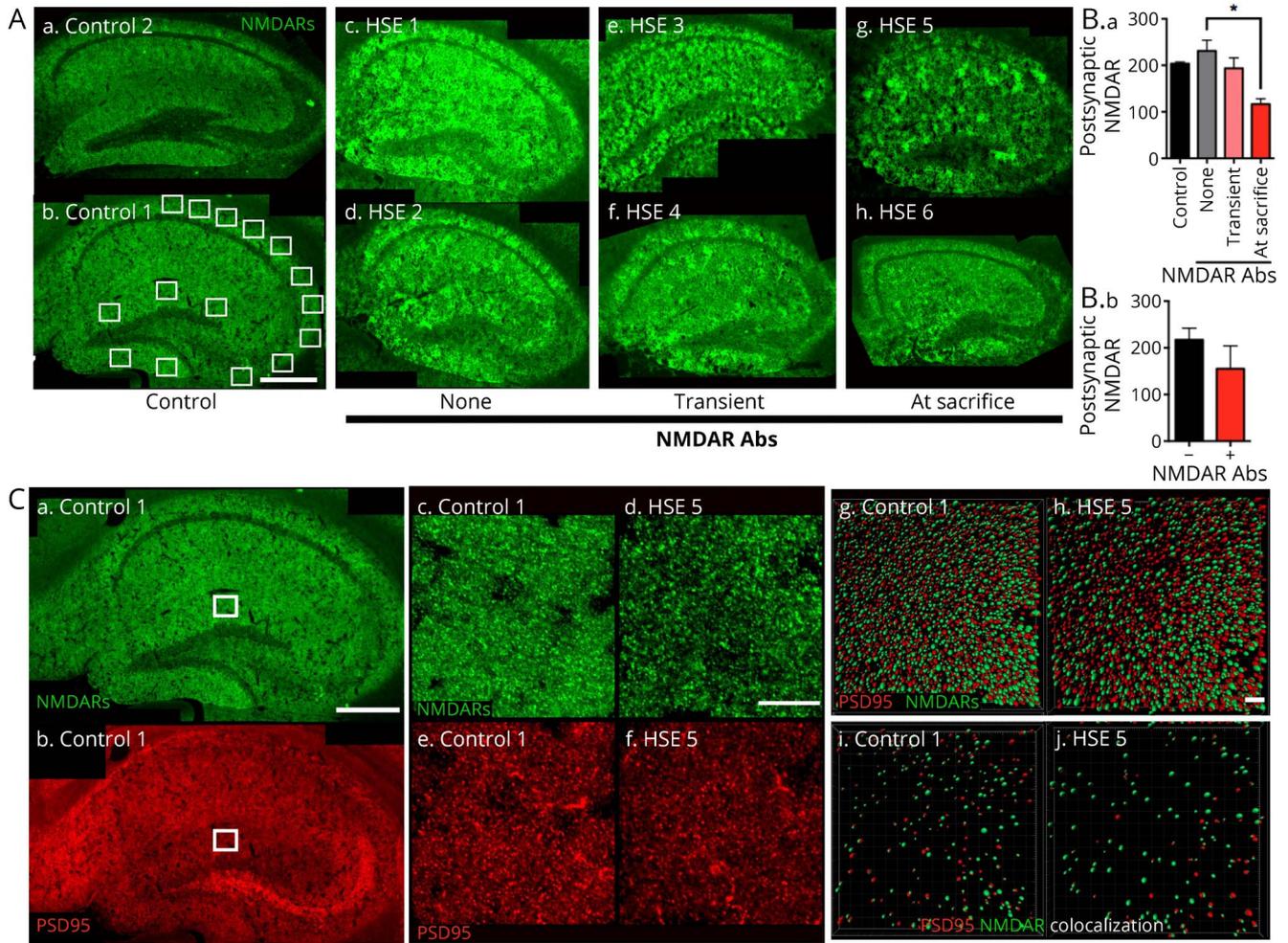
(A) Epifluorescence composites of hippocampi from mice inoculated with vehicle solution (control mice) 1 and 2 (b and a) and mice inoculated with HSV-1 (HSE) 1–6 (c–h) immunostained to assess the levels of membrane clusters of NMDARs. HSE mice 1 and 2 (c and d) did not develop serum antibodies (Abs) to NMDARs, HSE mice 3 and 4 (e and f) transiently made Abs at 6 and 3 weeks, and HSE mice 5 and 6 (g and h) had serum NMDAR Abs at the time of sacrifice (8 weeks). Boxes in b demonstrate sites that were further imaged under a confocal microscope for all mice. Scale bar = 500 μm. (B) Quantification of colocalized NMDAR and PSD95 clusters (= postsynaptic membrane NMDAR clusters) averaged from 3-μm stacks across the 15 hippocampal regions outlined in A.b for all groups (a) and then further grouped by serum NMDAR antibody status (b). Data are presented as mean ± standard error of the mean (SEM). Significance of NMDAR Ab presence on postsynaptic NMDAR cluster number was assessed by one-way ANOVA for all groups and with Mann-Whitney U test for pooled analysis; *p < 0.05. (C) Epifluorescence composites of NMDAR staining (a) and PSD95 staining (b) for control mouse 1 (scale bar = 500 μm). Square represents an area that was further imaged on a confocal microscope (c–j). Representative confocal 2D images of NMDAR clusters (c and d) and PSD95 clusters (e and f) for control mouse 1 and HSE mouse 5 from the region outlined in a and b. Note the decrease in NMDAR cluster numbers, with relatively preserved PSD95 cluster numbers for HSE mouse 5 compared with control mouse 1 (scale bar = 10 μm). Representative NMDAR (green) and PSD95 (red) clusters for 3D Z-stacks from the region outlined in a and b for control mouse 1 (g) and HSE mouse 5 (h). Note the decrease in NMDAR (green) clusters in stack from HSE mouse 5 compared with control mouse 1. (i and j) Colocalized NMDAR and PSD95 clusters (= postsynaptic membrane NMDAR clusters) in the Z-stacks from g and h for control mouse 1 and HSE mouse 5. Note the decrease in postsynaptic membrane NMDAR clusters in this region in HSE mouse 5 vs control mouse 1 (scale bar = 3 μm).

memory and behavioral deficits, their brains are bound by patient IgG, and they show a decreased density of postsynaptic membrane NMDARs, NMDAR-mediated currents, and synaptic plasticity. In the model described here, mice produce their own NMDAR antibodies after HSE, mirroring what has been observed in patients with autoimmune encephalitis post-HSE. Behavioral studies were not a focus of this proof of principle study. They are planned for future investigations.

A recent prospective study of patients with HSE showed that 27% developed autoimmune encephalitis within 3 months after the viral infection.[5] In most cases, the disorder associated with novel synthesis of NMDAR antibodies, but a few patients

had transient antibody synthesis without overt clinical manifestations, and patients often developed additional antibodies against yet unknown protein antigens. Transient antibody synthesis and an IgG to an unknown target were similarly noted in the current animal model, the significance of which is left for future studies to clarify. Of interest, the outcome of patients with autoimmune encephalitis post-HSE was substantially worse than that of patients with classic (not HSE triggered) autoimmune encephalitis.

The recent findings in the human disease[5] raise important questions related to where and how the immune response is triggered and potential measures to prevent the subsequent

Figure 3 Mice with antibodies to NMDARs have decreased hippocampal total protein levels of NMDARs
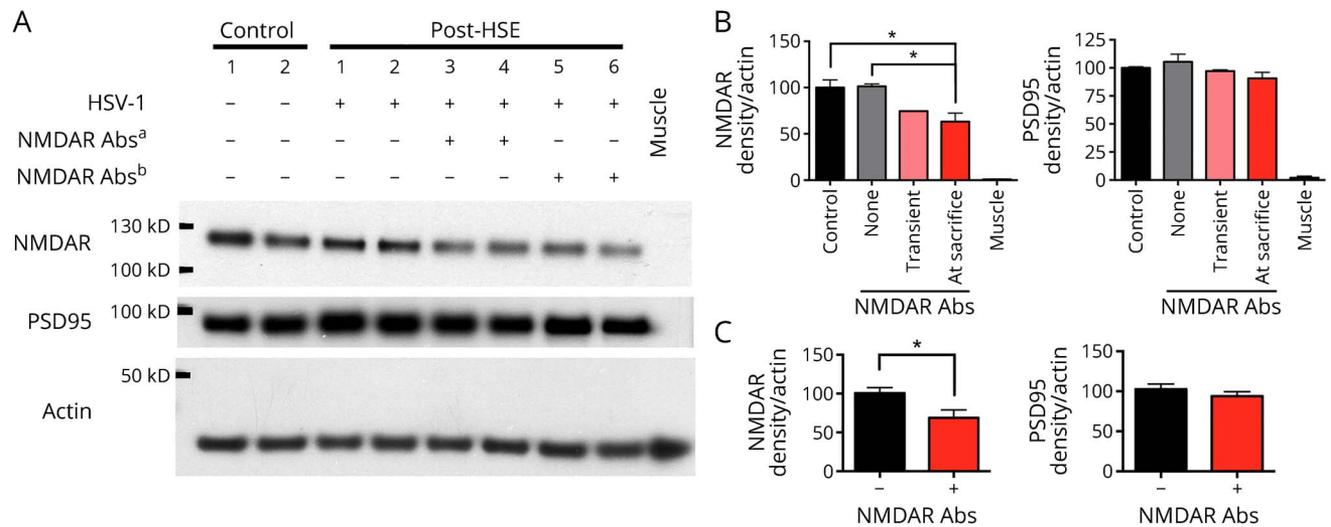
(A) Representative immunoblots of extranuclear proteins extracted from hippocampi of control mice and HSE mice, which were probed for NMDAR (GluN1 subunit), PSD-95, and actin (loading control). Muscle lysates were included as a control. Protein size is expressed as kilodaltons (kD). Note that there is less visible NMDAR protein in the hippocampi of mice that made antibodies (Abs) to NMDARs (mice 3–6), whereas the PSD-95 and actin protein amounts are similar in all the mice. Blots were run in duplicates. [a] NMDAR Abs transiently present. [b] NMDAR Abs present at the time of sacrifice. (B) Quantification of hippocampal NMDAR and PSD-95 in control mice, HSE mice that never made NMDAR Abs, HSE mice that transiently made NMDAR Abs, and HSE mice that made NMDAR Abs at the time of sacrifice (8 weeks). (C) Quantification of hippocampal NMDAR and PSD-95 based on serum NMDAR antibody status. Results in B and C were normalized to actin (loading control), and mean band density of control mice was defined as 100%. Data are presented as mean ± SEM. Significance of serum NMDAR Ab presence on hippocampal NMDAR protein amount was assessed by one-way ANOVA (B) and Mann-Whitney U test (C), respectively; *$p < 0.05$.

autoimmune disorder. To answer these questions, we need an animal model, similar to that reported here, which will allow for investigations into the pathogenesis of this disorder, with the potential to provide insights that are generalizable to non-parainfectious autoimmune encephalitides, as they likely share common mechanisms and pathways. The task is now to determine in our model the behavioral impact of the NMDAR antibodies, what other antibodies are produced in this model, where and how the immune system is primed to produce neuronal antibodies in response to HSE, and to develop a treatment strategy, adjuvant to ACV, that prevents neuronal antibody synthesis.

Disclosure
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Appendix 1 Author contribution

Name	Location	Role	Contribution
Jenny Linnoila, MD, PhD	Massachusetts General Hospital (MGH), Boston, MA	Author	Study concept and design, acquisition of data, analysis and interpretation of data, and writing of the manuscript
Benjamin Pulli, MD	MGH, Boston	Author	Study concept and design, implementation of experiments, analysis and interpretation of data, and critical revision of the manuscript for intellectual content
Thaís Armangué, MD, PhD	University of Barcelona, Barcelona, Spain	Author	Acquisition of data and critical review of the manuscript for intellectual content
Jesús Planagumà, PhD	University of Barcelona, Barcelona, Spain	Author	Implementation of experiments and critical review of the manuscript
Radha Narsimhan, PhD	MGH, Boston	Author	Acquisition of data and critical review of the manuscript
Stefan Schob, MD	University Hospital, Leipzig, Germany	Author	Study concept and design and critical review of the manuscript
Matthias Zeller, MD	Kepler University Hospital, Linz, Austria	Author	Implementation of experiments and critical review of the manuscript
Josep Dalmau, MD, PhD	University of Barcelona, Barcelona, Spain	Author	Study concept and design, analysis and interpretation of data, critical review of the manuscript for intellectual content, and study supervision
John Chen, MD, PhD	MGH, Boston	Author	Study concept and design, analysis and interpretation of data, critical review of the manuscript for intellectual content, and study supervision

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In the article " Mouse model of anti-NMDA receptor post–herpes simplex encephalitis" by Linnoila et al.,[1] first published online December 26, 2018, there should have been a link included to access online supplemental materials: links.lww.com/NXI/A92. The publisher regrets the error.

Reference

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