

SUPPORTING INFORMATION

Lazarini F, Lannuzel A, Cabié, et al., Olfactory Outcomes in Zika Virus-Associated Guillain-Barré Syndrome

Supplementary Methods

Diagnosis of acute inflammatory demyelinating polyradiculoneuropathy (AIDP) and acute motor axonal neuropathy (AMAN)

We used electrophysiological criteria of Uncini and Kuwabara (2012) to distinguish between the AIDP and AMAM subtypes of GBS.

Olfactory assessment

The T, D and I score

The Sniffin' Sticks test was used to assess olfactory functions. It provides a validated quantification of threshold (T) score, discrimination (D) score, identification (I) score and a total score (T+D+I=TDI total score).

- *The T score* was obtained in a 3 alternative forced choice paradigm (3 AFC) using 16 dilutions prepared from a *n*-butanol solution. Three felt-tip pens (two containing the solvent and the third the odorous solution) were presented in a randomized order using a single-staircase of increasing concentration. Subjects had to identify the odor-containing pen. Reversal of the staircase was triggered when the odorant was correctly identified in two subsequent trials. The T score was the mean of the last 4 of 7 staircase reversals, with the final score ranging from 1 to 16. Subjects were blindfolded during this test.

- *To assess the D score*, the same 3 AFC logic was used with 16 pairs of odorants. Triplets of pens were presented in a randomized order (two containing the same and one a different odorant). Patients asked to indicate which of three pens smelled differently. The score was the sum of correctly identified odorants, ranging from 1 to 16. Subjects were blindfolded during this test as well.

- *The I score* was assessed for 16 common odors. Subjects were presented with single pens and had to identify the smell, using a list of four descriptors for each pen. The I score was the sum of correctly identified pens, ranging from 1 to 16.

Scanning electron microscopy

Fixed samples were dissected and post-fixed by incubation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 1 h at room temperature then 12 h at 4°C. The samples were washed in 0.1 M cacodylate then several times in water and processed by alternating incubations in 1% osmium tetroxide and 0.1 M thiocarbohydrazide. After dehydration by incubation in increasing concentrations

of ethanol, samples were critical point dried, mounted on a stub, and analyzed by field emission scanning electron microscopy with a Jeol JSM6700F operating at 3 kV.

Immunofluorescence

Forty-micrometer coronal sections were obtained from fixed brains using a vibrating microtome (VT1000S, Leica). Epitope retrieval was performed by incubating free-floating sections for 20min in citrate buffer pH 6.0 (C-9999, Sigma-Aldrich) at 80°C. Sections were then blocked in 0.2% Triton X-100, 4% bovine serum albumin (BSA, Sigma-Aldrich), and 2% goat serum for 4 h at room temperature and incubated overnight with rabbit anti-ionized calcium binding adaptor molecule (Iba1) antibodies (1/500, Wako Chemicals, 016-20001) at 4°C. After rinsing, slices were incubated with with the appropriate secondary antibodies (Alexa Fluor 568-labelled anti-rabbit, A11036; Invitrogen) for 2 h at room temperature. All sections were then counterstained with Hoechst (H3570, Invitrogen), rinsed thoroughly in PBS and mounted in Fluoromount-G (15586276, Invitrogen) before observation with a Zeiss LM 710 inverted confocal microscope.

ZIKV RNA levels from mouse plasma and tissues

Blood samples were centrifuged to recover plasma from which viral RNA was extracted with a QIAamp viral RNA minikit (Qiagen). Nasal turbinate and brain tissue samples were homogenized in TRIzol reagent (Life Technologies), using ceramic beads and an automated homogenizer (PreCellys). Total RNA was extracted according to the manufacturer's instructions. cDNA synthesis was performed using reverse transcriptase (Life Technologies) in a Bio-Rad MyCycler thermocycler. ZIKV cDNAs were quantified by a TaqMan quantitative PCR (qPCR) in a ViiA7 instrument (Life Technologies), using standard cycling conditions. The following primer sets were used to detect ZIKV gene: forward primer 5'-CCG CTG CCC AAC ACA AG- 3' and reverse primer 5'-CCA CTA ACG TTC TTT TGC AGA CAT-3' with the probe 5'-6FAM-AGC CTA CCT TGA CAA GCA ATC AGA CAC TCA A-MGB-3' (Life Technologies) (where 6FAM is 6-carboxyfluorescein). The viral load is expressed as the number of viral genome copies per milliliter (plasma samples) or per number of total RNA micrograms (tissue samples) after comparison with a standard curve produced using serial 10-fold dilutions of a plasmid containing the corresponding fragment of ZIKV genome.

Supplementary Reference

Uncini A, Kuwabar S. Electrodiagnostic criteria for Guillain-Barrè syndrome: a critical revision and the need for an update. *Clin Neurophysiol.* 2012; 123(8):1487-1495.

Supplementary Table 1: Characteristics of the GBS-Zika+ and GBS-non-Zika Groups at the Disease Onset

Variable	GBS-Zika+	GBS-non-Zika Control group	P Value
n	19	9	n.a.
Zika: PCR confirmed ^a , No./ probable ^b , No./suspected ^c , No.	12 / 1 / 6	0 / 0 / 0	n.a.
Brighton level: 1, No. / 2, No./ 3, No. / 4, No.	12 / 2 / 1 / 4	3 / 6 / 0 / 0	n.a.
Hospitalization, No. (%)	19 (100)	8 (88.9)	0.321
Areflexia	10 (52.6)	7 (77.8)	0.250
Paresthesia, No. (%)	19 (100)	6 (66.7)	0.026
Ataxia	8 (44.1)	6 (66.7)	0.420
Persistent cranial nerve palsy	17 (89.5)	3 (33.3)	0.005
Facial palsy	2 (10.5)	2 (22.2)	0.574
Post-paretic facial hemispasm	2 (10.5)	0 (0.0)	>0.999
Sensory deficit	7 (36.8)	3 (33.3)	>0.999
Phonation disorders	4 (21.1)	1 (11.1)	>0.999
Swallowing disorders	1 (5.26)	1 (11.1)	>0.999
MRC scale ^d at onset, median (IQR)	52.0 (44.0-60.0)	53.5 (32.5-55.5)	0.451
Motor deficits at onset: MRC<60, No. (%)	12 (63.2)	8 (88.9)	0.214
Electrophysiological diagnosis			
Acute inflammatory demyelinating polyradiculoneuropathy, No.	16 (84.2)	7 (77.8)	>0.999
Acute motor axonal neuropathy, No. (%)	0 (0.0)	1 (11.1)	0.321
Unclassified ^e No.	3 (15.8)	1 (11.1)	>0.999

n.a., not applicable;

^aRT-PCR: positive for ZIKV in urine, plasma or CSF.

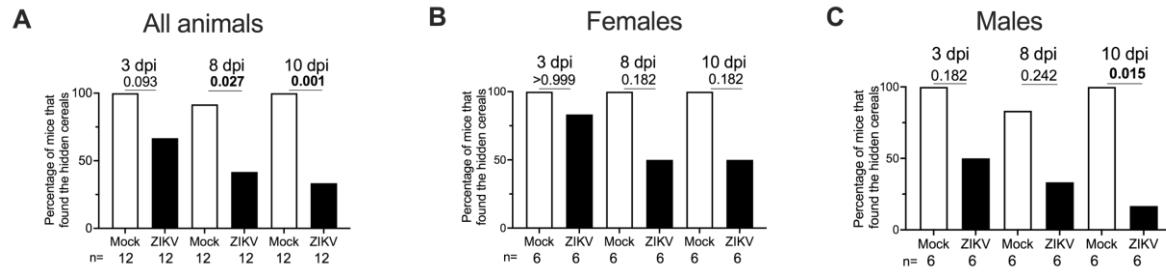
^bnegative RT-PCR for ZIKV in urine, plasma and CSF and detection of IgM for ZIKV in plasma

^ctypical symptoms of Zika (rash with 2 or more of the following signs or symptoms: fever, arthralgia, myalgia, conjunctivitis, edema), negative RT-PCR for ZIKV in urine, plasma and CSF, and detection of IgG for ZIKV in plasma without detection of IgM for ZIKV in plasma

^dThe MRC scale is defined as the sum of MRC subscores of 6 different muscles measured bilaterally (0: complete paralysis; 1: minimal contraction; 2: active movement with gravity eliminated; 3: weak contraction against gravity; 4: active movement against gravity and resistance; 5 normal strength), which results in a score ranging from 0 (tetraplegic) to 60 (normal);

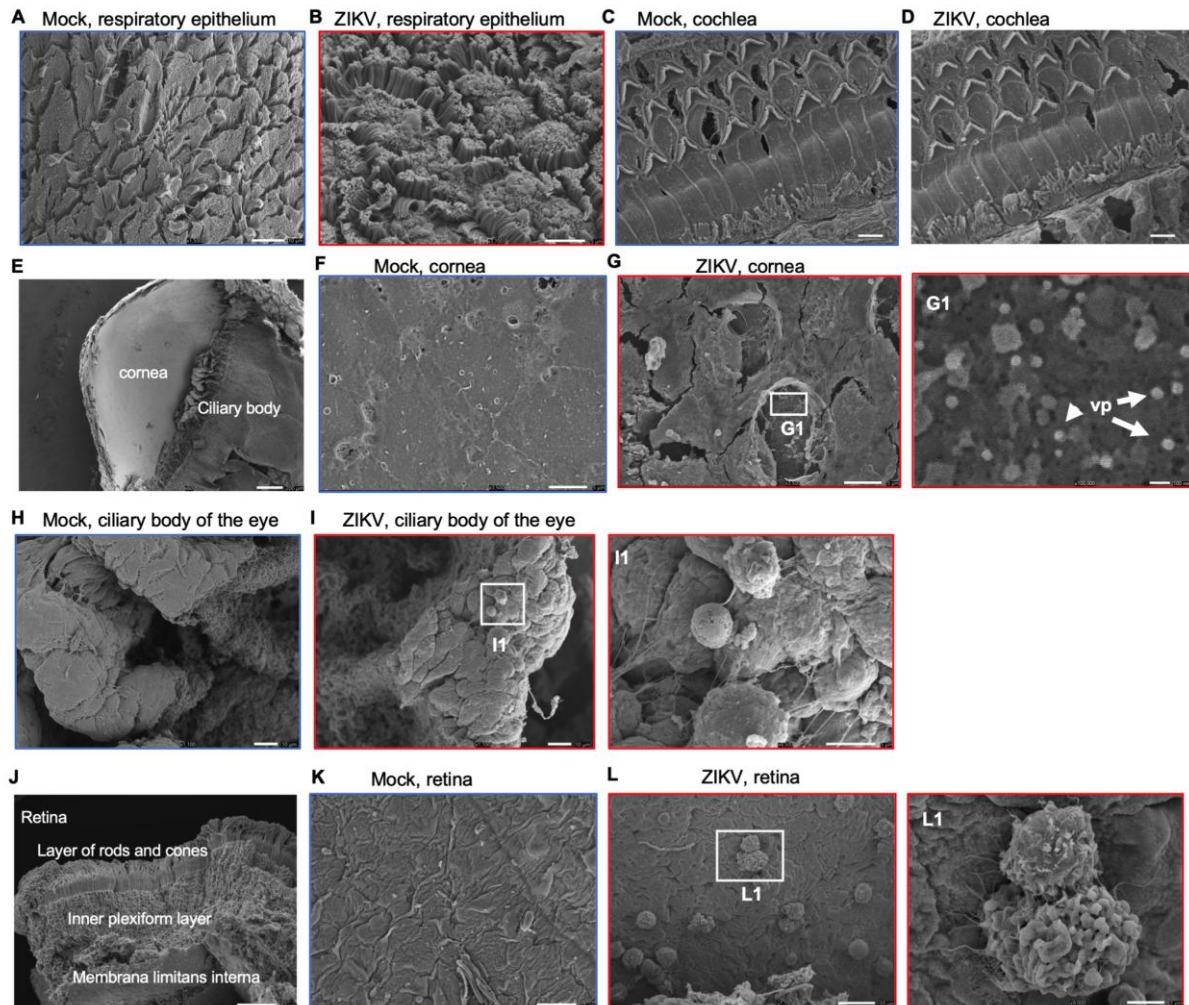
^ePatients which levels of certainty for the diagnosis of GBS were 2, 3 and 4 had equivocal studies
P values are calculated by Mann-Whitney and Fisher test when appropriate; In bold P values<0.05

Supplementary Figure 1: Fraction of mock or infected AG129 mice successfully finding hidden cereals in 15 minutes.



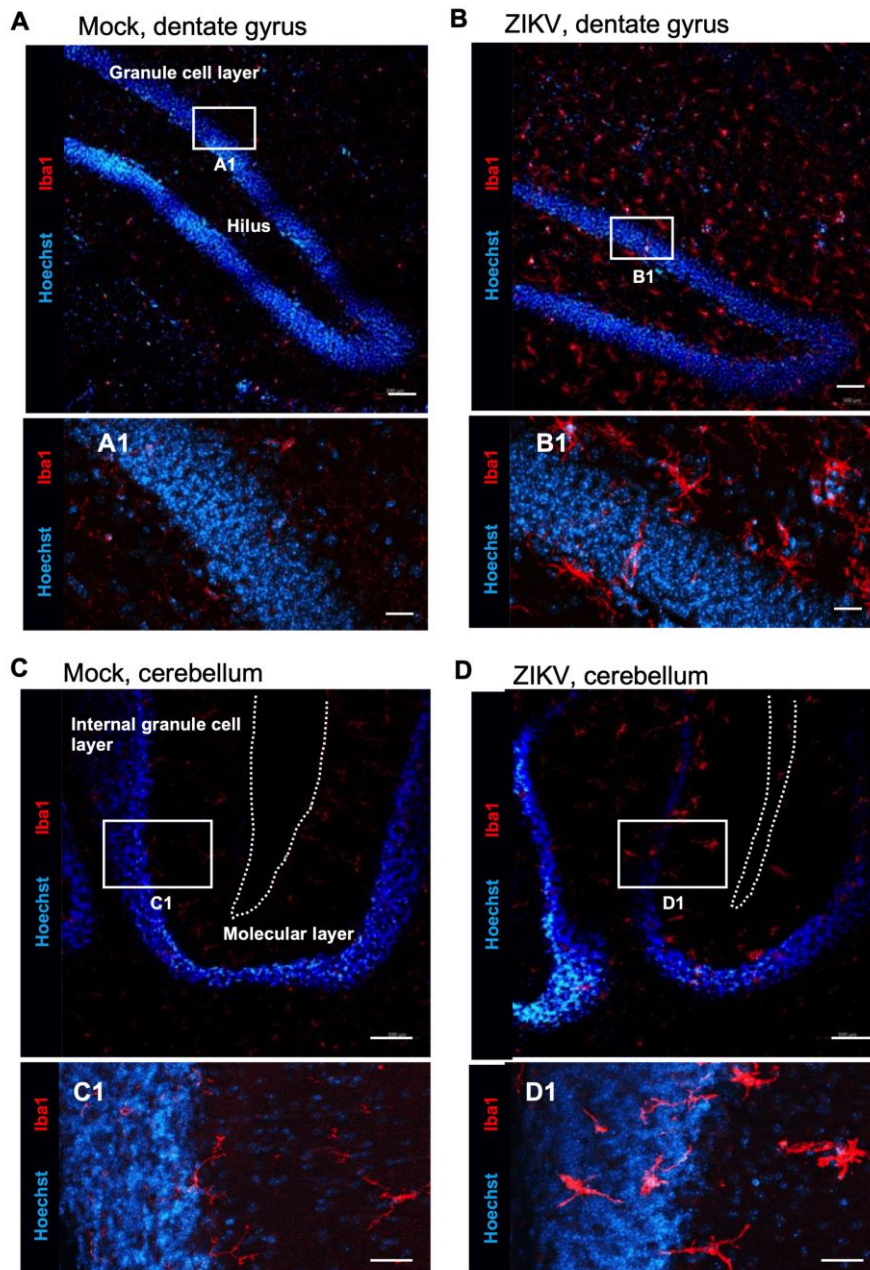
Food-finding assays were performed at 3, 8- and 10-dpi. Fisher's exact test. $n=12/$ timepoint in (A); $n=6/$ timepoint in (B, C); P value is indicated in bold when significant.

Supplementary Figure 2: Scanning electron microscope imaging of the respiratory epithelium, cochlea and eye following ZIKV infection.



Respiratory epithelium (A, B), cochlea (C, D) and eye (E-L) at 11dpi. Several immune cells are observed in the ciliary body of the eye and retina in infected mice (I1, L1). Viral particles (vp) are seen emerging from cornea cells (G1, white arrows). Scale bars: 200 μm (E) 10 μm (A, H, I, K, L), 5 μm (B-D, F, G, I1), 2 μm (L1), 100 nm (G1).

Supplementary Figure 3: ZIKV promotes brain neuroinflammation in AG129 mice.



(A-D) Coronal sections showing Iba⁺ cells in the dentate gyrus (A, B) and cerebellum (C, D) of mock and ZIKV infected mice at 11 dpi. Images are maximum intensity projection over a 6- μ m depth. Scale bars: 100 μ m (A-D), 5 μ m (A1-D1).

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