Supporting Information

Kopp et al. 10.1073/pnas.0908892106

SI Text

Effect of Mouse Genotype on Fusion of Herpes Simplex Virus (HSV) with Synaptosomes. Absence of HSV antigen expression in the brain parenchyma of nectin-1 KO and nectin-1/HVEM double KO mice, as described in the text, is likely due to failure of the virus to enter neurons and other cells of the brain. A previous study showed that fusion of HSV with rodent synaptosomes could serve as a surrogate assay for viral entry into neurons and as a way to visualize entry by cryoelectron tomography (1). Moreover, this fusion was observed only with the presynaptic, not postsynaptic, compartment of synaptosomes, consistent with findings that nectin-1 asymmetrically localizes to presynaptic sides of *puncta adherentia* junctions in the adult mouse hippocampus (2). To assess whether this fusion was dependent on nectin-1 or other receptors, synaptosomes were prepared from the brains of mice of each of the four genotypes. The synaptosomes, after incubation with HSV, were examined by cryoelectron tomography to determine whether fusion had occurred. Fusion events were evident from the presence of glycoprotein spikes on the synaptosome surfaces and nucleocapsids inside the synaptosomes (Fig. S1). Samples for each genotype were examined for the same amount of microscopy time (≈12 h), yielding five and four fusion events, respectively, for HVEM KO and wild-type mice and none for the nectin-1 KO or double KO mice. All fusion events observed were with the presynaptic compartment of synaptosomes. The images were indistinguishable from those recently reported (1).

Results obtained with the synaptosomes support the hypothesis that entry of virus into neurons from the brain requires nectin-1 expression and that the in vivo results can be attributed to an entry block resulting from the absence of nectin-1.

SI Methods. Groups of mice of each genotype were killed by CO_2 narcosis followed by decapitation. The brains were removed, coded, and immediately snap-frozen for shipment to the Max Planck Institute of Biochemistry in Martinsried, Germany. The frozen brains were homogenized in ice-cold buffer [0.32 M sucrose, 1 mM EDTA, 5 mM HEPES (pH 7.4), containing complete mini EDTA-free protease inhibitor mixture (Roche)] and synaptosomes were prepared as described (1). The activated synaptosomes were incubated with HSV (HSV-1, wild-type strain 17^+, 1.6×10^{10} PFU per brain) for 60 min on ice, then for 60 min at 37 °C, and processed for cryoelectron tomography, as previously described (1). The samples were examined in blinded fashion (coded, i.e., samples were examined without knowing the genotype), to search for events indicative of fusion between virions and synaptosomes.

The effects of freezing on the structural integrity of synaptosomes were tested in advance with rat brain and rat brain derived synaptosomes. Intact structures and synapses (pre- and postsynaptic parts connected via synaptic cleft) were found in both synaptosomes prepared from frozen brain and synaptosomes that had been frozen and thawed. However, structures were better preserved in synaptosomes derived from frozen brain; hence, experiments were carried out using these.

1. Maurer E, Sodeik B, Grünewald K (2008) Native 3D intermediates of membrane fusion in herpes simplex virus 1 entry. *Proc Natl Acad Sci USA* 105:10559–10564.

2. Mizoguchi A, et al. (2002) Nectin: An adhesion molecule involved in formation of synapses. *J Cell Biol* 156:555–565.

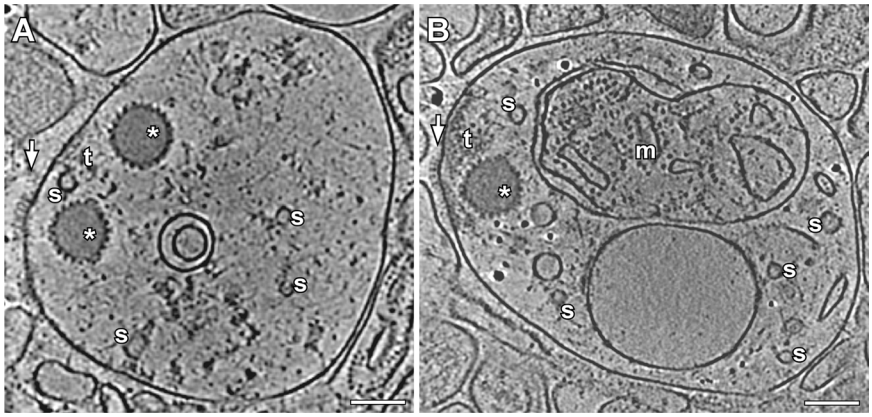

Fig. S1. Representative slices from tomograms (thickness ≈10 nm) with synaptosomes containing HSV capsids from wild-type mice (*A*) and HVEM KO mice (*B*). Capsids are highlighted by asterisks and glycoprotein spikes at the fusion site by white arrows. Synaptic vesicles (s), tegument protein (t), and a mitochondrion (m) are marked. (Scale bar, 100 nm.)