Supporting Information

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Fig. S1. Detected events for the entry states A to F. The diagram shows the number of events found for each depicted entry state of HSV-1 into synaptosomes. The numbers refer to the states A–F illustrated in Fig. 3. The structures combined in (X) denote a variety of heterogeneous structures which can be assigned as in between the states attachment (A) and fusion (B).



Fig. S2. HSV-1 has two function-related poles. Schematic drawing from HSV-1 (B) during secondary envelopment (A) and entry (C). (A) The post Golgi compartment (pGc) in the cytoplasm (cyt) of the host cell contains the viral glycoproteins and constitutes the future viral envelope. By enclosing the capsid, it creates the "assembly pole" of the virion (marked in green), in which tegument proteins and glycoproteins are highly concentrated. (B) Complete virion with its two function-related poles: entry pole (purple) with a small amount of glycoproteins and tegument, assembly pole (green) with a high amount of these. (C) The "entry pole" (purple) has the appropriate arrangement and type of entry-associated glycoproteins for fusion with the host's plasma membrane (pm).



Movie S1. Postfusion state of HSV-1. The tomogram shows the virus entry site in a PtK2 cell (compare with Fig. 1). Each frame corresponds to a 2.72-nm-thick section through the tomogram moving along the *z* axis. The incoming capsid (light blue) is separated from the majority of the tegument (orange) and found close to a dense actin network (red, only partially rendered).

Movie S1 (MOV)

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Movie 52. HSV-1 enters the presynaptic part of synaptosomes. The reconstructed volume from which the still image in Fig. 2 A was taken is presented as a series of x/y slices (2.72 nm thickness) running up and down along the z-direction followed by the rendering of selected features. Color coding is the same as used in Fig. 2: capsid (light blue), tegument (orange), glycoproteins (yellow), cell membrane/viral membrane (dark blue), actin (dark red), vesicles (purple), synaptic vesicles (only partially segmented; metallic green), synaptic cleft (light green), and postsynaptic density (green).

Movie S2 (MOV)



Movie S3. The fusion pore state of HSV-1. Tomogram of a virus fusing with the presynaptic part of a synaptosome (compare Fig. 3 B and Fig. 4). Each frame corresponds to a 2.72-nm-thick section through the tomogram moving along the z axis. Colors are maintained from Fig. 4.

Movie S3 (MOV)

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Table S1. Presence of cytosolic capsids in synaptosomes in dependence of time and temperature

Temperature, °C	Incubation time (min)						
	0	1	2	5	10	30	60
4	-	-	-	-	-	(-)	(-)
10	-			+	(+)	(+)	(+)
18	-	+	+	+	(+)	(+)	(+)
25	-	+	+	+	+	+	+
37	-	(+)	(+)	(+)	(+)	(+)	+

The table provides the combination of experimental conditions of temperatures and times which were sufficient (+) or insufficient (-) to enable HSV-1 entry into synaptosomes. Combinations without a symbol were not examined. Attachment was allowed for 60 min on ice before onset of incubation temperatures. 4°C corresponds to incubation on ice. To decelerate the entry process for catching intermediates, temperatures and times were lowered and shortened, respectively. Symbols in parentheses indicate experimental conditions not checked with assumed results (e.g., viruses entering after 5 min incubation time at 10°C are expected to have entered also after 20 min incubation time).

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