Supporting Information

Granstedt et al. 10.1073/pnas.1311062110

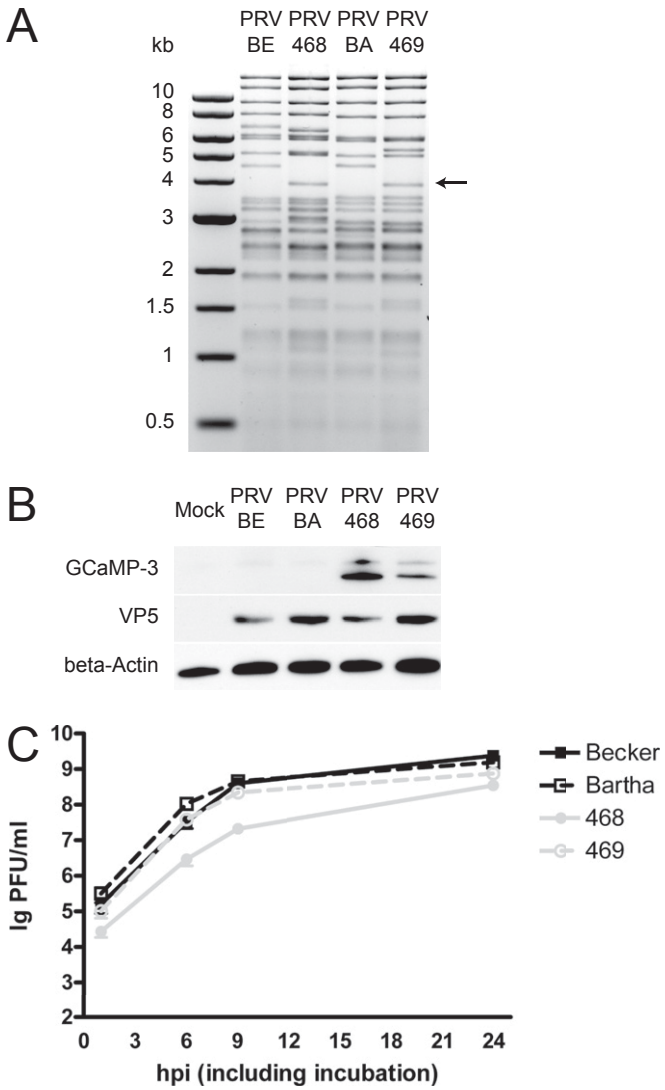


Fig. S1. Construction and characterization of pseudorabies virus (PRV) 468 and PRV 469. (*A*) To confirm correct genomic insertion after homologous recombination, nucleocapsid DNA from strains PRV Becker, PRV Bartha, PRV 468, and PRV 469 were digested with SalI and run on a gel. The size shift due to the insertion of the genetically encoded calcium indicator GCaMP3 is indicated with an arrow. (*B*) We confirmed protein expression of the GCaMP3 cassette with a Western blot. As a viral protein control, we probed for VP5, the major capsid protein, and as a cellular protein control, we probed for beta-actin. (*C*) PRV 468 and 469 have equivalent single-step growth kinetics of parental strains PRV Becker and PRV Bartha in epithelial cells in vitro. The average titer for each virus at each time point is plotted along with the SD.

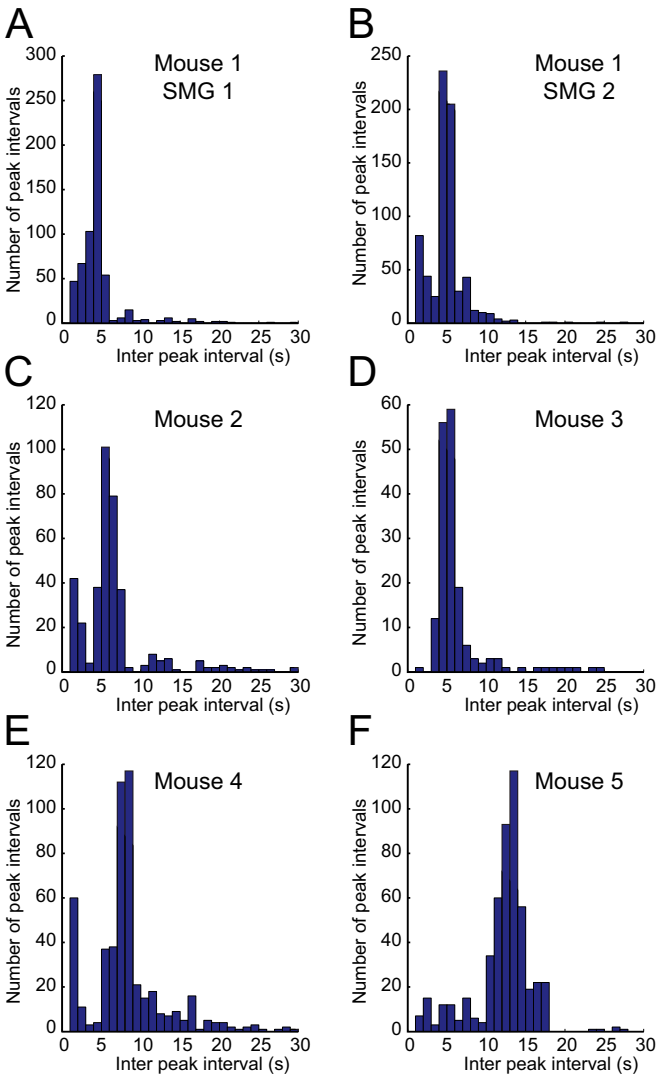


Fig. S2. Quantitative analysis of interpeak intervals across several mice. (*A–F*) The flashing phenotype was reproducible across many mice and the calcium flashes generally occurred every 5–10 s (*A–E*), although occasionally it was longer (*F*). We were able to acquire data from two different ganglia in one mouse and found that the ganglia were similar, but each still had a unique interpeak interval distribution (*A* and *B*).

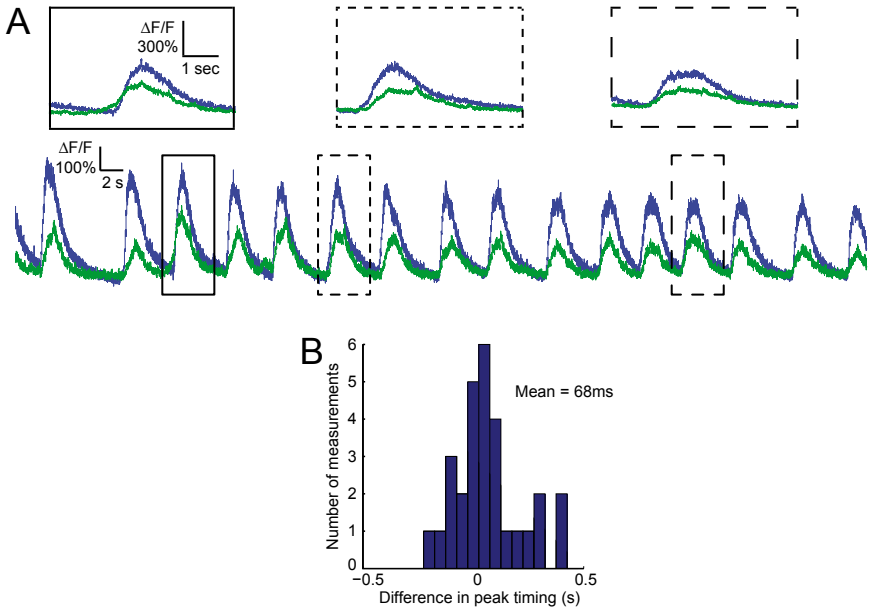


Fig. S3. Analysis of the extent of synchrony by repetitively imaging the same line. (*A*) Segments of traces from two cells that were imaged during a line scan. Inserts are magnifications of the traces, illustrating slight variations in the order that the cells flash. The relative fluorescence change (ΔF/F) and time scale (in seconds) are indicated. (*B*) Mean difference in peak timing during a line scan.

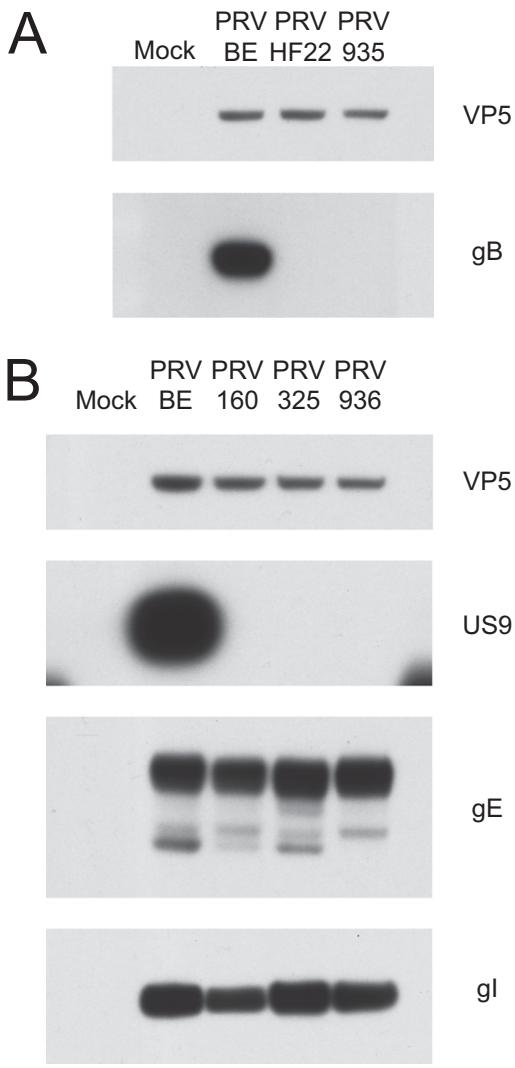
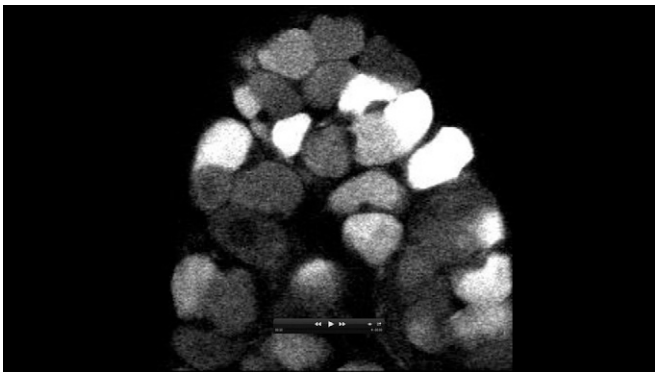


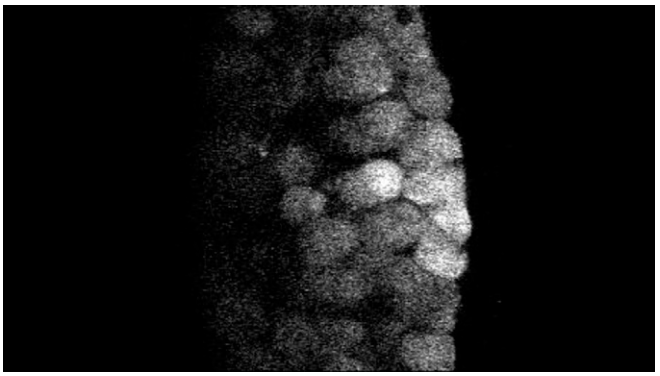
Fig. S4. Characterization of PRV 935 and PRV 936. (*A*) Swine epithelial (PK15) cells were mock infected or infected with PRV Becker (BE), PRV HF22 [glycoprotein B (gB) null] (1), or PRV 935 (gB null, GCaMP3) at a multiplicity of infection of 10 and incubated for 20 h. Afterward, cell lysates were analyzed by Western blotting and probed for the expression of gB. A monoclonal mouse antibody against the major capsid protein VP5 was used as loading control. (*B*) PK15 cells were mock infected or infected with PRV Becker, PRV 160 (Us9 null) (2), PRV 325 (Us9 null, mRFP-VP26) (3), or PRV 935 (Us9 null, mRFP-VP26, GCaMP3). Samples were prepared as in *A*. Polyclonal rabbit antisera against Us9, glycoprotein E (gE), and glycoprotein I (gI) were used to probe for the expression of Us9, gE, and gI, respectively. The expression of the major forms of gE and gI is unchanged, indicating no polar effects of the Us9 deletion.

1. Favoreel HW, Van Minnebruggen G, Nauwynck HJ, Enquist LW, Pensaert MB (2002) A tyrosine-based motif in the cytoplasmic tail of pseudorabies virus glycoprotein B is important for both antibody-induced internalization of viral glycoproteins and efficient cell-to-cell spread. *J Virol* 76(13):6845–6851.
2. Brideau AD, Card JP, Enquist LW (2000) Role of pseudorabies virus Us9, a type II membrane protein, in infection of tissue culture cells and the rat nervous system. *J Virol* 74(2):834–845.
3. Taylor MP, Kramer T, Lyman MG, Kratchmarov R, Enquist LW (2012) Visualization of an alphaherpesvirus membrane protein that is essential for anterograde axonal spread of infection in neurons. *mBio* 3(2).



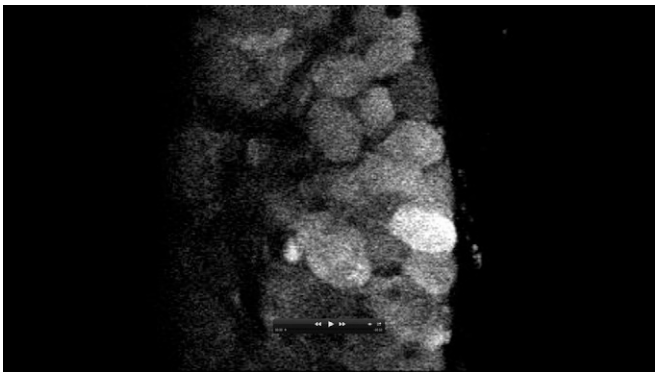
Movie S1. Spontaneous calcium activity during infection with the virulent strain PRV 468. The salivary glands of mice were inoculated with PRV 468. At 48 h postinfection, the animals were anesthetized and the submandibular ganglia were exposed for in vivo imaging. The majority of neurons in the ganglia were infected and many of them were firing synchronously.

Movie S1



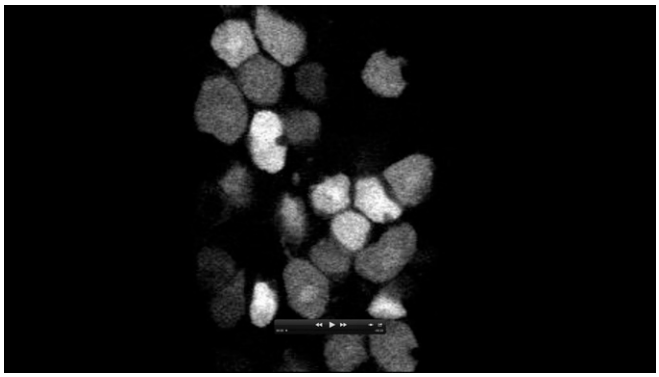
Movie S2. Spontaneous calcium activity during infection with PRV 468 before cutting the axon bundle to the brainstem. As before, the salivary glands of mice were inoculated with PRV 468. At 48 h postinfection, the animals were anesthetized and the submandibular ganglia were exposed for in vivo imaging. The majority of neurons in the ganglia were infected and many of them were firing synchronously.

Movie S2



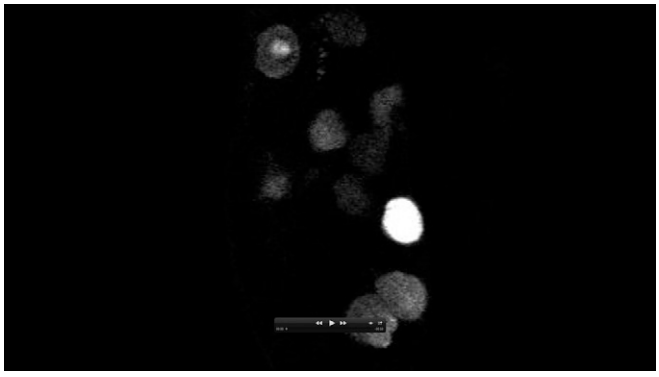
Movie S3. Spontaneous calcium activity during infection with PRV 468 after cutting the axon bundle to the brainstem. Once we detected the synchronous phenotype (Movie S2), we used a hemostat to clamp and hold the preganglionic bundle of axons and surrounding tissue and then cut everything below. We then immediately resumed our imaging in the same ganglion as before the cut and found that the synchronous phenotype was unaffected. We always verified that all tissue had been cut afterward by removing the hemostat and ensuring that nothing remained below.

Movie S3



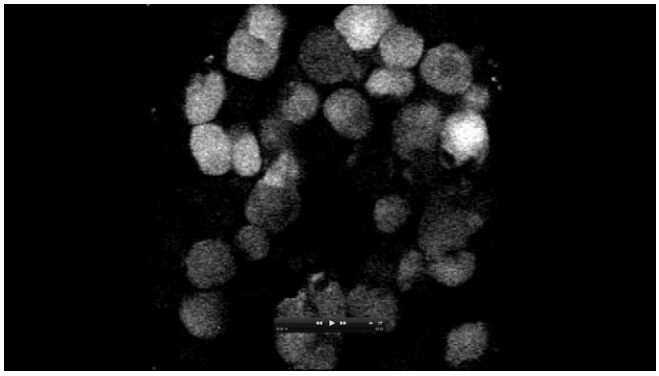
Movie S4. Spontaneous calcium activity during infection with the attenuated strain PRV 469. The salivary glands of mice were inoculated with PRV 469. At 72 h postinfection, the animals were anesthetized and the submandibular ganglia were exposed for in vivo imaging. None of the infected neurons in the ganglia fired synchronously; rather, each neuron exhibited complex calcium events that were unique to each cell and asynchronous.

Movie S4



Movie S5. Spontaneous calcium activity during infection with the gB null strain PRV 935. The salivary glands of mice were inoculated with PRV 935 grown on complementing cells. At 48 h postinfection, the animals were anesthetized and the submandibular ganglia were exposed for in vivo imaging. Few neurons in the ganglia were infected, yet none of the infected neurons in the ganglia fired synchronously; rather, each neuron exhibited complex calcium events that were unique to each cell and asynchronous.

Movie S5



Movie S6. Spontaneous calcium activity during infection with the Us9 null strain PRV 936. The salivary glands of mice were inoculated with PRV 936. At 48 h postinfection, the animals were anesthetized and the submandibular ganglia were exposed for in vivo imaging. None of the infected neurons in the ganglia fired synchronously; rather, each neuron exhibited complex calcium events that were unique to each cell and asynchronous.

Movie S6