Pathak et al., http://dx.doi.org/10.1085/jgp.201611672

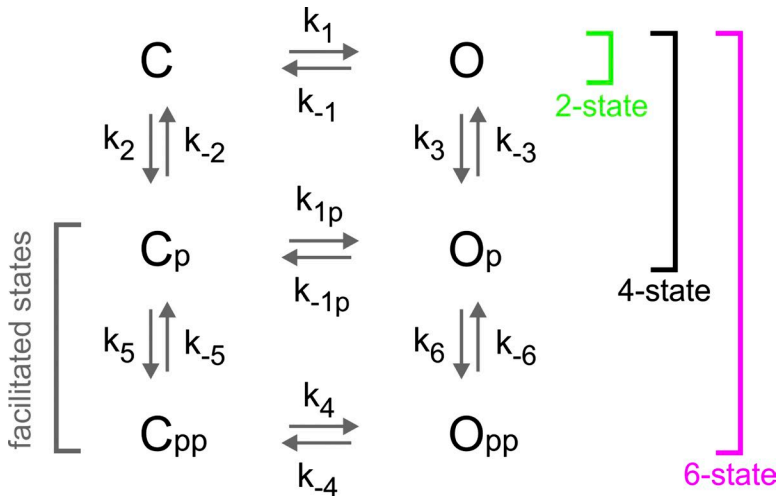

Figure S1. **Kinetic schemes used to simulate Hv1 mechanosensitive gating.** C = closed, O = open. Rate constants for "horizontal" transitions depend on both membrane voltage and mechanical stimulus (increase in membrane tension). Rate constants for "vertical" transitions (four-state and six-state schemes) are tension-sensitive only. Poststimulus values of all rates for the monomer are the same as their corresponding prestimulus values (quick reversibility). The $C_{pp} \leftrightarrow O_{pp}$ transition was added to properly simulate the slow recovery from the facilitated state in the Hv1 dimer (see section Models of Hv1 mechanosensitivity). C_{pp} and O_{pp} were treated as absorbing states (pre- and poststimulus k_5, k_{-5}, k_6, and $k_{-6} \approx 0$) because assigning rates consistent with the ~5 min "post-tension pulse" recovery time as characterized experimentally (Fig. 5 F) would not have visibly altered the simulations illustrated in Fig. 7 C. For rate values, see Tables S1, S2, and S3.

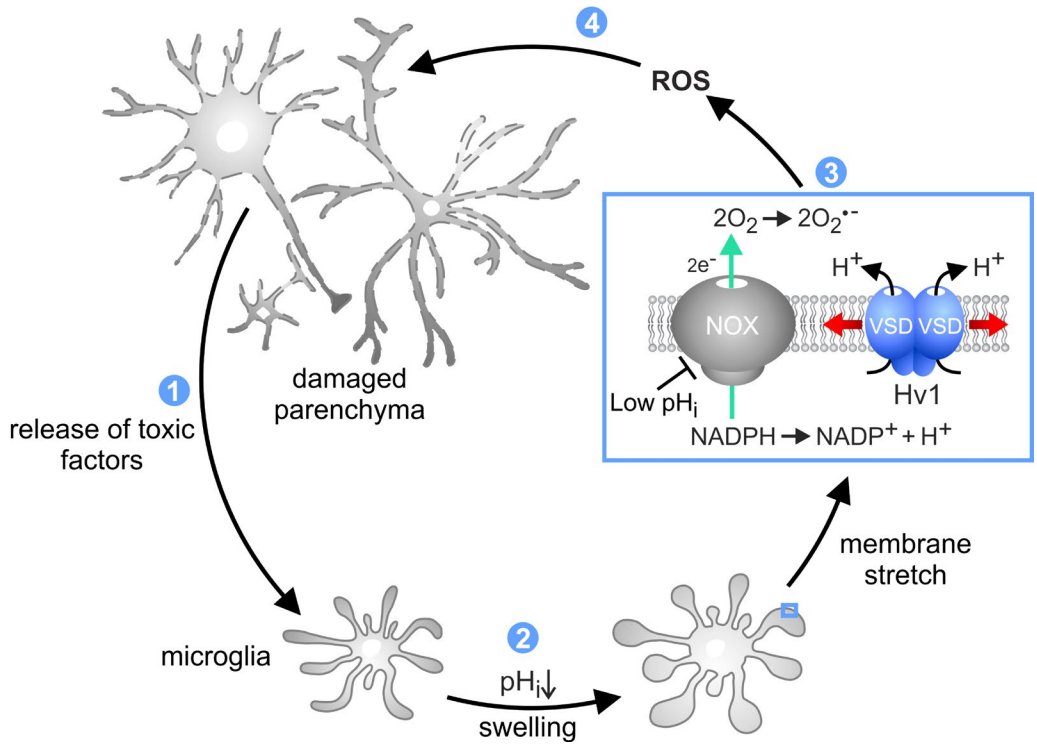

Figure S2. **Proposed vicious cycle connecting brain damage to Hv1 hyperactivation in the microglia.** Ischemic stroke causes damage in the parenchyma, leading to the release of toxic factors (1), such as ATP and glutamate. The toxic environment causes intracellular acidification and swelling of the microglia (2). Swelling-induced membrane stretch potentiates Hv1 activity to maximize proton extrusion and protect the cell. Hv1–NOX electrochemical coupling also causes ROS overproduction (3). Excessive levels of ROS further damage the parenchyma (4). NOX and Hv1 are not only present on the plasma membrane but also in intracellular compartments. Because NOX is inhibited by cytoplasmic acidification (Morgan et al., 2005), proton extrusion mediated by Hv1 on the plasma membrane may have the additional effect of enhancing the activity of intracellular NOX.

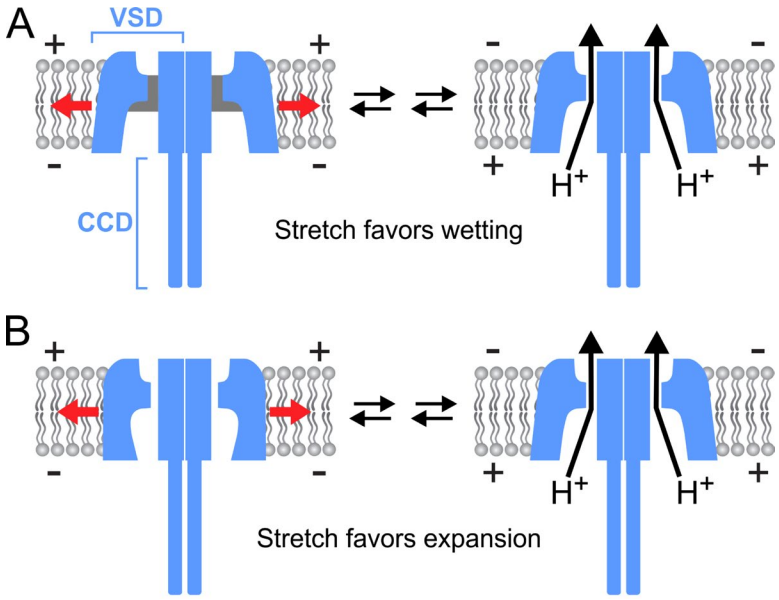

Figure S3. **Schematic representation of two processes proposed to be involved in the facilitation of Hv1 activation by membrane stretch.** (A) Wetting/dewetting of hydrophobic regions (gray blocks) in the VSDs. (B) Expansion/restriction of the intracellular vestibules of the VSDs. A combination of the two processes is also possible.

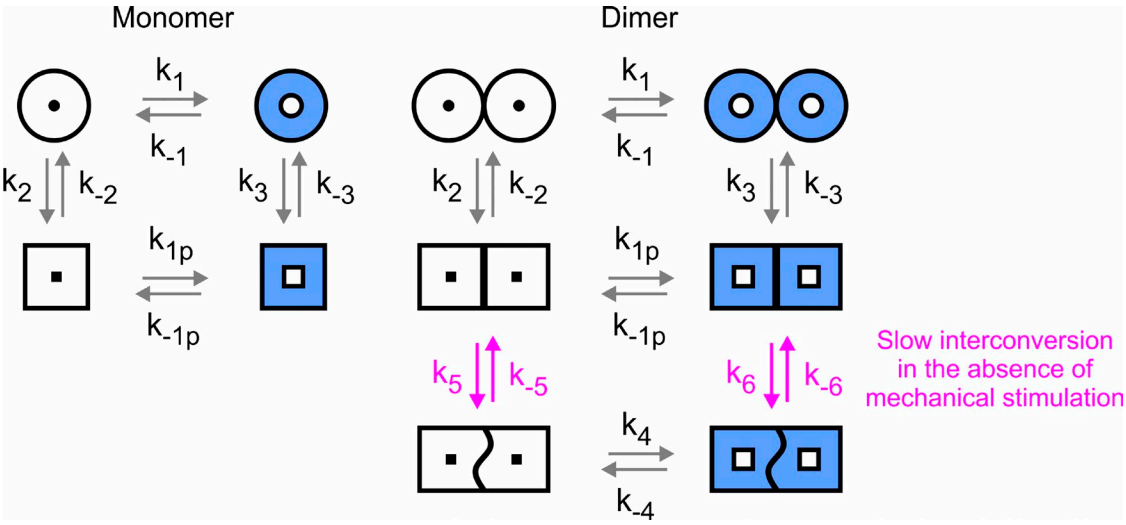

Figure S4. **Long-lasting facilitation observed in dimeric Hv1 is proposed to be linked to changes in the dimer interface.** The interface can only slowly revert to its original conformation once the mechanical stimulus is terminated. Changes in color indicate voltage-dependent conformational changes associated with channel opening. Changes in shape indicate tension-dependent conformational changes associated with short- and long-lasting facilitation.

Table S1. **Modeling the application of tension at −20 mV to the Hv1 dimer**

Rate constant	Prestimulus	Stimulus	Poststimulus
	s^{-1}	s^{-1}	s^{-1}
k_1 ($C \rightarrow O$)	0.003	0.04	0.003
k_{-1} ($O \rightarrow C$)	2.5	0.16	2.5
k_{1p} ($C_p \rightarrow O_p$)	0.003	0.04	0.003
k_{-1p} ($O_p \rightarrow C_p$)	2.5	0.16	2.5
k_2 ($C \rightarrow C_p$)	0.0033	100.0	0.0033
k_{-2} ($C_p \rightarrow C$)	200.0	100.0	50.0
k_3 ($O \rightarrow O_p$)	0.0033	100.0	0.0033
k_{-3} ($O_p \rightarrow O$)	200.0	100.0	50.0

Parameters are for the four-state model. Stimulus is increased membrane stretch.

Table S2. **Modeling the application of tension at 80 mV to the Hv1 dimer**

Rate constant	Prestimulus	Stimulus	Poststimulus
	s^{-1}	s^{-1}	s^{-1}
k_1 ($C \rightarrow O$)	0.221	0.3315	0.221
k_{-1} ($O \rightarrow C$)	0.045	0.03	0.045
k_{1p} ($C_p \rightarrow O_p$)	0.221	0.3315	0.221
k_{-1p} ($O_p \rightarrow C_p$)	0.045	0.03	0.045
k_2 ($C \rightarrow C_p$)	0.0033	100.0	0.0033
k_{-2} ($C_p \rightarrow C$)	200.0	100.0	50.0
k_3 ($O \rightarrow O_p$)	0.0033	100.0	0.0033
k_{-3} ($O_p \rightarrow O$)	200.0	100.0	50.0
k_4 ($C_{pp} \rightarrow O_{pp}$)	0.3315	0.9945	0.3315
k_{-4} ($O_{pp} \rightarrow C_{pp}$)	0.0075	0.01	0.0075
k_5 ($C_p \rightarrow C_{pp}$)	0	0.25	0
k_{-5} ($C_{pp} \rightarrow C_p$)	0	0.2	0
k_6 ($O_p \rightarrow O_{pp}$)	0	0.25	0
k_{-6} ($O_{pp} \rightarrow O_p$)	0	0.2	0

Parameters are for the six-state model. Stimulus is increased membrane stretch.

Table S3. **Modeling the application of tension at 80 mV to the Hv1 monomer**

Rate constant	Prestimulus	Stimulus	Poststimulus
	s^{-1}	s^{-1}	s^{-1}
k_1 ($C \rightarrow O$)	2.21	4.68	4.43
k_{-1} ($O \rightarrow C$)	0.45	0.095	0.90
k_{1p} ($C_p \rightarrow O_p$)	2.21	4.68	4.43
k_{-1p} ($O_p \rightarrow C_p$)	0.45	0.095	0.90
k_2 ($C \rightarrow C_p$)	0.0033	200.0	0.0033
k_{-2} ($C_p \rightarrow C$)	200.0	200.0	200.0
k_3 ($O \rightarrow O_p$)	0.0033	200.0	0.0033
k_{-3} ($O_p \rightarrow O$)	200.0	200.0	200.0

Parameters are for the four-state model. Stimulus is increased membrane stretch.

REFERENCE

Morgan, D., V.V. Cherny, R. Murphy, B.Z. Katz, and T.E. DeCoursey. 2005. The pH dependence of NADPH oxidase in human eosinophils. *J. Physiol.* 569:419–431. http://dx.doi.org/10.1113/jphysiol.2005.094748