Supplemental Information for Huang and Trussell “Presynaptic HCN channels regulate vesicular glutamate transport”

Supplemental Figure 1 “Calibration of SBFI fluorescence upon change in $[Na^+]_i$”, associated with Fig 2.

Supplemental Figure 2 “HCN channels contribute to resting membrane potential”, associated with Fig 3.

Supplemental Figure 3 “Actions of 8-Br-cAMP on miniature EPSCs”, associated with Fig 4.

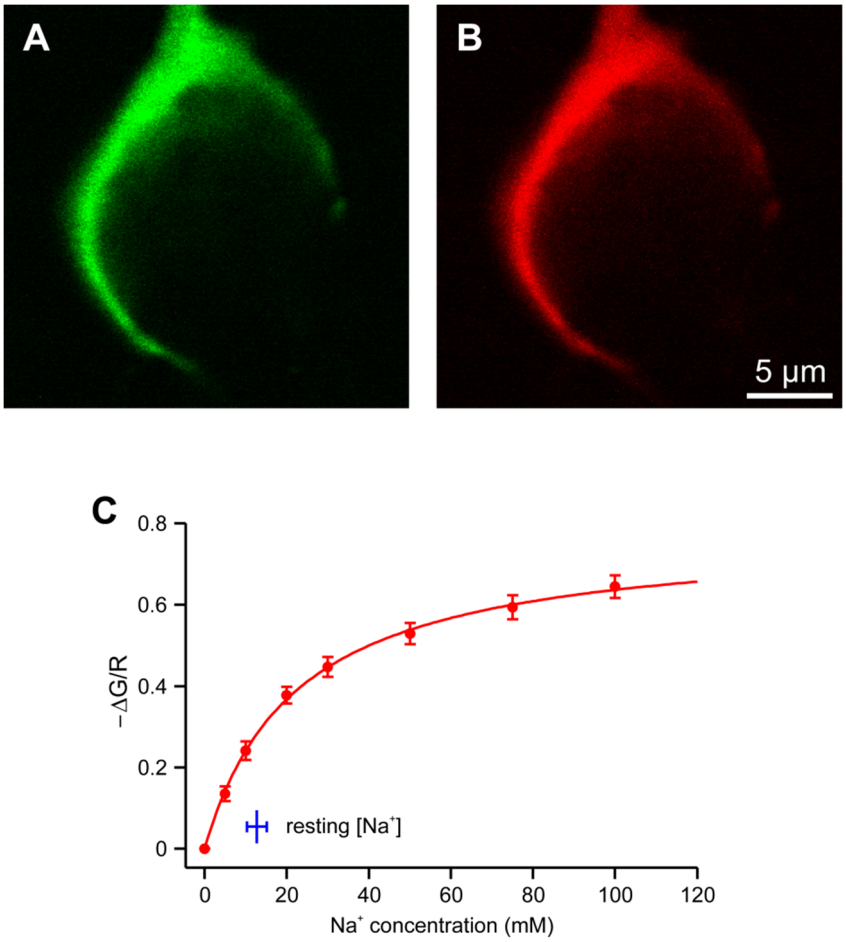

Figure S1 associated with Figure 2. Calibration of SBFI fluorescence upon change in $[Na^+]_i$.
(A-B) A single optical section of fluorescence from SBFI (A) and Alexa 594 (B) in a calyx of Held terminal. (C) Change in fluorescence with $[Na^+]_i$ (n = 5). The saline containing ionophores and ouabain (see Methods), changes in the extracellular $[Na^+]$ from 0 to 100 mM evoked decreases in the SBFI fluorescence. Data are normalized and fitted by the equation: $(\Delta G/R) = (G/R)_{max} \times [Na^+]_i / ([Na^+]_i + K_{app})$, where G/R is the ratio of green fluorescence relative to red fluorescence; (ΔG/R) is the change in fluorescence ratio measured at a given $[Na^+]_i$ divided by that at 0 mM $[Na^+]_i$; $(G/R)_{max}$ is the maximal change in fluorescence ratio and K_{app} is the apparent K_d of SBFI. The fitted curve yielded an apparent K_d (K_{app}) of 22 mM and $(G/R)_{max}$ of 0.78 for SBFI. The resting $[Na^+]_i$ is measured and shown in the insert. Error bars, ± S.E.M.

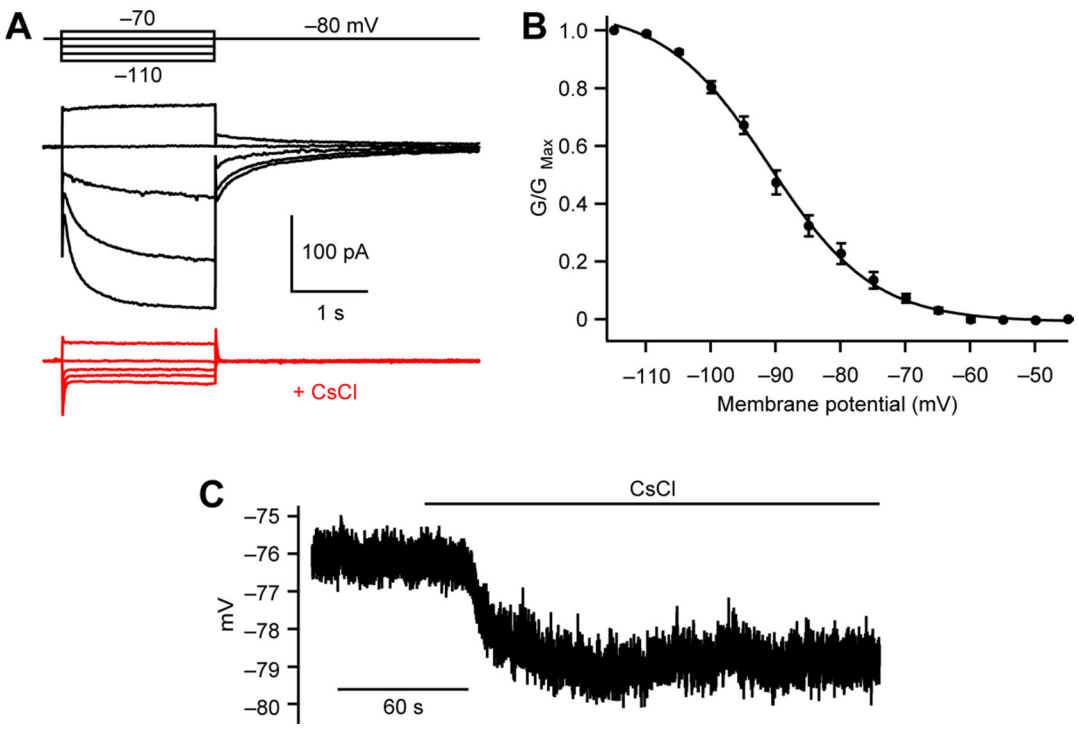

Figure S2 associated with Figure 3. HCN channels contribute to resting membrane potential. (A) Voltage steps (upper) from a holding potential of –80 mV revealed a hyperpolarization-activated inward current which has slow activation and deactivation kinetics (middle). This current was blocked by 2 mM CsCl (lower) (B) Activation curve of the HCN current. The tail current amplitude is plotted against the prior command voltage and is fitted to a Boltzmann function, yielding a V_{half} of = –91.8 ± 0.6 mV and a slope of 10.6 ± 0.5 mV (n = 10). Error bars, ± S.E.M. (C) Blocking HCN channels by CsCl (2 mM) hyperpolarized a calyx by 3 mV.

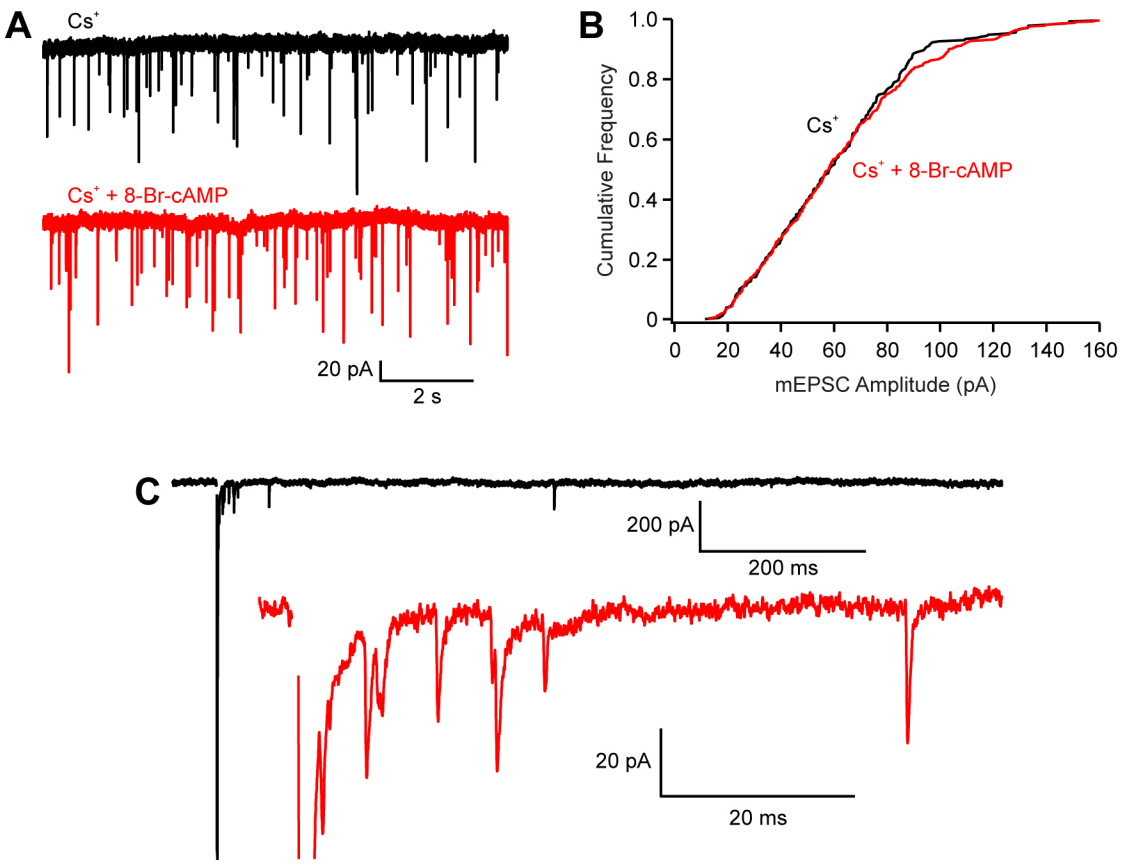

Figure S3 associated with Figure 4. Actions of 8-Br-cAMP on miniature EPSCs.
(A-B) 8-Br-cAMP showed no effects on mEPSC amplitude (A) or cumulative probability histograms (B) when HCN channels were blocked by CsCl. (C) Example trace showed prolonged asynchronous release (aEPSC) followed by the initial fast EPSC current. The insert depicts higher magnification of the asynchronous release. The extracellular Ca^{2+} was replaced by Sr^{2+} (8 mM). The presynaptic axons were stimulated with an extracellular theta electrode and the postsynaptic currents were recorded.