Van Hook et al., http://www.jgp.org/cgi/content/full/jgp.201411229/DC1

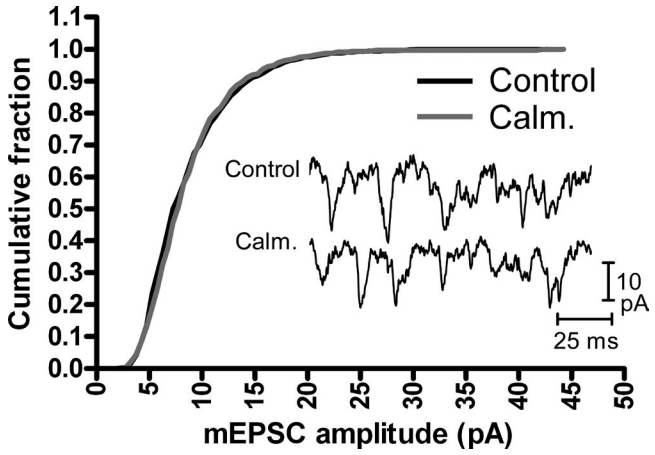

Figure S1. Miniature EPSC amplitude was not affected by calmidazolium. Cumulative frequency distribution of HC mEPSC amplitudes recorded in control conditions and in the presence of calmidazolium (Calm.; 20 µM). (inset) Example traces of mEPSCs from a single HC recorded before (control) and 5 min after application of calmidazolium.

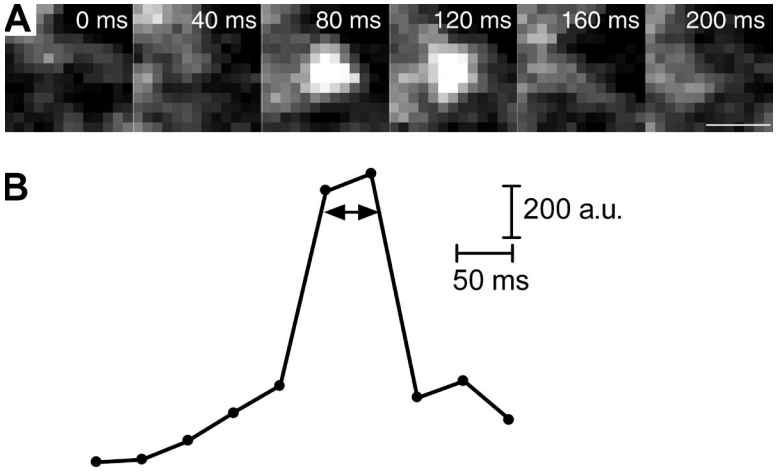

Figure S2. Imaging of single-vesicle fusion events. To test whether the time that vesicles spend at the membrane before fusion might limit the rate of replenishment in cones, we used TIRFM to image membrane approach, docking, and fusion of individual vesicles in isolated cone photoreceptors loaded with a dextran-conjugated, pH-sensitive form of rhodamine (pHrodo). (A) Consecutive images showing the appearance and disappearance of a single pHrodo-loaded synaptic vesicle in a cone terminal. Bar, 500 nm. (B) Change in fluorescence plotted as a function of time. The membrane dwell time, marked with the double arrow, is the interval from the 90% rise to 10% decline in pHrodo fluorescence intensity. This was measured as the number of frames in which fluorescence exceeded 90% of the peak pHrodo fluorescence value. Upon depolarizing stimulation, pHrodo-loaded vesicles entered the thin evanescent field of illumination (length constant = 64 nm; Chen et al., 2013), becoming progressively brighter as they approached the membrane. After spending a brief time near the membrane, vesicle fluorescence declined abruptly as pHrodo was released upon fusion and the fluorescence was quenched by the alkaline pH (7.8) of the extracellular environment. The membrane dwell times of single vesicles before fusion averaged 46.8 ± 2.4 ms (n = 41 fusion events), similar to that for vesicles in rods (Chen et al., 2013). The finding that membrane dwell times were substantially shorter than the measured τ_{fast} for replenishment (~800 ms) indicates that they do not limit the rate of replenishment and are thus unlikely to underlie Ca^{2+}/CaM acceleration of replenishment.

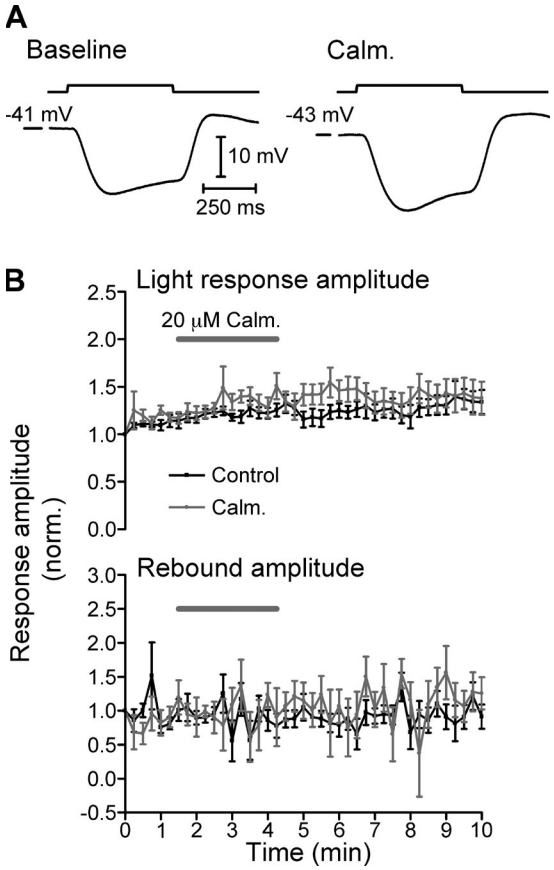

Figure S3. Cone light responses were not affected by calmidazolium. (A) Perforated patch current-clamp recording of responses of a cone to a 500-ms light flash delivered before (left) and during application of 20 μM calmidazolium (Calm.; right). (B) Group data. Cones were stimulated with a 500-ms flash of light every 15 s. Although the amplitude of the light-evoked hyperpolarization increased gradually over the course of the recording, it was not affected by calmidazolium when compared with controls. Normalized to the first light response, the amplitude was 1.32 ± 0.7 in the presence of CaM (n = 10) and 1.21 ± 0.05 when CaM was not added (n = 10; P = 0.2). Likewise, the amplitude of the depolarization at light offset (rebound) was not significantly different between control and calmidazolium conditions. Normalized to the first response, the amplitude of the rebound was 0.97 ± 0.18 (n = 10) in the presence of calmidazolium and 0.92 ± 0.08 when calmidazolium was not added (n = 10; P = 0.8). These results suggest that calmidazolium affects the light responses of HCs and Off BCs by affecting synaptic transmission rather than by affecting cone phototransduction. Mean ± SEM is shown.

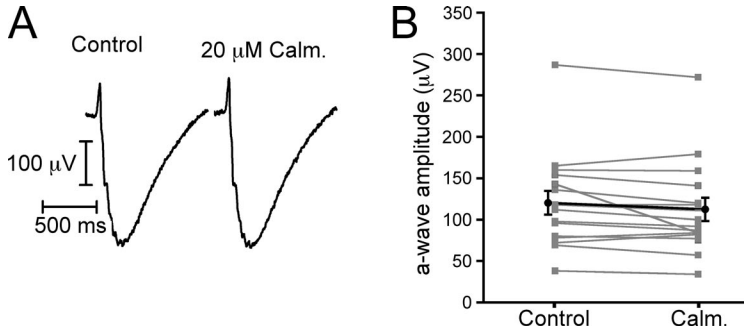

Figure S4. ERG a-wave was not affected by calmidazolium. The population cone light response was measured using intraretinal ERG recordings from salamander eyecups. (A) Example ERG traces from a single eyecup before and after application of 20 μM calmidazolium (Calm.). In this intraretinal recording configuration, the a-wave is the positive-going component that is followed by a slower, negative-going b-wave. (B) Population data showing that the a-wave amplitude was not affected by calmidazolium. Control amplitude = 120 ± 14 μV; calmidazolium amplitude = 112 ± 14 μV (n = 16; P > 0.05, paired t test). Mean ± SEM is shown.

REFERENCE

Chen, M., M.J. Van Hook, D. Zenisek, and W.B. Thoreson. 2013. Properties of ribbon and non-ribbon release from rod photoreceptors revealed by visualizing individual synaptic vesicles. *J. Neurosci.* 33:2071–2086. http://dx.doi.org/10.1523/JNEUROSCI.3426-12.2013