Supplementary Figure:

**Fig. S1 Trace-by-trace illustration of raw traces for Fig. 1A (20 µM FITC-cAMP in pipette solution).**

The trace shown in red was recorded at the time point indicated on the side. Traces recorded at other time points are shown in black. At 3 minutes, under the first light pulse, there was a significant increase in I_h but the increase in I_{inst} was minimal. At 5 minutes, under the last light pulse, the increase in I_{inst} became more prominent. At 5.5 minutes, with the light off, the increase in I_{inst} remained unchanged whereas the increase in I_h was reversed (to a lower level compared to control; likely due to rundown).

**Fig. S2 Trace-by-trace illustration of raw traces for Fig. 1B (20 µM FITC in pipette solution).**

The trace shown in red was recorded at the time point indicated on the side. Traces recorded at other time points are shown in black. At 9.5 minutes, under the first light pulse, there was a significant increase in I_h but the increase in I_{inst} was minimal. At 11.5 minutes, under the last light pulse, the increase in I_{inst} was still minimal. At 12 minutes, with the light off, the increase in I_h was reversed to a lower level compared to control (likely due to rundown).

**Fig. S3 Statistical analysis of FITC-cAMP, cAMP, and FITC results**

Values of absolute I_{inst} (relative to zero current) were first normalized to the I_{inst} and I_h of the last trace before light exposure: \((I_{inst} - I_{inst, before light}) / I_h, before light\). The I_{inst} component sensitive to Cs^+ refers to difference between the timepoint of 1 minute after light off and the timepoint of 1 minutes after Cs^+ application.

**Fig. S4 Light illumination applied to neurons loaded with 1 mM cAMP still increases I_h amplitude.**
To investigate the molecular mechanism behind this dramatic increase in I\textsubscript{h} amplitude and kinetics upon light exposure, we studied two chemical factors that are known to be able to enhance HCN channel opening, cAMP and PIP2. To test the possibility of a transient increase in cAMP upon light illumination, we added saturating concentration of cAMP (1 mM) into the pipette solution. However, light exposure still enhances the I\textsubscript{h} component, which excluded the involvement of cAMP (Number of experiments: N=5).

**Fig. S5 Light illumination applied to neurons loaded with PIP2 (47 µM) or cAMP (1 mM) + PIP2 (47 µM) still increases I\textsubscript{h} amplitude.**

To test the possibility of a transient increase in PIP2 upon light illumination, we added PIP2 (47 µM) into the pipette solution (Number of experiments: N=5). In addition, we tested the condition of adding both cAMP and PIP2 into the pipette solution (Number of experiments: N=4). Light exposure still enhances the I\textsubscript{h} component, which excluded the involvement of PIP2 and cAMP.

**Fig. S6 Light pulses induced an instantaneous and local temperature increase of 1.1°C.**

A glass recording pipette was filled with ACSF and mounted in the bath. A digital thermistor was mounted adjacent to the tip of the recording pipette and both were submerged in the bath solution. A voltage step of 5 mV was delivered to the recording pipette under the voltage-clamp mode. The current passing through the recording pipette and the readings from the thermistor were recorded simultaneously. Then the tip of the recording pipette was transferred to the middle of brain slice, at the same depth of typical whole-cell recordings.

Top: calibration of the recording pipette as a sensor for local temperature sensor. Raw data points for the current-temperature curve were fitted with a linear function. Grey, pipette tip in bath, slope = 28.88 µA/°C; black, pipette tip in brain slice, slope = 28.95 µA/°C.

Bottom: local temperature changes due to light on or off. The estimated change of temperature was estimated to be ~1.1°C.