recording (c). At light offset the dark current showed an inward shift which slowly decayed and flash responses returned to only 50% of control amplitude.

To determine the concentration of free Ca^{2+} required to induce desensitization of ON-bipolar cell light responses, whole-cell recordings were obtained with the free Ca^{2+} in the patch-pipette solution buffered with BAPTA to concentrations of 1 and 50 μM (Fig. 3). With 1 μM free Ca^{2+} there was little change in the dark current, peak flash responses or membrane conductance over the duration of 15 min recording (b). In contrast, with free Ca^{2+} buffered to 50 μM (a) a gradual decrease in inward dark current and input conductance was observed, which was accompanied by a decrease in light responses. The slow timecourse of response suppression was probably due to the time taken for equilibration of the dendrites with the patch-pipette solution. The absence of any effect with 1 μM Ca^{2+} suggests that this concentration may be close to the dark level, as is the case in photoreceptors.[12] Higher concentrations of free Ca^{2+} (>100 μM) induced a more rapid and complete block of light responses, whilst some reduction was observed with lower concentrations of 20 μM.

A well known characteristic of background light adaptation is the recovery of responses, but with a lower sensitivity, after a period of marked desensitization which may last tens of minutes, especially at low temperatures.[13,14] To demonstrate light adaptation occurring in ON-bipolar cells independently from rods, flash responses were superimposed on very dim backgrounds (1 $Rh^* s^{-1}$), too dim to induce significant desensitization in rods, in the absence of Ca^{2+}-chelator in the patch-pipette solution (Fig. 4). The intensity-response relation was measured in darkness and during the application of the dim background step. At step onset (a), there was a small inward current transient, after which the membrane current returned close to zero. Test flash responses were suppressed for 30 s by the dim background, then recovered to about half their initial amplitude, indicating a time-dependent recovery from desensitization. On increasing the test flash intensity, the same inward current response could be obtained at twice the light intensities as that elicited in darkness, which was suggestive of true adaptation. At

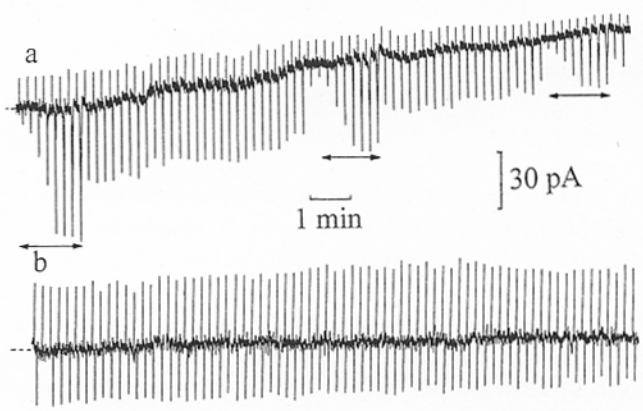

Fig. 3 Raising intracellular free Ca^{2+} desensitizes ON-bipolar cell light responses. Whole-cell recordings from ON-bipolar cells with 50 μM (a) (n = 4) and 1 μM (n = 3) (b) free Ca^{2+} in the patch-pipette solution (Cs-based). The records begin 30 s after going whole-cell, with the cells voltage-clamped to their dark potentials (−29 (a) and −26 mV (b)). Inward current responses were elicited by 1 Rh^* 0.2 ms test flashes (a) and 2 Rh^* 20 ms test flashes (b). In (a) the intensity-response relation was determined at intervals (horizontal arrows) by applying light flashes ranging from 0.25–16 Rh^*. The upward deflections are current responses to 0.5 mV voltage command pulses. There was a decrease in input conductance from 43 to 14 nS as Ca^{2+} diffused into the cell, which was accompanied by an outward current of 35 pA from the initial dark level. (Reproduced with permission from Shiells & Falk, 1999[39]).

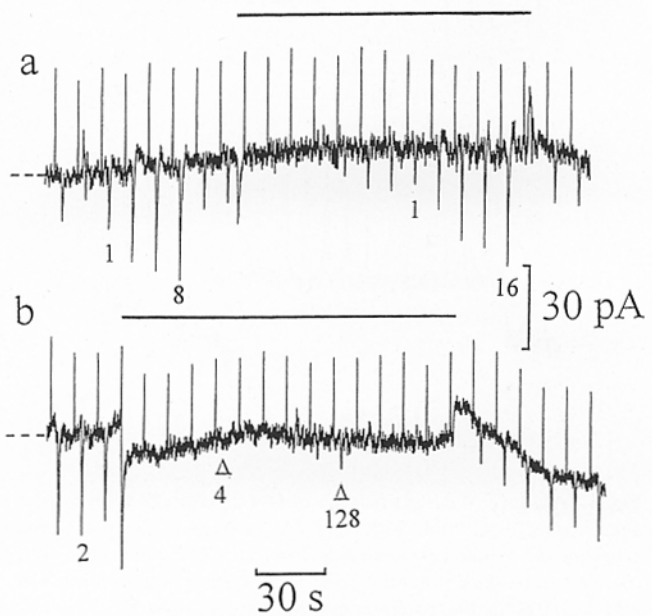

Fig. 4 Adaptation of ON-bipolar cell flash responses by dim light steps. Whole-cell recording from an ON-bipolar cell with no Ca^{2+}-chelator in the patch-pipette solution (K-based). The cell was voltage-clamped to its dark potential (−27 mV). (a) The test flash (2 ms) bleached 1 Rh^* and the intensity was increased by factors of 2 as indicated below the light responses. The upward deflections are current responses to 1 mV voltage command pulses. A light step bleaching 1 $Rh^* s^{-1}$ was applied for the duration indicated by the bar above the trace, and test flashes were superimposed periodically just after each voltage command pulse. The test flash intensity was then increased by factors of 2 from 1 to 16 Rh^* in the presence of the background. Trace (b) continues without break from (a), but the test flash was increased to 2 Rh^*. A background bleaching 10 $Rh^* s^{-1}$ was then applied with the same test flashes superimposed. At the arrow the test flash intensity was increased to 4 Rh^* and then raised by factors of 2 to 128 Rh^*. Even brighter test flashes were applied in the following 30 s, but these failed to elicit any responses. The input conductance remained relatively constant (30 nS) during the recording. (n = 3). (Reproduced with permission from Shiells & Falk, 1999[39])