Figures and figure supplements

Activity and Ca$^{2+}$ regulate the mobility of TRPV1 channels in the plasma membrane of sensory neurons

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Figure 1. Capsaicin induced sparklets in whole-cell voltage clamped dorsal root ganglion cells loaded with Fluo-4. (A) Images of immobile sparklet (white circle) in dorsal root ganglion cell at time points when inactive (1) and active (2) with corresponding intensity trace below (see Video 1). (B) Moving sparklet recorded for 5.6 s in dorsal root ganglion cell (see Video 2). Inset is of sparklet trace magnified fivefold. Inset scale bar is 1 µm. All other scale bars are 5 µm. (C) Mean fluorescence intensity ($\Delta F/F_0$) and (D) open probability ($P_{\text{open}}$) of dorsal root ganglion sparklets ($N = 5; 3$ cells) in Figure 1—figure supplement 1 A–E. Cut-off for ‘open’ is when intensity ($\Delta F/F_0$) exceeds 0.05. DOI: 10.7554/eLife.03819.003
**Figure 1—figure supplement 1.** All points histogram of immobile sparklets in dorsal root ganglion cells (N = 5). (A) Histogram of sparklet intensity trace shown in inset of Figure 1A (corresponding Video 1). (B, C) Histograms of two separate sparklets observed in a cell (see Video 3). (D, E) Histograms of two additional, separate sparklets in one cell.

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**Figure 1—figure supplement 2.** Dose-response relation for activation TRPV1 channels in isolated dorsal root ganglion neurons by capsaicin. Points are the means from four cells, and the currents for each cell were normalized by the current measured at 300 nM capsaicin. Error bars are standard error of the mean. Data is fitted to a Hill curve with Hill coefficient of 2.2. Blue arrow indicates the data point for 100 nM capsaicin.

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Figure 1—figure supplement 3. Increase in fluorescence intensity due to Ca\(^{2+}\) influx into F11 cell transfected with TRPV1-tagRFP, with Fluo-4 introduced via the whole-cell patch pipette. The two images of the F11 cell are a projection of bright pixels in a set of images before capsaicin was added (left) followed by a projection of bright pixels in a set of images after capsaicin was added (right) (see Video 5). Scale bars are 5 µm. DOI: 10.7554/eLife.03819.006

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Figure 2. Fluorescence intensity traces of immobile sparklet sites in cells loaded with Fluo-5F and extracellular buffer containing 20 mM Ca\(^{2+}\) and voltage-clamped as described in ‘Materials and methods’. (A) Without Ca\(^{2+}\) in the extracellular buffer very little green fluorescence was observed in the capsaicin-treated (100 nM) HEK293T/17 cell that was transiently transfected with TRPV1-tagRFP. The image in the top panel was acquired after a 30 s exposure to capsaicin. Addition of 20 mM Ca\(^{2+}\) to the extracellular buffer increased the fluorescence of the cell footprint dramatically and individual sparklet sites could be observed (indicated with white arrowheads in bottom panel). TIRF images have matching lookup table (LUT) values and have not been otherwise altered. Scale bar is 5 µm. (B) Representative trace of sparklet from TRPV1-tagRFP expressing HEK293T/17 cell. (C) All points histogram of trace in (B). (D) Mean fluorescence intensity (ΔF/ΔF\(_0\)) and (E) open probability (P\(_{\text{open}}\)) of dorsal root ganglion sparklets (Blue; same data as in Figure 1C,D) and TRPV1-tagRFP sparklets in HEK293T/17 cells (green; N = 4). Cut-off for ‘open’ is when intensity (ΔF/ΔF\(_0\)) exceeded 0.05. See Video 7. DOI: 10.7554/eLife.03819.013
Figure 2—figure supplement 1. Fluorescence intensity traces of TRPV1-independent Ca$^{2+}$ events in HEK293T/17 cells loaded with Fluo-5F, with extracellular buffer containing 20 mM Ca$^{2+}$, and studied with whole-cell voltage clamp as described in ‘Materials and methods’. (A) Representative trace of Ca$^{2+}$ events in an untransfected cell. (B) All points histogram of trace in (A).
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Figure 3. Photobleach step analysis of TRPV1-GFP in fixed HEK293T/17 cells. (A) Fluorescence time traces of six representative TRPV1-GFP features. Arrowheads indicate individual photobleach steps (B) Histogram of TRPV1-GFP bleach steps in fixed HEK293T/17 cells. TRPV1-GFP fluorescence is characterized as having 1–4 bleaching steps (blue). A zero-truncated binomial distribution fit by maximum likelihood estimate with $n = 4$ to give a probability of 0.45 (red) ($\chi^2$ goodness-of-fit is 0.1712 and the model is acceptable). (C) Fits to zero-truncated binomial distributions with $n = 5$ (cyan) or $n = 8$ (green) by maximum likelihood estimate gave probabilities of 0.35 and 0.21, respectively. Chi-squared goodness-of-fit tests were done with $n = 5$ and $n = 8$ models, yielding $\chi^2$ values of 4.0717 and 16.386, respectively.
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Figure 4. Distribution of TRPV1-GFP mobility in HEK293T/17 cell (not voltage-clamped; see ‘Materials and methods’). (A) TRPV1-GFP channel tracks in a 200 frame video (33 frames per second). Tracks are identified with the u-track algorithm (see ‘Materials and methods’) and are overlaid on the final image of the series (See Video 8). Extent of cell body is shown in grey. The inset is an isolated track from the bottom center of the larger panel labeled ‘3’. (B) Mean square displacements (MSD) at different time lags of three tracks identified as 1 (blue), 2 (green), and 3 (red) in (A). (C) A Histogram of effective diffusion coefficients derived from tracks in the full 15 s video for the cell shown in (A). Tracks identified as 1, 2, and 3 in (A) are indicated over their assigned bins. (D) Mean effective diffusion coefficients calculated from TRPV1-GFP tracks in HEK293T/17 cells (N = 5). Scale bar of image in (A) is 5 µm and inset scale bar is 1 µm.

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**Figure 5** TRPV1-GFP and sparklet observed in a moving channel in a voltage-clamped HEK293T/17 cell. (A) Track of TRPV1-GFP with increased sparklet intensity. Scale bar is 1 μm. (B) Fluorescence intensity trace of mobile TRPV1-GFP channel in (A) with sustained higher levels of fluorescence due to sparklet activity. The background image in (A) corresponds to the time annotated with an arrowhead in panel (B). (C) TRPV1-GFP and sparklet positions are shown as filled circles. Red circles are positions assigned to TRPV1-GFP and cyan circles are sparklet positions. Each circle's radius is 1/10 of the standard deviation attributed to the 2-D bivariate Gaussian fitted to the fluorescence feature at that position. Scale bar is 0.5 μm. See Videos 9 and 10.

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**Figure 4—figure supplement 1** Distribution of effective diffusion coefficient ($D_{\text{eff}}$) in different cells (A–D). Each event accounts for the effective diffusion coefficient ($D_{\text{eff}}$) calculated from a time lag of 1–10 frames in a single TRPV1-GFP track (See ’Materials and methods’).

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Figure 6. Mobility change in TRPV1-GFP channels after capsaicin treatment in cells not subject to voltage clamp. (A) HEK293T/17 cells expressing low numbers of TRPV1-GFP are initially imaged for 15 s and then a second 15 s video follows perfusion with a 1 μM capsaicin solution (described in ‘Materials and methods’). All three treatment conditions begin in HBSS (containing Ca²⁺) with no capsaicin. Average MSD (time lag of 90 msec) for all tracks shows no significant change in mobility from before to after capsaicin. (B) Using the same source data, we characterize mobility with the MSD difference ratio (RΔMSD, described in ‘Materials and methods’ and Figure 6—figure supplement 1). With the switch from no capsaicin to 1 μM capsaicin but maintaining the Ca²⁺ concentration at 1.8 mM, the RΔMSD drops a significant amount from the initial condition (N = 5, blue data points) as determined by a one-tailed paired t-test (p < 0.01). This significant drop in RΔMSD from the initial video to the second video is not observed under conditions where the second solution contains no capsaicin and no added Ca²⁺ (N = 6 cells, green data points; n.s.: not significant) nor is it seen when the second solution contains capsaicin (1 μM) but no added Ca²⁺ (N = 6 cells, red data points).

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Figure 6—figure supplement 1. Flow diagram detailing MSD difference ratio ($R_{\text{MSD}}$) calculation from top to bottom. Videos taken initially or after treatment (beginning 50 s after end of first video) are split into two parts. Each part has the MSD calculated for each trajectory with the time lag set to 90 or 120 msec (3 or 4 frames, respectively for Figure 6 or Figure 6—figure supplement 2) before averaging these to acquire a mean for the set of trajectories, giving MSD$_1$, MSD$_2$, MSD$_3$, and MSD$_4$. The MSD difference ratio is expressed as the difference between the first part MSD and the second part MSD (i.e. MSD$_2$-MSD$_1$ in initial video) divided by the first part (MSD$_1$ in initial video).

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Figure 6—figure supplement 2. Mobility change in MSD calculated for 120 msec with TRPV1-GFP channels after capsaicin treatment. Using the same source data as in Figure 6 but with the time lag set to 120 msec (4 frames), we characterized mobility with the MSD difference ratio ($R_{\Delta MSD}$, described in ‘Materials and methods’ and Figure 6—figure supplement 1). With the switch from no capsaicin to 1 μM capsaicin but maintaining the Ca$^{2+}$ concentration at 1.8 mM, the $R_{\Delta MSD}$ drops a significant amount from the initial condition ($N = 5$, blue data points) as determined by a one-tailed paired t-test ($p < 0.05$). This significant drop in $R_{\Delta MSD}$ from the initial video to the later video is not observed under conditions where the second solution contains no capsaicin and no added Ca$^{2+}$ ($N = 6$ cells, green data points; n.s.: not significant) nor is it seen when the second solution contains capsaicin (1 μM) but no Ca$^{2+}$ ($N = 6$ cells, red data points).

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Figure 7. Characterization of mobile TRPV1 sparklets in voltage-clamped HEK293T/17 cells. (A) Trajectory of sparklet with decreasing mobility. The total steps of the trajectory are divided into three equal parts representing the first third (red), the second third (blue) and the last third (green) of each track. Scale bar is 1 μm. Displacement (see ‘Materials and methods’) plot of sparklet in (A) is shown below. The blue line is the slope used to characterize the change in displacements over time. (B) Slopes corresponding to the change in displacements of mobile sparklets (N = 16) in density plot with the center of the distribution located below 0.0. DOI: 10.7554/eLife.03819.026

Figure 7—figure supplement 1. Characterization of mobile TRPV1 sparklets in voltage-clamped HEK293T/17 cells. (Left) A representative trajectory of sparklet with increasing mobility is shown with the same color coding for the track as in Figure 7. Scale bar is 1 μm. Displacement (see ‘Materials and methods’) plot of sparklet is shown to the right. The blue line is the slope used to characterize the change in displacements over time. DOI: 10.7554/eLife.03819.027