Supplementary Material

Population Coding of Capsaicin Concentration by Sensory Neurons Revealed Using Ca^{2+} Imaging of Dorsal Root Ganglia Explants from Adult *pirt-GCaMP3* Mouse

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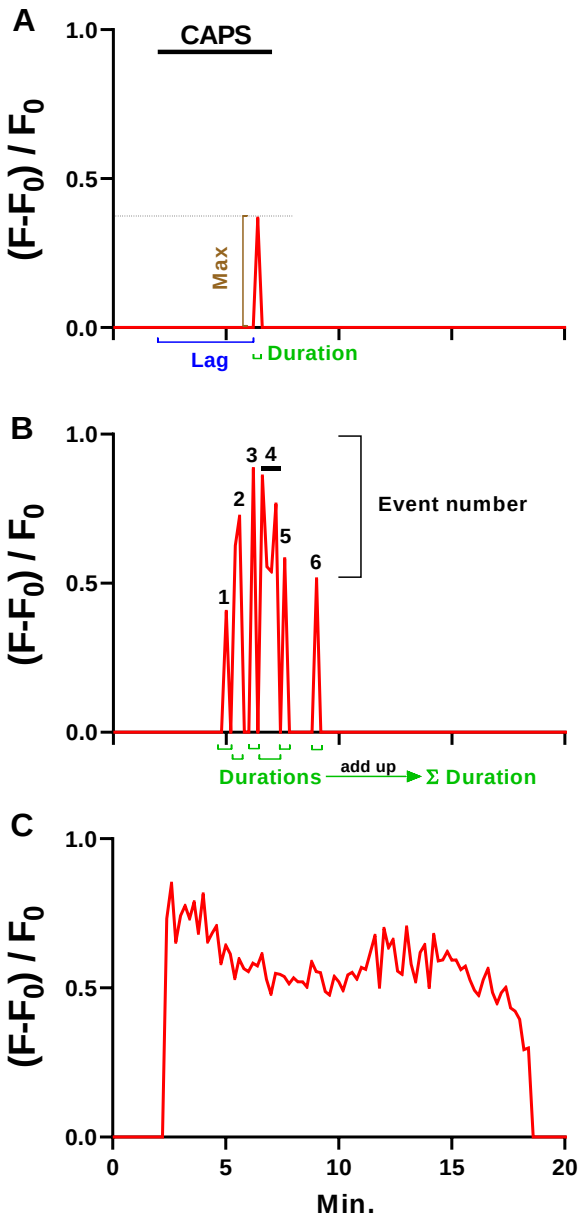
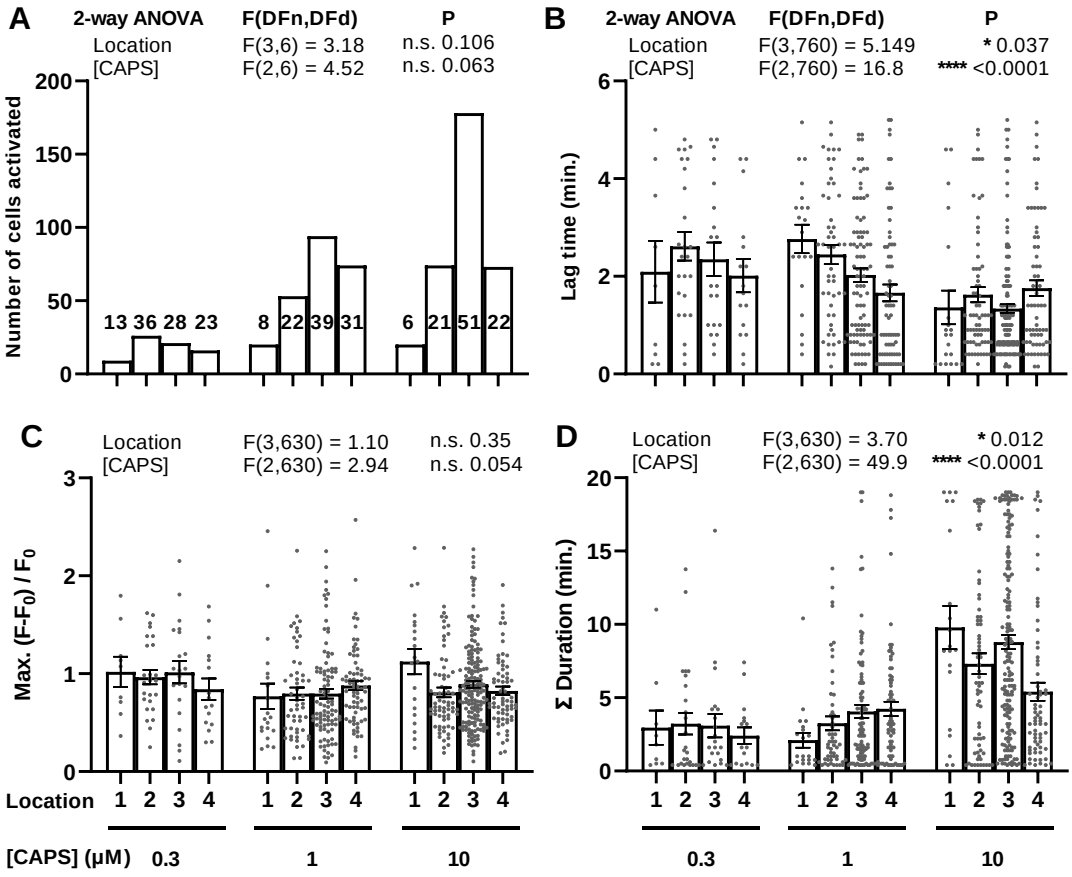
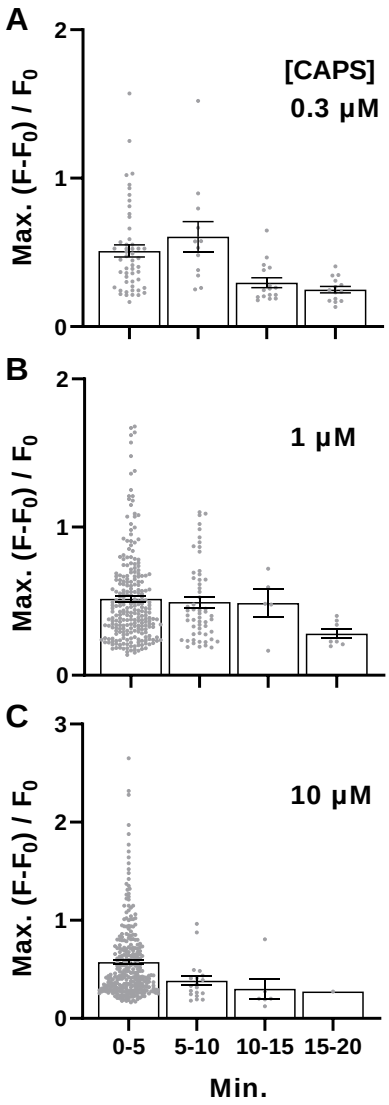
Supplementary Figures



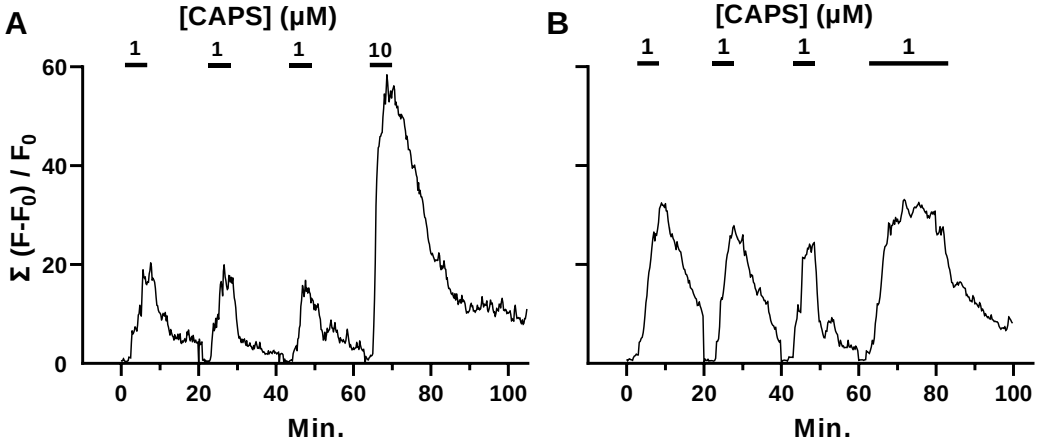
Supplementary Fig. 1. Examples of fluorescence responses to CAPS from individual cells. (A) One brief signal, (B) a signal that flickered between high and low intensity; in between high intensity flashes, the fluorescence often deteriorated below threshold (as defined earlier, see **Materials and Methods**) before illuminating again. Thus, discrete signals above threshold could be identified (numbers in B), using the peak analysis program. (C) Fluorescence that remained elevated for almost the entire recording period with only minor fluctuations in intensity.



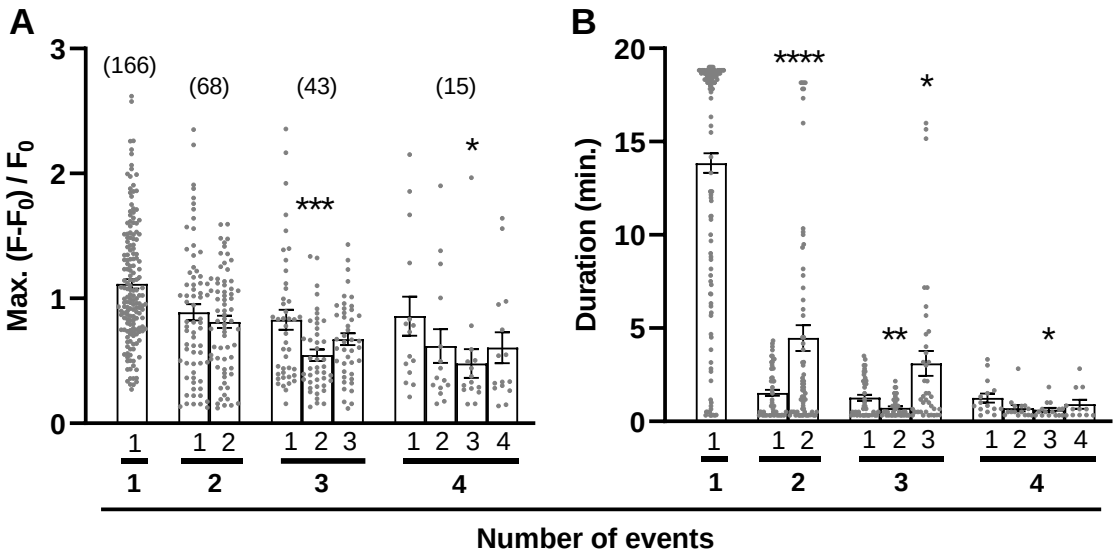
Supplementary Fig. 2. The location of neurons within DRG has little impact on their excitation by CAPS. Fiji was used to determine the x and y co-ordinates for ROIs centred over the bright spots in individual confocal slices, whilst z-axis values were determined from their positions in z-stacks. Only DRGNs that were excited during the first 5 min. were analysed. The centre point and peripheral boundary of the whole DRG was determined from the overall height of the confocal stack and from large ROIs encompassing the whole ganglia in collapsed confocal images; these were utilised to determine radial length along the x, y and z axes. Using the latter information, the co-ordinates of each ROI were converted to % of radial distance from the DRG centre along each axis. DRGNs were then assigned to location groups as follows: 1, x, y and z co-ordinates all < 25 % of their respective DRG radius; 2, 25-50 %; 3, 50-75 %; 4, 75-100%. Thus, DRGNs assigned to location 1 are closest to the centre of the DRG, followed by location 2 and then 3, so that those in location 4 are nearest to the outer edge of the DRG. (A) Histogram showing the number of DRGNs excited by 0.3, 1 and 10 μM, and assigned to each location category. Numbers above/inside columns indicate the fraction in each location expressed as a % of the total number excited by the requisite [CAPS]. Analysis tools were used to determine for each category the mean ± s.e.m. of (B) lag, (C) Max. and (D) Σ duration of signals in DRGNs assigned to them. The dependence of each measure on [CAPS] or DRGN location was assessed by 2-way ANOVA, with results presented above the requisite panels. This demonstrated that lag and Σ duration, but not Max., were strongly associated with [CAPS]. Although a weak association with location was indicated, the direction of change was inconsistent (i.e. random), by contrast to the uniform direction of [CAPS]-associated changes.



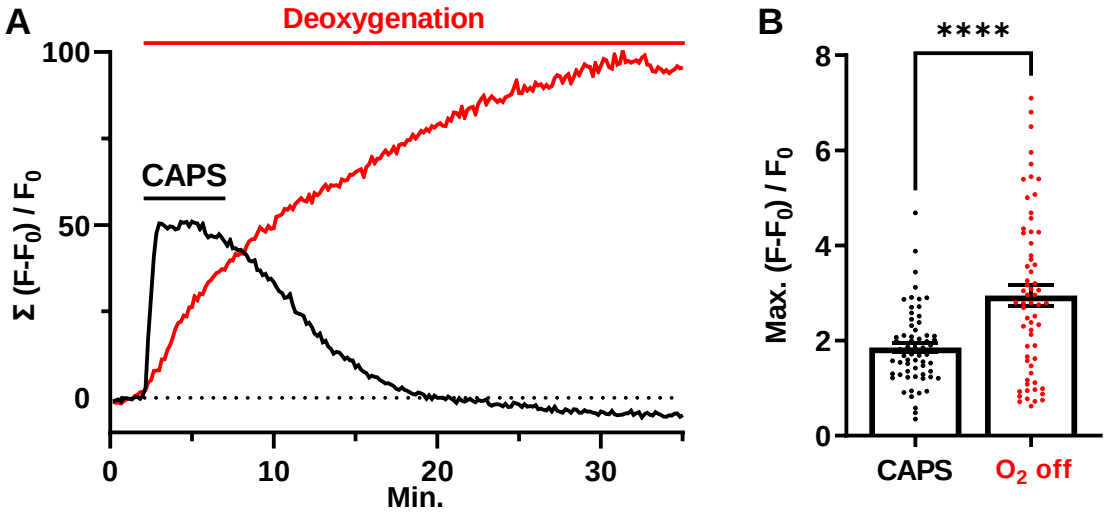
Supplementary Fig. 3. Late-activating DRGNs exhibit lower Max. signals. DRG were stimulated sequentially with 5 min. exposure to 0.3 (A), 1 (B) and 10 (C) µM CAPS, with 15 min. washouts between, as detailed for Fig. 1B. All excited neurons were categorised into groups depending on when their first signal above a threshold (F_0 + 10 x s.d.) was observed and their Max. was measured (irrespective of when the Max. value was reached) and plotted for 5 min. groups (each single grey dot in scatter plots represents the Max. measured in one DRGN). Raising [CAPS] increased the numbers of cells excited and skewed the distribution to the first 0-5 min., when CAPS was present. During washout, the number of neurons activating, and their average Max., declined progressively in consecutive 5 min. periods, with the exception of the 5-10 min. after 0.3 µM CAPS (first 5 min. of washout). The sparse numbers and low Max. of DRGNs with delayed activation might be due to slow washout of the vanilloid.



Supplementary Fig. 4. Raising [CAPS] increases signal density, whereas prolonging CAPS extends signalling at a uniform density. (A,B) Traces show Σ $(F-F_0)/F_0$ plots against time for three 5 min. stimulations with 1 µM CAPS, separated by 15 min. washout periods followed by (A) 5 min. stimulation with 10 µM CAPS and 35 min. washout or (B) 20 min. with 1 µM CAPS and 20 min. washout. In both cases, the signal density progressively declines for the three consecutive 5 min. stimulations with 1 µM CAPS before (A) massively increasing in response to 10 µM CAPS or (B) being maintained for more than 20 min. at a level similar to that observed for the very first 5 min. stimulation with 1 µM CAPS. Data from both traces for the first three stimulations with 1 µM CAPS were used in Fig. 4.



Supplementary Fig. 5. CAPS-induced fluctuations in $[Ca^{2+}]_i$ are preserved after pre-treatment of DRG with thapsigargin to empty internal Ca^{2+} stores. DRG (N=2) were exposed for 20 min. to 1 µM thapsigargin in aCSF-BOC prior to and during exposure for 5 min. to 10 µM CAPS, as well as for its 15 min. washout. Baseline fluorescence (F_0) was not altered significantly by thapsigargin. Signals initiated within 5 min. exposure to CAPS were categorised, analysed and (A) Max. and (B) duration were plotted, as described for Fig. 3. Numbers in brackets (in A) indicate the number of cells assigned to each number of events category.



Supplementary Fig. 6. GCaMP3 is not saturated by 10 µM CAPS-induced increases in $[Ca^{2+}]_i$. (A) Overlaid plots of summed changes in fluorescence plotted against time before, during and after stimulation of DRGNs for 5 min with 10 µM CAPS (black trace; the black bar indicates when it was applied), and in the same cells after subsequent deoxygenation (red trace), which was achieved by stopping the perfusion of warmed aCSF-BOC (red bar). (B) Scatter dot plots of the Max. induced in each ROI by 10 µM CAPS (black dots) and after deoxygenation (red dots; O_2 off), overlaid on histograms showing the mean ± s.e.m. of the Max. values. Asterisks (in B) represent the result of a paired Student's t-test; ****$p < 0.0001$.

Supplementary Movies

To access and watch the Supplementary Movies, please use the respective links.

Supplementary Movie 1. Video montage showing fluorescence signals evoked by sequential exposure of a DRG to increasing concentrations of CAPS**.** Three video segments are shown, for stimulations with 0.3, 1 and 10 µM CAPS, respectively, as labelled in the top left corner of each. The segments were created by projection of the requisite confocal slices into a flattened image time-series (as detailed in **Materials and Methods**). The resultant video clips have been arranged vertically for synchronised accelerated playback. The counter (top right) indicates real time (Min.:Sec.) corresponding to the starting time for acquiring the first image in each confocal stack. Temporary appearance of the 'CAPS' label below the time counter indicates the period during which the vanilloid was applied. Parallel lines of fluorescence to the left and right of the ganglion arise from the tissue holder. All supplementary video montages were created using Fiji.

https://www.cellphysiolbiochem.com/Articles/000394/SM/Supplementary_Movie_1.mp4

SMovie 2. Example of ROI placement for video analysis. This montage shows the flattened confocal video clip for stimulation with 10 µM CAPS, as shown in SMovie 1, presented in duplicate and arranged in a vertical stack for synchronised accelerated playback. The placement of ROIs used for fluorescence signal analysis (yellow circles) is indicated on the upper copy.

https://www.cellphysiolbiochem.com/Articles/000394/SM/Supplementary_Movie_2.mp4

SMovie 3. Video montage displaying signals evoked by 10 µM CAPS in individual confocal slices. This exemplary DRG was captured in 8 adjacent but non-overlapping optical slices of 31.6 µm from z1 at the lowest level to z8 uppermost and closest to the objective lens. Video segments created from images for each confocal layer have been arranged for synchronous playback but note that there was an approximately one second lag for the acquisition of each successive confocal layer and that the counter (upper right) has been set to the starting time for acquisition of slice z1.

https://www.cellphysiolbiochem.com/Articles/000394/SM/Supplementary_Movie_3.mp4