Activity-dependent modulation of dendritic excitability in starburst amacrine cells by metabotropic glutamate receptor type 2 (mGluR2) signaling

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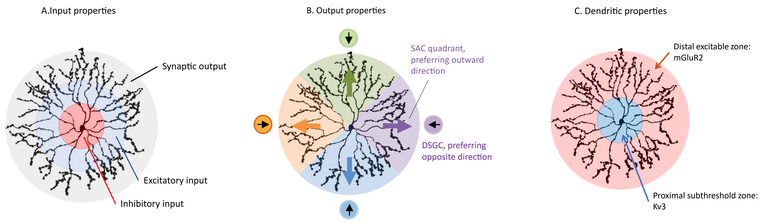
Introduction: Synaptic and dendritic properties of SAC



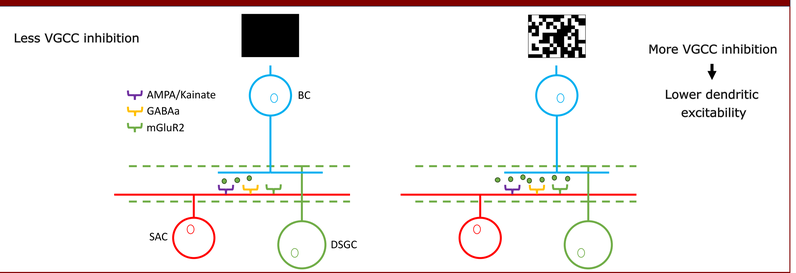
Ledesma et al. (2024)

A. Synaptic inputs and outputs organized along the radial dendritic axis.

B. Output of SAC into four dendritic quadrants with different preferred motion directions. Colored arrows represent preferred direction of SAC quadrant, black arrow represent the preferred direction of direction-selective ganglion cells.

C. Concerted action of dendritic mGluR2 and somatic Kv3 signaling maintains SAC dendritic direction selectivity.

Hypothesis: mGluR2 signaling links visual activity to SAC dendritic excitability

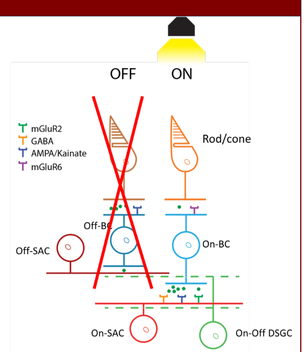


Methods

Visual stimulation of whole mount retina: A white organic light-emitting display (OLED, 400 x 600 pixels, controlled by Matlab) shows a moving bar 440 µm/s with dimensions of 400 µm x 600 µm in 3-5 repetitions per light intensity (up to 6.5 × 104 R*/rod/s) in 8 directions. Either a black background or flickering checkerboard (50 µm diameter, 15 hertz,1/4th of bar intensity) was used to modulate synaptic release of glutamate onto SAC dendrites.

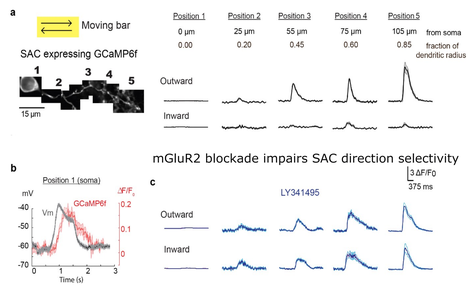
Whole cell patch clamp recordings: Floxed tdTomato mice (129S6-Gt(ROSA)26Sortm9(CAG-tdTomato)Hze/J) crossed with Chat-IRES-Cre mice.Recording with pClamp 10 software and a Multiclamp 700b amplifier. AP5, DNQX and GABAzine were used to isolate mGluR2 signaling (see illustration). Additionaly LY341395, a mGluR2 antagonist was used to confirm the role of mGluR2 in calcium channel activity.

Two-photon calcium imaging: Chat-IRES-Cre mice (129S6-Chat tm2(cre)Lowl/J) injected with AAV vector carrying floxed GCaMP6f. 4 repetitions of a moving bar in 8 directions was shown while imaging the dendritic layer (S4) of the SACs. A framerate of 15-20 hertz was maintained. Only dendritic regions with a transient response were used for analysis.

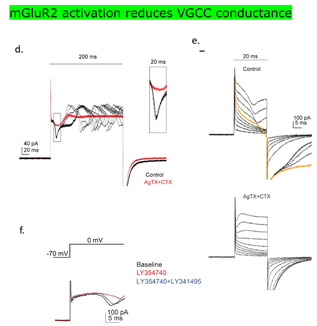


mGluR2 signaling is required for direction selectivity through inhibition of VGCCs

Starburst amacrine cell dendrites are direction selective for outward motion



Ledesma et al. (2024)



Koren et al. (2017)

a. Left: ROIs (#1-5) of a SAC dendritic branch expressing GCaMP6f from soma (1) to distal tip (5). Right: dF/F0 traces in ROIs on the left (Mean, SEM).

b. Overlay of Vm from current clamp recordings and GCaMP6f traces.

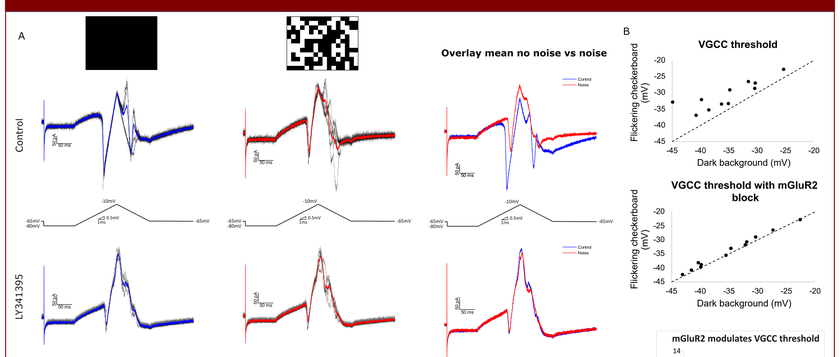
c. Same as in a, but after application of LY341395. Both a and c are scaled to same dF/F0

d. Traces of a SAC (cesium-based internal) at baseline and after addition of calcium channel antagonists (AgTX + CTX) during steps of depolarization from -70 mV to 0 mV for 200 ms. For each condition, traces represent the overlay of ten repetitions. Inset shows the boxed region at higher magnification.

e. Same as in (d), but with a shorter depolarization step of 20 ms. Orange trace represents the onset of the inward calcium transient.

f. Traces of a SAC at the baseline (black), mGluR2 agonist (red), and mGluR2 agonist + antagonist (blue)

Visual stimulation decreases VGCC activity in SACs through mGluR2 activation

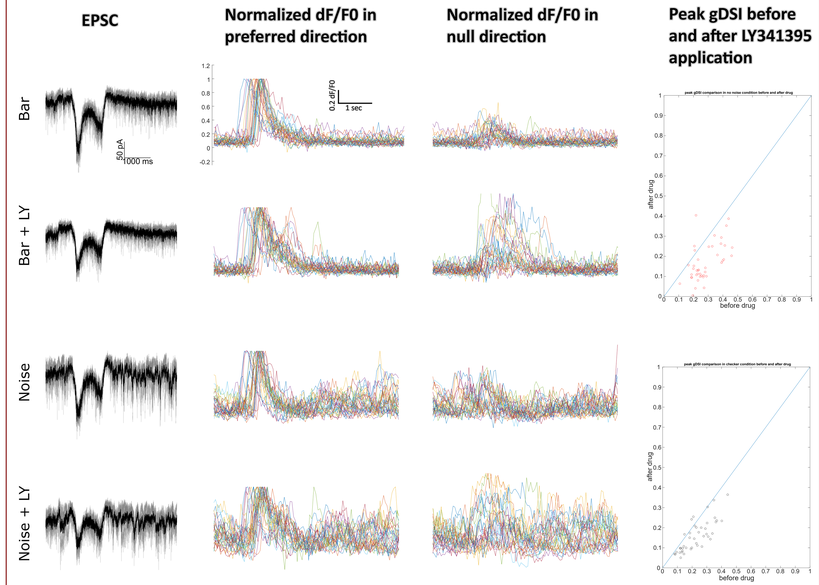


A. Middle; shows the voltage ramp protocol. Holding at -65 mV, stepping down to -80 mV for 100 ms. The up/downward part of the ramp is +/- 0.5 mV/ms with the peak at -10 mV. The left (zero background) and middle (flickering checkerboard) figure shows the individual trials (black) and the average of 5 trials (blue/red). The right figure shows the average of both left and middle figure. All recordings were performed in presence of DNQX, AP5 and GABAzine. Bottom; Same as control (top), but in addition of LY341395

B. Top, VGCC threshold is illustrated for 11 cells. Each point shows VGCC threshold of a SAC under the two stimulus conditions. Middle, same as the top but in presence of LY341395 for a total of 12 cells. The difference in membrane potential between the low and high glutamate conditions has been compared for both control and with LY341395. Student's paired t-Test with two tailed distribution has been used for the given p values.

**** Student's unpaired t-Test with unequal variance; P = 0.002**

The role of activity-dependent mGluR2 signaling in SAC direction selectivity



Four stimulus conditions were used for calcium imaging and whole-cell recordings: First row is in response to a moving bar, the second row to a moving bar with LY341395, third and fourth rows are the same as the first two rows, but in the presence of noise. The left column shows example EPSC recordings from SAC for the four conditions. In the second column, normalized calcium transients (GCaMP6f) from included ROIs in the preferred direction are shown, with each color the average of 4 repetitions (n=30 from XX mice). The third column is the same as the second column, but now for the null direction (opposite of the preferred). The last column shows the gDSI for the no noise (top) and noise condition (bottom) before and after mGluR2 antagonist (100nM). The blue lines represent the unity lines.

Summary

* Visual noise shifts the VGCC threshold of SACs.
* Shift in VGCC threshold is mediated by mGluR2 signaling.
* Direction selectivity is impaired when blocking mGluR2.

Acknowledgement

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