Profound desensitization by ambient GABA limits activation of δ-containing $GABA_A$ receptors during spillover

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Supplementary Figures

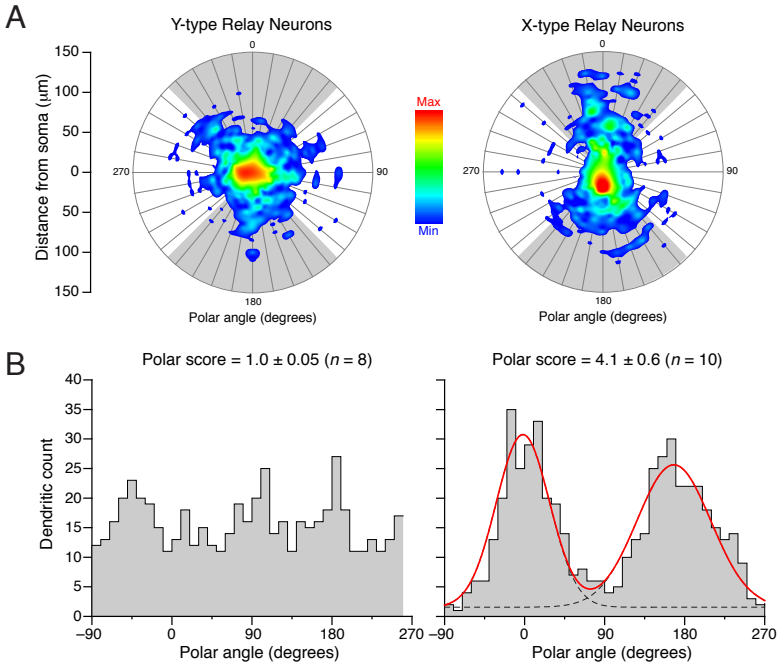

Figure 1 Morphological identification of X- and Y-type thalamic relay neurons. Neurons were initially identified as X- or Y-type based on visual examination; X-type neurons had obvious polar distribution of dendrites and, spine-like appendages close to dendrite branch points, reflecting the presence of triadic glomerular synapses (Rafols and Valverde, 1973). By contrast, Y-like neurons were characterized by their radial distribution of relatively smooth dendrites. A proportion of reconstructed cells was not easily assigned to either group and may represent W-type cells. To confirm X- or Y- type morphology, neurons were z sectioned into 1 μm optical sections using standard confocal microscopy (see **Methods**) and Reconstruct (version 1.0.9.6) (Fiala, 2005) was employed to three dimensionally render the soma, dendrite and axonal membrane. Line tracing through optical sections was performed to calculate dendrite length and to identify the location of branch points. The final reconstruction for each cell included a three dimensional representation of the soma, dendrite and axonal membrane along with line tracing data for the dendrite/axon to identify branch point and spine location. Spines were characterized as portions of dendrite shorter than 5 μm in length. Spine density and spine proximity to branch points was then quantified for each cell. (***A***) Contour plots of superimposed dendritic morphologies, showing distinct dendrite distribution in X- and Y-type thalamic relay neurons. This data was constructed by superimposing a two-dimensional projection of the full reconstruction onto a polar plot in order to measure the distance from the soma of dendrite crossings at 10° intervals. Dendrites clearly cluster around the 0° and 180° polar angles in the X-type relay neuron population (n=10), whereas dendrites are evenly distributed in the Y-type relay neurons (n=8). The polar score for each neuron was calculated from the number of crossings in the vertical quadrants divided by the number of crossings in the horizontal quadrants. From this data, X-type relay neurons had a polar score of 4.14 $\pm$ 0.57 compared to a value close to unity for Y-type neurons (1.02 $\pm$ 0.05; P = 0.0004). (***B***) Histograms of dendritic coverage as a function of polar angle for the X- and Y-type relay neuron populations. The plot from Y-type neurons lacks obvious peaks whereas two clear peaks are visible in the histogram constructed from X-type neurons. The histogram from X-type neurons was well described by two Gaussian functions (red lines) with peaks separated by 180°.

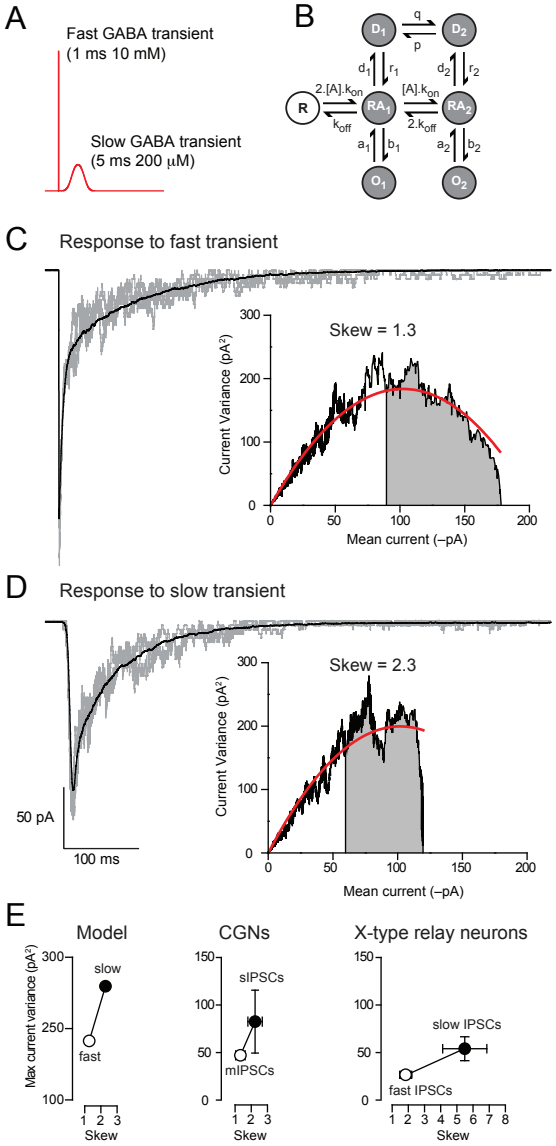

Figure 2 Simulation of fast- and slow-IPSCs following different GABA transients and the resulting current-variance plots. (***A***) Representation of the fast and slow GABA transients used to generate simulated IPSCs. The fast GABA transient was a step function of 1 ms duration reaching a concentration of 1 mM. The slow GABA transient was a filtered step response that peaked at 200 μM with a width at half amplitude of 5 ms. (***B***) The kinetic model used to simulate the response of $GABA_A$ receptors to these different transients. The kinetic scheme and the rate constants are from Jones *et al.* (1998). Simulation was performed with ChanneLab version 2 (Synaptosoft Inc.), using Monte-Carlo simulation with a time interval of 1 μs. Currents were calculated (for 100 $GABA_A$ receptors) from the sum of the two open states (O1 and O2), each with a single-channel conductance of 30 pS. Gaussian noise with a standard deviation of 0.025 was added to the single-channel simulations (***C***) Individual responses (grey traces) and average waveform (black trace) generated in response to the fast GABA transient. The inset is a plot of the mean current against current variance following peak scaled non-stationary fluctuation analysis.

The red curve is a parabolic fit to the entire current-variance relationship (see **Methods**). The open and grey areas of the plot were defined according to the median value of the mean current. The ratio of these two areas was used to calculate a fit-independent measure of skew in the current-variance relationship. (***D***) Same conventions as *C* but for simulated IPSCs generated in response to slow GABA transients. (***E***) Plots of the maximum current variance and skew of the current-variance relationships for simulated currents as well as sIPSCs and mIPSCs recorded form cerebellar granule neurons (CGNs) and fast-and slow-IPSCs recorded from X-type thalamic relay neurons. Note the increased skew and variance associated with data obtained from the model when the same numbers of $GABA_A$ receptors were exposed to slow GABA transients. A similar trend is observed when comparing sIPSCs with mIPSCs from CGNs and comparing fast-and slow-IPSCs in thalamic relay neurons.

References

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