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Supplementary Materials for

Tailoring of the axon initial segment shapes the conversion of synaptic inputs into spiking output in OFF-α T retinal ganglion cells

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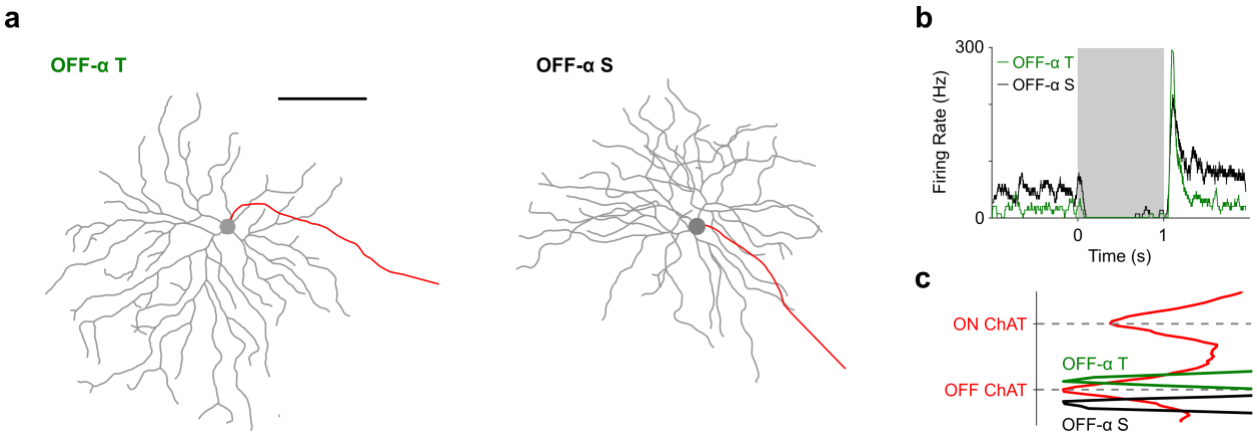

Fig. S1. Identification of OFF-α T RGCs. (**a**) Tracings of representative OFF-α T (left) and OFF-α S (right) RGCs. Axons are indicated in red. Scale bar: 100 μm. (**b**) Light responses of a typical OFF-α T (green) and OFF-α S (black) RGCs. The stimulus was a bright spot 300 μm in diameter on a grey background and was centered over the soma. (**c**) Distribution of fluorescent voxels of OFF-α T (green) and OFF-α S (black) dendrites relative to the ON and OFF ChAT bands (red peaks). All three traces are normalized to their peaks.

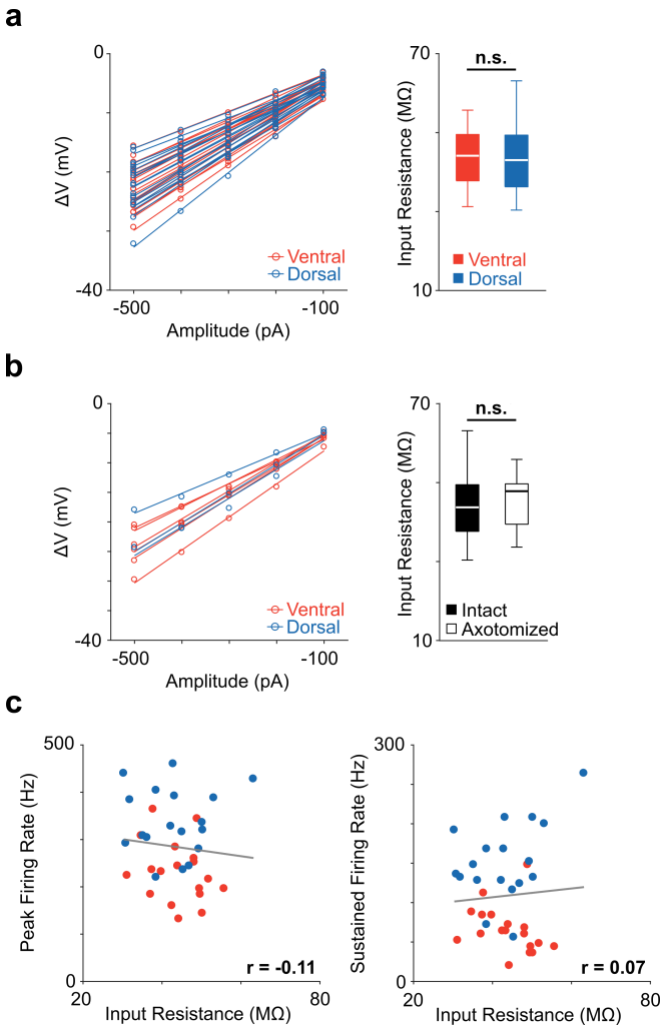

Fig. S2. Passive properties are similar in ventral and dorsal OFF-α T RGCs – (**a**, left) Each line is the best-fit linear regression for ΔV in response to hyperpolarizing current injections for an individual ventral (red, n=18) or dorsal (blue, n=18) RGC. The slope of each line was used as a measurement for input resistance. (**a**, right) Mean input resistance was similar in ventral and dorsal cells (44.2 ± 0.4 MΩ vs. 43.0 ± 0.5 MΩ, p=0.6487). (**b**, left) Each line is the best-fit linear regression for ΔV in response to hyperpolarizing current injections for an individual axotomized ventral (red, n=6) or dorsal (blue, n=3) RGC. The slope of each line was used as a measurement for input resistance. (**b**, right) Mean input resistance was similar in intact vs. axotomized cells (43.6 ± 0.4 MΩ vs. 45.7 ± 0.8 MΩ, p=0.4494). (**c**) Each point is a plot of peak firing rate (left) or sustained firing rate (right) vs. input resistance for an individual ventral (red, n=18) or dorsal (blue, n=18) RGC. The grey lines are the best-fit linear regressions (p=0.5397 and p=0.6820, respectively).

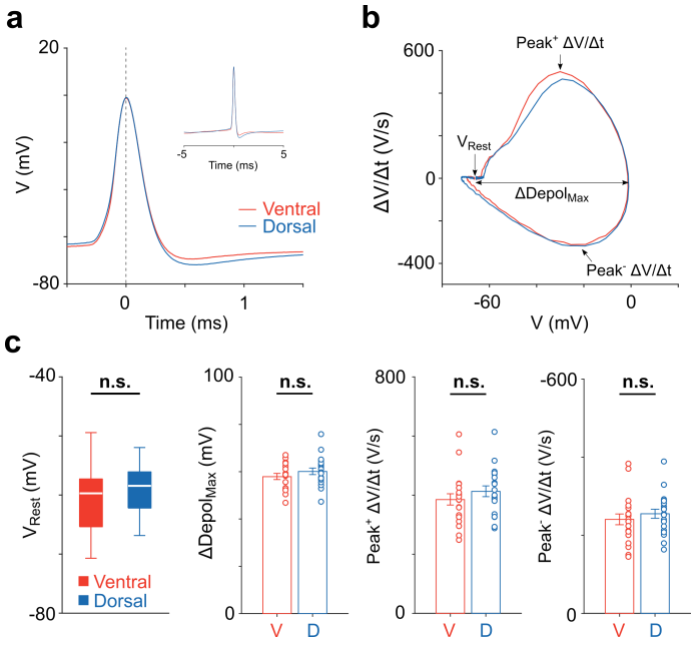

Fig. S3. Action potential dynamics are similar in ventral and dorsal OFF-α T RGCs – (**a**) Overlay of spontaneous spikes from OFF-α T RGCs in the ventral (red) and dorsal (blue) retina. Inset shows the same action potentials on a larger time scale. (**b**) Phase plots, i.e. time-derivative of membrane potential vs. membrane potential, for the spikes in (a). (**c**) Comparison of the resting membrane potential (V_{Rest}, -60.5 ± 1.3 mV vs. -59.1 ± 1.0 mV, p=0.4107), the maximum amplitude of depolarization ($\Delta Depol_{Max}$, 56.5 ± 1.4 mV vs. 59.0 ± 1.4 mV, p=0.1917) and the maximum rates during de- ($Peak_{+}$ ΔV/Δt, 386 ± 19 V/s vs. 413 ± 18 V/s, p=0.3038) and repolarization ($Peak_{-}$ ΔV/Δt, -241 ± 14 V/s vs. -255 ± 12 V/s, p=0.4311) between action potentials of ventral (red, n=20) and dorsal (blue, n=20) cells.

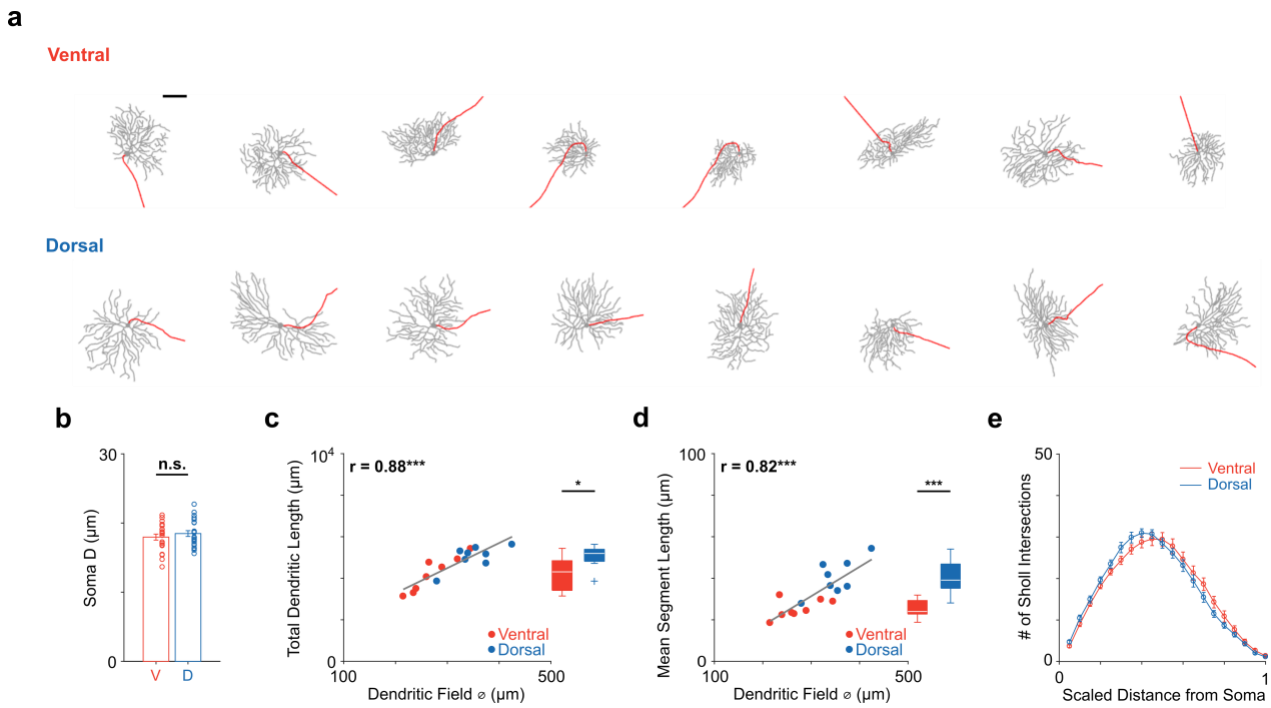

Fig. S4. Dendritic field sizes are different in ventral vs. dorsal OFF-α T RGCs. (**a**) Reconstructions of 8 ventral (top) and 8 dorsal (bottom) OFF-α T RGCs. Axons are indicated in red. Scale bar: 100 µm. (**b**) Soma diameter was compared between ventral (red, n=21) and dorsal (blue, n=22) cells (18.0 ± 0.4 µm vs. 18.5 ± 0.4 µm, p=0.4018). (**c**) Scatter plot of total dendritic length vs. dendritic field diameter in ventral (red, n=8) and dorsal (blue, n=8) reveals a linear correlation (p=0.0005). Total dendritic length was significantly larger in dorsal cells (p=0.0357). (**d**) Scatter plot of dendritic field diameter vs. mean segment length (p=0.0001). Mean segment length was significantly larger in dorsal cells (p=0.0005). (**e**) Sholl analysis of ventral (red, n=15) and dorsal (blue, n=14) cells (Two-way repeated measures ANOVA: interaction p=0.1164, subgroups p=0.9851).

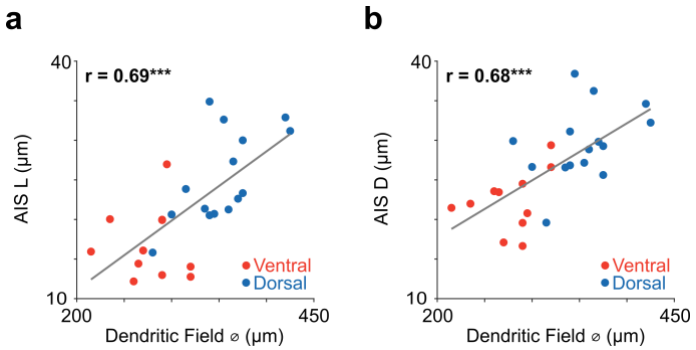

Fig. S5. AIS anatomy scales with cell size. Scatter plots of AIS length (**a**) and AIS distance (**b**) vs. dendritic field diameter for dorsal (blue) and ventral (red) RGCs. The lines are best-fit linear regressions (p=0.0001 and p=0.0001, respectively).

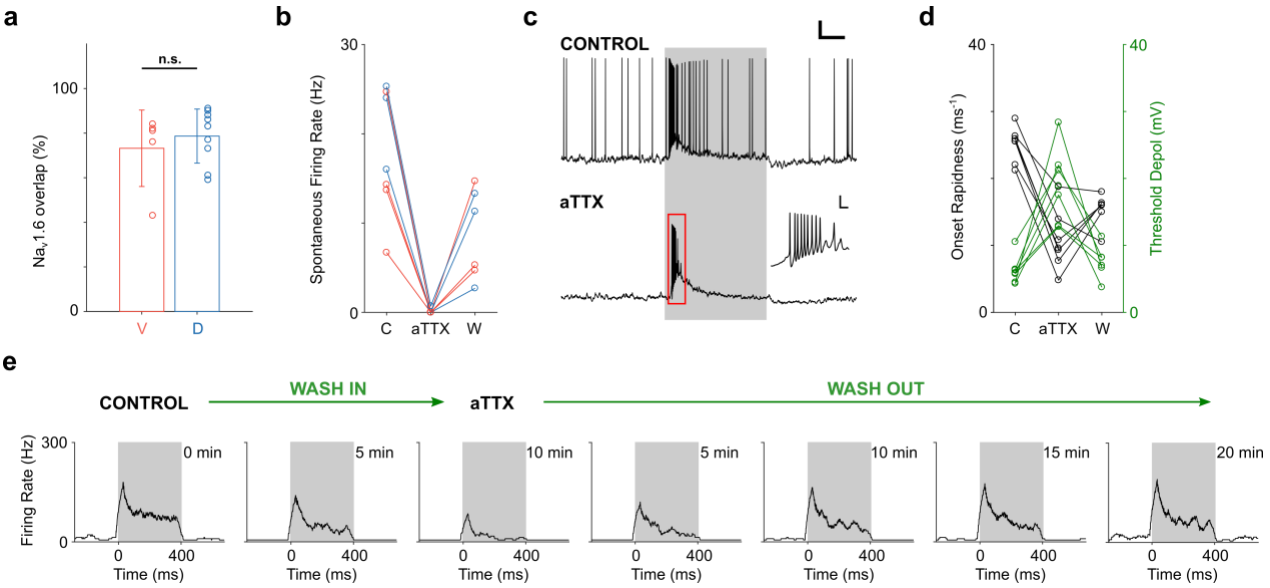

Fig. S6. Na$_v$1.6 channels contribute significantly to spiking responses in OFF-α T RGCs. (**a**) Average amount of overlap between Na$_v$1.6 and Ankyrin$_G$ staining in ventral (red, n=5) and dorsal (blue, n=9) RGCs (p=0.4969). Error bars represent one standard deviation. (**b**) Spontaneous firing rate under control conditions (C), during the application of aTTX and after washout of aTTX (W). (**c**) The response to visual stimulation under control and during the application of aTTX. Scale bar: 100 ms / 10 mV. The inset shows an expanded time scale of the transient burst during application of aTTX. Scale bar: 10 ms / 10 mV. (**d**) Onset rapidness (black) and threshold depolarization (green) under control (C), during the application of aTTX and after wash (W). (**e**) Responses over time under control, during the application of aTTX and after wash.

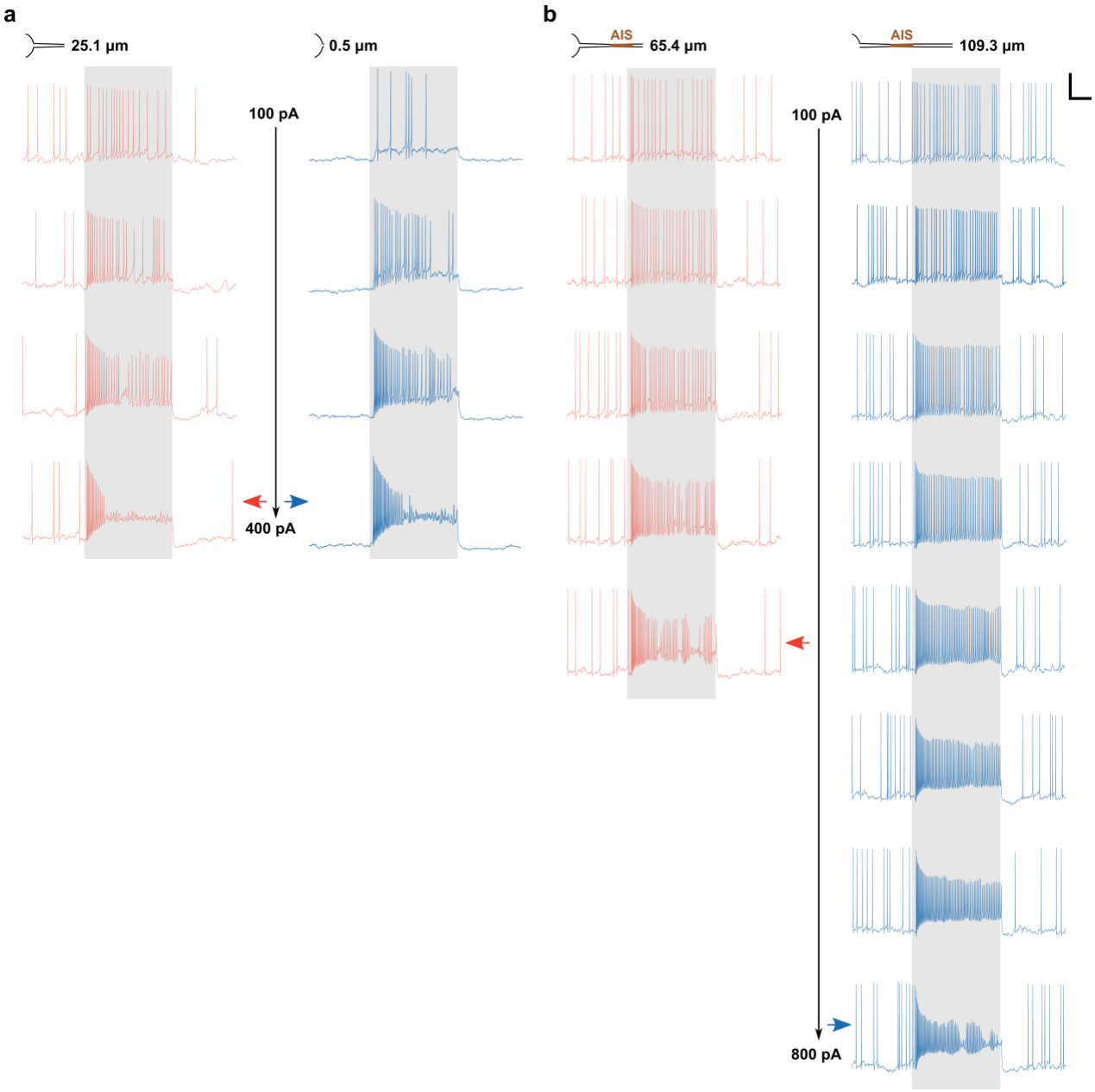

Fig. S7. Responses from axotomized RGCs – (**a**) Representative current clamp recordings from axotomized ventral (red) and dorsal (blue) OFF-α T RGCs when the axotomy was located between the soma and the AIS. (**b**) Representative current clamp recordings from axotomized ventral (red) and dorsal (blue) OFF-α T RGCs when the axotomy was located distal of the AIS. Pulse duration was 400 ms (grey shading), amplitudes ranged from 100-800 pA. Scale bar: 100 ms / 20 mV. Schematics on top indicate location of axotomy. Horizontal arrows indicate failure threshold.

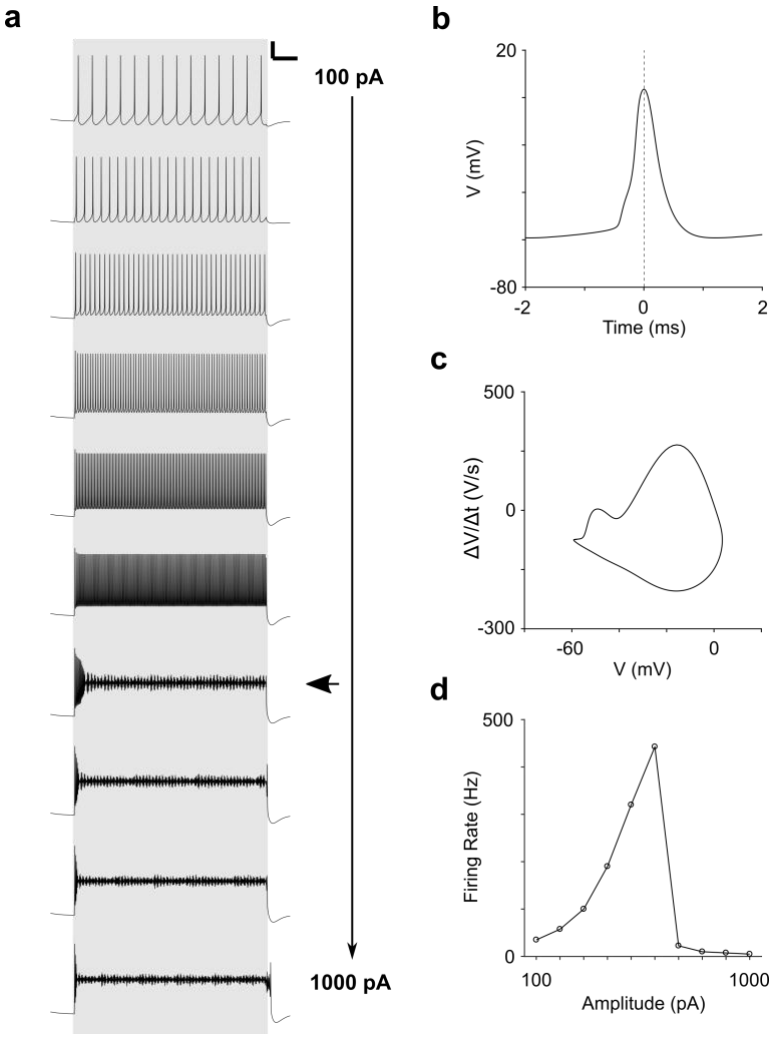

Fig. S8. Simulated RGC responses to somatic current injections – (**a**) Representative current clamp recordings from a modeled OFF-α T RGC. Pulse duration was 400 ms (grey shading), amplitudes ranged from 100-1000 pA. Scale bar: 50 ms / 20 mV. Horizontal arrow indicates failure threshold. (**b**) Membrane potential over time showing one action potential during current injection into the soma. (**c**) Phase plot, i.e. time-derivative of membrane potential vs. membrane potential, for the spike in (b). (**d**) The number of elicited spikes is plotted vs. injected current amplitude for the model cell.

	Dendrites	Soma	Soma-AIS	AIS	Axon
$g_{Nav1.1}$	65	65	130	0	70
$g_{Kv1.1}$	35	35	70	0	70
$g_{Nav1.6}$	0	0	0	325	0
$g_{Kv1.6}$	0	0	0	175	0
g_{Ca}	1.5	1.5	1.5	1.5	1.5
$g_{K,Ca}$	0.15	0.15	0.15	0.15	0.2
g_{L}	0.1	0.1	0.1	0.1	0.1

Table S1. Ion channel densities along the membrane of model neurons. Values are based on Fohlmeister et al. *(39)* with minor modifications. All values are given in mS/cm^2.