

# Modulation of mouse retinal ganglion cell excitability by mitochondrial reactive oxygen species

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## Background

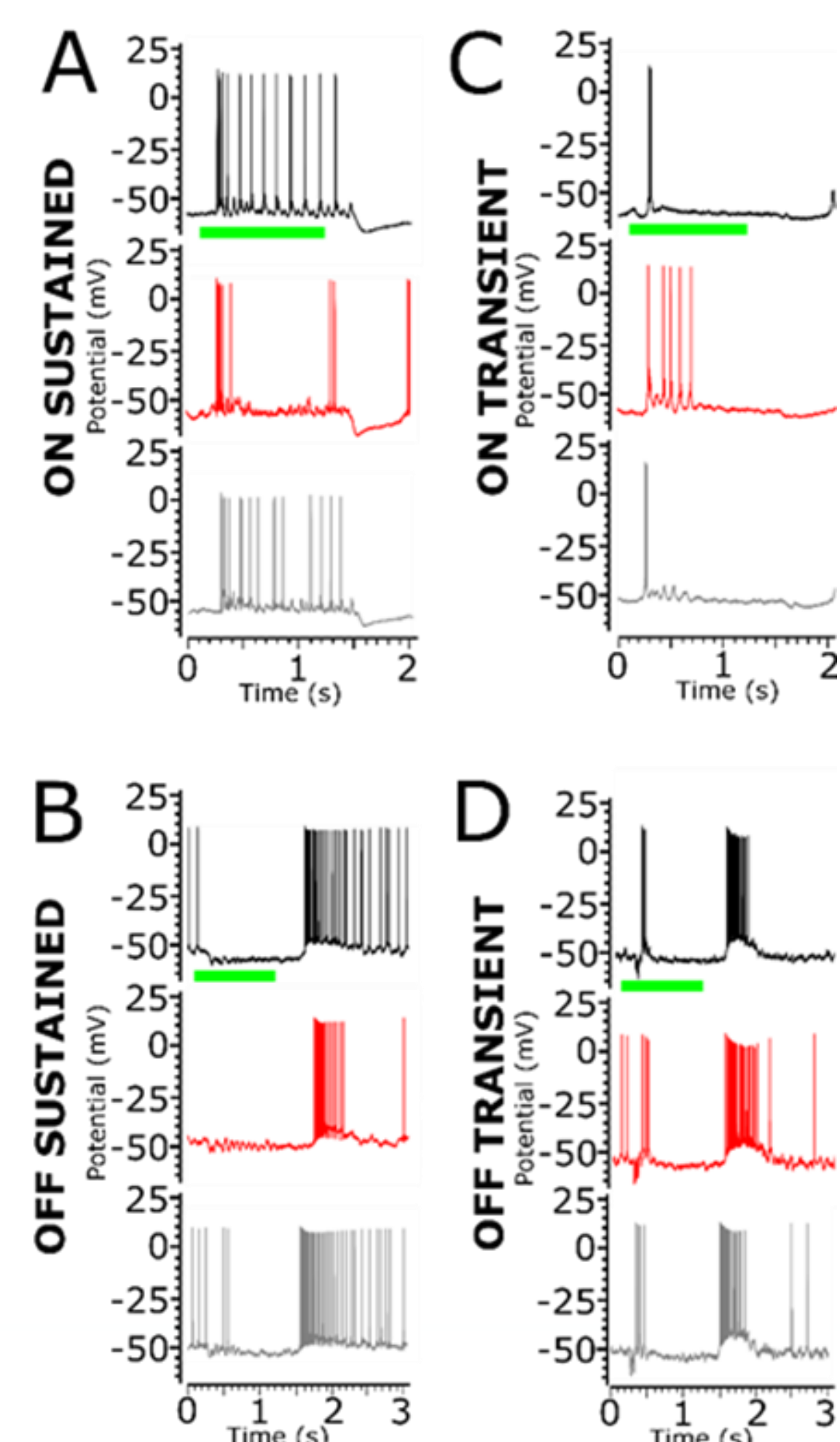
Hypothesis: Changes of retinal ganglion cell (RGC) excitability in response to mitochondria-derived reactive oxygen species (ROS) can be detected electrophysiologically and differs between RGC subtypes. Mitochondrial ATP synthesis increases ROS, alters RGC responses to visual stimuli, and regulates retinal energy use.

## Objectives

1. Define the effects of normal and elevated ROS levels on the responses of transient and sustained, ON-, OFF- and ON-OFF  $\alpha$  ganglion cells.
2. Characterize the ROS sensitivity of the membrane mechanisms responsible for ganglion cell action potential generation.
3. Show the net influence of ROS modulation caused by light intensity changes (contrast adaptation) on ganglion cell output.

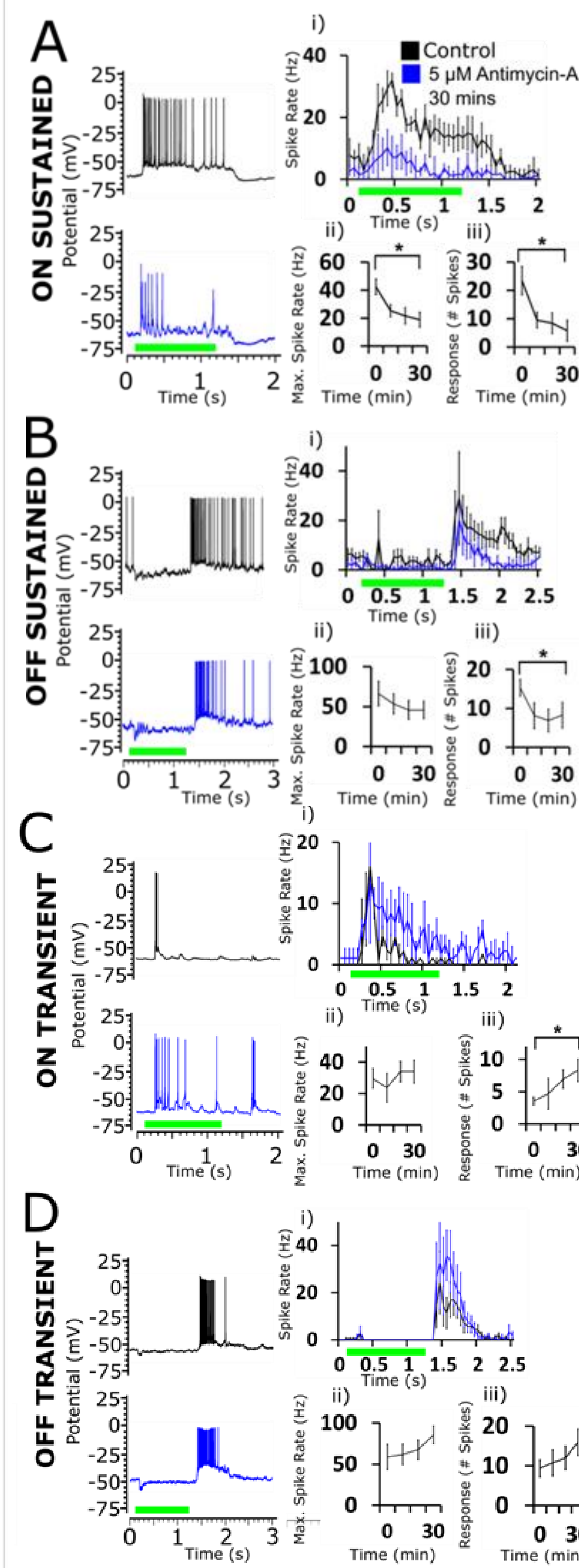
## Methods

Whole cell patch clamp recordings were made from fluorescently labeled  $\alpha$  RGCs in Kcng4 mice under infrared illumination at 35 C. Light stimuli discriminated sustained and transient ON-, OFF-, and ON-OFF  $\alpha$ RGCs. ROS levels were increased with mercaptosuccinate (MCS, 1 mM), Antimycin A (5  $\mu$ M), H<sub>2</sub>O<sub>2</sub> (400  $\mu$ M), or decreased with internal catalase (500 IU). Voltage-clamp was used in nucleated patches to measure Na<sub>v</sub> channel gating changes to assess spike efficiency.



**Fig. 1.** Reversible effects of extracellular 1 mM MCS, which blocks glutathione peroxidase, increases H<sub>2</sub>O<sub>2</sub>, reversibly reduces light-stimulated spiking in sustained ON- and OFF-RGCs (A,B) but increases spiking in transient ON- and OFF-RGCs (C,D).

## Results



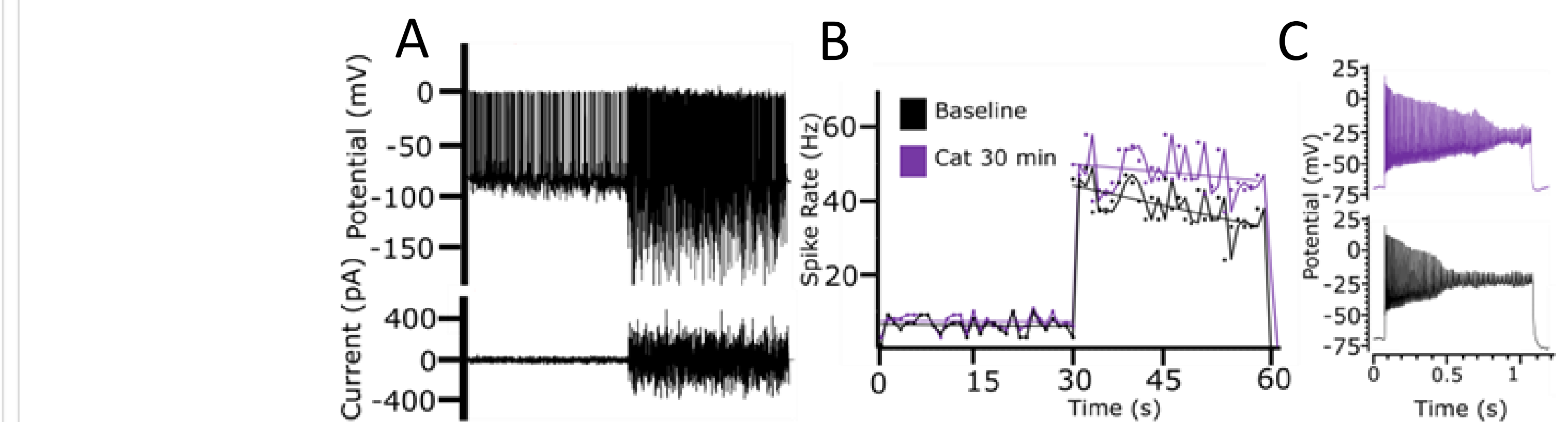
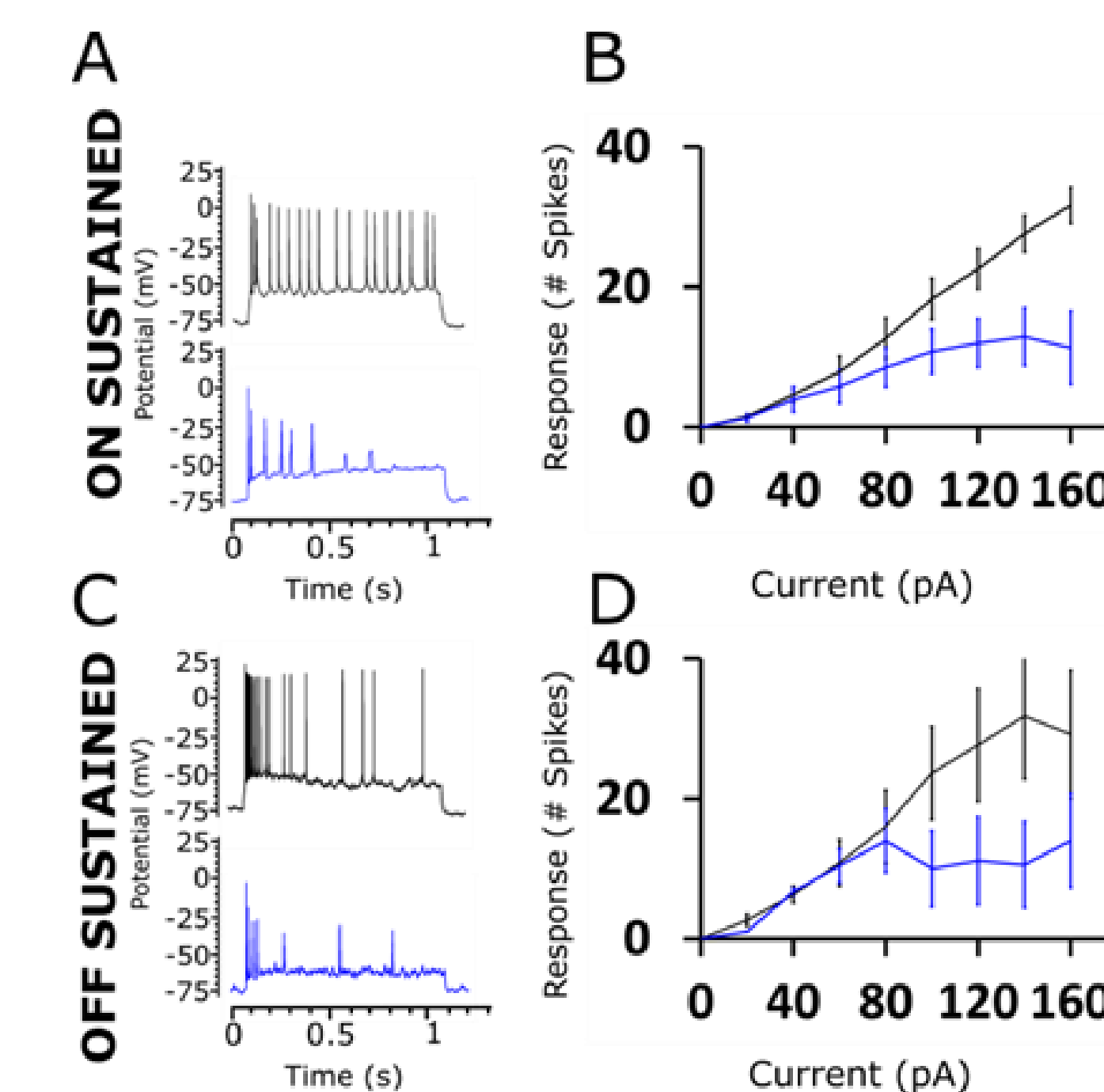
**Fig. 3.** Antimycin A also suppresses frequency response relations to injected current in sustained ON- and OFF- RGCs. Increased ROS levels inhibit spiking at levels of current injection that under control caused high spike rates. High spike rates increase energy demand and this leads to elevated ROS levels.

## Metabolically produced ROS modulate the response of RGCs to light stimuli.

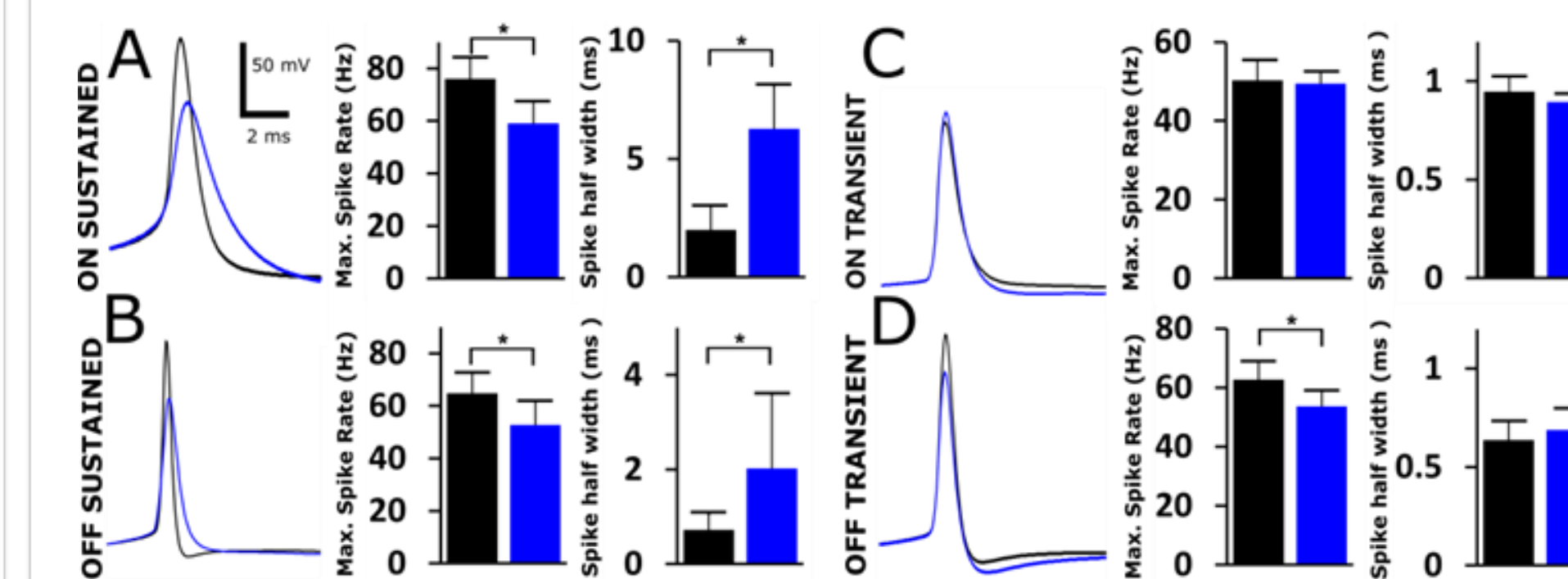
**Fig. 2.** Antimycin A (30 min), which blocks electron transport by Complex III of the ETC, also reduces light-induced (1.1 s) spiking in sustained ON- and OFF-RGCs (A,B), but increases spiking in transient ON- and OFF-RGCs (C,D).

## Dramatic actions of increased cell-generated ROS are seen in RGC firing properties, esp. with the differential responses of sustained vs. transient $\alpha$ RGCs.

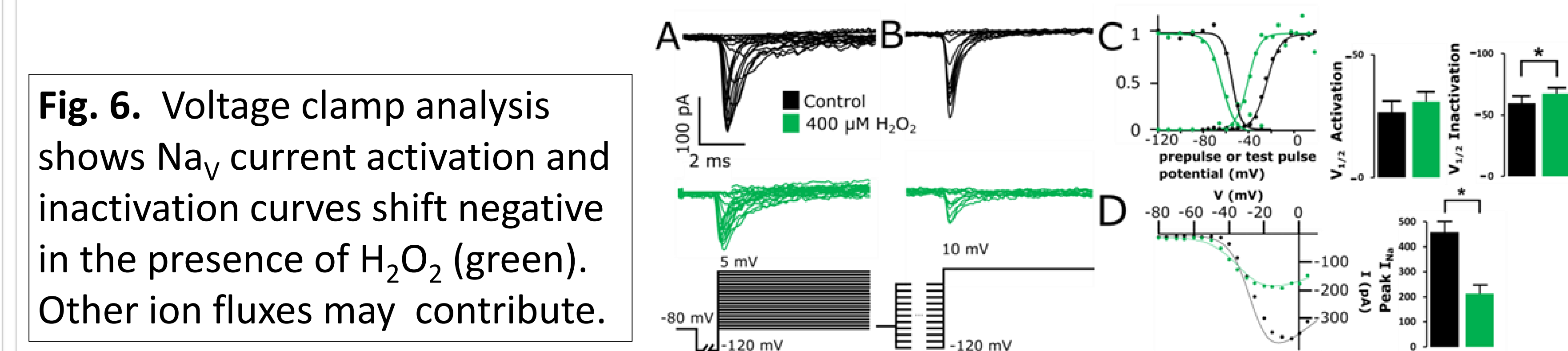
Comparison of light-induced changes (Fig. 2) with those from injected currents (Fig. 3), offers a novel perspective on RGC excitability (Fig. 4) and points to mechanistic bases for rapid metabolic feedback for testing (Figs. 5,6).



**Fig. 4.** A. Injection of low, then high variance white noise current (lower) induces a large increase in spiking and baseline V<sub>m</sub> fluctuations in an ON sustained RGC (upper). B. Changes in spike rate at high variance decay less in catalase. C. Catalase reduces adaptation during spiking.



**Fig. 5.** Spike width of sustained, not transient, RGCs increases in response to Antimycin A induced ROS. Slower spikes tend to increase metabolic efficiency of spikes.



**Fig. 6.** Voltage clamp analysis shows Na<sub>v</sub> current activation and inactivation curves shift negative in the presence of H<sub>2</sub>O<sub>2</sub> (green). Other ion fluxes may contribute.

## Conclusions

Links between normal physiological energy requirements and bioenergetic stress producing RGC dysfunction help identify sensitive, interpretable signals that may reveal developing metabolic crisis in the retina. Changes to voltage-gated Na<sub>v</sub> channels may underlie the oxidative modulation of excitability. This could aid development of diagnostic assessments that allow early detection using specific light stimuli and interventions to prevent vision loss due to RGC diseases such as glaucoma.

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