

# Low-dose atropine might affect alpha ganglion cell signaling in the mouse retina

**Author Block:** *Qin Wang*<sup>1</sup>, *Feng PAN*<sup>1,2</sup>

<sup>1</sup> The Hong Kong Polytechnic University, Kowloon, Hong Kong; <sup>2</sup> Centre for Eye and Vision Research Limited, Hong Kong, Hong Kong

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**Purpose:** Atropine was used to retard myopia progression in clinic, while its effect on retina is unclear. Therefore, we explored the impact of atropine from concentrations 0.05  $\mu$ M to 500  $\mu$ M on retinal ganglion cells (RGCs) in the mouse retina.

**Methods:** Adult C57BL/6J mice, Kcng4-YFP mice, Cx36-knockout mice were used in this study. Retinas (n=5) were removed and immersed in 800  $\mu$ M (0.05%) atropine sulfate for 30 minutes and liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to detect atropine concentration in retina. Alpha RGCs (n=10) were injected with Neurobiotin to show morphology. In electrophysiological recording, retinas were directly applied in atropine and stimulated with 525nm full-field light. ON (n=5) and OFF  $\alpha$ RGCs (n=5) were applied with 0  $\mu$ M, 100  $\mu$ M, 300  $\mu$ M, 500  $\mu$ M atropine subsequently for dose-dependent test. For time and concentration-dependent test, alpha RGCs were recorded before and after application of 0.05  $\mu$ M (n=8), 0.5  $\mu$ M (n=8), 10  $\mu$ M (n=8), 100  $\mu$ M (n=9) atropine respectively.

**Results:** Around 400-fold reduction was detected in retina after 800  $\mu$ M atropine applied in cornea and choroid side ( $1960.0 \pm 524.2$ nmol/L). No morphological changes were observed after superfusion in 1  $\mu$ M atropine for 30 minutes. Atropine over 100  $\mu$ M had a dose-dependent inhibition effect on light-evoked response in ON  $\alpha$ RGCs (300  $\mu$ M p=0.048, 500  $\mu$ M p=0.001) and OFF  $\alpha$ RGCs (300  $\mu$ M p=0.048, 500  $\mu$ M p=0.003). Application of 100  $\mu$ M, 10  $\mu$ M, 0.5  $\mu$ M, 0.05  $\mu$ M atropine had no effect on spike frequency and time latency of original ON or OFF light-evoked responses. Synchronized firing pattern between OFF RGCs was not changed in 0.5  $\mu$ M atropine. However, ON responses were induced in certain OFF  $\alpha$ RGCs (20% in 0.05  $\mu$ M, 37% in 0.5  $\mu$ M, 40% in 10  $\mu$ M, 33% in 100  $\mu$ M). Atropine of 50  $\mu$ M extended the threshold of joint inter-spike interval (ISI) distribution of  $\alpha$ RGCs.

**Conclusions:** Atropine of high concentration had inhibition effect on  $\alpha$ RGCs firing response, while low-dose atropine did not interfere with spike frequency,

synchronized pattern, and threshold of joint ISI distribution of ON and OFF  $\alpha$ RGCs. However, atropine induced ON responses from certain OFF RGCs, which suggested unintended consequences on retinal information processing.

**Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.:** Atropine is used to retard myopia progression in children and low-dose atropine was adopted to avoid side effects. Our study explored the effect of atropine on retinal ganglion cells (RGCs) over a range of concentrations in mouse retina. Although low-dose atropine had few effect on most of RGCs, it could induce ON responses from certain OFF RGCs. This may suggest the unintended consequences of atropine on retinal information processing.