Store operated calcium entry (SOCE) mediates insect nephrocyte clearance function

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INTRODUCTION

Insect nephrocytes are highly endocytic cells which remove proteins and xenotoxins from circulation (Figure 1). They express evolutionarily conserved 'slit diaphragms' at their surface; ultrastructures also expressed by mammalian kidney podocytes. Identifying nephrocyte signalling pathways is therefore important as they are relevant to clearance and renal function in many species, including humans. Establishing a method to investigate calcium signalling in nephrocytes was attempted in the current work.

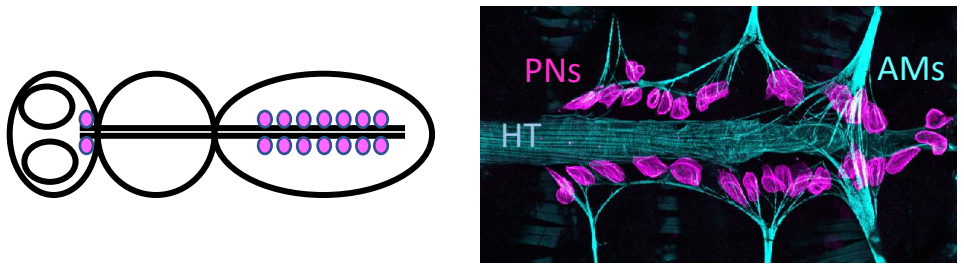

Figure 1. Anatomical location of nephrocytes in *Drosophila melanogaster*. In the schematic the garland cell nephrocytes and pericardial nephrocytes are highlighted in pink. In the fluorescence micrograph, pericardial nephrocytes (PNs) can be seen either side of the heart tube (HT) and in close proximity to alary muscles (AMs). PNs are stained with antisera to the slit diaphragm protein Dumbfounded (Duf) Nephrocytes are ~50 µm in diameter.

METHODS

Drosophila expressing the calcium reporter *GCaMP6* under the control of *Dot-gal4* were used to characterise nephrocyte calcium transients *in vivo* and *ex vivo*. The role in nephrocyte function of store operated calcium entry (SOCE) was analysed in *Stim* and *Orai* loss-of-function (LOF) models. Calcium transients in pericardial nephrocytes were imaged by widefield microscopy using immobilised larvae and dissected adults. Confocal microscopy was used to image endocytic clearance after incubating cells with labelled cargoes (albumin), fixing and counter-staining them with wheatgerm agglutinin (WGA).

RESULTS: *in vivo calcium transients*

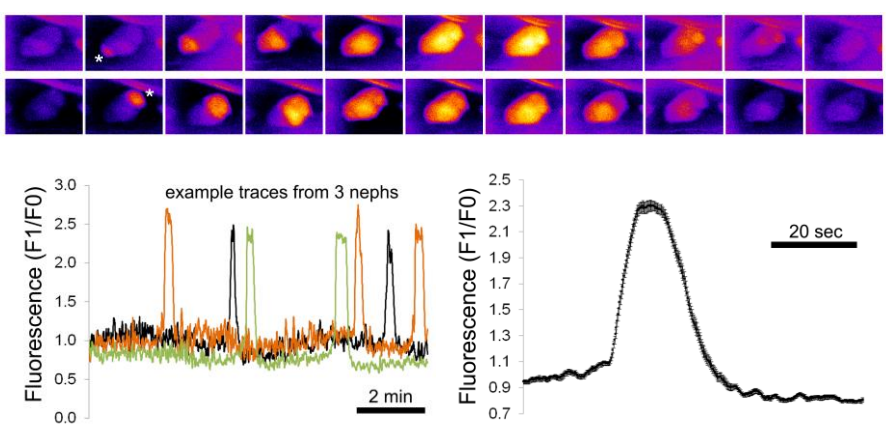

Figure 2. *In vivo* calcium traces. The upper panel shows two consecutive calcium waves initiating at opposite ends (asterisk) of a single nephrocyte over 20 seconds. The graphs show examples of individual traces from three different cells (left) and averaged traces from 17 cells (+/-SEM).

RESULTS: *modulating Orai and Stim*

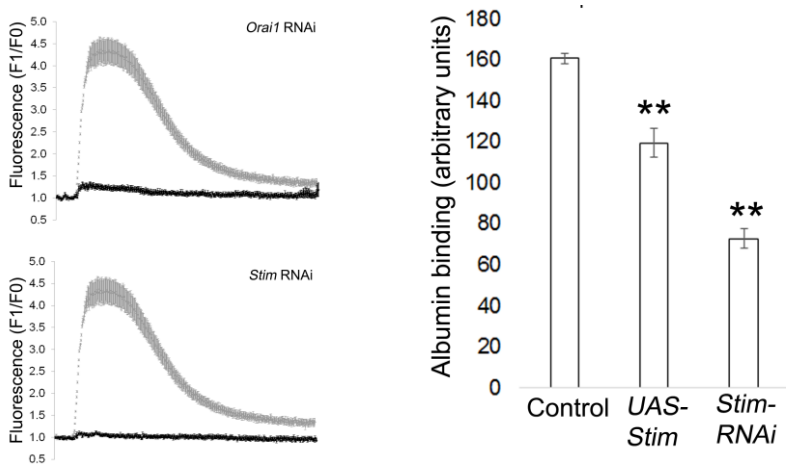

Figure 3. SOCE Loss of function impacts albumin binding. SOCE was modulated by modulating *Stim* or silencing *ORAI* in nephrocytes, which abrogated calcium traces initiated *in vitro (*control (grey) and experimental (black); *n* = >50 nephrocytes). The bar chart shows the impact on albumin binding when nephrocyte *Stim* expression was modulated. *n* = 20 nephrocytes per group; **P<0.001.

RESULTS: *imaging of nephrocytes*

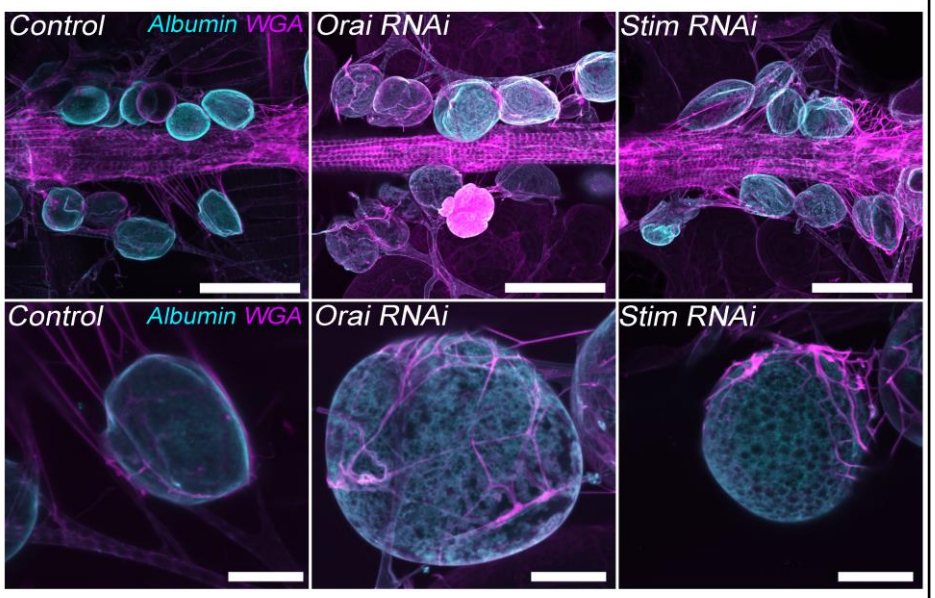

Figure 4. Impact of SOCE LOF on nephrocyte phenotype. Confocal images of nephrocytes stained with the endocytic cargo (albumin) and counterstained with WGA. *Orai* and *Stim* were silenced for three weeks and nephrocytes imaged. Scale = 20 µm.

DISCUSSION

Drosophila Nephrocytes exhibit regular calcium transients lasting approximately 20s *in vivo* (Fig 2). Store operated calcium entry appears to be linked with clearance function because modulating *Stim* reduced albumin binding (Fig 3 and 4). Silencing *Stim* or *Orai* altered nephrocyte morphology and led to an irregular pattern of albumin binding (Fig 4). These data establish for the first time that insect nephrocytes are highly dynamic cells whose clearance function is dependent SOCE. This model recapitulates aspects of mammalian renal physiology and offers a tractable means to identify factors both up and downstream of SOCE required for excretory function in a range of species.