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Mechanotransduction mechanisms in central nervous system glia

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See related article, Jo AO, Lakk M, Rudzitis CN, Križaj D (2022) TRPV4 and TRPC1 channels mediate the response to tensile strain in mouse Müller cells. Cell Calcium 104:102588.

Mechanical forces shape the development, function, and survival of every cell within the central nervous system (CNS) but are particularly important for astroglia, a subtype of glial cell that mediates communication between neurons and blood vessels. Astrocytes utilize changes in intracellular concentration of the 2nd messenger calcium $[Ca^{2+}]_i$ to integrate local electrical, chemical, and mechanical microenvironments, with Ca^{2+}-dependent release of gliotransmitters and cytokines implicated in the regulation of neurovascular coupling, short- and long-term synaptic plasticity, and neuronal excitability. These functions may be perturbed by age, tissue swelling (edema), ischemia, physical trauma, and chronic elevations in intraocular or intracranial pressure, to produce a reactive response that manifests as increases in hypertrophy, cell proliferation, and proinflammatory signaling. Astroglial activation by acute and chronic mechanical trauma compromises the integrity of blood-CNS barriers and neuronal function yet information about molecular sensors that transduce mechanical forces into astroglial $[Ca^{2+}]_i$ is surprisingly sparse. We know that large tissue deformations that activate astroglia and injure CNS induce $[Ca^{2+}]_i$ elevations (Rzigalinski et al., 1998; Lindqvist et al., 2010) but it is not known whether the cells are capable of responding to physiological deformations of the extracellular matrix (< 5% strain) and what such force transducers might be.

The goal of the work under discussion in this Commentary (Jo et al., 2022) was to characterize strain transduction in Müller cells, radial astroglia that constitutes ~90% of the retinal glial population, with critical functions in release/recycling of neurotransmitters, osmoregulation, metabolism, and maintenance of the retina-blood barrier (Reichenbach and Bringmann, 2020). We used a combination of genetic mouse models, pharmacology, and optical imaging to identify ion channels that mediate their sensitivity to a range of applied strain magnitudes, focusing on calcium-permeable channels as principal drivers of glial excitability in a dynamic biomechanical CNS milieu. Previous investigations showed that application of pressure induces a nonselective cation current in voltage-clamped human Müller cells (Puro, 1991), and that exposing guinea pig retinas to uniaxial 20% stretch elevates $[Ca^{2+}]_i$ (Lindqvist et al., 2010). To gain an insight into Müller glial strain sensing, we seeded cells purified from mouse retinas onto deformable silicon membranes and exposed them to cyclic stretch at a frequency that approximates intraocular pressure oscillations from pulsatile blood flow in the choroid vessel (~1 Hz). We found that 1% stretch and rapid cell indentation elevate $[Ca^{2+}]_i$. Dose-dependent increases in the amplitude of stretch-evoked signals were observed up to 12% applied strain, with larger stimulus amplitudes correlated with progressively longer recovery times. Stretch-induced Ca^{2+} responses tended to originate in the end foot compartment and propagated towards the cell body as Ca^{2+} waves. Analysis of the transcriptome of putative Ca^{2+}-permeable stretch-activated channels, showed the expression pattern: *Trpc1>>Piezo1>Trpv2>Trpv4>*Piezo2*>>Trpv1/Trpv3*.

The transient receptor potential (TRP) superfamily consists of proteins with six transmembrane domains that assemble as homo- or heterotetramers to (mostly) form nonselective Ca^{2+}-permeable cation channels. The channels are expressed in most if not all vertebrate cells, in which they function as key transducers of sensory (mechanical, chemical, thermal, nociceptive) information. Our previous studies implicated both vanilloid (TRPV4) and canonical (TRPC1) members of the family in Müller glial sensing of cell swelling and depletion of intracellular Ca^{2+} stores, respectively (Ryskamp et al., 2014; Molnar et al., 2016). Whether the two proteins contribute to force transduction has not been settled, with evidence supporting positive and negative conclusions. Given the gene expression, functional expression, and the presence of intraocular pressure-induced glial phenotypes in TRPV4$^{-/-}$ and TRPC1$^{-/-}$ retinas (Ryskamp et al., 2014; Molnar et al., 2016), we hypothesized that the two proteins also participate in Müller strain sensing. Stretching wild-type Müller glia in the presence of pharmacological inhibitors of TRPV4 and TRPC1 channels, and comparison of responses from wild-type cells to TRPV4$^{-/-}$ and TRPC1$^{-/-}$ glia showed significant decreases in stretch-evoked Ca^{2+} signals in KO cells, TRPV4$^{-/-}$ and TRPC1$^{-/-}$ glia showed 55% and 22% reductions in response amplitude, respectively. Despite the prominent *Trpv2* mRNA, TRPV2 inhibitor tranilast had no effect on the amplitude or time course of the stretch-evoked $[Ca^{2+}]_i$ signal. Thus, TRPC1 and TRPV4, but not TRPV2, subunits contribute to the glial stretch response, with the Piezo1 channel likely mediating the residual response in cells with blocked/ablated TRPV1/V4. *Trpc1* transcript levels were ~80-fold higher relative to *Trpv4* mRNA yet TRPV4 inhibition/knockdown was about twice as effective in suppressing stretch-evoked excitation compared to inhibition/knockdown of TRPC1. We interpret this as suggestive of greater sensitivity of TRPV4 subunits to membrane deformation, or less force-sensitive tethering of TRPC1 subunits to membrane lipids/proteins.

TRPC1 subunits do not form functional homomeric pores (Storch et al., 2012) and thus activation by stretch must reflect heteromerization with additional canonical, vanilloid, or polycystin TRP isoforms and/or functional coupling with non-TRP channels. A likely potential heteromeric partner is TRPV4, which was shown to interact with TRPC1 subunits in heterologously expressing HEK293 and endothelial cells (Ma et al., 2011). The transmembrane current in Müller cells, evoked by membrane stretch, exhibits a linearized I–V relationship (Ryskamp et al., 2014) that resembles the current signature of TRPC1:TRPV4 heteromers (Ma et al., 2011). TRPV4 antagonists halve the amplitude of stretch-evoked Ca^{2+} signals in TRPC1$^{-/-}$ cells whereas TRPC1 blockers did not lower the calcium signal in TRPV4$^{-/-}$ cells. One possible explanation is that homotetrameric TRPV4 channels exist independently of TRPV4:C1 heterotetramers. Orai channels collaborate with TRPC1 to mediate store-operated Ca^{2+} signaling in Müller cells (Molnar et al., 2016) but the Orai1-3 antagonist GSK-7975A has no effect on indentation-evoked Ca^{2+} responses in wild type or TRPV4$^{-/-}$ cells.

What is the functional significance of Müller cell force sensing? Müller processes ensheath every retinal neuron, cover the entire width of the retina (**Figure 1**) and continually experience tensile stretch from intraocular pressure, tug from vitreous fibers, volume changes due to activity-dependent shifts in local osmotic gradients, and compression/tension from fluctuating intraocular pressure (Križaj, 2019). TRP- and Piezo1-mediated Ca^{2+} influx may adjust local neurovascular coupling (Biesecker et al., 2016) and neuronal activity (Shibasaki et al., 2014) through stimulation of Ca^{2+}-dependent kinases, phosphatases, phospholipases, metabolic enzymes, and transcription factors. Overactivation of one or more of these channels by chronic mechanical stress in glaucoma, retinal detachment, diabetic retinopathy, traumatic ocular injury, and abnormal axial elongation (myopia) may result in reactive gliosis. Consistent with this, intravitreal injection of TRPV4 agonists induces a massive reactive response (Ryskamp et al., 2014), which may affect neuronal viability via TRPV4-dependent release of proinflammatory cytokines (Matsumoto et al., 2018). Just as significantly, deletion of the TRPV4 gene was associated with mild gliosis (Ryskamp et al., 2014), indicating that tonic TRPV4 activity, responding to small extracellular matrix displacements caused by intraocular pressure fluctuations, is required to maintain the homeostatic state. Jo et al. (2022) also found that Müller cells express the *Kcnk2* gene, which encodes the mechanosensitive, tandem pore potassium channel TREK-1. We propose that excitation, mediated by TRPV4, TRPC1, and Piezo1 channels is balanced by an opposing, hyperpolarizing, mechanosensitive K^+ efflux The remarkable sensitivity of Müller glia to modest strains is consistent with their function as retinal baroreceptors and early responders

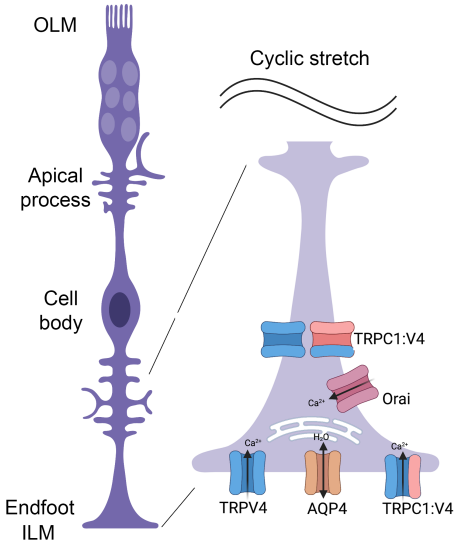

Figure 1 | **Schematic representation of the mammalian retinal Müller cell.**
Its processes form the outer retina-blood barrier at the outer limiting membrane (OLM) and the inner retina-blood barrier at the inner limiting membrane (ILM). Inset shows a prominent expression of calcium-permeable stretch-activated channels TRPV4 and TRPC1 in the end foot compartment. Functional coupling between TRPV4 and aquaporin 4 (AQP4), a bidirectional water channel, regulates swelling-induced TRPV4 activation (Ryskamp et al., 2014). Coordinated activation of TRPC1 and Orai channels mediates store-operated Ca^{2+} influx (Molnar et al., 2016). TRPC1: Transient receptor potential canonical 1; TRPV4: transient receptor potential vanilloid 4. Created with BioRender.

to mechanical stress (Križaj, 2019). Unlike brain astrocytes, which express TRPV4 in a limited subpopulation (~15–30%; Shibasaki et al., 2014; Pivonkova et al., 2018), all Müller glia strongly express TRPV4, and TRPC1 channels, which are therefore well placed to integrate the glial sensing of intraretinal blood flow and intraocular pressure with retinal neuronal signaling. Overactivation of these channels by mechanical stressors in glaucoma, retinal detachment, myopia, and other diseases, however, may lead to pro-inflammatory states that adversely affect the visual signal.

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