The role of PIEZO1 in Purkinje fibre-mediated arrhythmia development: A study from the whole heart to the nanoscale level

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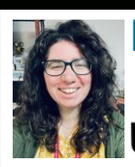

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INTRODUCTION

Purkinje fibres (PF) rapidly transmit electrical signals from the His bundle, into the ventricles to enable the heart to act as a functional syncytium. Their function is enabled by their specialised structure. Premature ventricular contractions originate from the PF network; however, their underlying mechanism and propagation is unknown. Such answers would be revolutionary for targeting arrhythmia development within ablation therapy. PIEZO1 is a non-selective cation mechano-sensitive ion channel (MSC) which rapidly opens upon mechanical stimulation, for example membrane stretch, to enable calcium influx. The aim of this study is to evaluate the role that PIEZO1 has within PF-mediated arrhythmia development upon mechanical stimulation.

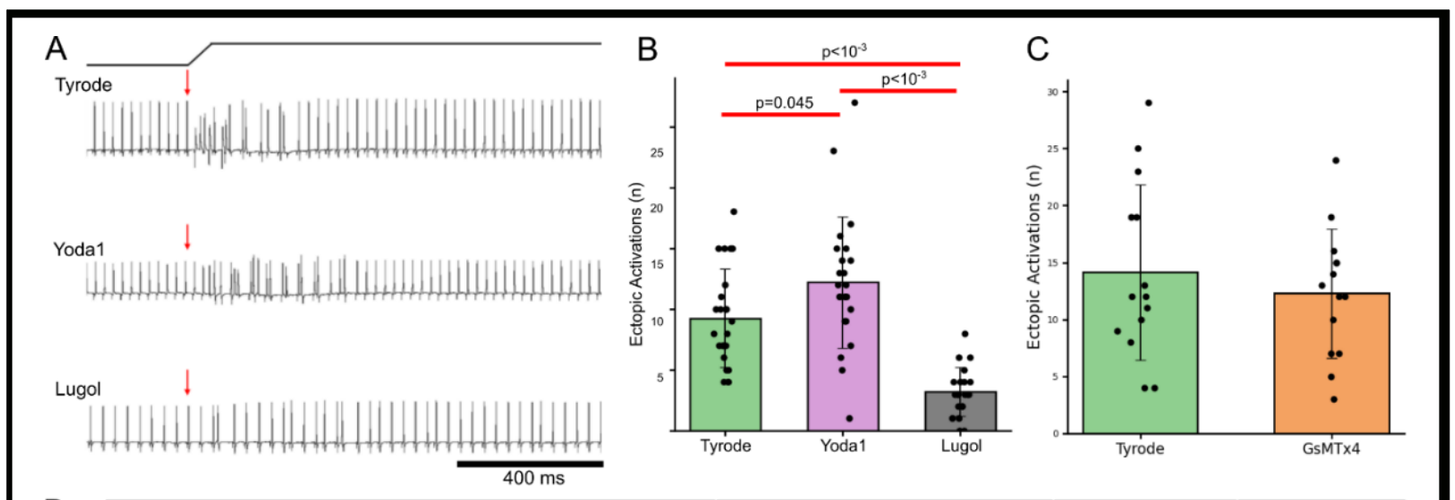
METHODS

In accordance with local ethical regulations, three experimental workflows were undertaken to evaluate the role of PIEZO1. Data presented as mean ± SD.

1. Langendorff-perfused, male Wistar rat hearts underwent mechanical stimulation by a left ventricular (LV) fluid-filled balloon (100 µL inflation over 2s; maintained for 38s). Mechanical stimulation was undertaken whilst the LV was perfused with Tyrode and then again after perfusion of either; 1 µM GsMTx4 (N=7), a non-specific MSC inhibitor for 3 minutes, or perfusion with 50 µM Yoda1 (N=8), a PIEZO activator, for 5 minutes, followed by Lugol's (IK/I_2) to preferentially ablate free-running PFs.

2. Isolated female Ovine PFs (N=3) were loaded with 10 µM Fura-2 and mounted at a fixed length onto a hook and force transducer whilst the bath was perfused with Tyrode at 37°C and PF was field stimulated. The PF was stretched to 10% of it's resting length and intracellular calcium measured in the absence and presence of 50 µM Yoda1 whilst the PF was at rest and stretched.

3. An isolated male Porcine PF (N=1) was sectioned (350 µm) and incubated with 15 µM Rhod2; 10 µM blebbistatin and 7.5 µM RH237 in pre-oxygenated BDM-Tyrode for optical mapping (0.5 Hz field stimulation; 35℃). Recordings were analysed with BrainVision, using a 0.4 Hz high-pass filter. All tissue underwent histological analysis using Haematoxylin and Eosin stain or immunofluorescence analysis with 4x expansion microscopy (ExM) for PIEZO1. Tissue sections were imaged using ZEN software with an Airyscan detector upon a Zeiss LSM880 Inverted microscope, 40x oil objective, 1.4 NA and 488 nm laser. Functional and structural datasets were aligned based upon cell geometry and regions of interest within the tissue using a custom-written script [Hurley et al., 2021; Methods 193(27-37)].



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	MSC inhibitor		PIEZO1 agonist		
	Tyrode	GsMTx4	Tyrode	Yoda	Lugol
Hearts (n)	7	7	4	4	4
Ectopic Activations (n)	14.14 ± 7.71	12.29 ± 5.65	9.25 ± 0.82	12.21 ± 1.10 *	3.09 ± 0.43 *
Ectopic Duration (s)	4.54 ± 2.61	6.41 ± 3.20	4.36 ± 0.87	7.45 ± 1.09 *	1.63 ± 0.31 *
QRS width (ms)	18.17 ± 7.00	15.83 ± 3.86 *	15.71 ± 1.62	17.13 ± 1.65	19.37 ± 1.43
LV developed pressure at rest (mmHg)	21.32 ± 12.86	29.87 ± 20.08	14.31 ± 3.93	20.76 ± 5.21	18.86 ± 4.87
SDNN during stretch (ms)	73.67 ± 26.32	98.69 ± 29.54 *	53.50 ± 4.25	81.35 ± 10.68 *	52.20 ± 6.54 *

Figure 1: PIEZO1 in whole-heart stretch-induced arrhythmias. Langendorff male rat hearts with lead II ECG and left ventricular (LV) indwelling fluid-filled balloon to measure developed pressure and upon inflation induced acute stretch. **(A)** ECG upon acute stretch (red arrow) in the presence of; Tyrode, 50 µM Yoda1 (PIEZO1 agonist) and Lugol (IK/I_2) to ablate free-running Purkinje fibres and **(B)** ectopic activations quantified. **(C)** In a separate experiment, acute stretch was undertaken whilst the LV was irrigated with Tyrode followed by 1 µM GsMTx4 (mechanosensitive ion channel inhibitor), and ectopic activations quantified. **(D)** Upon stretch, ECG and pressure was analysed for each perfusate. Data are mean ± SD; *p<0.05 (shown by red bar) for Independent t-test (GsMTx4; N=7) or One-Way ANOVA (Yoda1; N=8).

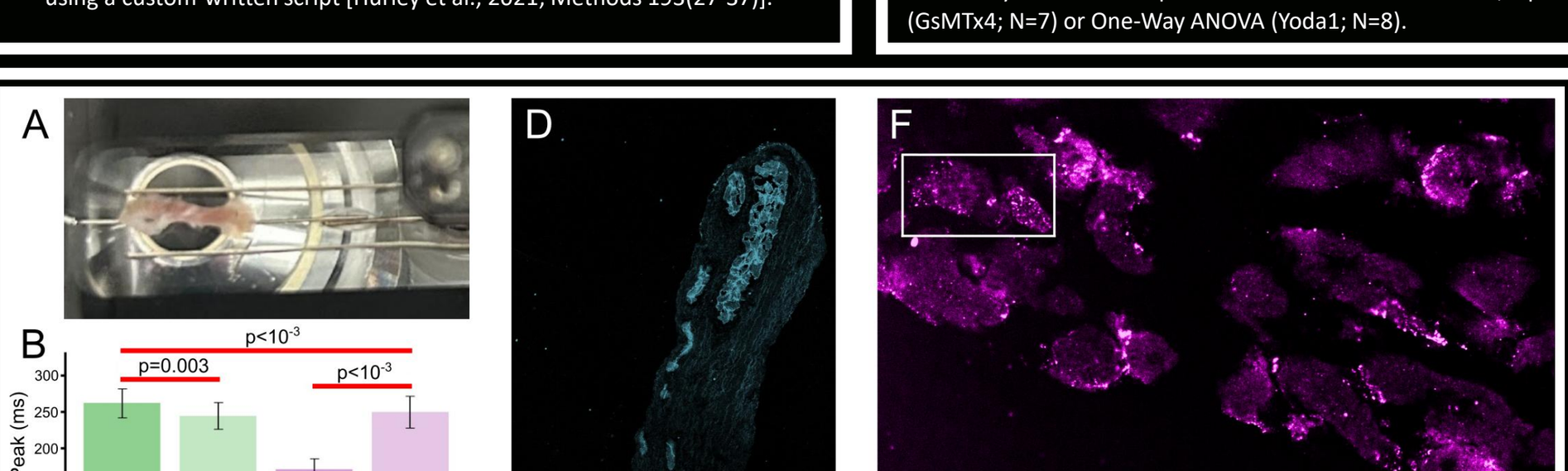

Figure 2: PIEZO1 in an isolated Purkinje fibre. (A) An isolated PF attached to a force transducer and loaded with 5 µM Fura-2. Calcium transients were analysed when the PF was stretched 10% of its resting length in the presence of Tyrode or 50 µM Yoda1 (PIEZO1 agonist) to assess **(B)** time to peak (ms) and **(C)** amplitude (V). Tissue was fixed (1% PFA) and snap frozen before confocal microscopy to stain for **(D)** Connexin 40, **(E)** TRPM4 and **(F)** PIEZO1, with 4x expansion microscopy then undertaken (inset). Data are mean ± SD; p<0.05 (shown by red bar) Linear Mixed Model (Female pig; N=4). Scale bar: **(D,E)** 100 µm; **(F)** 5 µm (15 µm).

Relevant Publications

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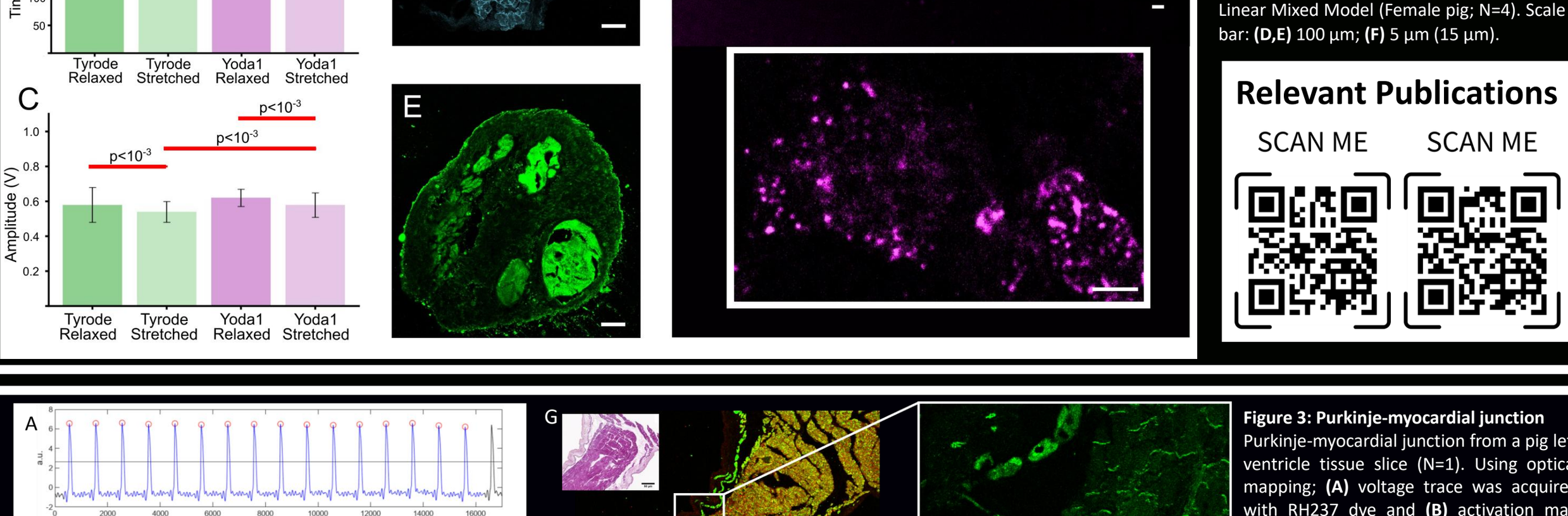

Figure 3: Purkinje-myocardial junction Purkinje-myocardial junction from a pig left ventricle tissue slice (N=1). Using optical mapping; **(A)** voltage trace was acquired with RH237 dye and **(B)** activation map produced to evaluate **(C)** action potential morphology, with an exemplar APD80 calculated. Simultaneously, **(D)** calcium signals were acquired with Rhod2 dye, to produce **(E)** activation map and **(F)** calcium transient morphology, with an exemplar CaTD80 calculated. **(G)** Histological analysis with H&E stain enabled cell type and overall tissue morphology to be studied at a gross level, with confocal acquisition to probe for PIEZO1 (green) and NCX (red), followed by ExM to study PIEZO1 at a 35 nm in-plane resolution. **(H)** Methodology to study function of a cardiac tissue slice and it's underlying structure. This includes; tissue preparation, optical mapping of the tissue slices and immunofluorescence staining to undertake confocal and expansion microscopy. Datasets were aligned based upon cell geometry using a custom-written script. Figure compiled using BioRender.

CONCLUSION

We have presented a case for how PIEZO1 is located within the PF network and is involved in the generation of stretch-mediated arrhythmias. This finding has the potential to lead to a new therapeutic target for conduction-based premature ventricular contractions.