Correct techniques for extracellular recordings of electrical activity in gastrointestinal muscle

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In their Perspectives (Problems with extracellular recording of electrical activity in gastrointestinal muscle. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 731–741; 2016), Sanders *et al.*¹ expand on previous claims that the gastrointestinal extracellular literature, together with related electrophysiology models, could be unreliable owing to contamination with movement artefacts. The essence of their claims seems to be that extracellular methods might not provide physiologically meaningful or mechanistically useful information. We feel that the authors are incorrect and misrepresent our work and other competing evidence. Similar previous claims have already been evaluated and disputed in previous research from our laboratory^{2–4}, and the reported concerns with extracellular recordings might have arisen simply owing to an incorrect application of extracellular techniques and misunderstanding of basic extracellular principles. Here, we clarify correct approaches to extracellular recordings.

Sanders *et al.*¹ performed their extracellular recordings *in vitro* on devitalised tissues; however, they have published that their tissue isolation process aberrantly elevates slow-wave frequencies, causing loss of intrinsic frequency gradients^{5,6}. Intrinsic frequency gradients are critical for slow-wave entrainment and generation of extracellular field potentials^{2,4}, and extracellular data cannot be recorded in their absence⁷. Extrapolating findings from devitalised tissue studies to all extracellular studies is inappropriate in this context.

Furthermore, we feel that Sanders *et al.*¹ have misrepresented basic extracellular physiology. Their representation of weak sharply oscillating biopotentials as extracellular potentials is misleading because these gastric signals do not resemble legitimate biphasic slow-wave data recorded by many research groups over a century. In their experimental studies, Sanders *et al.*¹ seem to have recorded movement artefacts and have then attributed problems to extracellular methods in general, rather than technical issues⁸.

A simple validation for the morphology of gastrointestinal extracellular slow-wave

potentials is achieved by performing suction and conventional contact recordings simultaneously (side-by-side) *in vivo* (FIG. 1). In accordance with known biophysical principles, suction extracellular recordings give a monophasic potential approximating the transmembrane potential, whereas contact electrodes give a biphasic potential². This biphasic potential coincides with the activation phase of the monophasic potential (FIG. 1); it is upgoing before arrival of the wavefront, steeply negative when the wavefront is under the electrode, then returns to baseline. This biphasic potential configuration has been repeatedly shown over the past century to precede contractions, and, therefore, cannot be a contraction artefact⁴. This signal of interest is not present in extracellular data currently offered by Sanders and colleagues^{1,8} and must be present in the raw signal traces, with minimal filtering used only to aid interpretation³ (FIG. 2a,b). The 3–100 Hz bandpass filter that Sanders *et al.*¹ advocate would grossly distort true slow-wave data because it eliminates

almost all signal content³ (FIG. 2c). For optimal recordings, researchers should also use an amplifier with no low cut-off frequency to avoid restricting wave morphology.

Attempts by Sanders *et al.*¹ to relate an extracellular waveform to the first-order derivative of the intracellular recording are inadequate. Continuum modelling approaches have been used for decades to relate extracellular and membrane potentials. Extracellular recordings do not record activity from a single cell, they spatially average a potential field arising from many cells². The time course of an extracellular potential is obviously much longer than the upstroke of a single cell's membrane potential. Moreover, the Perspectives authors also claim an excessive variability to extracellular recordings¹, but this issue has been overstated. Extracellular morphologies are highly consistent when recorded appropriately. The variability that does occur relates to several reproducible properties of extracellular fields, such as fractionation during slow-wave propagation or dysrhythmias, recovery phase heterogeneity and/or technical factors (for example, electrode types and configurations, filtering, hardware systems)⁴. Indeed, extracellular biophysics offers falsifiable hypotheses for the validity of extracellular recordings reflected in this 'variability', for example by predicting a positive linear correlation between velocity and extracellular amplitude due to rate of current entering the extracellular space⁹. We have confirmed this prediction experimentally, providing yet another validation for extracellular techniques⁹.

Figure 1 | Extracellular morphologies. **a** | Comparison of extracellular morphologies from a suction electrode and conventional serosal contact electrode employed simultaneously *in vivo* on adjacent regions of porcine gastric serosa. **b** | Suction electrode position. **c** | Serosal contact electrodes used for the comparison (flexible printed circuit board array). **d,e** | Comparison of experimental slow-wave data recorded by the two extracellular modes simultaneously. The suction electrode generates a monophasic potential, whereas the contact electrode generates a biphasic potential corresponding with the upstroke (activation / wavefront) phase of the monophasic potential. Reproduced with permission from Wiley © Angeli *et al.* *J. Physiol.* **591**, 4567–4579 (2013).

We conclusively demonstrated that extracellular slow waves are readily recordable *in vivo* even during complete motion suppression by intra-arterial nifedipine administration². In this Perspectives, the authors¹ misrepresented our methodology, incorrectly claiming we only assessed longitudinal tissue motion, undermining our validation study². However, our intestinal segments were not arranged in straight lines; we captured curved intestinal segments within each measured field, recording motion to single-pixel (sub-millimetre) resolution. There was no motion. The correct interpretation is that extracellular recordings are valid when performed and analysed correctly, and routine motion suppression is not required *in vivo*.

We disagree with the conclusions made by Sanders *et al.*¹ in their Perspectives. The ‘problems’ they describe are easily overcome if correct extracellular techniques are used,

and routine motion suppression is unnecessary *in vivo*. Indeed, the role of extracellular methods is currently expanding, as high-resolution electrical mapping is now contributing to substantial translational advances in human motility disorders¹⁰.

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Figure 2 | Comparison of filter effects on gastric serosal slow-wave signals (porcine data). **a** | Slow-wave signals from adjacent channels sampled at 512 Hz, with only the baseline wander removed (moving median window of 20 s). **b** | The same data following application of a Butterworth 2 Hz low-pass filter³. **c** | The same data following application of a 3–100 Hz band-pass Butterworth filter as advocated by Sanders *et al.*¹. True slow-wave data would be eliminated with this filter.

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Competing interests statement

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