

## Many parts make a whole: Calcium transients sum for slow waves

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New JGP study shows how calcium events drive long intestinal slow wave plateaus.

The peristaltic movements that propel food along the digestive tract require coordinated muscle contractions, which are governed by rhythmic electrochemical impulses termed slow waves: periodic membrane depolarization events with a plateau phase lasting ~1 s (1). In stomach and small intestine, slow waves originate in pacemaker cells called interstitial cells of Cajal of the myenteric plexus (ICC-MY; 2). In their paper this month in JGP, Drumm et al. explain how slow waves are generated within ICC-MY (3).

ICC-MY cells are arranged in a fine mesh across the muscle tissue of the small intestine and are connected via gap junctions to smooth muscle cells to convey membrane depolarization to the muscle. Slow wave membrane depolarization in ICC-MY is attributed to the opening of calcium ( $\text{Ca}^{2+}$ )-activated ANO1 chloride channels (4). Thus, slow wave generation ultimately depends on  $\text{Ca}^{2+}$  influx to the cytoplasmic space, which may occur via activation of voltage-gated plasma membrane calcium channels (5) and/or via  $\text{Ca}^{2+}$  release from ER intracellular stores (6). However, notes Salah A. Baker, Professor at the University of Nevada School of Medicine (Reno), this poses a conundrum:

“Typically when we think about mechanisms of calcium release events, we tend to think about  $\text{Ca}^{2+}$  sparks and puffs, which last a few hundred milliseconds at the most. So, addressing how calcium release events lead to the extended duration or plateau phase of an electrical slow wave was very intriguing,” says Baker.

Led by postdoc Bernard T. Drumm, and in collaboration with Grant W. Hennig and department head Kenton M. Sanders (also at UNR), Baker’s group set out to determine how such brief  $\text{Ca}^{2+}$  events prompt ICC-MY slow waves. To do this, the researchers expressed a genetically encoded  $\text{Ca}^{2+}$  sensor called GCaMP3 in mouse ICC-MY cells. The sensor glows in the presence of  $\text{Ca}^{2+}$ , allowing visualiza-

tion of cytoplasmic  $\text{Ca}^{2+}$  levels in intact tissue preparations from the mouse small intestine.

Initial observations at low magnification showed  $\text{Ca}^{2+}$  waves sweeping rhythmically across the tissue. However, higher magnifications revealed that  $\text{Ca}^{2+}$  events were highly localized within the cells, with each cell displaying numerous discrete sites where  $\text{Ca}^{2+}$  was briefly elevated. These events, called  $\text{Ca}^{2+}$  transients, occurred both within the cells’ somas and along their processes.

“These events occurred in asynchronous fashion and were temporally clustered within the time frame of the slow waves,” explains Baker. “The summation of these  $\text{Ca}^{2+}$  transients resulted in relatively uniform and prolonged  $\text{Ca}^{2+}$  responses from slow wave to slow wave.”

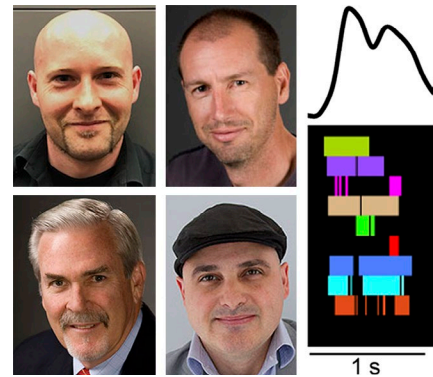
Unexpectedly, electrical recordings from muscle cells indicated that muscle cell depolarization preceded the observed  $\text{Ca}^{2+}$  transients in ICC. Baker suggests this could be because the muscle cells were connected to a dominant pacemaker ICC-MY outside the field of view, or because depolarization was triggered by  $\text{Ca}^{2+}$  entry mechanisms that could not be resolved by the researchers’ system.

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To identify how  $\text{Ca}^{2+}$  transients are generated, the researchers used pharmacological agents to block different calcium channels in the cell membrane and the ER. Consistent with other studies (5), these experiments showed that transients rely on T-type voltage-gated  $\text{Ca}^{2+}$  channels in the plasma membrane. The initial influx of  $\text{Ca}^{2+}$  from those channels was then amplified by activation of  $\text{Ca}^{2+}$ -activated ryanodine receptors on the ER, which release  $\text{Ca}^{2+}$  from intracellular stores. Inositol-3,4,5-triphosphate ( $\text{IP}_3$ )-stimulated



Co-first authors Bernard T. Drumm (top left) and Grant W. Hennig (top right), collaborator Kenton M. Sanders (bottom left), corresponding author Salah A. Baker (bottom right), and colleagues show how slow waves in the mouse small intestine emerge from spatially distinct, asynchronous calcium transients (indicated by different colors) that sum for a prolonged elevation in internal  $\text{Ca}^{2+}$  (trace at top). PHOTOS COURTESY OF THE AUTHORS.

ER  $\text{Ca}^{2+}$  channels also contributed to  $\text{Ca}^{2+}$  transients, although ryanodine receptors seemed more prominent. This contrasted with earlier work, which had pointed to a greater role for  $\text{IP}_3$  receptors in slow wave generation (6).

These data help explain how ICC-MY generate slow waves, but also raise new questions. For example, Baker and colleagues note that  $\text{Ca}^{2+}$  transients appear to occur at specialized sites within the cells. They plan to investigate which specific ryanodine receptors and  $\text{IP}_3$   $\text{Ca}^{2+}$  release channels amplify  $\text{Ca}^{2+}$  transients at these sites and also whether  $\text{Ca}^{2+}$  transients are affected by other  $\text{Ca}^{2+}$  sources, such as mitochondrial stores or sodium/calcium exchange.

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