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Background: Live Ca^{2+} imaging is a proxy for electrophysiological measurements and a valuable tool to analyze activity in multiple cells simultaneously. In the enteric nervous system (ENS), two main electrophysiological classes of neurons exist (AH and S). Although they have different Ca^{2+} handling mechanisms, they are rarely considered separately in Ca^{2+} imaging studies.

Aim: To investigate whether the characteristics of a $[Ca^{2+}]_i$ transient reflect the electrophysiological differences between murine AH and S neurons.

Methods: Primary ENS cultures were made from adult Wnt1-Cre;R26R-GCaMP6f mice. After 4-5 days in vitro, simultaneous Ca^{2+} imaging and patch-clamp recordings were performed. Cells were depolarized by either 10 or 500 ms current pulses or high K^+ (75 mM). DMPP (10 μM), a nicotinic receptor agonist, was used to mimic fast cholinergic transmission.

Results: We recorded from 40 neurons, classed as AH (16) and S (24) based on the presence or absence of an "inflection" in the action potential's (AP) repolarizing phase. Unlike previous studies performed in guinea pig, S neurons exhibited a prominent $[Ca^{2+}]_i$ transient accompanying a single AP. In response to a 10 ms depolarization pulse, both the AP and $[Ca^{2+}]_i$ transient amplitudes were significantly larger in AH neurons. However, with a 500 ms depolarization pulse or high K^+, no significant difference persisted. Interestingly, when tetrodotoxin (1 μM) was applied to block APs, a reduced but distinct $[Ca^{2+}]_i$ transient remained following the 500 ms depolarization pulse. In 10/12 AH neurons, DMPP did not elicit a membrane potential change or a $[Ca^{2+}]_i$ transient. In 14/16 S neurons, DMPP triggered both a $[Ca^{2+}]_i$ transient and either an AP or sub-threshold membrane potential change.

Conclusions: $[Ca^{2+}]_i$ transients were found to accompany single APs in both AH and S neurons. Although sub-threshold membrane depolarizations could also elicit $[Ca^{2+}]_i$ transients, these were generally amplified if an AP was present. The $[Ca^{2+}]_i$ response to DMPP was the most reliable way to optically distinguish between AH and S neurons.