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## Temporal Dynamics of Calcium Signaling: Clustering Adrenal Cells Producing Aldosterone through Ca2+ FURA-2 with the use of functional statistical analysis

The intricate regulation of calcium signalling plays a pivotal role in cellular processes, particularly in adrenal cells responsible for aldosterone production. Using calcium sensitive fluorescent ratiometric dye fura-2, this study investigates Angiotensin II (Ang II) induced intracellular calcium oscillations in adrenocortical cells, from primary normal tissue and aldosterone-producing adenomas. The aim is to understand the levels of cellular coordination between cell types and to provide proof of the existence of "functional" clusters where high levels of synchronicity in intracellular calcium oscillations are observed. With the use of a Functional ANOVA, the study ascertains the presence of clusters with a substantial level of synchronization and coordination among the cells within the same cluster, reflecting a more profound functional connection that leads to similar responses to Ang II stimulus. Additionally, a correlation-based hierarchical clustering algorithm and linear mixed-effects models find no statistically significant difference in the degree of synchronicity among cells categorized as primary normal tissue compared to those categorized as aldosterone-producing adenomas. Through the exploration of calcium bursts in adrenal cells, this study is able to provide evidence for the presence of "functional" clusters where cells exhibit high levels of temporal synchronicity in response to Ang II.

Primary author: FERNANDEZ DE VELASCO, Juan

Presenter: FERNANDEZ DE VELASCO, Juan

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