Activity-dependent regulation of synaptic neurotransmission by Ca2+ and tomosyn

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English summary

The work described in this thesis provides a tool to analyse Ca^{2+} imaging data, SICT (Chapter 2) and add new information about the occurrence of spontaneous Ca^{2+} events (SCE) and synaptic vesicles (SV) release events in cultured neurons (Chapter 3). Further findings on the association of tomosyn with moving synaptic vesicles are reported, which is at odds with the canonical inhibition of SNARE complex formation (Chapter 4).

Chapter 2 describes SICT: supervised inspection of fast Ca^{2+} transients. SICT is an informatics tool to automatically detect Ca^{2+} fluctuation events in time-lapse imaging data and to calculate the event parameters. SICT was mainly developed to detect low signal-to-noise fast Ca^{2+} events (SCTs). Several other Ca^{2+} waveforms were also detected, although in minor contribution. SICT presents a user-friendly interface which helps to visualise and to categorise Ca^{2+} events with different features by sorting out events based on different properties and allowing the spatial and temporal visualisation of the ROIs in both raw Ca^{2+} imaging data and the calculated $\Delta F/F_0$ signal. This step was validated by four human observers. SICT reduced the time of analysis to 10% compared to manual analysis. As biological application, we used SICT to show that caffeine increases the frequency and duration of SCTs. This demonstrates that SICT is a useful tool to study fast Ca^{2+} signals in living cells. Moreover, SICT is open source and can be adapted for many other purposes.

Chapter 3 aimed to study the Ca^{2+} dependence of spontaneous SV release using different procedures. First, we characterised SCTs in absence of an AP. Several Ca^{2+} indicators were tested and GCaMP6f identified to be the best probe to analyse SCTs compared to OGB-1, GCaMP6s and JRex. SCTs were enriched in synaptic compartments. Consistently, presynaptic targeting of GCaMP6f (called syGCaMP6f) detected more events than the globally expressed Ca^{2+} indicator. Other than SCTs, several other presynaptic SCEs were observed with different kinetics. The frequency of both SCEs and mEPSC was upregulated by increased $[Ca^{2+}]_e$ up to 10 mM. When we simultaneously measured presynaptic SCT/ SCEs and mEPSCs at 4 mM $[Ca^{2+}]_e$, no strict temporal coupling was observed. As an alternative procedure to assess the Ca^{2+} dependence of spontaneous SV release, sypHyJRex was used. SypHyJRex was validated to report vesicle fusion and $[Ca^{2+}]_i$ events with good optical separation of the dual emission channels. It detected Ca^{2+} events spatiotemporally coupled to spontaneous SV release events. Further analysis is required to evaluate how many events co-occur together, if they belong to a specific neuronal subtype and which Ca^{2+} pathway they are associated with. Together these results show the complexity of the neuronal Ca^{2+} pathways and the need to further dissect the role of SCT/ SCE at the presynapse and other neuronal locations. Moreover, our results merged with the literature suggest that spontaneous SV release is regulated by Ca^{2+} in several ways. Important to discriminate in the future studies will be the neuronal subtypes, the $[Ca^{2+}]_i$ needed for such events, the Ca^{2+} sources, and the Ca^{2+} sensors involved.

In Chapter 4, tomosyn localization was studied in wildtype hippocampal neu-

rons. Tomosyn presented both a cytosolic and punctate distribution, although, it does not contain a transmembrane domain. Tomosyn is expressed in both axons and dendrites and, it co-localized and co-migrated with both SV and LDCV markers, synapsin and NPY respectively. Field stimulation slightly reduced the velocity of the tomosyn puncta. Tomosyn immunoprecipitated together with syntaxin-1A, SNAP25 and syt1. Moreover, the overexpression of several tomosyn mutants showed a consistent vesicular binding which was not inhibited by the absence of syt1. Altogether these results suggest that tomosyn might bind vesicles by redundant mechanisms and this association may be important in regulating vesicle cluster and transport during synaptic plasticity to sustain repetitive stimulations.

In conclusion, the neuronal Ca^{2+} events happening independently of an AP are diverse and their roles and mechanisms need further elucidation. To this aim and beyond, the standardization of Ca^{2+} analysis would be advisable to better merge results of different investigations. SypHyJRex is a valuable tool to study the Ca^{2+}-dependence of spontaneous release and can be used to unravel the Ca^{2+} pathways, the neuronal subtype or subpopulation of SVs involved. Finally, tomosyn analysis suggests that tomosyn has multiple functions based on its neuronal localization and/or binding partners which might depend on the activity state of a neuron.