The endoplasmic reticulum (ER) forms a continuous network of tubules and cisterns in neurons. However, recent evidence suggests that neuronal ER does not represent a uniform Ca\(^{2+}\) pool but rather a spatially heterogeneous system organized into subcompartments. These ER domains, or calciosomes, are usually enriched in certain isoforms of Ca\(^{2+}\) pumps, Ca\(^{2+}\) binding proteins and Ca\(^{2+}\) permeant channels, and are supposed to unload and refill Ca\(^{2+}\) independently [1]. Ca\(^{2+}\) release from the ER is mediated by two families of Ca\(^{2+}\) permeable channels, namely the ryanodine receptors (RyRs) and the inositol 1,4,5-trisphosphate receptors (IP3-Rs), each with three major isoforms. Areas of the plasma membrane overlying calciosomes also form specialized microdomains that contain unique sets of ion channels. These plasma membrane domains together with the underlying calciosomes are proposed to build functional units, termed plasmerosomes [2].

This study was undertaken (i) to unravel exact morphological parameters of subsurface cisterns (SSCs), representing particular types of calciosomes in cerebellar Purkinje cells (PCs), and (ii) to analyze the molecular composition of SSCs as well as overlying plasma membrane domains with respect to Ca\(^{2+}\) release channels (IP3-Rs, RyRs), voltage-gated Ca\(^{2+}\) channels and Ca\(^{2+}\) sensors such as large-conductance Ca\(^{2+}\) activated K\(^{+}\) (BKCa) channels. The morphological parameters of SSCs are established by 3D-reconstruction plane-by-plane from series of ultrathin sections by using the software CAR (Contour Alignment Reconstruction). The molecular composition is studied by means of pre- and post-embedding immunogold electron microscopy and SDS-digested freeze-fracture replica immunolabeling.

SSCs are discoid flattened cisterns, 0.4-1.5 µm wide, with a luminal depth of 4-5 nm (widening at their lateral edges), situated beneath the inner leaflet of the plasma membrane at a regular distance of 10-15 nm. IP3-Rs and RyRs are both localized to SSCs indicating that these Ca\(^{2+}\) release channels share a common Ca\(^{2+}\) pool and dispose SSCs to the generation of both Ca\(^{2+}\) puffs and sparks. Clustered BKCa channels are always associated with plasma membrane domains overlying SSCs and likely facilitate the generation of small transient outward currents. These findings indicate that functional units exist in cerebellar PCs resembling plasmerosomes in myocytes [3], and these units may contribute significantly to spatial signalling in central principal neurons.


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Fig. 1: 3D-reconstruction of Purkinje cell subsurface cisterns by means of CAR (Contour Alignment Reconstruction; K. Sätzler, Univ. Ulster, UK). Green, axon terminal; purple, aspect of PC soma; dark-red, subsurface cistern; white, mitochondrion; orange and yellow, junctional and non-junctional ER, respectively.

Fig. 2: Clustering of BKCa channels at sites of subsurface cisterns in Purkinje cells. BKCa channels, immunolabeled with antibodies conjugated to 10-nm gold particles (arrowheads) in a post-embedding immunogold approach, are enriched at sites of SSCs. Scale bar = 180 nm.