Calcium Dynamics and Functional Hyperexcitability in Human iPSC-Derived Organoid Models of Neurodegenerative Disorders

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Presynaptic Ca^{2+} influx is a key factor coupling neuronal excitability with neurotransmitter release, and its dysregulation is increasingly recognized as an early, convergent event in Alzheimer's disease (AD). However, how presynaptic Ca^{2+} transport is disrupted in the context of pathology in both familial and sporadic Alzheimer's disease in human tissues remains unclear. Therefore, we integrated two human stem cell-based research programs into a single calcium imaging platform encompassing familial AD (FAD; APP, PSEN1 mutations), sporadic AD (SAD), and healthy controls.

Brain organoids (8–9 months; identification confirmed around day 90 by FOXG1, TBR2, SATB2, MAP2, PSD95/SNAP25) and iPSC-derived neurons (2–4 months) were studied using complementary Ca^{2+} indicators: (1) ratiometric Fura-2 (340/380) during the pharmacological cascade—NMDA (20 or 50 µM), KCl (59 mM), ionomycin (10 µM), EGTA (10 mM)—analyzing receptor-driven influx, depolarization-induced release from presynaptic stores, and total releasable pool size; (2) FLUO-4 AM; and (3) soma-targeted, hSyn1-driven jGCaMP8f (validated 10 days after transduction), involving time-lapse imaging from brain organoids with analysis of network synchronization.

FAD and SAD neurons exhibited altered NMDA-evoked Ca^{2+} responses, indicating impaired coupling between presynaptic Ca^{2+} influx and the pool of releasable vesicles. In the organoids, the AD groups showed a trend toward elevated peak ΔF/F and AUC for GCaMP8 (Cohen's d ≈ 1 for FAD vs. CTRL; p_adj > 0.05). Functional hyperexcitability was pronounced in AD: SAD organoids exhibited significantly increased network synchronization (0.39 vs. 0.20 for CTRL). Independently, synaptosomal proteomics revealed a remodeling of the presynaptic Ca^{2+} mechanism—downregulation of exocytotic Ca^{2+} sensors (SYT1/2/7), CaMKII-α, and voltage-gated Ca^{2+} channel subunits, along with upregulation of Ca^{2+} buffers such as S100, essential for Ca^{2+}-regulated exocytosis and calcium channel activity. This is consistent with a shift toward decreased presynaptic Ca^{2+} sensitivity and thus decreased synaptic vesicle release efficiency in AD.

Collectively, chemical and genetic markers point to presynaptic Ca^{2+} dysregulation and functional hyperexcitability as key phenotypes in human organoid and neuronal models of Alzheimer's disease, making our research platform mechanistically grounded for presynaptic drug screening targeting Alzheimer's disease.

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