Neuronal BIN1 expression is required for spatial learning and memory

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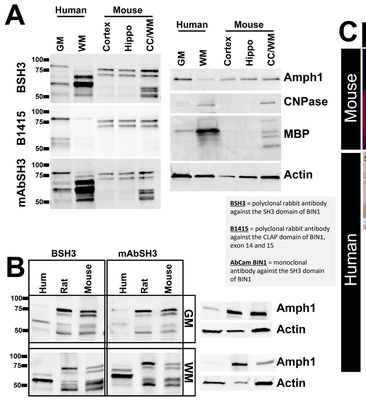
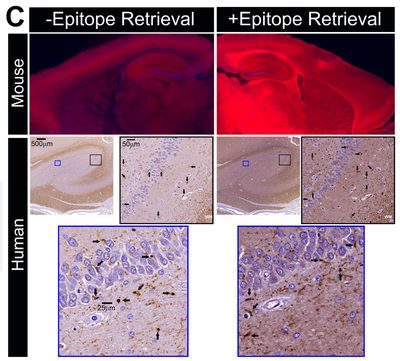
Abstract

Genome-wide association studies have recently identified *BIN1* as a major susceptibility locus for late-onset AD (LOAD). BIN1 was previously characterized for its role in endocytosis and membrane dynamics in non-neuronal cells and peripheral tissue. Previously, we characterized a predominant expression of BIN1 in mature oligodendrocytes and the white matter tracts. We reported that oligodendrocytes mainly expressed the BIN1 isoform9. In addition, the levels of a subset of BIN1 were found correlated with neuronal markers such as PSD95 and synaptophysin. This isoform was characterized as Isoform 1. Although the GWAS identification of BIN1 as a risk factor for LOAD has stimulated a burgeoning interest on BIN1 in the field, fundamental information about the role of BIN1 in the brain, especially in the neurons, is still lacking. Here, we ascertained BIN1 localization in cultured hippocampal neurons and mouse brain, using confocal and STORM microscopy and immunoEM approaches. Our results show an enrichment of BIN1 at presynaptic sites, which we confirmed using synaptosomal fractionation studies. We then generated conditional BIN1 knock-out models, to investigate the role of neuronal BIN1 in learning and memory. Although the loss of BIN1 expression did not affect the behavioral outcomes on a wide range of tests, we uncovered a select deficit in spatial learning memory in BIN1 cKO mice compared to their littermate controls. We are currently employing complementary approaches including electrophysiology, high-resolution imaging, and biochemistry to gain further insights and uncover mechanistic details. Altogether, our results demonstrate a non-redundant role for BIN1 in neurons, which plays a role in learning and memory and synaptic regulation.





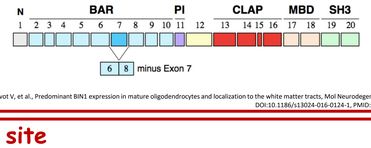
BSH3 = polyclonal rabbit antibody against the SH3 domain of BIN1

B1415 = polyclonal rabbit antibody against the CLAP domain of BIN1, exon 14 and 15

AbCam BIN1 = monoclonal antibody against the SH3 domain of BIN1

1. Brain BIN1 isoforms

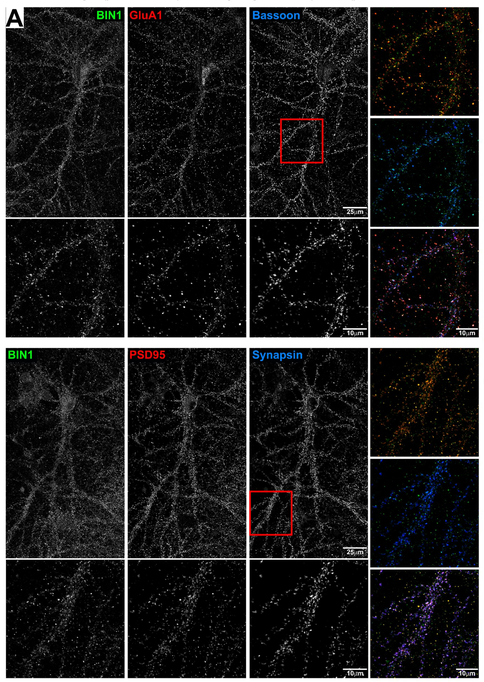
We first explored the expression of BIN1 in the human and rodent brain. **A)** We observe that BIN1 isoforms in the human brain differed from the rodent brain isoforms. We also observed that BIN1 isoforms expressing the CLAP domain (BIN1:H) were mostly expressed in the grey matter and those lacking the CLAP domain (BIN1:L) were expressed in the white matter. These observations confirmed our previously published results (De Rossi et al, 2016). **B)** By Western blot, both BSH3 and mAbSH3 antibodies revealed similar profiles. **C)** Immunohistochemical analysis of BIN1 expression using mAbSH3 antibody revealed neuronal and white matter BIN1 expression. Notably, the white matter staining predominates following epitope retrieval (De Rossi et al, 2016).

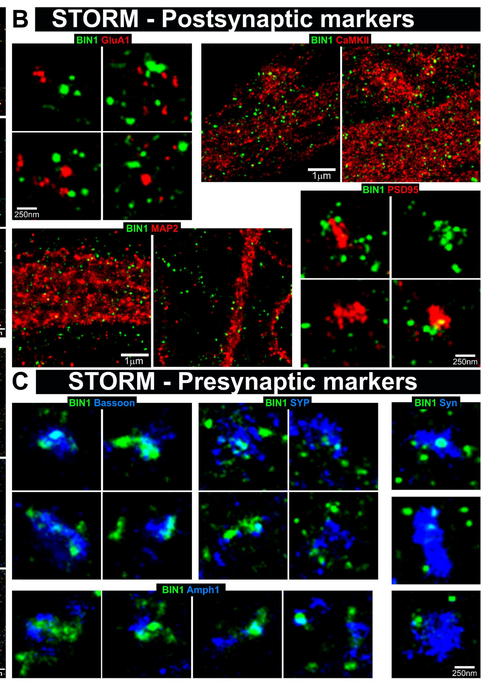


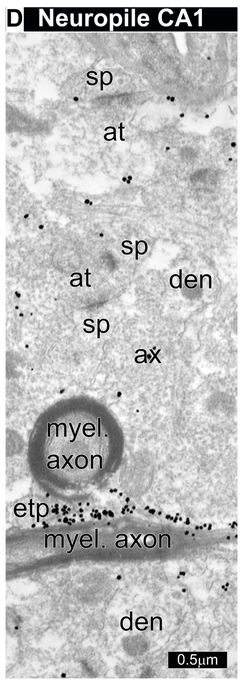
De Rossi P, Buggia-Prévot V, et al., Predominant BIN1 expression in mature oligodendrocytes and localization to the white matter tracts, Mol Neurodegener, 2016. DOI:10.1186/s13024-016-0124-1, PMID:27488240.

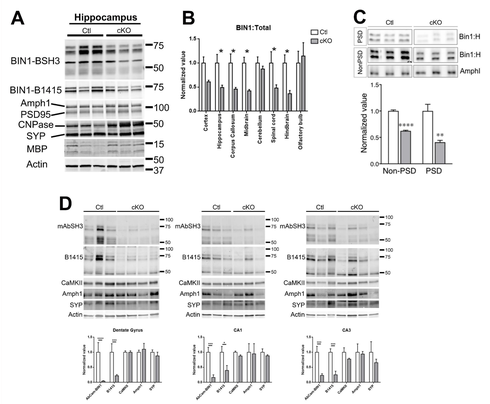
2. BIN1 localization at the synaptic site

We then explored BIN1 localization in neurons. A) Using primary hippocampal neuronal culture, we examined BIN1 localization compared to pre- (Bassoon and Synapsin) and post-synaptic markers (GluA1 and PSD95) using confocal microscopy. BIN1 colocalizes with both Pre- and Post-synaptic markers. B) To improve the spatial resolution we compared BIN1 localization to post- (PSD95, GluA1 or CaMKII), pre-synaptic (Synapsin, Synaptophysin, Bassoon or BIN1 homolog Amph1) and dendritic (MAP2) markers using Stochastic Optical Reconstruction Microscopy (STORM). We observe BIN1 in close proximity with the presynaptic markers rather than the postsynaptic ones. C) Finally, using immunoEM, we examined BIN1 localization in the neuropile of CA1. The expression of BIN1 in the neuropile was quite low compared to white matter tract expression. We confirmed BIN1 localization within the axons (ax), but not within the spine (sp) or the dendrites (den). Moreover, BIN1 was found concentrated at the external tongue process of myelinating oligodendrocytes (etp).



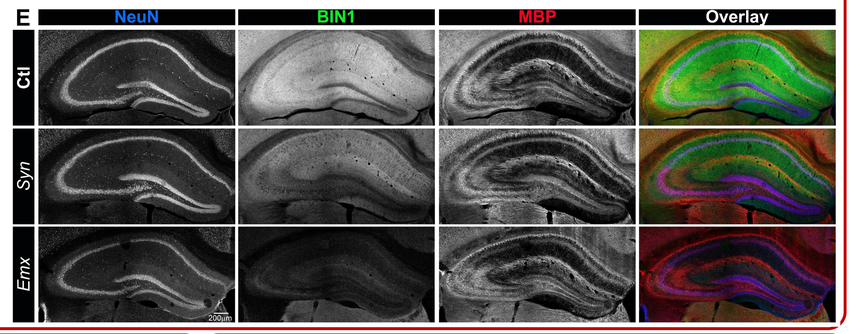




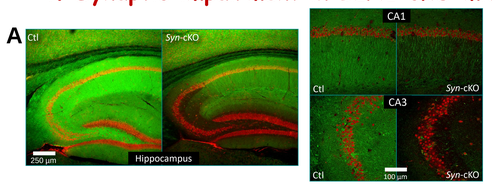


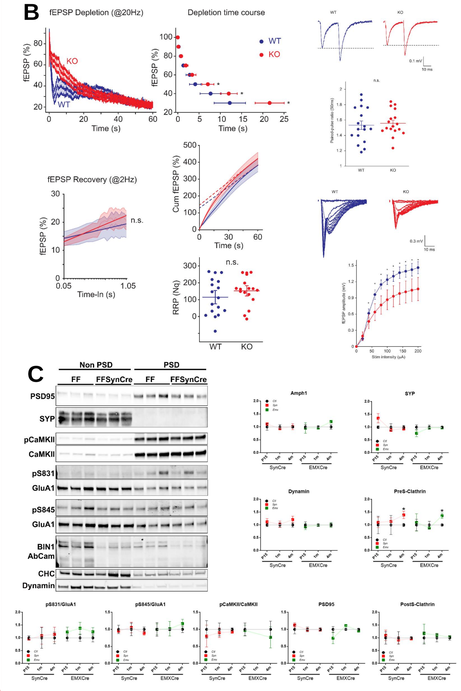
3. Characterization of BIN1 cKO mice

We generated neuronal BIN1 cKO mice using *Syn*-Cre or *Emx*-Cre drivers. Using biochemistry, **A)** Analysis of BIN1 levels in different areas of the brain and **B)** Quantification (*Syn*-Cre cKO model) shows significant reduction of BIN1 in most regions of the brain. **C)** In synaptosomal preparations high-levels of BIN1 was present in the non PSD fraction compared to the PSD. In both fractions, BIN1 was significantly decreased in the cKO mice. **D)** Microdissections of the hippocampus were used to quantify the loss of BIN1 in the different hippocampal regions - Dentate Gyrus (DG), CA1 and CA3. **E)** IF analysis of BIN1 expression in the hippocampus of control and cKO mice.



4. Synaptic impairment in BIN1 cKO mice





A) Using the *Syn*-cKO model, we explored the synaptic properties. BIN1 expression was reduced in both pre- and post-synaptic sites in the CA3. In the CA1, BIN1 seemed mainly decreased at the presynaptic site (Schaeffer's collaterals) with still some dendritic expression.

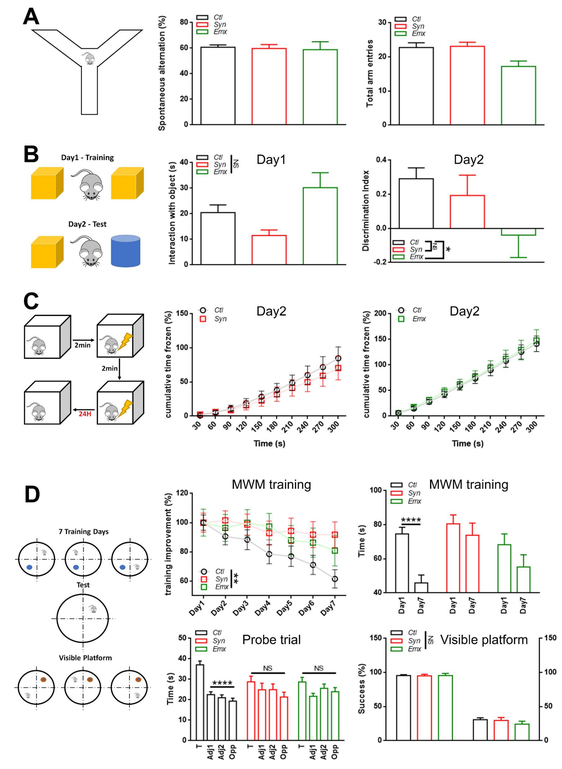
B) By slice electrophysiology, we first analyzed the CA1 fEPSP depletion over time. Our results showed that the depletion course in the cKO was slower than the controls. However, the paired-pulse facilitation was found normal. cKO also showed a reduction in the amplitude of the fEPSP.

C) In order to identify the reason of these impairment, we explored the expression of presynaptic markers and plasticity markers at the post-synaptic sites using synaptosomal preparations. Our results showed that most of the proteins are normally expressed and that synaptic plasticity markers were expressed at similar levels.

We concluded that the absence of BIN1 did not result in altered levels of synaptic proteins, but BIN1 is involved at some level in synaptic regulation.

5. Spatial and learning memory deficits in BIN1 cKO mice

We explored behavioral deficits induced by the loss of BIN1 in both cKO models. The cKO mice showed a normal behavior in most of the CA1-dependent learning tasks such as **A)** Y-Maze, **B)** Novel object recognition, and **C)** fear conditioning. However, both cKO models showed impairment in Morris Water Maze, a spatial learning task **(D)**. Both *Syn* and *Emx* cKO mice had deficits in acquisition and memory retention.



Conclusions

BIN1 is expressed in the neurons and mostly localized at the presynaptic site. In cKO models BIN1 levels are overall decreased in the brain, particularly in the hippocampus. Electrophysiology studies reveal weaker synapse strength in BIN1 cKO mice. Finally, the loss of BIN1 leads to selective impairment in learning and memory behavior in spatial learning and memory task.

To pursue this study, we will: 1) Confirm BIN1 localization at the synapse by immunoEM; 2) Identify the cellular / molecular mechanism by which BIN1 is involved at the synapse; 3) explore BIN1's role in amyloid and Tau pathology using cKO models, in the context of BIN1 as a risk factor for Alzheimer's disease.

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