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Calcium handling: a strategy to fight neurodegeneration in Alzheimer's disease

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In the last few years, preclinical and clinical studies identified the ventral tegmental area (VTA) as one of the first brain regions to be affected in the prodromal phase of Alzheimer's disease (AD).

The VTA is a deep midbrain nucleus rich in dopaminergic neurons that innervates and releases dopamine (DA) in several cortical and subcortical brain regions. DA plays a crucial role in the modulation of both cognitive and non-cognitive functions and failure of its release underlies both cognitive and neuropsychiatric symptoms in patients with AD.

DA neurons in the VTA are characterized by distinctive electrophysiological properties. Typically, VTA DA neurons exhibit phasic bursts of action potentials during novelty seeking and reward-related behaviors, while the low-frequency tonic spontaneous firing, called "pacemaking" activity, may contribute to maintaining awareness during learning and working memory functions.

DA neuron pacemaking activity requires high energy levels and a consequent efficient mitochondrial turnover; this is possible thanks to an efficient autophagy mechanism to remove damaged organelles including exhausted mitochondria. All these cellular processes rely on the tight regulation of intracellular calcium levels.

Calcium homeostasis mechanisms: The cytosolic calcium concentration depends on calcium influx from the extracellular space and efflux from intracellular stores, two finely regulated mechanisms. The extracellular calcium influx is mediated by different types of calcium channels as the voltage-gated calcium channels, N-methyl-D-aspartic acid, and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors. Calcium is also released into the cytosol from endoplasmic reticulum (ER) stores through the ryanodine receptors and the inositol 1,4,5-triphosphate receptors, and from the mitochondria via the sodium/calcium exchanger. This cytosolic calcium increase is counteracted by the sarcoendoplasmic reticulum calcium transport ATPase pump and the mitochondrial calcium uniporter. Neurons display also intrinsic calcium buffering ways to control calcium levels, thanks to the expression of calcium-binding proteins (i.e., calbindin and calretinin), calcium sensors (such as calmodulin), and a tied interplay between the ER and mitochondria. The functional consequence of this intricate machinery is the tight control of physiological calcium oscillations, crucial for the activity of DA neurons in VTA.

Calcium homeostasis dysregulation: from aging to neurodegenerative diseases: During aging, neurons progressively lose their ability to handle calcium homeostasis due to the dysfunction of calcium-regulating systems. This causes an increase in intracellular free calcium and impaired calcium-dependent cellular processes. Indeed, altered activity of calcium channels, decreased mitochondria ability to buffer calcium and the reduction of calcium-binding protein and sensor levels, linked to oxidative and metabolic stress, were observed in aged neurons.

The cytosolic calcium overload can also modulate the amyloid precursor protein (APP) processing, promoting the accumulation of amyloid-β (Aβ) peptide and the Aβ-related neurotoxicity, typical hallmarks of AD. Symmetrically, Aβ oligomers contribute to the calcium homeostasis perturbation, resulting in an excessive intracellular calcium influx from the extracellular space and intracellular stores.

Aβ oligomers can induce the increased calcium influx via different mechanisms: i) they interact with the cellular membrane and disrupt its permeability by forming Aβ pore-channels; ii) they can stimulate the activity of calcium channels located in the neuronal membrane, as the voltage-gated calcium channels or the N-methyl-D-aspartic acid receptor; iii) Aβ peptides interfere with ER calcium channels, inducing a high release of calcium from ER stores into the cytoplasm, or iv) facilitating mitochondrial calcium uptake with the consequent calcium overload.

High levels of intracellular calcium impair the activity of mitochondria, leading to their membrane permeabilization and the cytochrome c release. The abnormal calcium increase also induces the activation of calpain, a calcium-sensitive protease. Active calpain cleaves the apoptosis-inducing factor (AIF) from the mitochondrial membrane and elicits AIF translocation to the nucleus. Eventually, all these pathways, together with Aβ toxicity, result in the death of neurons. Many of these age-related calcium deficits are described in AD animal models and patients, supporting the hypothesis that calcium homeostasis dysregulation could be responsible for neuronal loss in neurodegenerative diseases.

In this context, the recent work (La Barbera et al., 2022) found that DA neurons in the VTA accumulate AIF in the nuclei and damaged mitochondria in their cytosol; this is linked to an impaired autophagy flux (La Barbera et al., 2021). These events result in early DA neurodegeneration of a validated AD mouse model (Tg2576), overexpressing a mutated form of the human APP (APPswe); the overexpression of APPswe in all neurons, including the DA ones of the VTA, leads to the accumulation of intracellular Aβ oligomers. However, a previous work in the same mouse model provided evidence that DA neurons in VTA are more susceptible to cell death, and probably this is determined by DA neuron intrinsic features underlying their functions (Nobili et al., 2017).

In Tg2576 mice, VTA DA neurons undergoing apoptosis are characterized by low levels of calcium-binding proteins, suggesting a global defective calcium-regulating system. By contrast, surviving VTA DA neurons show the overexpression of calcium-binding proteins, calbindin, and calretinin, to probably counteract the observed mitochondrial dysfunctions and potential calcium alterations. This explains why survived neurons display lower levels of cytosolic free calcium compared to controls (La Barbera et al., 2022; **Figure 1**). Taken together, the results suggest that preserving neuronal calcium homeostasis might be an attempt of DA neurons to fight physiological and pathological aged-related alterations.

In strict compliance with our observations in Tg2576 mice, other Authors studied the involvement of dopaminergic transmission in the early phase of the disease by using different AD mouse models. A recent study described the degeneration of dopaminergic midbrain and low levels of DA transporter in midbrain-projecting areas, all resulting in functional alterations (Vorobyov et al., 2019); similarly, other Authors reported an increase in DA receptor density in the 3xTg-AD model, as a compensatory response to low level of DA (Gloria et al., 2021).

All these preclinical results shed light on the role of VTA in AD patients. Functional and structural magnetic resonance imaging analysis demonstrated that mild cognitive impairment patients have a functional disconnection between VTA and its projecting areas, which worsens in AD (Serra et al., 2021); additionally, a tied correlation between the loss of VTA integrity and hippocampal size appears since the early phases of AD (De Marco and Venneri, 2018). As further proof, an *in vivo* DAT imaging with single-photon emission computed tomography showed that VTA DA projections to several brain areas are reduced starting from the mild-cognitive impairment stage (Sala et al., 2021).

These neuroimaging reports support the hypothesis that the degeneration of VTA and its disconnection from its projecting areas is a very precocious event in the progression of AD. Despite this, understanding why it occurs precociously in humans is still debated; however, preclinical results emerging from our work suggest that mitochondrial alterations and calcium-regulating system defects could be early events occurring in VTA DA neurons during the early stages of AD.

Calcium-handling strategies to counteract neuron dysfunctions: Calcium-handling defects are similar across different neurological disorders, suggesting a common underlying mechanism leading to neurodegeneration. New therapeutic strategies aimed to maintain calcium homeostasis appear to be a promising approach to slow down neurodegenerative processes.

Of note, experimental evidence demonstrated that blunting mitochondrial oxidative stress and overexpression of calcium-binding protein levels through antioxidants vitamins may be an important adjuvant therapy against neurodegenerative processes (Bhatti et al., 2016). Vitamin A levels and functions are reduced during physiological aging; not surprisingly, vitamin A is decreased in plasma and cerebrospinal fluid of AD patients. A diet supplementation with vitamin A was shown to reduce cognitive decline, improving memory performance and spatial learning in AD patients (Bhatti et al., 2016). Moreover, retinoic acid, the active metabolite of vitamin A, can increase calcium-binding protein levels in different models and prevent intracellular calcium overload with a neuroprotective effect (Wang and Christakos, 1995).

Promising results support the hypothesis that the modulation of calcium-permeable ion channels to maintain calcium balance could be helpful to delay the deterioration of cognitive impairments in moderate-to-severe AD patients. Indeed, the antagonism of N-methyl-D-aspartic acid receptors with memantine, a moderate-affinity non-competitive inhibitor, blocks excessively activated receptors in different preclinical models of AD (Folch et al., 2018). Further clinical trials demonstrated that best results are obtained when memantine is administrated in combination with acetylcholinesterase inhibitors (#NCT00322153; #NCT01921972), a tyrosine kinase inhibitor (#NCT00976118) and vitamin D (#NCT01409694) in AD patients.

The systemic administration of dihydropyridines (isradipine and nimodipine, voltage-gated calcium channel antagonists safely used for years to treat hypertension) is shown to have moderate benefits on AD-related cognitive deficits in both AD models and patients (Anekonda and Quinn, 2011).

These clinical trials targeted the late phases of AD, resulting in weak effects. However, recent works suggest that a precocious intervention would have a better chance to delay the AD progression and, probably, this is also due to the effectiveness of the intervention on VTA that is precociously affected.

VTA DA neurons share common features with the neighboring substantia nigra pars compacta DA neurons. Evidence demonstrated that calcium-regulating system defects occur in substantia nigra pars compacta DA neurons in Parkinson's

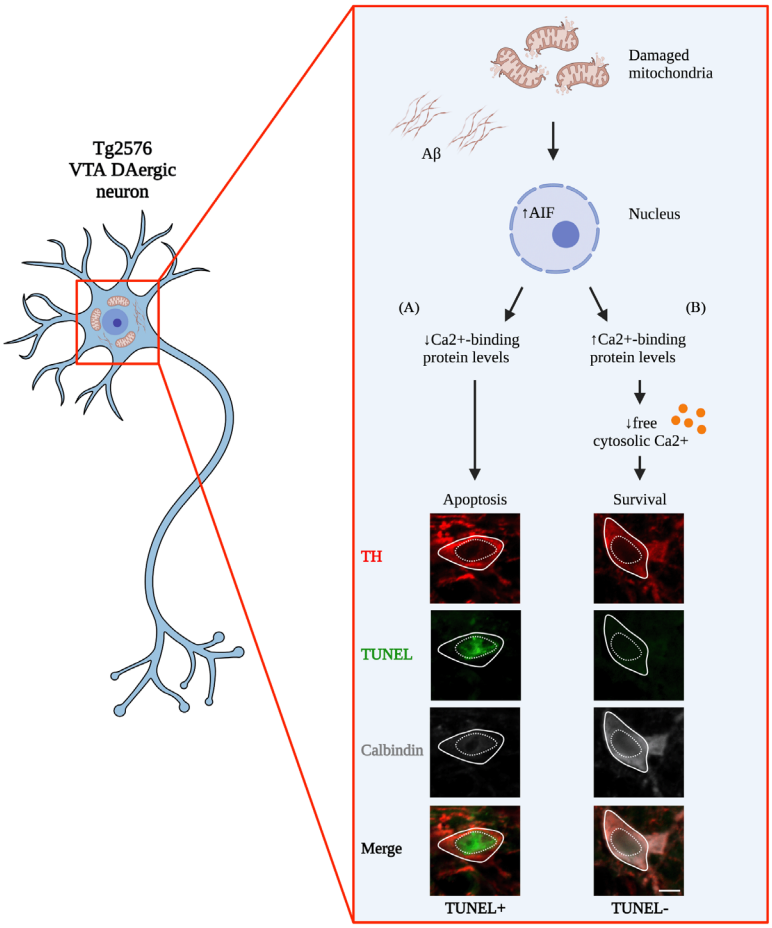

Figure 1 | **Calcium-binding protein overexpression contributes to VTA DA neuron survival in the Tg2576 Alzheimer's disease mouse model.**
DA neurons display damaged mitochondria and nuclear apoptosis-inducing factor (AIF) at the onset of the VTA neurodegeneration in Tg2576 mice (3 months of age). (A) DA neurons undergoing apoptosis (TUNEL^{+}) express low levels of calcium-binding proteins (i.e. calbindin), suggesting a defective calcium-buffering system. (B) Survived neurons overexpress calbindin protein, thus resulting in reduced cytosolic calcium levels. Aβ: Amyloid-β; AIF: apoptosis-inducing factor; Ca^{2+}: calcium; DA: dopamine; TH: tyrosine hydroxylase; VTA: ventral tegmental area. ↑: Increase; ↓: reduction. Unpublished data. Created with BioRender.com.

disease (PD) models and patients. Interestingly, pharmacological calcium-handling treatments prevent intracellular calcium overload and protect substantia nigra pars compacta DA neurons from cell death (Pchitskaya et al., 2018). Furthermore, results from epidemiologic and clinical studies (#NCT02168842 and #NCT00909545) reveal that dihydropyridines slow down PD progression since the early stages (Surmeier et al., 2022).

Thus, similarly to PD, an early intervention to handle calcium homeostasis could be a promising therapeutic approach to improve VTA DA neuron resistance to cell death and slow down the AD progression.

Conclusions: Overall, this work underlies that calcium is an important secondary messenger that requires a tight spatial and temporal regulation. DA neurons possess cellular functions to ensure physiological homeostasis in the short term (physiological functions); however, the prolonged dysregulation of cellular calcium homeostasis leads to a more global breakdown in cellular functions and structures, culminating in neuronal death. In neurodegenerative diseases, such as AD and PD, vulnerable brain regions exhibit patterns of calcium signaling alterations closely associated with the pathophysiology and disease symptoms. It would be promising to target the sources of calcium dysregulation as an effective therapeutic strategy to slow down the disease progression and provide a more complete understanding of the pathology from an etiological point of view.

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References

Anekonda TS, Quinn JF (2011) Calcium channel blocking as a therapeutic strategy for Alzheimer's disease: the case for isradipine. Biochim Biophys Acta 1812:1584-1590.
Bhatti AB, Usman M, Ali F, Satti SA (2016) Vitamin supplementation as an adjuvant treatment for Alzheimer's disease. J Clin Diagn Res 10:OE07-11.
De Marco M, Venneri A (2018) Volume and connectivity of the ventral tegmental area are linked to neurocognitive signatures of Alzheimer's disease in humans. J Alzheimers Dis 63:167-180.
Folch J, Busquets O, Ettcheto M, Sánchez-López E, Castro-Torres RD, Verdaguer E, Garcia ML, Olloquequi J, Casadesús G, Beas-Zarate C, Pelegri C, Vilaplana J, Auladell C, Camins A (2018) Memantine for the treatment of dementia: a review on its current and future applications. J Alzheimers Dis 62:1223-1240.
Gloria Y, Ceyzériat K, Tsartsalis S, Millet P, Tournier BB (2021) Dopaminergic dysfunction in the 3xTg-AD mice model of Alzheimer's disease. Sci Rep 11:19412.
La Barbera L, Vedele F, Nobili A, Krashia P, Spoleti E, Latagliata EC, Cutuli D, Cauzzi E, Marino R, Viscomi MT, Petrosini L, Puglisi-Allegra S, Melone M, Keller F, Mercuri NB, Conti F, D'Amelio M (2021) Nilotinib restores memory function by preventing dopaminergic neuron degeneration in a mouse model of Alzheimer's disease. Prog Neurobiol 202:102031.
La Barbera L, Nobili A, Cauzzi E, Paoletti I, Federici M, Saba L, Giacomet C, Marino R, Krashia P, Melone M, Keller F, Mercuri NB, Viscomi MT, Conti F, D'Amelio M (2022) Upregulation of Ca2+-binding proteins contributes to VTA dopamine neuron survival in the early phases of Alzheimer's disease in Tg2576 mice. Mol Neurodegener 17:76.
Nobili A, Latagliata EC, Viscomi MT, Cavallucci V, Cutuli D, Giacovazzo G, Krashia P, Rizzo FR, Marino R, Federici M, De Bartolo P, Aversa D, Dell'Acqua MC, Cordella A, Sancandi M, Keller F, Petrosini L, Puglisi-Allegra S, Mercuri NB, Coccurello R, Berretta N, D'Amelio M (2017) Dopamine neuronal loss contributes to memory and reward dysfunction in a model of Alzheimer's disease. Nat Commun 8:14727.
Pchitskaya E, Popugaeva E, Bezprozvanny I (2018) Calcium signaling and molecular mechanisms underlying neurodegenerative diseases. Cell Calcium 70:87-94.
Sala A, Caminiti SP, Presotto L, Pilotto A, Liguori C, Chiaravalloti A, Garibotto V, Frisoni GB, D'Amelio M, Paghera B, Schillaci O, Mercuri N, Padovani A, Perani D (2021) In vivo human molecular neuroimaging of dopaminergic vulnerability along the Alzheimer's disease phases. Alzheimers Res Ther 13:187.
Serra L, D'Amelio M, Esposito S, Di Domenico C, Koch G, Marra C, Mercuri NB, Caltagirone C, Artusi CA, Lopiano L, Cercignani M, Bozzali M (2021) Ventral tegmental area disconnection contributes two years early to correctly classify patients converted to Alzheimer's disease: implications for treatment. J Alzheimers Dis 82:985-1000.
Surmeier DJ, Nguyen JT, Lancki N, Venuto CS, Oakes D, Simuni T, Wyse RK (2022) Re-analysis of the STEADY-PD II trial-evidence for slowing the progression of Parkinson's disease. Mov Disord 37:334-342.
Vorobyov V, Bakharev B, Medvinskaya N, Nesterova I, Samokhin A, Deev A, Tatarnikova O, Ustyugov AA, Sengpiel F, Bobkova N (2019) Loss of midbrain dopamine neurons and altered apomorphine EEG effects in the 5xFAD mouse model of Alzheimer's disease. J Alzheimers Dis 70:241-256.
Wang YZ, Christakos S (1995) Retinoic acid regulates the expression of the calcium binding protein, calbindin-D28K. Mol Endocrinol 9:1510-1521.

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