New insights into physiology of aged-related cognitive disorders: the DNA repair protein ATR

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Background

Aging is a multifactorial process characterized by the slow decline of cellular physiology associated with a **lowdown of brain functions**.
Neurological complications mainly reflect **defects at the synaptic structure**. Indeed, preclinical studies demonstrate that preserving functionality of synapses delays the occurrence of aged-related neurological and cognitive defects and prevents the progressive neuronal degeneration.
Defects in DNA repair mechanisms have a critical role in aged-related neurological diseases and expression of core **DNA repair genes is downregulated during aging** across brain regions.

ATR (**Ataxia Telangiectasia mutated-Rad3 related**) is a **serine/threonine protein kinase**, belonging to the phosphatidylinositol 3-kinase-related kinases (PIKKs) family.
ATR is mostly known for its role at the peak of the signalling cascade mediating **DNA damage repair** upon single-strand breaks, however it also exerts many other functions unrelated to DDR. In the nucleus it is involved in meiotic silencing, in the maintenance of telomeres and in the regulation of perinuclear chromatin.
Furthermore, ATR localizes in the cytoplasm. Particularly in neurons, it was shown to participate in the **control of synaptic vesicles density** and **trafficking**, and to play a role in the **maintenance of the correct excitatory/inhibitory** (E/I) **balance.** Moreover, it has been reported that ATR deletion leads to the upregulation of the calcium sensor synaptotagmin2 (SYT2) and PROT (sodium-dependent proline transporter) at excitatory neurons conferring hyperexcitability,
Relevantly, mouse models characterized by reduced expression of ATR protein feature signs of **premature aging**.

Aim

To investigate the impact of **ATR protein** in synapse physiology and its potential role in aging neuropathology.

To do this, hippocampal neurons were treated with the **selective ATR kinase activity inhibitor AZD** and examined by electrophysiological, confocal and calcium imaging studies

Results - 1 -- ATR kinase activity inhibitor AZD enhances the inhibitory tone of mature hippocampal cultured neurons

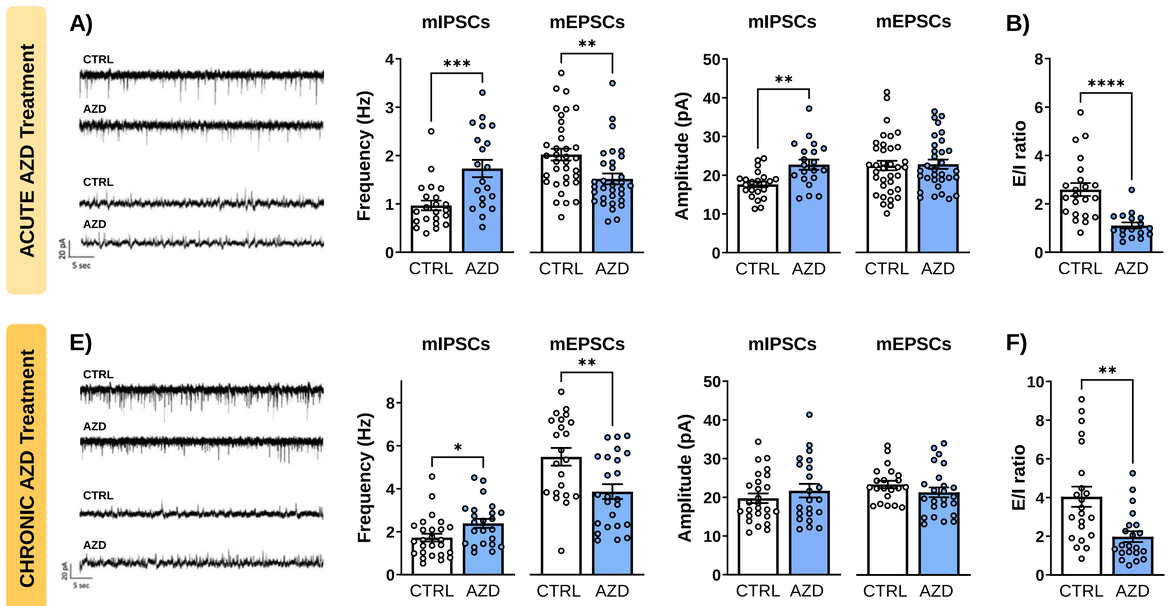

Figure 1. mPSCs electrophysiological recordings and vGAT and vGLUT immunofluorescence stainings of mature hippocampal neurons treated acutely or chronically with AZD

(A) Mature neurons (DIV 14) treated acutely with AZD (at DIV 13) show increased mIPSCs frequency and amplitude and decreased mEPSCs frequency
(C-D) Mature neurons treated acutely with AZD show increased density of vGAT-positive puncta (C) and decrease density of vGLUT-positive puncta (D) per unit length of dendrite (Tubulin-positive filament)
(E) Mature neurons (DIV 21) treated chronically with AZD (at DIV 14 and 17) show increased mIPSCs frequency and decreased mEPSCs frequency
(B-F) E/I ratios indicate an enhanced inhibitory tone in mature neurons treated acutely (B) or chronically (F) with AZD

*(A and E frequencies, B, C, D) Mann-Whitney U test ; (A and E amplitudes) Unpaired t test; *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

Results - 2 -- Chronic AZD impairs LTP in mature hippocampal neurons

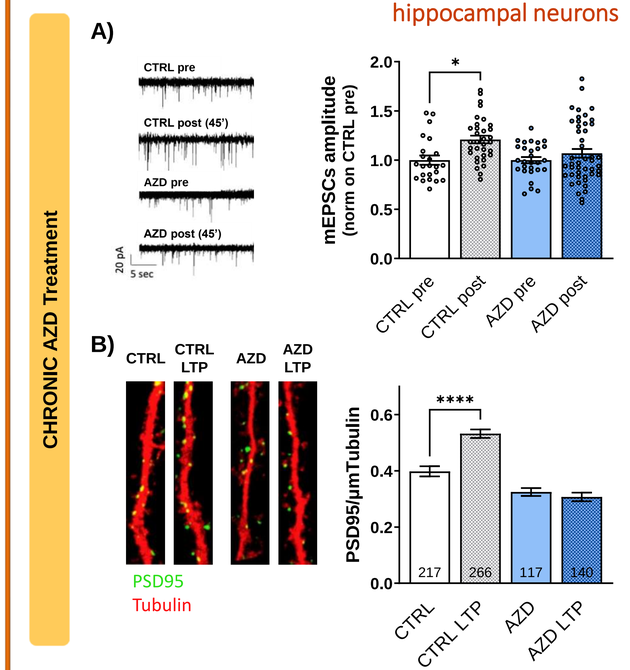

Figure 2. Electrophysiological recordings and PSD95 immunofluorescence stainings of mature hippocampal neurons treated chronically with AZD upon long-term potentiation (LTP)

(A-B) Mature neurons (DIV 17) treated chronically with AZD (at DIV 10 and DIV 13) show no differences in mEPSCs amplitude (A) and in the density of PSD95-positive puncta (green) per unit length of dendrite (β–tubulin-positive filament; red) (B) upon glycine stimulation indicating impaired LTP

*(A and B) Kruskal-Wallis test followed by Dunn's multiple comparisons test; *p<0.05, ****p<0.0001*

Results - 3 -- Chronic AZD reduces Ca^{2+} elevation upon NMDA in mature hippocampal neurons

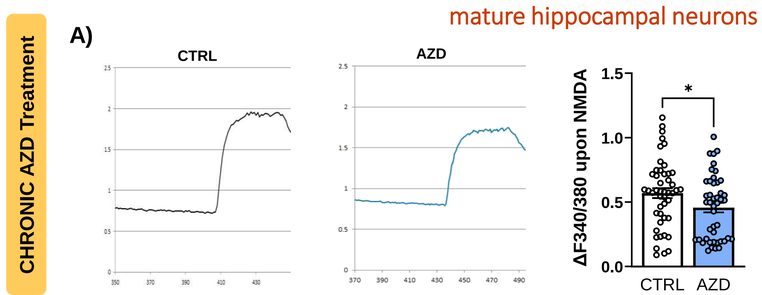

Figure 3. Calcium imaging recordings of mature hippocampal neurons treated chronically with AZD upon NMDA activation

(A) Mature neurons (DIV 16) treated chronically with AZD (at DIV 10 and DIV 13) show reduced Ca^{2+} elevations upon transient exposure to exogenous 100 µM NMDA

*Unpaired t test; *p<0.05*

Results - 4 -- Chronic AZD treatment promotes re-arrangement of AMPA receptors in mature hippocampal neurons

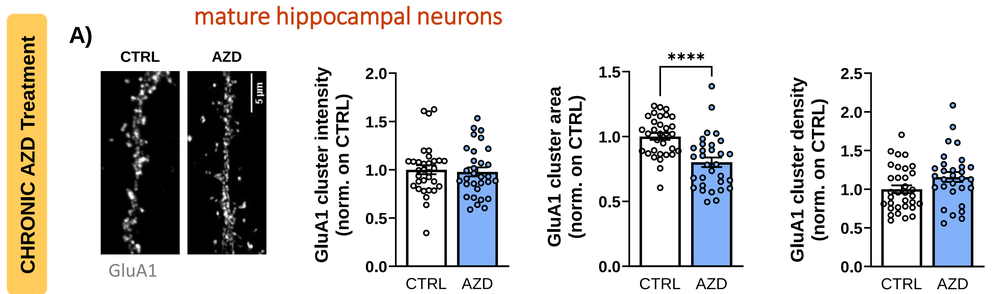

Figure 4. Immunolocalization of surface-expressed GluA1 AMPA receptor subunits in mature hippocampal neurons treated chronically with AZD

(A) Mature neurons (DIV 21) treated chronically with AZD (at DIV 14 and 17) show smaller clusters of surface-expressed GluA1 suggesting a decreased presence of AMPAR in the synapses

*(A, B and C) Unpaired t test; ****p<0.0001*

Conclusions

Our studies indicate **higher inhibition** in WT neurons treated with the ATR kinase activity inhibitor AZD. Also, the specific block of ATR **prevents the induction of long-term potentiation** upon glycine stimulation suggesting impaired NMDA-mediated processes. Indeed, calcium imaging recordings confirmed **reduced calcium elevations** in neurons with impaired ATR kinase activity upon transient exposure to exogenous NMDA.
Our data indicate that **ATR activity is essential to synapse functionality** and that alterations in its activation may affect neuronal health beyond its expected responses to DNA damages and oxidative stress.

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