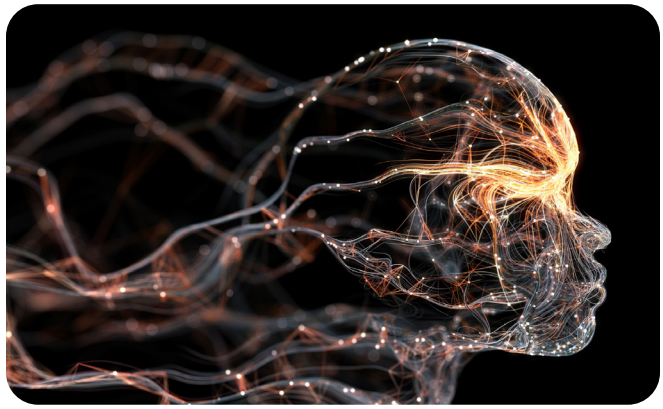
The modulatory effects of $A_{2A}R$ on NMDAR functionality using primary neurons and microglia cultures for potential neuroprotection in Alzheimer's disease

In the field of neurodegenerative disease therapies, there is continued interest in exploring not only the basic mechanism of how the drug is eliciting a protective effect against symptoms, but also synergistic cellular pathways that may have an additive effect in increasing the effectiveness of existing therapies or be applicable to other neurodegenerative diseases that may share similar symptoms. For those impacted by Alzheimer's Disease (AD), two main types of drugs exist on the market that either modulate the N-methyl D-aspartate ionotropic glutamate receptor (NMDAR) or are Acetylcholinesterase inhibitors (AchEIs). These drugs that act on NMDARs are purposefully designed to be weak negative allosteric modulators to elicit sufficient response without becoming toxic. At this time, all available therapies currently only delay progression of symptoms and are not disease modifying.

Adenosine is a purine nucleoside that functions as an autacoid that affects both metabolic and regulatory pathways in many tissues and cells, including the brain where it functions as one of the main neuromodulators acting on GPCRs. In particular, the A_{2A} receptor ($A_{2A}R$) is heavily expressed in the areas that control motor skills as well as other regions of the CNS. In fact, an antagonist of this receptor recently received approval for use in Parkinson's disease for not only combating motor symptoms but showed potential as a neuroprotective factor as well in *in vitro* and *in vivo* studies. The mechanism is still being explored though others have that $A_{2A}R$ is upregulated in activated microglia. Interestingly, the field has also explored the therapeutic application of $A_{2A}R$ antagonists outside of



Parkinson's, namely Alzheimer's, which is another common neurodegenerative disease that can present itself with similar symptoms like dementia. Many have shown that indeed, blocking the $A_{2A}R$ has shown neuroprotection in various research models, including taupathy mouse models and transgenic AD models such as APPswe/PS1dE9 and triple 3xTg-AD mice.

Dr. Franco and Dr. Navarro recently investigated the potential regulation of NMDAR function via $A_{2A}R$ pathway activation in both neurons and microglial cells to see if $A_{2A}R$ antagonists may be modulating NMDAR to offer the neuroprotection similar to that from NMDA blockers. Using fusion protein colocalization and BRET studies, they suggest a possible direct interaction between the NMDA and A_{2A} receptors (Figure 1).

revvity

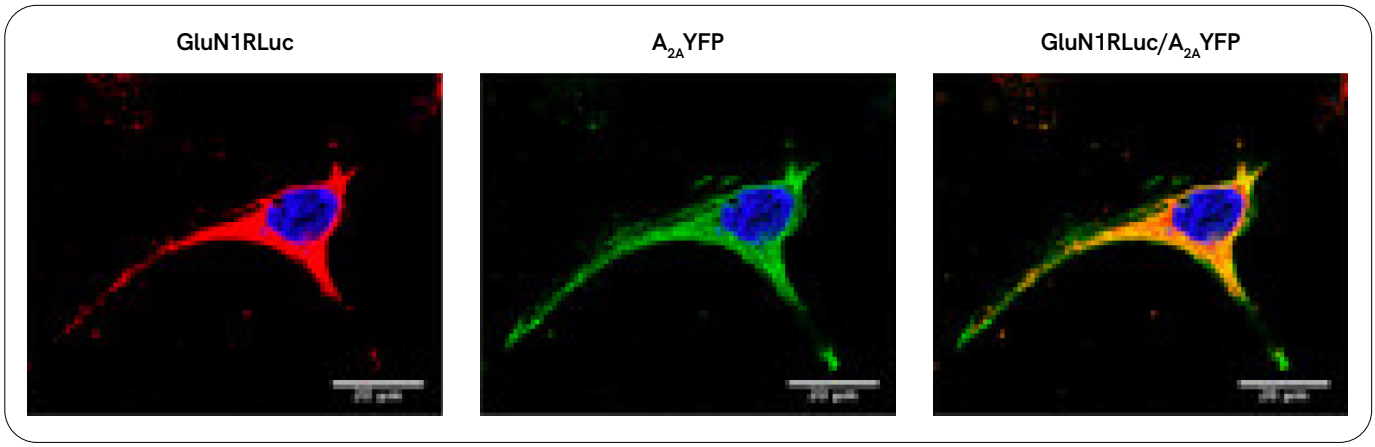


Figure 1: Exploring the interaction of $A_{2A}R$ and NMDAR using confocal microscopy. Additional method details can be found in the method and figure legend of https://doi.org/10.3390/cells9051075.

They further characterized the functional properties of the A_{2A}-NMDA receptor relationship, including cAMP levels and ERK 1/2 phosphorylation. They used $A_{2A}R$ selective ligands and antagonists to stimulate or block the corresponding receptors, and saw that receptor-mediated signaling was cross-antagonistic, where cAMP and ERK 1/2 phosphorylation increased as expected upon agonist treatment of either $A_{2A}R$ but this increase was blocked by both $A_{2A}R$ and NMDAR antagonists (Figure 2). Interestingly, this phenomenon of cross-antagonism was also observed in both resting and active microglia cells challenged with LPS and IFN-γ in primary cultures from mice.

Moreover, they saw that upon microglia activation using LPS and IFN-γ, the $A_{2A}R$-NMDAR complex expression increased by an eight-fold increase in dots/cell with more than 92% of the cells showing red dots versus the 23% of cells in resting state using the *in situ* PLA assay, indicating a potential role in activated microglia cells. They also observed marked increase in expression of the $A_{2A}R$ in the microglia from the hippocampus of $APP^{Sw,Ind}$ transgenic mice as compared to WT, indicating AD-mice may have more endogenous activated microglial cells compared to control animals. Additionally, increases in inducible nitric oxide synthase (iNOS), a marker of the M1 phenotype, and arginase-1 (Arg-1), a M2 phenotype marker in microglia in these primary cells from AD-mice were observed. When pre-treated with an $A_{2A}R$ antagonist, they saw that these same $APP^{Sw,Ind}$ -derived cells saw a significant decrease in iNOS with a significant but small increase in Arg-1, which indicated the potential effect of the $A_{2A}R$ antagonist in inducing the more neuroprotective M2 phenotype.

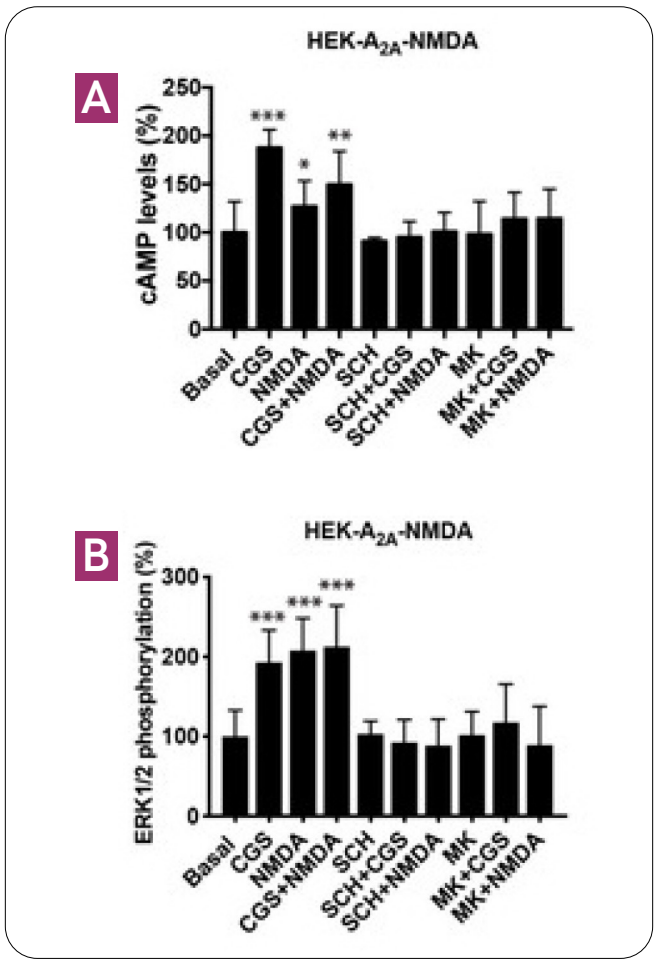


Figure 2: Assessing functional properties of $A_{2A}R$ and NMDAR, including (A) cAMP and (B) ERK 1/2 phosphorylation upon $A_{2A}R$ activation and either $A_{2A}R$ or NMDAR antagonist treatment. Additional method details can be found in the method and figure legend of https://doi.org/10.3390/cells9051075.

The lab's research provides evidence of the neuromodulatory potential of $A_{2A}R$ antagonists on NMDAR function, which is the target of several current AD therapies on market. Their work also explores the cross-antagonism and potential synergistic neuroprotection it may offer, particularly as it pertains to microglia activation. Taken together with the growing body of work in this area, the findings point to Adenosine A1 and A_{2A} receptors as potential targets for particularly dementia-related neurodegenerative diseases, not only Parkinson's but also Alzheimer's. It also suggests a potential application of a creative approach in countering the overactivity of NMDAR that in part, is thought to be responsible for accelerated neuronal death in AD where NMDAR itself is unable to be full blocked due to its requirement for neural cell viability.

Reference

1. Franco, R., Rivas-Santisteban, R., Casanovas, M., Lillo, A., Saura, C. A., & Navarro, G. (2020). Adenosine A2A Receptor Antagonists Affects NMDA Glutamate Receptor Function. Potential to Address Neurodegeneration in Alzheimer's Disease. Cells, 9(5), 1075. https://doi.org/10.3390/cells9051075 (and references included therein).

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com