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## Glutamate partner drives gamma-glutamyl-dipeptide effects on ionotropic glutamate receptors

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Valérie Rolland, Sandro Andreu, Victoria Gizzi, Mete Kayatekin, Fatiha Sebih, et al.. Glutamate partner drives gamma-glutamyl-dipeptide effects on ionotropic glutamate receptors. Quand Rabelais rencontre Balard, May 2024, Montpellier, France. hal-04740425

**HAL Id: hal-04740425**

**<https://hal.science/hal-04740425v1>**

Submitted on 16 Oct 2024

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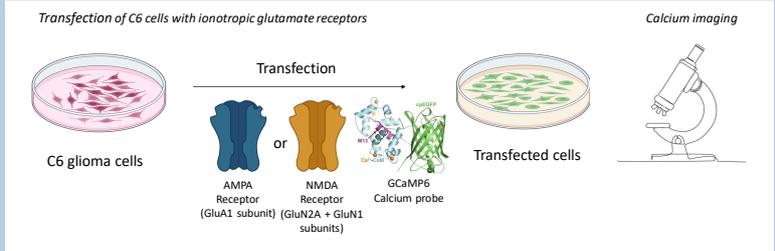
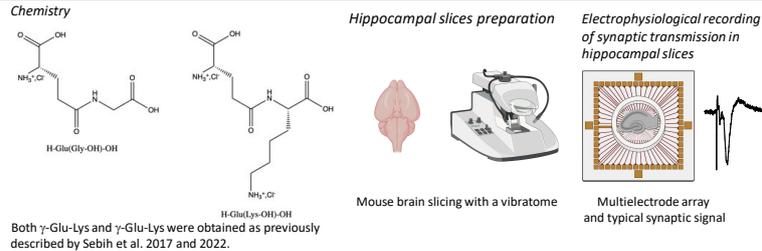
# QUAND RABELAIS RENCONTRE BALARD

Mardi 28 mai 2024 | 9h00 - 17h15

## Introduction

Gamma-glutamyl dipeptides are yielded by the condensation of glutamate with another amino acid on the  $\gamma$  position. Their synthesis results from the hydrolysis of glutathione (GSH ;  $\gamma$ -Glu-Cys-Gly) by  $\gamma$ -glutamyl transferase (GGT). In fact, while  $\gamma$ -glutamyl dipeptides have been detected in different organs and fluids of the body, especially in the brain, their biological activity remains elusive. Nevertheless, binding studies have revealed that they could bind glutamate ionotropic receptors, i.e. AMPA, kainate and NMDA subtypes. In the present work, we have examined the activity of two of them,  $\gamma$ -Glu-Gly and  $\gamma$ -Glu-Lys, which exhibit high affinity for ionotropic glutamate receptors. They were synthesized as previously described (Sebih et al., *Frontiers in Pharmacology*, 2022) and LC-MS analyses indicated that both dipeptides production by atrocytic C6 cell line is tightly linked to the integrity of GSH metabolism. They were further tested on both AMPA and NMDA receptors-expressing cells and acute hippocampal slices.  $\gamma$ -Glu-Gly displayed both co-agonist activity on NMDA receptor Glycine site and partial agonism on AMPA receptors. Interestingly,  $\gamma$ -Glu-Gly potentiated excitatory synaptic transmission.  $\gamma$ -Glu-Lys elicited antagonist action on AMPA receptors which resulted in short- and long-lasting synaptic depression. However,  $\gamma$ -Glu-Lys had no effect on NMDA receptors. Therefore, the amino acid associated to glutamate in  $\gamma$ -glutamyl dipeptides governs their selectivity towards ionotropic glutamate receptors. The accumulation of  $\gamma$ -glutamyl dipeptides within the brain under pathological conditions associated to oxidative stress and high GSH consumption could thus contribute to excitatory synaptic transmission and plasticity disruption which is a hallmark of neurodegenerative diseases.

## Methods



## Results

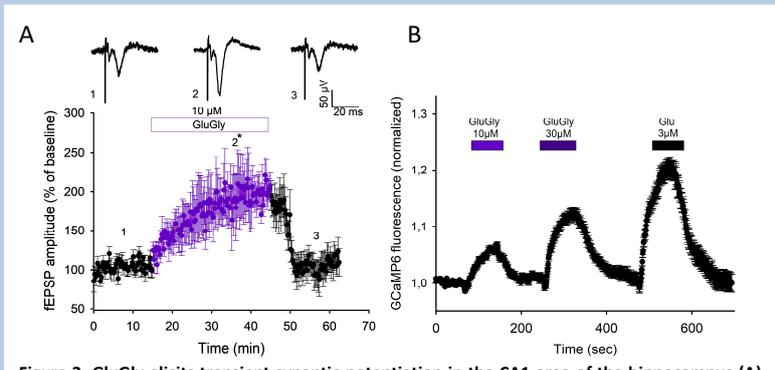
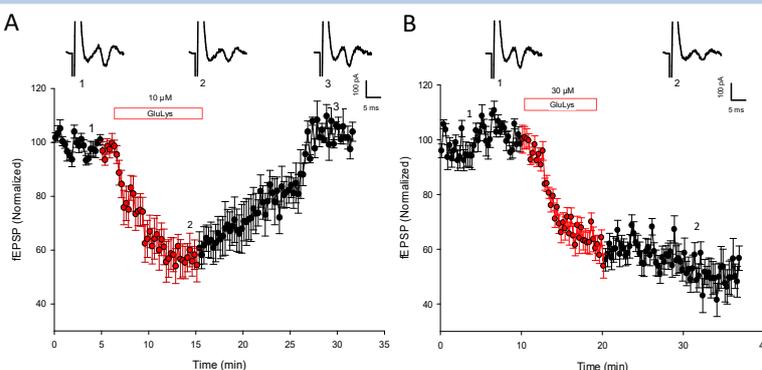


Figure 1. GluLys transiently depresses (A) excitatory synaptic transmission and induces long-term depression (B) in the CA1 area of the hippocampus according to the concentration tested.

Figure 3. GluGly elicits transient synaptic potentiation in the CA1 area of the hippocampus (A) and exhibits modest agonist action on AMPA receptors (B).

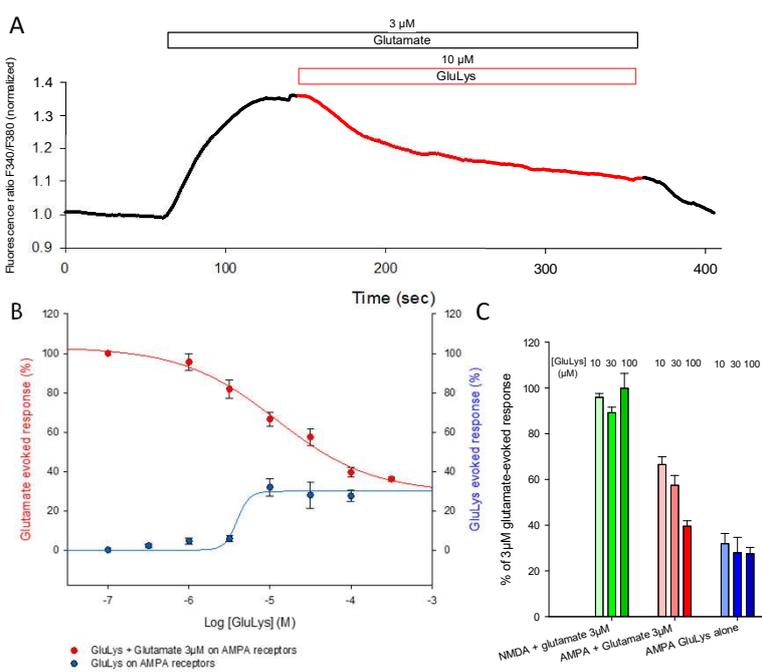


Figure 2. GluLys both antagonizes AMPA receptors-mediated responses and partially activates AMPA receptors  
 A, Illustrative example of the blockade of the response elicited by 3  $\mu$ M glutamate by GluLys in GluA1-expressing cells. B, Concentration dependent curve depicting GluLys antagonistic action on Glu-induced response (red) and per se effect (blue). C, Recapitulative graph plotting the effect of increasing concentrations GluLys on either NMDA or AMPA receptors-expressing cells in the presence or not of glutamate (3  $\mu$ M).

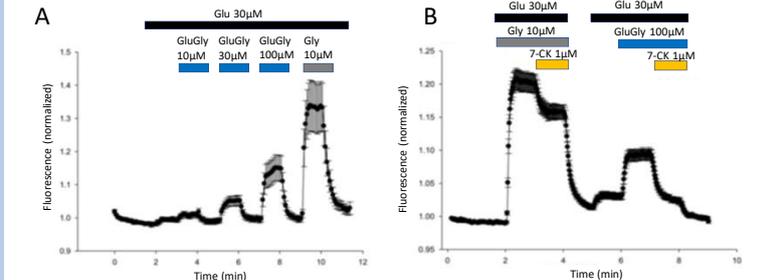


Figure 4. GluGly elicits co-agonist action on NMDA receptors (A) via binding glycine site (B) on these receptors. 7CK-7-Chloro-kynurenate is an NMDA receptors antagonist selective for the glycine binding site.

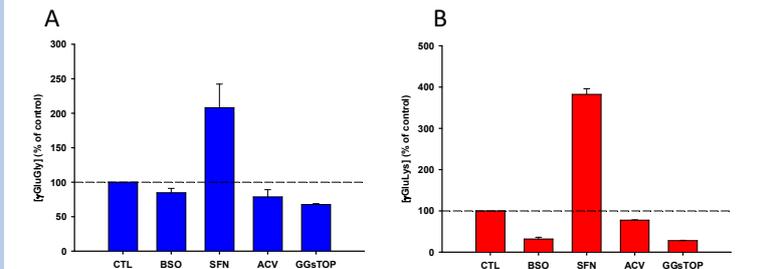


Figure 5. Both GluGly (A) and GlyLys (B) productions by C6 cells are tightly linked to GSH metabolic cycle. BSO (buthionine sulfoximine, 10  $\mu$ M) is a GSH synthesis blocker; ACV (acivicin, 100  $\mu$ M) and GGeTioP (20  $\mu$ M) are  $\gamma$ -glutamyl transferase inhibitors; SFN (sulforaphane, 1  $\mu$ M) is GSH synthesis booster via genomic action involving the activation of the Nrf2 transduction pathway. These modulators were applied for 24h in the culture medium of C6 cells. GluGly and GluLys concentrations were further determined by LC-MS (Sebih et al. 2022)

## References

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