

Modulation of NMDA and alpha7 nicotinic ACh receptor by tryptophan metabolites



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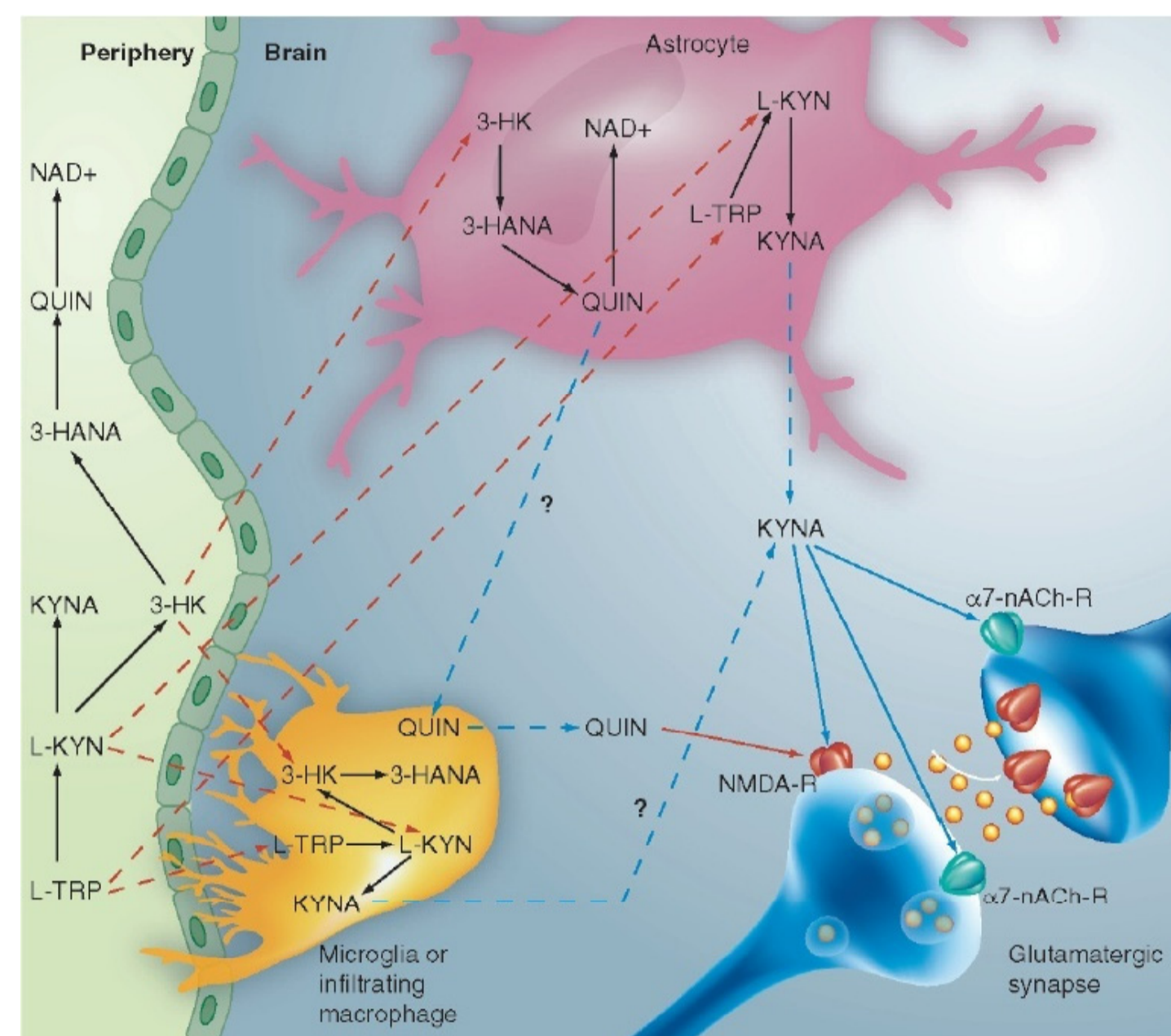
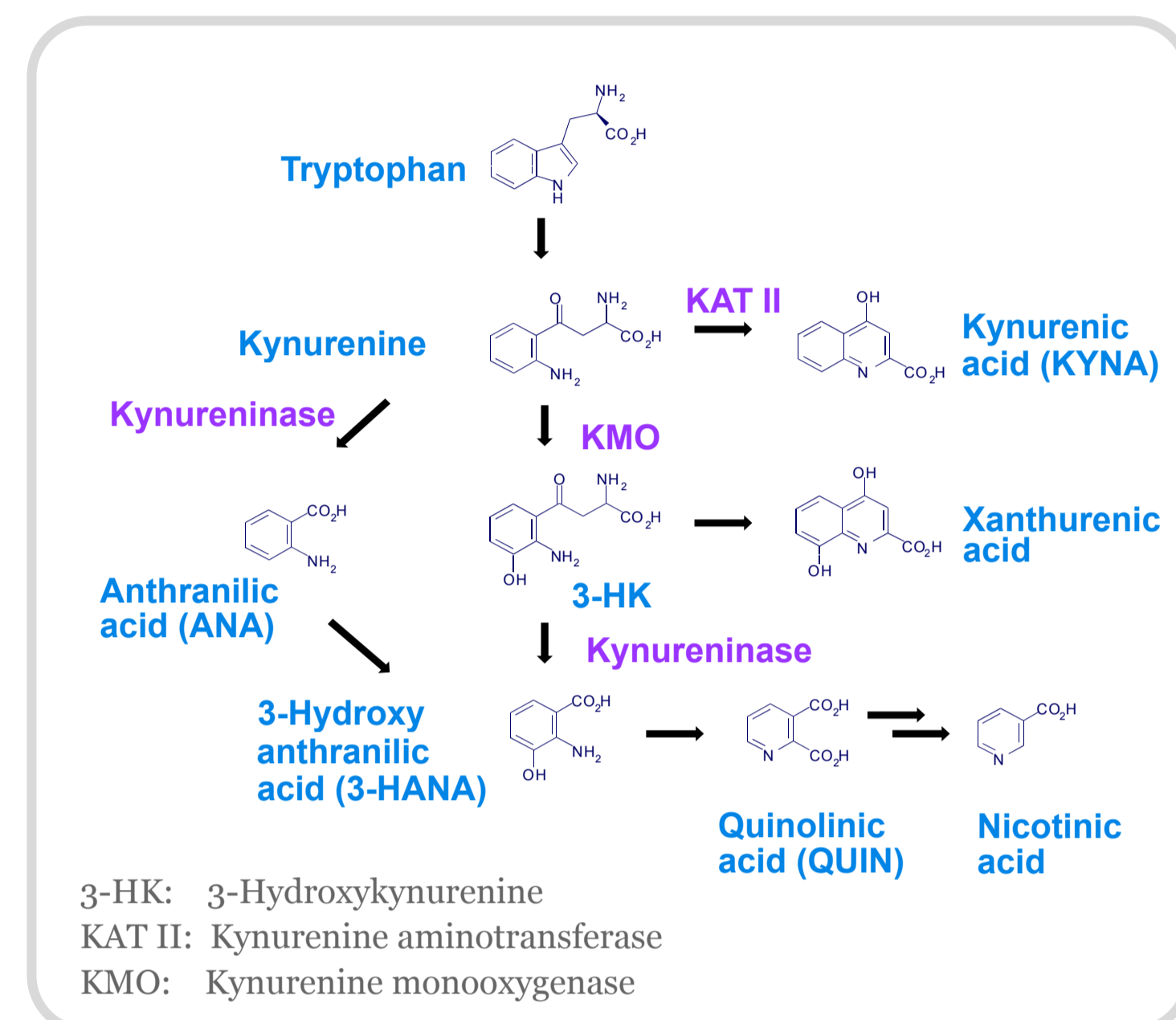
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Introduction

Huntington's disease (HD) is an inherited neurodegenerative disease with 1:10,000 incidence characterised by degeneration of the striatal neurons. Early stage HD patients and transgenic mouse models show increased levels of kynurenines (products of the tryptophan degradation pathway) in striatum and cortex and it is believed that neuroactive potential of these metabolites can account for HD neurodegeneration by directly modulating ligand-gated ion channels such as NMDA, AMPA and alpha 7 nicotinic receptors. As a result, there is a strong interest in targeting the enzymes involved in this pathway (such as KMO and KAT) as a way to manipulate the concentration of different neuroactive kynurenines. Nevertheless a detailed quantification of the activity of all tryptophan metabolites at the receptor level is missing, which renders an incomplete picture of the neuromodulatory potential of the intermediates and the enzymes involved in this pathway.

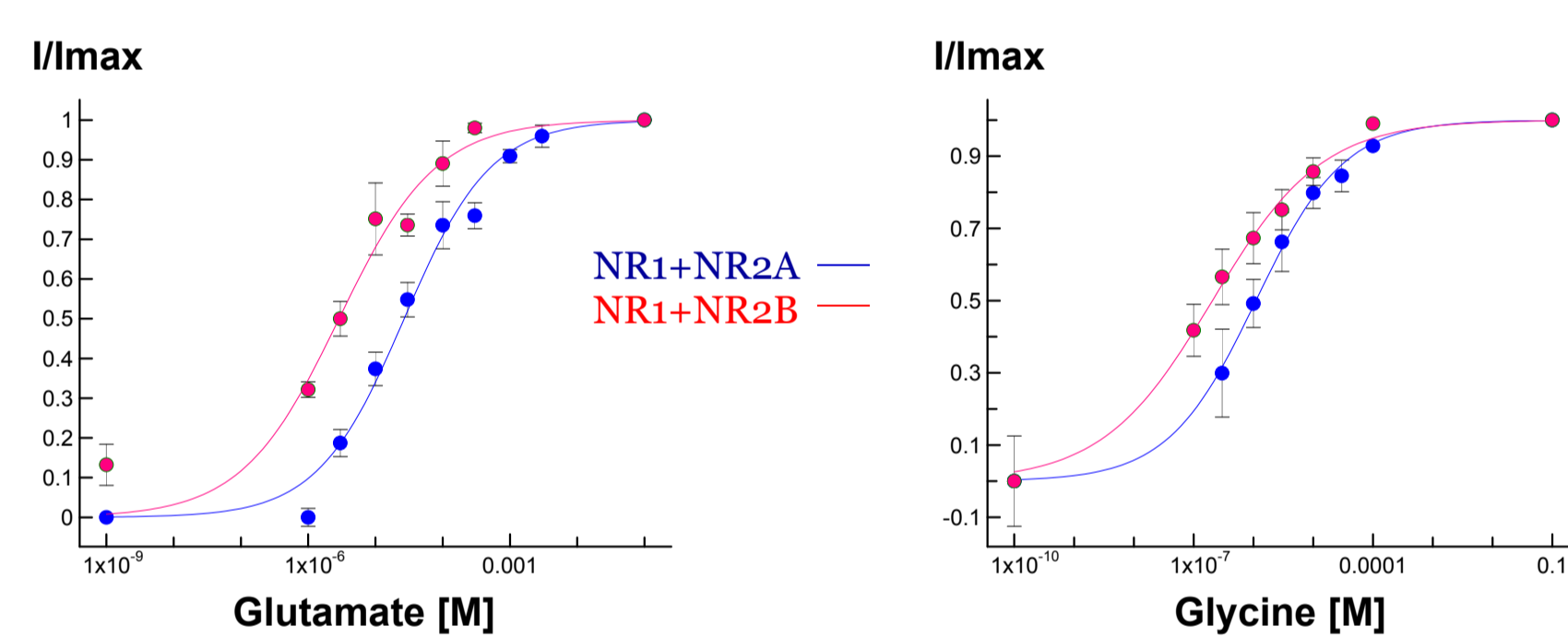
In this work we have investigated the potential of each metabolite of the kynurenine pathway to modulate NMDA and alpha 7 nicotinic receptors (nAChR) expressed in HEK cells using whole-cell patch-clamping and a fast-exchange perfusion system (Dynaflow).

Kynurenine pathway in the brain



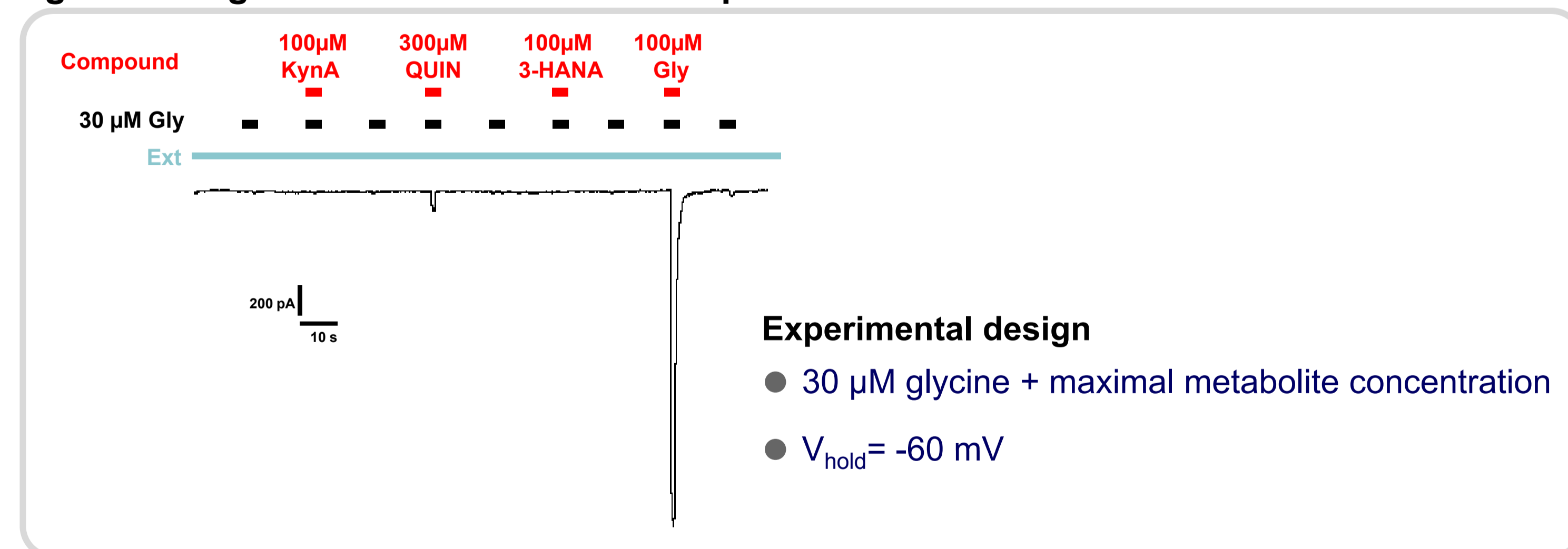
NMDA receptor activation

NR1 and NR2A/NR2B subunits of NMDA receptor were expressed in HEK cells and currents of 0.2-2nA with typical kinetics were elicited by co-application of both agonists: Glutamate (Glu) and glycine (Gly).



	NR1+NR2A EC ₅₀ (μM)	NR1+NR2B EC ₅₀ (μM)	Experimental Design
Glutamate	24 ± 4	9 ± 2	100 μM Gly
Glycine	1.2 ± 0.3	0.19 ± 0.02	100 μM Glu

Agonism at glutamate site of NMDA receptors



	NR1+NR2A (n = 4)		NR1+NR2B (n = 3)	
	Elicited current* (%)	Wash-out (%)	Elicited current (%)	Wash-out (%)
L-Kyn (300μM)	0 (no response)	0 (no response)	0	0
3-HK (100μM)	0 (no response)	0 (no response)	0	0
ANA (300μM)	0 (no response)	0 (no response)	0	0
3-HANA (300μM)	0 ± 0**	0 ± 0**	0 ± 1	-1 ± 1
QUIN (300μM)	1 ± 1**	0 ± 0**	5 ± 1	0 ± 1
KynA (100μM)	1 ± 0**	0 ± 0**	1 ± 1	0 ± 0

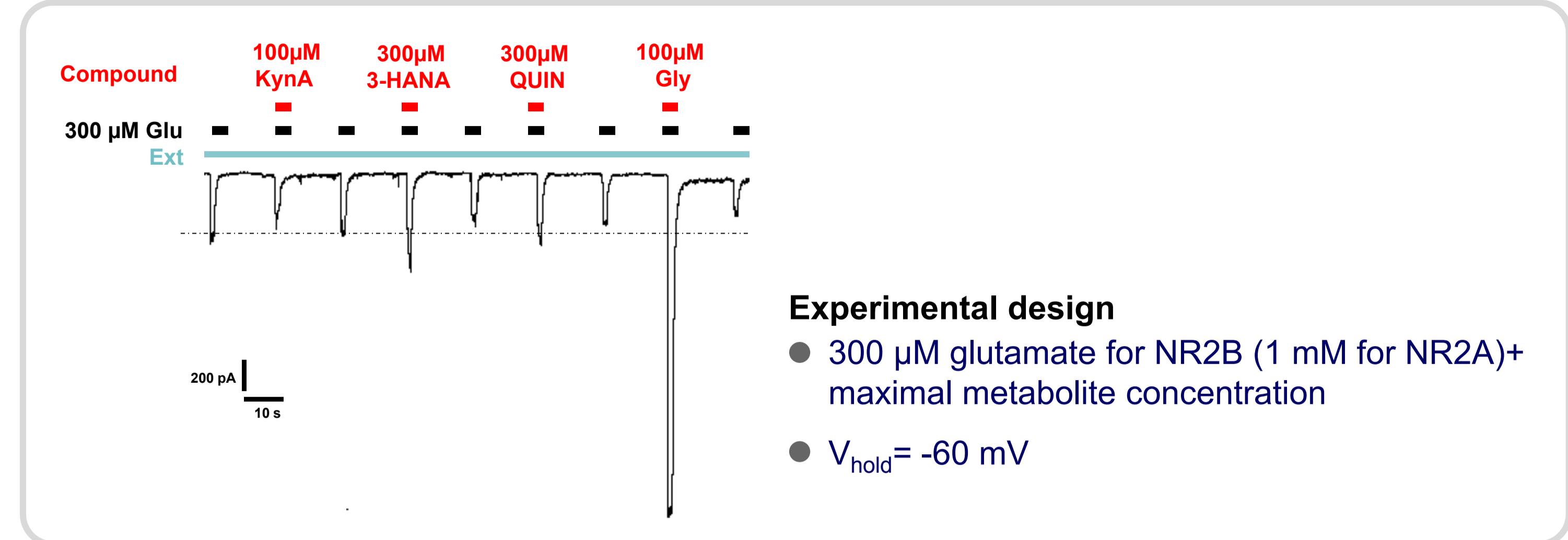
*Responses were normalised to amplitudes of the currents elicited by 100 μM Glu + 30 μM Gly (100%)

**In 50% of cells, Gly alone elicited a very small amplitude current (<5%)

Summary

- Kynurenic acid is the only active metabolite of the tryptophan degradation pathway that can interact directly with NMDA receptor as antagonist (in μM range)
- Kynurenic acid has higher affinity for NR2A than for NR2B receptors
- None of the tested tryptophan metabolites were active on alpha7 nicotinic receptors, suggesting that their effects on neuronal excitability are not mediated by direct interaction with these receptors

Agonism at glycine site of NMDA receptors



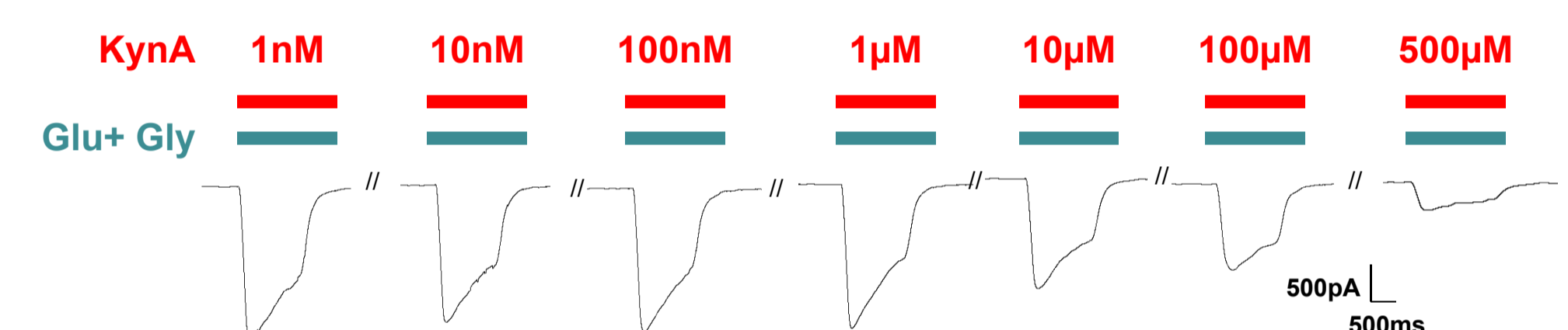
	NR1+NR2A (n = 3-5 cells)		NR1+NR2B (n = 5-8 cells)	
	Elicited current* (%)	Wash-out (%)	Elicited current (%)	Wash-out (%)
L-Kyn (300μM)	0 (no response)	0 (no response)	7 ± 4**	5 ± 3
3-HK (100μM)	0 (no response)	0 (no response)	3 ± 2	3 ± 3
ANA (300μM)	0 (no response)	0 (no response)	9 ± 4	-2 ± 2
3-HANA (300μM)	1.6 ± 0.5	2.0 ± 0.1	8 ± 2	0 ± 2
QUIN (300μM)	1.6 ± 0.8	1.1 ± 0.6	0 ± 0	0 ± 0
KynA (100μM)	2.7 ± 1.8	1.2 ± 0.7	0 ± 1	0 ± 1

*Responses were normalised to current amplitudes at 300 μM (1 mM) Glu + 100 μM Gly (100%)

**In 50% of cells, glutamate elicited current response in absence of glycine. Its amplitude is subtracted from the signal obtained with compound

Antagonism at NMDA receptors (both glycine and glutamate sites)

- Paradigm: 100 μM glutamate (EC₉₀) + 10 μM (EC₉₀) glycine + maximal metabolite concentration
- Kynurenic acid is the only kynurenine active at both NR2A and NR2B with different potencies

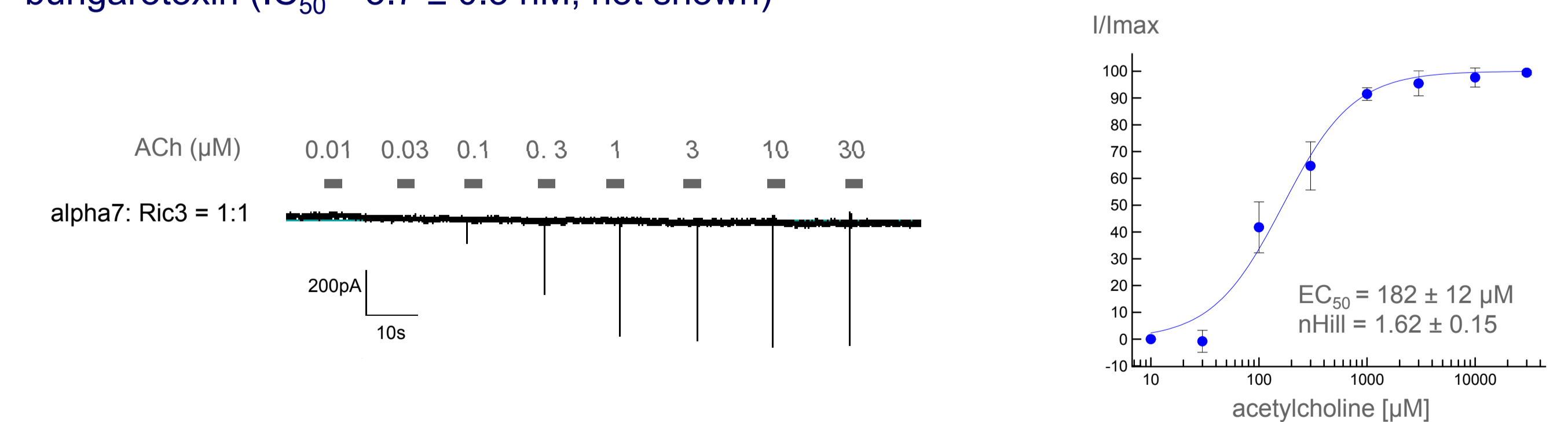


	NR1+NR2A (n = 4-5 cells)		NR1+NR2B (n = 3-4 cells)	
	Remaining current * (%)	Wash-out (%)	Remaining current * (%)	Wash-out (%)
L-Kyn (300μM)	74 ± 11	100 ± 23	93 ± 5	97 ± 5
3-HK (100μM)	83 ± 14	104 ± 19	109 ± 3	111 ± 5
ANA (300μM)	77 ± 12	80 ± 6	98 ± 13	95 ± 4
3-HANA (300μM)	93 ± 4	114 ± 18	105 ± 21	89 ± 2
QUIN (300μM)	92 ± 8	100 ± 2	93 ± 6	109 ± 22
KynA	IC ₅₀ = 25 ± 12 μM	90 ± 1%	IC ₅₀ = 489 ± 78 μM	103 %

*Responses were normalised to current amplitudes at 100 μM Glu + 10 μM Gly (100%)

Alpha7 nicotinic receptor (nAChR) functional expression

Functional alpha7 nAChR expression in HEK cells has been achieved by co-transfection with the chaperone ric-3, which is critical for trafficking to the surface. Short, fast-inactivating current responses of 0.5-1 nA following acetylcholine application (EC₅₀ = 182 ± 12 μM) were specifically blocked by alpha-bungarotoxin (IC₅₀ = 3.7 ± 0.3 nM, not shown)



Antagonism at alpha7 nAChR

- Paradigm: 1 mM Ach (EC₉₀) + maximal metabolite concentration
- None of the tested tryptophan metabolites is active at alpha7 nAChR

	Elicited current* (%)	Wash-out (%)
KynA (100μM)	94 ± 8	92 ± 7
QUIN (300μM)	93 ± 3	87 ± 8
3-HANA (300μM)	98 ± 5	101 ± 6
ANA (300μM)	90 ± 7	88 ± 7
3-HK (100μM)	78 ± 7	75 ± 10
L-Kyn (300μM)	73 ± 5	87 ± 11

*Responses were normalised to amplitudes of current at 1 mM Ach (100%); Current rundown accounts for 20% reduction