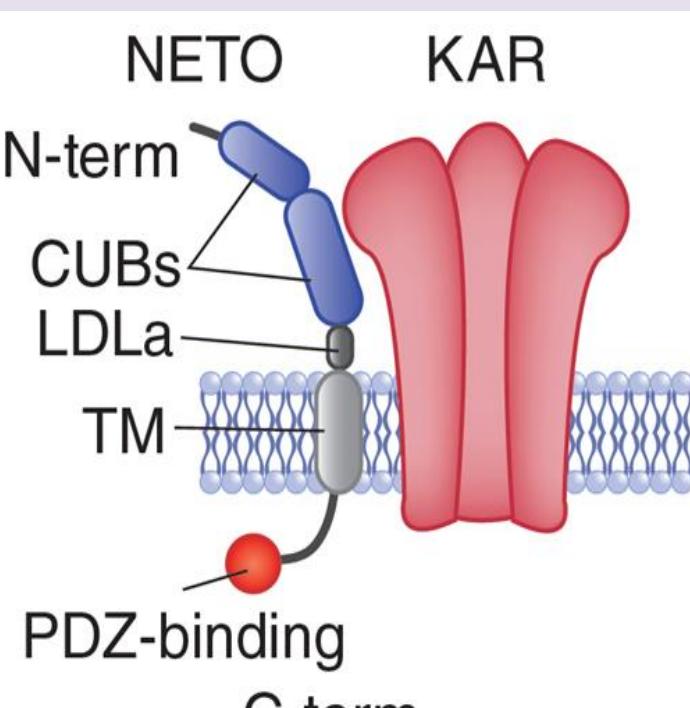


# Functional Impact of Auxiliary Proteins on Kainate receptors

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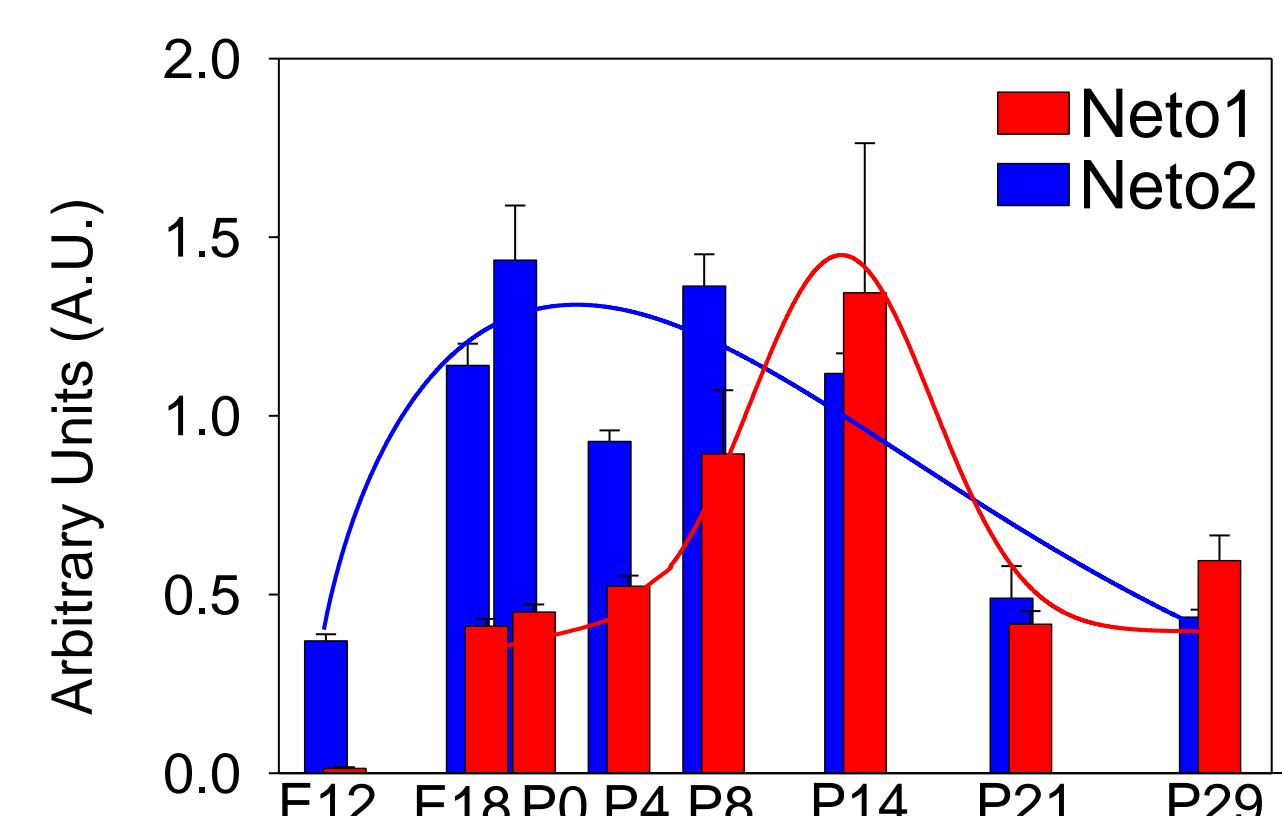
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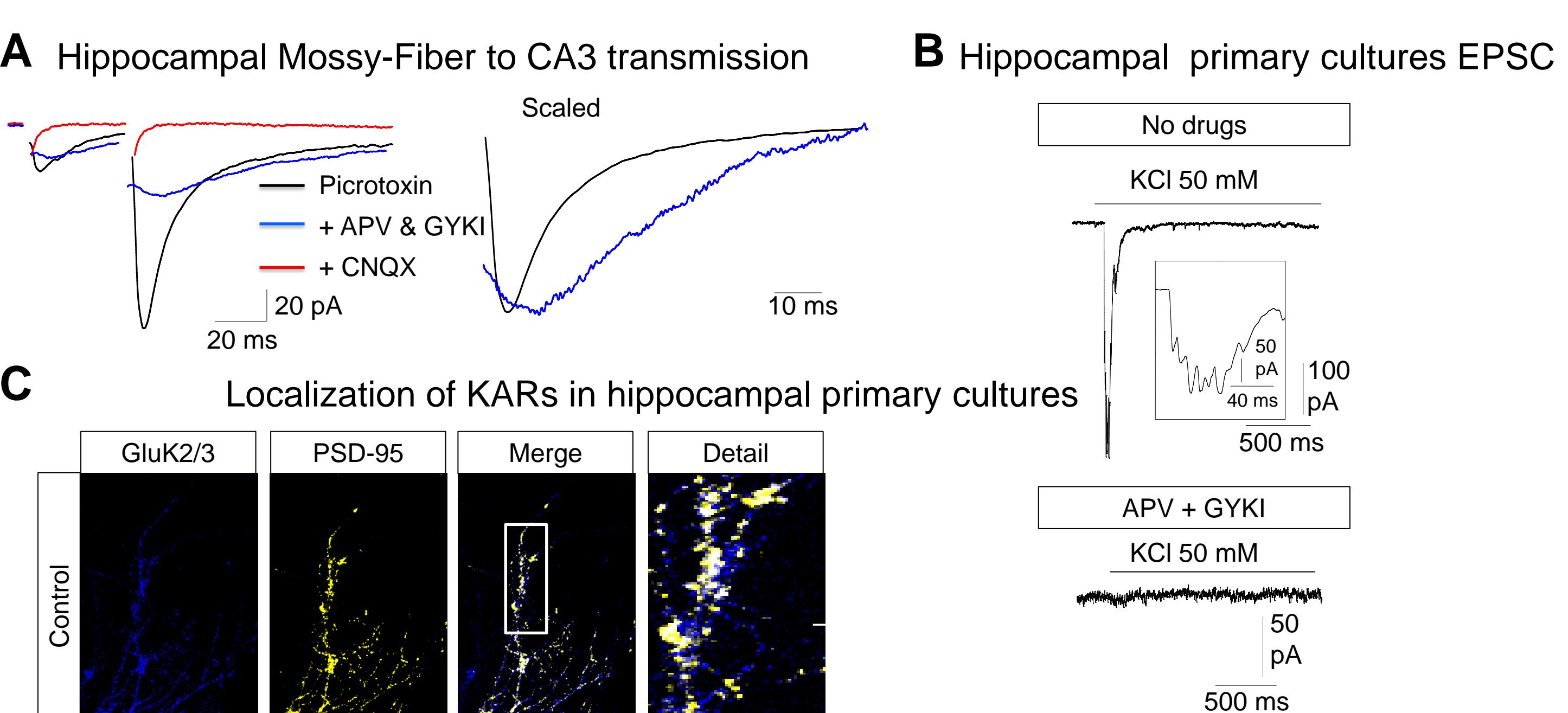
The existence of auxiliary proteins for Kainate receptors (KARs) has emerged as an important mechanism to explain receptor physiological properties. Indeed, there is a mismatch in their functional properties when native and recombinantly expressed receptors are compared. Recently, two auxiliary proteins for KARs, Neto1 and Neto2 (NEUropilin TOLloid-like 1 and 2) have been identified. GluK5, Neto1 and Neto2 are not expressed at hippocampal cultures while they are present at mature hippocampal neuron *in vivo*. We verify that cultured mature hippocampal neurons exhibit KARs mediated currents but not EPSC<sub>KAR</sub>. We transfected GluK5, Neto1 and Neto2 and forced synaptic activity by stimulating neurons with KCl. We showed that individually all the subunits confer synaptic responses mediated by KARs. We further verified the spontaneous activity of cultured hippocampal neurons and confirmed no synaptic KARs. In addition, expression of GluK5, Neto1 or Neto2 on cultured hippocampal neurons showed sporadic spontaneous activity. Unexpectedly, neurons expressing GluK5 subunit reveal more frequent spontaneous activity than Neto1 or Neto2 does, while these last subunits provide KARs synaptic responses with larger onset and decay kinetics. Altogether, our data demonstrate that different subunit of KARs set out singular functions, giving a preferential role to GluK5 subunit targeting KARs to the synapses and Neto1 or Neto2 setting the slow kinetics of these receptors.

Figure 1. Neto1 and Neto2 gene expression during development



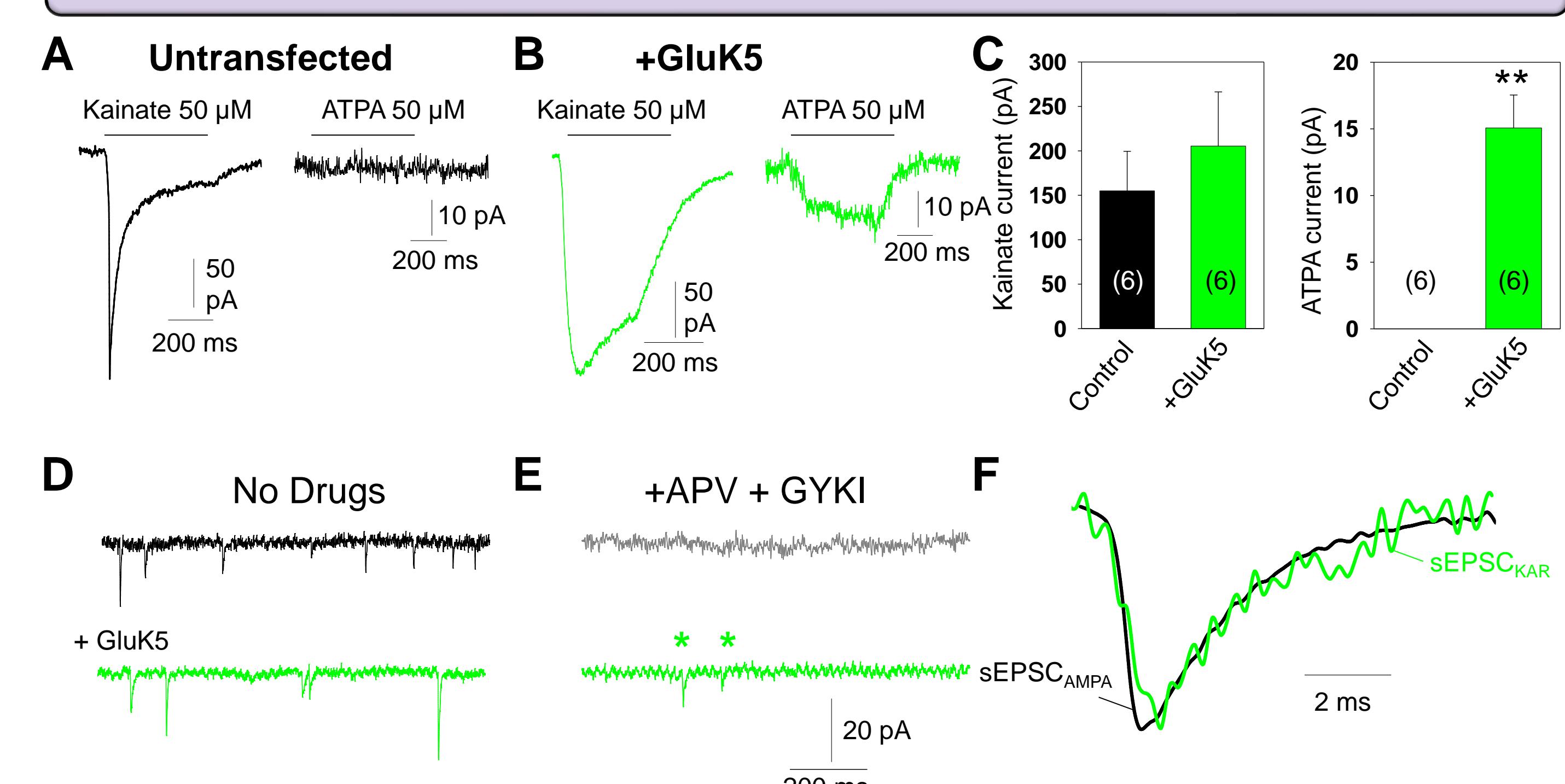
**Neto1 and Neto2 gene expression levels during development in mice brain.** Quantitative PCR analysis of Neto1 and Neto2 mRNA at different developmental stages of mouse brain. Curves were fitted (line plots) to bar values using a log normal peak equation. The analysis shows a peak of Neto2 mRNA during early development compared with Neto1. After P14 both proteins present similar levels of expression. Data are mean + SEM.

Figure 3. Synaptic Kainate Receptors at hippocampal models



**Differences in the “*in vivo*” and “*in vitro*” situation.** A, hippocampal Mossy-Fiber to CA3 AMPAin shown, where EPSC<sub>KAR</sub> are found. It is also shown a magnification of the second pulse scaled, where the slow kinetics of KARs are seen. In B, ionotropic synaptic activity of hippocampal primary culture is presented where KARs do not appear at postsynaptic sites. In C, DIV 15 hippocampal neurons, immunolocalization of GluK2/3 (blue) and PSD-95 (yellow) colocalized in the ~40% of immunolabeled puncta.

Figure 5. GluK5 KARs subunits allow fast synaptic responses



**Effect of transfecting GluK5 into hippocampal primary cultures.** DIV 15 neurons where used to check Kainate 50  $\mu$ M and ATPA 50  $\mu$ M evoked currents of control and GluK5 KARs subunit transfected conditions (A and B). In C, quantification of panel A and B. Afterwards, in D, spontaneous activity of both conditions was tested under inhibitory neurotransmission was block (50  $\mu$ M Picrotoxine). In E, spontaneous activity was observed after NMDA and AMPA receptors were blocked (APV 50  $\mu$ M and GYKI 50  $\mu$ M). In F, magnification of scaled spontaneous activity of picrotoxin blocked (black trace) and APV-GYKI blocked (green trace) of GluK5 transfected condition. Data are mean + SEM. \*p<0.01

Figure 7. Comparison of sEPSC<sub>KAR</sub> in cells expressing GluK5 and Neto proteins

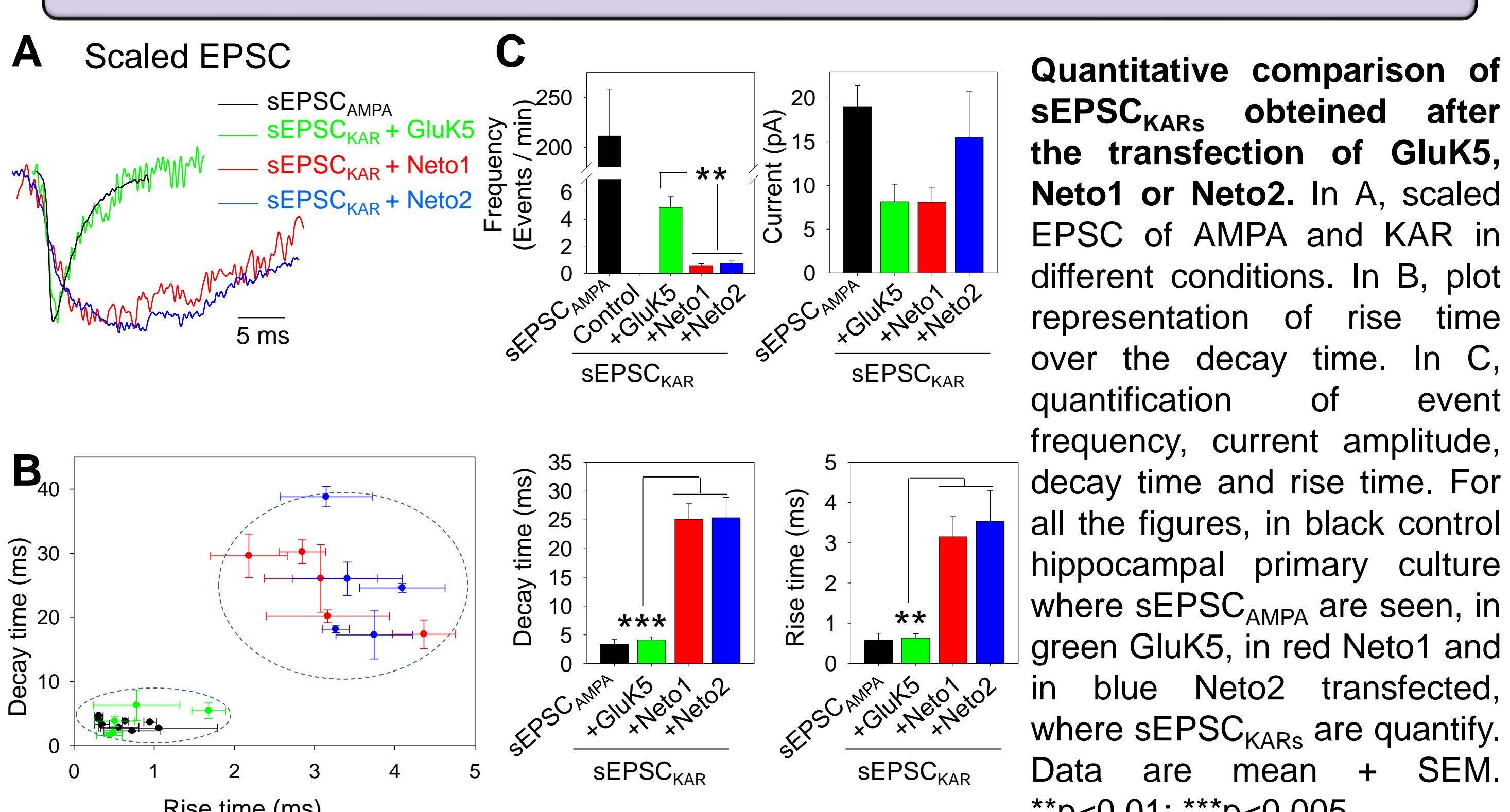
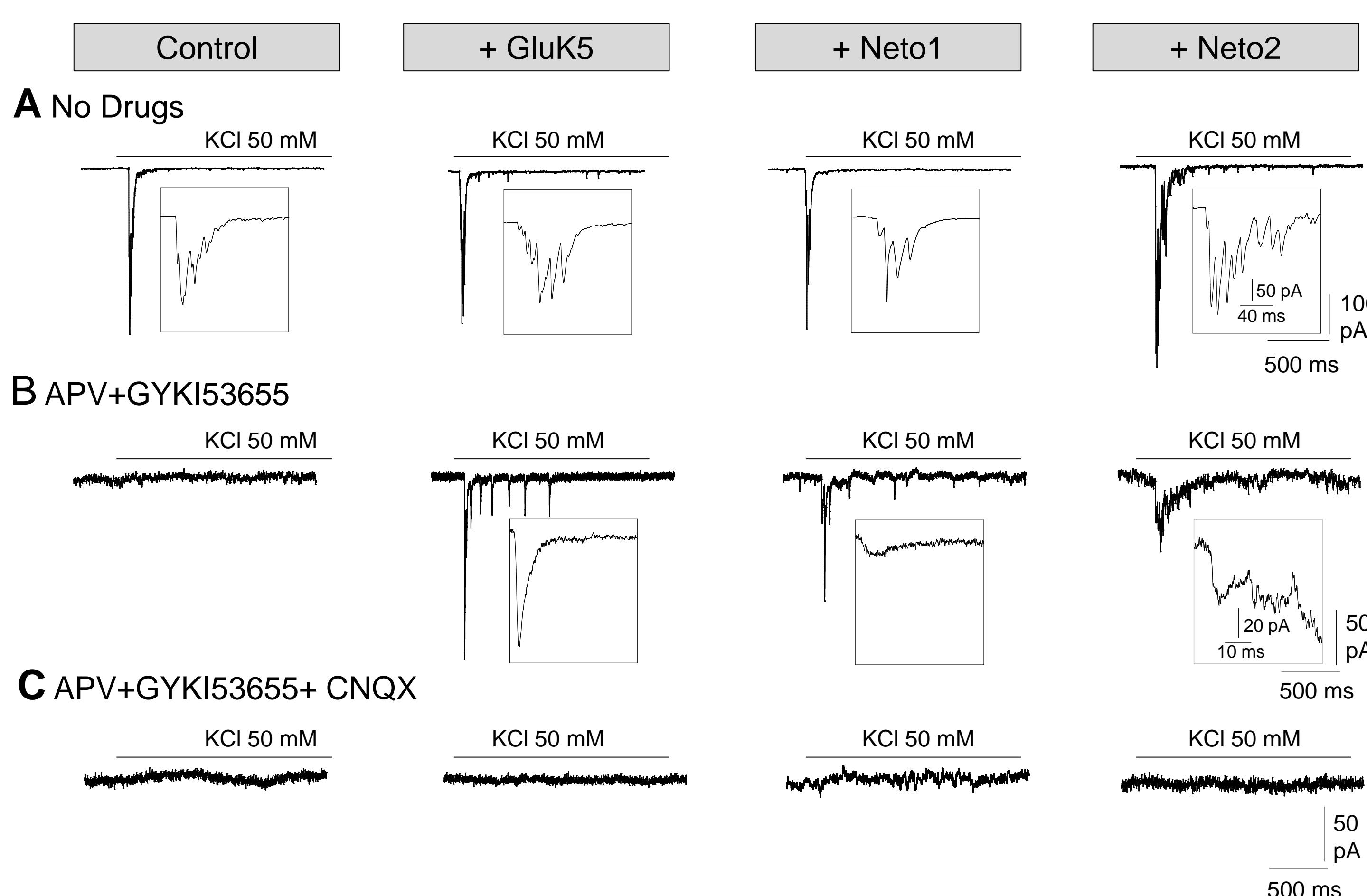


Figure 2. Summary of KARs properties altered by Neto proteins

	Current amplitude		Desensitization $\tau$		Agonist affinity		Recovery Rate from desensitization		Sodium dependence (current loss)	
	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2	Neto1	Neto2
GluK1	282%	573%	43%	242%	289%	758%	625%	95%	58%	31%
GluK2	143%	146%	125%	213%	433%	289%	370%	120%	42%	27%
GluK3	303%	1348%	71%	148%	↓	↑	270%	89%	nd	nd

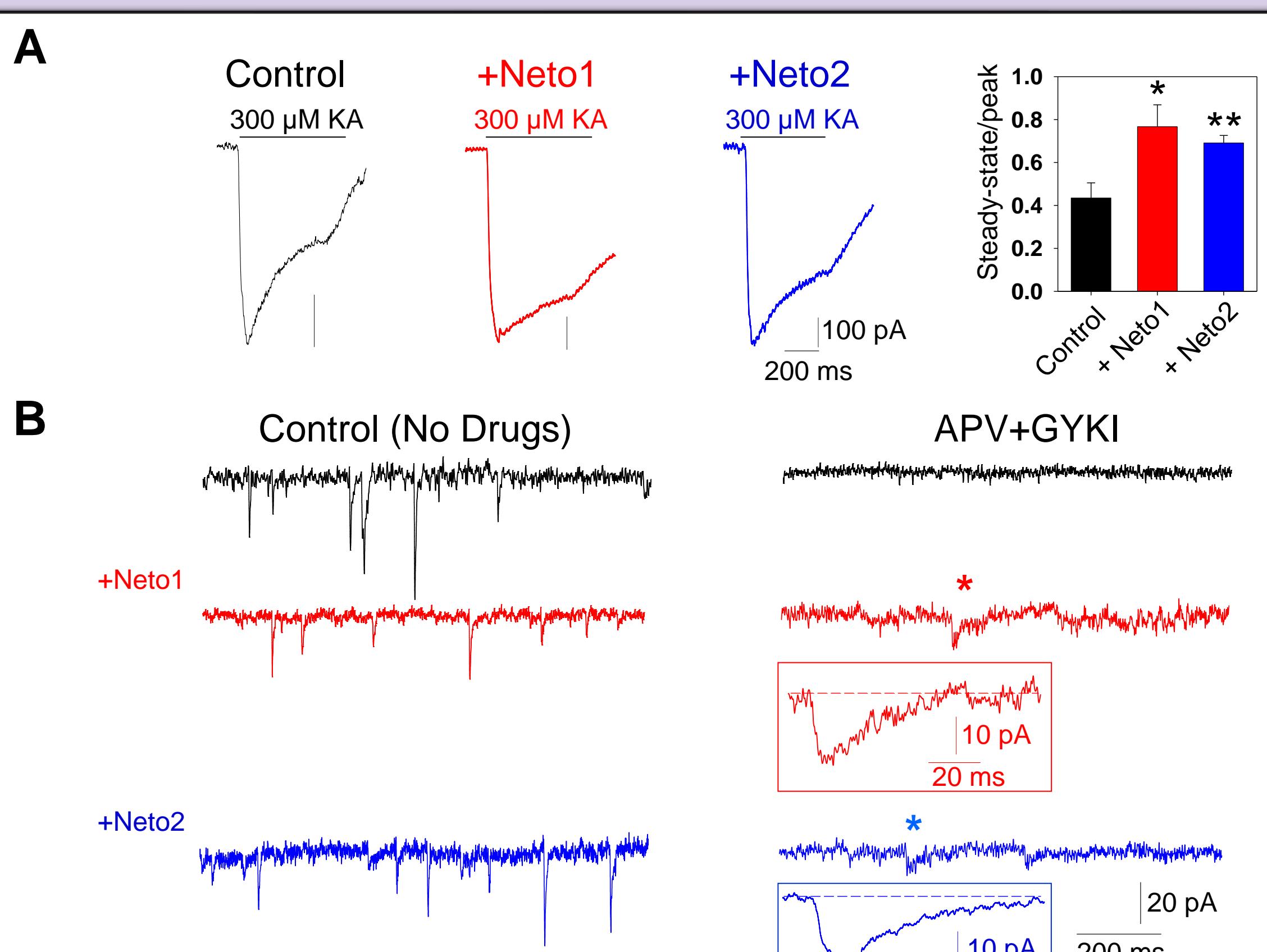
Neto1 and Neto2 functionally interact with and modulate the three main KAR subunits but the degree and sign of modulation depend on the type of receptor.

Figure 4. Molecular requirements for EPSC<sub>KAR</sub> at hippocampal cultures



**Forcing synaptic activity to study different EPSC.** Hippocampal cultures where used to test synaptic responses (DIV 15) transfecting KARs high affinity (GluK5) or auxiliary subunits (Neto1 or Neto2) cDNA. Synaptic responses, recorded under whole cell configuration where elicited by stimulation with 50 mM KCl of neighbor neurons (1 mm apart) while continually perfusing ringer (ACSF) to pachted cell. In A, blocking inhibitory activity (50  $\mu$ M Picrotoxin). In B, synaptic responses where challenged after blocking NMDA and AMPA receptors (APV 50  $\mu$ M and GYKI 50  $\mu$ M). In C, synaptic responses where totally block by CNQX 20  $\mu$ M.

Figure 6. Neto proteins set up slow onset and decay kinetics of EPSC<sub>KARs</sub>



**Effect of transfecting Neto1 or Neto2 into hippocampal primary cultures (DIV 15).** In A, responses evoked by Kainate 300  $\mu$ M to control (in black), Neto1 (in red) and Neto2 (in blue) transfected cultures. It is also shown a quantification of the steady-state (steady-state/peak ratio). In B, different cultures spontaneous activity, where only Neto1 and Neto2 exhibit APV and GYKI resistant currents. Magnification of those currents are also plotted. Data are mean + SEM. \*p<0.05, \*\*p<0.01.

- Neto1 and Neto2 show developmental differential expression in the brain.
- Neto1 and Neto2 functionally interact with and modulate the three main KAR subunits but the degree and sign of modulation depend on the type of receptor.
- Expression of either GluK5, Neto1 or Neto2 is required for targeting KARs to the synapse.
- Neto1 or Neto2 impose slow kinetics to EPSC<sub>KARs</sub>.