

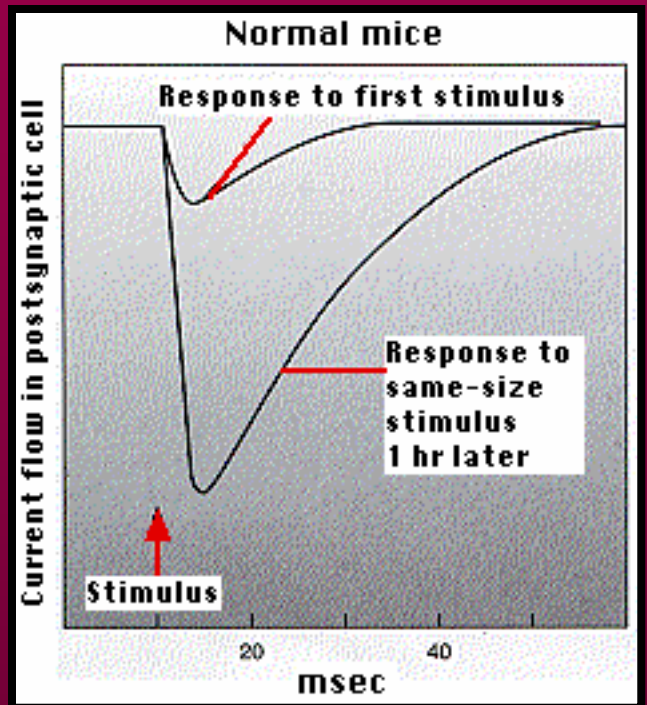
CONTEXT

LTP (long term potentiation) definition

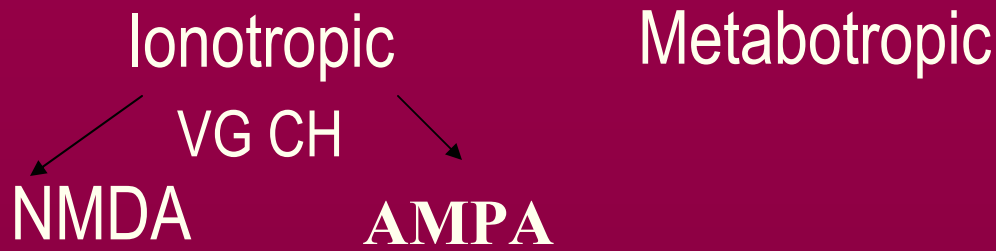
LTP as a interesting mechanism for learning and memory

LTP is due primarily to a pre or post- synaptic modification ?
(Increased Glut release or increased AMPA R responsiveness ?)

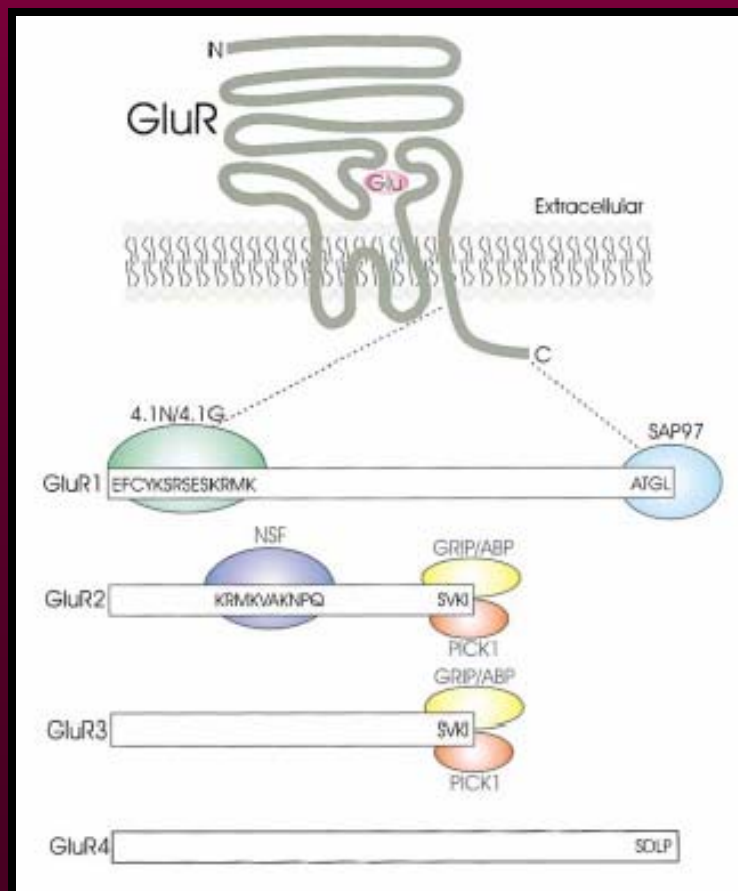
AMPA's "responsiveness" : either number or function.
This paper shows a mechanism that increases the number of AMPAR at the synapse in an activity dep. manner



Post-synaptic receptors



Heteromeric combination of GLUR1-4 subunits
GLUR1/2
GLUR2/3



Mutant receptor used

GLUR2 (R586Q): Inward rectifying

GLUR2 (R586E) : Dead

-IRES GFP

-IRES GFP-tCaMKII

GLUR2 (R586Q) +Y: unable to be recognized by PDZ

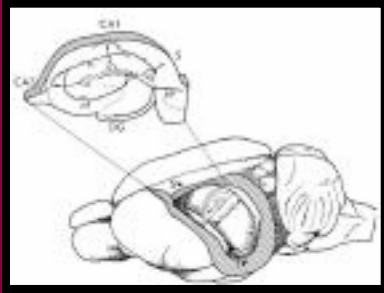
GLUR2(R586Q N839A P840A): unable NSF bind.

GLUR1 (1-822) -GLUR2 (827-862) : chimeric 1 and 2

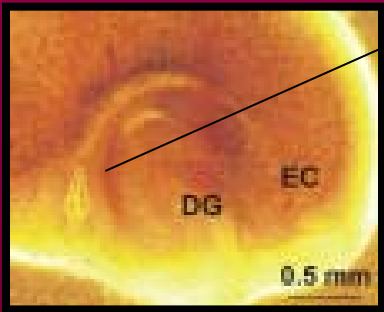
GLUR2 (1-826)-GLUR1 (823-899) : chimeric 2 and 1

System used:

Hippocampal slice cultures : “primary cultures”



E19-P7 Mouse/rat brain

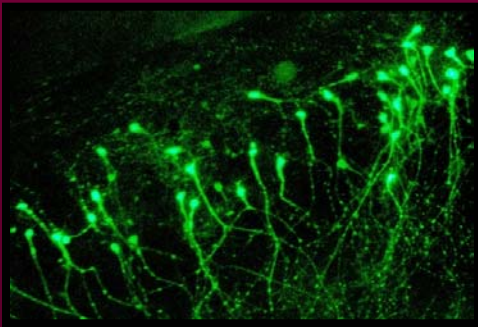


CA1 pyramidal neurons

Where therecording electrodes are places

300-400 microM thick

Slices on a membrane support
float on top of media



Transfected via sindnis virus
Or biolistic method

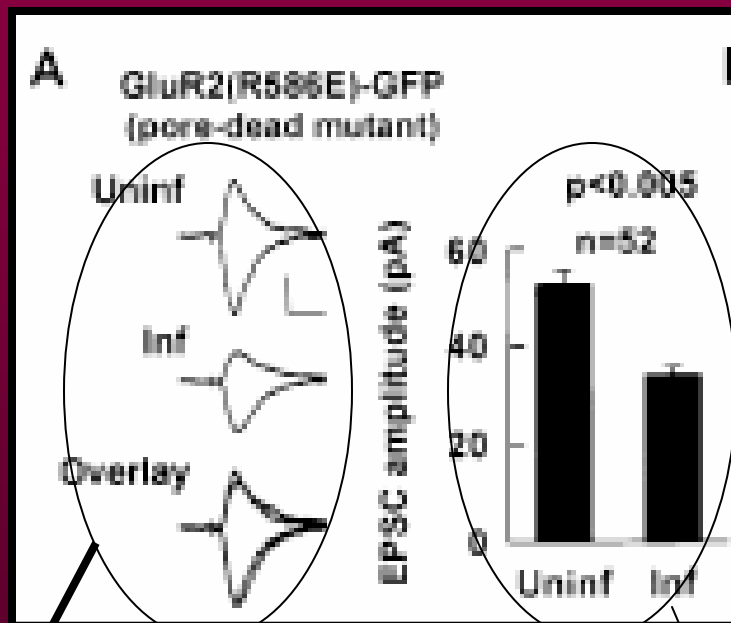
EGFP-Sindbis virus infection, CA1 neuron hippocampus.

From A. Terashima, A. Griffiths & F. Duprat, march 200

“ Read Out “

EPSC : Excitatory post synaptic currents

$$\text{EPSC} = \text{constant} \times (\text{Ca}^{+} \text{ influx})^n$$



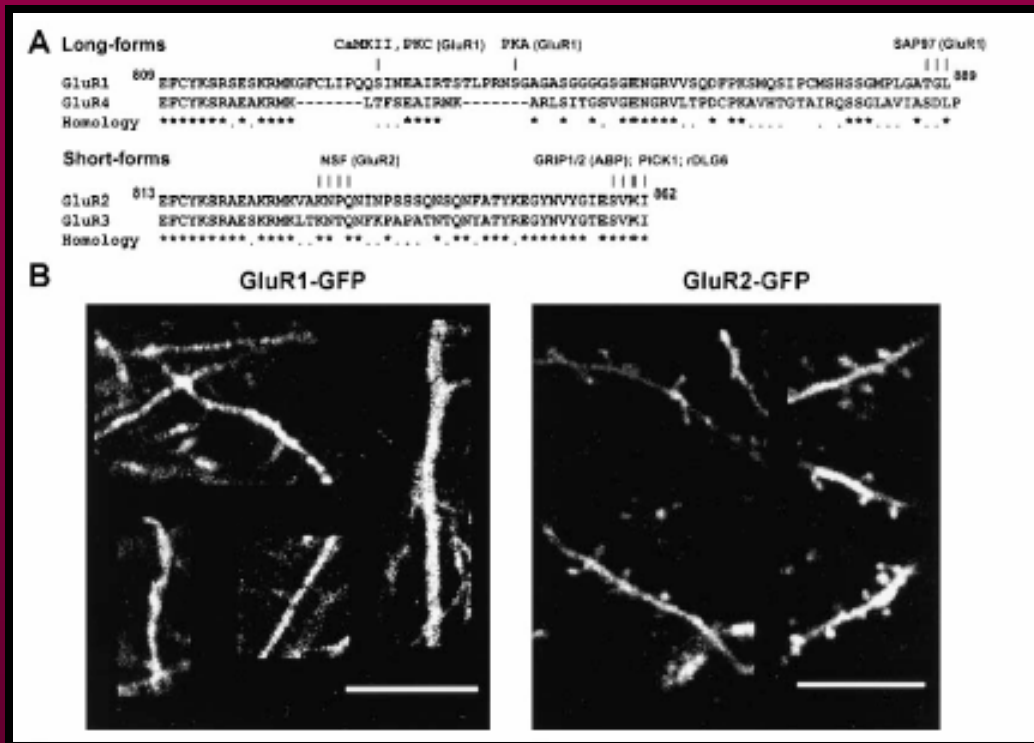
Sample of typical trace

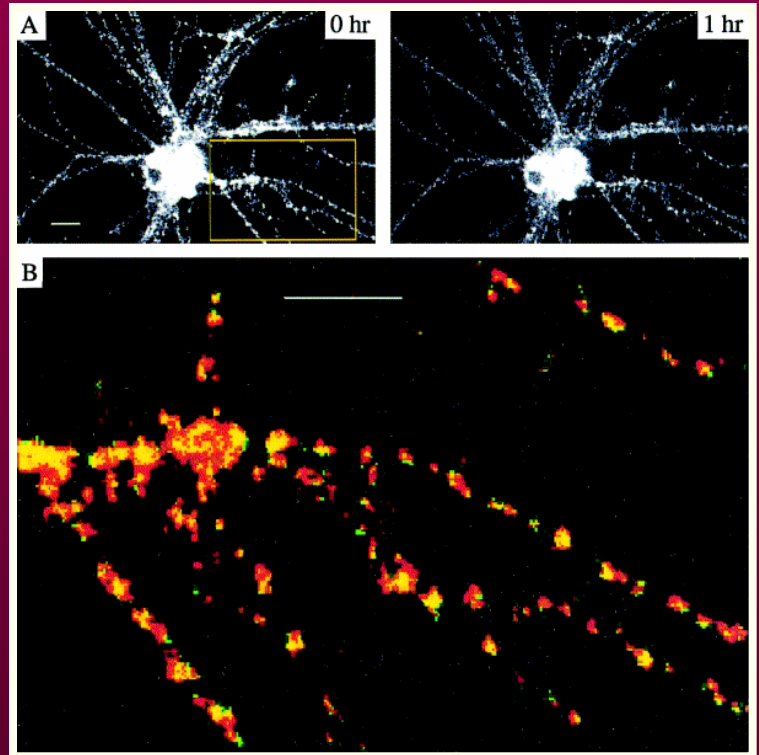
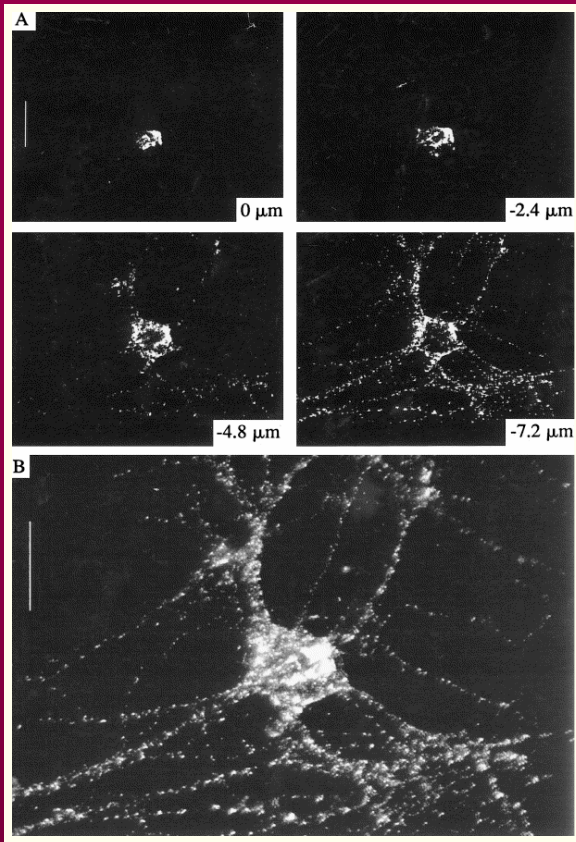
Average of all traces

Fig1A-B

Localization of the GLUR1 and GFP constructs

GLuR2 receptors are normally found in synapse while homo GluR1 are normally not.





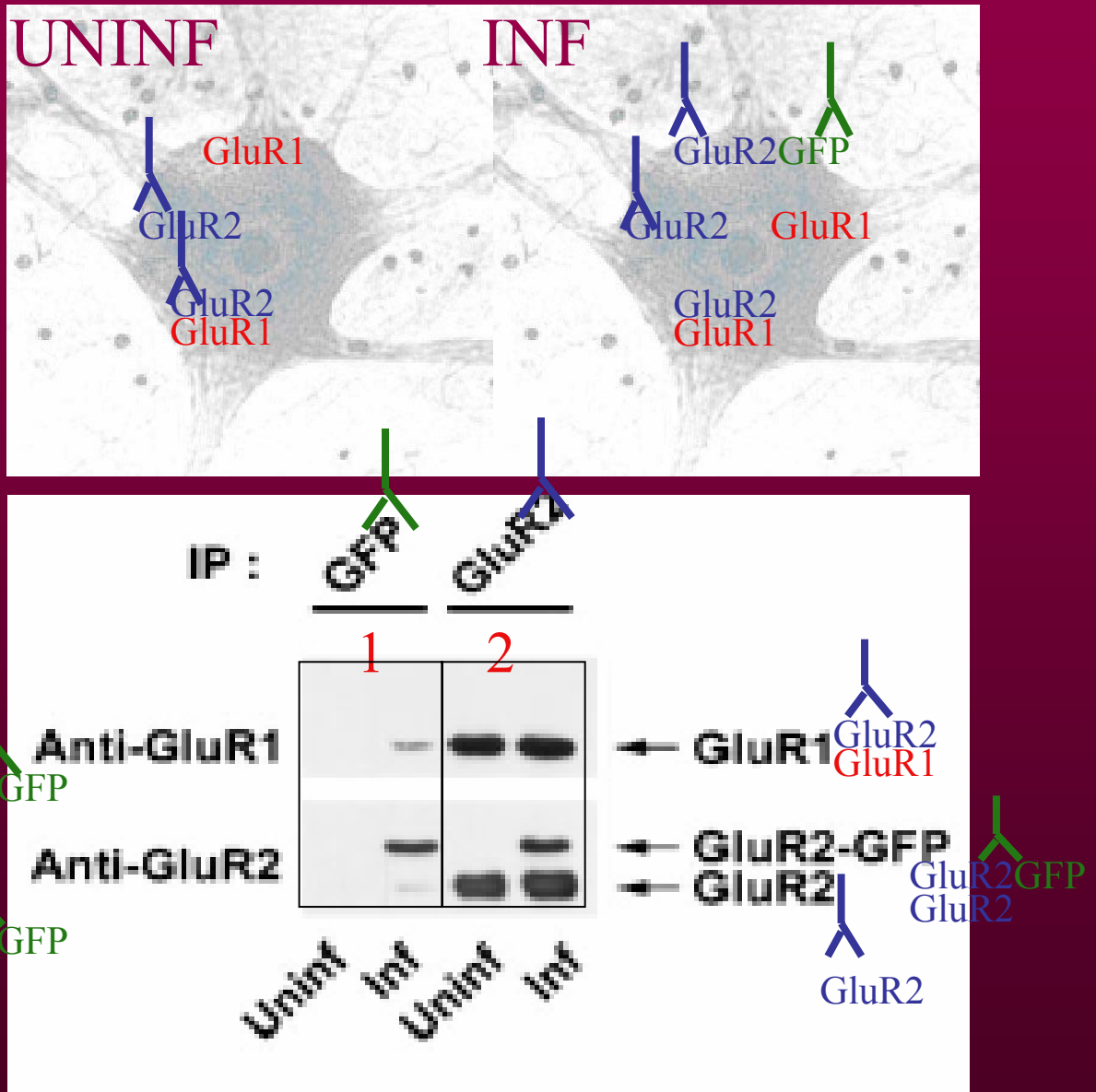
Neuroscience. 1996 Nov;75(1):69-82.

Localization of the glutamate receptor subunit GluR1 on the surface of living and within cultured hippocampal neurons.

Richmond SA, Irving AJ, Molnar E, McIlhinney RA, Michelangeli F, Henley JM, Collingridge GL.

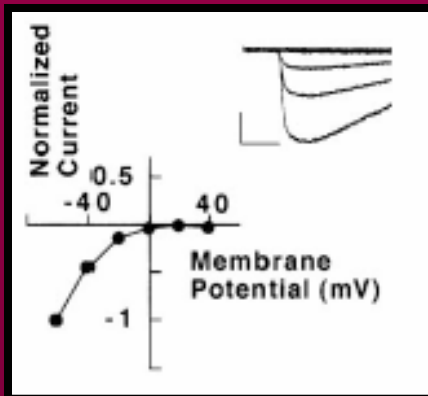
FIG1C

To show that their mutated **GLUR2(R586Q)-GFP** is able to form homodimers



To determine if homomeric receptors participate to synaptic transmission they tag them with an “Electrophysiological tag”

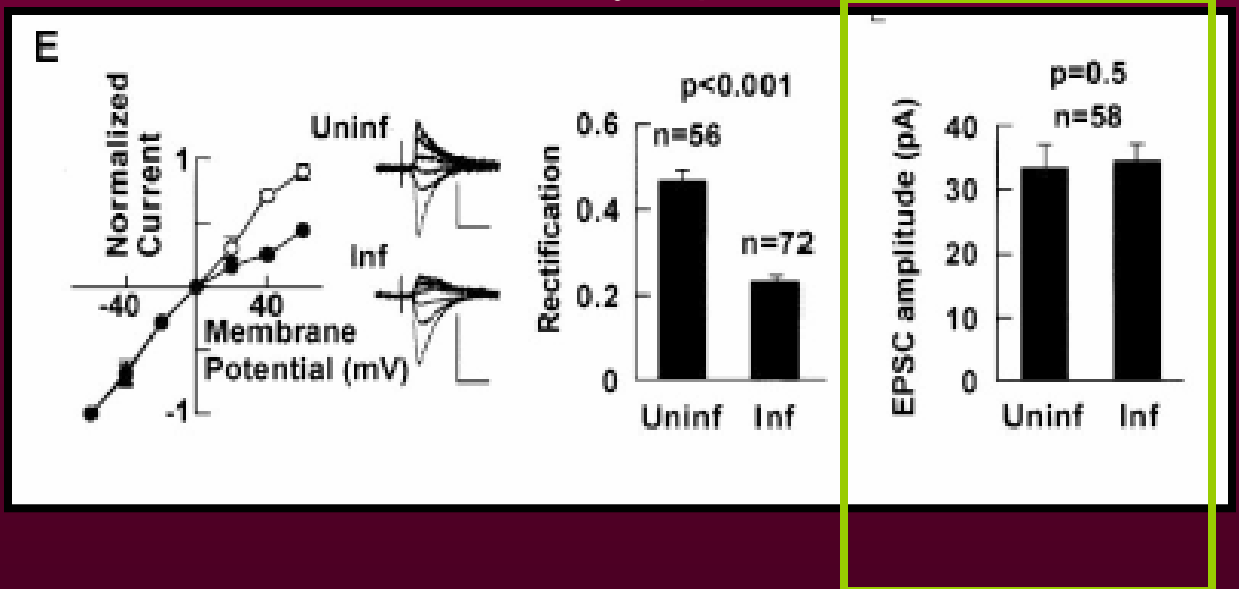
FIG 1D : ctrl for



Kainate evoked response in HEK cells
Shows inward rectification

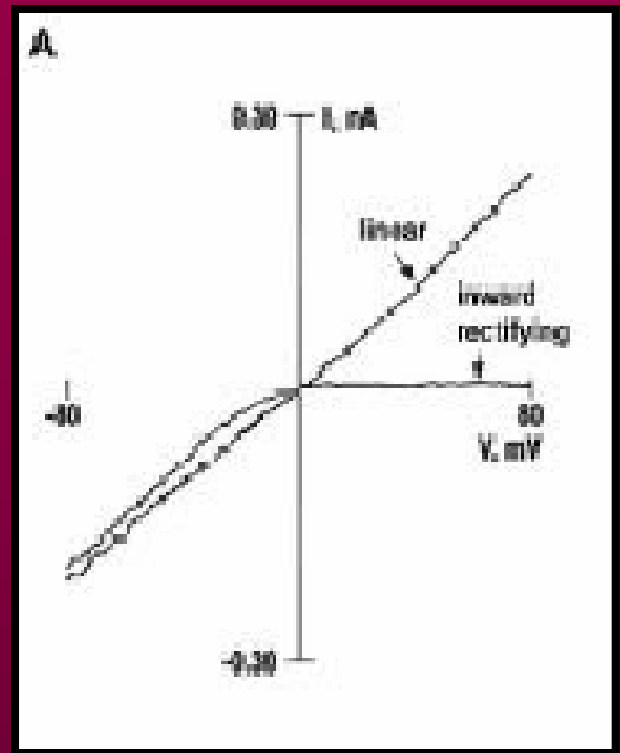
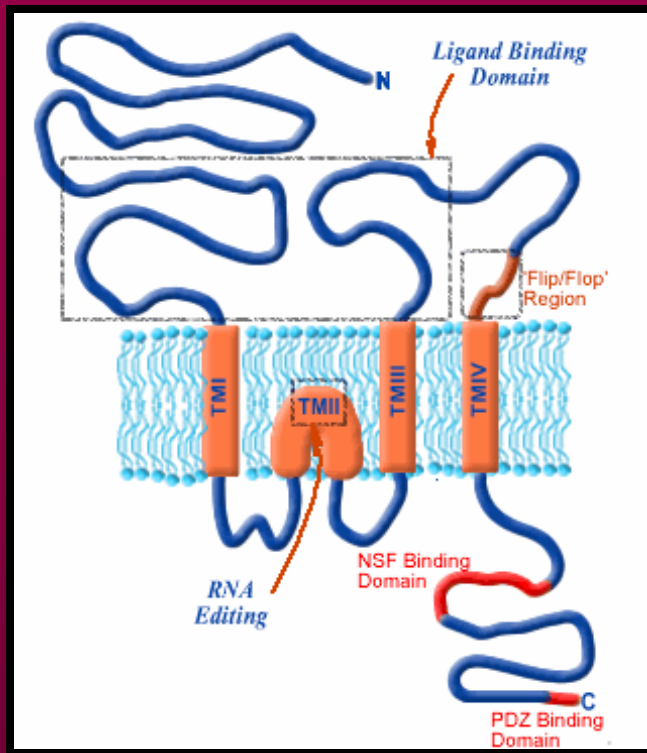
FIG 1E : experiment

In hippocampal neurons
Evoked AMPA-R mediated synaptic transmission



Same amplitude..
so seems that the recombinant GluRs are replacing the
endogenous ?

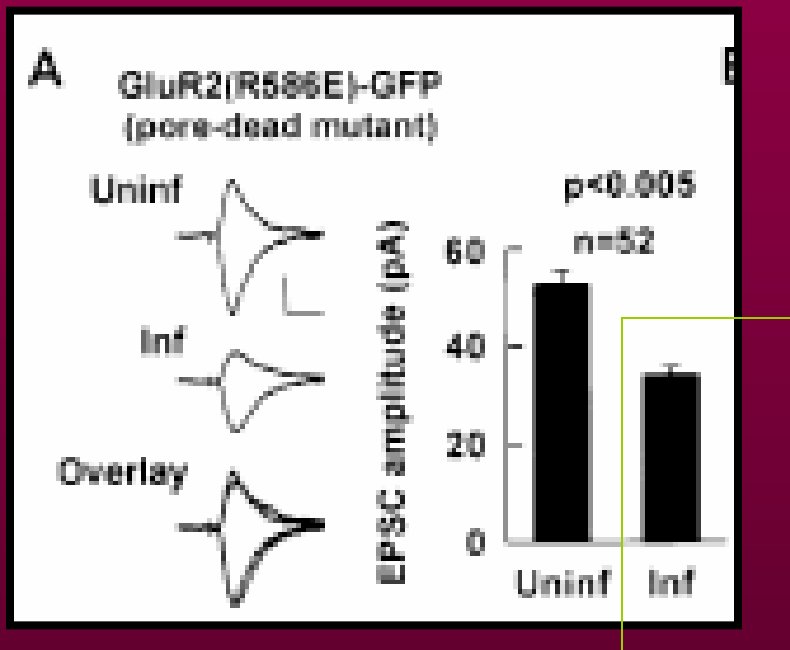
Electrophysiological tagging and Inward rectification



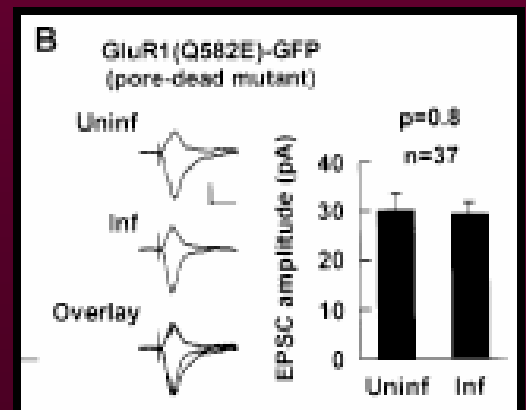
Native AMPAR are impermeable to Ca^{2+} , A function controlled by the GLUR2 subunit. The Calcium permeability of GLUR2 is determined by a postranscr. Editing of its mRNA, which changes a single aa in the TMII region from Glutamine (Q) to Arginine (R). The Q form is calcium permeable while R is not. Almost all the GLUR2 in the CNS are in the R form.

FIG.2 A and B

To test whether delivery of **GLUR2 (R586Q)-GFP** is followed by wtGluR2 replacement they make a “dead pore” (**R586E**) mutant (oligomerize but do not conduct). If they replace then amplitude should decrease, if they just add on should stay the same



In B, as a ctrl, the same mutation on GluR1 has no effect



Silent synapses

Silent synapses are those at which an NMDA current is identified at +50 mV, but no AMPA currents are observed at –60 mV.

Several years ago, two labs reported silent synapse activation in association with LTP (Liao et al., 1995; Isaac et al., 1995).

Failure rates

The action potential dependent release of neurotransmitter from a synapse occurs only about 10-40 % of the time. Therefore you record a mixture of quantal EPSC, or “success” and “failure”.

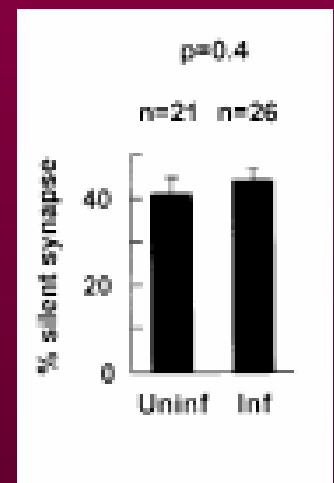
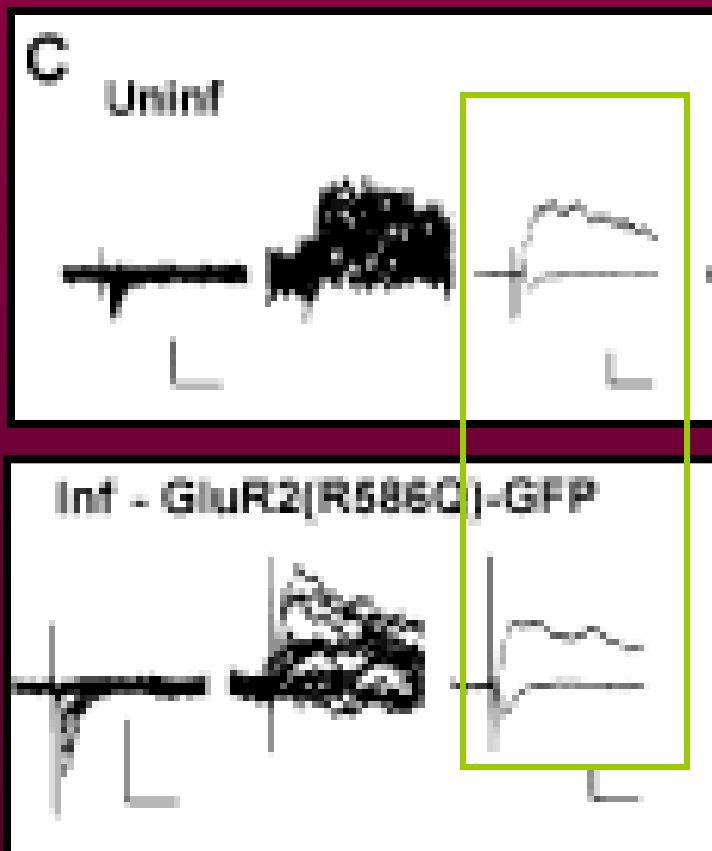
LTP is known to also reduce the failure rate

FIG. 2C

So recombinant GluR2 take the place of other endog. AMPAs :

Is this “replacement “ or “displacement”

If it is replacement ,they would only go where AMPAs are already present (active synapses) but not in synapses lacking AMPAs (silent)



If it would go to silent synapses
I'd “activate them” so that Inf. Cells will have
Increased response amplitude.
But it seems not to be the case

FIG.2C Bottom

Just another way to see it

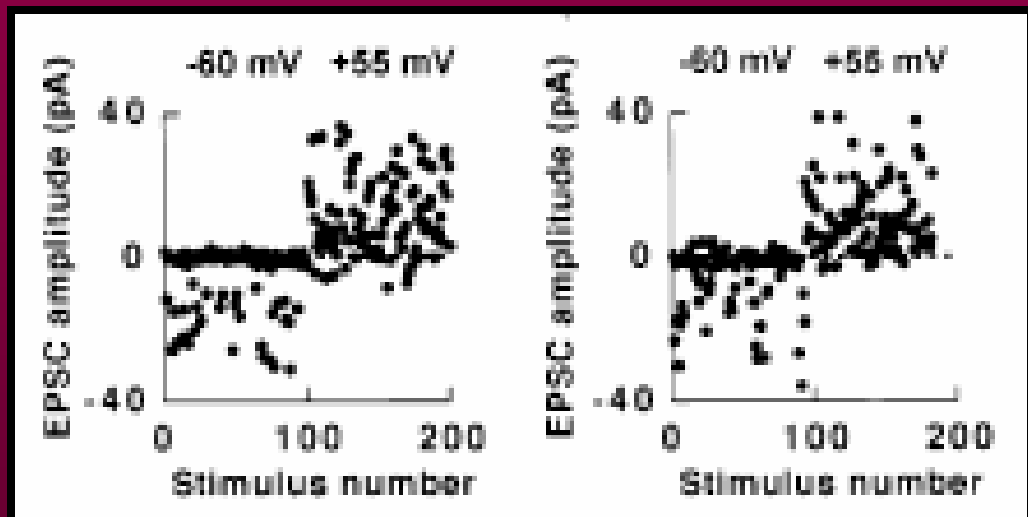
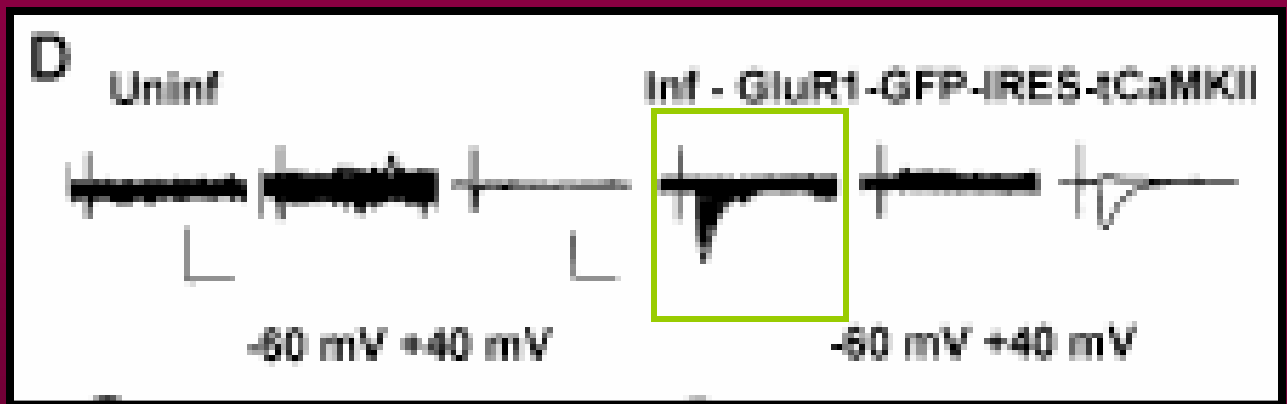


FIG 2D

Delivery of homo **GLUR1-IRES-GFP-tCMARKII**

To silent synapses. (From previous experiments they know that GluR1s go to the synapse only in the presence of CamkII in LTP ind.)



GLUR1 does get into silent synapses as they see
Increased synaptic transmission in the infected.
But only at hyperpolarized potentials.....

APV NMDA antagonist

They also notice a decreased synaptic failure at hyperpolarized potentials(-60). To test if the effect is specific, they measure failure rates at Depolarized (+40) potentials, where the mutant homomeric GLUR1 do not conduct. And Indeed the effect is not present, proving that the effect is specifically due to delivery of recomb. Homomer. GLUR1 receptors to silent synapses

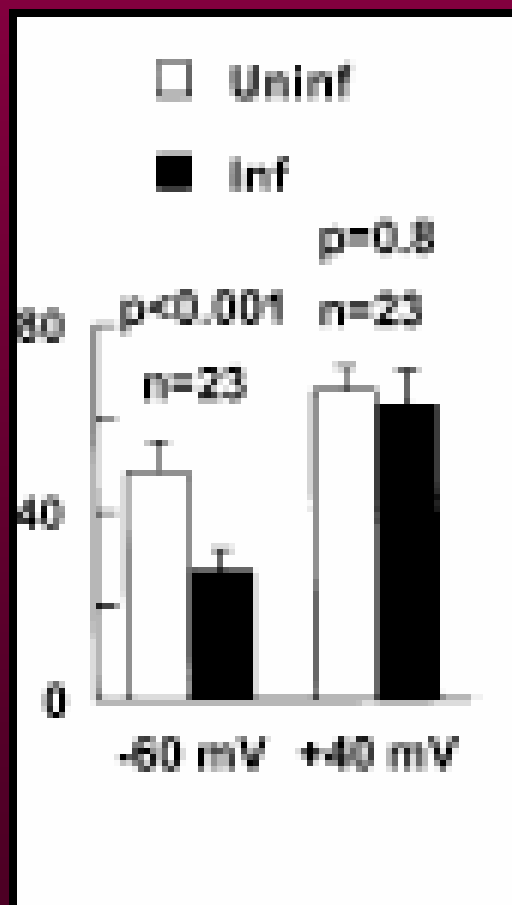


FIG.3

Going back to homo GLUR2, let's see if their synaptic delivery is also (like homo GLUR1) dependent on neuronal activity

Use the GLUR2 (R586Q) mutant, treat the culture with various drug and look if you Loose inward rectification.

Drugs: Tetrodotoxin (block voltage gated Na Ch)
High Mg^{2+} (depress synaptic activity)
DLAPV (block NMDAs)
NBQX (blocks AMPAs)

So the delivery is not activity dependent....

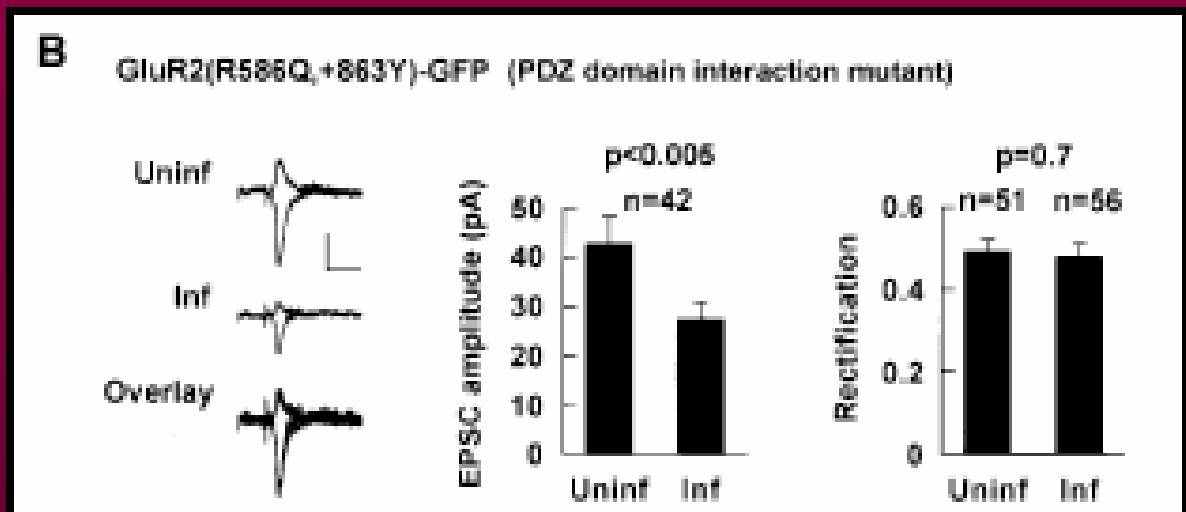
.... well then it could be dependent upon interaction with other proteins.

The C-term Of GLUR2 was known to contain a PDZ ligand domain and to be able to bind 4 PDZ containing proteins.

They then mutated the C-term (addition of a tyrosin) creating: **GLUR2 (R586Q +Y)** and repeated the recordings with this conditions.

FIG. 3B

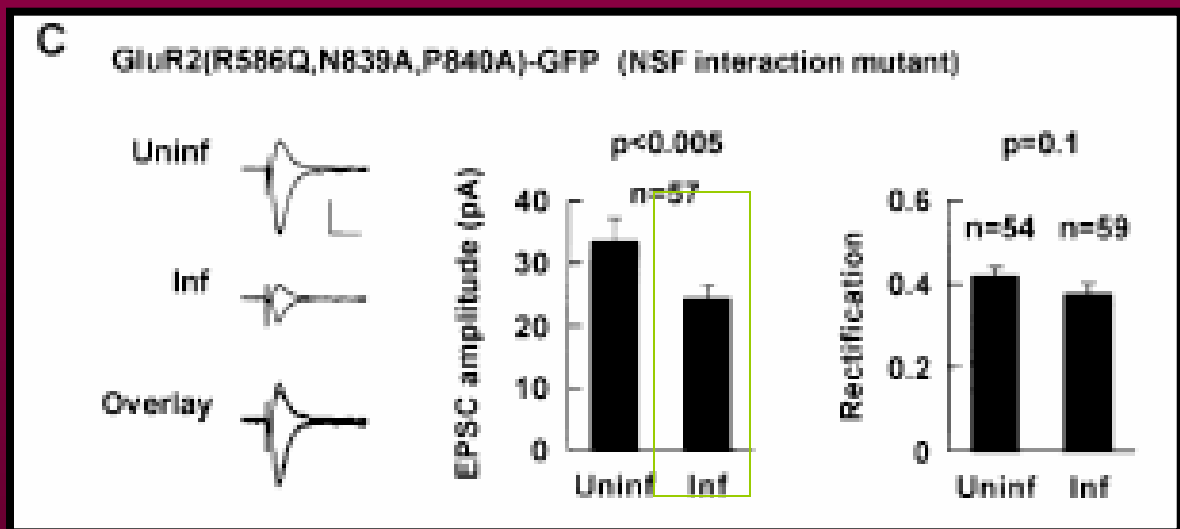
First they had to prove that their mutant wasn't a dead receptor. So they introduce it in HEK 293 cells. Then they introduce it in neurons and see no amplitude change and no inward rectification.



Meaning that the new mutant doesn't get delivered to synapses. Which in turns shows that the Interaction with PDZ proteins is necessary for delivery At the synapses.

FIG. 3C

Which of the PDZ proteins could be “the one”? From other Experiments they have the feeling that it could be NSF (protein involved in membrane fusion ..). SO they mutated the residues known to be involved in this interaction **GLUR2(R586Q N839A P840A)**



Amplitude goes down and no inward rectification. So the mutated receptors do not get to the synapses and even interfere with the wild type ones (dominant negative effect)

FIG 4A

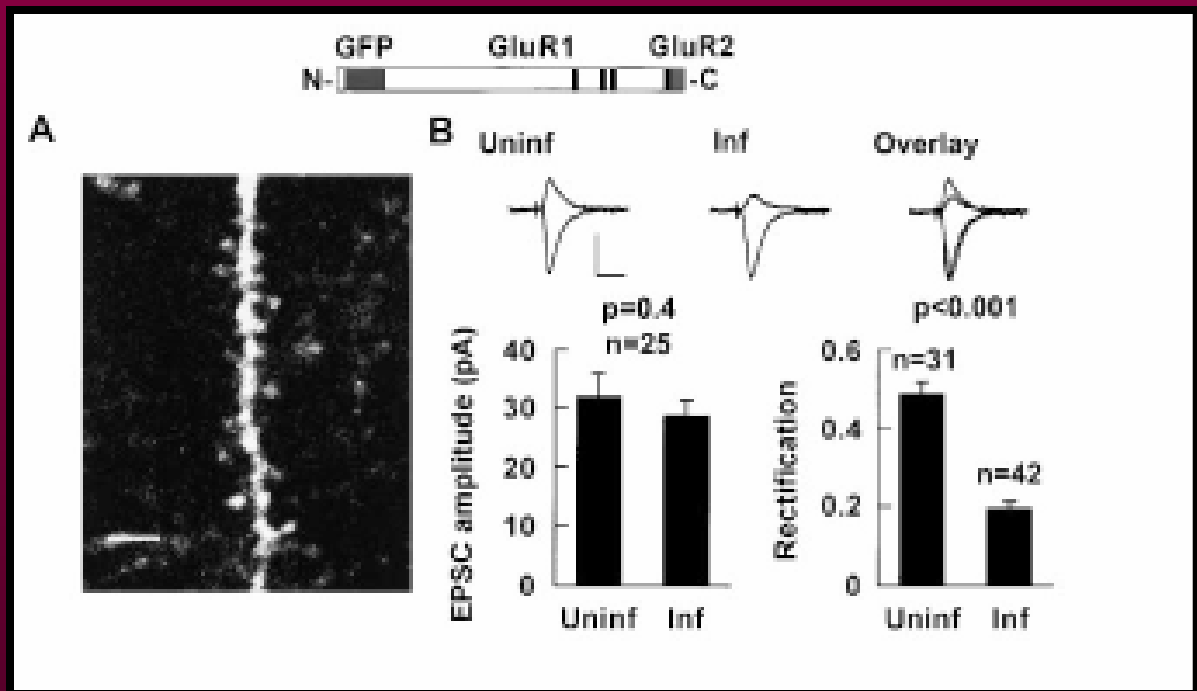
The C-term seems important for the localization/delivery event. GLUR1 and GLUR2 behave differently and have different C-term.

What happens if we swap the C-term?

GLUR1 (1-822) -GLUR2 (827-862)

GLUR2 (1-826)-GLUR1 (823-899)

Will the behavior of the receptor change too?

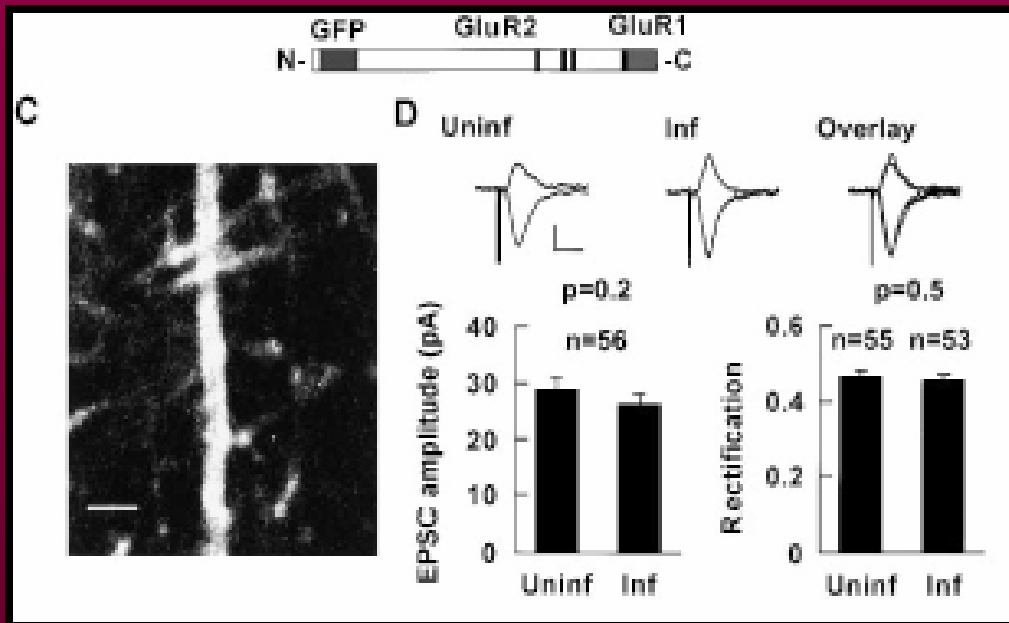


Indeed so.

GLUR1 Now goes to the Synapses , shown by confocal microscopy and by electrophysiology (Inward rectif)

FIG 4B

While chimeric GLUR2 behaves like wt GLUR1



By now they made their major points :

1- the existence of 2 different trafficking mechanisms for the 2 AMPA receptor

2- the different trafficking is controlled by different C-term tails

But..... All the experiments involved recombinant proteins. So they needed to show that what they found applies to the Wt receptors too.

To address this problem they decided to express in the culture only the GFP tagged C-terminal tails. So, in this new condition, **they only “functional” receptor present are the endogenous ones.**

So whatever physiological effect they'll measure will only be imputable to the wt receptors.

FIG. 5

What makes the C-term. tails very useful, in this case, is that they are not functional, of course, but they can still oligomerize with the Wt and therefore potentially interfere with their trafficking.

This will hold true only if the wt trafficking also relies on the C-tails.

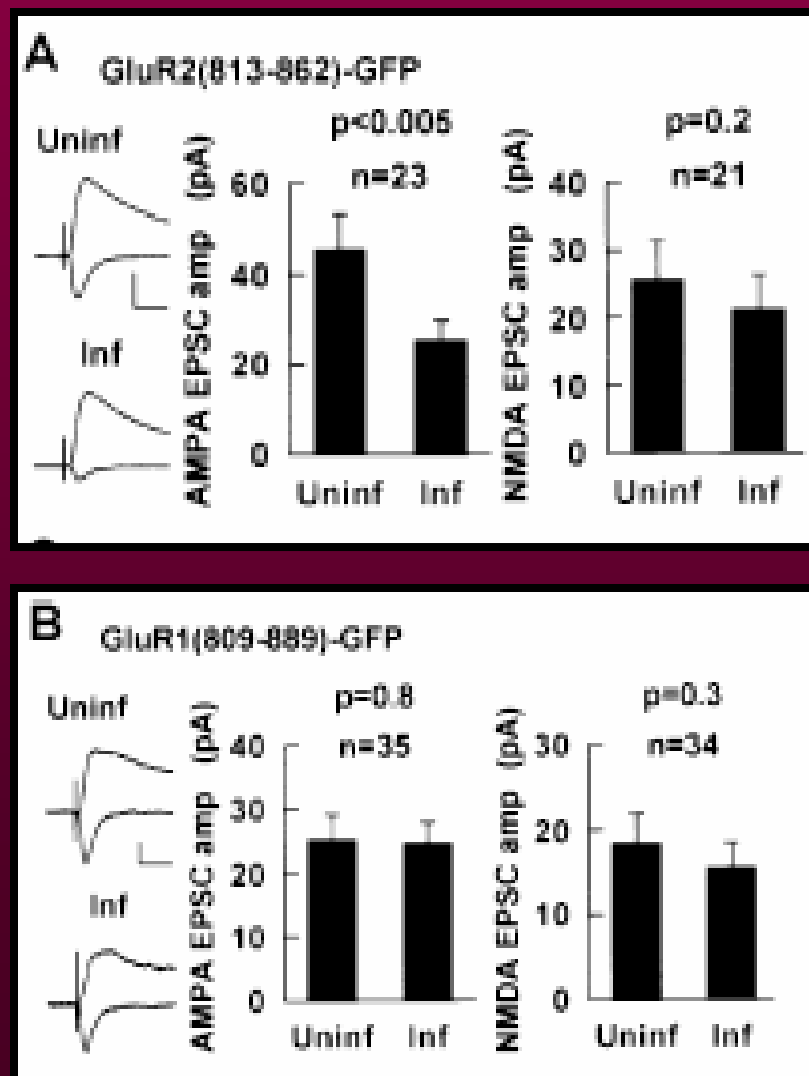


FIG. 5C

Another way to interfere with GLUR2 C-Term. Function is using a synthetic peptide (G10/pep2m) that MIMICS a NSF binding site. This peptide should compete with GLUR2 for NSF binding. Therefore we would expect less GLUR2/NSF and less GLUR2 synaptic delivery less

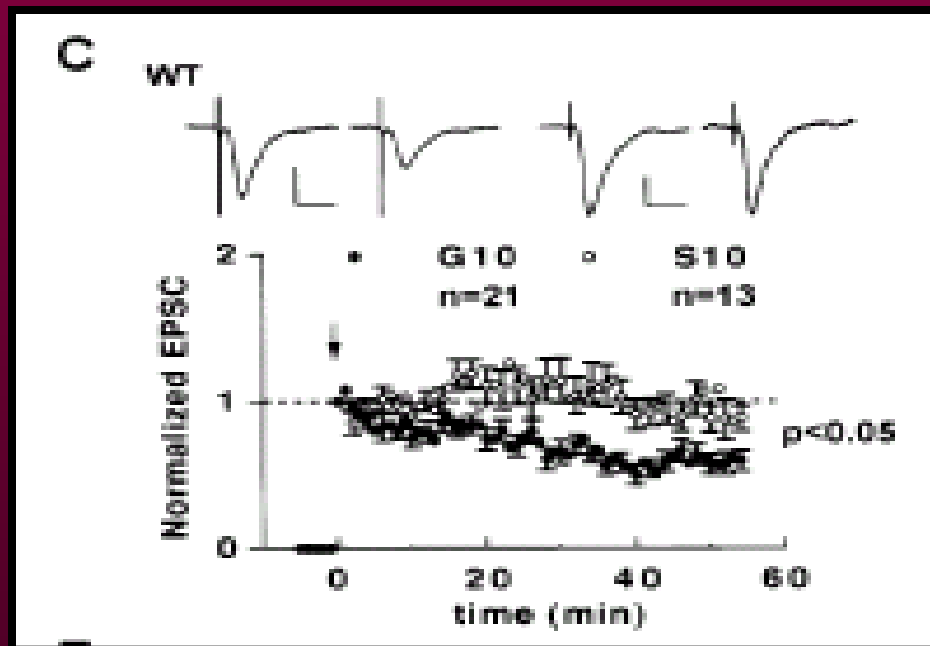


FIG 5D

And if they repeat the experiment in a GLUR2 null condition the amplitude of AMPA response does not change. This confirms that G10 is specifically interfering with GLUR2 only.

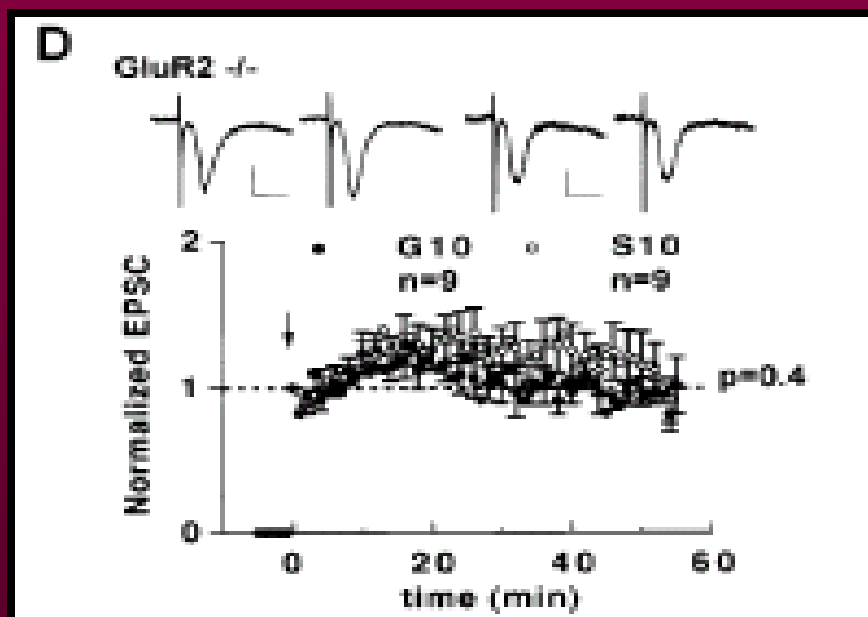
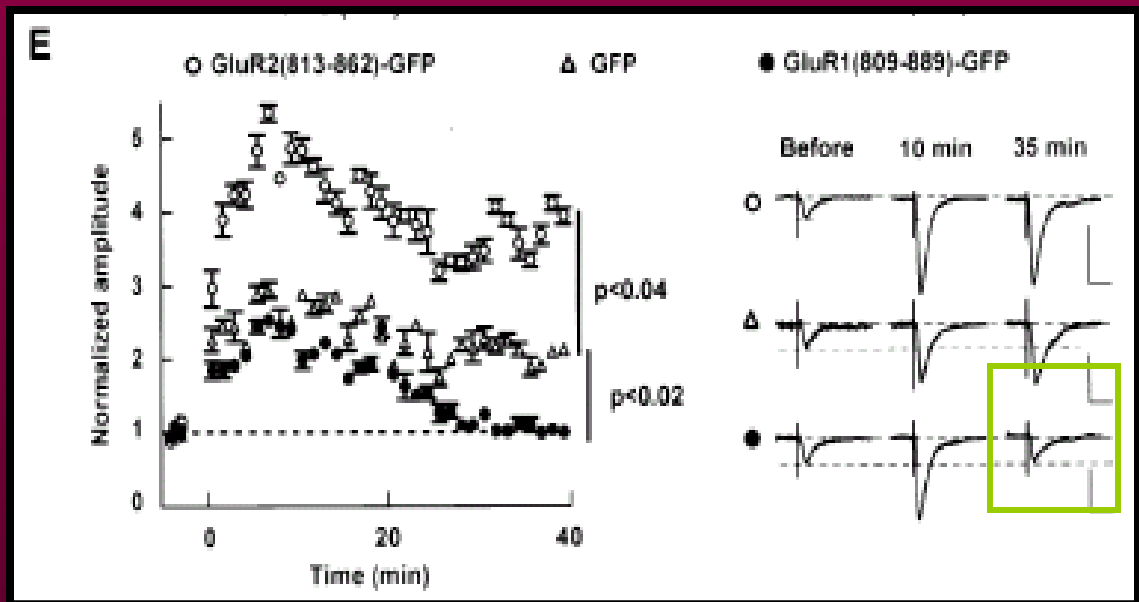


FIG 5E

The C-term. tail is important for GLUR2 localization, is this the case also for GLUR1 ?

GLUR1 localization to the synapses is activity depend as it occurs upon LTP induction.

They again transfect the cultures with the C-term tails and induced LTP

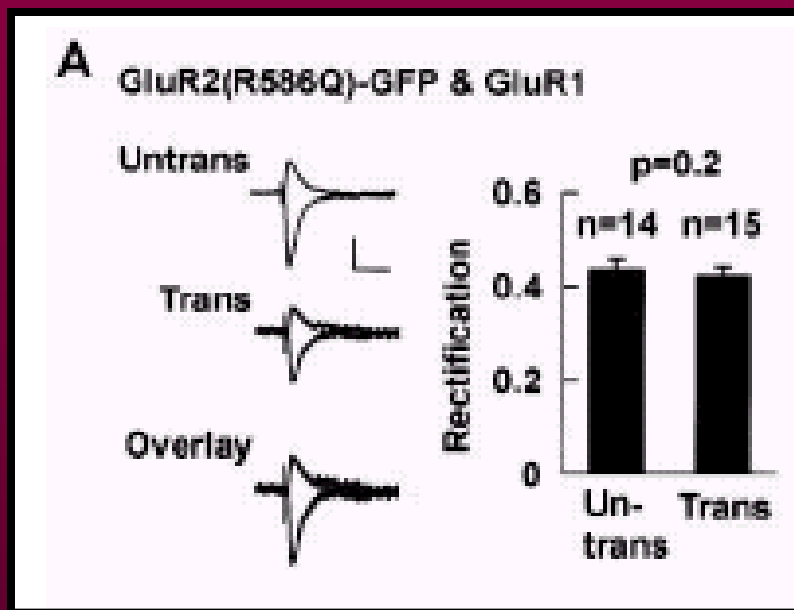


when they infect with GLUR1 tail, the LTP response is unstable.

Meaning that the C-terminus of GLUR1 is important for the LTP induced synaptic delivery.

FIG 6A

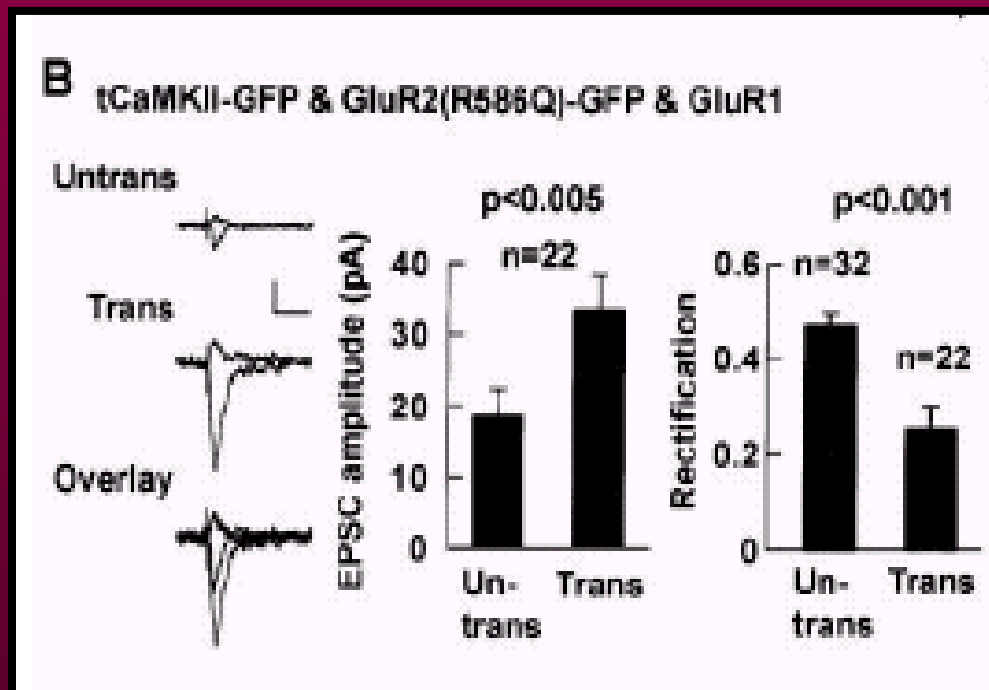
So far they studied the behaviour of homomers but if co-expressed the 2 receptor mainly form heterodimers and if GLUR1 is coexpressed with the GLUR2 (R586Q) There is no rectification effect meaning that they do not get delivered to synapse.



GLUR1 has a “dominant” effect, as the heterodimer behaves like a GLUR1 homomer and do not get to synapses unless CMAKII is present or during LTP

FIG 6B

So they coexpress along with GLUR2 (r586Q) a constitutively active CAMKII (t-CamKII) and in this case they do see rectification, meaning that the oligomers get delivered to synapses.



To summarize things at this point:

Behaviour of homomeric receptors

-**Homo GLUR1** does not go to the synapses (unless CAMKII or LTP is present)

-**Homo GLUR2** does go to the synapses and takes part into a AMPAs continuous “turnover”

but in reality most of the time they form HETERO OLIGOMERS

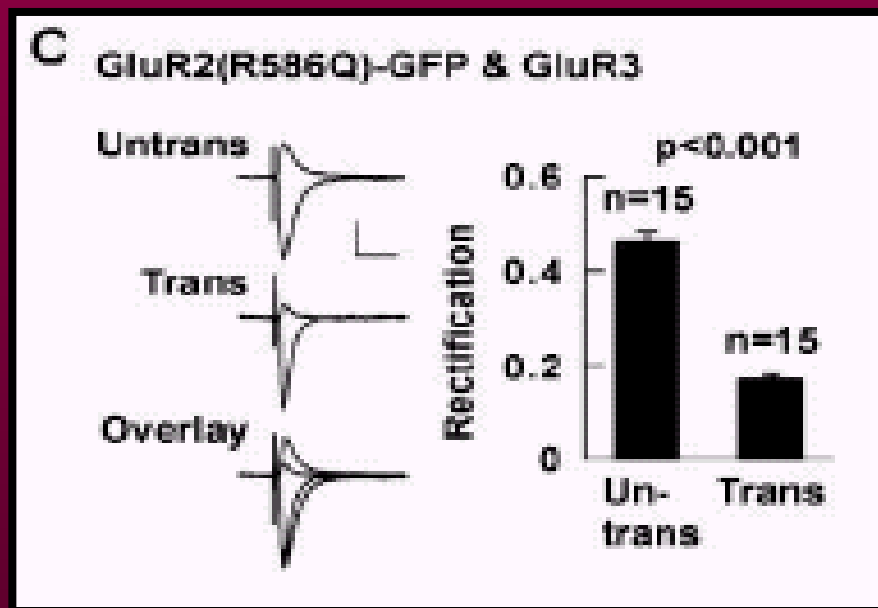
- **Hetero GLUR1/GLUR2** are delivered to synapses only upon LTP (activity) and once there they enhance transmission

.....so which is the heterooligomer that is continuously delivered to the synapses?

FIG.6C

Then they coexpress the GLUR2(R586Q) with GLU3
{FIG 6D is again to test that the GLUR3-GFP fusion
is functional in HEK cells and delivered to synapses
(2foton image 3fgE)}

Cells expressing the GLUR2(R586Q)-GFP and
GLUR3-GFP show inward rectification



So the Hetero oligomer continuously present at synapses
is GLUR2/GLU3

FIG. 6F

They also had a look at the behaviour of GLUR3 by transfecting GLUR3-GFP.

There was no rectification changes and there was a decreased AMPA-R mediated transmission.

The GLUR3-GFP were at the synapses (2 foton) but not active

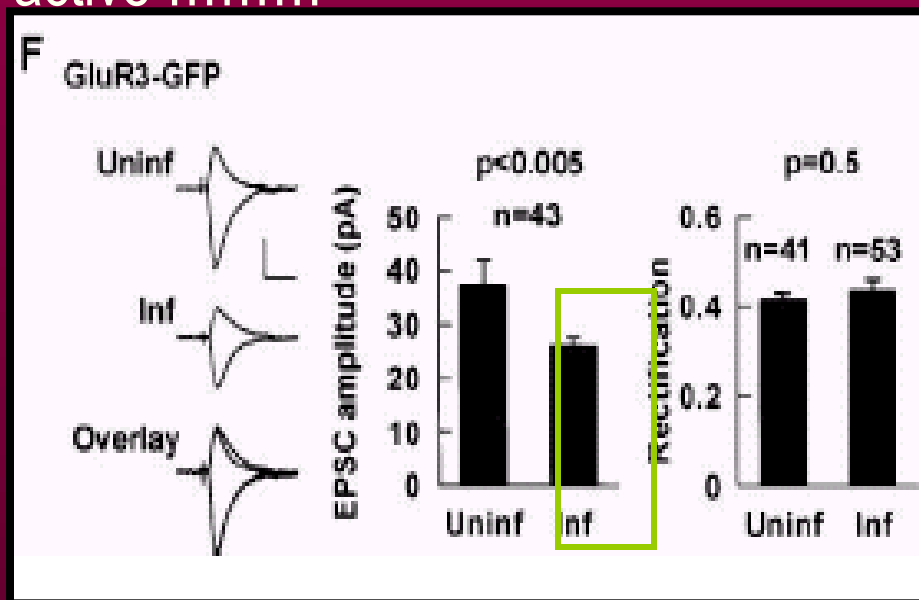
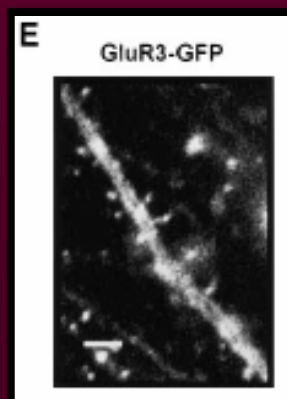
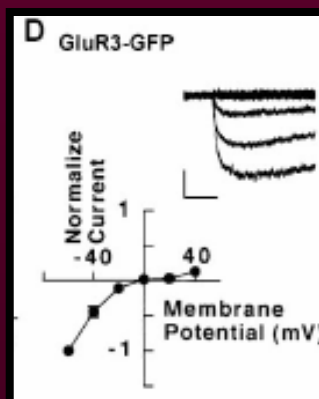


FIG. 6D and 6E , to prove that the GLUR3-GFP fusion is functional and localizes properly



MODEL PROPOSED

