LTP (long term potentiation) definition

LTP as an 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?)

AMPAs “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

Ionotropic
- VG CH
- NMDA
- AMPA

Metabotropic

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:
Hypocampal 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

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

Sample of typical trace

Average of all traces
Localization of the GLUR1 and GFP constructs. GLuR2 receptors are normally found in synapse while homo GluR1 are normally not.
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.
To show that their mutated GLUR2(R586Q)-GFP is able to form homodimers
To determine if homomeric receptors participate in synaptic transmission they tag them with an “Eltreophysiological 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?
Native AMPAR are impermeable to Ca+, a function controlled by the GLUR2 subunit. The Calcium permeability of GLUR2 is determined by a postranscri. Edition 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.
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-tCMAKII** To silent synapses. (From previous experiments they know that GluR1s go to the synapse only in the presence of CamkII in LTP ind.)

![Graph showing synaptic activity](image)

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 recombinant homomeric 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:  Tetrodoxin (block voltage gated Na Ch)  
        High  Mg2+ (depress synaptic activity)  
        DLAPV (block NMDAs)  
        NBQX (blocks AMPAs)
We do not loose Inward rectif. In any condition meaning that the delivery is continuos and activity indipendent
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.
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.
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 \textbf{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 )
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 rectifi
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 the 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.
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.

![Graphs showing AMPA and NMDA EPSC amplitudes for GluR2(813-862)-GFP and GluR1(809-889)-GFP](image)
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
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.
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.
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 “dominat “ effect, as the heterodimer behaves like a GLUR1 homomer and do not get to synapses unless CMAKII is present or during LTP.
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.

FIG 6B
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 in 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 heteroligomer 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
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.
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