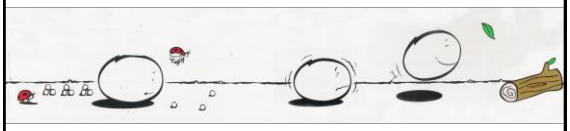
An atypical antidepressant drug regulates synaptic plasticity



Zhang, H., Etherington, L. A., Hafner, A. S., Belelli, D., Coussen, F., Delagrange, P., ... & Groc, L. (2013). Regulation of AMPA receptor surface trafficking and synaptic plasticity by a cognitive enhancer and antidepressant molecule. Molecular psychiatry, 18(4), 471-484.

Ed Shi
April 7, 2014

Molecular Psychiatry

- Nature Publishing Group
- Impact Factor: 14.897 (2012)
  - 1/135 Psychiatry
  - 7/251 Neuroscience
  - 5/290 Biochemistry & Molecular Biology

"Molecular Psychiatry" (2014). Retreived April 5, 2014. http://www.nature.com/mp/index.html

Laurent Groc

- Research Director at French National Center for Research (CNRS)
- Study of molecular mechanisms of neural connection formation
- Principal Investigator at Bordeaux Segalin Unversity
- Neuroscience PhD from Wayne State University, Michigan

Development and Adaptation of Neural Circuits. Retreived April 5, 2014. http://www.iins.u-bordeaux2.fr/research-teams/groc-team

Daniel Choquet

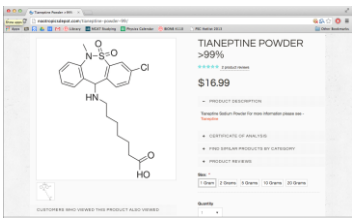
- Research Director at French National Center for Research CNRS
- Director of the Institute of Interdisciplinary Neuroscience – Bordeaux Segalin University
- Director of Bordeaux Imaging Center
- Study of receptor trafficking via high resolution imaging techniques
- PhD in neuroscience from Pasteur Institute, France

"Daniel Choquet Group" Retreived April 5, 2014. http://www.iins.u-bordeaux2.fr/research-teams/daniel-choquet-team

Others

- Honyu Zhang – Postdoc
- Anne-Sophie Hafner – Postdoc
- Francois Coussen – Researcher (now married to D. Choquet)

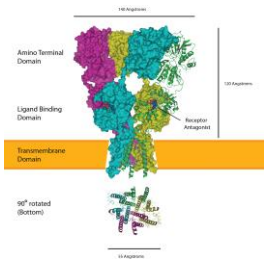
Tianeptine is an unregulated legal substance with an unknown site of action



- Not believed to directly modulate monoamine transmission
- Action may involve glutamate receptor transmission

"Tianeptine Powder"(2014). Retreived March 25, 2014. http://nootropicsdepot.com/tianeptine-powder-99/

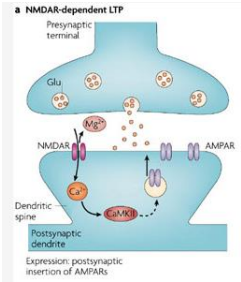
Ionotropic Glutamate Receptors (iGluRs)



AMPA receptor

- Ligand gated (glutamate) ion channels responsible for excitatory synaptic transmission
- AMPA Receptor (AMPAR)
  - Four subunits
  - GluA1-3 subunits
  - Excitatory
- NMDA Receptor (NMDAR)
  - Voltage dependent Mg^{2+} block
- Kainate Receptor

Mechanism of long term potentiation (LTP) synaptic plasticity at a glutamate synapse



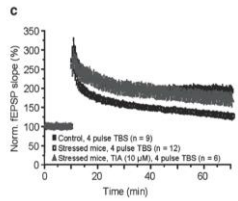
Kauer & Malenka 2007

1. Depolarization of postsynaptic membrane
2. Relief of NMDAR Mg^{2+} block
3. Ca^{2+} influx through NMDAR
4. Activation of Ca^{2+} Dependent Kinase
5. Phosphorylation of AMPAR facilitating surface delivery
6. More excitatory AMPAR, more excitable synapse

Compromised synaptic plasticity may underlie mood disorder

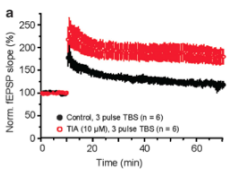
- Alterations in plasticity seen in stress-induced animal models of depression
- Synaptic plasticity mechanisms intimately related to denritic branching and brain region volume
- Regions affected include hippocampus, pre-frontal cortex, amygdala
- All important in cognitive and affective function
- **Tianeptine may exert its antidepressant effect by reversing compromised plasticity**

Tianeptine reverses stress induced decreases in LTP



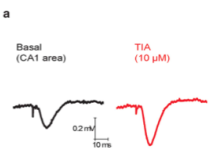
- LTP dependent on high frequency activation
- 4 pulse of high freq. stim results in subsequent higher fEPSP
  - Indicative of LTP
- Graph plot of fEPSP after LTP-inducing stimulation, as % of control
- Stress decreases magnitude of LTP
  - Effect reversed by tianeptine

Tianeptine decrease stimulation threshold for LTP



- 3 pulse stimulation produces change in fEPSP in control (black trace)
- Tianeptine administration results in significantly greater potentiation with 3 pulse stimulation (red trace)

Tianeptine reduces stress induced decrease in field EPSP



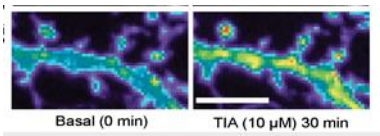
- Field EPSP
  - Extracellular recording of a population of neurons
  - A measurement of basal synaptic transmission strength

Tianeptine molecular mechanism involves AMPA receptor lateral diffusion

GluA1 AMPA receptor subunit is important in LTP

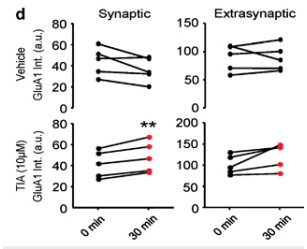
- Regulatory phosphorylation sites
  - Serine-831: receptor trafficking
  - Serine-845: modulate how often receptor can open
- AMPA receptors containing only GluA1 are trafficked to synapse in early LTP
  - Open "wider"
  - Permeability to Ca^{2+} may play later role in LTP

Tianeptine reduces stress induced decrease of surface GluA1 AMPA receptor subunit



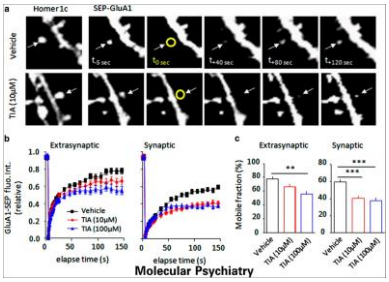
- Fluorescent imaging of GluR1 :: superEcliptic pHluorin (SEP) intensity
  - pH sensitive fluorescent protein
  - Strong emission indicative of extracellular environment (~neutral pH)
  - Low emission in acidified intracellular vesicles

Tianeptine increases GluA1 at the synapse



- Quantification of GluA1 at synapse by fluorescent intensity
- Postsynaptic protein homer1c is tagged with red fluorescent protein
  - Co-localization with tagged homer1c defines "synaptic"

Tianeptine decreases GluA1 AMPAR surface diffusion

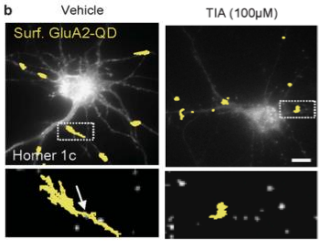


- GluA1 :: SEP used to visualize
- Light pulse is used to bleach SEP
- Unbleached tagged GluA1 diffuse laterally and florescence is recovered
- In (a) & (b) qualitative and quantitative fluorescence recovery is faster with tianeptine
  - Indicates greater lateral mobility

GluA2 AMPA receptor subunit quantum dot (QD) tracking

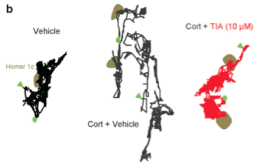
- Antibody specific for N-terminal domain of GluA2
  - QD secondary antibody
- Visualization using mercury lamp and regular CCD video camera
- High signal/noise ratio vs. fluorescent protein

Tianeptine decreases GluA2-AMPAR surface diffusion



- Yellow traces show path of a single GluA2 containing AMPA receptor
- Quantification of diffusion revealed significant differences

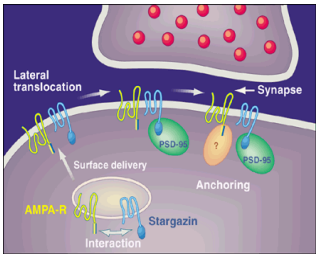
Tianeptine reduces glucocorticoid incubation induced AMPAR movement



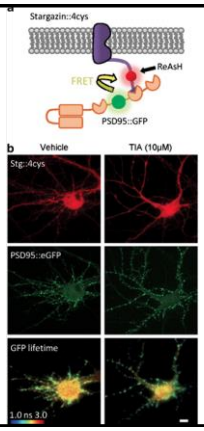
- Glucocorticoid (GC) is a stress hormone
- Activation of glucocorticoid receptors in brain implicated in effects of stress

- Figure shows trajectory of QD tagged GluA2
  - GC induced mobility increase is reduced by tianeptine

The molecular mechanism of tianeptine action is dependent on a CaMKII – Stargazin – PSD-95 interaction



- Dependent on CaMKII phosphorylation of Stargazin protein that associates with AMPARs
- Phosphorylated Stargazin must bind PSD-95 (a synaptic strutural protein)
- Inhibition of either of these steps by drug or mutation prevented the effects of tianeptine



Further evidence for affect on Stargazin – PSD95 interaction

- Energy emitted by GFP on PSD95 is absorbed by red fluorophore on Stargazin if two are close together
  - GFP will become dimmer
- Measurement of GFP "lifetime" quantifies stargazin-PSD95 binding
- GFP lifetime is significantly shorter in tianeptine administration

Summary

- Tianeptine reduces stress induced LTP changes
  - Perhaps through reduction of LTP-inducing stimulation threshold
- Tianeptine reduces lateral diffusion of AMPA receptors out of the synapse
  - No effect on endocytosis or exocytosis seen
- A CaMKII – Stargazin – PSD-95 interaction is necessary for the effects of tianeptine