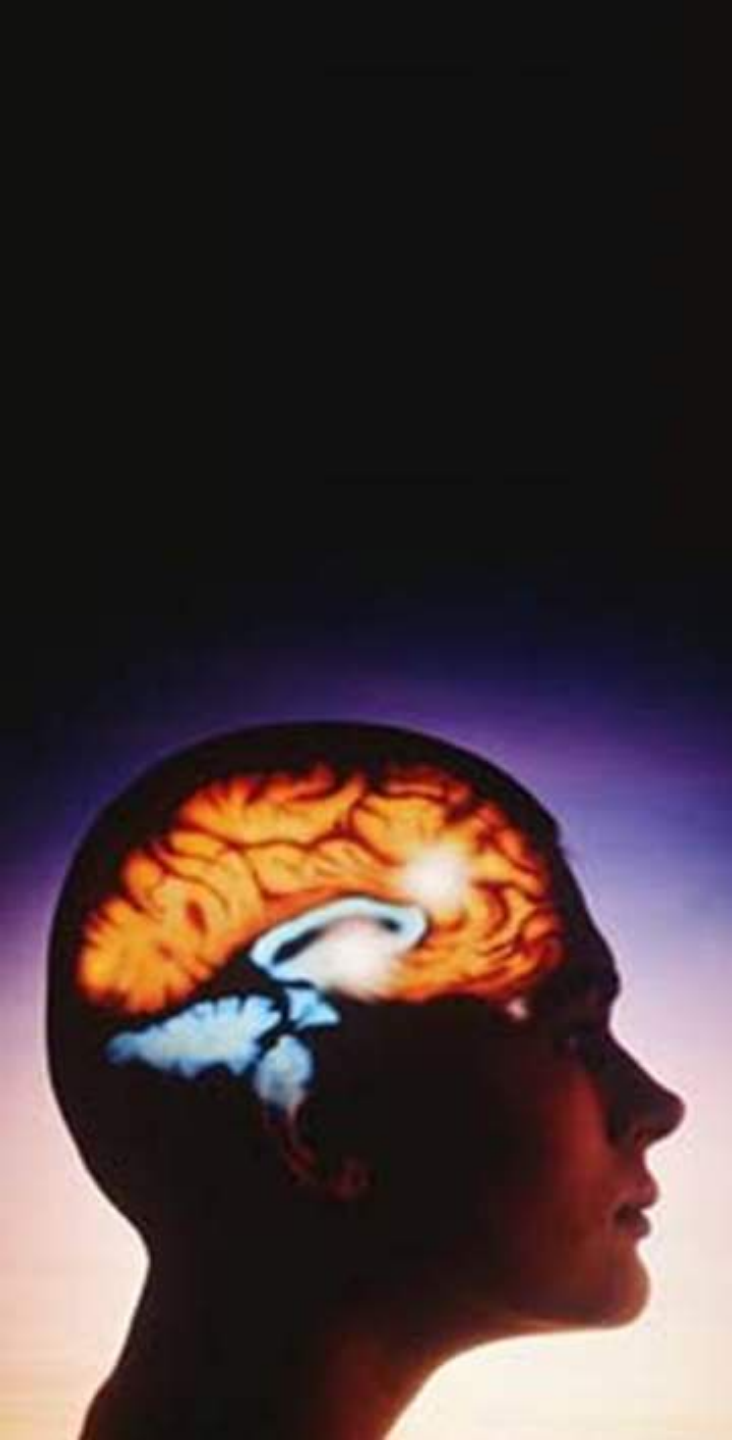

New Insights into the Pathophysiology of Major Depressive Disorder

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CIBER Salud Mental (CIBERSAM)

Major Depressive Disorder

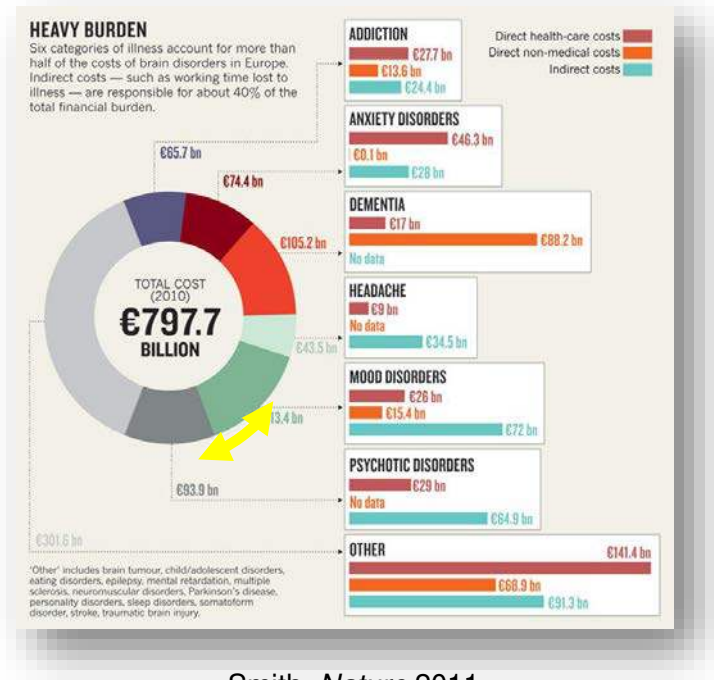
Leading cause of disability (years lived with disability; YLD) in 56 countries, second leading cause in another 56 countries and third cause in 34 countries (Global Burden of Disease Study Collaborators, *Lancet* 2015)

Lifetime prevalence of major depression: 20% women, 10% men. Long duration of depressive episodes; emergence during active periods of life

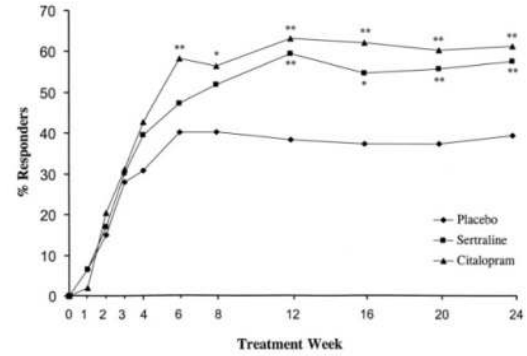
Slow clinical action and limited efficacy of AD treatments: 60% response, 40% remission after 6-8 wk of treatment in controlled trials

Real world figures (STAR*D): 80% patients are chronic or recurrent. Response and remission rates at 8 wk are 43% and 28%. Remission rates after 4 sequenced treatments (12 months) increase to 67% (Rush et al., *Am J Psychiatry* 2006; Trivedi et al., *Am J Psychiatry* 2006) → poor quality of life and increased suicide risk

Urgent need to understand pathophysiology of MDD and to improve treatments



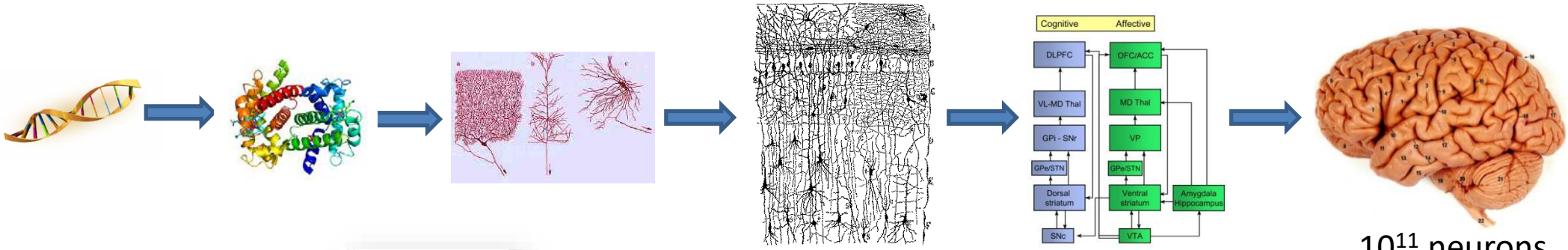
Smith, *Nature* 2011



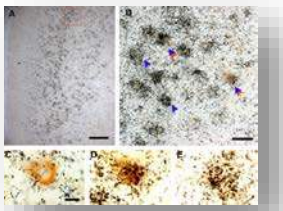
Stahl, *Biol Psychiatry* 2000

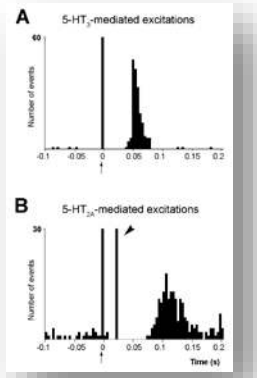
Mental Disorders and Brain Circuits

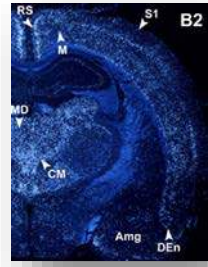
- Mental disorders are "*connectopathies*", with complex pathological mechanisms that apply at the level of circuits and their communication (Bargmann and Lieberman, *Am J Psychiatry* 2014)
- Alterations at different levels of complexity or of different neurobiological mechanisms may give rise to similar symptoms or disorders (e.g., psychotic symptoms can be evoked by an excess of dopaminergic activity or by blockade of NMDA receptors, among others)
- On the other hand, psychiatric drugs may produce their therapeutic effects by acting at different levels of complexity, yet they usually target neuronal communication by altering the efficacy of neurotransmitter systems. Different drugs can have a common clinical action by acting on different initial molecular/cellular elements

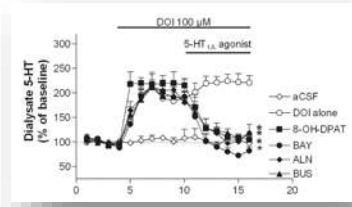


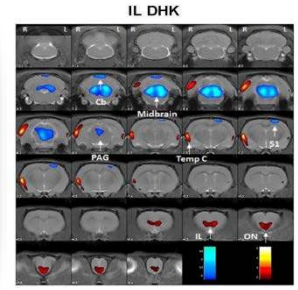
10^{11} neurons

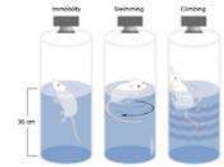






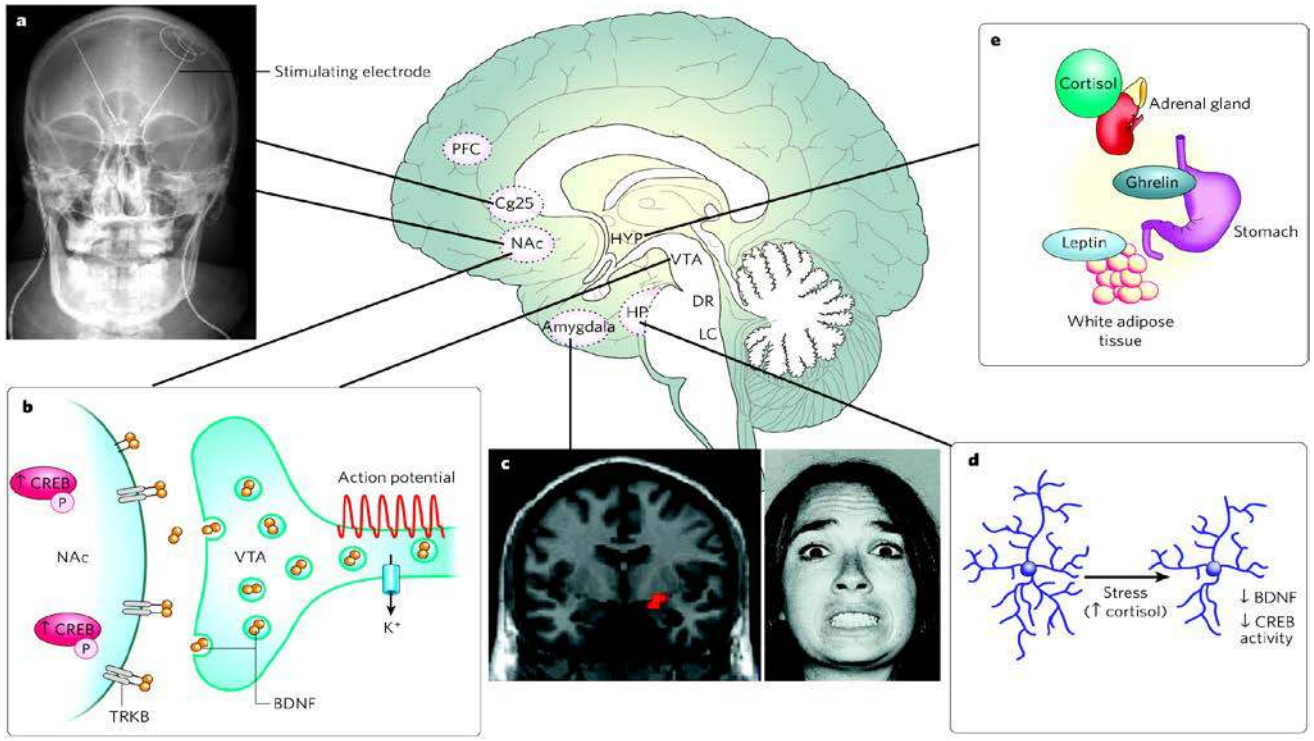






Major Depressive Disorder

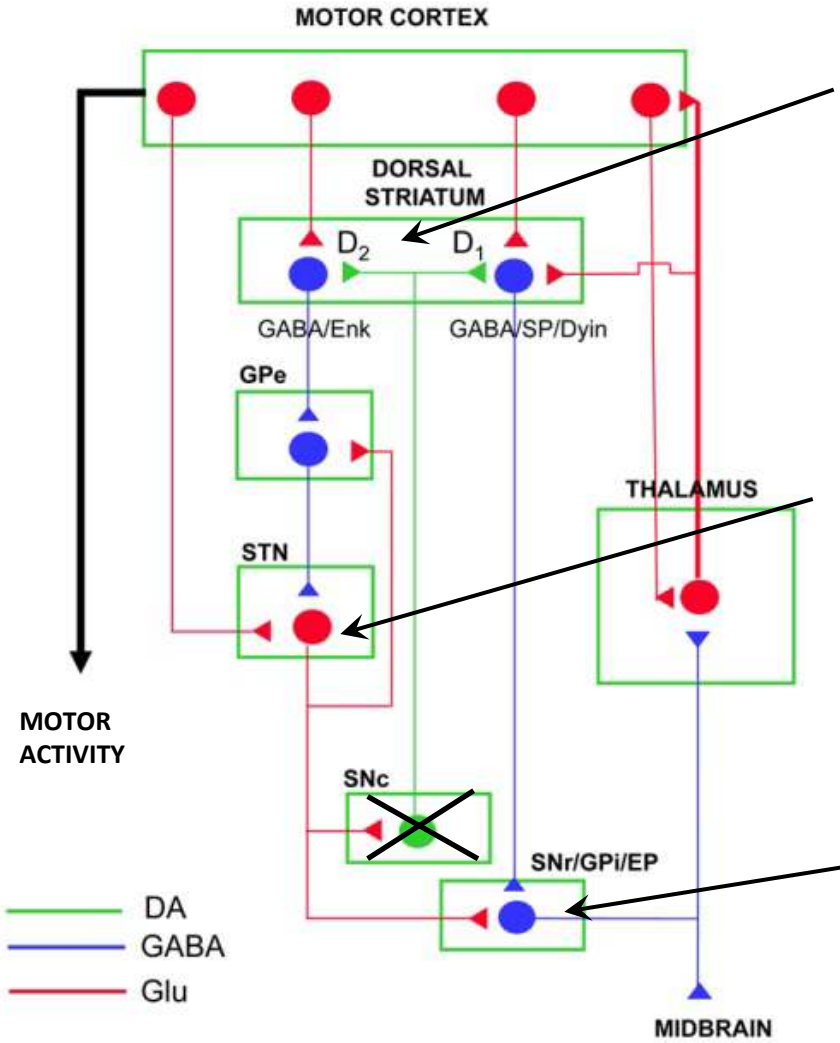
MDD arises from a complex interaction between multiple genes and environmental factors, including stress and aversive stimuli



- **Monoamine deficiency**
- **Neuroendocrine dysregulation**
- **Neurotrophic impairment**
- **Altered excitatory synapses**
- **Altered glutamate-astrocyte interaction**
- **…….**

Krishnan & Nestler. *Nature* 2008

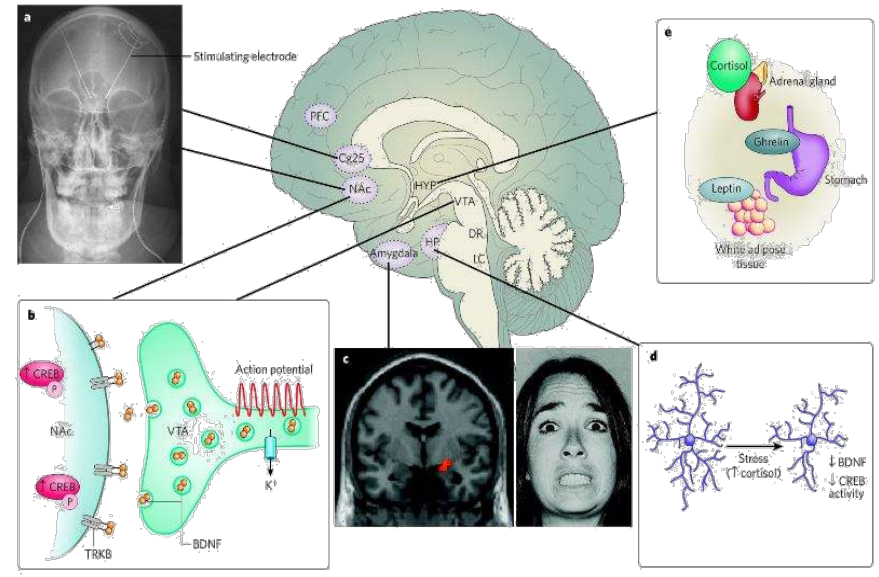
Parkinson's Disease vs. Major Depressive Disorder



L-DOPA
DA agonists

Deep brain stimulation

Future drugs?

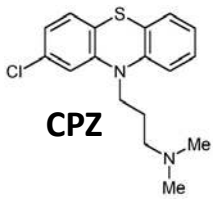


Krishnan & Nestler. *Nature* 2008

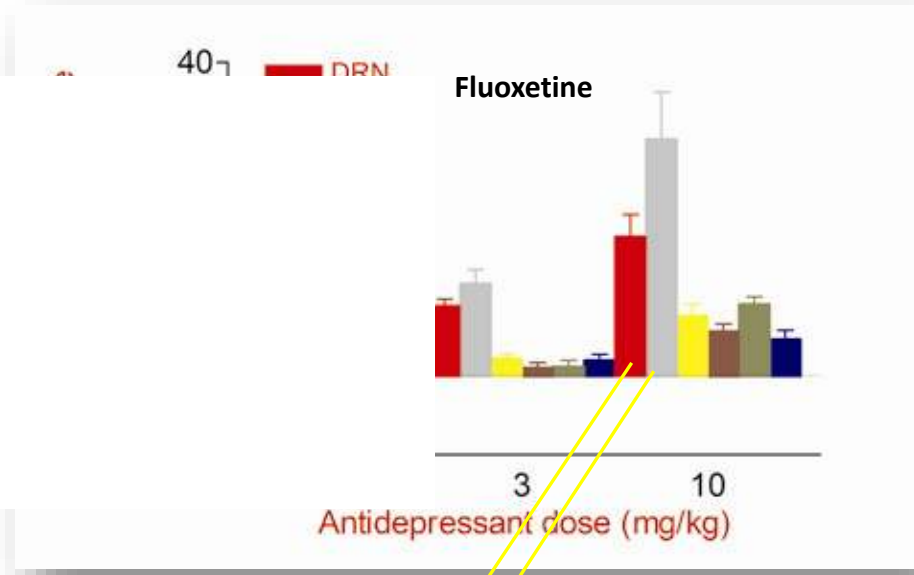
- **Monoamine deficiency**
- **Neuroendocrine dysregulation**
- **Neurotrophic impairment**
- **Neuroinflammation**
- **Altered excitatory synapses**
- **Alterations in glial cell number/function**
- **……**

Monoaminergic AD Drugs

- Current antidepressant drugs (SSRI, SNRI) are pharmacological refinements of TCA, discovered by serendipity 60 yr ago when searching for antipsychotic drugs (chlorpromazine-like tricyclic drugs)

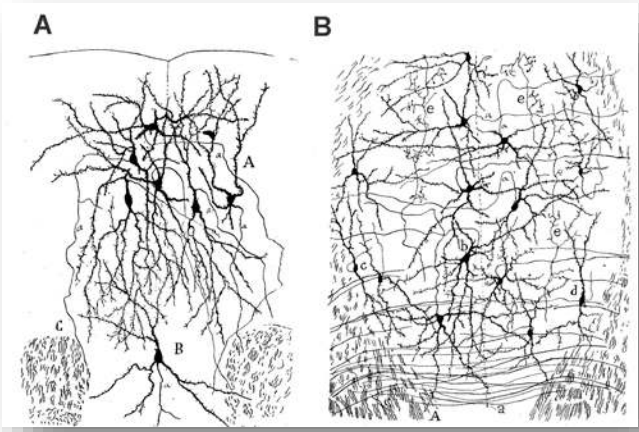


- Following reuptake blockade, AD drugs trigger presynaptic negative feed-back mechanisms through autoreceptor activation that delay and self-limit their pharmacological effects and therapeutic action



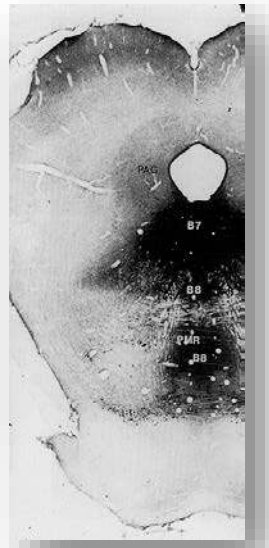
Hervás and Artigas, *Eur J Pharmacol* 1998

Anatomy



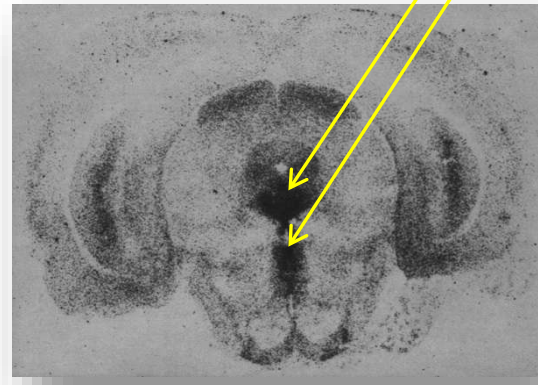
Cajal, 1905

5-HT fibers



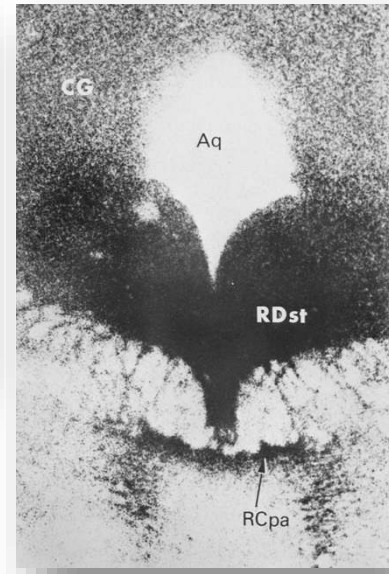
Paxinos
The Rat Nervous System, 2004

5-HT transporter (rat)



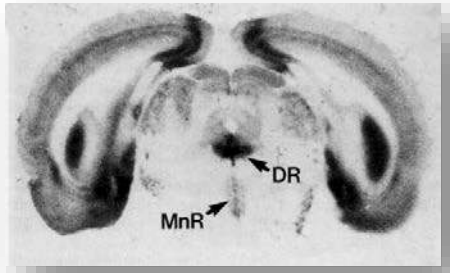
Fuxe et al., *PNAS* 1983

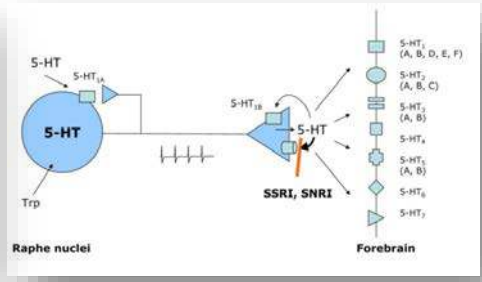
5-HT transporter (human)



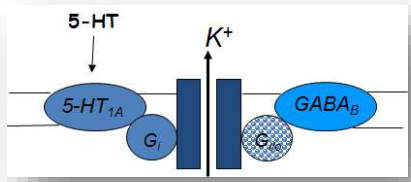
Cortés et al., *Neuroscience* 1988

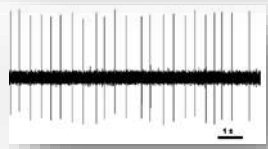
5-HT$_{1A}$ autoreceptor: The Main Brake of the 5-HT System





Pompeiano et al., *J Neursoci* 1992





Excitation — Glu (AMPA, NMDA)

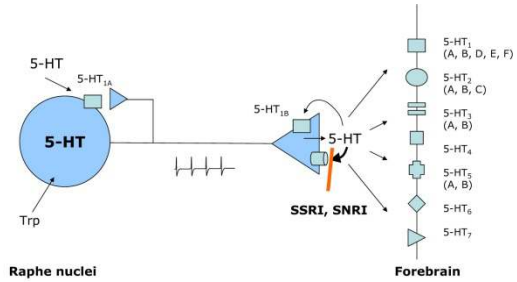
Self-inhibition — 5-HT$_{1A}$

- Low number of neurons: 250.000 5-HT neurons in the human brain (out of a total of 10^{11})
- Extensive arborisation (>10^6 nerve terminals/mm^3)
- Innervation of the whole neuraxis
- Control of activity by descending (prefrontal cortex, lateral habenula, hypothalamus, etc.) and ascending (locus coeruleus, spinal cord) inputs to the raphe nuclei
- Mutual control with monoaminergic cell groups
- Slow and regular discharge (pacemaker neurons): ***strong homeostasis***
- Neuronal activity dependent on sleep-wake cycles (REM-off neurons)
- Rich neurochemistry: 14 different postsynaptic receptors
- ***Very sensitive to self-inhibition by autoreceptors***
- Implication in a large number of physiological functions

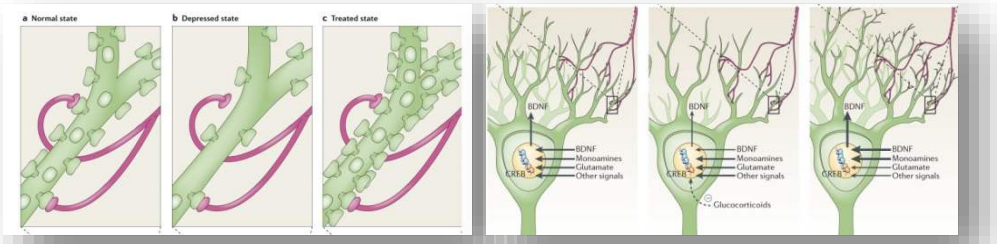
Artigas, *Pharmacol Ther* 2013

Serotonergic AD Drugs

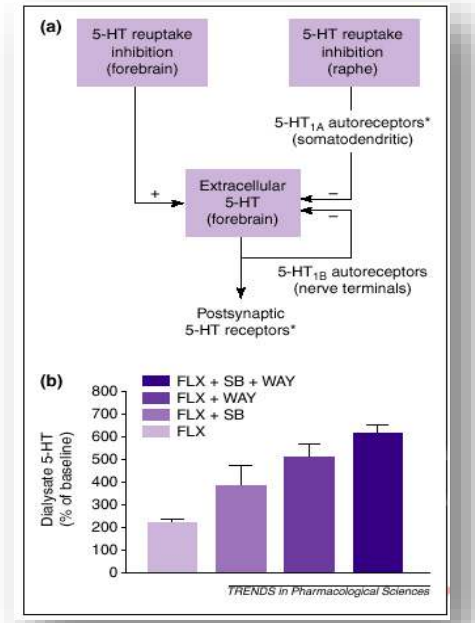
- They trigger presynaptic negative feed-back mechanisms through autoreceptor activation that self-limit their pharmacological effects and therapeutic action



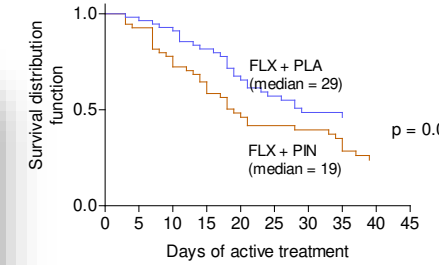
- They also trigger slow postsynaptic adaptive changes involved in the therapeutic action (increased expression of trophic factors, increased neurogenesis and neuronal complexity, etc.)

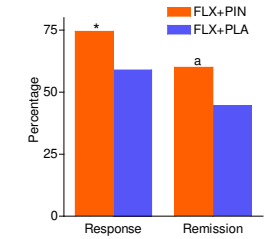


Berton and Nestler, *Nat Rev Neurosci* 2006

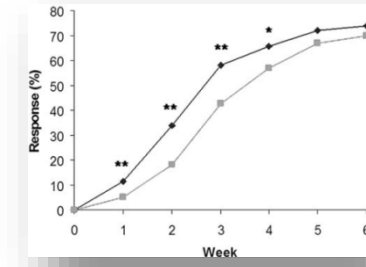


Artigas et al., *Trends Neurosci* 1996
Artigas et al., *Trends Pharmacol Sci* 2001





Pérez et al., *Lancet* 1997

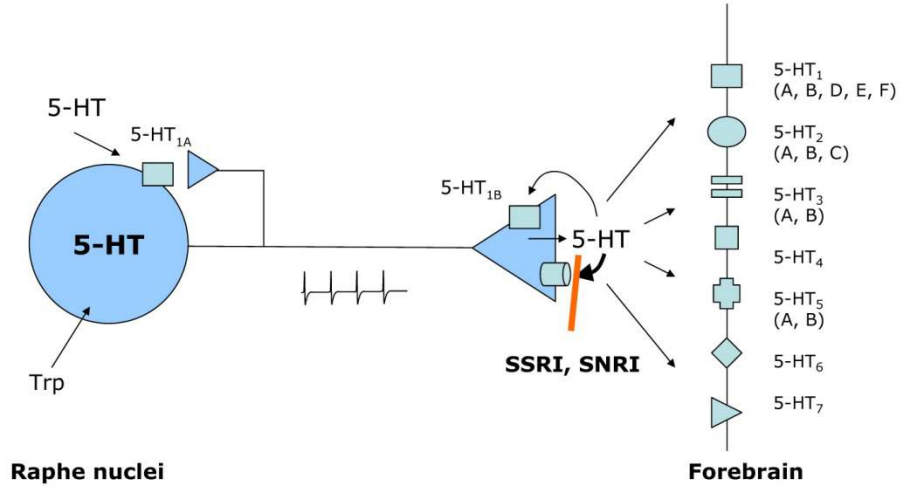


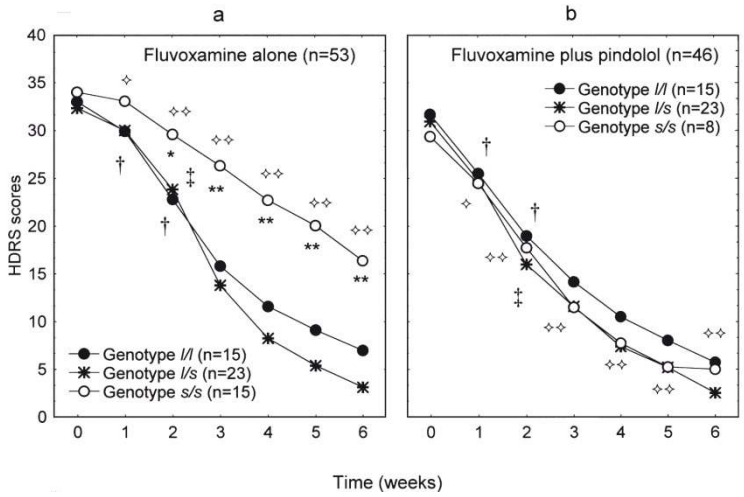
Ballesteros and Callado , *J Aff Disord* 2004
Whale et al., *J Psychopharmacol* 2010
Portella et al., *J Clin Psychiatry* 2011

Two new antidepressant drugs incorporate partial $5HT_{1A}$ agonist activity to SERT inhibition (vilazodone, vortioxetine). Vortioxetine also prevents $5\text{-}HT_3$ R–mediated inhibition of 5-HT release and increases pyramidal neuron discharge in mPFC

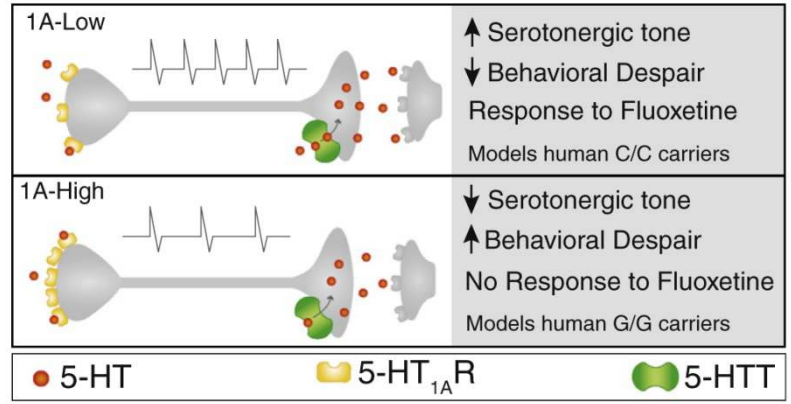
Monoaminergic AD Drugs: Genetic Factors

- Genetic factors of the two main players determining AD response (SERT and 5-HT_{1A} autoreceptors)
  - Patients with the short (s/s) form of SERT show lower response rates than those with long form (l/l) or heterozygotes (s/l)
  - Likewise, patients with over-expression of 5-HT_{1A} autoreceptors show reduced antidepressant response and increased incidence of suicide. Mice models mimic findings in patients





Smeraldi et al., *Mol Psychiatry* 1998



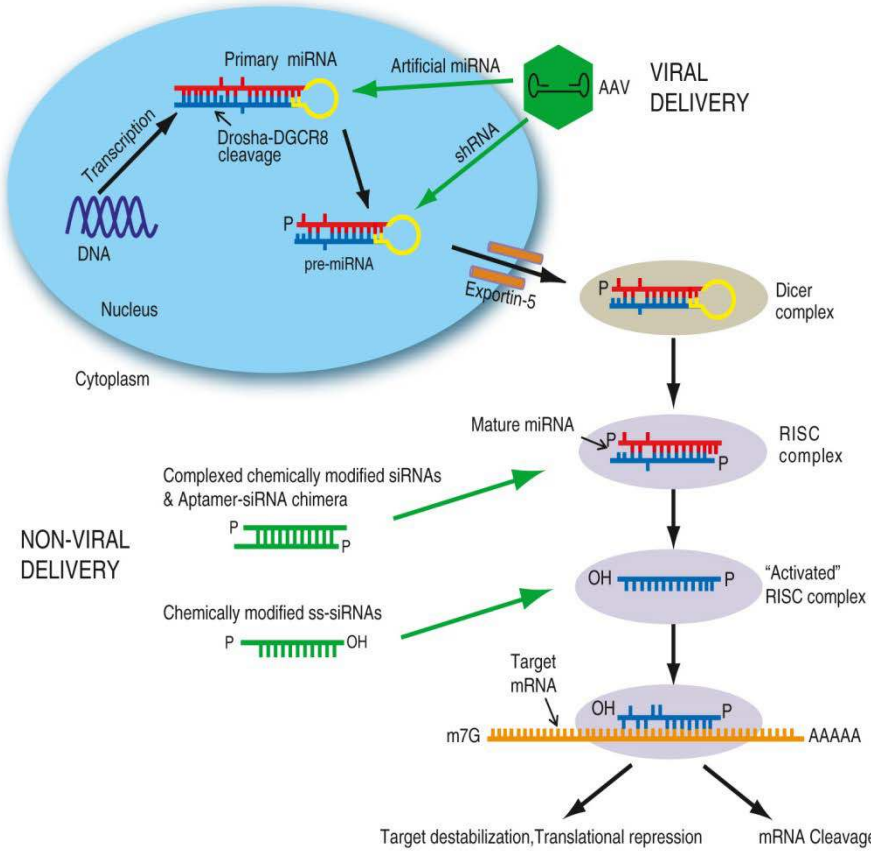
Richardson-Jones et al., *Neuron* 2010

Use of RNAi for CNS Disorders

- New therapeutic strategy for the treatment of mental disorders. The Nobel Prize in Physiology and Medicine 2006 was awarded to Andrew Z. Fire and Craig C. Mello "*for their discovery of RNA interference – gene silencing by double-stranded RNA*"
- A substantial percentage of protein-encoding genes may be regulated by microRNA (miRNAs): e.g., miR-16 regulates SERT expression (Baudry et al., *Science* 2010) and miR-135 regulates SERT and 5-HT_A receptors; (Issler et al., *Neuron* 2014). miR-186 over-expression reduces ß-amyloid formation (Kim et al., *J Neurochem* 2016)
- Ability to selectively target any protein-encoding mRNA with siRNA, avoiding unspecific drug actions
- Particularly useful for disorders lacking appropriate pharmacological treatments

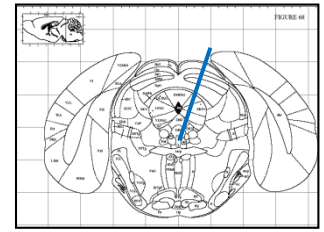
however…

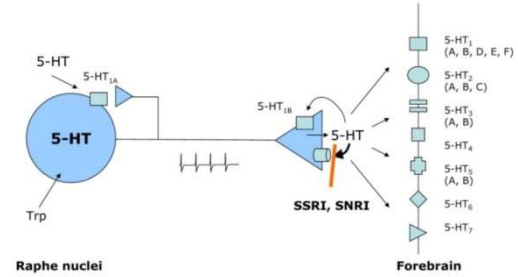
- **How to deliver siRNAs or miRNAs to the CNS?**
- **Which administration route? Need to avoid peripheral degradation by RNAses**
- **Once in CNS, how to target specific neuronal populations?**



RNAi Strategies as Fast-Acting AD Treatments

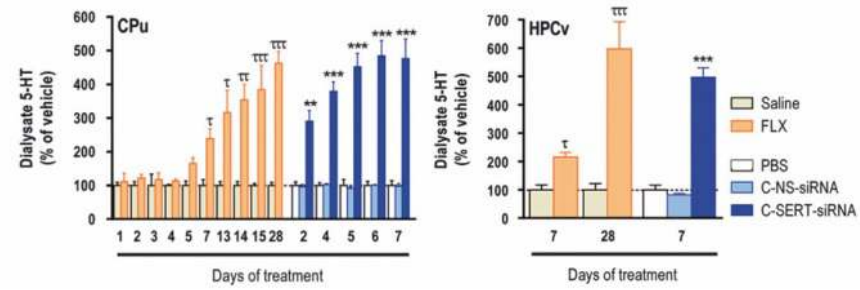
- Use of small interfering RNA (siRNA) targeting SERT or 5-HT_{1A} autoreceptors. Local application in mouse DR
  - Ferrés-Coy et al., *Psychopharmacology* 2012
  - Ferrés-Coy et al., *Transl Psychiatry* 2013



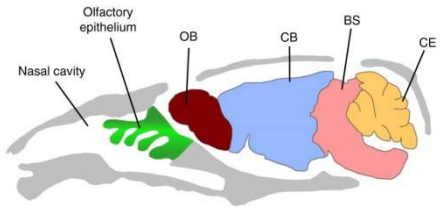


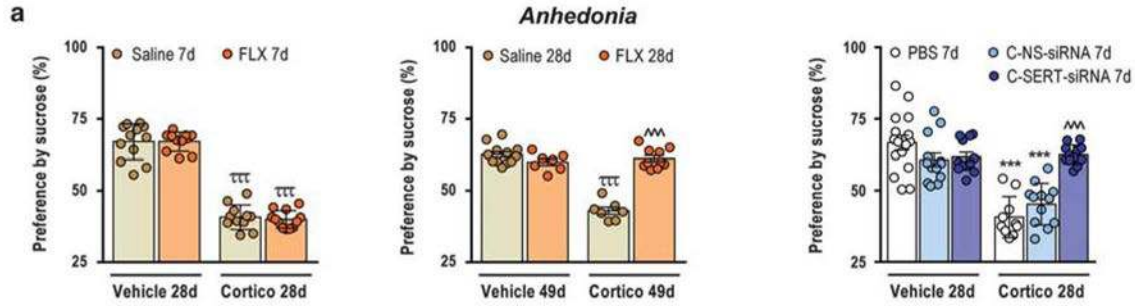
- Design of sertraline-conjugated siRNA that can be administered intranasally to target genes expressed in 5-HT neurons (5-HT_{1A} autoreceptors, SERT, TASK3 potassium channels)
  - Bortolozzi et al., *Mol Psychiatry* 2012
  - Ferrés-Coy et al., *Mol Psychiatry* 2016
  - Artigas and Bortolozzi, *Neuropsychopharmacology* 2017
  - Fullana et al., *Mol Neurobiol 2018*

1 wk SERT-siRNA ≡ 4 wk Fluoxetine

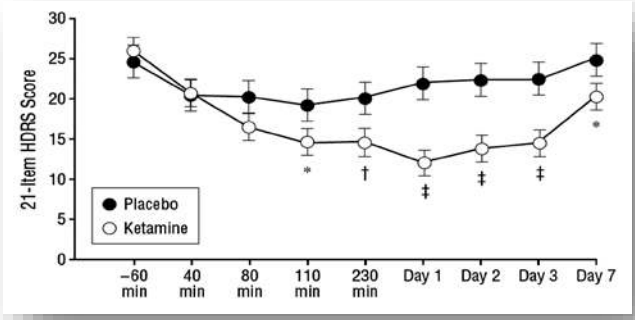


15 µg/5 µl in each nostril



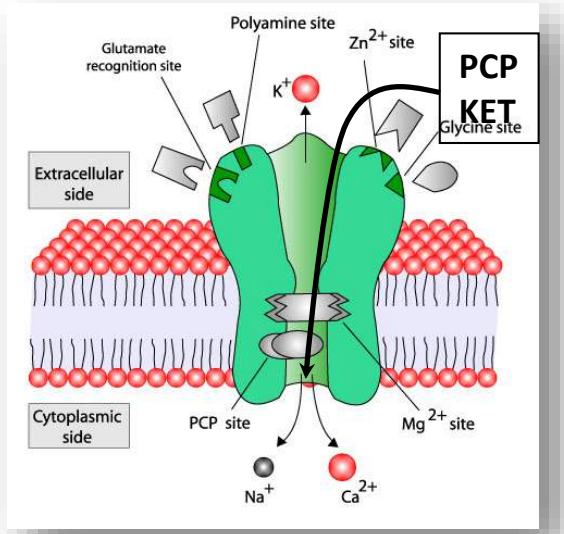


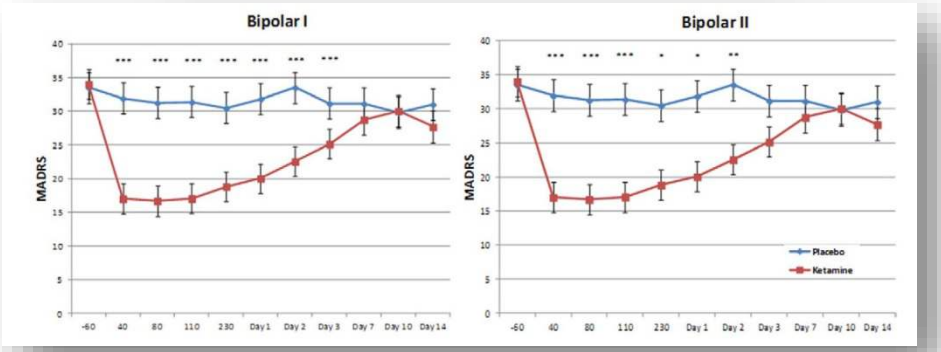
The Glutamatergic System as a New Target for AD Drug Development: Antidepressant Effects of Ketamine



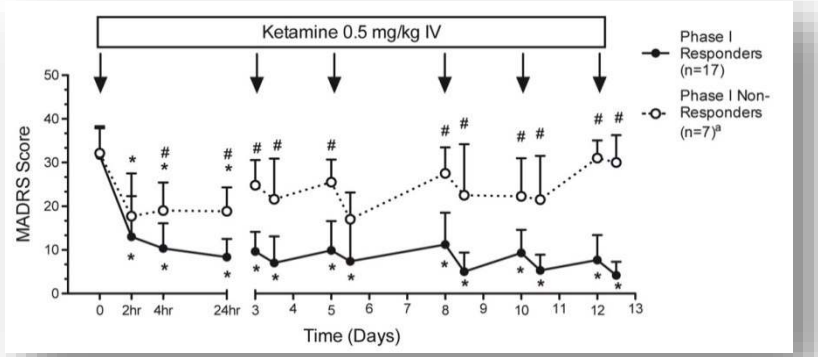
Zarate et al., *Arch Gen Psychiatry* 2006

FDA approved intranasal ketamine for treatment-resistant n MDD (March 2019)

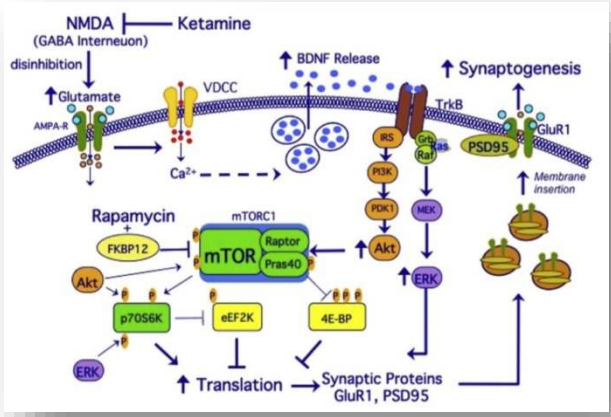




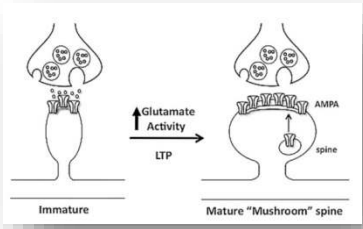
Zarate et al., *Biol Psychiatry* 2012



Murrough et al., *Biol Psychiatry* 2013



Duman et al., *Neuropharmacology 2012*



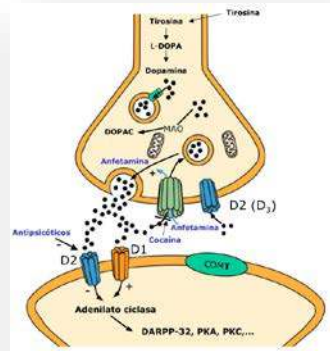
Synaptic Contacts and Neurotransmitters

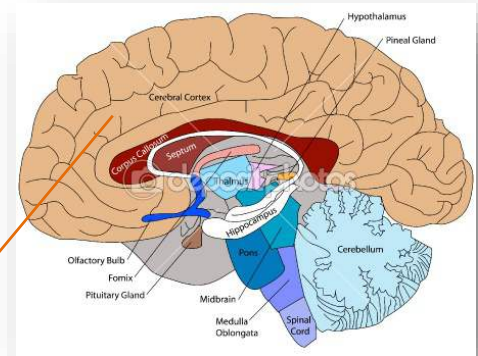
- Synapses are the active zones of communication between neurons

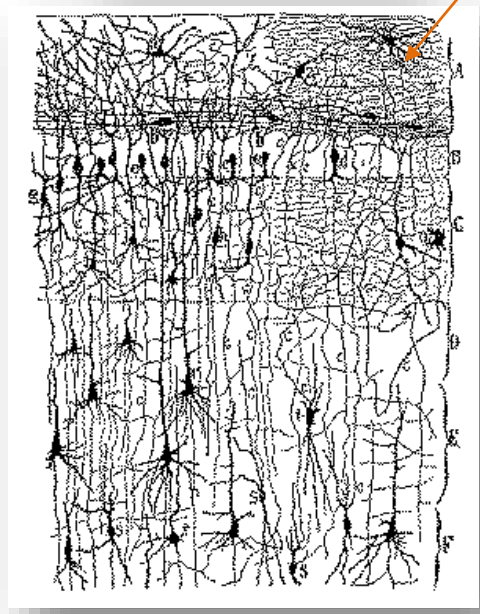
- Multiple chemical neurotransmitters
  - Glutamate (excitation; accelerator)
  - GABA (inhibition; brake)

  - ACh
  - Catecholamines (DA, NA)
  - Serotonin (5-HT)
  - Histamine
  - Peptides
  - Gases (NO, CO)
  - etc.

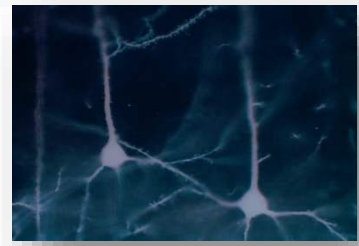
- Receptors: *I*) ionotropic (e.g., AMPA, NMDA, KA, 5-HT_3…), and *ii*) metabotropic (G-compled protein receptors)

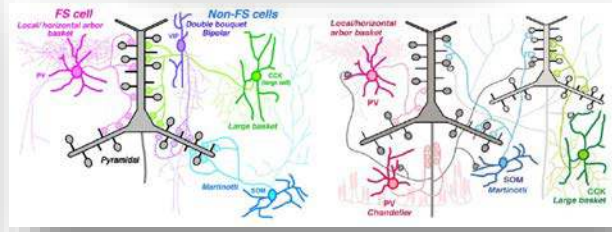






Cajal, 1905



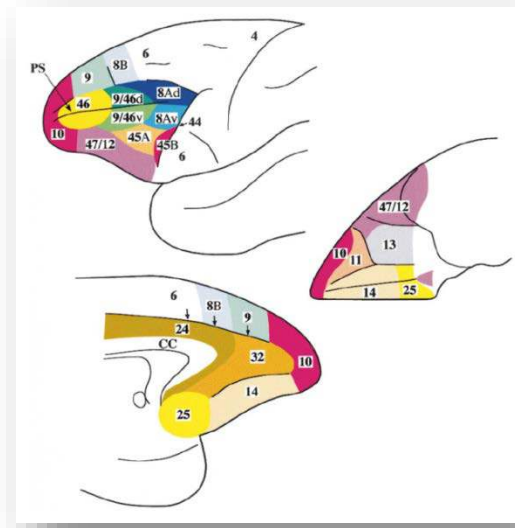


75-80% of pyramidal excitatory neurons

15-20% of GABAergic interneurons

The Prefrontal Cortex in Psychiatric Disorders

- Most rostral part of the frontal lobe: defined by its reciprocal connectivity with the mediodorsal thalamus
- The PFC is the highest association cortex and *exerts a top-down control of neuronal activity* in most other cortical and subcortical areas
- The PFC is involved in higher brain functions which are altered in psychiatric patients: cognition, mood and emotional control , behavioral inhibition, etc.
- Alterations of energy metabolism in PFC of MDD and schizophrenic patients
- Anatomical, cellular and biochemical abnormalities in PFC of schizophrenic patients
- The PFC contains a moderate-high density of neurochemical elements participating in antidepressant and antipsychotic drug action (e.g., SERT, NET, 5-HT_{1A}, 5-HT_{2A}, α_1-adrenoceptors, DA D1 D2..)

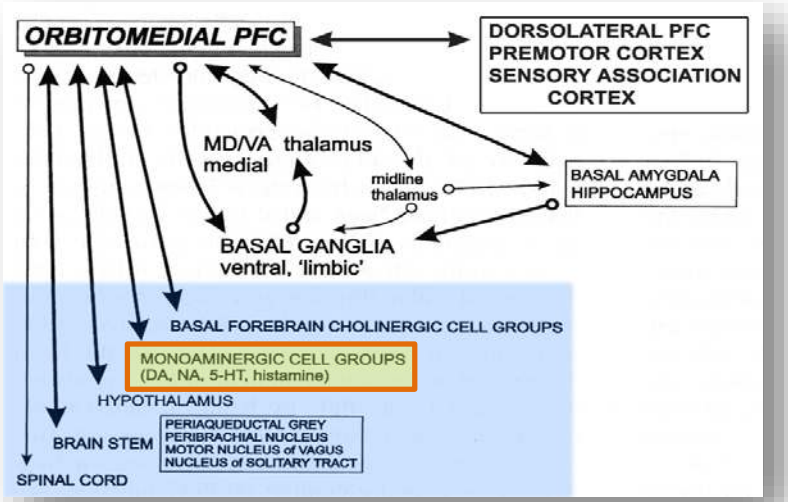


Fuster, *Neuron* 2001

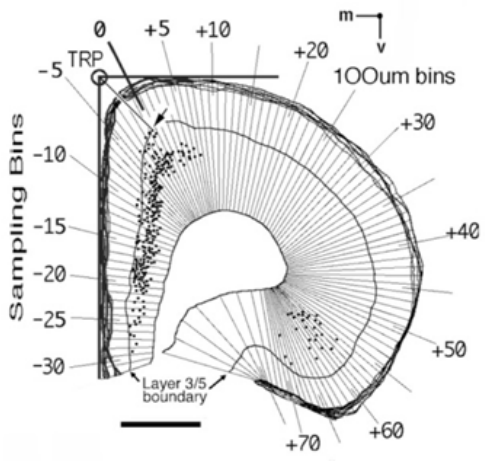
- **Orbito-frontal**– involved in behavioral inhibition
- **Dorsolateral**– involved in working memory, executive functions and action planning
- **Ventromedial**– involved in emotional processing
- **Dorsal anterior cingulate**– involved in attention and perception

Prefrontal Cortex: The Conductor of the Brain Orchestra

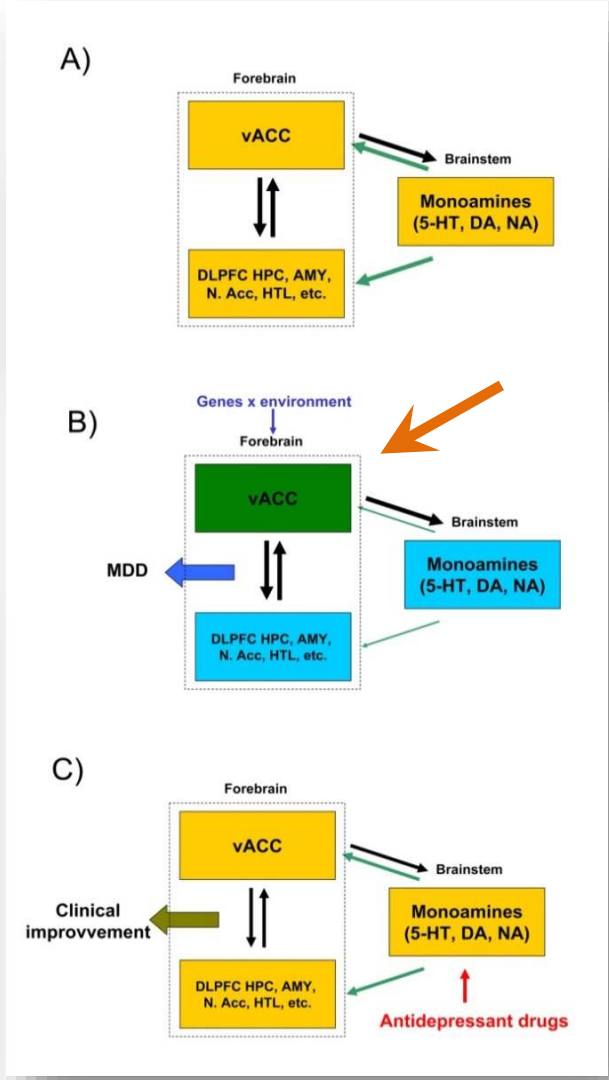
Dysfunctional Ventral Prefrontal Areas in MDD: Deleterious Impact on 5-HT Function?



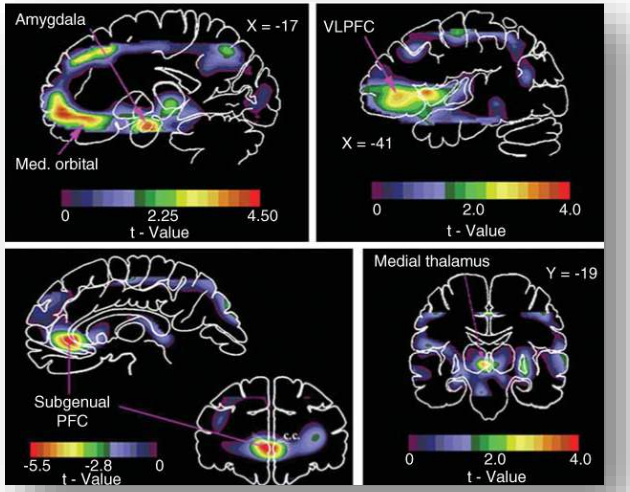
Groenewegen and Uylings, *Prog Brain Res* 2000



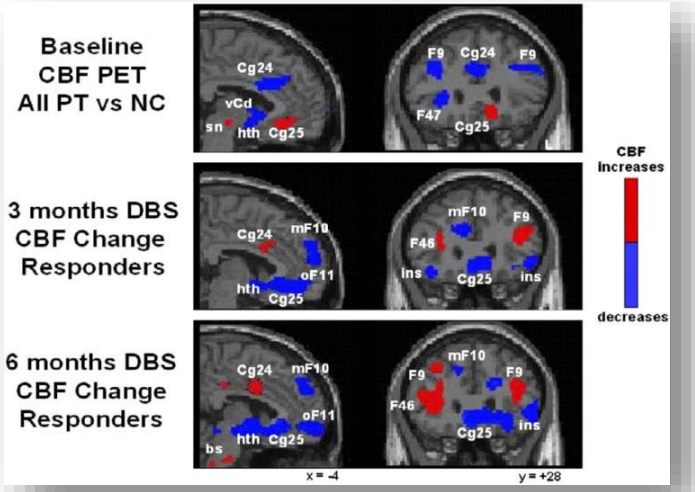
Gabbott et al., *J Comp Neurol* 2005



Artigas, *Eur Neuropsychopharmacol* 2015



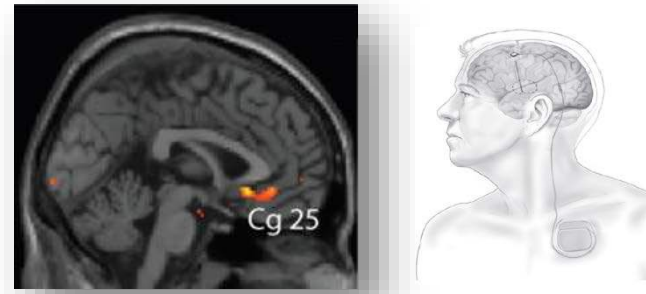
Price and Drevets, *Neuropsychopharmacology* 2010



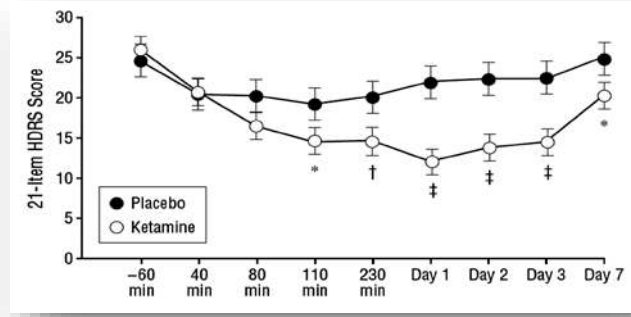
Mayberg et al., *Neuron* 2005

Fast-Acting Antidepressant Strategies: DBS and Ketamine

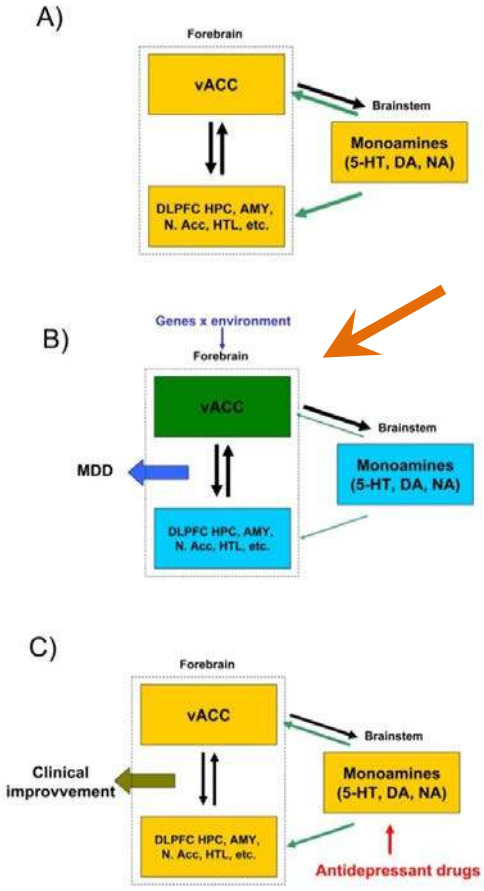
- Rapid antidepressant effects of DBS (Cg25) in treatment-resistant patients (Mayberg et al., *Neuron* 2005; Puigdemont et al., *Int J Neuropsychopharmacol* 2012)
- Rodent studies show that the effect of DBS is associated to an enhancement of 5-HT function via AMPA-R (e.g., Hamani et al., *Biol Psychiatry* 2010; Veerakumar et al., *Biol Psychiatry* 2014; Jiménez-Sánchez et al., *Cereb Cortex* 2016)



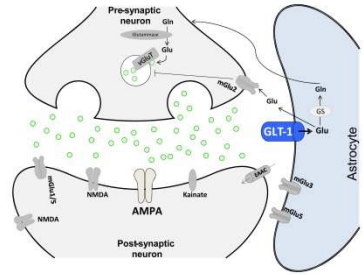
- Intravenous ketamine administration evokes *immediate* and *persistent* effects in subpopulations of treatment-resistant unipolar and bipolar patients (Zarate et al., *Arch Gen Psychiatry* 2006)
- Effects possibly mediated by AMPA-R stimulation and dependent on mTOR signalling (Duman and Aghajanian, *Science* 2012)
- *Optogenetic stimulation of IL* (rodent equivalent of vACC) mimics antidepressant effects of ketamine (Fuchikami et al., *PNAS* 2015)



Modulation of Glutamatergic Neurotransmission in Infralimbic Cortex by Attenuating Glial Glu Uptake. Downstream Effects on 5-HT Function



- Acute, short-term increase of excitatory neurotransmission in rat IL through the local blockade of GLT-1 with DHK.
- Persistent disruption of excitatory neurotransmission in mouse IL evoked by siRNA-induced knockdown of GLT-1 and GLAST.

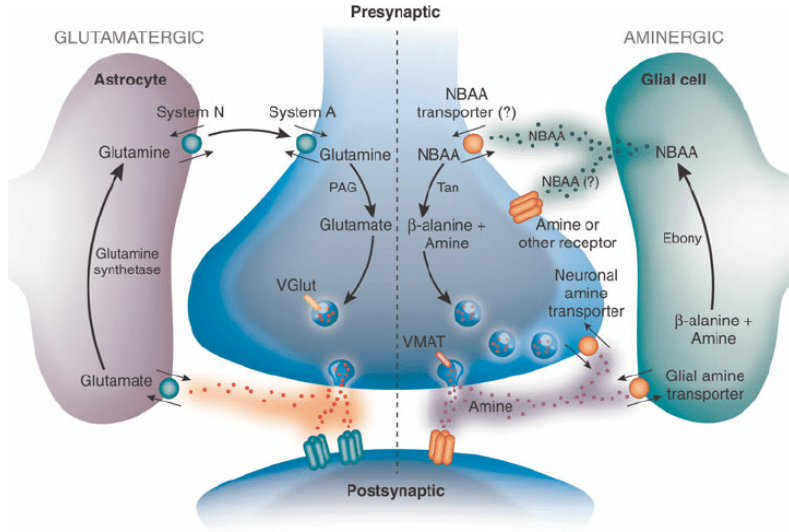


GLAST (EAAT1)
GLT-1 (EAAT2)

Artigas, *Eur Neuropsychopharmacol* 2015

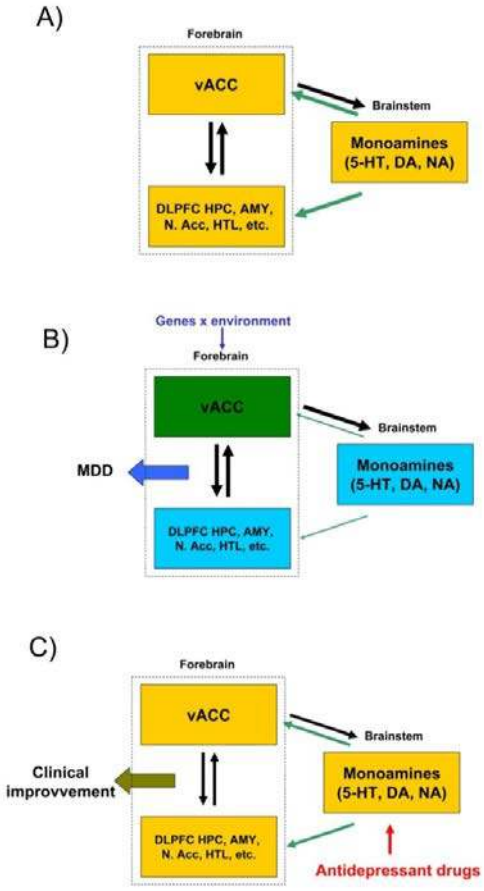
Glial Cells

- 10^{11} neurons, 10^{12} glial cells in human brain
- Provide support for neurons (structural, nutritional and metabolic)
- Can replace themselves
- Serve to clean up the brain, remove dead tissue and foreign objects
- Play a large role in brain development
- Different cellular types: microglia, oligodendrocytes, astrocytes, etc.
- Increasing role in neuronal communication by controlling synaptic neurotransmitters and by releasing gliotransmitters

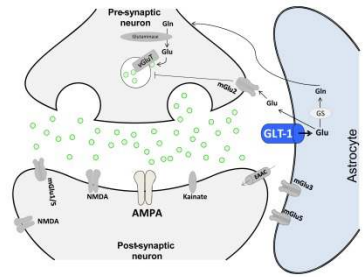


- **Alterations in glial cell number/function in MDD**

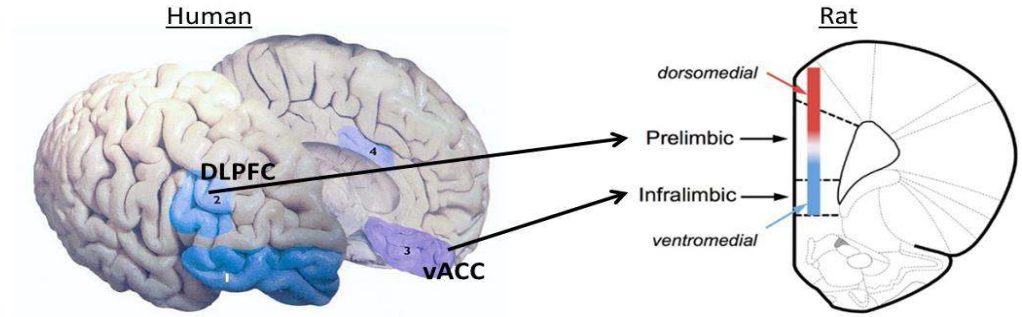
Modulation of Glutamatergic Neurotransmission in Infralimbic Cortex by Attenuating Glial Glu Uptake. Downstream Effects on 5-HT Function



- Acute, short-term increase of excitatory neurotransmission in rat IL through the local blockade of GLT-1 with DHK.
- Persistent disruption of excitatory neurotransmission in mouse IL evoked by siRNA-induced knockdown of GLT-1 and GLAST.
- Comparison with PrL in both models



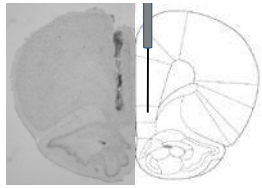
GLAST (EAAT1)
GLT-1 (EAAT2)

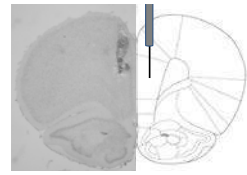


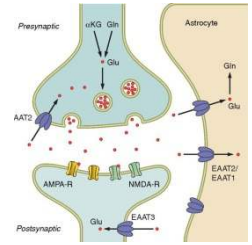
Artigas, *Eur Neuropsychopharmacol* 2015

Selective Blockade of GLT-1 in Infralimbic Cortex Induces AD-like Effects in Rats: Involvement of AMPA-R

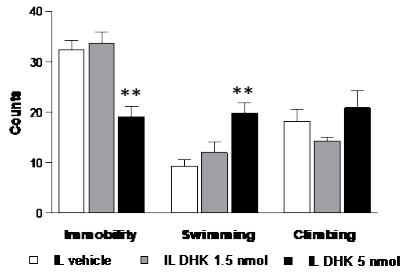
DHK (GLT1 inhibitor – glial Glu uptake)



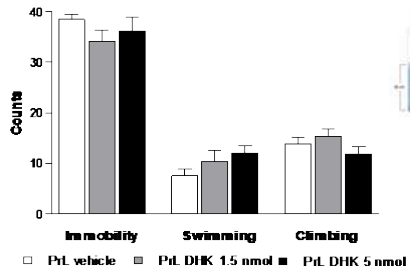


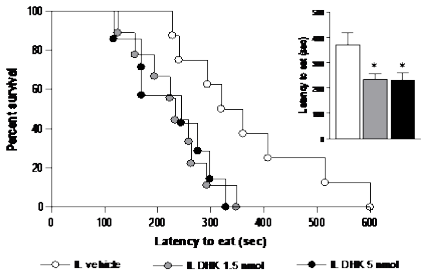


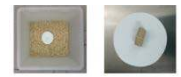
IL

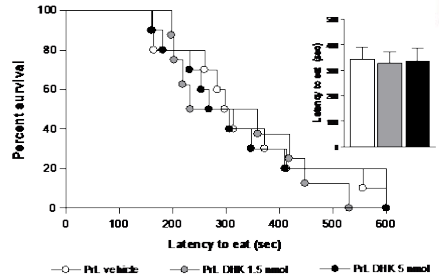


PrL



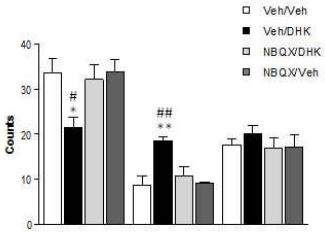






DHK effect blocked by NBQX and mimicked by S-AMPA

IL



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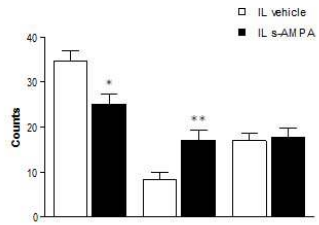
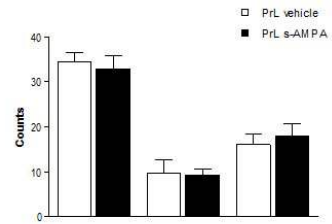
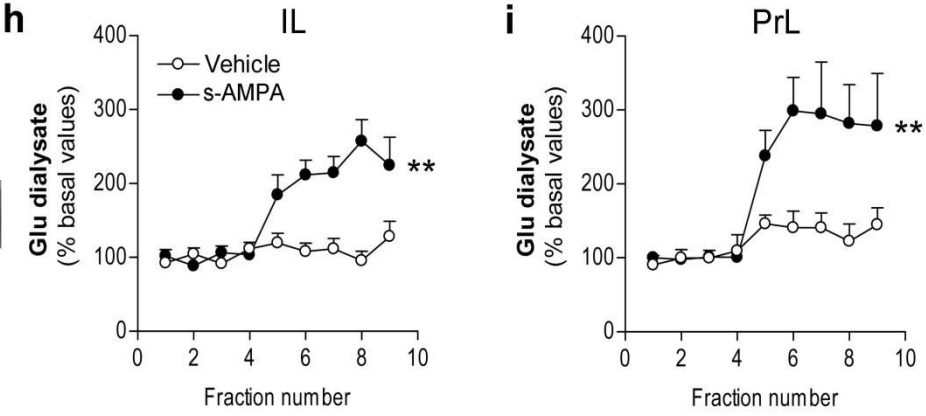


PrL

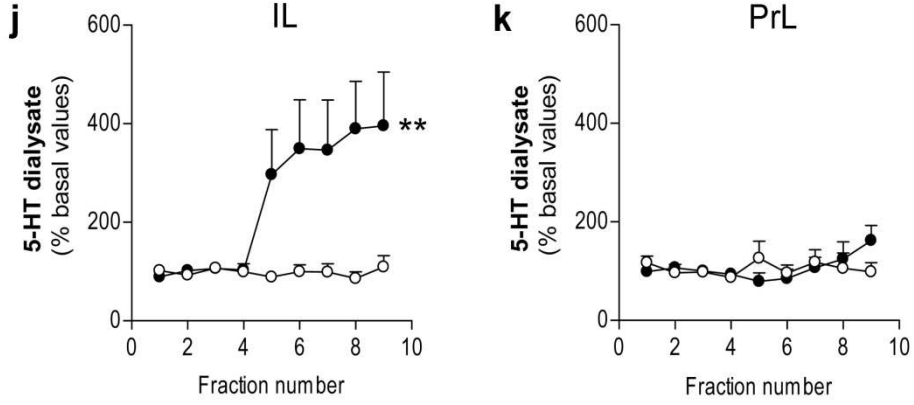


S-AMPA

Glutamate

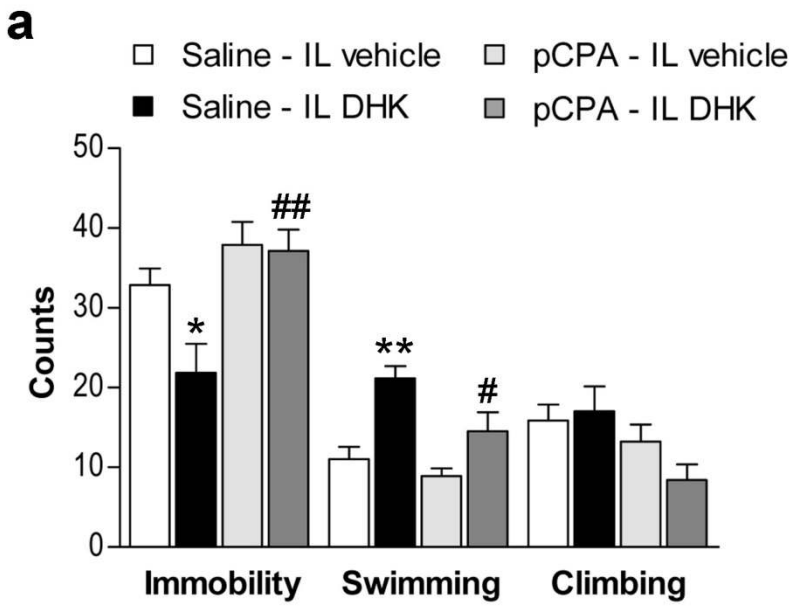


Serotonin

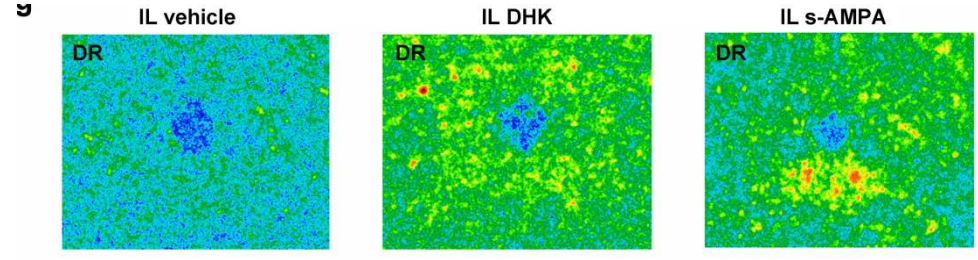


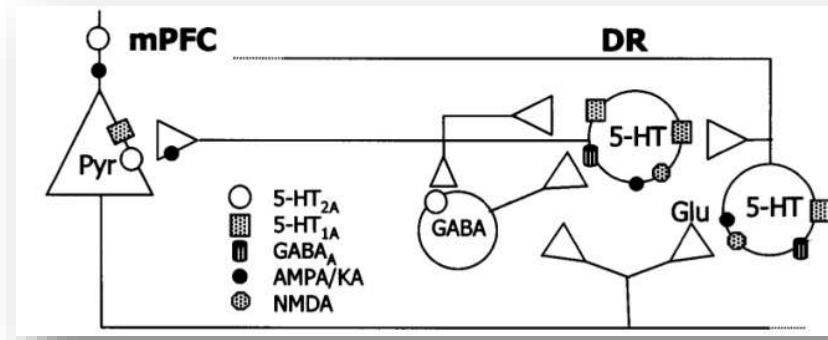
Gasull-Camos et al., *Transl Psychiatry* 2017

Glutamatergic mechanisms….. But in the end, 5-HT is required



Gasull-Camos et al., *Transl Psychiatry* 2017

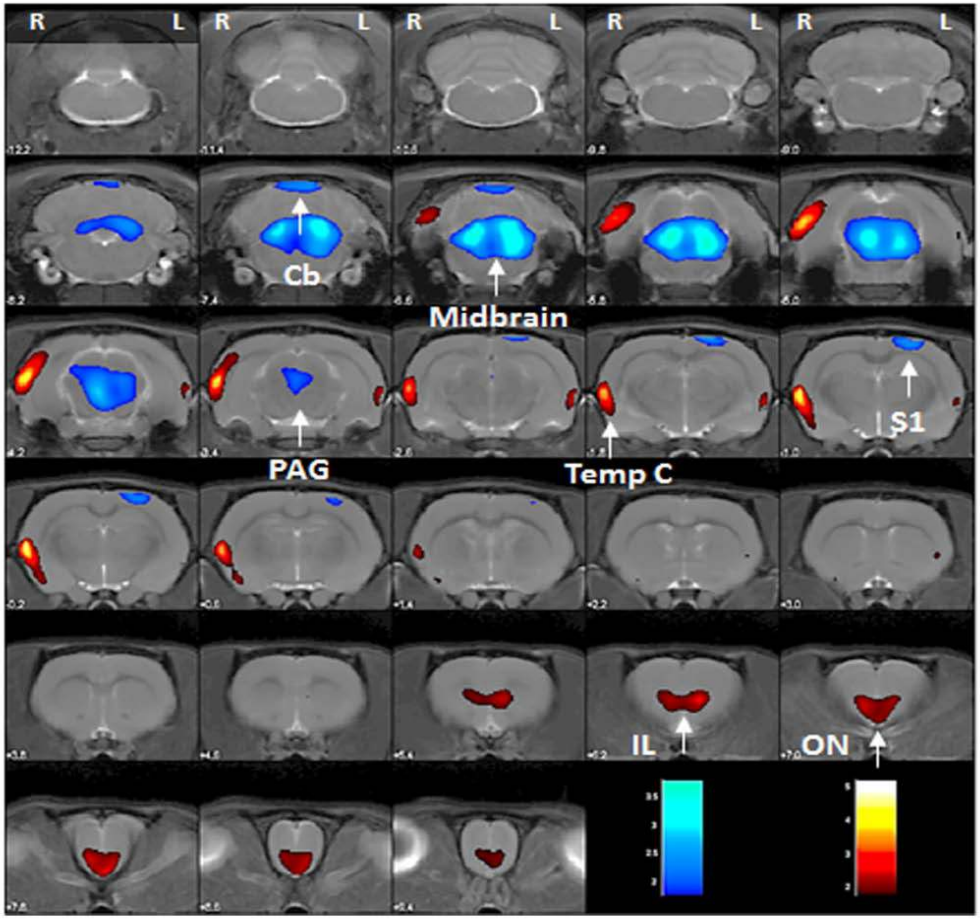




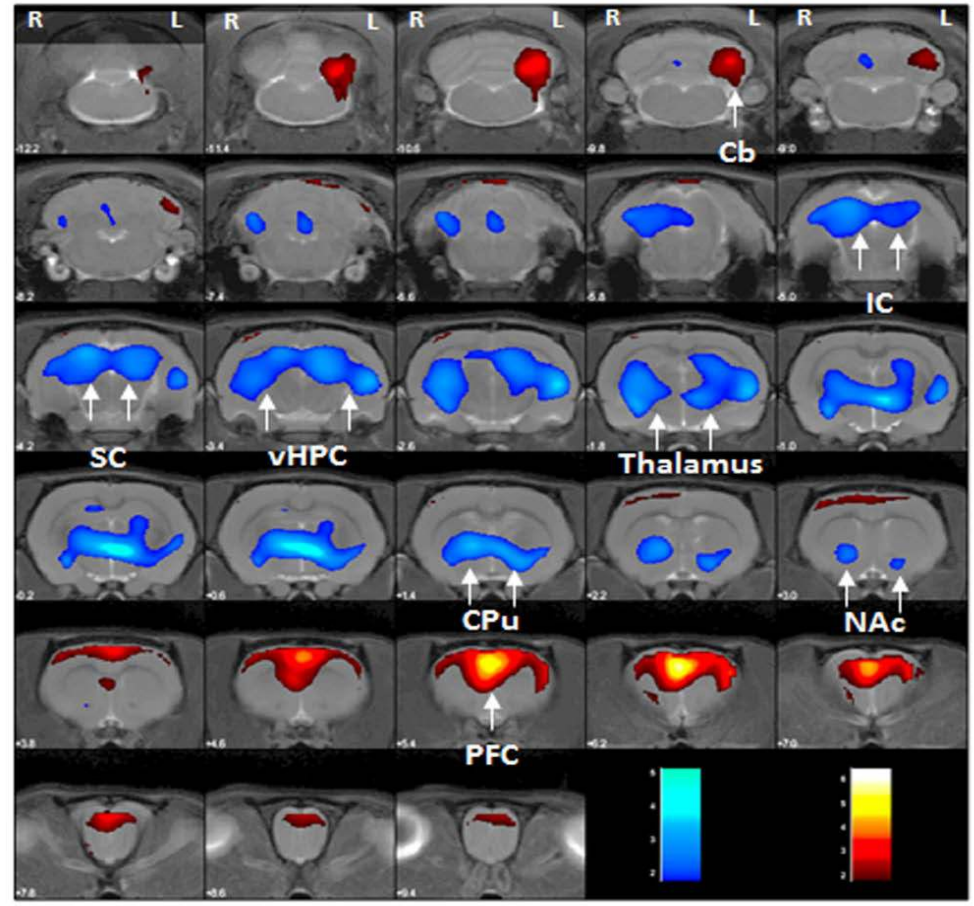
Celada et al., *J Neursoci* 2001
Martin-Ruiz et al., *J Neurosci* 2001

^{18}FDG-PET Scan Study

IL DHK



PrL DHK



Gasull-Camós *et al, Int J Neuropsychopharm 2017*

IL-DHK

PrL-DHK

Similar increase of local glutamate extracelular levels

Evokes immediate antidepressant responses mediated by AMPA-R

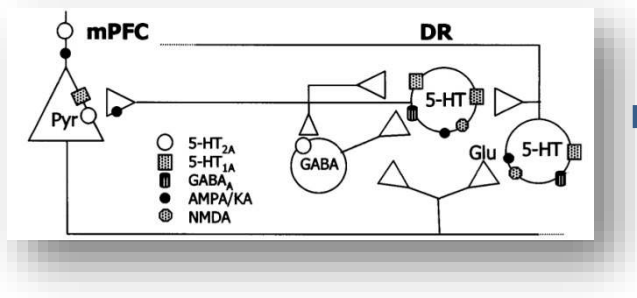
No antidepressant-like responses

Increases 5-HT release in IL

5-HT relase in IL is unaffected of slightly reduced

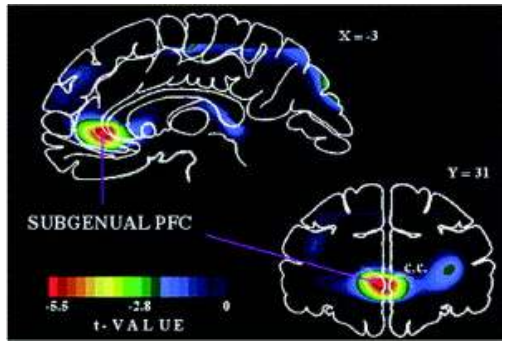
Changes glucose metabolism mainly in midbrain

Changes glucose metabolism in basal ganglia, vHPC, SC and IC

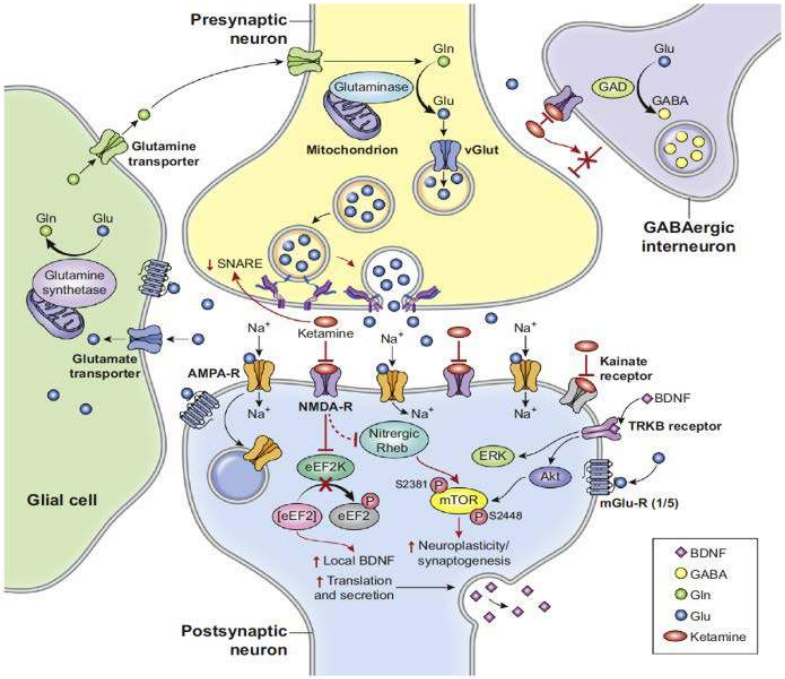


Differential control of GABA and 5-HT neurons in DR by PrL and IL?

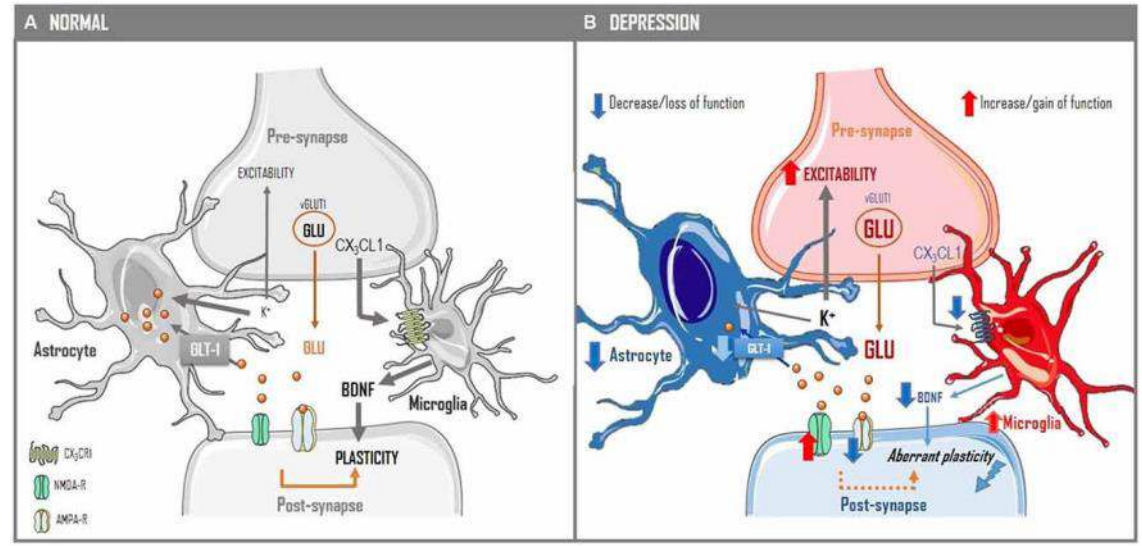
Major Depressive Disorder: a Glutamate/Glial Basis?



Drevets WC et al, 2009



Leber et al., 2016



Aim:

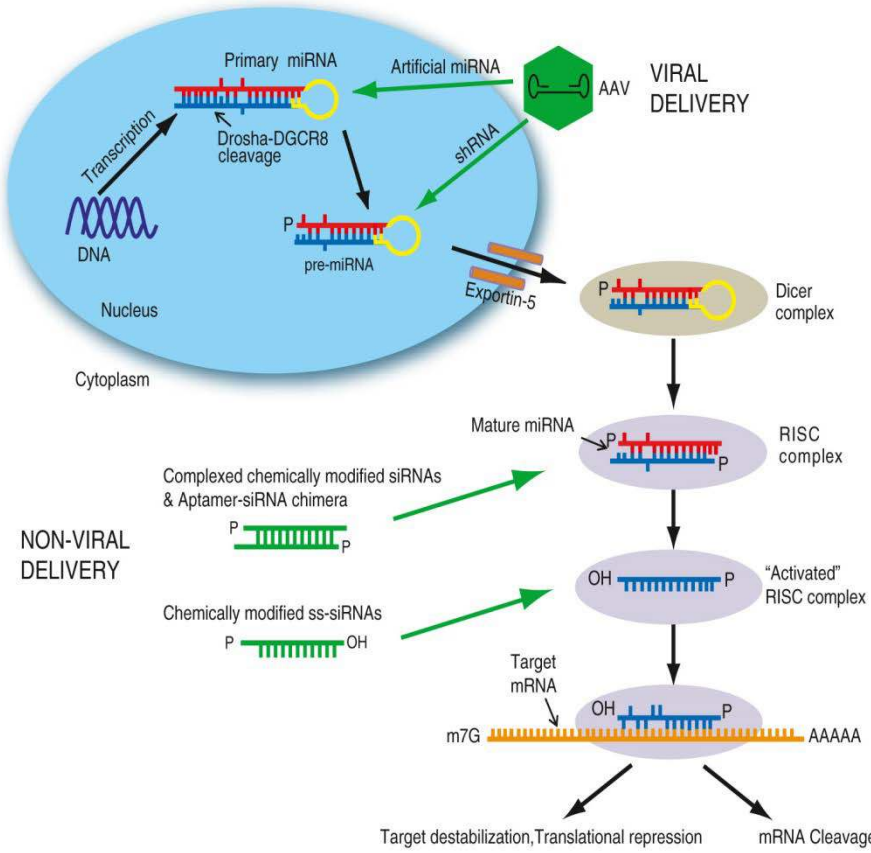
In an attempt to mimic astroglial dysfunction and vACC hyperactivity in MDD patients, we knocked-down the expression of the astrocytic glutamate transporters GLAST (EAAT1) and GLT-1 (EAAT2) in mouse IL, using small interfering RNA, siRNA.

Working hypothesis:

siRNA-induced knockdown of GLAST/GLT-1 in IL may evoke a persistent glutamatergic hyperactivity, deeply altering the local excitation/inhibition balance and therefore the descending excitatory input to subcortical structures (e.g. brainstem monoamine nuclei).

Use of RNAi for CNS Disorders

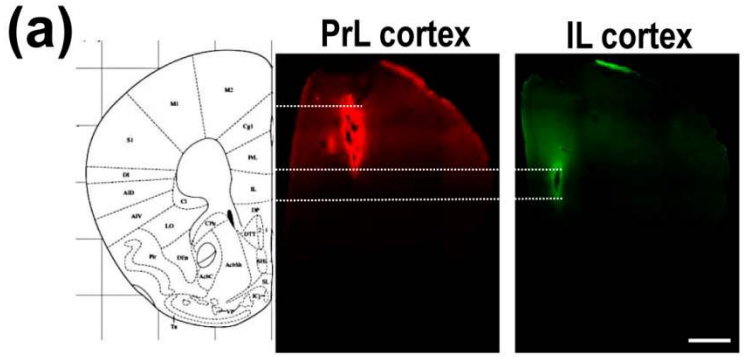
- New therapeutic strategy for the treatment of mental disorders. The Nobel Prize in Physiology and Medicine 2006 was awarded to Andrew Z. Fire and Craig C. Mello "*for their discovery of RNA interference – gene silencing by double-stranded RNA*"
- A substantial percentage of protein-encoding genes may be regulated by microRNA (miRNAs): e.g., miR-16 regulates SERT expression (Baudry et al., *Science* 2010) and miR-135 regulates SERT and 5-HT_A receptors; (Issler et al., *Neuron* 2014). miR-186 over-expression reduces ß-amyloid formation (Kim et al., *J Neurochem* 2016)
- Ability to selectively target any protein-encoding mRNA with siRNA, avoiding unspecific drug actions
- Particularly useful for disorders lacking appropriate pharmacological treatments

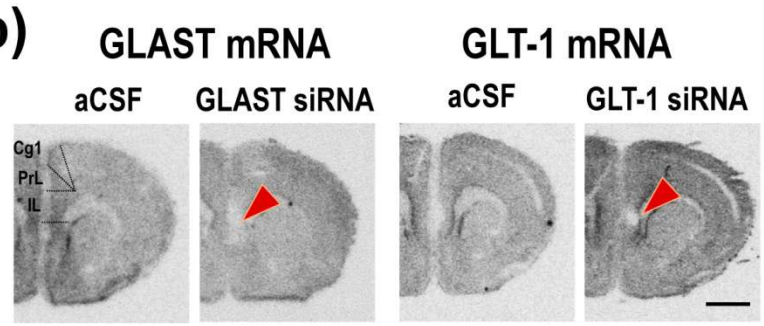


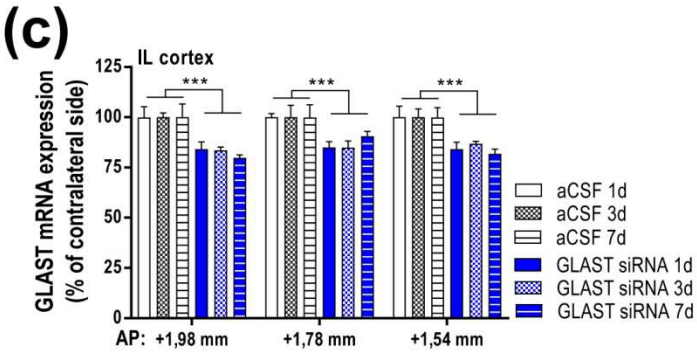
Local application of siRNA directed against GLAST and GLT-1 in mouse PrL and IL

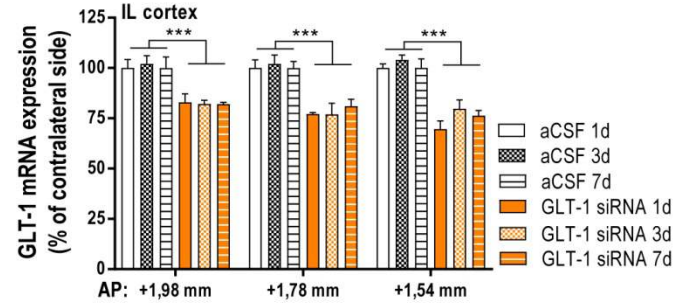
Unilateral GLAST/GLT-1 siRNA Infusion in IL Induces a Moderate Long-lasting Reduction of mRNA/protein Levels

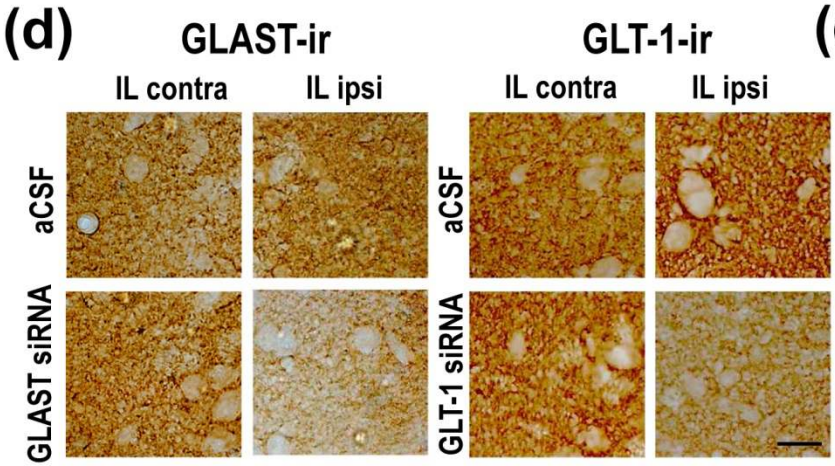
Unilateral Infusion
3 siRNA sequences
Dose=4.2 nmol siRNA

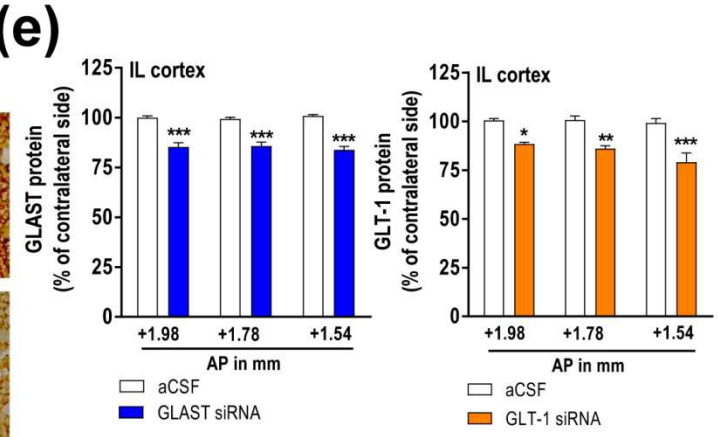




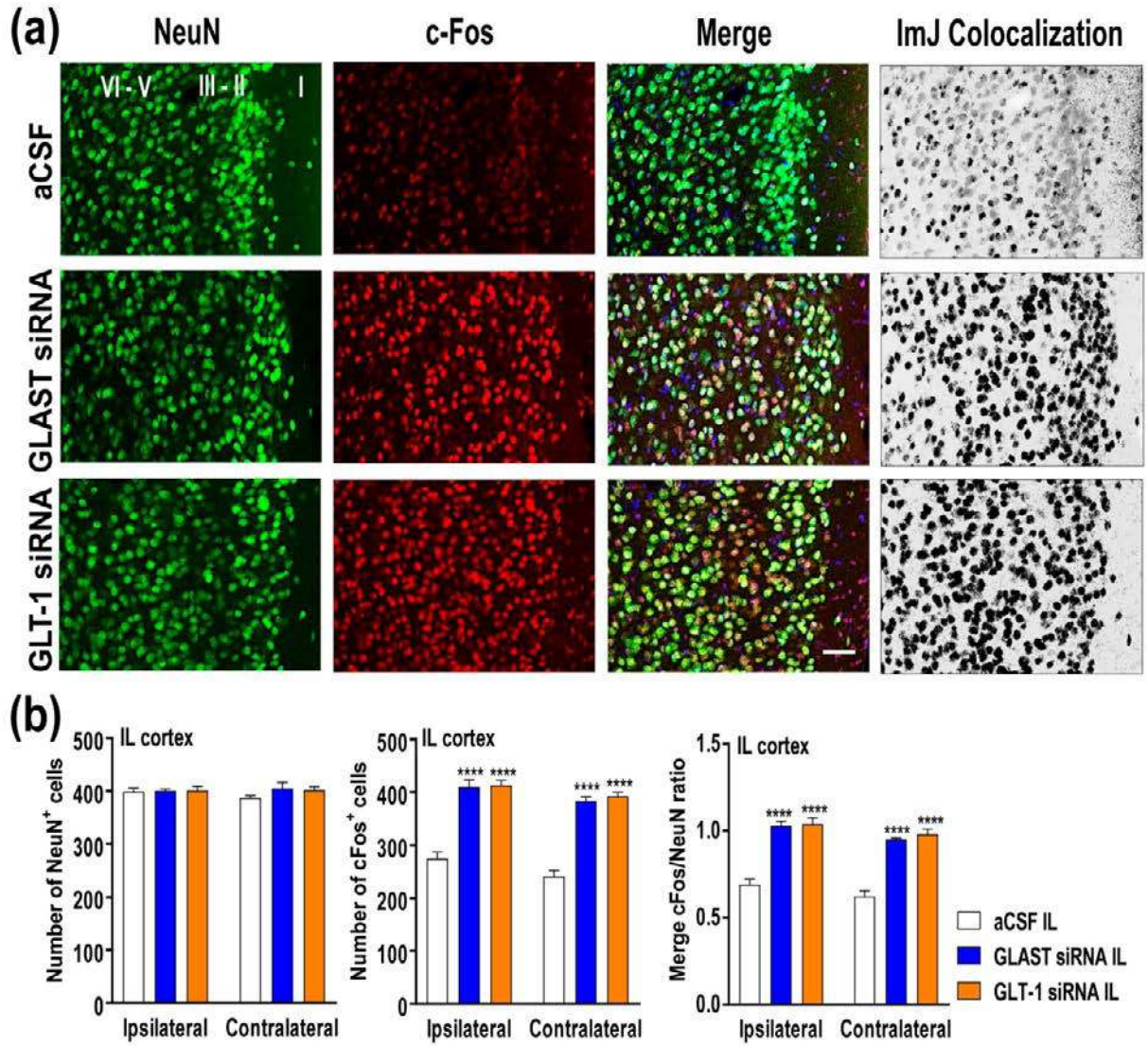




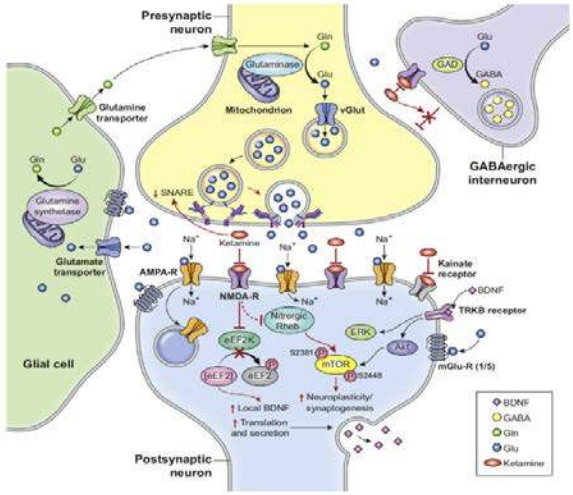




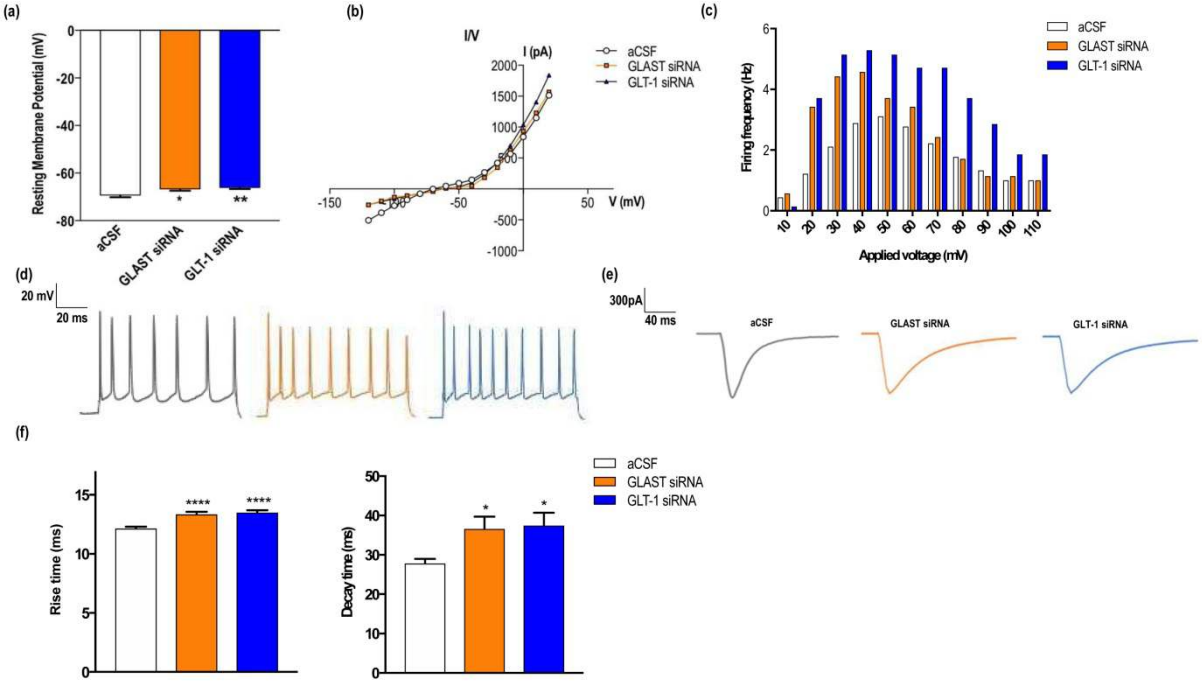
GLAST/GLT-1 siRNA Infusion in IL Evokes a Large Neuronal Activation (c-Fos Labelling)



Electrophysiological Characteristics and Synaptic Activity of IL Layer V Pyramidal Neurons in Mice after GLAST and GLT-1 Knockdown

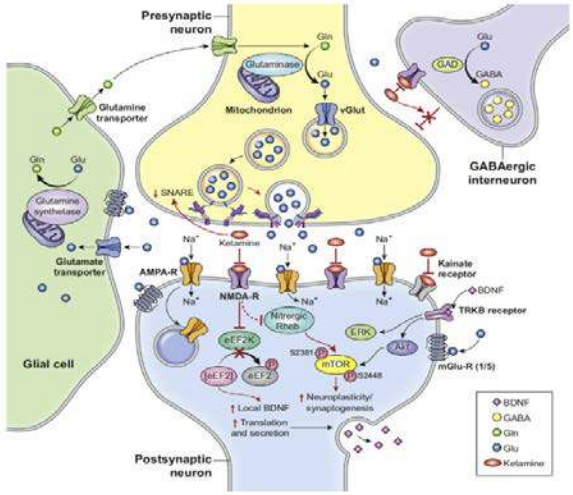


Leber et al., 2016

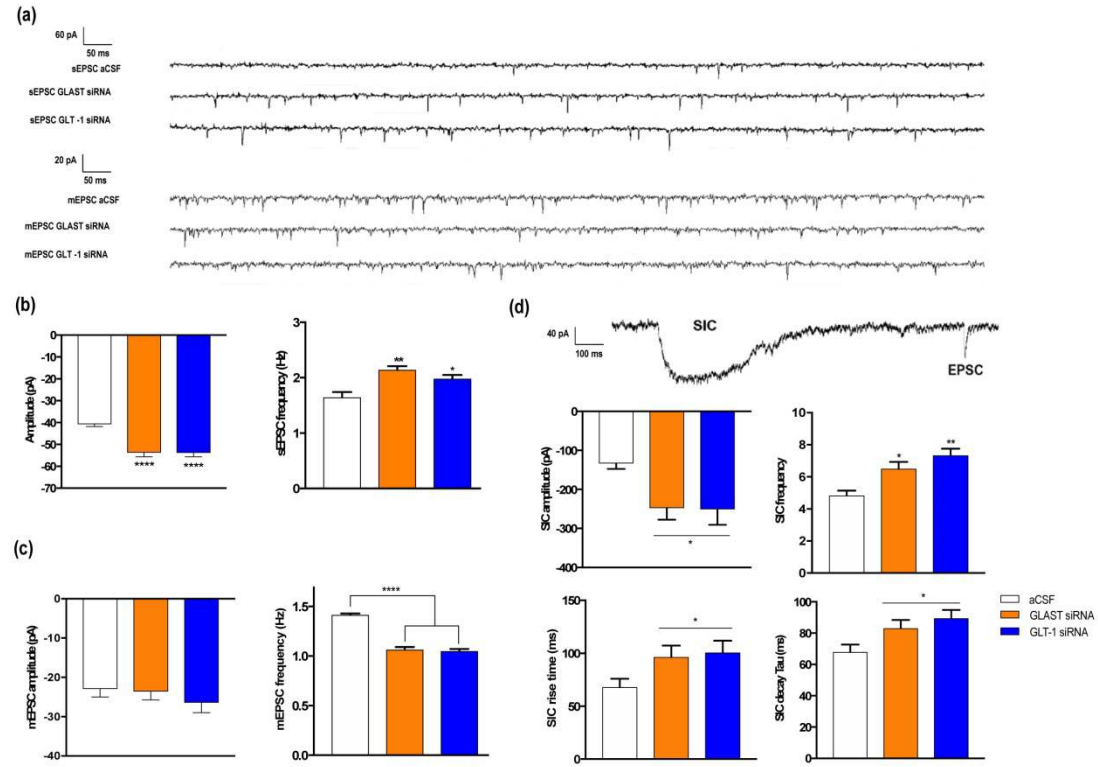


- Higher resting membrane potential
- Increased evoked neuronal discharge
- Prolonged time course of evoked EPSCs

Electrophysiological Characteristics and Synaptic Activity of IL Layer V Pyramidal Neurons in Mice after GLAST and GLT-1 Knockdown

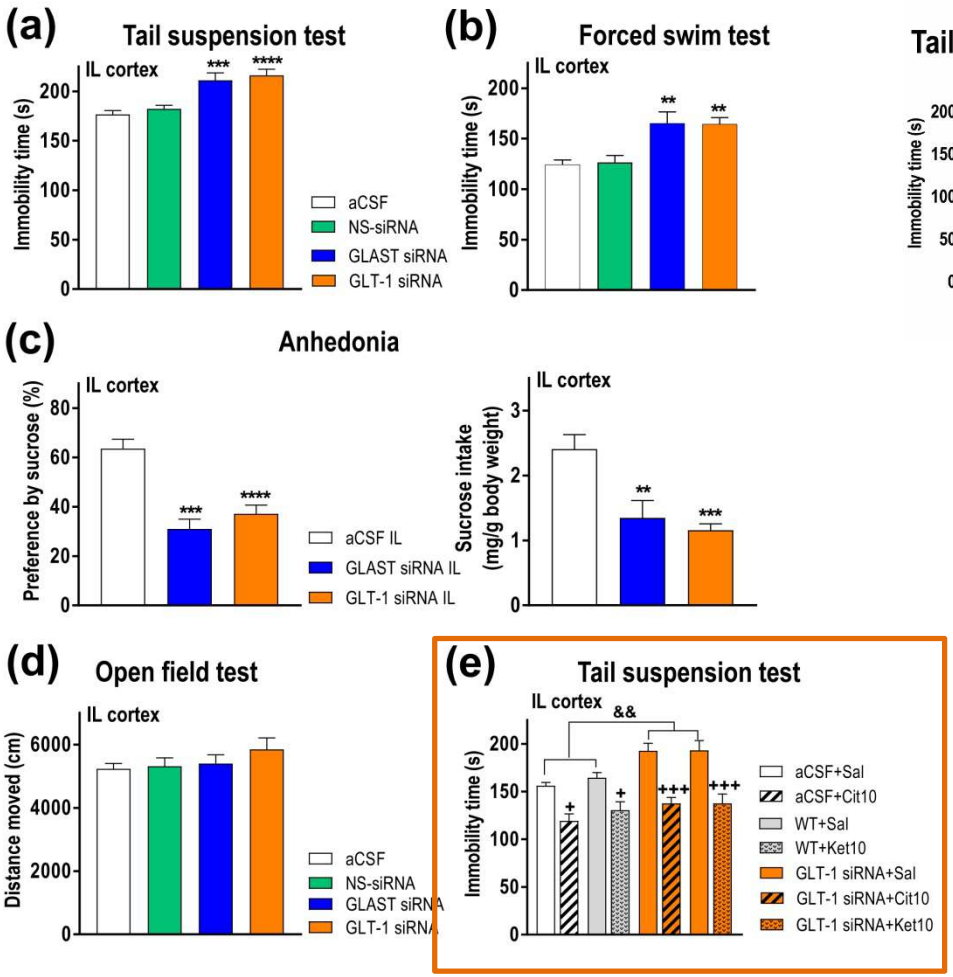


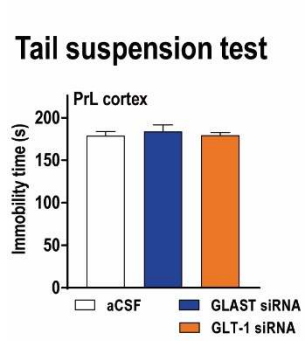
Leber et al., 2016



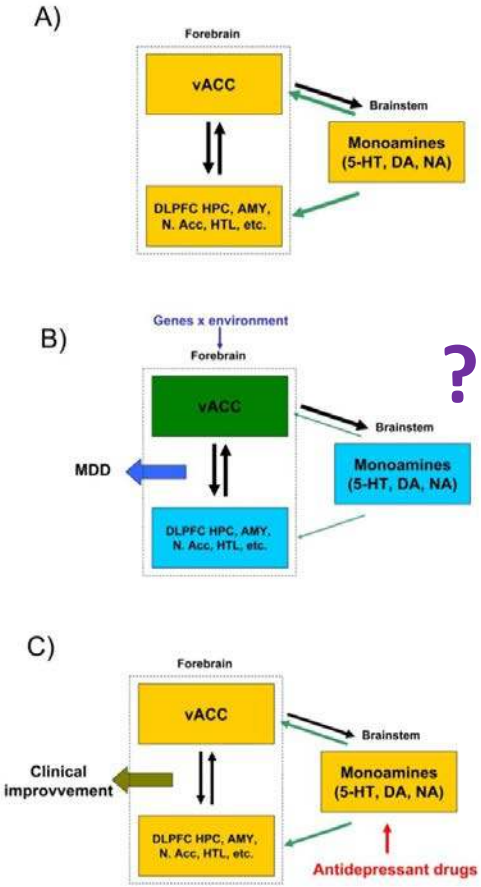
- **Greater amplitude and frequency of spontaneous EPSCs**
- **Lower frequency and same amplitude of miniature EPSCs (presynaptic component)**
- **Greater amplitude and frequency of slow inward currents, as well as prolonged waveform (mediated by extrasynaptic GluN2B receptors)**

GLAST or GLT-1 siRNA into IL, but not into PL, evoke a depressive-like phenotype in mice

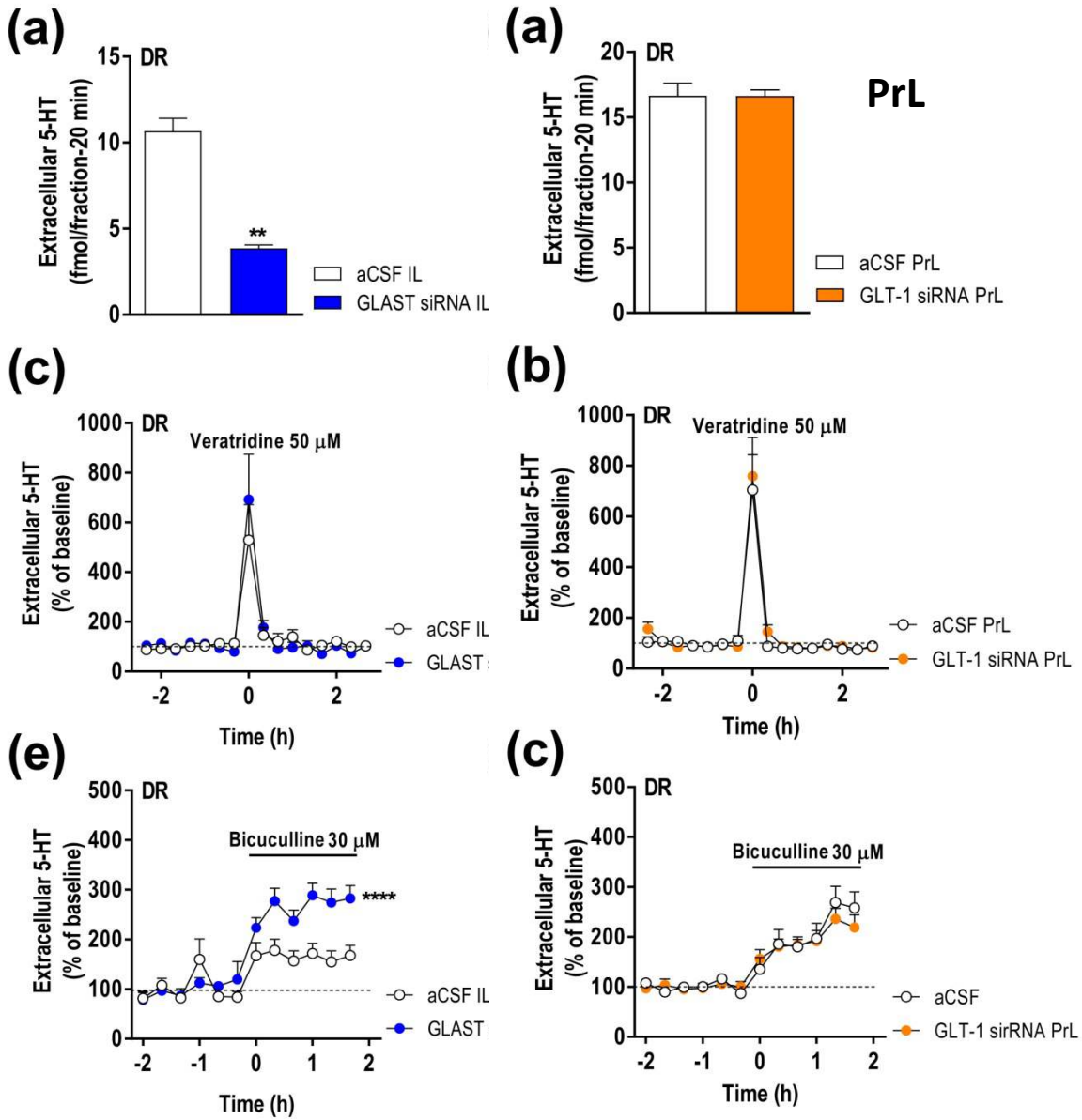




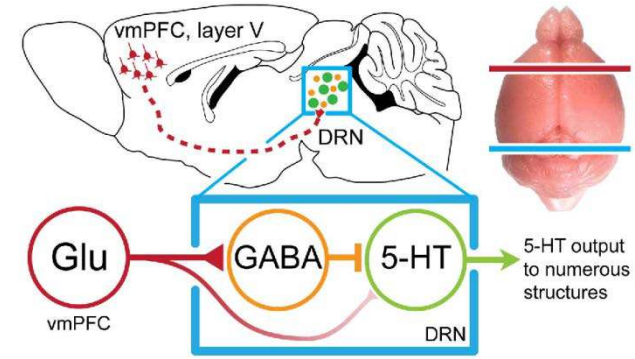
- Increased immobility times in TST and FST
- Anhedonia: Reduced preference and sucrose intake
- Depressive-like phenotype reversed by citalopram and ketamine



Effects of GLAST and GLT-1 Knockdown in IL on Serotonergic Function

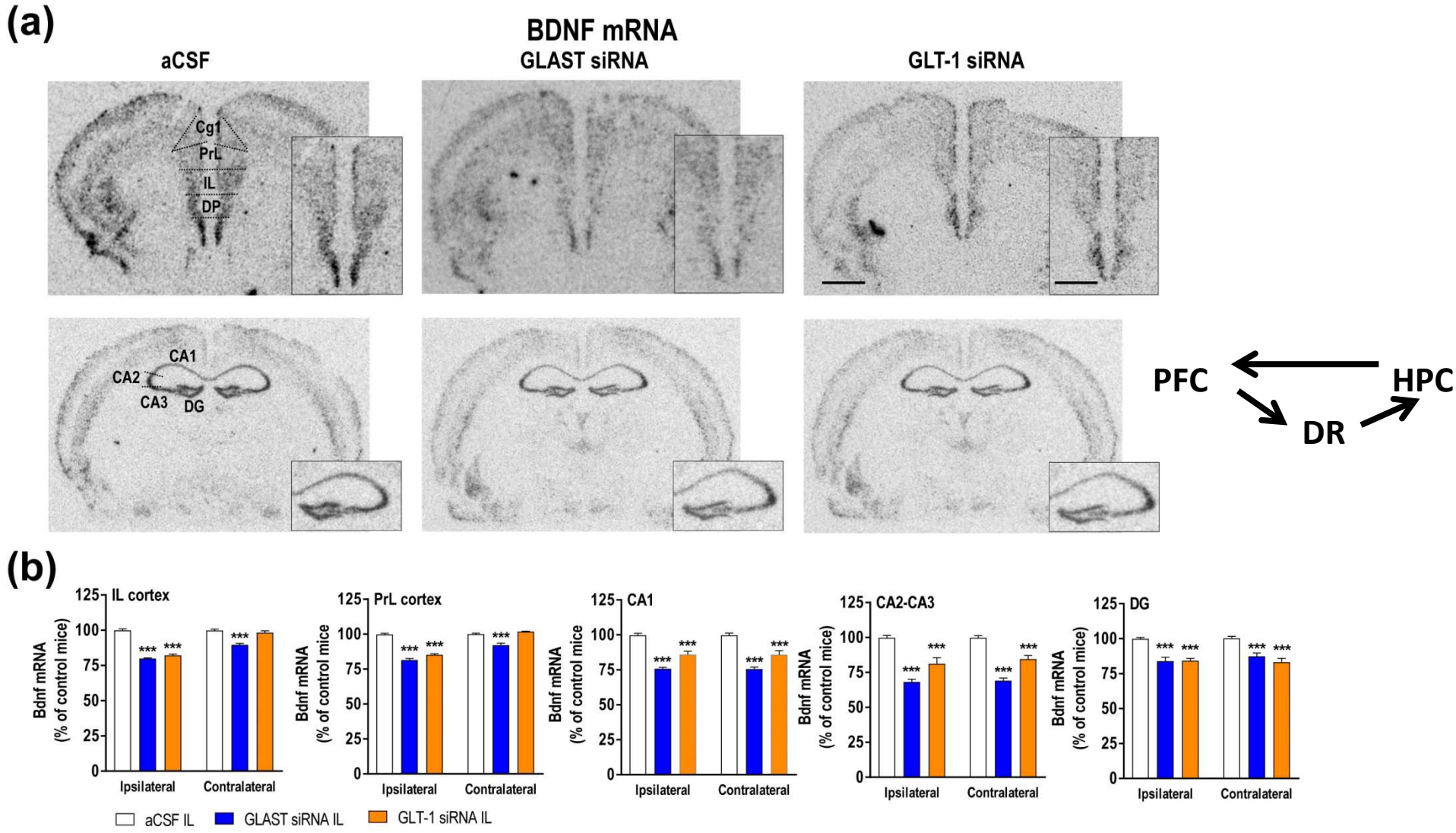


- **Reduced 5-HT baseline levels in DRN**
- **Similar responsiveness to Veratridine (intracellular 5-HT stores)**
- **Increased 5-HT release after local bicuculline infusion ($GABA_A$–R antagonist)**

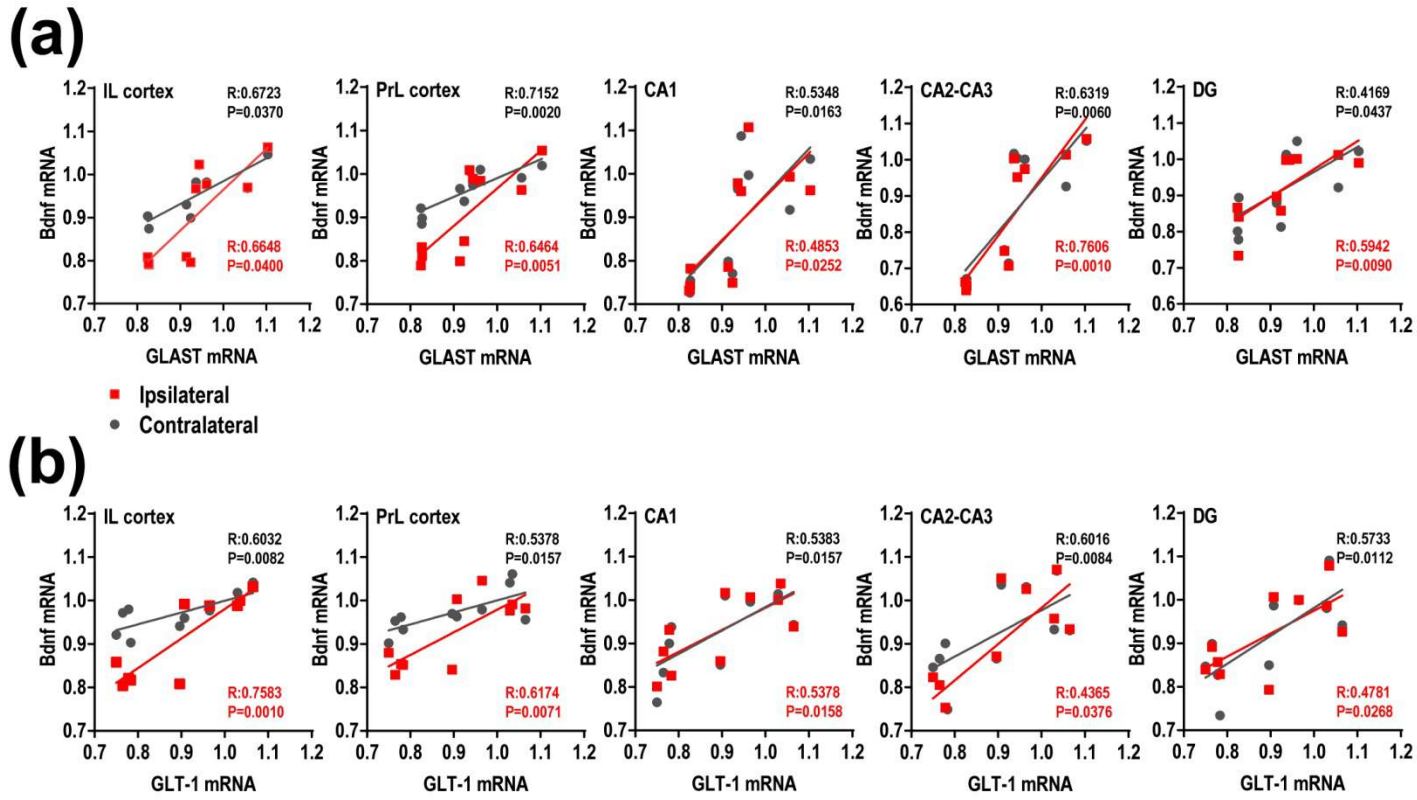


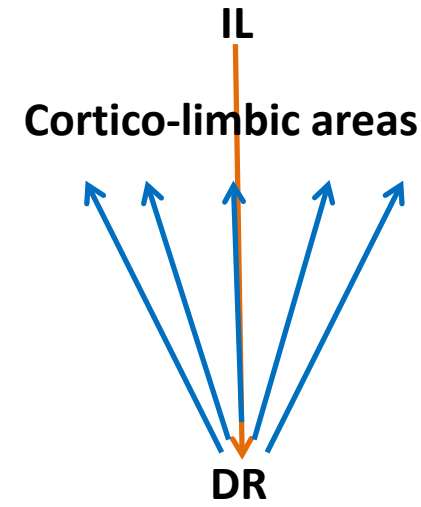
Adapted from Challis and Berton, 2015

Unilateral GLAST and GLT-1 Knockdown in IL Cortex Decreases BDNF mRNA Expression in mPFC and HPC (both Ipsilateral and Contralateral Hemispheres)



Highly Positive Correlations between GLAST/GLT-1 Knockdown in IL and BDNF Expression in mPFC and HPC of Ipsilateral and Contralateral Hemispheres





A *focal* glial change in IL translates into *global* change of brain activity by virtue of the descending projections from IL to DR and the subsequent attenuation of serotonergic activity, an effect perhaps related to the varied symptomatology of MDD.

Summary

- Increasing serotonergic neurotransmission has a clear antidepressant effect. However, the clinical action of serotonergic antidepressants is limited by autoreceptor-mediated negative feed-back mechanisms. Novel molecular strategies may circumvent this problem by directly modulating the expression of proteins involved in serotonergic function (e.g., SERT, 5-$HT_{!A}$-R, TASK3 potassium channels, etc.)

- Glutamatergic neurotransmission in ventral cingulate areas (IL in rodents) is emerging as a new neurochemical/regional target in the pathophysiology and treatment of MDD. Hence, acute blockade of GLT-1 in IL (but not in PrL) increases resilience to stress in naïve rats, associated to the stimulation of AMPA-R and to an enhanced serotonergic function.

- The prior 5-HT depletion prevents behavioral effects of GLT-1 blockade, thus supporting a major role for 5-HT in the present glutamatergic strategy. Overall, AMPA-R activation in IL may result in a direct activation of serotonergic function, thus overcoming the self-inhibitory actions induced by SERT blockade.

- On the other hand, a persistent dysfunction of glutamatergic neurotransmission evoked by unilateral GLT-1/GLAST knockdown in IL (but not PrL) induces a depressive-like phenotypes in mice. In parallel, there is a reduced serotonergic function and BDNF expression in cortical and hippocampal areas, in both ipsilateral and contralateral hemispheres.

- Hence, a *focal* change of the excitatory transmission in IL may translate into a *global* change in brain activity by virtue of the descending projections from IL to the midbrain raphe and the subsequent expansion of an abnormal neurochemical signal (low 5-HT → low BDNF) by the 5-HT system to forebrain areas involved in MDD symptoms.

- Differences between the two models are possibly related to the time course and degree of glutamatergic hyperactivity evoked. Hence, DHK effects are short-term and mediated by AMPA-R activation whereas siRNAs induce a persistent reduction of GLT-1 and GLAST expression/function and involve the additional activation of extra-synaptic GluN2B –containing NMDA-R. Species differences may also contribute.

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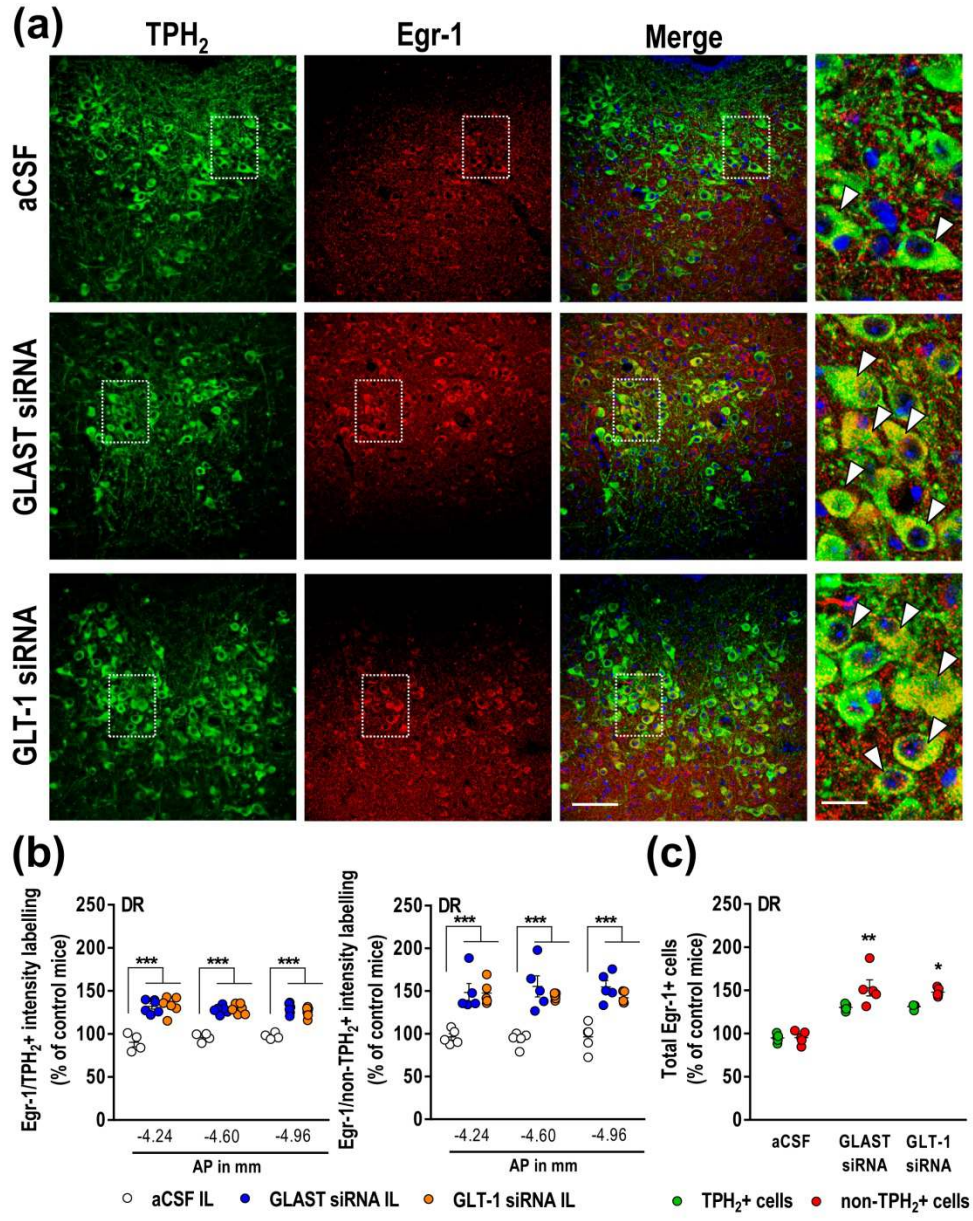
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

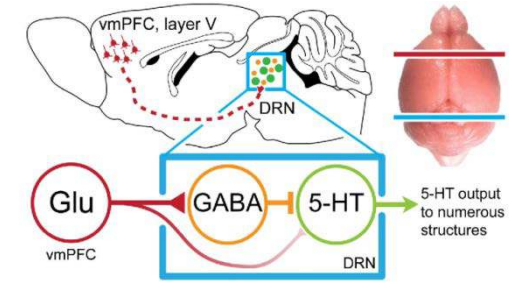
Instituto de Salud Carlos III

Thank you very much for your attention !!

GLAST and GLT-1 Knockdown in IL Cortex Increases Egr-1 Expression in DR Preferentially in non-TPH2 (Putative GABA) Cells



- **Greater number of cells expressing Egr-1 in the DR**
- **Greater number of non-5-HT cells expressing Egr-1**



Adapted from Challis and Berton, 2015