1. Animal learning and memory

    Classical conditioning

    Operant conditioning

    Memory

2. Emotional and cognitive stimulus processing

    Processing of rewarding stimuli

    Aversive stimuli, fear and the amygdala

    Stress, learning and memory

3. Animal models of human affective disorders

    Translational experimental psychiatry

    Manipulations and readouts

    Animal models relevant to anxiety and depression

    Immune system and depression

4. **Pre-clinical psychopharmacology**

    SSRIs and affective disorders

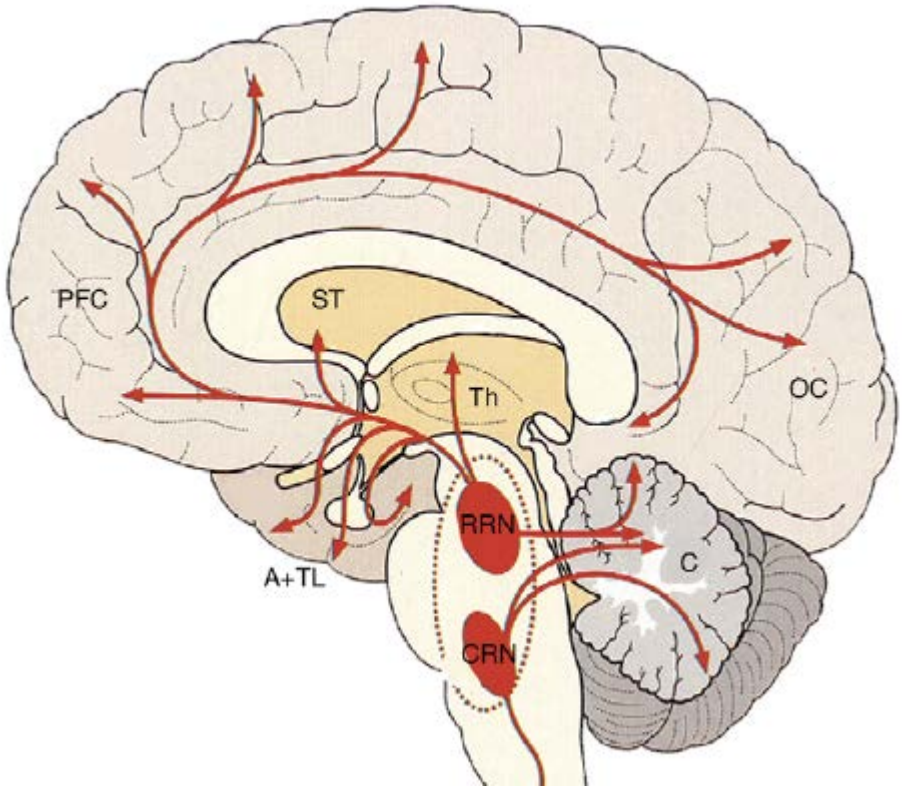
    Anti-depressants: the next generation?

Selective Serotonin Reuptake Inhibitors and Affective disorders

- Neurobiology of the serotonin system
- Changes in serotonin system in depression
- Past and present antidepressant treatments
- Selective serotonin reuptake inhibition (SSRI), 5-HTT/SERT blockers
- Possible mechanism of action of SSRIs:
    - Increased serotonin in synapse
    - Decreased post-synaptic 5-HT2A receptor signalling
    - Increased pre-synaptic or post-synaptic 5-HT1A receptor signalling
- Assessing SSRI mechanism of action in animal models
- 5-HTT gene polymorphism as a risk factor for depression: the *s* allele paradox
- Efficacy of SSRIs as depression pharmacotherapy

Neurobiology of the Serotonin (5-HT) system

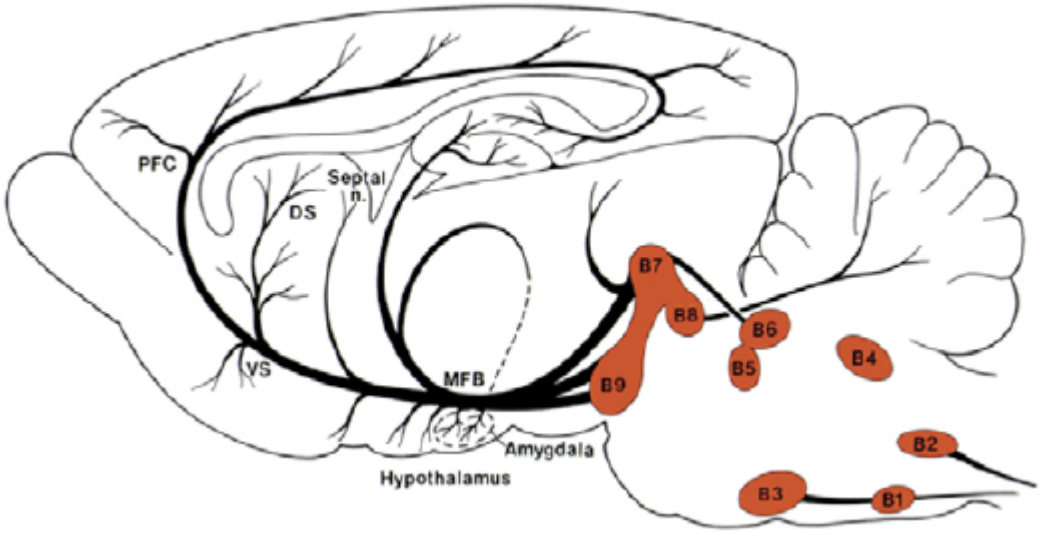
Cell bodies & Projections in Human brain



RRN = Rostral raphé nuclei = Dorsal RN (B7) + Medial RN (B8)

A: Amygdala, PFC: Prefrontal cortex, ST: Striatum, Th: Thalamus, TL: Temporal lobe,

Cell bodies & Projections in Rat brain



B7-B9 = Rostral groups of neurons
B7 = Dorsal raphé nucleus
B8 = Medial raphé nucleus
B9 = Pontine tegmentum

DS: dorsal Striatum, VS: ventral Striatum,
PFC: Prefrontal cortex

Cools et al. (2008) Trends Cogn Sci 12: 31

Serotonin Neurons and Synapses: Pre-synaptic

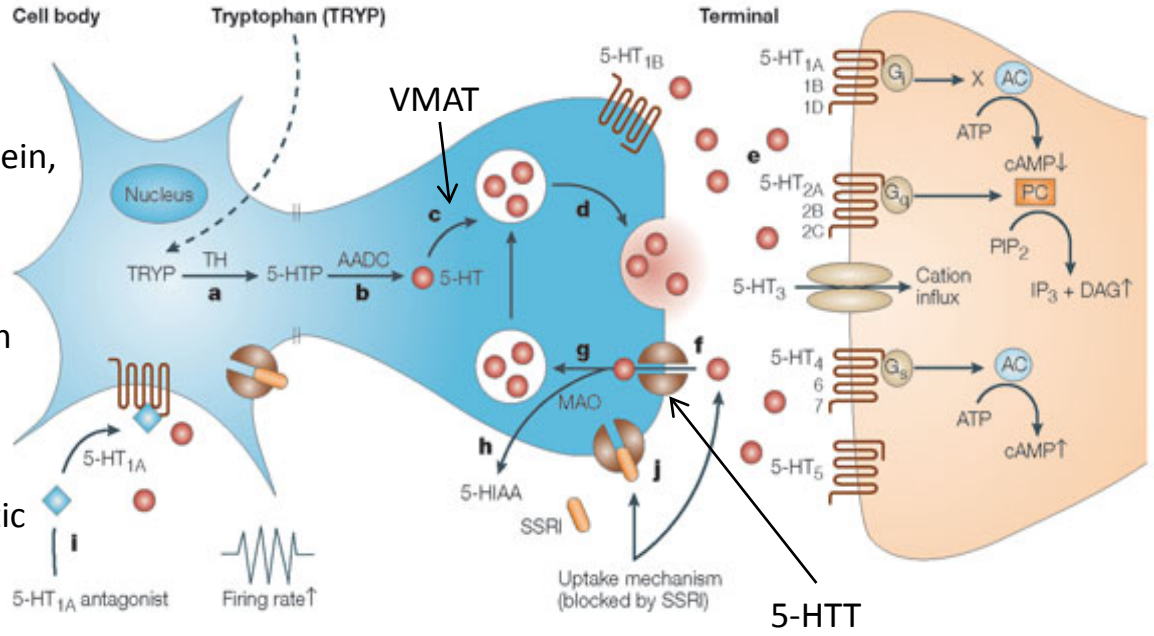
VMAT, Vesicular monoamine transporter protein, in synaptic vesicles

5-HTT/SERT, Serotonin transporter protein

MAO, Monoamine oxidase

5-HIAA, 5-Hydroxyindoleacetic acid

5-HT_{1A} Receptor



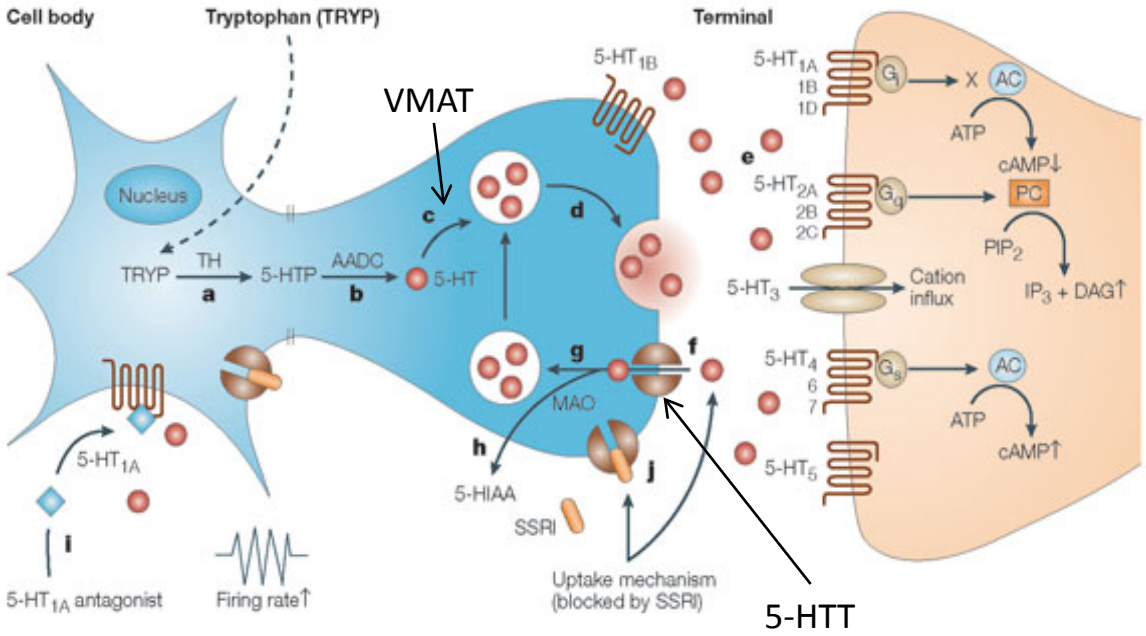
Tryptophan → 5-Hydroxy-L-Tryptophan → 5-Hydroxytryptamine

Tryptophan Hydroxylase

Aromatic L-amino acid Decarboxylase

Millan (2003) Progr Neurobiol 70: 83

Serotonin Neurons and Synapses: Post-synaptic

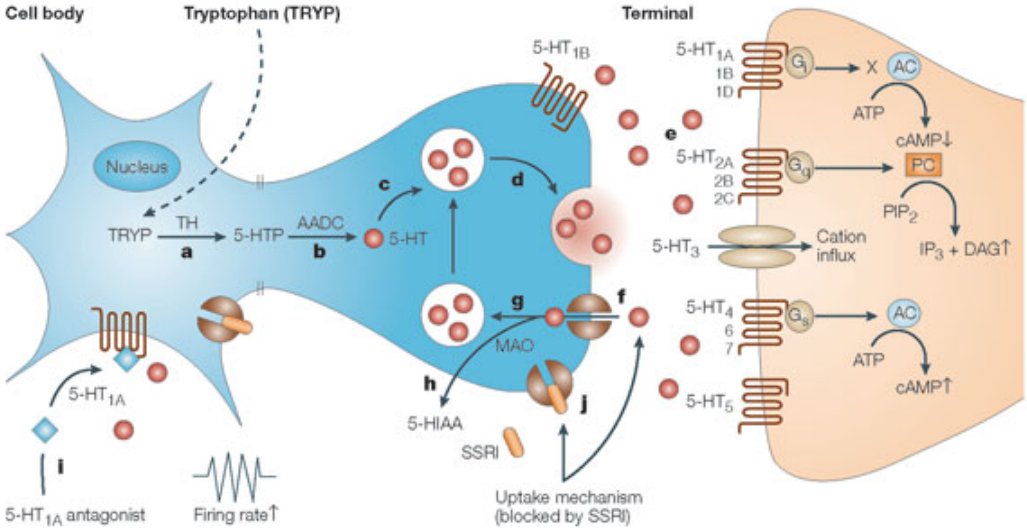


13 5-HT Receptors, 12 G protein-coupled receptors and 1 (5-HT_3) ionotropic receptor

5-HT1A	Hippocampus, Amygdala, Anterior cingulate cortex
5-HT1B	Nucleus accumbens, Dorsal Striatum, Substantia nigra
5-HT2A	Anterior cingulate cortex
5-HT2C	Ventral tegmental area, Nucleus accumbens, Dorsal Striatum, Substantia nigra
5-HT3	Hippocampus, Brain stem, Spinal cord
5-HT4	Nucleus accumbens, Dorsal Striatum, Hippocampus
5-HT5B	Habenula, Hippocampus
5-HT6	Nucleus accumbens, Dorsal striatum, Hippocampus, Anterior cingulate cortex
5-HT7	Hyopothalamus, Thalamus, Suprachiasmatic nucleus, Anterior cingulate cortex

Millan (2003) Progr Neurobiol 70: 83

Serotonin synaptic signalling and re-uptake/metabolism



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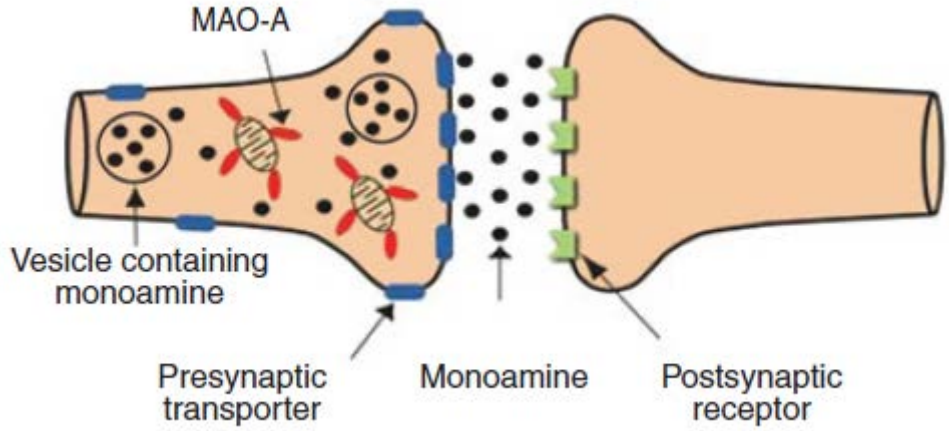
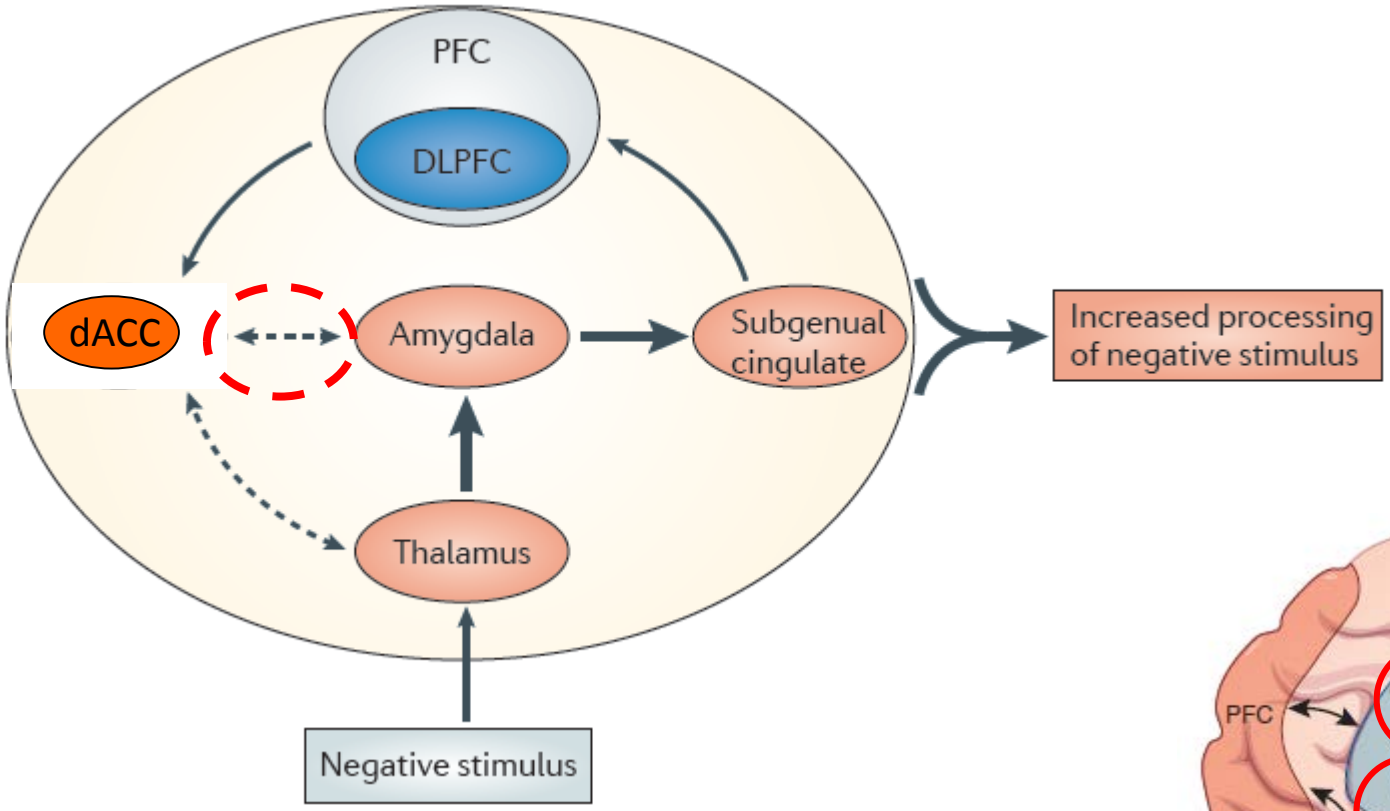
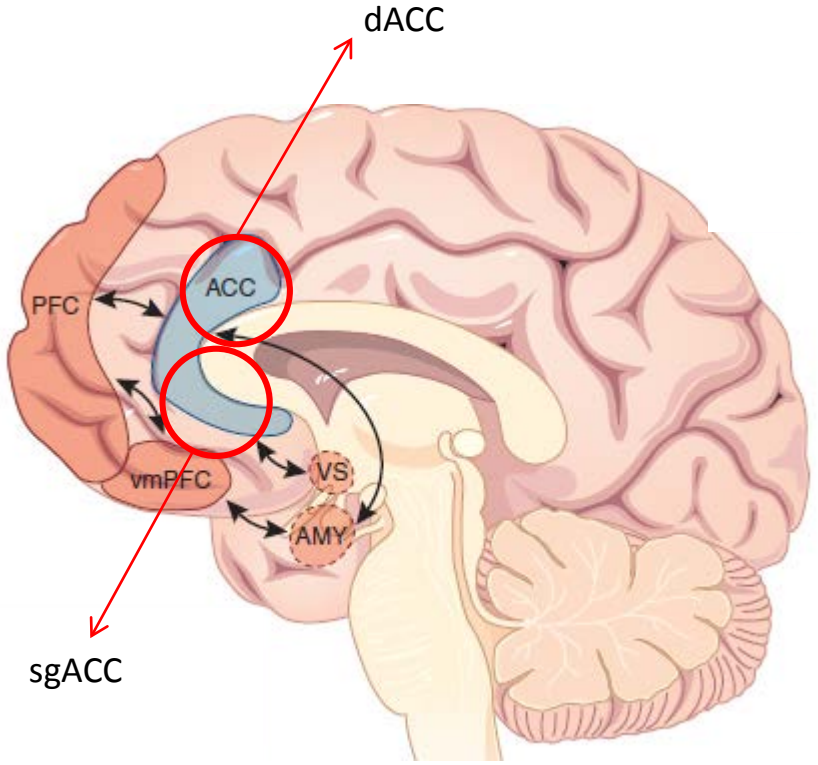


BOLD fMRI-based model of processing of negative stimuli in depression





Hyper-active region in MDD

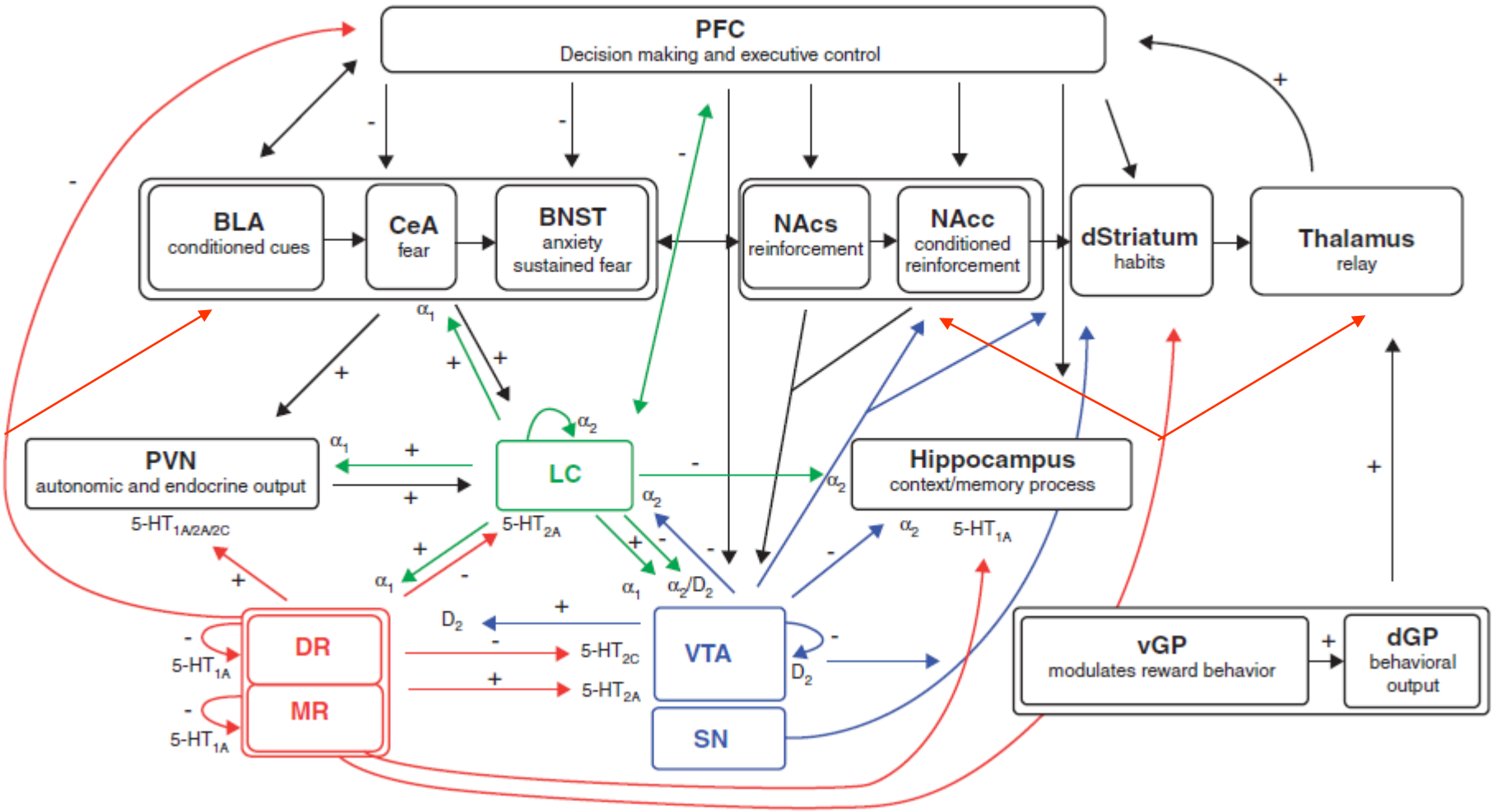
Hypo-active region in MDD

Intact connectivity

Reduced connectivity

Disner et al (2011) Nature Rev Neurosci 12: 467

Overview of brain regions involved in depression and their monoaminergic connections



+: Excitatory; -: Inhibitory.
BLA: Basolateral amygdala; BNST: Bed nucleus of stria terminalis; CeA: Central amygdala; DR: Dorsal raphe nucleus; dGP: Dorsal globus pallidus; LC: Locus coeruleus; MR: Median raphe nucleus; NAcs: Nucleus accumbens shell; NAcc: Nucleus accumbens core; dStriatum: Dorsal striatum; PFC: Prefrontal cortex; PVN: Paraventricular nucleus of hypothalamus; SN: Substantia nigra; VTA: Ventral tegmental area; vGP: Ventral globus pallidus.

Prins et al. (2011) Expert Opin Investig Drugs 20: 1107

Evidence for changes in Serotonin system in (some) depression studies

- High monoamine oxidase-A (MAO-A) density throughout brain
- High serotonin transporter (5-HTT) density in cortico-limbic regions in some patients
- Low 5-hydroxyindoleacetic acid (5-HIAA) levels in cerebrospinal fluid
- Low tryptophan levels in plasma (link to cytokine-kynurenine pathway)
- Tryptophan depletion may lead to relapse in recovered patients

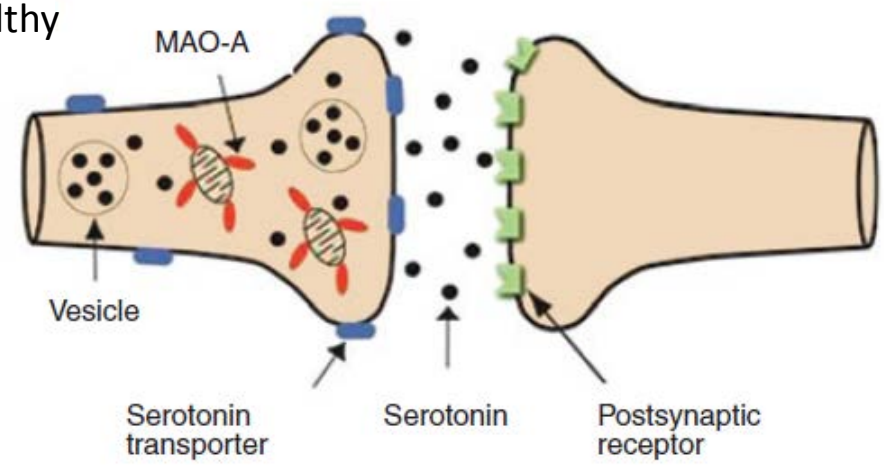
- Decreased numbers of 5-HT_{1A} receptors both pre- and post-synaptically
- Either increased or decreased numbers of 5-HT_{2A} and 5-HT_{2C} receptors post-synaptically
- Dysregulation in post-transcriptional (mRNA) 5-HT_{2C} receptor editing

HM van Praag et al. (2004) *Stress, the Brain and Depression*
Cambridge, Cambridge University Press

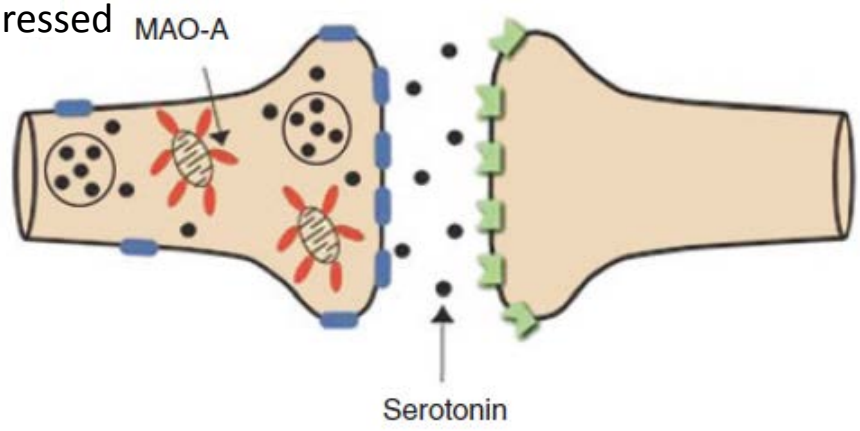
Pre-synaptic neuron (and glia) monoamine oxidase-A density is increased in depression

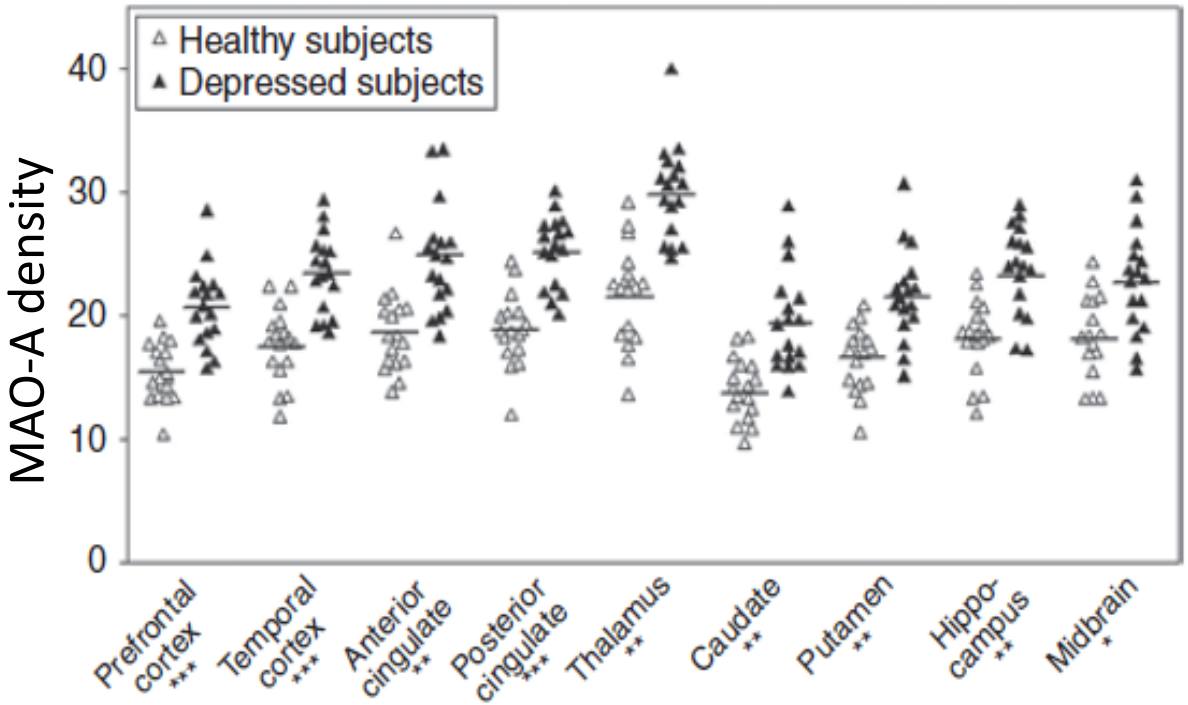
- MAO-A and MAO-B
- Catalyses the oxidation of monoamine (5-HT, DA, NE)
- Bound to outer membrane of mitochondria

Healthy



Depressed

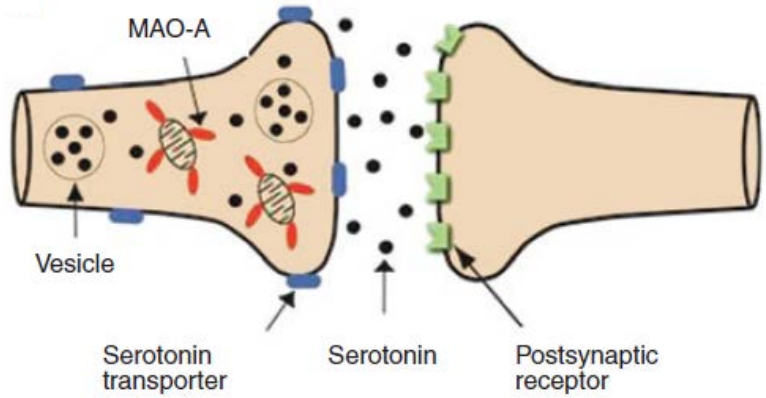




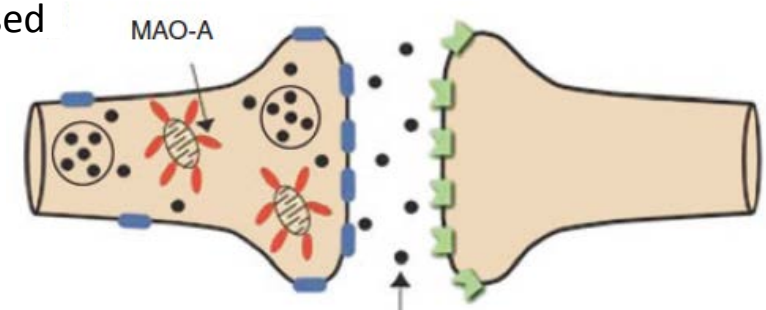
Meyer et al. (2004) Arch Gen Psychiatry 61: 1271
Meyer (2012) Clin Pharm Therapeut 91: 201

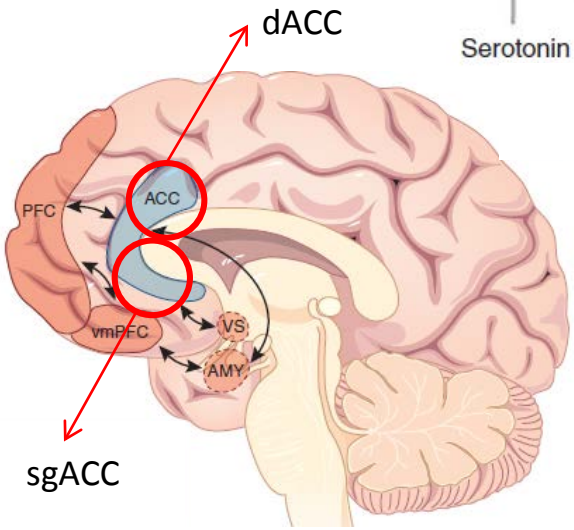
Serotonin transporter density is increased in depression-relevant regions - especially in patients with High Dysfunctional Attitude Scale scores (pessimism, hopelessness)

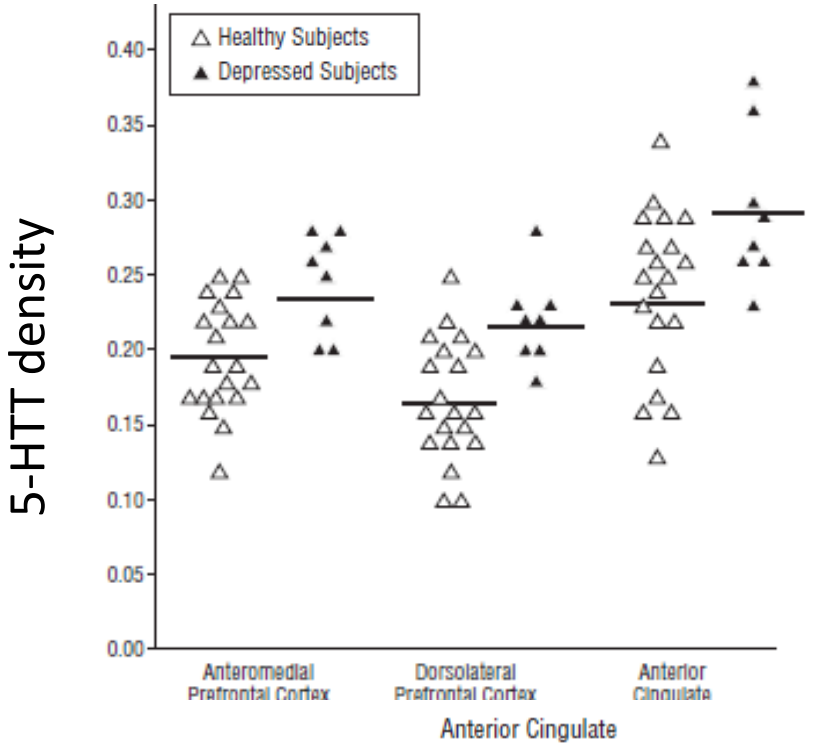
Healthy

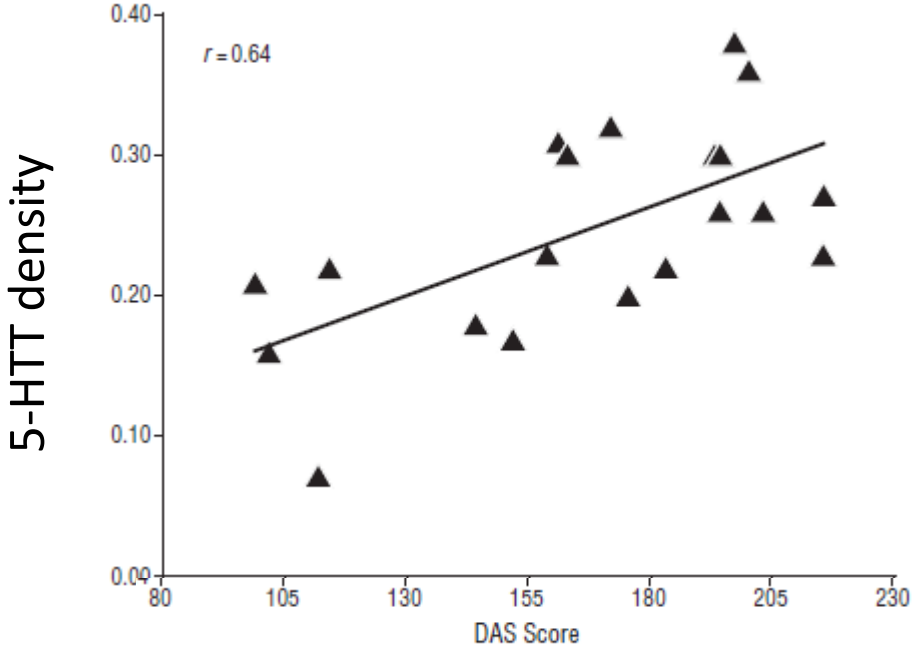


Depressed





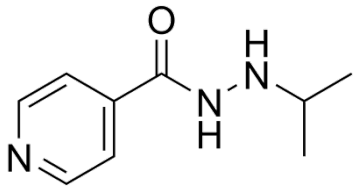




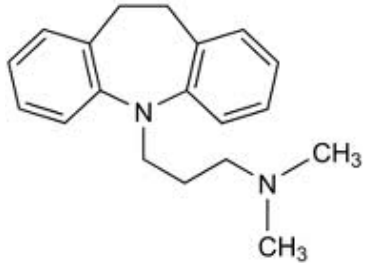
Meyer et al. (2004) Arch Gen Psychiatry 61: 1271
Meyer (2012) Clin Pharm Therapeut 91: 201

Chance discoveries: the basis of past and present anti-depressant treatments

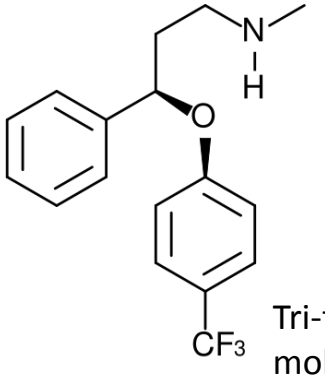
Tuberculosis drug development → Monoamine oxidase Inhibitor (MAOI) in 5-HT, DA, NA neurons → Iproniazid



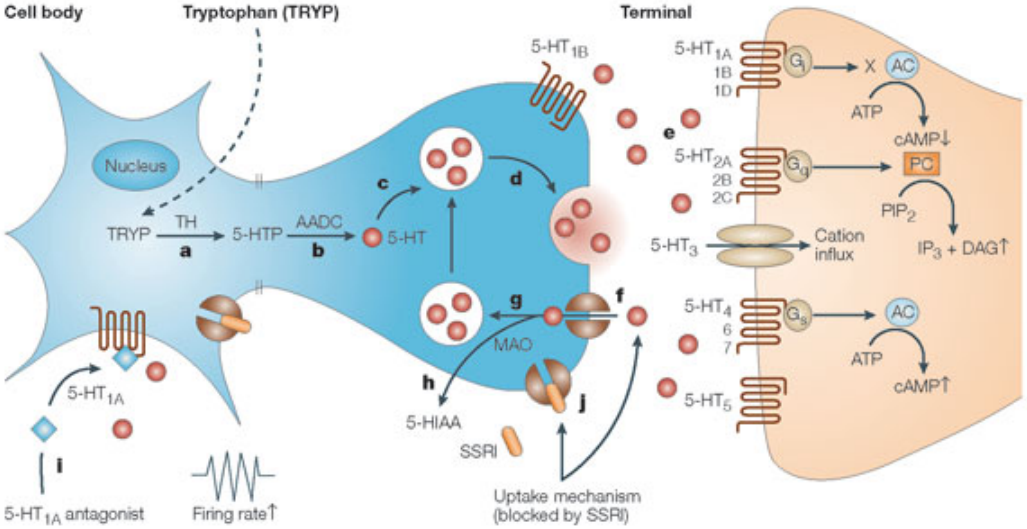
Anti-histamine drug development (antagonistic at H_1 receptor (sedation)) → Tertiary amines Tricyclic antidepressant (TCAs) Also NA, (DA) reuptake inhibition → Imipramine

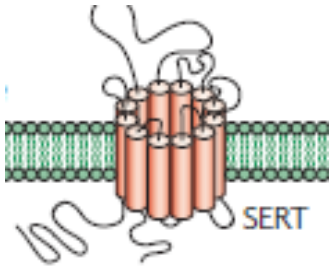


Anti-depressant drug development → Selective serotonin Reuptake inhibitors (SSRIs) → Fluoxetine



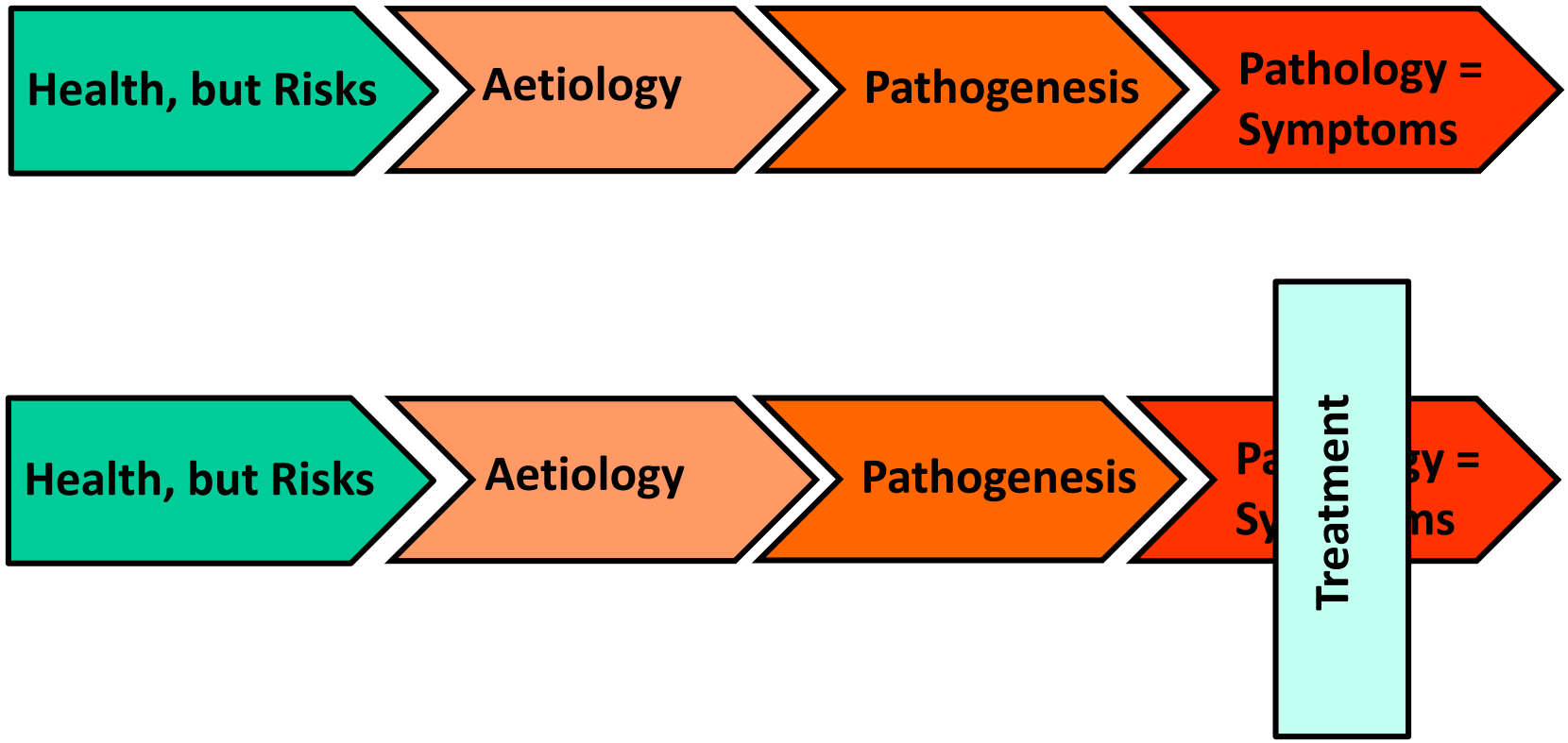
Tri-fluoro-methyl molecule



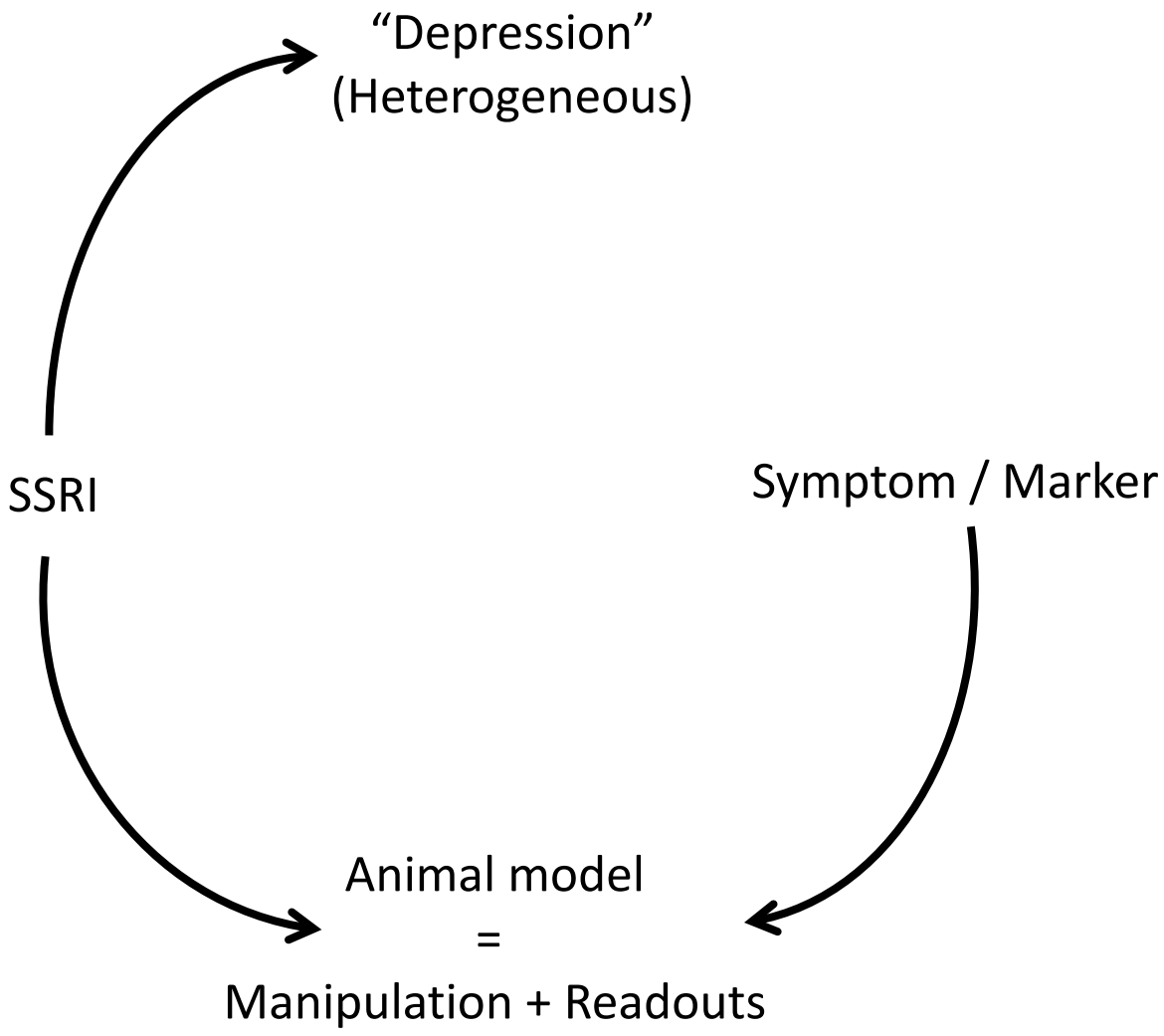


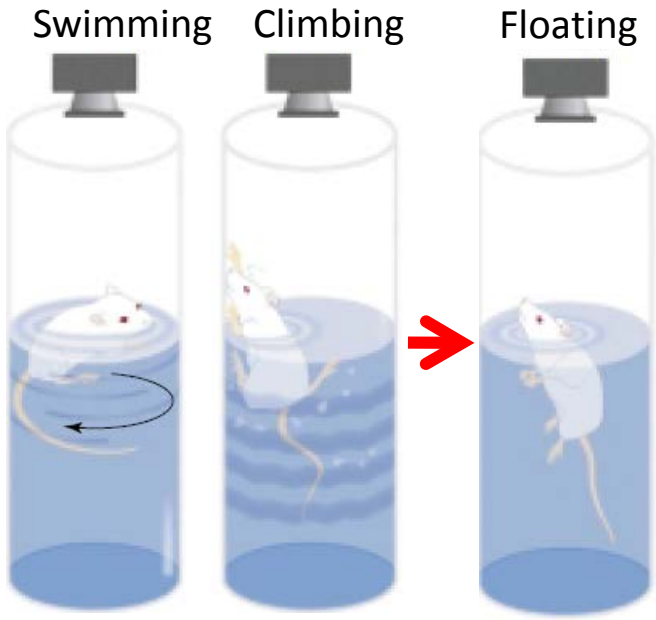
12 Transmembrane domains

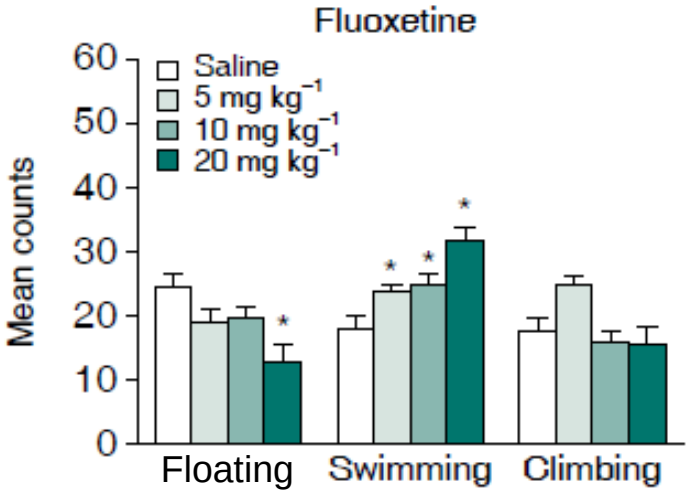
The scientific approach to drug discovery versus the chance approach



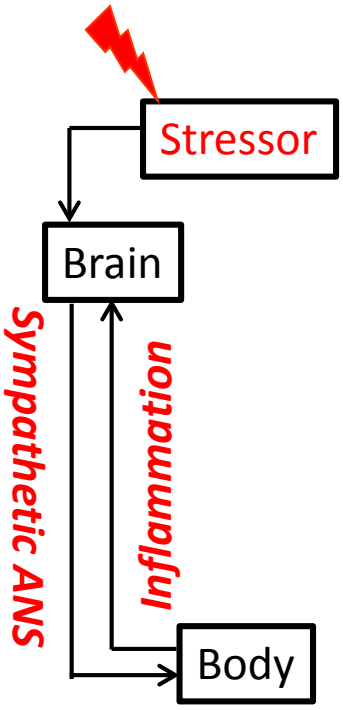
Back-to-front assessment of the predictive validity of animal models
In anti-depressant target discovery and development

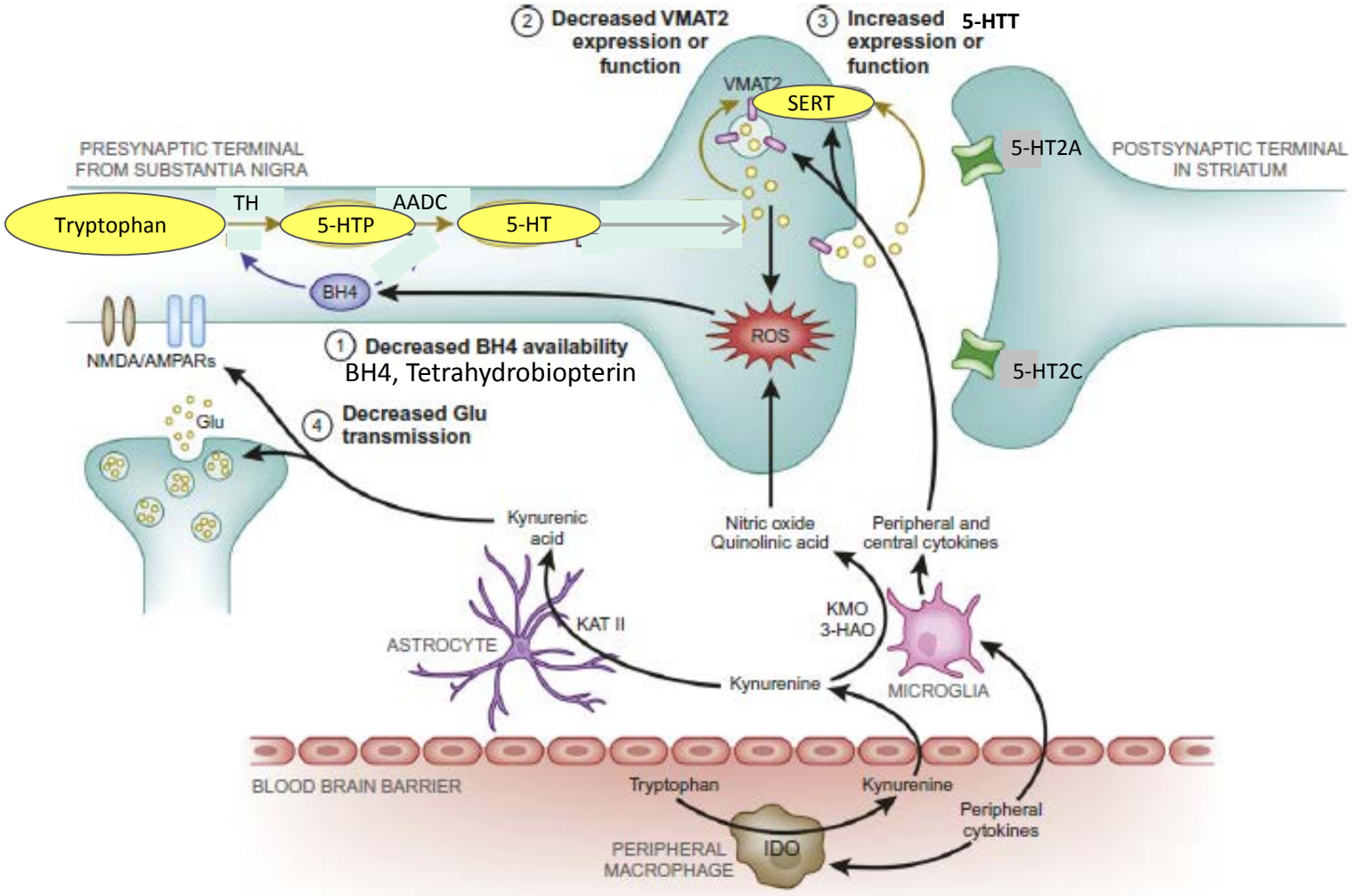






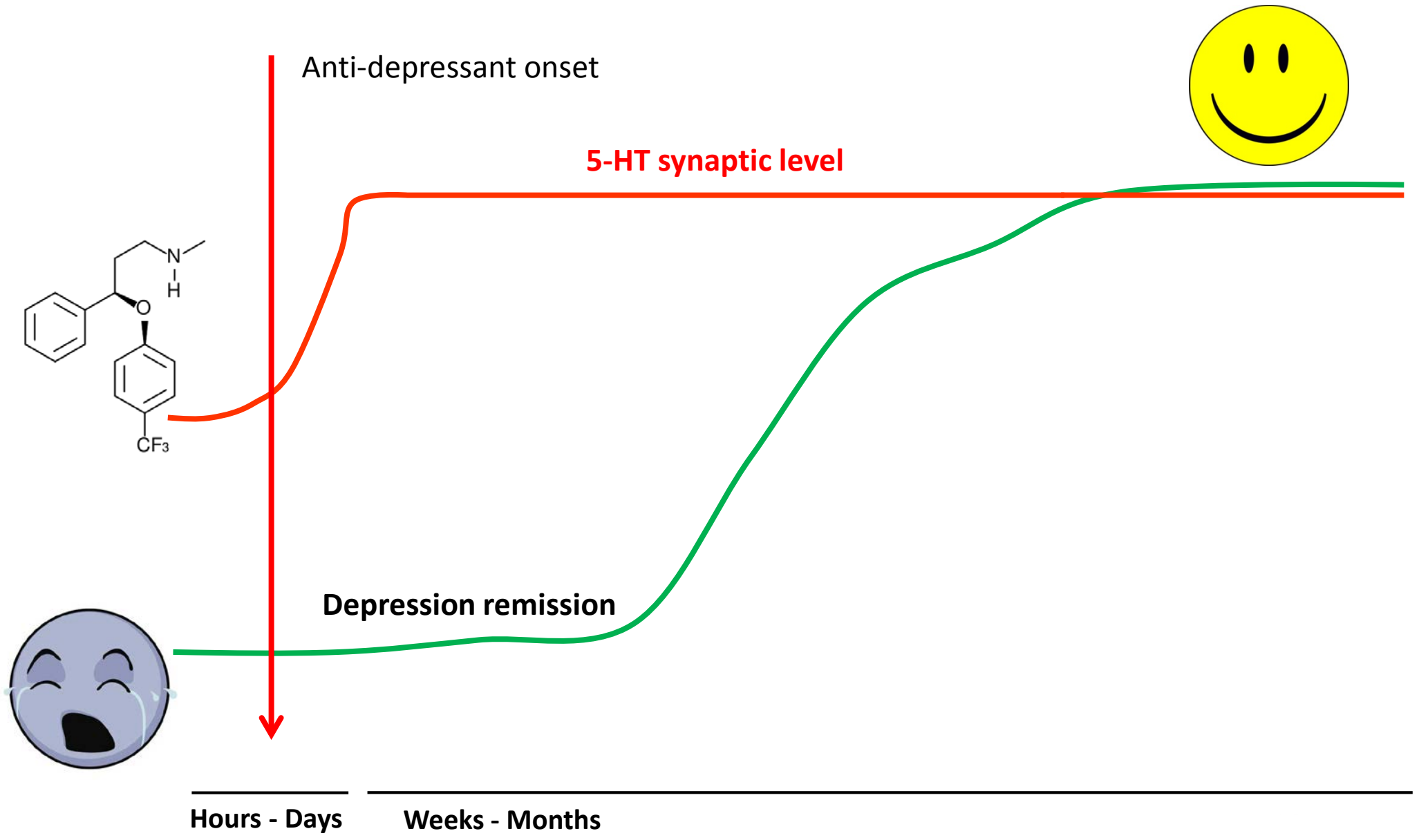
Inflammation Hypothesis: Kynurenine Pathway inhibits Serotonin and Dopamine neurotransmission



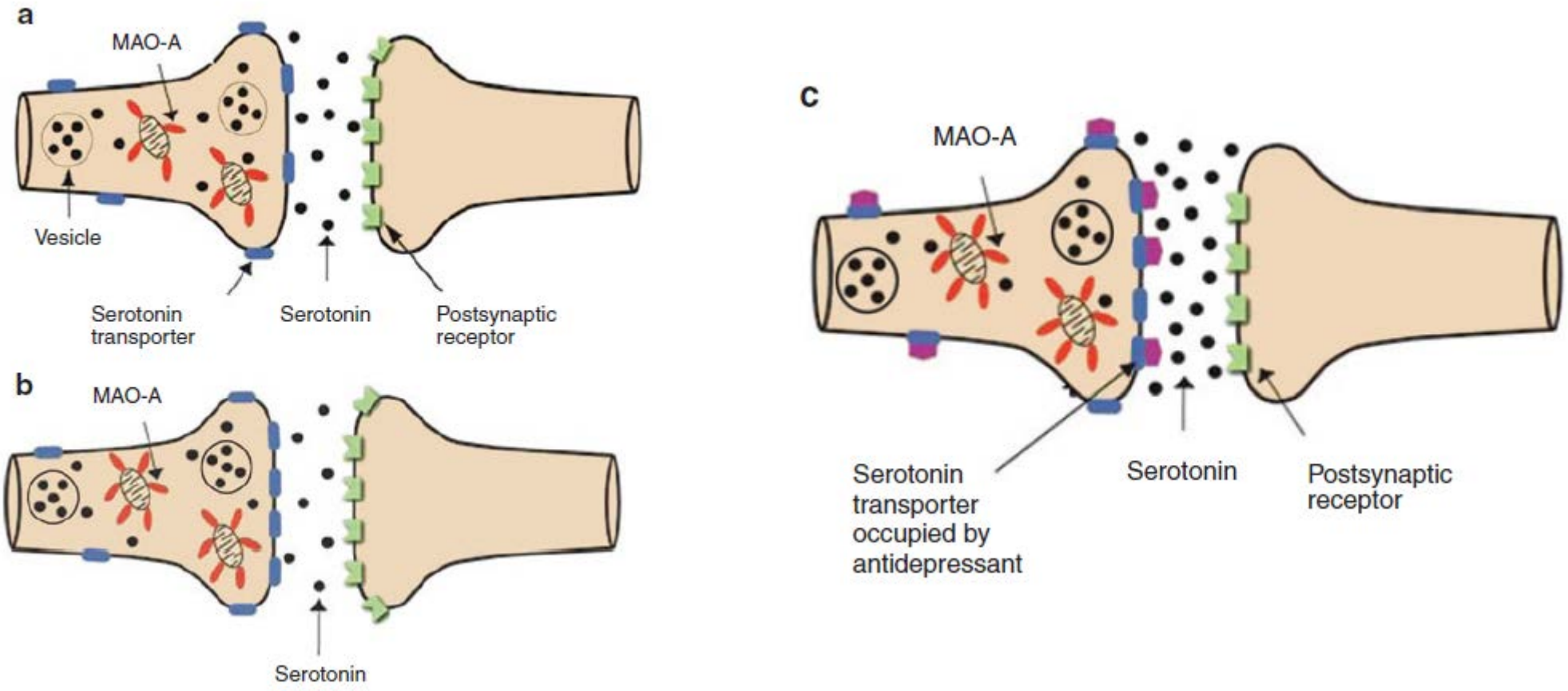


Felger & Miller (2012) Front Neuroendocrinol 33: 315

Possible mechanisms of action of SSRIs: Increased Serotonin in the synapse



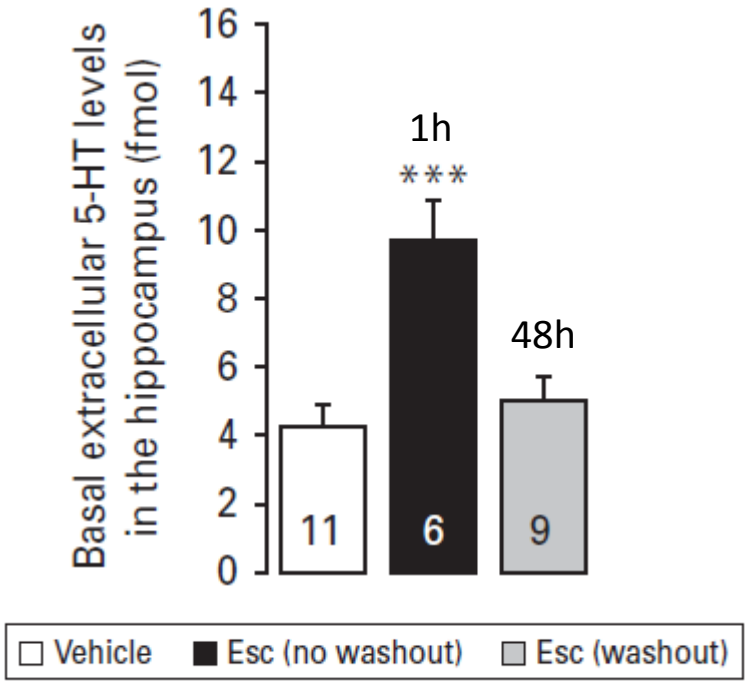
Pharmacotherapeutic strategy of blocking serotonin reuptake



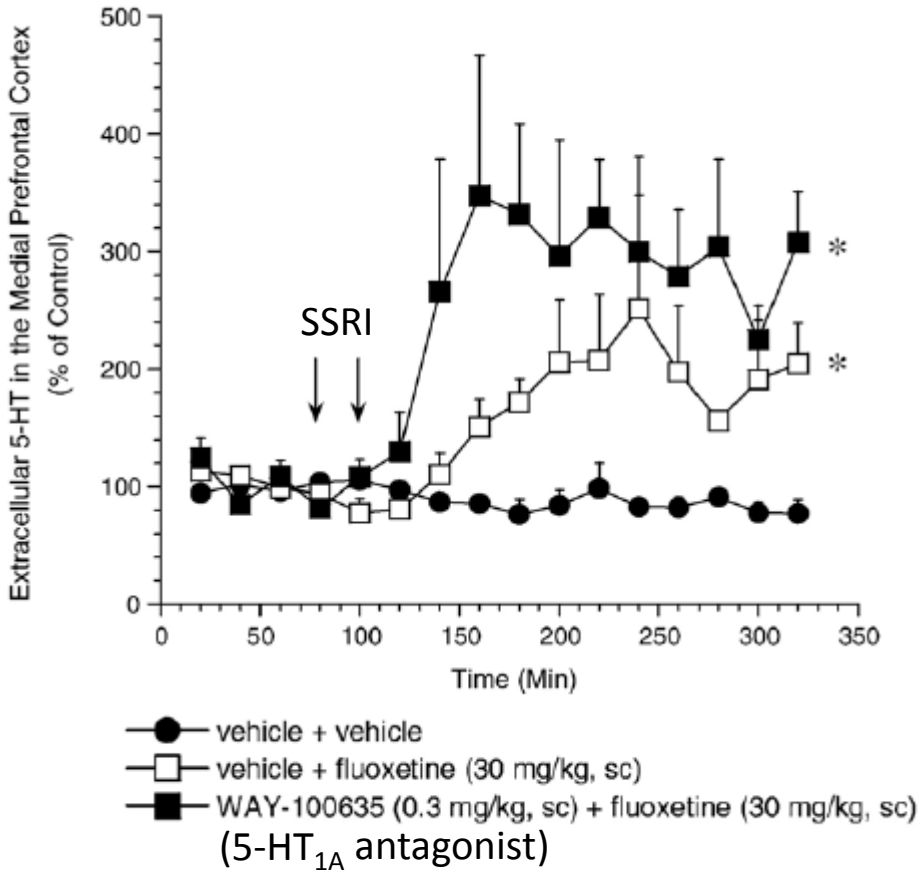
Meyer (2012) Clin Pharm Therapeut 91: 201

Acute SSRI leads to increased serotonin release in the rodent brain

Acute escitalopram effects on 5-HT in mouse hippocampus



Acute fluoxetine effects on 5-HT in rat medial prefrontal cortex



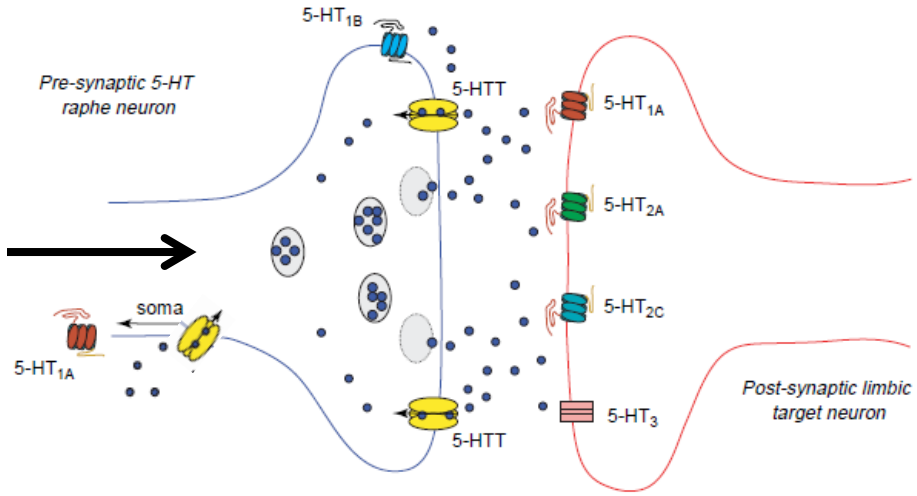
(5-HT_{1A} antagonist)

Guiard et al. (2012) Int J Neuropsychopharmacol 15: 349

Beyer & Cramers (2008) Eur J Pharmacol 580: 350

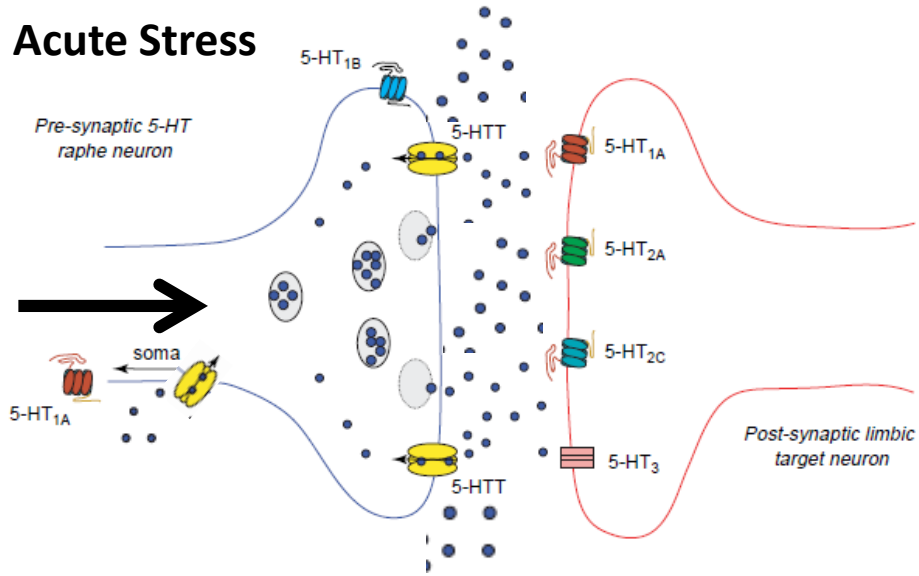
Changes in serotonin signalling in Stress and Depression:
Serotonin levels, 5-HTT levels, and pre- and post-synaptic 5-HT receptor function

(1) Homeostasis



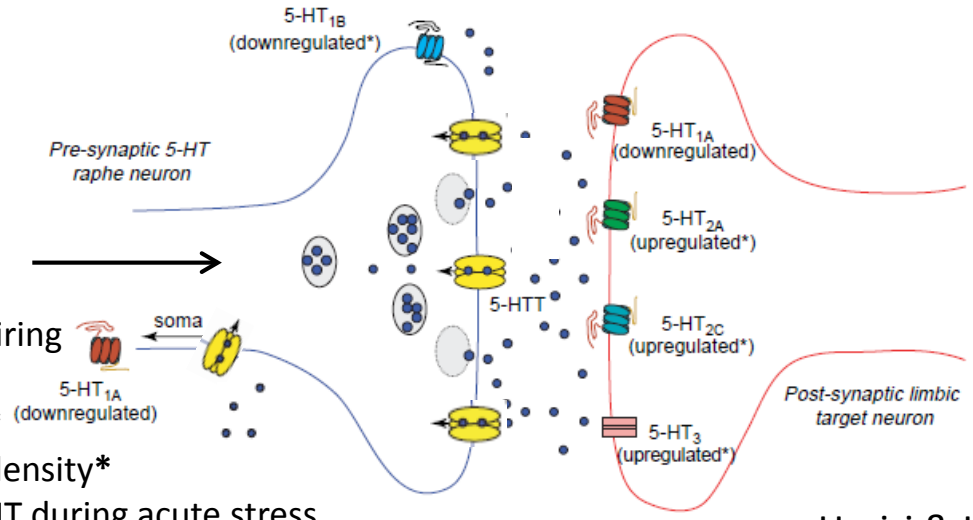
- Moderate 5-HT synthesis and neuron firing
- Moderate 5-HT level in synapse
- Moderate pre-synaptic 5-HTT density
- Post-synaptic 5-HT receptors normal

(2) Acute Stress



- High 5-HT neuron firing
- High 5-HT level in synapse
- Moderate pre-synaptic 5-HTT density
- Post-synaptic 5-HT receptors normal

(3) Depression/Chronic Stress



- Low 5-HT synthesis and neuron firing
- Low 5-HT level in synapse
- High pre-synaptic 5-HTT density*
- Altered 5-HT receptors binding/density*

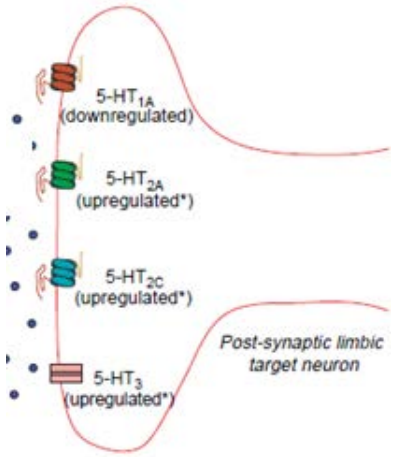
* To compensate for increased 5-HT during acute stress

<u>But, Conditions for</u>:

- Increasing 5-HTT density
- Modifying post-synaptic 5-HT receptors binding/density

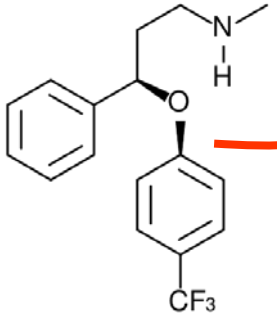
Hariri & Holmes (2006) Trends Cogn Sci 10: 182

Possible mechanisms of action of SSRIs:
5-HT2A/2C Receptor down-regulation



Anti-depressant onset

5-HT synaptic level



Depression remission

5-HT2A/2C Receptor sensitivity

Hours - Days

Weeks - Months

5-HT2A density is increased in depression-relevant regions
- especially in patients with High Dysfunctional Attitude Scale scores (pessimism, hopelessness)

FIGURE 4. 5-HT_2 Binding Potential in 22 Healthy Subjects and 22 Subjects With a Major Depressive Episode Secondary to Major Depressive Disorder and High or Low Scores on the Dysfunctional Attitude Scale[a]

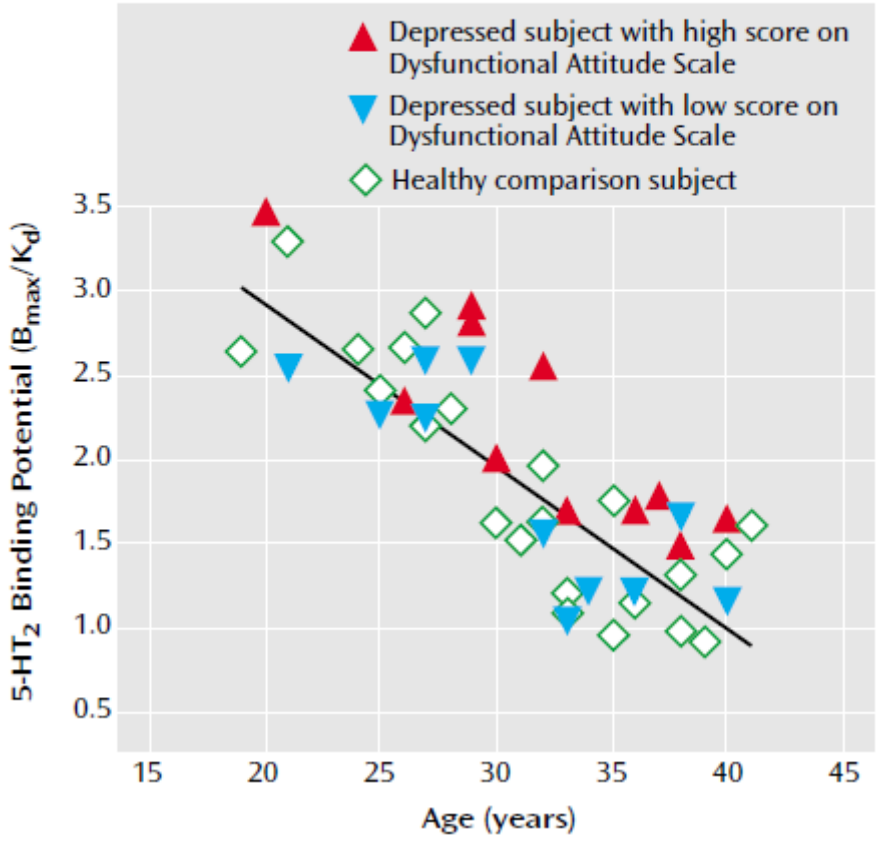
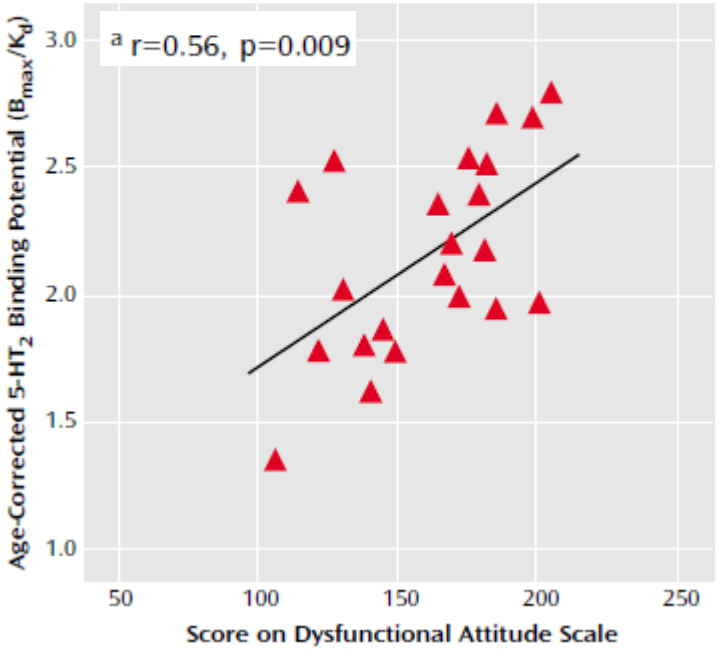
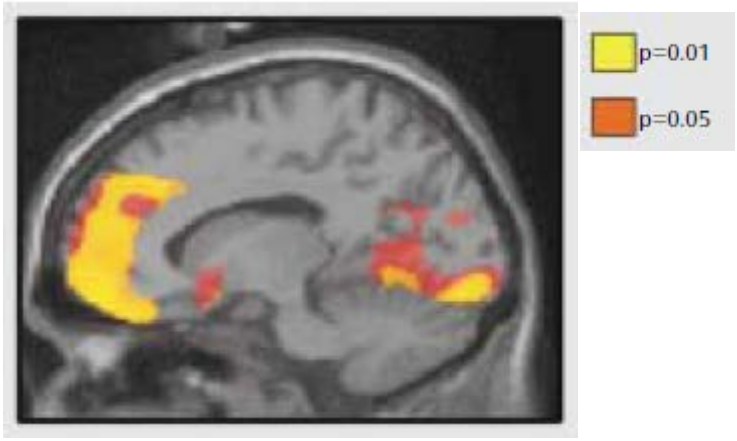


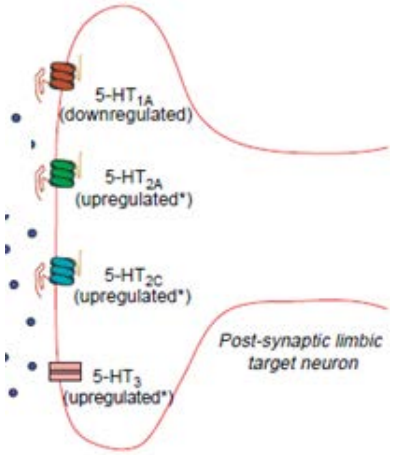
FIGURE 2. Correlation of Age-Corrected 5-HT_2 Binding Potential in the Prefrontal Cortex With Scores on the Dysfunctional Attitude Scale for 22 Subjects With a Major Depressive Episode Secondary to Major Depressive Disorder[a]





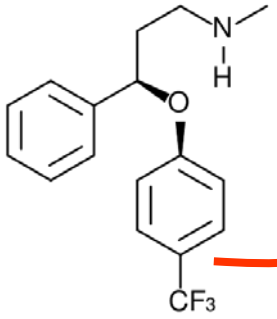
Meyer et al. (2003) Am J Psychiatry 160: 90

Possible mechanisms of action of SSRIs:
5-HT1A up-regulation



Anti-depressant onset

5-HT synaptic level



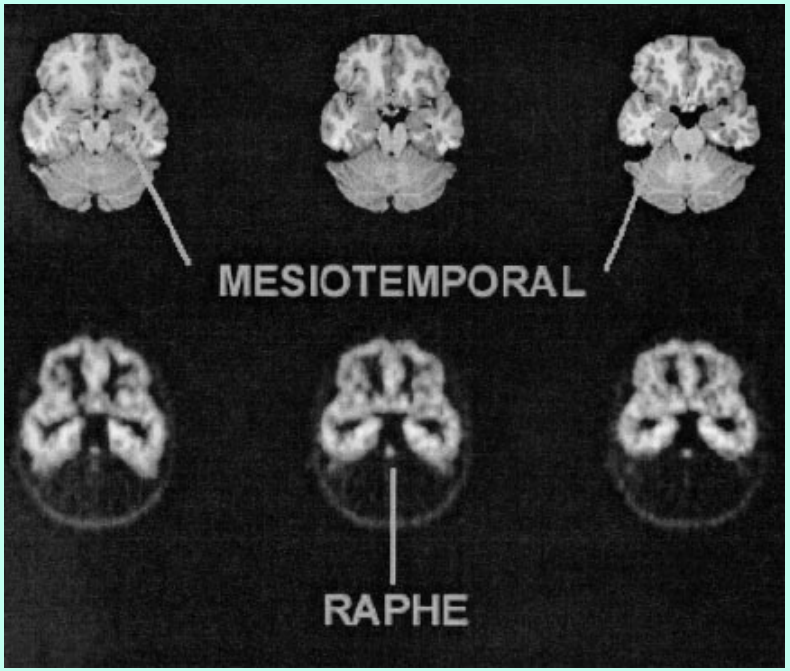
5-HT1A Receptor sensitivity

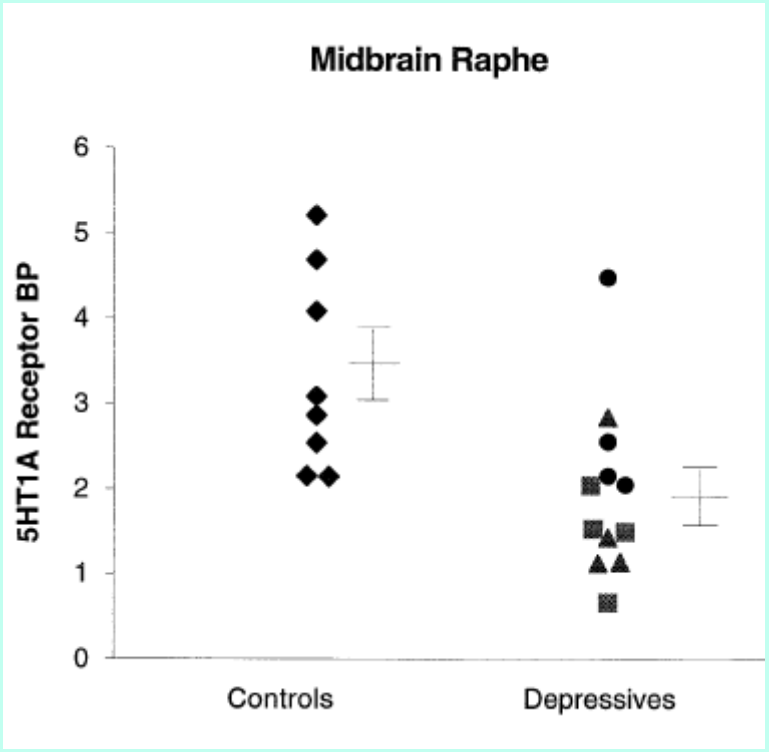
Depression remission

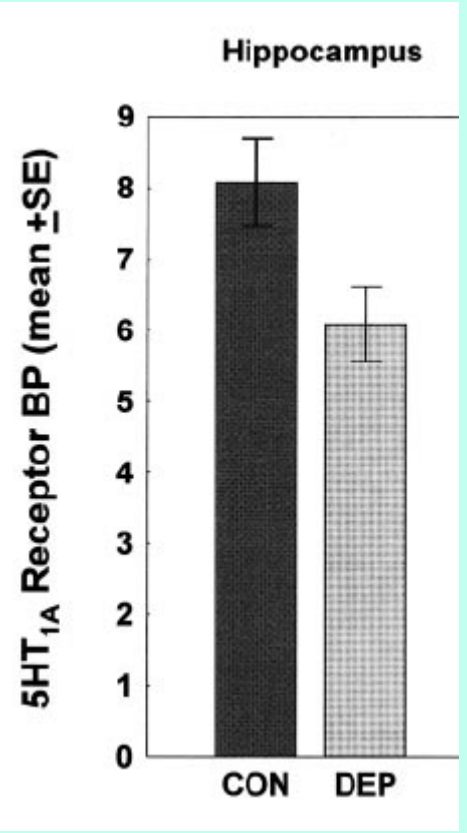
Hours - Days

Weeks - Months

Serotonin 1A receptor in depression using PET [^{11}C]WAY-100635 binding

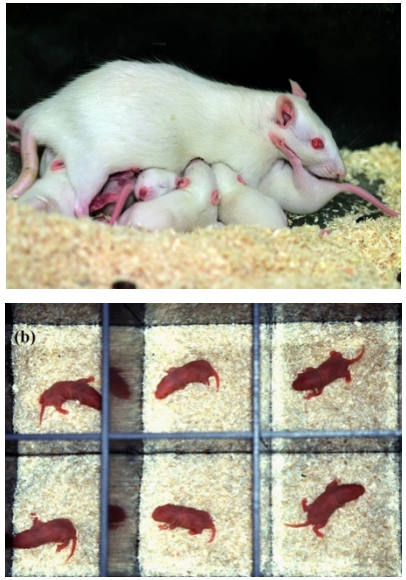


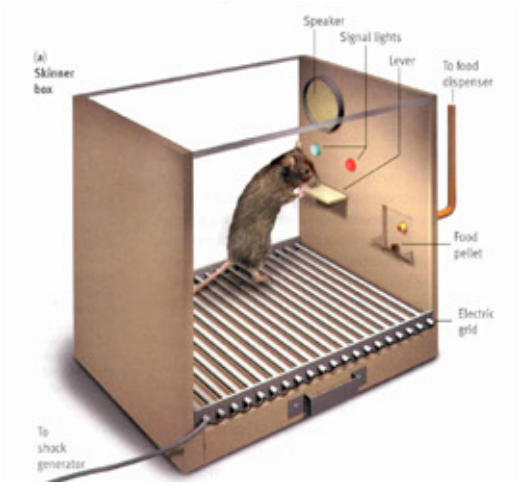


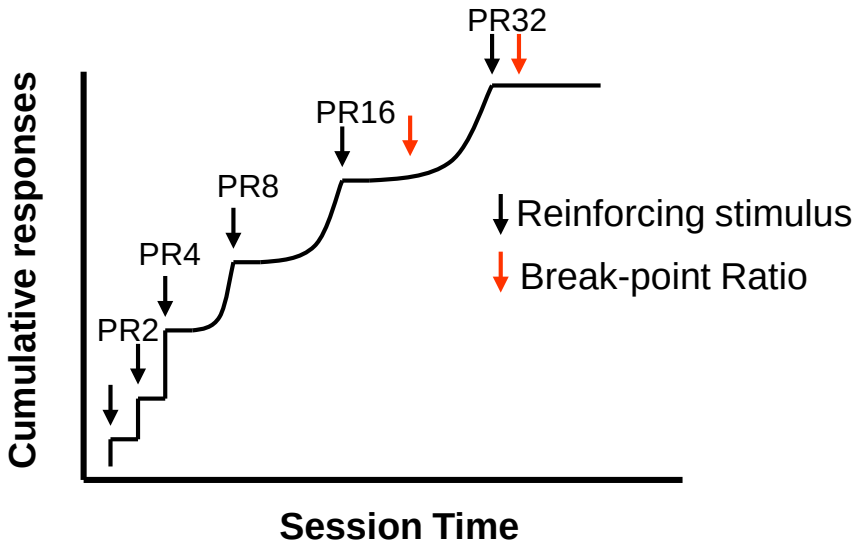


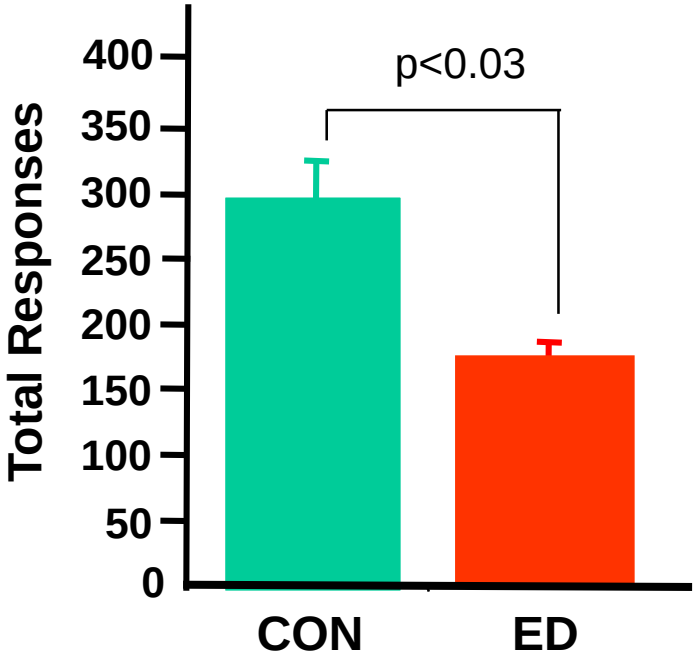
Drevets et al. (2000) Nucl Med Biol 27: 499

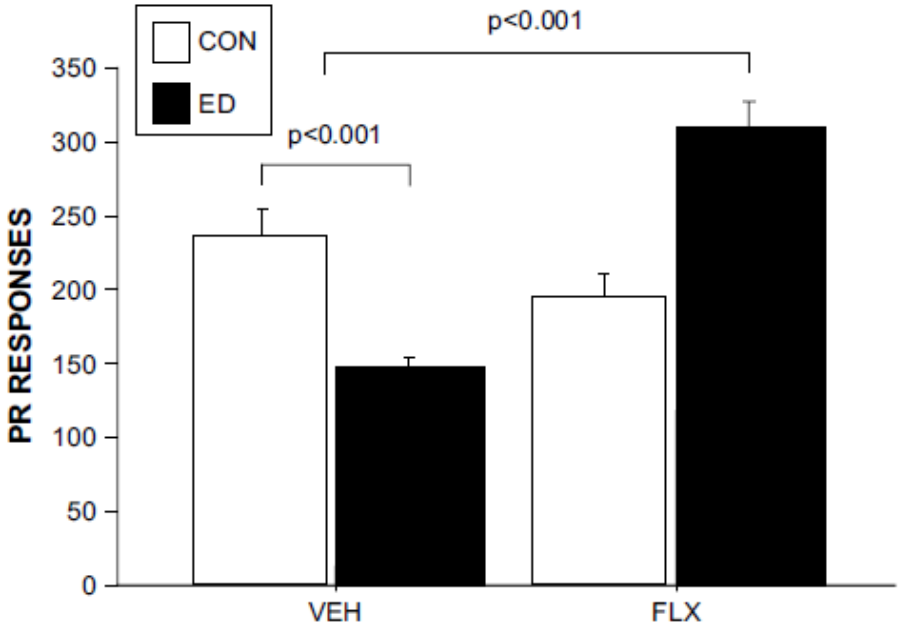
Long-term effects of early deprivation on reward wanting: progressive ratio reinforcement







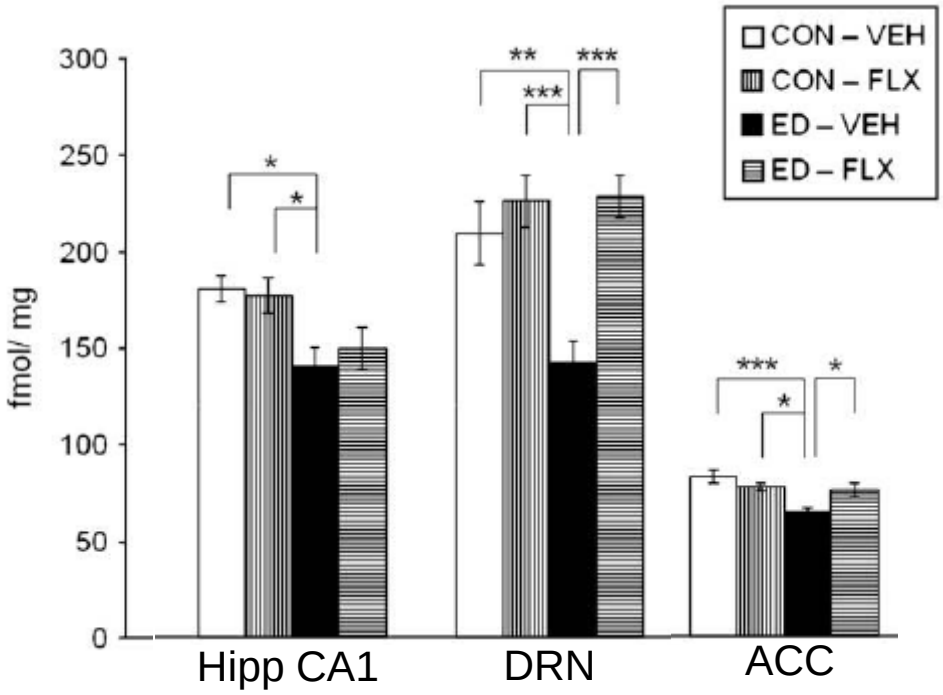


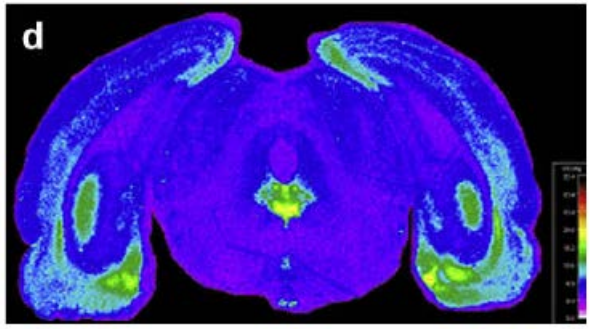


Rüedi-Bettschen et al. (2005) Behav Brain Res 156: 297

Leventopoulos et al. (2009) Neuropharmacol 56: 692

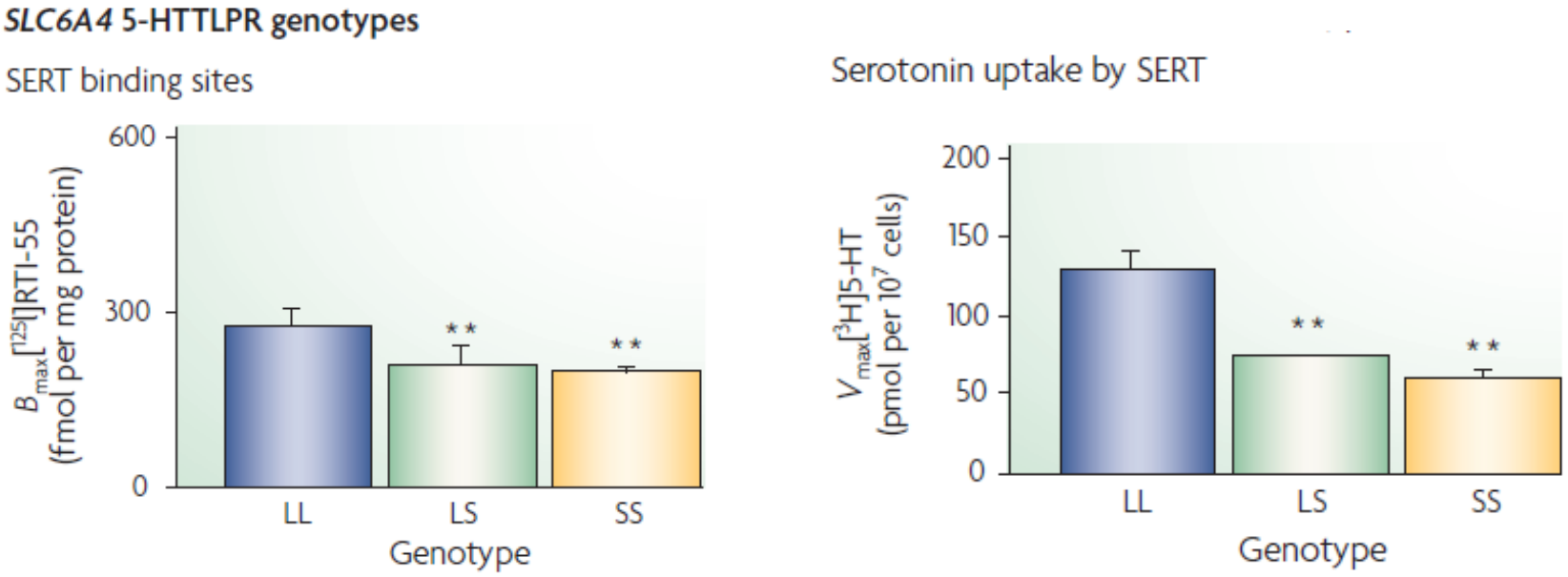
Effects of ED +SSRI on serotonin 1A receptor binding in rat: Reduced [^{3}H]WAY-100635 binding in ACC, Hippocampus and Dorsal raphé nucleus



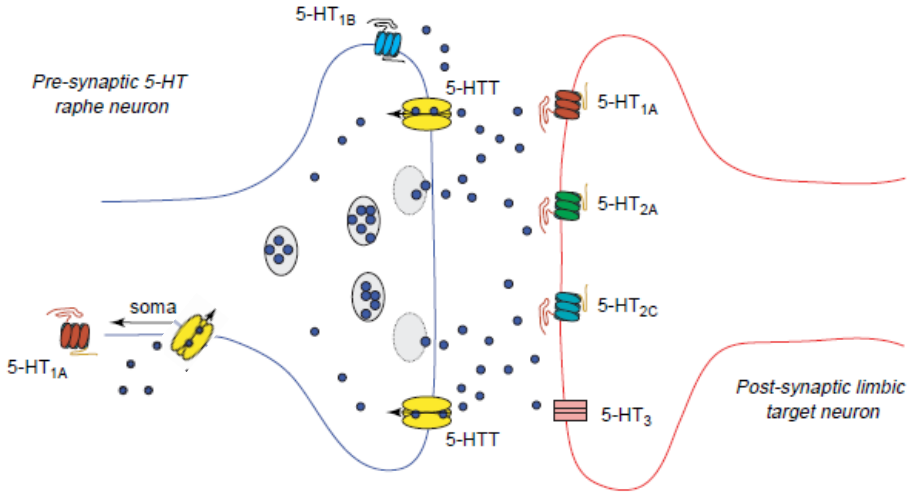


Leventopoulos et al. (2009) Neuropharmacol 56: 692

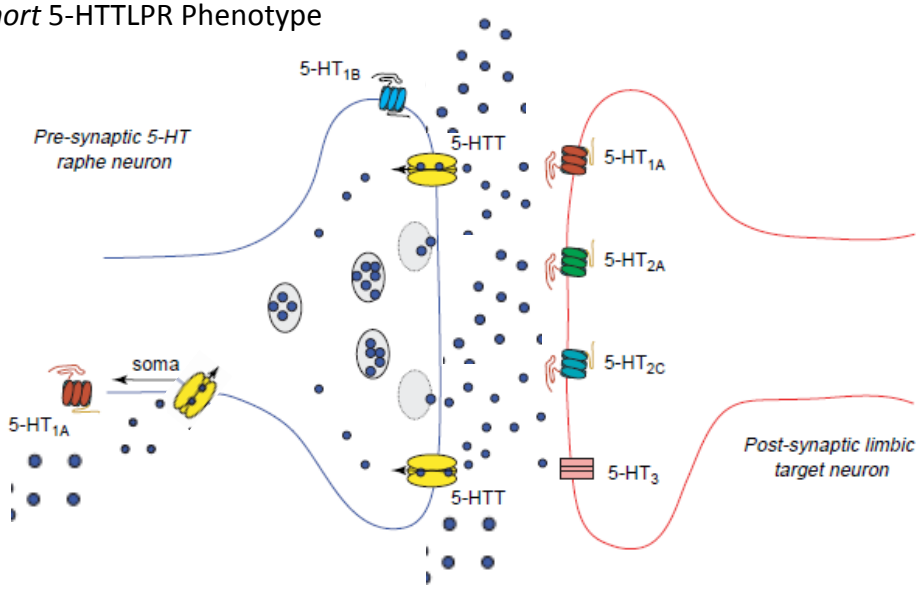
5-HTT promoter gene-linked polymorphic region (5-HTTLPR), (*s)hort* and (*l)ong* genotypes: Developmental effects of low 5-HT reuptake / high 5-HT signaling



Long 5-HTTLPR Phenotype



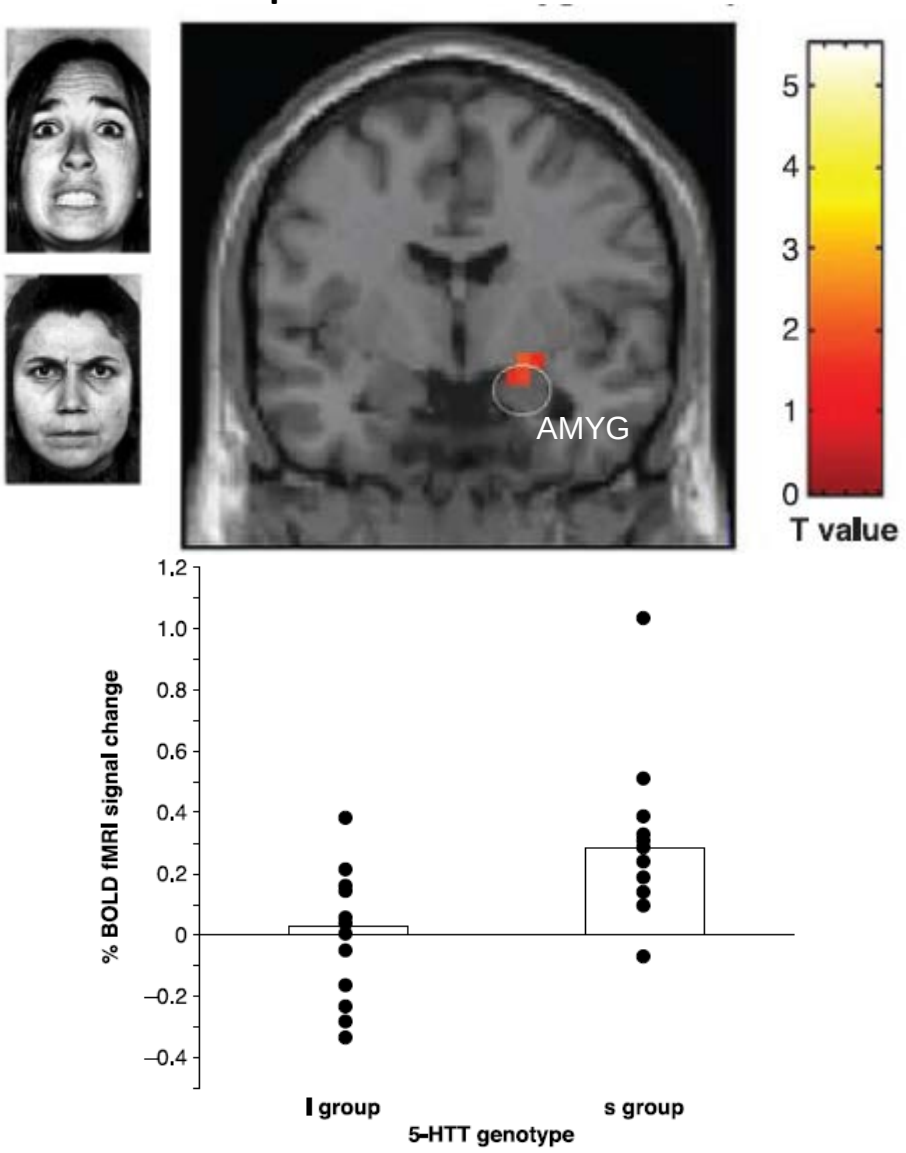
Short 5-HTTLPR Phenotype



Murphy & Lesch (2008) Nature Rev Neurosci 9: 85

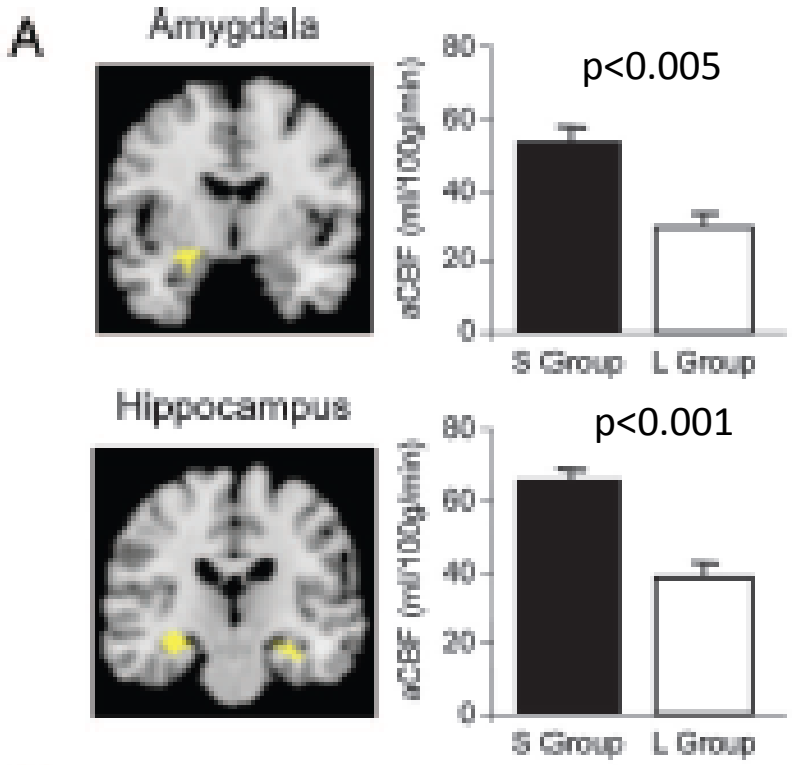
5HTTLPR genotype associated with potential neural endophenotypes of affective disorder – healthy subjects

BOLD fMRI response to fearful face



Hariri et al. (2002) Science 297: 400

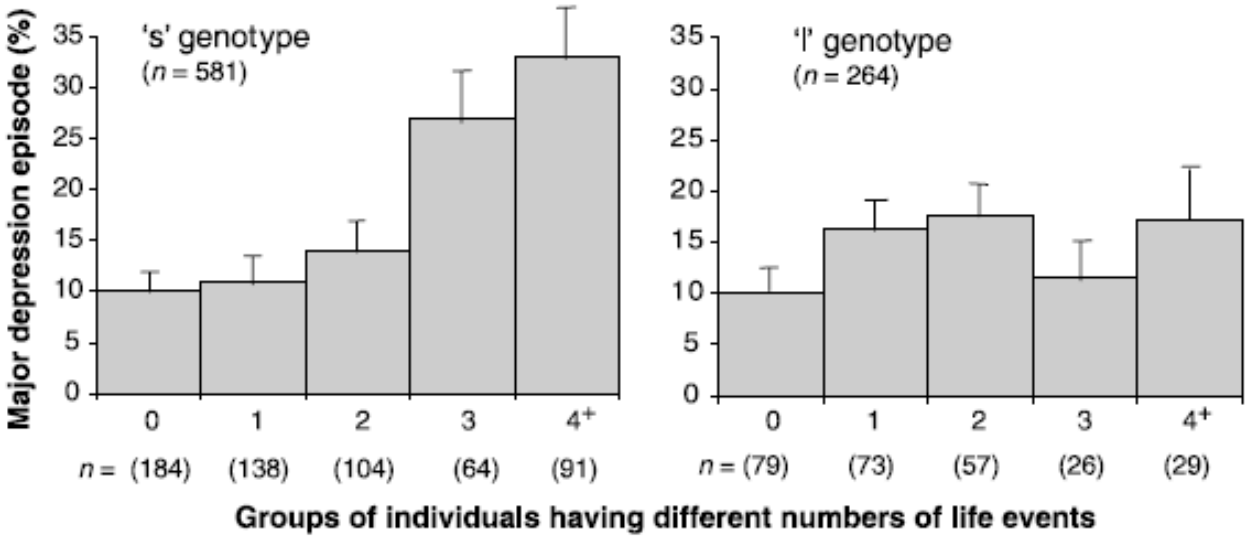
Absolute Cerebral Blood Flow at Rest



Canli et al (2006) PNAS 103: 16033

5-HTTLPR polymorphism interacts with stressful life events to increase prevalence of depression:

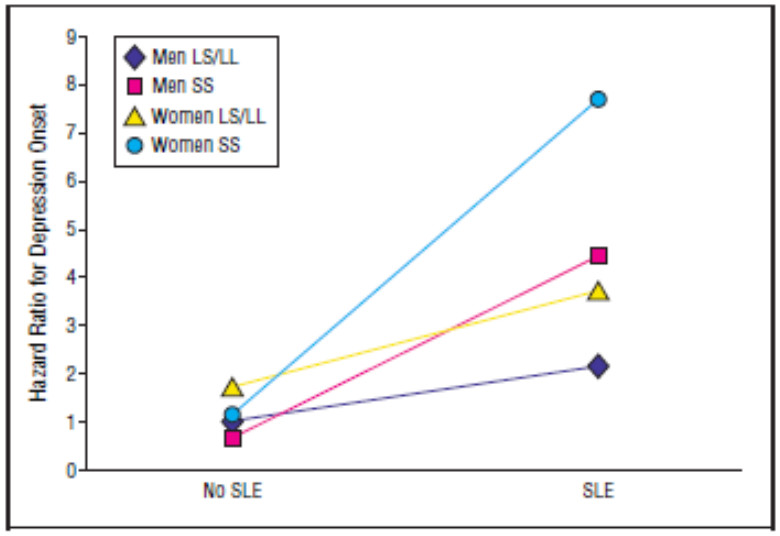
Paradox that the s allele which leads to decreased 5-HTT is the risk polymorphism



Caspi et al. (2003) Science 301: 386

Stressful life events:

- Employment
- Finance
- Health
- Housing
- Social relationships



Kendler et al. (2005) Arch Gen Psych 62: 529

Meta-analysis: 5-HTTLPR polymorphism interacts with stressful life events IN DEVELOPMENT to increase prevalence of depression

Table 2. Studies Included in the Childhood Maltreatment Group Meta-Analysis

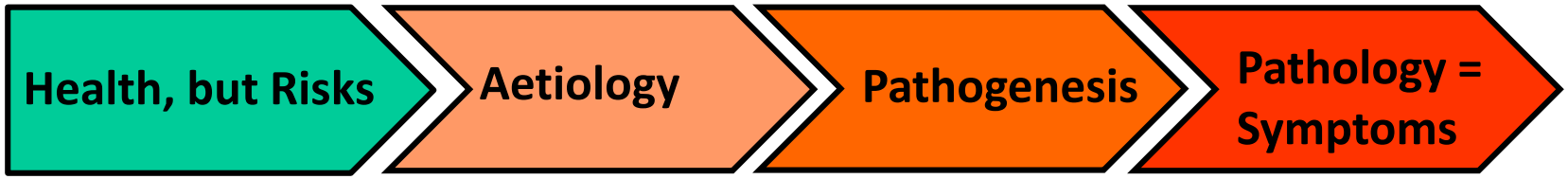
Source, Year	Total No. of Participants	1-Tailed *P* Value	Fisher *P* Value After Study Exclusion
Caspi et al,[1] 2003	845	.010	5.38×10^{-4}
Kaufman et al,[18] 2006	196	.023	1.17×10^{-4}
Cicchetti et al,[22] 2007	339	.252	8.72×10^{-5}
Wichers et al,[39] 2008	394	.200	9.71×10^{-5}
Aguilera et al,[23] 2009	534	5.0×10^{-5}	8.31×10^{-4}
Aslund et al,[40] 2009	1482	.008	1.40×10^{-3}
Ressler et al,[81] 2010	926	.500	2.97×10^{-5}
Benjet et al,[46] 2010	78	.005	9.27×10^{-5}
Kumsta et al,[47] 2010	125	.012	1.03×10^{-4}
Sugden et al,[49] 2010	2017	.160	7.42×10^{-6}
Total	6936		
Average sample size	694		.00007

Table 4. Studies Included in the Stressful Life Events Group Meta-Analysis

Source, Year	Total No. of Participants	1-Tailed *P* Value	Fisher *P* Value After Study Exclusion
Caspi et al,[1] 2003	845	.010	.054
Eley et al,[72] 2004	374	.258	.034
Kendler et al,[19] 2005	549	.007	.047
Jacobs et al,[20] 2006	374	.020	.040
Sjöberg et al,[21] 2006	198	.472	.032
Surtees et al,[74] 2006	4175	.500	.014
Taylor et al,[63] 2006	110	.028	.034
Wilhelm et al,[75] 2006	127	.118	.034
Zalsman et al,[64] 2006	79	.342	.033
Cervilla et al,[76] 2007	737	.014	.050
Chipman et al,[61] 2007	2094	.292	.039
Chorbov et al,[77] 2007	236	.99995	.025
Dick et al,[35] 2007	956	.004	.062
Kim et al,[78] 2007	732	.039	.046
Mandelli et al,[15] 2007	670	.011	.049
Middeldorp et al,[79] 2007	367	.500	.032
Scheid et al,[16] 2007	568	.080	.040
Lazary et al,[38] 2008	567	.002	.050
Power et al,[80] 2010	1421	.620	.026
Araya et al,[34] 2009	4334	.500	.013
Coventry et al,[42] 2010	3243	.500	.021
Bukh et al,[43] 2009	290	.035	.037
Laucht et al,[62] 2009	309	.500	.032
Ritchie et al,[82] 2009	942	.539	.030
Wichers et al,[83] 2009	502	.380	.033
Zhang et al,[45] 2009	792	.998	.016
Hammen et al,[13] 2010	346	.376	.034
Goldman et al,[50] 2010	984	.020	.055
Total	**26 921**		
Average sample size	961		.03

Karg et al. (2011) Arch Gen Psych 68: 444

Applying the scientific approach to antidepressants discovered using the chance approach



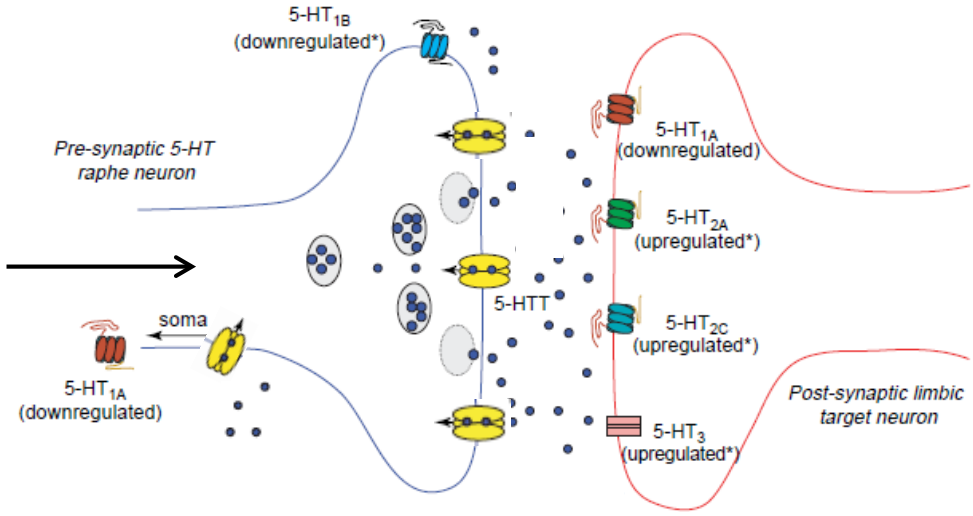
Polymorphism in serotonin genes:
• *s* 5-HTTLPR leads to endophenotypes of increased reactivity to aversive stimuli !!!
• *s* 5-HTTLPR a risk genotype in interaction with early-life stress specifically

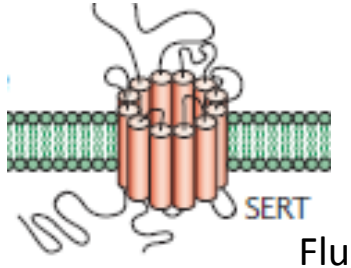
• Some evidence for stress-induced changes in 5-HT receptor levels
• Hypothesis that inflammation leads to changes in 5-HT/5-HT receptor levels

• No strong neurobiological evidence that any major symptom of depression is caused directly by 5-HT/5-HT receptor deficiency

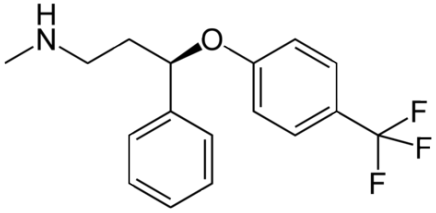
Current generation anti-depressant action: 5-HTT (SERT) blocker / reuptake inhibition

(3) Depression/Chronic Stress

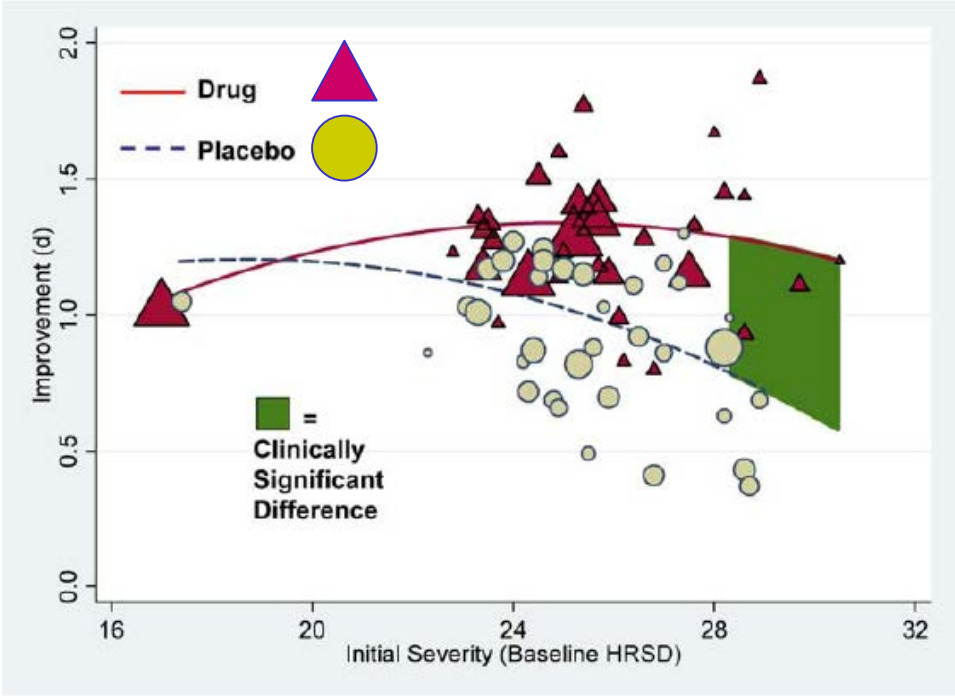




Fluoxetine



SSRI Efficacy: meta-analysis

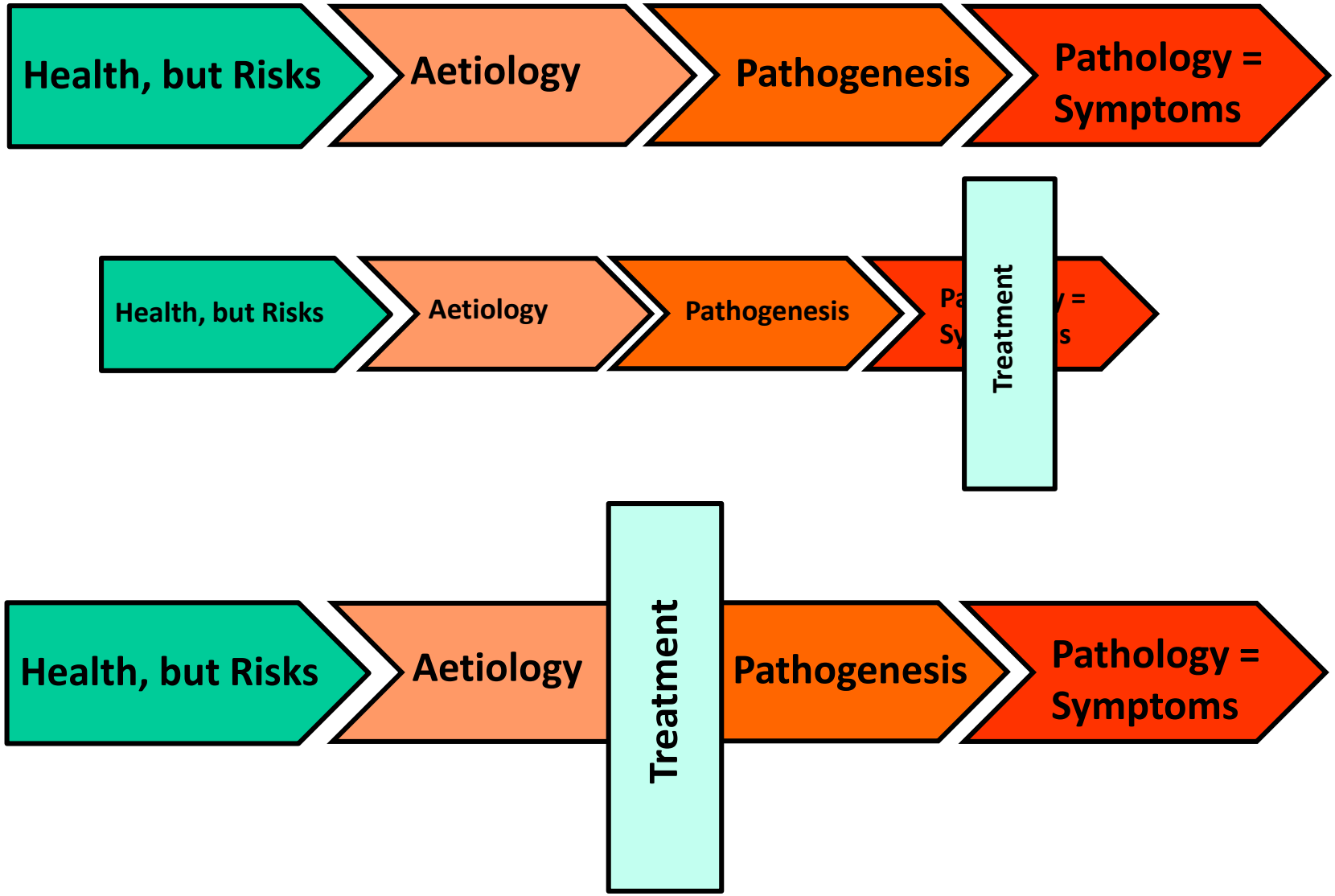


Despite SSRI's:

- Depression remains highly prevalent
- Patient non-response is high
- Onset of response is delayed
- Relapse is common
- Recurrence is common
- Hospitalisation and invasive treatments are common
  (e.g. ECT, experimental pharmacology)

Kirsch et al. (2008) PLoS Medicine 5(2): e45
Cipriani et al. (2018) The Lancet 391(10128):1357

The Aetiology-Pathogenesis Interface as the key to understanding depression:
Understanding the mechanism underlying a disease is essential to its treatment



Selective Serotonin Reuptake Inhibitors and Affective disorders

- Serotonin neuron cell bodies located in Raphe nuclei and project to structures important in emotional and cognitive processing
- 5-HT1A receptor, VMAT, 5-HTT and MAO are important pre-synaptic regulators of 5-HT neurotransmission
- Thirteen 5-HT receptors are important post-synaptic mediators of 5-HT neurotransmission. 5-HT1A, 5-HT2A, 5-HT2C are proposed to be particularly important in stress/depression
- Serotonin projects to several structures that exhibit altered activity in depression, including Anterior cingulate cortex, Amygdala, Hippocampus, Nucleus accumbens
- Evidence that several factors that regulate or mediate serotonin neurotransmission are altered in depression
- Chance findings that drugs that inhibit serotonin catabolism or reuptake are anti-depressant have led to SSRIs as the current generation of anti-depressant drugs
- The scientific approach of targeting the aetiology-pathophysiology provides only limited support for SSRIs as depression drugs
- SSRIs leading to decreased 5-HT2A and/or 2C signalling or increased 5-HT1A signalling have been proposed as possible mechanism of action
- There is a paradox that the current treatment of depression is SSRI (5-HTT blocker) but the major risk factor is a polymorphism that leads to decreased 5-HTT expression/function. This polymorphism is a risk factor in interaction with childhood stress
- SSRIs are comparable to placebo in mild depression and somewhat more efficacious than placebo in severe depression