A roadmap to PTSD treatment research

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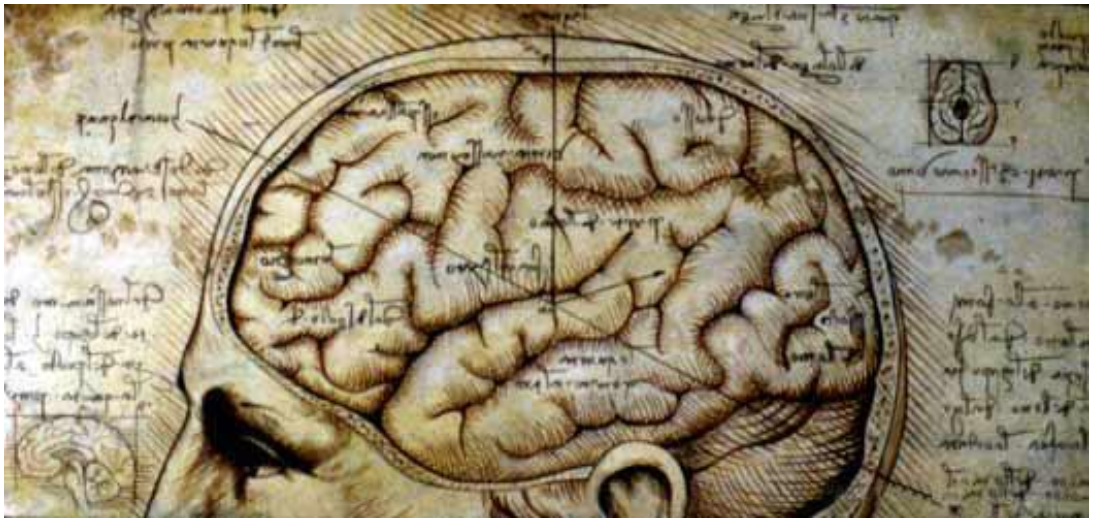
- 7 World Trade Center -

The tragic events that I eye-witnessed exactly eleven years ago while being not far from here and watching what happened have changed our world. We learned what we should have known before: there is no safe place on this planet earth and atrocities can happen everywhere.

This event and the increasing awareness of the high risk for post traumatic stress disorder, abbreviated PTSD, among combat veterans doing service in current and previous wars have rekindled public interest in this clinical condition. On the positive side we see how mutually fertilizing basic and clinical research in this area provided a new fundament on which we can build a roadmap for better treatment of PTSD:

The unique human brain

Max Planck Institute of Psychiatry



Leonardo da Vinci (1452 – 1519)

The human brain is unique with regard to its capacity to associate stored with new information and to its richness of new ideas, which seem to be inexhaustible.

This creativity, however, is unfortunately not limited to the arts and science, to social responsibility and ethical values. Man-made atrocities can reach dimensions that had been unimaginable until we learned that they can happen. Such atrocities and shocking events are

traumata, which comprise all functions of our body including a very specific form of anxiety disorder.

In the 1980ies the term posttraumatic stress disorder was coined and Rachel Yehuda, the president of our society has championed the public recognition of that clinical state in as much as she had been most influential and successful in the research of causality and treatment of trauma victims suffering from PTSD. Therefore, I am grateful for being her presidential lecturer today.
PTSD is not a disease of modern times, Shakespeare describes PTSD in his drama Henry IV quite precisely.

PTSD described by William Shakespeare

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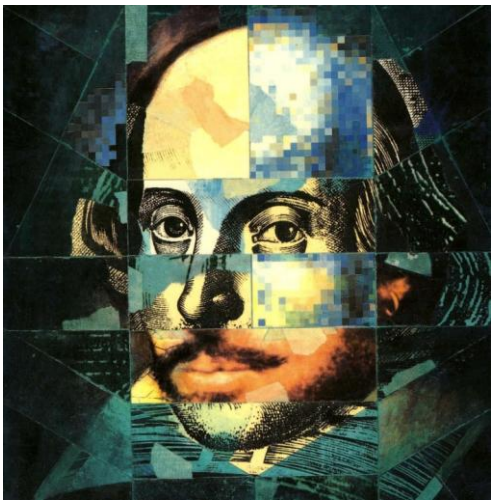
Lady Percy in Henry IV :

Tell me, sweet lord, what is 't that takes from thee
Thy **stomach, pleasure**, and thy **golden sleep**?
Why dost thou **bend thine eyes upon the earth**
And start so often when thou sit'st alone?
Why hast thou lost the **fresh blood in thy cheeks**
And given my treasures and my rights of thee
To thick-eyed musing and **curst melancholy**?



William Shakespeare (1564 – 1616)

Lady Percy recognizes that the king has severe nightmares in which he talks of a "tale of iron wars" and when he is awake she sees him acting like a ghost.

What Lady Percy describes are further symptoms of depression and PTSD, such as loss of appetite, anhedonia, dissociative thinking, autonomous nervous symptoms, such as musing and in particular sleep disturbances.

Leonidas at the Thermopylae (480 B.C.)

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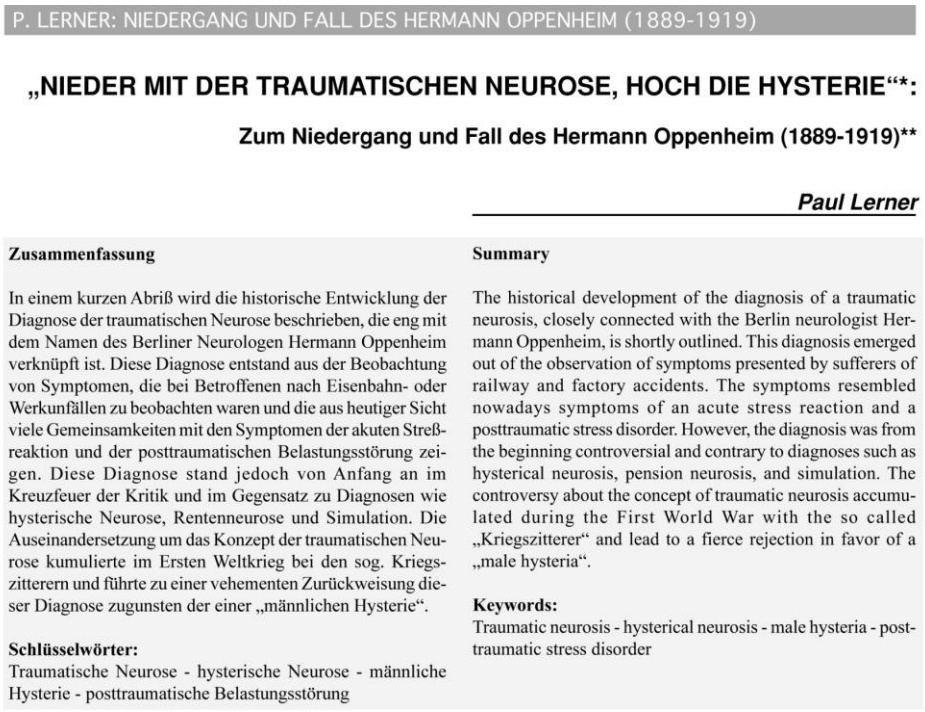
Jacques Loùis David, 1814

But also in antiquity combat experience had its toll and what we now call PTSD had been well described. For example, the Greek historian Herodotus (484-420 B.C.) wrote that the commander of the battle of Thermopylae in 480 B.C., Leonidas, has dismissed his men from going to combat because they were psychologically exhausted.

This clearly contrasts to the spirit of the painting by Jacques Loùis David that obviously idealizes the cruel war. Herodotus mentions that one soldier was so shaken by battle that he was nicknamed “The Trembler” – the soldier later hanged himself, perhaps in shame, perhaps in depressive desperation, we will never know. “The Trembler” became recognized two thousand years later, when, as a consequence of the brutalities of World War I, large numbers of combat men were suffering from what we call now PTSD. In Germany they were called “Kriegszitterer” similar to the description of “The Trembler” by Herodotus.



P. LERNER: NIEDERGANG UND FALL DES HERMANN OPPENHEIM (1889-1919)

„NIEDER MIT DER TRAUMATISCHEN NEUROSE, HOCH DIE HYSTERIE“*:

Zum Niedergang und Fall des Hermann Oppenheim (1889-1919)**

Paul Lerner

Zusammenfassung

In einem kurzen Abriß wird die historische Entwicklung der Diagnose der traumatischen Neurose beschrieben, die eng mit dem Namen des Berliner Neurologen Hermann Oppenheim verknüpft ist. Diese Diagnose entstand aus der Beobachtung von Symptomen, die bei Betroffenen nach Eisenbahn- oder Werkunfällen zu beobachten waren und die aus heutiger Sicht viele Gemeinsamkeiten mit den Symptomen der akuten Streßreaktion und der posttraumatischen Belastungsstörung zeigen. Diese Diagnose stand jedoch von Anfang an im Kreuzfeuer der Kritik und im Gegensatz zu Diagnosen wie hysterische Neurose, Rentenneurose und Simulation. Die Auseinandersetzung um das Konzept der traumatischen Neurose kumulierte im Ersten Weltkrieg bei den sog. Kriegszitterern und führte zu einer vehementen Zurückweisung dieser Diagnose zugunsten der einer „männlichen Hysterie“.

Schlüsselwörter:
Traumatische Neurose - hysterische Neurose - männliche Hysterie - posttraumatische Belastungsstörung

Summary

The historical development of the diagnosis of a traumatic neurosis, closely connected with the Berlin neurologist Hermann Oppenheim, is shortly outlined. This diagnosis emerged out of the observation of symptoms presented by sufferers of railway and factory accidents. The symptoms resembled nowadays symptoms of an acute stress reaction and a posttraumatic stress disorder. However, the diagnosis was from the beginning controversial and contrary to diagnoses such as hysterical neurosis, pension neurosis, and simulation. The controversy about the concept of traumatic neurosis accumulated during the First World War with the so called „Kriegszitterer“ and lead to a fierce rejection in favor of a „male hysteria“.

Keywords:
Traumatic neurosis - hysterical neurosis - male hysteria - posttraumatic stress disorder

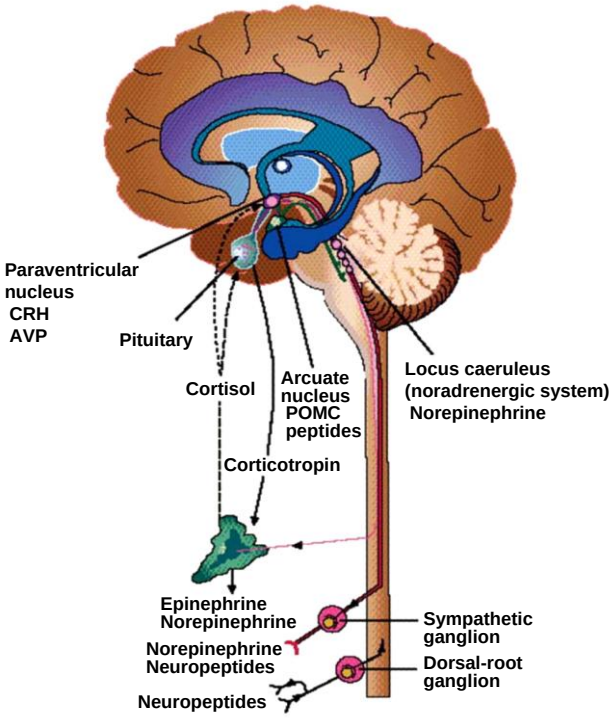
Already in the year 1889 the German neurologist Hermann Oppenheim published his concept of “traumatic neurosis”. He emphasized the psychological causality but notably he also suggested that – as he said “molecular rearrangements” may play a role. This idea is remarkable as it points to molecular events that we call today epigenetics.

Oppenheim’s position was not accepted by the science community and he remained a disliked outsider, a position which made him suffer throughout his life.

Now with the engagement of troops from European countries in Afghanistan, PTSD again receives much attention in Europe. But despite of all protestations to the contrary there are two sobering facts: Stigma and insufficient treatment continue to burden patients with PTSD.

To overcome these burdens is a pressing need and an important research task. In my talk today I will take a few examples to demonstrate that a wealth of information from clinical and basic research has been accumulated which justifies optimism. The achievements allow one to predict that both goals, elimination of stigma and optimized treatment as well as prevention are feasible in the future. It is my experience that whenever you have an objectifiable laboratory measure, the issue of stigma and discrimination gradually disappears:

Humoral responses to stress



Obviously, two prime candidates have been in the focus since the beginning of PTSD research of causality and treatment. They are the usual suspects in all stress physiology, namely catecholamines and cortisol.

I do not have to be very explicit to this audience as everyone knows that acute exposure to a stressor enhances sympathetic and decreases parasympathetic activity as is reflected by elevations of plasma catecholamines and a number of clinical signs and symptoms. This response to stress is swiftly followed by enhanced activity of the HPA axis which manifests by increased plasma cortisol and corticotropin (ACTH) concentrations. When the stress exposure is over, all these adaptive humoral responses come back to baseline. If this return to baseline is impaired stress-related diseases can develop particularly in individuals carrying risk factors.

Mason and colleagues reported already in 1986 that although combat veterans with PTSD demonstrated sustained elevations in urinary catecholamines, their plasma cortisol levels were decreased.

Stress-hormone response to stress

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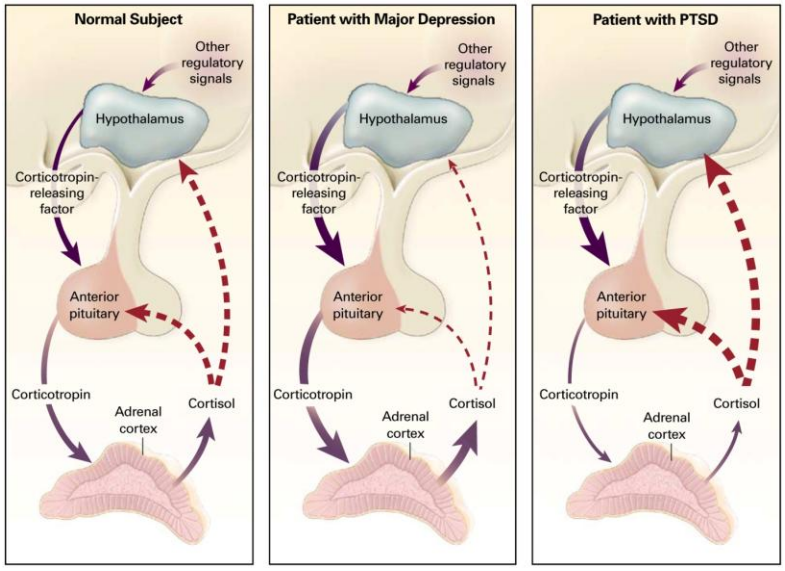

Development of hypercortisolism in depression and hypocortisolism in PTSD

R. Yehuda

This unexpected finding was confirmed and extended in well controlled studies by Rachel Yehuda, showing that throughout the diurnal cycle plasma cortisol levels were lower in combat veterans than in controls, although their corticotropin releasing hormone (CRH) concentrations in the cerebrospinal fluid were increased.

Similarly, in rape victims lower plasma cortisol levels but higher levels of plasma MHPG, a metabolite of epinephrine and norepinephrine were associated with the risk for PTSD.

Hair cortisol concentrations were higher in traumatized non-PTSD group than in traumatized PTSD patients after earthquake

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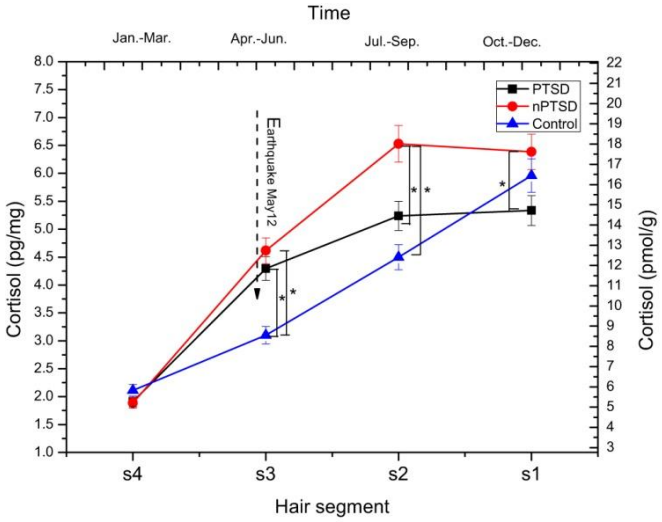


Analysis of cortisol levels in 3-cm hair segments (S1, S2, S3, or S4) from posttraumatic stress disorder (PTSD) (*n* = 32), non-PTSD (nPTSD; *n* = 32), and nontraumatized control (*n* = 20) groups (*$p<.05$ significance, error bar: 95% confidence interval). S1, period of 5 to 7 months after the earthquake; S2, period of 2 to 4 months after the earthquake; S3, period between 2 months before and 1 month after the earthquake; S4, period of 3 months before the earthquake.

Luo et al., 2012

Of particular interest is a study from China measuring cortisol in the hair of individuals that were exposed to the earthquake in Wenchuan 2008. The authors found lower hair cortisol concentrations in these individuals that were suffering from PTSD.

The hair cortisol concentration can be taken as a longterm integral of cortisol secretion. This study is a further evidence of a link between traumatized individuals, that suffer from PTSD and lower cortisol levels.

The studies of Rachel Yehuda and a number of other investigations including holocaust survivors, combat veterans and persons involved in motor vehicle accidents or physical assault all agreed that low cortisol levels are associated with increased PTSD risk. Nevertheless, some studies did not find this decrease of cortisol concentrations, which again indicates that patients assembled under the diagnostic umbrella of PTSD are not uniform with regard to underlying causality.

One interpretation of the association between low cortisol and PTSD is offered by McGaugh and Roozendaal, submitting that the failure to contain the sympathetic nervous system response to the traumatic stressor can lead to more strongly encoded memories.

Cortisol is believed to facilitate this containment of sympathetic nervous system response and consequently, decreased cortisol may result in excessive consolidation of memory at the time of the trauma.

This mechanism is believed to propel forward the intrusive memories and associated hyperarousal, which are key features of PTSD.

As both systems can be pharmacologically approached with drugs that interfere with catecholamines or cortisol a number of clinical trials were initiated.

Monoamingergic medications under investigation for efficacy in prevention and treatment of PSTD

Prazosin	**adrenergic α_1-receptor blocker**
Carvedilol	**adrenergic α_1- and β-blocker**
Propranolol	**adrenergic β-blocker**
Nepicastat	**dopamine ß-hydroxylase inhibitor**
PRX-03140	**partial 5-HT_4 receptor agonist**

In most clinical trials α1-antagonists and β-blockers were studied, however, mixed results emerged. However, prazosin, an α1-antagonist, was effective in reducing nightmares in PTSD.

A more recent report summarized uncontrolled trials where trauma activation was initiated under the influence of a β-blocker. The authors concluded that trauma reactivation sessions in propranolol pre-treated PTSD patients result in large symptom improvements. However, controlled trials confirming an approach that targets this kind of neuroplasticity are not yet available.

On the contrary, a randomized trial designed to test propranolol among veterans with PTSD, that were deployed in Iraq or Afghanistan failed to detect beneficial effects of the drug. Thus, the jury is still out to which extent β-blockers or other medications that block the sympathetic nerve system will be a therapeutic option.

HPA-axis directed medications under investigation for efficacy in prevention and treatment of PTSD

Cortisol (Hydrocortison)	**gluco- and mineralocorticoid-receptor agonist**
Prednison	**glucocorticoid receptor agonist**
Mifepristone	**glucocorticoid- and progesterone receptor antagonist**
GSK 561679	**CRH R1 antagonist**

Instead of blocking excessive catecholamine release, elevating cortisol is an alternative strategy and a recent study by Zohar and colleagues administered either a single intravenous bolus of cortisol at a fairly high dose (100-140 mg) or placebo within six hours after a traumatic event in a prospective randomized double blind study. Those trauma victims that had received cortisol had a lower risk to develop PTSD than those under placebo.

The picture, however is also not clear here, as in mice glucocorticoids when infused into the hippocampus after fear conditioning were found to induce PTSD-like memory impairments.

In line with these findings is a report by Tronel and Alberini from the Mount Sinai School of Medicine in New York that used mifepristone, a drug that blocks both glucocorticoid- and progesterone receptors.

This study found that a traumatic memory is persistently disrupted if immediately after its retrieval by trauma-related cues these receptors are blocked. The authors conclude that disrupting glucocorticoid signalling also disrupts traumatic memory consolidation.

A similar study confirmed this and found even better results than with the β-blocker. That finding initiated a placebo-controlled study in combat veterans with PTSD. The result of the study is to my knowledge yet not published.

These apparently contradictory approaches, enhancement of glucocorticoid receptor (GR) signalling by replacing low circulating cortisol by exogenous steroids versus blocking GR signalling by mifepristone tells us that there is not one single first-line treatment for each patient with PTSD at hand. At least, with regard to drugs that interfer with the HPA-axis.

PTSD prevention has yet not been limited to obvious targets, the sympathetic and humoral stress system.

Miscellaneous medications under investigation for efficacy in prevention and treatment of PTSD

Ketamine	**NDMA receptor antagonist (++)**
Benzodiazepines	**$GABA_A$-receptor agonists**
Pregnenolone	**$GABA_A$-receptor agonists**
Eszopiclone	**$GABA_A$-receptor agonists**
Ganaxolone	**$GABA_A$-receptor agonists**
Aprepitant	**Neurokinin 1-receptor antagonist**
GR205171	**Neurokinin 1-receptor antagonist**
Orveptitant	**Neurokinin 1-receptor antagonist**
Rapamycin (Sirolimus)	**Binds to FBKP12 and inhibits mTOR pathway FKBP5 antagonist**
D-Cycloserine	**Partial agonist at the glycine recognition site of the NMDA-R**

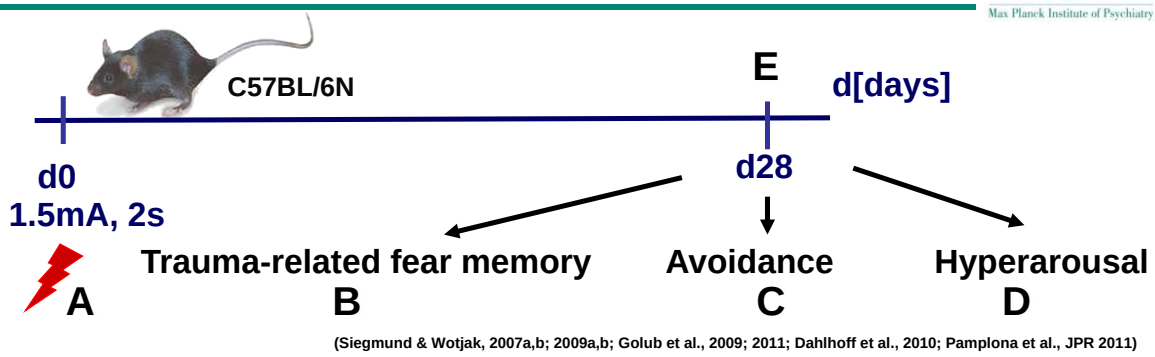
Soldiers were treated for burns and had received ketamine, as part of a multimodal anaesthetic plan that includes usually also an opioid. The authors had found markedly lower prevalence of PTSD among those that had received ketamine, an NMDA receptor antagonist, when compared to those which did not. However, there exist studies that failed to find this.

Other studies include $GABA_A$ –receptors, neurokinin receptors, and rapamycin. We have to admit that there is a mix of pharmacologies either preventing development of PTSD or attempting to erase overconsolidated traumatic memories, but no clear first line treatment for all patients exists.

Overall, this situation is anything but satisfying, calling for approaches for drug discovery that rely less on serendipity and obvious candidates, but more on basic science-driven approaches.

Memory formation is a multi-facetted process and the transformation of a newly acquired information into a stable memory involves many biochemical changes in distinct brain regions following a specific temporal pattern.

Shock sensitization – a mouse model of PTSD



(Siegmund & Wotjak, 2007a,b; 2009a,b; Golub et al., 2009; 2011; Dahlhoff et al., 2010; Pamplona et al., JPR 2011)

A Exposure to a traumatic event

B Trauma-related memories

C Avoidance

D Hyperarousal

E Symptoms > 1month

Fear conditioning in experimental animals is commonly used as a model of associative learning in rodents in which memory for a context-shock is stabilized via a hippocampus-dependent consolidation process. In contrast, memory for a cue-shock is stabilized through an amygdala-dependent consolidation processes. Translated into clinical conditions, that means that fear memories are essential components of PTSD and therefore animal models of PTSD are potentially useful for the discovery of new drugs preventing or healing this disease.

Carsten Wotjak from the Max Planck Institute of Psychiatry has developed such a mouse model by shock sensitisation and defined four different states: Exposure to a traumatic event, trauma-related fear memory, avoidance, and hyperarousal. The aim of his study was to test whether central blockade of the HPA-system might be an alternative strategy and whether there is an appropriate time-window.

CRHR1-antagonist: Window of therapeutic opportunity

Trauma → d28-d42 → PTSD (d50)

Therapeutic Treatment with CRHR1 antagonist

Starting 28d after shock and lasting for 14d

Trauma-related memories =

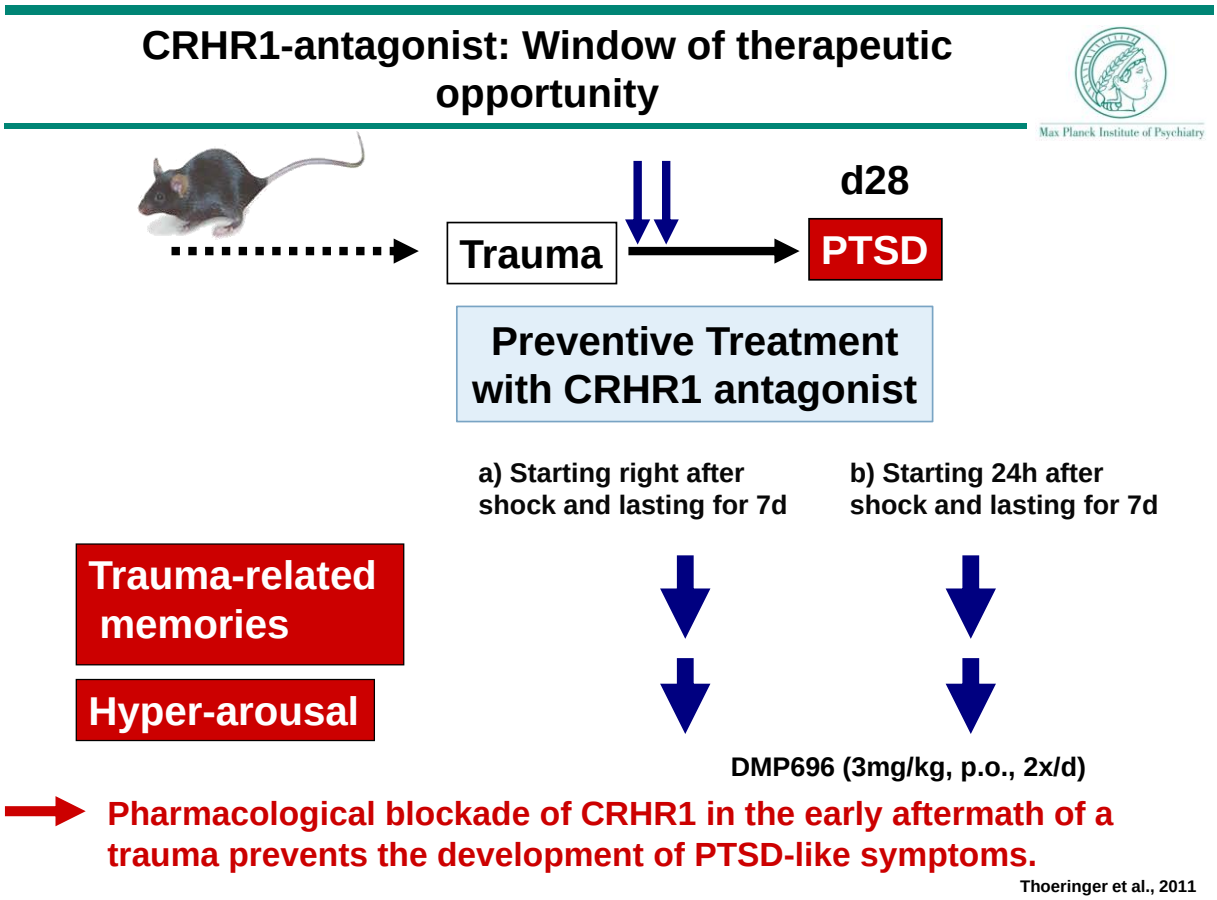
Hyper-arousal =

DMP696 (3mg/kg, p.o., 2x/d)

→ **Pharmacological blockade of CRHR1 starting 1 month after the trauma has no beneficial effects.**

Thoeringer et al., 2011

In fact, this study which administered the CRHR1 antagonist DMP696 one month after trauma exposure failed to find an effect on trauma-related fear memory and hyperarousal. Therefore, CRHR1-antagonists are perhaps not ideally suited to treat PTSD once this condition became chronic.



However, if the CRH R1 antagonist treatment starts immediately after trauma or at least within 24 hours after trauma and lasts for seven days both, trauma-related memories and hyperarousal are prevented. The lesson we can learn from this study is that CRHR1 antagonists are perhaps suited to prevent the emergence of PTSD. We also learn from Wotjak's study that CRHR1 antagonists work only if administered shortly after trauma exposure.

When talking about time frames that need to be utilized for drug treatment, we also need to address the issue when during the life span the trauma exposure occurs. Christine Heim and Charlie Nemeroff have shown in a series of elegant studies that individuals having experienced trauma in early childhood are more likely to suffer from depression in adulthood. Such acquired vulnerabilities compare well with those that are inherited.

Genetic load and early childhood trauma result in impaired HPA-regulation

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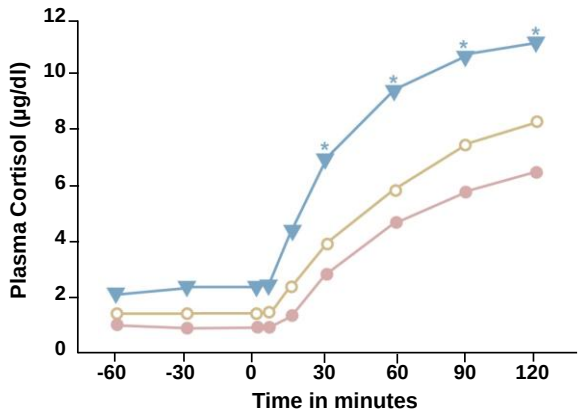
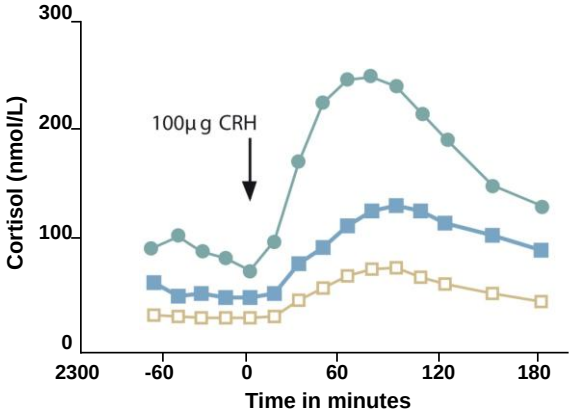


Plasma cortisol concentrations (mean) in the Dex/CRH-test in men

- with early life trauma and major depression
- with early life trauma without major depression
- without early life trauma and without current or previous psychiatric disorder

Heim et al., 2008



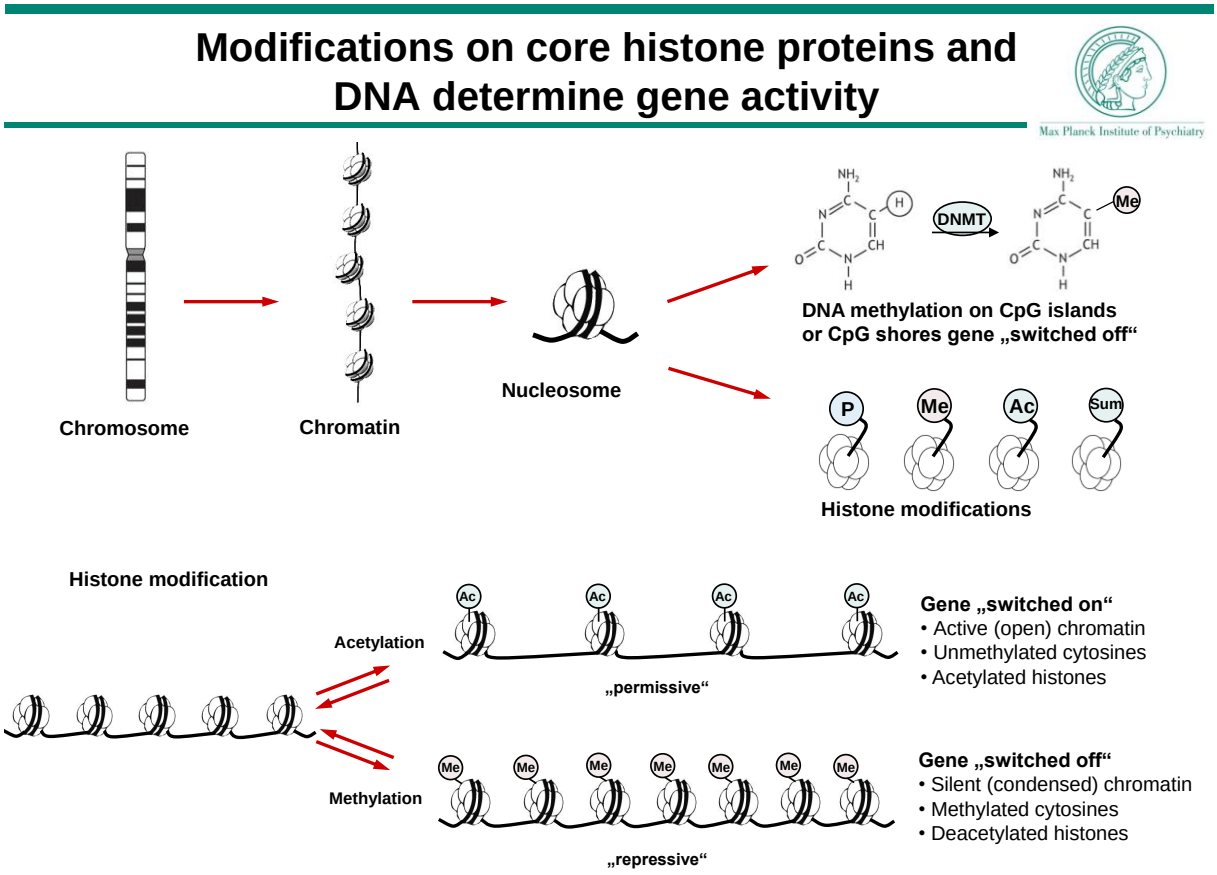
Plasma cortisol concentrations (mean) in the Dex/CRH-test in

- patients with major depression
- healthy controls with first degree relatives having major depression
- healthy controls without family history of psychiatric disorder

Holsboer et al., 1995

The effect of early trauma on neuroendocrine regulation assessed by the dex/CRH-test as administered in a study by Christine Heim was compared with our study on neuroendocrine HPA regulation of healthy individuals with a high genetic load for depression. Both, early traumatized individuals as well as those having high genetic load for depression have similar degrees of HPA impairment and both also have an increased risk for affective disorder.

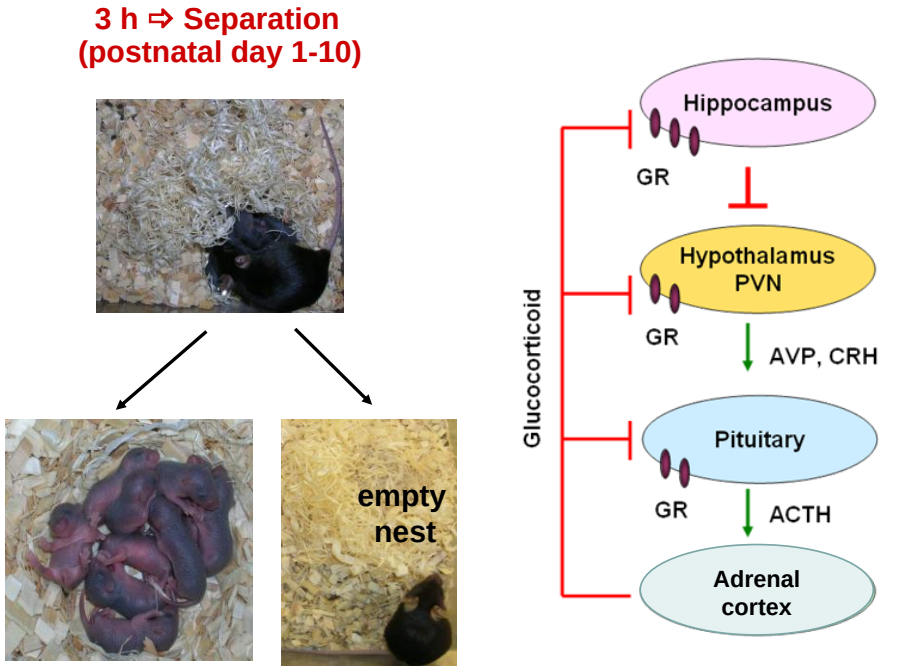
We now know that the physiologic changes following trauma involve epigenetic modifications that impact upon gene activity.



In case of DNA modifications, methyl groups or other small chemical substituents can be covalently bound to cytosine at cytosine and guanine-rich areas. These are called CpG islands and there are frequently methylation sites that are neighbouring CpG islands where also methylation can occur. These sites are called CpG shores.

Another possibility are epigenetic marks at the histone molecule, the protein around which the DNA is coiled. These can be modified by methylation, acetylation and other substituents. Those modifications can facilitate or prevent transcription factors from DNA binding and by that mode of operation genes are switched on or off.

Maternal separation as early-life stressor



Almeida et al., 2009

How that works had been demonstrated by Dietmar Spengler and Osborne Almeida at the MPI of Psychiatry. They have separated newborn mice from their mothers for three hours per day for ten days and found that this early-life stressor not only results in increased HPA-activity, but also in a number of changes which are taken as depressive-like behaviour that lasts permanently.

Persistent programming of the HPA-axis

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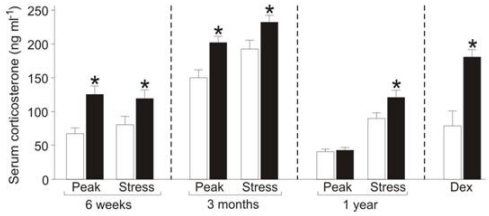
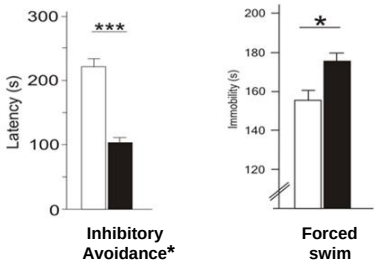
Hypercorticosteronism



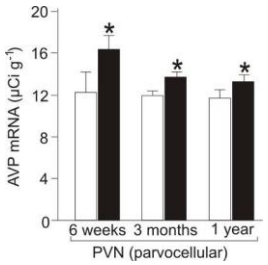
Depressive-like reactions

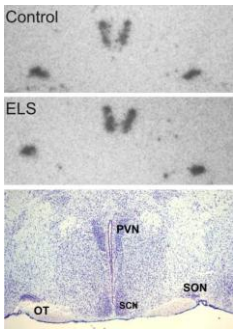


□ **control**

■ **early-life stress**

Vasopressin



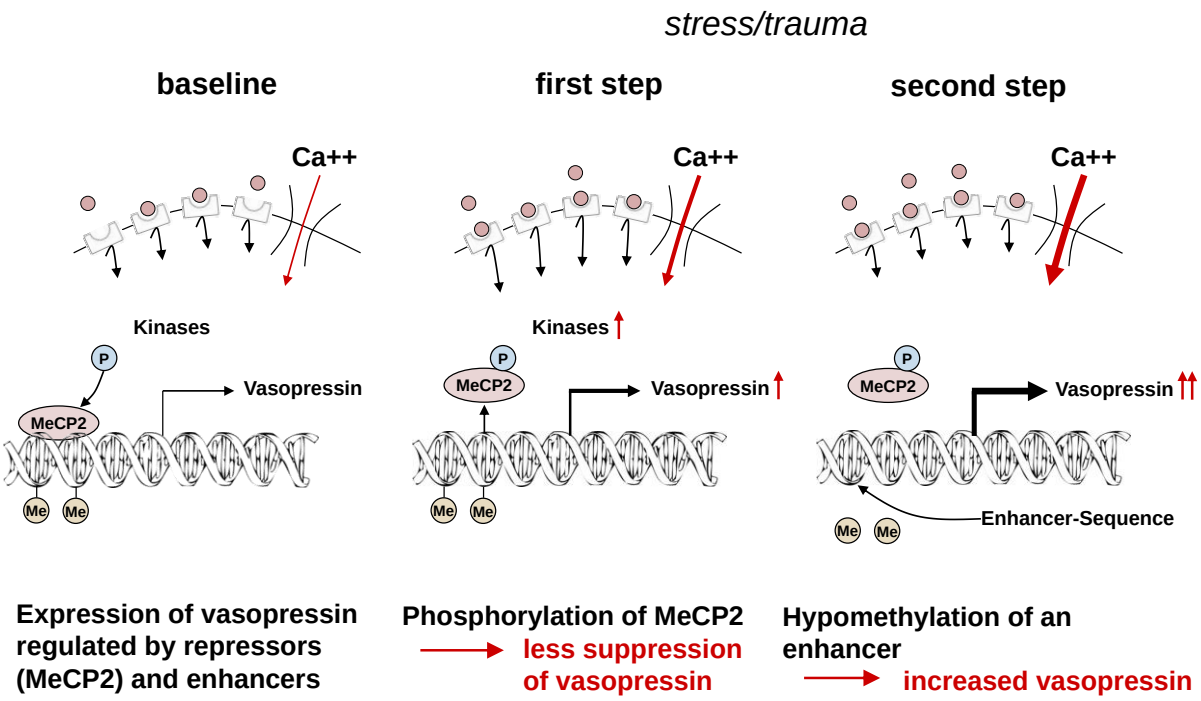


*** impaired hippocampus dependent memory**

Murgatroyd et al., 2009

The driving force behind this phenomenon was increased vasopressin and when these animals were treated with a vasopressin 1B –receptor antagonist these neuroendocrine and depressive-like signs and symptoms disappeared.

Postnatal trauma increases vasopressin-expression by a two step mechanism



Today we are in a very fortunate situation as we can analyse these experience-related phenomena on a biochemical level.

Under baseline conditions vasopressin gene activity is determined by metyl-CpG-binding protein 2, MeCP2, that acts as a repressor. Vasopressin is also regulated by DNA sequences that are normally muted by covalently bound methylgroups. Once an environmental stressor is experienced, this event gets translated into massive changes of homeostasis, in this case into activation of cell membrane located receptors and calcium channels. As a result intracellular kinases get activated and phosphorylate MeCP2 which dissociates from DNA and reduces vasopressin suppression.

At the next step demethylation is initiated and the resulting hypomethylation of enhancer sequences leads to permanently enhanced vasopressin gene activity.

Clearly, this is a complex cascade of events. When translating this new knowledge into treatment options the first important steps would be the blockade of MeCP2 phosphorylation and prevention of hypomethylation of enhancer sequences.

Only if this is not feasible or the intervention comes too late we may treat the condition with a vasopressin 1B receptor antagonist, which works at both levels, behavioural and neuroendocrine.

Brain derived neurotrophic factor, BDNF

BDNF a key player in development and course of depression and anxiety disorder; and specifically in memory and PTSD

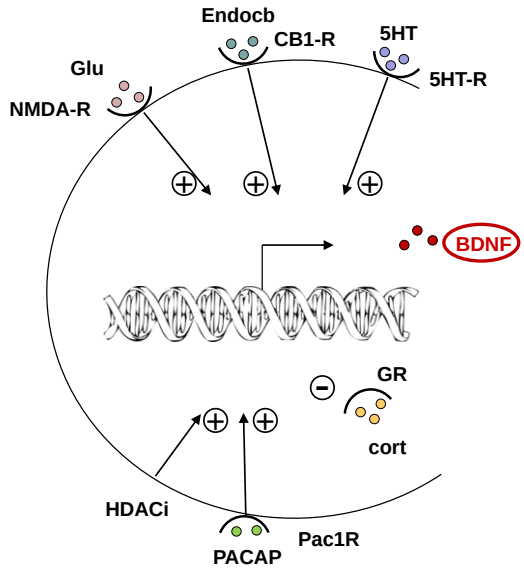
Hans Thoenen (1928 – 2012)

Let me turn to brain derived neurotrophic factor, BDNF,which has been implicated in synaptic plasticity processes that are required for long-term learning and memory and more specifically in the formation of fear memories and fear extinction.

I like to address this line of research also as a tribute to Hans Thoenen who passed away in June 2012. Hans Thoenen was a pioneer in neurobiology and BDNF was first discovered, cloned and characterized in his laboratory at the Max Planck Institute of Psychiatry in Munich.

The research on BDNF in the context of PTSD was developed by Kerry Ressler, David Sweatt and Eric Nestler, but also many other excellent laboratories have made major contributions.

Simplified scheme how BDNF is modulated by molecules relevant for fear extinction



NMDA rec
CB1 rec
5-HT rec
PAC R1 rec
→ BDNF ↑

GR
→ BDNF ↓

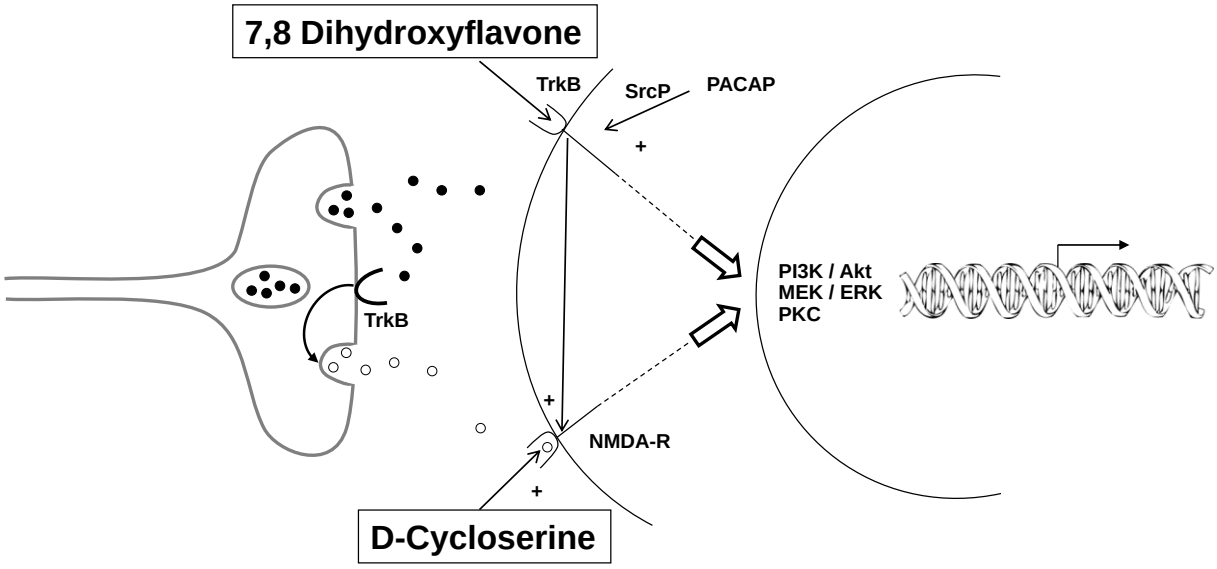
HDACi = Valproic acid
Trichostatin A
Sodium butyrate

modified from Andero and Ressler, 2012

In brief, studies showed that BDNF is needed for fear learning processes. Several mechanisms that are potentially involved, include glutamate, pituitary adenylate cyclase activating peptide, PACAP, its receptor PAC1 and endocannabinoids acting through cannabinoid type 1 (CB1) receptors.

More recently it was shown that also chromatin structural remodelling plays an important role in BDNF gene expression. In particular, the remodelling involved histonedeacetylase, HDAC, which removes acetyl-groups from the lysine residue of histones. It was observed that systemic administration of valproic acid, an HDAC inhibitor increases BDNF gene activity and at the same time enhances contextual fear extinction.

Simplified scheme how BDNF modulators act during fear extinction



Powerful activation of downstream signaling pathways by TrkB- and NMDA-receptor activation changes transcription and synaptic plasticity

modified from Andero and Ressler, 2012

BDNF administered to humans has, of course, no effect as it is metabolized quickly and fails to penetrate into the brain. Nonpeptide small molecules that act as agonists at the receptor through which BDNF acts are needed. This receptor is named TrkB and one molecule 7,8 dihydroxyflavone acting as TrkB-agonist has been studied in mice. After systemic administration to mice 7,8 dihydroxyflavone crosses the blood brain barrier and binds with high affinity at TrkB receptors. In behavioural paradigms it enhances both, fear acquisition and extinction in naïve mice.

The connection between BDNF/TrkB signalling and PTSD is also interesting from another perspective. I mentioned before the role of MeCP2 in the context of early trauma-induced vasopressin release. This gene is located at the X-chromosome and mutations in this gene cause a severe neurodevelopmental disease affecting almost exclusively females which live up to forty years at maximum. This disorder, an encephalopathy is called Rett syndrome. A transgenic mouse model for Rett syndrome has similar signs and symptoms as affected humans and BDNF enhancement acting through TrKB can increase the life span in these animals.

The recent finding that 7,8 dihydroxyflavone also exhibits therapeutic efficacy in mice with Rett syndrome is a strong argument that this compound has therapeutic potential by activating TrkB-receptors. We can only hope that the patent situation is not preventing further drug discovery and development along that line.

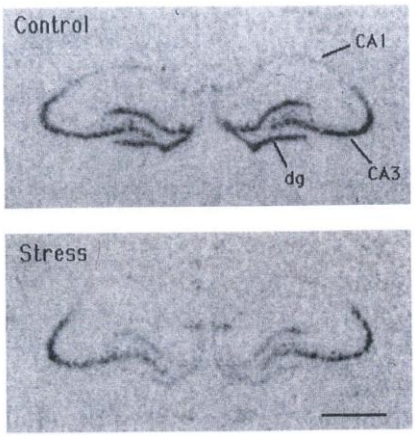
To date, perhaps the most substantial therapeutic progress made is that which specifically targets BDNF/TrkB-signalling. It was shown that patients with PTSD benefit when psychotherapy is combined with D-cycloserine administration. This compound acts as a partial NMDA-receptor agonist and BDNF and TrkB were found in glutamatergic neurons at

both pre-synaptic and post-synaptic sites. By administration of D-cycloserine BDNF-TrkB signalling is enhanced which may explain why it facilitates consolidation of fear extinction. Unfortunately, not all clinical data support the notion that D-cycloserine is beneficial in this disorder.

BDNF expression is suppressed by stress and corticosterone

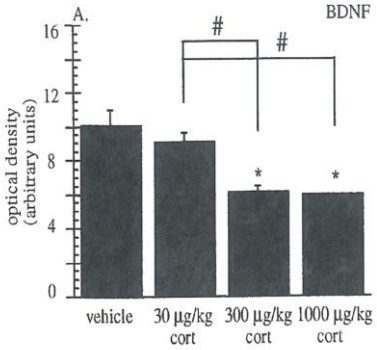
Max Planck Institute of Psychiatry

Stress



Autoradiographs of BDNF mRNA in the hippocampus. Note decrease in BDNF hybridization in the dentate gyrus (*dg*) from the rat stressed on 7 consecutive days compared to the unstressed control rat. Scale bar, 1.5 mm.

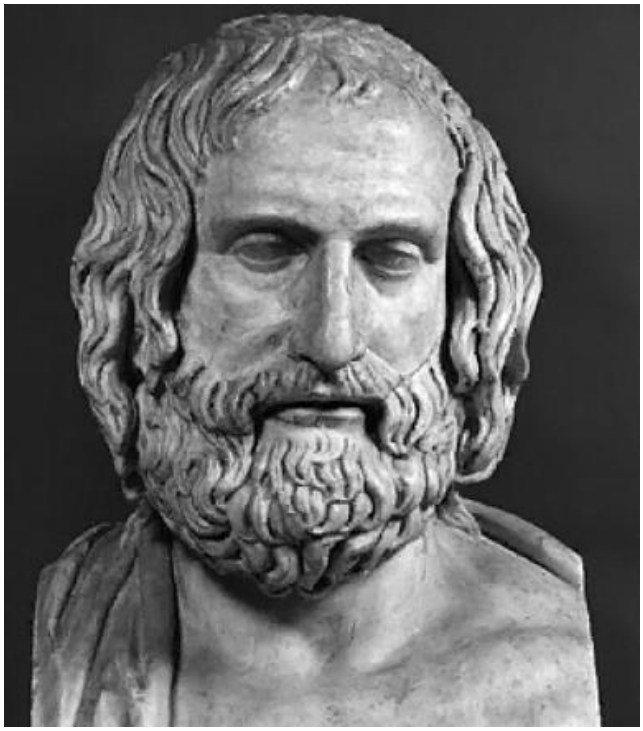
Corticosterone



Schaaf et al., 1997, Smith et al., 1995

The BDNF expression and the stress hormone system are substantially intertwined and the role of the HPA system in BDNF-TrkB signalling is obvious: the BDNF gene is a target of cortisol activated glucocorticoid receptors and it is well known that stress suppresses BDNF via increased cortisol, mainly in the hippocampus where BDNF is highly expressed. It is plausible to submit that those individuals that have excessive release of cortisol in the context of trauma exposure will suppress BDNF. And that interaction causes long-term impairment of BDNF-TrkB signalling and therefore overconsolidation of fear memories.
It is true for any psychiatric condition that DNA-sequence variations in single genes or their regulatory sites do not act alone. Our DNA is not a quiet place.

In fact, individual genes have only modest effects, but the interaction of many different minor genetic variants amongst themselves and with the outside world ultimately shape the individual phenotype. This important concept is known since ancient times.



MAN IS THE MEASURE OF ALL MEANS

Protagoras (490-411 B.C.)

In particular, the Greek philosopher Protagoras (490-411 B.C.) was well aware of gene-environment interactions when stating: "Man is the measure of all means."

But only today we have the tools to exploit this concept when designing better treatments for PTSD, for the individual patient.

In depression research we have learned that any two patients with depression, same age, same gender, even same genes, having the same signs and symptoms according to any rating scale for psychopathology you can think of, will respond differently to the same drug. In PTSD the situation is not unlike that of depression.

On the contrary, it is a very good example how diverse underlying mechanisms can be. It is a disorder which is defined as a consequence of a psychic trauma, but only a fraction of those who are exposed to trauma get PTSD. Again, it's a mantra: People are different regarding susceptibility and clinical conditions and despite identical phenotypes and diagnoses, they are different with regard to causal mechanisms.

Severe trauma can result in permanent changes of gene regulation

- **Disturbed placenta**
- **Childhood trauma**
- **Severe illness**
- **Malnutrition**
- **Natural disaster**
- **Physical assault**
- **Combat experience**
- **Terror attack**

Type of trauma and severity are emotionally handled in an individual way

Thus, there is also a strong genetic component that determines whether we are vulnerable or resilient to trauma. In addition, there is the question which type of trauma will lead to PTSD. A major factor is what the traumatic event may mean to the individual person. For example, victims of natural disasters are less prone to PTSD than those victims that fall to sexual assault.

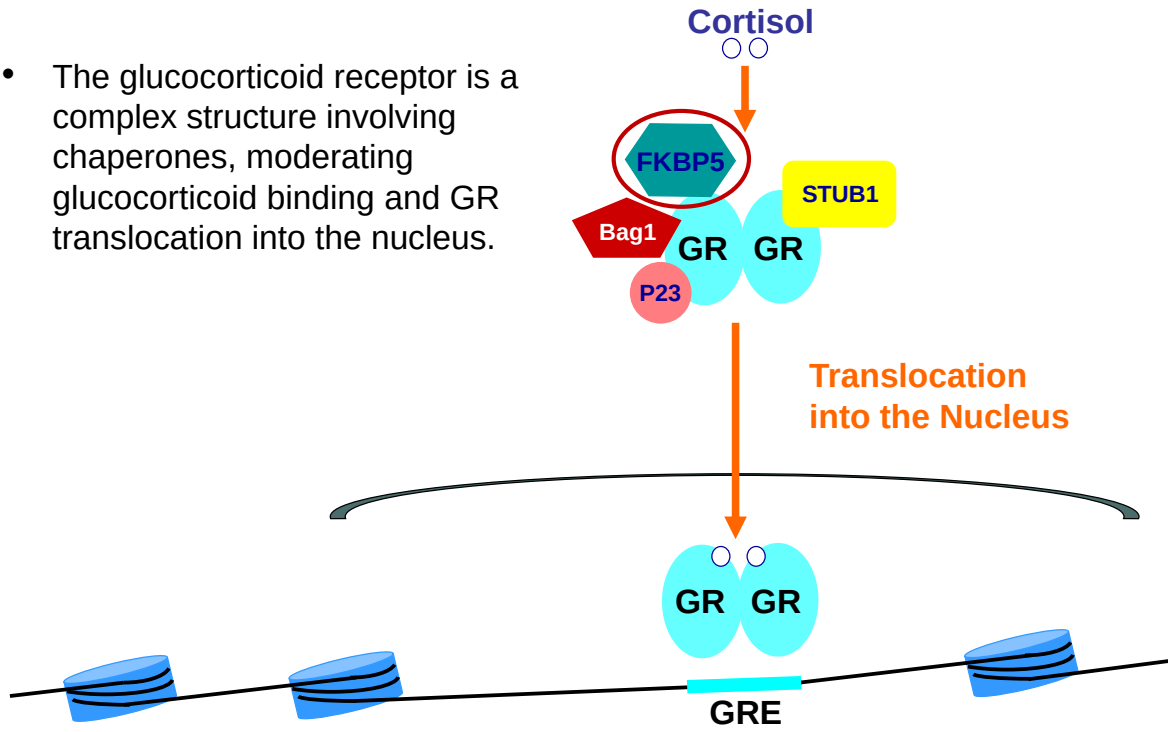
Of great importance is the time and intensity of trauma. In fact, a kind of dose-response-relationship exists as in combat veterans, for example, the duration and number of deployments and the intensity of experienced trauma increases the likelihood of PTSD.

A key to better understanding of causality of PTSD and inherited or acquired vulnerability for this disorder is in our view the corticosteroid receptor hypothesis that submits impaired corticosteroid receptor signalling as a major contributor to the pathogenesis.

The function of the glucocorticoid receptor complex is determined by chaperones and co-chaperones

Max Planck Institute of Psychiatry

- The glucocorticoid receptor is a complex structure involving chaperones, moderating glucocorticoid binding and GR translocation into the nucleus.

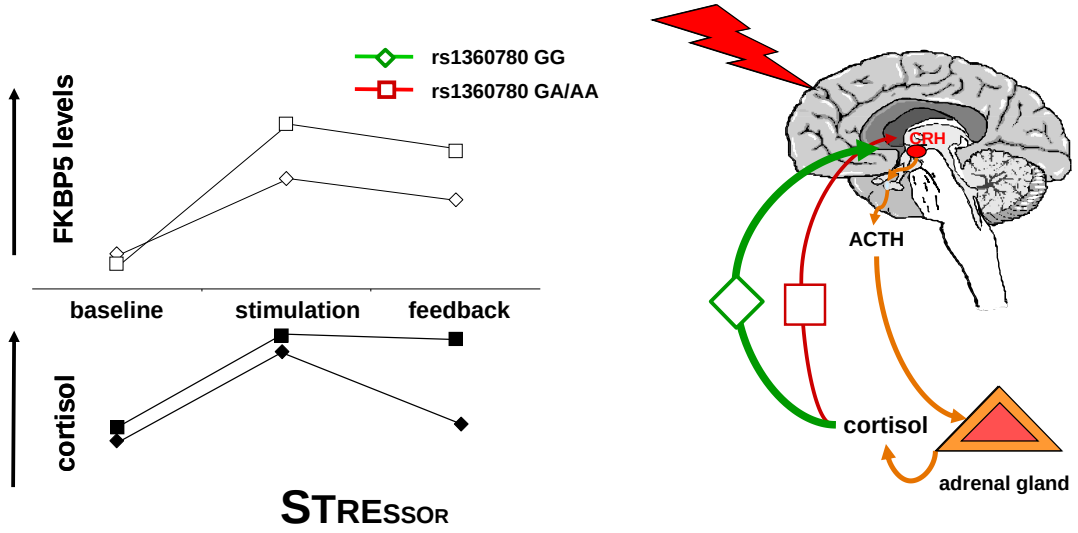


modified from Binder, 2009

Along that line we investigated a number of molecules and their coding genes that are potentially involved.

As many of you know the Max Planck Institute of Psychiatry has initiated many studies to understand the function of the FK506 binding protein, FKBP5, which is a co-chaperone of the heat shock protein Hsp90 folding machinery.

Activity of the HPA-axis depends on negative feedback of the FKBP5 genotype



More FKBP5 induction = more GR resistance = less negative feedback = higher cortisol levels

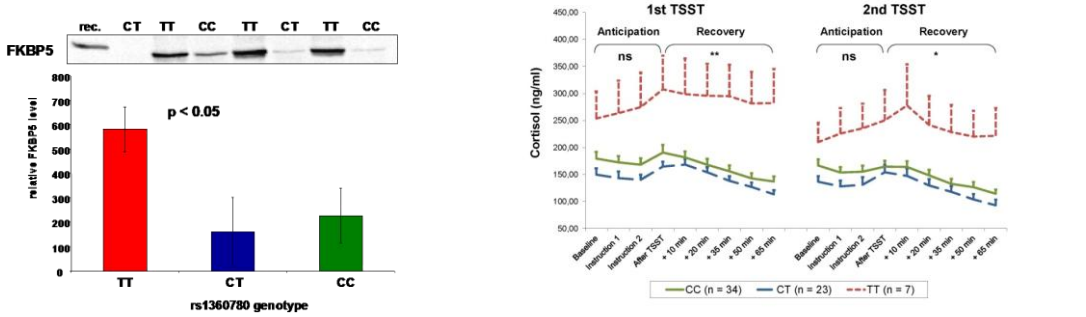
Binder et al., 2004, 2008; Ising et al., 2008

Studies of Elisabeth Binder and Marcus Ising from the MPI showed that a certain FKBP5 genotype is associated with increased HPA activity and increased FKBP5mRNA and protein levels. FKBP5 inhibits the function of the glucocorticoid receptor (GR) by changing the receptor structure in a way that decreases its affinity to corticosteroids. Another feature is that FKBP5 slows down the nuclear translocation of the ligand-activated GR. The interest in FKBP5 genotypes for clinical psychiatry stems mainly from Elisabeth Binder's initial finding that the FKPB5 genotype predicts antidepressant treatment outcome. Those depressed patients carrying the genotype associated with increased gene expression responded less well to antidepressants.

Polymorphisms in the FKBP5 Gene Affect Psychosocial Stress Response

Max Planck Institute of Psychiatry

- Polymorphisms in the FKBP5 gene previously identified as correlated with FKBP5 activity (Binder et al., 2004) affect also the cortisol response to psychosocial stress (TSST) in healthy subjects, which could be replicated in a second test.



Binder et al., Nat. Gen., 2004

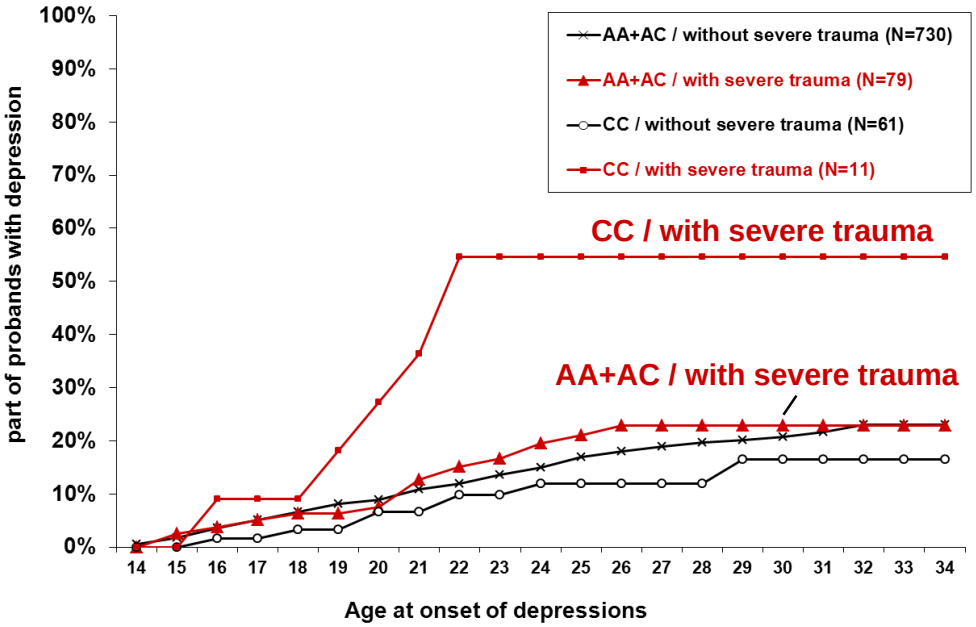
Ising et al., 2008

Also other clinically interesting results highlight the role of FKBP5.

Marcus Ising from the Max Planck Institute of Psychiatry showed that the previously described FKBP5 risk alleles for depression also predicted insufficient cortisol recovery and increased self-reported anxiety following an experimental psychosocial stress test, to the Trier Social Stress Test.

These findings support the view that individuals that are homocygous for alleles that activate FKBP51 gene expression which conveys increased stress susceptibility have increased risk to develop stress-related diseases. Also other studies were in line with the view that genetic variations in FKBP5 modulate the sensitivity to stressful life events throughout life.

Onset of depression is determined by FKBP5-Genotype and early Trauma



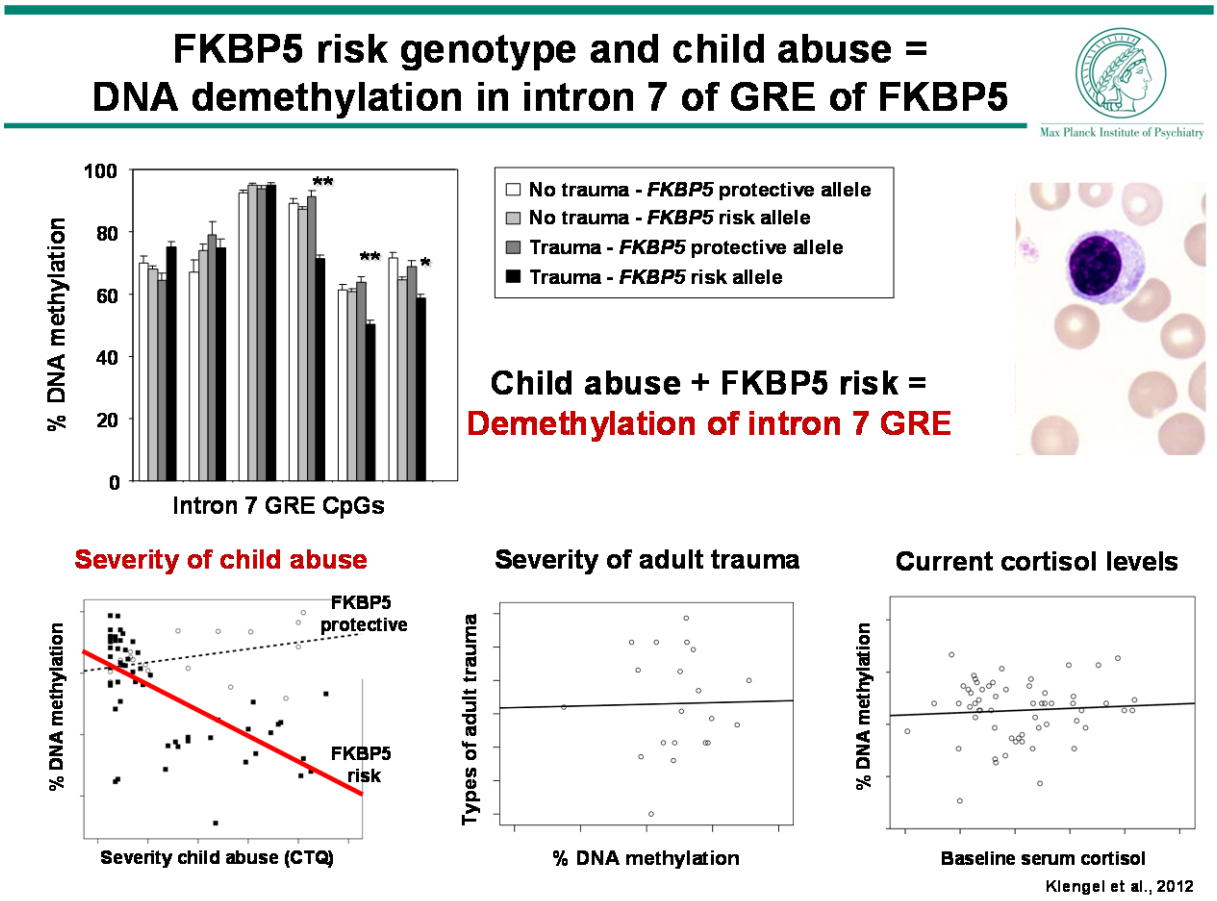
FKBP5 is a gene, encoding a cochaperone that regulates GR-function

Zimmermann et al., 2010

A study by Petra Zimmermann from the Max Planck Institute of Psychiatry showed that individuals with a specific FKBP51 polymorphism that had experienced a childhood trauma carry a much higher risk for early onset of depression than those with other FKBP5 alleles.

This study supports an earlier report by Binder showing that these SNPs interact with child abuse and predict risk for PTSD.

In a collaborative study between the Max Planck Institute of Psychiatry and Emory University, Elisabeth Binder and her co-workers investigated whether the trauma-induced changes in FKBP5 levels involve epigenetic alterations. She identified alleles that were protective and alleles that were risk conferring.



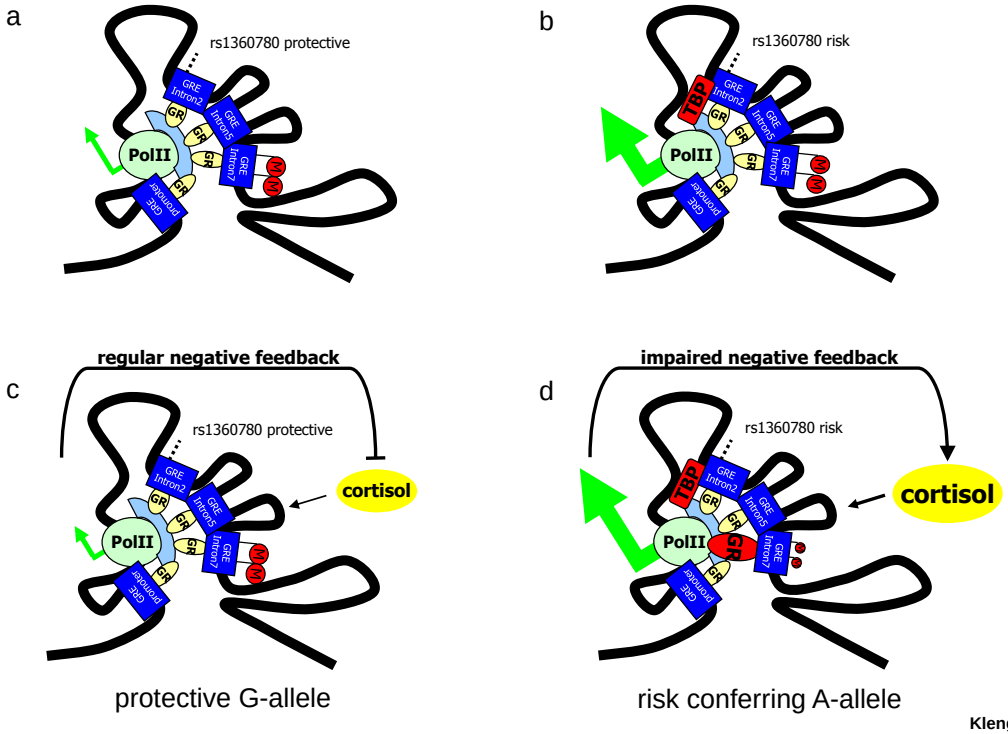
That study indicates that genetic variation in the FKBP5 gene may determine also the stress hormone response to trauma, which has implications for PTSD risk as glucocorticoid receptor sensitivity and function are related to overconsolidation of traumatic memories. This process is believed to be influenced by cortisol acting through GR.

A DNA methylation analysis was conducted by Elisabeth Binder and co-workers in CpG-islands that were located within the consensus sequence in intron 7 of the glucocorticoid response element, called GRE. They found that trauma had induced a significantly higher rate of demethylation in individuals exposed to child abuse and carrying the risk allele when compared to individuals carrying the protective allele. Indeed the degree of demethylation leading to higher FKBP5 corresponded with the severity of child abuse, but only in risk allele carriers.

It was further tested if this demethylation could be related to excessive GR activation and might also be seen in tissues other than peripheral blood which proved to be the case.
To cut a long and complicated story short, it was shown that in a human hippocampal progenitor cell line dexamethasone by activation of GR produces DNA demethylation in the same order of magnitude as trauma does in risk allele carriers. Importantly, these effects on DNA methylation are limited to childhood trauma, as they were absent in traumatized adults and independent from current plasma cortisol levels.

Long-distance interaction of Glucocorticoid Response Elements in FKBP5 and epigenetic mechanism of trauma-induced demethylation

Max Planck Institute of Psychiatry



Klengel et al., 2012

What we can conclude from their findings is that FKBP5 gene expression is induced by cortisol via an interaction with ligand-activated GR. In the presence of the risk allele of rs1360 780, the A-allele, a TATA box binding protein, TBP, is bound stronger to FKBP5. This results in enhanced FKBP5mRNA transcription in response to cortisol only in risk allele carriers, but not in carriers of the protective genotype.

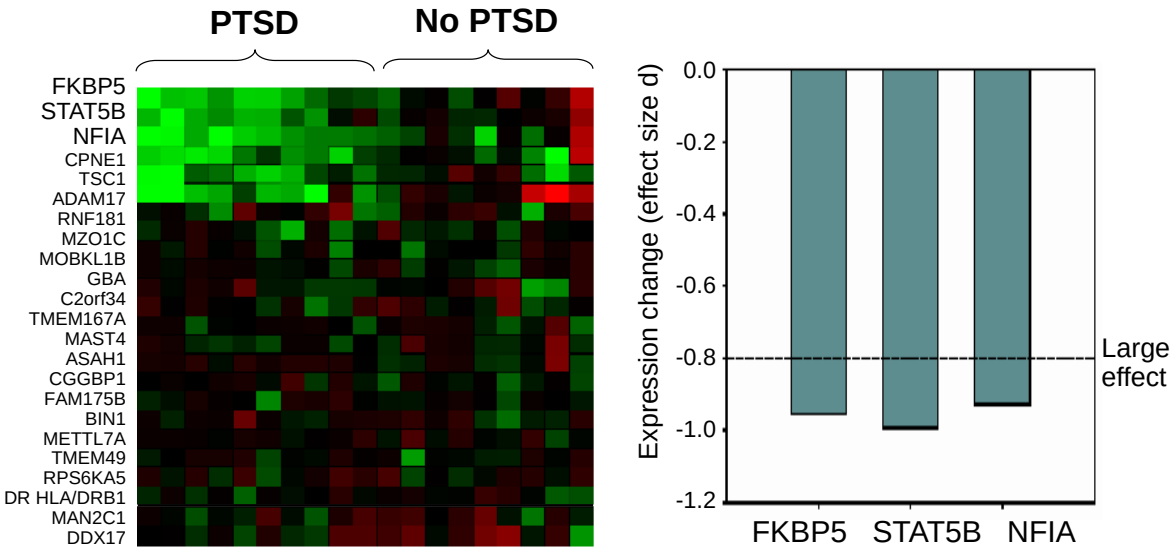
After trauma exposure the activated GR induces negative feedback regulation which results in an overall normalization of the activation of GR target genes.

In risk allele carriers, however, the initial rise in cortisol has prompted increased expression of FKBP5 resulting in prolonged elevation of cortisol which, as shown before, induces DNA demethylation around functional GREs in intron 7 of the GREs. This enhances the genetic predisposition of GR induced FKBP5 expression and finally results in changes of GR sensitivity.

Reduced expression of GR-regulated genes in PTSD

Max Planck Institute of Psychiatry

Results from a collaboration between the MSSM and the MPI of Psychiatry studying long-term consequences of the 9/11 WTC terror attack in NYC



Expression profile to identify a PTSD-subgroup?

Yehuda et al., 2009

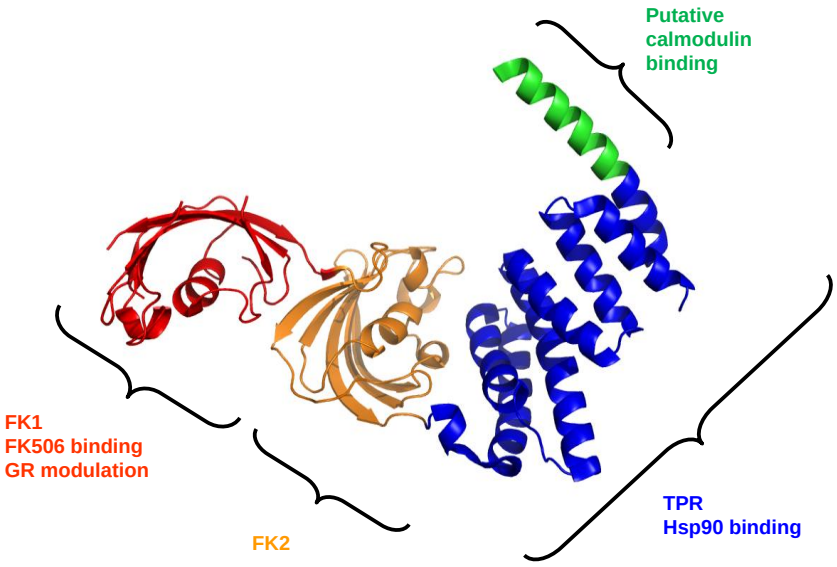
These findings help us to understand results of a joint study between the Mount Sinai School of Medicine and the Max Planck Institute in Munich where we could show that victims of the 9/11-terror attack who still suffered from PTSD five years after the event had different gene expression patterns when compared to those who were similarly exposed but remained unaffected.

One of the best predictors of PTSD was in fact low FKBP5 expression. FKBP5 is not only a gene that is central to the pathogenesis of PTSD, but also one of the most sensitive markers of GR activation. Gene expression of FKBP5 but also of a number of other HPA-axis related genes was in fact lower in PTSD patients than trauma exposed controls, possibly a reflection of lower cortisol levels often reported with PTSD. This seems to be in contrast with the previously mentioned hyper-responsiveness of FKPB5 as a result of risk genotype and early trauma exposure. Only longitudinal studies can address this issue of whether an initial hyper-responsiveness to trauma increases the risk for PTSD. Once a patient develops this disorder, additional regulatory mechanisms may counterbalance this even below a given baseline as was observed in PTSD victims five years after exposure to the 9/11-terror attack.

Genotyping and peripheral biomarkers where gene expression and hormone regulation are measured allow us now to define a clinical phenotype where excessive FKBP5 gene expression is a causative mechanism. The obvious question is whether or not we are able to target the mechanism that leads to FKBP5 excess?

That requires a major effort in drug discovery which as we all know is a high risk endeavour. Nevertheless we are trying that at the MPI in Munich and in the following I shall explain you how we proceed.

The prospect of FKBP51 as a drug target



from Zimmermann et al., 2011

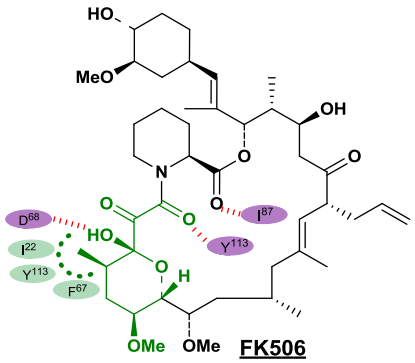
FKBP5 consists of an N-terminal FK506 binding domain, called FK1. This domain binds as its name suggests to FK506, also called tacrolimus, and to rapamycin, both are immunosuppressant drugs.

The FK1 structure is also essential for modulation of the glucocorticoid-receptor. FK2 is catalytically inactive and does not bind immunosuppressants. The large TPR domain forms the docking site for HSP 90 which represents the key feature of FKBP5 thus acting as a co-chaperone of HSP 90 heterocomplexes. HSP 90 complexes are determining the GR structure and thus its affinity to corticosteroids. In addition, there is a putative calmodulin binding domain.

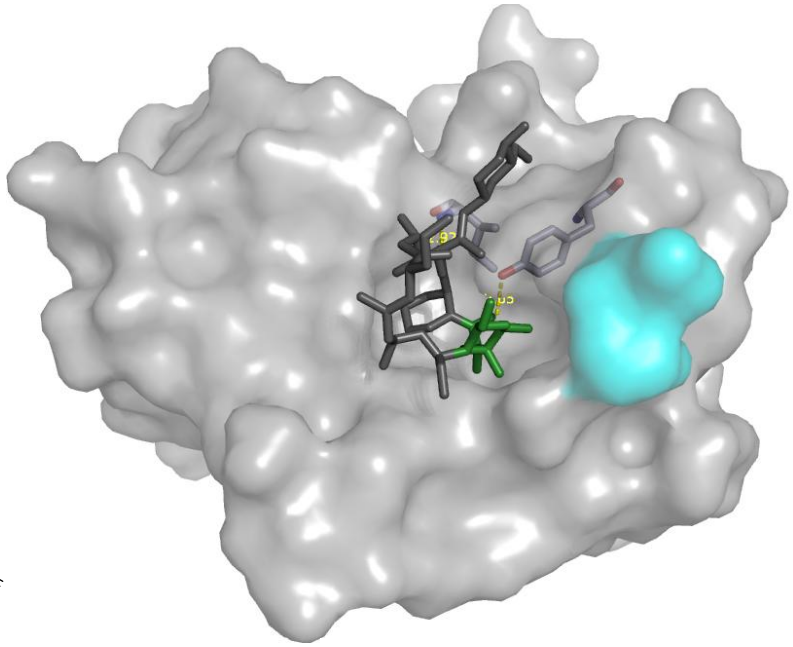
Chemically, FKBP51 is already a validated and druggable protein. It has a close homology to the prototypic FKBP12, for which a large number of ligands has already been reported. And there exist already clinically validated chemical molecules that bind to a defined domain of the FKBP51 protein. One is FK506, the other is rapamycin. Both are very unspecific and have a variety of diverse pharmacological effects which limit their usefulness. Rapamycin, for example, is an immunosuppressant that also targets mTOR which exacerbates hyperglycemic states, thus inducing diabetes.

The interaction of GR with FKBP51 demonstrates how the FK1 domain can be approached.

Co-crystal structure of **FK506** with **FKBP51 FK1** domain



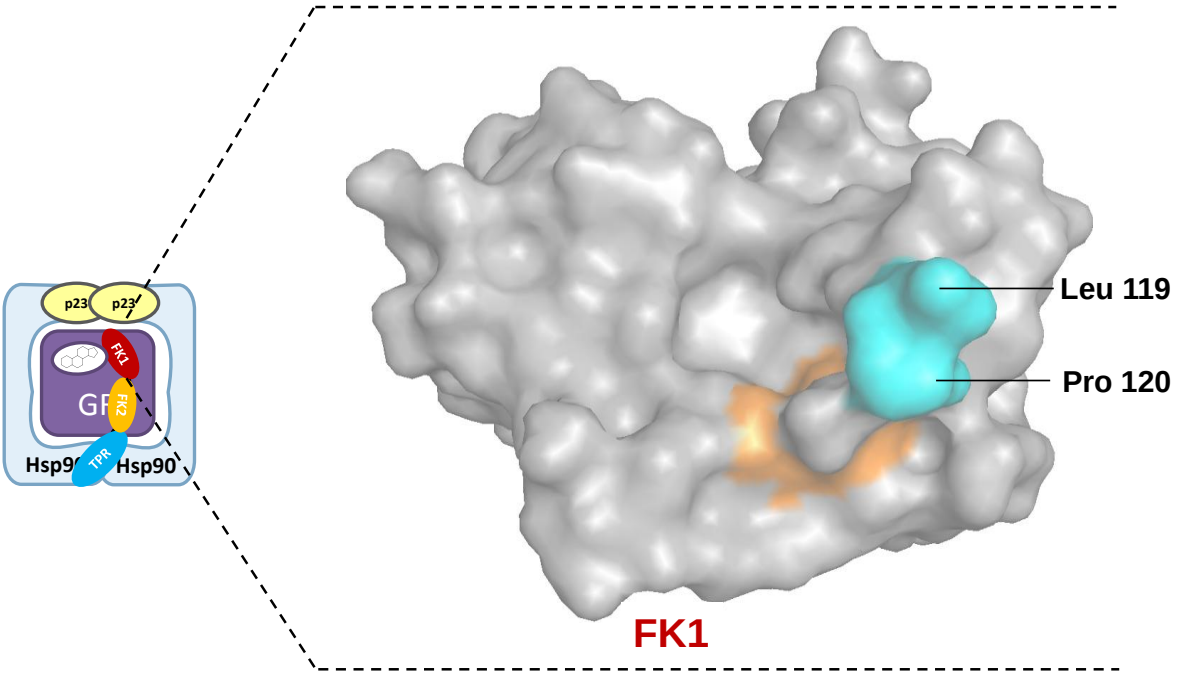
FK506



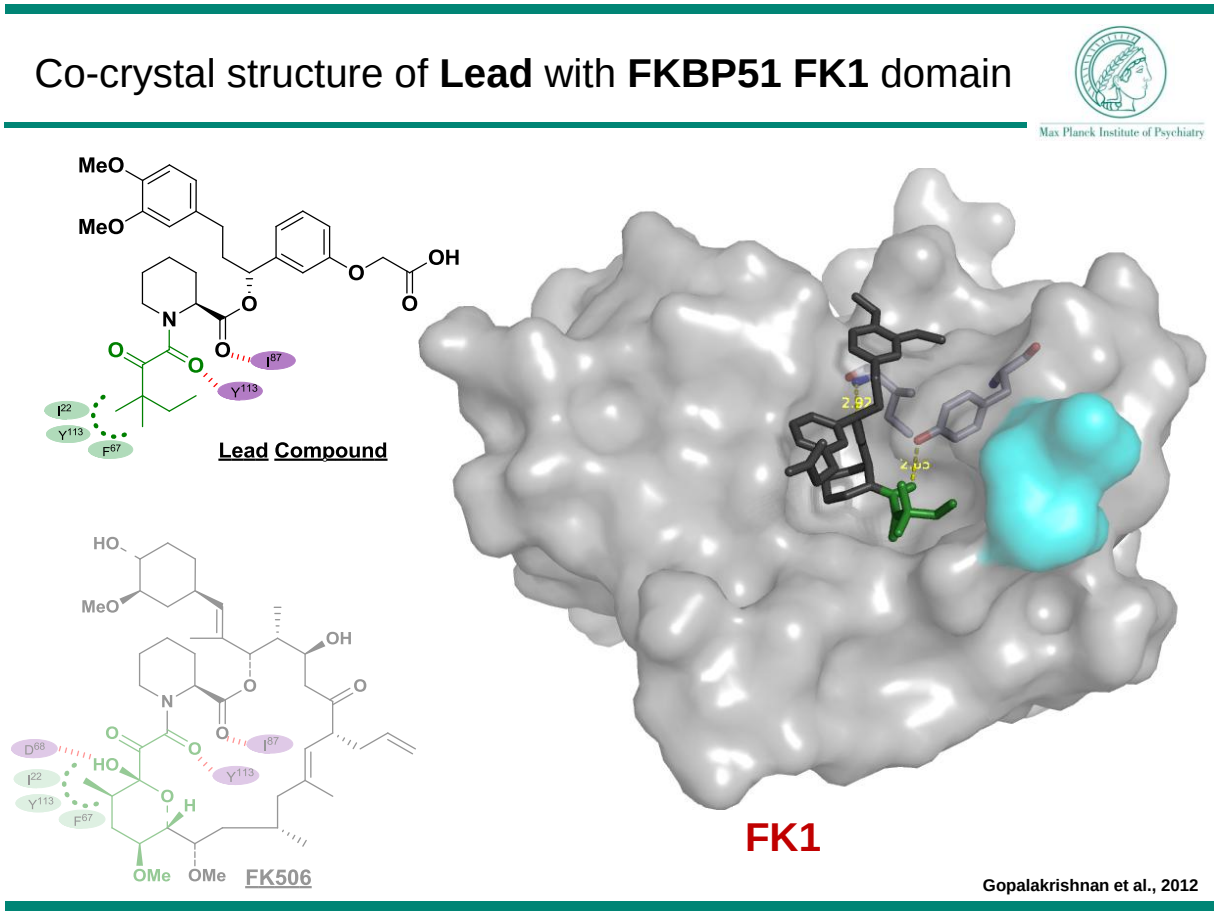
FK1

Bracher et al., 2011

Interaction of **GR** and **FKBP51**



A co-crystal structure of FK506 with the FK1 domain of FKBP5 gives an insight into the binding mode of FK506. From that structure we derived information on how to discover new non-immunosuppressive synthetic molecules that act as ligands capable to modify FKBP5.



Based on a simplified analogue a new lead compound was discovered at the Max Planck Institute of Psychiatry. The molecule was designed and synthesized by Felix Hausch and co-workers and this group conducted a first systematic structure activity relationship for FKBP51 ligands. It turned out from the medicinal chemistry campaign that one important issue still remains crucial:

That is the FKBP5 subtype selectivity because among other concerns the action of FKBP51 on GR is opposite to that of FKBP52.

At this point we can state that the decreased function of GR in the presence of FKBP5 can be overcome by FKBP5 antagonists.

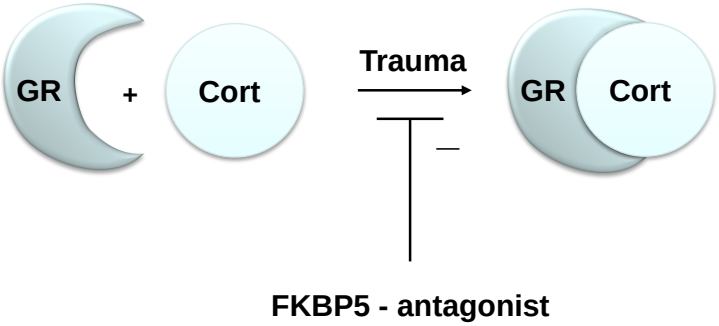
Several findings from animal experiments support the idea that targeted treatment of a specific subtype characterized by FKBP5 overexpression is a realistic goal.

A recent phase I clinical trial conducted in Japan administering a pan-specific FKBP ligand, FK1706 confirmed that FKBP blockade is tolerated in humans for longer times. Thus, FKBP5 ligands may not only be important for PTSD, but for all clinical conditions where negative feedback of the HPA axis is impaired by excessive FKBP5.

Personalized intervention after trauma by blocking FKBP5 in vulnerable individuals

Max Planck Institute of Psychiatry

In the presence of the risk (A-) allele of rs1360780 trauma exposure results in enhanced FKBP5 expression inducing DNA demethylation around functional GRE‘s



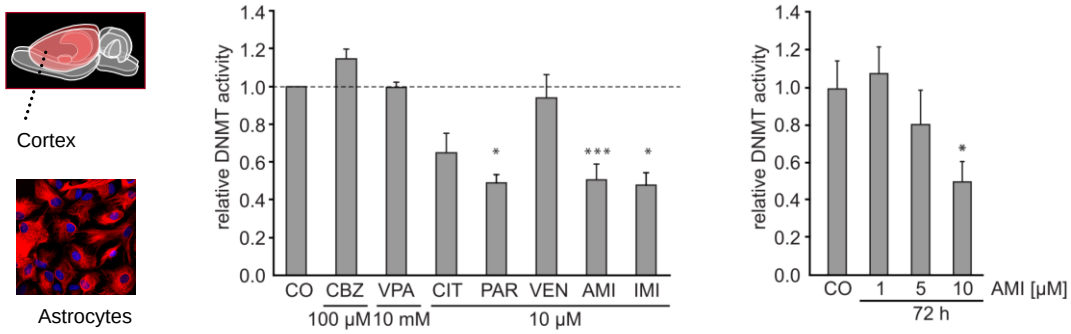
Translated into personalized prevention of PTSD we can submit the following: Those trauma victims that carry the risk alleles are those where FKBP5 might be overexpressed decreasing GR sensitivity. These individuals should be treated swiftly with an FKPB5 antagonist.

While not utopian, it is clear that the vision of discovery and development of an FKBP5 antagonist will take time. The pragmatic question is: Can we use drugs that are currently available for interfering with the development of epigenetic marks?

Antidepressants regulate DNMT activity

Max Planck Institute of Psychiatry

DNMT activity in primary astrocytes

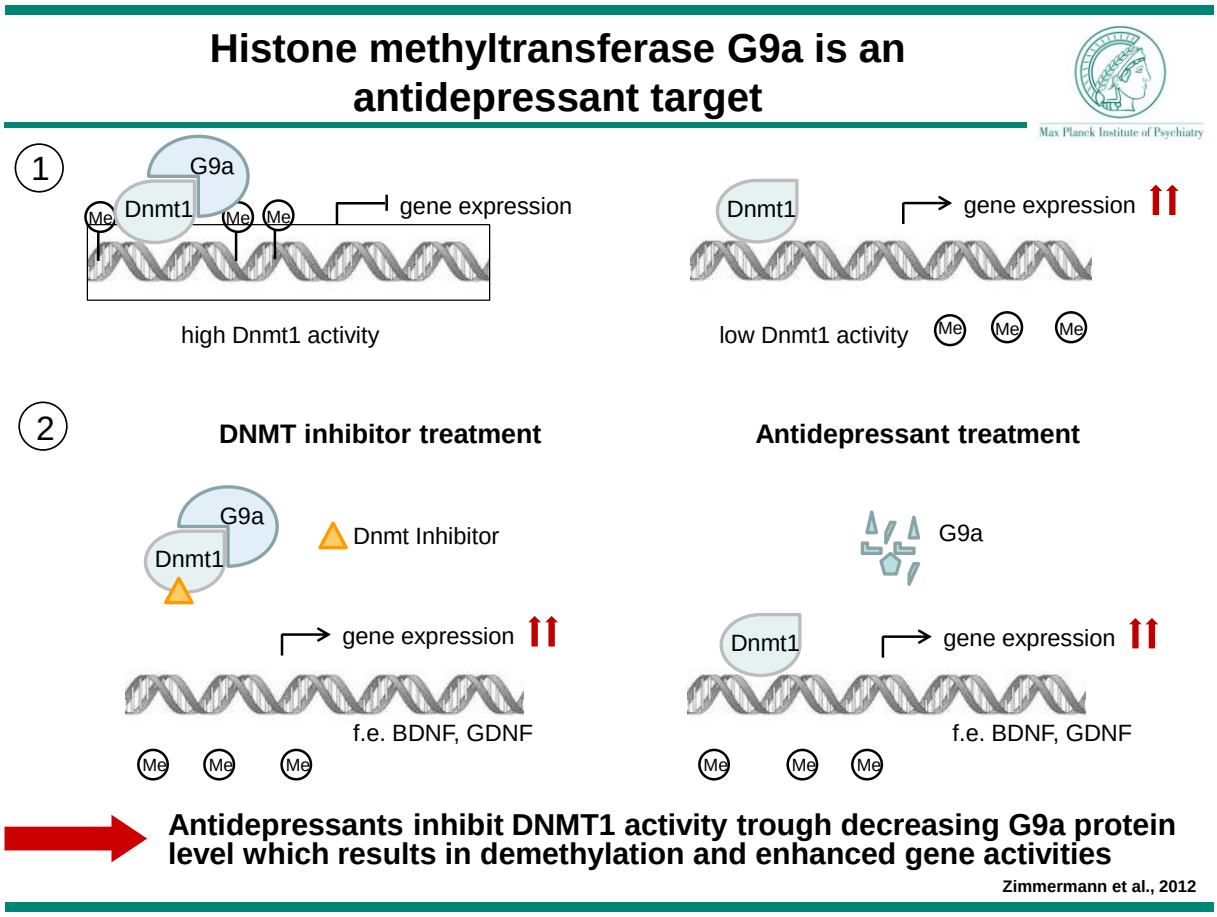


AMI, Amitriptyline; CBZ, Carbamazepine; CIT, Citalopram; IMI, Imipramine; PAR, Paroxetine; VEN, Venlafaxine

Zimmermann et al., 2012

Nicole Zimmermann from Theo Rein's group at the Max Planck Institute of Psychiatry used primary cortical astrocytes and studied the effect of a variety of currently available antidepressants and mood stabilizers on DNA-methyltransferase activity. This enzyme is abbreviated with DNMT and a number of isoforms of this enzyme exists.

She could show that amitryptiline suppresses DNMT-1 in a dose-dependent mode. She also showed that not all antidepressants exert this effect, for example, venlafaxine does not influence DNMT-1.



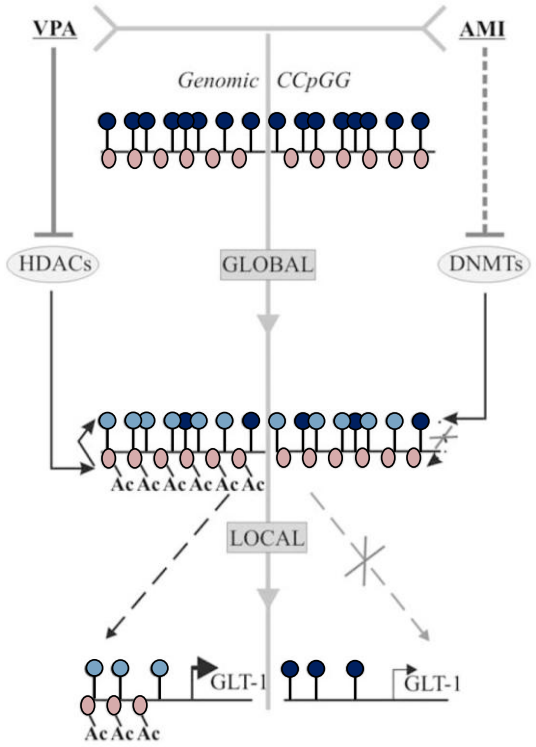
Under physiological conditions DNMT-1 activity is enhanced by interaction with the histone-methyltransferase G9a. By this protein-protein interaction DNA methylation is induced which results in repression of gene activity.

The mechanism leading to DNMT-1 decrease involves reduction of G9a protein levels. We conclude that antidepressants exert their effect on DNMT-1 via decrease of G9a levels, which impairs enhancement of gene activity by the DNMT1-G9a protein-protein interaction.

Thus, even without knowing clinicians have targeted epigenetic mechanisms with tricyclic and other antidepressants and mood stabilizers since many decades.

Tatjana Perisic, also from Theo Rein's group at the Max Planck Institute of Psychiatry focussed on glutamatergic signalling that is determined by glutamate release, its degradation and reuptake.

Psychoactive drugs impact differentially on epigenetic marks



AMI and VPA differentially influence global DNA demethylation

= CpG site

= highly metylated CpG site

= core histones

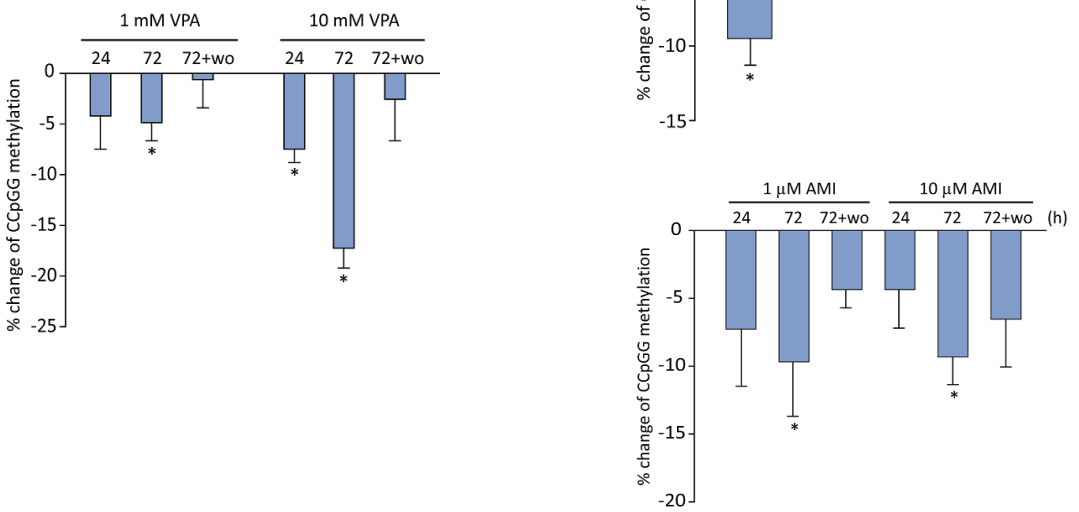
Perisic et al., 2012

She found that the gene activity of the glutamate uptake transporter GLT-1 can be epigenetically modified in astrocytes. After treatment of these cells with valproate which is an unspecific HDAC inhibitor demethylation and hyperacetylation of CpG shores at this location is found. CpG shores are methylation sites in close distance to CpG islands. In contrast to valproic acid amitriptyline does not show this effect.

CCpGG DNA methylation changes induced by VPA and amitriptyline

Max Planck Institute of Psychiatry

DNA methylation in astrocytes was measured by luminometric methylation analysis

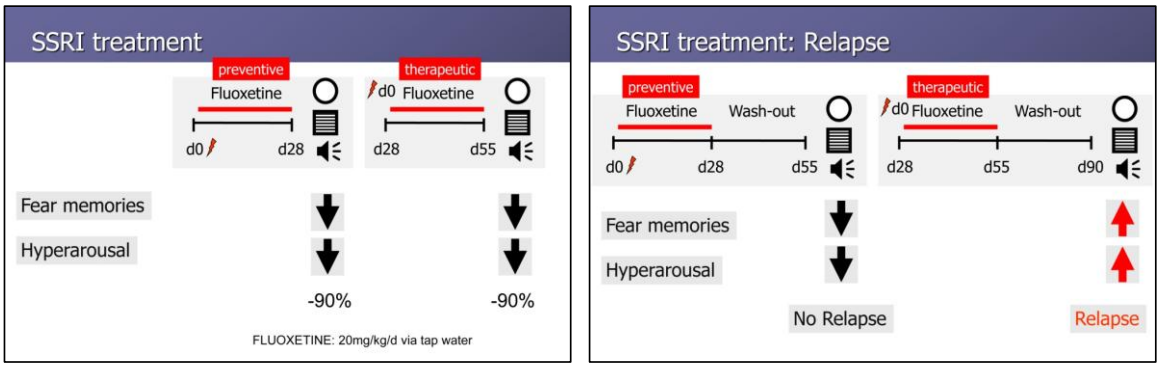


Perisic et al., 2010

However, on the genome-wide level she found that valproic acid as well as amitriptyline but not lamotrigin reduce the overall DNA methylation pattern.

When taken all these basic and clinical findings together we conclude that selective antidepressants administered right after trauma in the "emergency room" can be used to prevent the risk for PTSD. It needs to be determined which of all the antidepressants we currently have are efficient for this specific indication - that is prevention of PTSD after trauma.

Treatment with antidepressants must start shortly after trauma to prevent PTSD



Chronic AD treatment suppresses PTSD-like symptoms in animal models

BUT: only if initiated shortly after trauma PTSD can be prevented

TIME MATTERS !

Wotjak, 2011

This statement is strongly supported by animal experiments where long-term antidepressant treatment that is initiated immediately after trauma completely prevents the development of PTSD. Treatment started at later time can suppress PTSD symptoms. However, if an external cue is offered, after treatment cessation, fearful memories may emerge again.

At this point, before I finish, I want to clearly state that the development of epigenetic changes is extremely complex. We are only beginning to perceive the complexity of epigenetic marks that has evolved throughout development of all living organisms. Any attempt correlating environmental influences with overall methylation or acetylation patterns will lead to nothing as long as we remain uninformed about the biological function these epigenetic marks produce.

We need to understand how our mental state in general is shaped by the life we live and the world we live in right now. Our genes are switched on and off by what we experience and inasmuch as these switches are determined by inherited DNA variations. Another twist in the story is that experiences of fathers also matter as their traumatic experiences communicate to their offspring in a yet not understood way. Today there is little doubt that traumatic experiences of fathers, their life-style, age and nutrition leave biological traces in their children which can not entirely explained by genetic variants. It will take many more years until we will have a clearer picture how this nature-nurture puzzle works and how we can translate this into better prevention and treatment.

But clinician scientists working with patients cannot wait until everything is resolved, they need to act now. Therefore, it is not only pragmatic but also scientifically justified to translate some of the findings I mentioned into practice: A strong case is that early intervention with antidepressants is better than any “wait and see” attitude.

Finally, with gene tests and biomarkers we will soon be able to create patient subgroups that benefit from more specific interventions and those that are not in need of treatment. The prime candidates here are drugs that interfere with the HPA system, with NMDA receptor antagonists or with valproic acid – just to name a few.

I do hope this tour de force has convinced you that there is substantial progress in the field of PTSD treatment research justifying optimism.