



BenBedPhar Training School 2023
NRF2 in noncommunicable diseases:
From bench to bedside

June 26 - 30, 2023
Smolenice Castle, Slovakia

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Implication of NRF2 in Depression



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


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General concepts on Depression

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Depression
The symptoms of depression can vary slightly depending on the type and can range from mild to severe. In general, symptoms include:

 Low energy.	 Feeling very sad or hopeless.	 Thoughts of self-harm or suicide.
 Irritability.	 Changes in eating behavior.	 Sleep changes.
 Loss of interest in hobbies and activities.	 Difficulty concentrating or making decisions.	

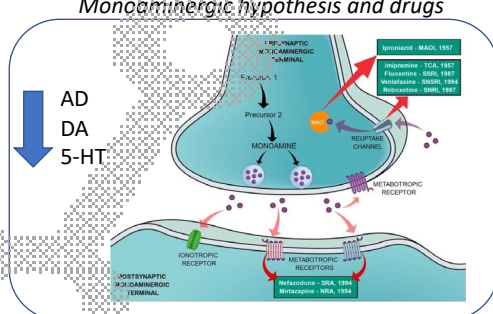
- ✓ **Leading cause of disability** worldwide
- ✓ Affects more than **300 million people** worldwide
- ✓ The **incidence of depression** increases
- ✓ More **women** are affected
- ✓ Depression can lead to **suicide**.
- ✓ Depression is **closely related** to and affected by physical health and **other disease** (cause or consequence)
- ✓ Current **treatments** take months to exert their effect
- ✓ Treatments: around **50% no efficacy** or **abandon treatment** because of side effects

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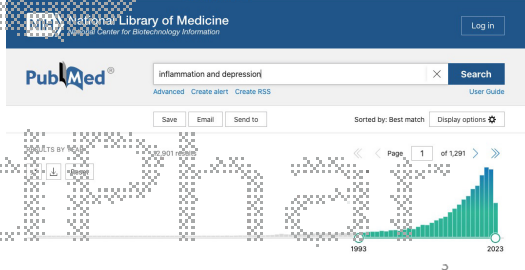
Theories implicated in the pathology of Depression

1. **Monoaminergic** (Schildkraut 1965)
 - ✓ Deficiency of **noradrenaline, dopamine, serotonin**
 - ✓ Most antidepressive increase these NTs
2. **Inflammatory** (Smith 1991, Maes 1990-1993)
 - ✓ Patients exhibit elevated blood and CSF levels of proinflammatory cytokines and cytokine receptors
 - ✓ 30-40% of refractory depressive patients have high levels of inflammatory markers (IL6, RPC)
3. **Stress:** (Blackburn-Munro, 2001; Gosben et al., 2008; Tafet & Bernardini, 2003)
 - ✓ The hypothalamic-pituitary-adrenal (HPA) axis: increased Cortisol
 - ✓ The sympathomedullary axis (or the locus coeruleus-norepinephrine [LC-NE] system)



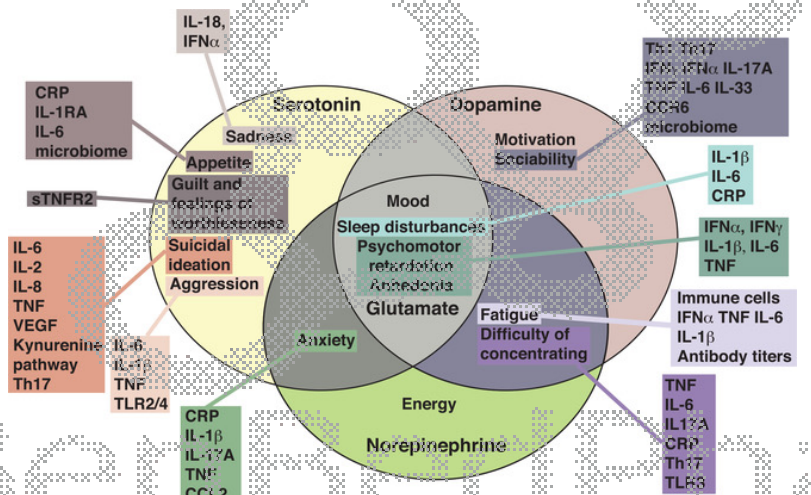
AD
DA
5-HT

MAO, 1967
 Imipramine - TCA, 1967
 Fluoxetine - SSRI, 1987
 Venlafaxine - SNRI, 1994
 Moclobemide - MAOI, 1987
 Mirtazapine - MIA, 1994
 Nefazodone - SRA, 1994
 Mianserin - MIA, 1994



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Evidences that support inflammation in mood disorders




Serotonin
 Appetite
 Guilt and feelings of worthlessness
 Suicidal ideation
 Aggression
 Anxiety
 Energy
 Norepinephrine

Dopamine
 Motivation
 Sociability
 Fatigue
 Difficulty of concentrating

Norepinephrine
 Anxiety
 Energy
 Norepinephrine

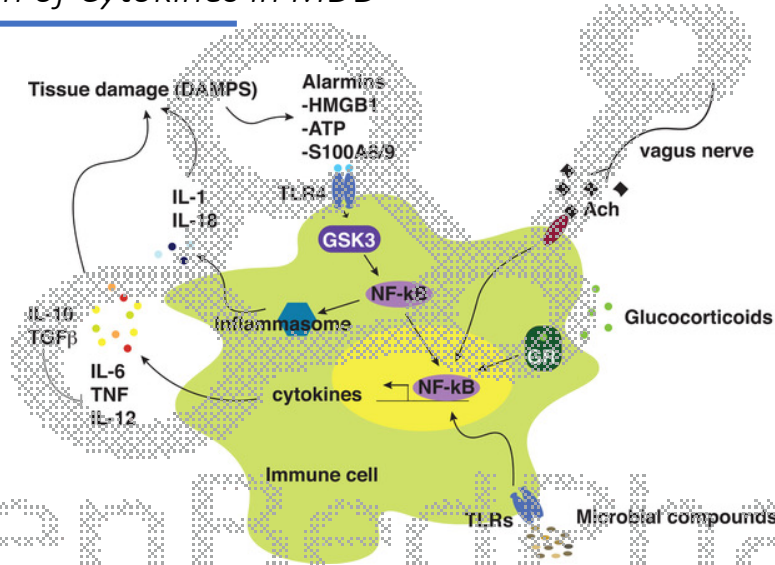
Central Inflammatory Markers:
 CRP, IL-1RA, IL-6, microbiome, sTNFR2, IL-6, IL-2, IL-8, TNF, VEGF, Kynurenine pathway, Th17, IL-18, IFN α , IL-1 β , IL-6, CRP, TNF, Th17, TLR2/4, IL-1 β , IL-17A, TNF, CCL2, IL-1 β , IL-6, CRP, TNF, Th17, TLR8, IL-1 β , IL-6, CRP, IFN α , TNF, IL-6, IL-1 β , Antibody titers, IL-1 β , IL-6, CRP, TNF, Th17, TLR8



Beurel et al., ,Neuron 107, 2020. <https://doi.org/10.1016/j.neuron.2020.06.002>

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Multiple Mechanisms May Contribute to the Dysregulation of Cytokines in MDD

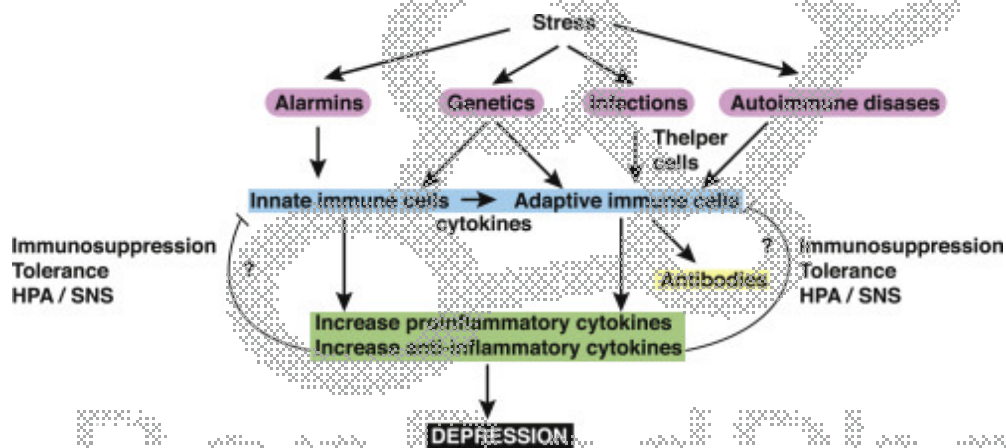


Beurel et al., ,Neuron 107, 2020. <https://doi.org/10.1016/j.neuron.2020.06.002>

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Potential Immune Response deregulation in MDD: link to stress

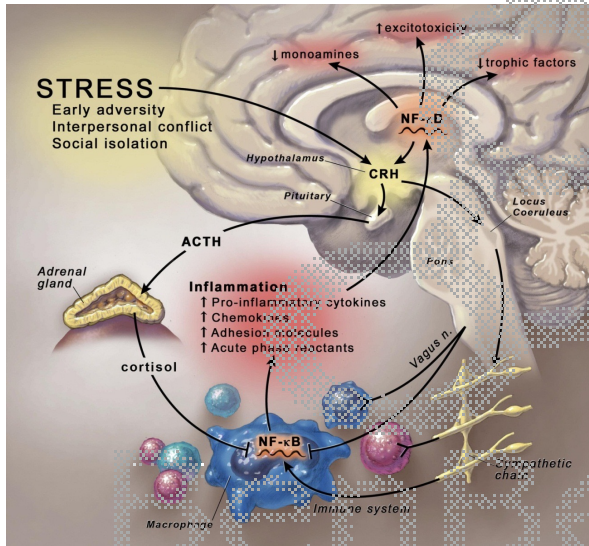


Beurel et al., ,Neuron 107, 2020. <https://doi.org/10.1016/j.neuron.2020.06.002>

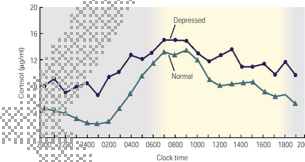
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The hypothalamic-pituitary-adrenal (HPA) axis and sympathetic system: Stress-Inflammation balance



Approximately 50% of mood disorder patients exhibit hypercortisolism. (Halbreich et al. 1985; Atil et al 1993; Checkley et al 1996)



Reduced

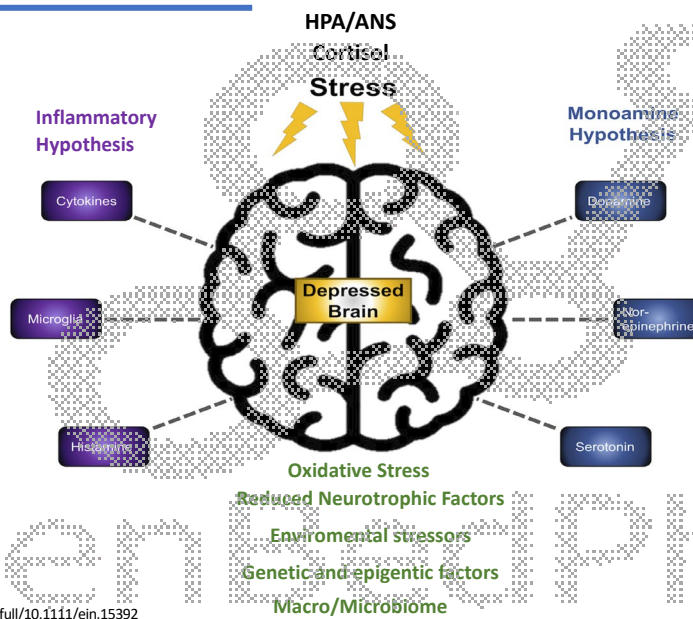
- Monoamines
 - NA
 - DA
 - 5HT
- Trophic factors
 - BDNF

Increased

- Excitotoxicity (glutamate)
- Inflammation
- Oxidative stress

Andrew H. Miller, Vladimir Maletic, Charles L. Raison. *Biological Psychiatry* e 65, 732-741.2009. *Biological Psychiatry* e 65, 732-741.2009

Main players implicated in depression



<https://onlinelibrary.wiley.com/doi/full/10.1111/ejn.15392>

Nrf2 regulates inflammation

Homeostasis
 HS SH
 KEAP1
 NRF2
 Ub
 Ub
 Ub
 Ub
 Proteasomal degradation

Stress
 (e.g. UV radiation, wounding)
 ROS, Electrophiles
 S-S
 KEAP1
 NRF2
 Cytoplasm

Nucleus
 NRF2
 MAF
 ARE
 Target genes
 ROS detoxification

Metabolic Pathways:

- Redox balance
 HMOX1, NQO1, TrxR, cGS, GCLC, GCLM, Gpx, GR ...
- Purine metabolism
 PPAT, MTHFD2
- Pentoses metabolism
 GAPDH, PGD, IDH1, ME
- Lipid metabolism
 ACC1, FAS, SCD1, ACL...
- Inflammation** (highlighted)
- Proteostasis
 Gpx8, P5MB7, SQSTM1, CALCOCO2, ULK1...

Cuadrado et al., Pharmacol Rev. 2018 Apr;70(2):348-383. doi: 10.1124/pr.117.014753.

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hypothesis

- ✓ NRF2 regulates inflammation
- ✓ Inflammation participates in depression

Can NRF2 be a target for the treatment of depression?

Approaches to proof our hypothesis:

- 1.- Animal models**
 - Nrf2-KO vs NRF2-WT
 - Induced models (LPS, cortisol, Stress..)
- 2.- How can we evaluate a depressive phenotype in animals?**
 - Behaviour
 - Inflammation
 - Neurotransmitters
 - Neurotrophic factor
- 3.- Will Nrf2 inducers improve depressive phenotypes**
- 3.- Do MDD patients have alterations in Nrf2 markers**

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1.- Do Nrf2-KO mice show a depressive phenotype ?



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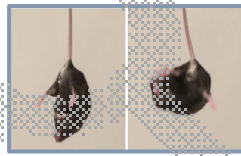
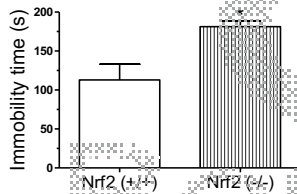
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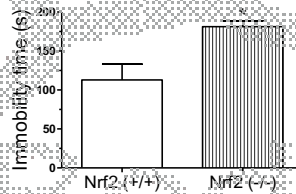
Nrf2-KO animals have a depressive behaviour



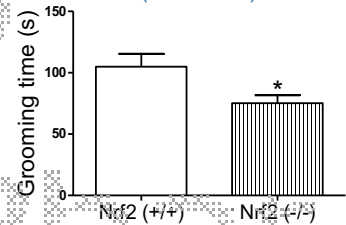
Forced swimming Test (Dispair)



Tail Suspension Test (Dispair)



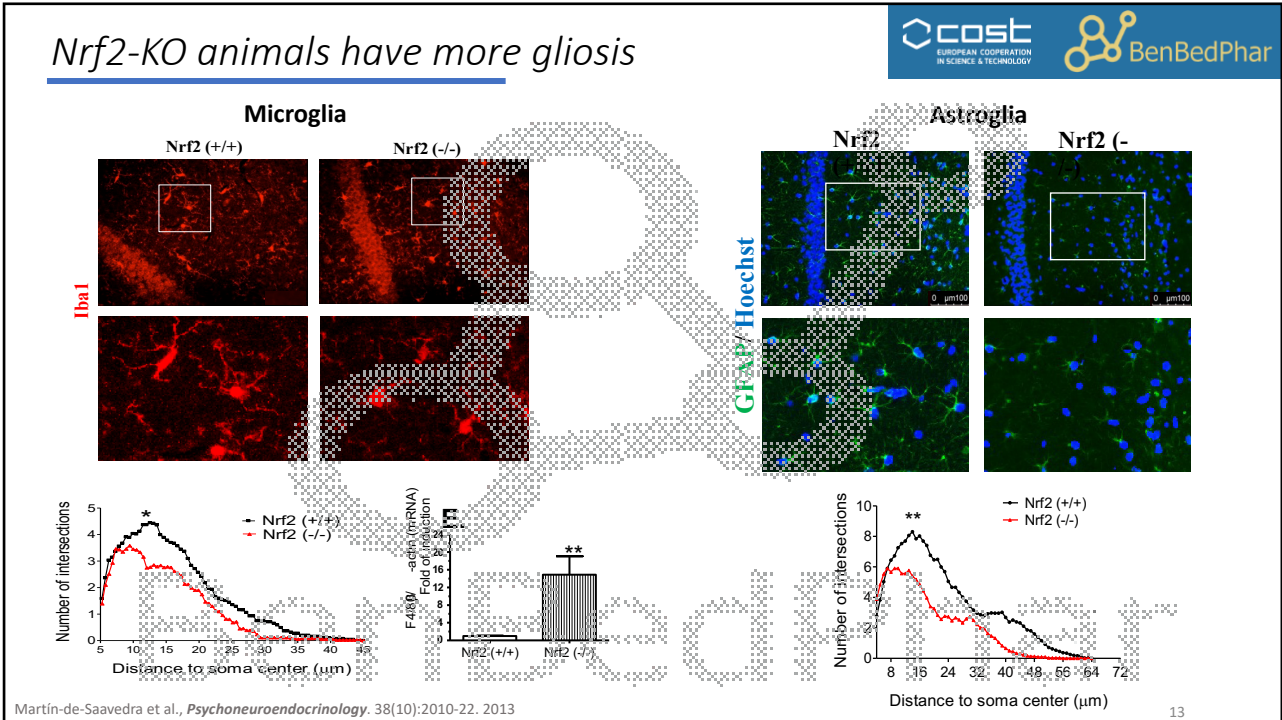
Sucrose Splash Test (Anhedonia)



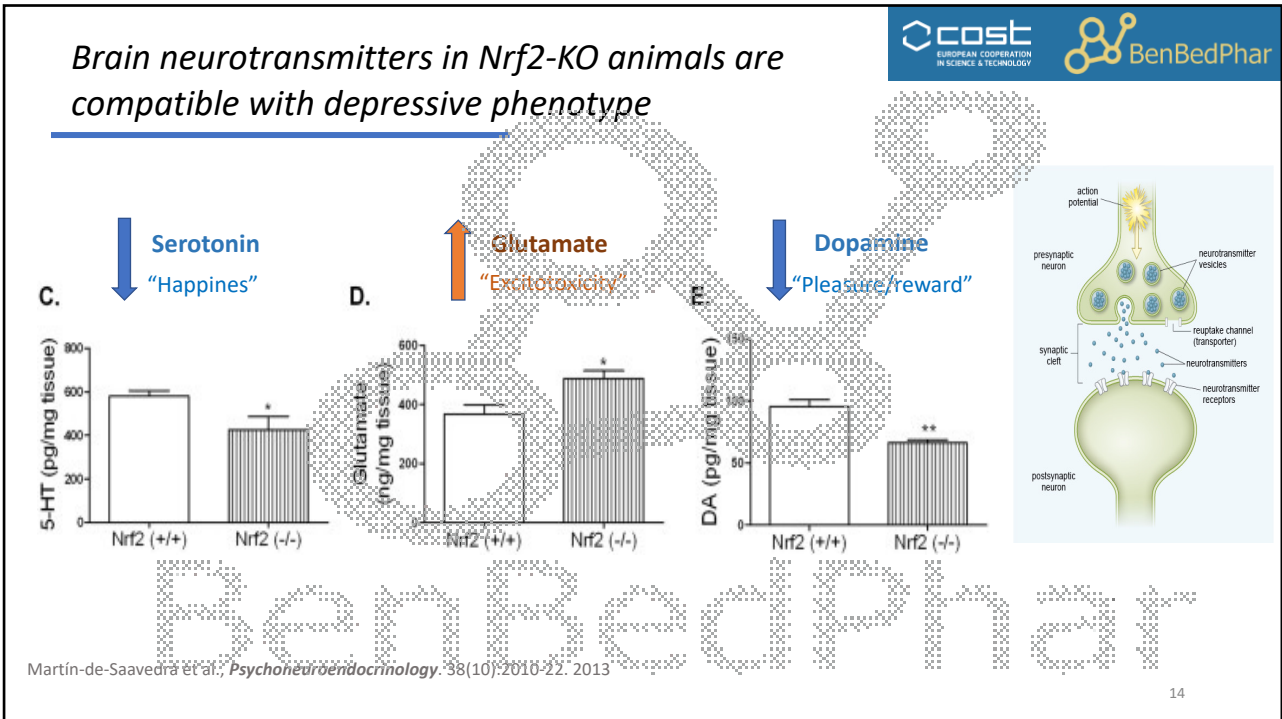
Martín-de-Saavedra et al., *Psychoneuroendocrinology*. 38(10):2010-22. 2013

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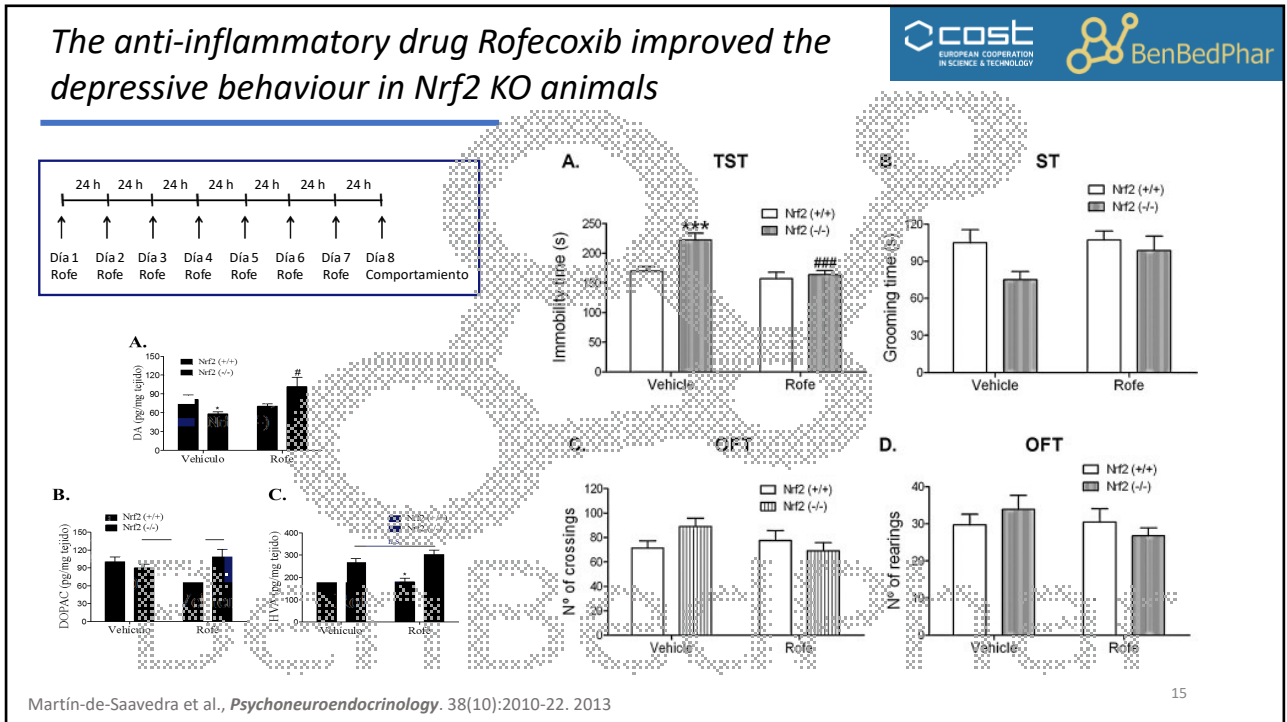
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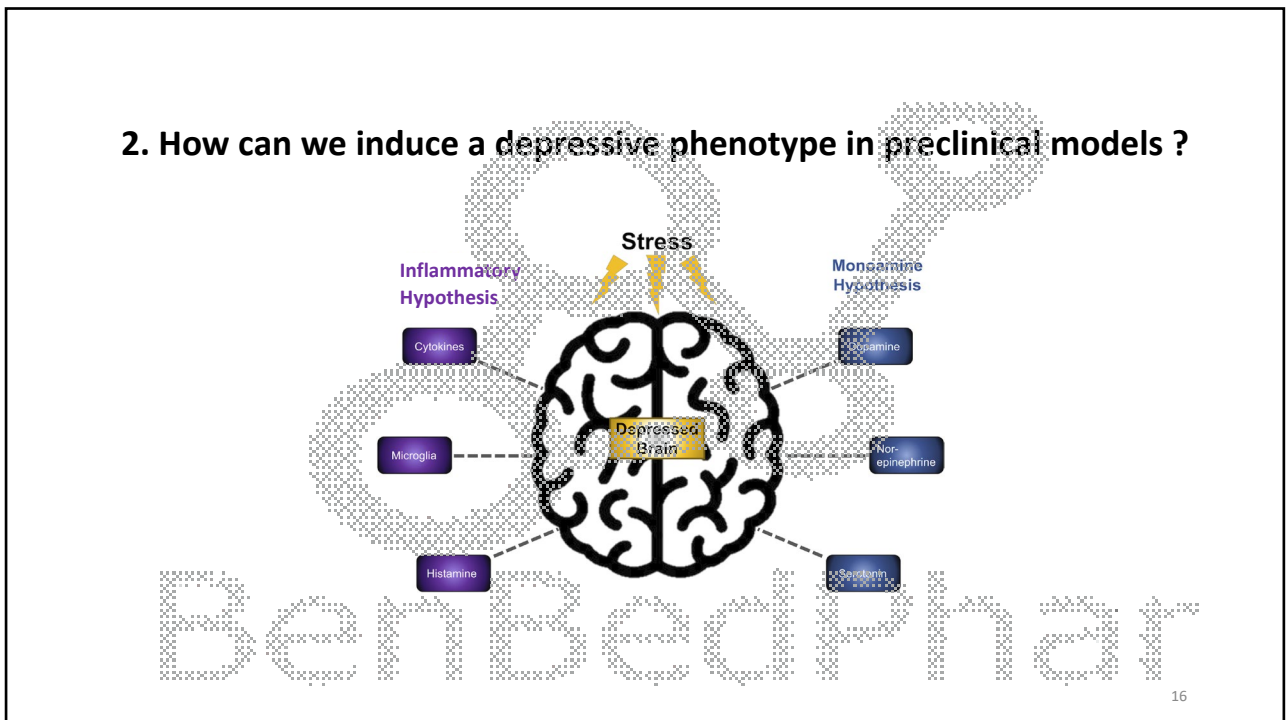
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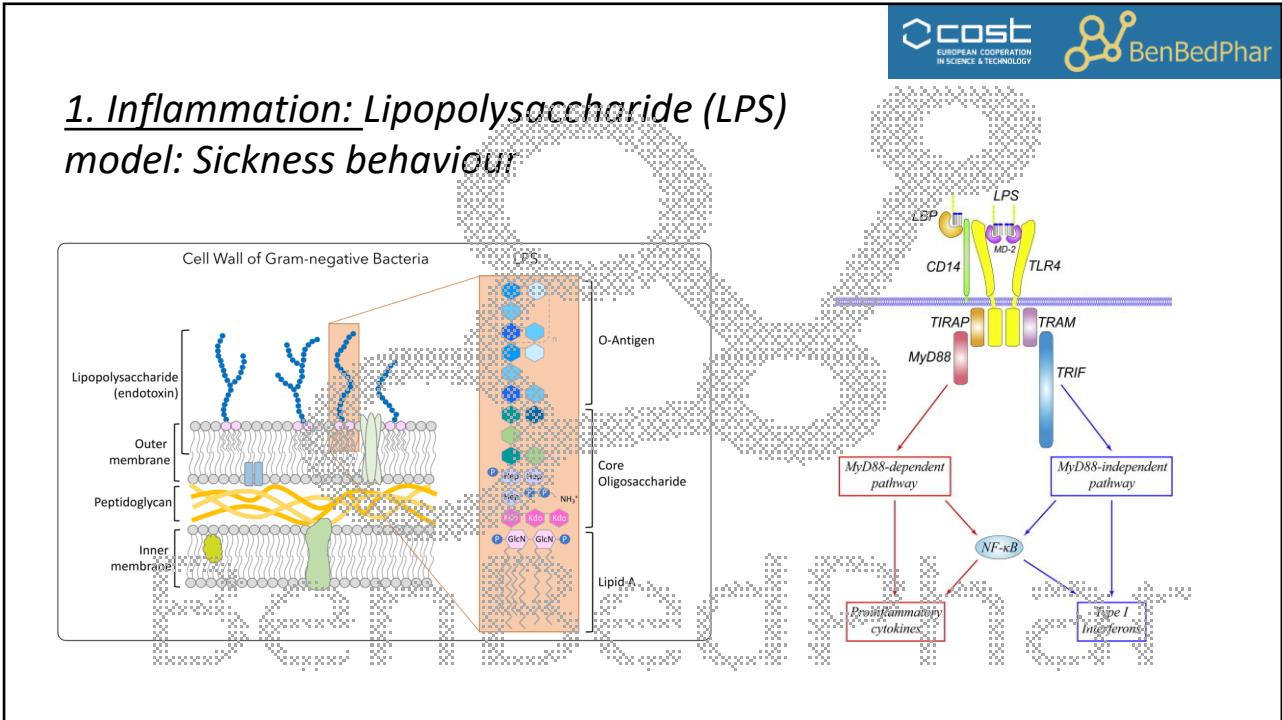
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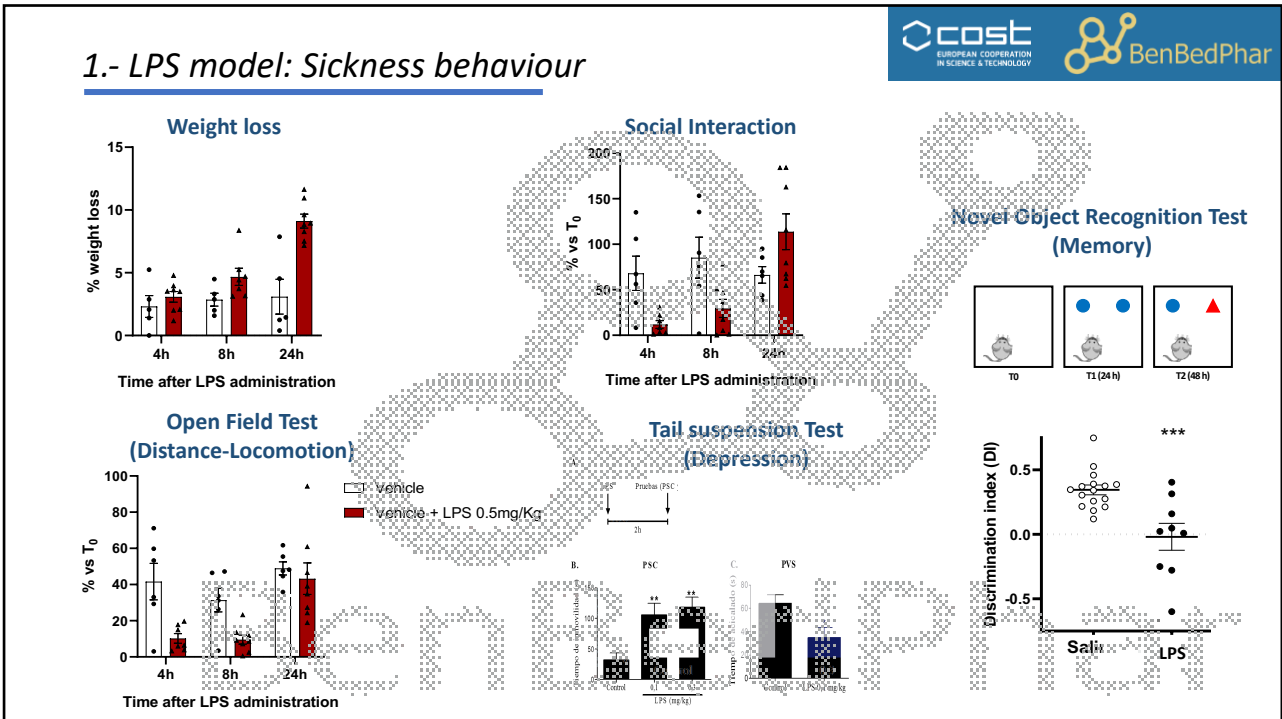
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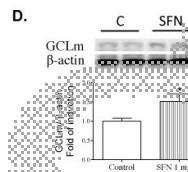
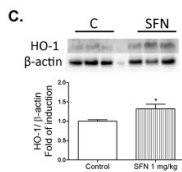
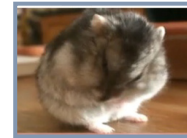
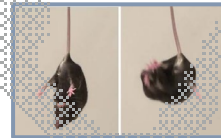
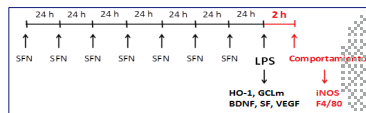


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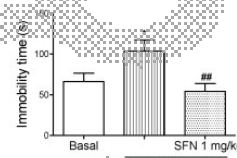


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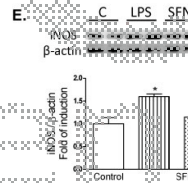
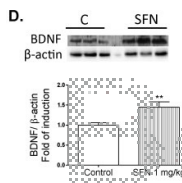
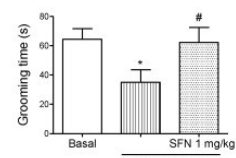
The Nrf2 inducer **sulforaphane** recovers the depressive phenotype in an LPS depression induced model



A. Tail Suspension Test

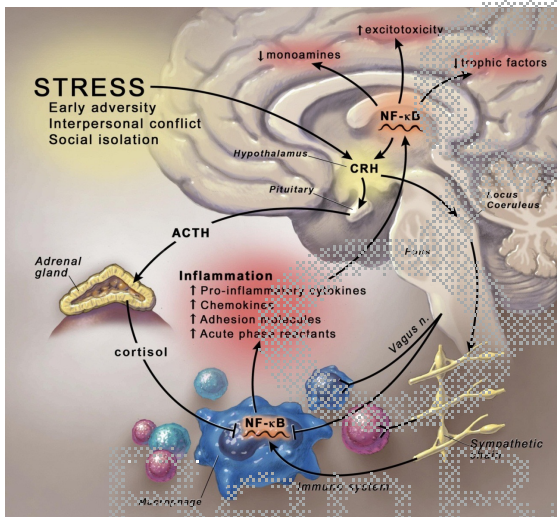


B. Sucrose Splash Test



Martin-de-Saavedra et al., *Psychoneuroendocrinology*. 38(10):2010-22. 2013

2.- Stress Models of depression

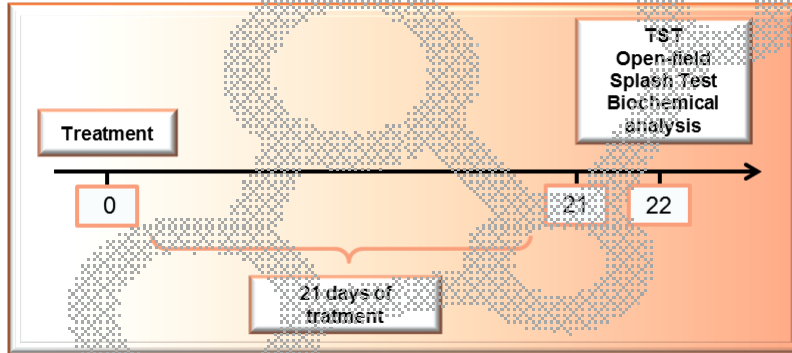


2.1- Subchronic Corticosterone

2.2- Social Defeat (SD)

2.3- Mild Chronic Stress (MCS)

2.1. Subchronic corticosterone model



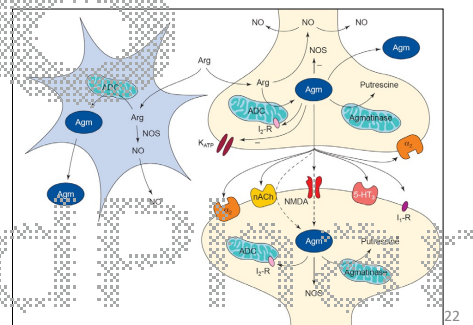
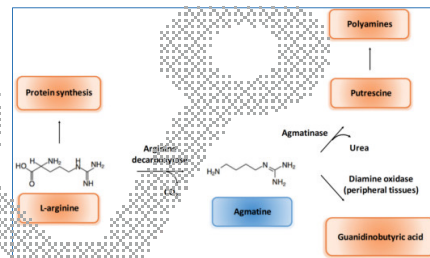
Treatments {
Corticosterone (20 mg/kg, p.o.)
Agmatine (0.1 mg/kg, p.o.)
Vehicle (10 ml/kg, p.o.)

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Agmatine an endogenous compound

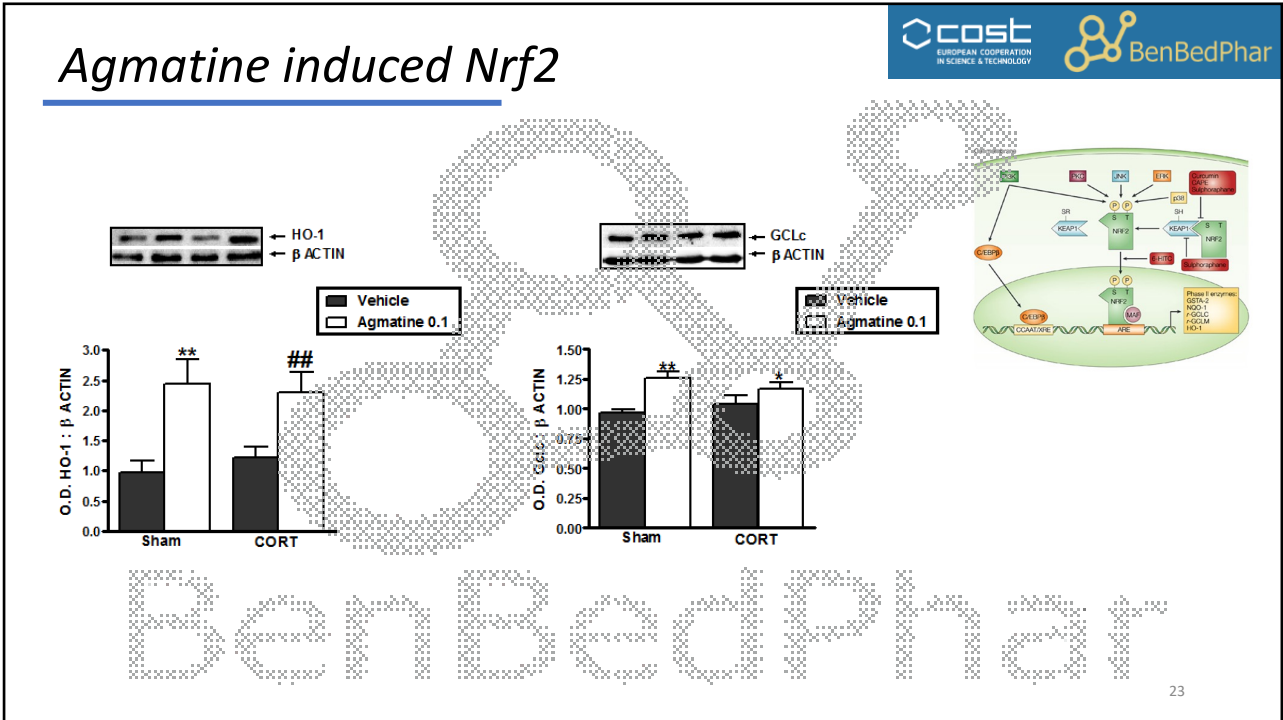
1. It is taken up by presynaptic axon terminals, stored in synaptic vesicles alone or with other NT, and released through membrane depolarization^[1, 2, 4]
2. It is inactivated by the enzyme agmatinase^[5]
3. It activates several postsynaptic membrane receptors, namely I₁R, and α2-adrenergic receptors, and 5HT₃ receptors^[1, 5]
4. It inhibits membrane Ca⁽²⁺⁾ channels, all isoforms of nitric oxide synthase (NOS) in the brain, and blocks N-methyl-D-aspartate (NMDA) receptors^[5]
5. Has shown antidepressant effects in predictive models by a mechanism that implicates NMDA receptors and L-Arginine NO pathway^[6]



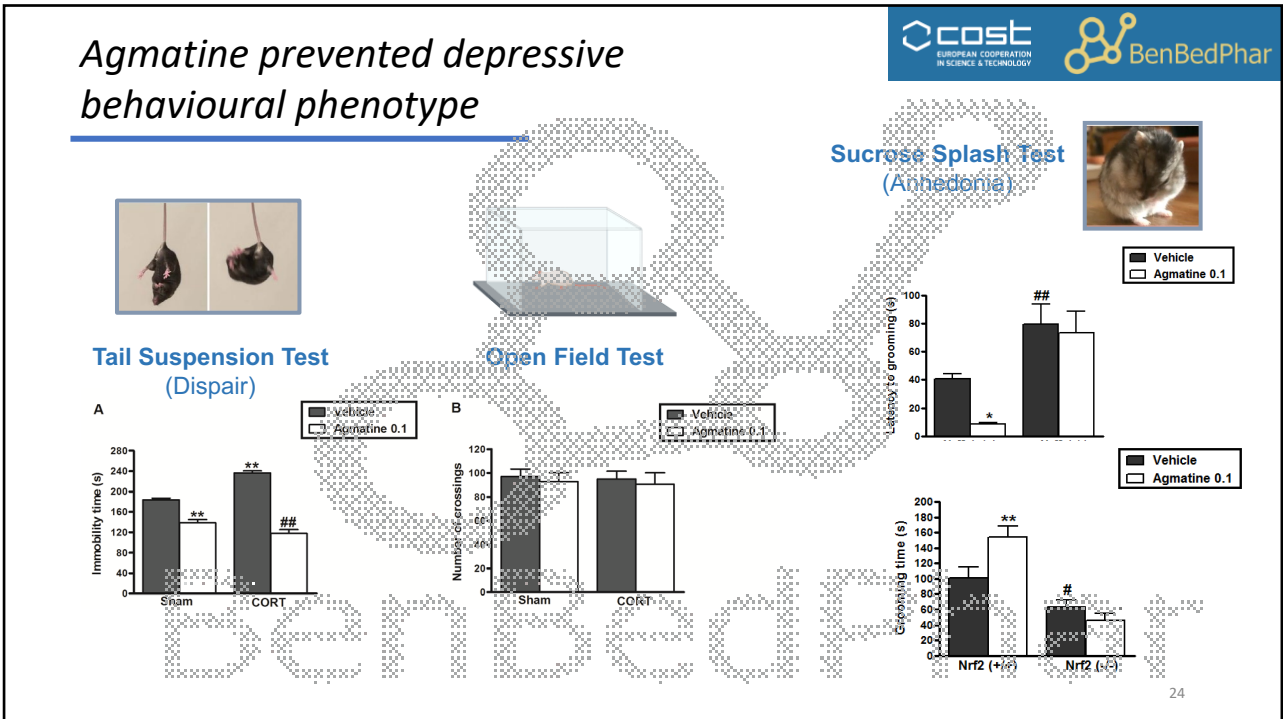
1. Reis DJ, Regunathan S (1999) Agmatine: an endogenous ligand at imidazoline receptors is a novel neurotransmitter. *Ann N Y Acad Sci* 881:65-80
 2. Goracke-Pastle CJ, Overland AC, Reul MC, Storm LC, Fairbanks CA (2007) Potassium- and capsaicin-induced release of agmatine from spinal nerve terminals. *J Neurochem* 102:1738-1746
 3. Todor CW, Todor H (1994) Polyamines. *Ann Rev Biochem* 63:745-774
 4. Seo S, Liu P, Leitch B (2011) Spatially and time-dependent regulation of agmatine and glutamate at hippocampal CA1 synapse terminals. *Neuroscience* 192:28-36
 5. Reis DJ, Regunathan S (1998) Agmatine: an endogenous ligand at imidazoline receptors may be a novel neurotransmitter in brain. *J Auton Nerv Syst* 72:80-85
 6. Freitas et al., *Prog. Neuro-psychopharmacology Biol Psychiatry* 50:143-150, 2012

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Agmatine increases NA, serotonin and dopamine levels in the brain



	NA	5-HT	DA	Glutamate
	(ng/g tissue)	(ng/g tissue)	(ng/g tissue)	(mg/g tissue)
Vehicle/Vehicle	44.9 ± 3.2	24.7 ± 2.5	1.5 ± 0.1	2.6 ± 0.4
Agmatine/Vehicle	96.5 ± 8.8**	41.7 ± 6.3**	14.6 ± 3.9**	1.7 ± 0.2
Vehicle/CORT	41.7 ± 4.7	11.3 ± 0.9*	1.4 ± 0.06	4.1 ± 0.3**
Agmatine/CORT	83.6 ± 11.3##	35.8 ± 5.5##	11.2 ± 2.1##	2.4 ± 0.4##

Data are expressed as mean ± S.E.M. (n = 5). Statistical analysis was performed by two-way ANOVA, followed by the Duncan's test. *p<0.05 and **p<0.01 as compared with the control group (vehicle-vehicle); #p<0.05 and ##p<0.01 as compared with the corticosterone vehicle group.

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Agmatine an endogenous compound that regulates stress and mood

- ↑ NA
- ↑ 5-HT
- ↑ DA

Monoamine Hypothesis

- ↑ pPKA
- ↓ pJNK1/2
- ↑ pAkt
- ↑ pCREB
- ↑ pGSK-3β
- ↑ BDNF
- ↑ ERK1/2
- ↑ SV2
- ↑ Neurogenesis

Neurotrophic Hypothesis

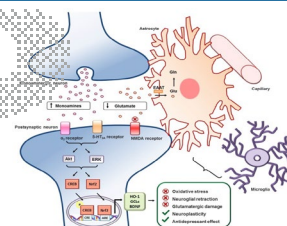
AGMATINE

- ↓ Glutamate
- NMDA receptor blockade
- NOS inhibition
- ↓ Neuroglial atrophy

Glutamate Hypothesis

- ↓ ROS
- ↓ Apoptosis
- ↓ Lipid peroxidation
- Nrf2 activation

Oxidative Hypothesis



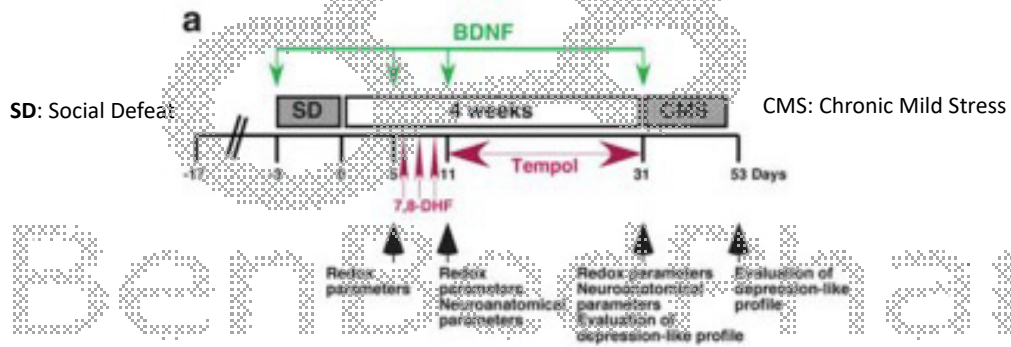
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2.2. Social Defeat and Chronic Mild Stress: Contribution of oxidative stress to depression

OPEN
Molecular Psychiatry (2017) 22, 1701–1713
www.nature.com/mp

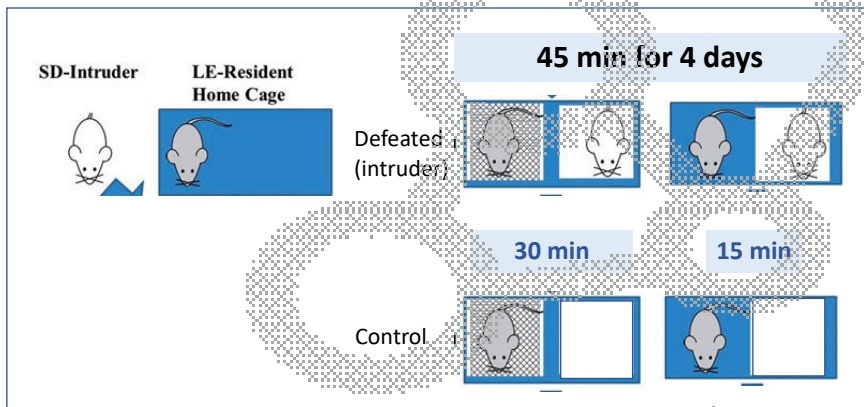
ORIGINAL ARTICLE
Nrf2-dependent persistent oxidative stress results in stress-induced vulnerability to depression
E Bouvier^{1,2,3}, F Brouillard^{4,5}, J Molero^{6,7}, D Clavier^{8,9,10}, J-H Cabungcal¹¹, N Grasso^{12,13}, N Doligez^{1,2,3}, C Rhyu^{14,15}, K Do¹⁶, C Bernard¹⁷, J-J Benoitel^{18,19,20} and C Becchi²¹



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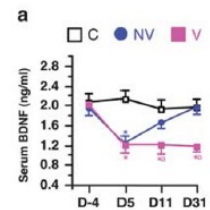
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2.2. Social defeat



Based on BDNF levels at 31 days, SD are divided:

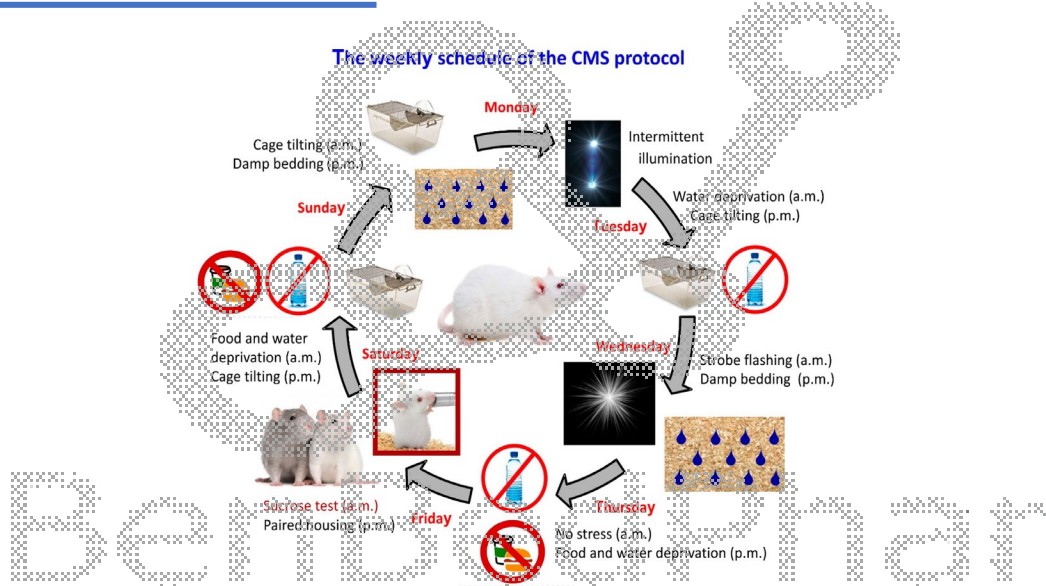
NV= Non Vulnerable
V= vulnerable



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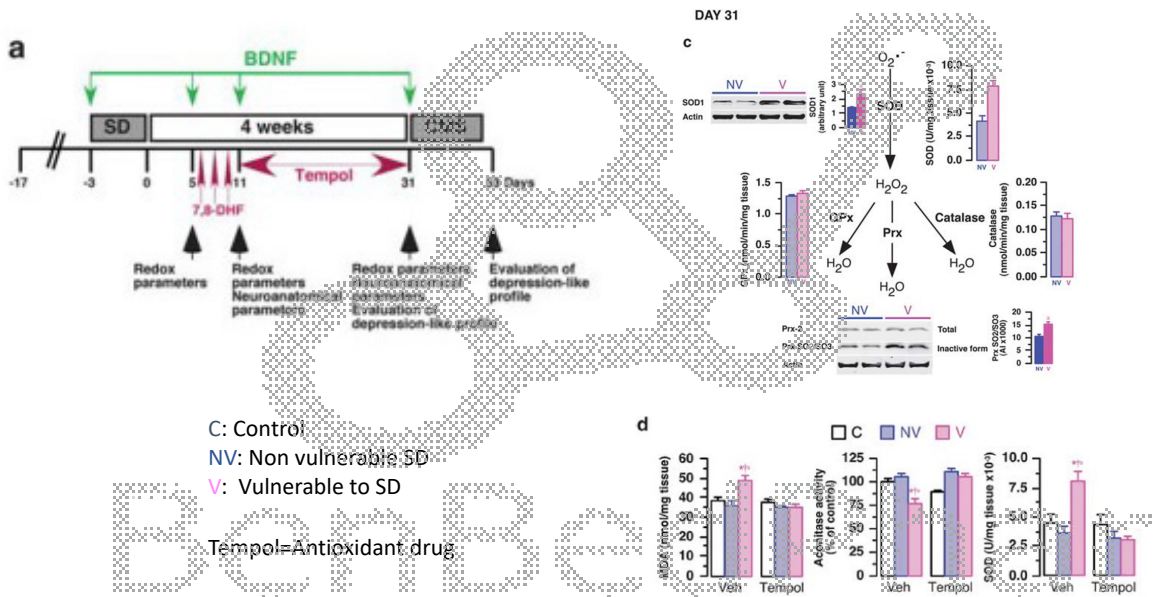
2.2. Chronic Mild Stress

The weekly schedule of the CMS protocol



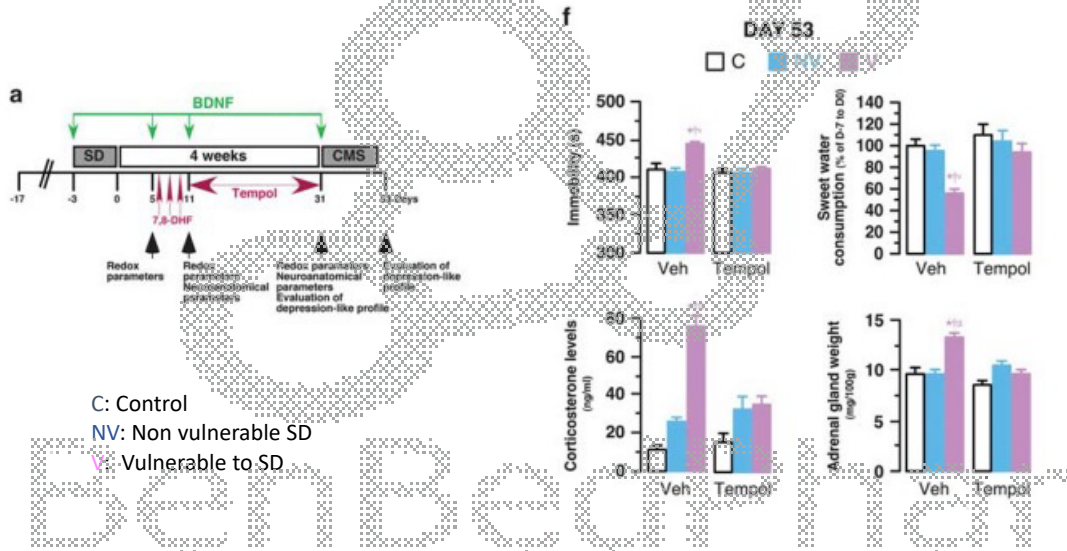
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2.2. Animals with SD have higher levels of oxidative stress



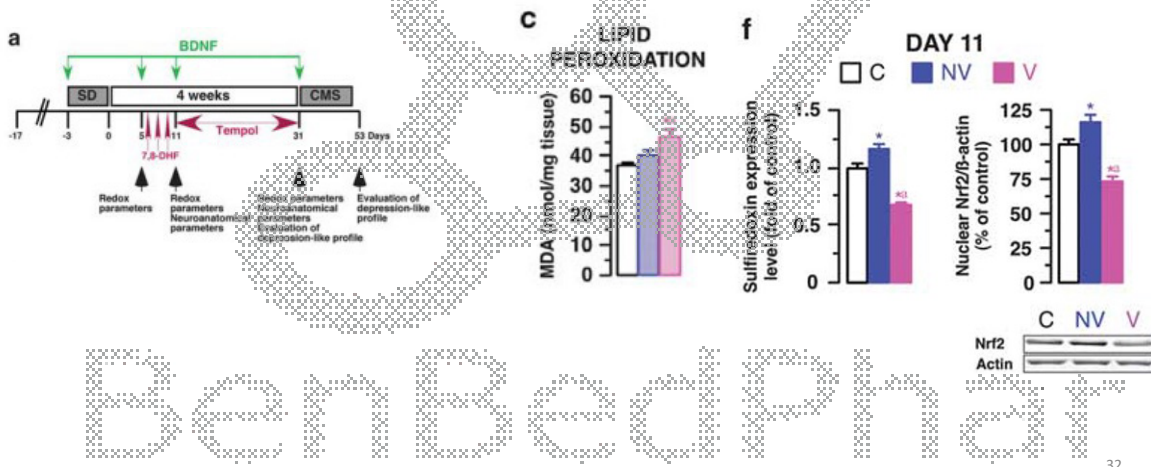
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Higher oxidative stress levels were related to depressive behaviour

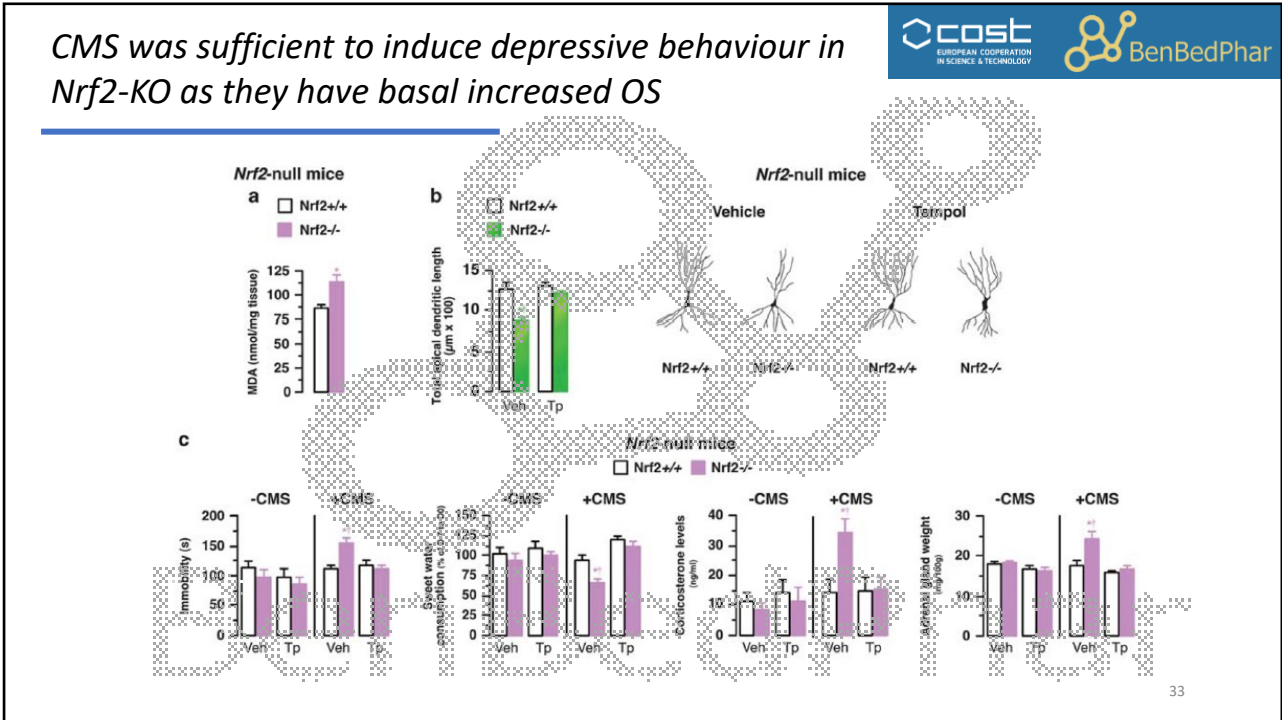


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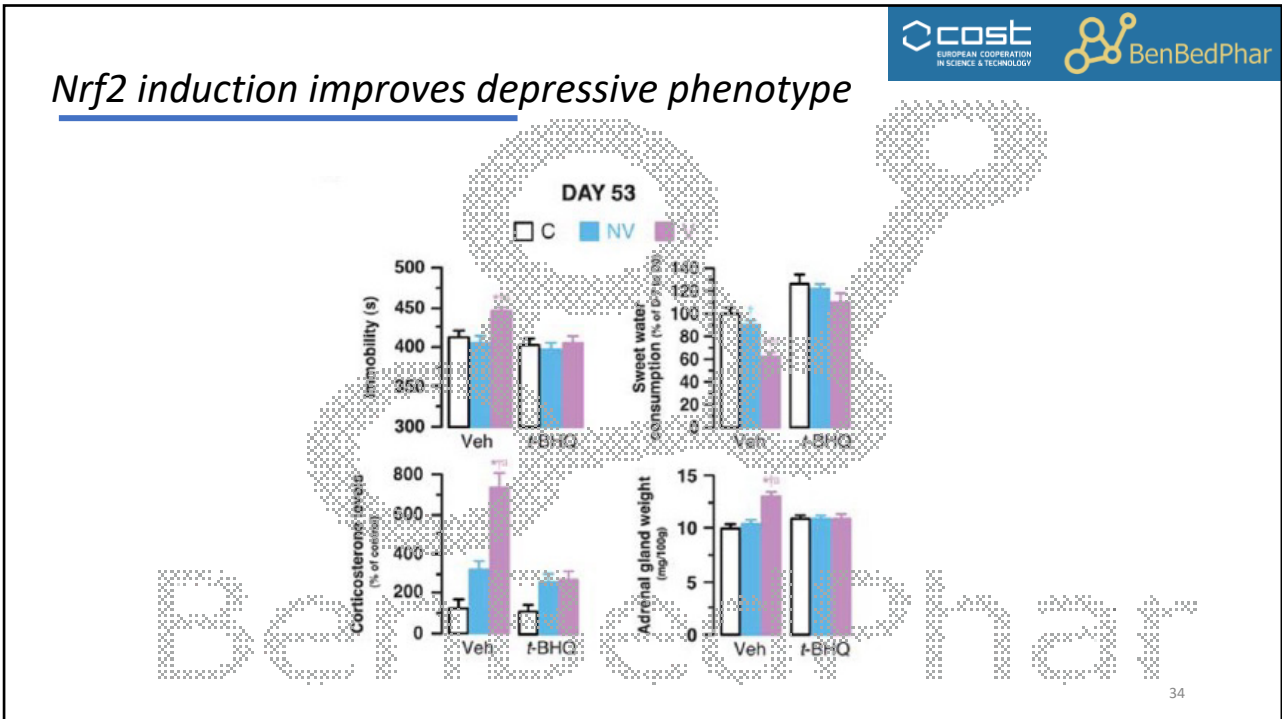
Vulnerable animals had more OS and less induction of Nrf2



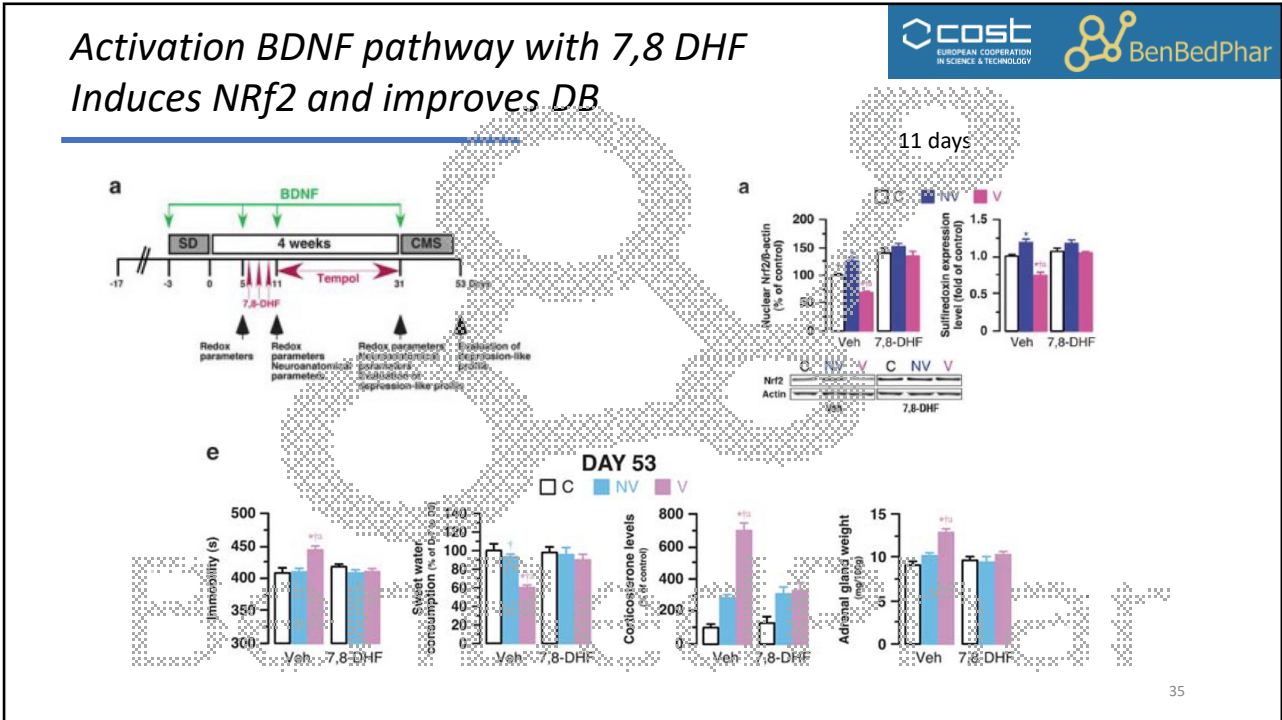
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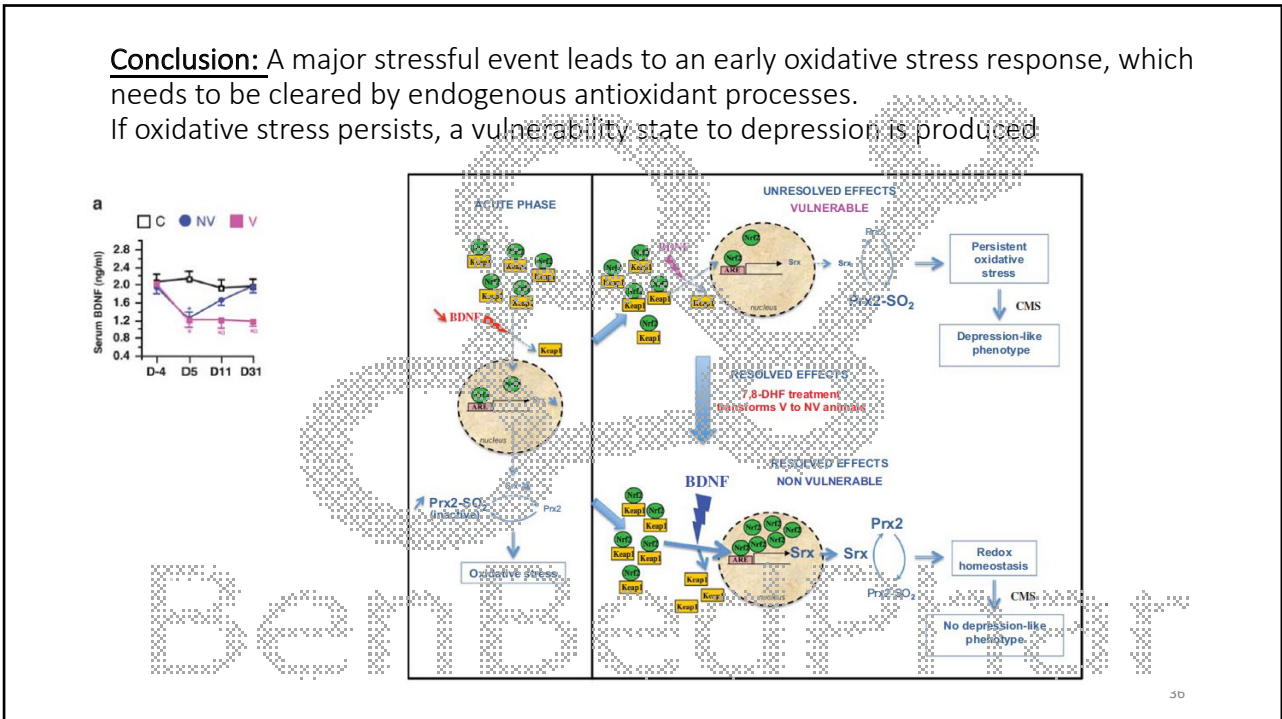
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What have we learnt?

1. Which theories are implicated in depression?

- Monoamine
- Inflammatory
- Stress: Sympathetic-HPA-cortisol
- Oxidative stress-BDNF

2. Induced Animal models of depression?

- LPS: inflammation
- Stress:
 - Subchronic Corticosterone
 - Social defeat
 - Chronic mild stress



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What have we learnt?

3. How can we evaluate a depressive phenotype in animals?

- Behaviour: FST, TST, ST
- Brain Neurotransmitters: DA, 5-HT, Glutamate
- BDNF levels brain
- Inflammatory markers



Major conclusions

- ✓ Nrf2 participates in depression
- ✓ Drugs targeting Nrf2 could be a promising therapeutic approach for depressive patients

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Thank you for your attention !

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