How do the homeopathic medicines work?

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Verona Integrative Medicine Research Group (y. 2015)

Clara Bonafini

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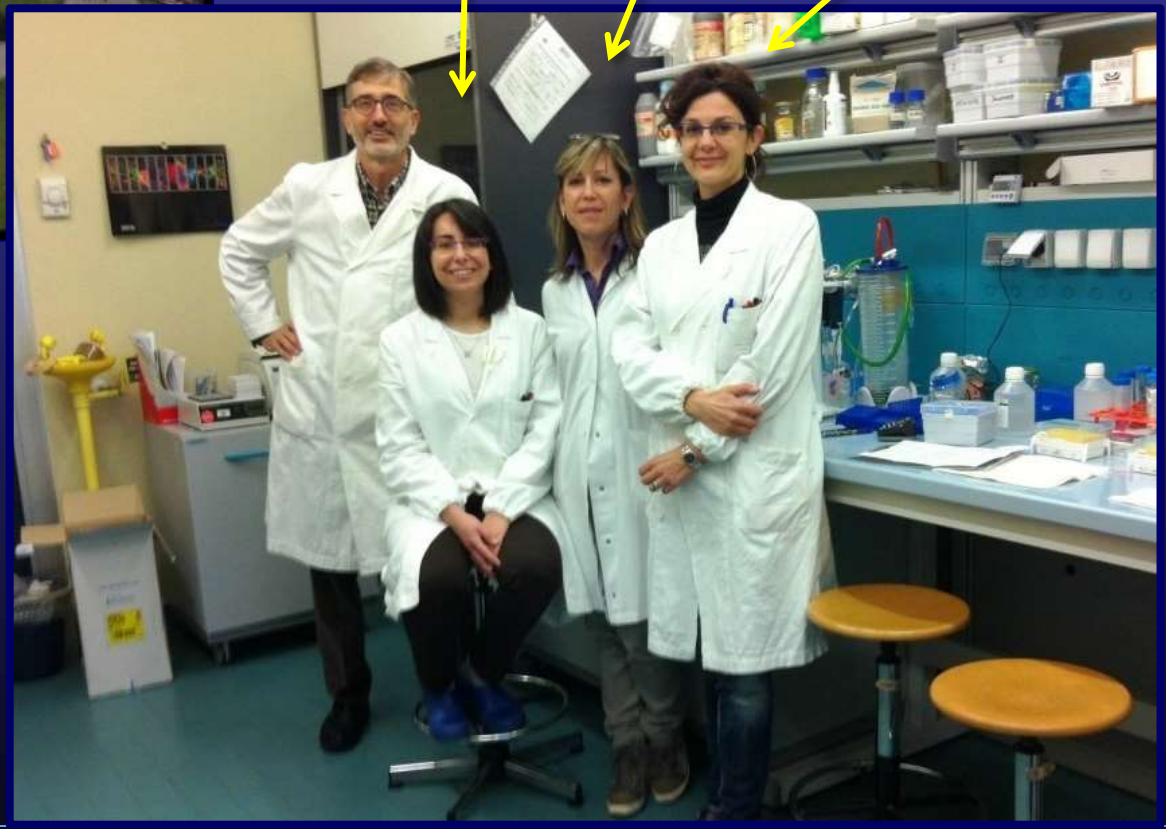

We thank for research grants:
- Boiron Laboratoires
- Italian Research Ministry

How do the homeopathic medicines work?

1. Introduction
2. Studies in animal and plant models
3. In vitro laboratory studies
4. General insights

How does NATURE work?

"There is no philosophical high-road in science, with epistemological signposts. No, we are in a jungle and find our way by trial and error, building our roads behind us as we proceed."

- Max Born (1882-1970), Nobel Prize-winning physicist,

PROBLEMS

We investigate (well) only some pieces of the whole mosaic…

1. **Most homeopathic medicines have several active principles**
2. **The active principles are very diluted! Often not even detactable**
3. **Each active substance has different cell targets and action mechanism**
4. **Non-linear or «bizarre» dose-dilution-effect relations in experimental models**

Pieces of a 6° century mosaic (Ravenna, Italy)

PROBLEMS

We investigate (well) only some pieces of the whole mosaic…

GENERAL ADVANCES IN THE LAST 25 YEARS:

1. **Knowledge of many pieces of the mosaic from laboratory studies of several medicines**
2. **General confirmation (with exceptions) of major principles of homeopathy that are working in several models**

Pieces of a 6° century mosaic (Ravenna, Italy)

The two major PRINCIPLES

- The same substance or similar substances can have opposite (inverse) effects in different conditions:
  a) **doses** or
  b) **sensitivity** of the target system
- Pharmacological power of the original substance is retained (or even enhanced?) in serial **dilutions with succussion**

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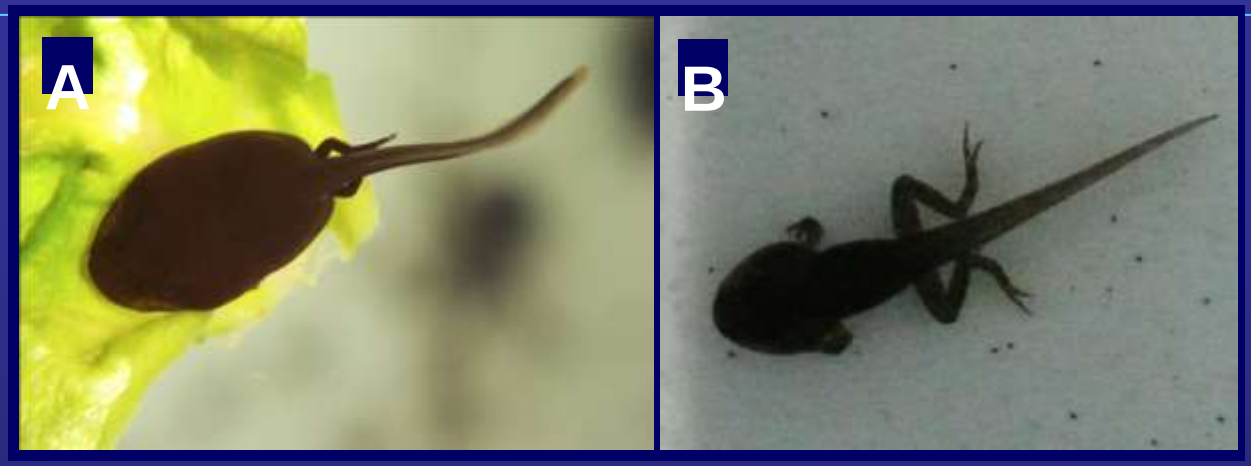
EXAMPLES OF STUDIES IN WHOLE ORGANISMS (ANIMALS AND PLANTS)

System	Agent	"Conventional" effect	"Homeopathic" effect	Ref.
Rat, Guinea pig	Histamine Lung Histamine Apis mell.	Pro-inflammatory agent	Histamine (30x), Lung histamine (18c) and Apis mellifica (7c/10c) reduce inflammation symptoms	Bastide 1975,Poitevin 1988, Bildet 1990 Conforti 1993
Rat, Mouse	Arsenic	Whole body and liver toxicity	Ars. high dilutions (7c-30c) protect from intoxication	Lapp 1955; Wurmser 1955; Cazin1987-1991; Banerjee,P, Khuda-Bukhsh 1998-2000
Rat	Nux vomica	Neurohinibition (strychnine)	Reduces alchool-indced sleeping time	Sukul et al., 1999
Rat	Aspirin	Antithrombotic	Aspirin 10^{-30} g/kg (15c) has pro-thrombotic effects	Beulogne-Malfatti,Doutreme-puich , Eizayag et al. 1998-2012
Rat	Phosphorus	Hepatotoxicity	Phosphorus high dilutions (30x) protects from toxic hepatitis	Bildet 1984, Guillemain 1987 Palmerini 1993
Tadpoles	Thyroxine	Increases the rate of metamorposis	Thyroxine high dilutions (up to 30x) inhibit metamorphosis	Endler 1990-2014, Lingg 2008, Weber 2008, Guedes 2011, Harrer 2013
Rat, Mouse	Gelsemium s.	Toxic and convulsivant	Anxiolytic effect (2c-30c) of Gelsemium s.	Magnani 2010, Venard 2011, Bellavite 2012
Wheat	Arsenic	Cell toxicity	Ars. high dilutions (45x) stimulate vitality	Betti et al. 1997-2014

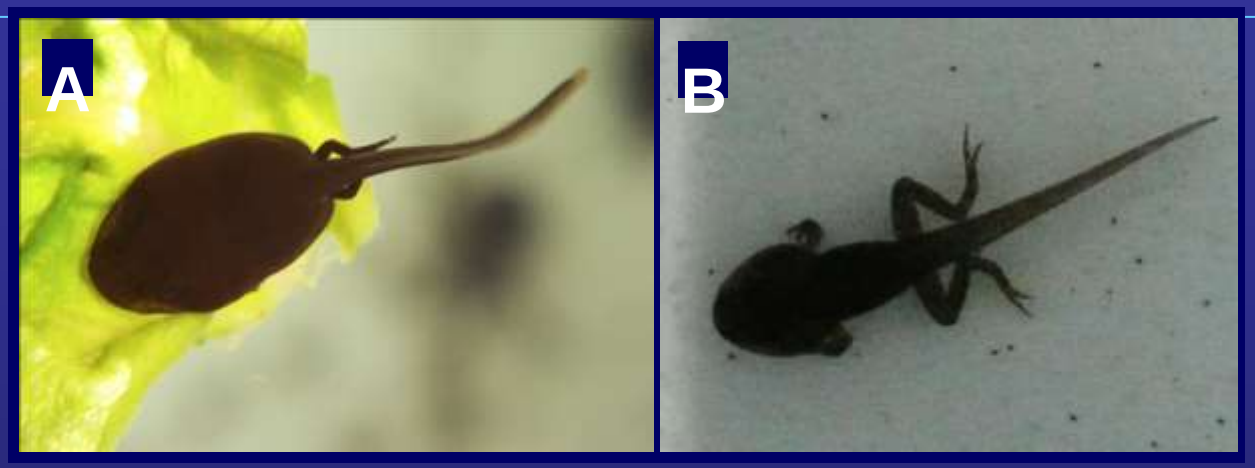
Christian Endler

Thyroxine/tadpoles studies (1990-2015!)



Christian Endler

Thyroxine/tadpoles studies (1990-2015!)



→ High dilution effect (30x)

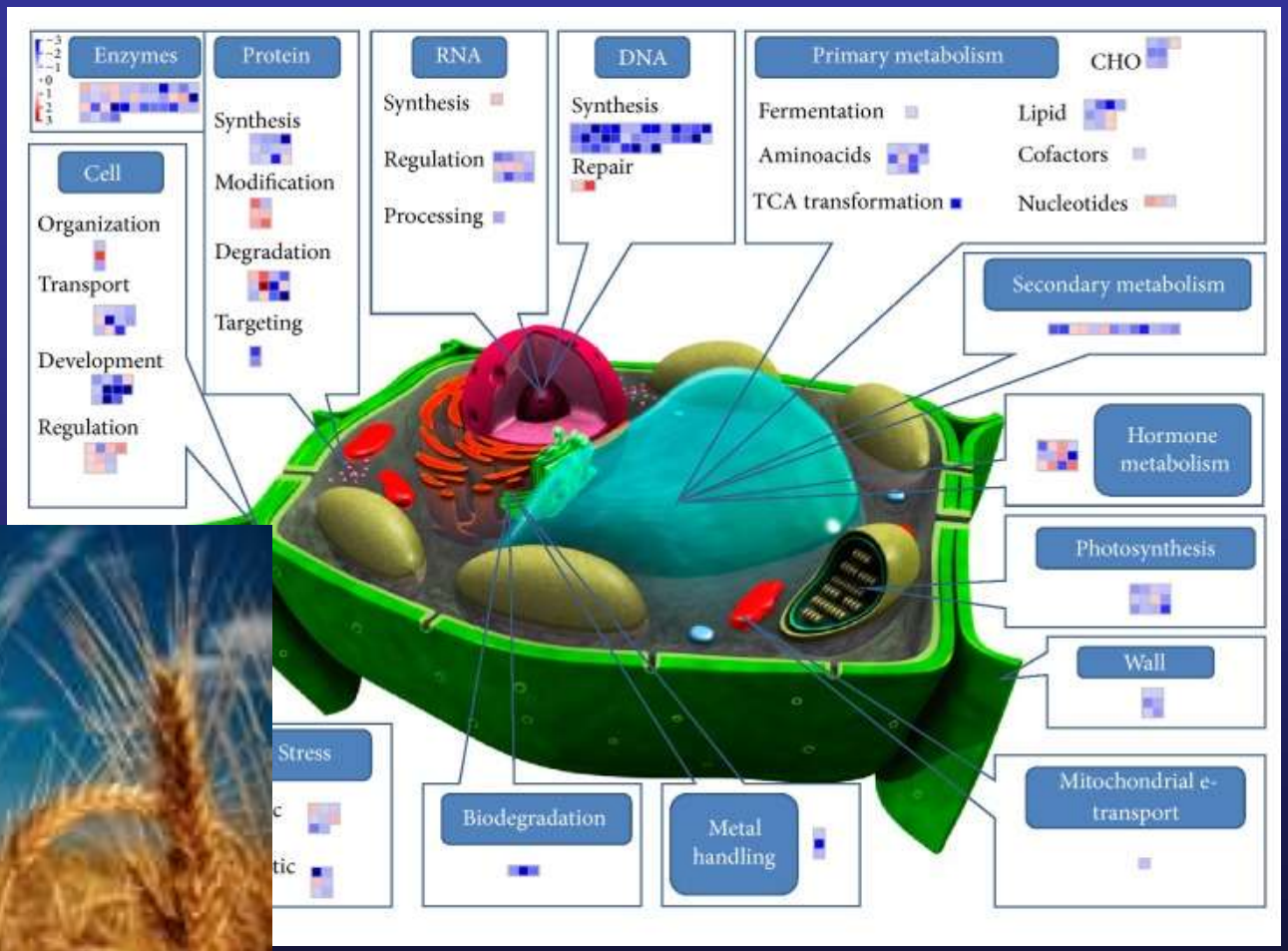
→ Inverse effects of Thyroxine ponderal/homeopathic

→ Information transfer in vials immersed in liquid

→ No inactivation by X-rays scans

Arsenic/plant studies (1994-2015!)

Lucietta Betti



«AGRO-HOMEOPATHY»?

Arsenic/plant studies (1994-2015!)

Lucietta Betti

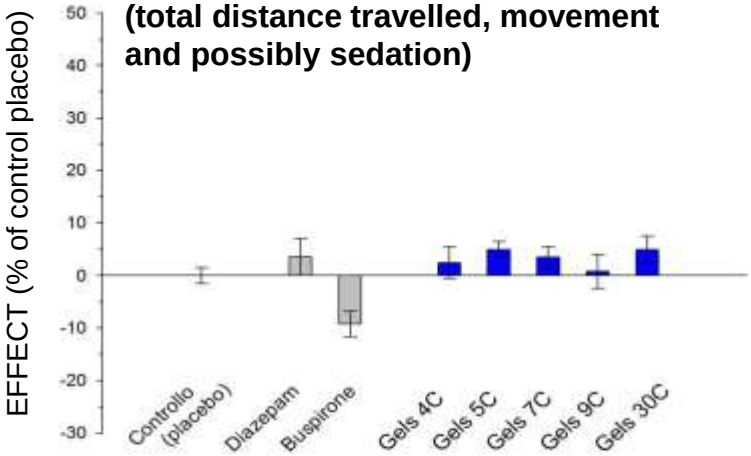
→ High dilution effect (45x)
→ Inverse effects of Arsenic ponderal/homeopathic
→ Action in several plant models
→ Multiple genetic effects

POOLED DATA ANALYSIS (14 complete experiments)
Evidence-Based Complementary and Altern. Med., 2012

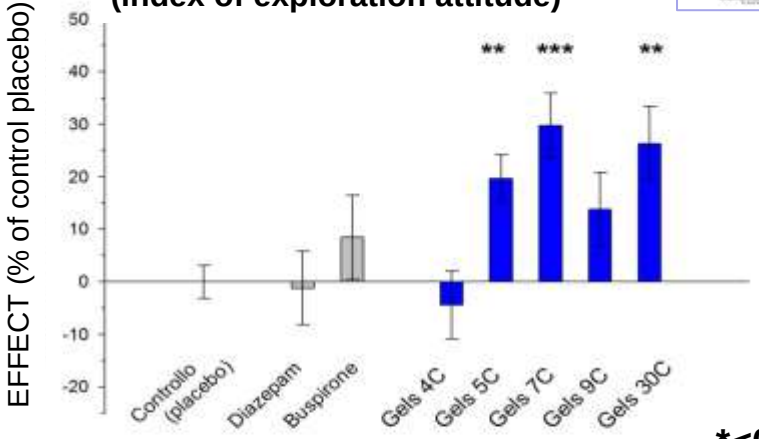
Open field

Light Dark

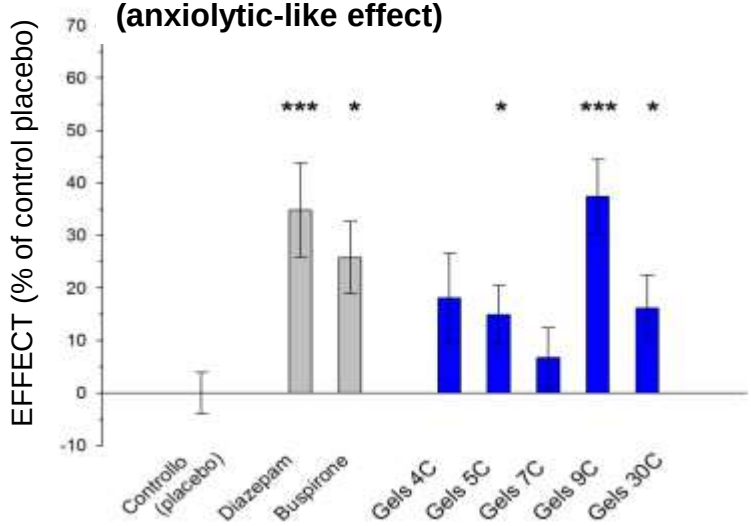
A. GENERAL MOVEMENT
(total distance travelled, movement and possibly sedation)



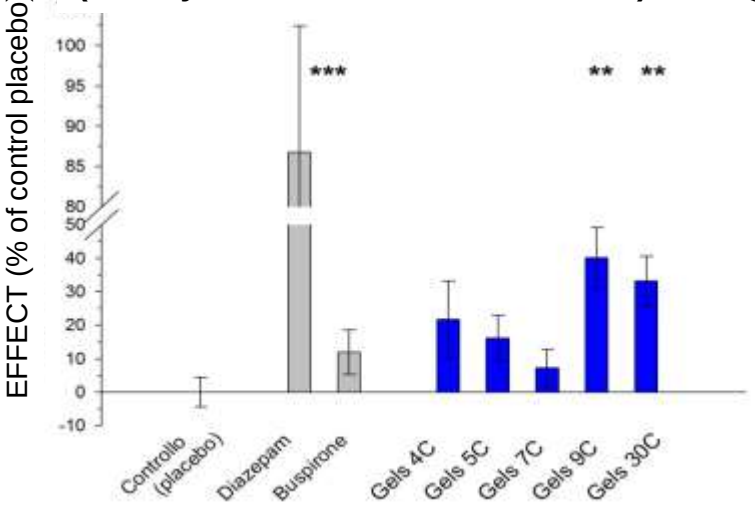
B. TIME SPENT IN CENTRAL AREA
(index of exploration attitude)



C. TIME SPENT IN LIT AREA
(anxiolytic-like effect)



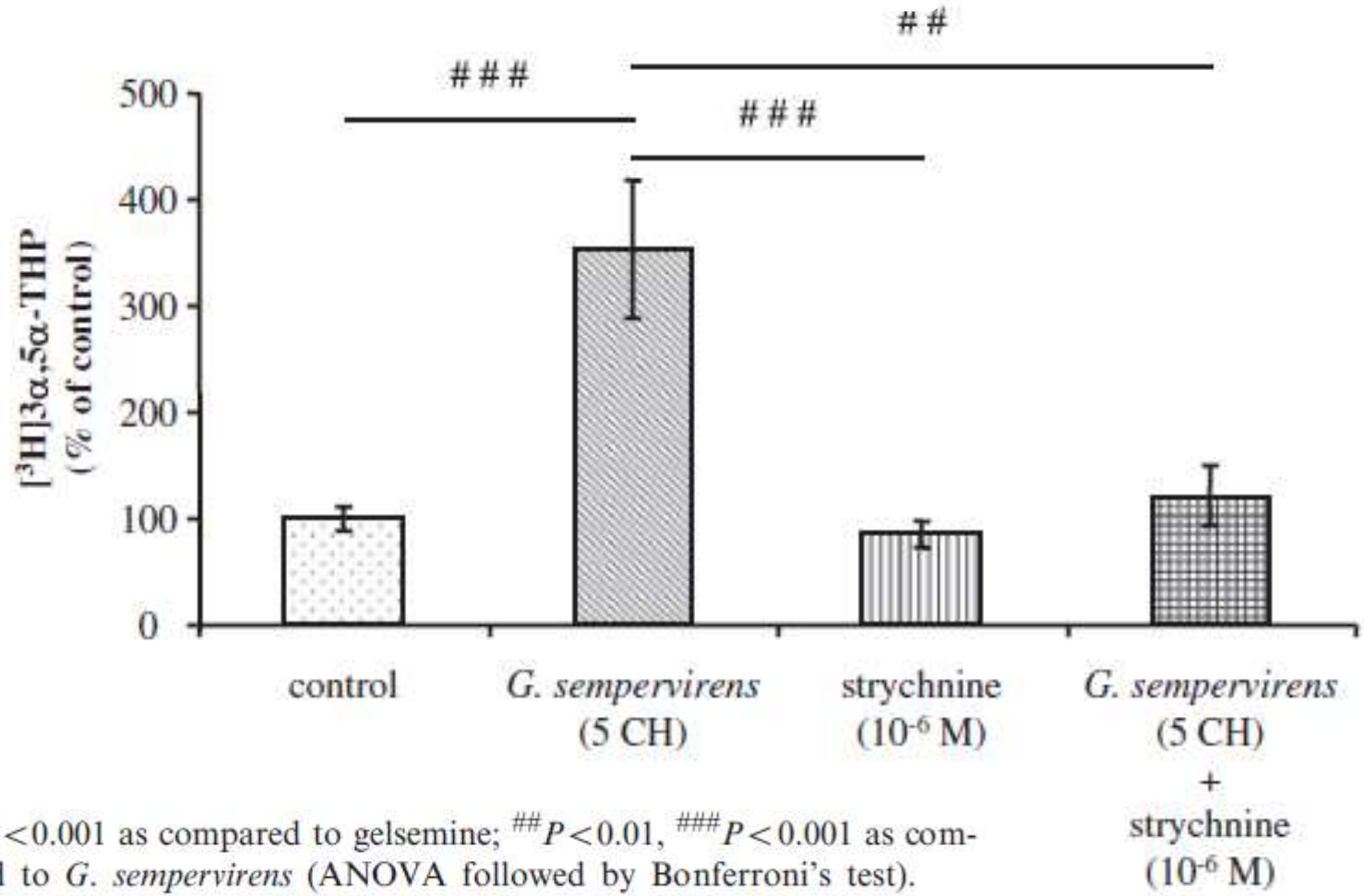
D. LIGHT-DARK transitions
(anxiolytic-like effect and movement)



***<0.05**
****<0.01**
*****<0.001**

Gelsemium sempervirens Activity on Neurosteroid Allopregnanolone Formation in the Spinal Cord and Limbic System of Rats

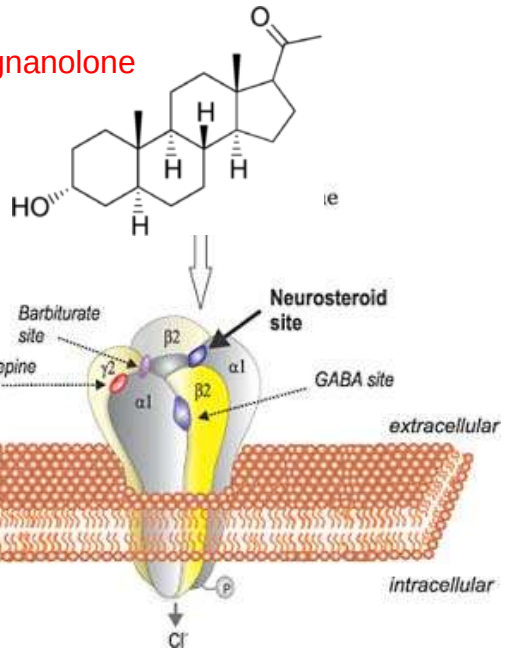
Adapted from: Christine Venard et al., ECAM-2011

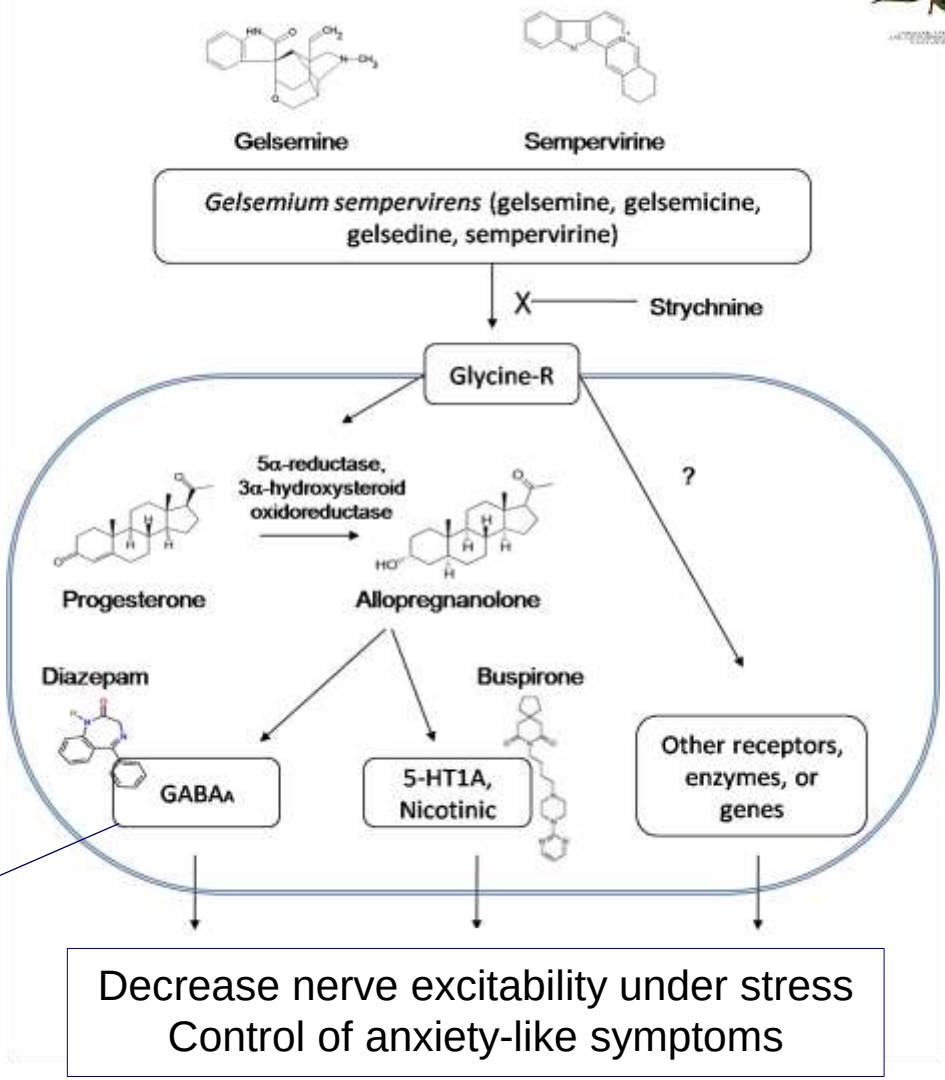


***$P < 0.001$ as compared to gelsemine; ##$P < 0.01$, ###$P < 0.001$ as compared to *G. sempervirens* (ANOVA followed by Bonferroni's test).

Working model of the mechanism of action of *Gelsemium sempervirens: ALLOPREGNANOLONE*

Allopregnanolone





Decrease nerve excitability under stress
Control of anxiety-like symptoms

Role of Prokinecitin receptor in neuronal function

Gelsemium sempervirens L.

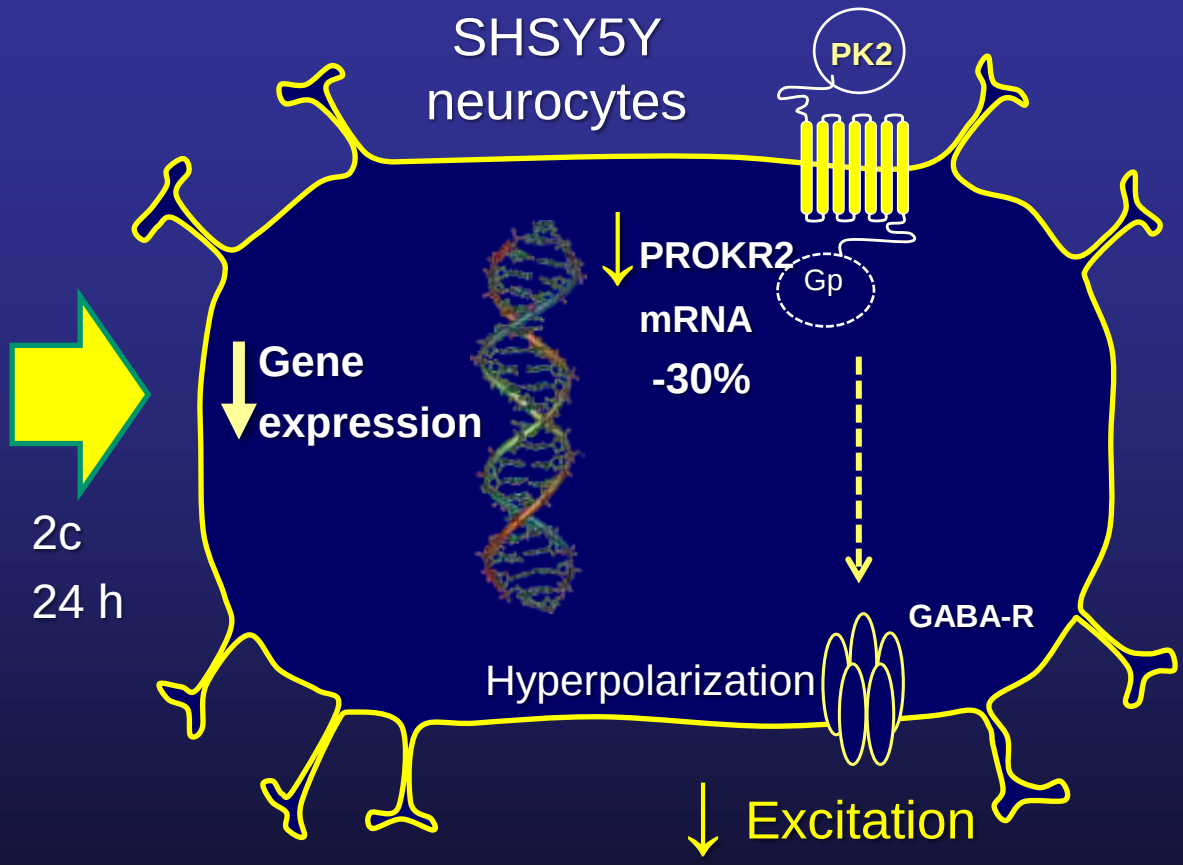


Figure from Olioso et al., 2014

Gelsemium s. actions in experimental models

- Reproducible and significant effects in behavioral models in mice, concerning a subset of homeopathic repertory "symptoms" : **aversion to open space, amelioration with movement, feeling in a danger, aversion to light.**

- **No adverse effects on general locomotion** (an effect shown by buspirone in chronic treatment)

- **Differences from conventional anxiolytics**:
  - Different responses in different models (Open Field versus Light dark)
  - Effect with high dilutions

- **NON-LINEARITY** (various activity peaks) with increasing potencies, BUT in general different potencies have **the same trend** of effects (important for practical purposes). 7c-9c-30c higher effects than 4c and 5c

- Hypotheses of action mechanism: **stimulation of glycine receptors and thus neurosteroid synthesis** with consequent increase of GABA inhibitory effects (Venard et al 2011). More recently we also showed an effect on **prokinecitine receptors** (Olioso et al. 2014)

UP-TO DATE CONCLUSIONS FROM ANIMAL AND PLANT MODELS

- **Confirmation of the "similia principle": homeopathic dilutions counteract toxicity of ponderal doses (e.g. *Arsenic*, *Phosphorus*)**
- **Confirmation in animals of some symptoms reported by Materia Medica (e.g. *Gelsemium, Apis, Histaminum*)**
- **Consistent evidence that high dilutions (even beyond Avogadro) have reproducible effects different from control solutions: end of "placebo story"**
- **Preliminary evidence of action on gene expression *(Canova, Arsenic)***
- **Hope of possible applications in agro-homeopathy**

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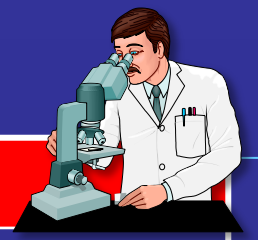
EXAMPLES OF HIGH DILUTION EFFECTS "*IN VITRO*"



System	Agent	Dilution	Effect	Ref.
Human basophils	Apis, Histamine	12CH-16CH $10^{-24} \rightarrow 10^{-32}$	Inhibition of activation markers	Poitevin 1988, Belon 1999-2009 (and Verona Group)
Human basophils	Adrenaline	12CH-16CH $10^{-24} \rightarrow 10^{-32}$	Inhibition of activation markers	Mannaioni et al. 2010
Cicken embrio	Bursin	15 CH (10^{-27}g)	Immunomodulatory and endocrine activity	Bastide, Youbicier-Simo 1993-97
Human neutrophils	Phosphorus	12 D to 30 D	Inhibition of superoxide production	Chirumbolo and Bellavite 1993
Wheat germination	Arsenic Silver nitrate	26 D (10^{-45})	Protect from toxicity Enhances growth	Betti 1997/2015 Pongratz 1998
Rat neurons	Glutamate	$10^{-18} \rightarrow 10^{-30}$	Protection from glutamate toxicity	Jonas et al., 2001
Bacteria	Arsenicum	30CH	Protects from toxicity	Das et al 2011, De et al 2012
Human Neurocytes	Gelsemium s.	2-30 CH	Prevalent gene down-regulation	Marzotto 2014, Olioso 2014
Colon cancer cells	Ruta grav.	MT-30CH	Decrease viability, apo-ptotic gene expression	Arora and Tandon 2015

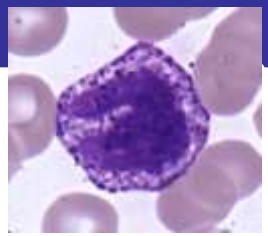
POSSIBLE MODELS EXPLAINING INVERSE EFFECTS (= SIMILIA PRINCIPLE) AT A CELLULAR LEVEL

References in www.paolobellavite.it
And recent papers in Journal «Homeopathy»

- Detoxification enzymes (gene expression and enzyme activation)
- Heat shock proteins (stress proteins, chaperonins)
- Various receptors (different affinity and different coupling with signal transduction pathways)
- Gating theory (signal transduction)
- "Hormesis" (includes all the previous mechanisms): YES for low dilutions of toxic substances (ponderal doses); doubts for high dilutions.

Philippe Belon

EFFECT OF HISTAMINE HIGH DILUTIONS ON BASOPHIL "DEGRANULATION"



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Inflammation Research

Inhibition of human basophil degranulation by successive histamine dilutions: Results of a European multi-centre trial

P. Belon[1], J. Cumps[2], M. Ennis[3], P.F. Mannaioni[4], J. Sainte-Laudy[5], M. Roberfroid[6] and F.A.C. Wiegant[7]

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[3] Department of Clinical Biochemistry, Institute of Clinical Science, The Queen's University of Belfast, Grosvenor Road, Belfast BT12 6BJ, UK, Fax +44 12 32 23 61 43, e-mail: m.ennis@qub.ac.uk
[4] Department of Pharmacology, Viale G. Pieraccini 6, I-50139 Florence, Italy
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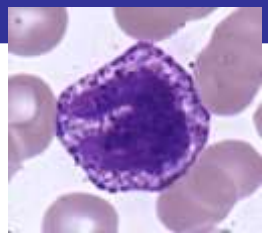
[6] Laboratoire de biotoxicologie, UCL 7369, 73 avenue Emmanuel Mounier, B-1220 Brussels, Belgium
[7] University of Utrecht, Department of Molecular Cell Biology, P.O. Box 80.056, NL-3508 TB Utrecht, The Netherlands

Laboratory	Control (% degranulation)	Histamine (% degranulation)	Number	p
1	45.8	36.5	123	0.0002
2	50.2	47.5	312	0.065
3	51.6	47.4	183	0.024
4	47.8	35.7	154	≤ 0.0001
All	48.8	41.8	772	≤ 0.0001

Statistical comparisons were made using MANOVA.

Table 1. Comparison of percentage degranulation induced by anti-IgE (0.04 µg/ml) in the absence and presence of histamine dilutions (15th–19th centesimal dilutions).

REPLICATION EXPERIMENTS OF HIGH-DILUTION EFFECTS



Inflamm. Res.
DOI 10.1007/s00011-009-0044-4

Inflammation Research

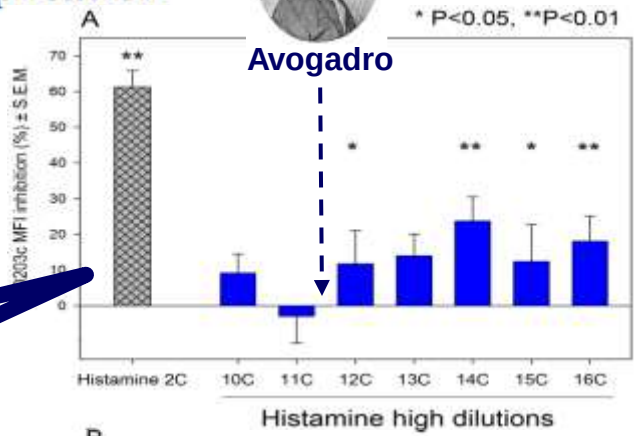
ORIGINAL RESEARCH PAPER

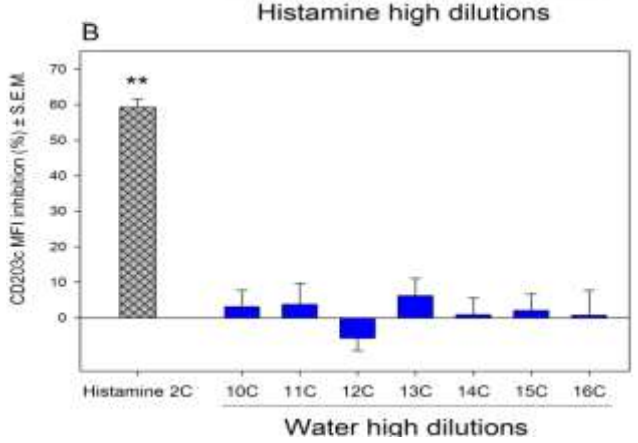
Inhibition of CD203c membrane up-regulation in human basophils by high dilutions of histamine: a controlled replication study

Salvatore Chirumbolo · Maurizio Brizzi · Riccardo Ortolani · Antonio Vella · Paolo Bellavite

Receive 009

Avogadro



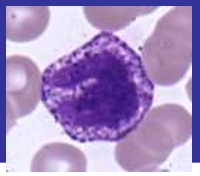


Note:

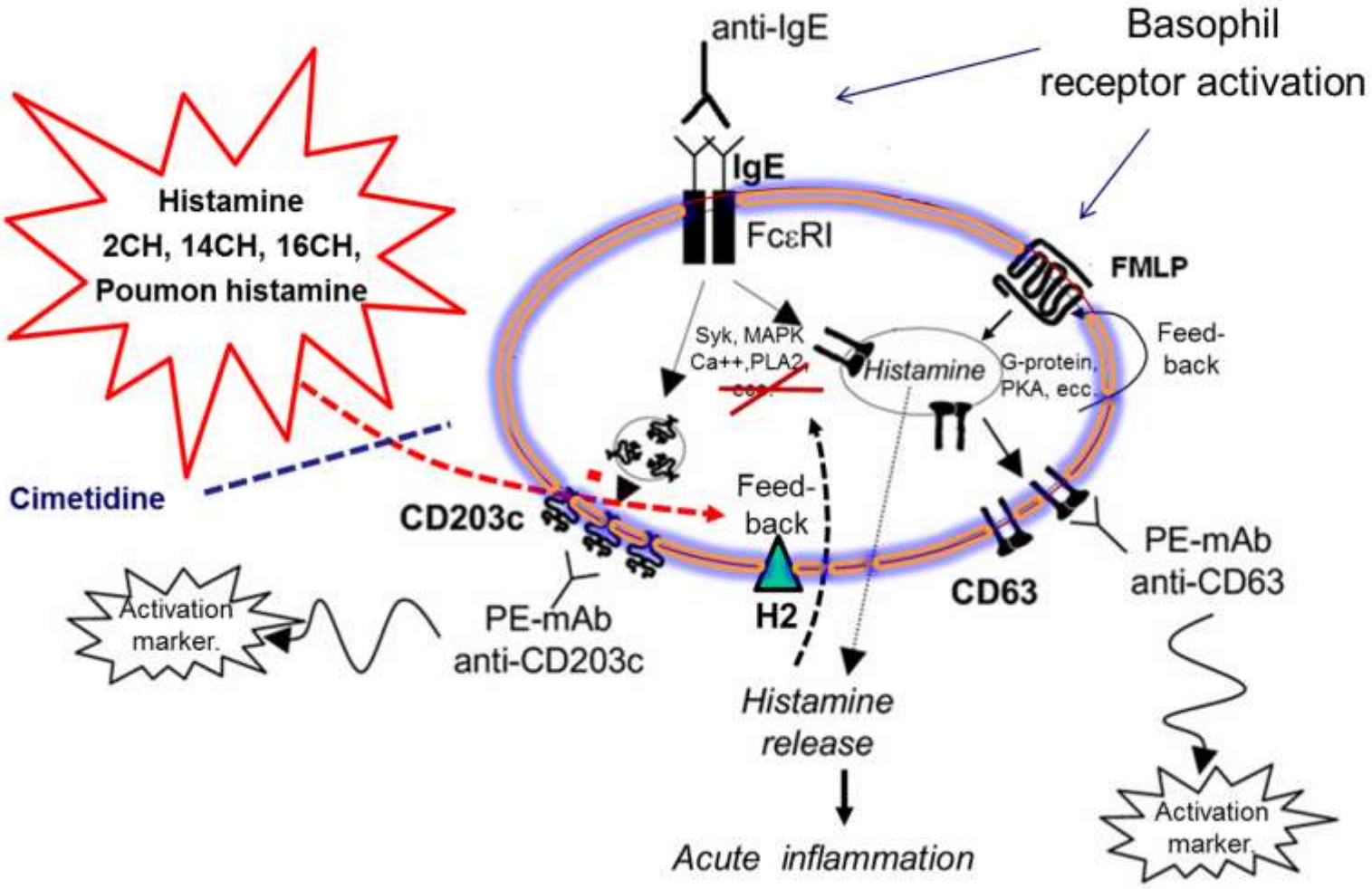
No inversion of effects

No hormesis

Striking non-linearity



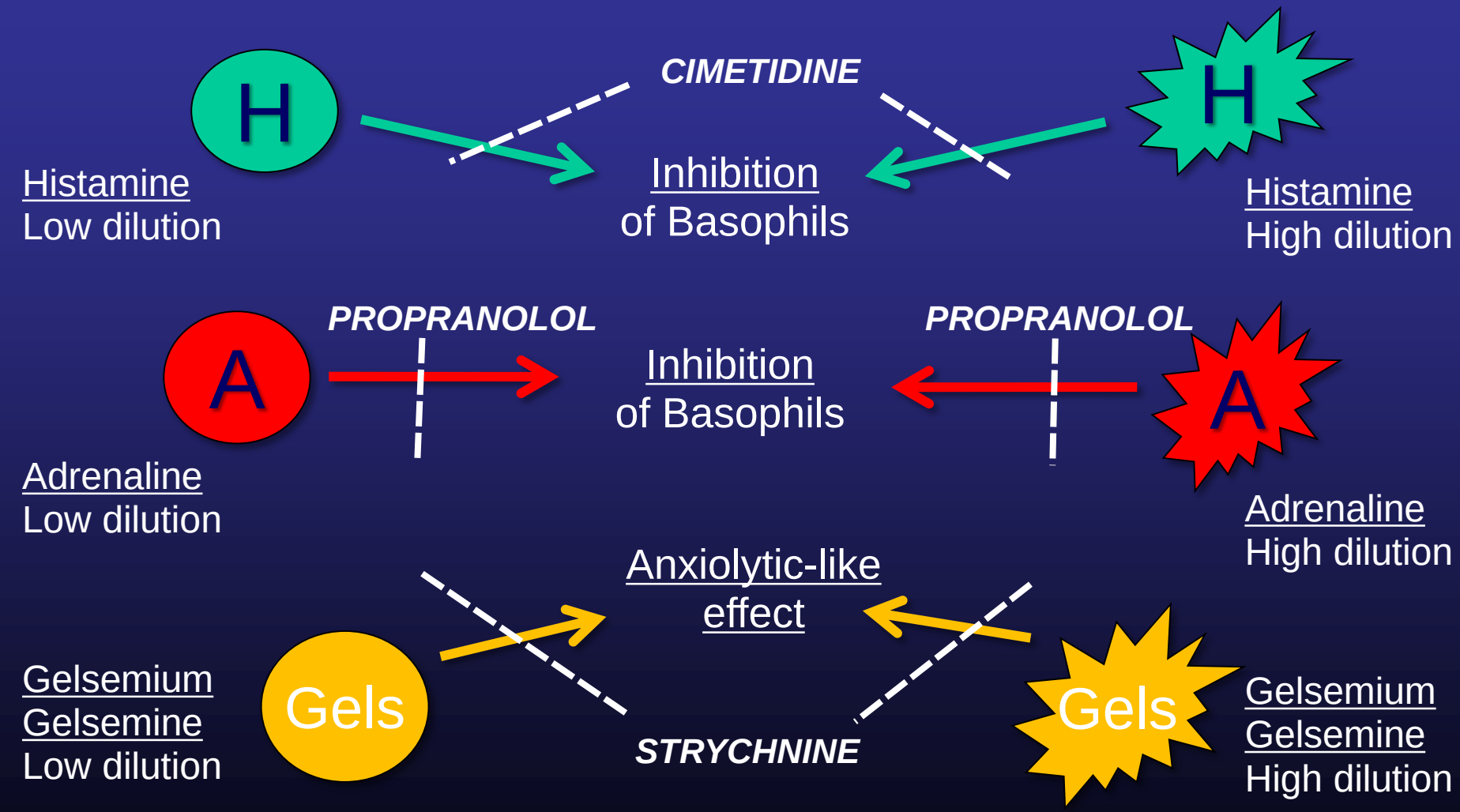
MODEL OF THE HISTAMINE ACTION ON BASOPHILS AND MAST CELLS



Action at receptor level

LOW DILUTIONS (e.g. 2CH-5CH)

HIGH DILUTIONS (e.g. 9CH-30CH)

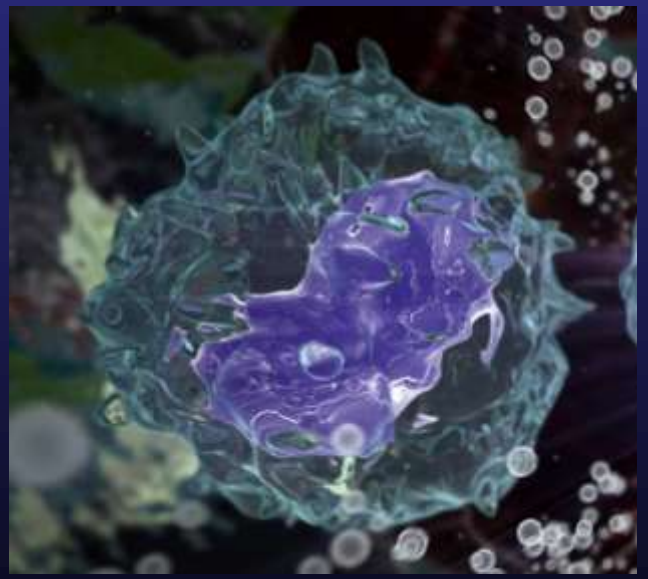


Recent evidence of Homeopathy and genome analysis on cellular models

	Potencies	Cell type	Effect	REF
Carcinosinum	MT, 30C, 200C	DLA cells	**↑ specific gene expression (p53 pro-apoptotic)**	**(Sunila et al. 2009)**
Arsenicum alb.	30C	Saccharomyces cerevisiae, E. coli	**↑ Resistance to arsenicum toxicity ↓↑ expression of specific genes (apoptotic, stress response proteins)**	**(Das et al. 2011; De et al. 2012 of Khuda-B.group)**
Carcinosinum, Hydrastis, Ruta or Thuja	200C	DLA cells	**↑ Apoptosis , ↓↑ Gene expression (whole genome analysis)**	**(Preethi et al. 2012)**
Gelsemium s.	2C, 3C, 5C, 9C, 30C	Human neurocytes SHSY5Y	**7 genes ↑ 49 genes ↓ expression (whole genome analysis) ↓ gene expression (RT-Array, 2C)**	**(Marzotto et al. 2014; Olioso et al. 2014)**
Apis mellifica	3C, 5C, 7C	Human prostate RWPE-1	**↑↓ expression of different groups of genes (whole genome analysis)**	**(Bigagli et al. 2014)**
Rhus tox.	30X	Primary cultured mouse chondrocytes	**↑ specific gene expression (COX-2), ↓ specific gene expression (collagen II; de-differentiation role)**	**(Huh et al. 2013)**
Arsenicum alb.	45X	Arsenic-intoxicated wheat seeds	**↑ Germination ↓ Gene expression levels**	**(Marotti et al. 2014)**
Condurango	30C	H460-non-small-cell lung cancer cells	**↓↑ expression of specific genes (apoptotic), ↑ Apoptosis, oxidative stress, mitochondrial depolarization**	**(Sikdar et al. 2014)**

Evaluation of Arnica montana dilutions on the expression of inflammatory genes by a human macrophage cell line

Olioso D., Marzotto M., Bonafini C., Brizzi M. and Bellavite P.



Arnica montana and Helenalin actions

***Arnica montana* L.**

- Pain Management
- Inflammation and trauma
- Postoperative setting

Helenalin

NF-κB/IkappaB complex

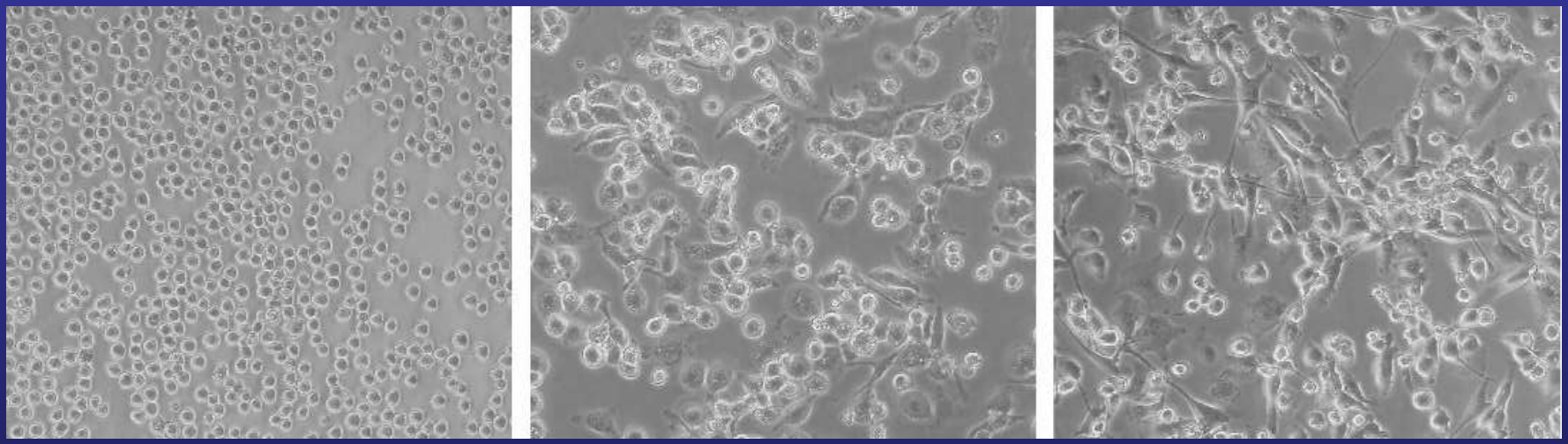
- T & B cells
- Macrophages
- Epithelial cells

↓ **NF-κB** ↓

- IL-1-b ↓
- TNF-α ↓
- 5-lipoxygenase, Leukotriene C4 synthase ↓
- NO ↓
- iNOS
- COX-2

Iannitti et al. 2014

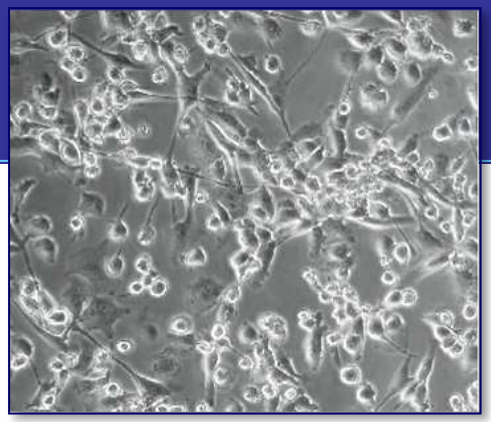
THP-1 human macrophages cell line



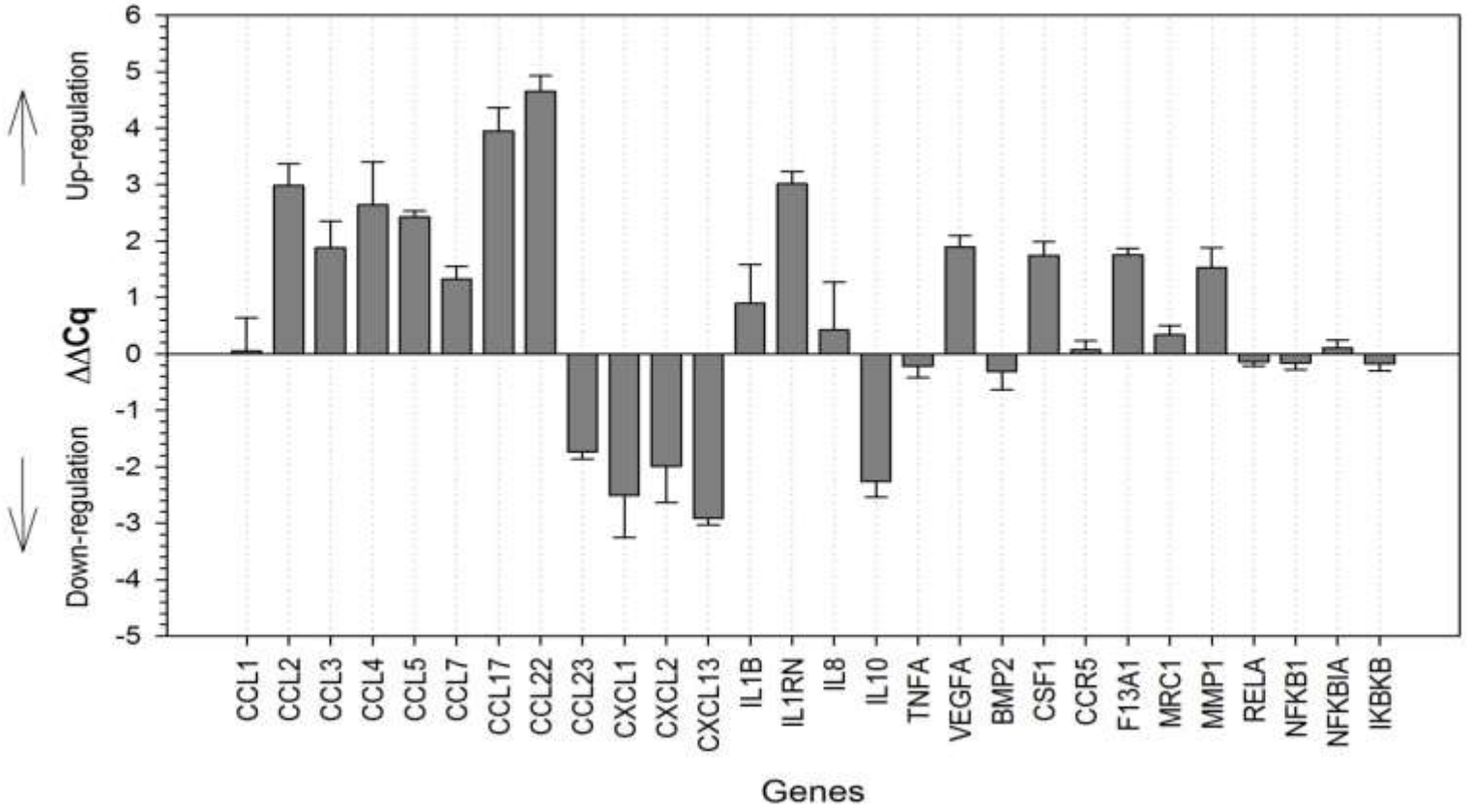
Normal THP-1 culture

PMA-activated macrophages

PMA-activated macrophages +IL-4

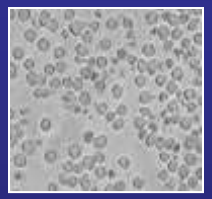


IL-4 polarization macrophages (M2, “wound-healing” phenotype)



Macrophages conditioning with *Arnica m.*

Day1 → Day2 → Day3 → Day4 → 2h or 24h



Seeding of THP-1 cells

PMA addition

Normal Mac

IL-4 addition

IL-4 Mac

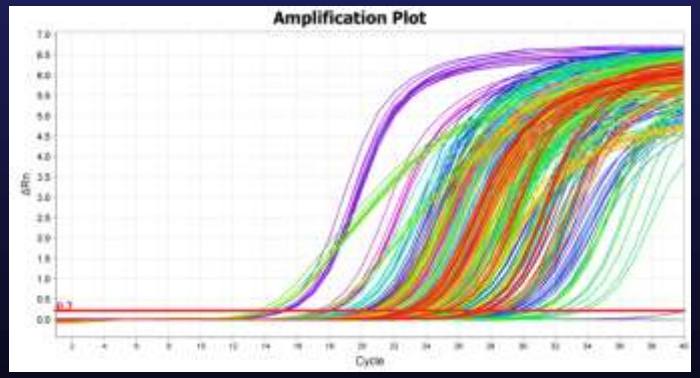
Arnica 2c, 3c, 5c, 9c, 15c or placebo

RNA extraction (24h)

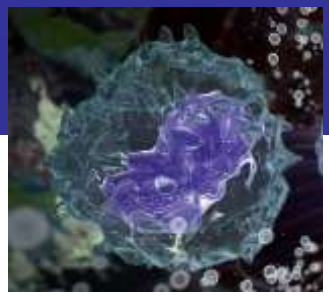
c-DNA synthesis

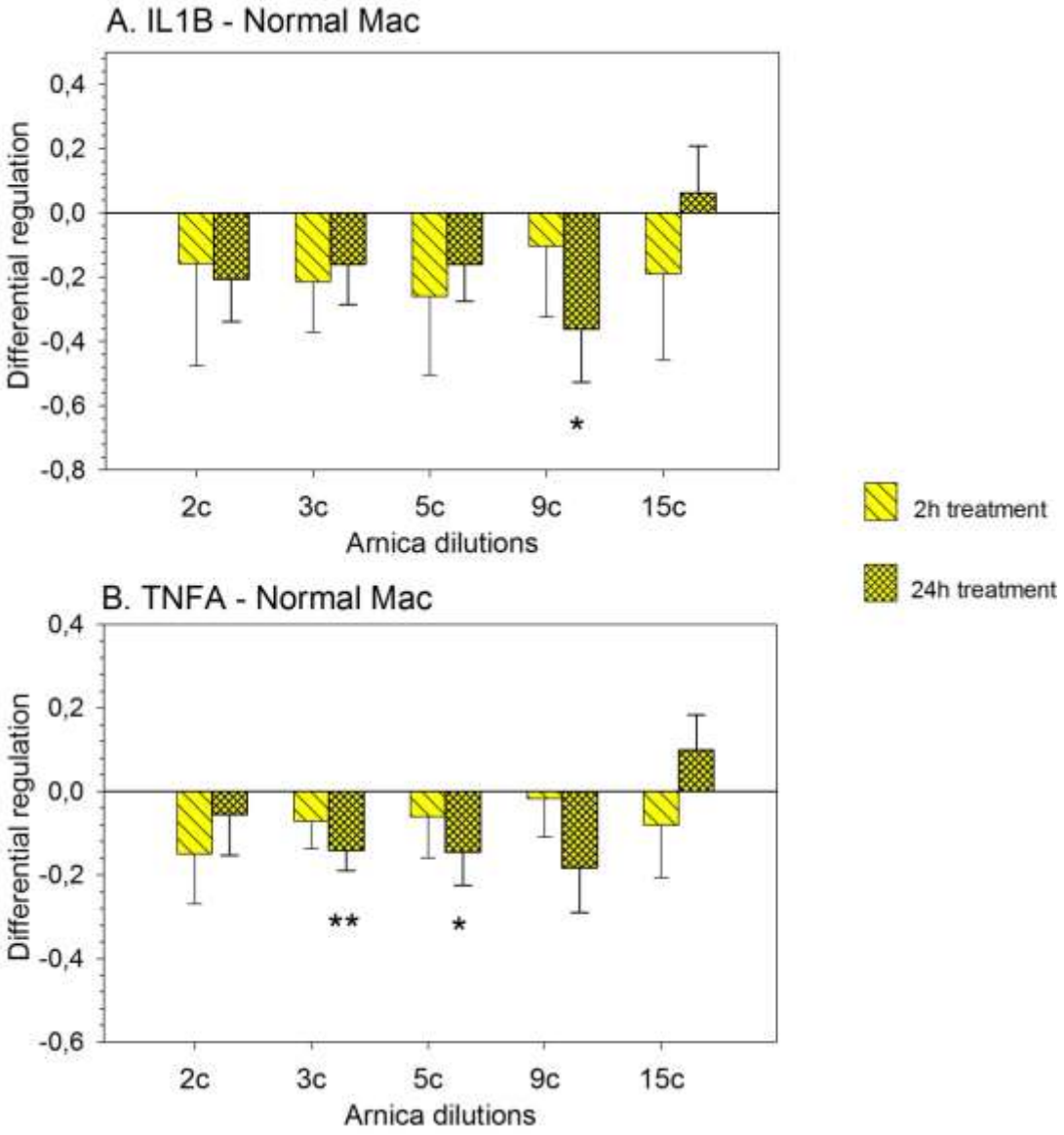
Real-time PCR

c-DNA amplification

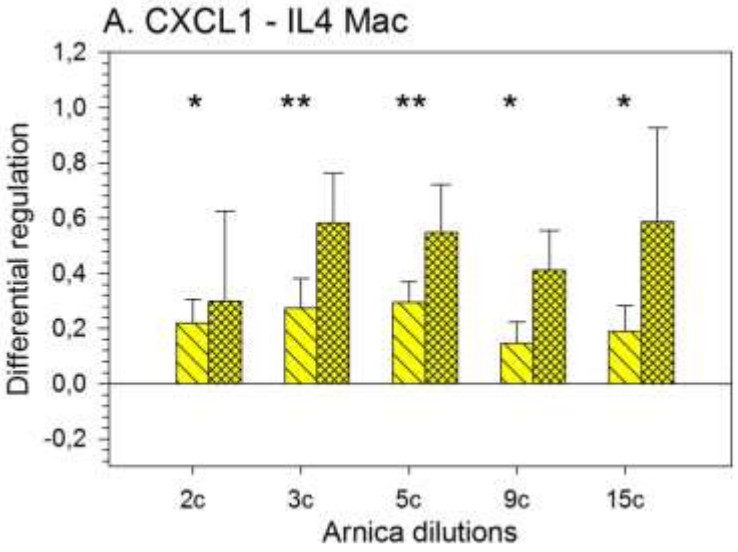


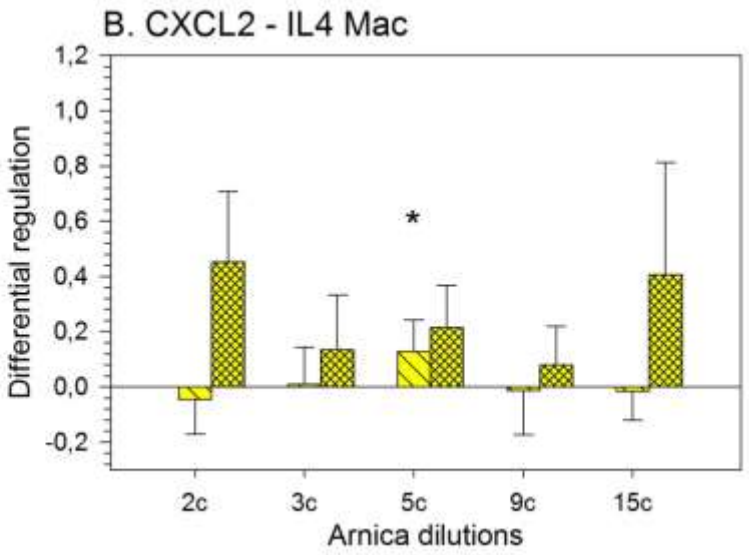
Arnica m. effects on Normal Macrophages

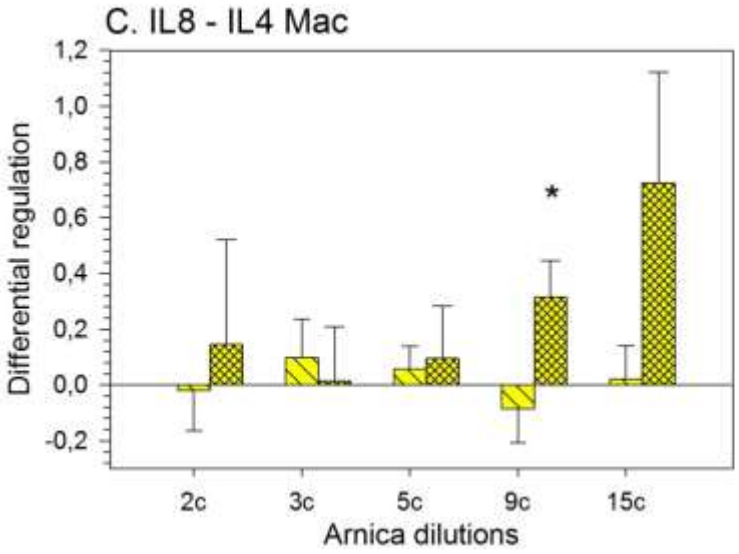


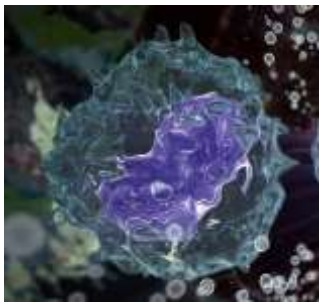


Arnica m. effects on IL-4 Macrophages

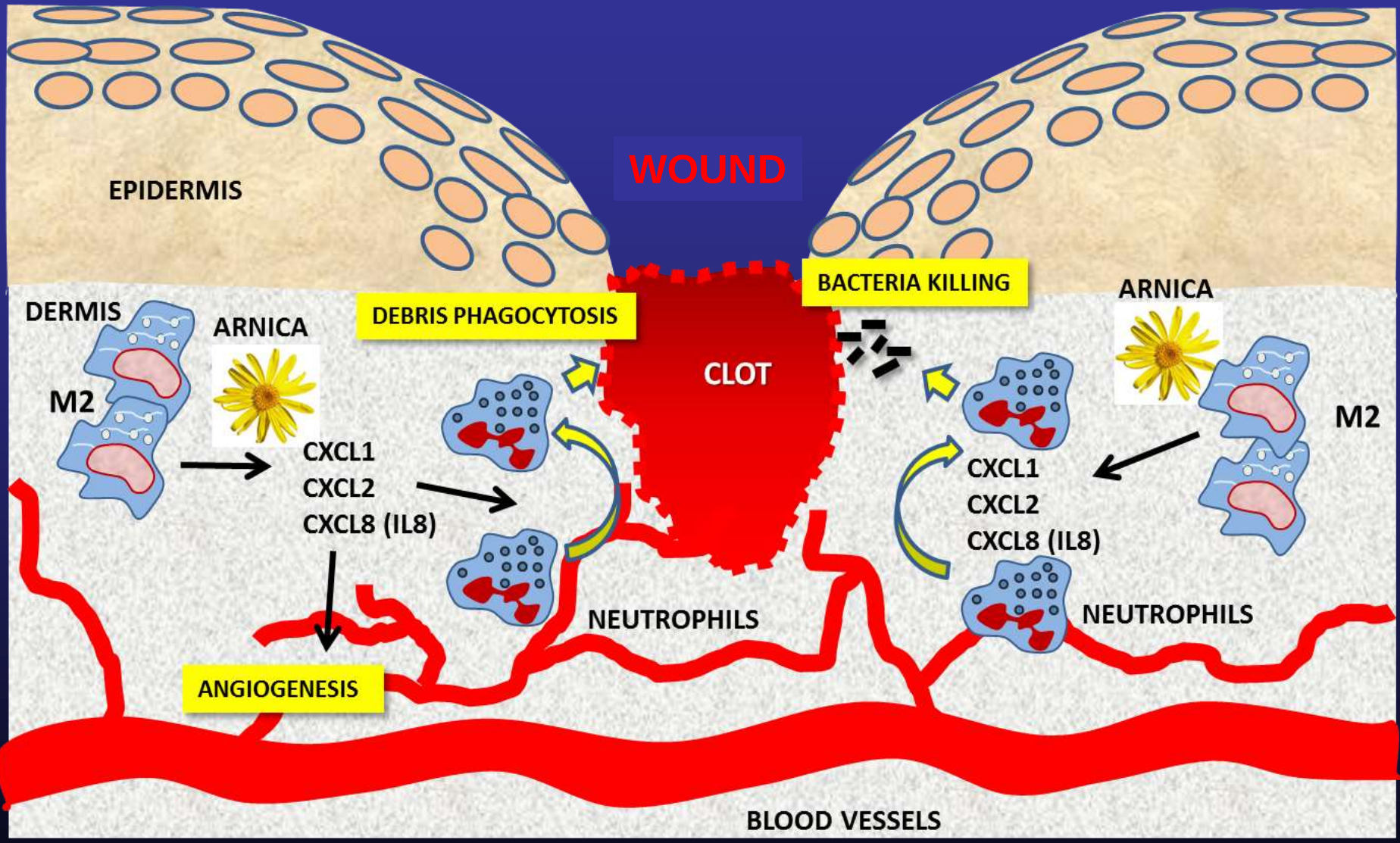








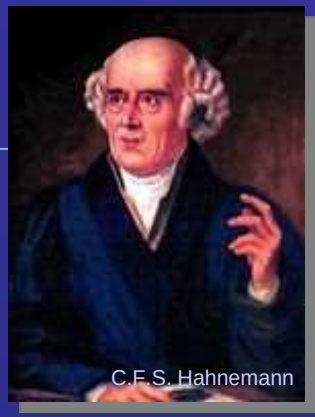
Hypothetical effects of *Arnica m.* in wound healing



How do the homeopathic medicines work?

1. Introduction
2. Animal and plant models
3. Laboratory studies
4. General insights

Targets and mechanisms of homeopathic drugs



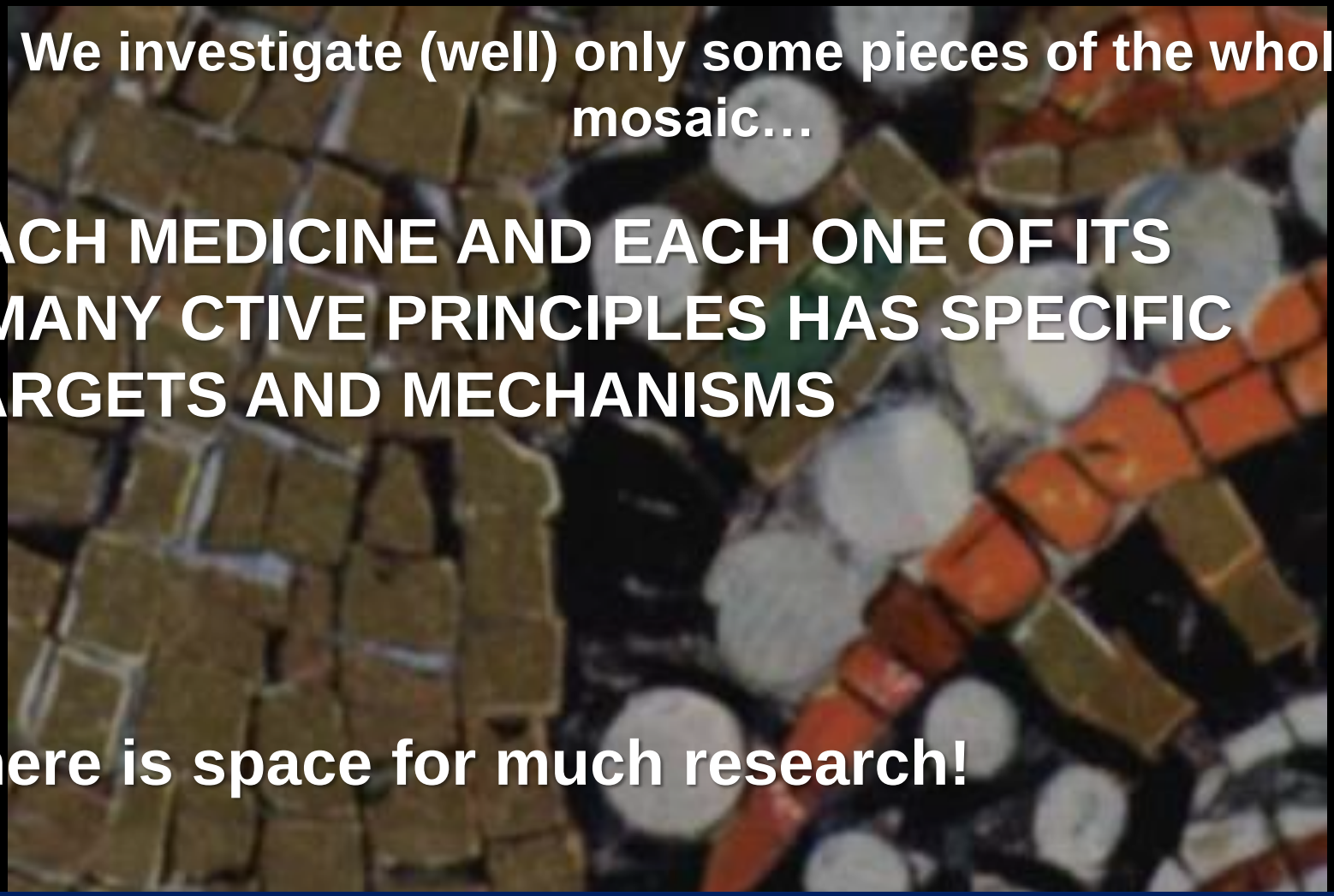
"LOCAL" TARGETS AND MECHANISMS

- Receptors (Histamine, Gelsemium, Adrenaline)
- Gene expression (Gelsemium, Canova, Apis, Arsenic)
- Cell functions (Neutropils, Basophils, lymphocytes, Macrophages)
- Nanoparticles, water clusters etc

"SYSTEMIC" TARGETS AND MECHANISMS

- Immune, Nervous, Endocrine systems
- Bioelectromagnetic interactions
- The role of water in signal transmission and amplification
- Complexity science and homeopathy (Self-organizing systems at the edge of chaos)

How do homeopathic medicines work?



We investigate (well) only some pieces of the whole mosaic…

EACH MEDICINE AND EACH ONE OF ITS AMANY CTIVE PRINCIPLES HAS SPECIFIC TARGETS AND MECHANISMS

…

There is space for much research!

Pieces of a 6° century mosaic (Ravenna, Italy)

Homeopathy and gene expression

DNA gene expression is sensitive to:

→Low energy information (Montagnier studies)

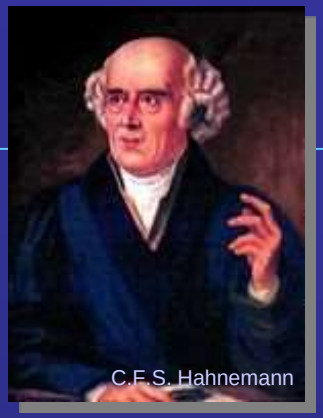
→High homeopathic dilutions

→Bioelectromagnetics

→Water clusters

The rapid development of new high-throughput technology platforms provides a methodological basis for deep understanding the action mechanisms and targets of homeopathic remedies.

In future: Help in prescription??



Future tasks

- Further development of laboratory models for
  a) *high dilutions/dynamizations* and
  b) *similia principle* (opposing effects in normal and stressed systems)
- Identification of variables and critical factors in reproducibility
- Evaluation of different preparations (liquid, granules, alcohol) and different dynamization procedures
- Integration of experimental models from molecules to humans for specific relevant remedies (“from bench to bedside”)

Teodora, Bizantine empress (6th Century)