Hypotheses and findings on the action mechanism(s) of homeopathic drugs

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We thank
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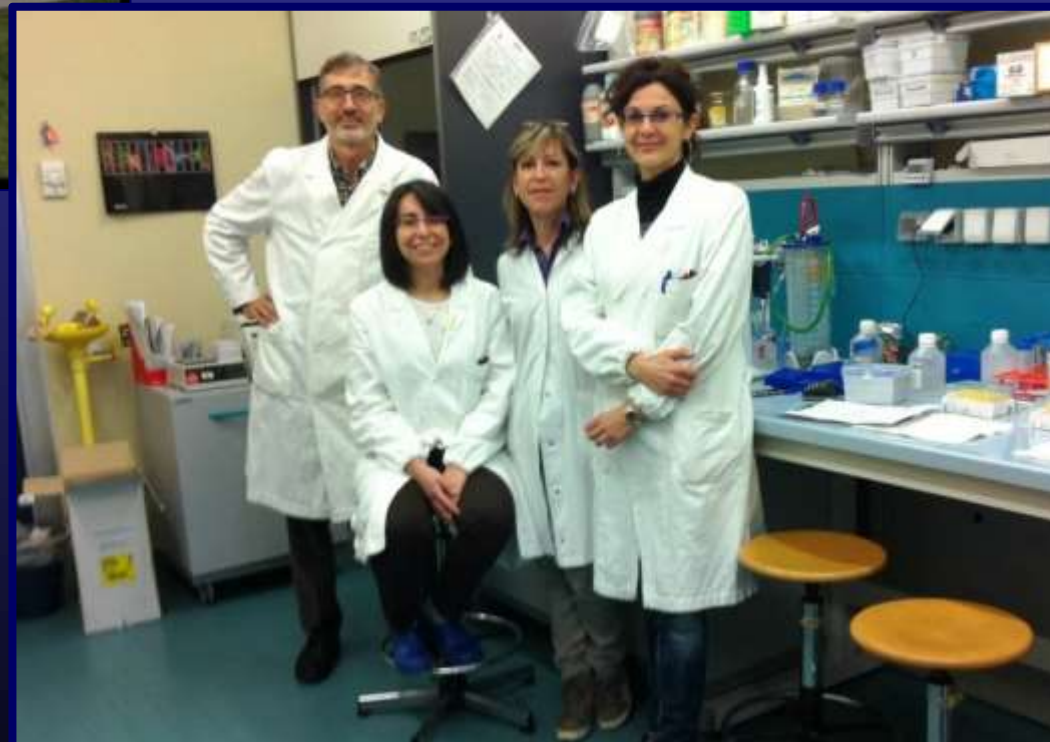
Italian Research Ministry



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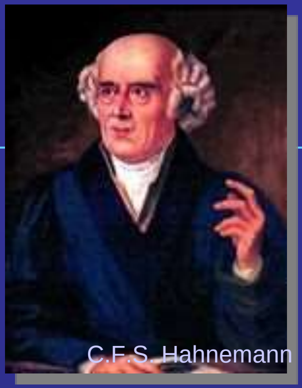
Debora Olioso
(2015)

Others: Andrea Signorini, Anita Conforti, Elisabetta Zanolin, Elisabetta Moratti

PROBLEMS OF UNDERSTANDING AND ACCEPTING HOMEOPATHY AS A SCIENTIFIC MEDICAL DISCIPLINE

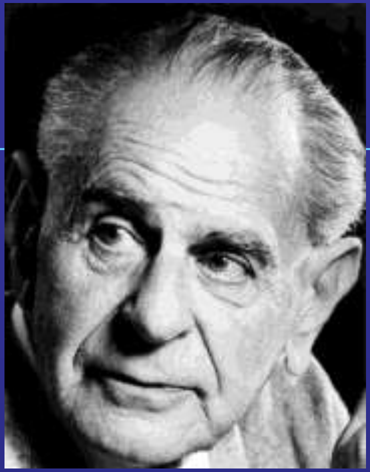


1. The structure of contemporary scientific thought inherited from positivism :
   a) everything is material,
   b) everything object can be reduced to its single parts,
   c) quantitative relation cause-effect.
2. Different OPINIONS on the evidence of clinical efficacy. Problem of suitable methods
3. Different OPINIONS on the plausibility of action mechanisms in terms of current pharmacological theories.

Point 1 belongs to philosophy (and history) of science

Points 2 and 3 are scientific matter!

IS HOMEOPATHY A SCIENCE?



- What is science?
- What is scientific?

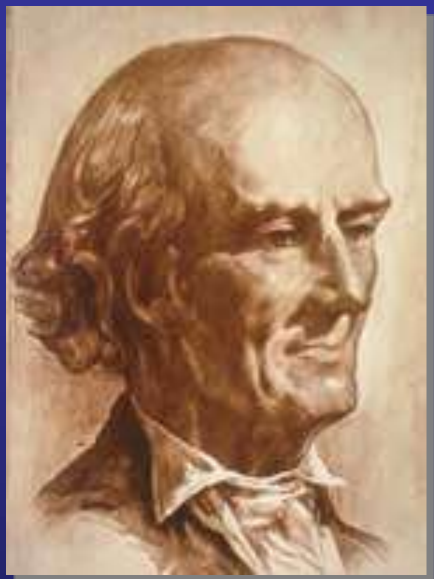
“We must not look upon science as a "body of knowledge", but rather as a system of hypotheses,

or as a system of guesses or anticipations that in principle cannot be justified,

but with which we work as long as they stand up to tests. . .”

- Karl R. Popper (1902-1994), *The Logic of Scientific Discovery*

HOMEOPATHY IS A <u>SCIENCE</u> JUST BECAUSE IT IS TESTABLE



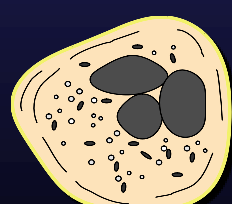
C.F.S. Hahnemann
(1755-1843)

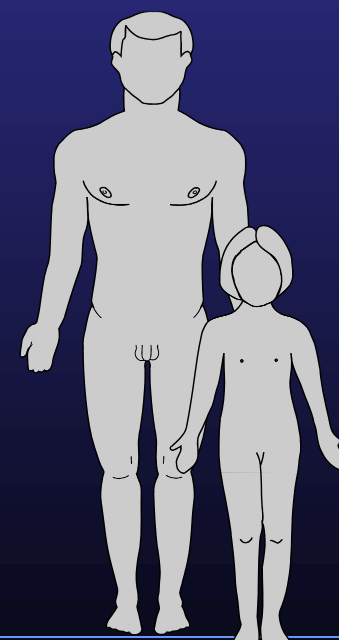
"The majority of substances have more than one action;
the first is a direct action, which gradually changes into the second,
which I call its indirect secondary action.
The second is generally the opposite of the first"

C.F.S. Hahnemann, 1796

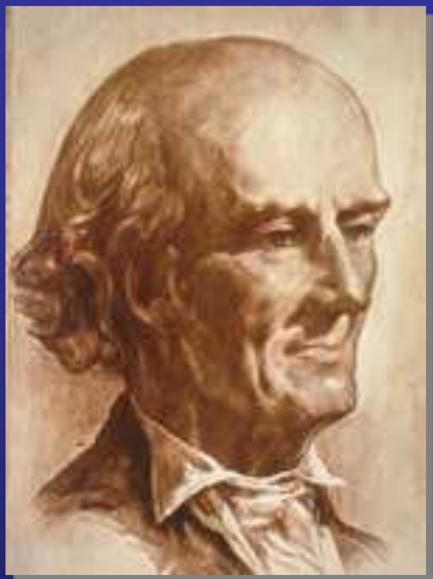
TRUE **FALSE**

SCIENTIFIC RESEARCH





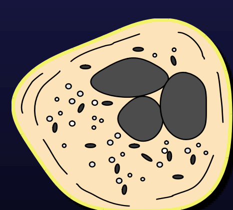
HOMEOPATHY IS A SCIENCE JUST BECAUSE IT IS TESTABLE



C.F.S. Hahnemann
(1755-1843)

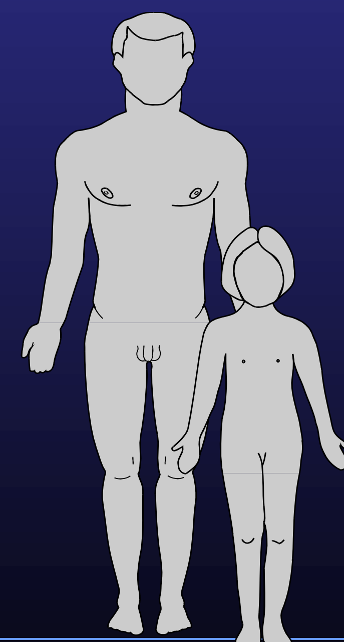
"A medicine whose selection has been accurately homoeopathic must be all the more salutary the more its dose is reduced to the degree of minuteness appropriate for a gentle remedial effect..."

C.F.S. Hahnemann, 1810
Organon, par. 277



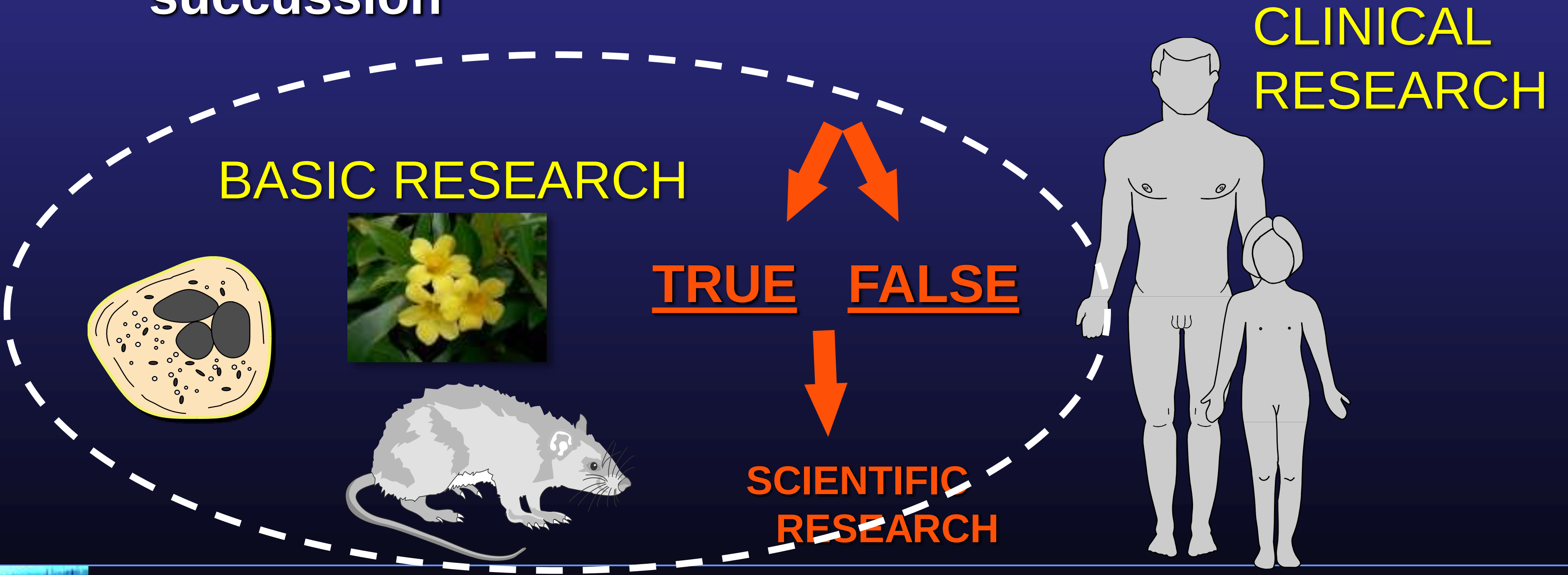
TRUE **FALSE**

SCIENTIFIC RESEARCH



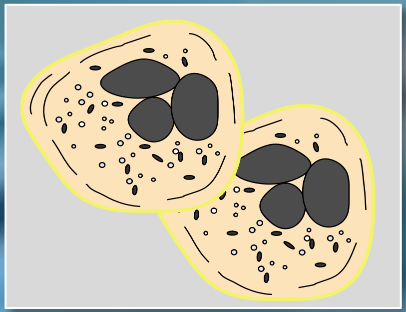
The two major SCIENTIFIC working hypotheses for Homeopathy

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

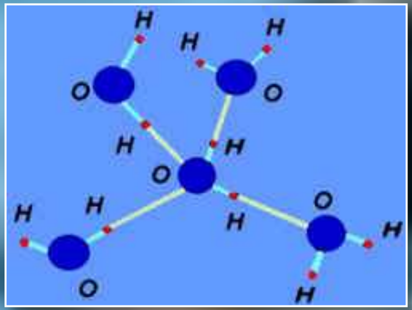


Basic research on Homeopathy

Animal and plant models



Cell models



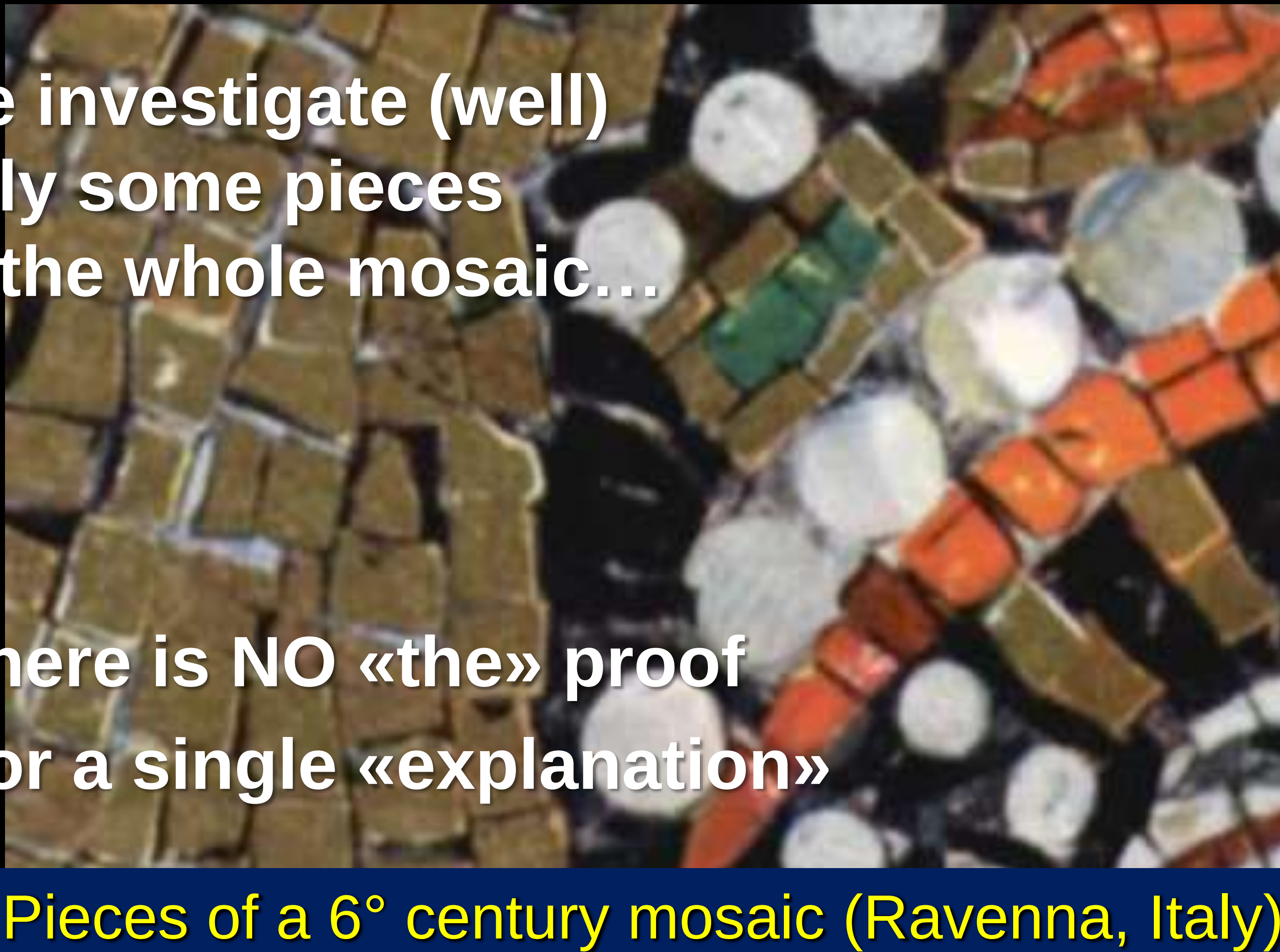
Physico-chemical models

Pre-clinical research (efficacy) in controlled trials

Drug targets and action mechanism(s)

Composition and nature of remedies

Basic research is always «reductionistic»



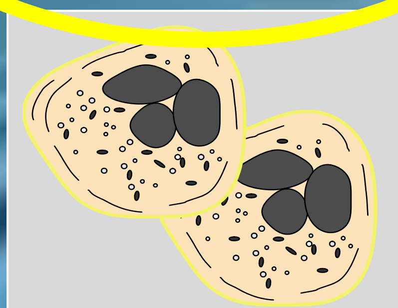
We investigate (well) only some pieces of the whole mosaic…

There is NO «the» proof nor a single «explanation»

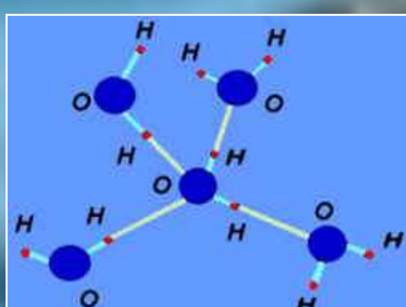
Pieces of a 6° century mosaic (Ravenna, Italy)

Basic research on Homeopathy

Animal and plant models



Cell models



Physico-chemical models

Pre-clinical research (efficacy) in controlled trials

Drug targets and action mechanism(s)

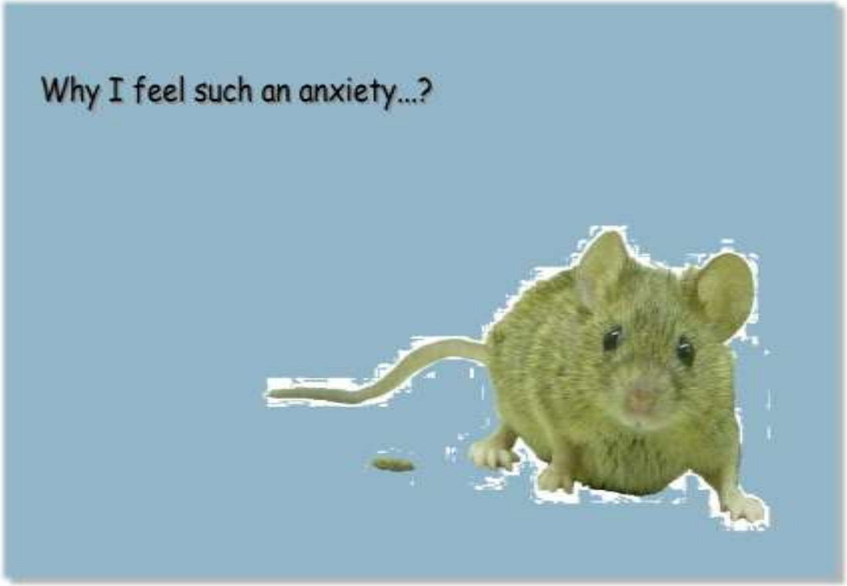
Composition and nature of remedies

THE “SIMILE” IN THE LIFE: EXAMPLES OF “OPPOSITE” or “INVERSE” EFFECTS 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

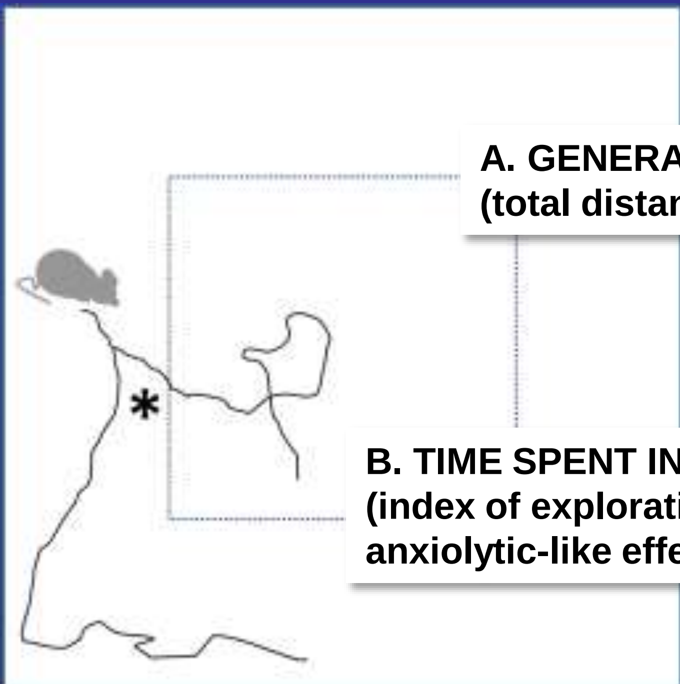
Animal models of psychopharmacology
Background

- Research in anxiety and psychopharmacology has a long history of development of animal models.
- The measurement of anxiety-related behaviour in animal models is based on the assumption that some emotional responses in animals are comparable to those in humans.
- Anxiolytic drugs are effective also in animals (tested mostly in rodents)



Models used

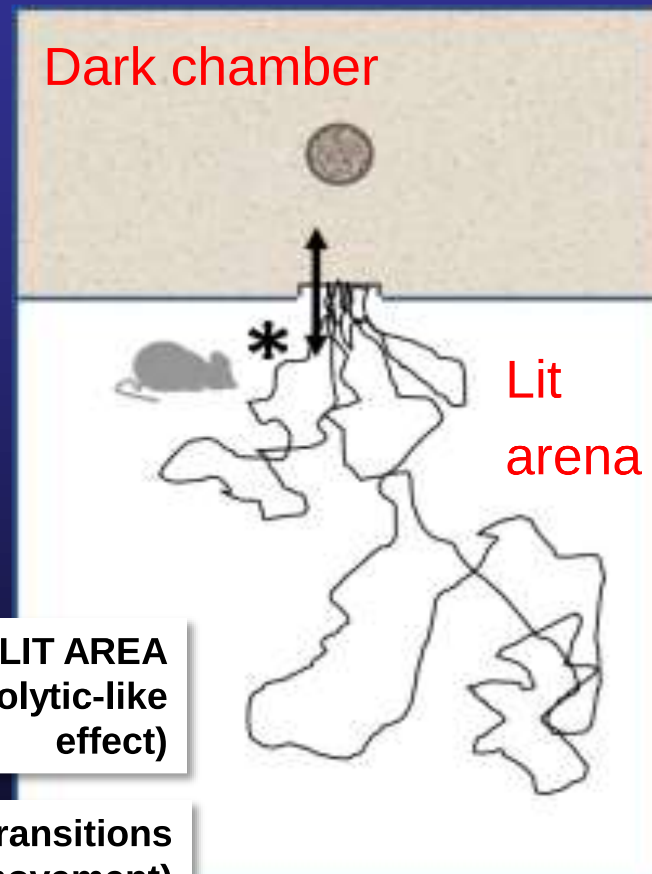
Open Field



A. GENERAL MOVEMENT
(total distance travelled)

B. TIME SPENT IN CENTRAL AREA
(index of exploration attitude, anxiolytic-like effect)

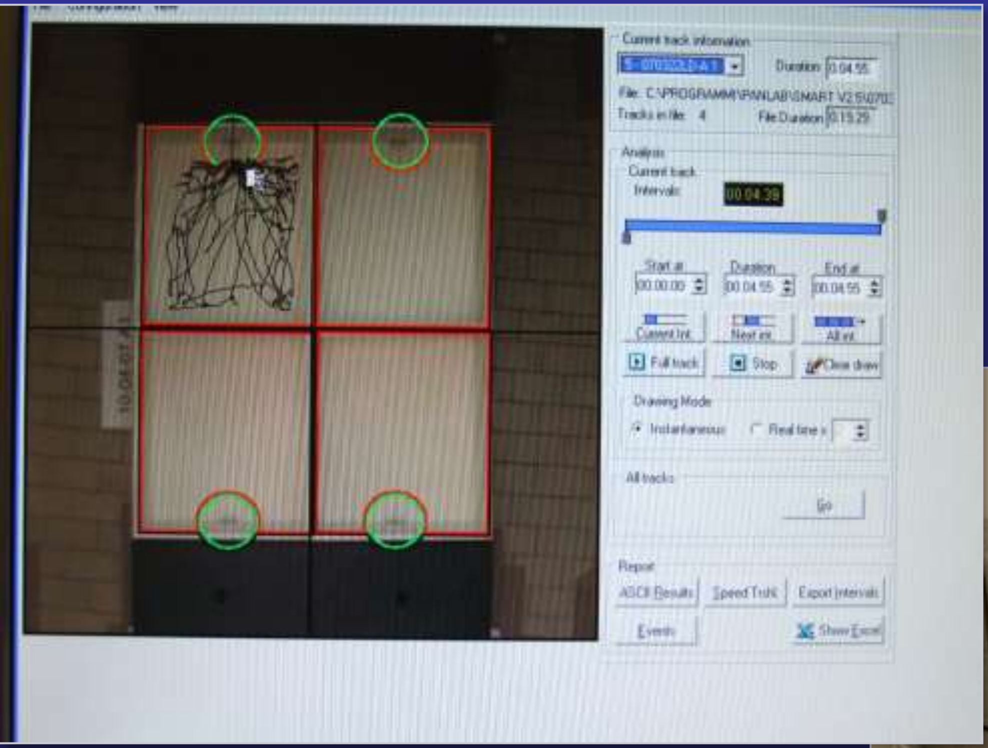
Light-Dark choice



C. TIME SPENT IN LIT AREA
(no aversion to light, anxiolytic-like effect)

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

VIDEO-TRACKING AND AUTOMATIC COMPUTATION OF BEHAVIOURAL SCORES



Tracking and Analysis with Smart software (Panlab Instruments)

Dr. Paolo Magnani

First drug screening

Homeopathic medicines

- ***Aconitum,***
- ***Belladonna,***
- ***Gelsemium,***
- ***Nux vomica,***
- ***Argentum nitricum,***
- ***Tabacum***
- **their control solvent hydroalcoholic (30%) solution**

We started with 5C potencies

Gelsemium sempervirens

TRADITIONAL MATERIA MEDICA

<u>Repertorial Materia Medica</u>: Result of search by index in all repertories: [root:WALK] AND [root:AMEL]

- ✓**MIND - ANXIETY - walking - air, in open – amel. 7**
- ✓**MIND - ANXIETY - walking – amel. 8**
- ✓ **MIND - WALKING - air; in the open – amel. 20**
- ✓**GENERALS - WALKING - air; in open – amel. 135**
- ✓**GENERALS - WALKING - rapidly – amel. 19**
- ✓**GENERALS - WALKING - slowly – amel. 15**

<u>Materia Medica (</u>Boenninghausen, Murphy):

- ✓**MIND: FEELING AS IN DANGER OF FALLING**
- ✓**MIND: DREAD/DESIRE OF BEING ALONE**
- ✓**MIND: IMPATIENT AND IRRITABLE**
- ✓**MIND: NERVOUS DREAD OF APPEARING IN PUBLIC**

Summary of two complete series of 14 experiments testing *Gelsemium s.* on mice behavior

Hindawi Publishing Corporation
Evidence-Based Complementary and Alternative Medicine
Volume 2012, Article ID 954374, 9 pages
doi:10.1155/2012/954374

Research Article

Testing Homeopathy in Mouse Emotional Response Models: Pooled Data Analysis of Two Series of Studies

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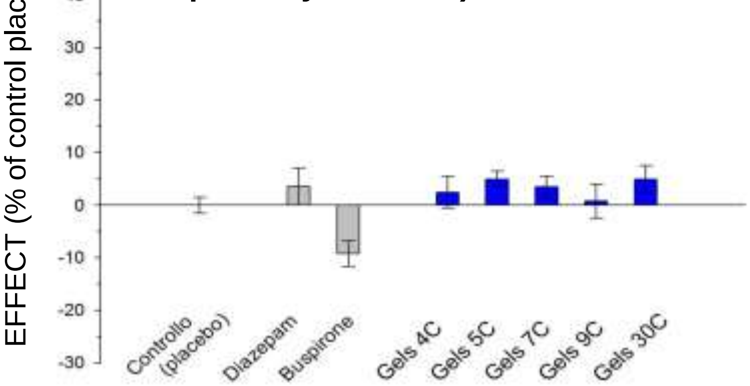
Received 21 November 2011; Accepted 29 January 2012

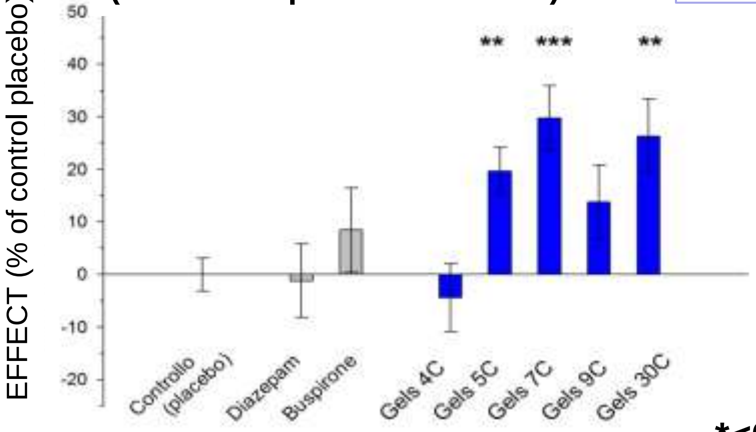
POOLED DATA ANALYSIS (14 complete experiments)
Evidence-Based Complementary and Altern. Med., 2012

Open field

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



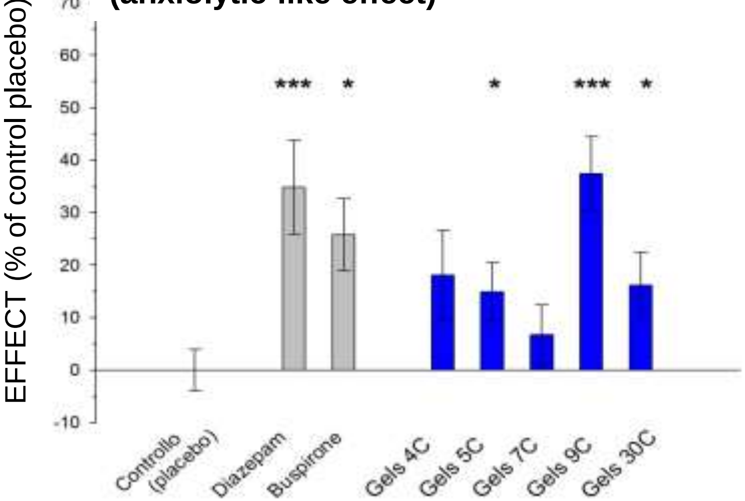
B. TIME SPENT IN CENTRAL AREA (index of exploration attitude)



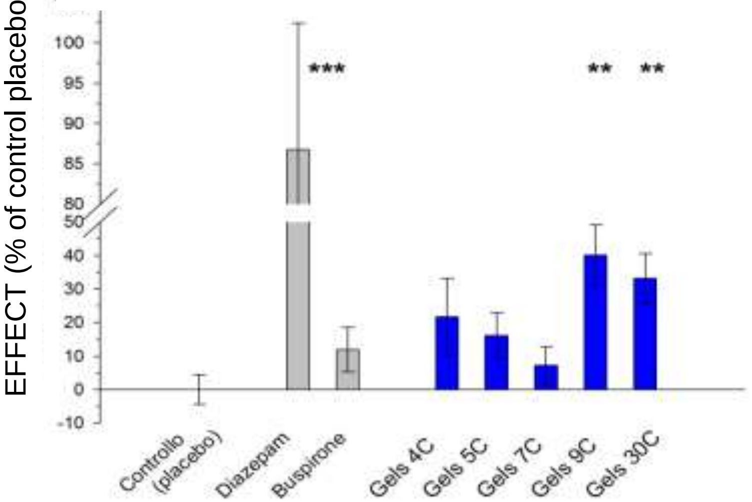
*<0.05
**<0.01
***<0.001

Light Dark

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

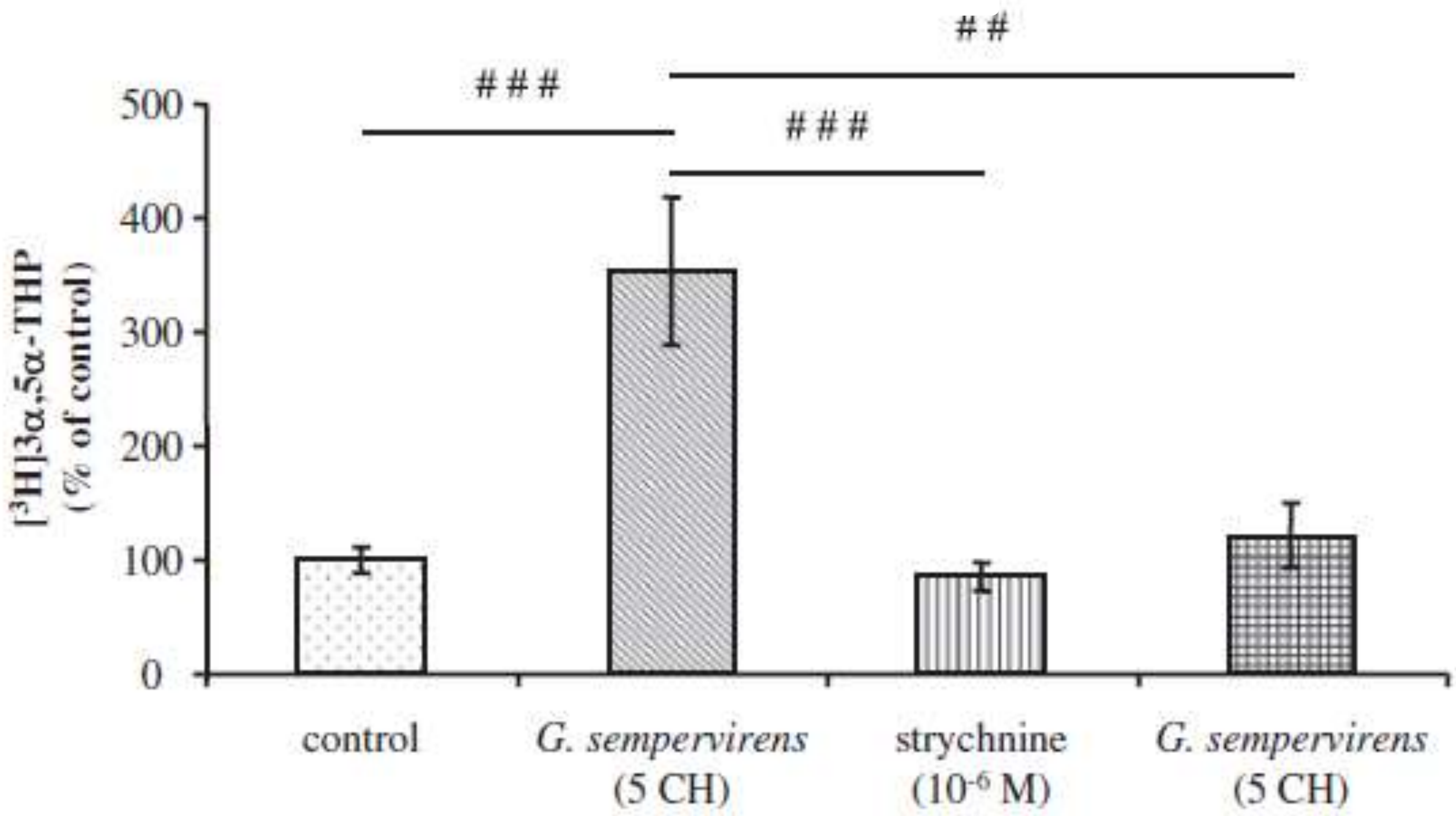


D. LIGHT-DARK transitions (anxiolytic-like effect and movement)



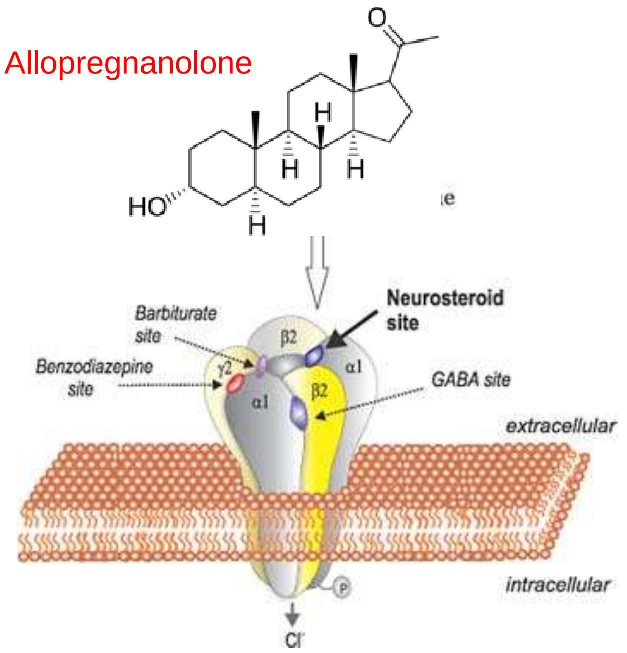
Gelsemium sempervirens Activity on Neurosteroid Allopregnanolone Formation in the Spinal Cord and Limbic System of Rats

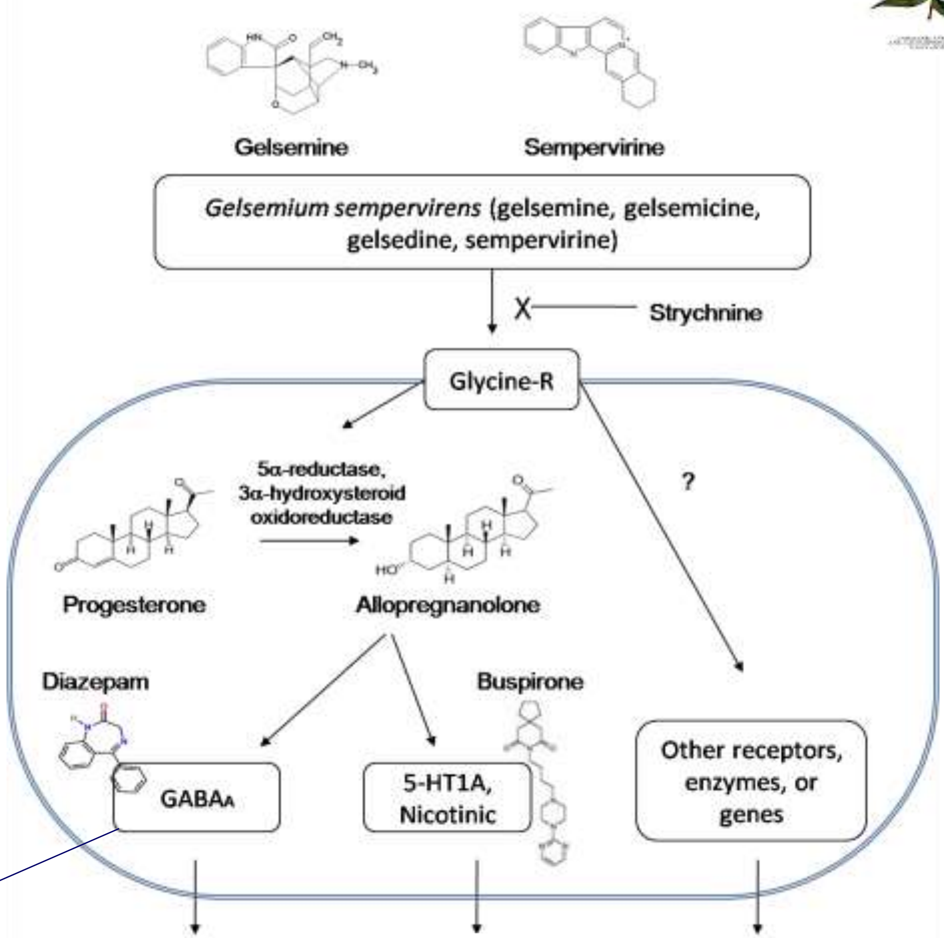
Adapted from: Christine Venard et al., ECAM-2011



***$P < 0.001$ 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*





Decrease nerve excitability under stress
Control of anxiety-like symptoms

Gelsemium s. in mice: KEY-NOTES

- Reproducible and significant effects in mice, concerning a subset of "symptoms" which have been tested in Open field and Light-dark:
  - 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)

- NON-LINEARITY (various activity peaks) with increasing potencies, BUT in general different potencies have the same trend of effects (important for practical purposes)

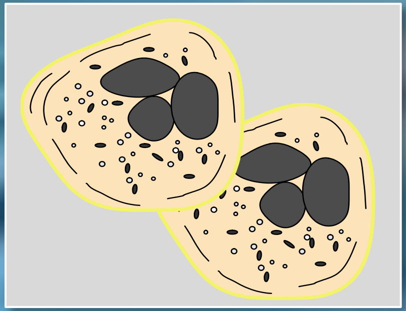
- First hypothesis of action mechanism: stimulation of glycine receptors and thus neurosteroid synthesis with consequent increase of GABA inhibitory effects

UP-TO DATE CONCLUSIONS FROM ANIMAL AND PLANT MODELS

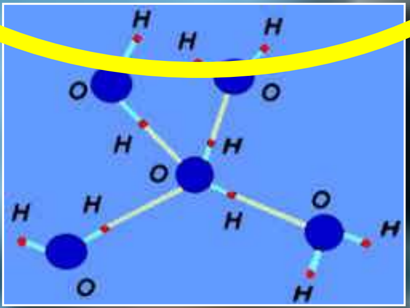
- **Consistent evidence that high dilutions (even beyond Avogadro) have reproducible effects different from control solutions: end of placebo hypothesis**
- **Confirmation of the "similia principle": homeopathic dilutions counteract toxicity of ponderal doses (e.g. *Arsenic*, *Phosphorus*)**
- **Confirmation in animals of some symptoms reported by Materia Medica (e.g. *Gelsemium, Apis, Histaminum*)**

Basic research on Homeopathy

Animal and plant models



Cell models



Physico-chemical models

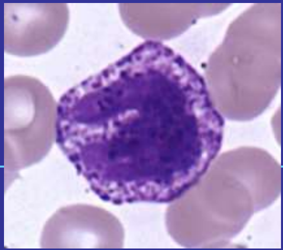
Pre-clinical research (efficacy) in controlled trials

Drug targets and action mechanism(s)

Composition and nature of remedies

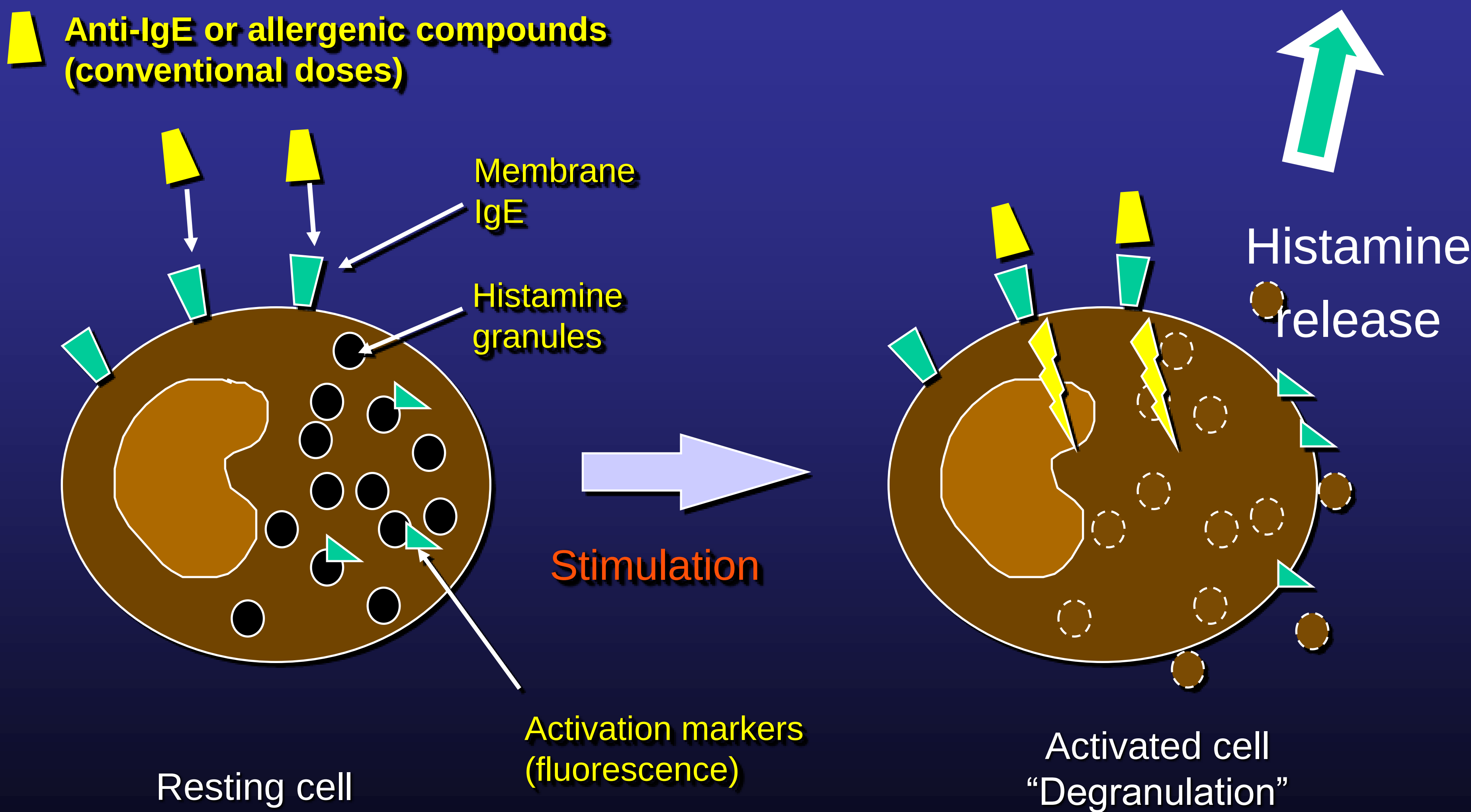
EXAMPLES OF HIGH DILUTION EFFECTS *"IN VITRO"*

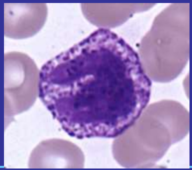
System	Agent	Dilution	Effect	Ref.
Human basophils	Histamine	12CH-16CH $10^{-24} \rightarrow 10^{-32}$	Inhibition of activation markers	Belon 1999-2009 (and Verona Group)
Human basophils	Adrenaline	12CH-16CH $10^{-24} \rightarrow 10^{-32}$	Inhibition of activation markers	Mannaioni et al. 2010
Cicken 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
Neurocytes	Cycloheximide	10^{-27}	Increases viability	Marotta 2002
Bacteria	Arsenicum	30CH	Protects from toxicity	Das et al 2011, De et al 2012
Neurocytes	Gelsemium s.	2-30 CH	Prevalent gene down-regulation	Marzotto 2014, Olioso 2014
Colon cancer cells	Ruta grav.	MT-30CH	Decrease cell viability, apoptotic gene expression	Arora and Tandon 2015



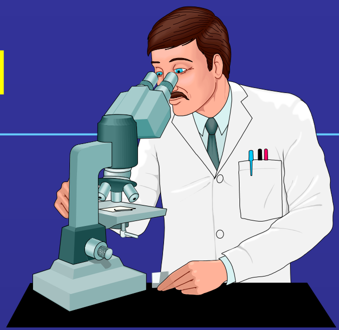
THE MODEL OF BASOPHIL ACTIVATION

ALLERGY SYMPTOMS





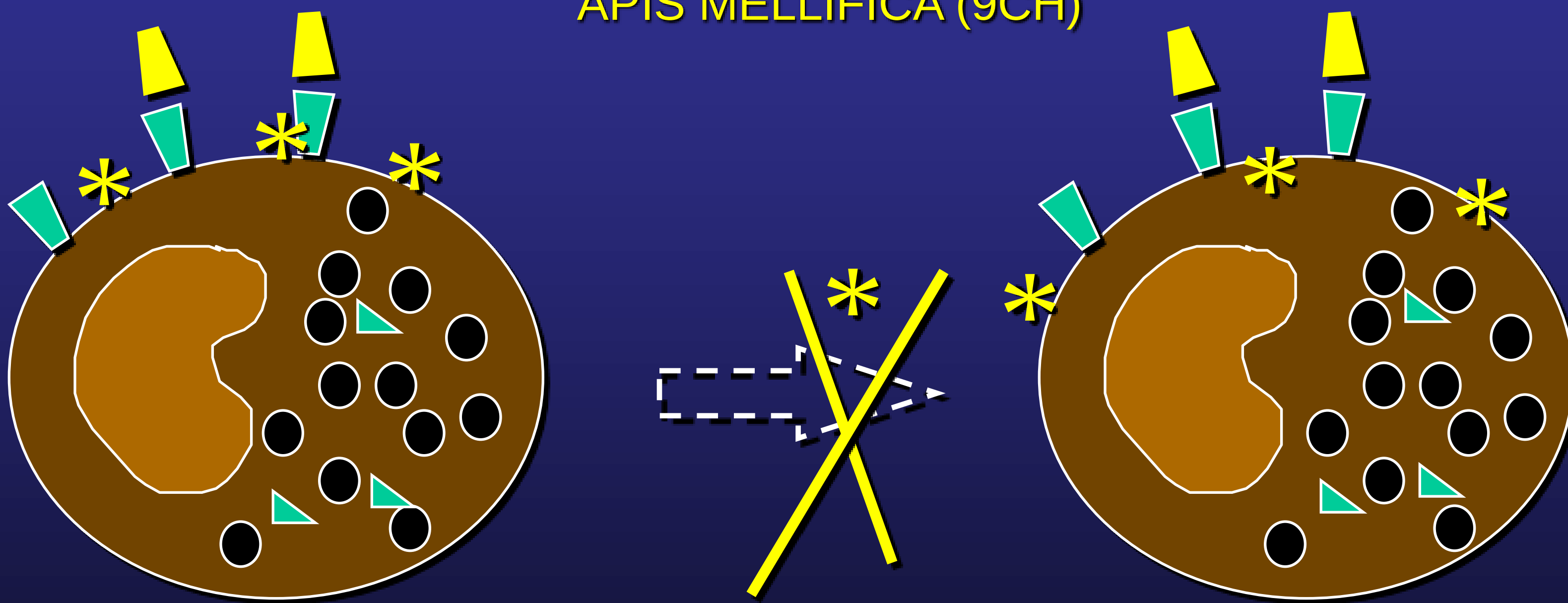
THE MODEL OF BASOPHIL ACTIVATION



EXPERIMENTS OF POITEVIN ET AL. (BR. J. CLIN. PHARM. 1988)

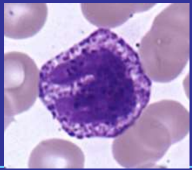
Anti-IgE
(Medium doses)

* + Homeopathic drugs
LUNG HISTAMINE (5CH, 15 CH)
APIS MELLIFICA (9CH)

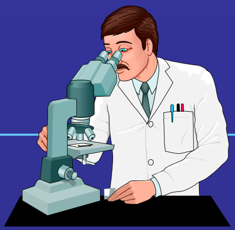


Resting cell

Inhibition of response
to Anti-IgE



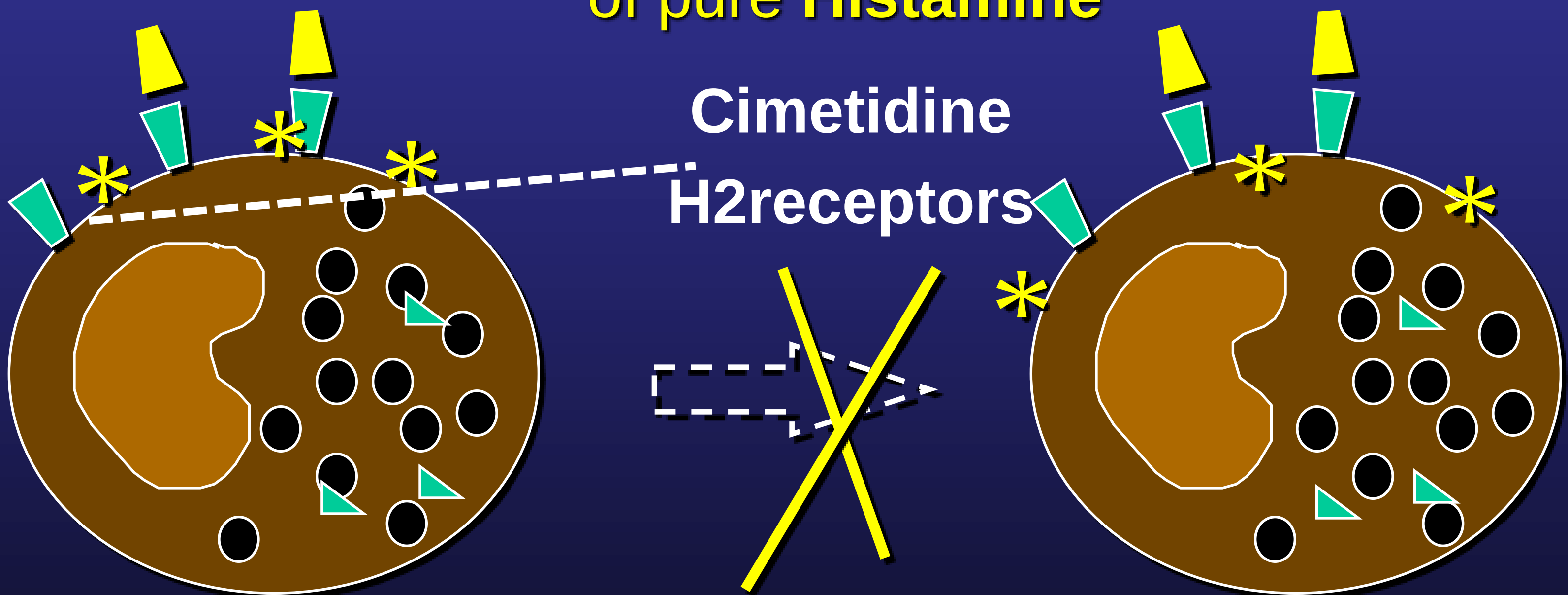
THE MODEL OF BASOPHIL ACTIVATION



EXPERIMENTS OF SAINTE LAUDY, BELON ET AL. (1989-2010)

Anti-IgE
(Medium doses)

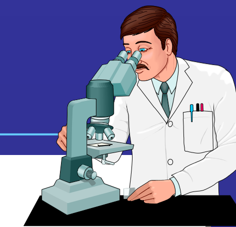
* Ultra-high dilution (10^{-36} M)
of pure **Histamine**



Resting cell

Inhibition of response
to Anti-IgE

EFFECT OF HISTAMINE HIGH DILUTIONS ON BASOPHIL "DEGRANULATION"



Inflamm. res. 48, *Supplement 1* (1999) S 17–S 18
1023-3830/99/010S17-02 $ 1.50+0.20/0

Inflammation Research

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

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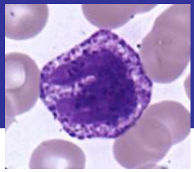
Table 1. Comparison of percentage degranulation induced by anti-IgE (0.04 µg/ml) in the absence and presence of histamine dilutions (15th–19th centesimal dilutions).

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.



REPLICATION EXPERIMENTS OF HIGH-DILUTION EFFECTS

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

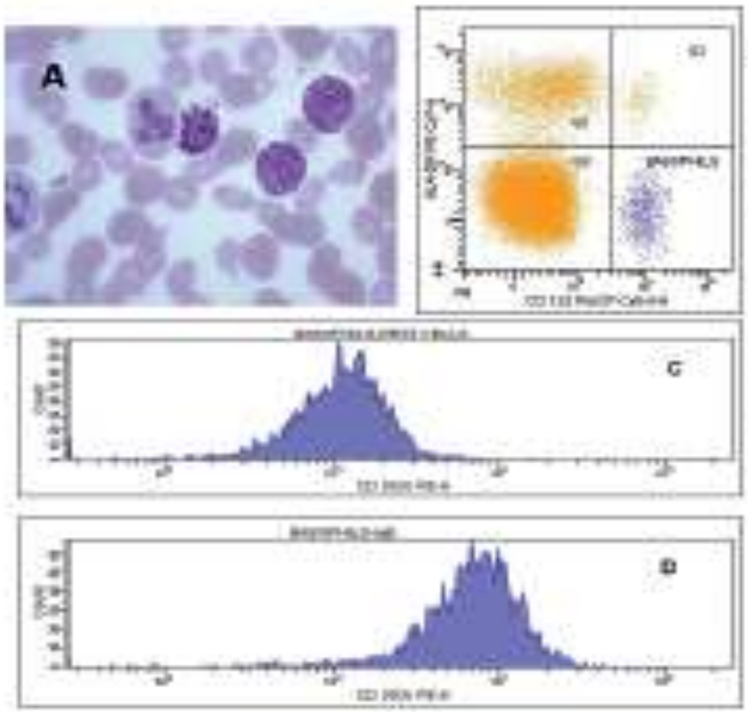
Inflammation Research

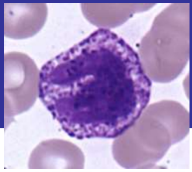
ORIGINAL RESEARCH PAPER

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

Received: 14 November 2008 / Revised: 3 April 2009 / Accepted: 9 April 2009





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

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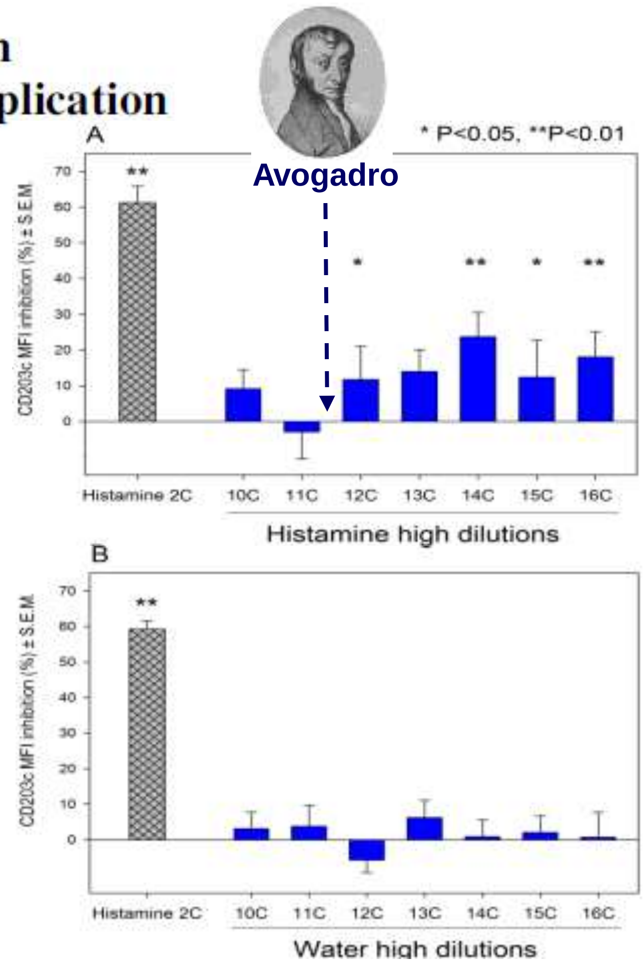
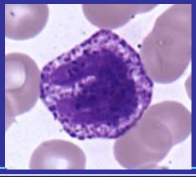




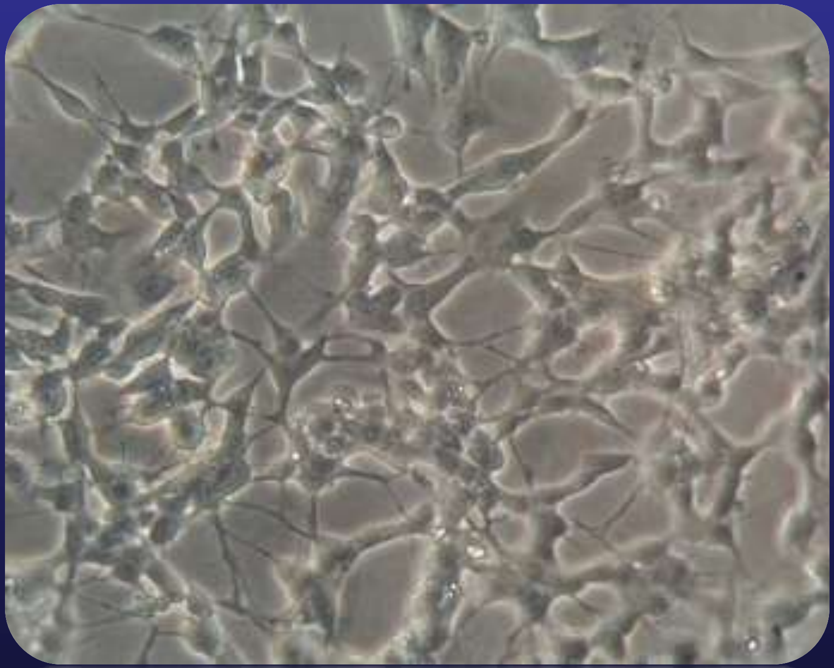
Reports concerning the effect of highly diluted/succussed histamine on human basophils published in the mainstream literature

➢IN SUMMARY:

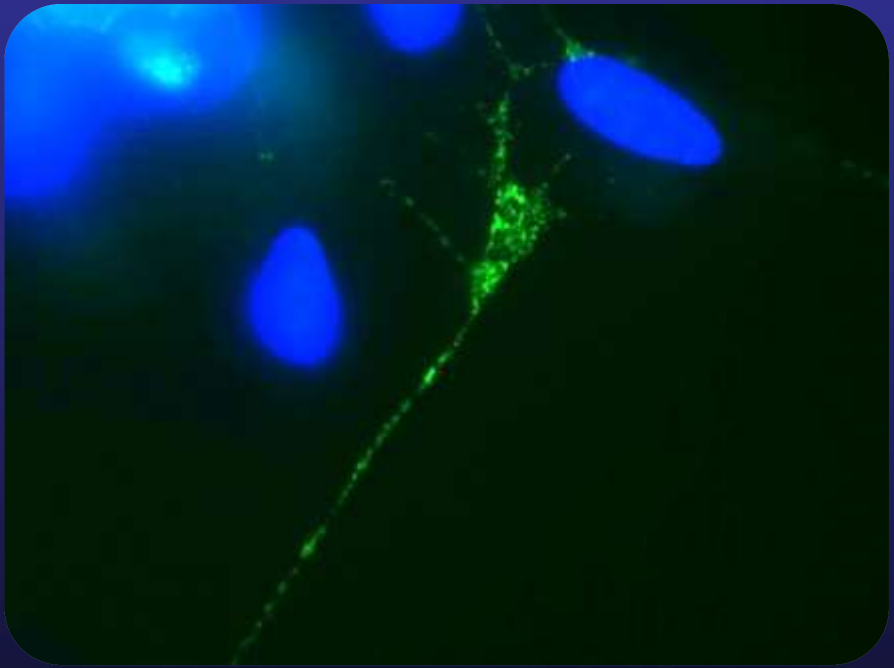
- **14 publications (2 with multicentre studies)**
- **4 independent laboratories involved**
- **12 papers with positive results**
- **1 negative**
- **1 uncertain**

Gelsemium s. in a neuronal model

SH-SY5Y neurocytes-human neuroblastoma cells



Inverted microscope image



Confocal immunofluorescent image

Marzotto et al. BMC Complementary and Alternative Medicine 2014, 14:104
http://www.biomedcentral.com/1472-6882/14/104

BMC Complementary & Alternative Medicine

RESEARCH ARTICLE | Open Access

Extreme sensitivity of gene expression in human SH-SY5Y neurocytes to ultra-low doses of *Gelsemium sempervirens*

Marta Marzotto[1], Debora Olioso[1], Maurizio Brizzi[2], Paola Tononi[3], Mirco Cristofoletti[1] and Paolo Bellavite[1*]

Abstract

Background: *Gelsemium sempervirens* L. (*Gelsemium s.*) is a traditional medicinal plant, employed as an anxiolytic at ultra-low doses and animal models recently confirmed this activity. However the mechanisms by which it might operate on the nervous system are largely unknown. This work investigates the gene expression of a human neurocyte cell line treated with increasing dilutions of *Gelsemium s.* extract.

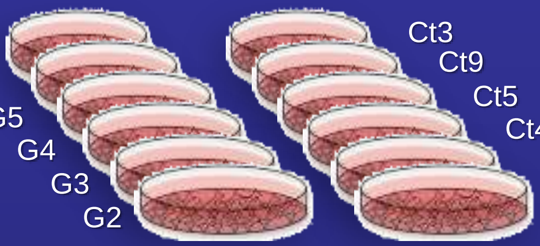
Methods: Starting from the crude extract, six 100 × (centesimal, c) dilutions of *Gelsemium s.* (2c, 3c, 4c, 5c, 9c and 30c) were prepared according to the French homeopathic pharmacopoeia. Human SH-SY5Y neuroblastoma cells were exposed for 24 h to test dilutions, and their transcriptome compared by microarray to that of cells treated with control vehicle solutions.

Results: Exposure to the *Gelsemium s.* 2c dilution (the highest dose employed, corresponding to a gelsemine concentration of 6.5×10^{-9} M) significantly changed the expression of 56 genes, of which 49 were down-regulated and 7 were overexpressed. Several of the down-regulated genes belonged to G-protein coupled receptor signaling pathways, calcium homeostasis, inflammatory response and neuropeptide receptors. Fisher exact test, applied to the group of 49 genes down-regulated by *Gelsemium s.* 2c, showed that the direction of effects was significantly maintained across the treatment with high homeopathic dilutions, even though the size of the differences was distributed in a small range.

Conclusions: The study shows that *Gelsemium s.*, a medicinal plant used in traditional remedies and homeopathy, modulates a series of genes involved in neuronal function. A small, but statistically significant, response was detected even to very low doses/high dilutions (up to 30c), indicating that the human neurocyte genome is extremely sensitive to this regulation.

Microarray analysis of gene expression changes in human neurocytes

24h



G30
G9
G5
G4
G3
G2

Ct3
Ct9
Ct5
Ct4
Ct3
Ct2

Gelsemium s. dilutions (G)

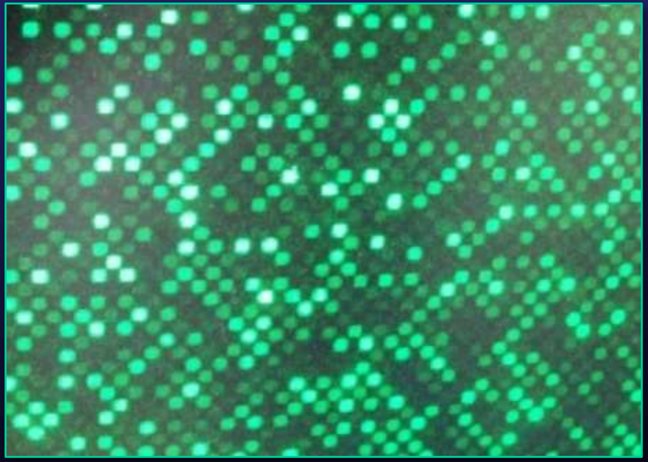
Control (solvent) Dilutions (Ct)

RNA → cDNA

12-samples microarray chip

4 independent replicate experiments

- **NimbleGen chip**
- Entire human transcriptome **(45034 genes!)**



Scanning and data analysis

BMC-Complementary Alternative Medicine

March 2014

Exposure to the Gelsemium s. 2CH promoted the significant down-expression of 49 genes

while 7 genes were overexpressed

Many of these genes belong to:

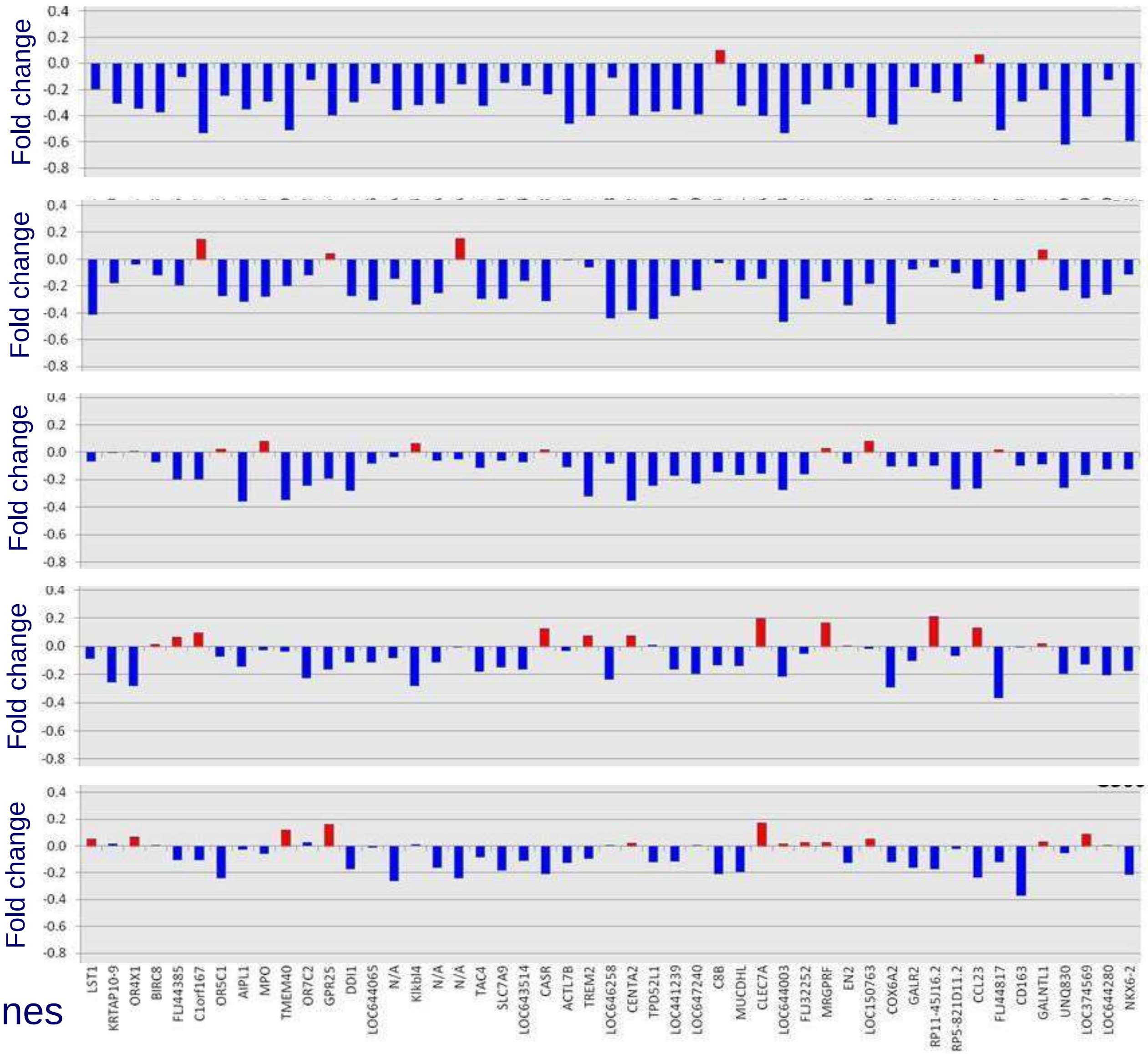
- neuropeptide/receptor systems
- calcium signalling
- G-protein coupled transduction systems
- inflammatory pathways

DOWN

UP

Gene ID	Transcript ID	Symbol	Log2 fold change	p[1]	Description
7940	AF000424	LST1	-0.84 ± 0.14	0.04	leukocyte specific transcript 1
390113	NM_001004726	OR4X1	-0.83 ± 0.06	0.01	olfactory receptor, family 4, subfamily X, member 1
23746	AJ830742	AIPL1	-0.82 ± 0.16	0.04	aryl hydrocarbon receptor interacting protein-like 1
284498	AL833920	C1orf167	-0.80 ± 0.17	0.05	chromosome 1 open reading frame 167
221191	AK058068	Klkbl4	-0.79 ± 0.12	0.04	plasma kallikrein-like protein 4
26658	NM_012377	OR7C2	-0.77 ± 0.07	0.01	olfactory receptor, family 7, subfamily C, member 2
112401	BC039318	BIRC8	-0.76 ± 0.11	0.00	baculoviral IAP repeat-containing 8
2848	NM_005298	GPR25	-0.75 ± 0.15	0.02	G protein-coupled receptor 25
55803	NM_018404	ADAP2	-0.75 ± 0.11	0.02	ArfGAP with dual PH domains 2
386676	NM_198690	KRTAP10-9	-0.73 ± 0.12	0.04	keratin associated protein 10-9
4353	X04876	MPO	-0.72 ± 0.15	0.04	Myeloperoxidase
N/A	AY358413	N/A	-0.71 ± 0.18	0.02	Homo sapiens clone DNA59853 trypsin inhibitor
392391	NM_001001923	OR5C1	-0.71 ± 0.05	0.04	olfactory receptor, family 5, subfamily C, member 1
N/A	AK094115	N/A	-0.70 ± 0.11	0.04	Homo sapiens cDNA FLJ36796 fis, clone ADRGL2006817
55287	BC020658	TMEM40	-0.70 ± 0.15	0.02	transmembrane protein 40
54209	NM_018965	TREM2	-0.69 ± 0.10	0.02	triggering receptor expressed on myeloid cells 2
150365	AK097834	RP5-821D11.2	-0.68 ± 0.17	0.02	similar to mouse meiosis defective 1 gene
400934	NM_207478	FLJ44385	-0.68 ± 0.09	0.04	FLJ44385 protein
255061	NM_170685	TAC4	-0.67 ± 0.14	0.01	tachykinin 4 (hemokinin)
644065	XM_931993	LOC644065	-0.65 ± 0.23	0.04	hypothetical protein LOC644065
1339	NM_005205	COX6A2	-0.64 ± 0.17	0.01	cytochrome c oxidase subunit VIa polypeptide 2
N/A	AK128093	N/A	-0.63 ± 0.09	0.04	Homo sapiens cDNA FLJ46214 fis, clone TESTI4012623.
53841	AY358368	CDHR5	-0.63 ± 0.11	0.04	mucin-like protocadherin
9332	NM_004244	CD163	-0.63 ± 0.18	0.03	CD163 molecule
441239	XM_499305	LOC441239	-0.63 ± 0.22	0.05	hypothetical gene supported by BC063653
7164	NM_001003397	TPD52L1	-0.62 ± 0.09	0.02	tumor protein D52-like 1
11136	NM_014270	SLC7A9	-0.62 ± 0.09	0.04	solute carrier family 7 member 9
389084	NM_206895	UNQ830	-0.62 ± 0.11	0.04	ASCL830
400224	XM_375090	FLJ44817	-0.62 ± 0.20	0.04	similar to pleckstrin homology domain protein (5V327)
647240	XM_934559	LOC647240	-0.60 ± 0.06	0.00	hypothetical protein LOC647240
846	BC104999	CASR	-0.59 ± 0.06	0.00	calcium-sensing receptor
116123	NM_138784	RP11-45J16.2	-0.58 ± 0.09	0.04	flavin-containing monooxygenase pseudogene
644280	XM_497769	LOC644280	-0.58 ± 0.06	0.05	hypothetical protein LOC644280
57452	AB032956	GALNTL1	-0.57 ± 0.17	0.05	alpha-D-galactosamine N-acetylgalactosaminyltransferase
414301	NM_001001711	DDI1	-0.56 ± 0.11	0.04	DDI1, DNA-damage inducible 1, homolog 1 (*S. cerevisiae*)
116535	BC016964	MRGPRF	-0.55 ± 0.17	0.01	MAS-related GPR, member F
8811	NM_003857	GALR2	-0.55 ± 0.07	0.04	galanin receptor 2
10880	NM_006686	ACTL7B	-0.55 ± 0.12	0.04	actin-like 7B
6368	NM_145898	CCL23	-0.55 ± 0.11	0.05	chemokine (C-C motif) ligand 23
64581	BC071746	CLEC7A	-0.54 ± 0.08	0.04	C-type lectin domain family 7, member A
644003	XM_927256	LOC644003	-0.54 ± 0.11	0.04	similar to Mucin-2 precursor (Intestinal mucin 2)
643514	XM_931594	LOC643514	-0.54 ± 0.10	0.03	hypothetical protein LOC643514
374569	XM_935431	LOC374569	-0.54 ± 0.07	0.04	Similar to Lysophospholipase
84504	BC101635	NKX6-2	-0.53 ± 0.13	0.03	NK6 transcription factor related, locus 2 (Drosophila)
732	NM_000066	C8B	-0.53 ± 0.06	0.05	complement component 8, beta polypeptide
146336	NM_182510	FLJ32252	-0.52 ± 0.03	0.01	hypothetical protein FLJ32252
150763	BC042847	LOC150763	-0.51 ± 0.10	0.04	hypothetical protein LOC150763
2020	NM_001427	EN2	-0.51 ± 0.08	0.04	engrailed homolog 2
646258	XM_929203	LOC646258	-0.51 ± 0.11	0.04	hypothetical protein LOC646258
154872	NM_001024603	LOC154872	0.51 ± 0.10	0.03	hypothetical LOC154872
400866	NM_001001789	C21orf24	0.52 ± 0.12	0.05	chromosome 21 open reading frame 24
9457	NM_020482	FHL5	0.55 ± 0.19	0.04	four and a half LIM domains 5
55816	NM_018431	DOK5	0.56 ± 0.04	0.03	docking protein 5
1446	NM_001890	CSN1S1	0.57 ± 0.09	0.04	casein alpha s1
285600	AK130941	KIAA0825	0.63 ± 0.06	0.01	KIAA0825 protein
57538	NM_020778	ALPK3	0.76 ± 0.10	0.01	alpha-kinase 3

Effects of *Gelsemium* increasing dilutions/dynamizations on the expression of 49 Gels C2-down-regulated genes



	Genes DOWN/UP	P (Fisher)
Gels 3c /Placebo 3c	2↑ 47↓	<0.001
Gels 4c /Placebo 4c	3↑ 42↓	<0.001
Gels 5c /Placebo 5c	3↑ 38↓	<0.001
Gels 9c /Placebo 9c	9↑ 30↓	<0.01
Gels 30c /Placebo 30c	7↑ 27↓	<0.01

Recent literature of Homeopathy and molecular biology (cited in Bellavite et al. Homeopathy 2015)

Test compound	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 gene, stress response proteins)	(Das et al. 2011; De et al. 2012 of Khuda-Bukhsh 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 mellifera	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 markers), ↑ Apoptosis, oxidative stress, mitochondrial depolarization	(Sikdar et al. 2014)

Homeopathy and moleculat biology

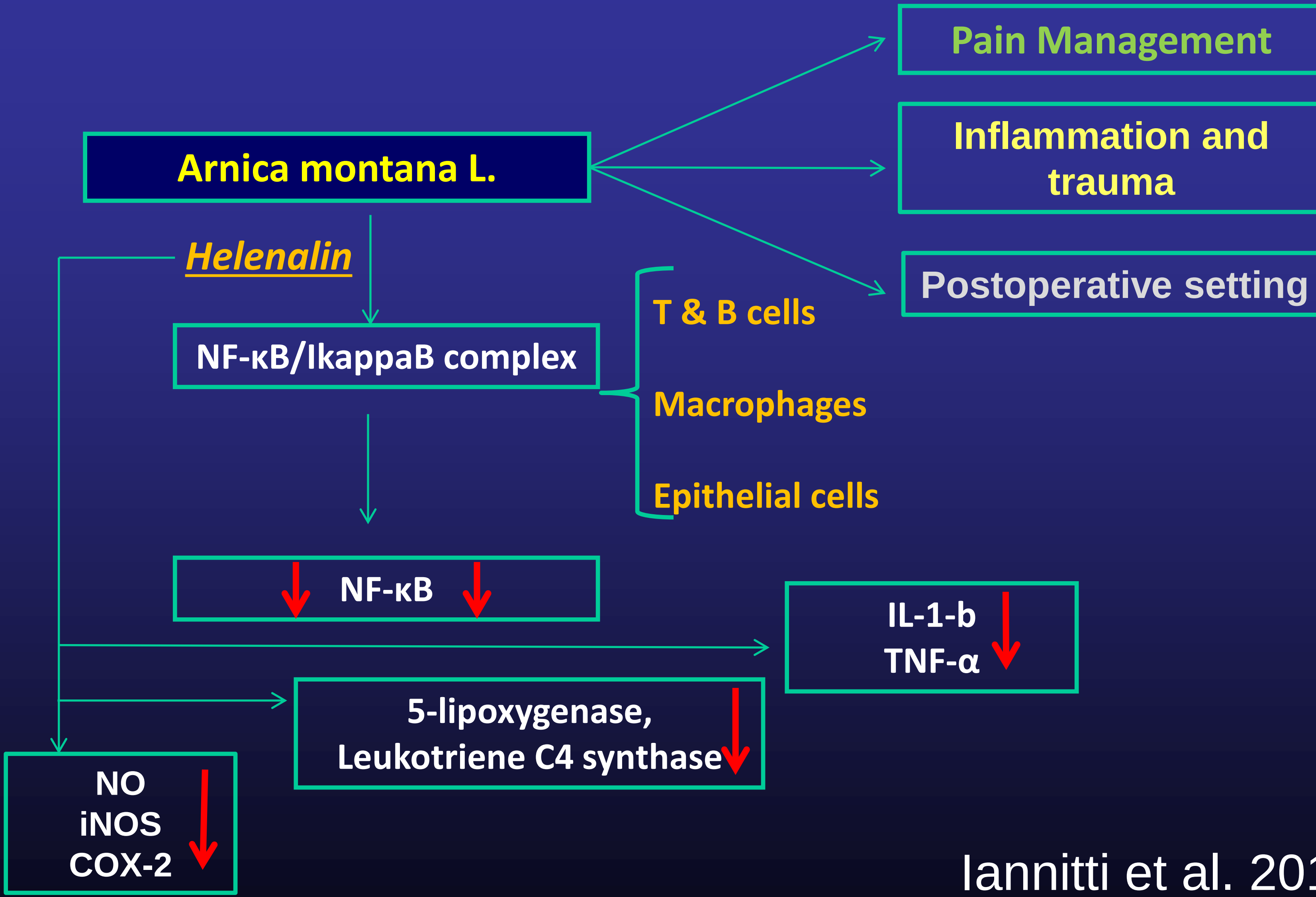
The rapid development of new technology platforms provides a methodological basis for deep understanding the action mechanisms and targets of homeopathic remedies.

DNA

→is SENSITIVE to low energy-information

→is COMPLEX and HOLOGRAPHIC

Arnica montana actions and possible targets of helenaline active principle at cell level



Iannitti et al. 2014

UP-TO DATE KEY FINDINGS FROM *IN VITRO* MODELS

- **Multicentre confirmation of high dilution effects (even beyond Avogadro) in rigorous cell models (e.g. *Histamine in basophils)***
- **According to different models, high dilutions may have protective effects (e.g. Arsenic), prevalent inhibitory (e.g. Gelsemium, Apis) or apoptotic (e.g. Carcinosinum)**
- **Dilution-effect studies show that the same trend is obtained with 2-3-5-9-30 CH (*Histamine*, *Arnica, Gelsemium*) with various peaks**
- **High dilutions act through membrane cell receptors as as shown by studies with inhibitors: cimetidine, propanolol, strychnine (not excluded a direct effect on gene regulation)**
- **Homeopathic high dilutions have effects on the expression of a number of genes and pathways, different for each remedy, that are revealed at the best by molecular biology high-throughput techniques**

Teodora, Bizantine empress (6th Century)