Studies of homeopathic medicines tested in animal behavioural models

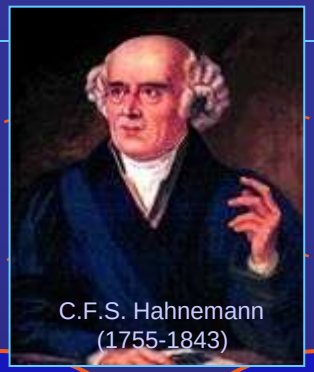
UFRJ 2 SEPT 2011

Paolo Bellavite, University of Verona

1. Introduction and literature review
2. Materials and Methods
3. Results and discussion

The figures can be seen and downloaded at: www.paolobellavite.it
(under "News")

THE MAJOR TENETS OF HOMEOPATHY


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

"By choosing a remedy for a given natural disease that is capable of producing a very similar artificial disease we shall be able to cure the most obstinate diseases"
S. Hahnemann, Hufeland's Journal 2: 381 (1796)

"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..."
S. Hahnemann, The Organon of Medicine (1810), par 277

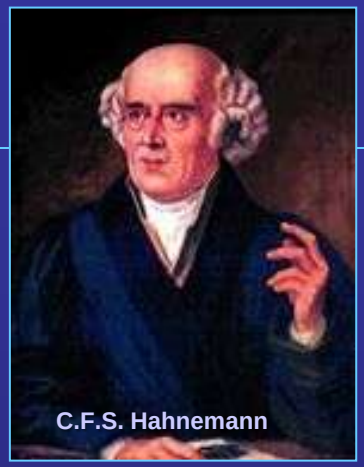
SIMILIA PRINCIPLE

MICRODOSE AND "POTENCY"

TOTALITY OF CURE

"Each individual case of disease is most surely, radically, rapidly and permanently annihilated and removed only by a medicine capable of producing (in the human system) in the most similar and complete manner the totality of its symptoms"
C.F.S. Hahnemann, The Organon of Medicine (1810), par. 27

PROBLEMS OF UNDERSTANDING AND ACCEPTING HOMEOPATHY



1. The structure of contemporary scientific thought inherited from positivism (everything is material, and each object can be reduced to its component parts).
2. The scarcity of demonstrations of efficacy based on methods shared by official medicine, which is due partly to intrinsic difficulties and partly to the fact that homeopathy has long been banned from western academic institutions
3. The scarcity of explanations that are plausible and understandable in terms of current pharmacological theories based on the molecular and quantitative paradigm.

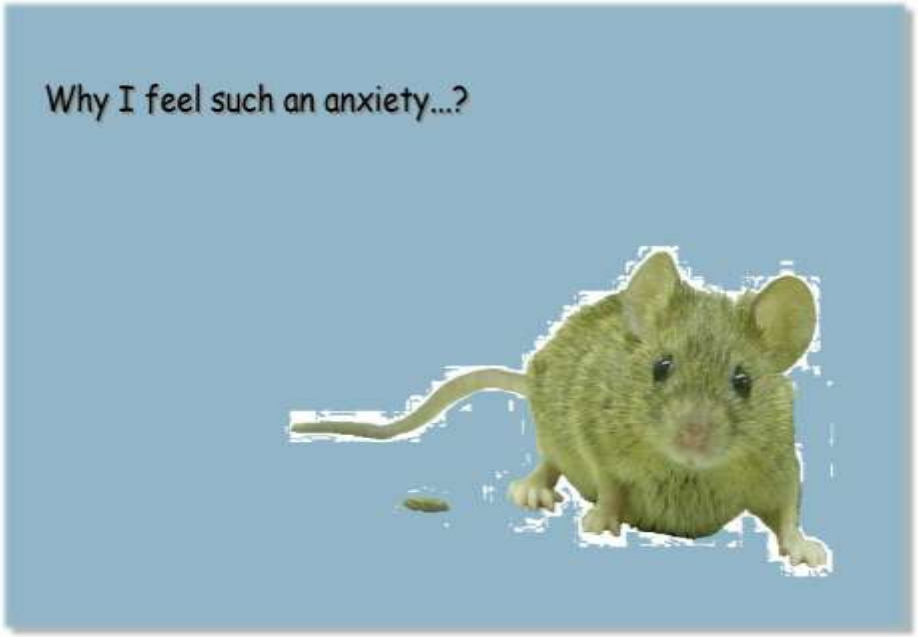
→→Points 2 and 3 can be scientifically investigated!!

SCIENCE FIELDS INVOLVED IN THE INVESTIGATION OF HOMEOPATHIC PHENOMENA

- ✓ **Clinical research (humans, animals, plants in field…)**
- ✓ **Physical research (water…)**
- ✓ **Biological research (cell and animal laboratory studies)**
  - → **Evidence of pharmacological activity in the absence of "placebo" effects**
  - → **Study of the active principles of drugs and their action mechanism(s)**
  - → **The problem of dose-dependence (non linearity) in reproducible conditions**

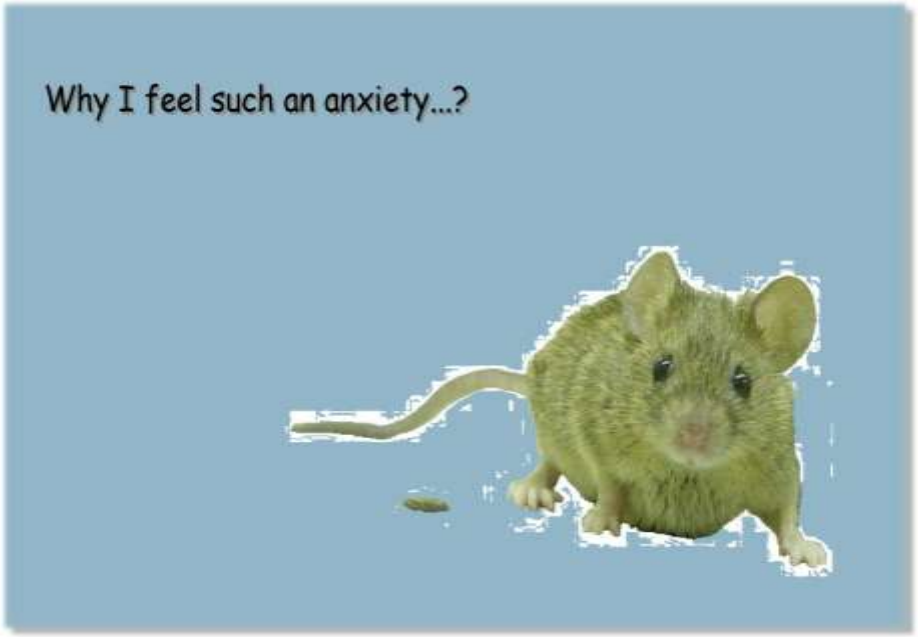
Animal models of psychopharmacology Background

- 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.



Animal models- Background

- Animal models have helped to elucidate the mechanisms of absorption, distribution, transformation and excretion of drugs, thereby allowing the active ingredients of medicinal plants and animal products to be identified.
- With allopathic drugs, dosages and adverse reactions are generally studied in animal models prior to undertaking human trials.
- In homeopathic research there are several problems that can be dealt with in animal models: reproducibility of effects, dose-dependence, action mechanism(s), drug formulations, way of administration, etc.



Reports on psychopathological and behavioural models of homeopathy in rodents - 1st part of 2

Date Author	Animal	Model	Remedy	Route	Main effects
1979 Binsard	Mouse	4 plates	Ignatia and Gelsemium 3C,4C,5C	i.p. 3 weeks	Anxiolytic (Ignatia 3C and Gelsemium 5C only) or sedative (Ignatia 5C)
	Rat	Staircase	Ignatia and Gelsemium 3C,4C,5C	i.p. 3 weeks	Sedative (Ignatia 4C and Gelsemium 3C and 5C only)
1980 Binsard et al.	Mouse	4 plates Escape test Rota-rod	Gelsemium 3C, 5C, 7C	i.p. 3 weeks	Gelsemium 3C reduces exploration, Gelsemium 7C increases it
1981 Guillemain et al.	Mouse	Strychnine. Induced convulsions	Ignatia 3D, 3,5,7,12C	i.p. 0.5 ml/20g single dose	Slight protective effect of 3D and 5C
1986 Sukul	Rat	Cataleptogenic effects of restraint	Gelsemium, Cannabis, Graphites and Agaricus Muscarius (30C and 200C)	Per os	Increase cataleptogenic effects of restraint.
1991 Sukul et al.	Rat (and cats)	Electrophysiology of SNC	Arnica 30C, Hypericum 200C, Arsenic 30C	Per os (0.5 ml)	Arnica and Hypericum decrease firing rate, Arsenic increase it.

Reports on psychopathological and behavioural models of homeopathy in rodents - 2nd part of 2 (** non-homeopathic journal)

Date Author	Animal	Model	Remedy	Route	Main effects
1997 Cristea et al.	Mouse	Behavioural tests	Chamomilla 5C and 30C	Per os 4 times/day for 1 day (5C) or 2 times/day for 3 days (30C)	Stimulating effects with 5C and tranquillizing effects with 30C
1999-2001 Sukul	Mouse	Loss of righting reflex due to ethanol	Nux vomica 30C	Per os 0.05 ml/2 ml water and given at 0.05 ml/individual.	Protective effect
2001 Bousta et al. **	Mouse	Electric stress Light-dark test Blood cell count Gastric lesions	Atropa belladonna Gelsemium s. Poumon histamine (5C,9C,15C)	I.p. 30 min before stress and test	Reversal of stress-induced alterations
2005 Ruiz-Vega	Rat	Sleeping behaviour	Coffea cruda 30C and 200C	Per os in feeding bottle	Coffea 30C changes spectral power of EEG Delta band
2008 Da Silva Rocha	Rat	Open field	Rhus toxicodendron 200C	Per os 24 h	Decreases locomotion in hyperactive rats
2008 Pinto	Mouse	Open field Forced swimming	Chamomilla 6C	Per os 7days	Prevents decrease of general activity. In O.F.

Literature on animal models of anxiety

For details see Bellavite et al, Homeopathy special issue (2009)

1. **Less than a dozen of papers published**
2. **Only one paper published in non homeopathic literature, only three published in peer-reviewed journals**
3. **Extremely heterogeneous as the methods employed**
4. **Only 4 employed blind conditions**
5. **Only a few medicines have been studied by multiple laboratories, and concern *Ignatia, Gelsemium, Chamomilla* (in homeopathic dilutions/potencies)**
6. **There are also anxiogenic findings (eg. Sukul's report in 1986)**
7. **Overall, the laboratory evidence in this field is little, of low quality and… unknown to medical scientists**

Animal models of behavior-our objectives

1. To set up **validated and reproducible models** in animal models of anxiety-behavior applicable to homeopathic research

2. To test the effects of several homeopathic medicines used in anxiety in humans (**screening** of 5C) using water as negative control (placebo) and allopathic drugs as positive controls

3. To perform **replication** experiments of most promising compounds

4. To test several **dilutions/dynamizations** in rigorous reproducible way (4-5-7-9-30 C)

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DRUGS

1. Homeopathic drugs (and control solvent) were provided by **Boiron Laboratories** (Lyon) in 30% hydroalchoolic solution.

2. **Stored** at room temperature in dark, wrapped in aluminium foil, in metal cupboard (Faraday cage)

3. Just before starting treatments, the solutions were 100-folds **diluted** in distilled sterile and apyrogenic water, then vigorously **succussed** by hand, thus lowering the alcohol concentration to 0.3 %.

4. All solutions were delivered by **intraperitoneal (i.p.) injection** using insuline needles (painless), 0.3 ml/mice per day. I.p. delivering was chosen in agreement with pharmacologists to guarantee the dosage.

Dilution and dynamization

↓ Produced by Boiron

↓ Used in treatments

0.4 ml **Gels 3C** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Gels 4C**

0.4 ml **Gels 4C** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Gels 5C**

0.4 ml **Gels 6C** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Gels 7C**

0.4 ml **Gels 8C** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Gels 9C**

0.4 ml **Gels 29C** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Gels 30C**

0.4 ml **EtOH 30%** + 39.6 ml **H_20** → ***shaking*** → 40 ml **H_2O+EtOH 0.3%**

0.4 ml **EtOH 30%** + 39.6 ml **H_20** → ***shaking*** → 40 ml **H_2O+EtOH 0.3%**

0.4 ml **Buspirone** + 39.6 ml **H_20** → ***shaking*** → 40 ml **Buspirone**
50mg/kg in EtOH 30% **5mg/kg in H_2O+EtOH 0.3%**

All solutions were **coded** by people not involved in the research

Placebo (Control)
= same hydro-alchoolic solution
(0.3% EtOH)

Allopathic drug
= Buspirone or Diazepam
in the same hydroalchoolic solution
(0.3% EtOH)

22.10.2008

Schema per la codifica delle provette con i medicinali

Medicinale	Numero originale	Lettera CODIFICATA (a sorte tra A, B, C, D,E,F,G,H)
Gels 4C	N.1	.....
Gels 5C	N.2	.....
Gels 7C	N.3	.....
Gels 9C	N.4	.....
Gels 30C	N.5	.....
Placebo Non Dinamizzato:	N.6	.....
Placebo Non Dinamizzato:	N.7	.....
Buspirone 0.5 mg/ml	N.8	.....

I codici sono inseriti in una busta chiusa e sigillata che è consegnata in custodia a:

……

Firma (leggibile) di chi ha effettuato la codifica: ……

Scheme of the standard experiment

- 8 groups of 8 animals randomized 2 x cage
- 5 receive dilutions of *medicines*, 1 Allopathic drug and 2 water placebo
- All medicines/control coded by independent people

Start
Housing
Animal
randomization

A	Medicine /Control1
B	Medicine/Control 2
C	Medicine/Control 3
D	Medicine/Control 4
E	Medicine/Control 5
F	Medicine/Control 6
G	Medicine/Control 7
H	Medicine/Control 8

-7 0 1 2 3 4 5 6 7 8

days

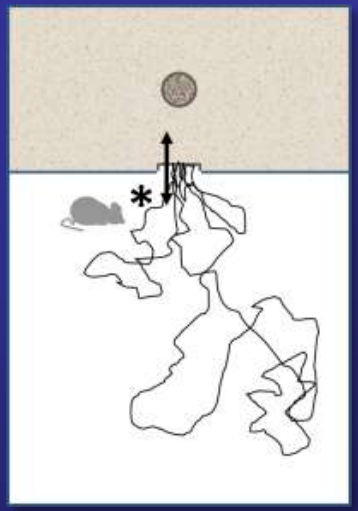
Tracking
Calculations
(in blind
conditions)

Open
-Field
Test

Light
-Dark
Test

Experiments approved
by ethical committee
No pain, no artificial stress

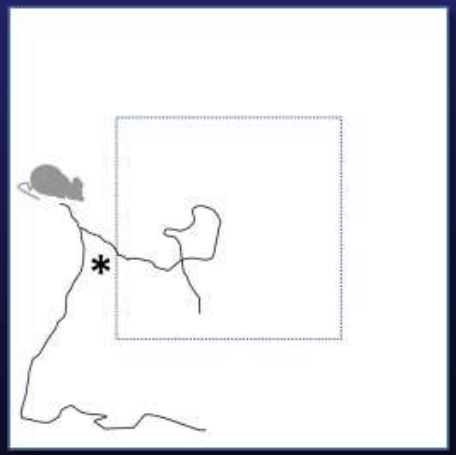
Models used



LD

Two validated animal models:
-the Light-Dark choice test (LD)
-the Open-Field test (OF)

Were used to acquire various behavioural parameters widely used in neuropsychopharmacology for drug screening



OF

Light-Dark ethological test

(anxiety-like response due to conflict between tendency to exploration and aversion to light and to be alone in open space)

Main parameters:

→% Time in Lit area

→ N. of light-dark transitions

Experiments approved
by ethical committee
No pain, no artificial stress

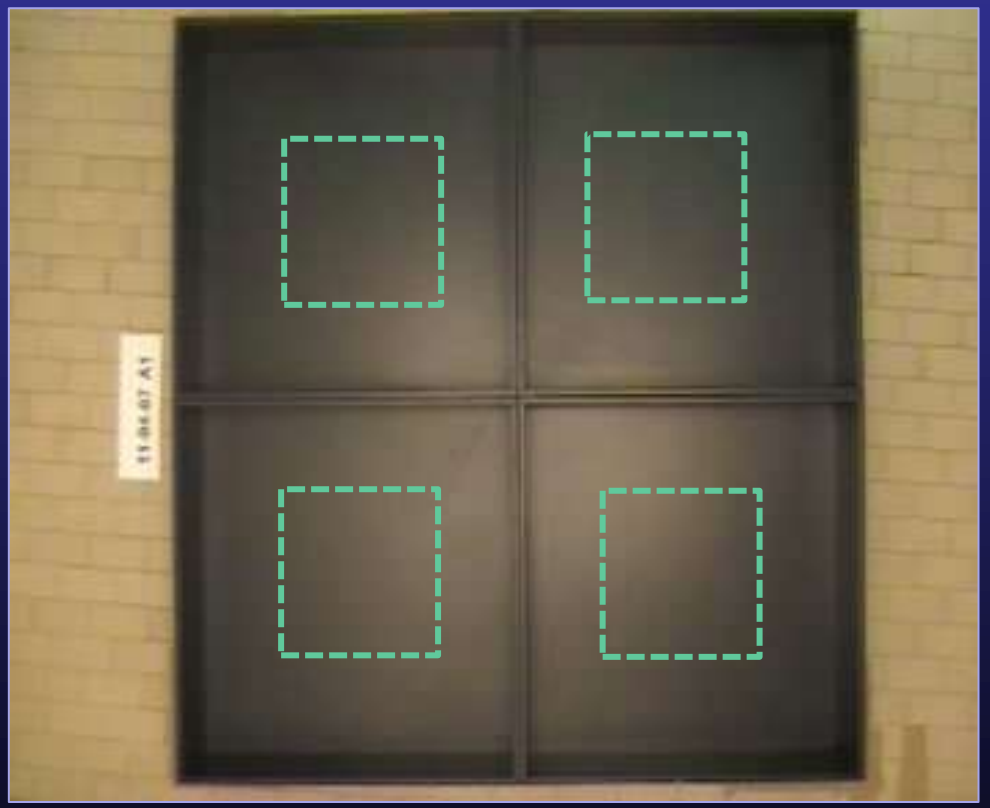
Open-Field

(anxiety-like response due to conflict between tendency to exploration and aversion to open space)

Main parameters:

→Total distance in 10 min.
("Locomotion", and/or "sedation" effects)
→% Time in central area
(exploration, anxiety like emotions)
→Distance traveled in centre
(exploration + locomotion)

→ Urine spots, stools
(Aconit and Ignatia studies)



Experiments approved by ethical committee
No pain, no artificial stress

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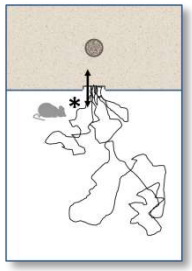
Drug screening

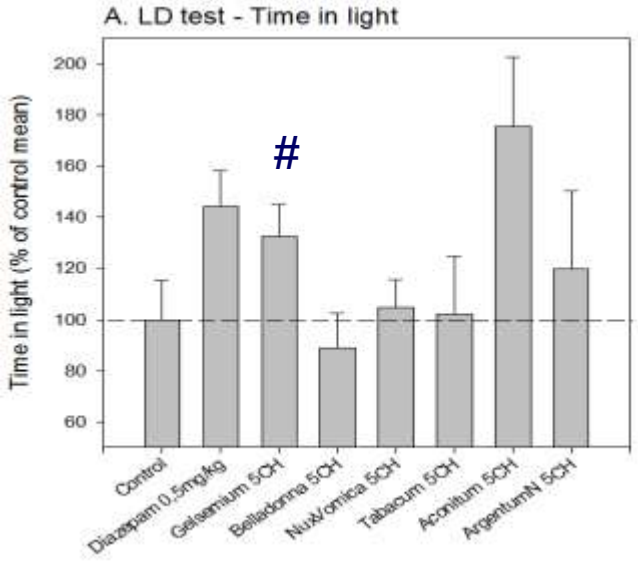
Homeopathic medicines

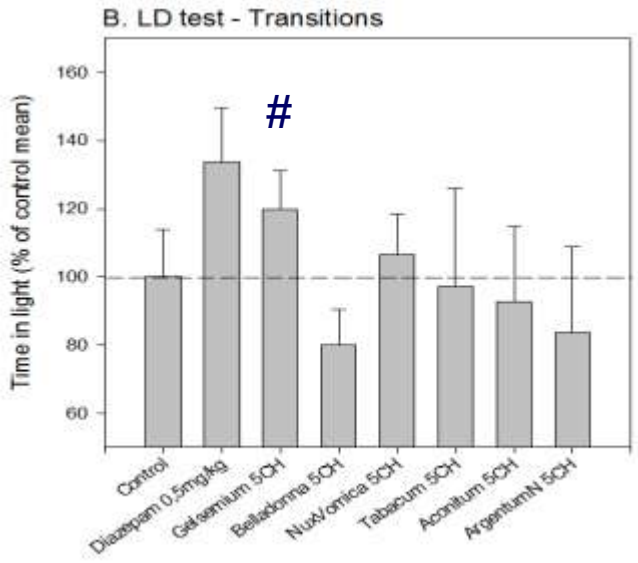
- **Aconitum,**
- **Belladonna,**
- **Gelsemium,**
- **Nux vomica,**
- **Argentum nitricum,**
- **Tabacum**
- **their control solvent hydroalcoholic (30%) solution**

We started with 5C potencies because they are high enough to be surely non-toxic and low enough in order to contain some (a few) molecules of active principle.
So, their possible action seemed more plausible to pharmacology colleagues who were available to collaboration.

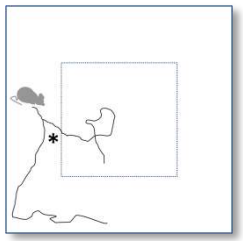
SCREENING TEST OF HOMEOPATHIC REMEDIES ON MICE BEHAVIOURAL MODELS

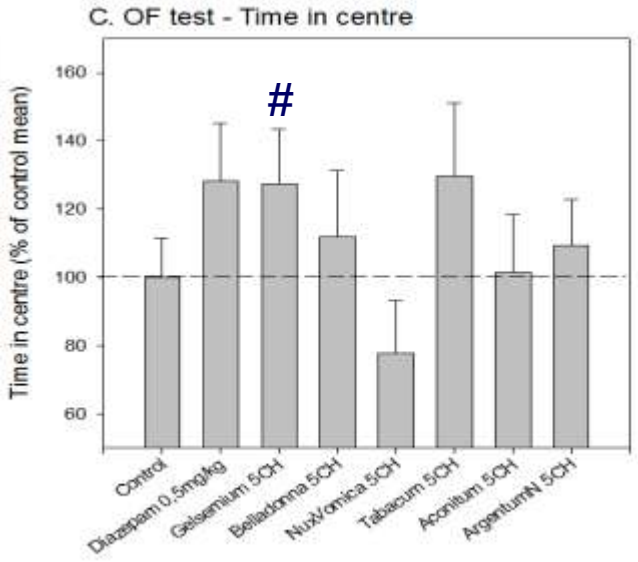


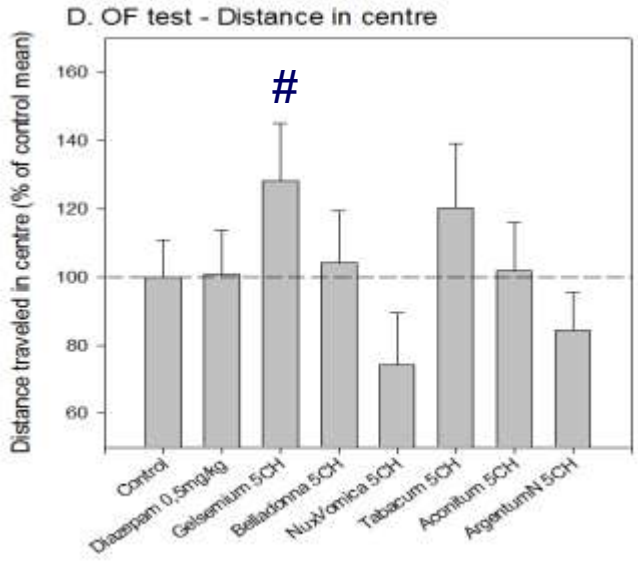




#Gelsemium.s 5C







Key points from screening

1. **Screening of six medicines suggested Gelsemium sempervirens as the most active in regulating anxiety-like behaviours in unconditioned experimental models.**

2. **High inter-individual variability of responses was found, even in the same group/strain, indicating that also in mice there is individual sensitivity to the medicine.**

3. **High inter-experiment variability was observed in Light-Dark test. We could not identify the responsible factor(s). For this reason screening test did not provide sufficient statistical power.**

4. **These preliminary studies prompted us to perform replication studies**

Replication studies

1. **First series (ECAM J 2009):**
   - →8 replication experiments with *Gelsemium s.* 5C
   - →3 replication experiments with *Gelsemium s.* 7C
   - →2 replication experiments with *Gelsemium s.* 30C
   - Positive control: 8 replications with Diazepam

2. **Second series (Psychopharmacology 2010):**
   - → 6 replication experiments with *Gelsemium s.* 4C, 5C, 7C, 9C, 30C
   - Positive control: 5 replications with Buspirone
     1 replication with Diazepam

Recent unpublished studies:

- → pooled data analysis of the two series With *Gelsemium s.*
- → 4 replication experiments with Aconitum 5C, 7C, 9C, 30C
- → 5 replications with Ignatia 4C, 5C, 7C, 9C, 30C

Note: each replication experiment lasts about 4 weeks

Gelsemium sempervirens

TRADITIONAL MATERIA MEDICA

Repertorial Materia Medica: 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**

Materia Medica (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**

Drug analysis

Fax reçu de : 0472164223
15-05-08 09:42 Pg: 1
CERTIFICAT D'ANALYSE
N° LIMS : 62477

20 rue de la Libération
F - 69110 SAINTE-FOY-LES-LYON

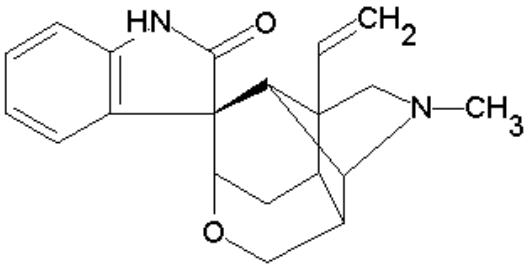
GELSEMIUM SEMPERVIRENS TM
G0B9.2TMGT1E

Date de fabrication	Quantité	N° de Lot
31 Mars 200	173.1 L	TH0082

Date de contrôle	N° de contrôle
26 Avril 20(4	C04046134

Partie Utilisée : Organes souterrains (1/10)
Description : Liquide jaune ambré, odeur aromatique.

ANALYSES	SPECIFICATIONS	RESULTATS
CARACTERES		
Couleur	conforme	conforme
IDENTIFICATION		
Chromatographie sur couche mince	conforme	conforme
ESSAI		
Teneur en éthanol	60 - 70 % V/V	63.7 % V/V
Méthanol	<0.05 %	<0.05 %
2-Propanol	<0.05 %	<0.05 %
Résidu sec	>0.50 %	1.37 %
DOSAGE		
Teneur en gelsémine	>0.010 %	0.021 %



Gelsemine
0.021% in MT

***Gelsemium* 9C: 10^{-22} Mol/L ~ 1 molecule/mouse!**
(10,000,000,000,000,000 times less than *diazepam* as control drug)
***Gelsemium* 30C: no "molecules" of gelsemine present!**

Summary of *Gelsemium s.* studies in mice

First series:

OF: significant positive effects of 5C, 7C and 30C

LD: non-significant positive effects of 5C and 30C

Diazepam active as anxiolytic in LD test, not in OF test

Second series:

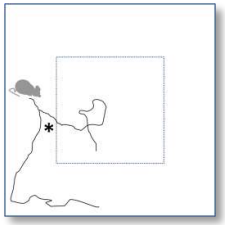
OF: non-significant positive effects of 5C, 7C , 9C and 30C

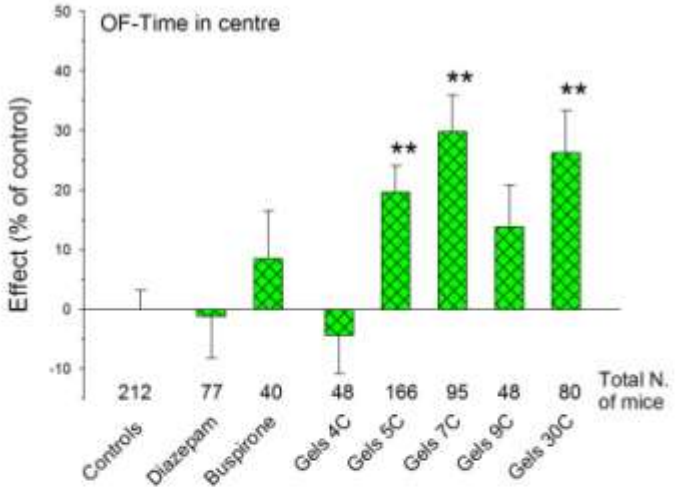
LD: significant positive effects of 5C, 9C and 30C

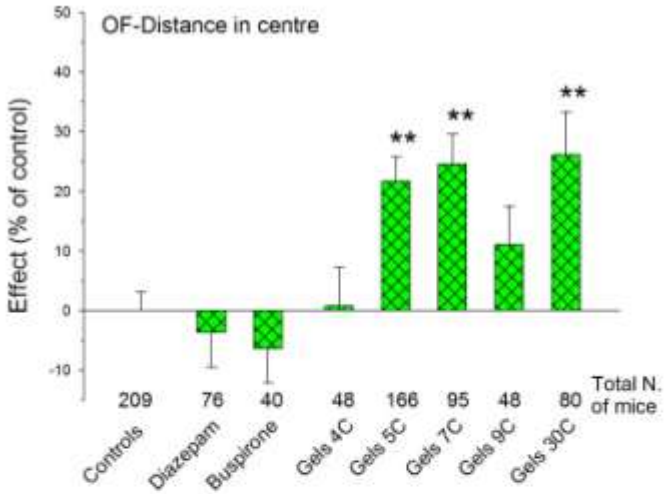
Diazepam and also Buspirone active as anxiolytic in LD test, not in OF test

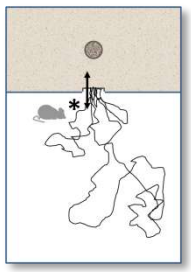
Buspirone decreases general motility (sedation effects?)

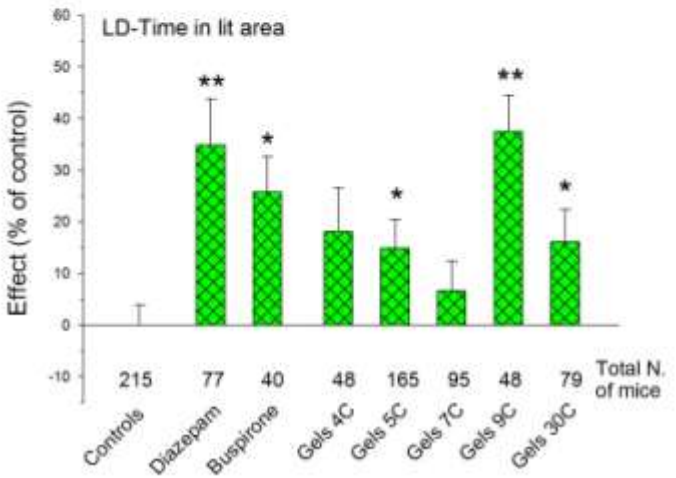
Pooled data analysis of the two series: effects on the OF and LD behavioral parameters

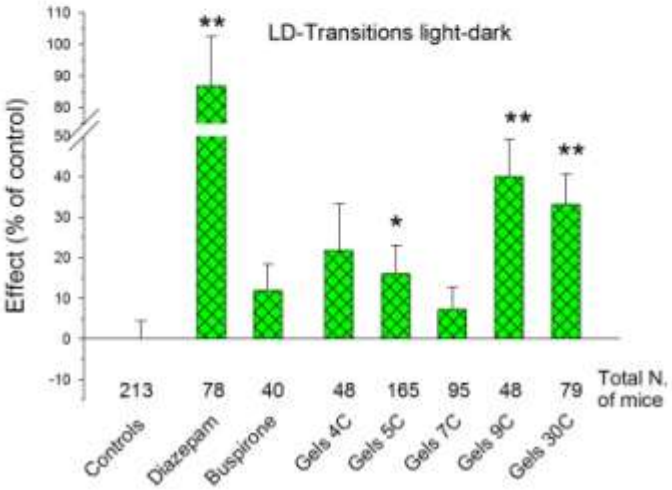








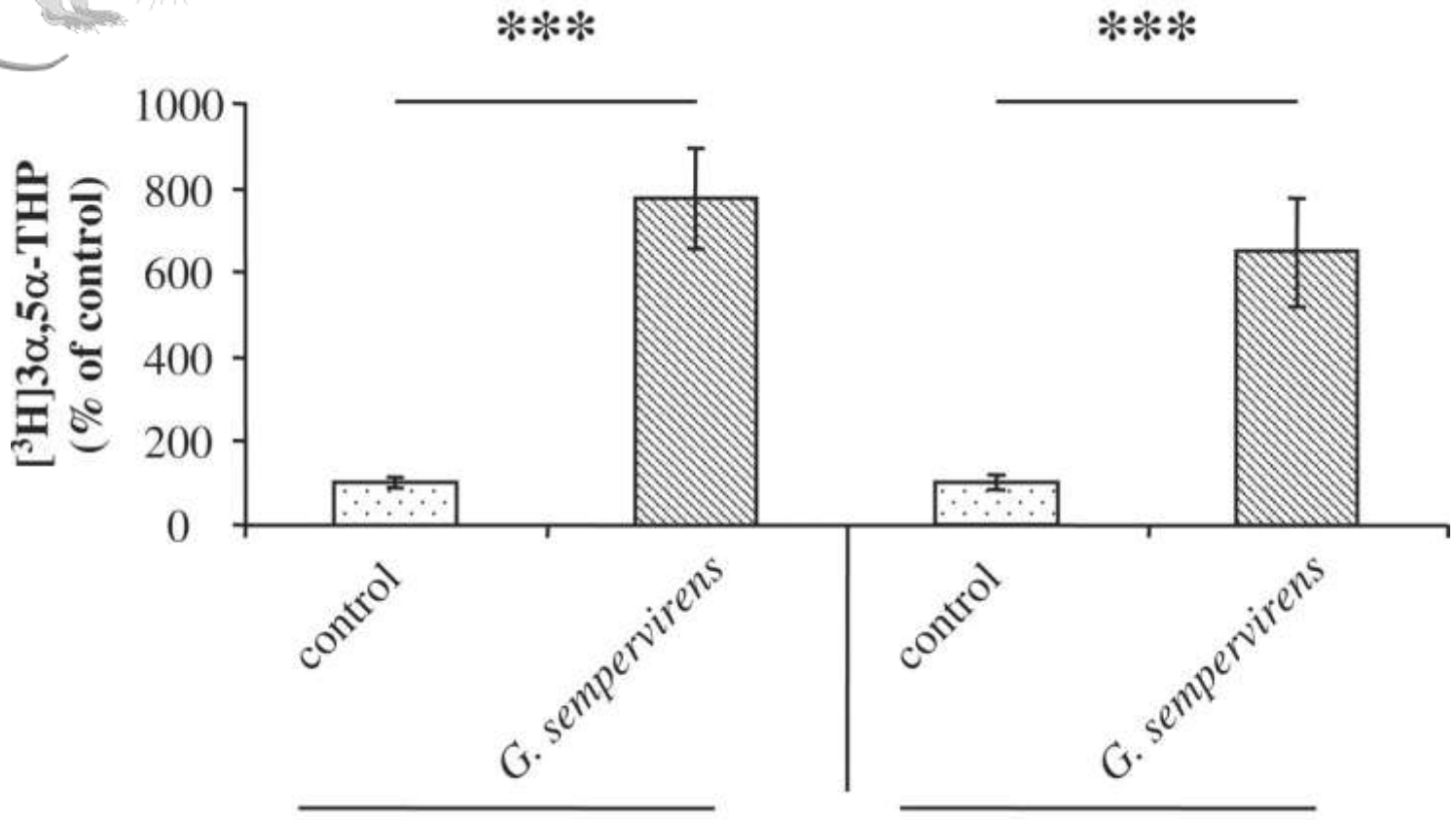




* p<0.05
** p<0.01
Significantly different from control

Neurosteroid Allopregnanolone Formation in the Spinal Cord and Limbic System

Christine Venard et al., ECAM-J 2011 (advance access online)

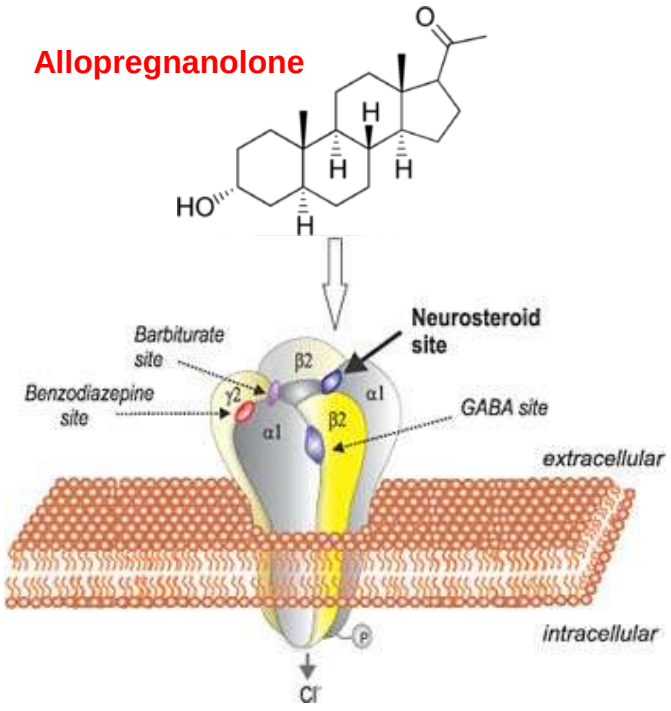


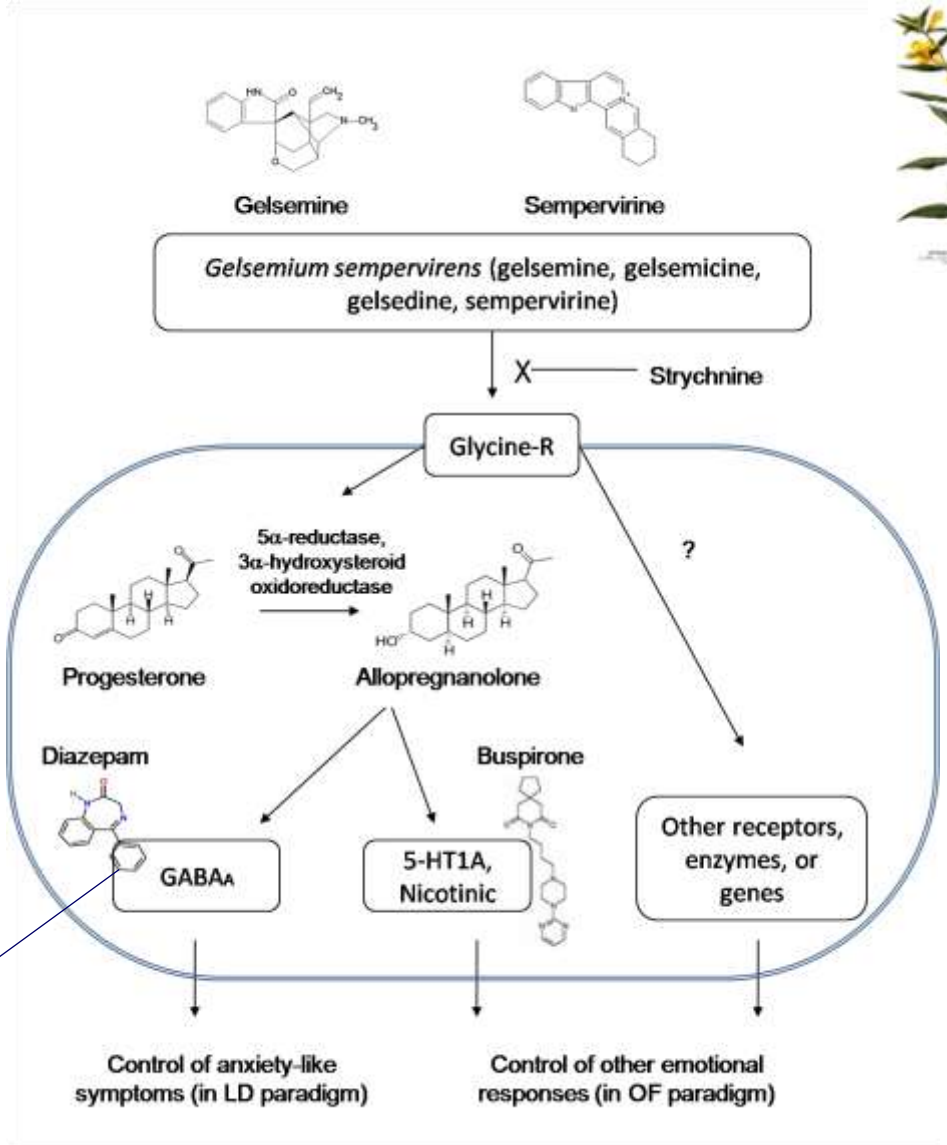
(≈10^{-22}mol/L)

(≈10^{-14} mol/L)

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

Allopregnanolone: an endogenous anxiolytic-like neurosteroid





KEY-NOTES: *Gelsemium* in mice models, pooled data analysis of 14 replications

1. ***Gelsemium sempervirens*** **improves some parameters of anxiety-like behavior significantly more than placebo in two validated test models and in rigorously blind conditions**

2. **The *Gelsemium s.* effects in mice concern a subset of emotions and symptoms which have been tested in our models:**
   aversion to open space (agoraphobia?), symptoms amelioration with movement, feeling in a danger, aversion to light

3. **The differences of results between the two series indicate that the system and/or the response to diluted/dynamized drugs are highly sensitive to experimental conditions**

4. **The effects of *Gelsemium s.* in Open Field are evident even in conditions where buspirone and diazepam are uneffective, indicating different targets and mechanisms at variance with allopathic drugs**

5. ***Gelsemium*** **has no adverse effects on locomotion nor causes sedation (an effect shown by buspirone in chronic treatment)**

6. **Thanks to the studies of Venard et al., a putative and provisional mechanism of action of *Gelsemium* is suggested at the level of the neurosteroid system**

KEY-NOTES: *Gelsemium* in mice models, pooled data analysis of 14 replications

NON-LINEARITY OF DOSE-RESPONSE (!!!)

a) All the gelsemium s. dilutions (but the 4C in OF) have positive effects in the same direction (anxiolytic-like).
This experimental evidence is encouraging for homeopathic practitioners (the choice of potency is not dramatically determinant)

b) The dilution-response patterns of pharmacological activity differ according to the experimental system:
→ In OF test, the peak of activity is 7C; moreover, 5C, 7C, 9C and 30C potencies are significantly more effective than 4C
→In LD test, the peak of activity is 9C; moreover, 9C is significantly more effective than 5C and 7C.
This evidence, if confirmed, should suggest that different symptoms could benefit of different dilutions of the same drug. In other words, the most effective dilution should be chosen in relation of the type of symptom(s).

Interesting coincidence

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Homeopathy for the panic attacks following the L'Aquila earthquake

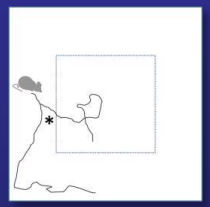
Dr. Tiziana Di Giampietro

I write to report my personal experience of treating children with panic attacks after the devastating earthquake (6.3 on the Richter scale), which struck L'Aquila, capital of the Abruzzo Region in the center of Italy at 3:32 a.m on the 6th April 2009. The city and many surrounding villages were severely damaged causing the death of 300 and injuring 1500 people. 65,000 people were forced to leave their homes for emergency camps. Many survivors had panic attacks and were emotionally disturbed.

A team of SIOMI homeopaths chose three drugs: *Arnica*, *Gelsemium* and *Ignatia*. I administered at *Arnica* 30 cH for physical and mental traumas, *Ignatia* for bitterness, restrained pain, *Gelsemium* for suppressed fear; it was very helpful for children, improving their nightmares and reducing their morbid attachment to their mothers.

The coincidence of the effect noted in our experience in the field and the evidence accumulating in rigorous laboratory studies on *Gelsemium sempervirens* is particularly provocative and stimulating for future controlled studies.[1,2] It suggested to me that I should communicate this experience to the scientific community to emphasize the need for further research.

SUMMARY OF EFFECTS OF DIFFERENT DRUGS ON LABORATORY MICE EMOTIONAL MODELS

		Buspirone	Diazepam	*Gelsemium* (14 replications)	*Aconitum* (4 replications)	*Ignatia* (5 replications)
Parameter						
 OF	Total distance	↓	↔↑	↔	↔	↔
	Time in Centre	↔	↔	↑↑ (peak 7C)	↔	↔
	Distance In centre	↔	↔	↑↑ (peak 7C)	↔	↔
 LD	Time in Light	↑↑	↑↑	↑↑(peak 9C)	↔	↑↑(9C)
	Transi-tions	↑	↑↑	↑↑(peak 9C)	↑ (5C)	↑↑(9C)
Urination		nt	↓	nt	↔	↓
Defecation		nt	↓	nt	↓	↔

The Verona “Gelsemium”study group (2008-11)

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