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](http://www.paolobellavite.it) (under “News”)
THE MAJOR TENETS OF HOMEOPATHY

“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

“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
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.

Why I feel such an anxiety...?
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 - 1\textsuperscript{st} part of 2

<table>
<thead>
<tr>
<th>Date</th>
<th>Author</th>
<th>Animal</th>
<th>Model</th>
<th>Remedy</th>
<th>Route</th>
<th>Main effects</th>
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</thead>
<tbody>
<tr>
<td>1979</td>
<td>Binsard</td>
<td>Mouse</td>
<td>4 plates</td>
<td>Ignatia and Gelsemium 3C, 4C, 5C</td>
<td>i.p. 3 weeks</td>
<td>Anxiolytic (Ignatia 3C and Gelsemium 5C only) or sedative (Ignatia 5C)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rat</td>
<td>Staircase</td>
<td>Ignatia and Gelsemium 3C, 4C, 5C</td>
<td>i.p. 3 weeks</td>
<td>Sedative (Ignatia 4C and Gelsemium 3C and 5C only)</td>
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<td>1980</td>
<td>Binsard et al.</td>
<td>Mouse</td>
<td>4 plates Escape test Rota-rod</td>
<td>Gelsemium 3C, 5C, 7C</td>
<td>i.p. 3 weeks</td>
<td>Gelsemium 3C reduces exploration, Gelsemium 7C increases it</td>
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<tr>
<td>1981</td>
<td>Guillemain et al.</td>
<td>Mouse</td>
<td>Strychnine. Induced convulsions</td>
<td>Ignatia 3D, 3,5,7,12C</td>
<td>i.p. 0.5 ml/20g single dose</td>
<td>Slight protective effect of 3D and 5C</td>
</tr>
<tr>
<td>1986</td>
<td>Sukul</td>
<td>Rat</td>
<td>Cataleptogenic effects of restraint</td>
<td>Gelsemium, Cannabis, Graphites and Agaricus Muscarius (30C and 200C)</td>
<td>Per os</td>
<td>Increase cataleptogenic effects of restraint.</td>
</tr>
<tr>
<td>1991</td>
<td>Sukul et al.</td>
<td>Rat (and cats)</td>
<td>Electrophysiology of SNC</td>
<td>Arnica 30C, Hypericum 200C, Arsenic 30C</td>
<td>Per os (0.5 ml)</td>
<td>Arnica and Hypericum decrease firing rate, Arsenic increase it.</td>
</tr>
<tr>
<td>Date Author</td>
<td>Animal</td>
<td>Model</td>
<td>Remedy</td>
<td>Route</td>
<td>Main effects</td>
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</tr>
<tr>
<td>1997 Cristea et al.</td>
<td>Mouse</td>
<td>Behavioural tests</td>
<td>Chamomilla 5C and 30C</td>
<td>Per os 4 times/day for 1 day (5C) or 2 times/day for 3 days (30C)</td>
<td>Stimulating effects with 5C and tranquillizing effects with 30C</td>
<td></td>
</tr>
<tr>
<td>1999-2001 Sukul</td>
<td>Mouse</td>
<td>Loss of righting reflex due to ethanol</td>
<td>Nux vomica 30C</td>
<td>Per os 0.05 ml/2 ml water and given at 0.05 ml/individual.</td>
<td>Protective effect</td>
<td></td>
</tr>
<tr>
<td>2005 Ruiz Vega</td>
<td>Rat</td>
<td>Sleeping behaviour</td>
<td>Coffea cruda 30C and 200C</td>
<td>Per os in feeding bottle</td>
<td>Coffea 30C changes spectral power of EEG Delta band</td>
<td></td>
</tr>
<tr>
<td>2008 Da Silva Rocha</td>
<td>Rat</td>
<td>Open field</td>
<td>Rhus toxicodendron 200C</td>
<td>Per os 24 h</td>
<td>Decreases locomotion in hyperactive rats</td>
<td></td>
</tr>
<tr>
<td>2008 Pinto</td>
<td>Mouse</td>
<td>Open field Forced swimming</td>
<td>Chamomilla 6C</td>
<td>Per os 7 days</td>
<td>Prevents decrease of general activity. In O.F.</td>
<td></td>
</tr>
</tbody>
</table>
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)
Studies of homeopathic medicines tested in animal behavioural models

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1. Introduction and literature review
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1. Homeopathic drugs (and control solvent) were provided by **Boiron Laboratories** (Lyon) in 30% hydroalcoholic 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

0.4 ml Gels 3C + 39.6 ml H₂O → shaking → 40 ml Gels 4C

0.4 ml Gels 4C + 39.6 ml H₂O → shaking → 40 ml Gels 5C

0.4 ml Gels 6C + 39.6 ml H₂O → shaking → 40 ml Gels 7C

0.4 ml Gels 8C + 39.6 ml H₂O → shaking → 40 ml Gels 9C

0.4 ml Gels 29C + 39.6 ml H₂O → shaking → 40 ml Gels 30C

0.4 ml EtOH 30% + 39.6 ml H₂O → shaking → 40 ml H₂O+EtOH 0.3%

0.4 ml EtOH 30% + 39.6 ml H₂O → shaking → 40 ml H₂O+EtOH 0.3%

0.4 ml Buspirone + 39.6 ml H₂O → shaking → 40 ml Buspirone 50mg/kg in EtOH 30%

5mg/kg in H₂O+EtOH 0.3%
All solutions were **coded** by people not involved in the research

<table>
<thead>
<tr>
<th>Medicinale</th>
<th>Numero originale</th>
<th>Lettera CODIFICATA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gels 4C</td>
<td>N.1</td>
<td>......</td>
</tr>
<tr>
<td>Gels 5C</td>
<td>N.2</td>
<td>......</td>
</tr>
<tr>
<td>Gels 7C</td>
<td>N.3</td>
<td>......</td>
</tr>
<tr>
<td>Gels 9C</td>
<td>N.4</td>
<td>......</td>
</tr>
<tr>
<td>Gels 30C</td>
<td>N.5</td>
<td>......</td>
</tr>
<tr>
<td>Placebo Non Dinamizzato</td>
<td>N.6</td>
<td>......</td>
</tr>
<tr>
<td>Placebo Non Dinamizzato</td>
<td>N.7</td>
<td>......</td>
</tr>
<tr>
<td>Buspirone 0.5 mg/ml</td>
<td>N.8</td>
<td>......</td>
</tr>
</tbody>
</table>

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

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

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22.10.2008

Schema per la codifica delle provette con i medicinali

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

……………………………….

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

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

Allopathic drug = Buspirone or Diazepam in the same hydroalcoholic solution (0.3% EtOH)
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 /Control 1
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

Experiments approved by ethical committee
No pain, no artificial stress

Tracking Calculations (in blind conditions)

Open-Field Test

Light-Dark Test

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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

A. LD test - Time in light

B. LD test - Transitions

C. OF test - Time in centre

D. OF test - Distance in centre

#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

Gelsemium 9C: $10^{-22}$ Mol/L ~ 1 molecule/mouse!
(10,000,000,000,000,000,000 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

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Neurosteroid Allopregnanolone Formation in the Spinal Cord and Limbic System

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

$[\text{H}]3\alpha,5\alpha$-THP (% of control)

control  G. sempervirens  control  G. sempervirens

$9 \text{ CH}$  $5 \text{ CH}$

$(\approx 10^{-22} \text{ mol/L})$  $(\approx 10^{-14} \text{ mol/L})$
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).
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.\textsuperscript{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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Buspirone</th>
<th>Diazepam</th>
<th>Gelsemium (14 replications)</th>
<th>Aconitum (4 replications)</th>
<th>Ignatia (5 replications)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total distance</td>
<td>↓</td>
<td>↔↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Time in Centre</td>
<td>↔</td>
<td>↔</td>
<td>↑↑ (peak 7C)</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Distance In centre</td>
<td>↔</td>
<td>↔</td>
<td>↑↑ (peak 7C)</td>
<td>↑↑</td>
<td>↑↑(9C)</td>
</tr>
<tr>
<td>Time in Light</td>
<td>↑↑</td>
<td>↑↑</td>
<td>↑↑(peak 9C)</td>
<td>↔</td>
<td>↑↑(9C)</td>
</tr>
<tr>
<td>Transitions</td>
<td>↑</td>
<td>↑↑</td>
<td>↑↑(peak 9C)</td>
<td>↑ (5C)</td>
<td>↑↑(9C)</td>
</tr>
<tr>
<td>Urination</td>
<td>nt</td>
<td>↓</td>
<td>nt</td>
<td>↔</td>
<td>↓</td>
</tr>
<tr>
<td>Defecation</td>
<td>nt</td>
<td>↓</td>
<td>nt</td>
<td>↓</td>
<td>↔</td>
</tr>
</tbody>
</table>
The Verona “Gelsemium” study group (2008-11)

Paolo Bellavite
Paolo Magnani
Elisabetta Zanolin
Marta Marzotto
Anita Conforti

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

Paolo Bellavite
Paolo Magnani
Elisabetta Zanolin
Marta Marzotto
Anita Conforti

E nós também

Nós garantimos que a homeopatia não é água!!!