

Systemic Pharmacology II (Practical)

For B. Pharm. Semester V

Course Code: BP510P

As per the new PCI syllabus 2026 (NEP 2020)

Dr. Saurabh Saklani

Assistant Professor

School of Pharmacy & Emerging Sciences, Baddi
University of Emerging Sciences and Technology,
Himachal Pradesh, India

Subhani Khan

Assistant Professor

Faculty of Pharmacy, Maharaja Agrasen Himalayan
Garhwal University, Pauri Garhwal, Uttarakhand,
India

Amisha Bhandari

Assistant Professor

Shree Dev Bhoomi Institute of Education, Science
and Technology, Poundha, Dehradun, India

Tripti Bisht

Assistant Professor

Shree Dev Bhoomi Institute of Education, Science
and Technology, Poundha, Dehradun, India

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Authors: Dr. Saurabh Saklani, Subhani Khan, Amisha Bhandari, Tripti Bisht

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Preface

Pharmacology is best understood when theoretical concepts are connected with practical observation, experimentation, and interpretation. The present book, *Systemic Pharmacology II (Practical)*, has been designed as a student-friendly practical guide for B.Pharm III Year students and is aligned with the New PCI Syllabus 2026 under NEP 2020 for BP510P.

The book aims to provide a systematic approach to understanding the practical application of pharmacological principles through carefully structured experiments, demonstrations, and simulation-based exercises. Particular emphasis has been placed on developing the student's ability to observe, record, analyse, interpret, and scientifically communicate experimental findings.

The practical exercises cover important areas of systemic pharmacology, including the study of drug effects on cardiovascular, respiratory, skeletal muscle, and gastrointestinal systems through appropriate experimental and simulation models. Experiments and demonstrations involving Langendorff's heart preparation, spasmogenic and spasmolytic activity, PA_2 value determination, analgesic and anti-inflammatory activity, antiepileptic screening, antidepressant and antipsychotic models, and other commonly used pharmacological techniques are presented in a clear and structured manner.

In keeping with contemporary trends in pharmacological education and the principles of 3Rs (Replacement, Reduction, and Refinement), appropriate emphasis has also been given to computer-based simulations and alternative experimental approaches. Students are introduced to simulation models, dose-response relationships, bioassay concepts, antagonist studies, and the interpretation of pharmacological data. The book also introduces modern approaches such as *in silico* pharmacology, ADME/toxicity prediction, pharmacological databases, and network pharmacology, helping students appreciate the evolving role of computational methods in pharmaceutical research.

Each practical has been developed to encourage conceptual understanding rather than mere procedural learning. Wherever appropriate, the content is organized around the principle, objective, requirements, procedure, observations, calculations, results, and discussion, enabling students to maintain their practical records effectively and prepare for viva voce and examinations.

We have made every effort to keep the language simple, precise, and academically appropriate, while retaining the scientific depth expected at the undergraduate level. The book is intended not only to help students successfully perform their practicals but also to develop the analytical and experimental skills that form the foundation of future pharmaceutical research and clinical understanding.

We sincerely hope that *Systemic Pharmacology II (Practical)* serves as a useful companion for students, teachers, and laboratory instructors and makes the study of practical pharmacology more structured, engaging, and meaningful.

Authors

Dr. Saurabh Saklani

Subhani Khan

Amisha Bhandari

Tripti Bisht

Course objectives

1. Provide understanding of the theoretical and practical aspects of different pharmacological experiments using virtual simulations and video demonstrations.
2. Develop skills to assess drug effects on various systems such as cardiovascular, respiratory, skeletal muscle and gastrointestinal using simulation models.
3. Familiarise the learners with experimental methodologies such as Langendorff's heart preparation, spasmogenic and spasmolytic effects, and pA₂ value determination using the Schild plot.
4. Provide knowledge of the modern techniques for drug evaluation, such as intraocular pressure measurement, nitric oxide estimation, neutrophil function tests and spirometry.
5. Explore clinical pharmacology aspects through case studies on drug interactions, management of cardiovascular and respiratory diseases, and plasma volume expanders.

Course outcomes

CO	Upon successful completion of this course, the student will be able to:
CO1	Explain the pharmacological principles and mechanisms of drug action involved in analgesic, antiepileptic, antidepressant, antipsychotic, antianxiety and antiulcer drugs using interactive computer-based experimental models.
CO2	Apply experimental and simulation-based methods to evaluate drug activity using models such as Eddy's hot plate, MES-induced seizures, tail suspension test, elevated plus maze, actophotometer, Morris water maze and ulcer models.
CO3	Analyse and interpret experimental pharmacological data, including bioassay results, dose-response relationships and estimation of agonist and antagonist concentrations using three-point and four-point bioassay, matching and bracketing methods, and simulated datasets.
CO4	Demonstrate advanced biomedical and laboratory techniques, including cell culture methods, electrophoresis of DNA and proteins, and antibacterial sensitivity testing, relevant to pharmaceutical and biomedical research.
CO5	Utilise bioinformatics tools, open-source pharmacological databases and network pharmacology software to predict drug targets, analyse ADME and toxicity profiles, and construct pharmacological networks.

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Experiment 1: Evaluation of Analgesic Activity by Eddy's Hot Plate, Tail Flick, Tail Immersion and Acetic Acid Writhing

Aim

To evaluate the analgesic activity of centrally and peripherally acting analgesics in the mouse using Eddy's hot plate, the tail flick, the tail immersion and the acetic acid-induced writhing methods, through interactive computer simulation.

Requirements

Simulation. The prescribed interactive pharmacology package with the four analgesic screening modules installed; a computer for each pair of students.

Apparatus modelled. Eddy's hot plate analgesiometer with thermostatic control and a transparent cylindrical enclosure; radiant heat tail flick analgesiometer with a photocell-triggered timer; a water bath with an immersion thermostat; stopwatch; observation chamber for writhing counts.

Drugs and solutions. Morphine sulphate 1 mg/mL; pentazocine lactate 2 mg/mL; aspirin suspension 20 mg/mL in 0.5 per cent w/v sodium carboxymethylcellulose; diclofenac sodium 2 mg/mL; naloxone hydrochloride 0.2 mg/mL; acetic acid 0.6 per cent v/v; normal saline as vehicle.

Animals modelled. Albino Swiss mice of either sex, 20 to 30 g, fasted overnight with free access to water for the writhing method.

Principle

Pain in a conscious animal cannot be measured. What can be measured is the latency of a defensive reflex to a noxious stimulus of fixed intensity, and an analgesic is detected as a lengthening of that latency. The choice of stimulus decides what kind of analgesic the method will find, and the four methods of this experiment were chosen by the syllabus precisely because they differ in that respect.

Thermal methods apply heat and measure a withdrawal reflex. In the **tail flick** method radiant heat is focused on the tail and the animal flicks it away; the reflex is organised at spinal level, and the latency is prolonged mainly by drugs acting on spinal opioid receptors. In **tail immersion** the tail is dipped in hot water, and the reflex is again spinal. In **Eddy's hot plate** method the mouse stands on a plate held at a fixed temperature and responds by licking a hind paw or by jumping; licking and jumping are organised supraspinally, so the hot plate detects drugs acting at higher centres. All three are sensitive to opioids such as morphine and pentazocine and are relatively insensitive to aspirin-like drugs, which is the diagnostic feature that identifies a centrally acting analgesic.

The chemical method applies an inflammatory stimulus instead. Dilute acetic acid injected into the peritoneal cavity releases arachidonic acid from phospholipids and generates prostaglandins locally, which sensitise nociceptive afferents and evoke a characteristic writhing: an abdominal constriction with arching of the back and extension of a hind limb. Because the response depends on prostaglandin synthesis, it is abolished by cyclo-oxygenase inhibitors, and the **acetic acid-induced writhing** test therefore detects peripherally acting analgesics. It is sensitive, and its weakness is that it is not specific, since any drug that reduces movement will also reduce the writhing count.

A cut-off time is imposed on every thermal method. An animal that has not responded by the cut-off is removed and its latency recorded as the cut-off value. The purpose is to prevent tissue injury, and the cut-off also fixes the denominator of the percentage analgesia calculation.

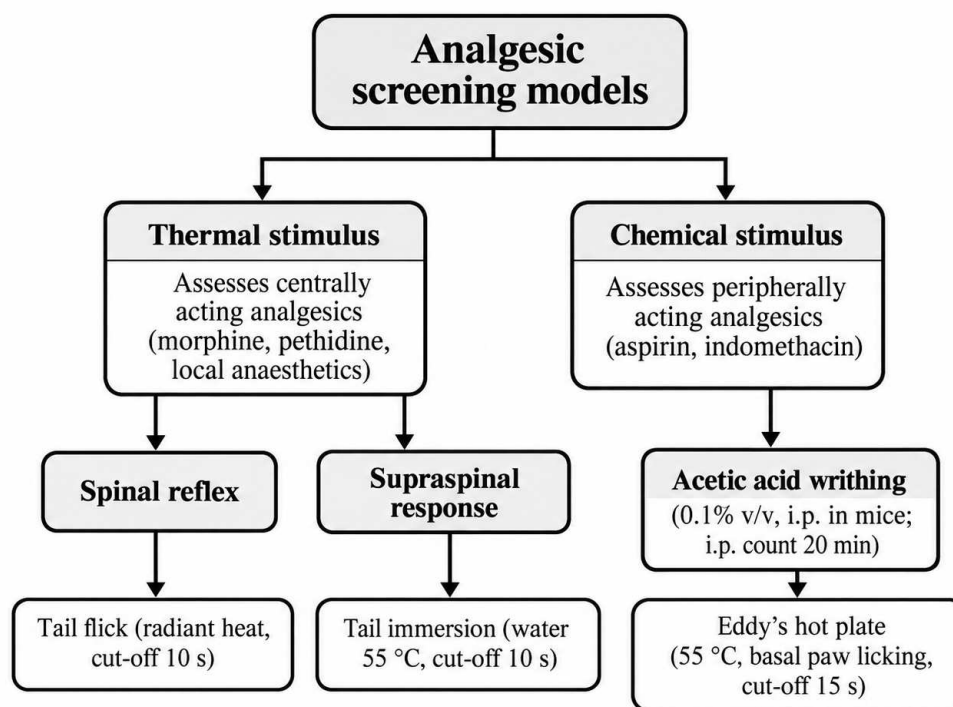


Figure 1.1 Classification of the analgesic screening methods of this experiment by the nature of the stimulus and the level at which the response is organised.

Procedure

A. Eddy's hot plate method

1. Set the plate temperature to 55 ± 1 °C and the cut-off time to **15 s**. Confirm both before the first animal.
2. Select eighteen mice and divide them into three groups of six: vehicle, standard (morphine sulphate 5 mg/kg i.p.) and test.
3. Place each mouse on the plate and record the **basal reaction time**, taken as the interval from placement to the first lick of a hind paw or a jump. Reject any animal with a basal latency outside 3 to 6 s.
4. Administer the assigned treatment.
5. Record the reaction time at **30, 60 and 90 min** after administration, removing the animal at 15 s if it has not responded.

B. Tail flick method

6. Set the radiant heat source to the intensity that gives a control latency of 3 to 5 s, and the cut-off to **10 s**.
7. Place the mouse in the holder with the distal third of the tail over the heat aperture and record the basal latency to the flick.
8. Treat as in step 4 and record latency at 30, 60 and 90 min.

C. Tail immersion method

9. Maintain the water bath at 55 ± 0.5 °C with a cut-off of **10 s**.
10. Immerse the distal 5 cm of the tail and record the time to withdrawal, before treatment and at 30, 60 and 90 min after it.

D. Acetic acid-induced writhing method

11. Divide fasted mice into groups of six: vehicle, standard (aspirin 100 mg/kg p.o., or diclofenac sodium 10 mg/kg i.p.) and test.

12. Administer the treatment and allow the stated pretreatment interval, 30 min for an intraperitoneal drug and 60 min for an oral one.
13. Inject **0.6 per cent v/v acetic acid, 10 mL/kg (i.p.)**.
14. Place the mouse in the observation chamber, allow **5 min** to elapse, then count the number of writhes over the next **20 min**. This is the usual convention; windows of 10, 20 and 30 min all appear in the literature, so the window used is stated in the record.

Observations

Table 1.1 Specimen observations, Eddy's hot plate (for reference).

Group	Treatment and dose	Basal reaction time (s)	At 30 min (s)	At 60 min (s)	At 90 min (s)
I	Normal saline, 10 mL/kg (i.p.)	3.6 ± 0.2	3.7 ± 0.2	3.5 ± 0.3	3.6 ± 0.2
II	Morphine sulphate, 5 mg/kg (i.p.)	3.5 ± 0.2	10.4 ± 0.6	13.1 ± 0.7	9.2 ± 0.5
III	Test compound, 20 mg/kg (i.p.)	3.4 ± 0.3	5.9 ± 0.4	7.6 ± 0.5	6.1 ± 0.4

Mean ± SEM, n = 6. Specimen values typical of the simulation; cut-off 15 s.

Table 1.2 Record of observations, thermal methods (to be completed).

Group	Treatment and dose	Basal latency (s)	At 30 min (s)	At 60 min (s)	At 90 min (s)	% analgesia at peak
I						
II						
III						

Table 1.3 Record of observations, acetic acid writhing (to be completed).

Group	Treatment and dose	Writhes in 20 min, animal 1	2	3	4	5	6	Mean ± SEM	% inhibition
I									
II									
III									

Calculations

Percentage analgesia for a thermal method, using the mean latency at the time of peak effect:

$$\% \text{ analgesia} = (T_{\text{test}} - T_{\text{control}}) / (T_{\text{cut-off}} - T_{\text{control}}) \times 100$$

Worked example. For the morphine group at 60 min in Table 6.1: $T_{\text{test}} = 13.1$ s, $T_{\text{control}} = 3.5$ s, cut-off = 15 s.

$$\% \text{ analgesia} = (13.1 - 3.5) \div (15 - 3.5) \times 100 = 9.6 \div 11.5 \times 100 = \mathbf{83.5 \text{ per cent.}}$$

For the test compound at 60 min: $(7.6 - 3.4) \div (15 - 3.4) \times 100 = 4.2 \div 11.6 \times 100 = \mathbf{36.2 \text{ per cent.}}$

Percentage inhibition of writhing:

$$\% \text{ inhibition} = (M_{\text{control}} - M_{\text{treated}}) / (M_{\text{control}}) \times 100$$

Worked example. Control mean 42.0 writhes, aspirin-treated mean 15.0 writhes. % inhibition = $(42.0 - 15.0) \div 42.0 \times 100 = 64.3$ per cent.

Result

The peak analgesic effect of the standard and of the test compound was observed at _____ min. Percentage analgesia at peak was _____ per cent for the standard and _____ per cent for the test compound on the hot plate, and percentage inhibition of writhing was _____ per cent and _____ per cent respectively.

Conclusion and interpretation

A compound that prolongs latency on the hot plate and the tail flick and whose effect is reversed by naloxone is acting centrally through opioid receptors. A compound that leaves thermal latency unchanged but reduces the writhing count is acting peripherally, most probably by inhibiting cyclo-oxygenase. A compound active in both is either a mixed agent or is producing sedation, and the actophotometer of Experiment 5 is the control that separates the two: a drug that reduces writhing only because the animal is barely moving will also reduce locomotor counts.

Precautions

1. Hold the hot plate at 55 ± 1 °C throughout. A drop of two degrees roughly doubles the control latency and destroys the comparison.
2. Observe the cut-off strictly. An animal left on the plate beyond 15 s sustains injury, and the latency ceases to be a measure of nociception.
3. Reject animals whose basal latency lies outside the acceptable range, since an insensitive animal cannot show a further increase.
4. Use the same observer for all counts and latencies in one session, because the endpoint is judged by eye.
5. Prepare the acetic acid fresh at 0.6 per cent v/v. A stronger solution causes peritoneal irritation severe enough to suppress movement altogether.
6. Allow the full 5 min latent period before counting writhes, and count for exactly 20 min.

Viva voce

1. Why is the hot plate said to detect centrally acting analgesics?

The endpoints scored, licking of a hind paw and jumping, are complex responses organised at supraspinal level, so a drug must act above the spinal cord to abolish them.

2. How does the tail flick differ from the hot plate in the level of the reflex?

The tail flick is a spinal reflex and can be elicited in a spinalised animal, whereas the hot plate endpoints require supraspinal integration.

3. Why is aspirin inactive on the hot plate but active in the writhing test?

Aspirin inhibits cyclo-oxygenase and reduces prostaglandin-mediated sensitisation of peripheral nociceptors. The hot plate applies a brief thermal stimulus that does not depend on prostaglandin sensitisation, so aspirin has little effect on it.

4. What is a writhing?

A constriction of the abdomen with arching of the back, extension of the hind limbs and inward turning of the hind paws, following intraperitoneal injection of an irritant.

5. Why is a 5 min latent period allowed before counting?

The response develops over the first few minutes after injection, and counting during that time gives an irregular and unrepresentative rate.

Experiment 2: Evaluation of Antiepileptic Activity by Maximal Electroshock-Induced Seizures

Aim

To evaluate the antiepileptic activity of a test compound against maximal electroshock (MES)-induced seizures in the mouse, through interactive computer simulation.

Requirements

Simulation. The prescribed package with the MES module; electroconvulsimeter with settings for current, duration and electrode type.

Apparatus modelled. Electroconvulsimeter delivering an alternating current of fixed intensity for a fixed duration through corneal or ear-clip electrodes; stopwatch; observation chamber.

Drugs and solutions. Phenytoin sodium 5 mg/mL; carbamazepine suspension 10 mg/mL; normal saline as vehicle; 0.9 per cent w/v saline drops for corneal electrodes.

Animals modelled. Albino Swiss mice of either sex, 20 to 30 g.

Principle

The maximal electroshock test is the standard laboratory model of the **generalised tonic-clonic seizure**, and a drug active in it is predicted to be active clinically against that seizure type. A current well above the threshold for convulsion is passed briefly through the brain, and the resulting seizure runs through four phases in a fixed order.

Tonic flexion, lasting about 2 to 3 s, in which the fore and hind limbs are drawn towards the body. **Tonic extension**, lasting about 10 to 12 s, in which the hind limbs are extended rigidly at an angle to the trunk; this is the **tonic hind limb extension (THLE)** phase. **Clonus**, a period of rhythmic jerking lasting 10 to 15 s. **Stupor and recovery**, during which posture and righting return over 1 to 2 min.

The endpoint is the tonic hind limb extension phase, and the criterion of protection is its **complete abolition**. This choice is deliberate. Shortening of the extensor phase is graded and hard to score reproducibly, whereas its presence or absence is unambiguous. The endpoint is therefore quantal, the result is expressed as the percentage of animals protected, and no mean is calculated for it.

Phenytoin and carbamazepine act by producing a use-dependent block of the voltage-gated sodium channel, which prolongs its inactivated state and limits the high-frequency repetitive firing that sustains the tonic phase. This is why they abolish tonic extension and why they are the standards for this test. **Note the exception:** ethosuximide, which is effective in absence seizures, is inactive against maximal electroshock, and the same drug protects against pentylenetetrazol in Experiment 3. The two experiments together separate the two seizure types, and that separation is the reason the syllabus prescribes both.

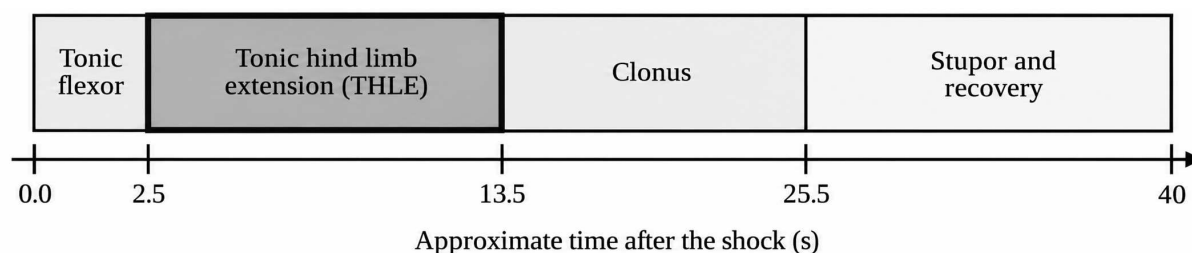


Figure 2.1 The four phases of a maximal electroshock seizure in the mouse, with the tonic hind limb extension phase identified as the scored endpoint.