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Review Article

LABORATORY MODELS FOR SCREENING ANALGESICS

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ABSTRACT

Pain is a complex unpleasant phenomenon composed of sensory experiences that include time, space, intensity, emotion, cognition and motivation. Analgesics are the agents, which selectively relieve pain by acting in the CNS or by peripheral pain mechanisms without significantly altering consciousness. Analgesics may be narcotic or non-narcotic. The study of pain in animals raises ethical, philosophical and technical problems. Philosophically, there is a problem that pain cannot be monitored directly in animals but can only be measured by examining their responses to nociceptive stimuli. The observed reactions are almost always motor responses ranging from spinal reflexes to complex behavior. The animal models employed for screening of analgesic agents, include Pain-state models based on the use of thermal stimuli, mechanical stimuli, electrical stimuli and chemical stimuli. The neuronal basis of most of the above laboratory models is poorly understood, however their application is profitable in predicting analgesic activity of newly discovered substances.

Keywords: Analgesics, Nociception, Thermal, Mechanical, Chemical.

INTRODUCTION

Pain is a complex unpleasant phenomenon composed of sensory experiences that include time, space, intensity, emotion, cognition and motivation originating from damaged tissue or abnormal physiological condition.

Table 1. Pain Terminology

Terms	Definition
Nociception	The neural processes of encoding and processing noxious stimuli.
Noxious stimulus	An actually or potentially tissue-damaging event.
Nociceptive pain	Pain arising from activation of nociceptors.
Neuropathic pain	Pain arising as a direct consequence of a lesion in the neuron or disease affecting the somato-sensory system.
Allodynia	Pain in response to a non-nociceptive stimulus.
Hyperalgesia	Increased pain sensitivity.
Pain threshold	The minimal intensity of a stimulus that is perceived as painful.

The animal models employed for screening of analgesic agents, include Pain-state models based on the use of thermal stimuli, chemical stimuli, mechanical stimuli and electrical stimuli. The neuronal basis of most of the above laboratory models is poorly understood, however their application is profitable in predicting analgesic activity of newly discovered substances. Analgesics are those agents, which selectively relieve pain by acting in the CNS or peripheral pain mechanisms without significantly altering consciousness. Analgesics may be Narcotic or Non-narcotic.

Table 2. Narcotic analgesics vs Non-narcotic analgesics

Narcotic Analgesics	Non-Narcotic Analgesics
Act centrally.	Act peripherally.
Cause addiction.	Do not cause addiction.
Produce CNS depression.	Do not produce CNS depression.
Do not produce gastric irritation.	Produce gastric irritation.
Show no anti-inflammatory effect.	Show anti-inflammatory effect.
e.g. Morphine, Tramadol Pethidine etc.	e.g. Diclofenac, Ibuprofen, Aspirin etc.

EXPERIMENTAL MODELS

The animal models employed for screening of analgesic agents include -

A) Pain-state models using Thermal stimuli

i) The tail-flick model using radiant heat/ Immersion of the tail in hot water.
ii) Paw-withdrawal test.
iii) Hot-plate test.
iv) Pain-state models using Cold- stimuli

B) Pain-state models using Mechanical stimuli

i) Strain gauges.
ii) Von-Frey filaments.
iii) Inclined-plane test.

C) Pain-state models using Electrical stimuli

i) Electrical stimulation of the tail.
ii) Grid-shock test.
iii) Stimulation of the tooth pulp.
iv) Monkey-shock titration test.
v) Stimulation of the limbs.

D) Pain-state models using Chemical stimuli

i) Formalin test.
ii) Acetic acid induced writhing test.
iii) Stimulation of hollow organs.

A) PAIN-STATE MODELS USING THERMAL STIMULI

Heat is a suitable stimulus for activating cutaneous receptors. The source of nociceptive stimulation can be distant from its target (e.g., radiant heat from a lamp) in direct contact with the skin. Radiant heat constitutes a relatively selective stimulus for nociceptors and has an advantage over the other modes of thermal stimulation in that it produces no tactile stimulus.

i) The Tail-flick models

"The tail-flick" method is a commonly accepted model for quantifying analgesic response in animals. In this model, radiant heat is applied to a small surface of the tail or the tail is immersed in hot water.

a) Tail-flick model using radiant heat

Principle: The tail-flick test with radiant heat is an extremely simplified version of the method used on human subjects[1]. Indeed, Hardy and his colleagues eventually used the

technique in rats[2]. The application of thermal radiation to the tail of an animal provokes the withdrawal of the tail by a brief vigorous movement[3]. The withdraw of the tail from the heat source is referred to as "tail-flick latency". In this model a timer is started at the same time as the application of the heat source and time taken by the rat to withdrawal its tail is recorded. Usually withdrawal time is within 2 to 10s. The lengthening of this reaction time by the animal seen after the administration of a drug is interpreted as an analgesic action. It is advisable not to prolong the exposure to radiant heat beyond 20s because the skin of the tail may be burnt. A rheostat is inserted in the apparatus so as to control the intensity of the current passing through the filament, which further controls the intensity of radiant heat. From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics but not of opioid partial agonists. This test is more sensitive to morphine, when the distal part of the tail is stimulated than, when a more proximal part of the tail is stimulated, with the middle part giving an intermediate effect (**Fig. 1).**

Merits

- This method is very effective for screening morphine-like analgesics.
- The technique is simple and does not require any special skill.
- The results of experiments are quite accurate and less time consuming.

Demerits

- The tail-flick response is prone to habituation. The tail-flick response is not consistent upon repetitive stimulation**.** Habituation is observed with a shortening of the inter-stimulus interval and increased intensity of heat.
- From a pharmacological point of view, there is a consensus that this test is truly efficient only for revealing the activity of opioid analgesics (but not of opioid partial agonists).
- It is advisable not to prolong the exposure to radiant heat beyond 20 s because the skin of the tail may be burnt.

Modification

- Ultrasound-induced tail-flick procedure.
- Ear withdrawal by applying radiant heat.
- The Tail-Flick Formalin Test in Rodents: Changes in Skin Temperature.



Fig. 1 Tail flick model

b) Tail-flick model using immersion of the tail

Principle: The use of immersion of the tail is apparently a variant of the test described above. The most obvious difference is that the area of stimulation is far greater. Immersion of an animal's tail in hot water provokes an abrupt movement of the tail and sometimes the recoiling of the whole body. Again, here it is the reaction time that is measured. This test can be used on monkeys and some investigators have used cold instead of hot stimuli.

ii) Paw-withdrawal test

Principle: This test is entirely comparable to the test of D'Amour and Smith (1941)[4] but offers the advantage that it does not involve the pre-eminent organ of thermoregulation in rats and mice, i.e., the tail [5]. In this test radiant heat is applied to a paw that had already been inflamed by a subcutaneous injection of carrageenin. An inflammation can also be produced by exposure to ultraviolet rays[6]. One advantage in these tests is that heat is applied to a freely moving animal. (**Fig. 2**)



Fig. 2 Paw withdrawal model

iii) Hot – plate model

Principle : This test consists of introducing a rat or mouse into an open-ended cylindrical space with a floor consisting of a metallic plate that is heated by a thermode or a boiling liquid[7]. A plate heated to a constant temperature produces two behavioral components that can be measured in terms of their reaction times, namely paw licking and jumping. Both are considered to be supra-spinally integrated responses. As far as analgesic substances are concerned, the paw licking behavior is affected only by opioids. On the other hand, the jumping reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol, especially when the temperature of the plate is 50°C or less or if the temperature is increased in a progressive and linear fashion, e.g., from 43 to 52°C at 2.5°C/min[8]. The specificity and sensitivity of the test can be increased by measuring the reaction time of the first evoked behavior regardless of whether it is paw-licking or jumping, or by lowering the temperature. The behavior is relatively stereotyped in the mouse but is more complex in the rat, which sniffs, licks its forepaws, licks its hind paws, straightens up, stamps its feet, starts and stops washing itself, among other things. These behaviors have been labeled "chaotic defensive movements" [9] (**Fig. 3**).

Demerit: The sedatives, muscle relaxants or psychomimetics yield false positive results, while partial opiate agonist-antagonists produce unreliable results.



Fig. 3 Hot plate model

iv) Pain-state models using cold stimuli

Cold stimuli is very rarely used to test acute pain, but it is more common to test cold allodynia in animal models of neuropathies.

B) PAIN- STATE MODELS USING MECHANICAL STIMULI

The preferred sites for applying nociceptive mechanical stimuli are the hind paw and the tail. Tests using constant pressure have been abandoned progressively for those applying gradually increasing pressures[10]. In the course of such a test, a pressure of increasing intensity is applied to a punctiform area on the hind paw or, far less commonly, on the tail. The paw or tail is jammed between a plane surface and a blunt point mounted on top of a system of cogwheels with a cursor that can be displaced along the length of a graduated beam[11]. These devices permit the application of increasing measurable pressures and the interruption of the test, when the threshold is reached. The measured parameter is the threshold (weight in grams) for the appearance of a given behavior. When the pressure increases, the reflex withdrawal of the paw, or a complex movement of the animal to release its trapped limb and finally a vocal reaction is observed. This type of mechanical stimulation has a certain number of disadvantages 1) it is sometimes difficult to measure the intensity of the stimulus with precision; 2) repetition of the mechanical stimulus can produce a diminution or conversely an increase in the sensitivity of the stimulated part of the body, in the latter case, this carries the risk that the tissues may be altered by inflammatory reactions that could call into question the validity of repeated tests; 3) the necessity of applying relatively high pressures, which explains the weak sensitivity of the method and the relatively small number of substances that have been shown to be active by this test; and 4) a non-negligible level of variability of the responses. With the aim of improving the sensitivity of the test, Randall and Selitto (1957) proposed comparing thresholds observed with a healthy paw and with an inflamed paw[12]. The inflammation was induced beforehand by a subcutaneous injection into the area to be stimulated of substances such as croton oil, beer yeast, or carrageenin, the last of these being the most commonly used today[13]. Even though it was found that the sensitivity of the method was improved, it was to the detriment of its specificity because, a priori, two different pharmacological effects viz. analgesic and anti-inflammatory could be confused. It is therefore quite difficult to state that there has been analgesic or even "analgesic" activity. However, a comparison in the same animal of responses triggered from a healthy and an inflamed paw allows this problem to be overcome: non-steroidal anti-inflammatory drugs (NSAIDs) are inactive on the former but do increase the (lowered) vocalization threshold when pressure is applied to the latter[14]. One can increase the discrimination between different analgesic substances with this test by reducing the rate at which the pressure applied to the paw is increased and by increasing the time limit for subjecting the animal to the stimulus (cut-off time).[15]

C) PAIN- STATE MODELS USING ELECTRICAL STIMULI

i) Electrical Stimulation of the tail

Electrical stimuli of gradually increasing intensities can be applied in sequence (lasting for some milliseconds) through subcutaneous electrodes placed in the tail of the rat or the mouse[16]. When such gradually increasing intensities of electrical stimuli are applied from constant voltages 40-50V, one can observe a reflex movement of the tail, vocalization at the time of stimulation, and then vocalization continuing beyond the period of stimulation. Morphine or morphine like drugs are effective in this model. There may be chances of death of the animal due to the electrical current.

Modification : Ultrasonic stimulation of the tail may be used in place of electrical stimulation. This method is fast, simple and precise. The stimulation can be applied repeatedly without causing injury to the tissue.

ii) Grid-shock model

Principle and procedure : Male mice weighing around 18 to 20 g are individually placed in clear plastic chambers. The floor of the box is wired with tightly strung stainless steel wire, spaced about 1 mm apart. The stimulus is given in the form of square wave pulses, 30 cycles per second with duration of 2 ms per pulse. The output of the stimulator has to be connected to alternate wires of the grid (**Fig 4**). A fixed resistance is placed in series with the grid and in parallel to an oscilloscope to allow calibration in milliamperes. With increasing shock intensities the mice flinch, exhibit a startling reaction, increase locomotion or attempt to jump. This behavior is accurately reflected on the oscilloscope by marked fluctuations of the displayed pulse and defined as pain threshold response. Pain thresholds are determined in each individual mouse twice before administration of the test drug and 15, 30, 60, 90 and 120 min after dosing .[17]

Modification: In a "fractional escape" procedure, animals are trained to press a lever to reduce the intensity of the shock delivered continuously through the floor grids of the experimental chamber. Each time, the rat depresses the lever, it reduces the intensity of the shock. An external timer is programmed to increase the intensity of the shock every few seconds. If the animal fails to press the lever, the shock continues to increase in intensity until lever-pressing behavior drives it down. Thus, the level of shock fluctuates depending on the rat's lever pressing. The action of an analgesic in altering the level of shock, which the rat will "tolerate" can then be measured by comparing the average level at which the rat maintains the shock under control conditions with the average level at which the rat maintains the shock after analgesic treatment.

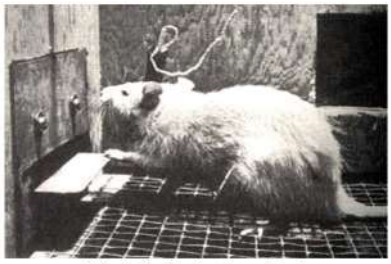


Fig.4 Grid-shock method

iii) Electrical stimulation of the Tooth-pulp

Principle and procedure: This method is based on the stimulation of the tooth-pulp of the animal by applying electric current. Stimulation of the tooth-pulp produces characteristic reactions such as licking, biting, chewing and head flick due to induction of pain, which can be observed easily. Rabbits of either sex are anaesthetized with 15 mg/kg thiopental or 0.2 mg/kg fentanyl-citrate i.v. Pulp chambers are exposed close to the gingival line in the lateral margins of the two front upper incisors with a high-speed dental drill. On the day of the experiment, clamping electrodes are placed into the drilled holes. After an accommodation period of

30min, stimulation is started to determine the threshold value. The stimulus is applied by rectangular current with a frequency of 50 Hz for duration of 1 s. The electrical current is started with 0.2 mA and increased until the phenomenon of licking occurs. In some cases, the current has to be increased and then decreased again in order to find the appropriate threshold.

Modification: The micro infusion of bradykinin solution on to the tooth pulp serves as a reliable method for evaluating analgesic potencies of drugs on trigeminal pain.

iv) Monkey - shock titration test

In this model, the monkeys are seated in restraining chairs. Electrical current is delivered by a Coulbourn Instrument Programmable Shocker through electrodes coupled to two test tube clamps, which are attached to a shaved portion of the tail. The current ranges from 0 to 4 mA through 29 progressive steps. The monkey presses a bar to interrupt the shock. A stable baseline shock level is established for each monkey on the day prior to drug administration. After drug administration, shock titration activity is rated according to the change in maximum level of median shock intensity attained for drugs as compared to control levels. Doses of 3.0 mg/kg i.m. morphine, 1.7 mg/kg i.m. methadone and 10 mg/kg i.m. pentazocine were found to be effective.[18]. However, the monkey shock titration test may be used for final evaluation of a new compound before administration to man. For screening activities the procedure can not be recommended since the test is too time consuming and the apparatus too complicated.

v) Stimulation of the Limbs

Principle : Electromyographic recordings of nociceptive limb reflexes have been used for pharmacological studies of nociception, but they are far less common than behavioral tests. These electromyographic studies have allowed the quantification of responses regardless of whether there is any movement.

D) PAIN-STATE MODELS USING CHEMICAL STIMULI

Chemical stimulation involving the administration of algogenic agents represents a slow, progressive and irreversible form of stimulation. Without doubt, these experimental models are the closest in nature to clinical pain.

i) Formalin test

Principle : The formalin test in rats has been proposed as a chronic pain model, which is sensitive to centrally active analgesic agents. Formalin is injected in to the front paw and reaction is recorded as excessive licking and biting of the paw. Analgesic response or protection is indicated, if both paws rest on the floor. The term formalin usually means a 37% solution of formaldehyde. 0.5 to 15% solution of formalin, when injected into the dorsal surface of the rat forepaw provokes a painful behavior that can be assessed on a four-level scale related to posture: 0 denotes normal posture; 1 denotes the injected paw remains on the ground but not supporting the animal; 2 denotes the injected paw clearly raised; and 3 denotes the injected paw being licked, nibbled, or shaken.

ii) Acetic acid induced Writhing

Principle : Pain is often induced in rats or mice by injecting certain irritants such as phenyl quinone or acetic acid into the peritoneal cavity. The animal reacts with a characteristic stretching behavior which is called writhing. ("Writhing Test"). The intraperitoneal administration of agents that irritate serous membranes elicits a stereotyped behavior in the mouse/rat, which is characterized by abdominal contractions, movements of the body as a whole (particularly of the hind paws), twisting of dorso-abdominal muscles, and motor in-coordination. Generally, the measurements are of the occurrence of abdominal cramps per unit time resulting from the injection of the algogenic agent. Unfortunately, the frequency and intensity of cramps decrease spontaneously with the passage of time to such an extent that it is impossible to evaluate the efficacy of an analgesic on a single animal[19] .

Modification: Many modifications have been made in the original test by employing phenylbenzoquinone and acetylcholine in place of acetic acid. Modifications have also been made to the concentration, temperature, and volume of the injected solution, the experimental conditions, and ways of monitoring behavioral changes so as to simplify the test and increase its sensitivity. Mechanical hyperalgesia was induced by injection of substance P and capsaicin in the rat knee joint and the download force tolerated by the injected leg was measured. Hyperalgesia could also be produced by intraplantar injection in the hind paw of rats of various agents, like bradykinin, carrageenin, LPS, PGE2, dopamine, TNFα, IL-1β, IL-6 and IL-8. A constant pressure of 20 mm Hg was applied to the hind paws and discontinued when the rats presented a typical freezing reaction. Sub-plantar injection of 0.1 µg of serotonin in the rat results in a brief period (up to 20 min) of increased pain sensitivity to an applied force (hyperalgesia), which precedes a longer period of decreased pain sensitivity (hypoalgesia). This phenomenon is used for pharmacological characterization of the algesic response. Similarly, a biphasic algesic behavior after sub-plantar injection of 250 µg of trypsin was described by Vinegar et al [20].

iii) Stimulation of Hollow Organs

True visceral pain can be produced in animals by injecting algogenic substances directly into hollow organs. For example, administration of formalin into the rat colon can produce a complex biphasic type of "pain behavior" involving an initial phase of body stretching and contraction of either the flanks or the whole body and a second phase that predominantly involves abdominal licking and nibbling.[21]. Intra-colonic infusions of glycerol also produce abdominal constrictions[22]. Similarly, a number of models have been developed for bladder pain, whereby reflexes and/or more complex behaviors have been observed following intra-vesical administration of capsaicin [23] or turpentine[24]. A model for inflammatory uterine pain was developed, whereby intrauterine injections of mustard oil produced complex behavior patterns in rats[25]. Arguably, a more natural noxious visceral stimulus is that produced by distension of hollow organs. In this context, colo-rectal distension by means of an inflatable balloon in the rat is the most commonly used stimulus. In addition, colonic distension has been used in models involving other species, including the rabbit[26].

CONCLUSION

Algesia implies experience related to pain, whereas hyper-algesia indicates increased sensitivity to pain. Analgesics are the agents, which selectively relieve pain by acting in the CNS or by peripheral pain mechanisms without significantly altering consciousness. Analgesics may be narcotic or non-narcotic. Non-narcotic analgesics such as aspirin, ibuprofen, paracetamol etc. possess not only anti-inflammatory properties but also antipyretic activity besides analgesic activity. For many of them the mode of action has been elucidated as an inhibition of cyclooxygenase in the prostaglandin pathway. Nevertheless, new peripheral

analgesics have to be tested not only for their in vitro activity on cyclooxygenase, but also for their in vivo analgesic potential. The most commonly used methods for measuring peripheral analgesic activity are the writhing tests in mice (various modifications) and the RANDALL SELITTO- test in rats. The study of pain in animals raises ethical, philosophical and technical problems. Philosophically, there is a problem that pain cannot be monitored directly in animals but can only be measured by examining their responses to nociceptive stimuli. Technically, the observed reactions are almost always motor responses ranging from spinal reflexes to complex behavior. The animal models employed for screening of newly discovered analgesic agents, include Pain-state models based on the use of thermal stimuli, mechanical stimuli, electrical stimuli and chemical stimuli. Although, the neuronal basis of most of the above laboratory models is poorly understood, their application is of immense value in predicting the analgesic activity.

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