Animal Experiments in Medicine

Anesthesia, pre- and postoperative care

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Pain and distress

Pain and distress are elements of a particular kind of biological adaptation in nature. Because of the difficulties in determining and measuring them, frequently only abnormal behavior patterns point to pain and distress. Distress is an aversive state in which an animal is unable to adapt completely to stressors and the resulting stress. Pain is normally defined as an unpleasant sensory and emotional experience associated with potential or actual tissue damage (human definition). Long-lasting (chronic) pain (usually produced by disease, injury or surgery) and long-lasting discomfort are important causes of distress, which can lead to anxiety and depression (passivity and apathy). Why is it important to recognize and reduce pain? Pain has different negative consequences ("stress response"), which lead to various physiological malfunctions, such as delayed wound healing, the delayed recovery of organic functions, decreases in immune functions, etc. Experimental data may therefore be false, or modified (unless a stressor, such as pain, is the subject of the experiment).

This topic is given high priority in animal welfare regulations: the pain and distress of animals should be minimized whenever possible: "all experiments shall be designed to avoid distress, unnecessary pain and suffering to the experimental animals." Accordingly, pain and distress must be reduced to a minimum for both ethical and scientific reasons.\textsuperscript{1,4,5}

1. Recognition of pain

It has been proved that animals can feel pain, even at a very young age. Pain is difficult to assess in animals because of the inability to communicate directly about what the animal is experiencing. In the absence of evidence to the contrary, it is assumed that something that is painful in a human will also be painful in an animal. Acute and chronic pains can give rise to different symptoms. The objective signs of pain, which can be measured (defined), are as follows (mainly in cases of acute pain):

- sudden vocalization,
- pupil dilatation,
- increases in respiratory and heart rates,
- sudden urination or defecation, etc.

Mostly in the event of chronic pain, the behavior of animals changes, but, it is difficult to recognize these alterations (and different species display different behavior patterns). The behavior patterns are as follows:

- an anxious appearance, and increased aggression and guarding,
- limited or/and slow movement, and a hunched position (back and neck),
- adoption of an unusual posture, e.g. a rigid posture,
- a weight loss,
- failure to groom,
- withdrawal, and extended sleep periods,
- a lack of appetite, refusal of food and water,
- apathy and depression,
- vocalization.\textsuperscript{1-3,4,5}
2. Alleviation of pain

There are two possible ways to alleviate pain: 1. anesthesia, or the exclusion of the perception or consciousness of pain, i.e. the absence of sensation, and 2. analgesia, i.e. the suppression, restriction or reduction of pain. As laid down in animal welfare regulations: "all experiments shall be carried out under general or local anesthesia". This does not apply in cases when:
- anesthesia is judged to be more traumatic to the animal than the experiment itself;
- anesthesia is incompatible with the object of the experiment.

In such cases, appropriate legislative and/or administrative measures shall be taken to ensure that no such experiment is carried out unnecessarily. If anesthesia is not possible, analgesics or other appropriate methods should be used in order to ensure as far as possible that pain, suffering, distress or harm are limited. Provided such action is compatible with the object of the experiment, an anesthetized animal which suffers considerable pain once the anesthesia has worn off shall be treated in good time with pain-relieving means.

3. Anesthesia

a. Local (regional) anesthetic drugs act to disrupt nerve conduction temporarily. When applied around a nerve, they produce analgesia in the region served by a nerve. However, these drugs exert no depressant effect on the brain. The major forms are: superficial (surface), conductive and infiltrative local anesthesia.

b. General anesthesia is defined as: all of the methods when the exclusion of the sensation of pain is accompanied by unconsciousness. The basic ways to perform general anesthesia (introduction and maintenance) can be:
- Injectable anesthetics can be administered by various routes, depending upon the specific compound. The most frequently used routes of administration in laboratory animals are intraperitoneal, intramuscular and intravenous.
- Inhalant anesthesia with anesthetic gases. Frequently, an injectable anesthetic is used to induce anesthesia, and the inhalation agent is used for maintenance.

4. Preanesthetic medications (premedication)

It is often advisable and sometimes necessary to premedicate an animal before the induction of anesthesia. In general, anticholinergic drugs, tranquilizers, opioid narcotics (general anesthetics), neurolept-analgesics and dissociative agents are used for premedication. These drugs are used to relieve anxiety and produce calmness, to reduce the dose of anesthetic needed and to provide postoperative pain relief. All premedication drugs, except the anticholinergics, are considered CNS depressants.

5. Assessing the depth of general anesthesia

Involuntary reflexes (such as the palpebral reflex, the ear pinna reflex, and the pedal / withdrawing / reflex, patellar reflex) and the objective signs of pain (such as increases in heart rate and respiratory rate, a rise in blood pressure, pupil dilatation, salivation, movements of limbs, head and tongue, etc.) are used primarily as a means of determining the depth of general anesthesia.
6. Basic tasks during anesthesia, recovery

A variety of things must be done to prepare for anesthesia:

- Withhold food and water from large animals for 12 h prior to anesthesia induction, and from small animals for 2 h to prevent regurgitation and aspiration. It is not necessary to withhold food and water from rodents prior to anesthesia.
- Intubate the trachea whenever possible, even if injectable anesthetics are being used. Intubation can be achieved in animals as small as a rat. This will prevent aspiration pneumonia and allow the assistance of respiration if the animal stops breathing.
- To minimize the effects of surgery and anesthesia on hydration (decreasing blood pressure, increasing fluid requirements and hemorrhage), an intravenous catheter is placed whenever possible to provide access for fluids and medication. Fluids are supplemented intravenously if possible, or otherwise intraperitoneally or subcutaneously during and after anesthesia.
- Animals frequently become hypothermic during anesthesia. Hypothermia depresses all physiological functions, and resulting in prolonged recoveries. Heat loss should be prevented by insulating cold surfaces with a blanket, and heat should be supplemented heat with a thermal blanket or with pre-warmed fluids.
- The depth of anesthesia must be monitored carefully and closely to ensure that the animals do not become too deeply anesthetized and die, and to ensure that they do not become too lightly anesthetized (see above) and experience pain from the surgical procedure. Evaluation of the normal physiological functions (vital signs) in combination with an evaluation of the reflexes is a means of assessing the animal’s relative health status under anesthesia. Vital signs routinely monitored are: the heart rate and rhythm, the respiratory rate, depth and rhythm, the arterial pulse, the body temperature, the mucous membrane color, the capillary refill time, the pupil size and the response to light and the muscle tone.
- Vital signs should be evaluated at least every minute during the induction stage, and as often as possible during the maintenance and recovery stages, but not less frequently than once every 5 min. Electrical and mechanical monitoring devices (e.g. ECG and pulse-oximetry) facilitate the monitoring of a greater number of vital signs, with constant checks on the anesthetized patient.
- Monitoring and support must continue until the animal has recovered completely from the anesthesia.

During the recovery period, it is important that attention be paid to the following:

- The prevention of heat loss (towels, heating pad, etc.).
- Checks on vital signs (at least every 5 min).
- Turning the animal from side to side once every 15 min unless contraindicated.

Complete recovery means that the animal is able to hold itself in a normal upright position, its body temperature has returned to normal and all physiological indices are within the normal limits. Anesthetic recovery can be rapid for gaseous agents and short anesthetic episodes. The recovery time can be prolonged if animals were anesthetized for a long time or if injectable agents were used.

7. Analgesia
The complete exclusion of the sensation (experience) of pain can be achieved only through general anesthesia, but with the use of analgesics it can be considerably decreased and the negative consequences of pain can be avoided. The basic principle of analgesia today is that the prevention of pain is more effective than its treatment ("preemptive analgesia"). This means that it is best if analgesia can be provided to animals prior to the painful procedure, rather than waiting until after the clinical signs of pain are observed. In this way, the hypersensitivity of the nervous system can be prevented. It is more efficient if two or more drugs are used in combination. The drugs tend to have synergistic effects.

Some anesthetics (narcotics) do have a pain-alleviating effect, but most of them do not. When the latter drugs are used, hypersensitivity of the nervous system (peripheral and central) likewise develops, in spite of the anesthesia (the absence of a pain sensation), and leads to clinical pain after recovery. Accordingly, prior to general anesthesia it should be considered what kinds of analgetic drugs are needed (see premedication above).

8. Drug groups which can be used as analgesics

These comprise opioids, local anesthetics, nonsteroidal anti-inflammatory drugs, alpha-2-agonists and dissociative agents. Some of these groups, when used in combination with each other or with other anesthetics, are suitable for the achievement of general anesthesia (see premedication above).
Administration of different agents. Basic principles of invasive (surgical) procedures

1. Handling and restraint of experimental animals

The use of proper restraint and handling techniques reduces the level of stress to the animals and also to the researcher. Handling stress is an experimental variable and should be minimized whenever possible. As a result of improper handling, animals can inflict serious injuries on humans and to themselves. If a study involves significant handling of animals, it is recommended that the animals be accustomed to the handling. The basic principles of handling are gentleness and firmness. A chemical restraint should be considered for any prolonged or potentially painful procedure.

- **Mouse**
  - Tail restraint is adequate for examining or lifting animals and transferring them to another cage. Grasping the loose skin of the neck and back may be used to perform minor, nonpainful procedures such as injections.

- **Rat**
  - A rat may be picked up by grasping the base of the tail, similarly as for mice. A suitable method to lift and restrain a rat is to place the hand over its back, slipping the thumb ventrally between the forelimbs into the intermandibular space. The hind legs should be grasped to restrain the animal for injections or other minor procedures.

- **Hamster**
  - As it does not have a tail, a hamster must be grasped firmly by the loose skin of the back, or handled in a manner similar to the rat.

- **Guinea pig**
  - The guinea pig is lifted by placing one hand around the animal’s trunk and supporting the hind limbs with the other hand. It is important to support the rear quarters.

- **Ferret**
  - Friendly ferrets can be lifted and handled by grasping the trunk. Active or biting ferrets may be restrained by grasping the skin of the neck firmly.

- **Rabbit**
  - To lift a rabbit, one should grasp a large fold of loose skin over the shoulders with one hand, and the rear feet with the other hand. Failure to grasp or support the rear feet may result in injury to the animal and/or to the handler (rabbits have powerful rear limbs). To carry a rabbit, it is useful to support the animal’s
body between the forearm and abdomen of the handler, with the rabbit’s face hidden under the handler’s elbow.

- **Dog**
  - A slip lead is recommended for working with dogs. The size of a dog determines the method used to lift it. The initial step is the same for all dogs. The dog is grasped above the neck, with one hand around its body, with the hand supporting the dog’s chest. The dog can be restrained in a lateral recumbent or sitting position for injections and minor procedures. An intractable dog may need to be muzzled with a commercial or a gauze muzzle.

- **Cat**
  - Cats are often cooperative enough to be restrained easily on a table by the loose skin at the back of the neck and hips, or with one hand restraining the body and the other restraining the head. A fractious cat should be wrapped in a heavy towel for restraint, with any needed limbs carefully withdrawn for treatment. A face mask (muzzle) can be used to avoid biting, and a squeezecage to restrain the animal for injections.

- **Swine**
  - Pigs in general are friendly and docile, but will react severely to poor handling. Handling and restraint in pigs relies greatly on treating the pigs in a humane manner. There are several levels of restraint and handling, from touching and coaxing the animal to restraining it for chronic procedures. When approaching a pig, be sure that it is made aware of your presence. If pigs are startled, they may cause injury to themselves or others in the pen. The giving of food is one of the most effective forms of basic restraint in the pig. Smaller pigs may be easily picked up with their body supported while their legs hang. To perform the procedure in larger pigs, place one arm under the chest cranial to the thoracic limbs and the other arm cranial to the pelvic limbs under the abdomen. When a pig is to be moved, a small board is used to apply pressure to its side. Several designs for slings to restrain pigs have been described and can be used.

- **Nonhuman primates (monkeys)**
  - No matter how small, these animals can be dangerous. Chemical immobilization is normally used. Injections can be given to a confined animal with the help of a squeeze cage. Tether systems are recommended if drugs must be administered to animals, or if blood must be collected frequently.
  - Laboratory dogs and monkeys can readily be trained through positive reinforcement to accept routine procedures such as an intramuscular injection, an intravenous injection and oral dosing. Such training helps to reduce the stress involved in these procedures; forceful restraint is not needed.¹,³,⁶

2. **Administration of different agents (fluids and drugs)**

   When drugs, vaccines, injectable anesthetics or other agents are to be administered, one or other of several different routes may be selected. The route is governed by the nature of the
agent being administered, the animal and the purpose of the administration, among other factors.

- **Enteral (gastrointestinal) administration.** The possible routes are oral (through the mouth), gavage (into the stomach via a gastric feeding needle), per rectum (through the anus into the rectum), or into the bowel via a tube.

- **Parenteral administration.** Parenteral routes of administration involve mainly injections into various compartments of the body: intradermal (into the skin), subcutaneous (under the skin), intramuscular (into a muscle), intravenous (into the vascular system through a vein), intraarterial (into the vascular system through an artery), intraperitoneal (into the abdominal cavity), into the spinal cavity, into the trachea, or into the nasal cavity. Sites used for the collection of blood from veins may also be used for intravenous administration. Intraperitoneal administration is one of the most frequently used parenteral routes in rodents.

- **Special procedures:** transplantation (*e.g.* embryo transfer, skin-grafting), implantation and blood transfusion.\(^1\text{-}^3\)
3. Basic requirements of invasive interventions (injections, blood collection and surgery)

- **Painlessly**, as far as possible. If pain can be avoided, this is a must! Animals cannot understand why they have to bear pain. Invasive experimental interventions are accompanied by anxiety, fear and suffering; rewarding and praising are therefore important after every regularly repeated intervention.

- **Aseptic intervention.** This is necessary in order to avoid contamination of the samples, and to avoid infection of an animal, even if it is euthanized after the experiment (intervention).
  - The animal should be surgically prepared by careful shaving to remove all hair from the surgical field.
  - The surgical field should be cleaned and disinfected with an appropriate preparation.
  - All surgical instruments and chronic instrumentation must be sterilized with steam or gas. Cold chemical sterilization is appropriate for minor surgical procedures.
  - Investigators should follow standard surgical practices (scrubbing, cap, mask, gloves, etc.).
  - Sterile drapes should be positioned on the animal to define the surgical field.
  - During the course of surgery, procedures for preserving sterility should be strictly followed.

- **Postsurgical care**
  - Surgical wounds should be protected by plastic collars and/or dressings (or in some other way).
  - Surgical wounds and sites of instrument entry into the body should be observed, cleaned and treated daily. Topical antibiotics can be applied. Surgical dressings should be changed every day.
  - Basic biological functions, including urination, defecation and appetite, are good indicators of an animal’s overall physical well-being. These are easy to observe and should be monitored regularly and often.
  - Stitches should be taken out after 10–14 days.
  - Postsurgical (clinical) pain must be relieved.¹³
Euthanasia

This is defined as a method of killing an animal rapidly and painlessly. Experimental animals must be euthanized painlessly when this is decreed by the rules. Another reason for euthanasia is to gain organs and tissues or to examine morphological changes caused by an experiment. The legal animal welfare rules lay down that: "At the end of any experiment, it shall be decided whether the animal shall be kept alive or killed by a humane method, subject to the condition that it shall not be kept alive if, even though it has been restored to normal health in all other respects, it is likely to remain in lasting pain or distress".

The method used must produce rapid unconsciousness and subsequent death without evidence of pain or distress, or the animal must be anesthetized before being killed. This should be carried out by properly trained personnel. The method used should be safe for the attending personnel, easy to perform, and cause death without producing changes in tissues. Careful, gentle handling is important in order to minimize distress to the animal. Whenever possible, animals should not be exposed to the euthanasia of others, especially of their own species. The resultant distress (because of the frightened behavior and the release of certain odors, or pheromones) may lead to physiological changes in other animals, which may affect research results. The basic methods and agents are as follows.

1. Pharmacological – chemical methods
   - The injection of a lethal substance, usually a large dose of a general anesthetic agent (pentobarbital) i.v.
   - An overdose of potent inhalant anesthetic gas (halothane or isoflurane).
   - Carbon dioxide inhalation (compressed CO₂ from cylinders). This can be suitable for all species, but in practice, its use is limited to rodents and other mammals weighing less than about 500 g.

2. Physical methods
   - Decapitation (small animals and rodents); this requires special equipment. Dislocation of the cervical vertebrae (mice and rats).
   - Exsanguination (farm animals and swine).
   The use of sedation or anesthesia prior to these methods is recommended.

3. Not recommended methods
   Freezing, the use of ether, chloroform, strychnine, magnesium sulfate, muscle paralyzers, etc.
References