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Pain expression in different laboratory animal species

by *Per Svendsen,* dr. med. vet.
Biomedical Laboratory, Odense University, DK-5230 Odense, Denmark.

Introduction

Acute pain is a complex physiological phenomenon of ultimate importance for the survival of animals in the wild. Wounds and fractures heal best if immobilized. The pain experienced from the affected region prevents the animal from moving, and thus supports healing.

Chronic pain as a result of prolonged disease seems to have no clear biological significance. The condition has mainly been studied clinically, but animal models of human chronic pain are being developed, for instance induction of arthritis by various chemicals or subcutaneous injection of long-duration, painproducing substances (*Sternbach* 1976, *Dubner* 1983). It is a characteristic of chronic pain that the pain tolerance to minor injuries is lowered (*Sternbach* 1984).

Pain is the result of a noxious stimulus, i.e. a stimulus that threatens or produces damage to tissue. This may be a mechanical, chemical, thermal or electric stimulation. The receptor that responds to the noxious stimulation is known as a nociceptor. It may be unimodal and respond to only one form of energy, or polymodal responding to several types of stimulation. The lowest level of noxious stimuli required to generate nerve impulses in a nociceptor is known as the nociceptor threshold. The nociceptive reflex is a stereotype movement of a part of the body in response to noxious stimuli. The pain detection threshold is the minimal amount of stimulation perceived, and the pain tolerance threshold is the highest intensity of a series of stimuli that a subject will tolerate. Pain tolerance varies between species and within an individual under varying circumstances. Fear and mild stress are known to decrease the pain tolerance threshold, whereas it is increased by reducing fear and by motivation and reward (*Radouco-Thomas et al.* 1961).

The pain detection threshold and the pain tolerance threshold of animals can be detected by training the animal to press a bar to avoid a noxious stimulus. The level of stimulus evoking a 50 % response is defined as the pain detection threshold. If the animal is rewarded for not pressing the bar the intensity of the stimulus can be increased, and the maximum stimulus the animal will receive without pressing the bar, is defined as the pain tolerance threshold. In humans pain levels just above the pain detection threshold are not disruptive, whereas repeated neartolerance levels of pain produce an intense emotional reaction. A similar phenomenon may occur in animals, and it may be this emotional stress, more than the pain as such, that ought to be prevented in laboratory animals (*Vierck* 1976, *Spinelli* 1987).

Experimental surgery is often more extensive than curative surgery, and the laboratory animal is more likely to experience severe post-operative pain than the clinical patient. It is therefore of great importance for the experimental surgeon to realize which surgical procedures and complications are likely to cause pain in the animals. It is also important for the experimentator to know the different stages of pain development. Different species of animals may express pain symptoms differently, and knowledge of normal and abnormal behaviour of the different species of laboratory animals is important when the degree of pain is to be evaluated.

Acute pain in laboratory animals is most likely caused by experimental surgery, whereas other types of experiments, like

toxicological testing or experimental infections, are more likely to cause chronic pain and suffering. Pain following an experimental operation may be unavoidable because of the extent of the surgical procedure, for instance in case of experimental fractures, but generally pain is caused by an imperfect surgical technique resulting in inflammation of the wound or the serous membranes, ischaemia or necrosis due to ligation of major vessels, extensive tissue damage caused by poor dissection or suture technique, or dehydration following inadequate fluid administration.

Post-operative pain is thus primarily a matter of prevention rather than of treatment. Properly carried out the majority of surgical operations performed for experimental purposes are unlikely to cause severe pain to the laboratory animals. Poor techniques, however, especially lack of proper aseptic techniques, are likely to cause considerable post-operative pain. It is still common for many investigators to perform surgery on small rodents using the so-called "clean technique", meaning operating with unautoclaved instruments, using bare hands, and performing the operation on an ordinary laboratory bench. The argument for this behaviour is that rodents survive just as well as they do if an aseptic technique is used because, for some unknown reason, they "resist infections" better than other species. There is no scientific proof for this statement, and other healthy animals, including man, are also likely to survive an operation where the "clean technique" is used. Post-operative infections, however, are more likely to occur, and the infection will certainly cause post-operative pain in all species.

Ligation of a major vessel, for instance the femoral artery, when performing arterial catheterization is commonly performed in large animals. The argument is that collateral arteries will supply the leg adequately. This is correct since gangrene does not occur, but there can be little doubt that severe pain accompanies the opening of collaterals. Using an alternative technique for arterial puncture, ligation becomes unnecessary.

Poor suturing technique, like too tight sutures, is likely to cause post-operative ischaemia in the suture line. This condition is painful. The animal may react by scratching the wound and tearing the suture line apart, leading to herniation or rupture of the wound. This is certainly a very painful complication, which may even cause the death of the animal.

During surgery the animal loses water because of evaporation from internal surfaces. If the loss is not compensated by intravenous or subcutaneous administration of isotonic fluid, the animal will become severely dehydrated. This condition is life threatening, and from humans it is known that dehydration is accompanied by severe headache. Whether this is the case in animals we do not know, but if so, dehydration during surgery could cause post-operative pain in laboratory animals.

Besides surgery other procedures may cause severe pain in laboratory animals. Immunization of animals – mainly rabbits – for the production of polyclonal antibodies is usually a less harmful procedure. If Freund's complete adjuvant is used, and the volumes injected are too large, skin necroses may develop. Some investigators use the cruel technique of injecting the rabbits in the hind footpad, arguing that this technique gives rise to a better antibody response. There is no doubt that a chemically induced abscess in the footpad is a very painful condition, especially when the rabbit is housed in a wire-floor cage.

Production of monoclonal antibodies by transferring hybridoma cells to the peritoneal cavity of mice has great advantages. The consequent peritonitis, however, may be a very painful experience for the mouse. Certain guidelines have been made for the fluid volume produced by the animal, but it is likely that the inflammation of the peritoneal membrane itself is a very painful condition.

Sensitivity of different organs and tissues

Organs and tissues react differently to noxious stimuli. Common to all tissues, however, is that any procedure that causes stretch, intense pressure, heat or cold, or cellular damage is likely to cause some degree of pain. Inflammation, however caused, will produce localized pain and hypersensitivity.

Pain receptors of the skin are sensitive to heat, cold, and mechanical stimuli. The density of the receptors varies, particularly sensitive areas are found around the eyes, ears, mouth, at the digits, and in the perineal region. Animals react to acute cutaneous pain by reflex movements. Longer lasting painful conditions in the skin lead to licking and biting of the affected area, which may give rise to self-mutilation. Injections and simple incisions cause only transient pain. If, however, inflammation occurs, for instance caused by too tight sutures or contamination of the wound, the animal is likely to experience severe pain.

Teeth and gums are sensitive to heat and cold. Pain originating from the oral cavity leads to abnormal eating and drinking behaviour. The animal may stop eating completely, or it may show difficulty in mastication. Other signs of oral pain are excessive salivation and rubbing of the head against the cage or the paws.

The respiratory tract is sensitive to inhaled substances. The irritation provokes coughing and excessive bronchial secretion. Irritation of the pleura causes pain, and the animal will show shallow abdominal respiration.

Animals appear to react less to abdominal procedures than man does, and post-operative recovery is usually fast and uncomplicated. Traction and distension of abdominal viscera is very painful, and may arouse the animal if anaesthesia is not sufficiently deep. The placement of permanent catheters in abdominal organs usually causes little pain unless an inflammatory condition develops. Symptoms of abdominal pain differ among animal species. Some species will kick or bite at the abdomen, lie down and roll, whereas other species will show reduced motility, lie streched out or adopt a hunched position.

Tearing and distension of muscle fibres causes pain in the affected area. Most commonly, however, muscular pain is caused by intramuscular injection of irritant solutions. Symptoms of muscular pain include swelling of the affected area and pain reaction by palpation. The animal will attempt to immobilize the affected region and resist manipulation.

Nervous tissue does not contain pain receptors. Denervation is associated with dysaesthesia, which may provoke self-mutilation.

Irritation to the retina and conjunctiva may cause pain of various degree. Drying of the cornea during anaesthesia can also lead to a painful condition. Symptoms of ocular pain include closing of the eyes and rubbing of the eyes with the front legs. This may lead to inflammation of the eye and an increase in the intensity of the pain.

Procedures involving the auditory canal and the middle ear are likely to produce severe pain, especially if inflammation occurs. The animal will scratch the ear region, keep its head tilted with the affected side down, move in circles and resist handling of the affected area.

Regional ischaemia due to arterial occlusion is considered a very painful condition. Obstruction of the venous drainage will cause swelling and oedema of the region. The associated pain is regarded as moderate.

Damage to bone, periost and joints is considered painful. The affected animal will show various degrees of lameness and resist palpation and manipulation of the affected region (*Soma* 1987).

Stages of pain

Acute pain may be divided into three phases (*Craig* 1984, *Wall* 1984). In the immediate phase the relationship between injury and

pain expression is very variable. In humans a pain-free period is frequently experienced after severe injuries (*Melzack et al.* 1982), and in animals a similar pain-free period after injury has been seen in race horses that have completed races with fractured bones of the extremities. This pain-free period seems to be an instantaneous analgesia, and not an initial pain that is brought under control. This indicates that the pain control mechanism comes into action very rapidly after the injury, maybe even before the injury. The analgesia is localized to the injured region only, indicating that this phenomenon is caused by a localized internal control system, and not by a generalized system. Following the initial pain-free period, pain is always experienced.

Some time after the injury, when the sensation of pain is obvious, man will become anxious and seek treatment. Animals react in a similar way and become agitated and aggressive and try to protect the affected region.

In the recovery stage man becomes quiet, and the affected region is kept immobile, social contact is avoided, appetite is poor, and sleep is prolonged. This condition may occasionally be prolonged into a condition of chronic pain. In animals similar stages are recognized. The animal is quiet, solitary, loses appetite, moves little and sleeps much.

Clinical examination

The majority of animals used for experimental purposes are small rodents. The usual procedure in clinical examination of larger animals cannot be directly applied to these species. The first stage in assessing the clinical condition of small rodents is to become familiar with their normal behaviour. A number of problems are obvious in small rodents. Many inbred strains possess individual behavioural characteristics; some are docile, others are aggressive. Mice, rats and hamsters are nocturnal, and their behaviour will vary at given times of the day. A detailed physical examination will require the animals to be restrained, which in itself will cause a change in behaviour.

In general laboratory rodents in good health and free from pain are active, alert and inquisitive when approached. Their coat is clean and well-groomed. The skin is elastic and will retract quickly if lifted. Normal animals eat, drink, defaecate and urinate regularly. Attempts to capture and handle healthy animals will cause resistance, but not aggression. The healthy animal will have clear open eyes, dry nostrils and a clean anal region.

Acute pain in mice is best recognized when the animal is approached. It will show aggressiveness, and when touched it will try to bite. Left alone the mouse will bite the affected area. Abnormal locomotion with circling movements is symptomatic of acute pain in the inner and middle ear. Chronic pain is more difficult to recognize. The coat will appear rough with piloerection. The mouse will lose body weight, stay separated from the other animals in a hunched position. The abdomen is sunken indicating an empty gut, the skin is unelastic and the eyes are sunken because of dehydration, and the body weight is reduced.

Rats are usually less aggressive than mice. Acute pain is accompanied by constant vocalization and licking or guarding the painful area. Abdominal pain is associated with a stilted gait, whereas circling movements are seen in animals suffering from pain in the ear. Initially the rat will show increased awareness, aggressive behaviour and a tendency to bite. Later the animal will be resting in lateral recumbency with its head tucked up into the abdomen. Chronic pain is associated with weight loss, piloerection, dehydration, and unresponsiveness.

The healthy guinea-pig is timid and will avoid capture. Acute pain increases this behaviour. Animals suffering from acute pain may show aggressiveness toward cage mates, resulting in wounds. Guinea-pigs that suffer chronic pain gradually become unresponsive, lose weight and become dehydrated.

Diagnosing pain and disease in rabbits represents a problem as this species reacts little to pain and distress. The most common response to acute pain is photophobia – the animal will sit in the back of the cage facing a corner. Acute pain in the ear causes torticollis. When handled a rabbit in acute pain will often vocalize for short periods. Signs of chronic pain are non-specific, but loss of appetite, weight loss and dehydration are common (*Wallace et al.* 1990).

Acute pain in the dog causes aggression or fear depending on the temper of the animal. The dog may howl or bark and its behaviour indicates distress (tail between legs, anxious glances and frequent urination). Pain such as that manifested by an animal with a fracture or a wound, can easily be identified by producing a response to palpation of the affected area. In other cases, where the pain does not interfere with the animal's normal activities, the manifestations of pain will be less apparent, and only demonstrated by behavioural changes (*Yoxal* 1978). A clinical examination will reveal increased heart rate and weak pulse, superficial respiration with increased frequency, raised body temperature and dilated pupils. Chronic pain causes reduced food intake, reduced body weight and dehydration. The dog will have an unsteady gait and be reluctant to move (*Morton & Griffiths* 1985).

The cat reacts to acute pain by showing an aggressive behaviour. Its ears will be flattened, it will resist being handled and hiss or spit. A cat in chronic pain will try to hide and lie with "tucked in" limbs and hunched head and neck.

A pig suffering from pain will appear drowsy and react little when approached. It will stop eating and drinking, lose body weight and become dehydrated.

Conclusion

Research with animals should ideally be performed without the risk of causing pain or suffering. However, even the most experienced experimentator may fail and the best planned experiment may go wrong. It is therefore important for everybody working with laboratory animals to be able to diagnose a painful condition in an animal. The symptoms, however, differ among species and individuals, and the signs may be weak and difficult to interpret. The most important aspect is to know the normal behaviour of the different species of laboratory animals and to react to any changes in this behaviour. Animal technicians responsible for the daily care of the animals form the first line of defence against unnecessary suffering. The experimentator, however, should not rely on the technicians alone, but seek to become familiar with the normal behaviour of his animals, and closely follow any changes in their behaviour during the course of the experiment.

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