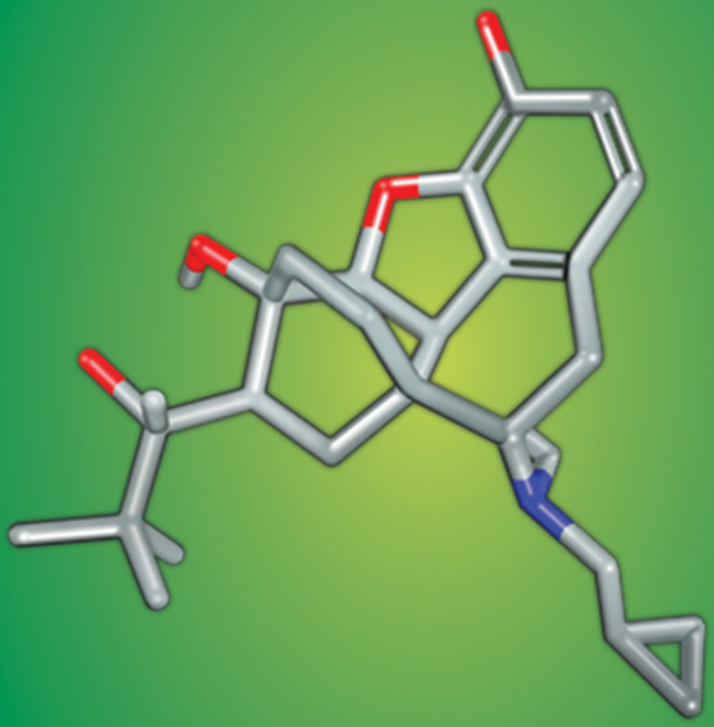
Fourth Edition

Laboratory Animal Anaesthesia

Paul Flecknell



Laboratory Animal Anaesthesia

Fourth Edition

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Preface

The front cover of this new edition displays a different molecule – buprenorphine. Unlike sevoflurane, which illustrated the cover of the last edition, this isn't a new drug, but it is a compound that continues to shape strategies for managing pain in laboratory animals. The last few years have seen further data on the efficacy buprenorphine in alleviating post-operative pain in a range of species. New slow release formulations offer potential advances in providing long term alleviation of severe pain, and the agent continues to be the most widely used analgesic in laboratory animals. It is a great disappointment, however, that reported use of post-operative analgesics continues to be very low, even though their use in veterinary clinical practice is now widespread. To try to address this problem, the section on pain assessment and pain management has been revised, updated and expanded to encourage not only greater use of analgesics, but also more structured assessment of pain.

The anaesthetic methods used in many research facilities continue to improve as a result of the introduction of newer anaesthetic agents and new techniques. Sophisticated monitoring devices are widely available and their cost continues to fall. The introduction of newer techniques often represents significant refinements of research methodologies but this is not invariably the case. Some research projects still benefit from the use of older agents, since these may produce fewer interactions with the body systems being studied. For this reason, these older agents are still described in this new edition. The new edition continues to provide information on new methods and more complex procedures, but also emphasizes the basic principles of good anaesthetic practice for less experienced research workers. To emphasize these basic principles, the introductory chapters have been rearranged into a single section.

As in earlier editions, the number of references has been increased and updated, and these are used, as previously to support contentious statements, to indicate conflicting opinions and to provide a starting point for searching the more specialist scientific literature. Where older references continue to provide essential information they have been retained.

Paul Flecknell

Glossary

Inevitably, a number of specialist terms are used throughout this book and these are defined below.

Anaesthesia a state of controllable, reversible insensibility in which sensory perception and motor responses are both markedly depressed
Analgesia the temporary abolition or diminution of pain perception
Analeptic drug which stimulates respiration
Anoxia complete deprivation of oxygen for tissue respiration
Apnoea temporary cessation of breathing
Arrhythmia (cardiac) alteration in the normal rhythm of the heart
Asystole lack of cardiac muscle contractions
Ataxia lack of co-ordination, 'wobbliness'
BMR basal metabolic rate
Bradycardia slowing of the heart rate
CNS central nervous system
CNS depressant any agent which modifies function by depressing sensory or motor responses in the CNS
Cyanosis blue or purple colouring of the skin or visible membranes due to the presence of an increased concentration of reduced haemoglobin in capillary blood, symptomatic of hypoxia
Dosages mg of drug per kg body weight (mg/kg) except for the neuroleptanalgesic combinations which are more conveniently expressed as ml of commercial or diluted premixed solution per kg body weight (ml/kg)
Dosage schedules
u.i.d. once daily
b.i.d. twice daily
t.i.d. three times daily
q.i.d. four times daily
Dyspnoea laboured breathing
ECG electrocardiogram
Hypercapnia elevated blood carbon dioxide content
Hyperpnoea fast or deep breathing
Hypertension elevated (arterial) blood pressure
Hypnotic a drug which induces a state resembling deep sleep, but usually with little analgesic effect
Hypocapnia reduced blood carbon dioxide content
Hypopnoea slow or shallow breathing
Hypotension a fall in (arterial) blood pressure
Hypothermia a fall in body temperature

Hypovolaemia a fall in circulating blood volume
Hypoxia depressed levels of oxygen
Induction (of anaesthesia) the initial establishment of a state of anaesthesia
Injection routes
i.v. intravenous
i.m. intramuscular
i.p. intraperitoneal
sc subcutaneous
Laryngospasm spasm of the vocal cords, producing complete or partial obstruction of the airway
Minute volume the volume of gas breathed in 1 min, that is, the product of tidal volume and respiratory rate
Narcosis a state of insensibility or stupor from which it is difficult to arouse the animal
Normovolaemic having a normal circulating blood volume
PCO_2 partial pressure of carbon dioxide
Per os by mouth
PO_2 partial pressure of oxygen
Polypnoea rapid, panting breathing
Pulmonary ventilation the mechanical expansion and contraction of the lungs in order to renew alveolar air with fresh atmospheric air
Tachycardia an increase in heart rate
Tachypnoea rapid respiration
Tidal volume the volume of gas expired with each breath

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Acknowledgements

This book has continued to develop in scope and content as a result of the advice and helpful criticism from colleagues I have worked with during my career. The discussions and questions raised by colleagues on the email lists COMPMED and VOLE have stimulated inclusion of additional material in this edition, and thanks are due to Ken Boschert, AALAS and Peter Nowlan for their establishment and management of these groups.

Thanks are due to Jon Gledhill for providing many of the new illustrations for this edition, and to Eddie Clutton, for reading the text and providing many helpful comments.

I am particularly grateful to Ruth, my wife, for her constant assistance during all stages of the preparation of this book.

During the final stages of preparation of this edition, Dr Charles Coid passed away. Charles had a major influence on my early career, and without his assistance the first edition of this book would not have been published. This latest edition is dedicated to the memory of this outstanding veterinary surgeon and scientist.

Introduction

Providing the most appropriate and effective anaesthetic regimen is an essential part of good experimental design. Anaesthesia has profound effects on the physiological processes of animals and this can have a marked effect on experimental data. These effects can arise as a direct result of the anaesthetic agents used, for example hyperglycaemia caused by dexmedetomidine. Other effects such as hypothermia may be secondary to the depression of various body systems. Some effects persist only during the period of anaesthesia, other effects may continue for hours or days. Minimizing and controlling these effects can be frustrated by the use of inappropriate anaesthetics and a failure to provide high standards of perioperative care. Sometimes the effects of a poor choice of anaesthetic agent can be dramatic, for example ileus (gut stasis) after administration of chloral hydrate. More usually, the effects are less obvious, but even subtle changes can result in an increase in the variability of study data. This increased variability can require an increased number of animals to be used to demonstrate treatment effects (Festing et al., 2002).

Aside from the requirements to carry out studies as efficiently as possible, it is widely accepted that when it is still necessary to use animals in experiments, research procedures should be refined to minimize pain and distress. A requirement to comply with the principles set out by Russell and Burch (1959), of Reduction, Refinement and Replacement, now forms part of the legislation controlling use of animals in research in the European Union and elsewhere. Reviewing our anaesthesia and perioperative care so that they are the most appropriate for a particular study will contribute to both reduction and refinement of animal use. Achieving this is not always straightforward and it is important that attention should be given not only to the anaesthetic agents used, but also to the measures adopted to minimize the unwanted side-effects of anaesthesia and surgery. Use of effective analgesia following surgical procedures is particularly important, yet recent reviews of current practices in rodents suggest that analgesic use is still relatively low (Richardson & Flecknell, 2006, Coulter et al., 2009). This is somewhat ironic, since almost all of the analgesic and anaesthetic techniques currently used in man were developed and assessed in laboratory animals, before being accepted for clinical use in man. We therefore have a very wide range of techniques and anaesthetic and analgesic agents available for use in laboratory animals. Careful consideration of the options available can lead to improvements both in the quality of scientific data obtained and in the welfare of the animals involved.

Chapter 1

Basic Principles of Anaesthesia

To provide anaesthesia of the standard required in modern research laboratories, adequate preparations must be made before attempting to anaesthetize an animal. Good pre-operative care will reduce the incidence of many of the complications that can occur during anaesthesia, and thorough preparation of facilities and equipment contributes to the smooth running of a research protocol. It is important to consider preparation of the animals that are to be anesthetized and also the equipment, drugs, facilities and personnel involved in the procedure. This section provides important background information on the process of anaesthesia. It also explains the preparation and maintenance of the equipment needed when anaesthetizing animals. It includes a description of the use of items such as anaesthetic chambers, endotracheal tubes, breathing circuits and anaesthetic machines. It also includes a brief description of the various agents used in inducing and maintaining anaesthesia.

INTRODUCTION: WHAT IS ANAESTHESIA AND HOW DO WE PRODUCE IT?

Anaesthesia means ‘‘loss of sensation’. This can involve loss of consciousness (general anaesthesia), or the loss of sensation can be restricted to a small area of the body (local anaesthesia). Larger body areas can be rendered anaesthetic by injecting drugs around nerve trunks, to produce regional anaesthesia. Each of these techniques can be used in laboratory animals, but general anaesthesia is the most common approach. This is because it provides a loss of awareness, as well as a loss of sensation, and so prevents any distress associated with the procedures that are to be undertaken during the anaesthetic period. It also ensures the animal remains largely immobile, produces muscle relaxation and suppresses reflex activity. General anaesthesia is produced using either injectable or inhalational agents, or a combination of the two methods. Often a single drug can be given to produce all of these required features of general anaesthesia: loss of consciousness, analgesia, suppression of reflex activity and muscle relaxation. Alternatively, a combination of agents can be given, each making a contribution to the overall effect. The advantage of this approach is that the undesirable side effects of anaesthetic agents can often be minimized. The side effects of anaesthetics are usually dose-dependent. Giving several drugs in combination, at

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Chapter 4

Analgesia and Post-Operative Care

Successful anaesthesia requires careful attention to the entire peri-operative period. It is particularly important to provide effective post-anaesthetic care, as it is in this period that most anaesthetic-related problems occur. The recovery area environment must be appropriate for the species and the procedure involved. The continued provision of supplemental warmth, fluid and nutritional support and nursing care is often necessary. Of particular importance following surgical procedures is the continued maintenance of effective analgesia. This requires careful assessment of the animal and efforts to determine if any signs of pain or discomfort are present. If pain is present, then the analgesic regimen used can be modified to improve the degree of pain control. The initial choice of analgesic regimen depends not only upon the species of animal involved and the nature, duration and intensity of the pain that might otherwise be experienced, but also upon the nature of the specific research procedure. Balancing these factors is complex. Although analgesic agents may interact with a range of physiological processes, pain itself can have numerous confounding effects on research procedures, in addition to being a major ethical and animal welfare concern.

Post-operative care must be considered a natural and essential extension of good anaesthetic practice. Failure to attend to the animal's needs during this critical period will inevitably complicate recovery from anaesthesia and is in any case inhumane. Poor post-operative care will exacerbate and prolong the metabolic disturbances caused by surgery, and if seriously neglected, the animal may die. The results of a survey of anaesthetic-related mortality in small animal veterinary practice has shown that the majority of deaths (>50%) occurred in the post-operative period (Brodbelt et al., 2008). Although some risk factors in veterinary practice will differ from those in a laboratory animal facility, these results highlight the critical importance of good post-operative care.

All animals will require some degree of additional attention in the post-operative period, and this is often best achieved by providing a special recovery area. This will simplify the provision of the most appropriate environmental conditions, which will frequently differ from those present in a standard animal holding room. It will also highlight the special needs of animals placed in the recovery area and encourage extra attention from animal husbandry and nursing staff.

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THE RECOVERY ROOM ENVIRONMENT

The recovery area for most laboratory mammals should be warm and quiet. Lighting should be subdued but adequate to allow easy observation of the animal. Higher intensity lighting must be readily available to enable more detailed examination and to allow procedures such as intravenous injection. In the immediate post-operative period, when the normal physiological mechanisms that maintain body temperature are depressed and the animal is still recovering from anaesthesia, the ambient temperature should be 27–30°C for adult animals and 35–37°C for neonates. Once the animal has recovered from the major depressant effects of the anaesthetic, the temperature can be reduced to 25°C for adults, but should be maintained at 35°C for neonates. This gradation in temperature can be achieved by maintaining a general room temperature of 21–25°C and providing supplemental heating using warming lamps or heating pads. Ideally, an animal incubator should be used: this will allow careful control of the ambient temperature and enable easy administration of oxygen. Unfortunately, many commercially available incubators do not maintain stable temperatures. This is less of a problem with larger animals (>2 kg) but can result in transient hypothermia in small rodents. If available, paediatric incubators designed for use with human neonates provide excellent conditions for small rodents. These incubators are often available when being replaced by hospitals with new models. One practical point to note is that most of these infant incubators have a gap around the inner tray to allow air circulation, so small rodents need to be confined in an inner cage. Stable temperatures can also be provided using forced air warming systems placed around a standard rodent cage (Rembert et al., 2004).

Although hypothermia is a potentially serious problem in the post-operative period, care must be taken not to overheat the animal, and both the animal's rectal temperature and the temperature of its immediate environment should be carefully monitored. In small experimental units it may be impracticable to allocate space permanently for use as a recovery room. This should not lead to the concept being abandoned, as a temporary area within a laboratory can be set aside for this purpose. This can most easily be achieved by equipping a suitably sized trolley with an animal incubator and other necessary equipment. This can then be moved around the unit to wherever it is required. In some instances, when performing surgery on a large number of rodents, use of a portable incubator to provide a suitable recovery area is the most practicable solution. It has the added advantage of allowing the theatre assistant to regularly observe animals that are recovering from anaesthesia and surgery, as well as providing technical support to the surgeon and anaesthetist.

With larger species, such as small ruminants and pigs, use is often made of warming lamps, since heat pads placed in the pen or cage can be easily damaged. It is advisable to place a thermometer in the position that the animal will occupy, at a height equivalent to the animal's back or flank when lying down.

FIGURE 4.1 Specialized warming rack for longer term maintenance of small rodents (Techniplast).

Switch on the lamp an hour or so in advance and check the temperature – this will avoid inadvertently overheating or even burning the animal's skin. If practicable, use of warm air blowers, particularly in the immediate post-operative period is preferable, as the degree of warming is easier to control.

Following major surgical procedures, an alternative to a separate recovery room for small rodents is the use of a specialized warming rack (Fig. 4.1). This can maintain animals at warmer temperatures (e.g. 25–26°C) for several days following surgery, and can be of considerable benefit in reducing mortality and morbidity after major surgery.

Caging and Bedding

In most instances, small rodents can be allowed to recover in their normal cages, placed either in a recovery room or inside an incubator, but sawdust or wood shavings must not be used as bedding. This type of bedding will often stick to the animal's eyes, nose and mouth and so should be replaced by more suitable materials. A synthetic bedding with a texture similar to sheepskin (fleece) has proven particularly useful for all animal species and can be obtained from a number of different suppliers ('Vet-Bed', 'Dry-Bed'). It is washable, autoclavable and extremely durable and appears to provide a comfortable surface for the animal. If such material is unavailable, towelling or a blanket should be used. Tissue paper is relatively ineffective as animals usually push it aside during recovery from anaesthesia and end up lying on the bottom of a plastic cage, soiled with urine and faeces. Shredded paper of a type that will not stick to the

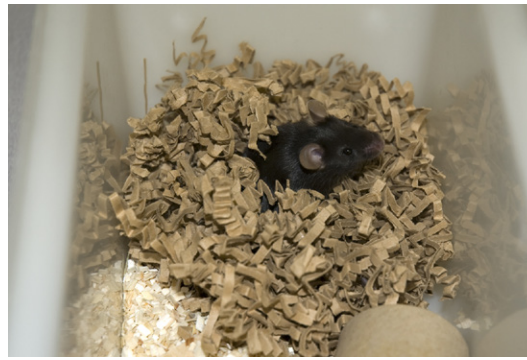

FIGURE 4.2 Mouse using shredded paper as nesting material post-operatively.

animals' orifices or wounds should also be provided, since it provides a warm and comfortable nesting material (Fig. 4.2) (e.g. 'paper shavings', RS Biotech, Appendix 4). Good quality hay can be provided for rabbits and guinea pigs, as this will provide both insulation and a readily available source of high fibre food. Animals should not be placed in grid-bottomed cages to recover from anaesthesia, but should be placed either directly in an incubator or in a temporary plastic or cardboard holding box.

Larger species will require a recovery cage or pen. Synthetic sheepskin (see above, this section) can be used with most species for the immediate post-operative period, but deep straw beds are also suitable for small ruminants and pigs.

Nursing Care

The response to human contact varies considerably among different animal species and is influenced by previous experiences. Excessive contact may have adverse, stressful effects in some small rodents and rabbits, but other species will benefit from some degree of nursing care carried out in a calm and reassuring manner. The degree of alarm caused to the animal can be reduced if it has been gradually familiarized to regular handling in the pre-operative period. This process forms an important part of pre-operative acclimatization in all species (see Chapter 1) and should be considered essential when planning any series of experiments.

Most cats, dogs and many pigs will respond positively to stroking or scratching and to a reassuring, familiar voice. If the recovery period is prolonged and normal grooming activity not resumed, some animals, particularly dogs and cats, may respond favourably to regular grooming by nursing staff. Time should be provided to encourage any dogs and cats that are reluctant to eat following surgery. Most can be tempted by hand feeding. Warming the food will often make it more appetizing. Very often, the presence of a familiar staff member to encourage eating will greatly affect the animal's appetite. Similar techniques

FIGURE 4.3 Normal pelleted diet can be made more palatable simply by soaking in water.

can also be useful in pigs and non-human primates, provided they have been properly familiarized to human contact in the pre-operative period.

All species, including rodents and rabbits, should be checked at least once a day. In the immediate post-operative period, constant attention may be needed, followed by observation every 1–4 h for the first 8–12 h. Particular attention should be given to cleaning the eyes, nose and mouth, which can become clogged with dried mucus or other debris. Monitoring of body weight and checking of wounds and surgical implants are also an important part of post-operative care. Rodents may be offered food pellets softened with warm water in bowls placed on the cage bottom, as many may be reluctant to reach up to food hoppers at this time (Fig. 4.3).

It is important that a daily routine is followed as far as possible. It will be an advantage if some staff are assigned specifically to the care of post-surgical animals throughout the peri-operative period, as they are more likely to notice subtle changes that may take place on a day-to-day basis. Careful record keeping is essential, so that other staff attending the animal, for example during weekends and out-of-hours, will be aware of all treatments given and the animal's progress. It is important to record not only all active interventions, but also that the animal has been examined and found to be progressing satisfactorily. Records of clinical observations and treatments must be readily available, and use of electronic record systems can be of great assistance in providing legible, easily accessible and up-to-date information.

PROBLEMS DURING THE RECOVERY PERIOD

The Immediate Recovery Period

The swallowing and cough reflexes are usually suppressed during anaesthesia, and these gradually return as the animal recovers consciousness. If an endotracheal tube is present, it should be removed when the animal begins to swallow

spontaneously or attempts to cough. If the tube has been tied in position, the ties should be loosened in anticipation of the need to remove the tube. Care must be taken that the tube is not pulled out too soon, for example, when the animal is repositioned as surgical drapes are removed.

In this immediate recovery period, use of a pulse oximeter to assess respiratory function is extremely useful, particularly in larger species (see Chapter 2). Changes in oxygen saturation after disconnection from the breathing system should be assessed. An observation period of approximately 2 min is usually sufficient to assess the fall in oxygen saturation that occurs when changing to breathing room air rather than the higher oxygen concentration in anaesthetic gases. If saturation falls below 85%, then oxygen should be administered and ventilation supported or stimulated. If an endotracheal tube is in use, if it is a cuffed tube, deflate the cuff and gently remove the tube as the protective pharyngeal and laryngeal reflexes return. After removal of the endotracheal tube (if one has been used), maintain the animal on a face mask and administer oxygen. If respiratory movements and oxygen saturations are judged to be adequate, remove the mask and continue to monitor the animal. Removal of the endotracheal tube often causes a fall in oxygen saturation, as the airway is not as well-maintained. Before removal of the tube, or if one is not used, before recovery of swallowing and cough reflexes, the mouth should be inspected and any secretions removed using suction. A soft tipped catheter (e.g. a feeding tube) connected to a large syringe allows this to be carried out in small rodents. Monitoring of adequacy of ventilation can also be assessed using a capnograph, and this is particularly useful following prolonged periods of anaesthesia, especially if the animal has been mechanically ventilated.

During this initial recovery period, drapes and surgical equipment should be removed, together with any non-essential monitoring devices. This will allow the animal to be moved rapidly to a more comfortable environment if it regained consciousness more rapidly than expected.

The respiratory depression produced by most anaesthetic agents often persists into the post-operative period. The degree of depression may also increase post-operatively, and this may go unnoticed until severe hypercapnia and hypoxia have developed. For this reason, it may be advisable to continue monitoring the respiratory system, and the use of a pulse oximeter is ideal for this purpose, particularly in larger species (see Chapter 2). If not already in use, the probe can be attached to the animal in the operating theatre and a battery-operated instrument used to monitor the animal during movement to the recovery area. The probe can be left taped in place on the tail or on a digit until the animal has regained its righting reflex.

If a pulse oximeter is unavailable, then other forms of respiratory monitor can be used, for example, positioning a sensor close to the animal's nose. At the very least, regular clinical observation of the animal should be made and the respiratory rate recorded. If respiratory depression is noted, it should be treated using a respiratory stimulant such as doxapram and by the administration of

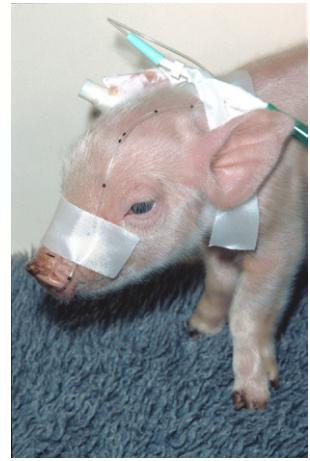

FIGURE 4.4 Administration of oxygen using a nasal catheter in the post-operative period.

oxygen. Since doxapram has a relatively short duration of action (10–15 min), it may be necessary to administer repeated doses or to establish a continuous infusion of the drug.

Many animals appear to benefit if oxygen administration is continued into the immediate post-operative period. This is best achieved in small animals by piping the gas into an animal incubator, but in large animals, it is often more practicable to tape a small, soft-ended catheter at the external nares and use this to administer the gas (Fig. 4.4).

Non-ruminant species should be placed on their sides, with head and neck extended, to try to minimize the risk of airway obstruction. If the animal is recumbent for more than 4 h, then it should be repositioned to lie on its other side, to prevent passive congestion of the lungs and the development of hypostatic pneumonia. In large animals such as dogs and farm animals, it may be necessary to massage areas such as the elbow and hock, to prevent pressure sores developing. If prolonged recumbency is anticipated, it may be advisable to protect these areas with padded bandages.

If the animal begins to vomit, it should be positioned so that its head is below the level of the thorax and abdomen, to try to prevent aspiration of the vomit. If practicable, the mouth and pharynx should be cleared using a vacuum suction, or a piece of suitably sized tubing attached to a 50-ml syringe. Oxygen should be administered, and if inhalation of vomit could have occurred, corticosteroids should be administered (30 mg/kg i.v. of methyl prednisone), together with a broad-spectrum antibiotic.

Ruminants (sheep, goats and cattle) can present particular problems during recovery from anaesthesia. They should be propped up on their sternums to minimize the risk of over-distension of the rumen with gas (rumenal tympany)

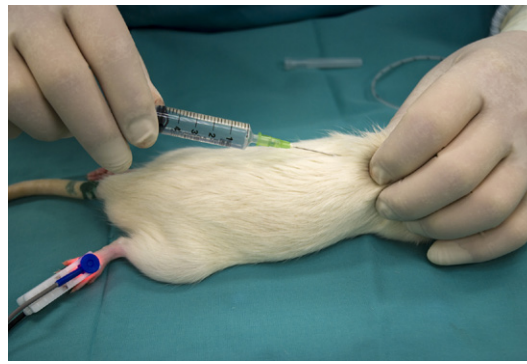

FIGURE 4.5 Administration of subcutaneous fluids to a rat.

and to reduce the risk of inhalation of regurgitated rumen contents. If rumenal tympany develops, it should be relieved immediately either by passing a stomach tube or by puncturing the rumen through the left abdominal wall with a large-bore trochar. If a trochar is not available, the largest possible needle (preferably 12 SWG or larger) should be used. If the member of staff involved is not familiar with this technique, veterinary advice should be sought immediately.

Fluid Therapy

The voluntary water intake of all animals should be recorded post-operatively, even if this consists simply of making a rough estimate based on the level in a water bottle. Fluid intake is frequently reduced post-operatively, and if dehydration is allowed to develop, it can seriously compromise the recovery of the animal. Fluid requirements of most species are approximately 40–80 ml/kg/24 h, but the presence of vomiting or diarrhoea or other abnormal losses will increase this requirement.

If the animal is fully conscious, supplemental fluid is best given by the oral route. If the animal is unable or unwilling to accept oral administration, then dextrose–saline (4% dextrose, 0.18% saline) or saline (0.9%) can be given quickly and easily by the subcutaneous or intraperitoneal routes (Fig. 4.5, Table 4.1). Severe dehydration causes loss of skin tone that causes it to tent and tend to remain elevated when a fold is twisted between the fingers. In larger animals, dehydration will result in the mucous membranes becoming dry to the touch. If this degree of dehydration has inadvertently been allowed to develop, fluids must be administered intravenously. If the animal is severely depressed, then it may not interfere with the intravenous line; however, as the beneficial effects of rehydration occur, it will become more active. Various bandaging and splinting techniques can be used (Fig. 4.6), and Elizabethan collars (Fig. 4.7) can be used in rats, rabbits, dogs and cats to prevent interference with catheter sites.

TABLE 4.1 Approximate Volumes for Fluid Replacement Therapy by Intraperitoneal or Subcutaneous Administration

Species	Subcutaneous (ml)	Intraperitoneal (ml)
Cat (3 kg)	50	50–100
Gerbil (60 g)	1–2	2–3
Guinea pig (1 kg)	10–20	20
Hamster (100 g)	3	3
Marmoset (500 g)	5–10	10–15
Mouse (30 g)	1–2	2
Rabbit (3 kg)	30–50	50
Rat (200 g)	5	5

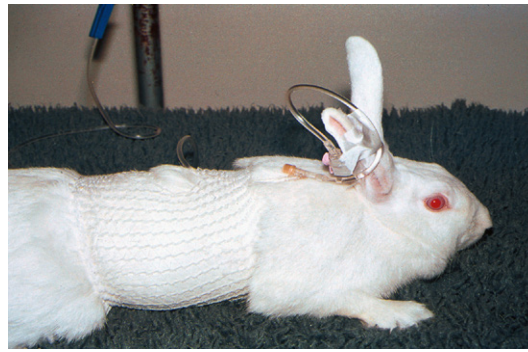

FIGURE 4.6 Stockinette bandage used to secure an intravenous line.

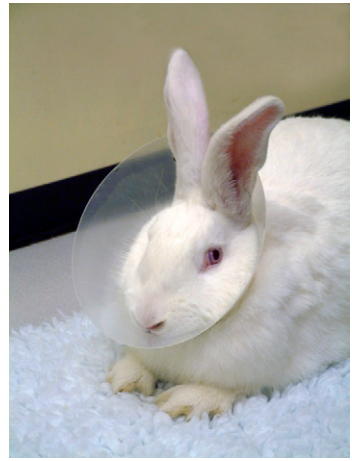

FIGURE 4.7 Elizabethan collar placement in the rabbit.

These bandages and collars may interfere with the animals' normal activities, and may delay resumption of voluntary food and water intake. They also prevent coprophagy in rats, guinea pigs, rabbits, and other species and may cause significant distress to some individuals. It is therefore important to monitor the animals closely, to ensure the benefits outweigh possible problems.

If peripheral vessels are too constricted to catheterize, the intraosseous route can be used in small mammals. A hypodermic needle can be inserted into the proximal end of the femur or tibia, in the same manner as when taking a bone marrow biopsy or performing a marrow transplant (Zehnder, 2008). If the animal is severely depressed, this can be performed under local anaesthesia; otherwise, a light general anaesthetic using an agent such as isoflurane is required.

The monitoring of body weight in the pre- and post-operative periods can provide a good indication of the adequacy of fluid intake. Although a small fall in body weight (<1–3% reduction) will be recorded because of the almost inevitable reduction in food intake that occurs post-operatively, most weight loss usually represents a fluid deficit.

Besides assessing food and water intake, the urinary and faecal output of the animal should be recorded and any abnormalities investigated. As with most of these variables, a meaningful judgement can only be made if the animal has been observed in the pre-operative period. A reduction in urine output may be the result of dehydration, urinary tract injury, or the animal suffering pain. If the bladder is full, it may require catheterization to empty it, although in some instances *gentle* pressure through the abdominal wall will trigger urination. Catheterization is a relatively simple technique, but requires some degree of expertise, and it will usually be preferable to consult a veterinary surgeon or experienced animal technician. If catheterization is not possible, it may prove necessary to drain the bladder by direct puncture through the body wall. This procedure should only be attempted by individuals who have undergone training in the technique. Catheterization of the bladder of most laboratory species requires induction of a brief period of general anaesthesia, or heavy sedation.

Food Intake and Bowel Function

If the animal fails to pass faeces, this may be due simply to an absence of faecal material because of pre-operative fasting. It may also be caused by a loss of normal peristalsis (ileus), or the animal may be constipated and require administration of an enema (e.g. Microlax, SmithKline Beecham). Defaecation may also be suppressed if the animal is in pain, particularly following a laparotomy.

Ileus (gut stasis) can be a serious post-operative complication, and can be life-threatening in rabbits and guinea pigs. Ileus is particularly common after laparotomy, but can also occur after any surgical procedure. Minimizing handling of the bowel can reduce the incidence of ileus following abdominal

surgery. When displacing and handling the viscera is unavoidable, ensure they are kept moist and handled gently. Pigs seem particularly sensitive to handling of the intestines, and we have found 'bowel bags' designed for use in humans to protect the intestines during surgery to be of great value in preventing problems.

If ileus is suspected, then motility stimulants (metoclopramide and cisapride) can be administered to stimulate gut function. In rabbits, ranitidine (2–5 mg/kg by mouth, daily) has been used for managing post-operative inappetance and gut stasis as it promotes gut motility (Kounenis et al., 1992). Pain must be controlled, since this can increase the severity of ileus. The surgical notes should be reviewed to check that a swab was not inadvertently left in the abdomen.

In some species (e.g. rabbits and guinea pigs), inappetance due to other causes can lead to the development of ileus, since normal gut function appears to depend to some extent on regular intake of fibre. Supplemental feeding, using nasogastric tubes if necessary, may be beneficial. A range of specialist dietary preparations is now available for veterinary use in 'exotic' companion species, and these can be of considerable benefit in laboratory animals.

It is important that food and water intake and the other observations described above are recorded carefully. It is helpful to provide a standardized paper or electronic record for each animal, which will encourage nursing staff to complete the required observations. It will also allow easy and rapid reference by staff who may be called in to deal with any problems that might arise. It is always preferable to obtain measures of pre-operative body weight and, if possible, of food and water consumption, so that the progress of an animal can be assessed accurately in the post-operative period. Obtaining daily weights for 3–4 days pre-operatively both familiarizes the animal with handling, and provides a base-line growth curve to allow interpretation of post-operative changes.

Prevention of Wound Infection

Provided careful aseptic surgical techniques have been employed, it may be considered unnecessary to administer antibiotics routinely to animals in the post-operative period. In addition, some species appear to show a remarkable resistance to the development of wound sepsis and appear to tolerate standards of cleanliness that would be totally unacceptable in human medical practice. This apparent resistance to infection must not be used as an excuse for poor surgical standards, and every effort should be made to adopt aseptic techniques for all animal surgical procedures (www.procedureswithcare.org.uk). It has been demonstrated, for example, that rats are not only susceptible to infection but also show behavioural changes following the establishment of wound infections (Bradfield et al., 1992). It is therefore important that all animal species should be monitored carefully for any signs of infection (Morris, 1995).

Since animals will almost inevitably soil their wounds with faeces and urine, administration of prophylactic antibiotics may be useful in minimizing the risk

of infection. One problem of providing peri-operative treatment with antibacterials is the risk of inducing enterotoxaemia in some species, particularly the guinea pig, hamster and rabbit. The use of antibacterial agents in rodents and rabbits has been reviewed by (Morris, 1995), and provided care is taken in the choice of agent, such problems can be avoided. Suggested dose rates of antibiotics for each species are given in Tables 4.2 and 4.3.

MANAGEMENT OF POST-OPERATIVE PAIN

Pain in laboratory animals is a major animal welfare problem that must be addressed if we are to apply Russell and Burch's principle of refinement (Russell & Burch, 2009) – 'to reduce to an absolute minimum the pain and distress experienced by those animals that are used' (in research procedures). In order to provide effective analgesia, it is essential that we have a good knowledge and understanding of animal pain. We need to know when pain might occur and how long it might last, and assess how well it responds to therapy. We also need to consider the advantages and the disadvantages of the various methods of managing pain, and how we can best apply these in different situations. If we are to manage pain relief optimally, and monitor the effects of our therapy, then we will need to recognize the presence of pain and assess its severity. When developing our understanding of this area, we will also need some information about the basic mechanisms involved in pain perception. More fundamentally, we must accept that pain occurs in animals – that it can result in suffering, in a similar way to pain in humans – and so become convinced that its avoidance and alleviation need to be given a high priority.

Despite the emphasis given to humane treatment of laboratory animals in the national legislation of many countries, analgesics may still not be administered routinely in the post-operative period. This omission is particularly common when the animals concerned are small rodents (Richardson & Flecknell, 2005; Coulter et al., 2009; Dorward, 2015). Even when analgesics are given, the assessment of their efficacy in alleviating pain is often based on highly subjective criteria. In many instances, research workers simply assume that administering an analgesic at a recommended dose rate will provide effective pain relief and do not even attempt to assess the degree of pain relief provided. This approach in people was recognized as being one of the major factors in provision of poor post-surgical pain management, with over 50% of patients reporting inadequate analgesia (Owen et al., 1990; Smith, 1991).

A lack of easy to use, reliable and objective method of assessing animal pain leads to use of subjective, often anthropomorphic approaches. Although starting from the assumption that pain in animals is similar to pain in people is appropriate, assuming that animals in pain will therefore behave like people in pain is rarely correct. Different species will behave in different ways when in pain, and this behaviour can be strongly influenced by the presence of a human observer (see 'Pain assessment' below). But in order to manage pain effectively,

TABLE 4.2 Antibiotic and Antibacterial Drug Doses for Laboratory Animals (small animals)

	Mouse	Rat	Hamster	Gerbil	Guinea pig	Rabbit
Cephalexin	15 mg/kg i.m. b.i.d.	15 mg/kg sc b.i.d.	–	25 mg/kg sc u.i.d.	15 mg/kg sc b.i.d.	15 mg/kg sc b.i.d.
Chloramphenicol	50 mg/kg sc b.i.d.	10 mg/kg i.m. b.i.d.	30 mg/kg sc b.i.d.	30 mg/kg sc b.i.d.	20 mg/kg i.m. b.i.d.	15 mg/kg i.m. b.i.d.
Enrofloxacin	10 mg/kg sc b.i.d.	10 mg/kg sc b.i.d.	10 mg/kg sc b.i.d.	10 mg/kg sc b.i.d.	5–10 mg/kg sc b.i.d.	5–10 mg/kg sc b.i.d.
Neomycin	2 mg/ml in drinking water	2 mg/ml in drinking water	250 mg/kg per os in divided doses	100 mg/kg per os in divided doses	5 mg/kg per os b.i.d.	0.2–0.8 mg/ ml in drinking water
Co-trimazine 40/200	30–50 mg/kg sc b.i.d.	30–50 mg/kg sc b.i.d.	30 mg/kg sc b.i.d.	30 mg/kg sc b.i.d.	30–50 mg/ kg sc b.i.d.	30–50 mg/kg sc b.i.d.
Tylosin	–	10 mg/kg sc u.i.d.	10 mg/kg sc u.i.d.	10 mg/kg sc u.i.d.	–	–

Note that the majority of these doses are based solely on clinical experience, since only limited pharmacokinetic data are available for these species (with the exception of enrofloxacin). Before administering any of these compounds, research workers are strongly advised to consult their laboratory animal veterinarian for advice on drug selection and the duration of treatment. For a comprehensive review of the effects of antibiotics in laboratory species, see Morris (1995).

TABLE 4.3 Antibiotic and Antibacterial Drug Doses for Laboratory Animals (larger species)

	Ferret	Cat	Dog	Pig	Sheep	Primate
Amoxycillin	7 mg/kg sc u.i.d.	7 mg/kg sc u.i.d.	7 mg/kg sc u.i.d.	7 mg/kg i.m. u.i.d.	7 mg/kg i.m. u.i.d.	7 mg/kg sc u.i.d.
Cephalexin	10 mg/kg sc u.i.d.	10 mg/kg sc u.i.d.	10 mg/kg sc u.i.d.	10 mg/kg i.m. u.i.d.	10 mg/kg i.m. u.i.d.	10 mg/kg i.m. b.i.d.
Chloramphenicol	25 mg/kg sc u.i.d.	25 mg/kg sc u.i.d.	50 mg/kg sc u.i.d.	11 mg/ kg i.m. u.i.d.	–	20 mg kg i.m. b.i.d.
Enrofloxacin	5–10 mg/kg sc b.i.d.	5 mg/kg sc u.i.d.	5 mg/kg sc u.i.d.	2.5 mg/kg sc u.i.d.	–	5 mg/kg sc b.i.d.
Neomycin	10 mg/kg per os u.i.d. in divided doses	10 mg/ml per os u.i.d. in divided doses	10 mg/kg per os u.i.d. in divided doses	11 mg/kg per os b.i.d.	11 mg/kg per os b.i.d.	10 mg/kg per os b.i.d.
Trimethoprim/ sulphonamide	15–30 mg/kg sc b.i.d.	30 mg/kg sc u.i.d.	30 mg/kg sc u.i.d.	15–24 mg/kg i.m. u.i.d.	15–24 mg/kg i.m. u.i.d.	30 mg/kg sc u.i.d.

Note although some of these doses are based on manufacturer's recommendations, others are based solely on clinical experience, since pharmacokinetic data are not available for all agents in all species. Before administering any of these compounds, research workers are strongly advised to consult their laboratory animal veterinarian for advice on drug selection and the duration of treatment. For a comprehensive review of the effects of antibiotics in laboratory species, see Morris (1995).

we need to be able to assess it; otherwise we cannot know if our analgesics have proved effective.

Pain in humans is defined as 'an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage' (IASP, 1979).

So pain in humans is a sensory and psychological experience with several components:

- Sensory discriminative – where the pain is, how intense it is, what type it is and when it occurs.
- Affective and emotional – the 'feeling' of pain, which is unpleasant and distressing.
- Cognitive – people can think about what their pain means to them and what it could indicate (Am I having a heart attack? Do I have cancer?), and this can change the intensity of pain (Jackson, Wang, & Fan, 2014) and their need for analgesics.

All of these aspects of pain can cause behavioural responses.

The experience of pain is largely subjective, so different people will respond to similar causes of pain differently, have differing experiences and require different treatments. It seems likely that the subjective experience of pain in animals will differ from that in humans, and that different species of animals will experience pain in different ways. In order to accept that animals can experience pain, we have to accept that animals have a conscious awareness of their emotional states – in other words, that they have 'feelings' (Duncan, 1996).

This remains a controversial topic. We can demonstrate relatively easily that animals can experience the sensory components of pain – they have very similar mechanisms for detecting damaging or potentially damaging stimuli (with nociceptors), and this information is transmitted to spinal and higher brain centres in similar ways in animals and humans (Viñuela-Fernández et al., 2007). However, the demonstration of equivalent anatomical structures and physiological processes does not provide conclusive evidence that both the sensory and emotional components of the experience of pain are similar in animals and humans. It is possible that the relative significance, magnitude and duration of pain in response to particular types of injury may all vary in animals (Sneddon et al., 2014). Whether animals possess the same, or a similar capacity as humans, to experience emotions such as pain has been extensively debated for centuries. The main reason for the continued debate is that it is impossible to investigate such emotional states directly – we can only draw inferences from other, indirect measures, such as investigation of behavioural responses. Some philosophers and scientists have firmly asserted that animals cannot experience pain (Bermond, 2001) but only respond to noxious stimuli, without being consciously aware. Others argue in support of the presence of emotional states such as pain (see, e.g. Duncan, 1996; Weary et al., 2006; Fraser, 2009; Panksepp, 2011). This uncertainty regarding animal pain is similar to that relating to pain

in human neonates, a debate that still generates controversy especially in relation to pre-term infants (Bartocci et al., 2006; Bowsher, 2006). The inferences drawn from neuroanatomy, neurophysiology and behaviour that have been used to argue for the capacity to experience pain in neonatal humans (Simons & Tibboel, 2006) have clear parallels in the debate relating to animal pain.

Since pain is a subjective experience, it is doubtful that we will ever be able to demonstrate its presence in animals conclusively, but the growing body of evidence supporting conscious emotional states suggests we should assume a capacity for pain in animals. Certainly, most members of the public have no hesitation in stating that animals experience pain. On reflection, many would agree that the experience might not be exactly the same as the pain they might experience themselves, but nevertheless would have no doubt that it was 'pain'. This view is reflected in the legislation that controls our treatment of animals.

Irrespective of whether animals experience pain or simply respond to activation of sensory nerve (nociceptor) pathways, this process results in major physiological and pathophysiological changes. Consequently, pain or nociception will represent a source of uncontrolled variation in research and may introduce specific confounding factors in some studies. We can therefore advocate the control or elimination of both pain and nociception on both scientific and welfare grounds (Gebhart et al., 2009). Although we would wish to alleviate pain either because of concerns for animal welfare or to reduce a potential confounding factor in a research project, a number of counter-arguments have been advanced to justify withholding analgesics.

- 'Alleviation of post-operative pain will result in the animal injuring itself'.

  Pain has a protective function and is of value in warning of tissue damage in an individual. Pain arising from injured tissues often results in the animal or human immobilizing the affected area since this will help to prevent further injury. However this response is also harmful since the immobility and muscle spasm it produces can cause muscle wasting and weakness. Thoracic and abdominal pain may reduce ventilation and cause hypoxia and hypercapnia. Pain may also cause a marked reduction in food and water consumption (Liles & Flecknell, 1993). Pain in humans has been shown to prolong the metabolic response to surgery (Kehlet & Dahl, 2003), to increase the requirement for hospital care following operative procedures and to have a range of other detrimental effects (Breivik, 1994).

  Provided that surgery has been carried out competently, administration of analgesics to encourage resumption of normal activity by controlling pain rarely results in problems associated with the removal of pain's protective function. Claims that analgesic administration results in skin suture removal are unsubstantiated, and contrary to findings in our laboratory. In certain circumstances, for example, after major orthopaedic surgery, additional measures to protect and support the operative site may be required, but this

is preferable to allowing an animal to experience unrelieved pain. All that is required in these circumstances is to temporarily reduce the animal's cage or pen size, or to provide additional external fixation or support for the wound. It must be emphasized that these measures are very rarely necessary, and in our institute, administration of analgesics to laboratory animals after a wide variety of surgical procedures has not resulted in any adverse clinical effects.

- 'Analgesic drugs have undesirable side-effects such as respiratory depression'.

  In medical clinical practice, analgesic drugs were frequently withheld because of fears of their undesirable side-effects such as respiratory depression and addiction (Cousins et al., 2000). This attitude has changed significantly (Bonnet & Marret, 2007). It is also important to note that the side-effects of opioids, such as respiratory depression, are generally less marked in animals than in people and should rarely be a significant consideration when planning a post-operative care regimen. It is, however, important to consider the potential interactions between analgesic therapy and research protocols, and this is discussed in more detail below.

- 'We do not know the appropriate dose rates and dosage regimens'.

  Another factor that may limit the use of analgesic drugs is a lack of knowledge of appropriate dose rates and dosage regimens. This is primarily a problem of poor dissemination of existing information. Virtually every available analgesic drug has undergone extensive testing in animals, both for safety, and for efficacy in a range of nociceptive tests (e.g. hot-plate and tail flick tests, see section "Assessment of Acute Pain Responses – Nociceptive Tests" below). Safe dose rates are therefore available for a range of drugs in several common laboratory species (Flecknell, 1984; Liles & Flecknell, 1992). The main problem that we currently face is extrapolating available dose rates from one species to another and translating dose rates that are effective in nociceptive tests into those that are appropriate for clinical use. Nevertheless, in many instances, a reasonable guide as to a suitable and safe dose rate can be obtained.

- 'Pain-relieving drugs might adversely affect the results of an experiment'.

  It is clear that some research scientists are reluctant to administer pain-relieving drugs because their use might adversely affect the results of an experiment. Although there will be occasions when the use of one or other type of analgesic is contra-indicated, it is extremely unlikely that there will be no suitable analgesic that could be administered. More usually, the reluctance to administer analgesics is based upon the misconceived idea that the use of any additional medication in an experimental animal is undesirable. The influence of analgesic administration in a research protocol should be considered in the context of the overall response of the animal to anaesthesia and surgery. As discussed in Chapter 1, the responses to surgical stress may overshadow any possible adverse interactions associated with analgesic

administration. In many instances the intra-operative support provided to animals fails to control variables such as body temperature, respiratory function and blood pressure. It seems illogical to assume that these changes are unimportant, but that administration of an analgesic will be of overriding significance. It is an ethical responsibility of a research worker to provide a reasoned, scientific justification if analgesic drugs are to be withheld. It is also important to realize that the presence of pain can produce a range of undesirable physiological changes, which may radically alter the rate of recovery from surgical procedures (Wu & Raja, 2011). In animals, post-surgical pain can reduce food and water consumption, interfere with normal respiration (e.g., after thoracotomy) and reduce a whole range of 'self-maintenance' behaviours. The immobility caused by pain can lead to muscle spasm, can cause atrophy of areas and can slow healing. Prolonged immobility can also cause pressure sores, urine scalding and faeces soiling and can greatly complicate animal care routines. Immobility in mice also results in a fall in body temperature (Refinetti & Menaker, 1992; Weinert & Waterhouse, 2007), and this can have significant and wide-ranging metabolic effects. Positive effects of analgesics have also been reported, for example administration of NSAIDs improved outcomes from embryo transfer in mice (Schlapp et al., 2015).

- Legal constraints.

  In many countries, the use of the majority of opioids is controlled by legislation (e.g. the Misuse of Drugs Act in the United Kingdom). Complying with this legislation often requires careful record keeping of the purchase, storage and dispensing of opioids and may restrict the persons who are able to dispense and administer these substances. In some countries, the degree of record keeping required can act as a strong disincentive to the use of these analgesics in animals. Legislative control, together with genuine safety concerns, may also limit the dispensing of this class of analgesics for use by investigators or technicians. These issues can be addressed by using non-opioid analgesics when these would be appropriate, and by evolving systems of prescribing and supply that make it easier to meet legislative requirements.

PAIN ASSESSMENT

The approach to animal pain based on comparative biology, outlined at the start of this section, leads naturally to the assumption that conditions which would cause pain in people would also lead to the production of pain in animals. When examining animals, we interpret certain clinical signs as suggesting the presence of pain. Following a surgical procedure, a dog might howl or whimper, perhaps guarding the surgical wound, and show signs of avoidance or aggression when the area is handled. These types of behaviour are easy to equate with the behaviour of humans in pain, so we readily diagnose animals showing these

clinical signs as being in pain and may then give analgesics. Unfortunately, this anthropomorphic view of pain is flawed. Many animals do not respond to conditions and procedures that would cause pain in humans in a way that is immediately apparent as pain-related behaviour. For example, to an untrained observer, rats do not appear to show obvious signs of pain following routine laparotomy. This is an apparent contradiction of assumptions based on comparative biology – people do experience significant pain after abdominal surgery, most complain about their pain and most require opioids or other forms of analgesia. This discrepancy between the apparent behaviour of animals and the behaviour that would be predicted from human experience gives rise to the view that, although pain may occur in animals, it is less severe than that in people. It also leads to the assumption that the more resilient nature of animals results in more rapid recovery with less experience of pain. The natural consequence of this is to assume that animals do not require analgesics as frequently as people, and perhaps may not even require them at all. The key to introducing effective pain control is therefore to improve our methods of pain recognition and assessment. For example, in the laparotomy example mentioned earlier, if the animal is observed closely and its behaviour analysed carefully, then more subtle changes become apparent (Roughan & Flecknell, 2001). These changes may be normalized by administration of an analgesic, and this supports the view that they are related to the presence of post-operative pain.

Pain assessment is important not simply because it would encourage greater use of analgesics, but because it would also encourage more appropriate use of these drugs. In many animal research units, national legislation requires that pain is assessed based on the assumption that procedures that are painful in people will be equally painful in animals. Following a surgical procedure, it is therefore assumed that an analgesic will be required. The choice of analgesic should be determined in some part by the degree of pain that is present, since inappropriate use of potent analgesics may lead to the undesirable side-effects of these agents outweighing any benefits arising from alleviation of pain (Blaha & Leon, 2008). Similarly, the use of low-potency agents in circumstances in which severe pain is present will result in insufficient pain relief. Simply assuming that after identical surgical procedures, the degree of pain present in all animal species and in people will be identical is highly unlikely to be correct. Even if this broad comparison were possible, it would also be necessary to assume that the duration of pain, and hence requirement for pain relief, was identical in all animal species and humans in equivalent circumstances. It also fails to account for individual variation in response to analgesics. Following identical surgical procedures, different human patients can have markedly different analgesic requirements (Yoshida et al., 2015). This has become clearly apparent with the introduction of patient-controlled analgesia, which removed some of the obstacles to analgesic administration (Skues et al., 1993). Although equivalent data in laboratory animals are limited, data from nociceptive tests in research animals show similar variation among individuals (Cowan et al., 1977a; Cowan et al., 1977b), and both age

and sex have been shown to influence the responses to analgesics (Frommel & Joye, 1964; Kest et al., 2000). Major variations in the behavioural and endocrine responses to surgery have been reported in mice (Wright-Williams et al., 2007), and similar changes in nociceptive thresholds occur (Mogil et al., 1999, 2006). Different strains of rats and mice also differ in their responses to different analgesics (Elmer et al., 1998; Morgan et al., 1999). Selection of an arbitrary dose of analgesic will therefore almost certainly lead to overdosing of some animals, and provision of inadequate analgesia for others. Development of reliable methods of pain assessment would enable analgesic treatment to be tailored to suit the needs of each individual animal.

This problem, of varying analgesic responses in different individuals is encompassed in medical practice in the concept of 'number needed to treat' (NNT) (McQuay et al., 2012). In the context of analgesic use, this is defined as the number of patients that need to receive a particular analgesic in order for one patient to experience a 50% reduction in pain. NNT is therefore a measure of the effectiveness of a particular analgesic. Rankings of analgesics based on systematic reviews of controlled trials assigns NNTs ranging from 1.5 for eterocoxib (an NSAID) to 17 for codeine. Morphine has an NNT of 2.9. NNT is rarely reported in animal studies, but applying the concept would help better explain the variability seen in responses of individuals to painful procedures and the effects of analgesics. This in turn would give a more realistic expectation of outcomes, and most importantly it would reinforce the need for careful assessment of the efficacy of analgesic therapy. An animal that does not respond to treatment requires either additional doses of the analgesic, or administration of a different analgesic.

Methods of Assessing Animal Pain

Assessment of Acute Pain Responses – Nociceptive Tests

During drug development programs, an acute noxious stimulus is often used to determine the efficacy of different analgesics. The majority of these investigations have been carried out in rodents, and use mechanical, thermal or electrical stimuli to produce brief painful stimuli (reviewed by Le Bars et al., 2001). Most studies are designed in such a way that the animal can terminate the stimulus. These assessment methods enable determination of the analgesic potency of different drugs, but the neurological mechanisms involved do not fully reflect those involved in clinical pain. In addition, the dose rates required vary depending upon the test and the analgesic used (Flecknell, 1984; Liles & Flecknell, 1992). For example, NSAIDs are generally relatively ineffective in tests using thermal and electrical stimuli, and the test systems used require some modification when assessing this class of analgesics. This relative lack of efficacy in response to acute brief noxious stimuli, in comparison with opioids, is sometimes misinterpreted as showing that NSAIDs are therefore unlikely to be effective in controlling clinical pain. This is clearly not the case. It is also

difficult to relate the dose rates that are effective in these test systems with those that are needed to control clinical pain (Roughan & Flecknell, 2002). In all analgesics, both the potency and the duration of action vary depending on the test used and the strain and the sex of the animal. Results from these tests indicate a likely effective dose range, and data from studies of adverse effects indicate the likely dose ranges that could cause significant clinical problems. Taken together, these results enable initial suggestions of dose rates to be made, but objective scoring systems that can be applied to the assessment of post-operative pain are needed. These enable assessment of an individual animal to confirm that an appropriate and effective dose of analgesic had been administered following a given surgical procedure. If appropriate pain scoring schemes cannot be used, then dose rates are probably best estimated from the results of tonic analgesiometric tests such as the late-phase formalin test (Roughan & Flecknell, 2002).

Assessment of Post-Operative Pain

A number of different approaches to pain assessment in animals have been suggested, but progress in developing and validating scoring systems has been slow. Although suggestions for assessing pain were published over three decades ago (Flecknell, 1984), these were largely based on subjective clinical criteria that had not been subjected to any form of validation. A proposal to develop more robust scoring schemes was published by Morton and Griffiths (1985). This paper influenced a large number of other groups, who modified the original hypothesis, but retained the central notion of identifying pain-specific behaviours and rating them in some way. However, attempts to apply this were largely unsuccessful (Beynen et al., 1987), primarily because the variables selected for inclusion were not fully identified and the scales used (0–3) not sufficiently well characterized. The scheme has proven much more successful when applied as a means of developing more humane endpoints for studies. These problems were identified by the original authors, but indiscriminate application of the system seems to have led to failure in identifying animals in pain, and pain scoring systems are almost certainly still under-used in most facilities (Hawkins, 2002). This is to be regretted, since when applied carefully, the Morton and Griffiths scheme provides a structured method for assessing animals, and can be a useful aid for developing endpoints in a range of different situations.

Measurements of body weight and food and water intake have been proposed as potential indicators of post-operative pain and the efficacy of analgesic therapy (Liles & Flecknell, 1993; Jacobsen et al., 2012). These latter measures are objective, but they are retrospective measures and so could not be used to modify analgesic therapy for a particular animal. They are also relatively non-specific measures that can be influenced by numerous factors in addition to post-operative pain. They can, however, be used as a simple measure of post-operative recovery, and as a means of adjusting future analgesic and post-operative care regimens for similar animals undergoing similar surgical procedures.

Behaviour-Based Pain Scoring Systems

More recently, behaviour-based schemes for assessing animal pain have been developed (Roughan & Flecknell, 2001). Further development of the system in rats showed it could be used successfully by placing the animal in an observation cage for a brief period of time (5–10 min) (Roughan & Flecknell, 2003). Staff can learn to apply this scoring system after a short period of training (Roughan & Flecknell, 2006). A problem with applying behaviour-based systems is that it relies on animals recovering relatively rapidly from anaesthesia. When recovery is delayed, or is associated with prolonged sedation, then animals may fail to express pain behaviour. At present, it is not certain whether this is because the animals are not experiencing pain, or whether the heavy sedation prevents them showing signs of pain. The scoring system may also be influenced by other factors, such as fear and apprehension, and unexpected variations in behaviour between different strains of the animal may be encountered. Nevertheless, this approach offers a step forward in developing a practically useful scoring system to evaluate pain and analgesic efficacy after at least some types of surgery in rats. Studies in mice (Wright-Williams et al., 2007), rabbits (Leach et al., 2009) and guinea pigs (Ellen, personal communication) suggest it may be possible to develop similar systems in other laboratory species.

Pain Faces

The assessment of pain by evaluating facial expressions has been widely used in infants and children (Craig et al., 1993; Grunau et al., 1998). Recently, a means of analysing pain faces in mice, using a Mouse Grimace Scale, was described (Langford et al., 2010) and the method applied successfully to assess post-surgical pain (Leach et al., 2012; Matsumiya et al., 2012; Faller et al., 2015). Grimace scales have been described for the rat (Sotocinal et al., 2011; Liao et al., 2014), rabbit (Keating et al., 2012) and horse (Costa et al., 2014), and studies are in progress in other species. In rats and mice, a series of separate action units have been identified, and each of these is scored separately and then combined to produce an overall grimace score (Figs 4.8 and 4.9). Most studies using the technique have obtained high resolution images, and the scored them retrospectively, but initial work in a number of establishments has indicated that 'cage-side' assessment is possible.

It is important to appreciate that use of this approach is still at an early stage of development. It is not yet certain what factors other than pain can influence facial expression in many species, and sedation persisting after anaesthesia appears to have significant effects in increasing grimace scores. It is also clear that different strains of mice have varying grimace scores when pain-free and varying degrees of response following surgery (Miller, personal communication). Using grimace scoring as the sole means of evaluating animals after surgery may lead both to a failure to detect animals in need of analgesic treatment,

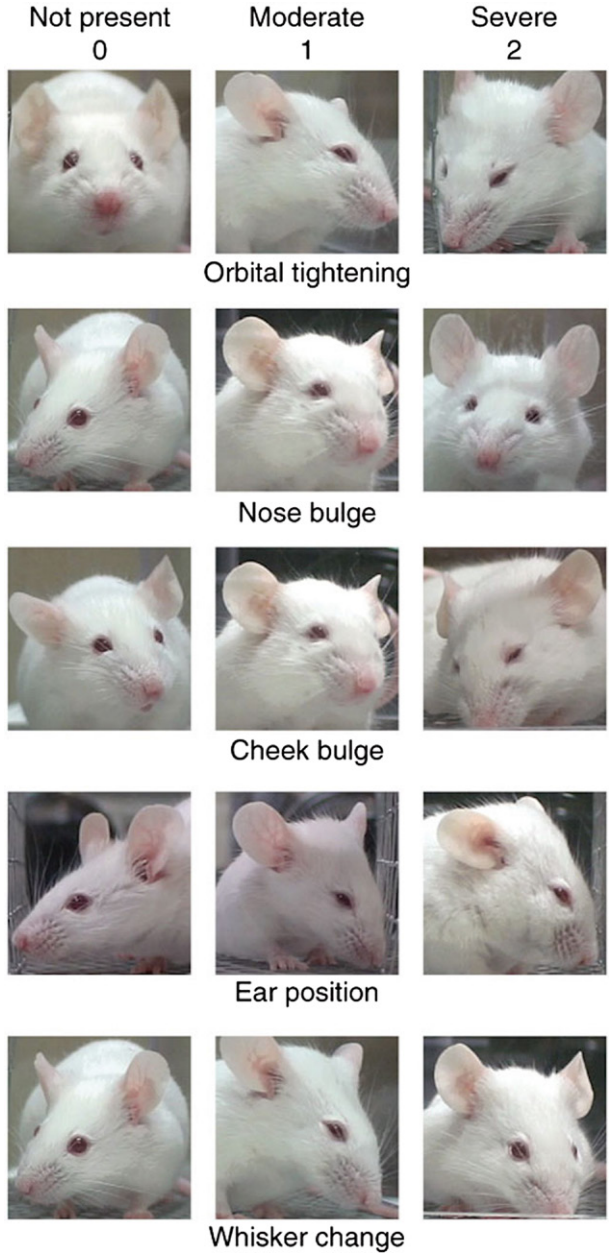

FIGURE 4.8 Mouse grimace scale.

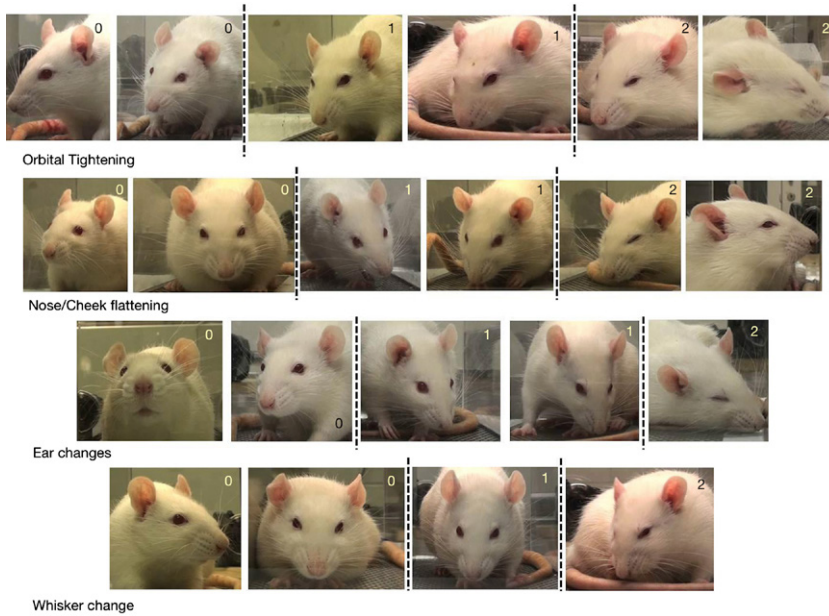

FIGURE 4.9 Rat grimace scale.

and administration of analgesics to animals that do not require them. However, when used in combination with other measures, grimace scoring is likely to prove valuable. It has, for example, been used as a rapid means of determining the need for analgesic intervention (Oliver et al., 2014). It also has the advantage of being relatively unaffected in normal animals by opioid analgesics, that have been shown to markedly influence normal behaviour. Grimace scores may also be a method of pain assessment that can be applied following a wide range of different surgical procedures.

Other Behavioural Assessments

Unalleviated post-operative pain is likely to inhibit performance of a range of normal behaviours, and some highly motivated behaviours may be sensitive measures of the efficacy of analgesic use. Use of these measures is also at an early stage, but some studies suggest that nest-building in mice (Arras et al., 2007), or the time to commence nest building (Jirkof et al., 2013; Rock et al., 2014), may be useful assessments. Similarly, burrowing behaviour has been shown to be both a measure of general welfare in rats and mice (Deacon, 2006, 2009) and also, in rats, to be reduced in frequency by pain, with this effect reversed by analgesics (Andrews et al., 2012; Whittaker et al., 2014). Burrowing may also be a useful measure of post-operative pain in mice (Jirkof et al., 2010). As with the other measures described above, a number of factors other than pain may influence both nest-building and burrowing, and in mice considerable

FIGURE 4.10 Mouse with a well-formed nest.

strain variation occurs (Deacon et al., 2007). However, it is relatively easy to provide these assessments in rodents, by including nesting material and burrowing opportunities within their cages (Deacon, 2012). For burrowing, a water bottle filled with food pellets provides an easy means of assessment in mice. Although more sophisticated means of using these assessments are likely to be developed, at present they can form a useful overall assessment of animals following surgery. For example, if a mouse was forming a good quality nest pre-operatively (Fig. 4.10), and is failing to do so post-surgery, this should trigger careful evaluation. Similarly, a reduction in burrowing activity should give cause for concern.

General Assessments of Behaviour

Although detailed well-controlled studies are lacking in most animals, a number of behavioural changes may occur following surgery, and some of these may be pain related. The general types of changes that may be assessed are described below, and more specific descriptions of abnormal, pain-related behaviours for the rat, mouse and rabbit are listed in Table 4.5.

Activity

As mentioned above, the overall level of activity of an animal suffering pain is often reduced, and most laboratory species will tend to remain motionless in a corner of their cage. Occasionally, an animal may show unusual restlessness and may seem unable to relax. When the animal moves, its posture or gait may be altered. This is most obviously seen when limb pain is present, but is often noted following laparotomy, when the back may be arched to reduce tension on the abdominal muscles. This altered posture, coupled with a tendency to shorten the length of each stride, can be seen both in rodents and rabbits, and also in dogs, cats and farm animals. Pain from an abdominal incision may also affect the frequency of urination and defaecation in species in which this process requires marked

abdominal muscle contraction. Particular behaviours such as climbing, rearing up onto the hindlimbs, stretching and scratching may also be affected, but careful observation by an experienced assessor may be necessary before such changes are noticed. A further complication in assessing behaviour is that the animal may change its responses in the presence of an unfamiliar observer. In addition, some species are nocturnal, and observation of normal behaviour will require attendance during the dark phase of its photoperiod. Both of these problems can be solved to some extent by using video cameras to monitor the animal's behaviour.

Appearance

Even when at rest, the animal's overall appearance may be altered. The animal may adopt a hunched-up posture and position itself in a corner of its cage or pen. Pain may result in a reduction in grooming activity, which leads to the development of an unkempt appearance of the coat and soiling of the anus. Lack of grooming may also lead to the build-up of an encrusted discharge around the eyes, nose and mouth. Rats may develop dark encrustations around the eyes or nose. This material is porphyrin excreted from the Harderian glands, and if wiped with moist cotton wool, it has a red colour. The presence of porphyrin staining is a non-specific stress response, but should alert the observer to the possibility that the stress involved may be pain (Fig. 4.11).

Temperament

Changes in temperament often occur in animals experiencing pain. Previously tractable animals may become uncharacteristically aggressive and may bite or

FIGURE 4.11 Rat with porphyrin staining around the eyes and nose. This is a non-specific stress response, but can be associated with the presence of pain.

scratch. Alternatively, a previously active animal that showed obvious interest in its handler may appear completely apathetic. The animal may cower away from the handler and attempt to avoid being restrained. The interpretation of any of these types of behaviour will require not only a knowledge of the normal predicted behaviour of an animal of that particular age, sex and species, but also prior knowledge of the normal behaviour of that particular individual. Clearly, close liaison with animal care staff is essential in attempting to assess the behaviour of an animal in the post-operative period.

Vocalizations

Acute pain can make an animal cry out, and handling an animal that is in pain may provoke such a response. The pitch of the cry may be abnormal and may be accompanied by attempts to bite the handler or to escape. Animals in pain rarely cry continuously, although on occasions dogs may howl or whimper for long periods and sheep and cattle may also make prolonged vocalization. When assessing pain in rodents, it is important to appreciate that many of their cries are at high sound frequencies that are inaudible to humans.

Feeding Behaviour

Food and water intake are often markedly reduced if an animal is in pain. Severe pain is often associated with a complete cessation of eating and drinking. These changes in feeding may go unnoticed if the animal is fed *ad libitum* from a hopper, or if other animals that are feeding normally are present in the cage or pen. A reduction in body weight as a consequence of this inappetance can usually be readily detected, but normal day-to-day variations in body weight must also be appreciated. To improve the detection of changes in food and water intake, weighed quantities of food and water should be dispensed and daily intake measured. Weighing the food hopper and the water bottle provides a satisfactory means of monitoring intake in larger rodents. Care must be taken that spillage of food by the animal does not result in intake being erroneously assessed as normal. In addition to recording food consumption, the animal should be weighed each day to determine any changes in body weight.

A reduction in food and water intake will also be reflected in a reduction in faecal and urine output, but the latter may be difficult to detect. The onset of dehydration will be reflected in the clinical appearance of the animal. Loss of skin tone will cause it to tent and tend to remain elevated when a fold is twisted between the fingers.

Alterations in Physiological Variables

Pain generally causes changes in the respiration pattern and rate. This can be dramatic following thoracic surgery, when the reduction in the depth of respiration can cause considerable concern. In other instances, the change may be less

obvious and masked by the normal tendency of animals such as rodents or rabbits to respond to restraint or close observation with an increase in their respiratory rate. Pain may also affect the cardiovascular system. Frequently, the heart rate is increased, but the natural responses to handling may mask these changes. The other factors influencing these cardiorespiratory variables may render them of little use for routine assessment of post-operative pain (Conzemius et al., 1997; Ledowski et al., 2012).

Severe pain may cause the development of circulatory failure (shock), with blanching and chilling of the extremities and a decrease in the strength of the peripheral pulse.

A Practical Approach to Pain Assessment

Although well-validated, quantitative methods of post-operative pain assessment have yet to be developed for all species, virtually all studies of post-operative pain in animals have demonstrated a beneficial effect of analgesic therapy (Flecknell, 1994). It is not unreasonable, then, to suggest that most animals require some analgesics post-operatively. When they are available, pain assessment should be made using species-specific scoring systems (e.g., using the Glasgow Pain Scale in dogs) (Reid et al., 2013). Abnormal behaviours following specific types of surgery can also be assessed (see Table 4.4). Suggestions as to means of evaluating pain in a number of species are available (Harvey-Clark et al., 2000; Hay et al., 2003; Ashley et al., 2005; Fitzpatrick et al., 2006; Weary et al., 2006; Hudson et al., 2008) and these descriptions can help develop appropriate scoring systems in research facilities.

As a general approach, it is recommended that attempts are made to assess pain using a modification of the Morton and Griffith scheme, using a range of variables to develop a scoring scheme adapted to the specific type of surgery and animal species concerned. The selection of variables should be made after observing a small number of animals following the particular surgery, to determining which variables are most affected by the procedure. The assessment may include the general types of changes outlined above, and should incorporate specific pain-related behaviours when appropriate. These measures can be expanded to include use of facial expression, and behaviours such as nest building and burrowing.

Many of the changes that will be observed are primarily indicators of ‘abnormality’ and are not necessarily indicative of pain – they could be caused by the general response to surgery or anaesthesia, or could be the result of dehydration, hypothermia or other factors. However, noting a positive response to analgesic therapy helps indicate which measures can be useful, but remember that some analgesics (e.g. opioids) can alter behaviour.

Developing a scoring scheme takes time and effort, and it is important to involve all of those who may carry out the assessments, so that a standardized approach can be developed. If several staff assess the same animal together,

TABLE 4.4 Pain-Related Behaviour Following Abdominal Surgery in Rats, Mice and Rabbits

Species	Behaviours
Rat	Back arching (vertical stretch from crouched position as in felines upon waking); belly-press (muscular contraction where the ventral abdomen is pressed upon bedding – occurs immediately prior to or during ambulation); fall/stagger (stagger or fall during ambulation – a rapid transition to crouch from high or low rear. More often a partial loss of balance during grooming, resulting in lateral lying position from which recovery to balanced crouched posture occurs almost immediately); writhing (writhing involving lateral contortion of flank abdominal muscles, usually when crouching but also during transient break in walking or grooming); twitch (brief, seemingly spasmodic contraction, usually of the muscles of the back, travelling in an anterior–posterior direction)
Mouse	Writhing (slow contraction of abdominal muscles); rear-leg lift (momentary lifting of rear paw, often associated with writhe or press); belly-press (pressing of abdomen to cage floor, often associated with hindlimb extension); flinching (rapid contraction of muscles of back, as in twitching, but also involving other areas of the body)
Rabbit	Twitch (rapid movement of fur on back); wince (rapid movement backwards in a rocking motion, accompanied by eye closing and swallowing); stagger (partial loss of balance); flinch (body jerks upwards for no apparent reason); press (abdomen pushed towards floor, usually before walking); writhe (contraction of the abdominal muscles)

For further details, see Roughan and Flecknell (2003, 2004), Wright-Williams et al. (2007) and Leach et al. (2009).

then grading of the degree of abnormality will become more consistent. This approach is particularly valuable when different staff are involved in assessments on different occasions. Applying a scoring scheme, once it has been developed, also takes time, and the staffing resources needed should be included in the general infrastructure of the facility or the specific budget for each research project.

Pain assessment, whether based on formal scoring systems, or more subjective evaluations will be facilitated by:

- A good knowledge of the species-specific behaviours of the animal being assessed.
- A knowledge and comparison of the individual animal's behaviour and appearance before and after the onset of pain (e.g. pre- and post-operatively).
- The use of palpation or manipulation of the affected area and assessment of the responses obtained.

- Examination of the level of function of the affected area, for example, leg use following injury or limb surgery, together with a knowledge of any mechanical interference with function.
- The use of analgesic regimens or dose rates that have been shown to be effective in controlled clinical studies, and evaluation of the changes in behaviour this brings about.
- A knowledge of the non-specific effects of any analgesic, anaesthetic or other drugs that have been administered.

PAIN RELIEF

Leaving aside the problems of pain assessment, empirical treatment of presumed painful conditions will continue, and it is not unreasonable to assume that analgesic therapies shown to be effective in people are also likely to be effective in animals. A growing body of data from well-controlled trials of analgesic efficacy in animals is now also becoming available, but even when these have not been completed, results of nociceptive tests can be used to guide dosing regimens. Analgesics can be broadly divided into two groups, the opioids or narcotic analgesics and the NSAIDs such as aspirin. Local anaesthetics can also be used to provide post-operative pain relief by blocking all sensation from the affected area. Suggested dose rates of analgesics are given in Tables 4.5–4.8.

Analgesic Agents

NSAIDs

Traditionally, NSAIDs have been considered low-potency analgesics, suitable for the control of mild pain, or as agents primarily for use in conditions such as arthritis, where the inflammatory component of the disease process was responsible for some or all of the pain. The perception of NSAIDs has changed with the introduction of a number of compounds that have been shown to have considerable analgesic potency (Gaynor & Muir, 2014). In laboratory species, data from a number of nociceptive tests provide a basis for estimating appropriate dose rates for clinical use in these species (Liles & Flecknell, 1992), and some investigations of their use for post-operative pain control are now available. Estimating the frequency of administration is much more difficult, since there are very considerable variations in elimination times for NSAIDs in different species (Lees et al., 1991; Busch et al., 1998; Baert & De Backer, 2003; Turner et al., 2006; Shukla et al., 2007). Despite these problems, there are now a range of NSAIDs with clear indications for use in alleviating pain in animals (Lees et al., 2004; Papich, 2008).

NSAIDs exert their main effects by inhibiting the action of the enzyme cyclooxygenase (COX). COX is an enzyme that catalyses the conversion of arachidonic acid to prostaglandin H2, the first step in the synthesis of prostanoids. The prostanoids are important mediators of inflammation, and both

TABLE 4.5 Suggested Dose Rates for Non-Steroidal Anti-Inflammatory Drugs in Laboratory Animals (small animals)

Drug	Mouse	Rat	Guinea pig	Rabbit	Ferret
Aspirin	120 mg/kg per os	100 mg/kg per os	87 mg/kg per os	100 mg/kg per os	200 mg/kg per os
Carprofen	5 mg/kg sc	5 mg/kg sc	4 mg/kg sc ? once daily	1.5 mg/kg per os u.i.d., 4 mg/kg sc u.i.d.	4 mg/kg sc u.i.d.
Diclofenac	8 mg/kg per os	10 mg/kg per os	2.1 mg/kg per os	–	–
Flunixin	2.5 mg/kg sc or i.m.? 12 h	2.5 mg/kg sc or i.m.? 12 h	2.5 mg/kg sc or i.m.? 12 h	1–2 mg/kg sc i.m.? 12 h	0.5–2 mg/kg sc 12–24 h
Ibuprofen	30 mg/kg per os	15 mg/kg per os	10 mg/kg i.m.? 4 h	10 mg/kg i.v.? 4 h	–
Indomethacin	1 mg/kg per os	2 mg/kg per os	8 mg/kg per os	12.5 mg/kg per os	–
Ketoprofen	5 mg/kg sc	5 mg/kg sc	–	3 mg/kg i.m.	3 mg/kg i.m.
Meloxicam	5 mg/kg sc or per os	1 mg/kg sc or per os	0.1–0.3 mg/kg sc or per os every 24 h	0.6–1 mg/kg sc or per os	0.1–0.2 mg/kg sc or per os
Paracetamol (acetominophen)	200 mg/kg per os	200 mg/kg per os	–	–	–

Note that considerable individual and strain variation in response may be encountered and that it is therefore essential to assess the analgesic effect in each individual animal.

TABLE 4.6 Suggested Dose Rates for Non-Steroidal Anti-Inflammatory Drugs in Laboratory Animals (larger animals)

Drug	Pig	Sheep	Primate	Dog	Cat
Aspirin	10–20 mg/kg per os, 4–6 h	50–100 mg/kg per os, 6–12 h	20 mg/kg per os, 6–8 h	10–25 mg/kg per os, 8 h	10–25 mg/kg per os, every 48 h
Carprofen	2–4 mg/kg i.v. or sc, once daily	2–4 mg/kg sc or i.v., once daily (? 2–3 days)	3–4 mg/kg sc u.i.d.	4 mg/kg i.v. or sc, once daily 1–2 mg/kg b.i.d. per os, for 7 days	4 mg/kg sc or i.v.
Flunixin	1–2 mg/kg i.v. or sc, once daily	2 mg/kg i.v. or sc, once daily	0.5–2 mg/kg sc or i.v. daily	1 mg/kg i.v. or i.m., 12 h; 1 mg/kg per os, daily for up to 3 days	1 mg/kg sc, daily for up to 5 days
Ibuprofen	–	–	7 mg/kg per os	10 mg/kg per os, 24 h	–
Ketoprofen	1–3 mg/kg i.v., i.m., sc, per os, 12 h	–	2 mg/kg sc daily	2 mg/kg sc, i.m. or i.v., once daily for up to 3 days 1 mg/kg per os, daily for 5 days	1 mg/kg sc, once daily for up to 3 days; 1 mg/kg per os, once daily for up to 5days
Meloxicam	0.4 mg/kg sc, once daily	0.5 mg/kg i.v., sc up to b.i.d. for 1 day, then 0.5 mg/kg per os u.i.d. for 5 days	0.1–0.2 mg/kg u.i.d. sc or per os	0.2 mg/kg u.i.d. sc or per os, then 0.1 mg/kg sc or per os	0.2 mg/kg u.i.d. sc or 0.3 mg/kg per os, then 0.1 mg/kg sc or per os
Tolfenamic acid	–	–	–	4 mg/kg sc daily for 2 days	4 mg/kg sc daily for 2 days
Paracetamol (acetominophen)	–	–	–	15 mg/kg per os, 6–8 h	Contra-indicated

Note that considerable individual and strain variation in response may be encountered, and that it is therefore essential to assess the analgesic effect in each individual animal.

TABLE 4.7 Suggested Dose Rates for Opioid Analgesics in Laboratory Animals (small animals)

Drug	Mouse	Rat	Guinea pig	Rabbit	Ferret
Buprenorphine	0.05–0.1 mg/kg sc 12 h	0.01–0.05 mg/kg sc or i.v., 8–12 h 0.1–0.25 mg/kg per os, 8–12 h	0.05 mg/kg sc, 8–12 h	0.01–0.05 mg/kg sc or i.v., 8–12 h	0.01–0.03 mg/kg i.v., i.m. or sc, 8–12 h
Butorphanol	1–2 mg/kg sc, 4 h	1–2 mg/kg sc, 4 h	1–2 mg/kg sc, 4 h	0.1 2 0.5 mg/kg i.v., 4 h	0.4 mg/kg i.m., 4–6 h;
Hydromorphone	–	–	–	0.1–0.2 mg/kg ? 6–8 h	0.1–0.2 mg/kg ? 6–8 h
Morphine	2.5 mg/kg sc, 2–4 h	2.5 mg/kg sc, 2–4 h	2–5 mg/kg sc or i.m., 4 h	2–5 mg/kg sc or i.m., 2–4 h	0.5–2 mg/kg i.m. or sc, 6 h
Nalbuphine	2–4 mg/kg i.m.? 4 h	1–2 mg/kg i.m., 3 h	1–2 mg/kg i.v., i.p. or i.m.	1–2 mg/kg i.v., 4–5 h	–
Oxymorphone	0.2–0.5 mg/kg sc ? 4 h	0.2–0.5 mg/kg sc ? 4 h	0.2–0.5 mg/kg sc ? 4 h	0.05–0.2 mg/kg sc ? 6–8 h	0.05–0.2 mg/kg sc ? 6–8 h
Pentazocine	5–10 mg/kg sc 3–4 h	5–10 mg/kg sc, 3–4 h	–	5–10 mg/kg sc, i.m. or i.v., 4 h	–
Pethidine (Meperidine)	10–20 /kg sc or i.m., 2–3 h	10–20 mg/kg sc or i.m., 2–3 h	10–20 mg/kg sc or i.m., 2–3 h	5–10 mg/kg sc or i.m., 2–3 h	5–10 mg/kg i.m. or sc, 2–4 h
Tramadol	5 mg/kg sc, i.p.?	5 mg/kg sc, i.p.?	–	–	–

Note that considerable individual and strain variation in response may be encountered, and that it is therefore essential to assess the analgesic effect in each animal.?, duration of action uncertain.

TABLE 4.8 Suggested Dose Rates for Opioid Analgesics in Laboratory Animals (larger animals)

Drug	Pig	Sheep	Primate	Dog	Cat
Buprenorphine	0.01–0.05 mg/kg i.m. or i.v., 6–12 h	0.005–0.01 mg/kg i.m. or i.v., 4 h	0.005–0.01 mg/kg i.m. or i.v., 6–12 h	0.005–0.02 mg/kg i.m., sc or i.v., 6–12 h	0.005–0.01 mg/kg sc or i.v., 8–12 h
Butorphanol	0.1–0.3 mg/kg i.m. or i.v., 4 h	0.5 mg/kg i.m. or i.v., 2–3 h	0.01 mg/kg i.v.,? 3–4 h	0.2–0.4 mg/kg sc or i.m., 3–4 h	0.4 mg/kg sc, 3–4 h
Hydromorphone	–	–	–	0.05–0.2 mg/kg i.m., sc 2–4 h	0.1 mg/kg i.m., sc 0.2 2–4 h
Morphine	0.2–1 mg/kg i.m., ? 4 h	0.2–0.5 mg/kg i.m.,? 4 h	1–2 mg/kg sc or i.m., 4 h	0.5–5 mg/kg sc or i.m., 4 h	0.3 mg/kg sc, 4 h
Nalbuphine	–	–	–	0.5–2.0 mg/kg sc or i.m., 3–4 h	1.5–3.0 mg/kg i.v., 3 h
Oxymorphone	0.15 mg/kg i.m. 4 h	–	0.15 mg/kg i.m. 4–6 h	0.05–0.22 mg/kg i.m., sc or i.v., 2–4 h	0.2 mg/kg sc or i.v.
Pentazocine	2 mg/kg i.m. or i.v., 4 h	–	2–5 mg/kg i.m. or i.v., 4 h	2 mg/kg i.m. or i.v., 4 h	–
Pethidine (meperidine)	2 mg/kg i.m. or i.v., 2–4 h	2 mg/kg i.m. or i.v., 2–4 h	2–4 mg/kg i.m. or i.v., 2–4 h	10 mg/kg i.m., 2–3 h	2–10 mg/kg sc or i.m., 2–3 h
Tramadol	–	–	? 1–2 mg/kg sc or i.v., 2 mg/kg orally ? b.i.d.	2–5 mg/kg i.v. or sc, 2–5 mg/kg orally t.i.d.	2–4 mg/kg sc

Note that considerable individual and strain variation in response may be encountered, and that it is therefore essential to assess the analgesic effect in each animal.?, duration of action uncertain.

directly and indirectly influence the degree of pain associated with tissue injury and other inflammatory processes. COX exists in two isoforms: COX-1 and COX-2. A third isoform, COX-3, has now been described (Botting & Ayoub, 2005; Chandrasekharan et al., 2002). COX-1 mediates essential physiological responses in a wide range of body tissues; in contrast, COX-2 is expressed by cells that are involved in inflammation (e.g. macrophages), and it has emerged as the isoform primarily responsible for the synthesis of prostanoids involved in acute and chronic inflammatory states. It was initially thought that developing NSAIDs with effects only on COX-2 would avoid any undesirable side-effects; however, this relatively simple view of the functions of COX and the effects of COX-1 and COX-2 inhibition have become steadily more complex (Hotz-Behofsits, Simpson, Walley, & Bjarnason, 2010).

One of the problems that arise when trying to interpret information about new and older NSAIDs is the wide variation of assays used to determine COX-1 and COX-2 inhibitory effects. This is further compounded by the use of both EC_{50} and EC_{80} for comparison, and the failure in some studies to link these data to the tissue concentrations that are likely to be produced when the drug is used clinically. A further problem is that the drug concentration produced, the relative COX-1–COX-2 inhibition and the duration of action of the drug are likely to vary between species. It is therefore important to balance an enthusiasm to provide the most effective pain management, with the need for caution when using drugs in different species. It is clear, however, that the new generation of highly selective COX-2 NSAIDs, in particular the coxibs such as deracoxib and firocoxib, are likely to provide effective pain relief with a reduced risk of side-effects, particularly those involving the gastrointestinal tract (McCann et al., 2004), although systematic reviews of clinical veterinary data have been inconclusive (KuKanich et al., 2012; Monteiro et al., 2013). Information is also becoming available on the use of these agents in less familiar species (e.g. birds; Baert & De Backer, 2003). The most significant problems associated with NSAID administration are gastrointestinal disturbances, notably ulceration and haemorrhage, nephrotoxicity and interference with platelet function (MacPherson, 2000; Mathews, 2000). Other problems such as blood dyscrasias and liver toxicity can also occur (Lees et al., 1991). These side-effects are seen primarily following prolonged administration, and are rarely of significance when treatment is for 2 or 3 days post-operatively. It should be noted that some NSAIDs (e.g. aspirin) have been reported as causing foetal abnormalities, so it is often recommended that they should not be administered to pregnant animals, although the likelihood of adverse effects may be low (Cook et al., 2003). In the research environment, the non-specific effects of NSAIDs may preclude their administration in certain research protocols. For example, carprofen and other NSAIDs have been shown to increase the risk of leakage after intestinal anastomoses in rats (van der Vijver et al., 2013). Consideration of the nature of the research study and potential interactions with analgesics allows a logical choice of analgesic agent to be made.

Drugs Available

Aspirin

Aspirin can be used to alleviate mild pain. It is most effective in humans for musculoskeletal pain, and is less effective for visceral pain. Dispersible tablets and enteric-coated tablets are available. Injectable formulations are generally available only for research use. There are few reports of the use of aspirin for the control of post-operative pain in animals, although it appeared to have some positive effects in rats (Jablonski & Howden, 2002).

A wide range of preparations that combine aspirin with other analgesics [e.g. paracetamol (acetominophen), codeine, dextropropoxyphene] are available for use in humans, but their efficacy in animals for use in post-operative pain has not been evaluated.

Paracetamol (Acetaminophen)

Paracetamol has similar analgesic efficacy as aspirin but has little anti-inflammatory activity. It causes less gastrointestinal irritation, but overdosage causes liver toxicity. These analgesics should not be administered to cats, because of problems of toxicity. Tablets and oral suspensions are available for human use, and these may be used in a wide range of laboratory species, although very little data concerning efficacy in post-operative pain in animals are available. In acute pain models in mice and rats, paracetamol has clear analgesic effects (Mickley et al., 2006; Miranda et al., 2008), and its use for post-operative pain relief has been suggested (Bauer et al., 2003). Assessment of its analgesic efficacy in mice, indicated that it had very limited effects after surgery (Dickinson et al., 2009; Matsumiya et al., 2012).

Ibuprofen

Ibuprofen is effective against mild pain in human beings, but very few controlled clinical trials in animals have been undertaken (Hayes et al., 2000). Both tablets and suspensions are available for human use.

Phenylbutazone

Phenylbutazone has been widely used for controlling mild pain in larger species. Nociceptive testing enables initial estimates of dose rates for small rodents, but no clinical trials have been carried out in these smaller laboratory species. Injectable (intravenous only) and oral preparations (tablets and powder) are available.

Flunixin

Flunixin has been reported as being effective in controlling post-operative pain in dogs (Reid & Nolan, 1991), and it has been widely used as an analgesic in larger species (cattle and horses). It also appears to be an effective analgesic

in pigs, sheep and cats, but no controlled trials have been undertaken in these species. It has been reported to have little efficacy in mice (Goecke et al., 2005; Tubbs et al., 2011). Both injectable and oral preparations are available. The most significant problem reported has been nephrotoxicity either when administered together with a known nephrotoxic agent (Mathews et al., 1987), or in circumstances when renal blood flow was likely to have been compromised (McNeil, 1992). The mechanism of action has been suggested to be inhibition of the normal prostaglandin regulation of renal blood flow, resulting in a failure of renal perfusion during periods of hypotension. Good anaesthetic practice, appropriate fluid therapy and administration of flunixin after the completion of surgery are likely to minimize this risk. Administration to conscious, healthy animals appears not to be associated with any significant risk, but more recently developed NSAIDs should be used when practicable.

Carprofen

Carprofen can provide effective post-surgical pain relief in the dog, cat and rat (Nolan & Reid, 1993b; Slingsby and Waterman-Pearson, 2000; Roughan & Flecknell, 2001), and has also been used in a number of different species with apparent success (Allison et al., 2007; Paull et al., 2007). Both oral and injectable preparations are available. Recent studies have demonstrated efficacy following surgery in mice (Jirkof et al., 2010), but relatively high dose rates were needed to produce significant changes in the Mouse Grimace Scale (MGS) (Matsumiya et al., 2012).

Ketoprofen

Ketoprofen is a non-selective COX inhibitor that provides moderate pain relief in rats, dogs, cats and horses. Its efficacy in other species is uncertain, although likely effective dose rates can be suggested from analgesiometric data. Both oral and injectable formulations are available. However significant side effects have been reported at clinically effective dose rates in rats (Lamon et al., 2008; Shientag et al., 2012), so its use in this species should be avoided.

Ketorolac

Ketorolac is used in humans to control moderate to severe post-operative pain, and it may be effective in the dog (Mathews et al., 1996) and rat (Martin et al., 2004) for the control of post-operative pain. Data on its pharmacokinetic in a number of species are available (Mroszczak et al., 1987). Both injectable and oral formulations are available (Mroszczak et al., 1990). As with other NSAIDs, ketorolac is best not administered to animals with pre-existing renal disease or fluid deficits.

Meloxicam

Meloxicam is available in the United Kingdom as an oral suspension and an injectable preparation for use in dogs, cats and cattle. It has been shown to

be effective for alleviating post-operative pain in rats (Roughan & Flecknell, 2003), mice (Wright-Williams et al., 2007; Tubbs et al., 2011; Rätsep et al., 2013), dogs, cats and cattle. It is effective against mild to moderate pain, and the palatable oral preparation makes it particularly useful when additional doses of drugs are required. Relatively high doses may be necessary in mice (Wright-Williams et al., 2007; Matsumiya et al., 2012), and these high dose rates can produce acute toxicity in some strains (e.g. Balb/c, Ellen, personal communication). For this reason, lower dose rates are suggested in Table 4.5 as a starting point when using this agent in mice. Higher dose rates than originally suggested may also be required in rabbits (Turner et al., 2006; Leach et al., 2009; Delk et al., 2014). A slow release preparation of meloxicam has recently become available in the United States, but no controlled trials in laboratory species have yet been published.

Naproxen

Naproxen is unusual in having an exceptionally long half-life in the dog (35 h) and has been used to alleviate moderate pain in this species, although, as with most analgesics, no controlled clinical trials have been undertaken. Naproxen is available as tablets and as an oral suspension.

Coxibs – Deracoxib, Eterocoxib, Firocoxib, Paracoxib, Roficoxib

The coxibs are a group of NSAIDs with high selectivity for COX-2 (Hinz et al., 2007). The degree of inhibition of COX-1 and COX-2 may vary in different species, but generally, when used at therapeutic dose rates, their effect is primarily on COX-2. Initially, these agents were only available as oral preparations, but injectable formulations are now available. Since these agents have minimal effects on COX-1 in most species, there are no effects on platelet function. The coxibs still have adverse effects on the gastrointestinal system when administered for prolonged periods, but these effects are less than those produced by older NSAIDs (Fiorucci & Distrutti, 2011; Kim & Giorgi, 2013).

Opioids (Narcotic Analgesics)

A wide range of different opioid analgesics is available for use in animals. The different drugs vary in their analgesic potency, duration of action and also effects on other body systems. Opioids are classified by their activity at specific opioid receptors. The most clinically important of these are the mu and kappa receptors. Morphine and other opioids such as pethidine (meperidine), fentanyl and alfentanil are mu agonists (they bind to, and activate mu receptors). The analgesic action of full agonists increases with increasing dose rates. Other opioids (e.g. buprenorphine) are classed as partial mu agonists. Increasing the dose of these agents eventually reaches a plateau, with no further analgesia being produced. These effects are most relevant when considering the use of analgesics as components of balanced anaesthetic regiments, when mu agonists can prevent responses to surgical stimuli. Partial agonists may not achieve as

great a degree of analgesia, however in the post-operative period, the degree of analgesia provided by both groups of agents is usually sufficient to control post-surgical pain.

Some analgesics are agonists at kappa receptors, but antagonists at mu receptors. These agents are generally referred to as mixed agonist/antagonist analgesics (nalbuphine, butorphanol, pentazocine). This property is useful since these agents can be used to antagonize full mu agonists such as fentanyl, used as part of balanced anaesthetic regimens. Using a specific mu antagonist, such as naloxone, would be possible, but this would potentially result in the animal being in pain during recovery. Using a mixed agonist/antagonist ensures analgesia continues to be provided because of the drug's action at the kappa receptor (see Chapter 1).

Opioid agonists and partial agonists relieve pain without impairing other sensations. However, they can cause some undesirable side-effects. All opioid agonists can produce some degree of respiratory depression, but when administered at clinically effective dose rates for post-operative pain relief, this is rarely a serious problem in animals. Opioids may also cause sedation or excitement, their effects varying considerably in different animal species (Le Bars et al., 2001). The effects on behaviour also depend upon the dose of the drug which has been administered (Flecknell, 1984).

When administered at dose rates appropriate for providing post-operative analgesia, opioids have minimal effect upon the cardiovascular system (Martinez et al., 1997; Bowdle, 1998). Higher dose rates, such as those that might be administered when using opioids as part of a balanced anaesthetic regime, can cause bradycardia (Pugsley, 2002), although this can be prevented by administering atropine. In addition, morphine, pethidine (meperidine) and some other opioids can stimulate histamine release and produce a peripheral vasodilatation in some species. Clinically significant hypotension is usually seen only after administration of high dose rates or after rapid intravenous administration.

Opioids can cause vomiting in some animal species, notably in non-human primates and dogs. This side-effect is seen primarily when opioids are administered to pain-free animals (e.g. as pre-anaesthetic medication), and is less frequent when administered post-operatively. Apart from causing vomiting, opioids may delay gastric emptying, increase intestinal peristalsis and cause spasm of the biliary tract. These effects may preclude the use of opioids in certain experimental procedures, but generally, the effects are of minimal clinical significance in animals. The detailed pharmacology of opioids has been extensively reviewed; general introductions to the field can be found in a number of sources (Pasternak, 2012, 2014; Tranquilli et al., 2013; Vuong et al., 2013).

Drugs Available

Opioid Agonists

Morphine Morphine is obtained from opium and has been used as an analgesic in humans for many years. It has been extensively studied in a range of experimental animals and is also used in veterinary clinical practice). Its duration of

action in most animals is 2–4 h (Gades, Danneman, Wixson, & Tolley, 2000), but a slow-release injectable preparation (Duromorph) and a slow-release oral preparation (MST, Napp; Oramorph SR, Boehringer Ingelheim; Appendix 4) are also available. Initial trials of oral slow-release morphine in rats indicated it had a prolonged duration of action in anti-nociceptive tests (Leach et al., 2010), and it may be of value for providing prolonged post-operative pain relief. Rapid intravenous injection in the dog can cause transient hypotension, because of histamine release, but this is not a problem if the drug is given by continuous intravenous infusion (see section "Pain Relief-Problems" below). Although morphine remains one of the most useful and potent analgesics, it is relatively short acting in many species (<4 h), and its administration after neuroleptanalgesic anaesthetic techniques in laboratory species can, not surprisingly, result in severe respiratory depression. It is also a drug with significant abuse potential.

Pethidine (Meperidine) Pethidine (meperidine) has been widely used as an analgesic in veterinary practice in the United Kingdom, but it has a relatively short duration of action in many species (<2 h). It has a spasmolytic action on smooth muscle in some species, and this has led to its recommendation for use in specific clinical situations such as colic in horses. Both oral and injectable formulations are available.

Methadone Methadone has been used clinically as an analgesic in the horse, dog (Ingvast Larsson et al., 2010) and cat (Dobromylskyj, 1993), and dose rates for use in other species can be extrapolated from the results of experimental analgesiometry (Flecknell, 1984). Methadone does not tend to cause vomiting and has a slightly more rapid onset than does morphine. In addition to its activity at mu receptors, it is an NMDA antagonist, and this may add to its efficacy as an analgesic (Holtman & Wala, 2007). Both injectable and tablet formulations are available and it is currently marketed for veterinary use in Europe.

Oxymorphone Oxymorphone has actions similar to morphine and has been reported to be an effective analgesic in dogs and cats (Pypendop et al., 2014). Its pharmacokinetics and activity in anti-nociceptive tests has been evaluated in rats (Lemberg et al., 2006) and other species (Kelly et al., 2011). Because of its relatively short duration of action, oxymorphone offers no particular advantages in comparison with morphine; however, slow-release preparations of this analgesic have been produced and evaluated in rodents and other species (Krugner-Higby et al., 2003, 2009; Smith et al., 2013). If these slow-release preparations become available commercially, then they may be of considerable value in providing prolonged post-operative pain relief (Prommer, 2006).

Hydromorphone Hydromorphone, like oxymorphone, is a pure mu agonist, with greater potency than morphine but a similar duration of action. It is used for

analgesia in dogs and cats in veterinary practice in the United States (Bateman et al., 2008). Pharmacokinetic and antinociceptive data are available in a number of species (Robertson et al., 2009; KuKanich & Spade, 2013). Slow-release injectable formulations have been produced for research purposes (Smith et al., 2013), and shown to be effective in rats (Smith et al., 2006). Oral slow-release capsule preparations of hydromorphone are available commercially, and these may be of value for providing prolonged pain relief in larger species.

Codeine and Dihydrocodeine Codeine and dihydrocodeine are morphine derivatives of low and moderate potency, respectively. Codeine is used in combination with paracetamol for the relief of mild to moderate pain. Limited data on the efficacy of codeine to treat post-operative pain in animals are available (Martins et al., 2010). Dihydrocodeine is also available as an oral preparation, and is an effective analgesic in human beings. To date, no information concerning its clinical efficacy in animals is available, however extensive information on its antinociceptive properties in laboratory rodents and other species is available (Miranda et al., 2013; Steagall et al., 2015).

Fentanyl Fentanyl is a potent, relatively short acting synthetic opiate. Its main use in laboratory animal anaesthesia is in the neuroleptanalgesic combinations Hypnorm (fentanyl/fluanisone) and Innovar-Vet (fentanyl/droperidol). Because of its short duration of action (under 30 min in most species (Tranquilli et al., 2013); fentanyl is most widely used for providing analgesia during surgical procedures. If it is to be used to control post-operative pain, it should be administered as a continuous infusion or transdermally (see section "Pain Relief - Problems" below).

Alfentanil Alfentanil is a synthetic opioid related to fentanyl. It has pharmacodynamic properties similar to fentanyl, but has a more rapid onset and shorter duration of action. Alfentanil can be administered by continuous infusion to provide analgesia during surgical procedures, and its short duration of action enables good moment-to-moment control of the intensity of the analgesic effect.

Sufentanil Sufentanil is a highly potent mu opioid (approximately 60 times more potent than fentanyl in humans) used primarily for the provision of analgesia as part of balanced anaesthetic regimens (Camu & Vanlersberghe, 2002).

Remifentanil Remifentanil is a very short acting mu agonist. Its short duration of action is primarily due to hydrolysis by non-specific blood and tissue esterases. This has led to its use to provide analgesia as part of balanced anaesthetic regimens in humans and animals (Murrell et al., 2005; Komatsu et al., 2007). Even after very prolonged periods of administration, its effects are absent within a few minutes of ceasing intravenous infusion. Administration by other routes are unpredictable and not recommended (Alves et al., 2007).

Opioid Mixed Agonists/Antagonists and Partial Agonists

Pentazocine Pentazocine has been reported to provide effective analgesia in a range of animal species (Taylor & Houlton, 1984; Craft & McNiel, 2003; Adetunji et al., 2009). It has been reported to produce dysphoria in humans (Zacny et al., 1998), but it is uncertain whether similar effects occur in animals. Generally, the sedative effect of pentazocine is less than that of morphine. Both oral and injectable formulations are available.

Butorphanol Butorphanol has a veterinary product licence as an analgesic in several countries, and is believed to provide post-operative analgesia in a variety of species (Flecknell & Liles, 1990; Waterman et al., 1991; Carroll et al., 2005). It has marked mu opioid antagonist properties and can be used to reverse the action of fentanyl while maintaining some analgesic effects by its action at kappa receptors (Hedenqvist et al., 2000). Its relatively short duration of action (<2 h) limits its value for post-operative pain relief. It is useful as a component of balanced anaesthetic mixtures (e.g. ketamine/medetomidine/butorphanol) in a range of species (see Chapters 1 and 5).

Buprenorphine Buprenorphine is a potent partial mu agonist that has the advantage of having a prolonged duration of action in many species (Cowan et al., 1977a; Cowan et al., 1977b; Roughan & Flecknell, 2002). The drug has been used in veterinary clinical practice in dogs and horses (Hunt et al., 2013), cats (Sramek et al., 2015) and a wide range of laboratory animal species (Roughan & Flecknell, 2002). It is available as an injectable formulation, and as tablets for sublingual administration in humans.

Buprenorphine has been reported to cause pica (eating of bedding) in rats (Clark et al., 1997; Bosgraaf et al., 2004) a behaviour that may reflect nausea (Mitchell et al., 1977). This uncommon but undesirable side-effect may occur with other opioids and may be preventable with methylnaltrexone (Aung et al., 2004); however, it is best managed by avoiding the use of opioids in susceptible strains of rat.

Since buprenorphine is a partial agonist that has been shown to be effective in reversing the effects of full mu agonists (Flecknell et al., 1989b), it has been suggested that mu agonists such as morphine cannot be given after buprenorphine has been administered. Paradoxically, this does not appear to be the case, and it has been demonstrated that within the analgesic dose range, a switch from buprenorphine to morphine is possible, without a loss of analgesic efficacy. There also appeared to be no refractory period between termination of buprenorphine analgesia and onset of the effects of morphine (Kögel et al., 2005).

Recently, slow-release formulations of buprenorphine have been marketed, and these have been shown to provide analgesia for up to 3 days in rodents (Foley et al., 2011; Carbone et al., 2012; Chum et al., 2014; Jirkof et al., 2014). This is potentially an extremely useful means of ensuring effective pain relief

following major surgery. However, there may be disadvantages if this agent is administered to animals after minor procedures when the duration of pain may be much shorter and its intensity mild. Further studies are needed to confirm the risk/benefit profile of these agents in rodents following a range of surgical procedures. At present it is advisable to use the slow-release formulation only when pain assessment confirms that moderate to severe pain is likely to persist for 2–3 days.

Nalbuphine Nalbuphine has been reported to provide effective analgesia in dogs (Flecknell et al., 1991) and rats (Flecknell & Liles, 1991). It has a duration of action of 2–4 h in most species. It rapidly and effectively antagonizes the effects of mu agonists such as fentanyl, while maintaining an analgesic effect at kappa receptors. It is therefore particularly suitable for reversal of opioid-based anaesthetic regimens.

Other Agents

Tramadol Tramadol is an opioid agonist that also has an analgesic action mediated via the inhibition of serotonin and noradrenaline reuptake in the spinal cord. Both oral and injectable formulations are available. Although it has been advocated for use in animals as an alternative to more potent opioids because of its second mode of action (Giorgi, 2008), it is rapidly metabolized in several species. The main metabolite has moderate opioid activity but no effects on serotonin and noradrenaline. It has been assessed in animal models relevant to post-surgical pain (Affaitati et al., 2002; Dürsteler et al., 2007; Guneli et al., 2007; Martins et al., 2010; McKeon et al., 2011; Rätsep et al., 2013) and its pharmacokinetics have been studied in dogs (Kukanich & Papich, 2004), rats (Garrido et al., 2003), mice (Beier et al., 2007), goats (De Sousa et al., 2008), rabbits (Souza et al., 2008) and piglets (Vullo et al., 2014). Tramadol may provide a useful alternative to opioids in a range of species, but variations in metabolism may produce considerable variations in analgesic efficacy. Initial trials in post-operative pain have produced variable results, but the high oral bioavailability of tramadol have led to its extensive use for the control of chronic pain in dogs.

Tapentadol Tapentadol is a relatively recently marketed analgesic, which in people has a lower incidence of adverse effects in comparison to equivalent doses of opioids such as morphine (Giorgi, 2012; Hartrick & Rodríguez Hernandez, 2012). It acts at mu opioid receptors and also has noradrenaline reuptake inhibitory activity. Pharmacokinetics of tapentadol have been reported in dogs (Giorgi et al., 2012), cats (Lee et al., 2013) and goats (Lavy et al., 2014) and its efficacy has been assessed in rodent antinociceptive models (Schiene et al., 2011). Preliminary studies indicate it may be of value for the control of postoperative pain in rabbits (Giorgi et al., 2013).

Local Anaesthetics As discussed in Chapter 1, local anaesthetics can be used both as adjuncts to general anaesthesia and to provide post-operative pain

relief. This is likely to be more effective if a longer lasting local anaesthetic (e.g. bupivacaine) is used. These techniques are well established in larger species (Tranquilli et al., 2013) but only limited data are available in rodents. This is presumably due to the practical difficulties associated with the use of the very small volumes of drug that can safely be administered (see Chapter 1). Nevertheless, this offers a potentially valuable means of providing analgesia when concerns related to drug interactions and a particular scientific protocol preclude the use of NSAIDs and opioids. The use of local anaesthetic can also be incorporated into a multimodal analgesic regimen. Mixtures of lidocaine and bupivacaine have been shown to provide effective analgesia after local infiltration at the time of surgery in mice (Leach et al., 2012) and guinea pigs (Ellen personal communication), and numerous studies in larger species have shown similar results.

When using local anaesthetics in small rodents, the volume of solution for injection can be increased by diluting the mixture. The author's preference is to mix 1% lidocaine with 0.25% bupivacaine, as a 50:50 mixture, and to dilute this mixture with no more than an equal volume of water for injection. Greater dilution reduces the duration of local anaesthesia (Grant et al., 2000). Toxicity of these two agents is additive, so the dose limits suggested in Chapter 1 should be reduced. However, in mice, 3 mg/kg bupivacaine plus 10 mg/kg lidocaine has proved safe and effective in our laboratory. Using local anaesthetics as part of a multimodal analgesic regimen (see section "Multi-Modal Pain Therapy" below) is a very effective means of improving the quality of post-operative pain relief.

Clinical Use of Analgesics

When formulating an analgesic regimen for a particular animal, several factors need to be considered:

- What is the likely severity of pain, and what is its anticipated duration?
- Which drug or drugs should be administered, and at what dose rates?
- Are there any special factors that will influence the choice of analgesic, for example, the animal species, any pre-existing abnormalities and any particular features of the current project or the type of pain?
- What facilities are available for management of the animal? What level of post-operative care and monitoring of the animal is available? Can staff attend throughout a 24-h period? Are there facilities for continuous infusion of analgesics?

Timing of Analgesic Administration

One of the most important advances in the control of post-operative pain has been the realization that the timing of analgesic intervention may have a significant bearing on the intensity of post-operative pain. The concept was originally formulated early in the twentieth century, by Crile (1913), based on clinical

observations. Crile suggested using regional blocks with local anaesthetics, in conjunction with general anaesthesia, to prevent post-operative pain in humans and the 'formation of painful scars caused by alterations in the CNS as a result of the noxious stimulation caused during surgery'. The discovery that changes in the central processing of noxious stimuli occurred in response to peripheral injury (Coderre et al., 1993; Woolf & Chong, 1993) increased interest in this concept. After demonstration that the changes in the CNS were suppressed to a greater extent by administration of opioids before rather than after injury (Woolf & Wall, 1986; Kissin, 2000), the concept of 'pre-emptive analgesia' was developed. This advocated administration of analgesics before noxious stimulation began to prevent the adverse CNS changes that this stimulation induces. To be most effective, pre-emptive analgesia must prevent the noxious stimuli from reaching the CNS. It should also aim to reduce or eliminate peripheral inflammation, which in itself increases input into the CNS and so aggravates central hypersensitivity.

The clinical application of this approach in humans has had mixed results (Grape & Tramèr, 2007), but it has been recognized that administering analgesics before the return of consciousness has significant advantages. As a result, the approach has been broadened and incorporated into a concept of 'preventive analgesia'. This approach aims to ensure that post-operative pain treatment starts before surgery and lasts long enough after surgery to avoid pain-induced sensitization of nociceptive processes (Katz et al., 2011). In animals, a positive effect of pre-emptive drug administration has been found experimentally (Woolf & Wall, 1986; Lascelles et al., 1995) and clinically in animals that are given opioids (Lascelles et al., 1997) and NSAIDs (Welsh et al., 1997). In addition, as in humans, ensuring pain relief has been provided before the animal recovers consciousness is clearly preferable to not administering analgesics until pain is experienced.

It is important to appreciate that a single dose of analgesic, administered prior to surgery, will not usually be all the analgesia that will be required. Additional analgesic medication will still be needed in the post-operative period, but this pain will be more easily controlled because preventive analgesia has been used. A further practical advantage of preventive analgesia is that it will often reduce the dose of anaesthetic drugs required, and by integrating analgesic therapy into a balanced anaesthetic technique, the potential adverse effects of anaesthesia can be improved, in addition to providing more effective pain relief.

Adopting preventive analgesia does not necessarily imply administration of opioids or NSAIDs before surgery. Crile's original concept, of using local anaesthetics, should also be considered. In addition, the use of anaesthetic agents with analgesic properties will produce 'preventive' analgesia. Of particular relevance to laboratory animals are the effects of ketamine. This drug has the potential to reverse central hypersensitivity because of its actions as an NMDA antagonist. Experimental studies have confirmed its efficacy, but clinical trials in humans have given mixed results (Visser & Schug, 2006). Its clinical benefit

in animals is uncertain, but it is reasonable to suggest that the very high doses used in rodent anaesthesia should have beneficial effects in reducing the severity of post-operative pain.

Administration of NSAIDs pre-operatively could potentially increase the risk of adverse side-effects related to renal function. However, these concerns are most relevant to clinical veterinary practice when animals may have chronic renal disease (Lascelles et al., 2007). The adverse effects are also only of concern during periods of hypotension. Pre-operative administration in healthy human patients is not considered to be a significant risk and it seems likely that this will apply to healthy animals. NSAIDs with significant COX-1 activity decrease platelet function and so could increase bleeding times during surgery; however, this has not proven to be a significant problem (Lewis et al., 2013; Mathiesen et al., 2014; Moss et al., 2014). In some circumstances, it may not be possible to administer analgesics preventively; nevertheless, administering analgesics as soon as practicable is of significant benefit. The longer pain is established, the greater will be the degree of central hypersensitivity, and the more difficult pain management becomes.

'Multi-Modal' Pain Therapy

Post-operative pain arises from the activation of a multiplicity of pathways, mechanisms and transmitter systems. Administering a single class of analgesic often fails to suppress all of these mechanisms, even when high dose rates are used. Multi-modal pain therapy advocates the use of several different analgesics to provide more effective analgesia. In humans, this concept has been widely adopted, and has the advantage that lower doses of each different analgesic can often be used, when they are given in combination (Ong et al., 2010). There are good experimental data in animals to support this concept (e.g. Martin et al., 2004; Miranda et al., 2008), and some evidence for efficacy in alleviating post-operative pain in animals is now available (Steagall & Monteiro-Steagall, 2013). It is an easy technique to use, and the balance of evidence suggests it will be of benefit. For example, the use of an opioid such as buprenorphine can be combined with an NSAID such as carprofen. The opioid acts centrally to limit the input of nociceptive information into the CNS and so reduces central hypersensitivity. In contrast, the NSAID acts centrally to limit the central changes induced by the nociceptive information that does get through. In addition, the NSAID peripheral actions decrease inflammation during and after surgery and limit the nociceptive information entering the CNS, as a result of the inflammation. By acting on different points of the pain pathways, the combination should be more effective than either drug given alone. Adding a local anaesthetic to this regimen can provide additional analgesia by blocking specific nerve pathways and so further improve the degree of pain control.

Using combinations of different classes of analgesics can also overcome some of the problems associated with differences in the speed of onset of action of the various agents. In a study comparing the degree of post-operative

analgesia provided by pethidine and carprofen in cats, animals which received pethidine had good analgesia immediately following recovery from anaesthesia, compared to animals which received carprofen (Lascelles et al., 1995). In contrast, dogs receiving carprofen had better analgesia later in the post-surgical period. Clearly, combining the two analgesics would produce a more effective approach for controlling post-operative pain – immediate pain relief due to the opioid, and more prolonged analgesia provided by the NSAID.

Pain Relief – Problems

A number of clinical problems arise when analgesics are administered to control post-operative pain. The most important problem is the short duration of action of most of the opioid (narcotic) analgesics. Maintenance of effective analgesia with, for example, pethidine may require administration every 1–3 h, depending upon the species. Continuation of such a regimen overnight can cause practical problems. One method of avoiding this difficulty is to use buprenorphine as the analgesic, since there is good evidence in humans, rodents, rabbits and pigs that it has a duration of action of 6–12 h, depending upon the dose administered (Cowan et al., 1977; Dum & Herz, 1981; Hermansen et al., 1986; Flecknell & Liles, 1990). In clinical use in a wide range of animal species, it appears to provide effective pain relief for 4–8 h, depending upon the dose administered. Its duration of action in the sheep appears to be considerably less, although of longer duration than pethidine and morphine (Nolan et al., 1987).

An alternative approach is to adopt the well-established clinical technique used in people, of administering analgesics as a continuous infusion. Infusions of analgesics have the advantage of maintaining effective plasma levels of the analgesic, and so providing continuous pain relief. This is in contrast to intermittent injections, where pain may return before the next dose of analgesic is administered. This technique obviously poses some methodological difficulties in animals, but if an indwelling catheter and harness and swivel apparatus are available, then this can be arranged quite simply. In larger species (>3–4 kg body weight), a light-weight infusion pump (Smiths Medical, Appendix 4) can be bandaged directly to the animal and continuous infusion made simply by means of a butterfly-type needle anchored subcutaneously or intramuscularly. When analgesics are to be administered by continuous infusion, the infusion rate can be calculated from knowledge of the pharmacokinetics of the analgesia to be used (Chapter 3). If these data are not readily available, an approximation that appears successful in clinical use is as follows: calculate the total dose required over the period of infusion, reduce this by half and set the pump infusion rate accordingly; administer a single, normal dose of the drug as an initial loading dose and start the infusion. The rate can then be adjusted depending upon the animal's responses.

Prolonged analgesia can also be provided by the use of slow-release patches that are placed on the animal's skin. Both fentanyl and buprenorphine patches are available, and they have been used with some success in a range of species

(Harvey-Clark et al., 2000; Shafford et al., 2004; Malavasi et al., 2006; Egger et al., 2007; Thiede et al., 2014). The patches are manufactured for use in humans, so the rate of drug release varies in different animal species (Riviere & Papich, 2001; Mills & Cross, 2006; Heikkinen et al., 2015). Measurement of plasma concentrations of drugs has shown that considerable individual variation occurs (Davidson et al., 2004). For this reason, it is best to consider these patches as providing basal analgesia, and to assess the animal regularly to ensure sufficient analgesia is being provided. Patches need to be placed on the skin for approximately 24 h before adequate plasma concentrations of analgesic are attained. Fentanyl is also now available as a prolonged duration topical preparation (Savides et al., 2012; Martinez et al., 2014). At present data are only available in dogs, but potentially the produce could be used in other larger species. Lidocaine patches have also been used in dog, cat and horse, with the patch placed close to the site of the surgical wound. Relatively little efficacy data are available, and the use of this approach is likely to be restricted to larger species (Ko et al., 2007; Ko et al., 2008; Andreoni & Giorgi, 2009).

Slow-Release Formulations

A number of slow-release preparations of analgesics have been marketed for use in humans (e.g. morphine, oxycodone, hydromorphone). These may be useful in larger species, but should be used with caution pending establishment of the pharmacokinetics of these agents in the particular species. In smaller animals, the tablets and the capsules would need to be divided into smaller doses, and in most instances, this results in a loss of the slow-release properties of the formulation. An exception appears to be an oral morphine preparation ('Oramorph SR', Boehringer Ingelheim) which has been shown to produce prolonged antinociception in rats (Leach et al., 2010). A range of slow-release preparations have been developed specifically for use in laboratory animals. Some of these formulations are simple to prepare and have proven effective in rodents. An excellent review of these potential approaches to providing long-lasting analgesia is available (Krugner-Higby et al., 2008). Slow-release formulations of buprenorphine are now available commercially (see earlier), and these can provide effective plasma concentrations of analgesics for up to 3 days (Chum et al., 2014). These formulations may be particularly valuable when prolonged moderate to severe pain is anticipated.

Oral Administration

The need for repeated injections of analgesics is time consuming and may be distressing to the animal, particularly smaller species that require firm physical restraint to enable an injection to be given safely and effectively. In addition, the need for repeated injections requires veterinary or other staff to attend the animal overnight. To circumvent this problem, the possibility of incorporating analgesics in food or water has been investigated (Kistler, 1988). Long-term analgesia

can be produced by this route: Kistler (1988) reported that rats had demonstrable analgesia for a 2-week period when buprenorphine was administered continuously in drinking water. Unfortunately, several practical problems limit the use of this technique. Some animals eat and drink relatively infrequently, or may only do so in the dark phase of their photoperiod. In addition, food and water intake may be depressed following surgery, and this, coupled with wide individual variation in consumption, makes routine application of the technique difficult. If opioids are used, the high first-pass liver metabolism following oral administration requires that high dose rates are given, and this can represent a significant cost if all of the animals' drinking water or food is medicated. Finally, there may be problems of palatability (Speth et al., 2001; Bauer et al., 2003). Despite these problems, encouraging results have been obtained with paracetamol (acetaminophen) in rats (Mickley et al., 2006), and with buprenorphine (Kalliokoski et al., 2011) and the approach deserves further evaluation in a range of different circumstances. Since fluid consumption can vary both due to varying husbandry conditions and between different strains of animals (Bachmanov et al., 2002; Tordoff et al., 2007) both over-dosing as well as under-dosing is possible, so it is important to measure the animals' actual fluid consumption. It is also important to provide effective analgesia by other means in the period before the animal commences drinking (Christy et al., 2014).

Medication of the feed has also been suggested as a means of providing repeated dosing with analgesic, and palatable preparations of a number of NSAIDs are available. Administration of small quantities of medicated food does not avoid the need for repeated attendance overnight, but does remove the need for repeated subcutaneous or intramuscular injections in small rodents. Provision of analgesia with buprenorphine in flavoured gelatin ('Buprenorphine Jello') has been recommended as a means of providing post-operative pain relief in rats; however, the efficacy of this approach has been questioned. Using thermal anti-nociceptive tests, the oral dose of buprenorphine required to produce analgesia equivalent to the recommended subcutaneous dose too high to be clinically useful (Martin et al., 2001; Thompson et al., 2004). In contrast, other studies have indicated oral administration is effective (Flecknell et al., 1999; Roughan & Flecknell, 2004; Godlkuhl et al., 2010). It is clear that in some animals, this route of administration is likely to be ineffective, so it should only be used if a reliable pain assessment system is in place (Leach Forrester and Flecknell, 2010). With all medicated foodstuffs, rats are initially cautious of jelly pellets, but once a few pellets have been consumed, subsequent pellets are eaten as soon as they are offered. It is therefore advisable to commence administering pellets, which do not contain analgesic, 2–3 days before surgery. After surgery, analgesic-containing jelly can be given. The flavoured gelatin used is domestic fruit-flavoured jelly, reconstituted at double the recommended strength. Other highly palatable foodstuffs, for example Nutella (Godlkuhl et al., 2010; Jacobsen et al., 2011) or standard pelleted diet (Molina-Cimadevila et al., 2014) have also been shown to be suitable vehicles for oral analgesic administration.

Techniques for administration of food pellets at intervals to experimental animals are well established, and it would be a relatively simple procedure to introduce an automated means of delivering pellets at appropriate time intervals. The technique could also be used with larger species, and need not be restricted to opioids, or indeed analgesics. Provided that the animal is eating or drinking, small quantities of highly palatable material could be provided at appropriate intervals. Simple timer devices to achieve this are already marketed for delayed feeding of pet dogs and cats.

Epidural and Intrathecal Opioids

Epidural and intrathecal opioids have been shown to have a prolonged effect in humans, and to provide effective analgesia (Glynn, 1987). In animals, both clinical and experimental studies have indicated that the technique can be used in a number of species (Dyson, 1993; Pablo, 1993; Pascoe & Popilskis et al., 1993; Duke, 2000). Although used as a research tool in laboratory species (Yaksh et al., 1988), this route of administration has yet to be exploited as a means of controlling post-operative pain. The necessary techniques of epidural or intrathecal injection have been described in rabbits (Kero et al., 1981; Hughes et al., 1993), guinea pigs (Thomasson et al., 1974) and small rodents (Mestre et al., 1994; Fairbanks, 2003). In larger species such as the cat, dog, sheep and pig, descriptions of the injection technique can be found in most veterinary anaesthesia texts and a number of other publications (e.g. Tranquilli et al., 2013).

As mentioned above, the administration of opioids by any route can be associated with the development of respiratory depression. It must be emphasized that this is rarely of clinical significance in animals, unless high doses of pure mu agonists (e.g. fentanyl) are used. If respiratory depression occurs, it can be treated by the administration of the opioid antagonist drug naloxone. Administration of naloxone will also reverse the analgesic effects of the opioid, and it may be preferable to correct the respiratory depression by the use of doxapram. Alternatively, if a mu agonist opioid such as morphine or fentanyl has been used, the respiratory depression can be reversed using nalbuphine or butorphanol, and some analgesia maintained because of the action of these latter two agents at kappa receptors. Repeated administration of these agents may be required, and the animal should be observed carefully for several hours to ensure adequate respiratory function is maintained.

Additional Considerations in Pain Relief

Although the use of analgesic drugs remains the most important technique for reducing post-operative pain, the use of these drugs must be integrated into a total scheme for peri-operative care (Carli & Asenjo, 2003). As discussed in Chapter 1, pain relief in the immediate recovery period can be provided by including an analgesic drug in any pre-anaesthetic medication. Alternatively, if a neuroleptanalgesic combination has been used to produce anaesthesia, it can

be reversed by the use of buprenorphine, nalbuphine or butorphanol, rather than naloxone. These agents have been shown not only to reverse the respiratory depressant effects of opioids such as fentanyl but, in contrast to naloxone, to provide effective prolonged analgesia (Flecknell et al., 1989b).

The expertise of the surgeon can also greatly influence the degree of post-operative pain. A good surgical technique that minimizes tissue trauma and the prevention of tension on suture lines can considerably reduce post-operative pain. The use of bandages to pad and protect traumatized tissue must not be overlooked and forms an essential adjunct to the use of analgesic drugs.

Aside from measures directed towards alleviating or preventing pain, it is important to consider the overall care of the animal and the prevention of distress. Distress is used in this context to describe conditions which are not in themselves painful, but which are unpleasant and which the animal would normally choose to avoid. For example, recovering from anaesthesia on wet, uncomfortable bedding in a cold, unfamiliar environment would be likely to cause distress to many animals. It is essential to consider the methods described for the control of pain, in conjunction with the techniques discussed earlier in this chapter, aimed at providing good post-operative care.

Recommendations

It is difficult to make firm recommendations concerning which analgesics to use routinely, and how often to give them, because of the various factors outlined above. Nevertheless, as a general guide, the following techniques are used routinely in the author's research facility.

When carrying out any surgical procedure, buprenorphine is administered either pre-operatively or immediately following the induction of anaesthesia, if a volatile anaesthetic is used. If neuroleptanalgesic regimens are used, or mu opioids are given as part of a balanced anaesthetic technique, then administration of buprenorphine is delayed until completion of surgery. If the procedure is relatively minor, for example, jugular or carotid cannulation, then only a single dose of analgesic is administered. In some circumstances, a potent NSAID, such as meloxicam or carprofen, may be used as an alternative to buprenorphine.

Following more invasive surgical procedures, such as laparotomy, orthopaedic surgery or craniotomy, opioid administration is continued for 8–48 h, depending upon the species and the expertise of the surgeon (since this has a major influence on the degree of tissue trauma). When undertaking major surgery, particularly in larger species when the degree of tissue trauma tends to be greater, analgesic administration may continue for 72 h. In addition, local anaesthetics (e.g. bupivacaine combined with lidocaine) may be infiltrated into the wound margins, or used to provide a localized nerve block of the area. Frequently, the technique chosen consists of an opioid (buprenorphine) in combination with an NSAID for 8–24 h, followed by NSAID alone for further 24–36 h (see Tables 4.5–4.8 for suggested dose rates).

It is important to note that extended use of opioids can produce significant negative effects on animals (Cooper et al., 2005). In rats, the major signs of pain are present for only 6–8 h following laparotomy (Roughan & Flecknell, 2004), although more subtle effects persist for longer in both rats and mice (Arras et al., 2007). This suggests that a single dose of a long-acting opioid such as buprenorphine, combined with a longer acting NSAID, may provide sufficient pain relief after mild and moderate surgical procedures in some species. In all instances, it is important to establish the intensity and the duration of pain, and the efficacy of analgesic therapy by the use of pain assessment systems.

CONCLUSIONS

Attention to the suggestions made in this section concerning post-operative care can have a dramatic effect on the speed with which animals return to normality following surgical procedures. It has been repeatedly demonstrated in humans that the provision of effective analgesia reduces the time taken for post-operative recovery (Joshi & Ogunnaike, 2005). The provision of good post-operative care should be considered essential both because of a concern for the animal's welfare and also because it is good scientific practice.