

Protocol for Anesthesia Animal Model in Biomedical Study

by Rachmat Hidayat

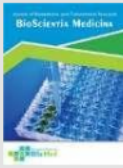
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Protocol for Anesthesia Animal Model in Biomedical Study

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ABSTRACT

Recognition of pain depends upon intact pathways from pain receptors to the thalamus and cerebral cortex, as well as functional cerebral cortex and subcortical structures. Thus any means that renders the cerebral cortex nonfunctional, such as hypoxia or drug depression, prevents pain. When this happens, stimuli that evoke motor nerve reflexes that may be painful to the conscious animal are not painful in the unconscious animal. Equally painful stimuli administered to animals chemically paralyzed by curare or succinylcholine will not evoke a motor reflex simply because of paralysis, but will cause pain because of the conscious state. Hence, it is possible that unconscious animals may feel no pain but respond to certain stimuli, and paralyzed animals may feel pain but cannot respond.

1. Introduction

Recognition of pain depends upon intact pathways from pain receptors to the thalamus and cerebral cortex, as well as functional cerebral cortex and subcortical structures. Thus any means that renders the cerebral cortex nonfunctional, such as hypoxia or drug depression, prevents pain. When this happens, stimuli that evoke motor nerve reflexes that may be painful to the conscious animal are not painful in the unconscious animal. Equally painful stimuli administered to animals chemically paralyzed by curare or succinylcholine will not evoke a motor reflex simply because of paralysis, but will cause pain because of the conscious state. Hence, it is possible that unconscious animals may feel no pain but respond to certain stimuli, and paralyzed animals may feel pain but cannot respond. Thus, movement is not a reliable indicator of pain, and paralyzing agents (i.e.,

succinylcholine and curare) are strictly prohibited as euthanatizing agents. The methods used for prevention or relief of pain and distress in scientific experimentation with living animals will be dependent upon the kind of procedures used on the animals. Selection of an appropriate anesthetic, analgesic, or tranquilizer require the assistance of an experienced professional.

Anesthesia

The definition is the local or general loss of sensation. General anesthesia is achieved by depressing the brain receptors of pain, thus producing a general anesthetic effect, although not necessarily blocking local responses such as spinal cord reflex arcs. Therefore, it is possible to have good levels of

general anesthesia but still have motor reflexes such as pinch-pad and corneal reflexes present. These should not be mistaken for purposeful responses to pain. They can however, be abolished by deepening the level of anesthesia. Great care must be exerted when general anesthesia is made too deep since not only are pain receptors depressed, but also the vital centers of the brain and brain stem including respiratory, cardiac, hypothalamic, etc. When depressed for too long, heart and respiratory function cease and death ensues unless heroic measures are taken--if they are available.

Regardless of the species involved, some principles of general anesthesia are universal and worth keeping in mind. They include:

- a. Maintain patent airway. This is essential if trouble arises and the subject is to survive. Nothing must block the ability to breathe freely and easily. With small rodents that are obligate nose breathers, a patent airway is easily maintained if the nostrils are not blocked.
- b. Avoid hypothermia. Core body temperature can fall alarmingly, particularly in small animals, during the course of prolonged general anesthesia. Hypothermia added to other factors can produce an irreversible sequence of events leading to death. Thermostatically controlled heating pads should always be used in animal surgery.
- c. Administer anesthetic to effect. Technically, because of wide variation within and between species, there is no such thing as predetermined anesthetic dose of a drug. General anesthesia must be given to effect, as measured by physiological parameters and response to stimuli. Most anesthetic deaths can be attributed to not following this principle. This is especially true for parenterally administered drugs such as barbiturates. Once they are injected, there is little the anesthetist can do to control the outcome; therefore, great care is necessary when administering these drugs.

Criteria for the Administration of Analgesics in Laboratory Animals :

Rodent analgesia

Pain in rodents may be identified by observing the animal's reluctance to move about, eat or drink, weight loss, salivation, hunched posture, piloerection, respiratory sounds (chattering in mice) and by vocalization with handling.

Guinea pig and chinchillas

Pain in guinea pigs and chinchillas may be identified by observing the animal's reluctance to move, vocalization with handling, decreased food and water intake and postural abnormalities.

Ferret analgesia

Pain in ferrets may be identified by observing the animal's reluctance to move, spontaneous vocalization or vocalization upon handling, reluctance to eat and drink, avoidance behavior, depression, postural abnormalities, increased respiratory rate and abnormal pattern.

Rabbit analgesia

Pain in rabbits may be identified by observing the animal's reluctance to move about, eat or drink, postural abnormalities, increased respiratory rate and/or abnormal pattern and by vocalization with handling.

Nonhuman primate analgesia

Pain in nonhuman primates may be identified by depression, guarding of painful part, avoidance behavior, spontaneous vocalization or vocalization upon handling, teeth grinding, lying down and getting up repeatedly, abnormal posture, increased respiratory rate and abnormal pattern, reluctance to move or inappetence.

Canine analgesia

Pain in dogs may be identified by depression, guarding of painful part, spontaneous vocalization upon handling, avoidance behavior, recumbency, inappetence, muscle tremors, attraction to painful area

(licking, biting, scratching), and abnormal posture.

Swine analgesia

Pain in pigs may be identified by depression, recumbency, vocalization when painful area is manipulated or spontaneous vocalization (e.g. grunting), abnormal posture, inappetence, increased respiratory rate and abnormal pattern, lying down and getting up repeatedly and avoidance behavior.

Sheep/goats

Pain in sheep and goats may be identified by depression, recumbency, vocalization when painful area is manipulated or spontaneous vocalization, abnormal posture, inappetence, increase respiratory rate and/or abnormal pattern, lying down and getting up repeatedly (especially in ruminants) and avoidance behavior.

Feline analgesia

Pain in cats may be identified by depression, guarding of painful part, spontaneous vocalization or vocalization upon handling, avoidance behavior, loss of appetite, reluctance to move, abnormal posture, muscle tremors, and attraction to area of pain (licking, biting, scratching).

Analgesia is insensitivity to pain without loss of consciousness. This is a general effect and involves depression of brain receptors as well as brain centers. A variety of drugs have analgesic properties when given in the proper dosage. Some categories of drugs do not produce analgesia, therefore, a list of commonly used terms is provided below for clarification.

a. Analgesic: Drugs like morphine, meperidine

(DemerolR) and codeine which alleviate pain without causing a loss of consciousness.

- b. Anesthetic: A drug or agent that is used to abolish the sensation of pain. Sodium pentobarbital, when injected intravenously or intraperitoneally, depresses the central nervous system and induces deep sleep during which the sensation to pain is lost.
- c. Cataleptic: A drug like ketamine hydrochloride which produces a trance-like state of hyporesponsiveness which is known as dissociative anesthesia. Because of the nature of its activity, ketamine does not produce analgesia for pain which accompanies abdominal, thoracic or CNS surgery or manipulation of fractured bones. In the latter cases, a tranquilizer or sedative must be used in conjunction with ketamine.
- d. Sedative: An agent which allays activity and excitement by producing a mild degree of central nervous system depression in which the patient is awake but calm and free of nervousness. Xylazine (RompunR) acts as an analgesic and a sedative but it is not a tranquilizer or an anesthetic.
- e. Tranquilizer: Drugs like promazine, acetylpromazine, and diazepam (ValiumR) act on the emotional state to calm and quiet the patient. These drugs increase the threshold to environmental stimuli and depress many physiological functions but do not produce sleep, analgesia or anesthesia. When used in combination with dissociative anesthetics, a degree of general anesthesia is effective for certain and procedures in small laboratory animals.
- f. Narcotic: Any of a class of addictive substances, such as opium and morphine, that blunt or distort the senses and in large quantities produce euphoria, stupor or coma.

Injectable anesthetics in mice (remember to provide heat to anesthetized rodents)

Drug	Mouse dose range	Route of administration	Comments
Sodium Pentobarbital (Nembutal®)	30 – 90 mg/kg	IP	Useful for immobilization, not surgical anesthesia, when used alone
¹⁰ Ketamine / xylazine	100 mg/kg ketamine + ¹⁶ 10 mg/kg xylazine	IP	Anesthesia; only redose with ketamine if needed
Ketamine / midazolam	100 mg/kg ketamine + ¹⁴ 5 mg/kg midazolam	IP	Anesthesia; only redose with ketamine if needed
Ketamine / diazepam	100 mg/kg ketamine + 5 mg/kg diazepam IP	IP	Anesthesia; only redose with ketamine if needed
Tribromoethanol (Avertine ®)	200 – 300 mg/kg Or 0.2 ml per 10 g BW of 1.25 % solution	IP	Requires storage in lightproof container under refrigeration; is an irritant, especially at high doses, high concentrations, or with repeated use. Adhesions are sometimes seen in the abdominal cavity after IP injections

Injectable anesthetics in rats (remember to provide heat to anesthetized rodents)

Drug	Rat dose range	Route of administration	Comments
¹³ Sodium Pentobarbital (Nembutal ®)	40 – 50 mg/kg	IP	Light anesthesia
Ketamine / xylazine	40 – 80 mg/kg ketamine + 5 – 10 mg/ kg xylazine	IP	Surgical anesthesia
Ketamine / midazolam	75 mg/kg ketamine + 5 mg/ kg midazolam	IP	Light anesthesia
Ketamine / diazepam	75 mg/kg ketamine + 5 mg/ kg diazepam	IP	Light anesthesia
Chloral hydrate	300 mg/kg	IP	Dilute as much as possible. Concentration > 2% causes ileitis-peritonitis

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