Laboratory animal anesthesia classification (LAAC)

Siavash Ahmadi-Noorbakhsh (s.noorb@gmail.com)
Preclinical Core Facility (TPCF), Tehran University of Medical Sciences, Tehran, Iran

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Abstract

Anesthesia and analgesia (AA) are major components of animal research. However, various studies have shown an undesirable quality of AA reporting and use in research proposals and even high impact articles. Part of the problem may be related to a lack of special training in animal anesthesia among researchers. This causes an absence of a common language for AA among researchers, grant reviewers, ethics committees, animal welfare bodies, and journal reviewers. Herein, I present a laboratory animal anesthesia classification (LAAC) system that facilitates identification and grouping of various procedures requiring AA. The LAAC classes are developed according to the distress level and type of tissue injury caused by each procedure. Thereby, procedures causing similar types of pain or distress are grouped together and a certain AA approach could be applied to each group. LAAC classifies procedures into 12 classes and three categories with neuropathic pain being a separate component that could be added to each of the classes. Category A comprises LAAC1 to LAAC4 and requires no anesthesia. Category B is comprised of LAAC5 to LAAC8 and mainly includes non-surgical procedures. Category C includes surgical procedures classified under LAAC9 to LAAC12. Generally, a higher class of LAAC requires more potent AA regimen. In conclusion, LAAC provides a common language among various research bodies. A certain LAAC class conveys information regarding the nature of the same-group procedures and required intraoperative and postoperative AA for those procedures. LAAC also enables comparison of various procedures according to their nature and AA requirements.

Introduction

The need for a classification system

Anesthesia and analgesia (AA) are inseparable parts of many procedures on laboratory animals. They also have direct effect on the quality of research projects and the welfare of animals. However, research shows that a large number of even high impact articles [1–6] or research proposals [7], insufficiently or inaccurately report the use of AA. In some cases, this is not merely insufficient reporting, but the nature of the used drugs reveals an inappropriate selection of the AA method [8,9]. The extent of this phenomenon is such that we could say we are facing a “pseudo-anesthesia crisis” in our published literature, let alone the unpublished uses of laboratory animals.

Anesthesia and analgesia in animals are *per se* major branches of veterinary science. However, many laboratory animal researchers are not basically veterinarians or veterinarians specially trained in laboratory animal anesthesia [10]. The lack of proper education and training in this field has been suggested by others [7], as well. Therefore, improper reporting or use of AA in laboratory animal research may not be a surprise in many cases. Since new research are built on the reporting of previous research, it would also not be a surprise to see a domino of inappropriate AA methods [8] in a chain of related articles.

As discussed, animal AA is a specialized topic and this makes another problem. When it relates to AA, there is not a common language among researchers, ethics committees, animal welfare bodies, animal

care staff, grant reviewers, and journal reviewers. Accordingly, a researcher may declare using anesthesia for a complex procedure, while he/she is only using a hypnotic. Or there are many research projects truly declaring the use of anesthesia, while they lack the analgesic component [7]. This lack of a common language causes a chaotic condition, in which improper reporting or use of AA occurs.

Therefore, there is a need for a common language that is simple enough to be understood by people without specialized knowledge in laboratory animal anesthesia. This common language should integrate various concepts of AA into meaningful classes. Certain procedures should fall into each class based on how they meet a set of criteria. For each class,

pertinent methods of AA should be described. Therefore, a user of this classification system can determine the class of a given procedure and find out the pertinent method of AA for it. Furthermore, by referring to the name of each class, a large amount of information related to that class would also be delivered. This facilitates scientific communications between people with various backgrounds. It also increases the accuracy of the scientific communication regarding AA in laboratory animals.

Presumptions

For developing this classification system, several concepts in laboratory animal AA should be considered. I have presented these concepts in Supplement 1 to this paper. According to these concepts, the proposed classification system assumes that both the pain sensation and pain perception should be suppressed in painful procedures. In this regard, analgesic drugs should be used according to the type and amount of pain, to provide a hypoalgesic state. For procedures with the pain similar to surgical procedures, surgical anesthesia should be used. This may be achieved via a balanced anesthetic technique combining general anesthesia with analgesia. Accordingly, preemptive analgesia and multimodal analgesia techniques should be considered. If possible, it may be preferrable to use local anesthesia or regional anesthesia instead of general anesthesia.

Results

LAAC system classifies various procedures into 12 classes and three main categories with an overall increasing level of AA from LAAC1 to LAAC12 (Fig. 1). Category A comprises non-surgical procedures with discomfort up to the discomfort threshold (DT). Category B, mainly includes non-surgical procedures, and category C includes surgical procedures. Below each of these classes are discussed. The choice of the medication in each class depends on several factors, such as: 1) the species and strain of the animal; 2) type and extent of the procedures; 3) clinical state of the animal; 4) effect of the medication on the research parameters; 5) familiarity of the researcher with a medication/technique; and 6) availability of the medication to researchers. The aim of this paper is not to provide a comprehensive list of medications for various species of animals and research protocols. Rather, it provides a guide for proper selection of potential drugs and techniques that could be considered in each class. It should also be noted that some medications may have contradictory effects depending on the animal species,

interspecies differences, and dose. For example, a sedative drug like diazepam may cause excitement in some species (dogs and cats) [11]. Readers are encouraged to consult a relevant textbook for more details on the properties of a specific drug [12], or anesthetic/analgesic method for various species of animals [11,13,14].

Category A

LAAC1

Sample procedures

Handling and minimal restraint of trained animals; sexing; displacing habituated animals; inserting a hypodermic needle; oral gavage.

Intraoperative anesthesia/analgesia

No anesthesia is required. Analgesia is not essential. However, topical anesthetics may be used to reduce the pain of needle insertions. Distraction techniques may be used for reducing the discomfort of the animal.

Post-operative analgesia

Post-operative analgesia is not necessary.

LAAC2

Sample procedures

Using an agitated animal for minimal restraint, sexing, displacing, injecting; pre-anesthetic preparations in distressed animals.

Intraoperative anesthesia/analgesia

No anesthesia is required. Occasionally, analgesia may be provided as discussed for LAAC1. Tranquilizers may be used to calm down the animal. In this regard, low dose phenothiazines (chlorpromazine, acepromazine, promazine), benzodiazepines (diazepam, midazolam), α2-adrenergic agonists (xylazine, detomidine, romifidine, medetomidine, dexmedetomidine), or butyrophenones (droperidol, fluanisone, azaperone) may be used [11,13].

Post-operative analgesia

Post-operative analgesia is not necessary.

LAAC3

Sample procedures

Short-term immobilization in a non-stimulating environment; pre-anesthetic preparations in agitated animals.

Intraoperative anesthesia/analgesia

No anesthesia is required. Analgesia may be achieved as discussed in LAAC1. Sedatives may be used to create a state of sedation and immobilization. In this regard, higher doses in the therapeutic window of LAAC-2 drugs may be used. Additionally, opioids (alfentanil, buprenorphine, butorphanol, etorphine, fentanyl, hydromorphone, methadone, morphine, nalbuphine, oxymorphone, pentazocine, pethidine (meperidine), remifentanil, sufentanil) may also be used to induce a state of moderate sedation accompanied with varying levels of hypoalgesia [11].

Post-operative analgesia

Post-operative analgesia is not necessary.

LAAC4

Sample procedures

Immobilization and sleep in a moderately stimulating environment; non-invasive imaging.

Intraoperative anesthesia/analgesia

Unconsciousness can be induced by hypnotics or anesthetics. These include alpha-chloralose, the barbiturates (e.g., thiopental, thiamylal, and methohexital, thiobutabarbital, and pentobarbital), chloral hydrate, etomidate, metomidate, propofol, the steroid anesthetics (alphaxalone/alphadolone), tribromoethanol, inhalational anesthetics, and injectable anesthetics.

Post-operative analgesia

Post-operative analgesia is not necessary.

Category B

LAAC5

Sample procedures

Conditions with non-somatic origin causing discomfort more than the DT and psychosomatic conditions; procedures leading to anxiety, fear, distress, depression; activity anorexia; developing thin sow syndrome; compulsive behaviors (stereotypical behaviors); addiction and withdrawal.

Intraoperative anesthesia/analgesia

Anesthesia may not be required. However, depending on the cause of the condition, various types of medications or non-medical methods may be used to alleviate the symptoms. For example, a highly distressed animal may receive sedatives and placed in a quiet and dim environment, or an animal in depression may benefit from anti-depressants.

Post-operative analgesia

Since there is no somatic pain in this class, the use of analgesics is questionable.

LAAC6

Sample procedures

Non-surgical procedures that involve visceral organs; procedures through natural body orifices (e.g., endoscopies); intravisceral injection or sampling (not thorax; since it induces discomfort more than the DT); models of sepsis, neoplasia, and inflammation that are induced non-surgically and involve visceral organs.

Intraoperative anesthesia/analgesia

The use of anesthesia may or may not be necessary. For endoscopies and intravisceral injection/sampling, depending on the type and length of the procedure, deep sedation or hypnosis may be used. Therefore, LAAC3 or LAAC4 provisions may be undertaken.

Models of sepsis in this class can be induced by injection of bacterial toxins, purified live bacterium, or fecal-derived bacteria (polymicrobial infection), which do not require anesthesia. Neoplastic models in this class may also be developed by inoculation of neoplastic cell lines and may not require anesthesia.

Post-operative analgesia

Visceral analgesia may be required if any of the aforementioned procedures lead to visceral pain. In this regard, local anesthetics (e.g., lidocaine) may be used for inhibiting pain transduction, transmission, or modulation. Opioids and tramadol may be used for inhibiting pain modulation or perception [10].

There are controversies regarding the need of analgesia for non-surgical models of sepsis [15]. However, the latest findings recommend considering analgesics (such as nalbuphine, buprenorphine, morphine, fentanyl) and closely monitoring the clinical signs of pain in animals [15,16]. The use of non-steroidal anti-inflammatory drugs (NSAIDs) in sepsis models is discouraged due to the risk of bleeding and renal dysfunction. Moreover, the anti-inflammatory effect of NSAIDs could interfere with the pathobiology of the sepsis model and negatively influence the study [16].

Analgesic treatment for inflammatory and neoplastic visceral pains depends on the tissue involved, the pathogenesis and severity of pain, and the species of the animal. For example, opioids such as buprenorphine, butorphanol, hydromorphone, meperidine, or methadone are recommended for pancreatitis pain in dogs [17]. Other analgesic modalities may be found in related references [18].

Neoplasia may influence the animal by pain and general ill-being (sickness syndrome) resulted from the paraneoplastic syndrome [19,20]. The neoplastic pain has several components as: pain resulted from the tumor, pain caused by disease progression, pain due to side effects of treatments, pain from other sources not related to malignancy [21]. Few studies have investigated the neoplastic pain in laboratory animals [13], but as a general rule, neoplastic pain evolves quite differently than acute procedural pain. Neoplastic pain may be acute, chronic, or intermittent [21] but in general it may start gradually, and may last for a longer time and may involve coping mechanisms that could suddenly fail [13]. Opioids and tramadol are used to alleviate cancer pain in animal models [19], and it is shown that certain nonsteroidal anti-inflammatory drugs may have synergy with opioids [13]. Antiepileptics (such as gabapentin and pregabalin) and antidepressants have used for managing chronic pain in humans [19]. Occasionally, NMDA receptor antagonists and local anesthetics have also been used for managing chronic pain [19].

LAAC7

Sample procedures

Intraosseous access; infection or neoplasia that involves hard tissues.

Intraoperative anesthesia/analgesia

The procedure of intraosseous access or induction of bone infection/neoplasia may be performed without general anesthesia. However, category A modalities may be undertaken to reduce the animal distress and facilitate the procedure. For intraosseous access, infiltration with local analgesics (e.g., lidocaine, bupivacaine) should also be used [22].

Post-operative analgesia

Analgesia is required. Bone infection models [23–25] leading to osteomyelitis may induce inflammatory pain. In this regard, NSAIDs such as celecoxib (a selective COX-2 inhibitor) has been used to alleviate the pain of the condition [26].

Bone cancer is a very painful condition and is one of the most common models of cancer pain in laboratory animals [27]. The mechanism of bone cancer pain are partially understood [28] and are described elsewhere [29,30]. Since bone cancer pain results from different mechanisms than bone inflammation pain, a higher dose of analgesics may be required for alleviating it [28]. The choose of analgesics depends on the species of the animal, extent of the neoplastic lesions, the severity of pain, and the objectives of the study. Morphine is shown to produce short-term analgesia (2-3 hr.) for bone cancer pain in mice. Concurrent use of acetaminophen and morphine is shown to produce even higher level of analgesia in this animal model [13]. Other complementary approaches include eradicating the tumor, stabilizing the affected bones, reducing bone loss, and using NSAIDs [31]. Analgesic therapy should be devised such that it does not negatively influence the study objectives, as described elsewhere [32].

LAAC8

Sample procedures

Sepsis or neoplastic conditions that involve the hard tissues and viscera.

Intraoperative anesthesia/analgesia

Anesthesia may not be required to induce the neoplastic or septic condition. However, category A modalities may be applied to reduce the distress of the animal.

Post-operative analgesia

A combination of LAAC6 and LAAC7 analgesic modalities may be used. In this regard a number of new modalities are also under investigation [21].

Category C

LAAC9

Sample procedures

Biopsies from skin, muscle, mucosa, or conjunctiva; incisional or excisional wound models; models of dead space, flap surgery, or burns; catheterization in major blood vessels (carotid artery, jugular vein, femoral artery and vein); vascular ligature models; neoplasms involving skin, muscle, mucosa, or conjunctiva.

Intraoperative anesthesia/analgesia

- Short term general anesthesia may be used. In this regard, inhalation anesthetics may be more appropriate due to their rapid onset of action and fast recovery. Analgesia should be provided by administration of systemic opioids, NSAIDs, or local anesthetics.

Or

- Category A modalities may be applied along with local anesthesia at the site of tissue injury.

Or

- LAAC3 may be applied along with systemic opioids to induce neuroleptanalgesia.

Post-operative analgesia

Depending on the extent of the injury, one or a combination of the below modalities may be used:

- For limited injuries, a long-acting local anesthetic (e.g., bupivacaine) may be used short-term [33] by injecting at the injury site before termination of the procedure.
- One dose [33] of NSIADs (e.g., carprofen, meloxicam) or opioids may be used for minor injuries. In more severe cases, less than one week use of NSAIDs may be warranted [33].
- Systemic or topical opioids may be used short-term (less than 10 d) without considerable effect on wound healing [33]
- Local infiltration of tramadol at the incision site may be used for analgesia without negative effect on wound healing [33].
- Gabapentin may be used as an adjunct analgesic in wound models [33]
- Acetaminophen may be used as a part of the post-surgical analgesia for wound models [33].
- For neoplastic lesions, LAAC6 and LAAC7 modalities may be used.

Systemic or topical steroids should not be used in wound healing models [33].

LAAC10

Sample procedures

Procedures on visceral organs in the abdominal cavity; thoracic interventions that do not involve hard tissues (ribs or sternum); thoracoscopies; ophthalmic procedures; neoplasms involving skin, muscle, mucosa, conjunctiva, and visceral organs.

Intraoperative anesthesia/analgesia

- For abdominal cavity procedures, injectable general anesthesia along with opioids, NSAIDs, and/or local anesthetics may be used.
- For cardiac puncture (as a terminal procedure), deep general anesthesia may be achieved using higher doses of injectable or inhalational anesthetics.
- For thoracic surgeries and thoracoscopies, deep general anesthesia with positive pressure ventilation, and proper multimodal analgesia (opioids, NSAIDs, and/or local anesthetics) should be considered.
- For ophthalmic procedures, one should consider the length of the procedure; goal of the research; depth of anesthesia; effect of the anesthetic on intraocular pressure, extraocular muscles relaxation, retinal ultrastructure, ocular position during surgical plane of anesthesia, and intraocular air volume [13]. Topical ophthalmic anesthetics may be used as an adjunct to general anesthesia for corneal procedures. Oculocardiac reflex may be inhibited using anticholinergic drugs such as atropine or glycopyrrolate [13]. To maintain a central position of the eyeball during intraocular surgeries, neuromuscular blocking agents (e.g., atracurium) may be used by people with special training on the use of these agents.

In large ruminants, enucleation may be performed by regional anesthesia (retrobulbar block of deep orbital nerves) or Peterson block [13].

Post-operative analgesia

- For survival procedures, a combination of opioids and NSIADs (8-72 hr. for non-thoracic procedures) depending on the species of the animal and the extent of the tissue injury may be considered [11].
- For thoracic procedures, LAAC12 postoperative analgesia should be applied.
- Combination of local anesthesia (e.g., lidocaine, bupivacaine) with systemic analgesics may be used [34].
- For neoplastic conditions, LAAC6 and LAAC7 modalities may be applied.

LAAC11

Sample procedures

Orthopedic procedures; surgically induced osseous conditions (e.g., osteotomy); chemically induced skeletal conditions such as arthritis; neoplasms involving skin, muscle, mucosa, conjunctiva, and bones.

Intraoperative anesthesia/analgesia

Deep general anesthesia along with potent analgesia is required. Furthermore, muscle relaxants (i.e., neuromuscular blockers or spasmolytics) may be used to facilitate orthopedic surgeries in animals with large muscular mass. Neuromuscular blockers must only be used by people specially trained in using these agents. Local and regional anesthesia may be used to provide preemptive analgesia and relaxing the muscles.

Post-operative analgesia

NSAIDs are excellent analgesics for orthopedic pain. The efficacy of the propionic acid derivatives (e.g., ibuprofen, fenoprofen, flurbiprofen, flunoxaprofen, ketoprofen, naproxen, and carprofen) to ameliorate orthopedic pains, is comparable with some opioids (such as buprenorphine, butorphanol, pethidine, and codeine) [13]. Overall, there are controversies regarding the effect of NSAIDs on bone healing [33]. There is some evidence that NSAIDs have a potential to alter fracture healing in microscopic levels [13], though it seems a short term use of some NSAIDs does not affect the long term healing rate of the bones [33,35]. However, the use of nonselective COX inhibitors (indomethacin, ibuprofen, ketorolac, parecoxib) are not recommended in orthopedic models of rodents, because of the negative effect of these drugs on bone healing [33]. Also, long term use (10 days- 4 months) of selective COX2 inhibitors (diclofenac, carprofen, rofecoxib) have negative effect on bone healing, but this has not been observed by their short term use (less than 10 days) [33]. Acetaminophen may be used along with other analgesics for treating the pain of orthopedic models in rats [33].

Orthopedic models usually cause chronic pain [7,33]. Therefore, a 2-3 week analgesic therapy using opioids may be warranted following extensive orthopedic surgeries without negatively affecting bone healing [33]. In this regard, sustained release medications such as fentanyl patch, fentanyl gel, or sustained release buprenorphine injection may be considered [35]. It should be noted that buprenorphine seems to have a ceiling analgesic effect, meaning that increasing the dose of the drug could not provide more analgesia after a ceiling point of analgesic effect is achieved [36]. Long term use of opioids (i.e., 8 weeks in rats), may negatively affect the remodeling and resorption of the fracture callus [33]. Combining NSAIDs with opioid agonists provide a synergistic effect. Therefore, if NSAIDs are omitted from the postoperative analgesic regimen, the dose of the opioid should be considerably increased.

Postoperative local and regional anesthesia may also be achieved by placing catheters for continuous anesthetic drug infusion (e.g., ropivacaine, bupivacaine). However, this should not be considered as the sole method of postoperative analgesia [13]. Low dose ketamine may be used as a preventive analgesic modality in orthopedic models [33].

Multimodal analgesia in shown to effectively reduce postoperative pain in spine surgery. It may comprise gabapentinoids, acetaminophen, regional anesthesia by placing local anesthetics close to the spinal nerves, and slow-release local anesthetics [35].

Steroids (e.g., prednisolone, methylprednisolone, dexamethasone) should be avoided in orthopedic models [33]. Gabapentin is also not a suitable analgesic for orthopedic models in rats [33]. Information on the general anesthetic medications used in orthopedic research on large laboratory animals are provided elsewhere [37]. For neoplastic pains, LAAC7 provisions should be applied.

LAAC12

Sample procedures

Thoracic surgeries involving sternum (sternotomy) or ribs (costotomy); brain surgeries through craniotomy; metastatic neoplasms involving skin, muscle, mucosa, conjunctiva, bone, and visceral organs.

Intraoperative anesthesia/analgesia

Deep general anesthesia and potent analgesia are required. Thoracic surgeries require positive pressure ventilation. If the anesthetic depth, analgesia, and ventilator settings are adjusted properly, the ventilation could usually be performed without the need of neuromuscular blocking agents [13]. When using neuromuscular blocking agents, special care should be paid to the monitoring of the anesthetic depth [11].

Various anesthetic protocols are described for thoracotomy in larger animals [14,38] and pets [39]. However, available literatures usually do not report specialized anesthesia/analgesia for thoracic surgeries in smaller animals such as mice and rats. For example, there are reports of using common anesthetics such as ketamine/xylazine [40–42], or isoflurane/carprofen [43], and narcotics such as sodium pentobarbital [44,45]. However, these agents could not provide an adequate depth of anesthesia and enough analgesia for thoracic surgery as one of the most painful operations. The same holds true for rabbits, in which sodium pentobarbital [46,47] or sodium pentobarbital/isoflurane [48] are used. There are also reports of proper anesthesia but inadequate perioperative analgesia [49,50] and inadequate postoperative analgesia [51] on rabbits.

Therefore, for this class of procedures, I recommend anesthetic protocols that incorporate cardiac-safe balanced anesthesia with multimodal analgesia (i.e., concurrent use of opioids, NSAIDs, and intercostal nerve block) as it could be exemplified in some previous works [52–54]. Cardiac effects of various anesthetics and analgesics are discussed elsewhere [55]. Nitrous oxide should be avoided due to its effect on developing pneumothorax after the surgery [13]. Craniotomy may be performed under general anesthesia and multimodal analgesia, along with a splash block of lidocaine on the skull periosteum.

Post-operative analgesia

Thoracic surgeries are accompanied with severe post-operative pain. Left untreated, this pain could lead to difficult breathing, hypoxia, and pneumonia. Pain of thoracotomy procedure may last longer than 48 hours [7]. Therefore, pure agonist opioids must be used parenterally for the first 48 hours after the surgery. During which time, the respiratory rate and quality should be monitored to identify and correct opioid-mediated respiratory depression. Other analgesics such as NSAIDs, intercostal nerve block with local anesthetics or intrapleural local anesthetics, epidural opioids, and ketamine may be used in combination with systemic opioids to provide multimodal analgesia. This multimodal analgesic therapy reduces the required dose of opioids and their side effects on respiration [13,56]. Some post-operative analgesic provisions could be adopted from companion animal practice [39].

Neuropathic pain (+)

Neuropathic pain (NP) may develop as a side effect of an intervention on laboratory animals [57], or it may be intentionally modeled on animals [57–60]. Proper detection of NP is a challenge in veterinary patients and laboratory animals [61–63]. Currently, there is no efficient analgesic drug to fully counteract NP [58,64]. However, it is believed that the same concepts of preemptive analgesia and multimodal analgesia also apply to NP [61].

The first line drugs for treatment of NP includes certain anticonvulsants (e.g., gabapentin and pregabalin), some tricyclic antidepressants (e.g., imipramine, amitriptyline), and topical lidocaine [61]. Other analgesics such as opioids, tramadol, and NSAIDs, if used alone, are usually less effective in treating NP. However, they may be considered as part of a multimodal analgesic approach in this condition [61]. A number of nonchemical approaches are also suggested as an adjunct therapy for alleviating NP, such as: providing environmental enrichment [65], using long particles as bedding material, reducing the soy and adding the taurine content of the feed, group housing the animals to encourage social buffering [66], massage, and thermotherapy [61]. It is shown that in animal models of NP, postoperative analgesic therapy may not adversely affect the development of NP [67].

Discussion

The current state of reporting and use of AA in animal research is concerning. There is an essential need for a regulating system to assist researchers to choose proper methods of AA and enables reviewers to detect improper reporting or use of AA. In this regard, the LAAC system classifies research procedures on animals into three categories and 12 classes. Each class includes several procedures affecting the same parts of the body, which may require similar methods of AA. The depth of anesthesia and intraoperative analgesia required for each class, generally increases from LAAC1 to LAAC12.

In veterinary anesthesia, there is an adopted classification system from medicine called American Society of Anesthesiologists Physical Status classification (ASA PS). ASA PS classifies the physical status of patients into 6 classes in medicine, and 5 classes in veterinary medicine [10]. Higher classes of ASA PS predict a higher risk of anesthetic mortality for a given patient [68,69]. In contrast to LAAC, the ASA PS does not consider the type of procedure that is intended to be performed on animals.

Another classification system used in laboratory animal research is the severity scoring system [70]. This classifies the severity of procedures into four classes, based on the level of pain, suffering, and distress that a procedure may cause [70]. In comparison, LAAC considers the type of tissues and pain involved in a procedure and specifically addresses the AA requirement of procedures.

Working with the LAAC system does not require special knowledge in laboratory animal anesthesia and people from various backgrounds can use it. For example, a researcher could determine the LAAC of a procedure in his/her research and using the instructions related to that LAAC, devise a proper AA regimen for it. LAAC also facilitates communication. For example, a researcher could cite the LAAC of a certain procedure in his/her paper and thus clearly communicate a large amount of information regarding the AA requisites of that procedure. This would avoid potential misunderstandings about the methodology of the published research.

Grant reviewers and ethics committees could use LAAC to classify the procedures and determine the best AA for each procedure. This may improve the quality of AA in research proposals. LAAC may also improve the quality of journals' peer review. Journals' reviewers could use LAAC system to determine if an AA protocol used in a manuscript is scientifically and ethically sound. Animal welfare bodies and animal care staff could also use LAAC as a measure of the severity of procedures and the level of care an animal need.

LAAC may have a limitation. It could not provide a quantitative measure for the extent of injury for a given procedure. However, it should be noted that the aim of LAAC is to provide a means for classifying procedures according to the body parts they involve and associated type of pain and distress they produce.

In conclusion, LAAC provides a simple language for communicating the type of procedure and methods of AA in laboratory animal science. It also assists researchers in deciding which anesthetic or analgesic method is appropriate for a given procedure. For future studies I suggest developing specific anesthetic/analgesic protocols according to the LAAC system for each species of animals.

Methods

The LAAC classes are defined according to the level of distress or the type of tissue injury that are accompanied with each type of procedures. For each class of LAAC, proper methods of anesthesia and analgesia are devised to properly address the anxiolytic, anesthetic, or analgesic needs. At the first level of classification, the procedures are classified according to the level of discomfort that they may cause

(Fig. 1). In this regard, a discomfort threshold (DT) is defined as the discomfort resulted from the insertion of a hypodermic needle into the skin of an animal, where the gauge and length of the needle are appropriate to the animal size and species, and the needle is inserted into the skin according to good veterinary practice. Hence, procedures could be classified under two main categories: 1) procedures with cumulative discomfort equal to or less than the DT, and 2) procedures with cumulative discomfort more than the DT.

For procedures with discomfort up to the DT (Class A procedures), the pain management is not the primary concern to properly perform the procedure. Therefore, further classification will be performed according to the level of unconsciousness that is required for properly performing the procedure. Figure 1 shows the first four classifications (LAAC1 to LAAC4), with LAAC1 requiring no chemical tranquilization, and LAAC4 requiring the use of hypnotics.

For procedures causing discomfort more than the DT, the classification is performed according to the type and level of pain. Figure 2 shows a pain classification diagram mainly based on the type of pain, in which somatic pain is further subdivided according to the level of pain arise from each type of tissues.

Since many surgical procedures involve skin/muscle/mucosa/connective tissues (SMMC) to varying degrees, I used SMMC involvement to generate two distinctive classes (Fig. 1): Class B) procedures that does not involve SMMC and are mainly performed non-surgically; Class C) procedures that may involve the SMMC and are mainly surgical. In each of the B or C classes, further classification is performed according to whether bones/joints (somatic pain) or viscera (visceral pain) are involved. In Class B, generally from LAAC5 to LAAC8, the level of involvement of various body parts and its accompanying pain gradually increases, thus requiring more potent intraoperative anesthesia/analgesia. The same holds true for Class C, in which the extent of involvement of various tissues (and their corresponding pain) increases from LAAC9 to LAAC12.

Neuropathic pain (Fig. 2) is a consequence of the pathology of the nervous system and it is assumed to cause discomfort more than the DT. Therefore, it could be a component to the overall pain in each class of the categories B or C. In LAAC system, neuropathic pain would be indicated with a (+) sign that could be added to each of the LAAC classes. For example, class LAAC8 with neuropathic pain would be shown as LAAC8+.

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Declarations

The author declares no competing interests.

Figures

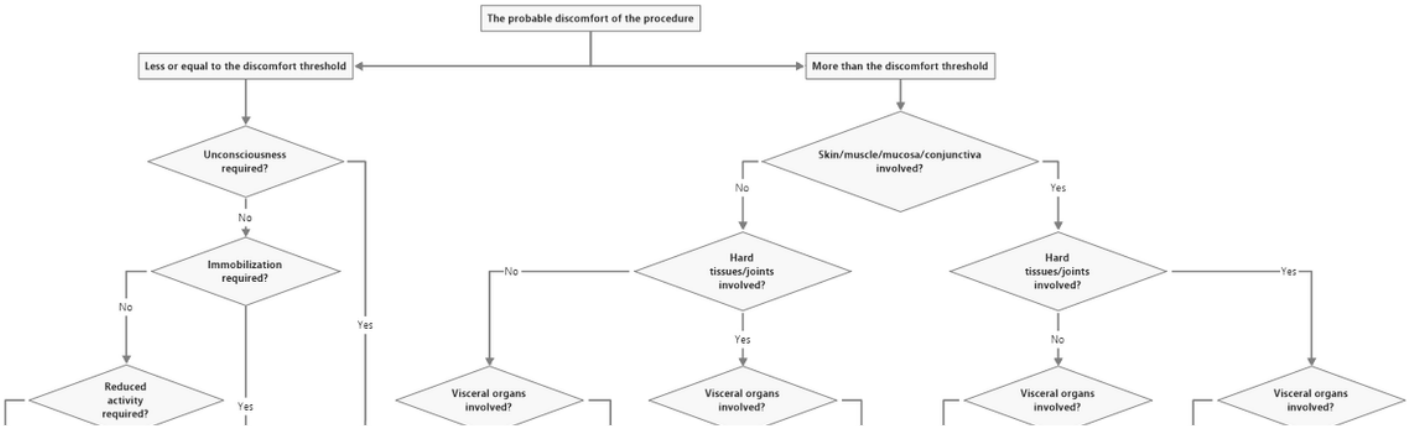

Figure 1

The LAAC system

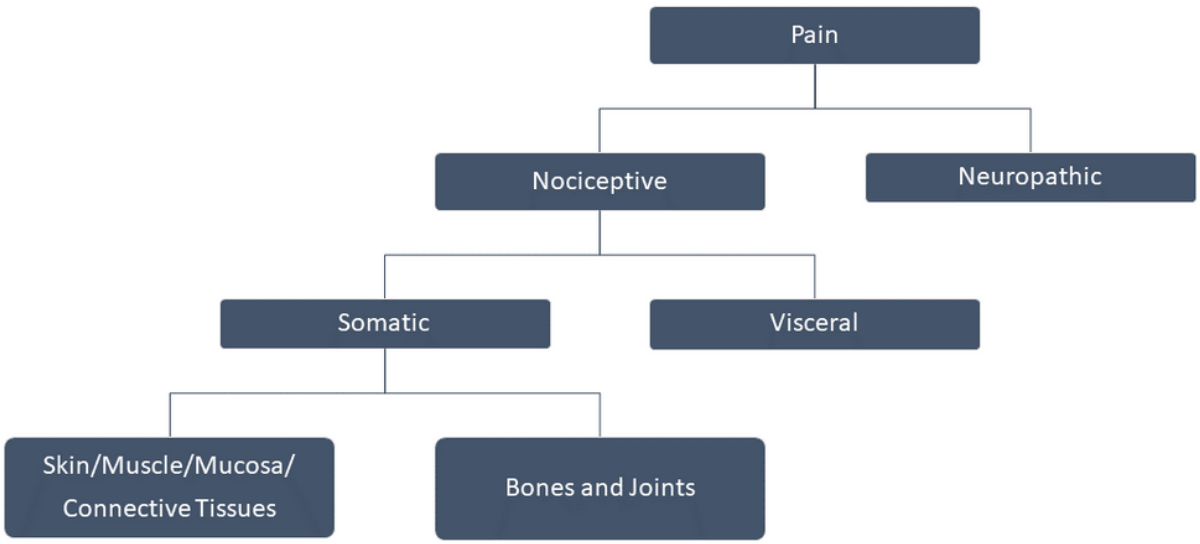

Figure 2

Classification of pain according to the type and level of pain

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