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**SPECIAL TOPIC SECTION**

***Management***

**Beversdorf and Adams. Attitudes toward animal research among medical students in the United States****, pp. 120-126**

Domain 6: Education

SUMMARY: The authors surveyed student members of the American Academy of Neurology (AAN) regarding their attitudes toward animal research.  The authors examined factors that may influence the attitude toward research and whether the attitudes represented rigidly held beliefs.  The survey consisted of 14 questions with 7 positively biased and 7 negatively biased statements regarding animal research.  The questions were written with a specific focus on understanding the role of animal research in the development of new therapeutics and surgical procedures.  The students were asked to take the survey and then watch a 14 minute educational video generated by Americans for Medical Progress.  Following the viewing of the video, the participants were asked to take the survey again.  The final sample size was 1,167 individuals.  The response rate was 14.4% with 168 individuals completing the initial survey.  One hundred and eight individuals completed the survey again after watching the video.  After viewing the video, the group’s overall attitude changed to be more positive toward animal research.

QUESTIONS

1. What were two factors having a statistically significant relationship with attitude score?
   1. Gender and Farming Experience
   2. Gender and Prior Research Experience
   3. Pet Ownership and Gender
   4. Pet Ownership and Prior Research Experience
2. How has the attitude toward animal research changed in the general public?
   1. Increase in public support of animal research in the last decade
   2. No change in public support of animal research in the last decade
   3. Decrease in public support of animal research in the last decade
3. T/F  Support for animal research is greater in men than women among the general public.

ANSWERS

1. b
2. c
3. T

**Pritt and Smith. Institutional Animal Care and Use Committee postapproval monitoring programs: a proposed comprehensive classification schemes, pp. 127-131**

Domain 5: Regulatory Responsibilities

SUMMARY: The goal of this article is to provide more detailed and robust discussions of IACUC’s PAM (Postapproval Monitoring programs), including how outcomes and successes should be evaluated. Such precise descriptions can benefit animal care and use programs and animal research oversight at large. PAM can be defined as a mechanism for assuring that animal activities are conducted in a manner consistent with the IACUC-approved protocol. This brief definition allows each IACUC to craft their own PAM program, which is crucial since the use of the term PAM is not directly included in the most commonly referenced regulations and guidelines that outline IACUC responsibilities. Required continuing IACUC oversight of animal activities is mentioned but no explicit obligation is provided. Therefore PAM is a very institutionally specific activity due to the lack of exacting requirements.

The IACUC community has created many classifications and descriptions for PAM. Many of the newer descriptors have not yet been documented in the peer-reviewed literature and are presented in a binary fashion:

* Protocol or administrative review compared with procedural observations: protocol and administrative monitoring is conducted through a meeting and procedural observations include observation of the previously discussed procedure by a trained person
* Formal/active compared with informal/passive PAM: PAM is not federally required. Postapproval activities are conducted within the confines of what IACUCs are required to do (informal) as compared with the undertaking specific activities outside of those required with the sole purpose of identifying compliance to IACUC-approved procedures (formal). The informal approach can be labelled as passive approach (that is, occurring passively through ongoing IACUC actions) and the formal approach as active PAM (that is, engaging in additional, nonmandated activities).
* Announced/scheduled vs unannounced/unscheduled monitoring: IACUC who conduct formal PAM should determine whether they will use unannounced PAM visits. Unannounced PAM can serve as an opportunity to substantiate or not allegations of impropriety.
* Random vs targeted/selected review: formal APM activities could be administrative or observational and then announced or unannounced. If randomization is used, it should be purposefully organized. IACUC could categorize studies in risk categories and decide on a % of studies to be selected per category.
* Regularly scheduled vs heightened monitoring: regular scheduling ensures the frequency of PAM prescribed by the IACUC is followed. Heightened PAM for a researcher as a result of previous PAM outcomes or noncompliance issues should be announced to that person. The researcher should be told why they are on a heightened PAM plan, for how long or until expectations are met.

IACUCs should describe their PAM program methodologies, ideally in a PAM policy or policies for required IACUC activities. These policies are useful during regulatory inspections, accreditation visits and training. The classification scheme proposed can standardize the way with which the animal research community discusses PAM, aid IACUC training, and create the ability of IACUC administrators to consistently benchmark PAM programs and outcomes across institutions.

QUESTIONS – True or False

1. The persistent use of the acronym PAM in IACUC vernacular has become synonymous with formal or active PAM

2. Using USDA pain classification (B, C, D or E) is a good method to assess the risk of a study

ANSWERS

1. True. Most likely as a result of the need to distinguish required monitoring activities from nonrequired activities

2. False. It does not correspond to risk. Pain, invasiveness of the procedure, competency of the researchers, species used, compliance history, newness to the institution as well as the history of the procedural outcomes should be included.

**Engel et al. Cognitive dissonance in laboratory animal medicine and implications for animal welfare, pp. 132-138**

Domain 5: Regulatory Responsibilities

SUMMARY: The authors assert that Cognitive Dissonance (CD), defined as psychological distress when a person’s beliefs and actions are in conflict, may manifest in veterinarians and veterinary technicians working in lab animal medicine. This may lead to mental strain affecting employee retention and the potential for both positive and negative effects on animal welfare. The goal was to detect CD if it existed; how severe it was; what negative emotions were experienced; and compensatory actions taken to relieve negative emotions and how these may or may not affect animal welfare.

The premise that veterinary professionals experience CD was based on the assumptions that they care about the wellbeing of the animals, they feel responsible for the animals, and they support the use of animals in research.

The method they used to detect Cognitive Dissonance was a survey. They compared veterinarians to veterinary technicians; small animal only, mixed, and large animal only categories; and time on the job to look for differences in CD between these categories. They also asked about specific ways that animal care professionals compensate by either placing responsibility outside themselves, devaluing the animals, or distancing themselves emotionally.

The results of the survey support the hypothesis that lab animal veterinary professionals experience emotional discomfort associated with their work.

Junior staff experience greater CD than senior staff. This finding may be because people who are frustrated by the work leave the profession prematurely or that as they work longer in the field they can compensate either by working to improve welfare or by justifying. Junior personnel anthropomorphize more and spend more time interacting with the animals which could also account for increased CD over senior staff.

CD intensity is positively correlated to time spent interacting with animals (vet techs more than vets) and with time spent working with large animals (dogs, pigs, primates) than small animals (rodents, fish) – which may be due to the increased time it takes to care for large animals or that it is easier to identify with larger animals such as primates and pigs than with mice and fish.

The most common way that animal care professionals compensated was to shift responsibility to IACUC and institutional roles, but this was seen more strongly with the small-animal workers than large-animal possibly due to less emotional attachment to rodents. The second most common way to compensate was to devalue the animals and this was seen more in small-animal caretakers than large – again likely due to the amount of time caretakers must spend caring for large animals. The least used compensation mechanism was emotional distancing by becoming indifferent to the animals’ welfare over time. This suggests that CD does not necessarily result in reduced animal welfare since apathy was not a strong compensatory mechanism.

The most effective way an institution can address CD is by advocating refinement practices. The authors suggest that by recognizing CD in their animal caretakers and knowing that CD is not in opposition to research goals, institutions can improve animal welfare and thus research outcomes buy supporting caretakers' sense of control to improve the welfare of the animals under their care.

QUESTIONS

1. Cognitive Dissonance is  \_\_\_\_\_\_.

a. A feeling of well-being

b. Learned helplessness

c. When one's beliefs and actions are in conflict

d. When one's beliefs and actions are in agreement

2. T/F: Animal caregivers often experience cognitive dissonance

3. T/F: Institutions that identify cognitive dissonance in its animal caregivers should move those employees to a non-animal care role to prevent serious harm to the research objectives.

ANSWERS

1. c

2. True

3. False

**Pavan et al. Using a staff survey to customize burnout and compassion fatigue mitigation recommendations in a lab animal facility, pp. 139-147**

Doman 4, Task 3

SUMMARY: Burnout (BNO) refers to emotional exhaustion that results from chronic or severe stress and compassion fatigue (CF) refers to strain that results from exposure to trauma of others. Both of these emotional conditions are frequently associated with professions that require high levels of empathy and exposure to moral stressors such as laboratory animal medicine. Consequences of BNO and CF include decreased quality of animal care, increased worker turnover and low employee morale. Increasing compassion satisfaction (Csat) is a critical intervention to offset BNO and CF.

In this study, a survey was administered to lab animal employees at a large academic institution to assess prevalence of BNO, CF and Csat, identify potential risk factors, assess employee awareness of existing resources and build a customized program to minimize BNO and CF. 82% reported BNO and CF alone or in combination with Csat while only 18% reported only experiencing Csat. The highest rates of BNO and CF were reported in those with 0-4 yrs experience in the field and those in the 30-39 YO age range. 50+ YO participants had an 89% risk reduction in experiencing BNO and/or CF. Respondent recommendations to ameliorate BNO/CF and improve Csat included increasing positive feedback, providing new work experiences and providing more workplace resources to help address these issues. While BNO and CF will never be fully eradicated, workplace programs have the potential to help staff members cope with negative components of a caregiving career.

QUESTIONS

1. Implementation of cross training and job rotation is an example of a BNO/CF mitigation strategy at what level of the social-ecological model?

a. Individual

b. Interpersonal

c. Institutional

d. Community

e. Policy

2. Which of the following is NOT considered an example of an engineering control measure that can be used to address laboratory animal allergens?

a. Increased room air exchanges

b. Use of robotics to reduce animal handling

c. Use of dust-free bedding

d. Use of individually ventilated HEPA-filtered caging with HEPA-filtered cage change and bedding dump enclosures

ANSWERS

1. c. The social-ecological model is used in public health programming and recognizes that wellness changes must be implemented at the individual, interpersonal, institutional, community and policy level. Implementation of cross training is an example of an institutional intervention (p. 146, Figure 5)

2. b. Use of robotics to reduce animal handling is considered an administrative/work practice control measure. BB Chapter 30, pp. 1389-1390

**ORIGINAL RESEARCH**

***Husbandry***

**Krueger et al. Enrichment preferences of single housed zebrafish (*Danio rerio*), pp. 148-155**

Domain 4 Animal care

Primary Species: Zebrafish (*Danio rerio*)

SUMMARY: Little is known about environmental enrichment preferences of zebrafish. This study included an inanimate and animate enrichment assessment. 10 inanimate forms of enrichment were tested on individually housed mature females: white PVC pipe, white tulle, dark paper on the cage bottom, multicolored marbles, green and brown plastic plant, image of marbles on the cage bottom, dark paper on the side of the tank, image of plants on the side of the tank, image of other zebrafish on the side of the tank, and mirrored paper on the side of the tank. A crossover study design was used so each animal was exposed to each enrichment. Tanks were divided in half using a partition with a passthrough so the fist could freely access either half. During acclimation with no additional enrichment, it was determined that 70% of all fish preferred the front half of the tank so enrichment was placed in the back half to account for this bias and see how strongly the enrichment was preferred. Fish location was assessed using video recordings. Fish were found to prefer the front of the tank regardless of enrichment at the beginning of each assessment point when the observer entered the room and turned off the lights. Fish had the strongest preference for the back of the tank with mirrored paper but also interacted with PVC pipe, tulle, and marbles. For the animate study, singly housed fish were placed next to a tank containing another singly housed fish, group housed fish, or an empty neighbor tank. Fish were found to prefer to be on the social side of the tank when next to singly or group housed fish. This is in accordance with previous studies establishing zebrafish preference for cohousing with conspecifics. Simulated movement is important as the fish had no increased preference for static images of other zebrafish.

QUESTIONS

1. What are shoaling fish?
2. What does scototaxic mean? Does this apply to zebrafish?

ANSWERS

1. A group of fish that stay together for social reasons
2. A preference for, and subsequent movement towards darkness; yes

***Animal Health Surveillance***

**Pettan-Brewer et al. Adoption of exhaust air dust testing in SPF rodent facilities, pp. 156-162**

Domain 3

SUMMARY:The use of soiled-bedding sentinel animals for health surveillance is widely accepted. However, there are some agents that are not efficiently transmitted by this method and the number of sentinels necessary is considerable. An alternative method is highly sensitive PCR arrays that detect DNA from environmental samples such as dust from inside IVC tops or exhaust air dust (EAD) from IVC racks. The article describes the transition to EAD PCR monitoring. 2 sampling methods were used: environmental sticky swabs and in-line EAD media strips. 116 racks were used to compare both methods. Differences between traditional sentinel serology and EAD testing were the improve detection of various excluded infectious agents with the latter. EAD testing concerns are false-positive result for LCMV due to genetically modified mice containing LCMV genomic DNA and residual nucleic acids from ineffective washing IVC is a concern for false positives. Cost and labor analysis indicated that is greater for the media compared with swabs although no evidences suggest that either is more effective. EAD PCR testing proved to be an optimal alternative with the concern of a proper SOP for rack washing.

QUESTIONS (True or False)

1. All excluded agents are acceptably transmitted via soiled-bedding transfer to sentinel animals.

2. EAD sampling offers a reliable alternative to replace live soiled-bedding sentinels to detect excluded agents.

3. Media is more effective thanks to the improved detection of intermittent infection over a period of time.

ANSWERS

1. False

2. True

3. False

***Anesthesia***

**Zude et al. Use of flavored tablets of gabapentin and carprofen to attenuate postoperative hypersensitivity in an incisional pain model in rats (Rattus norvegicus), pp. 163-169**

Domain 2: Management of pain and distress; Task 2: Minimize or eliminate pain and/or distress

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: This study investigated non-opioid postoperative analgesia options in rats, with the goal of attenuating pain and reducing stress associated with handling for drug administration. The study split 48 Sprague-Dawley rats into 5 treatment groups: 1) placebo tablet, 2) placebo tablet + subcutaneous dose of sustained release buprenorphine (bup-SR), 3) gabapentin 90mg tablet (gaba), 4) carprofen 2mg tablet (car), and 5) gabapentin/carprofen combo tablet at 90mg/2mg respectively (gaba/car). Tablets were administered 3 days prior to surgery (days -3, -2, and -1) for drug preloading and to decrease effects of neophobia, on the day of surgery (day 0), and 2 days post-op (days +1 and +2), with any pieces of tablet from the previous day collected and weighed to gauge dose ingested. The surgery used was a previously established hindpaw plantar surface incision with the flexor digitorum brevis transected. Daily Von Frey testing counting number of paw withdrawals was used to gauge mechanical hypersensitivity, and the latency to withdrawal from a radiant heat source was used to gauge thermal hypersensitivity. All of the following post-op results are in comparison to Day -1. The placebo group demonstrated significantly higher mechanical hypersensitivity on all 3 post-op days. Mechanical hypersensitivity in the BupSR group was higher on Day +2, but not on Days +1 and +3. Mechanical hypersensitivity in both the gaba and the car group was not higher on any post op day, and the gaba/car group showed increased mechanical hypersensitivity on Day +1, but not on Days +2 and +3. The placebo group showed significantly lower thermal hypersensitivity all 3 days postop. Thermal hypersensitivity in the BupSR group was lower on all postop days. For both the gaba and the car group, thermal hypersensitivity was lower on Days +1 and +2, but not Day +3. Thermal hypersensitivity was significantly lower on all 3 postop days for the gaba/car group. Average tablet consumption was 90% for placebo, 48% for gaba, 91% for car, and 42% for gaba/car. Weight gains were not statistically significant between groups and no lesions or overt gastric ulcerations were noted on gross necropsies.

Results showed that bupSR did not attenuate thermal hypersensitivity. Gabapentin and carprofen each attenuated mechanical but not thermal hypersensitivity. The gabapentin/carprofen combo group did not attenuate mechanical hypersensitivity on Day +1, but did on Days +2 and +3, which was likely due to decreased ingestion immediately post op and could be related to tablet palatability. Overall, the study demonstrated the successful use of nonopioid analgesics in the form of flavored gabapentin, carprofen, or combo tablets, voluntarily consumed, to reduce post-op pain without animal handling.

QUESTIONS

1. Which of the following is a structural analog of gamma-aminobutyric acid?

a.  Buprenorphine

b. Gabapentin

c.  Carprofen

d.  None of the above

2.  Buprenorphine is a:

a.  Partial mu opioid agonist

b.  Full mu opioid agonist

c.  Mixed opioid agonist/antagonist

d.  Partial kappa opioid agonist

3. True or False: Carprofen is an NSAID that preferentially inhibits cyclooxygenase 1 secondarily to prostaglandin synthesis.

ANSWERS

1.  b

2.  a

3. False

**Heng et al. Continuous rate infusion of alfaxalone during ketamine-xylazine anesthesia in rats, pp. 170-175**

Domain 2: Management of pain and distress

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY: Ketamine-Xylazine (K-X) combination has been widely used in rodent anesthesia. This anesthetic cocktail has limitations as the variability of its effects in time for induction, time of duration or plane of anesthesia. This report studies if the addition of a CRI of Alfaxolone, a safe and effective anesthetic, to the K-X cocktail would improve its performance. 32 Sprague-Dawley rats (50% males, 50% females) underwent general anesthesia and thigh surgery (exposition of underlying muscle). Two anesthetic protocols were assessed: 1) KX;  Ketamine (80mg/kg) plus Xylazine (8mg/kg) (SC route) and 2) KXA; Ketamine and xylazine (same doses and route as protocol 1) plus alfaxalone CRI (10mg/kg/h, IV, syringe pump). Alfaxalone was given 10min after loss of righting reflex and was discontinued 45min later. Loss of front and hindlimb withdrawal reflex was achieved in 100% of animals in the KXA protocol while in KX protocol were less consistent (not all the animals lost that reflex). KXA achieved a surgical plane of anesthesia during the 45min in 100% of animals  while KX only achieved that 50-75% of animals. Return to righting reflex was shorter in KXA than KX protocol. Heart rate, respiratory rate, SpO2, body temperature did not differ between protocols. Hypersalivation was noted in 75% males and 63% females in the KXA protocol. In conclusion, including alfaxalone in a KX protocol, provides a deeper and longer plane of surgical anesthesia.

QUESTIONS

1.  Median Time to loss of hindlimb withdrawal reflex was:

a. Shorter in KXA protocol

b. Longer in KXA protocol

c. Equal between KXA and KX protocols

d. Longer only in females undergoing to KXA protocol

2.   Median Time to return to righting reflex was:

a. Shorter in KXA protocol

b. Longer in males vs. females

c. Equal between KXA and KX protocols

d. Longer in KXA protocol

3.  In the recovery during the 3 days following anesthesia

a. Hypersalivation still occurred in KXA rats

b. Increase in weight had a normal evolution

c. Some rats had diuresis

d. Severe ulcers were observed in the site of injection

ANSWERS

1. c

2. d

3. b

**Sayce et al. Continuous rate infusion of ketamine hydrochloride and dexmedetomidine for maintenance of anesthesia during laryngotracheal surgery in New Zealand White rabbits (*Oryctolagus cuniculus*), pp. 176-185**

Domain 2: Management of pain/distress; T3 - Anesthesia

Primary Species: Rabbit (*Oryctolagus cuniculus*)

SUMMARY

Goal: Exploration of this anesthetic protocol for extended-duration procedures that require extensive manipulation of the trachea and larynx.

Animals: 58 male, 4-6m.o. NZW rabbits, 3kg.

Model/Surgical Procedure: Phonotrauma is the leading cause of dysphonia/hoarseness. Phonotrauma involves excessive or abusive vocal behaviors leading to vocal cord trauma. Phonotrauma induced changes, structural, molecular and functional consequences of hoarseness (dysphonia) and vocal cord wound healing are investigated experimentally.

Studies using the rabbit phonation model have characterized mRNA changes in response to phonotraumatic behaviors, described changes elicited in epithelial barrier function and elucidated timelines for the recovery of the vocal-fold epithelium after phonotrauma.

Rabbit Model Advantages: Surgically induced vocal fold phonation. Dose and duration can be controlled. Can be visualized endoscopically. Rabbits have similar vocal cord histology to humans. Rabbits don’t usually vocalize (non-habitual vocalizers).

This Study: Rabbit surgical model to characterize safety and efficacy of glucocorticoid steroid use for the treatment of phonotraumatic vocal-fold damage. Rabbits are anesthetized, trachea-larynx surgically exposed, ET tube placed after tracheotomy 4cm below larynx to maintain spontaneous respiration and supply oxygen. Animals surgically phonated by using cricothyroid electrical stimulation and forced, humidified airflow through the glottis for 120 min. Laryngoscope placement during the procedure for visual evaluation. Non-survival phonation.

Concern: Autonomic laryngeal response (e.g. laryngospasm) to upper airway stimulation during general anesthesia is widely reported, particularly in the presence of upper airway manipulation. Could have considerable negative effect on functional vocal-fold vibration and related downstream biologic processes. Anesthetic regimen should provide depth of anesthesia sufficient to prevent surgical pain responses (e.g. movement, increased heart rate in response to surgical manipulation) and to mitigate break-through, local, autonomic laryngeal responses during sustained phonation.

Anesthetic Protocol: Induction through 20 mg/kg IM ketamine hydrochloride and 0.125 mg/kg IM dexmedetomidine, followed by CRI intravenous maintenance anesthesia of 300 to 350 μg/kg/min ketamine hydrochloride and 1.5 to 1.75 μg/kg/min dexmedetomidine, with prophylactic topical 2% lidocaine administered every 40 min. Adverse events were best managed with a combination of CRI dosage increase paired with additional application of 2% topical lidocaine.

Adverse Events/Comments

* Autonomic laryngeal response was the leading complication (25/58 animals). In most animals it was avoided and in the rest it was managed successfully with additional lidocaine and anesthesia (20/25 animals). This is expected in such studies because of the continuous strain on the tissue. Insufficient depth of anesthesia and superior laryngeal nerve activation are the most likely causes of intraoperative laryngospasm. Interindividual differences seem to also play a role. In study design half animals received prophylactic local lidocaine.
* Vital signs were relatively stable. MAP was more stable than ketamine/xylazine IV. Few cases of acute increase of HR (8) or RR (1) were managed.

Advantages Of This Anesthetic Protocol:

* Advantages of ketamine/dexmedetomidine combination (stable HR/RR/BP compared to others)
* Advantages of CRI infusion maintenance
* Avoiding to use cuffed ET tube which would be required for inhalation anesthesia. Tissue damage would be significant because the procedure is long.
* Avoiding pentobarbital cardiorespiratory depression and immunosuppression.
* No effect on endogenous steroid levels.

Limitations:

* This study included only male rabbits due to differences in laryngeal architecture and functional vocal-fold vibration between sexes; however. But females may have different response to this anesthesia regimen. Female rabbits require more isoflurane supplementation when anesthetized with ketamine and medetomidine IM. Females are more susceptible to the antidepressant effects of low-dose ketamine.
* This study was acute – recovery studies will follow.
* Muscle relaxants were not considered because moderate tone of the thyroarytenoid and cricothyroid muscles is required for normal phonation.
* Dose selection was driven by need to maintain sufficient anesthesia depth to minimize laryngeal responses and maximum doses were not investigated.

QUESTIONS

1. Which is the leading complication of anesthesia during surgeries for phonotrauma studies in rabbits?

a. Decreased blood thickness

b. Decreased heart rate

c. Autonomic laryngeal response of laryngospasm

d. Decreased temperature

2. Which of the following is NOT an advantage of the rabbit model of surgical phonation?

a. Surgically induced vocal fold phonation with controlled dose and duration.

b. Can be visualized endoscopically.

c. Rabbits have similar vocal cord histology to humans.

d. Rabbits vocalize very often (habitual vocalizers).

3. True/False: According to the study by Sayce et al. 2020. induction through 20 mg/kg IM ketamine hydrochloride and 0.125 mg/kg IM dexmedetomidine, followed by CRI intravenous maintenance anesthesia of 300 to 350 μg/kg/min ketamine hydrochloride and 1.5 to 1.75 μg/kg/min dexmedetomidine, with prophylactic topical 2% lidocaine administered every 40 min is successful for non-survival phonation studies using male rabbits.

4. Which is NOT a procedure of surgical phonation trauma rabbit model.

a. Cricothyroid electrical stimulation

b. Forced, humidified airflow through the glottis

c. Laryngeal visualization

d. Vocal cord radiation

ANSWERS

1. c

2. d

3. True

4. d

**Llanguez et al. Quantitative and qualitative behavioral measurements to assess pain in axolotls (*Ambystoma mexicanum*), pp. 186-196**

Domain 2

Tertiary Species: Other Amphibians

SUMMARY:Regenerative medicine research usually undergo surgical injury for the assessment of healing process in axolotls. Providing appropriate pain relief is important for maintaining animal welfare and adhering to the law. NSAID usually are avoided, whereas opioids are the first-line drugs. Nevertheless, no choice of drug is without complications.

In the current study, the authors observed and recorded baseline behavior in all the subjects to establish each animal's normal status. Then, they used this baseline as an index of the resulting behavior after each animal received each analgesic compound. The objective is to ascertain whether the observed changes are purely associated with the administration of the analgesics.

Devices relying on thermal stimuli to induce pain are difficult to adapt to aquatic species. Chemical nociceptive sensitivity can easily be assessed by using the acetic acid test (AAT).

Given that endogenous opioids receptors are present in amphibians, the authors aimed to evaluate 2 opioid-based drugs. Buprenorphine and butorphanol.

Prior to any testing of analgesics, they tested the repeatability and reproducibility of the 2 quantitative pain assessments in axolotls: AAT and von Frey fiber system.

Male, adult wild-type axolotls were used in this study. See the whole paper for experimental design details.

Assessing 50 mg/kg buprenorphine and 0.5 mg/L butorphanol some behavioral test at each time point showed significant differences when behavior was compared between treated and control animals.

However, no consistent trend in overall behavioral responses according to analgesia usage over time was apparent for either study drug. During experiment some adverse effects occurred. After a 1 week wash out 0.75 mg/L butorphanol was compared with controls, and there were significant differences at 1, 6, and 25 h after. However, no consistent trend in overall behavioral responses was clear.

In conclusion, AAT is a well-established tool to quantitatively assess pain in laboratory animals, and it can be effectively adapted for use in axolotls. Future studies should explore additional doses of butorphanol and the efficacy of other opioids and non-NSAID-based analgesics as additional options.

QUESTIONS

1. Which one is false?

a. A- and C-type nociceptive fibers are present in most vertebrate animals

b. Amphibians does perceive pain

c. Scientific name of axolotls is *Ambystoma mexicanum*

d. Axolotls can recover from any fatal injury

2. Axolotls are very versatile as an animal model. Which one is false regarding research models for axolotls?

a. Immunological studies

b. Developmental studies

c. Regeneration studies

d. Evolutionary studies

3. What can determine the election of one or another opioid in the treatment of pain?

a. Clinical outcome

b. Degree of expected pain

c. Effect of the drug on the scientific outcome

d. All the above

ANSWERS

1. d is false. They can recover from any nonfatal injury

2. a is false

3. d

***Experimental Use***

**Eckley et al. 2020. Acepromazine and chlorpromazine as pharmaceutical-grade alternatives to chlorprothixene for pupillary light reflex imaging in mice, pp. 197-203**

Domain 1: Maintenance of Spontaneous and Experimentally Induced Diseases and Conditions; K6 - Pharmacology

Primary Species: Mouse (*Mus musculus*)

SUMMARY: This article looked using acepromazine and chlorpromazine in replacement of chlorprothixene. This antipsychotic drug the thioxanthene class is no longer produced in the USA and is only available in Europe. Chlorprothixene antagonizes dopamine, serotonin, histamine, muscarinic acetylcholine and a-adrenergic receptors.  In the USA, chlorprothixene is not available in pharmaceutical grade an appropriate replacement may be adequate for use in this study.  Acepromazine is also an antipsychotic drug, used mainly as a sedative and antiemetic in animals..  Likewise, chlorpromazine is used in a similar fashion in human medicine. The study compared the three premeditations and their abilities to lower the use of isoflurane and at the same time preserve the ability to measure pupillary light reflex (PLR) and found that acepromazine and chlorpromazine can be used as alternatives to chlorprothixene, but further studies are needed to refine the dosage.

QUESTIONS

1. The use of a combination of ketamine and xylazine may

a. Cause acute reversible lens opacities

b. Causes dose dependent muscle relaxation

c. Be metabolized by the liver and excreted by the lungs

d. Be nephrotoxic to rabbits

2. According to The Guide for the Care and Use of Laboratory Animals

a. Pharmaceutical grade chemicals MUST be used at all times

b. Non-pharm grade chemicals MUST be approved and justified in a protocol and approved by IACUC.

c. Grade, purity, sterility, pH, pyrogenicity and osmolality only need to be considered if cost is a factor

d.  Expired pharmaceuticals can be used for survival surgeries

3. Acepromazine and chlorpromazine are best described as

a. Benzodiazepines

b. Provide excellent analgesia

c. Cause biphasic hypertension then hypotension

d. Cause hyperglycemia and decrease GI secretions

4. Which statement pertaining isoflurane is correct?

a. Increase in cerebral blood flow can be prevented if hyperventilation is instituted prior to the administration of isoflurane

b. It does not have a significant effect on respiratory or cardiovascular depression

c. It is exclusively metabolized by the liver and excreted in urine

d. It very rarely causes breath holding in species such as the rabbit

ANSWERS

1. a

2. b

3. d

4. a (pg. 91 in AABB)

**Smith et al. 2020. Female urine-induced ultrasonic vocalizations in male C57BL/6J mice as a proxy indicator for postoperative pain, pp. 204-211**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Mice can produce ultrasonic vocalizations which are greater than 20KHz. Mice more commonly produce USV during nonaggressive same-sex and heterosexual interactions and not spontaneously. Three types of USV are easily reproducible: female urine-induced male mice USV (FiUSV), pup isolation-induced USV, and intruder-induced USV. FiUSV are produced by adult males specifically in the presence of adult female urine and are associated with

courtship and mating.

FiUSV produced by male C57BL/6J mice were assessed for 5 d before and after vasectomy or sham surgery with or without sustained-release buprenorphine. Postoperative pain was assessed by monitoring vocalization using an ultrasonic microphone and by evaluating orbital tightness, posture, and piloerection at postoperative time points. Male mice that produced 0 FiUSV after the addition of the urine stimulus for more than one of the 5 baseline time points were removed from study; 66% of mice on this study produced sufficient FiUSV for inclusion.

Significantly fewer FiUSV were produced by surgery-no treatment mice at the 4-h time point compared with baseline. Surgery-treatment mice had significantly decreased activity at 4 h after surgery compared with baseline, while surgery-no treatment mice had a trend toward having decreased activity at the 4-h time point after surgery compared with baseline. The results obtained from this study showed that FiUSV can indicate postoperative pain at least 4 h after surgery.

Previous studies assessing FiUSV as a proxy indicator of animal wellbeing showed that male mice presenting sickness behaviors after injection of LPS produced zero FiUSV. The production and quantity of FiUSV are dependent on many factors such as mouse strain, social status, and previous sexual experience. C57BL/6J mice have a high prevalence of producing FiUSV.

FiUSV could be advantageous as a proxy indicator of postoperative pain because they are objectively measured, noninvasive, quantifiable, and possibly more sensitive than visual examination.

QUESTIONS

1.  Which of the following is not a common form of murine ultrasonic vocalization?

a.  Pup isolation-induced USV

b.  Intruder-induced USV

c.   Female urine-induced male mice USV

d.  Male urine-induced female USV

2.  An animal producing zero FiUSV could be indicative of which of the following:

a.  Illness

b.  Adequate analgesic efficacy

c.   Normal strain variation

d.  Low social status

e.  All of the above

ANSWERS

1.  d

2.   e (this is not a pretty question – but that’s how things go sometimes)

**Whiteside et al. 2020. Elevated arsenic and lead concentrations in natural health clay applied topically as a treatment for ulcerative dermatitis in mice, pp. 212-220**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Various topical applications have been used to treat murine ulcerative dermatitis. Some institutions have used topical healing clays such as green and bentonite clays as a natural treatment for ulcerative dermatitis. These clays' healing effects are attributed to their mineral/chemical compositions, ion exchange, antibacterial, and adsorptive properties. The authors discovered that several commercially available healing clays have elevated concentrations of arsenic and lead, and they designed this study to determine if treatment with these clays contaminated with arsenic and lead would have a biologic effect in mice and cause unwanted research variability. Three brands of healing clays reported to be used in the laboratory animal field were analyzed via graphite furnace atomic absorption spectrometry (GFAAS) for total arsenic and lead levels and compared with mouse feed, bedding, water, enrichment, saline, and rodent tissues.

Adult male CD1 mice (7-8 weeks) were housed singly and dosed daily for 2 weeks with either saline or Brand 1 (Natural French Green Clay Facial Treatment Mask, Rainbow Research) green clay paste on shaved, dermatitis free skin. 2 cohorts of 20 mice (10 mice on green clay treatment, 10 mice on saline control) were evaluated approximately 12 months apart. Green clay was mixed with saline in a 1:1 v/v ratio to create the topical paste, and 0.1 to 0.4 g was applied daily with a sterile cotton swab. Mice were observed to have ingested the entire topical treatment within 30 minutes of application. After 2 weeks of treatment, mice were euthanized via CO2, and samples of blood, liver, kidney, and the skin under the application site were collected. In vitro bioaccessibility assay (IVBA) was performed to estimate the relative bioavailability of arsenic and lead in the clay and animal feed that is absorbed across the gastrointestinal tract. The activity of delta-aminolevulinic acid dehydratase (ALAD), which is involved in heme biosynthesis, was measured in liver and kidney tissues to assess lead toxicity. Lead reversibly replaces zinc at the active site of ALAD, resulting in enzymatic inhibition, which can be restored in vitro with the addition of dithiothreitol (DTT), allowing for assessment of inhibition by comparing activity with and without DTT. Statistical analyses were performed in Prism 7.03 for Windows. Data meeting normal distribution with equal standard deviation were analyzed with unpaired parametric t tests, and data with normal distribution with unequal standard deviation were analyzed with a t test with Welch correction. Mann-Whitney test was utilized for unpaired nonparametric data. ALAD activity was analyzed via one-way ANOVA with a Tukey post-hoc analysis.

The US Pharmacopoeia limits for bentonite clay products are 5 ppm (5000 ppb) for arsenic and 40 ppm (40,000 ppb) for lead. GFAAS revealed elevated concentrations of arsenic and lead in all 3 tested brands of clay products. Arsenic levels ranged from 8,483 to 31,607 ppb and lead levels ranged from 21,457 to 54,754 pp. The clay brand labeled "ultra-pure pharmaceutical grade" had the highest concentration of total lead. GFAAS revealed significantly lower levels of arsenic (272-1,332 ppb) and lead (693-774 ppb) in rodent feed and less than 5 ppb arsenic and lead in the rodent water, bedding, enrichment, and saline. None of these bentonite clays in this study met the standards for a pharmaceutical grade product.  IVBA revealed the bioaccessibility of arsenic in the clay to be 22% and the bioaccessibility of lead to be 43%.Oral ingestion was the primary route of exposure because mice groomed the topical application site immediately after application. GFAAS of kidney and liver demonstrated significantly higher concentrations of lead in the clay-treated mice compared with the saline-treated mice. ALAD enzyme activity was not significantly different between saline- and clay-treated mice.

QUESTIONS

1. When exposed to toxic levels of lead, in which tissues does lead accumulate?

a. Liver

b. Kidney

c. Brain

d. Bone

e. All of the above

2. One acute effect of lead toxicity is the inhibition of delta-aminolevulinic acid dehydratase (ALAD), which is involved in \_\_\_ biosynthesis and can result clinically in \_\_\_.

3. True or False: Arsenic is classified as a human carcinogen.

ANSWERS

1. e

2. heme, anemia

3. True

**Farrar et al. 2020. Comparison of rectal and infrared thermometry temperatures in anesthetized swine (*Sus scrofa*), pp. 221-226**

Domain 2: Management of Pain & Distress

Primary Species: Pig (*Sus scrofa*)

One Line Summary: Compared to rectal thermometers, infrared thermometry (IRTM) is an inadequate alternative to accurately measure internal temperature in swine.

SUMMARY

This paper looked into determining whether infrared thermometry (IRTM) could act as a noninvasive alternative to monitor internal temperature in healthy, unstressed swine. Accurately measuring an animal’s body temperature is an important parameter in assessing an animal’s health status in veterinary medicine. Currently, rectal temperature measurement is the most common method used to measure internal temperature, but is considered invasive due to stress caused from handling. As a result, stress from handling can promote alterations in both core and surface body temperature.

To determine the accuracy of IRTM compared to rectal temperature, twenty six healthy, juvenile female Yorkshire-cross swine were sedated with Tiletamine-zolazepam (0.5mg/kg). While sedated, both rectal and IRTM temperatures were measured and recorded, concurrently. Rectal temperatures were measured using a SureTemp Plus 690 Thermometer, approximately 10cm within the rectum of anesthetized pigs. Anatomic locations to take thermal measurements for IRTM were based on CT scan results, which demonstrating two prominent vasculature regions. The region location classified as the ‘Eye’ involved tissue located next to the lateral canthus and the region classified as ‘Neck’ referred to the region at the base of the ear along the neck. IRTM measurements were measured using a FLIR E5 thermal imaging camera, approximately 24-32 inches away from previously described anatomic sites.

Based on their findings, IRTM taken at either the eye or neck regions, would underestimate temperatures at lower values, and overestimate temperatures at higher values, when compared to corresponding rectal temperatures. Unfortunately, IRTM had two major limitations:

* + 1. Depth of penetration- restricted to measurement of the temperature of the body surface, with limited penetration into the skin to 2 – 3mm from the surface. Any alteration in the targeted skin region used to acquire temperature (e.g. Scarring, scabs, etc.) could potentially affect ability to measure internal temperature
    2. Required controlled environment, especially with positioning of the camera to measure the temperature. This would make taking thermal measurement difficult to acquire in non-sedated, active pigs.

Thus, IRTM should not be considered an accurate replacement for rectal temperature measurement. It can act as a potential quick, noninvasive temperature screening tool

QUESTIONS

* 1. Which anatomical sites were found on Computed Tomography to demonstrate prominent vasculature in swine? Select all that apply:
     1. Snout
     2. Medial canthus of the eye
     3. On the neck at the base of the ear
     4. Thoracic inlet
     5. Pinnae
     6. Lateral canthus of the eye
  2. T/F: The depth of penetration of the FLIR E5 thermal imaging camera is around 4-5mm from the skin surface. This reduces the impact skin alteration, such as scars, have on its ability to measure internal temperature and makes it a useful screening tool to monitor temperature of swine in research setting.
  3. Classify the type of anesthetic agent Tiletamine-Zolazepam are. Pick all that apply:
     1. Anticholinergic
     2. Phenothiazine tranquilizer
     3. Benzodiazepines
     4. Alpha 2 agonist
     5. Opioid
     6. Dissociative Agent

ANSWERS

1. On the neck at the base of the ear, Lateral canthus of the eye

2. False: IRTM has limited skin penetration, at about 2-3mm from the skin surface. As a result, any alteration to the skin surface can impede IRTM from accurately reading the internal temperature

3. Tiletamine = Dissociative agent, Zolazepam= Benzodiazepine