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**POSITION STATEMENT**

**Association of Primate Veterinarians’ Humane Endpoint Guidelines for Nonhuman Primates in Biomedical Research, pp. 6-8**

Domain 2: Management of Pain and Distress

SUMMARY: This article from the APV, lists 6 guidelines in relation to humane endpoints when working with NHP’s in research.

1. Strongly recommend the development of humane endpoint, monitoring parameters, and Quality of Life committees
2. Moribund conditions and death should be avoided as study endpoint unless scientifically justified and approved by the IACUC.
3. Veterinarians have the authority to euthanize any animal that has become moribund, has reached a humane endpoint, or when professional judgement deems it necessary. They should make every practical attempt to contact research teams before taking action.
4. Clearly defined humane endpoint criteria must be created and approved by the IACUC for all studies involving NHP’s.
5. Research and animal care personnel should be trained to recognize changes in behavior and clinical conditions to recognize pain and distress.
6. Scoring sheets are valuable tools to evaluate humane endpoints. They should include a clear description of when a humane endpoint is reached and emergency contact information. They should be available for review.

QUESTIONS

1. True or False: Quality of Life (QOL) committees should primarily be made up of primate experts from outside of the organization to offer a non-biased evaluation.
2. True or False: For new studies, humane endpoints may be developed prior to beginning research or, often, are established during the research as clinical changes are noted.
3. What does the Animal Welfare Act state in regard to pain and distress?

ANSWERS

1. False: QOL committees are composed of members who know the animals well (i.e.: vets, behavioral and animal care staff, researchers as applicable, etc.)
2. False: Humane endpoints must be established and approved by the IACUC prior to initiation of a study.
3. AWA requires animal pain and distress to be minimized.

**OVERVIEW**

**Gaskill and Garner. Power to the People: Power, Negative Results, and Sample Size, pp. 9-16**

Domain 3: Research

SUMMARY: This paper reviews and makes recommendations on statistical concepts related to power and sample size. The authors discuss how negative results may falsely occur due to issues with experimental design and analysis, namely underpowered experiments with insufficient sample sizes. IACUCs generally require researchers to justify their sample sizes before the start of an experiment based on study power. Power is the proportion of experiments that will produce a significant result if the alternative hypothesis (which predicts a significant difference between experimental groups) holds true. These prospective or *a priori*power calculations must occur before the experiments begin; post-hoc power calculations conducted after experimental data is obtained can lead to a “post-hoc power fallacy” that incorrectly interprets whether non-significant results are true-negatives or false-negatives. For all negative data, confidence intervals, observed effect sizes, and test statistics should be reported to allow for further meta-analysis.

Power equations focus on the relationship among the significance criterion (acceptable Type I error rate, typically ɑ <0.05), sample size, population effect size (measure of how much the outcome variable is affected by the predictor variable), and power. Effect size should be biologically relevant and should be reported in published literature. If there are multiple predictors in an analysis (e.g. mouse weight as predicted by age and genotype), the proportion of variation within dataset explained by each predictor (known as partial eta squared) should be reported. Pilot studies should not be used to estimate effect sizes, as they generally use small sample sizes and therefore require very large effects to be significant. This leads to effect size being overestimated.

Power is usually arbitrarily fixed at 80%, and classical power calculations require large sample sizes. The authors describe changing experimental design to maximize power without significantly increasing sample size, including the use of factorial studies utilizing multiple small treatment groups. These studies reduce nuisance variables, which cause variability in data and mask the true treatment effect, and also utilize heterogeneous study populations that more closely mimic human populations. The authors recommend moving away from traditional calculations of sample size and using Mead resource equation. This is based on curves of diminishing returns as sample size increases, and acceptable amounts of error. By altering study design and calculations of effect size and power, smaller sample sizes can be used while still retaining statistical power.

QUESTIONS

1. Accepting the alternative hypothesis when the null hypothesis is true is a Type \_\_\_ error.
	1. I
	2. II
	3. ɑ
	4. β
2. Rejecting the alternative hypothesis when it is true is a Type \_\_\_\_ error.

a. I

b. II

c. ɑ

d. β

1. T/F: Alpha is the proportion of experiments that will incorrectly, by chance, yield a significant result.

ANSWERS

1. a

2. b

3. True

**ORIGINAL RESEARCH**

***Biology***

**Restrepo-Gallego and Diaz. Influence of Dietary Vitamin A and Iron Deficiency on Hematologic Parameters and Body Weight of Young Male Wistar Rats, pp 17-23**

Domain 3: Research; T3: Design; K3: Animal models

SUMMARY: Anemia is linked to an inadequate supply of oxygen to the tissues and often is a result of iron deficiency, specifically when ferritin levels are below 12ug/L and Hgb is below established cut-off points. Vitamin A deficiency has been implicated in the metabolism of iron, and for this reason has been included in supplementation strategies to reduce prevalence of anemia. To characterize effects on iron and vitamin A deficiencies on hematologic parameters, including Hgb, HCT, RBC, MCV, MCHC and reticulocytes as well as weight gain, this study randomized 9-week-old Wistar rats into 5 groups fed diets of varying quantities of iron (in the form of Ferric citrate) and vitamin A (in the form of retinol acetate), for 6 weeks to create insufficient iron and/or vitamin A diets. The study found that even with adequate iron in the diet, insufficient vitamin A can cause serum iron to decrease proportionally, but iron content does not influence serum retinol levels. Additionally, the following hematologic findings were reported: No direct relationship between Hgb and vit A unless iron is deficient. Iron deficiency and not Vit A decreases MCV. Both iron and Vit A are necessary for adequate erythropoiesis, as indicated by a decrease in reticulocyte counts in deficient diet fed rats, and both iron and vit A deficiencies required for hypochromic anemia formation. Body weight gain was less in all experimental diet groups compared to the control group, implying that a lack of either vit A or iron can influence animals’ growth. The authors conclude that vitamin A must be considered in the assessment of iron-related nutritional status and in the identification of some types of anemia.

QUESTIONS

1. Iron in plasma is bound to which protein?
	1. Ceruloplasmin
	2. Transferrin
	3. Ferritin
	4. Ferroportin
2. Vitamin A is responsible for the production of rhodopsin, which is required for:
	1. Low-level light vision
	2. Retinal development
	3. Color vision
	4. High intensity light vision
3. An elevated MCHC indicates:
	1. Hyperchromic anemia
	2. Macrocytic anemia
	3. Artifact
	4. Spherocytosis

ANSWERS

1. b. Transferrin
2. a. low level light vision
3. c. Artifact as RBC cannot contain more Hgb than normal. BUT truly increased MCHC usually occurs in hereditary d. spherocytosis

**Brownlee et al. Blood Pressure Reference Intervals for Ketamine-sedated Rhesus Macaques (*Macaca mulatta*), pp. 24-29**

Domain 1: Management of Spontaneous and Experimentally Induced Diseases and Conditions
Primary Species: Macaques (*Macaca spp.*)

SUMMARY: Blood pressure parameters and reference intervals are important physiologic data used by researchers and clinicians alike. Establishing reference intervals requires careful consideration of any potential confounding variables. Invasive devices, such as telemetry, have been used by researchers to obtain blood pressure information on awake macaques, but this is technically more difficult and requires surgical placement. Non-invasive techniques, like oscillometry, are the mainstay for clinicians, due to the ease of obtaining blood pressures quickly.

While non-invasive techniques are the most commonly used, rarely is it performed on awake, unanesthetized rhesus macaques. While reference intervals have been established for other primate species, including chimpanzees and cynomolgus macaques, no such reference has been determined for sedated (ketamine), apparently healthy rhesus macaques.

Monkeys were selected if they were deemed healthy on physical exam, and were monitored for respiratory, renal, cardiac diseases. Using standard oscillometry and high definition oscillometry, the systolic arterial pressure (SAP), diastolic arterial pressure (DAP), mean arterial pressure (MAP) and pulse rate were obtained, at three locations on each monkey. Locations included the bicep, leg and tail.

Findings demonstrated a positive correlation between blood pressure and age, as well as BP and body condition score. Other findings included an inverse relationship between males and females in association with pulse rate and body weight. Blood pressure tended to be greater in the leg and tail compared to the arm.

It is important when obtaining or monitoring blood pressure, that the method is standardized and that the cuff is appropriate for that animal. This will provide the researcher or clinician with the most accurate data.

QUESTIONS

1. All of the following are advantages of using indirect blood pressure measurements except:

a.  Ease of use

b.  Accuracy

c. Speed

d. Affordability

2. T/F: Ketamine use in NHP has been shown to potentially lower BP and heart rate.

3. T/F: Age and BCS are positively correlated with BP in rhesus macaques.

ANSWERS

1. b. Accuracy

2. False; studies have shown ketamine potentially increases HR and BP

3. True

***Husbandry***

**Gjendal et al. Effect of Repeated Exposure to Isoflurane on Nest Building and Burrowing in Mice, pp. 30-36**

Domain 2: Management of Pain and Distress

Primary Species: Mouse (*Mus musculus*)

SUMMARY:Nest building activity (NBA) and burrowing behaviors are spontaneous, highly motivated and species-specific behavior that can be observed noninvasively in mice to aid in the assessment of welfare in mice when combined with other welfare parameters such as body weight and fecal corticosterone metabolites. NBA has been shown to be affected by both pain and stress in mice. This group hypothesized that burrowing and NBA would decrease in female mice after exposure to 3 specific stressors: grid-floor housing, anesthesia with isoflurane, and IP saline injections. They also hypothesized that these decreases would correlate to decreases in fecal corticosterone metabolites, body weight, fur status, and sucrose preference. As a final measure, they aimed to determine the agreement between observers in scoring NBA via a refined scoring system. They found that the refined scoring method for nest complexity had good inter-observer agreement. The system revealed a decrease in NBA and sucrose preference after a single exposure to isoflurane anesthesia. Burrowing activity was NOT reduced after exposure to grid-floor housing, repeated isoflurane anesthesia, or IP injections. IP injections did result in a decrease in sucrose preference. Their conclusions were that the NBA test proved objective and sensitive to the effect of a single exposure to isoflurane anesthesia, but the burrowing test was unable to detect the effect of repeated exposure to isoflurane anesthesia, which questions the applicability of the test in mice welfare assessments.

QUESTIONS

1. What is a known risk of using Nestlets as nesting material in nude or hairless rodent strains?
2. What type of bedding is generally discouraged in rodent housing and why?

ANSWERS

1. Fibers from the Nestlets can impact in the conjunctival sac, which can lead to conjunctivitis.

2. Softwood (pine or cedar) due to the volatile amines in the bedding which alter hepatic microsomal enzyme concentrations

**Gallo et al. Tell-tale TINT: Does the Time to Incorporate into Nest Test Evaluate Postsurgical Pain or Welfare in Mice?, pp. 37-45**

Domain 2

Primary Species: Mouse (*Mus musculus*)

SUMMARY: Nest building has been established as an important normal behavior in male and female mice across a variety of genetic backgrounds. The Time to Incorporate to Nest Test (TINT) was developed as a tool to quickly assess pain by determining the presence of absence of gathering behavior for nest building. A small amount of additional nesting material is introduced to a mouse cage and a positive result occurs when the mouse retrieves the new nesting material and incorporates it into an existing nest within 10 min. Mice were randomly assigned to one of the following experimental combinations:  Positive nesting treatment groups were provided with 10 grams of crinkle paper nesting material and negative nesting treatment groups were not given any nesting material. The 2 analgesia conditions included an analgesia control where mice were given a saline injection and anesthetized on the day of surgery and did not receive analgesia, and an analgesic group where mice received anesthesia as well as buprenorphine on the day of surgery. The surgery was carotid artery catheterization. All TINT observations were made between the hours of 0700 and 1000. The nest from each cage was scored using a naturalistic 0 to 5 point nesting scale. Mice were also assessed using the Mouse Grimace Scale (MGS). All cages were monitored daily for the animal body and food weights after experimental manipulations.  Mice receiving surgery were significantly more likely to fail the TINT than shams. Mice who did not receive additional nesting material were more likely to fail the TINT. The main effect of analgesia treatment did not affect the TINT. Mice that did not receive buprenorphine or undergo surgery were faster to interact with the TINT material if they were given additional nesting material.  Almost all mice, regardless of surgical or analgesia treatments, failed the TINT after receiving buprenorphine the day after surgery. Mice who received additional nesting material also earned a significantly higher NQS.  Mice who experienced anesthesia alone had significantly higher nest quality scores than mice who received surgery. Mice that had the surgery had significantly higher MGS scores directly after surgery. Mice who received surgery ate less and lost more weight than those who were only anesthetized. Results suggest that the TINT can identify mice that have undergone surgery and other sources of distress but that disruption of the TINT is not necessarily specific to pain resulting from a surgery. Possible uses can now extend to any research scenario that asks the question 'Is a mouse's normal behavior disrupted?'

QUESTIONS – True or False

1. Mice who experienced anesthesia alone had a significantly higher nest quality score than mice who received surgery

2. Disruption of the TINT was found to be specific to pain result from a surgery

3. Almost all mice, regardless of surgical or analgesia treatments, failed the TINT after receiving buprenorphine the day after surgery

ANSWERS

1. True

2. False: The TINT can identify mice that have undergone surgery but disruption is not specific to pain resulting from a surgery

3. True

**Johnston et al. Utility of Automated Feeding Data to Detect Social Instability in a Captive Breeding Colony of Rhesus Macaques (*Macaca mulatta*): A Case Study of Intrafamily Aggression, pp. 46-57**

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

Domain 4: Animal Care

Primary Species: Macaques (*Macaca spp.*)

SUMMARY

Objective:Describe changes in feeding patterns and trauma scores associated with an incident of intrafamily aggression within a large breeding groups of rhesus macaques (*Macaca mulatta*).

Background: A primary management goal of breeding groups is to detect emerging social unrest before the onset of significant fighting and wounding. Several factors contribute to the social stability, including clear dominance hierarchy, cohesive kinship structure within families, and presence of adult males/high-ranking animals that effectively ‘police’ social conflicts. Social aggression can also lead to increased psychosocial stress. Glucocorticoids are elevated in response to acute psychosocial stress, and the same central neuroendocrine pathways that control these hormonal changes also induce appetite suppression. Transient inhibition of food intake is a normal adaptive response to acute stress mediated by the hypothalamic neuropeptide corticotropin-releasing factor through the HPA axis. Glucocorticoids induced by acute stress also affect leptin and insulin release, which can modulate feeding behavior through corticotropin-releasing factor and other hypothalamic appetite regulatory neuropeptides.

An incident of intrafamily aggression occurred in a breeding group at the Yerkes National Primate Research Center. This site was equipped with an automated feeding system that tracks caloric intake of individual animals via radiofrequency identification microchips implanted subcutaneously in each hand of individual animals, so feeding data was analyzed to determine whether significant reduction in caloric consumption occurred prior to the onset of aggression.

Rhabdomyolysis is a serious consequence of crushing wounds because elevated blood levels of creatine kinase, myoglobin, electrolytes, purines, and other enzymes caused by trauma-inflicted muscle damage can lead to acute kidney injury, marked metabolic acidosis (hallmark of rhabdomyolysis), and decreased survival. Primary lab parameters evaluated in these cases included venous blood pH, lactate, base excess, bicarbonate, and serum creatine kinase levels.

Methods

* + - Caloric intake data was quantified using an automated feeding system at 24, 48, 72, or 96 h prior to the onset of fighting and compared with baseline values (prior 30-d daily average).
		- All trauma cases were scored using a 5-point severity scale.

Results

* Incident of social unrest resulted in crush trauma, moderate male and female-inflicted trauma, and mild male-inflicted trauma
* Greater percent decreases in caloric intake from baseline to –24 h predicted higher severity scores of wounds
* Targeted subfamily showed a marked  decrease (58%) in average caloric intake in the 24 h prior to the fighting incident

Discussion/Summary:Automated feeding data can be used to enhance the social management of captive macaques by providing supplemental information to behavioral and trauma data. Stress-induced inappetence and/or increased night-feeding may help management detect emerging social instability prior to the onset of significant aggression and wounding. Early detection of social instability may lead to improvements in animal welfare via reduced wounding and pain/distress.

QUESTIONS

1. What type of injury/wound can lead to rhabdomyolysis?
2. What organ system is effected by rhabdomyolysis/trauma-inflicted muscle damage, leading to acute injury in the organs?
3. What is the hallmark blood work finding for rhabdomyolysis?

No answers were provided.

***Animal Health Surveillance***

**Gallo et al. Comparing Mouse Health Monitoring Between Soiled-bedding Sentinel and Exhaust Air Dust Surveillance Programs, pp. 58-66**

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

Tasks 1-4: Prevent/control spontaneous or unintended disease or condition, diagnose/treat as appropriate

Primary Species: Rat (*Rattus norvegicus*)

SUMMARY:   Per the World Health Organization, micronutrient deficiencies are a major public health problem in developing countries, especially when deficiencies include vitamin A and iron, as both are associated with anemia.  Researchers suspect that vitamin A plays a role in the metabolism of iron, but the mechanism is not well known and has not been demonstrated to date.  Nonetheless, many countries have developed supplementation strategies for both iron and vitamin A to reduce the prevalence of anemia.  The authors conducted this project to evaluate if both vitamin A and iron must be taken together to ensure adequate weight gain and hematologic parameters, and they tested that hypothesis in growing young male Wistar rats. Using 21-day old subjects, the study compared the development and bloodwork between 5 dietary test groups (n = 7 per group) after a treatment course of 6 weeks.  With iron in the form of ferric citrate and vitamin A in the form of retinol acetate, the 5 test groups were fed modified diets as follows:

* Adequate in iron and vitamin A (control)
* Adequate in iron but low in vitamin A (fesvai)
* Adequate in iron but lacking vitamin A (fesvad)
* Low in iron but adequate in vitamin A (feivas)
* Low in both iron and vitamin A (feivai)

After 6 weeks, all rats in the four ‘deficient’ diet groups showed significant differences in serum iron relative to the control group, with the FesvAd (adequate in iron but lacking vitamin A) exhibiting the lowest serum iron values. The groups with insufficient (FesvAi) or no dietary vitamin A all (FesvAd) had significantly lower levels of serum retinol than the control group, but the two groups fed diets with low vitamin A (FesvAi and FeivAi) showed no significant intergroup difference.  The groups fed low dietary iron (FeivAs and FeivAi) had decreased levels of Hgb, MCV, and Hct compared to the control group, and all 4 deficient groups showed significant differences in reticulocyte count compared to control. Only the FeivAi (low in both iron and vitamin A) group showed a significantly different MHC versus the control group, and there were no significant intergroup differences in RBC count or MCHC.  At the end of the study, all four deficient diet groups showed significantly less weight gain versus the control group (~30 gm +/- 10 gm), but there were no significant differences between the four deficient diet groups.  Based on their results, the authors concluded that, in young male Wistar rats, both iron and vitamin A are essential to cause increases in body weight and various hematologic parameters.

QUESTIONS

1. Among the five test groups of young male Wistar rats in this study, which diet group exhibited the lowest serum iron values after 6 weeks?

a.  Adequate in iron and vitamin A (control)

b.  Adequate in iron but low in vitamin A (FesvAi)

c.  Adequate in iron but lacking vitamin A (FesvAd)

d.  Low in iron but adequate in vitamin A (FeivAs)

e.  Low in both iron and vitamin A (FeivAi)

2.  Among the four deficient diet test groups of young male Wistar rats in this study, which deficient diet group showed a significantly different MHC versus the control group after 6 weeks?

a.  Adequate in iron but low in vitamin A (FesvAi)

b.  Adequate in iron but lacking vitamin A (FesvAd)

c.  Low in iron but adequate in vitamin A (FeivAs)

d.  Low in both iron and vitamin A (FeivAi)

3. T/F: Among the five test groups of young male Wistar rats in this study, there were no significant intergroup differences in RBC count or MCHC after 6 weeks?

4.  T/F: At the end of the study, all four deficient diet groups showed significantly less weight gain versus the control group (~30 gm +/- 10 gm), but there were no significant differences between the four deficient diet groups?

ANSWERS

1.  c.  Adequate in iron but lacking vitamin A (FesvAd)

2.  d.  Low in both iron and vitamin A (FeivAi)

3.  True

4.  True

***Experimental Use***

**Huss et al. Evaluation of 3 Alcohol-based Agents for Presurgical Skin Preparation in Mice, pp. 67-73**

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

T2: Control spontaneous or unintended disease or condition

Primary Species: Mouse (*Mus musculus*)

SUMMARY:The Guide states that rodents are to be aseptically prepped for surgical procedures; traditional human surgical preps should be validated on rodents because their skin differs in several key ways: 1) Mice have a panniculus carnosus and more hair follicles, 2) the mouse epidermis comprises 2-3 layers of keratinocytes, compared to humans, who have 5-10; this results in decreased barrier capacity and greater absorption, 3) Human – but not mouse – neutrophil granulocytes produce antimicrobial peptides, and 4) Practically speaking, most mice live in environments that expose surgical wounds to fecal contamination. The authors aimed to evaluate products to determine the most effective, efficient, and economical aseptic prep.

The study compared 3 commercial products: 1) Iodine povacrylex + isopropyl alcohol, aka Duraprep solution 2) 2% w/v Chlorhexidine gluconate + isopropyl alcohol, aka Chloraprep solution, and 3) 4% w/v Chlorhexidine, aka Hibiclens solution (#3 not previously evaluated in veterinary patients) with the standard preparation of 3 alternating povidine-iodine and 70% isopropyl alcohol wipes. Antimicrobial efficacy was assessed with RODAC cultures immediately after prep and again after 20 minutes. Post-op histologic samples were examined for granulation tissue, fibrosis, epidermal hyperplasia, mononuclear infiltration, ulceration, and neutrophilic inflammation. Animals’ body temperatures were also noted to address the concern of rodent hypothermia resulting from surgical prep agents.

Results

* There were no significant differences in effects of preparation solution on core body temperature.
* There was no histologic evidence of contact dermatitis, skin irritation, or other postoperative complications that may affect wound healing.
* There were no significant differences in bacterial counts either immediately after prep or after 20 minutes.

It should be noted that product 1 was most expensive at $4.60 per preparation, followed by product 2 at $1.60, and product 3 at $0.10 per mouse. Traditional preparation is estimated at $0.42/mouse. Product 3 is applied with a syringe to the center of the surgical site, and then painted outwards with a cotton-tip applicator. This process is then repeated, and the site is dried with gauze. The authors state that, considering both ease of use and cost, compliance may be highest with product 3 (Hibiclens solution).

QUESTIONS

1.  T/F: The *Guide* states that alcohol is neither a sterilant nor a high-level disinfectant, but may be acceptable for some procedures if prolonged contact times are used.

2.   Match the agent combination with its MOA:

A. Iodine povacrylex + isopropyl alcohol                                        1. Disrupts bacterial membranes

B. 2% w/v Chlorhexidine gluconate + isopropyl alcohol               2. Denatures protein and disrupts bacterial membranes 3. Denatures proteins and causes

C. 4% w/v Chlorhexidine damage to bacterial DNA

3. Choose one of each underlined pair: Surgical scrubs/solutions contain an ionic detergent and are designed for preoperative preparation of intact skin before surgical incisions. Antiseptic agents without the detergent component are terms scrubs/solutions.

4. What is the panniculus carnosus?

ANSWERS

1. TRUE
2. A – 3, B – 2, C – 1
3. Scrubs…solutions
4. The panniculus carnosus muscle is deep to the dermal fat layer and on top of the subcutaneous adipose tissue and fascia. It is a fast-twitch muscle vestigial in humans, but otherwise present in mammals. It is characterized as containing higher than usual regenerative myofibers; mainly composed of type II fibers, the muscle is thought to provide rodent loose skin with twitching and thermoregulation capacities, as well as promoting contraction and supporting revascularization of full-thickness excisional wounds.

**Alstrup et al. Effects of Long-term Anesthesia, Blood Sampling, Transportation, and Infection Status on Hearts and Brains in Pigs Inoculated with *Staphylococcus aureus* and Used for Imaging Studies, pp. 74-84**

Domain 3: Research

Primary Species: Pig (*Sus scrofa*)

SUMMARY:Using a pig model to test novel radioactive tracers for imaging studies to diagnose osteomyelitis, this study was undertaken to determine if long-term anesthesia, frequent blood sampling and transportation of up to 1 hour would result in more damage to the heart and brain of pigs compared to shorter duration of anesthesia, less frequent blood sampling and no transportation. Pigs were inoculated with *Staphylococcus aureus*one week prior to imaging. Animals in group 1 were anesthetized up to 18 hours, imaged in 2 facilities that were ~1.5 hours away from each other, and had ~20 ml/kg blood sampled. Animals in group 2 were anesthetized up to 14 hours, imaged at either one of the two institutions, and had ~14 ml/kg blood sampled. After imaging, the pigs were euthanized and tissues collected for histologic evaluation. There was a temporary increase in heart rate during transportation of the pigs in group 1, as well as a drop in paO2 that did not rebound and an increase in lactate that came down slowly over the last few hours of anesthesia. Blood glucose and Hct slowly decreased over time in group 1. Atelectasis of the lungs was present in 16 of the 18 pigs (no difference between groups). Heart histology findings were minimal to marked myocardial necroses in 17 of 18 pigs. Histology scores did not differ between groups and no signs of hypoxia or neuronal degeneration were seen in brain sections from any of the pigs. The authors recommend streamlining demanding anesthetic protocols to mitigate adverse physiological effects and potential organ pathology.

QUESTIONS

1. After an extended period of anesthesia, most pigs in this study developed what lesion(s)?

a.  Decubitus ulcers and pulmonary emboli

b.  Atelectasis and myocardial necrosis

c. Renal infarction

d.  Neuronal degeneration

2.   Which imaging modality requires the most amount of time to capture, making it very important that the patient remains still?

a. Ultrasonography

b.   Radiography

c.   Computerized tomography

d.  Positron emission tomography

ANSWERS

1.   b

2.   d

**Hampton et al. Effect of Cranial Flexion of Pelvic Limbs on Interlaminar Length of the Lumbosacral Space in Sternally and Laterally Recumbent Juvenile Duroc and Adult Yucatan Pigs, pp. 85-89**

**Domain 2:** Management of pain and distress

**Primary Species:** Pig (*Sus scrofa*)

SUMMARY: Pigs are often used as a model for experimental and surgical procedures involved with translational research.  Epidural administration of medications can be done to provide regional anesthesia and analgesia, especially for procedures involving pain to the abdominal region or urogenital tract.  Epidural administration, however, can be challenging in swine due to the narrow vertebral spaces, large size and orientation of the spinous processes, and increased distance between the skin and he dorsal lumbosacral space.  This study looked to determine the most ideal positioning of swine (Adult male Yucatan and juvenile mixed-sex Duroc) to provide for the largest lumbosacral space (LSS) and lumbosacral angle (LSA).  Positions compared were sternal or lateral with or without limb cranial hyperflexion (LN – lateral neutral, LF – lateral flexed, SN – sternal neutral, SF – sternal flexed).

Results

* Cranial hyperflexion of limbs improved/increased LSS in laterally recumbent animals (30% in Duroc, 65% in Yucatan) and sternal animals (9% in Duroc, 22.9% in Yucatan).
* The greatest increase was in adult Yucatan comparing LN to SF (78% increase in LSS).
* LF, LN, and SF all had increased LSS compared to LN.
* In juvenile Duroc, LSS did not differ between lateral and sternal if limbs were hyperflexed.
* In adult Yucatan, only sternal positioning increased LSS (SF compared to LF).
* LSA was more optimal in LF, SN, and SF (compared to LN), with SF creating a larger angle than SN in Yucatan adults

Overall, in all age and breed groups, cranial flexion of limbs (in either position) should facilitate epidural administration by increasing the LSS and improving the LSA.  Sternal positioning with limb flexion may be more ideal for adult animals.

**QUESTIONS**

1. Which of the following is NOT an indicator that the epidural site has been accessed?

a.   CSF is visualized in the needle

b. CSF is seen free-flowing out of the inserted needle

c.   A small tint of blood is seen in the epidural needle

d.   Nothing is visualized in the needle, but here is minimal resistance while injecting

e.   All of the above can be signs of successful epidural placement

2. How many lumbar vertebrae do swine typically have?

3. What are the anatomical landmarks for lumbosacral epidural administration?

4. True or false:  It is acceptable to perform an epidural injection after cleaning the site with alcohol.

5. In the below picture, what color line (pink, blue, black, red) represents the following:

a.   Lumbar intraluminar space

b.  Lumbosacral angle

c.  Lumbosacral space

d. Last lumbar vertebrae length



**ANSWERS**

1. c

2. 6-7, although some in this study had 5

3. The last lumbar spinous process, and an imaginary line that joins the iliac crest

4. False:  Epidural injections should be performed aseptically / after complete aseptic preparation

5. a-pink, b-blue, c-red, d-black

**Bogh et al. New Device for Noninvasive Telemetric Monitoring of Vital Signs in Healthy and Newly Operated Piglets, pp. 90-93**

**Domain 2:** Management of Pain and Distress

**Primary Species:** Pig (Sus scrofa domesticus)

**SUMMARY:** Non-invasive devices can be used for long term monitoring in the awake laboratory animal, however, due to size, requirements for surgical implantation, or limitations with physiologic data recording options, novel telemetric technology devices are needed. One such device that is commercially available for humans, Cortrium C3, has the ability to monitor and record ECG, respiration curves, surface temperature, and accelerometer data. The author’s hypothesized that noninvasive telemetry using this device would provide easy and reliable, long-time monitoring of vital signs in large animal research.

Eight, 14-16 week old, female Landrace piglets underwent a surgical procedure that resulted in pulmonary insufficiency in order to test and validate the reliability of the C3 single unit telemetric device. Two days prior to surgery, the device was fixed to the piglets’ dorsum (under sedation) (see Fig 1) and subsequently removed 48 hours post-surgery. Midazolam and azaperone was administered for premedication; propofol for induction of anesthesia and for maintenance; and fentanyl and bupivacaine for analgesia. In addition, rocuronium was administered at deep anesthesia. Postoperative analgesia and infection prophylaxis consisted of penicillin, acetaminophen and meloxicam. The animals were exsanguinated under anesthesia at the conclusion of the study.

Study results indicated heart rate measure of the telemetry device showed good agreement with a clinical pulse oximeter across a variety of heart rates. In addition, all vital sign monitoring recordings in awake pigs were successful (Fig 2). The author’s demonstrated the feasibility of a new commercially available device for noninvasive and continuous monitoring of ECG, respiration, surface temperature, and movement in awake pigs. Moreover, noninvasive telemetry on freely moving animals facilitated the monitoring of vital signs without inducing stress.

Fig. 1 The monitoring device is secured to the posterolateral thorax.



Figure 2. Non-invasive monitoring of vital signs before (n=4) and after (n=3) brief surgery in piglets. The break in the x axis indicates when surgery was performed. (A) Heart rate. (B) Respiration rate. (C) Surface temperature. (D) Accelerometer-derived movement.

**QUESTIONS**

1. True/False. A non-invasive, commercially available telemetry device, Cortrium C3, has the ability to continuously monitor and record ECG, respiration, surface temperature, and movement in awake pigs.
2. Midazolam is a benzodiazepine drug listed under which controlled substance schedule.
	1. Schedule I
	2. Schedule II/IIN
	3. Schedule III/IIIN
	4. Schedule IV
	5. Schedule V
3. This non-steroidal anti-inflammatory drug is commonly used as a treatment for arthritis:
	1. Robenacoxib
	2. Tramadol
	3. Meloxicam
	4. Acetaminophen

**ANSWERS**

1. True
2. d (Schedule IV)
3. c (Meloxicam)

**Shirai et al. Pharmacologic Modulation of Noxious Stimulus-evoked Brain Activation in Cynomolgus Macaques Observed with Functional Neuroimaging, pp. 94-104**

Domain 2: Management of Pain and Distress

Primary Species: Macaques (Macaca spp.)

SUMMARY: Areas of the brain related to pain perception may still be active while under deep anesthesia and can lead to patients experiencing pain. fMRI was utilized to assess the activation of the parts of the brain involved in pain perception (secondary somatosensory cortex (SII) and insular cortex (Ins)) during the application of peripheral noxious stimuli on anesthetized cynos. 5 anesthetic regimens were tested and included propofol at 2 different doses, medetomidine/midazolam/butorphanol (MMB), isoflurane, and pentobarbital.

Methods:8 healthy male cynos with normal species specific behavior were anesthetized for the MRI with either Propofol 20 or 30 mg/kg/hr IV, MMB 0.012mg/kg, 0.16mg/kg, 0.2mg/kg IM respectively (0.6mg/kg atipamezole given as reversal), isoflurane via face mask (no prior sedative given), or pentobarbital 20mg/kg IV. A 1kg filled water bottle used as a weight was placed on a kitchen grater with projections to induce noxious stimuli on the dorsum of the foot. While in the fMRI, the stimulus was applied for 30 secs on and 30 secs off for a total of 10 sets of OFF-ON stimulations. Scans lasted for approximately 15 min and no observable movement or reflex to noxious stimuli was observed. Scans were analyzed with SPM12 software and resliced onto the mean echo-planar imaging and coregistered to corresponding T1-weighted anatomic image and normalized to a macaque brain template.

Results:Noxious pressure applied to the foot caused significant bilateral activation of SII/Ins in animals under either propofol or pentobarbital sedation. No activation with animals under isoflurane or MMB. Even with lack of visible behavioral symptoms of pain, brain activation suggests presence of pain depending on anesthetic agent used

Discussion: MMB combo reduces noxious stimulus evoked SII/Ins by direct inhibition of its neurons through several mechanisms including alpha 2 adrenoceptor activation, enhancing GABAA receptor activation, and partial agonist activity at the mu and K opioid receptors. Isoflurane reduces noxious stimulus via activation of CNS GABA receptors and blockade of glutamate receptors and voltage-gated cation channels that leads to decreased presynaptic neurotransmitter release and enhanced postsynaptic inhibition with an overall inhibition of synaptic neural transmission and transmission of pain signaling within the pain matrix. Propofol and pentobarbital share the common mechanism of modulation of GABA binding to the GABAA receptor and enhancing the opening of the GABAA receptor’s chloride channel, reducing the neurotransmitter release and postsynaptic neurotransmission. The lack of effect of either drugs could be due to increased baseline activity or sensitivity of SII/Ins neurons induced by these anesthetics.

Cyno fMRI differ from rats in that propofol in rats is antinociceptive. It prevents movement in 50% of rats and prevents wind-up(phenomenon of persistent neural hyperactivity following brief intense peripheral stimulation, in spinal horn neurons). Pre-treating rodents with propofol prior to noxious chemical stimulation decreased presence of wind up. Rodent findings also suggest a sex-based sensitivity to noxious stimuli and analgesics. Human females are more sensitive to pressure pain and butorphanol analgesia than males. Sex based differences in NHP are not as robust.

QUESTIONS

1.   T/F: The Isoflurane and propofol caused significant bilateral activation of SII/Ins.

2.  MMB reduced noxious stimulus by

a. Blockade of glutamate receptors

b. Enhancing GABAA receptor activation

c. GABA binding to the GABAA receptor

3. What drug can be given to rats to prevent wind-up

4.  T/F: Response to noxious stimuli and analgesics is sex dependent in NHP

ANSWERS

1. False, Propofol and pentobarbital
2. b
3. Propofol
4. False, No robust studies to prove this