Pain Level Classification and Perioperative Management of Animal Experimental Procedure

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Outline

- **Animal Experiment Pain Categories**

  Scientists Center for Animal Welfare（SCAW）classification：A classification of the degree of pain caused by experimental procedures, widely applied in Japan and overseas.

- **Relief of pain during surgery on laboratory animals**

  Explains perioperative management (preoperative management, intraoperative management, postoperative management).

  Explains the depth of anesthesia that is essential for surgery..

Painfulness of animal experiments

【 Refinement】

It is the responsibility of the researcher to minimize the pain and suffering caused by the experiment. In addition, pain and distress can affect the physiological responses of animals and modify experimental results. Reducing pain and suffering improves the accuracy and reproducibility of experiments.

【 Pain level classification 】

SCAW Classification: Classifies the degree of pain caused by animal experimental procedures into 5 levels.

→ It will be the criterion for the pain level category felt by the experimental animal due to each experimental treatment.

Category A: Experiments without individual organisms
Category B: Experiments and manipulations that cause little discomfort
Category C: Experiments that cause minor stress or distress
Category D: Experiments that cause unavoidable severe stress or distress
Category E: Experiments that inflict as much or more pain as can be tolerated
(Category E experiments are basically not permitted.)

Examples of pain severity categories

[Pain Classification of Animal Experiment Procedures] (SCAW classification)

Category A: Experiments without individual organisms

- Experiments using cultured cells, post-euthanasia animal organs and tissues
- Experiments using embryonated chicken eggs (not allowed to emerge after treatment)

Category B: Experiments/manipulations that are likely to cause little or no discomfort to animals

- Animal restraint
- Administering less harmful substances by injection
- Blood sampling
- Physical examination of animals
- An experiment in which the patient was treated under deep anesthesia and euthanized without awakening
- No food or water for 2-3 hours
- Euthanasia treatment to rapidly lose consciousness

Category C: Experiments that cause mild stress or pain on animals

- Experiment to expose blood vessels under anesthesia
- Experiment to place a catheter under anesthesia
- Experiment in which conscious animals are restrained with stress for a short period of time
- Immunization experiment using incomplete adjuvant
- Painful, but avoidable experiments
- Experiment in which some discomfort remains after the surgical procedure under anesthesia

Examples of pain severity categories

Category D: Experiments involving severe stress or pain that cannot be avoided

- **Experiments that intentionally add behavioral stress (forced running, forced swimming, exercise restriction)**
- **Experiment with significant discomfort after surgical procedure under anesthesia**
- **Experiments that cause painful anatomical and physiological deficiencies or disorders through surgical procedures under anesthesia**
- **An experiment in which the body of an animal is restrained for a long period of time**
- **Immunization experiment using complete adjuvant**
- **Experiment giving an inappropriate surrogate mother (primates)**
- **Experiments in which the animal itself or other individuals of the same species are damaged by aggressive behavior**
- **Experiments inflicting pain without using anesthetics (carcinogenesis experiments, tumor transplantation experiments)**
- **Experiments using disease model animals with severe symptoms**

Category E: Experiments that inflict pain at or above the maximum pain the animal can tolerate while conscious.

- An experiment in which surgery is performed using muscle relaxants or paralytic agents for the purpose of immobilization without using anesthetics
- Experiments that cause severe burns and trauma to unanesthetized animals
- Experiments that induce psychosis-like behavior (excluding rodents)
- Experimental killing using microwave ovens and strychnine
- Experiments that give animals unavoidable severe stress
- Experiments in which animals die due to stress

Example of pain classification by experimental treatment

Kagiyama & Mizushima, from Nichipharmacology 141 (2013)

Classification	Treatment	Degree of pain
Individual identification	Pigment application	B
	Hair clipping	B
	Ear punch / Ear cut	B
	Ear piercing/Tag/Earring	B
	Tattoo	B
	Microchip	B
Restraint/Constraint	Manual	B
	Pohlman cage	C
	Monchy Chair	C
	Retention with devices	B
	Constraint with equipment	C
Feeding/water restrictions	Feeding restriction (more than half a day and less than 1 day)	C
	Feeding restriction (more than a day)	D
	Limited pleural effusion (more than 2 hours and less than half a day)	C
	Limited water supply (more than half a day)	D
Body Measurement (Awake)	Weight /physical measurement	B
	Body temperature measurement	B
	Grip strength measurement	B
	Momentum measurement (no coercion)	B
	Behavioral measurement	B
	EEG measurement	B
	Ultrasonic echo	B
Body Measurement (under anesthesia)	Blood pressure measurement	B
	ECG examination	B
	MRI	B
	CT	B
	PET	B
	Ultrasonic echo	B
	Fluorescence/Luminescence Imaging	B
Blood sampling / sampling (when awakened)	Venous (single)	B
	Artery (single)	B
	Venous (over time)	C
	Orbital venous plexus	C
	Ascitic fluid	B
	Urine collection	B
	Excrement	B
	Fur	B
	Hair root	B
	Skin biopsy	C
	Semen	B
	Swap	B
Blood collection/collection (under anesthesia)	Venous (single)	B
	Venous (over time)	C
	Orbital venous plexus (single)	B
	Orbital venous plexus (plural)	C
	Heart	C
	Urine collection	B
	Tail cut	C
Dosing (Awake)	Inhalation	B
	Nose drops	B
	Oral	B
	Gastric tube/catheter	B
	Transcutaneous (patch)/transtympanic	B
	Intradermal	B
	Subcutaneous	B
	Intramuscular	B
	Intravenous	B
	Intra-arterial	B
	Intraperitoneal	B

Example of pain classification by experimental treatment

Kagiyama & Mizushima, from Nichipharmacology 141 (2013)

Dosing (Awake)	Rectal	B
	in the footpad	D
	mixed feed	B
	Dissolve/suspend in drinking water	B
Dosing (under anesthesia)	Nasal drops	B
	Gastric tube/catheter	B
	Intratracheal	B
	Intravenous	B
	Intra-arterial	B
	Intraocular	C
	Intracerebral/spinal cord	C
	Intracerebroventricular	C
	in the portal vein	C
	in the digestive system	C
	Intraperitoneal	B
	in the organ	C
	Transtympanic membrane	B
	Orbital venous plexus	C
	Eye drop	B
Euthanasia (without anesthesia)	Cervical dislocation	B
	Decapitation	B
	carbon gas	B
	Anesthetic overdose	B
Euthanasia (under anesthesia)	Bloodletting	B
	Whole blood collection	B
	Decapitation	B
Surgery/Transplant /Surgical Procedure	Endotracheal intubation	B
	Pump indwelling (no movement restriction)	B
	Pump indwelling (with movement restriction)	C
	Intra-arterial cannulation	C

Surgery/Transplant /Surgical Procedure	Intravenous cannulation	C
	Intracerebral cannulation	C
	Balloon catheter	C
	Arterial ligation (deep)	C
	Venous ligation (deep)	C
	Vasculature ligation	C
	Egg retrieval	C
	Embryo transplant	C
	Ovary transplant	C
	Intratesticular cell transplant	C
	Subcutaneous implant	B
	Intravenous transplant	B
	ntraperitoneal transplant	B
	Transplant within an organ	C
	Organ transplant	D
	X-ray radiation (disruption of spinal cord function)	D
	X-ray radiation (immunosuppression)	C
	Telemetry embed	C
	Electrode embed	C
	Electrical stimulation	B
	Cesarean section	C
	Neonatal reanimation	B
	Artificial feeding/fostering	B
	Sensory stimulation (light/sound/pain/taste/olfaction)	B
	Electroporation	B/C
	Abrasion/incision	B/C
	organ extraction	C
	Immunization	C

Example of pain classification by experimental treatment

Kagiyama & Mizushima, from Nipharmacological Journal 141 (2013)

Disease models	Myocardial infarction/ischemia	D
	Cerebral infarction/ischemia	D
	Spinal cord injury	D
	Peripheral nerve injury	D
	Peripheral neurodegeneration	D
	Parkinson's	D
	Dementia	C
	Autoimmune disease	D
	Obesity	C
	Diabetes	D
	Hypertension	D
	Muscular dystrophy	D
	Vomiting	C
	Cancer	D
	Prion diseases	D
	Allergy	C/D
	Immunodeficiency	D
	Carcinogenesis	D
	Graft versus host disease	D
	Pulmonary hypertension	D
	Anemia (transient)	C
	Anemia (chronic)	D
	Polycythemia	B
	Parallel fusion	D
Pharmacological toxicity	Tail flick	B
	Hot plate	C
	Single dose toxicity	D
	Repeat dose toxicity	D
	Reproductive and developmental toxicity	C
	Carcinogenicity	D

Tumor	Carcinogenesis	D
	Drug administration	B/C
Infection parasitism	Overt infection (including fatality)	D
	Inapparent infection	C
	Antibody production	C

- ✓ Restrictions on feeding and water, and surgical procedures (surgery and transplantation) are highly painful.
- ✓ It should be noted that the use of disease model animals is highly distressing.

Responsibilities of animal experiment supervisors (experimenters)

【 Planning stage of animal experiments plan 】

At the stage of drafting an animal experiment plan, predict the degree of pain that will be caused by the treatment given to the animal and describe it in the plan. In doing so, the following matters should be considered.

- ◆ Treatments that cause pain to humans should also cause pain to experimental animals.
- ◆ Transition to the least painful treatment as long as the purpose of the experiment can be achieved.
- ◆ Specify appropriate pain relief or relief measures.
- ◆ For the purpose of education, etc., when a practitioner with low skill performs, the pain level category is set one level higher.
- ◆ The setting of the degree of pain is based on the degree of pain in humans when similar treatments are performed.

【 Animal testing in progress (after) 】

Observe animals carefully during and after treatment to determine if they are inflicting unanticipated distress. If you determine that you are in pain, take appropriate pain relief and relief measures. If it is determined that the pain cannot be eliminated, the decision to stop the experiment should be made.

⇨ Setting up a humane endpoint

*It is recommended to set in advance even for treatments with low pain category.

About perioperative management

Surgery is highly likely to cause pain to experimental animals, and appropriate management is required.

	Major surgery	Minor surgery
	・ Invasion into the body cavity ・ Exposure of the body cavity ・ Induction of physical disability ・ Extensive tissue excision	・ No invasion of body cavities ・ No physical disability
Non-survival surgery (terminal surgery) ・ Euthanasia without awakening	Major terminal surgery	Minor terminal surgery
Survival surgery ・ Observation after awakening	Major survival surgery Open surgery, open heart surgery, joint replacement surgery, limb amputation	Minor survival surgery Trauma suturing, peripheral cannulation, percutaneous biopsy

Perioperative management (preoperative management, intraoperative management, postoperative management) is important to reduce pain.

Preoperative management

Surgical preparation
- Surgical planning
-Information sharing of practitioner members
-Confirmation of division of roles
-Technical training
-Securing operating rooms and surgical tools

Animal preparation
-Animal health check
-Acclimatization (acclimation to the surgical environment and perioperative rearing environment)
-In the perioperative period, animals should be kept alone. -Possibly premedication with antibiotics.

No food and water: To prevent vomiting during general anesthesia and to prevent impediments to operation.
-Dogs and monkeys should be fasted from 8 to 12 hours before anesthesia.
-Small animals often do not fast. (Prevention of dehydration)
-Confirmation of the health status of fasted and water-fasted animals is essential.

General anesthesia depth

【 Phase 1 】 Spontaneous motion period

Administration of anesthetic - unconsciousness

【 Phase 2 】 Excited period

Loss of consciousness - Stabilization of respiratory rhythm

↓

Responsive to external stimuli.

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【 Phase 3 】 Appropriate time for surgical anesthesia

- Decreased breathing (regular chest-abdominal breathing)
- Stabilize blood pressure and heart rate
- Pain reflex, laryngeal reflex, eyelid reflex, loss of withdrawal reflex due to visceral traction
- State of muscle relaxation

【 Phase 4 】

Suppression of the central nervous system, respiratory arrest, blood pressure drop → cardiac arrest

Intraoperative management

1. Aseptic operation

Large-scale survival surgery is based on aseptic technique.

-Use a specific room or area.
-Restrict the entry and exit of people during the operation. (Make it invisible from the outside.)
-Hair removal and disinfection of the surgical site
-Preparing for the operator (hand washing/disinfection, surgical wear/mask/gloves, etc.)
-Preparation of surgical tools and materials

2. Intra-operative monitoring

Monitor the depth of anesthesia and animal physiology at all times.
Monitoring items: body temperature, heart rate, respiratory rate, electrocardiogram, arterial blood saturated oxygen concentration, etc.

3. Normothermia maintenance

It is performed to suppress circulatory and respiratory system disorders during general anesthesia.
Especially effective for small and juvenile animals.

4. Others

Wet the exposed body cavities and organ surfaces with warm saline or the like.
Perform fluid replacement to replenish water and electrolytes.

Postoperative management

1. Breeding environment
They should be kept in clean, well-controlled areas of temperature and humidity.

2. Observation
Keep warm immediately after awakening and observe frequently.
Observe the surgical site and check for self-harm.

3. Dosing
Administer analgesics for pain control.
Administer antibiotics to prevent infection.

4. Others
Fluid replacement is performed to replenish water and electrolytes.
Give highly palatable supplementary food.
Properly adjust the position of feeders/waterers.

Summary

1. Pain assessment

At the planning stage, determine the animal's degree of distress from the experimental procedure.

"Keep an eye out for unexpected pain during experimental procedures.“

2. Surgical operation

Recognize that surgery is highly likely to inflict pain on animals, and take necessary measures.

"Confirm the depth of anesthesia."

Questions

Please check the correct answer below.

1. Considerations in predicting the severity of experimental procedures include:

- ☐ Experimental animals are less likely to feel pain, so the pain level should be one level lower than that expected to be felt by humans.
- ☐ Since model animals that are born with symptoms do not feel pain, there is no need to set the degree of pain.
- ☐ Judgment should be made as objectively as possible, referring to the SCAW classification and other materials.

2. When performing surgical procedures on experimental animals,

- ☐ Check the animal's health condition before surgery and decide to cancel if the condition is poor.
- ☐ During surgery, focus on the procedure rather than the depth of anesthesia.
- ☐ After waking up from anesthesia, the animal should be returned to the breeding room as soon as possible and observation should be refrained so as not to stimulate it.