Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Shabari Girishan
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
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Lecture – 43

Welcome back to our session on euthanasia and rodents. As I mentioned earlier, it's crucial to provide a humane death to the animals under our care. The fundamental principle behind euthanasia is to end an animal's life with minimal pain and distress. In our previous session, we covered the general methods of euthanasia, the different types, and preliminary aspects of carbon dioxide asphyxiation - the preferred method for euthanasia.

Today, we will delve into the specifics of carbon dioxide asphyxiation. We'll discuss the procedure, dos and don'ts, and how to ensure a humane death with the least amount of pain and distress.

Let's begin by examining the technique itself. As carbon dioxide is heavier than air, incomplete filling of the chamber is a common issue. Rats can potentially escape if they avoid coming into contact with the carbon dioxide, particularly if the chamber is large and the flow rate or concentration is inadequate. To ensure an appropriate dosage of carbon dioxide, we need to adhere to specific guidelines based on the size of the cage and the flow rate.

These two factors - cage size and flow rate - are critical in ensuring a successful procedure. We have pre-calculated flow rates that are suitable for specific cage sizes and different types of mice. However, if a standard-sized cage is not used, we need to calculate the flow rate using a specific formula.

This calculation typically aims for 50% displacement of air in the chamber, although a range of 30-70% is acceptable. To calculate the required flow rate to achieve this displacement, we need the length, width, and height of the cage in inches. Once we have these measurements, we can calculate the total cubic inches of the cage, or in other words, the size of the euthanasia chamber.

The process involves converting cubic inches to liters by dividing the total cubic inches of the euthanasia chamber by 61.02 cubic inches per liter. The desired flow rate is calculated by multiplying the cage size in liters by 50%, aiming for a displacement of 50% of the cage volume per minute.

Ensuring the accuracy of the flow rate is vital, even when using pre-filled cylinders, to achieve the appropriate displacement rate (within the 30-70% range).

The technique of carbon dioxide asphyxiation involves handling carbon dioxide cylinders. Familiarization with the different parts of the cylinder simplifies the process. Commercially available carbon dioxide cylinders with pre-set volumes are readily accessible; the user only needs to calculate and adjust the flow rate using the provided knobs.

Although there is a learning curve associated with this method, once mastered, euthanasia becomes a smooth process, making it the preferred method. The procedure entails opening the carbon dioxide cylinder using the knob, regulating the volume within the cylinder, and adjusting the flow rate at the cylinder's outlet using the flow meter.

Before initiating the procedure, the rat must be transferred from its housing cage to the euthanasia chamber. It is important to avoid using the same housing cage for other living rats. The only exception to this is when containing an infection. In all other scenarios, the rat is transferred to the euthanasia chamber. The personnel will then verify the carbon dioxide level in the cylinder, a crucial step before proceeding. Utilizing a cylinder used multiple times can lead to an inadequate flow rate, causing significant distress to the rat.

Before commencing the procedure, it is imperative to verify the carbon dioxide level in the cylinder and ensure it is adequately filled for the euthanasia process. The gas delivery valve and tubing from the cylinder should then be securely attached to the chamber lid.

Once the animal is placed inside the euthanasia chamber, the lid should be tightly closed to prevent any air leaks. The gas delivery pressure needs to be meticulously adjusted to a range of 20 to 30 psi, ensuring a smooth and controlled flow rate that is neither too low nor too high.

Following this, the animals are gradually exposed to increasing carbon dioxide levels by carefully turning on the flow meter. The final flow rate is adjusted using the valve knob, typically set at 2 to 4 liters per minute for mice and 8 to 10 liters per minute for rats. It's important to note that if the chamber size deviates from standard dimensions, the flow rate should be calculated independently based on the chamber's measurements.

The chamber should be filled with gas at a rate that results in a displacement rate of 30 to 70 percent of the chamber volume per minute, as determined by prior calculations. It is crucial to consider the holding capacity of the chamber, ensuring it is appropriate for the number of animals being euthanized. Generally, it is recommended not to exceed 5 mice per cage. For rats, the limit is 2 per cage if their weight is below 500 grams, and only 1 per cage for rats weighing more than 500 grams.

The animals should be exposed to carbon dioxide for a minimum of 3 minutes, or longer if necessary, until they cease breathing. Once unconsciousness is observed, the flow rate should be increased for an additional 2 minutes to guarantee the cessation of breathing and heartbeat. Only then should the carbon dioxide delivery valve be turned off and the euthanasia chamber opened.

It is advisable to visually monitor the animals' breathing through the glass chamber. The animals should only be removed from the chamber once complete cessation of breathing is confirmed. While specialized equipment can be used to monitor respiratory rates within the chamber, it is generally not necessary and can be quite expensive.

To definitively verify death, the cessation of both respiratory and cardiovascular movements must be meticulously observed for a minimum of 10 minutes after removing the rat from the chamber. This observation period is critical as it allows for the detection of any signs of recovery, particularly if the initial exposure was inadequate. If any movement is observed within this timeframe, secondary euthanasia methods, such as cervical dislocation, decapitation, or exsanguination, can be employed to ensure complete and irreversible death.

While these secondary methods are effective, they are not recommended as the primary means of euthanasia in all scenarios. Carbon dioxide asphyxiation remains the preferred method, and its effectiveness must be diligently confirmed through a combination of criteria. The absence of a pulse, breathing, corneal reflex, and a response to a firm toe pinch collectively indicate death. The corneal reflex is typically tested by gently touching the cornea with a wisp of cotton; a blink response indicates a live animal. Monitoring breathing and the absence of a pulse through a vital monitoring system or a stethoscope offer reliable confirmation.

Additionally, the graying of mucous membranes and the onset of rigor mortis can serve as indirect indicators of death. However, it is crucial to remember that, with the exception of rigor mortis, no single sign definitively confirms death. If rigor mortis is present, it unequivocally indicates death. In the absence of rigor mortis, a combination of the aforementioned signs must be used to ascertain death.

In essence, the objective is to ensure a humane death with minimal pain and distress. If carbon dioxide asphyxiation is the sole method employed, the aforementioned criteria must be comprehensively utilized to confirm the animal's demise. The heartbeat, in particular, necessitates an assessment period of at least 5 minutes or longer. This extended observation is vital as a momentary check may fail to detect the potentially long pauses between heartbeats, a condition known as bradycardia. The most reliable assessment involves either direct palpation of the pulse in the carotid or femoral artery or direct palpation of the heart itself. The pulse can be felt in the neck or hip region, while the heartbeat can be assessed by gently feeling the animal's chest.

In instances where doubts persist regarding the animal's status, the thorax can be opened to directly expose and observe the heart or palpate it to verify the absence of activity. This serves as an additional confirmatory measure. To access the heart, the rat is positioned supine, and a skin incision is made along the rib cage. The tip of the sternum is then lifted, and a lateral cut is made to open the thoracic cavity. The diaphragm and abdominal organs will be visible, and upon lifting the rib cage further, the heart will be directly exposed for inspection.

It is important to note that the arterial pulse may be faint and difficult to palpate, necessitating direct observation of cardiac mechanical activity. This is one reason why euthanasia is frequently followed by organ harvesting, as it not only confirms death but also facilitates histopathological analysis.

In addition to direct observation, other sophisticated methods such as ECG monitoring can be employed to confirm death. Within the central nervous system, the pupillary response to light can be assessed. If the iris fails to constrict when exposed to light, it indicates brain death.

Observing the respiratory pattern can also be helpful, but it is important to exercise caution. A profoundly anesthetized animal may exhibit shallow and irregular breathing, which can be easily mistaken for the absence of spontaneous breathing, especially after acute surgery. Therefore, relying solely on respiratory patterns can be misleading, emphasizing the importance of utilizing multiple signs to confirm death.

Once death is confirmed, the rat should be removed from the chamber, and the euthanasia chamber must be thoroughly cleaned before proceeding with the next animal. After organ harvesting, if applicable, the carcass should be disposed of in a designated area using the provided trash bags. It is crucial to adhere to the animal facility's disposal guidelines to prevent any potential hazards to research personnel. The euthanasia area should also be meticulously cleaned and disinfected.

While carbon dioxide asphyxiation is the preferred method, physical methods of euthanasia exist but are generally not favored.

It's important to emphasize that physical methods of euthanasia should only be resorted to in specific circumstances where they are deemed absolutely necessary, such as when carbon dioxide asphyxiation might interfere with research protocols, for instance, when studying a drug whose concentration in organs could be affected by carbon dioxide exposure.

Cervical dislocation is one such physical method that, when executed by a skilled individual, can be considered humane. It is typically employed for rats weighing less than 200 grams and is not recommended for larger animals. In the case of mice, cervical dislocation is performed only after the animal has been rendered unconscious through inhalation of carbon dioxide or injectable anesthetics.

The technique involves placing the animal on a flat surface while securely holding its tail. The thumb and index finger are positioned on either side of the neck, at the base of the skull. Alternatively, a rod or scalpel can be used to apply pressure at the base of the skull. Simultaneously, the base of the tail or hind limb is swiftly pulled in the opposite direction, creating traction and counter-traction. This forceful motion separates the cervical vertebra from the skull, resulting in rapid loss of consciousness and instantaneous death.

Decapitation, another physical method, albeit less preferred, entails severing the neck. This procedure is carried out using specialized guillotines designed to ensure a swift and precise severance at the correct location. Decapitation is primarily employed when pharmacological agents or carbon dioxide are contraindicated. However, it is worth noting that cervical dislocation is generally favored over decapitation.

In addition to these physical methods, adjunctive methods such as exsanguination can be utilized to ensure death in animals that have been stunned or rendered unconscious by other means. These secondary methods are typically employed only when there is uncertainty regarding the animal's death following the primary euthanasia procedure.

Given that anxiety is closely linked to extreme hypovolemia, exsanguination should never be employed as the sole method of euthanasia. It is invariably considered a secondary procedure due to the significant anxiety it induces as a result of hypovolemic shock. In essence, exsanguination involves the withdrawal of blood from the body. While it can be utilized to obtain blood products, it should only be performed on animals that have been sedated, stunned, or anesthetized.

The removal of a substantial blood volume, which is precisely what exsanguination entails, can effectively ensure the death of an animal and prevent any possibility of resuscitation. Typically, this necessitates the removal of at least 25 percent of the animal's estimated blood volume. Death occurs due to hypovolemia, a state of reduced blood volume, which leads to circulatory arrest. This happens because the reduced blood volume results in a diminished stroke volume of the heart, causing the heart itself to suffer from ischemia (lack of oxygen) and ultimately stop functioning. It's important to remember that the heart, like any other organ, requires a constant supply of blood and oxygen to operate. In cases of severe hypovolemia, the blood supply to the heart muscle is compromised, leading to the cessation of this vital "motor pump."

This concludes today's session, during which we have comprehensively covered various euthanasia techniques, their advantages, and disadvantages. We delved into the specifics of carbon dioxide asphyxiation, which is widely regarded as the most preferred method for euthanasia. In the upcoming sessions, we will shift our focus to the process of organ harvesting. Given the neural engineering focus of this project, we will specifically discuss the harvesting of the brain and spinal cord. We will explore the necessary steps to ensure the preservation of brain structure for subsequent histopathological analysis. Maintaining the integrity of this delicate, jelly-like structure is of paramount importance. For instance, if an implant has been used, preserving the implant's trajectory is crucial for accurate histopathological examination.

It is imperative to remember that organ harvesting always follows euthanasia. We will explore this process in greater detail in our next session. Thank you all for your dedicated attention.