Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Welcome back to the module on Rodent Neurosurgery. Today, we will delve into a crucial session on Euthanasia in Rodents. This topic holds immense significance within the framework of animal ethical guidelines established by the CPCSCA, and today's session adheres to those guidelines. It's also important to understand the techniques involved in harvesting various organs. So, we will first explore the aspect of euthanasia, followed by an examination of organ harvesting.

It's important to understand why we dedicate an entire session to euthanasia. We strive to ensure that rats are treated with compassion and that their lives are ended humanely. The simple definition of humane killing is the induction of death in an animal using a method that results in a rapid loss of consciousness with minimal pain and distress. The key phrases here are "minimal pain and distress." Another term used for euthanasia is "mercy killing," and this term is also applicable to humans. While euthanasia is not legalized in India, it is used in some Western countries for humans suffering from terminal illnesses or in situations where there is no hope for recovery, such as when a patient is in a deep coma and on life support.

In the context of animals, we aim to induce death with the least possible pain and distress, adhering to humanitarian principles. This humane killing is referred to as euthanasia. It's essential that all research personnel receive adequate and appropriate training in euthanasia techniques. This training is crucial for understanding the various methods available and ensuring that the correct technique is used for each specific animal. It's imperative that none of the guidelines are overlooked. These guidelines are in place to ensure that pain and distress are minimized at the time of death for these laboratory animals.

It's also necessary to evaluate the vital signs of the animal to confirm death. Merely observing that the animal is unconscious is not sufficient, as it may still be breathing. The entire purpose of euthanasia is defeated if you cannot ascertain that the animal has indeed passed away. Therefore, understanding how to evaluate vital signs is critical. Another important consideration is that euthanasia should never be performed in the animal housing rooms, except in exceptional circumstances, such as when an animal has contracted an infectious disease.

To prevent the transmission of infectious agents to other areas, euthanasia should ideally be conducted in a designated euthanasia chamber or a specifically equipped facility, rather than within the animal housing areas. The only exception to this rule is in special circumstances where the animal has contracted an infectious disease. Therefore, the animal must be carefully transferred from its housing or the experimental area to the designated euthanasia area for the final procedure.

Now, let's delve into the reasons why euthanasia becomes necessary. In general, animals are euthanized at the conclusion of an experiment or if they are exhibiting persistent adverse effects that compromise their well-being. There are a couple of key reasons behind this practice. Firstly, these animals are often subjected to a variety of potentially harmful agents or chemicals during the course of the experiment. If these animals were allowed to continue living, they could pose a risk to other animals within the facility or even to the research personnel themselves. Secondly, small animals such as rodents are specifically bred for the purpose of scientific research. Once the experiments they are involved in have been completed, they are typically euthanized.

Moreover, euthanasia serves the purpose of enabling researchers to procure tissues for scientific analysis. This is of paramount importance in understanding the full impact of the experiment on the animal. It allows researchers to examine the precise placement and effects of implanted electrodes, assess any potential damage that may have occurred beyond the original scope of the study, and investigate any toxic effects resulting from the implants or the chemicals administered.

Euthanasia is also ethically justified when it becomes evident that the animal's pain, distress, or suffering is likely to surpass acceptable levels or when it is necessary to alleviate existing suffering. If, during the course of the procedure, an animal experiences complications or infections that lead to a level of suffering that extends beyond the boundaries of what is considered acceptable within the experimental methodology, it is deemed more humane to euthanize the animal to bring an end to its suffering.

Finally, as previously mentioned, preventing the spread of infection constitutes another critical reason for euthanasia. In instances where an animal has developed a full-blown infection, it becomes imperative to euthanize it in order to curtail the transmission of the infectious agent to other animals within the facility. In essence, the two primary motivations behind the practice of euthanasia are to alleviate the suffering of the animal and to safeguard the health and well-being of the other animals in the research environment.

Furthermore, if there's a risk of an infection spreading throughout the entire colony, euthanasia becomes a necessary measure to safeguard the remaining animals. Additionally, animals that are no longer suitable for breeding purposes or those exhibiting undesirable characteristics might be euthanized. For instance, if the specific type or sex of an animal is not required for research, it is common practice to euthanize them. Lab animals are brought into the laboratory environment for specific experimental purposes, and if they are not suitable for any ongoing or planned studies, it is considered

more ethical and efficient to euthanize them rather than allowing them to live without a clear purpose. This practice also helps in conserving valuable lab resources and minimizing the potential for these animals to contract diseases that could jeopardize the health and safety of other lab animals.

Now, let's explore the different methods available for performing euthanasia. It's of paramount importance to have a comprehensive understanding of these methods, as this will enable you to appreciate the specific techniques that have been selected and approved by the CPSCSA committee for use in euthanasia procedures.

Broadly speaking, these methods can be classified into two main categories: non-physical methods and physical methods. The choice of which method to employ typically hinges on several factors, including the age and species of the animal, the quantity of animals involved, and the specific objectives of the research protocol. Additionally, your experimental methodology can also play a role in guiding the selection of the appropriate euthanasia method. These factors are closely intertwined, and it is imperative to carefully choose the most suitable method before proceeding. Furthermore, it's crucial to clearly outline and document the chosen euthanasia method within your study protocol before submitting it for review by the animal ethical committee. Once approved, it's expected that the same method will be consistently employed throughout the duration of your experiment.

Generally speaking, there are two primary ways in which a chemical agent can be introduced into the animal's body to induce euthanasia: inhalation or injection. These methods are generally favored over physical methods such as cervical dislocation or decapitation, as the latter are often perceived as being less humane. Thus, non-physical methods are typically the preferred choice. When it comes to non-physical methods, you have the option of using inhalants such as anesthetic agents or carbon dioxide. Alternatively, you can administer injectable drugs like barbiturates.

Let's take a look at this table, which has been extracted from the CCSA guidelines - the most recent version, which was published in 2017. This table provides an overview of the various methods available for euthanasia across a range of species, starting from mice, rats, and hamsters, all the way up to non-human primates. As you can see, the available options can vary significantly depending on the specific animal species in question. Generally, there tends to be a wider array of techniques at our disposal for rodents, while the choices become more limited when dealing with non-human primates.

This table offers a comprehensive overview of the spectrum of available methods. For the purpose of today's session, let's concentrate on the methods applicable to rodents. It's important to note that electrocution is strongly discouraged for any species, as it is considered to be an inhumane method of euthanasia. While physical methods do exist, they are utilized very sparingly. In the majority of cases, we employ inhalational methods for small animals, utilizing agents such as carbon monoxide, carbon dioxide, or a combination of carbon dioxide and chloroform. Furthermore, there is a range of

injectable drugs that can be used for rodents, including barbiturates, chloral hydrate, ketamine overdose, and sodium pentobarbital.

If you're wondering why these methods are specifically listed, it's because the primary goal is to minimize pain and distress to the animal. Another crucial factor is to avoid any interference with the experimental methodology. If you're studying a specific drug, the euthanasia method should not interact with that drug and affect your study analysis. This is an essential consideration when choosing a euthanasia method.

Methods that are completely unacceptable include physical methods like decompression. Decompression chambers, which were used in the past, create low ambient air pressures without supplemental oxygen, causing extreme distress to the animal and defeating the purpose of humane euthanasia. Stunning, involving a forceful blow to the head, causes significant brain injury and is also unacceptable. Inhalation of gases like nitrogen or argon, or flushing, are equally unacceptable. Additionally, certain drugs are strictly prohibited for euthanasia and are completely banned. It's vital to avoid such drugs and methods when planning your euthanasia protocol.

Let's delve into the non-physical methods. These involve using drugs or chemicals that act on the animal's system to suppress brain activity, leading to the cessation of breathing and circulation, and ultimately resulting in a humane death. These agents can be administered through inhalation or injection, either intravenously or intraperitoneally. It is strongly recommended to use only pharmaceutical-grade compounds, as these ensure a smooth and rapid death when administered in the correct dosage. It's important to avoid over-the-counter chemicals or rat poisons, as these can cause increased secretions, prolonged suffering, heightened pain and distress, thus negating the very purpose of humane euthanasia.

When it comes to the use of inhalant anesthetic agents, we employ a device known as a euthanasia jar. This is a highly recommended method. However, the agent that is generally preferred for this purpose is carbon dioxide, rather than other inhalant anesthetic agents. We will cover both methods for the sake of completeness, but it's worth noting that the underlying methodology remains the same, regardless of whether you are using an anesthetic gas or carbon dioxide.

The euthanasia jar itself is a relatively simple apparatus. It consists of a small container, which can be made of either plastic or glass, and has a known volume. The jar is equipped with a secure lid to prevent the animal from escaping. Inside the jar, you'll find a wire mesh or perforated floor, and beneath this, there is a layer of cotton padding. The anesthetic agent that you will be using will be soaked into this cotton padding, ensuring that the animal is exposed to the appropriate concentration of the agent.

So, typically, we utilize either isoflurane or a mixture of isoflurane and propylene glycol in the following proportions: 20% isoflurane for mice and 30% isoflurane for rats. The recommended mixture is approximately 1.5 to 2 ml per 500 ml volume of the jar. It is crucial to adhere to these proportions. The ultimate objective is to induce a swift and

painless death for the animal. Therefore, you need to ensure that you are using the correct amount of the anesthetic agent or carbon dioxide gas so that it adequately fills the given volume of the euthanasia chamber and causes a rapid cessation of breathing. If the flow rate or dosage is incorrect for the size of the chamber, the animal's suffering will be unnecessarily prolonged.

It's also essential to prevent any direct contact between the animal and the anesthetic agent. This is the purpose of the cotton pad at the bottom of the jar, along with the perforated floor. The volatile gas evaporates from the cotton pad and rises into the chamber, where the animal inhales it. The exposure time within the container should be limited to approximately 3 to 5 minutes, or until the cessation of breathing is observed. Once breathing has stopped, the animal can be removed from the jar, and the process of organ harvesting can commence.

The most preferred and currently recommended method for euthanasia, according to the CPCSEA guidelines, is carbon dioxide asphyxiation. Anesthetic agents and drugs can potentially interfere with your experimental results by causing organ changes. To avoid such complications, carbon dioxide asphyxiation is the favored methodology.

We will discuss carbon dioxide asphyxiation in detail because it's essential for all of you to understand and be trained in handling carbon dioxide gas and the associated euthanasia chambers. This is a technique you'll need to employ at the end of your experiments for the purpose of euthanasia. In this session, we will cover the advantages and disadvantages of this method. In the next session, we will delve deeper into the specific dos and don'ts, techniques, and other relevant details.

One of the primary advantages of carbon dioxide asphyxiation is that it induces rapid depression of the central nervous system, accompanied by analgesic and anesthetic effects, through a process known as carbon dioxide narcosis. Essentially, as carbon dioxide levels build up, the brain, which is highly dependent on oxygen, experiences a decrease in oxygenation, leading to a depression of overall CNS function. This results in a reduction of pain and a loss of consciousness, making it a humane method of euthanasia.

Furthermore, carbon dioxide is readily available and can be easily obtained in compressed gas cylinders. It is also non-flammable and non-explosive, posing minimal risk to personnel handling it. Carbon dioxide is a naturally occurring gas, and at normal concentrations, it does not have any direct adverse effects on humans, unlike anesthetic agents, which can cause drowsiness and depress the central nervous system in humans exposed to them. The non-flammable and non-explosive nature of carbon dioxide is particularly important in laboratory settings where you might be using various heat-generating or electrical equipment, including thermocoagulative devices and cautery tools.

Furthermore, the use of inhalational agents such as ether, which were once commonly employed for anesthesia, can pose a significant safety hazard within a laboratory setting.

These agents are highly flammable and, in the presence of heat sources or electrical equipment like cautery tools, can create a risk of fire or explosion, potentially endangering the entire laboratory. Carbon dioxide, being non-flammable and non-explosive, offers a much safer alternative in this regard.

An additional advantage of carbon dioxide asphyxiation is that it does not leave behind any residual chemical traces in the tissues that you intend to harvest from the animal. This is particularly important if your research necessitates comprehensive pathological analysis of various organs, such as the liver and lungs, or if you need to collect blood samples. It is crucial to ensure that the method of euthanasia does not introduce any foreign substances that could potentially alter the pathological parameters that you are planning to study. Carbon dioxide, as a naturally occurring gas that is already present in the body, does not interfere with these parameters, making it an ideal choice for such investigations.

However, it's important to acknowledge that carbon dioxide asphyxiation does have its drawbacks. This is precisely why we encounter specialized carbon dioxide euthanasia chambers. It's crucial to recognize that carbon dioxide is denser than air, which can result in incomplete filling of the chamber. This, in turn, can lead to some animals attempting to avoid exposure to the gas. For instance, if the chamber is not adequately sealed and the carbon dioxide has not been evenly distributed throughout, animals experiencing distress might try to escape the area where the gas is concentrated. Furthermore, neonatal rodents exhibit a certain degree of resistance to carbon dioxide-induced euthanasia. This necessitates the use of entirely different euthanasia procedures for these young animals.

Another potential issue is that the induction of loss of consciousness at lower concentrations of carbon dioxide can lead to the development of lesions. These lesions are essentially pathological alterations in the structure of the upper respiratory tract, and they can occur if the calculated concentration of carbon dioxide is not appropriate. Therefore, it is of utmost importance to ensure complete filling of the chamber with an adequate concentration of the gas. This requires careful attention to the flow rate and the concentration of carbon dioxide being used. On the other hand, excessively high concentrations of carbon dioxide can cause distress to the animal, thereby undermining the very purpose of humane euthanasia.

In conclusion, it is imperative to bear in mind all of these factors when determining the appropriate dosage and flow rate of carbon dioxide for euthanasia.

We will address that in the next session. Now, let's discuss the important do's and don'ts associated with the carbon dioxide asphyxiation methodology.

Firstly, the only acceptable source of carbon dioxide for this procedure is from readily available gas cylinders. Other sources, such as dry ice, fire extinguishers, or various chemical methods, should be strictly avoided to prevent causing unnecessary pain and distress to the animals.

It's also crucial to ensure that only animals of the same species are placed together in the chamber for euthanasia at any given time. For instance, if you need to euthanize multiple rats, you should not mix Long-Evans rats with Wistar rats; the animals within the chamber must belong to the same species.

Additionally, overcrowding the animals within the euthanasia chamber must be avoided. If your chamber is relatively small and you introduce too many animals into it, the intended purpose of the procedure will be compromised. The animals will experience undue suffering due to an insufficient amount of carbon dioxide being available to effectively induce unconsciousness and death, as multiple animals will be sharing the same limited space and gas supply.

Moreover, it is considered both unethical and inhumane to place live animals in a chamber that already contains deceased animals during the euthanasia process.

To minimize stress and ensure a humane death, it is advisable, whenever feasible, to carry out the euthanasia procedure within the animals' home cages. This familiar environment can help reduce their anxiety and fear during their final moments.

It's also crucial to be mindful that sudden exposure to high concentrations of carbon dioxide can be distressing for some species. Therefore, it's imperative to gradually introduce the gas into the chamber. Pre-filling the carbon dioxide chamber is strongly discouraged, as this can lead to the gas escaping and diminishing the effective concentration within the chamber. It can also inadvertently contribute to the very distress that we are striving to prevent.

These are some of the key do's and don'ts that you need to keep in mind before employing this methodology.

That concludes today's session. In our upcoming session, we will explore the various techniques available for employing carbon dioxide asphyxiation, including the proper methodology to be followed, the calculation of the flow rate, and the appropriate way to introduce carbon dioxide to ensure a humane and peaceful end for the animal. Following that, we will shift our focus to discussing physical methods of euthanasia.

Thank you for your attention.