Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
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Week - 10
Lecture – 40

Hello everyone, welcome back to the course on Rodent Neural Experiments for Neural Engineering. So, from the last session, we are dealing with the anesthesia aspects of rodent surgery, which is equally important. This knowledge can be useful for any surgeries that require long-term monitoring of the vitals and long-term administration of anesthesia. In the last session, we covered all the basic principles, definitions, abbreviations, dosages, and so forth. Now, we will look at the required types of equipment, which can be a bit expensive. But I emphasize that it is worthwhile and a one-time investment.

We have covered the definitions, abbreviations, pre-anesthetic evaluation, pre-anesthetic care, and preparation, how we would select an animal, the scoring system for body conditioning, and how to make out whether your subject is healthy or not so that you know your experimental outcomes are met as expected. In today's session, I will broadly cover general anesthesia equipment, the inhalational part of the administration, and injectable anesthesia. And in the next session, we will cover the remaining aspects of vital monitoring and post-operative care.

Coming to the materials and equipment, which is an important aspect, we need to know what every piece of equipment is; how it plays a big role in the administration of anesthesia and monitoring of the vitals, and their advantages and disadvantages. So, the first one is the general materials: the warm water circulating pad, or if you have an electronic warmer that is fine, or a heating disk or pad, or even gauze pads. These are some of the pieces of equipment required to conserve body heat. We will come to the body temperature aspect while discussing vital monitoring and the role it plays.

I introduced the concept of hypothermia and the possible issues arising from it. Another important aspect is that the eyes should be kept closed and moist so that the cornea of the eye does not get dried out. An ophthalmic ointment is also pretty important. So, the gas anesthesia machine is the major component of the general anesthesia endeavour, along with an adequate gas scavenging system or filter.

Last time we covered a bit of the scavenging system. What it does is, whenever the rat inhales the anesthetic gas, there is some amount of gas that is expelled from the circuit. So, that has to be removed from the atmospheric air to prevent inhalation by lab personnel; that is very important. So, to remove the air, this scavenging system or filter, which is a canister that is shown here is very important. And then you have the induction chamber, which is constructed of a see-through material, either glass or polycarbonate. So, before we transfer the animal into the stereotactic apparatus or the surgical system where the actual tubing of the anesthetic delivery machine is installed, you need to have an induction chamber where the induction of anesthesia happens.

The term "induction" is the onset of action of these anesthetic gases on the central nervous system. So, when you put the rat into an unconscious state, that is where the process of induction comes in. It is the first delivery of the anesthetic gases, where the rat is sedated or put to unconsciousness, and you will transfer the rat to where the maintenance of the anesthesia happens in the surgical apparatus where the gas delivery is assembled. So, induction is the first part, maintenance is the second part; that is very important to understand.

You will also have a lot of sterile isotonic saline or sterile water for injection, then crush ice or an ice pack, which is optional, and gloves for surgery and handling of the animals. So, these are the basic pieces of equipment that are required. When it comes to the inhalant anesthesia setup, which is the workhorse of the entire workflow of general anesthesia, you have two types of delivery: one is high-flow delivery, and another one is low-flow delivery.

This is the traditional vaporizer where there are high-pressure and high-flow gases that pass through the tubing, and here you have low flow. The difference is that the standard procedure will have a set flow rate in this high-flow vaporizer to a minimum of 500 ml per minute, and is important to know. The range is up to 1000 ml per minute, which is the flow rate, and the weight is calculated as 1.5 to 2 times the minute volume, which is calculated as per the body weight of the rat. For example, in a mouse that weighs around 30 grams, you need 26 ml per minute delivery, and that would come to approximately twice that or 50 to 60 ml per minute.

So, when you look at the minimum rate in this high-flow vaporizer, it is 500. So, this sort of vaporizer is used only for rats and is not compatible with the recommended flow rate of mice. That is an important thing to notice here. If your experiment involves mice, then this sort of wick-style vaporizer, where there is direct high pressure where the liquid is converted into a gas with a high flow of 500 to 1000 ml per minute, is not useful if your subject in the experiment is a mouse and not a rat, and this sort of vaporizer is always for rats and above. So, what do you do for small animals, something like mice?

This uses a syringe-driven digital vaporizer where the drug is loaded into the syringe rather than the canister. This is turned into a gas with minimal pressure compared to the high-pressure wick-style vaporizer. The drug is loaded into the syringe, and this sort of vaporizer is used for small animals. It uses room air or compressed gas, unlike the wick style, and it uses small amounts of liquid anesthetic; another huge difference. And it produces undetectable amounts of waste anesthesia gas. So, here, even if there is no scavenger, we can manage because the waste anesthetic gas is negligible, unlike the high flow. It delivers precise amounts of anesthesia and is useful if your delivery is only 0.3 to 0.5 ml per hour of isoflurane on a 30-gram mouse. Flow rates as low as 25 ml per minute can be achieved with the digital vaporizer. So, these are some of the specifics to be remembered before choosing your anesthetic setup for the experiment. We will not be using the drugs ketamine and xylazine, which are injectable anesthetics; isoflurane and sevoflurane are inhalational anesthetics.

So, this is the workflow in a nutshell, and an example of the SomnoSuite System has been used by the company Kent Scientific. The first step is to place the rat. Of course, before this, you will go through the process of selecting the animal for surgery, ruling out all the disease conditions, and choosing the healthiest subject. Once all that preliminary workup is done, you will let the rat acclimatize to the chamber for the initial 2 to 3 days; then try to consider the animal for the anesthetic workflow, provided all your surgical setups are ready.

Only then will you begin the anesthetic process, not the other way around, as I explained earlier. You will place the animal in the induction chamber, which is an acrylic box, glass, or polycarbonate, containing an inlet and an outlet. The inlet delivers the gas for the first time, and the rat inhales it and then goes into an unconscious state. That depends on the onset of action of that particular drug and the body weight of the rat. Once that induction happens, you are to switch to the actual behavioral setup or the experimental platform where the surgery will be performed.

Everywhere you see, an outlet is provided whenever there is an inlet. It does both scavenging and then this is the scavenger board, a system where the extra anesthetic gases are removed from the circulation and that is let out as waste. Using room air it creates the flow rate and maintains it. That, in a nutshell, is how the anesthetic flow works. And before that, you will be loading the drug into the syringe, which is digitally driven with a precise set flow rate. These are the different sizes in which the induction chamber is available, and this is how it would look. It affects the time required to induce the animal, and the user's exposure; this is very important to understand. The smaller the size, it holds a smaller amount of the anesthetic gas, and it might take a longer time to induce the rat. But it is an important component that you need to have.

So, now another major apparatus that is to be checked is the delivery system. How do you sort of deliver the anesthetic gas to the rat? There are various techniques, with the preferred one being to have a cone, which is an anesthetic mask, and this also comes in different sizes. Choose an appropriate size depending on the size of the rodent that you are planning to use. This will help to reduce staff exposure, along with the scavenger system, because the gas flows directly into the nostrils of the rat, and it also maintains the animal's plane of anesthesia with a constant flow rate. The distance is important. So, that is how the size is maintained. There should be a significant distance between the nostrils and the delivery system to prevent excessive wastage.

So, that is how the system works. The inflow happens along with oxygen, a major feature of all inhalation anesthesia, where oxygen is delivered along with the anesthetic gases, and the excess gas gets scavenged out. So, along with this, these are all monitoring systems. You will monitor the rectal temperature, and you will give fluid intake through the intravenous catheter if the surgery is long or if there are signs of dehydration—for that matter, for any surgery, it is always better to have intravenous access through which isotonic saline can be given—and the temperature and oxygen saturations are all monitored. It is good to have a properly fitting anesthetic mask with the general anesthesia equipment.

Another method of delivery is the stereotactic anesthetic mask. If your surgery is going to be on the brain or the spine, this is for the cervical cord. It gives very smooth anesthesia if you have this sort of setup, which is fitted into the stereotactic apparatus. If you remember from the previous classes, these are the nose and jaw bar sections of the stereotactic

apparatus. Right in front of it, the nostrils, or the nose of the rodent, go and fit in snugly where the anesthetic gas and oxygen are delivered.

These are the different ways of using it. So, this is where the nose goes, and then the nose bar and jaw bar are used for rigid fixation. So, this is another modification generally recommended for all your neural experiments, to use the stereotactic anesthetic mask, which does not take up extra space. It gives good maintenance of anesthesia throughout the procedure. When you are using injectable anesthesia, if the rat is fixed rigidly onto this stereotactic frame, you have to lift the hind paw to find the intraperitoneal space and inject it, which is very cumbersome, and you may not hit the intraperitoneal space if you are a beginner.

And then the rat wakes up, and it is difficult when the rat comes off the fixation points of the ear bars. It damages the brain, and probably even damages the implants. It is good to have the stereotactic anesthetic mask and the entire system built into the stereotactic apparatus. In inhalation anesthesia, a non-flammable liquid will be administered by vaporizing it into a usable gas, and that is done with the precision vaporizer in a low-flow system. For induction, the mouse or rat is placed in the anesthetic chamber, and a mixture of oxygen, which is 2 to 3 liters per minute for a rat, and isoflurane, which is 4 percent, is pumped into the chamber from the vaporizer.

Then the animal is anesthetized within 1 to 2 minutes; that is the usual duration for the onset of action, by which time the rat is usually put to sleep or unconscious state. Then the animal is removed from the induction chamber and then you transfer it to the stereotactic apparatus. And if anything needs to be maintained, which is the case most of the time, we will be using isoflurane if it is more than 15 to 20 minutes, if your surgery is prolonged; then it is always better to use inhalation anesthesia.

They can be maintained using a face or nose mask with isoflurane at 1 to 2.5 percent, and oxygen should be at 0.5 to 1 liter per minute. All these flow rates, dosages, and the strength of the drugs, have to be noted and these have to be used appropriately. Any difference in the mixture, or flow rate, affects the oxygenation of the rat and proves detrimental to the rodent's life. So, that is how, in a nutshell, inhalation anesthesia is maintained.

In this slide, I am going to explain the role of MAC. This is an important parameter when you are considering general anesthesia. It refers to the minimum alveolar concentration wherein 50 percent of the animals lose a motor response to a noxious stimulus. And that motor response is when you pinch the hind paw, usually, there is a withdrawal reflex. So, when you pinch the hind paw, there is a withdrawal, a flexion. That means the rat is still active and can perceive painful stimuli. In the lungs, the trachea becomes bronchi, principal bronchi, secondary, tertiary, small bronchioles, multiple bronchioles, and then come the alveoli. That is the end unit of the respiratory system where gaseous exchange happens.

MAC is the concentration of the anesthetic gas that flows down and reaches the alveoli where gas exchange is happening. The minimum concentration of that gas in the alveoli which will sedate or make the animals—50 percent of the animals—lose the motor response, being the withdrawal reflex, is the concept of minimum alveolar concentration. When you check that, it is different for different aspects of cerebral function. For example, if it is just, says, blunted autonomic reflexes, if it is about loss of motor response,

consciousness, loss of memory etc. So, this is how the plane of anesthesia becomes deeper and deeper.

Initially, it is just about the memory of events, then it loses consciousness, then it loses motor response to a noxious stimulus, and even autonomic functions like heart rate and respiratory rate—all this will get blunted, which is a dangerous level of anesthesia. Generally, we stop at the point where there should not be any motor response to noxious stimuli. What is the change that is happening? An increase in the MAC value from 0.25 to 1.5. So, as the MAC value keeps increasing, the plane becomes deeper and deeper, from loss of memory to blunted autonomic reflexes. If you see the vaporizer dial setting, the concentration of the gases that are used in the vaporizer is also going up. It starts from 0.3 to 0.5 percent required for loss of memory; it goes up to 2 to 2.5, 2.7 percent for blunted autonomic reflexes. This is the preferred setting, 1.3 to 1.8 percent of the vaporizer dial setting, which will only lead to loss of motor response to a noxious stimulus. These are the various parameters that can be looked at where cerebral function and spinal reflexes are anesthetized, but autonomic reflexes are intact. So this is important; whenever blood pressure falls, the heart rate increases.

We need that autonomic reflex, all right, not only to pick up that something is going wrong with the blood pressure, but it also compensates on its own, automatically. Automatically, the body switches to a higher heart rate when the blood pressure falls; that is the autonomic reflex which needs to be maintained throughout the surgery. But then you can bring the heart rate down if you can replace the volume with either isotonic fluid or, for that matter, even blood can be used. That is the importance of the minimum alveolar concentration.

As you all remember, the method of delivery, place, the animal needs to be placed in the clear-walled induction chamber, then you will turn the airflow to a level of 0.8 to 1.5 ml per minute, which will have 3 to 5 percent isoflurane; that is the dosage. And then, for continued delivery—this is for induction, this is for maintenance—for maintenance via the nose cones which are in the stereotactic apparatus, you will reduce the airflow to 0.4 to 0.8 ml per minute, and the concentration will also come down to 2 to 2.5 percent while monitoring for continued loss of reflexes along with sustained, steady tidal respiration; that is very important.

You have to look for the motor reflex, not the autonomic reflex. If, for example, with 2.5 percent itself the rat is losing respiration and blood pressure, which is the vitals, then immediately the concentration should be reduced to bring down the MAC, as I explained in the previous slide. It is important that vital monitoring is parallel with the maintenance aspect of the anesthesia. Then the entire experiment will go smoothly. These are the commonly used injectable general anesthetic drugs. The most common and favourite drug combinations are ketamine and xylazine, and the dose is 90 to 120 milligrams per kilogram of ketamine and 10 milligrams per kilogram of xylazine.

It can be given IP or IM. You have to remember from the abbreviations that we discussed, that it is intraperitoneal or intramuscular. We use the intraperitoneal route for these anesthetics. So, these are the other drugs, are dexmedetomidine, which is used along with ketamine, or you can add acepromazine, or pentobarbital. Acepromazine, diazepam, and xylazine are for sedation, that is, to put to sleep. However, "put to sleep" means where the animal is calm and relaxed, but not unconscious.

It is conscious; it will perceive pain. So, this may be preferred for the positioning stage, or putting the rat in the induction chamber, or if the animal is too wild and violent and not cooperative for a smaller procedure like drawing blood or giving an injection; then only these sedative drugs are used, where only sleep is induced, but consciousness is intact.

So, an injectable anesthetic is most commonly used if the required anesthesia time is only around 20 to 30 minutes. If it is anything beyond that, then it has to be either topped up with repeat injections, or you will have to use inhalational anesthesia. So, the problem with these injectable anesthetics is that they are pretty unpredictable.

We went through the disadvantages of injectable anesthesia in the last session, and that it is unpredictable and can have effects on the vital parameters, which is also unpredictable, where it can suppress the heart rate and bring down the blood pressure, and it can raise intracranial pressure. So, these are some of the side effects. The major difficulty is that if the rat is fixed to the stereotactic apparatus, to use this, the site to repeat the dosages is a big hassle, and it is a cumbersome procedure to repeat it again and again. And then those effects will vary from one injection to another injection.

The combination of ketamine and xylazine is a good combination of drugs if the procedure is only around 20 to 30 minutes because the entire preparation for inhalation anesthesia is huge, if you do not have the setup for general anesthesia, this is one thing that can be used as a last resort, especially if your procedure is only going to last for 15 to 20 minutes, preferably. This is how it is prepared: in a sterile 10 ml bottle with a rubber stopper, you need to mix 1 ml of ketamine with 0.1 ml of xylazine, then mix it with 8.9 ml of sterile water, and label it. This is very, very important because if you are using isotonic saline injection, and if you do not label it, you will switch the medicine, and give this beyond the recommended dosage, and the rat dies instantaneously. So, it is important to label all the drugs used in anesthetic procedures, or for that matter, in your entire surgical endeavor. Generally, the dose for a mouse is 0.1 ml per 10 grams of this mixture. First, you need to prepare this mixture, and then you will use 0.1 ml per 10 grams, preferably intraperitoneal, for comparatively smooth anesthetic properties. For a rat, you will be using 0.05 to 0.1 ml per 100 grams intraperitoneal, and you can use 0.1 ml per 100 grams of body weight intramuscularly as well. You will be using ophthalmic ointment before you begin the procedure. So, that is the end of discussing all the drugs used in anesthesia, both injectable and inhalational. These are all the pieces of equipment that are used, and these are the minimal requirements for general anesthesia usage, without which your setup is going to be compromised, and you will endanger the life of the rodent if any of these pieces of apparatus are lacking. If it is expensive, and if you cannot afford it, ketamine-xylazine is a wonderful combination of injectable anesthetics, especially if your procedure is a short one, lasting for around 20 to 30 minutes. So, let us look at the other aspects of anesthesia, especially vital monitoring and post-operative care, which are equally important, in the next session. Thank you.