Microsensors, Implantable Devices and Rodent Surgeries for Biomedical Applications
Course Instructor: Dr. Shabari Girishan
Department of Electronic Systems Engineering
Indian Institute of Science, Bangalore
Week - 09
Lecture - 36

So, hello everyone, welcome back to the session on Rodent Surgeries and Rodent Experiments for Neural Engineering. In today's session, we will try to cover a very important aspect of rodent experimental methodologies: anaesthesia in rodents. It is very important to understand what sort of agents need to be administered, what risks to the rodent's life arise from the drugs we use, and what methodology you need to employ that is suitable for your experiments. It is vital to understand the entire apparatus, how it works, and that there is a significant learning curve. There will be a lot of trial and error before you finalize your setup, and hopefully, this session should help you in choosing the right way to anaesthetize your rat. Since it requires a lot of investment in the setup, whether, for monitoring or anaesthesia (whether through injection or inhalation), it's essential to know if you need that apparatus and what the advantages and disadvantages are of each method of anaesthesia. As I said, this will be just a primer on the entire aspect of anaesthesia.

Hopefully, this session will help you set up a fairly good system for rodent anaesthesia. Here's how we're going to decide on the talk: In this particular session, let's try and look at the various definitions and abbreviations, then the pre-anesthetic evaluation, and pre-anesthetic care and preparation. All these techniques involve a lot of medical and anatomical terminology and jargon. However, you'll still be able to do it because these are techniques you need to replicate, which comes with practice.

In contrast, here you have to remember a lot of terms, and unless you are used to these regularly, it can be disastrous. Any mistake in dose calculation or miscalculation of drug timings, or if you choose the wrong drug and administer it incorrectly, can instantly kill the rat. That's why I cover this topic a bit more extensively. If you take away even 30 to 40 per cent from this session, it will benefit you in the long term. In the next session, we will cover anaesthetic equipment, administration, and anaesthesia vital monitoring. This is a big module similar to surgery because it is equally important; it will decide the morbidity and mortality of the rat. In surgery, if something goes wrong, you can pinpoint it.

However, in anaesthesia, if something goes wrong, it is very difficult to detect unless you have the right apparatus and setup. So, it is not only important to learn the techniques but

also essential to have the right equipment and tools to ensure the anaesthesia process goes smoothly.

With that brief introduction to this module, I will quickly take you through various definitions and abbreviations because we will be using them throughout the session, and you need to understand every aspect of this session. So, what is anaesthesia? The latter part of the word, “esthesia,” means sensation.

Anaesthesia is the loss of sensation. It is often accompanied by a loss of consciousness, brought about by controlled, reversible intoxication of parts of the nervous system. That’s the textbook definition, but in simpler words, there are two ways to induce anaesthesia. That’s why it’s essential to know what your objective is. If you want to make some part of the body numb, that’s local anaesthesia.

If you want to put the rat to sleep and make it unconscious, that’s general anaesthesia. General anaesthesia has three components. The most important component is that the rat is unconscious with adequate analgesia. These are mutually exclusive. It doesn’t mean that just because the rat is unconscious, it is not experiencing pain. This can be indicated by an increase in pulse rate.

All of you are familiar with the pulse rate—there’s a fixed rate, so many beats per minute—which we will discuss during the monitoring section. There must be adequate analgesia, and the rat must be unconscious. Another important component is that there is enough relaxation to allow for surgery—muscle relaxation. In rodent surgery, muscle relaxation is not usually a big deal except for spinal surgeries. In spinal surgeries, when you expose the spinal column, you will be dissecting a lot of muscles around it.

It is nice and easy to approach the spinal cord if the muscles are relaxed. This is where muscle relaxation matters. In cranial surgeries, there are not many muscles, practically none on the top of the skull. It is just skin and subcutaneous tissue, then the pericranium, and right through to the bone. But for the spinal canal, there are a lot of muscles along the spinal column, which you will see in the session on spinal surgery and spinal anatomy.

There are a lot of muscles around the area, so once you dissect them, you need to maintain them with retractors. You need adequate muscle relaxation. These are the three important components that distinguish general anaesthesia from local anaesthesia. I am sure all of you have visited the dentist before—what they give you is local infiltration anaesthesia, where only the gum is made numb.

So, that they can extract the tooth or perform some sort of root canal therapy or various surgical procedures. So, that is a classical example of local anaesthesia. In general,

anaesthesia, when they make the patient breathes those anaesthetizing gases, the patient or the animal goes unconscious, and that is classical general anaesthesia. But having said that, there are various injectable general anaesthetics. So, we will come to that in a moment.

So, that is about anaesthesia. How exactly is sedation different from anaesthesia, all right? In sedation, if you take a spectrum of consciousness, this falls in the lower limit of depression, all right? The higher the central depression, the higher the extent of unconsciousness, ok. You are just putting the animal to sleep, slightly unconscious, but still, it can perceive pain. Maybe the muscles are not fully relaxed, but it is sleepy—I mean, that is the right word.

So, a mild degree of central depression in a conscious but calm animal—that is sedation, all right? The animal is conscious, but it generally becomes more calm, activity goes down, and that is sedation. There is something also referred to as tranquilization, where you make the animal sort of, you know, cooperate—not generally out of its own will but because of the sedation. The animal will generally maintain a limb in a particular position, and you can maintain the body in a particular position when the animal is sedated. That is the whole point of using the word sedation. Another important aspect that you all need to learn and be familiar with is these abbreviations, which are standard and most commonly used in all medical practice and veterinary practice.

You will also see these in literature. So, I selected a few of them which are important. So, IM—these are all routes, all right? Routes of administration of various drugs. Intramuscular (IM), intraperitoneal (IP), intravenous (IV), simple.

This can be SQ or SA. PO is per os in Latin. So, oral drug, all right? When they say NPO, that is nil per os or oral, all right? That means you are going to keep the rat fasting, nil per oral. So, PO is also a pretty standard abbreviation. Q refers to every. So, where does this Q come in the dosage?

This particular drug has to be given every hour. So, do you say QH or just QH? So, this Q refers to every hour or every day. Once every hour or once a day—that is QH and QD. So, BID, SID—all these have come from Latin terminologies where BID stands for bis in die, all right?

SID refers to semel in die. These are Latin terminologies adopted in England. There are a lot of Latin usages in medical jargon, and that is how it becomes complex. But then, if you try to look at the literal meaning of what those Latin words mean, it makes more sense, and it is easier to remember. So, BID is twice, as in giving medication twice a day.

This can be written as BD or BID. BD refers to twice a day. SID is semel in die, so every medication is given once a day, right? SID is once a day. So, PRN refers to "as and when the situation demands," which is similar to SOS.

So, pro re nata and si opus sit. That is the actual expansion of these abbreviations, PRN and SOS. It is not the SOS of sailors, which is very different, all right? So, "save our souls" is another emergency beacon that comes in various situations. In medical terminology, SOS is si opus sit, which is giving medication as the situation demands. When they say, "You take this painkiller on an SOS or PRN basis," this is what it means: "as and when the situation demands."

NR simply means "not recommended," all right? So, these are some of the definitions and abbreviations that will keep coming your way while learning these new techniques, all right? So, anaesthesia—what exactly is it? Where exactly do the drugs act? As I said, it differs and depends on where you are going to use the drugs, for how long, and what route you are going to use. So, generally, if you take this classification, we use the classification as general and local, all right.

In general anaesthesia, you will have some sort of sedation or complete general anaesthesia. Whether you are injecting the drug intraperitoneally, intramuscularly, IV, or inhalational—whatever route you choose, based on the drug and based on the site of action of those drugs, it is divided into general and local, all rights. So, for general anaesthesia to act, it has to act on the central nervous system—I mean, always on the brain. It has to numb the brain and reduce activity in the brain to cause general anaesthesia, which will take care of analgesia and unconsciousness. The additional effect of these drugs is that muscle tone also comes down. The simple reason is that muscle tone is maintained by the higher-order neurons, wherein the motor cortex impulses reach the ventral horn cells in the spinal cord—in this case, the reticular activating system, specifically the vestibulospinal tract and the reticulospinal tract become numb. That is how muscle relaxation is also brought about.

There is also a direct action of muscle relaxants that are used along with general anaesthetic drugs, which will aid muscle relaxation, all right. So, in rodents, it is good to know that these are the injectable drugs: ketamine, acepromazine, dexmedetomidine—all these are injectable drugs. Isoflurane and sevoflurane are the gaseous forms of anaesthesia, all right. These are inhalational, others are injectable, and these drugs are generally depressants—not really that strong, but depressants. So, instead of using morphine, tramadol, and buprenorphine, these cause central analgesia, all right. So, this is the general breakdown for general anaesthesia and local anaesthesia. If the drugs act in the spinal subarachnoid space, then these are local anaesthetics.

So, you all remember the spinal anatomy we discussed. There is the spinal subarachnoid space; if the drug is injected into the spinal arachnoid space, it will act on the spinal cord and numb the second-order neuron, okay? Just to give you a recap of that anatomy: from the periphery to the spinal cord is the first order; the spinal cord to the brain is the second order, all right? No, sorry—the second order is until the thalamus, then from the thalamus onwards is the third-order neuron, all right? So, if it is acting on the first-order neuron or second-order neuron, you can use the spinal cord and the spinal subarachnoid space to numb the limbs, but the animal will still be conscious, all right. So, if you want to give a local infiltration, that will act by transduction, wherein the local nerve endings are anaesthetized, and then you can operate only in those areas where the anaesthesia has been infiltrated.

If you use it in the subarachnoid space, then both hind limbs will get anaesthetized; if you use it in the cervical space, all four limbs will be anaesthetized. If you are going to use it on the brain, then it is going to cause general anaesthesia, all right. So, that is an overview of the entire anaesthetizing agent, which we are going to discuss in detail. So, let us look at the differences between inhalational and injectable methods. In inhalational, as it is self-explanatory, the rat is going to inhale the various anaesthetic gases, and in an injectable method, you are going to supplement it with an additional drug that will act on the brain and cause general anaesthesia.

As I said, it can be used with inhalant gases or injectable drugs. Inhalant gas is the preferred method of anaesthesia whenever possible. We will come to the disadvantages and advantages of these two methods, but they give very smooth anaesthesia for a longer time—that is the basic advantage of inhalation anaesthesia. When inhalants are not possible, then various drugs are combined to induce anaesthesia. We have already gone through general and local anaesthesia, and within general anaesthesia, you have inhalation and injection methods. So, what are the advantages of injectable anaesthesia? It is definitely quick-acting, and there are many routes that we can use, but commonly we use intraperitoneal, wherein it causes systemic absorption. That is, from the peritoneum, it goes into the blood circulatory system, and through that, it reaches the brain, all right, to cause general anaesthesia.

Whatever route you use, what is going to be different is the bioavailability—that is, the extent of the drug reaching the site of action. In this case, the site of action is the brain, all right. If you give it subcutaneously, what is going to be different is the rate of transfer of the drug into the blood, which is going to be slow. So, there is a slow onset of action and slow delivery of the drug into the system. It might give us the benefit of long action, but then it is unpredictable, and we do not know when it is going to act because the thickness of subcutaneous fat is going to vary from rat to rat, all right.

So, generally, we prefer the intraperitoneal route, which will give us smooth induction as compared to the other techniques of IV and IM. IV is pretty dangerous because you are injecting a large amount of the drug into the blood directly, which can suppress the brain so much that breathing can also get compromised, all right. So, there are many routes, and it is easy to administer and does not require any special equipment—that is another major advantage of using injectable anaesthesia. Unfortunately, injectable anaesthesia is less preferred because of its significant physiological impact. For example, the drugs commonly used are ketamine and xylazine, all right.

These are the two most commonly used injectable general anaesthetic drugs, which have a significant impact on heart rate, all right, and intracranial pressure. Especially when you are doing brain surgery, it is going to matter a lot when you use ketamine and xylazine. That is the reason why, generally, inhalational anaesthesia is used for various central nervous system surgeries or neural surgeries, because it is going to have a big hindrance, and it can even cause the death of the rat if not used judiciously. Because it is going to cause central nervous depression, and you are usually going to operate on that depressed system, which is going to be detrimental to survival. And, of course, as I said, there is less control of variables.

You do not know to what extent it is going to suppress the heart or to what extent intracranial pressure is going to rise. It is difficult, really difficult unless you have a very good health monitoring system. Despite that, many rat deaths have been reported when such drugs are used, and they tend to take longer to recover because some drugs are going to stay in the system for a longer time, all right. Also, there is a variable effect from a single dose. The literature says the drug will stay for probably 45 minutes, but sometimes it quickly metabolizes, and the rat will start waking up in the middle of surgery within 15 to 20 minutes.

So, that sort of variable effect is reported with single doses and injectable anaesthesia. And, of course, there are significant differences in the dosing and in how much you need to dilute and where to inject based on the strain and gender of the rodent you are going to use, all right. Now, let us look at the advantages and disadvantages of inhalant anaesthesia. There is significantly less physiological impact, but there is good control over the anaesthetic depth, which is very important. We will look at the term called MAC—that is, minimum alveolar concentration, okay.

Alveoli, for the non-biological category of students, are the endpoint where the air ends and where the gaseous exchange happens. This is simple basic biological knowledge, where the lungs start from the trachea, then divide into bronchi, then divide into bronchioles, and terminate in the alveolar sac, where the gaseous exchange happens. So, there, the concentration of the drug matters a lot, and the exchange is smooth. So, the depth can be controlled when you look at the various parameters, and various neural

signals can be used to control the anaesthetic depth. Delivery usually involves oxygen, so the oxygen is taken care of, all right. That is a major attractive feature where it is generally mixed with oxygen. There is no worry about oxygen supply, especially when you give injectable anaesthetics and have to supplement oxygen, whereas in this case, delivery happens with oxygen, which makes this method very attractive, all right. The recovery time is quicker; as soon as you stop the flow of the gas, generally, the animal recovers within a predictable time range. Also, there is greater consistency between strains and genders. Of course, the major disadvantage is the cost of the equipment, which is the entry cost. It is a one-time investment, which eventually becomes cheaper to use if you look at the rats you are going to lose with injectable anaesthetics, the number of times you are going to use the drugs, and, of course, the delays and damages that can happen if the rat wakes up in the middle of the surgery and you need to give the injections again.

Another major difference in the injectable anaesthetic is that you are going to fix the rat in the stereotactic apparatus. We will deal with this when we discuss the method of delivery. We always say, look at the advantage offered by inhalation, but yes, the cost is a major problem, and it does require a scavenger system. This is something where the extra gases that spill over have to be scavenged by the various anaesthetic apparatuses that are required. If that does not function, it affects the operator, that is, the user, which is you. If your scavenger system is not good, you end up inhaling the doses, though they will be small since it is a small animal experiment. However, it has to have a robust system where the scavenger system takes up all the excessive anaesthetic gases, and then it requires good maintenance with annual recalibration.

So, those are the advantages and disadvantages. Let’s briefly have a walkthrough on the general anaesthesia aspect, where you need to have a state of total unconsciousness. Pre-anaesthetic care and evaluation are significantly important to achieve this sort of outcome and result. Choosing the animal and taking care of all these steps is a simple and doable task that will result in a really good outcome. Acclimatization is very important. You plan your experiments, and the animals need time to settle before you proceed. At least 48 hours is required after transportation to prevent the stress-related hormones from affecting the performance of the various anaesthetic drugs that you will give.

Generally, if you are transporting an animal, there is a step called quarantine. By the time you quarantine the animal and then subject it to experiment, this generally takes care of the acclimatization. Try to acclimatize the rat within your behavioural set of boxes once in a while and put the rat in the induction chamber itself. I am introducing the word "induction chamber," where the gases are introduced with oxygen to put the rat to sleep before you take it up and fix it in the stereotactic apparatus, where the continuous flow of anaesthesia happens. Acclimatization is very important, and how you select the animal for anaesthesia is very important.

Here comes the nutritional status of the rat. When the anaesthetic drug is given to the rat, there has to be a good overall nutritional score, which is important. It's called body conditioning. For example, BC-1 is a completely emaciated rat where you can see all the skeletal structures extremely prominently. This classification is available in the literature, and it helps a lot in choosing a well-conditioned BC-3 category, wherein your anaesthesia becomes smooth.

Obesity is also a problem. Emaciation is also a problem. Obese rats will have a lot of fat, and that takes in a lot of these gaseous agents, which stay in the fat. They recover slowly; induction is slow, there are a lot of changes, and obese rats are difficult to handle surgically. So, the rat should be neither over-conditioned nor under-conditioned, as emaciation will not withstand any extra physiological stress. The surgical and anaesthetic steps are quite stressful, and if the rat is already emaciated, it will not withstand any of these procedures.

So, it's very important to choose the right body conditioning. The rat you have chosen must be healthy and disease-free. How do you confirm that? There are various parameters, though some parts of the slides are missing. These parameters include overall general appearance, respiratory function, mucous membrane and skin condition, extent of hydration, and a body conditioning score between 2.5 and 3, which we just covered. The overall general appearance should be active, curious, and have a smooth fur coat, all of which indicate that the rat is healthy.

On the other hand, lethargy, a hunched posture, and a ruffled fur coat indicate that the rat is under some sort of stress or possibly disease. Breaths should not be noticeable, and there should be no discharge from the nares. For example, tachypnea refers to an increased respiratory rate. Tachypnea indicates increased respiratory effort, with deep, abnormal, shallow breaths, open-mouth breathing, or gasping. All of these symptoms indicate a poor physical state, likely due to systemic disease. The colour of the mucous membrane, especially around the nostrils, and inside the mouth (lips or tongue) should be pink.

If it is pale, blue, or bright red, it indicates systemic disease. The skin turgor, which we will cover during health monitoring, refers to tenting. When you pinch and release the skin, and the tent persists, it indicates dehydration. Piloerection, where the hairs are ruffled, and sunken eyes are also indicators.

Then there is the body condition scoring, which we discussed earlier. These are some of the physical examinations you must go through methodically, make a note of, and exclude the rat from the experimental procedure if necessary.

How do you prepare the rat for anaesthesia? There's something known as preoperative preparation and pre-anaesthetic preparation. Just before surgery, you paint and drape the

skin, and then drape the entire body of the rat. This is preoperative preparation. Similarly, for anaesthesia, you need to take care of the eyes. The eyes will remain open throughout the procedure when the rat is anaesthetized. You need to apply ophthalmic ointment and cover the eyes to prevent dryness and damage to the cornea. If the surgery is prolonged, you need to reapply it. Some part of the text is covered, but basically, you are infiltrating the local field with injectable anaesthesia (infiltration anaesthesia) to supplement and reduce the need for general anaesthesia. Pain can cause hyperventilation, leading to increased breathing, and activate the autonomic nervous system, causing the heart rate to go up.

To prevent this, it's always good practice, even if the rat is already anaesthetized, to infiltrate before cutting the skin. Another factor to consider is sensitivity to various anaesthetic agents. Age and body weight matter a lot. This is why you need to maintain all your rats at a particular age and body weight, so you don't have to calculate doses all the time, and inter-subject variation is minimal.

For example, bupivacaine requires dilution, and the dosage is different for mice and rats. Based on the weight of the rat, bupivacaine is diluted to 2.5 mg/ml with a maximum dose of 8 mg/kg. These are the various dosages available for bupivacaine. As you can see, as the weight increases, the demand for this agent also increases. So, maintaining the correct body weight consistently across experimental subjects is important.

You need to keep the animal warm throughout the surgical procedure because heat loss is rapid in anaesthetized rodents. As they breathe, they lose a lot of body heat. Warming the body is a very important aspect, and body temperature must be maintained. Hypothermia is one of the parameters in the triangle of death. The other two are acidosis and disseminated intravascular coagulation (DIC). These three features form the triangle of death. Hypothermia must be avoided at any cost by using an appropriate warmer. Most importantly, you should never leave an anaesthetized animal unattended.

If your surgical setup is not ready by the time of anaesthesia, you should ensure the entire surgical apparatus is ready so that you can quickly transfer the animal to the surgical apparatus and start the procedure. You don't want the anaesthetized animal to inhale excessive anaesthetic gases while you prepare the surgical setup after inducing anaesthesia.

That concludes today's session on pre-anaesthetic evaluation and care, which must be done before subjecting a rodent to general anaesthesia. In the next session, we will discuss in detail the anaesthetic setup required before you begin neural experiments or any rodent surgery.

This session is useful even if you're not doing full-fledged neural surgery, as any surgery requires a similar anaesthetic setup. It's important to have a vital monitoring setup and

general anaesthetic equipment. In the next session, we will go through the setup and then proceed to the actual general anaesthesia procedures. Thank you all.